

**Design and Fabrication of Small Molecule-Based
Supramolecular Systems for the Selective
Transmembrane Transport of Ions**

विद्या वाचस्पति की
उपाधि की अपेक्षाओं की आंशिक पूर्ति में प्रस्तुत शोध प्रबंध

*A thesis submitted in partial fulfilment of the requirements of the
degree of
Doctor of Philosophy*

द्वारा / By
Avisikta Upadhyay
अविसिक्ता उपाध्याय

पंजीकरण सं. / Registration No.: 20152022

शोध प्रबंध पर्यवेक्षक / Thesis Supervisor:

Prof. Pinaki Talukdar
प्रो पिनाकी तालुकदार



भारतीय विज्ञान शिक्षा एवं अनुसंधान संस्थान पुणे
INDIAN INSTITUTE OF SCIENCE EDUCATION AND RESEARCH
PUNE 2023

Certificate by Supervisor

I certify that the thesis entitled *Design and Fabrication of Small Molecule-Based Supramolecular Systems for the Selective Transmembrane Transport of Ions* presented by Ms. Avisikta Upadhyay represents her original work which was carried out by her at IISER, Pune under my guidance and supervision during the period from 01.01.2017 to 09.09.2023. The work presented here or any part of it has not been included in any other thesis submitted previously for the award of any degree or diploma from any other University or institutions. I further certify that the above statements made by her in regard to her thesis are correct to the best of my knowledge.

Date: 09. 09. 2023



Signature of the supervisor
(Prof. Pinaki Talukdar)

Declaration by Student

I declare that this written submission represents my idea in my own words and where others' ideas have been included; I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

The work reported in this thesis is the original work done by me under the guidance of Prof. Pinaki Talukdar.

Date: 09.03.2023

Arisikta Upadhyay
Signature of the student

Acknowledgment:

***This thesis is dedicated to my parents (Mr. Achintya Upadhyay and Mrs. Dipsikha Upadhyay), to those who dreamt for me,
AND The Almighty.***

I can never thank them enough for their unwavering love and support throughout my academic carrier. It is due to their unconditional trust and faith in me, that has inspired me to reach this position.

I am also immensely thankful to Sushama Di, who has always been like my second mother and my grandparents, Mrs. Jharna Roy, late Mr. Satyendra Nath Upadhyay, late Mr. Deepak Kumar Roy, late Mrs. Reba Upadhyay.

The road traversed from **Miss to Dr.** became an unforgettable journey of my life with a lot of memories. I would like to express my sincere gratitude to my supervisor Prof. Pinaki Talukdar for his support, encouragement, and his priceless suggestions during my entire Ph.D. tenure. The freedom he provided me with to execute my research projects in the best possible ways and the way he helped me overcome whatever problem I faced during this long journey is something worth remembering. During our conversations, I have grasped a lot about the planning and carrying out of my research, which has enabled me to develop a greater interest in doing research in the future. I can only state that without his leadership and steadfast encouragement, this thesis would not reach its finishing point. I would also like to express my sincere gratitude to my Research Advisory Committee (RAC), Prof. Harinath Chakrapani (IISER Pune) and Dr. Jayaraj Nithyanadhan (NCL, Pune), for their valuable suggestions during the RAC meetings. Through their suggestions, it became possible for me to push my boundaries beyond my thinking. The Department of Chemistry at IISER Pune will always hold a very special position in my heart for reasons galore. I express my sincere thanks to all my past and present lab members Dr. D.K., Dr. Sharad, Dr. Dinesh, Dr. Tanmoy, Dr. Arundhati, Dr. Sopan, Dr. Harshali, Dr. Javid, Dr. Debashis, Dr. Manzoor, Nihar, Naveen, Swati, Abhishek, Akram, Sandip, Anjana, Anurag, Debarati, Umesh, Parappa, Ravi, Paras, Kshitij, Ranjeet, Debraj, Soumyajit, Neelam, Shah Rukh, Ankit, Vishakha, Shweta, Shraddha, Tirth, Ronedy, Arjun, Anirban, Ahan, Shreya, Kavan, Sachin, Nileema, and Abhishek for maintaining a healthy and friendly environment in the lab.

I would like to express my special gratitude to Dr. Javid and Dr. Sopan who trained me during the initial phase of my Ph.D. tenure and were always a constant source of inspiration for me. I have always felt encouraged by them. The conversations we had greatly aided me in honing my research skills. I'm immensely grateful to Rashmi Di, for providing me with immense emotional and professional support throughout this journey. I extend my sincere acknowledgment to all the non-teaching staff including Ms. Swati, Mr. Mahesh (MALDI), Ms. Megha, Mr. Chinmay, Dr. Sandeep and Mr. Nitin (NMR), Mr. Tushar, Mr. Sandeep (HRMS), Mr. Mayuresh, Mr. Ganesh and Mr. Sanjay, for their constant support. Gourab and Javed gave me insightful inputs for me to scrutinise the details of the crystallographic structures. I'm especially thankful to Dr. Babu Varghese from IIT Madras who helped me in refining and finalising the crystal structures and Dr. Shilpy Sharma from University of Pune for all the biological studies.

I am also thankful to my batchmates from IISER, especially Shruti, Unmesh, Debesh, Deepak, Farhan, Rinku, Arindam, Naveen, Shailendra, Sunny, Yamini, Angira, Joyeeta, Shikha, Susovan, and Rutwik, with whom I have spent some precious and unforgettable moments. I am also very grateful to Sudeshna, Susmita, Abhirup, Moni Di, Udit Di, Nisarga, Kaustabh, Ayanendu, Swastika, Kittu, Sanchayita, Sukanya, and my IPhD brothers at IISER Pune who helped me weave a '*life outside the lab*'. Vineet and Puja, two of my school friends, deserve special mention, in this acknowledgement.

I am very grateful to all of my school teachers from Patha Bhavan, South Point and DPS, Ruby Park, for shaping me up. Rajiv Sir, Nishant Sir, Lokesh Sir and Manish Sir from my mentoring institution, Edudigm, deserve special mention among my teachers. St. Xavier's College, Kolkata, has bestowed me with a handful of amazing teachers, namely Dr. Rina Ghosh, Dr. Sanjib Ganguly, Dr. Ankur Roy, Dr. Ashish Nag, Dr. Indranil Chakraborty, Dr. Rahul Sharma, Dr. Manabendra Saha, and Dr. Dipankar Das, who have always encouraged me to thrive and embark on this difficult journey.

Finally, I would like to thank all those people whose names I might have missed here, for their unconditional support.

A good PhD is a finished PhD.

This LONG roller-coaster ride was indeed a very memorable one!!!

Table of Contents

1. Membrane Transport

1.1. Cell

1.2. Cell Membrane

1.2.1. Components of cell membrane

1.3. Mechanism of ion transport

1.3.1. Active ion transport

1.3.1.1. Primary active ion transport

1.3.1.2. Secondary active ion transport

1.3.2. Passive ion transport

1.3.2.1. Diffusion

1.3.2.2. Facilitated diffusion

1.3.3. Uniport

1.3.4. Antiport

1.3.5. Symport

1.4. Supramolecular interactions (non-covalent interactions)

1.4.1. Hydrogen bonding

1.4.2. van der Waal's interaction

1.4.3. Electrostatic interactions

1.4.4. Hydrophobic interactions

1.4.4.1. π - π Stacking interactions

1.4.4.2. Metal-ligand coordination

1.4.4.3. Host-guest interactions

1.4.4.4. π - π^* interactions

1.4.4.5. Anion- π^* interactions

1.5. Natural ion transporters

Table of Contents

- 1.6. Design principles for synthetic ion transporters
 - 1.6.1. Mimicking the structure of natural carriers
 - 1.6.2. Selectivity
 - 1.6.3. Hydrophobicity
- 1.7. Types of ion channels
 - 1.7.1. Unimolecular ion channels
 - 1.7.2. Barrel-hoop ion channels
 - 1.7.3. Barrel-stave ion channels
 - 1.7.4. Barrel-rosette ion channels
- 1.8. Importance of transmembrane ion transport
- 1.9. Techniques to study ion transport mechanism
 - 1.9.1 Vesicles mimicking the lipid bilayer membrane
 - 1.9.2 NMR-based assay
 - 1.9.3. Ion transport study in planar bilayer
 - 1.9.4. ISE-based assay
- 1.10. References
- 2. Triazol-benzamide-Based Small Molecules as Nontoxic Cl⁻ Transporters**
 - 2.1. Introduction
 - 2.2. Results and discussion
 - 2.2.1. General methods
 - 2.2.2. Physical measurements
 - 2.2.3. Synthesis
 - 2.2.4. NMR titration studies
 - 2.2.5. Theoretical calculations
 - 2.2.6. Ion transport studies

Table of Contents

- 2.2.6.1. Buffer and stock solution preparation
- 2.2.6.2. Preparation of EYPC-LUVs $\Rightarrow$ HPTS
- 2.2.6.3. Ion transport activity
- 2.2.7. Determination of ion selectivity by HPTS assay
 - 2.2.7.1. Buffer and stock solution preparation
 - 2.2.7.2. Preparation of EYPC-LUVs $\Rightarrow$ HPTS for cation selectivity
 - 2.2.7.3. Preparation of EYPC-LUVs $\Rightarrow$ HPTS for anion selectivity
 - 2.2.7.4. Anion and cation selectivity assays
- 2.2.8. Chloride Selectivity of 1d with the ion-selective electrode (ISE)
 - 2.2.8.1. Preparation of EYPC-LUVs for chloride efflux assay
 - 2.2.8.2. Determination of chloride efflux by ISE
- 2.2.9. Ion transport mechanism study by HPTS assay
 - 2.2.9.1. Preparation of buffer and stock solution for FCCP and Valinomycin assays
 - 2.2.9.2. Preparation of vesicles for FCCP and Valinomycin assays
 - 2.2.9.3. Ion transport activity in the presence of FCCP
 - 2.2.9.4. Ion transport activity in the presence of Valinomycin
- 2.2.10. Evidence of membrane integrity using CF assay
 - 2.2.10.1. Preparation of EYPC-LUVs $\Rightarrow$ CF
 - 2.2.10.2. CF leakage assay
- 2.2.11. Electrogenic mechanism of ion transport by compound 1d
 - 2.2.11.1. Preparation of EYPC-LUVs for chloride efflux assay
 - 2.2.11.2. Determination of chloride efflux by ISE
- 2.2.12. Carrier mechanism of ion transport, the U-tube experiment

Table of Contents

2.2.13. Critical aggregation concentration studies

2.2.13.1. UV-vis studies

2.2.14. Biological studies

2.2.14.1. Cell culture protocol

2.2.14.2. Measurement of intracellular Cl^- concentrations

2.2.14.3. MTT-based cytotoxicity assay

2.2.15. NMR spectra

2.3. References

3. Quinoline-based Small Molecules as Transmembrane K^+/H^+ Transporters

3.1. Introduction

3.2. Results and discussion

3.2.1. General methods

3.2.2. Physical measurements

3.2.3. Synthesis

3.2.4. Single crystal X-ray diffraction (SCXRD) studies

3.2.5. Theoretical studies

3.2.6. Ion transport studies

3.2.6.1. Buffer and stock solution preparation

3.2.6.2. Preparation of EYPC-LUVs $\Rightarrow$ HPTS

3.2.6.3. Ion transport activity

3.2.7. Determination of ion selectivity by HPTS assay

3.2.7.1. Buffer and stock solution preparation

3.2.7.2. Preparation of EYPC-LUVs $\Rightarrow$ HPTS for cation selectivity

3.2.7.3. Preparation of EYPC-LUVs $\Rightarrow$ HPTS for anion selectivity

Table of Contents

- 3.2.7.4. Anion and cation selectivity assays
- 3.2.8. Ion transport studies in the presence of KCl buffer
 - 3.2.8.1. Buffer and stock solution preparation
 - 3.2.8.2. Preparation of EYPC-LUVs $\Rightarrow$ HPTS
 - 3.2.8.3. Ion transport activity
- 3.2.9. Chloride transport activity across EYPC-LUVs $\Rightarrow$ Lucigenin vesicles
 - 3.2.9.1. Preparation of EYPC-LUVs $\Rightarrow$ Lucigenin vesicles
 - 3.2.9.2. Ion transport activity by Lucigenin assay
- 3.2.10. Ion transport mechanism study by HPTS assay
 - 3.2.10.1. Preparation of buffer and stock solution for FCCP and Valinomycin assay
 - 3.2.10.2. Preparation of vesicles for FCCP and Valinomycin assays
 - 3.2.10.3. Ion transport activity in the presence of FCCP
 - 3.2.10.4. Ion transport activity in the presence of Valinomycin
- 3.2.11. Evidence of membrane integrity using CF assay
 - 3.2.11.1. Preparation of EYPC-LUVs $\Rightarrow$ CF
 - 3.2.11.2. CF leakage assay
- 3.2.12. UV-visible titration studies
- 3.2.13. Liquid-liquid extraction experiment to validate K⁺ transport
 - 3.2.13.1. UV-visible studies
 - 3.2.13.2. ¹H-NMR and ESI-MS studies
- 3.2.14. ³⁹K NMR experiment to validate K⁺ transport
 - 3.2.14.1. Preparation of vesicles and shift reagent

Table of Contents

3.2.14.2. Experimental procedure for ^{39}K NMR experiment with 1a

3.2.15. NMR spectra

3.3. References

4. Mandelic-acid Based Small Molecules as K^+/Cl^- Symporters

4.1. Introduction

4.2. Results and discussion

4.2.1. General methods

4.2.2. Physical measurements

4.2.3. Synthesis

4.2.4. Ion transport studies

4.2.4.1. Buffer and stock solution preparation

4.2.4.2. Preparation of EYPC-LUVs $\Rightarrow$ HPTS

4.2.4.3. Comparison of ion transport activities

4.2.4.5. Calculation of EC_{50} and Hill-coefficient (n) values

4.2.5. Determination of ion selectivity

4.2.5.1. Buffer and stock solution preparation

4.2.5.2. Preparation of EYPC-LUVs $\Rightarrow$ HPTS for cation selectivity

4.2.5.3. Preparation of EYPC-LUVs $\Rightarrow$ HPTS for anion selectivity

4.2.5.4. Anion and cation selectivity assays

4.2.6. Determination of mechanism of ion transport by HPTS assay

4.2.6.1. Preparation of vesicles, buffer and stock solutions for FCCP and Valinomycin assays

Table of Contents

4.2.6.2. Ion transport mechanism study in the presence of FCCP and valinomycin

4.2.7. Chloride transport activity across EYPC–LUVs⊃Lucigenin vesicles

4.2.7.1. Preparation of EYPC–LUVs⊃Lucigenin vesicles

4.2.7.2. Ion transport activity by Lucigenin assay

4.2.8. Determination of mechanism of ion transport using Lucigenin assay

4.2.7.1. Preparation of EYPC–LUVs⊃Lucigenin vesicles

4.2.7.2. Determination of the symport mechanism

4.2.9. Estimation of integrity of bilayer membrane by CF leakage assay

4.2.9.1. Preparation of EYPC–LUVs⊃CF

4.2.9.2. CF leakage assay

4.2.10. Single crystal X-ray diffraction (SCXRD) studies

4.2.11. Planar bilayer conductance measurements

4.2.11.1. Calculation of average ion channel diameter

4.2.12. Biological data

4.2.12.1. Cell culture and cell lines used

4.2.12.2. Assessment of cellular viability by MTT assay

4.2.13. NMR data

4.3. References

List of Abbreviations

A

α	Alpha
Å	Angstrom
abs	Absorbance
A ⁻	Anion
Aq.	Aqueous
Ar	Aromatic
Ac	Acetyl
ACN	Acetonitrile
ADP	Adenosine di-phosphate
ATP	Adenosine tri-phosphate

B

β	Beta
br	Broad singlet
BLM	Bilayer lipid membrane or Black lipid membrane
<i>BE</i>	Binding energy
BSSE	Basis set superposition error

C

<i>c</i>	Concentration
Δ	Change in
Conc.	Concentration
Calc	Calculated
CF	Carboxyfluorescein
CHCl ₃	Chloroform
CH ₂ Cl ₂	Dichloromethane
CDCl ₃	Deuterated chloroform
CH ₃ CN	Acetonitrile
CLC	Chloride channel
CLCA	Calcium-activated chloride channel

List of Abbreviations

cm	Centimeter
CsCl	Cesium chloride

D

δ	Delta (chemical shift in ppm)
°C	Degree celsius
<i>d</i>	Diameter
d	Doublet
dd	Doublet of doublet
DPhPC	2-diphytanoyl- <i>sn</i> -glycero-3-phosphocholine
DMSO	Dimethylsulfoxide
DMF	Dimethylformamide
DCM	Dichloromethane
DFT	Density functional theory
dt	Doublet of triplet
dd	Doublet of quartet
DMEM	Dulbecco's Modified Eagle Medium
DMAP	4-Dimethylaminopyridine

E

EYPC	L- α -phosphatidylcholine from egg-yolk
<i>EC</i> ₅₀	Effective concentration at half maximal activity
e.g.	<i>Exempli gratia</i>
em	Emission
EtOAc	Ethylacetate
EtOH	Ethanol
Et ₃ N	Triethylamine
EDC·HCl	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
ESI	Electrospray ionization

List of Abbreviations

F

F_t	Fluorescence intensity at time t
FCCP	Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone

G

g	Gram
g	Corrected conductance
G	Free energy
G_{hyd}	Free energy of hydration

H

Hz	Hertz
h	Hour
HPTS	8-Hydroxypyrene-1,3,6-trisulfonate trisodium salt
HOBt	Hydroxybenotriazole
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
HF	Hartree fock

I

I_f	Normalized fluorescence intensity
IR	Infrared spectroscopy
ISE	Ion selective electrode

J

J	Coupling constant
-----	-------------------

List of Abbreviations

K

k	Rate constant
kcal	Kilocalorie
KBr	Potassium bromide
KCl	Potassium chloride
KOH	Potassium hydroxide
k_{obs}	Observed rate constant

L

λ	Lambda
LAH	Lithium aluminium hydride
LiCl	Lithium chloride
LUV	Large unilamellar vesicle
logP	Partition coefficient
ln	Log
LiOH	Lithium hydroxide

M

m	Multiplet
M	Molar
μM	Micromolar
μL	Microliter
M.P.	Melting point
MHz	Mega hertz
min	Minute(s)
max	Maximum
mg	Milligram(s)
MD	Molecular dynamics

List of Abbreviations

mol	Mole(s)
mmol	Millimole(s)
mM	Millimolar
mL	Milliliter
MeOH	Methanol
m/z	Mass to charge ratio
mV	Millivolt
M.P.	Melting Point
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
Me	Methyl

N

n	Hill coefficient
N	Normal
nM	Nanomolar
NMR	Nuclear magnetic resonance
Na ₂ SO ₄	Sodium sulfate
NaBr	Sodium bromide
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NaF	Sodium fluoride
NaNO ₃	Sodium nitrate
NaOAc	Sodium acetate
NaOH	Sodium hydroxide
NaClO ₄	Sodium perchlorate
NaI	Sodium iodide

O

obs	Observed
-----	----------

List of Abbreviations

ORTEP Oak ridge thermal ellipsoid plot

P

pA Picoampere

P *para*

Ph Phenyl

ppm Parts per million

pM Picomolar

p*K*_a Acid dissociation constant

Pi Inorganic phosphate

R

R Ideal gas constant

ρ Resistivity

RT Room temperature

RbCl Rubidium chloride

S

S Second

SCXRD Single crystal X-ray diffraction

T

T Triplet

Td Triplet of doublet

t Time

T_x Triton X-100

TBA Tetrabutyl ammonium

TLC Thin layer chromatography

THF Tetrahydrofuran

List of Abbreviations

TFA	Trifluoroacetic acid
TBAPF ₆	Tetrabutylammonium hexafluorophosphate

U

UV	Ultra-violet
----	--------------

V

V_m	Membrane potential
vis.	Visible
V	Volt
Val	Valinomycin

W

wt.	Weight
-----	--------

Y

Y	Fractional fluorescence intensity
-----	-----------------------------------

Z

ZPE	Zero-point energy
-----	-------------------

The thesis titled “***Design and Fabrication of Small Molecule-Based Supramolecular Systems for the Selective Transmembrane Transport of Ions***” comprises of four chapters.

Synopsis:

The goal of the thesis is to create artificial molecules that can recognize and transport ions across phospholipid membranes. Ion transporters are important throughout many facets of life and those that are naturally occurring have numerous biological uses like antibiotics and antimalarials. The use of natural and synthetic ion transporters as anticancer medicines has become possible because of recent advancements in the field of ion transport research. The goal of the thesis work was to understand the mechanism by which these molecules transport ions across vesicular membranes by evaluating the behaviour of specific small organic molecules as anion or cation receptors or as co-transporters by ^1H NMR, ^{39}K NMR and dye-entrapped vesicular methods. We also attempted to evaluate the cell viability of some cancerous and some non-cancerous cell lines in the presence of these compounds and how the chloride ion is being uptaken by these compounds in the cell.

Chapter 1: Introduction to Membrane Transport:

Membrane transport in cellular biology refers to a group of mechanisms that control the passage of solute particles through biological membranes, including ions, tiny, uncharged polar molecules, and larger charged and uncharged molecules. Lipid molecules make up biological membranes, which serve as the protective coverings for cells and cellular organelles. Simple diffusion allows tiny, uncharged, polar or nonpolar molecules to flow through the membrane with ease. Examples are O_2 , CO_2 , H_2O , and NH_3 . On the other hand, due to the high thermodynamic barrier of the lipid membrane, ions and highly charged compounds like sugars or amino acids cannot get through the membrane. However, the movement of these molecules is crucial to the efficient operation of live cells. Since these crucial molecules must be transported through the lipid bilayer membrane, nature has created specific molecules called ion channels, ion carriers, ion pumps, and ion pores. Ion transport is facilitated by molecules called ion carriers that may move back and forth across the membrane. On the other hand, ion channels offer the static hydrophilic route across the lipid bilayer membrane where ion transport takes place. Additionally, a process known as passive ion transport involves the movement of ions through ion carriers and ion channels down the concentration gradient without the use of energy. It is also possible for the ion transport to take place against the concentration gradient via a procedure known as active transport. Ion pumps are used for this

particular sort of ion transfer. Larger molecules such as amino acids and carbohydrates are transported through ion pores, which are channels with a larger lumen. The transport of chloride, which is the most prevalent ion among all others and is crucial for maintaining the membrane potential, cell volume regulation, trans epithelial salt transfer, the cell cycle, apoptosis, and other processes, typically takes place through a particular chloride ion channel. Cystic fibrosis, Bartters syndrome, myotonia congenita, and other serious disorders known as "channelopathies" can result from malfunction of chloride channels or the genes that control these channels. In this context, "channel replacement therapy" is a method for treating these disorders that involves replacing these defective ion channels with artificial ion carriers. The potassium ion plays an important role in cell membrane physiology and helps regulate fluid balance, muscle contractions and nerve signals. Imbalance in the level of potassium can cause fatal diseases such as Hypokalaemia, high blood pressure, constipation, muscle weakness, and fatigue.

Prodigosin, tembjamine, and sphingolipids are examples of natural ion transporters that have demonstrated promising anticancer applications. There are also several reports of man-made mimics that exhibit potential therapeutic applications as anticancer agents.

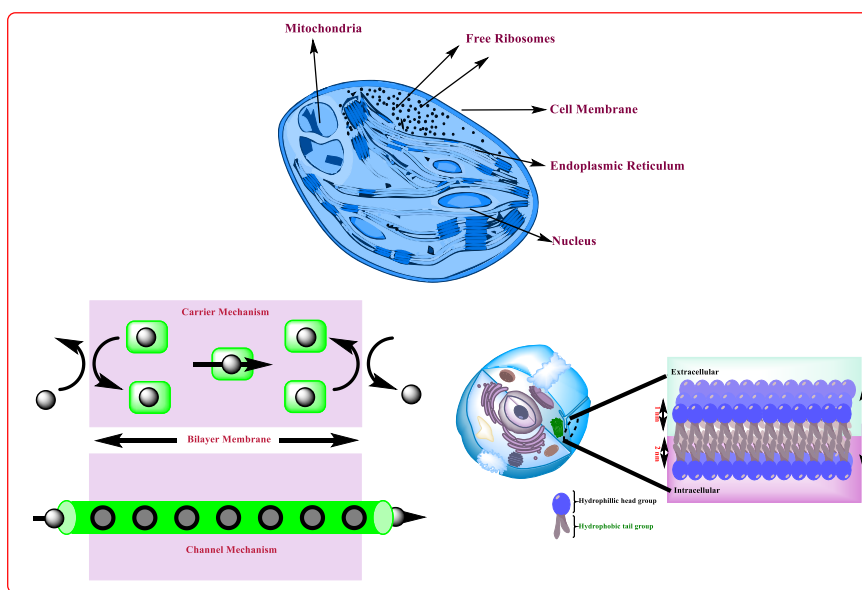


Figure 1. Schematic of the components of the cell and the types of ion transport through the cell membrane.

2: Triazole-benzamide-Based Small Molecules as Nontoxic Cl⁻ Transporters:

In this chapter, a series of triazole-appended benzamide-based anion receptors **1a-1e** were designed and synthesized. The lipophilic balance was varied by changing the substitution (R

group) on the aryl ring. ^1H NMR titration experiment of **1d**, the most efficient ion transporter, with tetrabutylammonium chloride was carried out to confirm the chloride recognition and involved interactions in the binding of the ion. The ion transport activity study was performed across large unilamellar vesicles with entrapped 8-hydroxypyrene-1,3,6- trisulfonic acid trisodium salt as a fluorescent dye (i.e., EYPC-LUVs $\Rightarrow$ HPTS) and ion-selectivity studies performed by varying the extravesicular anions and cations indicated that the transporters were Cl^- selective. The chloride selective ion transport was determined by ISE based assay. Monensin and valinomycin-coupled assays confirmed the electrogenic transport mechanism. Moreover, the antiport mechanism of chloride transport was confirmed by FCCP and valinomycin assays. Evidence of the membrane integrity was also confirmed by the CF-leakage assay. UV-Vis studies were also carried out to eliminate the possible self-aggregation as the cause of non-toxicity at elevated concentrations. At elevated concentrations, these transporters, which were proved to act as carriers by a well-known U-tube assay, were shown to uptake Cl^- ions in the cells by the MQAE assay and show considerably less toxicity to the cancerous MCF-7 and HeLa cell lines. Further, we investigated the effect of such Cl^- transport on cell viability. The HEK293T, HeLa, and MCF-7 cells were incubated with **1b**, **1c**, and **1d** for 24 hours, followed by evaluation of cell viability using MTT assay. The results showed the less toxic behavior of **1b**, **1c**, and **1d**, while, when the viability of MCF-7 cells was evaluated in presence of the **2**, significant cell death was observed. These results indicated that the substitution of one carboxamide moiety with its bioisostere 1,2,3-triazole, leads to the lesser toxic behavior of the 3-(1*H*-1,2,3-triazol-1-yl) benzamides.

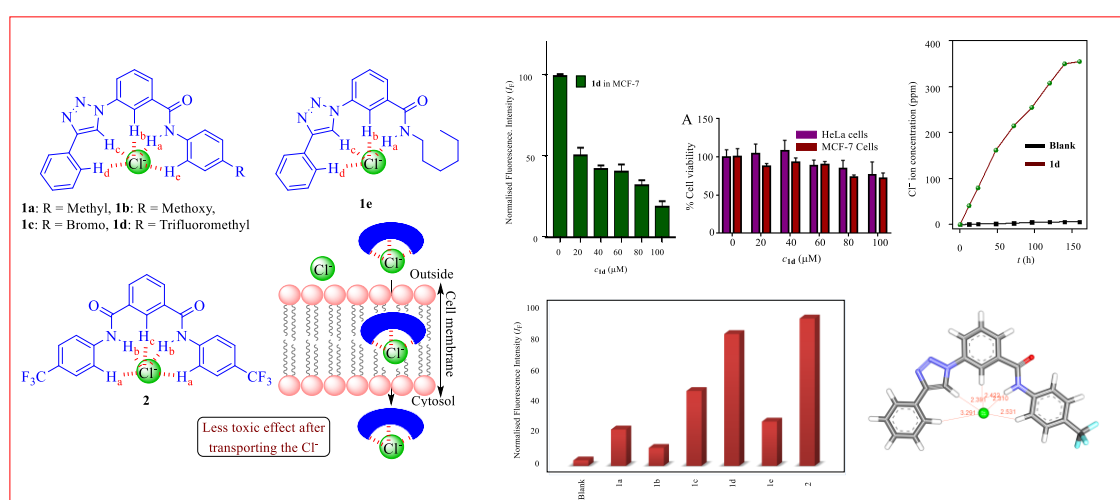


Figure 2. Structures of transporter molecules **1a**–**1e** and representative anion transport across the cell membrane.

3: Quinoline-Based Small Molecules as Transmembrane K^+/H^+ Transporters:

In this chapter, we report a series of small molecules **1a-1c** possessing a quinoline core that selectively bind to and aid the transmembrane transport of K^+ ion. In order to achieve a better tunability in terms of the partition coefficient values, the different substituents on the phenyl ring was varied, to aid the transmembrane transport of these small molecules. The ion transport activity study was performed across large unilamellar vesicles with entrapped 8-hydroxypyrene-1,3,6- trisulfonic acid trisodium salt as a fluorescent dye (i.e., EYPC-LUVs $\supset$ HPTS) by varying the NaCl and KCl buffers. The ion transporting abilities corroborated to the selectivity order obtained by varying the extravesicular cations and anions. The fact that Cl^- influx did not occur was further confirmed by the LUVs entrapped with a halide sensitive dye called Lucigenin. (i.e., EYPC-LUVs $\supset$ HPTS). Binding to the biologically relevant potassium and sodium cations was reiterated through UV-Vis studies with binding constants of $1.25 \text{ M}^{-1} \pm 7 \%$ in 1:1 receptor to cation (K^+) binding model, $1.26 \times 10^7 \text{ M}^{-2} \pm 6 \%$ in 2:1 receptor to cation (K^+) binding model and $113.33 \text{ M}^{-1} \pm 2 \%$ in 1:1 receptor to cation (Na^+) binding models. The process of K^+/H^+ antiport was assessed via FCCP and Valinomycin assays. Evidence of the membrane integrity was also confirmed by the CF-leakage assay. Further, the binding and transport of K^+ by the most efficient transporter **1a** was validated through 1H NMR, ESI-MS and the very coveted ^{39}K NMR studies.

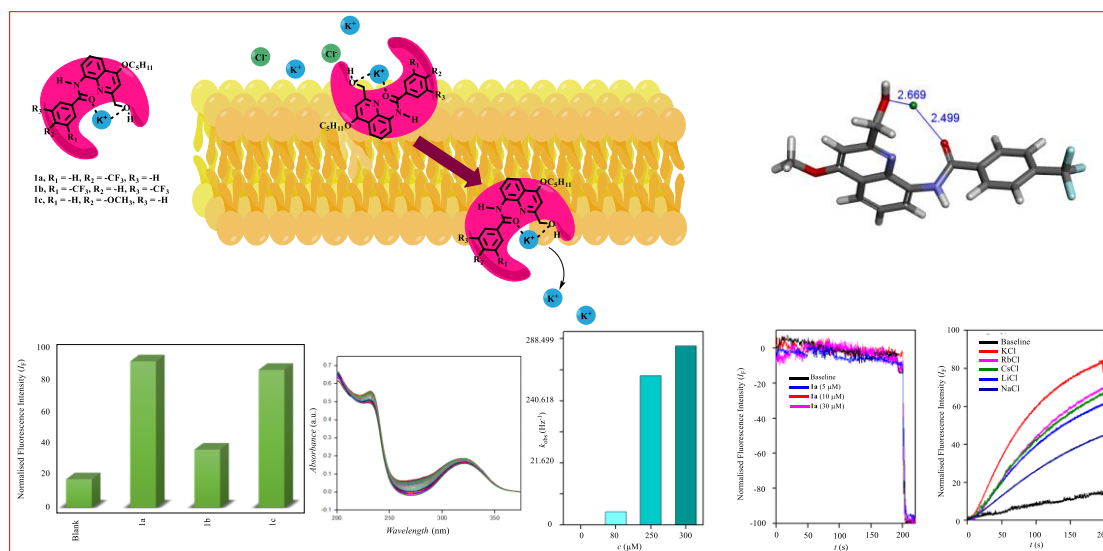


Figure 3. Structure of transporter molecules and their representative cation transport across the cell membrane.

4: Mandelic Acid-Based Small Molecules as K^+/Cl^- Symporters:

Intrigued by the hexameric crystal structures that were obtained with Mandelic acid as the core as reported by Haino and co-workers, we reflected on using the same moiety, to report a series of small molecules **1a-1c** based on the same core, which were hypothesized to form self-assembled barrel-rosette channel systems. It was proposed that the intermolecular hydrogen bonding and the π - π interactions would provide a pre-organization to the individual structures that would form an active channel system in the bilayer membrane. The possible mode of binding and co-transport of the cation and the anion could be through the cation- π interaction of the cation to the pi cloud of the aromatic benzene ring and the hydrogen bonding interactions of the anion with the hydroxyl $-OH$ and the amide $-NH$. The ion transport activity study was performed across large unilamellar vesicles with entrapped 8-hydroxypyrene-1,3,6- trisulfonic acid trisodium salt as a fluorescent dye (i.e., EYPC-LUVs $\Rightarrow$ HPTS) and ion-selectivity studies performed by varying the extravesicular anions and cations indicated that the transporters were preferentially selective towards K^+ and Cl^- ions. This mechanism of symport was further confirmed by HPTS-coupled FCCP, Valinomycin and Lucigenin-coupled Valinomycin assays. Indication of formation of a channel could be seen through preliminary BLM studies. The three tested compounds (**1a-c**) were found to be cytotoxic to the breast cancer MCF-7 cells and to the non-cancerous HEK293T cell. Thus, the compounds did not show selective toxicity in the cancerous cells. However, these studies are preliminary and further detailed biological studies need to be planned in the future to assess the mechanism of cell death in the MCF-7 cells

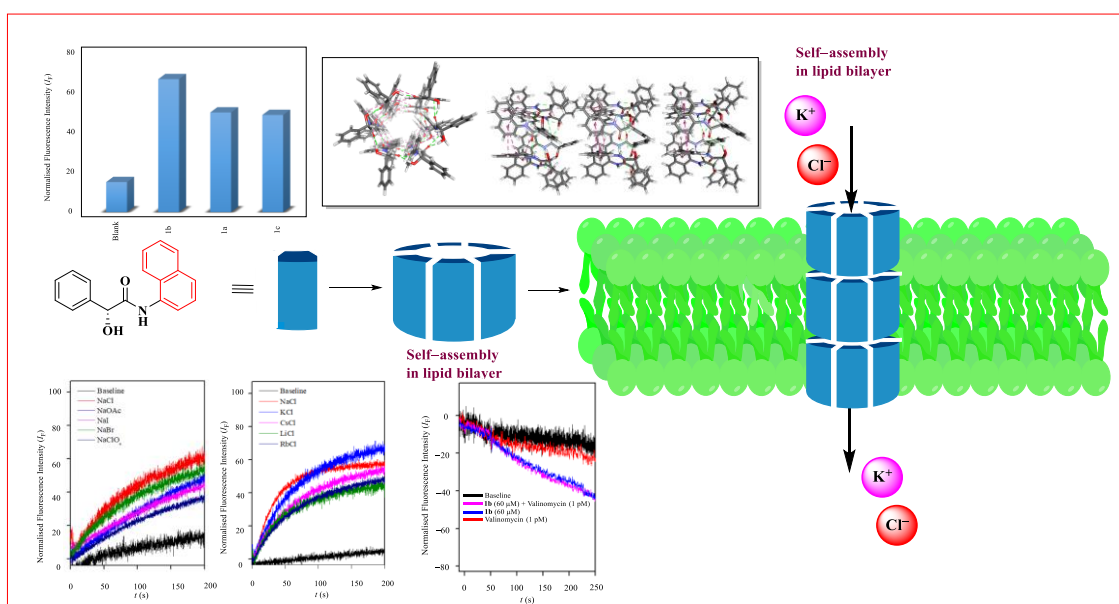


Figure 4. Structure of the channel molecules and their representative co-transport across the cell membrane.

List of publications:

Included in the Thesis:

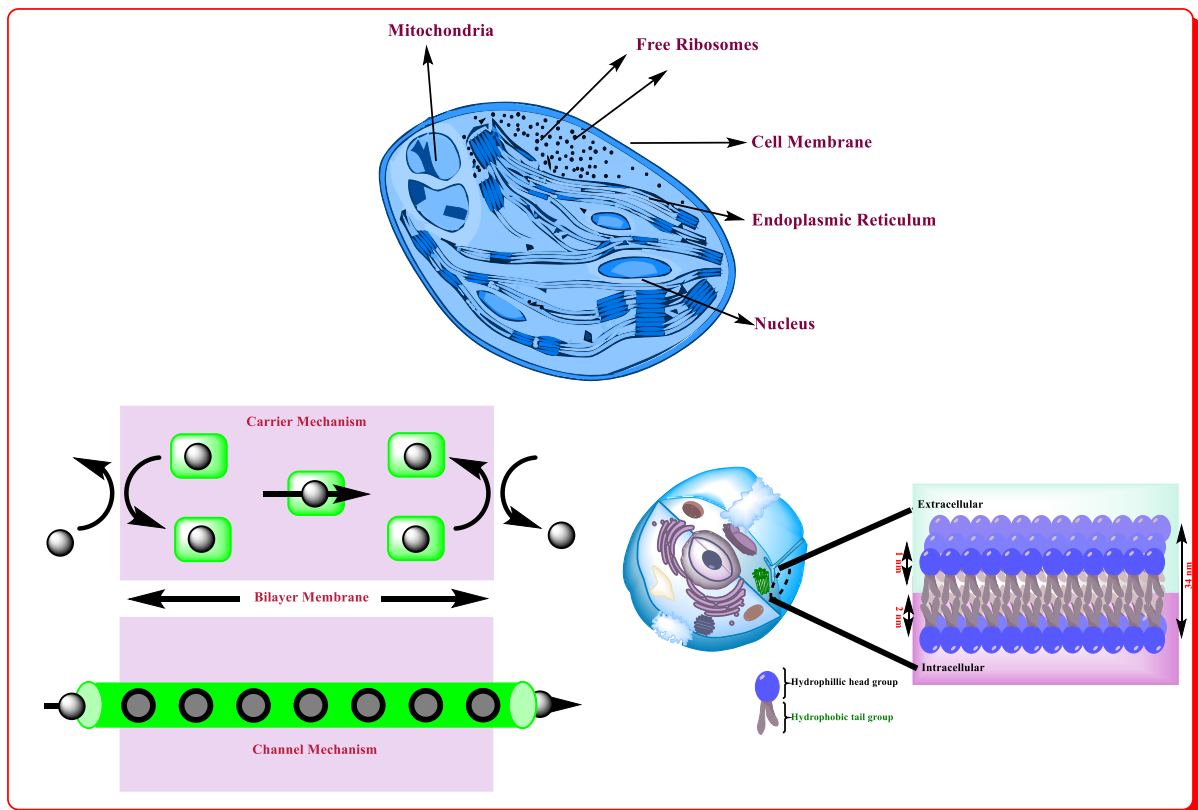
- Chloride Transport across Liposomes and Cells by Non-toxic 3-(1*H*-1,2,3-Triazol-1-yl) benzamides; Javid Ahmad Malla, **Avisikta Upadhyay**, Pulak Ghosh, Debashis Mondal, Abhishek Mondal, Shilpi Sharma, Pinaki Talukdar*, *Org. Lett.* **2022**, 24(23), 4124–4128.
- Quinoline-Based Small Molecules as Transmembrane K⁺/H⁺ Transporters; Avisikta Upadhyay, Sandip Chattopadhyay, Pinaki Talukdar*, *Manuscript under preparation*.
- Mandelic Acid-Based Small Molecules as K⁺/Cl[−] Symporting Channels; Avisikta Upadhyay, Sandip Chattopadhyay, Rashmi Sharma, Pinaki Talukdar*, *Manuscript under preparation*.

Not included in the Thesis:

- Anion Recognition through Multivalent C–H Hydrogen Bonds: Anion-Induced Foldamer Formation and Transport across Phospholipid Membranes; Debashis Mondal, Manzoor Ahmad, Prakash Panwaria, Avisikta Upadhyay, *J. Org. Chem.* **2022**, 87(1), 10–17.

Chapter 1

Introduction



1. Membrane transport:

1.1. Cell:

Small basic unit cells make up every type of organism on the planet earth, whether they are tiny prokaryotic cells or massive multicellular organisms. The essential structural and operational building blocks of living things are these biological cells. They contain essential elements, such as proteins, lipids, carbohydrates, and nucleic acids. Every living entity is comprised of cells, some of which are genuine organisms, examples being yeast or bacteria. On the other hand, multicellular organisms differentiate their cells into specialised tissues that perform the vital metabolic tasks of the body.¹ The generation and storage of energy, the transmission of genetic information, the induction of metabolic reactions, the promotion of development via mitosis, etc., are among the crucial tasks. The nucleus, cytoplasm, and cell membrane are the three main compartments that make up a cell. Genetic information is crucial for cell growth and reproduction happens in the nucleus. On the other hand, the cytoplasm is made up of several structures known as cell organelles, namely, the endoplasmic reticulum, mitochondria, Golgi apparatus, lysosomes, and ribosomes.²

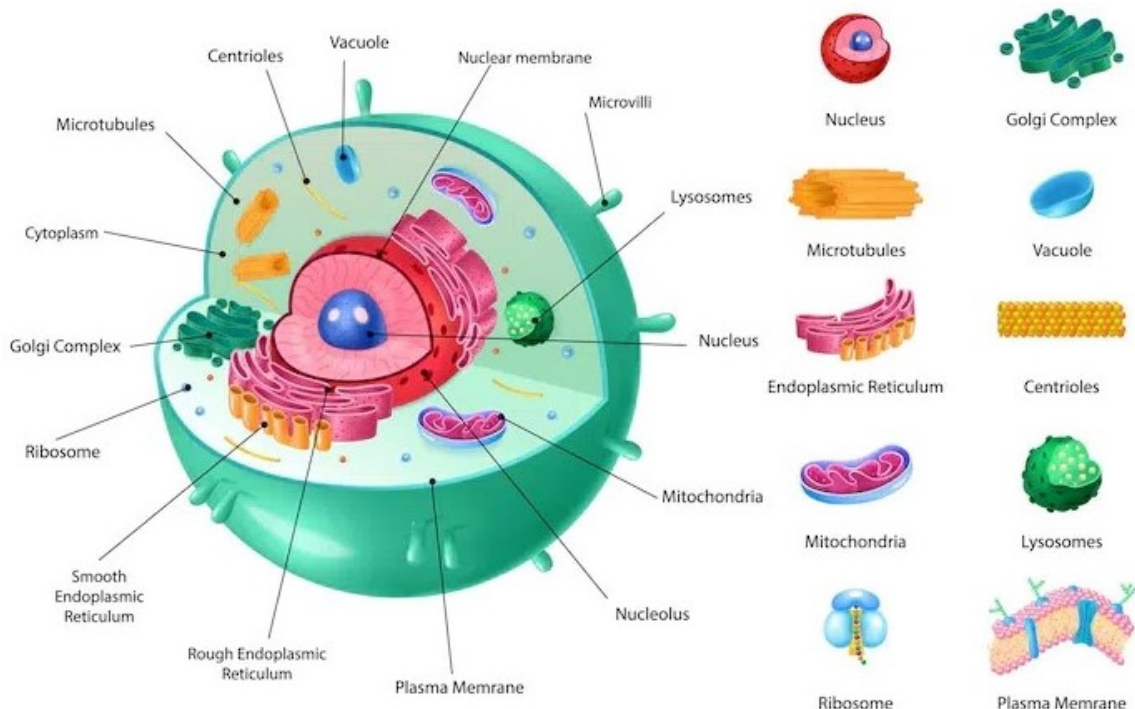


Figure 1.1. Components and structure of the cell. (<https://media.geeksforgeeks.org/wp-content/uploads/20230321113439/Animal-Cell.webp>)

These ionic concentrations enable the establishment of precise ionic homeostasis in cells and throughout the subcellular organelles, which maintains a definite potential. The equilibrium ionic membrane potential can be determined by the Nernst equation:

$$V_m = (RT/F) \ln ([ion]_{out}/ [ion]_{in}) \quad \text{Equation 1}$$

where, V_m = membrane potential; R = ideal gas constant (joules/ kelvin /mole); T = temperature(kelvin); F = Faraday's constant (coulomb per mol); $[ion]_{out}$ = concentration of ions outside cell; $[ion]_{in}$ = concentration of ions inside cell.

The membrane potential of any biological membrane is primarily regulated by ion flux through ion channels, and the potential at which there is no net ion flow is known as the resting potential. Chloride channels in mammalian cells have a resting potential of 30–60 mV, which results from a chloride gradient across the cell membrane (5–10 mM inside and 110–150 mM outside). The mitochondrial membrane also maintains a voltage between 150 and 220 mV.

Every cell organelle is trained to carry out a particular cell function, such as the creation of cell energy by mitochondria, the digestion of waste products by lysosomes, and internal organization by the Golgi apparatus and endoplasmic reticulum. A flexible protective covering of the lipid bilayer membrane known as the cell membrane or plasma membrane keeps the nucleus and all of the cytoplasmic components inside the cell from the outside. This protective lipid bilayer membrane that separates them from the cytoplasmic compartment also encloses the cellular sub-organelles. The cell membrane is very essential for the proper functioning of the cell because it permits only certain solutes to pass through and restricts others. Small, nonpolar molecules can easily diffuse through the lipid bilayer, whereas bigger, charged molecules need specialized transport proteins to penetrate the membrane. Examples of these molecules are oxygen and carbon dioxide.³

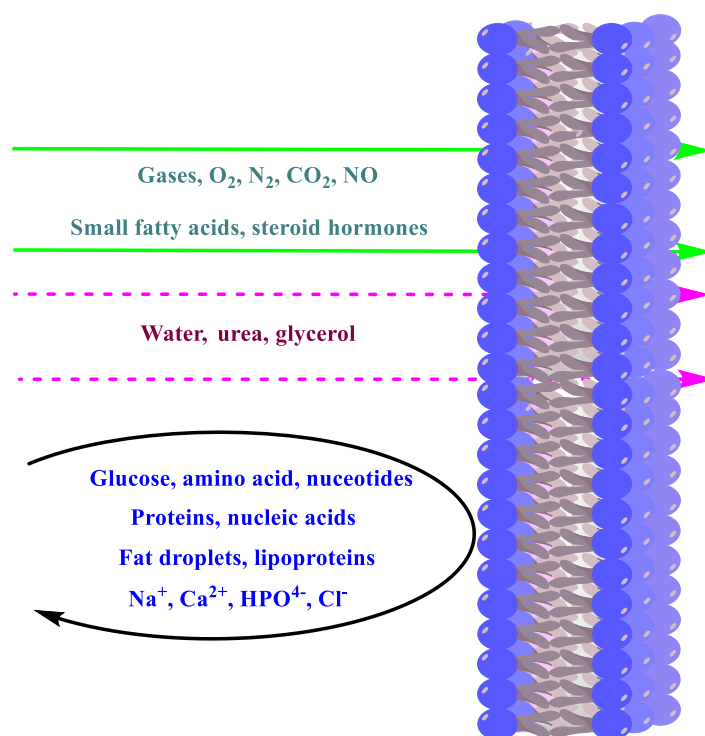


Figure 1.2. Representative example of passage of different kinds of solutes through the lipid bilayer membrane.

1.2. Cell membrane:

The cell membrane is a permeable protective covering that divides the cellular sub-organelles from the cytoplasmic compartment while also protecting the cytoplasmic components from the outside of the cell. The phospholipid molecules that make up the cell membrane or plasma membrane of the cell are divided into two parts: (i) a hydrophilic head group and (ii) a hydrophobic alkyl tail. Through self-assembly, these phospholipid molecules create a lipid bilayer membrane with a typical diameter of around 34 Å (Figure 1.3).⁴ Because the lipid bilayer membrane is selectively permeable, some chemicals can enter the cell while others cannot. The ion homeostasis of the cell depends on this selective transport. Variations of the hydrophilic head and hydrophobic tail groups constitute a wide variety of phospholipids in our cell membrane (Figure 1.3), and among all, phosphatidylcholine is the most abundant.

1.2.1. Components of lipid bilayer (cell) membrane:

The lipid bilayer is made up of two layers of phospholipids, each of which has two hydrophobic fatty acid tails and a hydrophilic head group. A charged or polar phosphate group and the three-carbon alcohol molecule glycerol make up the hydrophilic head group. Long, water-insoluble chains of hydrocarbons make up the hydrophobic fatty acid tails. The hydrophilic head groups

of the phospholipids are exposed to the aqueous environment on either side of the membrane, while the hydrophobic tails of the phospholipids form the interior of the lipid bilayer. The fatty acid chains of one layer face the fatty acid chains of the other layer in a tail-to-tail arrangement of the hydrophobic tails. Among several others, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, and sphingomyelin are the four most prevalent phospholipids in the membrane. With such an arrangement, the lipid bilayer can create a stable, impenetrable boundary between the cell and its surroundings. The hydrophobic area of the bilayer makes up around 2 nm of the thickness of the average biological membrane, whereas the hydrophilic head groups on either side make up about 1 nm. (Figure 1.3) The position of the membrane within the cell can also affect the thickness of the bilayer, with certain membranes being thicker or thinner than others. The thickness of the lipid bilayer affects which molecules and how quickly they can pass the membrane, which is crucial for its ability to act as a barrier. Since thicker membranes are often less fluid and more difficult to penetrate, the thickness of the bilayer membrane can also have an impact on its fluidity and permeability.⁵

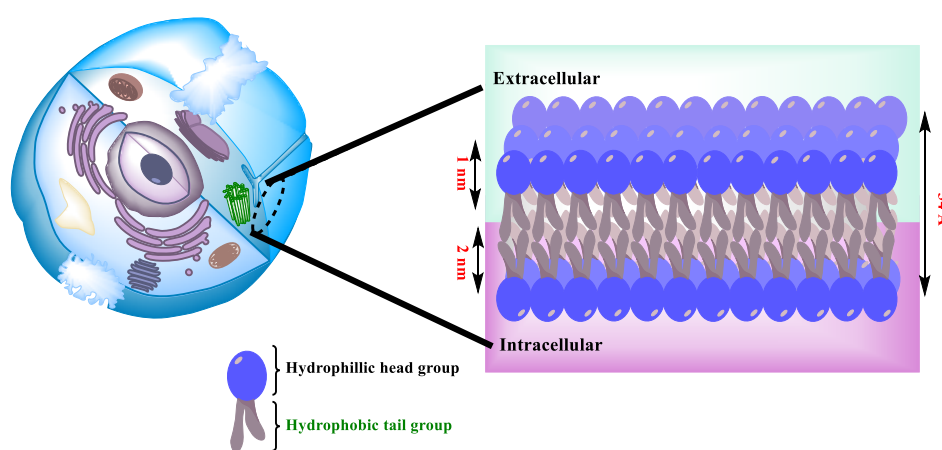


Figure 1.3. Schematic representation of the lipid bilayer membrane.

1.3. Mechanism of ion transport:

Active and passive ion transport are two distinct mechanisms that are distinguished based on the energy required for the transportation process across cellular membranes.

1.3.1. Active ion transport:

1.3.1.1. Primary active ion transport:

In order to move ions against their electrochemical gradient, this method of transport directly uses the energy produced by ATP hydrolysis. The sodium-potassium pump (Figure 1.4.), which

pumps three Na^+ ions out of the cell and two K^+ ions into the cell, and the calcium pump, which pumps Ca^{2+} ions out of the cell or into the endoplasmic reticulum, are two examples of primary active transport.

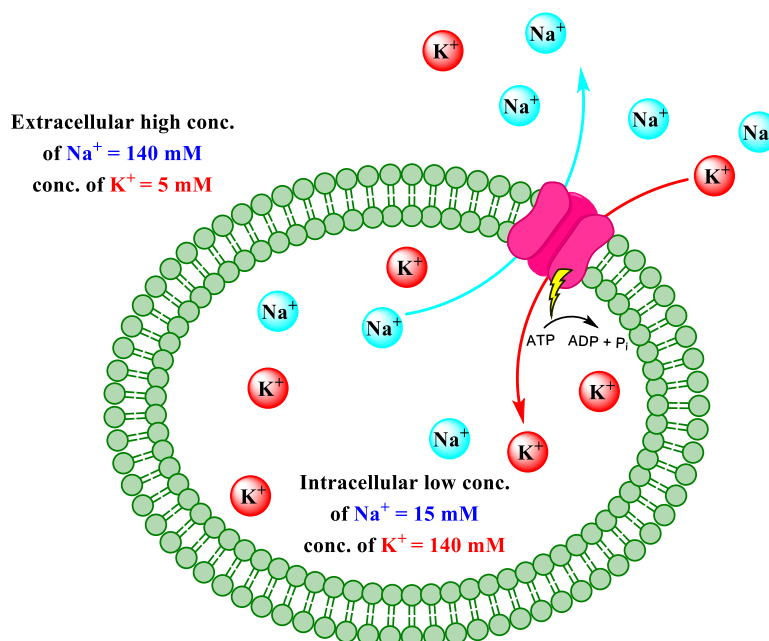


Figure 1.4. Schematic representation of the Na^+/K^+ pump.

1.3.1.2. Secondary active ion transport:

This type of transport indirectly utilizes energy stored in the electrochemical gradient of one ion to transport another ion against its electrochemical gradient. Examples of secondary active transport include the sodium-glucose transporter, which moves glucose into cells against its concentration gradient, and the sodium-calcium exchanger, which moves calcium ions out of cells against their gradient, both by using the energy stored in the sodium gradient.^{6, 7}

1.3.2. Passive ion transport:

1.3.2.1. Diffusion:

Ions transition from a concentrated region to a considerably more dilute region. The simple diffusion of oxygen and carbon dioxide over a cell membrane and the passage of potassium ions through potassium channels are two examples of diffusion⁸ (Figure 1.5.).

1.3.2.2. Facilitated diffusion:

With the aid of a transporter protein, ions travel across the membrane in this form of transport. Ion exchangers and ion channels are two examples of transporter proteins. Facilitated diffusion can happen via carriers, channels, or pores. The entry of glucose into cells via the glucose transporter and the passage of chloride ions through chloride channels are two examples of facilitated diffusion (Figure 1.5.).

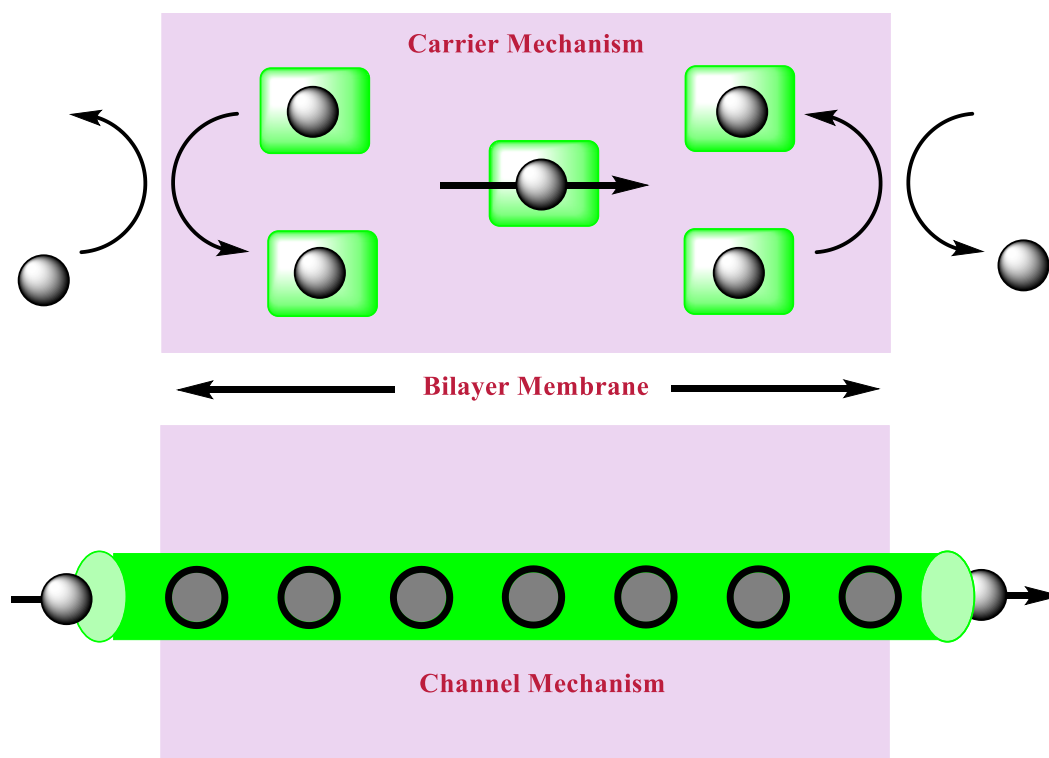


Figure 1.5. Schematic representation of transmembrane ion transport.

1.3.3. Uniport:

A single ion or molecule can pass the membrane only in the direction of the uniport. As a passive mode of transportation, it does not need external energy. Transmembrane proteins called uniporters allow ions or molecules to diffuse down concentration gradients. The glucose transporter is an illustration of a uniporter since it permits glucose molecules to permeate into the cell along their concentration gradient. (Figure 1.6.)⁹⁻¹²

1.3.4. Antiport:

Antiport refers to the opposite simultaneous transport of two distinct ions or molecules across the membrane. Antiporters are transmembrane proteins that transfer ions or molecules against concentration gradients using energy, typically ATP. The sodium-calcium exchanger is an

illustration of an antiporter since it expels calcium ions from the cell using the energy stored in the sodium gradient. The chloride-bicarbonate exchanger, which exchanges chloride ions for bicarbonate ions across the membrane, is another illustration of an antiporter.

1.3.5. Symport:

The simultaneous translocation of two different ions or molecules through the membrane is referred to as symport. Another name for this type of transportation is co-transport. Symporters are transmembrane proteins that transfer one ion or molecule against the concentration gradient of another ion or molecule by utilising the energy stored in the electrochemical gradient of one ion or molecule. The sodium-glucose transporter is an illustration of a symporter because it uses the energy stored in the sodium gradient to transfer glucose molecules into the cell. (Figure 1.6.)

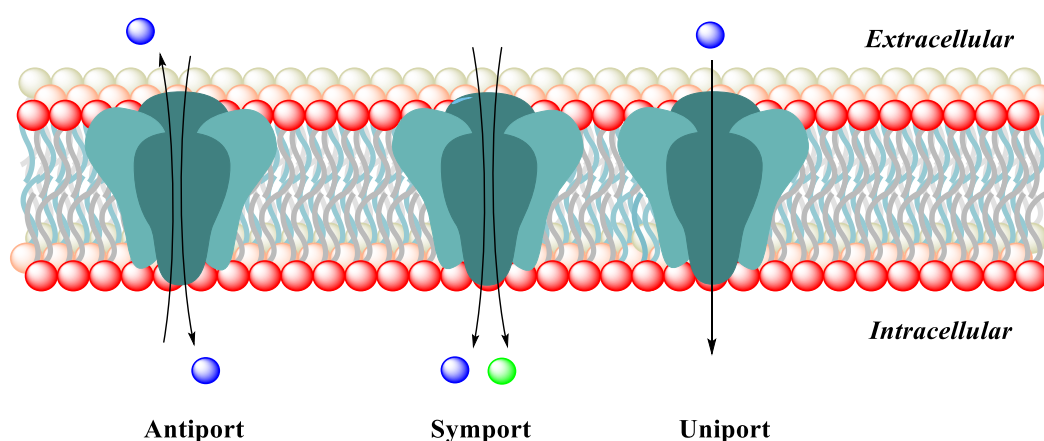


Figure 1.6. Modes of ion transport across the lipid bilayer system.

1.4. Supramolecular interactions (noncovalent interactions):

Noncovalent interactions between molecules or ions that result in the creation of bigger, more intricate structures are referred to as supramolecular interactions. These interactions play an important role in the operation of biological systems, materials science, and nanotechnology. They are responsible for the self-assembly of molecules. Hydrogen bonding, van der Waals interactions, electrostatic interactions, hydrophobic interactions, and π - π stacking are a few examples of supramolecular interactions.¹³⁻¹⁷

1.4.1. Hydrogen bonding:

Hydrogen bonding happens when a hydrogen atom electrostatically attracts another atom bearing a single pair of electrons. If the hydrogen atom is connected to an atom that is highly electronegative, hydrogen bonding may be possible. One hydrogen atom sandwiched between two oxygen atoms forms a strong link with the next water molecule in water. Hydrogen bonds are primarily strong, unlike other common forces. However, they are not as strong as actual covalent or ionic bonds.^{18, 19}

The necessary criteria for hydrogen bonding are:²⁰

- The hydrogen atom is connected to a highly electronegative atom, which makes the bond appropriately polar. With increased electronegativity, the molecule becomes more polarised.
- The hydrogen atom should be attached to a small electronegative atom because greater electrostatic attraction points to a smaller atomic size.

1.4.2. Van der Waals interactions:

When two atoms are quite close to each other for the outermost electron clouds to slightly touch each other, it gives rise to van der Waals interactions.²¹ This causes charge oscillations that produce a unidirectional, nonspecific attraction. When compared to the average kinetic energy of a molecule in solution, which is 2.5 kJ mol^{-1} , the energy of every single interaction is only about 4 kJ mol^{-1} .²² This energy is only important when several interactions are aggregated, such as when complementary surfaces meet. These types of interactions can provide bonding energies up to 40 kJ mol^{-1} under ideal conditions.

1.4.3. Electrostatic interactions:^{23, 24}

Electrostatic interactions or hydrogen bonds are the two main categories used to describe interactions among and between polar and ionisable groups. Many aspects of protein folding, stability, and activity depend on electrostatic interactions and hydrogen bonds. In order to interact with polar and ionic elements of their environment, such as water, protons, and salts, macromolecules must first exhibit electrostatic attraction. The electrostatic potential controls the electrostatic characteristics of a protein. The interactions between proteins and elements of their environment, such as water, ions, other macromolecules, and tiny molecular weight metabolites, are mediated by charged atoms at the surface of proteins.

1.4.4. Hydrophobic interactions:

The tendency of nonpolar compounds to assemble in an aqueous solution while repelling water molecules occurs due to the hydrophobic interactions.^{25, 26} It is by far the most significant biological force. The hydrophobic interaction between methane and water, is generally considered to be a short-ranged one, essentially a force of contact.²⁷

1.4.4.1. π - π stacking:^{28, 29}

The complex chemical connections between the drug and carrier in conventional nano drug-delivery systems frequently make their production more difficult and make them less likely to disintegrate after the drug enters the target. We have a viable alternative in the form of the weak contact between the aromatic rings known as " π - π - stacking." The creation of self-assembly based on this kind of stacking between drugs and carriers can improve the stability and loading capacity of the drug in addition to its lipophilicity and biosafety. This is due to the fact that the majority of the antitumor hydrophobic drugs have aromatic π -conjugated structures.

1.4.4.2. Metal-ligand coordination:

A range of covalence and strength can be seen in the bonding between metals and ligands through the donation of electron pairs from the ligand to the metal ion, as in the case of the binding and transport of oxygen by haemoglobin.³⁰ While some metal-ligand interactions are covalent, others are similar to ionic interactions. "Coordinate covalent" bond is a term given to the interactions between metals and ligands.

1.4.4.3. Host-guest interactions:

In host-guest chemistry, a big molecule or network host binds a smaller molecule guest in a binding pocket through noncovalent interactions.³¹ The manner in which antibodies bind antigens and many enzymes bind to their substrates is comparable to how host-guest binding works. Host-guest interactions are necessary for numerous biological processes, such as the catalysis of enzymes and the binding of receptors to ligands.

1.4.4.4. π - π^* interactions:

This kind of interaction happens when an empty π orbital (π^*) and a filled π orbital (π) come in contact with each other. The stabilisation of a transition state in an organic reaction usually happens via the π - π^* interactions between the catalyst and the substrate molecule.

1.4.4.5. Anion- π interactions:

The anion- π interaction is a distinct class of noncovalent force between anionic entities and electron-poor arene systems that is energetically favoured (~ 4 – 16 kcal mol⁻¹).^{32, 33} It is significant for both chemical and biological processes. This form of interaction involves a negatively charged anion and an aromatic ring.

1.5. Natural ion transporters:

In recent years, several efforts have been made for the efficient construction of small-molecule ion carriers for selective ion identification and transport. The naturally occurring valinomycin, a selective K⁺ ion transporter, and prodigiosin, which functions as a Cl⁻ ion transporter as well as an H⁺/Cl⁻ symporter, served as models for the creation of artificial ion carriers. An ion carrier should generally be designed with a stiff scaffold that has numerous ion binding sites for ion recognition.³⁴

A variety of ion channels made up of one or more protein subunits can be found in nature. For instance, the voltage-dependent anion channel (VDAC) is a single-protein unimolecular ion channel that may flip between particular on-and-off states in response to the membrane potential. Another instance is Gramicidin A, a dimeric ion channel with a preferential structure for cations that is created by the self-assembly of two identical helical peptides.³⁵ Another example is the potassium-selective KcsA channel, which is made up of four identical α -helical peptide units that self-assemble into an hourglass-shaped channel structure. Prodigiosin and valinomycin are examples of naturally occurring ion carriers that may move the ions Cl⁻ and K⁺, respectively. Another example of an amphiphilic nonpeptide molecule that forms an oligomeric cation-selective ion channel and has a hydrophilic pore made up of hydroxyl functionalities and a hydrophobic outer surface made up of polyene moieties is the natural antibiotic Amphotericin-B.³⁶ Additionally, there are naturally occurring ion carriers such as prodigiosin³⁷ and valinomycin,³⁸ which can transport Cl⁻ and K⁺ ions, respectively. Lastly, the heptameric structure of α -hemolysin, found in *Staphylococcus aureus*, forms a larger size pore in the membrane for the transport of solutes like water, DNA, and toxins.³⁹

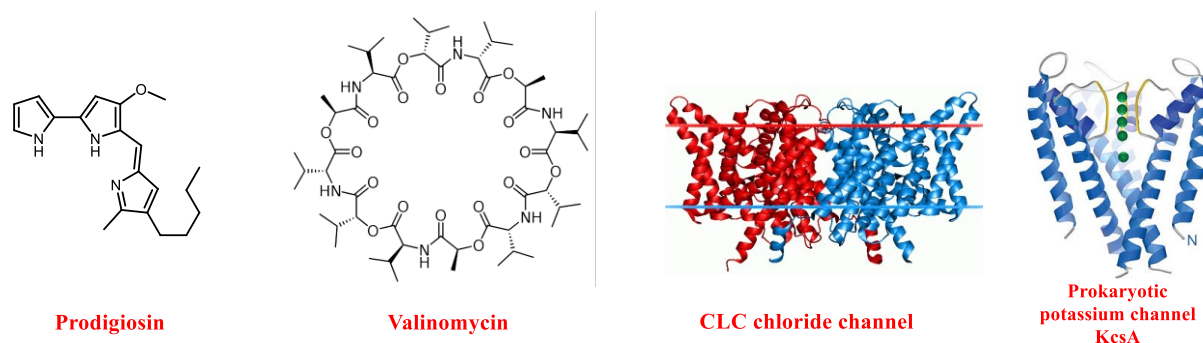


Figure 1.7. Some examples of naturally occurring carriers and channels.

1.6. Design principles for synthetic ion transporters:

Designing principles for synthetic ion carriers have been inspired by natural ion carriers. Some of the principles include:

1.6.1. Mimicking the structure of natural carriers:

The structure of natural carriers can be imitated in the creation of synthetic ion carriers. This involves studying the structure and function of natural carriers and using that knowledge to create artificial carriers with comparable characteristics.

1.6.2. Selectivity:

Natural carriers are extremely ion-selective. By including particular functional groups that interact with particular ions, synthetic carriers can be created to have a selectivity that is similar to natural carriers.

1.6.3. Hydrophobicity:

Ion selectivity can also be regulated by the hydrophobicity of the carrier. While hydrophilic carriers are more selective for polar ions, hydrophobic carriers are often particularly suitable for nonpolar ions. Examples include tripodal anion carriers⁴⁰, cholapod-based transporters⁴¹, trans-decalins,⁴²⁻⁴⁴, squaramides,⁴⁵⁻⁴⁸ and others. (Figure 1.8)

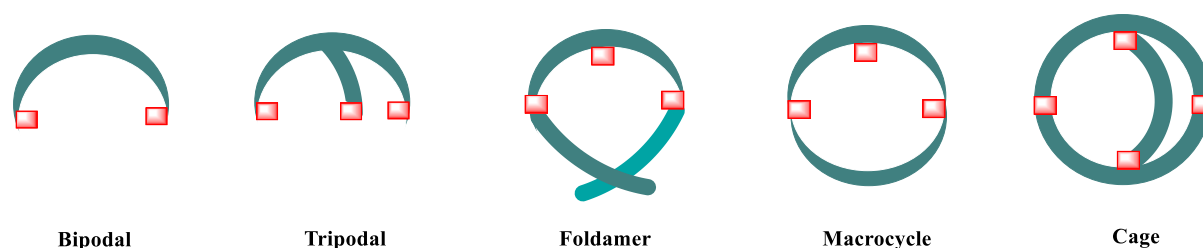


Figure 1.8. Schematic representation of types of ion carriers.

Due to the above observations, scientists have been compelled to undertake various strategies for the construction of artificial ion channels. These strategies include unimolecular, barrel stave, barrel hoop and barrel rosette ion channels.

1.7. Types of ion channels:

1.7.1. Unimolecular ion channels:

The transmembrane pathway for ion transport is provided by the ion channels, which are made up of a single molecular unit. Cyclodextrins⁴⁹ and calixarene⁵⁰ derivatives attached to suitably substituted hydrophobic tails are common examples. It is also known that peptide-based nanostructures create unimolecular ion channels.⁵¹

1.7.2. Barrel-hoop ion channels:

The pre-organized macrocyclic scaffolds that assemble to form columnar stacks inside the lipid bilayer membrane give rise to the barrel hoop ion channels. Ghadiri and colleagues described a family of peptide macrocycles that self-assemble to produce this type of ion channels in lipid membranes, which are being used as the standard examples.⁵²

1.7.3. Barrel-stave ion channels:

Few transmembrane staves self-assemble to create the barrel stave ion channels, which have a cylinder-shaped scaffold and a well-defined hydrophilic conduit for ion flow across the membrane. Bolaamphiphiles⁵³ and derivatives of bis-cholic acid⁵⁴ serve as classic examples.

1.7.4. Barrel-rosette ion channels:

Very discrete molecules self-assemble into supramolecular macrocyclic structures to create the barrel rosette ion channels, which in turn self-assemble into columnar stacks to create

transmembrane ion channels. Matile and colleagues' self-assembly of G-quartets into efficient ion channels is one of the oldest instances.⁵⁵ Other examples are the self-assembled chloride ion channels that are created by properly substituting mannitol⁵⁶ and vicinal diol moieties⁵⁷. (Figure 1.9)

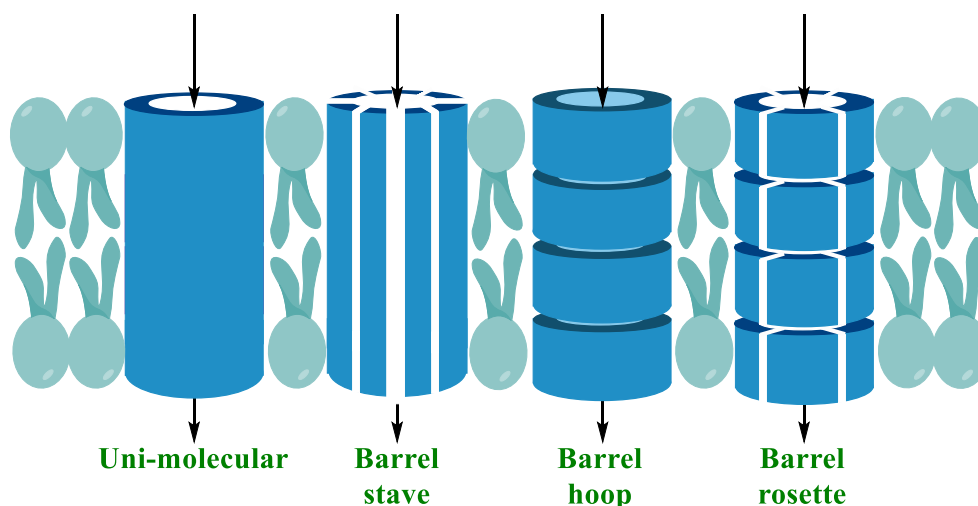


Figure 1.9. Schematic representation of different types of ion channels.

1.8. Importance of transmembrane ion transport:

Transmembrane ion transport is a crucial function for regulating fundamental biological processes like sensory transmission, cell growth, osmotic stress, and others.⁵⁸⁻⁶⁰ The study of chloride channels became increasingly important after it was discovered that they were involved in maintaining the membrane potential, and after a decade, the finding that cystic fibrosis is caused by an aberrant CFTR made it a fascinating topic among structural biologists.⁶¹⁻⁶⁴ The chloride ion and chloride channels are the two most prevalent anion species in physiological systems. The CLC family, CFTR, CLIC, CLCA, and other chloride ion channels are found throughout nature. The maintenance of the physiological pH of intracellular compartments, control of cell volume, trans-epithelial salt transport, cell cycle, and apoptosis are all regulated by the selective Cl^- transport.⁶⁴ In cancer cells, the Cl^- transport is an extremely critical function. The voltage-gated chloride ion channel family member CLC-3 is extensively expressed in cancer cells, which promotes cell growth, treatment resistance, and apoptosis.⁶⁵ Increased Cl^- absorption by CLC-3 leads to an enhancement in the volume of the cell, thereby promoting cell proliferation and the development of tumours. So the proper functioning of CLC-3 channels is very vital for normal physiological working of living organisms. (Figure 1.11). Transmembrane proteins, which can be categorised as channels and carriers and which

assure these transport processes for sustaining normal cellular activities, are specialized pieces of equipment that nature has provided cells with, to ensure the movement of these vital nutrients.

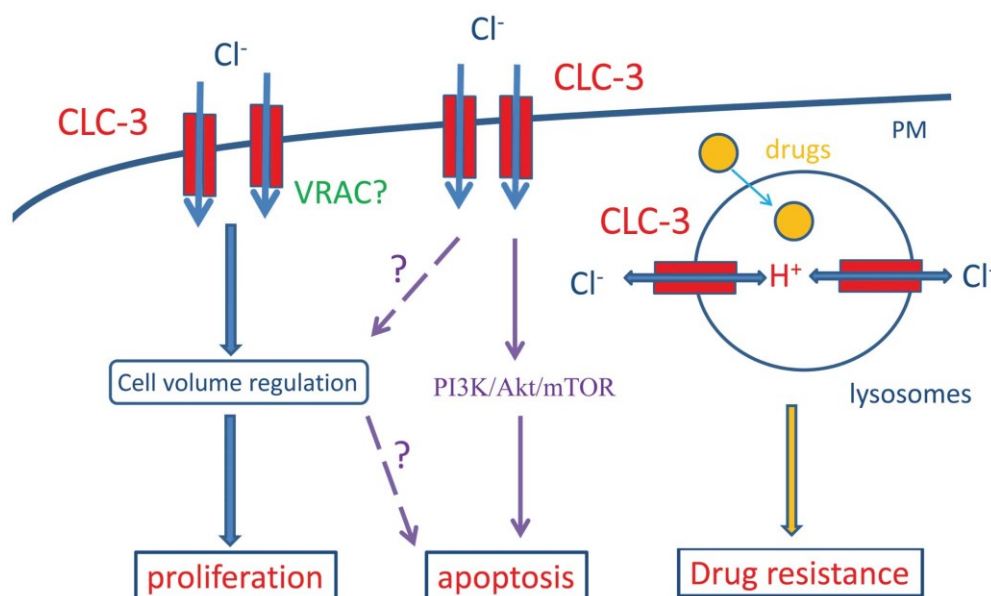


Figure 1.11. Schematic representation of effects of CLC-3 channel in cancer cells. The image has been adapted from https://www.spandidos-publications.com/article_images/or/33/2/OR-33-02-0507-g00.jpg.

Ion-channel malfunction caused by genetic or acquired mutations known as channelopathies is the other part.^{61, 62} The pathology for which the medicinal benefits of ion-channel activity are commonly acknowledged is cystic fibrosis (CF). The natural anionic channel, which transports Cl⁻ and HCO₃⁻, is encoded by the cystic fibrotic transmembrane conductance regulator (CFTR) gene (Figure 1.12), and abnormalities of this gene lead to poor mucus hydration and clearance.⁶⁶ The treatment of cystic fibrosis (CF) is currently challenging, and new methods are being investigated.⁶⁷ Similar renal disorders brought on by malfunctioning chloride ion channels include Bartters syndrome, Dent's disease, and others. Channel Replacement Therapy is receiving interest from researchers in addition to other therapeutic modalities for various channelopathies.

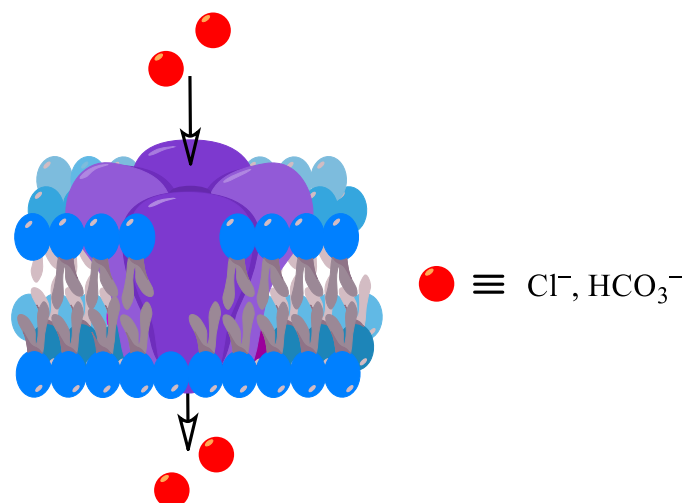


Figure 1.12. Schematic representation of the CFTR channel with $\text{Cl}^-/\text{HCO}_3^-$ antiport mechanism.

We must first evaluate the significance of anion in order to comprehend the role of chloride in transport through the cell membrane. Anions are pervasive and essential to a wide range of bodily processes. People at risk of radioactive exposure frequently take potassium iodide (KI) pills to prevent their thyroids from absorbing radioactive iodine (^{131}I), as iodide (^{127}I) is necessary for the thyroid glands to produce hormones.⁶⁸ Cystic fibrosis is brought on by the body's malfunctioning or insufficiently created chloride transport channels.⁶⁹ Fluoride is routinely added to toothpaste and water sources because it can successfully prevent tooth decay.⁷⁰ High quantities of nitrate and phosphate are present in nearby lakes and rivers as a result of the improper use of nitrate- and phosphate-rich fertilisers in intensive agricultural farming, which has resulted in eutrophication, typified by an excessive development of algae.⁷¹ Cigarette smoke and other types of industrial waste include the very toxic anion cyanide.⁷² Some naturally occurring anions, in addition to those that are released, are dangerous to human health. Due to arsenic contamination in the form of H_2AsO_4 or HAsO_4^{2-} in their groundwater, more than 300 million people worldwide are at danger.⁷³ The field of anion recognition has advanced rapidly as more individuals have understood the importance of anions in biological and environmental processes. An increasing variety of applications, including sensing, extraction, and catalysis, make use of the increasing number of anion receptors that have been identified.⁷⁴⁻⁷⁶ The quantities of chloride ions in the body are limited and greatly influence numerous physiological processes. Chloride, the biggest extracellular anion, helps maintain the pH balance and osmotic pressure of body fluids. The plasma of healthy people contains between 95 and 105 mmol/L of chloride. Particularly in neurons and muscle cells, chloride ions are necessary for the maintenance of cell electrical potential. Certain ion channels allow

chloride ions to permeate the cell membrane, maintaining the resting membrane potential and causing action potentials. The production of stomach acid, which is necessary for food digestion, depends on chloride ions. The proton-potassium ATPase pump, which transports potassium ions in exchange for hydrogen ions into the stomach lumen, and chloride ions, aids in the transfer of chloride ions into stomach epithelial cells. Additionally, the production of stomach acid, which is necessary for food digestion, depends on chloride ions. By pumping protons into the stomach lumen in exchange for K^+ ions and Cl^- ions, the proton-potassium ATPase pump helps chloride ions enter the stomach epithelial cells.⁷⁷ Another function of chloride ions is the regulation of the body's fluid and electrolyte balance. One hormone that regulates the amount of chloride in the body is aldosterone, which encourages the reabsorption of chloride ions in the kidneys.

Among the physiologically important cations, the K^+ ion plays an important role in cell membrane physiology and helps regulate fluid balance of fluids and neural signals. Organellar K^+ transport regulates osmotic balance in addition to regulating membrane potentials.⁷⁸ Within cells, a variety of K^+ transport pathways co-occur with H^+ or Ca^{2+} transport, which in turn regulates organellar processes that affect metabolism, enzyme activity, and protein release.⁷⁹ A rising body of evidence suggests that the exceptionally high propensity for cancer cells to proliferate is associated with K^+ channel instability.⁸⁰ For example, low K^+ channel expression and hyperpolarized mitochondrial membrane potentials (MMPs) are found in a range of human cancer cell types, including cancer stem cells (CSCs), which both increase the resistance of cells to apoptosis.^{81, 82} The transmembrane ion transport and that of other solutes is a necessary process to ensure the normal physiological functioning of cells. However, this transmembrane transport is sometimes deferred by the selective nature of the cell membrane which allows passage of certain nonpolar species but restricts the movement of polar species like ions.^{61, 83,}

84

When compared to regular phospholipid vesicles, liposomes which are fluorinated have much lesser membrane permeability, and hemolysis, more detergent resistance, and altered capacity to recognize negatively charged species such as tetraphenylborate, TPB. When hydrogen is replaced by fluorine in molecules such as drugs, anaesthetics, antibiotics, etc., it affects their cellular interactions apart from their metabolism, cytotoxicity, and pharmacological activity in a significant way. Compared to other ions, lipophilic ions easily flow through membranes and contribute to the addition of intrinsic charges, which have a

considerable impact on the membrane polarizability and conductance. Charge-pulse tests show that derivatives of TPB with phenyl groups substituted by fluorine (-F) or trifluoromethyl (-CF₃) evince significantly rate of transmembrane transport than the original compound. Inferring that the transmembrane mobility drastically rises with fluorine substitution, the analogue with the substitution of fluorine translocate much higher, almost twenty time, and the -CF₃ substituted analogue roughly four times more efficiently than the original TPB.⁸⁵

1.9. Techniques to study ion transport mechanism:

1.9.1. Vesicles mimicking the lipid bilayer membrane:

The majority of methods make use of unilamellar vesicles, which have a diameter of 0.1 to 1 μ M and are small, spherical membranes. Numerous techniques, such as running lipid liquids through a nano-porous filter, can be used to produce these vesicles. Ions and markers that are water-soluble are typically confined inside the vesicles. When components that remain outside the vesicles are removed, as in the case of dialysis, and replaced with a different set of components, active transporters can release substances from a gradient of concentration across the membrane. Several techniques are available to detect anion transport, such as the use of fluorescent dyes that respond to the appearance or disappearance of anions. This technique uses phospholipids such as 1-palmitoyl-2-oleoylsn-glycero-3-phosphocholine (POPC) or egg yolk phosphatidylcholine (EYPC) to create spherical vesicles that are then filled with a specific dye. After then, the transport activity is assessed and quantified by seeing how a certain dye changes fluorescence in response to changes in pH, an anion gradient, or electrical potential. 8-Hydroxypyrene-1,3,6-trisulfonic acid Trisodium salt (HPTS), N, N'- Dimethyl-9,9'-acridinium dinitrate (Lucigenin), Safranin O, and Carboxyfluorescein (CF) are among the several dyes that are frequently utilised in such kind of investigations. With a pK_a value of 7.2, the highly water-soluble, membrane-impermeable dye HPTS serves as a pH indicator in aqueous buffers. It can exist in two states, namely, protonated (excitation wavelength: 403 nm) and non-protonated (excitation wavelength: 460 nm). Nevertheless, emission occurs at the same wavelength of 510 nm when both wavelengths are excited. Compared to the protonated form, the deprotonated form emits light with a higher intensity. The pH gradient across the lipid vesicles is created during ion transport studies, and its dissipation when an ion transporter is present, observed as the enhancement in fluorescence intensity of the HPTS dye at 510 nm, is indicative of the ion transport, which occurs either via H⁺/A⁻ efflux or OH⁻/A⁻ exchange process. Another type of dye, lucigenin, exhibits fluorescence when there are halide anions

present. In this test, the lucigenin dye is contained in lipid vesicles that also contain a NaNO_3 solution. The addition of NaCl solution to the extravesicular solution results in the formation of a chloride gradient. A decrease in fluorescence in the presence of transport molecules is a sign that the $\text{Cl}^-/\text{NO}_3^-$ antiport mechanism is causing a chloride influx. Similarly, safranin O dye is sensitive to membrane potential. It is a cationic dye that sticks to the membrane, and the addition of a transport molecule will boost the membrane potential, increasing the fluorescence of safranin O. Carboxyfluorescein is a fluorescent dye that is employed in differentiating ion channels from transmembrane pores by employing membrane-leakage assays. (Figure 1.13)

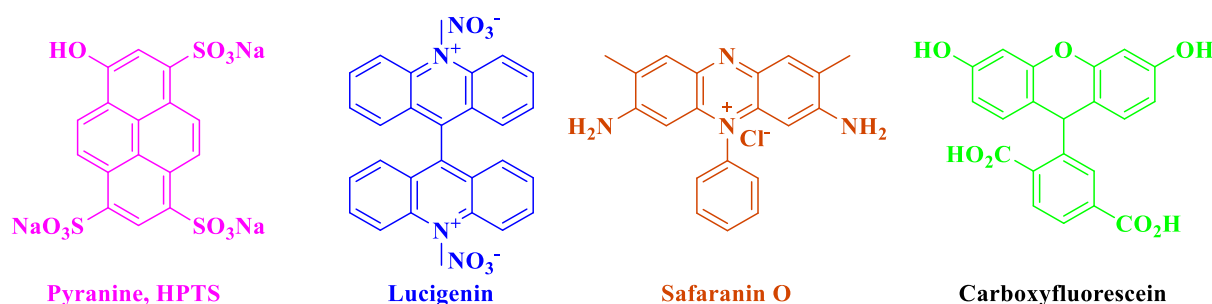


Figure 1.13. Structure of some of the dyes used in the vesicle method.

1.9.2. NMR-based assay:

The species being carried have to be necessarily NMR active (for example, ^{35}Cl , ^{23}Na , ^{39}K etc). In a typical experiment, unilamellar vesicles are prepared with NaA or (where, $\text{A}^- = ^{35}\text{Cl}^-$, or $^{33}\text{SO}_4^{2-}$, and NMR inactive anion), and paramagnetic shift reagents like Co^{2+} , Mn^{2+} are added to the extravesicular buffer.^{86,87} The paramagnetic shift reagent outside the vesicular membrane cause a significant difference in either the NMR chemical shift and/or the line-broadening of the peak for intra- and extravesicular anions/cations (Figure 1.14). Hence, in the presence of an ion transporting agent, the line broadening or chemical shift change of the peak for extravesicular NMR active ion can be monitored to check the transmembrane ion transport.⁸⁸

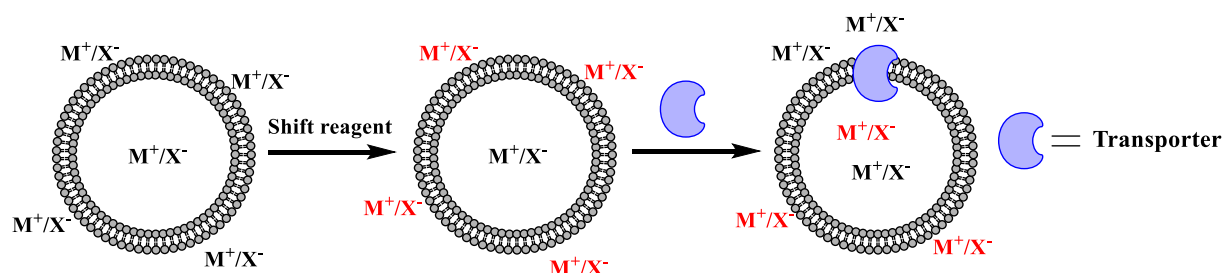


Figure 1.14. Schematic representation of NMR-based assay.

1.9.3. Ion transport study in planar bilayers:

One of the major practices in supramolecular chemistry is to employ planar lipid bilayer membranes (BLMs) as a model system for analysing the characteristics of ion channels, by measuring the ion conductance across them, which can reveal important details about their structure and functioning. The typical setup consists of an electrolyte chamber which consists of a cis and trans chamber, separated by a Teflon barrier with a tiny aperture of 150 - 250 μM , which is used to connect the two chambers. The lipid that separates the two chambers, typically 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC), is painted onto this aperture to create a planar lipid bilayer. This bilayer serves as an insulator, preventing any ion transfer between chambers, which prevents the observation of current flow. However, the channel that is created as a result of the insertion of the channel-forming molecule restores the link between the two chambers, allowing ionic flow and producing the measured conductance across the circuit. The parallel plate capacitor can be used to rationalize the operation of this type of planar bilayer conductance measurement since it generates current on application of a voltage across the membrane, which is a measure of the behaviour of ion channels. The regular variation of present values. The periodic fluctuation of current values (picoampere values) is the characteristic signature of the opening and closing of the channel. Channels and transporters are distinguished separately from other cellular components owing to the BLM approach, which is one of its benefits. As a result, it is now possible to explore their characteristics in a controlled setting, which can reveal important details about their structure and function. Ion channels and transporters of all types, including voltage-gated, ligand-gated, mechanosensitive, and ion transporters, have been studied using the BLM approach.⁸⁹ (Figure 1.15)

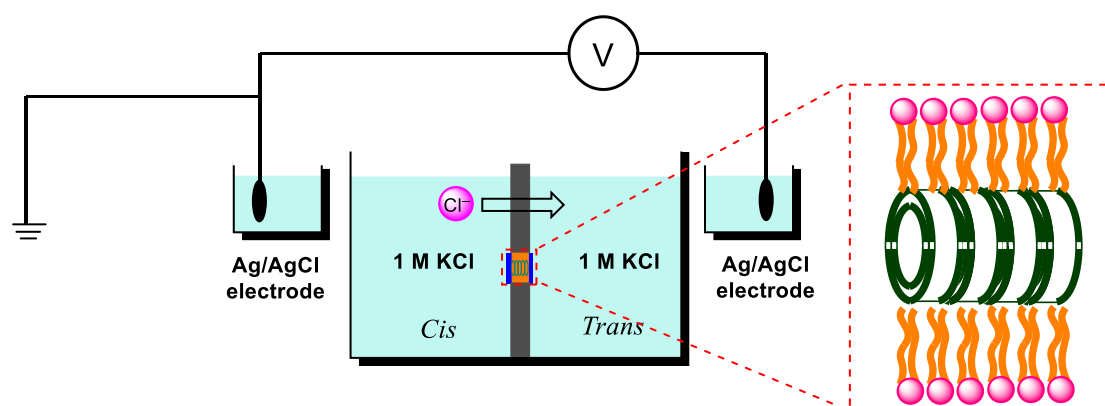


Figure 1.15. Schematic representation of black lipid membrane (BLM) experiment.

1.9.4. ISE- based assay:

The efflux of a specific ion trapped inside lipid vesicles is investigated using ion-selective electrodes by observing changes in the conductivity of an external solution. In this essay, the chloride ions are trapped inside of vesicles and their efflux is triggered by the addition of a transporter molecule. Chloride selective electrodes are one of the types of electrodes that are utilised to assess the conductivity of chloride ions in the external aqueous solution.^{90, 91} (Figure 1.16)



Figure 1.16. Schematic representation of chloride based ion-selective electrode (ISE) and its complete setup.

1.10. References:

1. Chaffey, N.; Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K; and Walter, P., Molecular biology of the cell. 4th edn. *Annals of Botany*. **2003**, *91* (3), 401.
2. Fyles, T. M., Bilayer membranes and transporter models. *Curr. Opin. Chem. Biol.* **1997**, *1* (4), 497-505.
3. Nagel, G.; Ollig, D.; Fuhrmann, M.; Kateriya, S.; Musti, A. M.; Bamberg, E.; Hegemann, P., Channelrhodopsin-1: A light-gated proton channel in green algae. *Science*. **2002**, *296* (5577), 2395-2398.
4. H. Lodish; S. L. Zipursky; P. Matsudaira; D. Baltimore and J. Darnell., *Molecular cell biology*, W. H. Freeman. **2000**.
5. Hille, B., Ion channels of excitable membranes. *Sinauer*. **2001**, 3rd Edition
6. Sano, R.; Shinozaki, Y.; Ohta, T., Sodium–glucose cotransporters: Functional properties and pharmaceutical potential. *J Diabetes Investig.* **2020**, *11* (4), 770-782.
7. Blaustein, M. P.; Lederer, W. J., Sodium/calcium exchange: Its physiological implications. *Physiol Rev.* **1999**, *79* (3), 763-854.

8. Alberts B J. A.; Lewis J., Membrane transport of small molecules and the electrical properties of membranes. *Molecular Biology of the Cell, Chapter 11, New York: Garland Science. 2002, 4th edition.* .
9. D. L. Nelson; a. M. C. M., Lehninger principles of biochemistry, W. H. Freeman. **2017, 7th edition.**
10. D. Voet; J. G. V.; and C. W. Pratt., Fundamentals of biochemistry: Life at the molecular level. *Wiley. 2016, 5th edition.*
11. G. M. Cooper; R. E. Hausman.; and C. Hausman., The cell: A molecular approach. *Sinauer Associates. 2017.*
12. E. R. Kandel; J. H. S.; T. M. Jessell; S. A. Siegelbaum; and A. J. Hudspeth., Principles of neural science. *McGraw-Hill Education 2012, 5th edition.*
13. Schneider, H.-J.; Strongin, R. M., Supramolecular Interactions in chemomechanical polymers. *Acc. Chem. Res. 2009, 42 (10), 1489-1500.*
14. Hao, Z.; Li, H.; Wang, Y.; Hu, Y.; Chen, T.; Zhang, S.; Guo, X.; Cai, L.; Li, J., Supramolecular peptide nanofiber hydrogels for bone tissue engineering: From multihierarchical fabrications to comprehensive applications. *Adv Sci (Weinh). 2022, 9 (11), 2103820.*
15. Reinhoudt., D. N., Supramolecular chemistry: From molecules to nanomaterials. *J. Nanopart. Res. 2007, 9, 983-1001.*
16. Whitesides, G. M.; Grzybowski, B., Self-assembly at all scales. *Science. 2002, 295 (5564), 2418-2421.*
17. Thakuria, R.; Nath, N. K.; Saha, B. K., The nature and applications of π - π interactions: A perspective. *Cryst. Growth Des. 2019, 19 (2), 523-528.*
18. Grabowski, S. J., What is the covalency of hydrogen bonding? *Chem. Rev. 2011, 111 (4), 2597-2625.*
19. Hunt, P. A.; Ashworth, C. R.; Matthews, R. P., Hydrogen bonding in ionic liquids. *Chem. Rev. 2015, 44 (5), 1257-1288.*
20. George A. Jeffrey, W. Saenger., Hydrogen bonding in biological structures. *Springer Nature 1991.*
21. Hermann, J.; DiStasio Jr. R. A.; Tkatchenko, A., First-principles models for van der Waals interactions in molecules and materials: Concepts, theory, and applications. *Chem. Rev. 2017, 117 (6), 4714-4758.*
22. Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J.; Johnson, G. T., *Cell Biology (Third Edition), Eds. Elsevier, 2017, 53-62.*

23. Zhou, H.-X.; Pang, X., Electrostatic interactions in protein structure, folding, binding, and condensation. *Chem. Rev.* **2018**, *118* (4), 1691-1741.
24. Hattori, Y.; Kaneko, K.; Ohba, T., *Adsorption Properties. Comprehensive Inorganic Chemistry II (Second Edition)*, Eds. Elsevier, Amsterdam, **2013**; 25-44.
25. Berruex, L. G.; Freitag, R., Separation and purification of biochemicals. *Encyclopedia of Physical Science and Technology (Third Edition)*, Ed. Academic Press: New York, **2003**, 651-673.
26. Bogunia, M.; Makowski, M., Influence of ionic strength on hydrophobic interactions in water: dependence on solute size and shape. *J. Phys. Chem. B.* **2020**, *124* (46), 10326-10336.
27. Meyer, E. E.; Rosenberg, K. J.; Israelachvili, J., Recent progress in understanding hydrophobic interactions. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103* (43), 15739-15746.
28. Chen, T.; Li, M.; Liu, J., π - π stacking interaction: A nondestructive and facile means in material engineering for bioapplications. *Cryst. Growth Des.* **2018**, *18* (5), 2765-2783.
29. Timmer, B. J. J.; Mooibroek, T. J., Intermolecular π - π stacking interactions made visible. *J. Chem. Educ.* **2021**, *98* (2), 540-545.
30. Balcerek, B.; Steinach, M.; Lichti, J.; Maggioni, M. A.; Becker, P. N.; Labes, R.; Gunga, H.-C.; Persson, P. B.; Föhling, M., A broad diversity in oxygen affinity to hemoglobin. *Sci. Rep.* **2020**, *10* (1), 16920.
31. Yang, H.; Yuan, B.; Zhang, X.; Scherman, O. A., Supramolecular chemistry at interfaces: Host-guest interactions for fabricating multifunctional biointerfaces. *Acc. Chem. Res.* **2014**, *47* (7), 2106-2115.
32. Chifotides, H. T.; Dunbar, K. R., Anion- π interactions in supramolecular architectures. *Acc. Chem. Res.* **2013**, *46* (4), 894-906.
33. Rather, I. A.; Wagay, S. A.; Ali, R., Emergence of anion- π interactions: The land of opportunity in supramolecular chemistry and beyond. *Coord. Chem. Rev.* **2020**, *415*, 213327.
34. Fyles, T. M.; Osa, T.; Atwood, J. L., Principles of artificial membrane transport of ions by synthetic ionophores. Inclusion aspects of membrane chemistry,. Eds. Springer Netherlands: Dordrecht, **1991**, 59-110.
35. Roux, B.; Karplus, M., Ion transport in a model gramicidin channel. Structure and thermodynamics. *Biophys J.* **1991**, *59* (5), 961-981.
36. Baas, B.; Kindt, k.; Scott, A.; Scott, J.; Mikulecky, P.; Hartsel, S. C., Activity and kinetics of dissociation and transfer of Amphotericin B from a novel delivery form. *AAPS PharmSci.* **1999**, *1* (3), 10.

37. Seganish, J. L.; Davis, J. T., Prodigiosin is a chloride carrier that can function as an anion exchanger. *Chem. Commun.* **2005**, *46*, 5781-5783.
38. Stark, G.; Ketterer, B.; Benz, R.; Läuger, P., The rate constants of valinomycin-mediated ion transport through thin lipid membranes. *Biophys. J.* **1971**, *11* (12), 981-994.
39. Song, L.; Hobaugh, M. R.; Shustak, C.; Cheley, S.; Bayley, H.; Gouaux, J. E., Structure of staphylococcal α -hemolysin, a heptameric transmembrane pore. *Science*. **1996**, *274* (5294), 1859-1865.
40. Busschaert, N.; Wenzel, M.; Light, M. E.; Iglesias-Hernández, P.; Pérez-Tomás, R.; Gale, P. A., Structure–activity relationships in tripodal transmembrane anion transporters: The effect of fluorination. *J. Am. Chem. Soc.* **2011**, *133* (35), 14136-14148.
41. McNally, B. A.; Koulov, A. V.; Lambert, T. N.; Smith, B. D.; Joos, J.-B.; Sisson, A. L.; Clare, J. P.; Sgarlata, V.; Judd, L. W.; Magro, G.; Davis, A. P., Structure–activity relationships in cholapod anion carriers: Enhanced transmembrane chloride transport through substituent tuning. *Chem. Eur. J.* **2008**, *14* (31), 9599-9606.
42. Hussain, S.; Brotherhood, P. R.; Judd, L. W.; Davis, A. P., Diaxial diureido decalins as compact, efficient, and tunable anion transporters. *J. Am. Chem. Soc.* **2011**, *133* (6), 1614-1617.
43. Jurček, O.; Valkenier, H.; Puttreddy, R.; Novák, M.; Sparkes, H. A.; Marek, R.; Rissanen, K.; Davis, A. P., Anion recognition by a bioactive diureidodecalin anionophore: solid-state, solution, and computational studies. *Chem. Eur. J.* **2018**, *24* (32), 8178-8185.
44. Valkenier, H.; Dias, C. M.; Butts, C. P.; Davis, A. P., A folding decalin tetra-urea for transmembrane anion transport. *Tetrahedron Lett.* **2017**, *73* (33), 4955-4962.
45. Busschaert, N.; Kirby, I. L.; Young, S.; Coles, S. J.; Horton, P. N.; Light, M. E.; Gale, P. A., Squaramides as Potent Transmembrane Anion Transporters. *Angew. Chem. Int. Ed.* **2012**, *51* (18), 4426-4430.
46. Vega, M.; Martínez-Crespo, L.; Barceló-Oliver, M.; Rotger, C.; Costa, A., Light-driven photoconversion of squaramides with implications in anion transport. *Org. Lett.* **2023**, *25* (19), 3423-3428.
47. Picci, G.; Carreira-Barral, I.; Alonso-Carrillo, D.; Busonera, C.; Milia, J.; Quesada, R.; Caltagirone, C., The role of indolyl substituents in squaramide-based anionophores. *Org. Biomol. Chem.* **2022**, *20* (40), 7981-7986.
48. Bao, X.; Wu, X.; Berry, S. N.; Howe, E. N. W.; Chang, Y.-T.; Gale, P. A., Fluorescent squaramides as anion receptors and transmembrane anion transporters. *Chem. Commun.* **2018**, *54* (11), 1363-1366.

49. Chui, J. K. W.; Fyles, T. M., Cyclodextrin ion channels. *Org. Biomol. Chem.* **2014**, *12* (22), 3622-3634.
50. Sidorov, V.; Kotch, F. W.; Abdrakhmanova, G.; Mizani, R.; Fettingner, J. C.; Davis, J. T., Ion channel formation from a calix[4]arene amide that binds hcl. *J. Am. Chem. Soc.* **2002**, *124* (10), 2267-2278.
51. Clark, T. D.; Buehler, L. K.; Ghadiri, M. R., Self-assembling cyclic β 3-peptide nanotubes as artificial transmembrane ion channels. *J. Am. Chem. Soc.* **1998**, *120* (4), 651-656.
52. Yudin, A. K., Macrocycles: lessons from the distant past, recent developments, and future directions. *Chem. Sci.* **2015**, *6* (1), 30-49.
53. Eggers, P. K.; Fyles, T. M.; Mitchell, K. D. D.; Sutherland, T., Ion channels from linear and branched bola-amphiphiles. *J. Org. Chem.* **2003**, *68* (3), 1050-1058.
54. Kobuke, Y.; Nagatani, T., Transmembrane ion channels constructed of cholic acid derivatives. *J. Org. Chem.* **2001**, *66* (15), 5094-5101.
55. Forman, S. L.; Fettingner, J. C.; Pieraccini, S.; Gottarelli, G.; Davis, J. T., Toward artificial ion channels: A lipophilic G-quadruplex. *J. Am. Chem. Soc.* **2000**, *122* (17), 4060-4067.
56. Saha, T.; Dasari, S.; Tewari, D.; Prathap, A.; Sureshan, K. M.; Bera, A. K.; Mukherjee, A.; Talukdar, P., Hopping-mediated anion transport through a mannitol-based rosette ion channel. *J. Am. Chem. Soc.* **2014**, *136* (40), 14128-14135.
57. Saha, T.; Gautam, A.; Mukherjee, A.; Lahiri, M.; Talukdar, P., Chloride transport through supramolecular barrel-rosette ion channels: Lipophilic control and apoptosis-inducing activity. *J. Am. Chem. Soc.* **2016**, *138* (50), 16443-16451.
58. Davis, J. T.; Okunola, O.; Quesada, R., Recent advances in the transmembrane transport of anions. *Chem. Soc. Rev.* **2010**, *39* (10), 3843-3862.
59. Becchetti, A.; Munaron, L.; Arcangeli, A., The role of ion channels and transporters in cell proliferation and cancer. *Front. Physiol.* **2013**, *4* (312), 312.
60. Shinde, S. V.; Talukdar, P., An anion receptor that facilitates transmembrane proton–anion symport by deprotonating its sulfonamide N–H proton. *Chem. Commun.* **2018**, *54* (73), 10351-10354.
61. Stoltz, D. A.; Meyerholz, D. K.; Welsh, M. J., Origins of cystic fibrosis lung disease. *N. Engl. J. Med.* **2015**, *372* (4), 351-362.

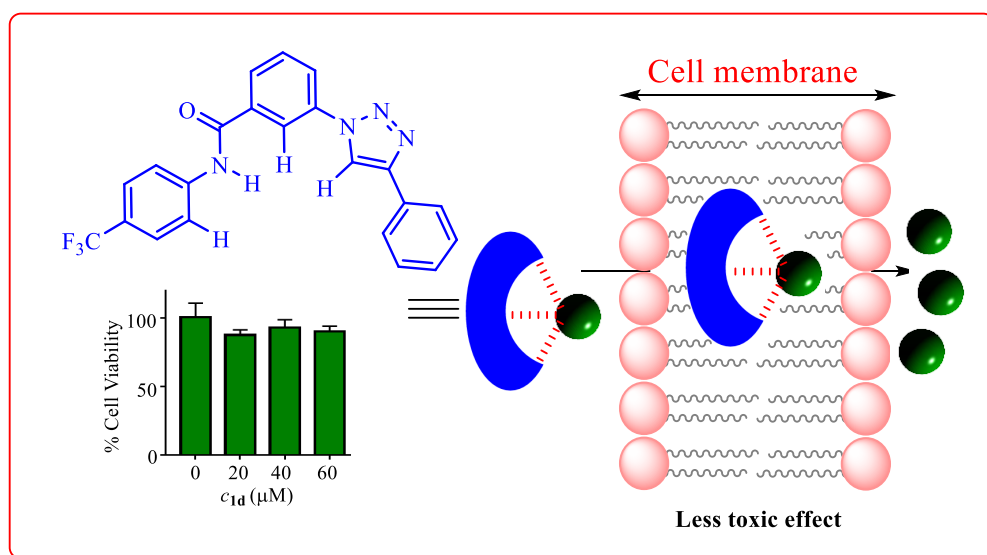
62. Hernando, E.; Capurro, V.; Cossu, C.; Fiore, M.; García-Valverde, M.; Soto-Cerrato, V.; Pérez-Tomás, R.; Moran, O.; Zegarra-Moran, O.; Quesada, R., Small molecule anionophores promote transmembrane anion permeation matching CFTR activity. *Sci. Rep.* **2018**, *8* (1), 2608.
63. Deriy, L. V.; Gomez, E. A.; Zhang, G.; Beacham, D. W.; Hopson, J. A.; Gallan, A. J.; Shevchenko, P. D.; Bindokas, V. P.; Nelson, D. J., Disease-causing mutations in the cystic fibrosis transmembrane conductance regulator determine the functional responses of alveolar macrophages. *J. Biol. Chem.* **2009**, *284* (51), 35926-35938.
64. Rao S.G.; Patel, N. J. S., H., Intracellular chloride channels: Novel biomarkers in diseases. *Front. Physiol.* **2020**, *11*.
65. Poroca, D. R.; Pelis, R. M.; Chappe, V. M., ClC channels and transporters: Structure, physiological functions, and implications in human chloride channelopathies. *Front. Pharmacol.* **2017**, *8*.
66. Duran, C.; Thompson, C. H.; Xiao, Q.; Hartzell, H. C., Chloride channels: Often enigmatic, rarely predictable. *Annu. Rev. Physiol.* **2010**, *72* (1), 95-121.
67. Somaraju, U. R. R.; Solis-Moya, A., Pancreatic enzyme replacement therapy for people with cystic fibrosis. *Cochrane Database Syst. Rev.* **2020**, *8*, 10.1002/14651858.CD008227.pub4.
68. Phan, G.; Rebière, F.; Suhard, D.; Legrand, A.; Carpentier, F.; Sontag, T.; Souidi, M.; Jourdain, J.-R.; Agarande, M.; Renaud-Salis, V., Optimal KI prophylactic dose determination for thyroid radiation protection after a single administration in adult rats. *Dose Response.* **2017**, *15* (4), 1559325817746558.
69. Cutting, G. R., Cystic fibrosis genetics: from molecular understanding to clinical application. *Nat Rev Genet.* **2015**, *16* (1), 45-56.
70. de Leeuw, N. H., Resisting the onset of hydroxyapatite dissolution through the incorporation of fluoride. *J. Phys. Chem. B* **2004**, *108* (6), 1809-1811.
71. Schindler, D. W., Recent advances in the understanding and management of eutrophication. *Limnol. Oceanogr.* **2006**, *51*, 356-363.
72. Johnson, C. A., The fate of cyanide in leach wastes at gold mines: An environmental perspective. *Appl. Geochem.* **2015**, *57*, 194-205.
73. Mazumder, D. N. G., Handbook of Arsenic Toxicology. **2015**.
74. Evans, N. H.; Beer, P. D., Advances in anion supramolecular chemistry: from recognition to chemical applications. *Angew. Chem. Int. Ed. Engl.* **2014**, *53* (44), 11716-11754.

75. Davis, J. T.; Gale, P. A.; Quesada, R., Advances in anion transport and supramolecular medicinal chemistry. *Chem. Soc. Rev.* **2020**, *49* (16), 6056-6086.
76. Molina, P.; Zapata, F.; Caballero, A., Anion recognition strategies based on combined noncovalent interactions. *Chem. Rev.* **2017**, *117* (15), 9907-9972.
77. Shin, J. M.; Munson, K.; Vagin, O.; Sachs, G., The gastric HK-ATPase: Structure, function, and inhibition. *Pflugers Arch - Eur J Physiol* **2009**, *457* (3), 609-622.
78. Xu, H.; Martinoia, E.; Szabo, I., Organellar channels and transporters. *Cell Calcium*. **2015**, *58* (1), 1-10.
79. Garlid, K. D.; Paucek, P., Mitochondrial potassium transport: the K⁺ cycle. *Biochim. Biophys. Acta - Bioenerg.* **2003**, *1606* (1), 23-41.
80. Prevarskaya, N.; Skryma, R.; Shuba, Y., Ion channels and the hallmarks of cancer. *Trends Mol Med.* **2010**, *16* (3), 107-121.
81. Kim, H. K.; Noh, Y. H.; Nilius, B.; Ko, K. S.; Rhee, B. D.; Kim, N.; Han, J., Current and upcoming mitochondrial targets for cancer therapy. *Semin. Cancer Biol.* **2017**, *47*, 154-167.
82. Sancho, P.; Barneda, D.; Heeschen, C., Hallmarks of cancer stem cell metabolism. *Br J Cancer* **2016**, *114* (12), 1305-1312.
83. Carlström, M.; Wilcox, C. S.; Arendshorst, W. J., Renal autoregulation in health and disease. *Physiol Rev.* **2015**, *95* (2), 405-511.
84. Löscher, W.; Rogawski, M. A., How theories evolved concerning the mechanism of action of barbiturates. *Epilepsia.* **2012**, *53*, 12-25.
85. Kürschner, M.; Nielsen, K.; von Langen, J. R. G.; Schenk, W. A.; Zimmermann, U.; Sukhorukov, V. L., Effect of fluorine substitution on the interaction of lipophilic ions with the plasma membrane of mammalian cells. *Biophys J.* **2000**, *79* (3), 1490-1497.
86. Gokel, G. W.; Daschbach, M. M., Coordination and transport of alkali metal cations through phospholipid bilayer membranes by hydrophile channels. *Coord. Chem. Rev.* **2008**, *252* (8), 886-902.
87. Gokel, G. W.; Ferdani, R.; Liu, J.; Pajewski, R.; Shabany, H.; Uetrecht, P., Hydrophile channels: Models for transmembrane, cation-conducting transporters. *Chem. Eur. J.* **2001**, *7* (1), 33-39.
88. Hall, A. C.; Suarez, C.; Hom-Choudhury, A.; Manu, A. N. A.; Hall, C. D.; Kirkovits, G. J.; Ghiriviga, I., Cation transport by a redox-active synthetic ion channel. *Org. Biomol. Chem.* **2003**, *1* (16), 2973-2982.

89. Sebaaly, C.; Greige-Gerges, H.; Charcosset, C., Chapter 11 - Lipid membrane models for biomembrane properties' investigation. *Current Trends and Future Developments on (Bio-) Membranes*, Eds. Elsevier, **2019**, 311-340.
90. Schlesinger, P. H.; Gross, A.; Yin, X.-M.; Yamamoto, K.; Saito, M.; Waksman, G.; Korsmeyer, S. J., Comparison of the ion channel characteristics of proapoptotic BAX and antiapoptotic BCL-2. *Proc. Natl. Acad. Sci.* **1997**, *94* (21), 11357-11362.
91. Silberstein, A.; Mirzabekov, T.; Anderson, W. F.; Rozenberg, Y., Membrane destabilization assay based on potassium release from liposomes. *Biochimica. et. Biophysica. Acta.* **1999**, *1461* (1), 103-112.

Chapter 2

3-(1*H*-1,2,3-Triazol-1-yl) benzamide-Based Small Molecules as Nontoxic Cl⁻ Transporters



2.1. Introduction:

Ion transport across cell membranes is an essential procedure for the maintenance of osmoregulatory functions, neural transmission for nerve coordination, muscular excitation, cell survival, cell differentiation, and apoptosis, among numerous diverse phenomena occurring in the realm of cellular systems.¹⁻³ Nature has provided the cells with a specific type of membrane transport proteins known as ion channels because the hydrophobic portion of the cell membranes acts as a barrier for the penetration of these polar substances.⁴⁻⁶ and carriers. Since these transport proteins are essential to cells, any defect or malfunctioning of these systems can induce "channelopathies" such as Bartters syndrome, Dent's disease, Best disease, Startle disease, and cystic fibrosis (CF), which can disrupt normal cellular functioning.^{7, 8} CF is a hereditary and life-limiting disorder⁹ that is caused by the genetic mutation of the gene coding for the protein cystic fibrosis transmembrane conductance regulator (CFTR).¹⁰ CFTR is a transmembrane channel that transports chloride and bicarbonate ions in the apical membrane of the epithelium, and also regulates the function of other channels.¹¹ The primary objectives of current treatments are to alleviate disease symptoms and lower the risk of complications. These include anti-inflammatories, potent antibiotics, and pancreatic enzyme replacements.¹² Recently, Schultz and co-workers advocated a peptide-based channel replacement therapy as a new approach where a channel-forming peptide was applied to the apical surface of CF airway tissues to promote anion secretion and surface hydration. The approach was further investigated by Yang and co-workers by using a small-molecule channel forming molecule.¹³ Very recently, the amphotericin B, a non-selective ion channel forming molecule, was used by Burke and co-workers to retain the host defense in cystic fibrosis airway epithelia with a genotype independent mechanism.¹⁴ In principle, synthetic ion carriers have the potential to be used in this replacement therapy for CF disease, however, their toxicity in healthy cells becomes an issue. Davis and co-workers reported a decalin bis-urea anion transporter which was non-toxic to cells up to 30 μ M concentration.⁹ This outcome inspired us to explore new non-toxic chloride selective ion transport systems. Herein, we report a class of 3-(1*H*-1,2,3-triazol-1-yl) benzamides as newly designed synthetic chloride carriers that show low cytotoxicity even at elevated concentrations. In the literature, benzene-1,3-dicarboxamides are reported as efficient anion transporters.¹⁵⁻¹⁹ In the design of the new transporter, we replaced one of the carboxamide linkages with its bioisostere 1,2,3-triazole because the moiety is known to be less toxic but still possesses anion binding ability.²⁰ The second amide moiety was retained in the structure to

ensure strong anion binding and optimum water solubility.²¹ The structures of the 3-(1*H*-1,2,3-triazol-1-yl)benzamides **1a–1e** are illustrated in Figure 2.1. The substitution on the 1,2,3-triazole ring was kept identical while the tail of the amide moiety was varied with 4-methylphenyl, 4-methoxyphenyl, 4-bromophenyl, 4-(trifluoromethyl) phenyl, and *n*-hexyl to modulate the p*K*_a of the amide N-H bond and log*P* of the molecules (Table 1). The *N*¹, *N*³-bis[4-(trifluoromethyl) phenyl] benzene-1,3-dicarboxamide **2** was also designed to compare its anion transport and cytotoxicity with the 3-(1*H*-1,2,3-triazol-1-yl) benzamide system.

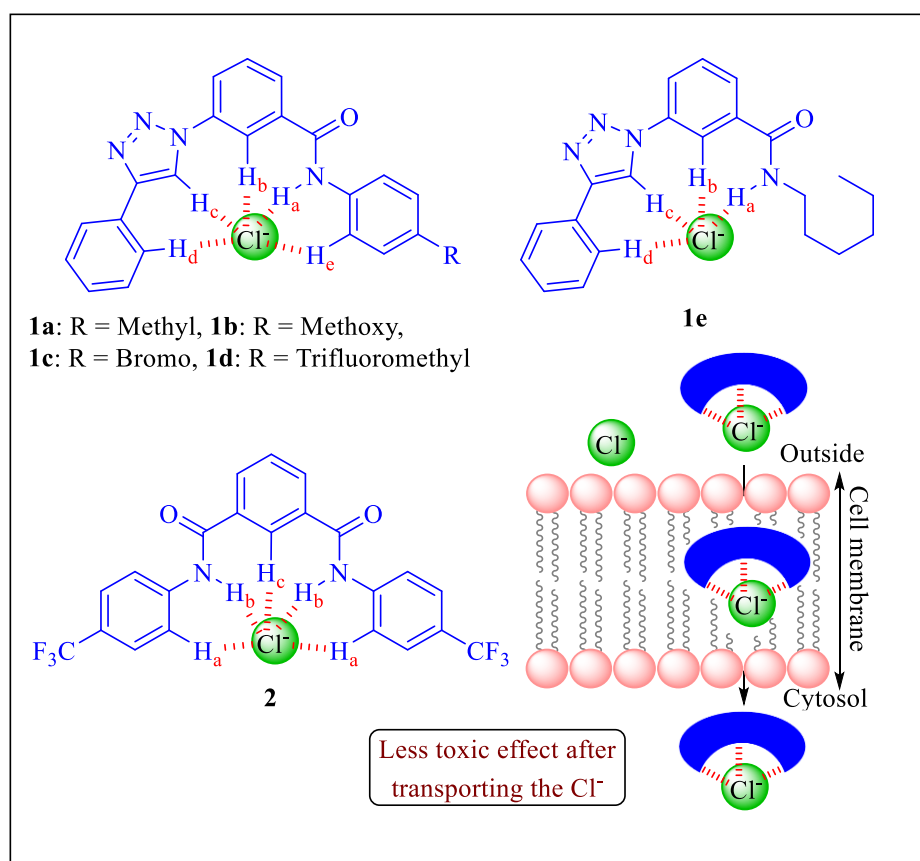


Figure 2.1. Structures of transporter molecules **1a–1e** and representative anion transport across the cell membrane.

Table 1. The p*K*_a and log*P* values of transporters **1a–1e** and **2**:

Compound	R	p <i>K</i> _a of (N-H)	log <i>P</i>
1a	-CH ₃	12.27	5.32
1b	-OCH ₃	11.89	4.63
1c	-Br	11.77	5.57

1d	-CF ₃	11.73	5.68
1e	---	15.14	5.00
2	---	11.15, 12.04	5.91

2.2. Results and discussion:

2.2.1. General methods:

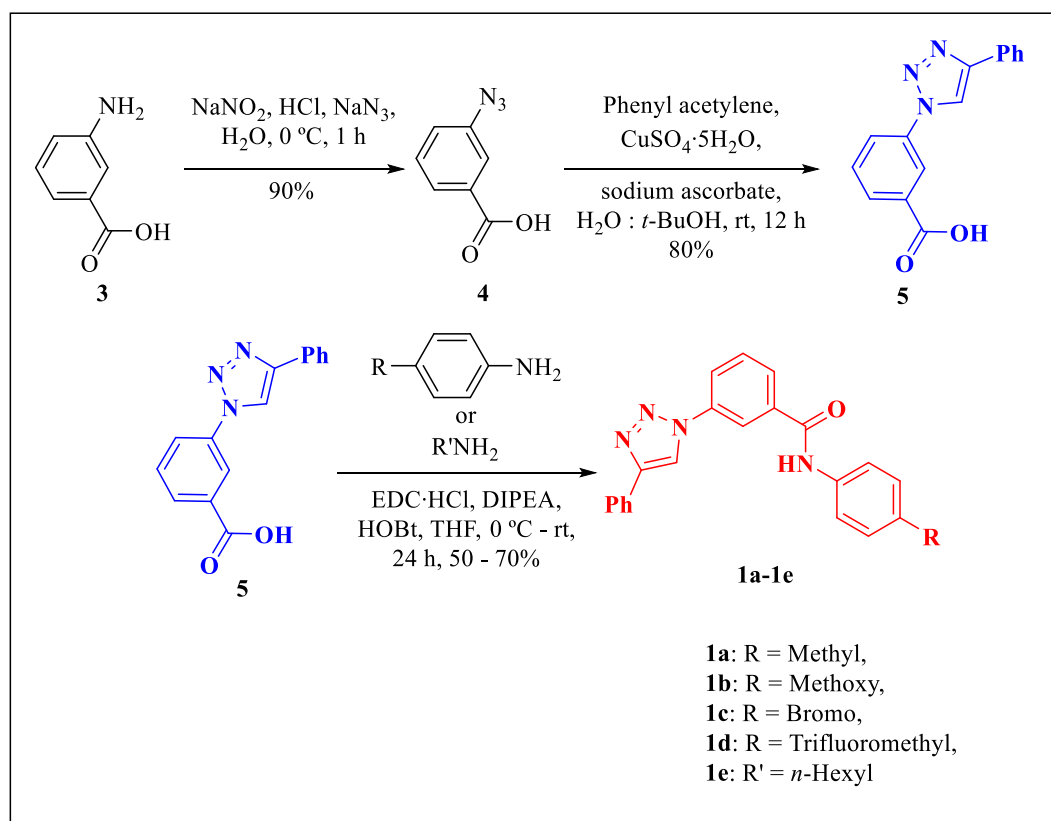
All chemical reactions were performed under a nitrogen atmosphere. All synthesized reagents and solvents were purchased from commercial sources (Sigma Aldrich, Spectrochem) and used further without purification. The column chromatography was carried out using Merck silica (100–200 or 230–400 mesh size). The thin-layer chromatography was performed on E. Merck silica gel 60–F254 plates. Egg yolk phosphatidylcholine (EYPC) as a solution of chloroform (25 mg/ mL), mini extruder, polycarbonate membrane of 100 nm and 200 nm were purchased from Avanti Polar Lipid. HEPES, HPTS, Lucigenin, NaOH, Triton X–100, FCCP, Valinomycin and all inorganic salts were obtained as molecular biology grade from Sigma Aldrich.

2.2.2. Physical measurements:

The ¹H NMR spectra were recorded at 400 MHz whereas ¹³C NMR was recorded at 101 MHz. The residual solvent signals were considered as an internal reference (¹H NMR CDCl₃: δ 7.26 ppm; ¹³C NMR CDCl₃: δ 77.2 ppm; ¹H NMR CD₃OD: δ 3.31 ppm; ¹³C NMR CD₃OD: δ 49.2 ppm; ¹H NMR DMSO-*d*₆: δ 2.5 ppm; ¹³C NMR DMSO-*d*₆: δ 39.5 ppm) to calibrate spectra. The chemical shifts were reported in ppm. Following abbreviations were used to indicate multiplicity patterns m: multiplet, s: singlet, d: doublet, t: triplet, q: quartet, dd: doublet of doublet, ddd: doublet of doublet of doublet, td: triplet of doublet, dt: doublet of triplet. Coupling constants were measured in Hz. Melting points were measured on micro melting point apparatus. High-resolution mass spectra (HRMS) were recorded on electrospray ionization time-of-flight (ESI-TOF). Fluorescence experiments were recorded on Fluoromax-4 from Jobin Yvon Edison equipped with an injector port and magnetic stirrer in a microfluorescence cuvette. All buffer solutions were prepared from the autoclaved water. The Helmer pH meter was adjusted to the pH of buffer solutions. The extravesicular dye was removed by performing

gel chromatography using Sephadex-G. The fluorescence studies were conducted using Origin 8.5. MTT assay and MQAE assay readings were carried out using a microplate reader (Varioskan Flash). Graphpad Prism 7 was used for plotting the data from biological assays.

2.2.3. Synthesis:



Scheme 2.1. Synthetic scheme of **1a-1e**.

The synthesis of compounds **1a-1e** was started from 3-aminobenzoic acid **3**, which was converted to corresponding azide **4** by a reported protocol.²² The azide **4** was subjected to the Cu(I)-catalyzed click reaction²³ with phenylacetylene to get compound **5**, which was finally coupled with corresponding amines to get the final products **1a-1e** (Scheme 2.1). The compound **2** was synthesized by coupling isophthaloyl dichloride **6** with 4-(trifluoromethyl) aniline (Scheme 2.2).²⁴

3-(1-phenyl-1H-1,2,3-triazol-4-yl) benzoic acid ($\text{C}_{15}\text{H}_{11}\text{N}_3\text{O}_2$) **5**:

The compound **4** (360 mg, 2.2 mmol) and phenyl acetylene (150 mg, 1.47 mmol) were suspended in the 1:1 mixture of H_2O and $t\text{-BuOH}$ (4 mL each) followed by the addition of sodium ascorbate (29 mg, 0.1 mmol) in H_2O . Then, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (2.3 mg, 0.01 mmol) was

added and the reaction mixture was allowed to stir at room temperature overnight. The reaction mixture was then cooled on ice and diluted with water, followed by the extraction with ethyl acetate. The combined organic phases were washed with brine and dried over sodium sulfate and concentrated *in vacuo*. The compound was directly used for another step.

General method for the synthesis of 1a–1e:

The compound **5** (1 equiv, 0.56 mmol) was dissolved in dry tetrahydrofuran (5 mL), followed by the addition of the respective amine (1.5 equiv, 0.85 mmol), HOBt (2 equiv, 1.13 mmol), and diisopropylethylamine (2 equiv, 1.13 mmol). The reaction mixture was allowed to cool at 0 °C. Then, EDC·HCl salt (2 equiv, 1.13 mmol) was added to the reaction mixture at 0 °C. The reaction mixture was allowed to stir at room temperature for 24 hours. Then, water was added to the reaction mixture followed by extraction with EtOAc (10 mL × 3 times). The combined organic phases were washed with brine and dried over sodium sulfate and concentrated *in vacuo*. The individual compounds were purified using silica gel chromatography over silica gel (Eluent: 20% EtOAc in petroleum ether). The compounds were characterized by melting point, ¹H NMR, ¹³C NMR, and HRMS.

3-(1-phenyl-1*H*-1,2,3-triazol-4-yl)-*N*-(*p*-tolyl)benzamide (C₂₂H₁₈N₄O) 1a: The crude product was purified by column chromatography over silica gel (20% EtOAc in petroleum ether) to furnish **1a** as a white solid (47 mg, 55% yield); **M.P.:** 251–253 °C; **¹H NMR (400 MHz, DMSO-*d*₆):** δ 10.44 (s, 1H), 9.47 (s, 1H), 8.55 (t, *J* = 1.8 Hz, 1H), 8.22 (ddd, *J* = 8.1, 2.2, 0.9 Hz, 1H), 8.15 – 8.07 (m, 1H), 8.02 (dd, *J* = 5.1, 3.2 Hz, 2H), 7.82 (dd, *J* = 23.7, 15.8 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.56 (dd, *J* = 10.5, 4.7 Hz, 2H), 7.50 – 7.41 (m, 1H), 7.23 (d, *J* = 8.3 Hz, 2H), 2.54 (dt, *J* = 3.6, 1.8 Hz, 3H); **¹³C NMR (101 MHz, DMSO-*d*₆):** δ 164.2, 147.5, 136.8, 136.7, 136.4, 133.0, 130.2, 129.1, 129.0, 128.3, 127.7, 125.4, 122.7, 120.5, 119.8, 119.2, 30.7, 20.5; **HRMS (ESI):** Calc. for C₂₂H₁₈N₄O [M+Na]⁺: 319.1294; Found: 319.1289.

***N*-(4-methoxyphenyl)-3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzamide (C₂₂H₁₈N₄O₂) 1b:** The crude product was purified by column chromatography over silica gel (20% EtOAc in petroleum ether) to furnish **1b** as a greyish white solid (50 mg, 60% yield); **M.P.:** 250–252 °C; **¹H NMR (400 MHz, DMSO-*d*₆):** δ 10.84 (s, 1H), 9.46 (s, 1H), 8.56 (s, 1H), 8.22 (d, *J* = 8.0 Hz, 1H), 8.11 (d, *J* = 7.9 Hz, 1H), 8.05 (d, *J* = 8.5 Hz, 2H), 7.98 (d, *J* = 7.3 Hz, 2H), 7.83 (t, *J* = 7.9 Hz, 1H), 7.77 (d, *J* = 8.6 Hz, 2H), 7.52 (t, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 1H), 3.32 (s, 3H); **¹H NMR (400 MHz, Acetone-*d*₆):** δ 9.69 (s, 1H), 9.12 (s, 1H), 8.52 (t, *J* = 1.8 Hz,

1H), 8.17 (ddd, $J = 8.1, 2.2, 1.0$ Hz, 1H), 8.11 (dt, $J = 7.7, 1.2$ Hz, 1H), 8.03 (dt, $J = 8.1, 1.6$ Hz, 2H), 7.78 (m, 3H), 7.50 (t, $J = 7.6$ Hz, 2H), 7.39 (t, $J = 7.4$ Hz, 1H), 6.95 (d, $J = 9.1$ Hz, 2H), 3.81 (s, 3H); **¹³C NMR (101 MHz, DMSO-*d*₆)**: δ 163.9, 155.7, 147.5, 136.8, 136.7, 131.9, 130.2, 129.1, 128.4, 127.7, 125.4, 122.7, 122.1, 120.9, 119.9, 119.1, 113.8, 55.2; **HRMS (ESI)**: Calc. for C₂₂H₁₈N₄O₂ [M+H]⁺: 371.1508; Found: 371.1512.

***N*-(4-bromophenyl)-3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzamide (C₂₁H₁₅BrN₄O)**

1c: The crude product was purified by column chromatography over silica gel (20% EtOAc in petroleum ether) to furnish **1c** as a white solid (48 mg, 70% yield); **M.P.**: 277–278 °C; **¹H NMR (400 MHz, DMSO-*d*₆)**: δ 10.61 (s, 1H), 9.45 (s, 1H), 8.53 (t, $J = 1.8$ Hz, 1H), 8.25 – 8.17 (m, 1H), 8.12 – 8.03 (m, 1H), 8.01 – 7.96 (m, 2H), 7.83 (d, $J = 6.8$ Hz, 1H), 7.83 – 7.76 (m, 2H), 7.62 – 7.57 (m, 2H), 7.52 (d, $J = 7.8$ Hz, 2H), 7.44 – 7.37 (m, 1H); **¹³C NMR (101 MHz, DMSO-*d*₆)**: δ 164.5, 147.5, 138.3, 136.7, 136.4, 131.6, 130.3, 130.1, 129.1, 128.4, 127.8, 125.4, 123.0, 122.3, 119.9, 119.2, 115.7; **HRMS (ESI)**: Calc. for C₂₁H₁₅BrN₄O [M+H]⁺: 419.0507; Found: 419.0502.

3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-*N*-(4-(trifluoromethyl)phenyl)benzamide

(C₂₂H₁₅F₃N₄O) 1d: The crude product was purified by column chromatography over silica gel (20% EtOAc in petroleum ether) to furnish **1d** as a grey solid (53 mg, 65% yield); **M.P.**: 264–265 °C; **¹H NMR (400 MHz, DMSO-*d*₆)**: δ 10.84 (s, 1H), 9.46 (s, 1H), 8.56 (s, 1H), 8.28 – 8.19 (m, 1H), 8.11 (d, $J = 7.9$ Hz, 1H), 8.06 (d, $J = 8.5$ Hz, 2H), 8.04 – 7.95 (m, 2H), 7.84 (t, $J = 7.9$ Hz, 1H), 7.78 (d, $J = 8.6$ Hz, 2H), 7.53 (t, $J = 7.6$ Hz, 2H), 7.41 (t, $J = 7.4$ Hz, 1H); **¹³C NMR (101 MHz, DMSO-*d*₆)**: δ 165.46, 148.04, 143.1, 137.2, 136.7, 130.8, 130.6, 129.6, 128.9, 128.5, 126.6, 125.9, 124.6, 124.3, 123.7, 120.8, 120.4, 119.8; **HRMS (ESI)**: Calc. for C₂₂H₁₅F₃N₄O [M+H]⁺: 409.1276; Found: 409.1279.

***N*-hexyl-3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzamide (C₂₁H₂₄N₄O) 1e**

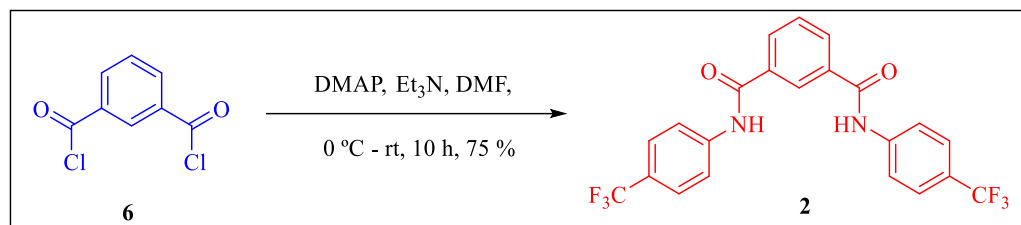
The crude product was purified by column chromatography over silica gel (20% EtOAc in petroleum ether) to furnish **1e** as an orange solid (53 mg, 50% yield); **M.P.**: 196–197 °C; **¹H NMR (400 MHz, DMSO-*d*₆)**: δ 9.42 (s, 1H), 8.72 (t, $J = 5.5$ Hz, 1H), 8.45 (t, $J = 1.8$ Hz, 1H), 8.14 (ddd, $J = 8.1, 2.2, 0.9$ Hz, 1H), 8.00 (ddd, $J = 7.8, 3.5, 1.4$ Hz, 3H), 7.77 (t, $J = 7.9$ Hz, 1H), 7.60 – 7.50 (m, 2H), 7.47 – 7.39 (m, 1H), 3.35 – 3.30 (m, 2H), 1.69 – 1.50 (m, 2H), 1.44 – 1.28 (m, 6H), 0.91 (t, $J = 6.8$ Hz, 3H); **¹³C NMR (101 MHz, DMSO-*d*₆)**: δ 164.9, 147.4, 136.6, 136.4,

130.2, 130.0, 129.0, 128.3, 127.2, 125.4, 122.4, 119.8, 118.8, 31.0, 29.0, 26.2, 22.1, 13.9;

HRMS (ESI): Calc. for C₂₁H₂₄N₄O [M+H]⁺: 349.2028; Found: 349.2030.

Synthesis of *N*¹, *N*³-bis(4-(trifluoromethyl) phenyl) isophthalamide (C₂₂H₁₄F₆N₂O₂)

2:



Scheme 2.2. Synthesis of **2**.

Compound **6** was purchased from a commercial source and used without further purification. Compound **2** was prepared following the literature reported procedure according to Scheme 2.2, and its ¹H and ¹³C NMR data were matched with the reported data.²⁴

To a solution of isophthaloyl dichloride **6** (63 mg, 0.031 mmol) in DMF (1.5 mL) at 0 °C were added 4-(trifluoromethyl) aniline (40 μL, 0.32 mmol), 4-dimethylaminopyridine (42 mg, 0.34 mmol) and triethylamine (86 μL, 0.68 mmol). The reaction mixture was allowed to come to room temperature and stirred for 10 hours. Then, water was added to the reaction mixture followed by extraction with ethyl acetate (10 mL × 3 times). The combined organic phase was washed with brine, dried over sodium sulfate and concentrated *in vacuo*. The crude compound was purified using silica gel chromatography (Eluent: 5% MeOH in CHCl₃) to get compound **2** as a white solid (105 mg, 75%). **M.P.:** 225–226 °C; **¹H NMR (400 MHz, DMSO-*d*₆):** δ 10.77 (s, 2H), 8.57 (t, *J* = 1.6 Hz, 1H), 8.19 (dd, *J* = 7.8, 1.8 Hz, 2H), 8.04 (d, *J* = 8.5 Hz, 4H), 7.75 (d, *J* = 9.2 Hz, 5H); **¹³C NMR (101 MHz, DMSO-*d*₆):** δ 165.3, 142.5, 134.6, 130.9, 128.6, 125.8, 123.8, 123.4, 122.8, 120.0; **HRMS (ESI):** Calc. for C₂₂H₁₄F₆N₂O₂ [M+H]⁺: 453.1038; Found: 453.1040.

2.2.4. NMR titration studies:

The stock solutions of the compounds (2 mM) and tetrabutylammonium chloride (200 mM) were prepared in acetonitrile-*d*₃. ¹H NMR spectra were recorded on a Bruker AscentTM 400 spectrometer and calibrated with respect to residual solvent peak in acetonitrile-*d*₃. ¹H NMR titrations were performed by stepwise addition of tetrabutylammonium chloride (TBACl) to

the solution of the compounds in acetonitrile-*d*₃. Upon addition of TBACl, the changes in the amide N-H chemical shifts were used for calculating the binding constant values. The ¹H NMR stacked plots were processed using MestReNova 6 software. The association constant was obtained by fitting the data either in 1:1 or 2:1 binding model of Bindfit program.²⁵

At first, we evaluated the chloride binding mode of compound **1d** by titrating it with tetrabutylammonium chloride (TBACl) in acetonitrile-*d*₃ (CD₃CN).²⁶ The titration data confirmed the downfield shifts for phenyl C-H (H_d), triazole C-H (H_c), carboxamide-linked aromatic C-H (H_b), and amide N-H (H_a) protons, indicating the interaction of these protons with the chloride ion (Figure 2.3-2.12). Moreover, the trifluoromethyl-containing aromatic ring also participated in hydrogen bonding interaction with the anion by involving the C-H (H_e) at the ortho-position. (Figure 2.8) Further analysis of the chemical shift of the amide N-H (H_a) of **1d** versus chloride concentration by the Bindfit program²⁵ confirmed a 1:1 host-guest binding model with a binding constant of 803 M⁻¹ ± 11%. For compounds **1a**, **1b**, and **1c**, the chloride binding modes were similar, and binding constants of 222 M⁻¹ ± 4%, 245 M⁻¹ ± 9%, and 531 M⁻¹ ± 10%, respectively (Figure 2.3-2.10). For **1e**, no significant participation of the aliphatic protons was observed, and a relatively lower binding constant of 160 M⁻¹ ± 1% was calculated for this compound (Figure 2.11 and 2.12). As expected, compound **2** showed hydrogen bond interactions with a chloride involving H_a, H_b, and H_c protons and the binding constant values of 32314 M⁻¹ ± 2% (1:1) and 2744 M⁻² ± 3% (2:1) (Figures 2.13 and 2.14) were obtained.

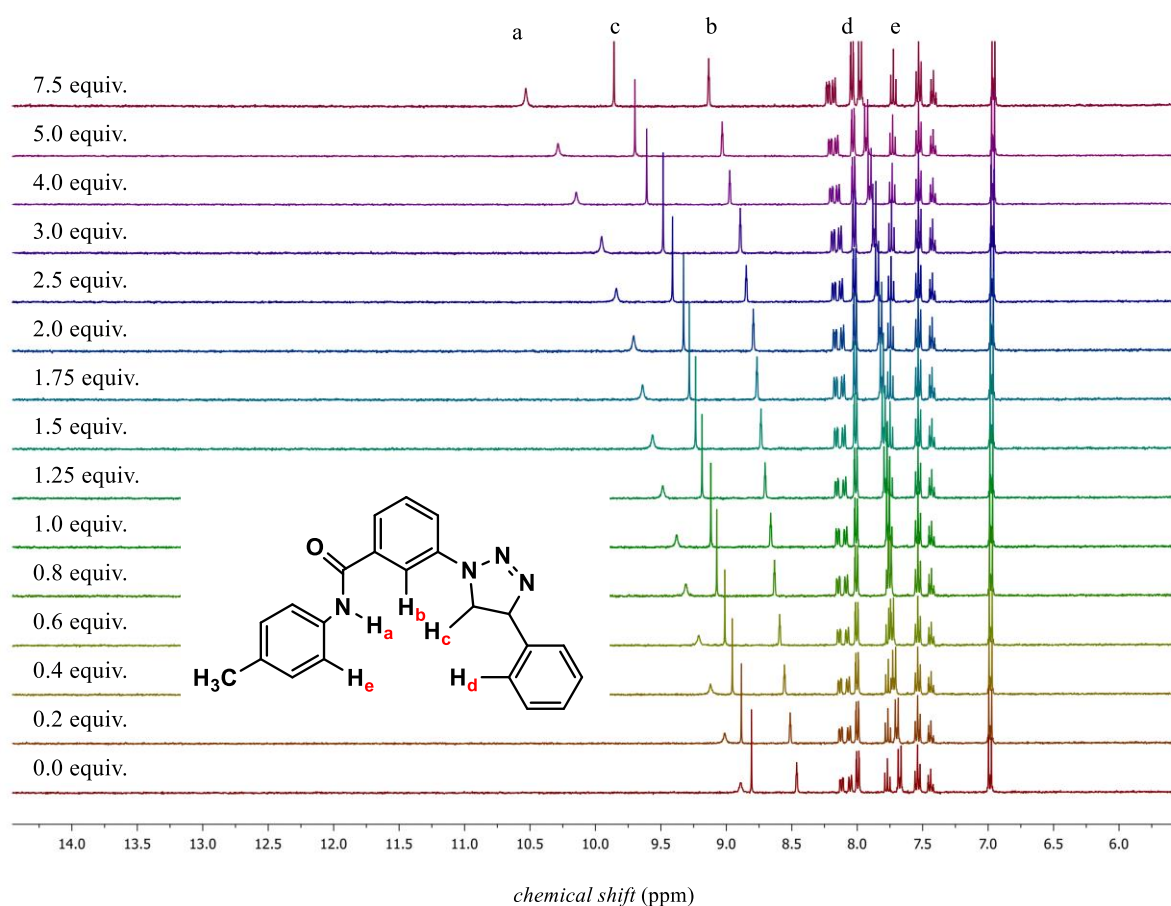


Figure 2.3. Stacked plot of ¹H NMR titration experiment (400 MHz, acetonitrile-*d*₃) for compound **1a** (2.0 mM) with the successive addition of TBACl.

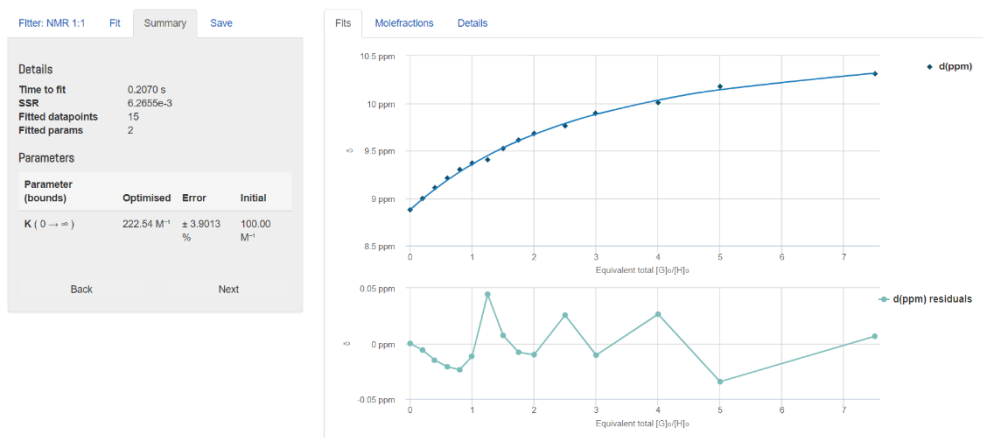


Figure 2.4. Screenshot of the fitted data plot from supramolecular.org. The binding constant was found to be 222 M⁻¹ ± 4% in 1:1 receptor to anion binding model (left side). The changing pattern of chemical shift and chemical shift residuals with the increasing equivalent of TBACl (right side). The Bindfit URL for this experiment is: <http://app.supramolecular.org/bindfit/view/580d84dc-f4ab-49a1-9050-0a297b0cf0f1>.

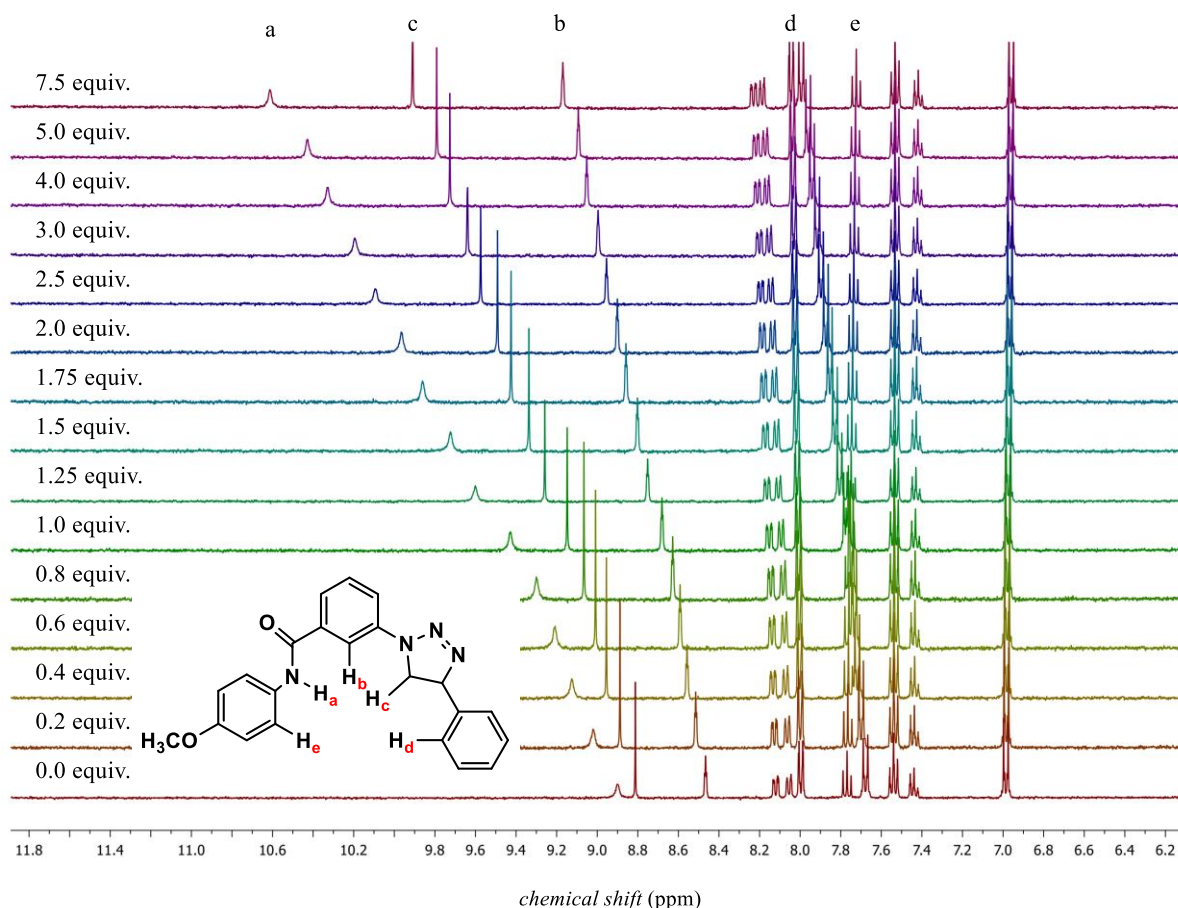


Figure 2.5. Stacked plot of ¹H NMR titration experiment (400 MHz, acetonitrile-*d*₃) for compound **1b** (2.0 mM) with the successive addition of TBACl.

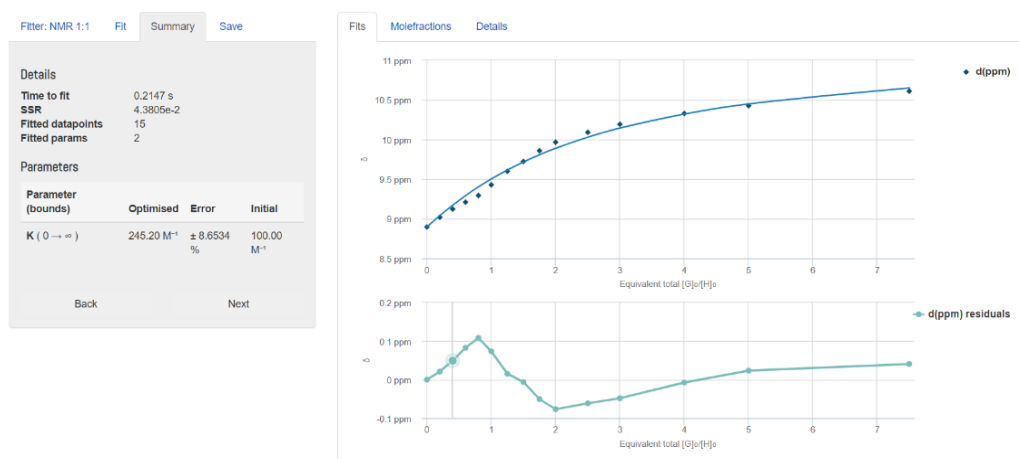


Figure 2.6. Screenshot of the fitted data plot from supramolecular.org. The binding constant was found to be 245 M⁻¹ ± 9% in 1:1 receptor to anion binding model (left side). The changing pattern of chemical shift and chemical shift residuals with the increasing equivalent of TBACl (right side). The Bindfit URL for this experiment is <http://app.supramolecular.org/bindfit/view/76a0abe0-4399-41f0-8e3f-274dfd3461e6>.

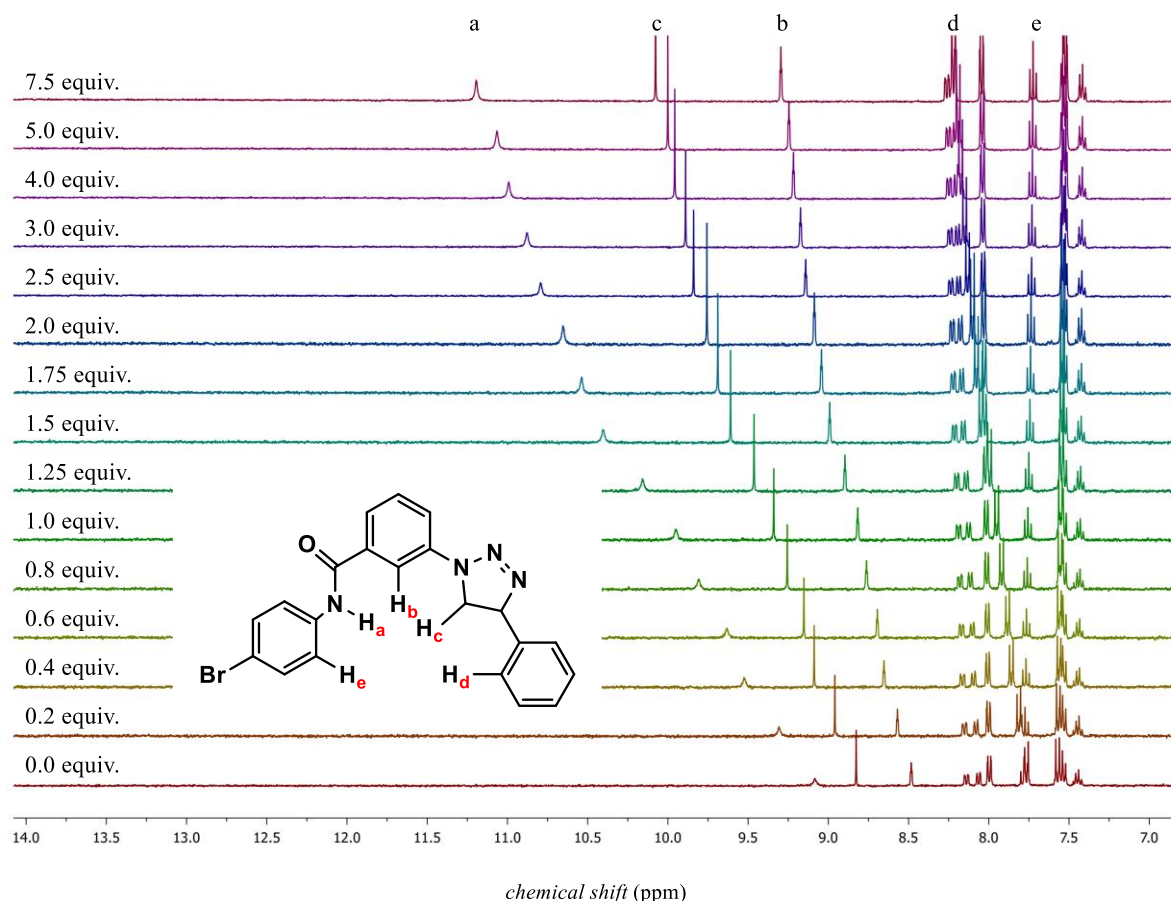


Figure 2.7. Stacked plot of ¹H NMR titration experiment (400 MHz, acetonitrile-*d*₃) for compound **1c** (2.0 mM) with the successive addition of TBACl.

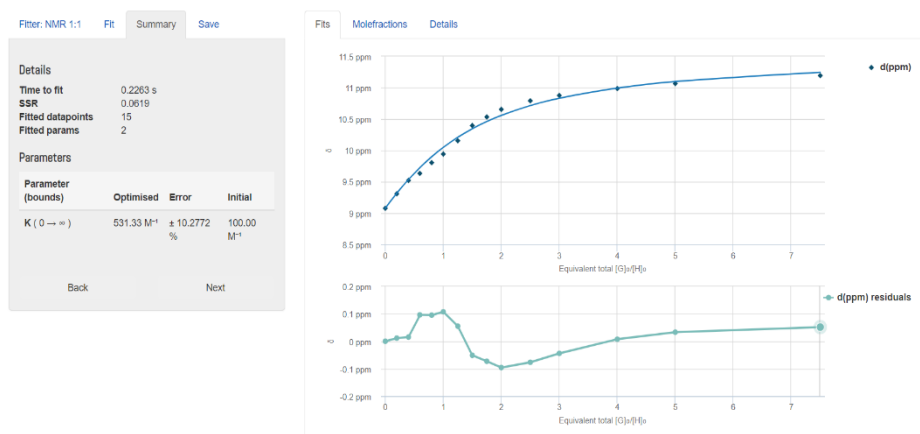


Figure 2.8. Screenshot of the fitted data plot from supramolecular.org. The binding constant was found to be 531 M⁻¹ ± 10% in 1:1 receptor to anion binding model (left side). The changing pattern of chemical shift and chemical shift residuals with the increasing equivalent of TBACl (right side). The Bindfit URL for this experiment is <http://app.supramolecular.org/bindfit/view/14092ac5-1845-4e1b-9b56-a3b8f5fdb04f>.

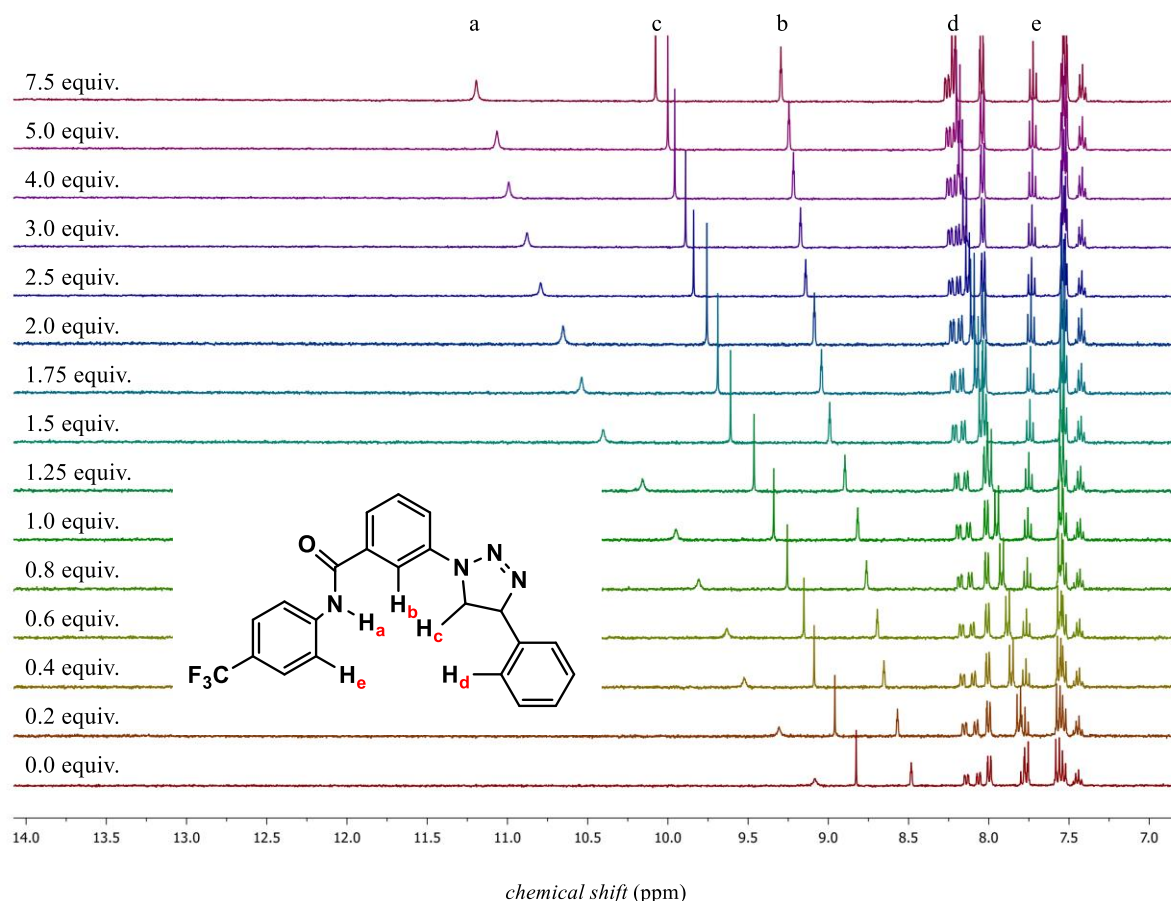


Figure 2.9. Stacked plot of ¹H NMR titration experiment (400 MHz, acetonitrile-*d*₃) for compound **1d** (2.0 mM) with the successive addition of TBACl.

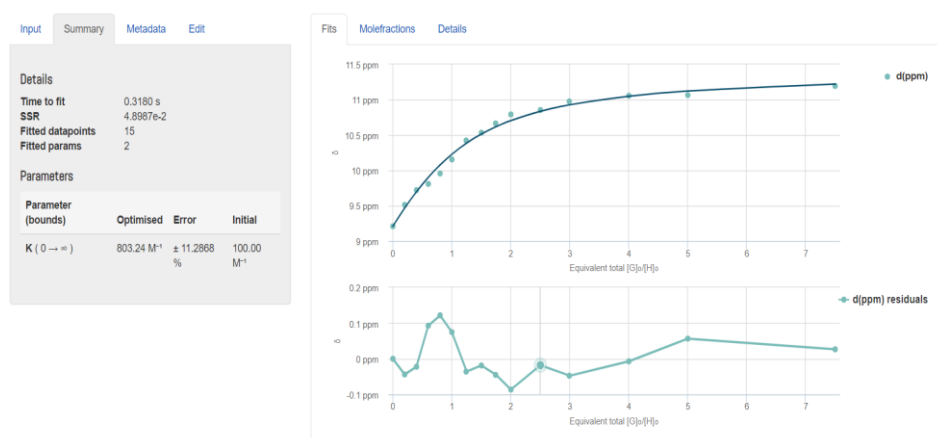


Figure 2.10. Screenshot of the fitted data plot from supramolecular.org. The binding constant was found to be 803 M⁻¹ ± 11% in 1:1 receptor to anion binding model (left side). The changing pattern of chemical shift and chemical shift residuals with the increasing equivalent of TBACl (right side). The Bindfit URL for this experiment is <http://app.supramolecular.org/bindfit/view/c6a6c8df-b82d-4dea-b900-e03ddc336d40>.

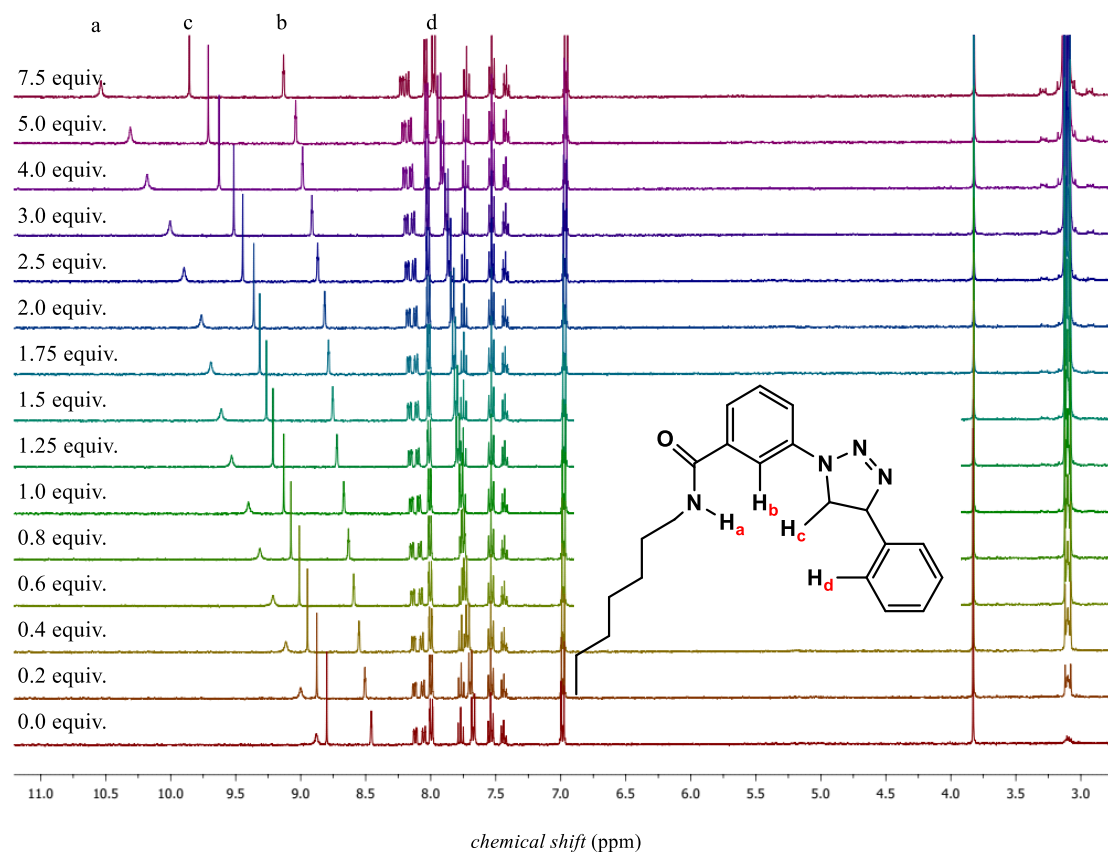


Figure 2.11. Stacked plot of ¹H NMR titration experiment (400 MHz, acetonitrile-*d*₃) for compound **1e** (2.0 mM) with the successive addition of TBACl.

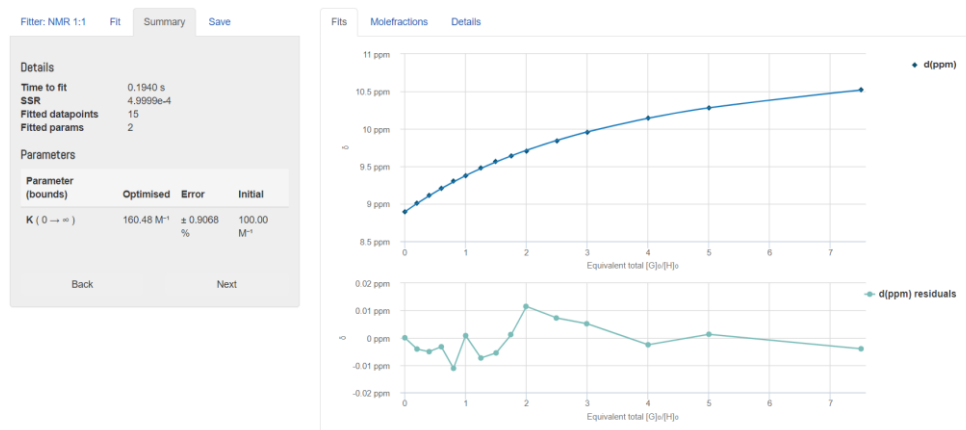


Figure 2.12. Screenshot of the fitted data plot from supramolecular.org. The binding constant was found to be 160 M⁻¹ ± 1% in 1:1 receptor to anion binding model (left side). The changing pattern of chemical shift and chemical shift residuals with the increasing equivalent of TBACl (right side). The Bindfit URL for this experiment is <http://app.supramolecular.org/bindfit/view/7144b3b7-a5dd-4d1a-87b8-671dfd3e5f82>.

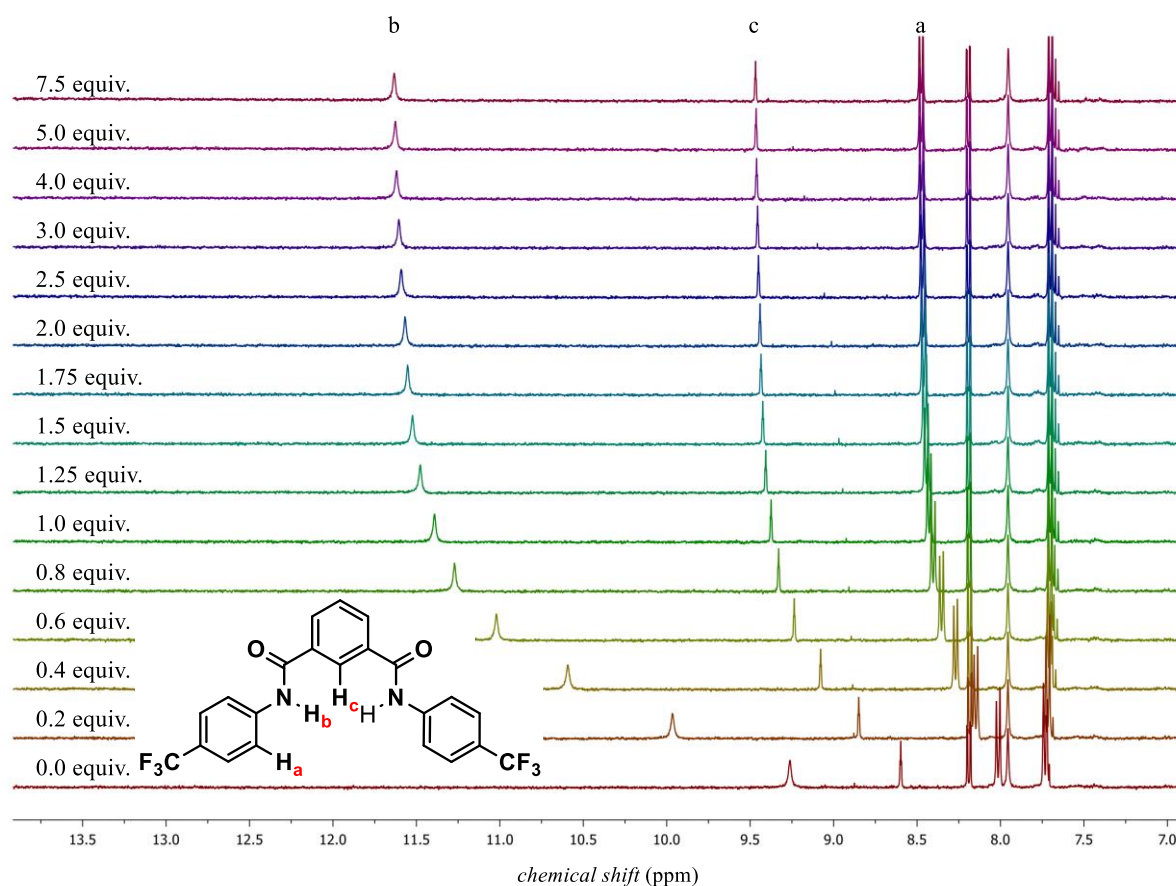


Figure 2.13. Stacked plot of ¹H NMR titration experiment (400 MHz, acetonitrile-*d*₃) for compound **2** (2.0 mM) with the successive addition of TBACl.

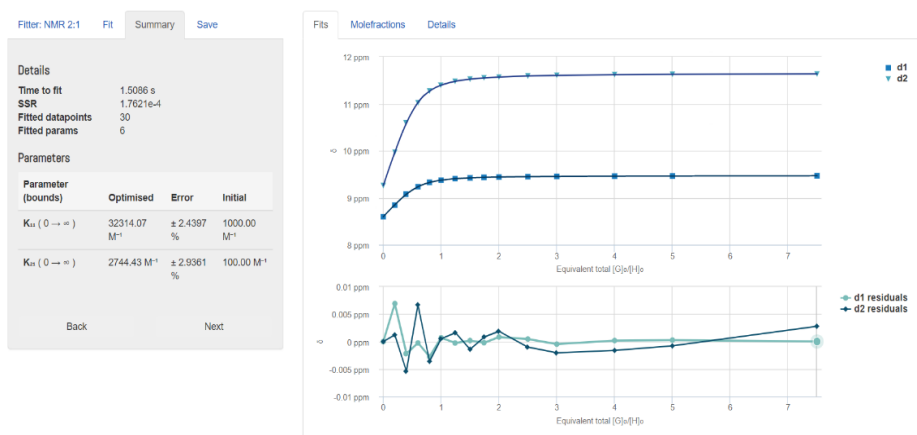


Figure 2.14. Screenshot of the fitted data plot from supramolecular.org. The binding constant was found to be $32314\text{ M}^{-1} \pm 2\%$ in 1:1 receptor to anion binding model and $2744\text{ M}^{-2} \pm 3\%$ in 2:1 receptor to anion binding model (left side). The changing pattern of chemical shift and chemical shift residuals with the increasing equivalent of TBACl (right side). The Bindfit URL for this experiment is <http://app.supramolecular.org/bindfit/view/a578a92f-ec95-4bfe-b7bf-33d65b917b22>.

2.2.5. Theoretical calculations:

Based on the stoichiometry obtained from ¹H NMR titration (1:1) and ion transport-based Hill coefficient value of ~ 1 , geometry optimization for the **1d** and [**1d**+Cl⁻] complex was performed.^{27, 28} The most probable conformations of **1d** and [**1d**+Cl⁻] complex were obtained from the CONFLEX 8 software program^{29, 30} and subsequently, their geometry optimization studies were performed (Figure 2.14). The resulting structure of [**1d**+Cl⁻] complex confirmed that each receptor participates in the anion recognition by hydrogen bonding interactions with H_a (H_a⋯Cl⁻ = 2.31 Å), H_b (H_b⋯Cl⁻ = 2.42 Å), H_c (H_c⋯Cl⁻ = 2.39 Å), H_d (H_d⋯Cl⁻ = 3.29 Å), and H_e (H_e⋯Cl⁻ = 2.53 Å) protons (Figure 2.15B). The binding energy for the [**1d**+Cl⁻] complex was calculated and found to be -72.99 kcal/mol.

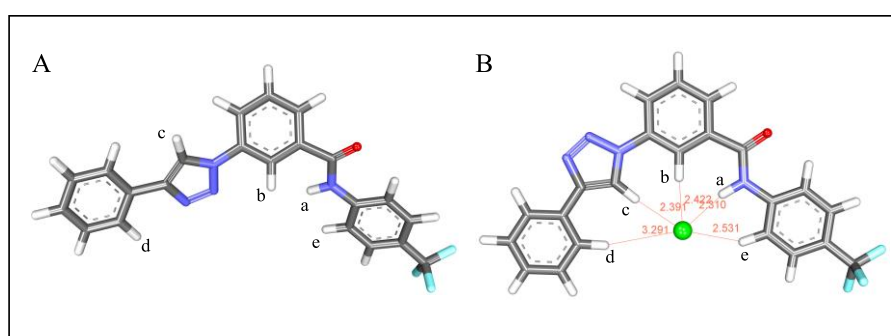


Figure 2.15. Geometry optimized structures of (A) **1d** and (B) [**1d**+Cl⁻] complex.

To get an idea about the different conformation of **1d**, several initial geometries of the complex were generated using the CONFLEX 8 software package.³⁰ using MMFF94s force field. The Boltzmann population with their percentage of populations are **Conf-1** with 24.84%, **Conf-2** with 24.74%, **Conf-3** with 22.96%, **Conf-4** with 22.90%, **Conf-5** with 1.22%, **Conf-6** with 1.21%, **Conf-7** with 1.07%, and **Conf-8** with 1.06% populations (Figure 2.16).

The structure with 22.96% Boltzmann populations was used for geometry optimization. The geometry optimization was carried out by Gaussian 09 program²⁷ package using B3LYP functional and 6-311++G(d,p) basis set.²⁸ For the structure **1d**, the vibrational frequency calculation during the geometry optimization has not shown any imaginary frequencies, which indicated that all optimized structures are ground state minima.

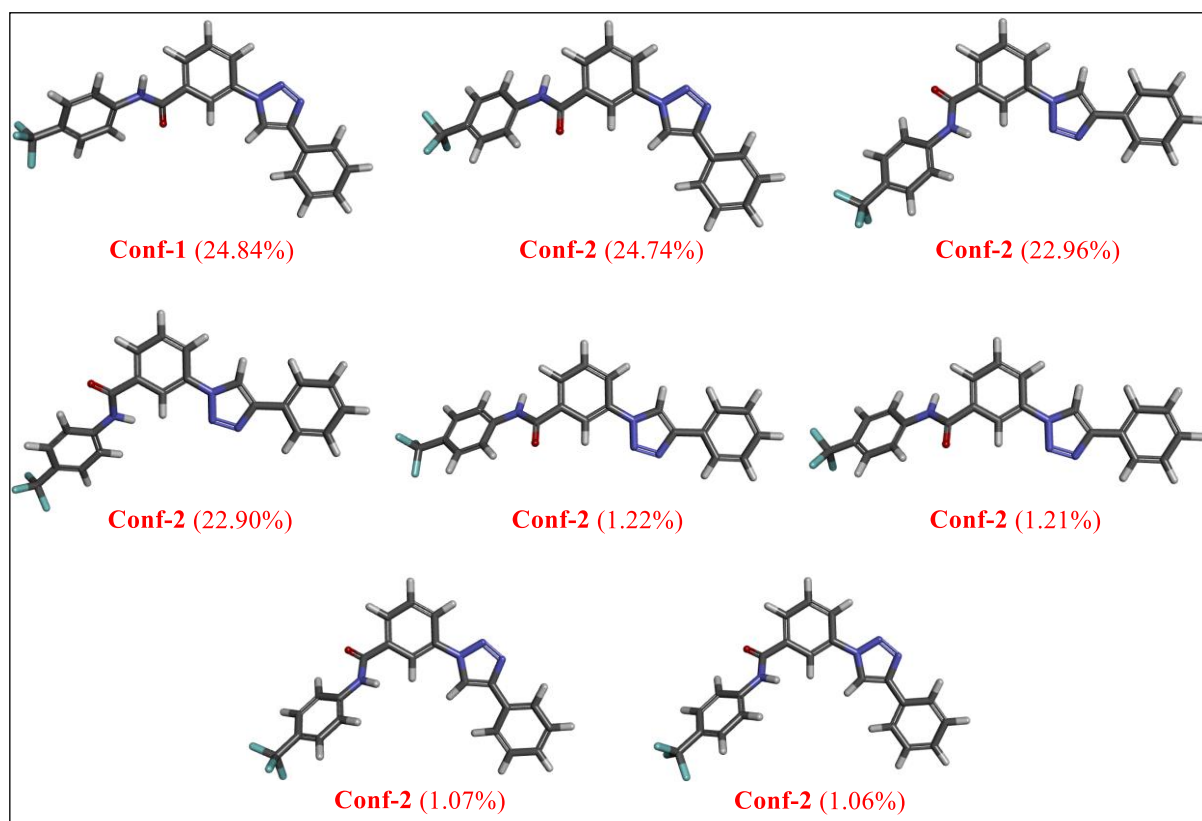


Figure 2.16. Initial geometries **Conf-1** and **Conf-8** for **1d**, optimized by CONFLEX 8 software using MMFF94s force field.

To get an idea about the different conformation of [**1d**+Cl⁻], several initial geometries of the complex were generated using the CONFLEX 8 software package³⁰ using MMFF94s force field. The calculation provided 319 conformers. The Boltzmann population of the two highest populated conformations are **Conf-1** with 50.08% and **Conf-2** with 49.87% populations (Figure 2.17).

Among all the conformations, the highest Boltzmann populated structure i.e. **Conf-1** (50.08%) was used for geometry optimization. The geometry optimization was carried out by Gaussian 09 program²⁷ package using B3LYP functional and 6-311++G(d,p) basis set.²⁸ For the anion complex, the vibrational frequency calculation during the geometry optimization has not shown any imaginary frequencies, which indicated that all optimized structures are ground state minima.

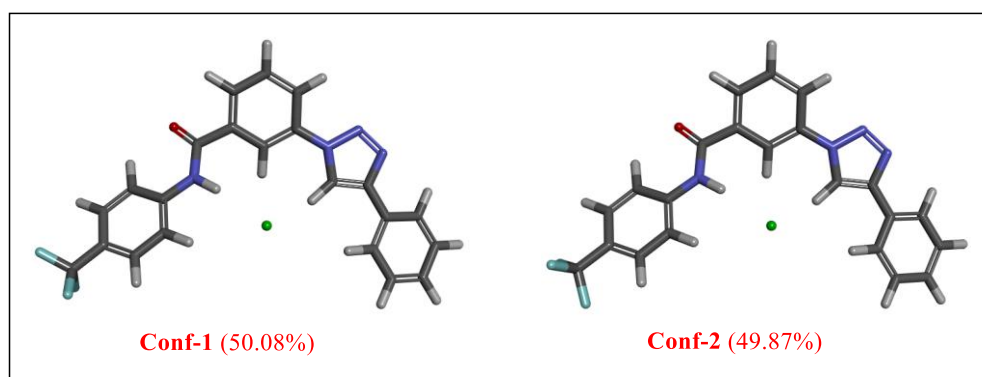


Figure 2.17. Initial geometries **Conf-1** and **Conf-2** for [1d+Cl⁻], generated by CONFLEX 8 software using MMFF94s force field.

The Gaussian 09 program was used to calculate the zero-point energy (ZPE) and basis set superposition error (BSSE) corrected bonding energy of [1d+Cl⁻] which was used for the calculation of binding energy (*BE*) using the following equations.

$$BE = [HF_{[1d+Cl^-]} + ZPE_{[1d+Cl^-]} + BSSE_{[1d+Cl^-]}] - [HF_{1d} + ZPE_{1d}] - [HF_{Cl^-}] \quad (\text{Equation 1})$$

where, $HF_{[1d+Cl^-]}$ = electronic energy of [1d+Cl⁻] complex, $ZPE_{[1d+Cl^-]}$ = zero-point energy of [1d+Cl⁻] complex, $BSSE_{[1d+Cl^-]}$ = BSSE of [1d+Cl⁻] complex, HF_{1d} = electronic energy of the receptor **1d**, ZPE_{1d} = zero point energy of the receptor **1d**, HF_{Cl^-} = electronic energy of anion Cl⁻.

Table 2. The electronic energy (HF), zero point energy (ZPE), basis set superposition error (BSSE) corrected energy (in Hartree unit) for all structures and complexes are calculated at the DFT B3LYP/6-311++G(d,p) level of theory.

Structures	Energy
$HF_{[1d+Cl^-]}$ (in Hartree)	-1901.897133
$ZPE_{[1d+Cl^-]}$ (in Hartree)	0.3322422
$BSSE_{[1d+Cl^-]}$ (in Hartree)	0.001835904

HF _{1d} (in Hartree)	-1441.52588140
ZPE _{1d} (in Hartree)	0.33139100
HF _{Cl-} (in Hartree)	-460.2522333
BE (in Hartree)	-0.11633067
BE (in kcal/mol)	-72.99853994

Table 3. Atomic coordinates of 1d after geometry optimization by Gaussian 09 program using B3LYP functional and 6-311++G(d,p) basis set.

Charge = 0 Multiplicity = 1

Atom number	Atom Type	x	y	Z
1	C	0.177885	3.732613	0.132978
2	C	-0.212822	2.395256	0.001966
3	C	0.752351	1.387250	0.025877
4	C	2.094225	1.717224	0.218175
5	C	2.481893	3.046137	0.383381
6	C	1.516912	4.049749	0.327009
7	N	3.059532	0.674913	0.245921
8	C	-1.679308	2.120884	-0.208498
9	N	-2.099452	0.871810	0.196410
10	O	-2.417799	2.963969	-0.687729
11	C	-3.382529	0.300445	0.109684

12	C	-3.553506	-0.974821	0.669979
13	C	-4.784189	-1.610639	0.618861
14	C	-5.869141	-0.979161	0.009189
15	C	-5.702322	0.287347	-0.549550
16	C	-4.472207	0.933664	-0.504250
17	C	4.377499	0.706214	-0.090696
18	C	4.831924	-0.578216	0.129764
19	N	3.768757	-1.317182	0.582625
20	N	2.712546	-0.574424	0.654111
21	H	4.853376	1.588427	-0.479584
22	H	-1.416176	0.307145	0.678515
23	H	0.503183	0.346370	-0.131611
24	C	6.163525	-1.163102	-0.065942
25	C	7.275995	-0.360148	-0.355081
26	C	8.530955	-0.929078	-0.548177
27	C	8.697466	-2.310006	-0.450107
28	C	7.597952	-3.115837	-0.157028
29	C	6.341010	-2.550446	0.033451
30	H	-4.351145	1.917615	-0.928512
31	H	-2.715625	-1.470750	1.149454
32	C	-7.191535	-1.680837	-0.098637
33	F	-7.372875	-2.590201	0.887029

34	F	-7.316214	-2.357668	-1.271431
35	F	-8.234565	-0.820462	-0.044530
36	H	-0.579737	4.503885	0.080783
37	H	3.519441	3.296054	0.567181
38	H	1.815958	5.084043	0.448816
39	H	-4.901694	-2.592626	1.059029
40	H	-6.541947	0.784128	-1.019896
41	H	7.167249	0.716903	-0.422562
42	H	9.380594	-0.293187	-0.770154
43	H	9.675629	-2.752943	-0.598711
44	H	7.718798	-4.190411	-0.077913
45	H	5.485515	-3.174183	0.259823

Table 4. Atomic coordinates of [1d+Cl⁻] after geometry optimization by Gaussian 09 program using B3LYP functional and 6-311++G(d,p) basis set.

Charge = -1 Multiplicity = 1

Atom Numbers	Atom Type	x	y	Z
1	C	0.160951	4.193342	0.150168
2	C	-0.070864	2.816748	0.037197
3	C	1.015376	1.941929	-0.051619
4	C	2.315806	2.448478	-0.007598
5	C	2.549557	3.818846	0.112325
6	C	1.461214	4.683501	0.186323

7	N	3.422123	1.552583	-0.078949
8	C	-1.519841	2.384632	-0.001235
9	N	-1.782722	1.039593	0.070779
10	O	-2.398216	3.238562	-0.087521
11	C	-3.047024	0.425090	0.038856
12	C	-3.072589	-0.980087	0.128082
13	C	-4.276505	-1.664729	0.105317
14	C	-5.485144	-0.969670	-0.002179
15	C	-5.463998	0.421570	-0.093934
16	C	-4.263292	1.122351	-0.072709
17	C	3.425940	0.199466	0.038201
18	C	4.752870	-0.155175	-0.099217
19	N	5.480138	0.991972	-0.288627
20	N	4.687484	2.017224	-0.278050
21	H	2.525926	-0.384086	0.203668
22	H	-1.004659	0.373723	0.176122
23	H	0.864084	0.870465	-0.141095
24	C	5.364772	-1.487541	-0.060833
25	C	4.572407	-2.637376	0.087324
26	C	5.161661	-3.897702	0.124608
27	C	6.545624	-4.036614	0.014562
28	C	7.338317	-2.898466	-0.134409

29	C	6.755470	-1.634930	-0.171488
30	H	-4.256640	2.197782	-0.135982
31	H	-2.133220	-1.517297	0.216335
32	C	-6.780537	-1.708297	-0.087267
33	F	-6.755342	-2.896285	0.567895
34	F	-7.138146	-2.003895	-1.373223
35	F	-7.823104	-1.002935	0.426392
36	H	-0.693033	4.855538	0.205764
37	H	3.566483	4.184781	0.143861
38	H	1.634636	5.750100	0.278167
39	H	-4.274029	-2.745415	0.178879
40	H	-6.393981	0.971599	-0.174633
41	H	3.493875	-2.548852	0.169572
42	H	4.533252	-4.774279	0.239165
43	H	7.000685	-5.020985	0.043773
44	H	8.415812	-2.994467	-0.221264
45	H	7.366977	-0.748367	-0.286622
46	Cl	0.394041	-1.457205	0.340939

2.2.6. Ion transport studies:

2.2.6.1. Buffer and stock solution preparation: HEPES buffer (10 mM) was prepared by dissolving solid HEPES in autoclaved water, NaCl (100 mM) was added and followed by

adjustment of pH = 7.0 by adding NaOH solution. Stock solutions of all compounds were prepared in either HPLC grade DMSO or ACN.

2.2.6.2. Preparation of EYPC-LUVs⊃HPTS: Vesicles were prepared according to the reported protocol.^{31, 32}

2.2.6.3. Ion transport activity: 1975 μL of HEPES buffer (10 mM HEPES, 100 mM NaCl, pH = 7.0) was taken in a clean fluorescence cuvette followed by the addition of 25 μL of EYPC-LUVs⊃HPTS in the same cuvette and was placed on the fluorescence instrument (at $t = 0$ s) equipped with a magnetic stirrer. The fluorescence emission intensity of HPTS dye, F_t was monitored at $\lambda_{em} = 510$ nm ($\lambda_{ex} = 450$ nm) with time. After that, a pH gradient between the intra and extra vesicular system was created by adding 20 μL of 0.5 M NaOH to the same cuvette at $t = 20$ s (Figure 2.18). All compounds were added at $t = 100$ s in different concentrations and finally, at $t = 300$ s, 25 μL of 10% Triton X-100 was added to destroy all vesicles which resulted in the destruction of pH gradient (Figure. 2.18), which resulted in saturation in fluorescence emission intensity. The fluorescence intensity was normalized according to Equation 2:

$$I_F = [(F_t - F_0) / (F_\infty - F_0)] \times 100\% \quad (\text{Equation 2})$$

where, F_0 = Fluorescence intensity just before the compound addition (at $t = 0$ s). F_∞ = Fluorescence intensity at saturation after complete leakage (at $t = 330$ s). F_t = Fluorescence intensity at time t .

The time of compound addition can be normalized to $t = 0$ s and the time of Triton X-100 addition was normalized to $t = 200$ s.

Fluorescence intensities (F_t) were normalized to fractional emission intensity I_F using Equation 3:

$$Y = Y_\infty + (Y_0 - Y_\infty) / [1 + (c / EC_{50})^n] \quad (\text{Equation 3})$$

where, Y_0 = Fluorescence intensity just before the channel addition (at 0 s). Y_∞ = Fluorescence intensity with excess channel concentration, c = Concentration of channel-forming molecule.(Figure 2.18)

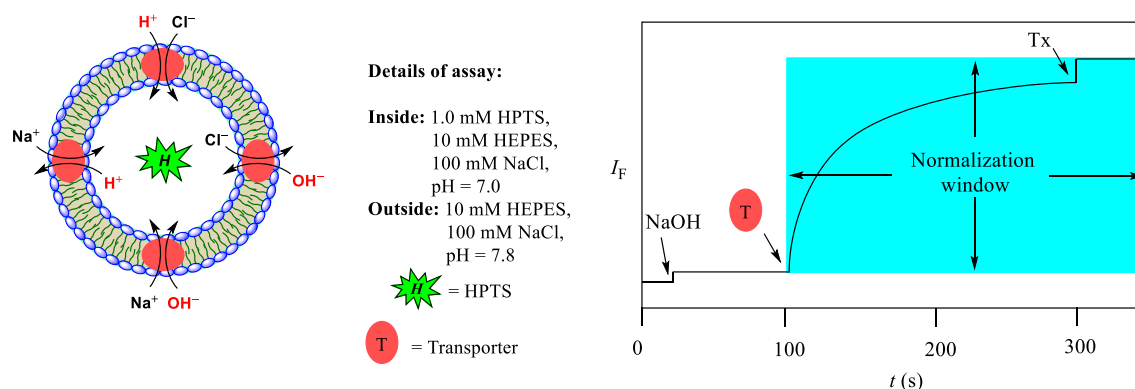


Figure 2.18. Representations of fluorescence-based assay across EYPC-LUVs $\Rightarrow$ HPTS.

All the above experiments inspired us to evaluate transmembrane ion transport activities of compounds **1a–1e** and **2** using EYPC-LUVs $\Rightarrow$ HPTS. Compound **1d** showed the highest activity among the entire triazole series. However, its activity was low compared to benzene-1,3-dicarboxamide **2** (Figure 2.19) although, its chloride binding strength was significantly lower. Together, these results corroborate that strong anion binding is not always necessary for better transport of the anion.

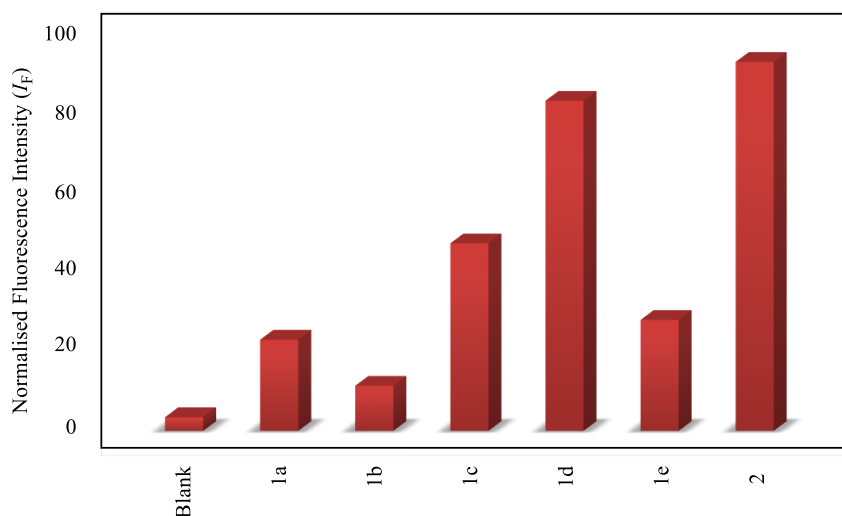


Figure 2.19. Comparison of ion transport activities of **1a–1e** and **2** across EYPC-LUVs $\Rightarrow$ HPTS.

Ion transport kinetics was studied at different concentrations for each compound. The change of HPTS emission intensity in this process was monitored with time. The concentration profile data were analyzed by Hill Equation (Equation 3) to get the effective activity at half-maximal concentration (EC_{50}) and Hill coefficient (n).

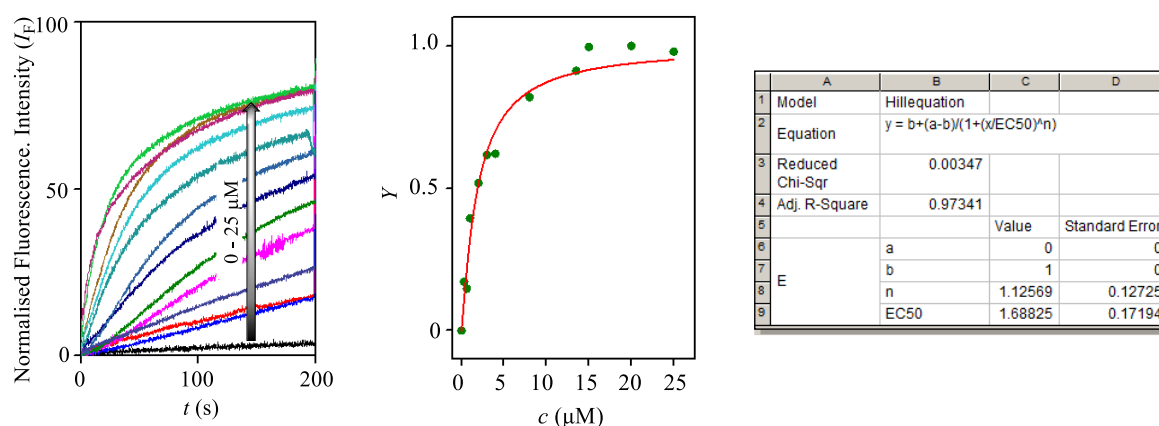


Figure 2.20. Dose-response curves and Hill analysis of compound **1d** across EYPC-LUVs $\rightarrow$ HPTS at $t=190$ s.

The dose-dependent transport study of **1d** suggested the half-maximum activity (EC_{50}) value of $1.68 \mu\text{M}$, i.e., 2.5 mol%, relative to the lipid concentration, and the Hill coefficient (n) equal to 1 (Figure 2.20) which suggests that a single molecule forms the active transport complex.

The dose-dependent transport study of **2** suggested the half-maximum activity (EC_{50}) value of 4.1 nM , and the Hill coefficient (n) equal to 2 (Figure 2.21) which suggests that two molecules forms the active transport complex.

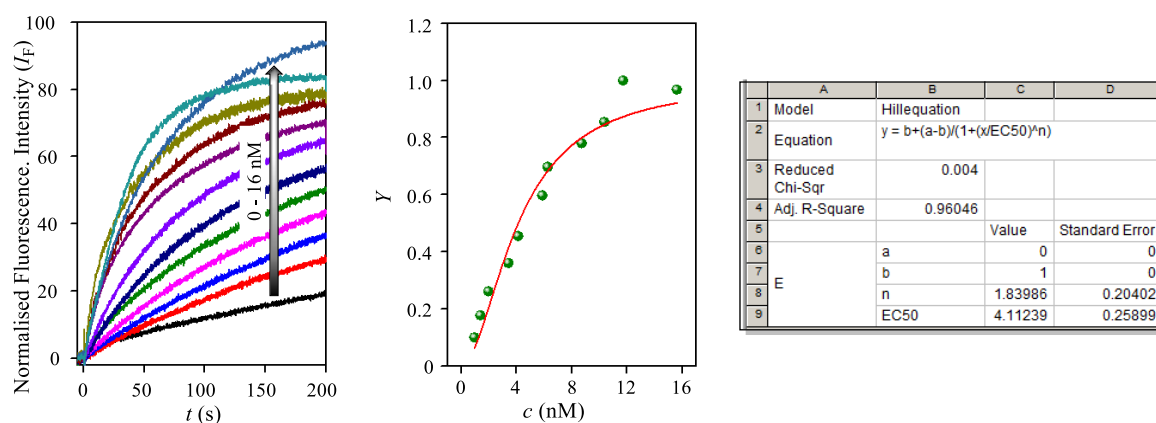


Figure 2.21. Dose-response curves and Hill analysis of compound **2** across EYPC-LUVs $\rightarrow$ HPTS at $t=190$ s.

2.2.7. Determination of ion selectivity by HPTS assay:³³

2.2.7.1. Buffer and stock solution preparation: HEPES buffers with all salts were prepared by dissolving solid HEPES (10 mM) followed by the addition of appropriate salt (100 mM) in autoclaved water and adjustment of pH (pH = 7.0) was done by dropwise addition of NaOH solution.

2.2.7.2. Preparation of EYPC-LUVs $\supset$ HPTS for cation selectivity:

EYPC-LUVs $\supset$ HPTS (~ 5.0 mM EYPC, inside: 1 mM HPTS, 10 mM HEPES, 100 mM MCl, pH = 7.0 and outside: 10 mM HEPES, 100 mM MCl, pH = 7.0 where $\text{M}^+ = \text{K}^+, \text{Na}^+, \text{Rb}^+, \text{Cs}^+$ and Li^+) were prepared. (Figure 2.22)³¹⁻³³

2.2.7.3. Preparation of EYPC-LUVs $\supset$ HPTS for anion selectivity:³⁴

EYPC-LUVs $\supset$ HPTS (~ 5.0 mM EYPC, inside: 1 mM HPTS, 10 mM HEPES, 100 mM NaX, pH = 7.0 and outside: 10 mM HEPES, 100 mM NaX, pH = 7.0; where, $\text{X}^- = \text{Cl}^-, \text{Br}^-, \text{ClO}_4^-, \text{NO}_3^-$ and I^-) were prepared. (Figure 2.22)

2.2.7.4. Anion and cation selectivity assays: In a clean fluorescence cuvette, 1975 μL of buffer solution (100 mM NaX or MCl, 10 mM HEPES, pH 7.0;), 25 μL of above-prepared vesicles solution were taken and it was placed in a fluorescence instrument equipped with a magnetic stirrer at $t = 0$ s. The fluorescence emission intensity (I_t) of the HPTS dye was measured at $\lambda_{\text{em}} = 510$ nm (where $\lambda_{\text{ex}} = 450$ nm) from 0 to 350 s. At $t = 50$ s, 20 μL of 0.5 M NaOH solution was added to the same cuvette to generate a pH gradient ($\Delta\text{pH} = 0.8$) between the intra and extra vesicular medium which leads to the increase in the fluorescence intensity. At 100 s, 0.75 μM of transporter molecule **1d** was added. Finally, Triton X-100 (25 μL of 10% solution in water) was added at $t = 300$ s to destroy all the vesicles for destructing the pH gradient. Fluorescence intensities (F_t) were normalized to fractional emission intensity I_F using Equation 2.

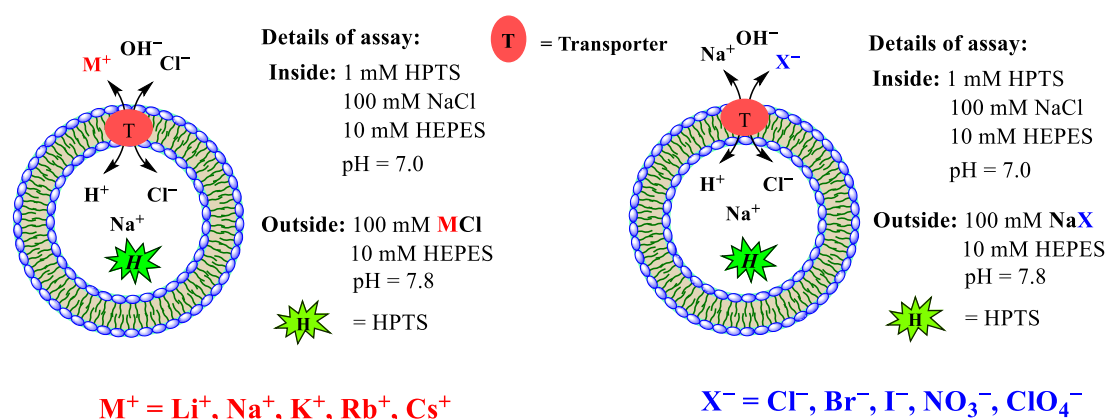


Figure 2.22. Representations of fluorescence-based cation selectivity (A) and anion selectivity (B) assays across EYPC-LUVs $\supset$ HPTS.

To assess the preferred transport among cations and anions, the transmembrane ion transport of **1d** was checked using EYPC-LUVs $\Rightarrow$ HPTS by varying the extravesicular ionic buffers.^{35, 36} The insignificant differences in the transport rate of **1d** in presence of different external cations (Figure 2.22) rules out the involvement of any cation, i.e., neither H^+/M^+ antiport nor M^+/OH^- symport is preferred during the ion transport. This was followed by evaluation of anion selectivity by varying the intravesicular and extravesicular anionic buffers,³³ followed by monitoring change in ion transport rate of **1d**. Upon addition of different anions, a considerable change of the transport rate of **1d** was observed for each NaX salt used (where $\text{X}^- = \text{Cl}^-$, Br^- , I^- , NO_3^- , and ClO_4^-) with the sequence as $\text{Cl}^- \sim \text{Br}^- > \text{I}^- > \text{NO}_3^- > \text{ClO}_4^-$ (Figure 2.23).

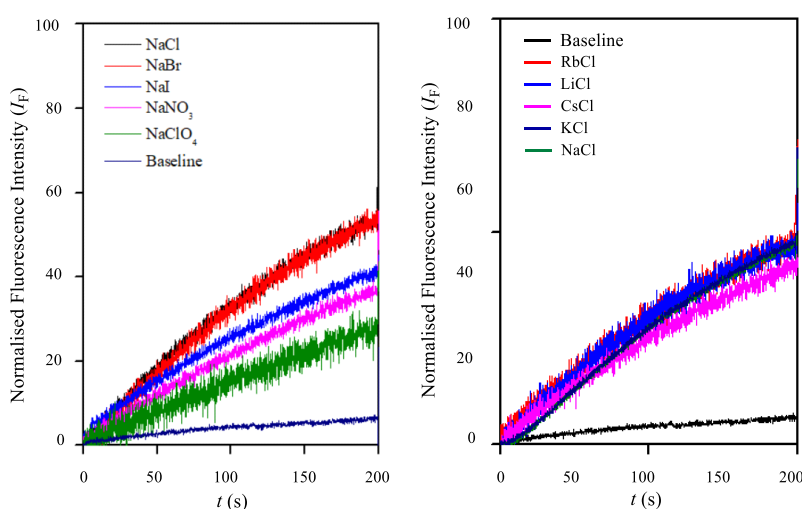


Figure 2.23. Anion selectivity (A) and cation selectivity (B) of **1d** (0.75 μM) across EYPC-LUVs $\Rightarrow$ HPTS.

2.2.8. Chloride selectivity of **1d** with the ion-selective electrode (ISE):³⁷

2.2.8.1. Preparation of EYPC-LUVs for chloride efflux assay: In a clean and dry small round bottom flask 1 mL of egg yolk phosphatidylcholine (EYPC, 25 mg/mL in CHCl_3) was dried by purging nitrogen gas with continuous rotation to form a transparent thin film of EYPC. The transparent film was kept under a high vacuum for 4–5 h to remove all traces of CHCl_3 at room temperature. The resulting film was hydrated with 1 mL buffer (5 mM phosphate buffer, 300 mM NaCl) solution for 1 h with occasional vortexing at 10-minute intervals and subjected to a freeze-thaw cycle (≥ 15 times). Extrusions were done 19 times (must be an odd number) by a mini-extruder with a polycarbonate membrane of pore size 200 nm (Avanti). The extravesicular chloride ions were removed by dialysis (using MWCO 1 kD membrane) against NaNO₃ (300 mM) solution. The external dialysis solution was changed three times to ensure

the complete removal of Cl^- ions from the extravesicular media. The lipid solution obtained after dialysis was diluted to a standard volume.

2.2.8.2. Determination of chloride efflux by ISE: In a clean and dry glass tube, 1950 μL of NaNO_3 (300 mM) buffer solution and 50 μL of vesicle suspension (containing 300 mM NaCl) was taken and kept in slowly stirring condition by a magnetic stirrer (at $t = 0$ s). Compound **1d** (in variable concentrations) was added at $t = 30$ s and Triton X-100 (25 μL of 10%) was added at 300 s to ensure the complete leakage of Cl^- ions. The amount of extravesicular Cl^- before the addition of compound **1d** has been normalized to 0% and after the addition of Triton X-100 has been normalized to 100%.

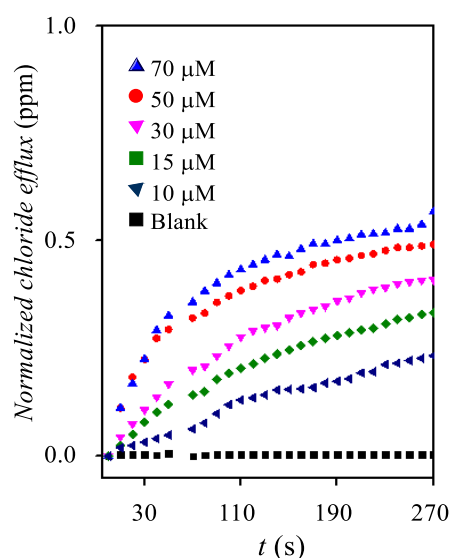


Figure 2.24. Concentration-dependent chloride efflux by **1d** from EYPC-LUVs $\Rightarrow$ NaCl .

The transport of Cl^- ion was further confirmed by monitoring its efflux at varied concentrations of **1d** using a chloride ion selective electrode (ISE) (Figure 2.24).

2.2.9. Ion transport mechanism study by HPTS assay:³⁴

2.2.9.1. Preparation of buffer and stock solution for FCCP and Valinomycin assays:

The buffer solution was prepared using autoclaved water of strength 100 mM of NaCl and 10 mM of HEPES. The pH of the solution was adjusted to 7.0 by the addition of a required amount of 0.5 M NaOH solution. Then 1 mM of HPTS solution was prepared from the same buffer solution. Similarly, buffer solution for valinomycin assay was prepared using autoclaved water

of strength 100 mM of KCl and 10 mM of HEPES. The stock solutions of **1d**, FCCP, and valinomycin for the HPTS assay were prepared using HPLC grade DMSO.

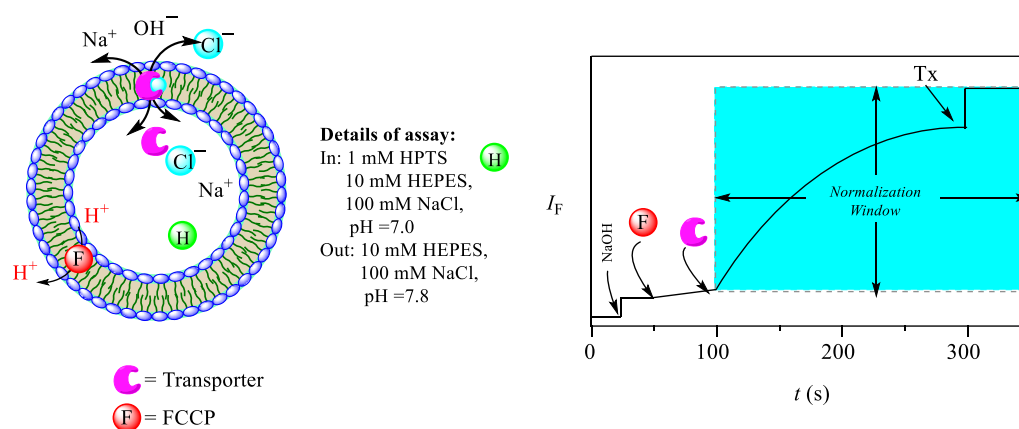


Figure 2.25. Representations of fluorescence-based FCCP assay across EYPC-LUVs with HPTS.

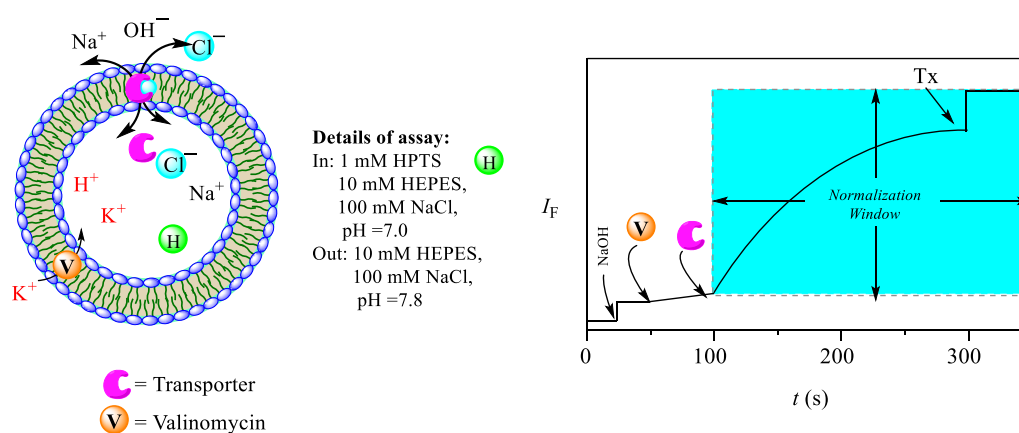


Figure 2.26. Representations of fluorescence-based Valinomycin assay across EYPC-LUVs with HPTS.

2.2.9.2. Preparation of vesicles for FCCP and Valinomycin assays:

The vesicles for the FCCP assay were prepared following the same procedure as mentioned earlier.

2.2.9.3. Ion transport activity in the presence of FCCP: In a clean and dry fluorescence cuvette 1975 μL of HEPES buffer (10 mM HEPES, 100 mM NaCl, pH = 7.0) was taken followed by the addition of 25 μL of EYPC-LUVs with HPTS in stirring condition by a magnetic stirrer equipped with the fluorescence instrument (at $t = 0$ s). HPTS fluorescence emission intensity was monitored with time and F_t was observed at $\lambda_{\text{em}} = 510$ nm ($\lambda_{\text{ex}} = 450$ nm). At $t = 20$ s, 20 μL of 0.5 M NaOH was added to the cuvette to make the pH gradient between the intra

and extra vesicular solution. FCCP (0.5 μM) was added at $t = 50$ s (whenever necessary) and transporter molecule was added at $t = 100$ s (whenever necessary) and finally at $t = 300$ s, 25 μL of 10% Triton X-100 was added to that cuvette resulting in destruction of the pH gradient. Fluorescence intensities (F_t) were normalized to fractional emission intensity I_F using Equation 2. (Figure 2.25)

2.2.9.4. Ion transport activity in the presence of Valinomycin: In a clean and dry fluorescence cuvette 1975 μL of HEPES buffer (10 mM HEPES, 100 mM NaCl, pH = 7.0) was added followed by the addition of 25 μL of EYPC-LUVs $\Rightarrow$ HPTS in slowly stirring condition by a magnetic stirrer equipped with the fluorescence instrument (at $t = 0$ s). The time course of HPTS fluorescence emission intensity, F_t was observed at $\lambda_{\text{em}} = 510$ nm ($\lambda_{\text{ex}} = 450$ nm). 20 μL of 0.5M NaOH was added to the cuvette at $t = 20$ s to make the pH gradient between the intra and extra vesicular system. Valinomycin (1 pM) was added at $t = 50$ s and transporter molecule was added at $t = 100$ s and finally at $t = 300$ s. 25 μL of 10% Triton X-100 was added to lyse those vesicles destroying pH gradient. Fluorescence intensities (F_t) were normalized to fractional emission intensity I_F using Equation 2. (Figure 2.26)

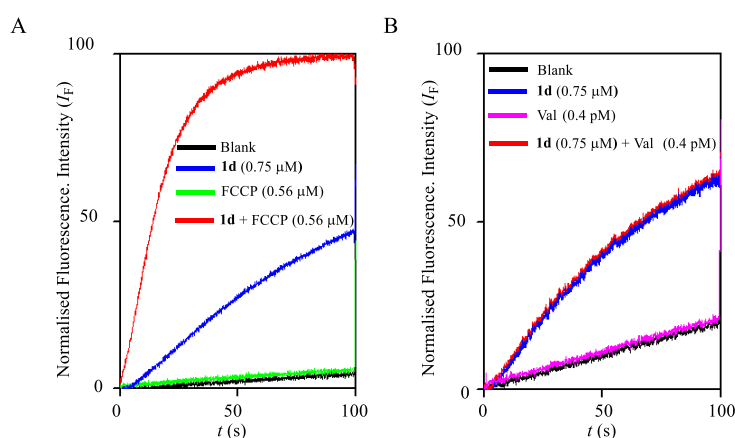


Figure 2.27. Ion transport rate of **1d** (0.75 μM) in presence of FCCP (0.56 μM) (A) and Valinomycin (0.4 pM) (B) across EYPC-LUVs $\Rightarrow$ HPTS.

At first, FCCP, a selective H^+ carrier, was used. Under the applied conditions of the HPTS assay, the FCCP is expected to facilitate the intravesicular H^+ efflux to the extravesicular medium. When the compound **1d** was coupled with FCCP, the transport rate enhanced to almost double compared to that of **1d** alone (Figure 2.27), indicating that a **1d** shows a cooperative effect with FCCP. This study suggested that the X^-/OH^- antiport mechanism is operating during ion transport. Subsequently, valinomycin, a K^+ selective carrier, was coupled

with **1d** in the HPTS assay to compare the preferential selectivity of compound **1d** between OH^- and Cl^- . Under iso-osmolar KCl (extravesicular) versus NaCl (intravesicular) and upon application of NaOH gradient in the extravesicular buffer, compound **1d** did not show any significant difference in the transport rate in the absence and presence of valinomycin, suggesting a faster rate of transport of Cl^- compared to OH^- (Figure 2.27).

2.2.10. Evidence of membrane integrity using CF assay:

2.2.10.1. Preparation of EYPC-LUVs $\rightarrow$ CF:³⁷ A thin lipid film was prepared by evaporating a solution of 12.5 mg EYPC in 0.5 ml CHCl_3 in vacuo for 4 h. After that lipid film was hydrated with 0.5 mL buffer (10 mM HEPES, 10 mM NaCl, 50 mM Carboxyfluorescein (CF), pH 7.0) for 1 h with occasional vortexing of 4–5 times and then subjected to freeze-thaw cycle (≥ 20 times). The vesicle solution was extruded through a polycarbonate membrane with 100 nm pores 19 times (has to be an odd number), to give vesicles with a mean diameter of ~ 100 nm. The extracellular dye was removed with size exclusion chromatography (Sephadex G-50) with 10 mM HEPES buffer (100 mM NaCl, pH 7.0. Final) Final concentration: ~ 2.5 mM EYPC lipid; intravesicular solution: 10 mM HEPES, 10 mM NaCl, 50 mM CF, pH 7.0; extravesicular solution: 10 mM HEPES, 100 mM NaCl, pH 7.0. (Figure 2.28)

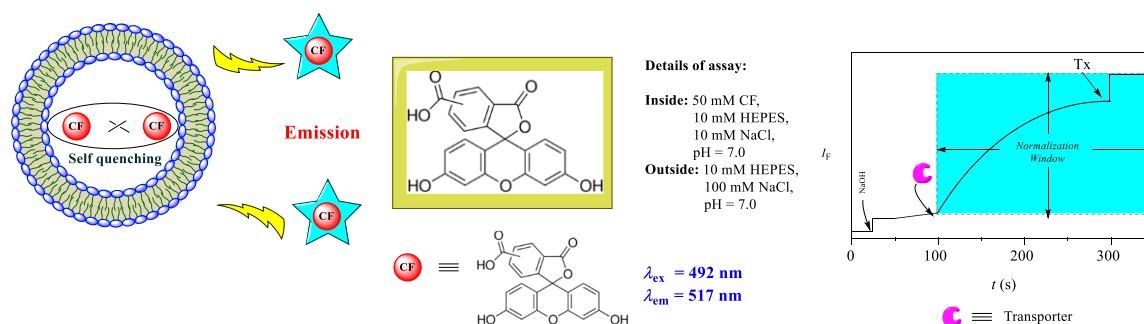


Figure 2.28. Representations of fluorescence-based assay across EYPC-LUVs $\rightarrow$ CF.

2.2.10.2. CF leakage assay:

In a clean and dry fluorescence cuvette 25 μL of above lipid solution and 1975 μL of 10 mM HEPES buffer (100 mM NaCl, pH 7.0) was taken and kept in slowly stirring condition by a magnetic stirrer equipped with the fluorescence instrument (at $t = 0$ s). The time course of CF fluorescence emission intensity, F_t was observed at $\lambda_{\text{em}} = 517 \text{ nm}$ ($\lambda_{\text{ex}} = 492 \text{ nm}$). Compound **1d** was added at $t = 100$ s and finally at $t = 300$ s, 25 μL of 10% Triton X-100 was added to lyse those vesicles for 100% chloride influx. Fluorescence intensities (F_t) were normalized to

fractional emission intensity I_F according to Equation 2. This study confirmed that neither the bilayer membranes are defected nor large transmembrane pores are formed by **1d**. (Figure 2.29)

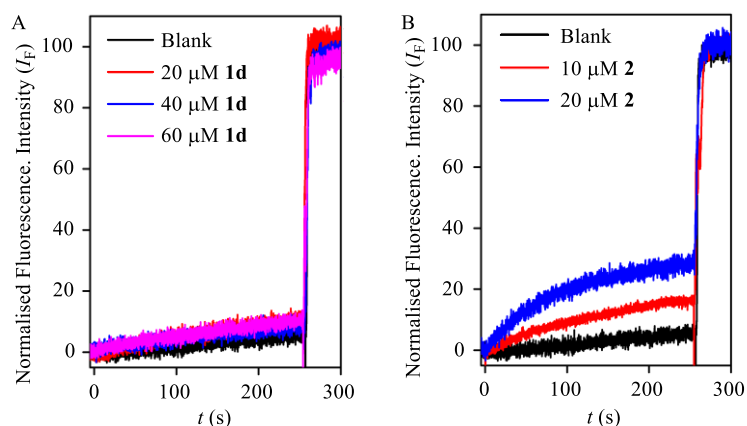


Figure 2.29. The carboxyfluorescein leakage assay of **1d** (A) and **2** (B) across EYPC-LUVs $\supset$ CF.

2.2.11. Electrogenic mechanism of ion transport by compound **1d**.³⁸

The buffer solution was prepared by adding 5 mM KH_2PO_4 and KCl (300 mM) or K-Gluconate (300 mM) to milli-Q water. The pH was adjusted to 7.0 by adding the 0.5 N KOH.

2.2.11.1. Preparation of EYPC-LUVs for chloride efflux assay: In a clean and dry small round bottom flask 1 mL of egg yolk phosphatidylcholine (EYPC, 25 mg/mL in CHCl_3) was dried by purging nitrogen gas with continuous rotation to form a transparent thin film of EYPC. The transparent film was kept under a high vacuum for 4–5 h to remove all traces of CHCl_3 at room temperature. The resulting film was hydrated with 1 mL buffer (5 mM phosphate buffer, 300 mM KCl) solution for 1 h with occasional vortexing at 10-minute intervals and subjected to a freeze–thaw cycle (≥ 15 times). Extrusions were done 19 times (must be an odd number) by a Mini-extruder with a polycarbonate membrane of pore size 200 nm (Avanti). The extravesicular chloride ions were removed by dialysis (using MWCO 1 kD membrane) against K-Gluconate (300 mM) solution. The external dialysis solution was changed three times to ensure the complete removal of Cl^- ions from extravesicular media. (Figure 2.30)

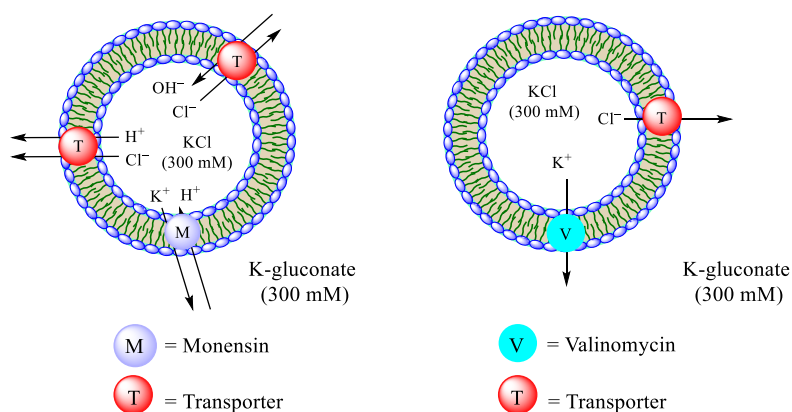


Figure 2.30. Representations of EYPC-LUVs $\Rightarrow$ Monensin (A) or Valinomycin (B) to check chloride efflux by ISE.

2.2.11.2. Determination of Cl^- efflux by ISE: In a clean and dry glass tube, 1950 μL of K-Gluconate (300 mM) solution and 50 μL of vesicles was taken and kept in a slowly stirring condition by a magnetic stirrer (at $t = 0$ s). At 30 s, either the electrogenic K^+ carrier valinomycin or the K^+/H^+ antiporter monensin was added. The Cl^- ion-selective electrode was dipped into the glass tube and the concentration of Cl^- ions was monitored with time. **1d** in respective concentration was added at $t = 50$ s and 25 μL Triton X-100 was added at 250 s to ensure the complete leakage of vesicles. The amount of extravesicular Cl^- before the addition of compound **1d** was normalized to 0% and after the addition of Triton X-100 was normalized to 100%.

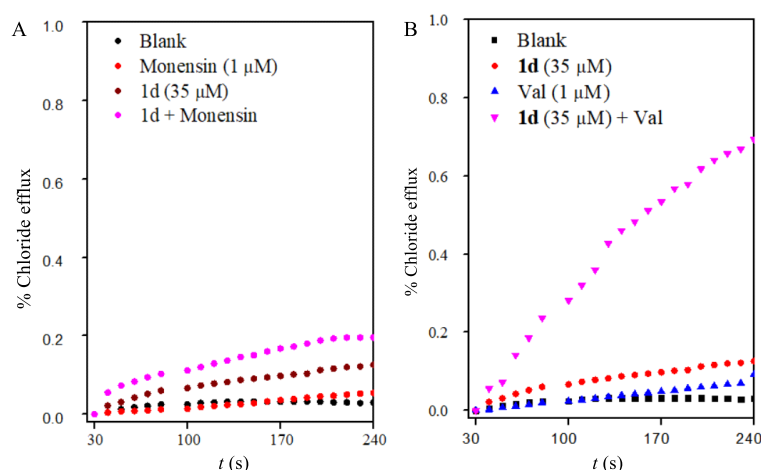


Figure 2.31. Representative scheme showing the net K^+/Cl^- efflux from coupled non-electrogenic transport processes (A), and coupled electrogenic transport processes, K^+ efflux by valinomycin and Cl^- efflux by transporter **1d** at 35 μM (B).

To confirm the electrogenic transport behavior of compound **1d**, the transport studies were performed in the presence of monensin (an electroneutral H^+/K^+ antiporter) and valinomycin (an electrogenic K^+ carrier).³⁸ No significant Cl^- efflux from KCl-loaded vesicles suspended in the potassium gluconate (K-Glu) buffer by **1d** in the presence of monensin (0.625 mol%) by ion selective electrode, indicates the occurrence of electrogenic mechanism. To validate this result, the transport activity of **1d** was assessed in the presence of valinomycin (0.625 mol%), which showed enhancement in the Cl^- efflux rate by **1d**, which further confirms that the transport occurs via an electrogenic transport mechanism. (Figure 2.31)

2.2.12. Carrier mechanism of ion transport, the U-tube experiment:³⁹

The U-tube experiments were carried out in a U-shaped glass tube, partially filled with CHCl_3 as an organic phase. Two aqueous layers were created at the donor and receiver end of the tube. 0.5 M NaCl and equimolar NaNO_3 were placed at the donor and receiver end, respectively. It has been assumed that the ion channel connecting two aqueous layers is not favourable as the long organic layer of about 10 cm would not favour its self-assembled structure to remain intact. 2 mM of TBAPF_6 was added in the organic phase to maintain the ionic balance. Delivery of Cl^- ions to the receiver aqueous layer was monitored in the absence and presence of **1d** (30 μM). The experiments were carried out at room temperature under slow stirring conditions in the organic phase. The concentration (ppm) of Cl^- ions in the receiving phase was measured by chloride ion-selective electrode (ISE) with time. (Figure 2.32)

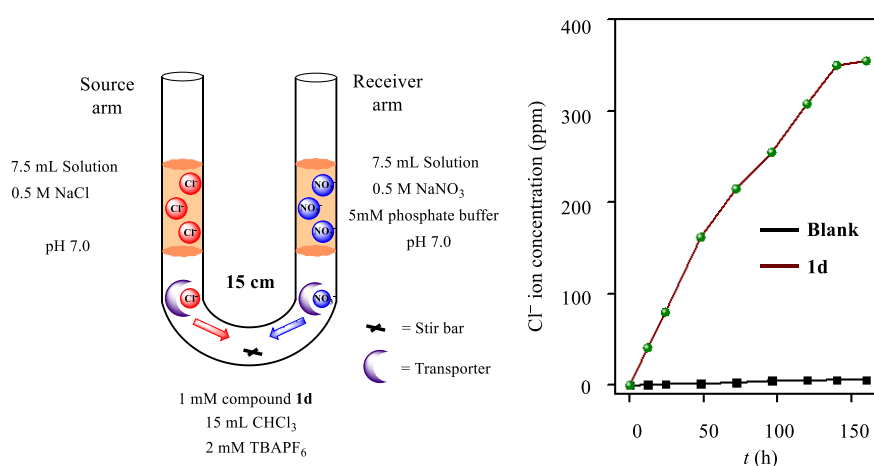


Figure 2.32. (A) Schematic representation of U-tube experiment, and (B) Cl^- transport from the donor end to the receiver end by **1d** (30 μM) with time.

To ascertain whether compound **1d** acts as an anion carrier or forms a self-assembled channel, U-tube experiment was carried out,³⁹ where the lipid bilayer membrane was substituted by a chloroform layer, separating two aqueous layers. In the presence of **1d**, a significant transport of Cl^- through the chloroform layer was observed with time (Figure 2.32). Since spanning a self-assembled ion channel across the chloroform layer is not feasible, the observed Cl^- transport to the receiving end from the donor end is happening via the shuttling of the transporter across the lipid bilayer membrane, i.e., a carrier mechanism is being followed.

2.2.13. Critical aggregation concentration (CAC) studies:

2.2.13.1. UV-vis studies:

Vesicles were prepared using the reported protocol as mentioned above without encapsulating the HPTS dye. Initially, stock solutions of these compounds (2 mM in DMSO) were prepared in different vials. Absorption spectra of compounds **1d** and **2** were recorded. (Figure 2.34 and Figure 2.35)

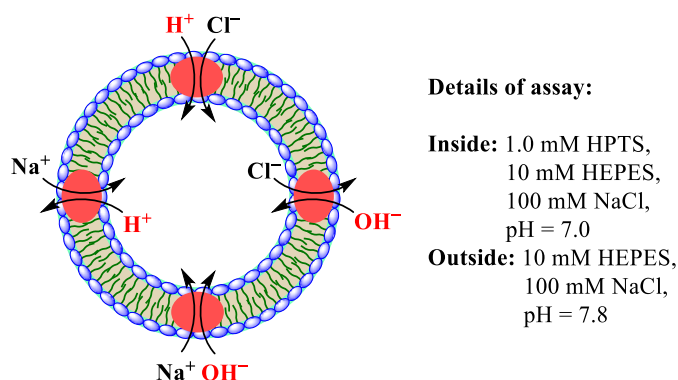


Figure 2.33. Representations of EYPC-LUVs without any encapsulated dye.

The UV-vis experiments were performed in a 3 mL UV cuvette where 1 mL of HEPES Buffer was placed and into that 25 μL of the above-mentioned vesicles were added. Next, absorbance intensity of the vesicles solution was recorded by adding increasing concentration of **1d** or **2**. The UV-vis absorbance data of only the vesicles solutions were considered as the baseline data.

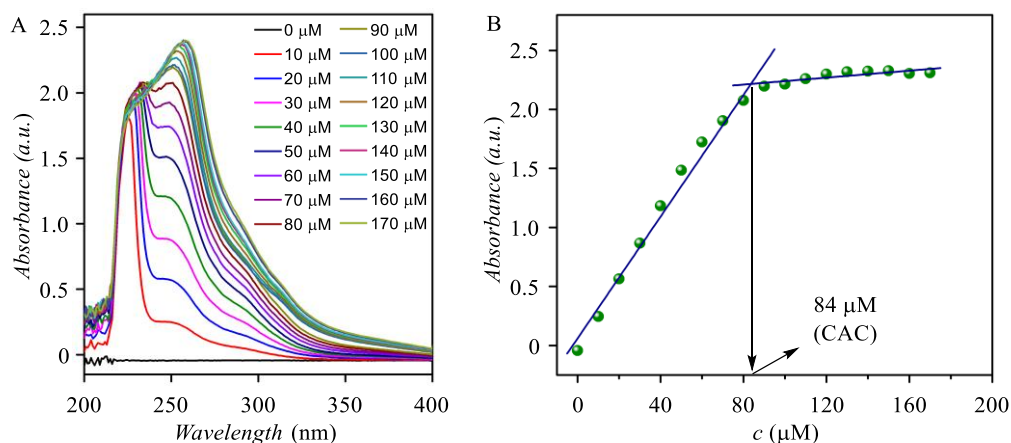


Figure 2.34. The UV-vis absorbance spectra of vesicles solution with increasing concentration of **1d** (0-170 μM) (A) and the absorbance (at 254 nm) vs concentration plot to calculate the critical aggregation constant (CAC) value of **1d** (B).

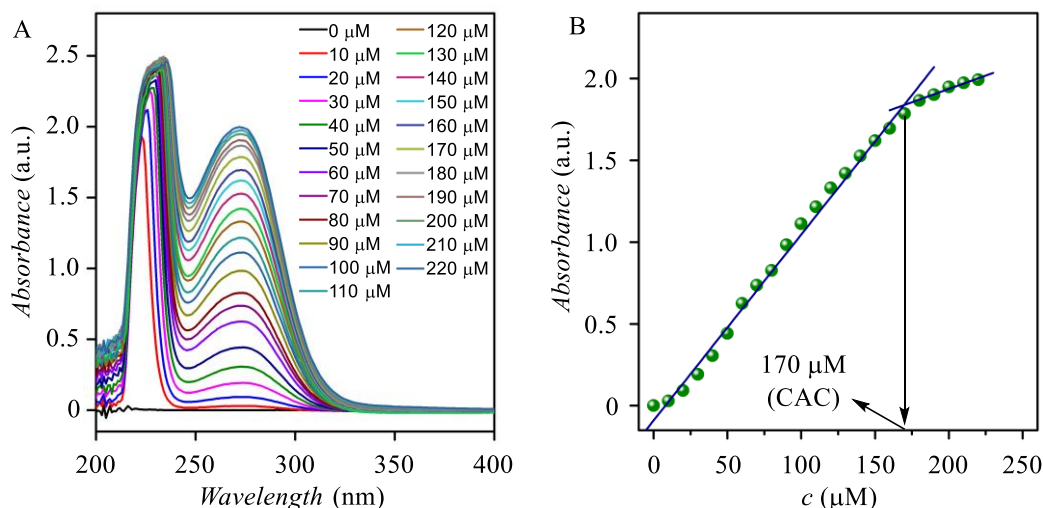


Figure 2.35. The UV-vis absorbance spectra of vesicles solution with increasing concentration of **2** (0-220 μM) (A) and the absorbance (at 273 nm) vs concentration plot to calculate the critical aggregation constant (CAC) value of **2** (B).

The absorbance values at a particular wavelength were plotted against the concentration. The concentration at which the compound starts aggregating will start showing saturation in terms of increase in absorbance represents the CAC for a particular compound. To check the possible self-aggregation of the transporter molecules in the vesicular media, the UV-Vis experiments were carried out, which were further used to determine the critical aggregation constant values as 84 μM for **1d** (Figure 2.34) and 170 μM for **2** (Figure 2.35), respectively.

2.2.14. Biological studies:

2.2.14.1. Cell culture protocol:

The cells were grown in High Glucose Dulbecco's Modified Eagle Medium (DMEM; Invitrogen or Lonza) containing 10% fetal bovine serum (FBS; Invitrogen), 2 mM L-glutamine (Invitrogen) and 100 units/mL penicillin-streptomycin (Invitrogen). Cells were maintained in 100 mm tissue culture treated dishes (Corning) at 37 °C in humidified 5% CO₂ incubator (Thermo Scientific).

2.2.14.2. Measurement of intracellular Cl[−] concentrations:⁴⁰

Time-dependent influx data of Cl[−] ions was measured in MCF-7 cells by using chloride selective cell permeable fluorescent dye N-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide (MQAE). Cells were seeded in a 96-well flat bottom tissue culture treated plates (Corning) at density of 10⁴ cells/well (per 100 μL) and incubated at 37 °C, 5% CO₂ for 24 h. MQAE was added to each well by maintaining final concentration as 5 mM for 3 h. Extracellular dye was removed by washing with PBS and compound **1d**, and **2** were added in DMEM at various concentrations and cells were incubated for 1 h. The MQAE fluorescence was measured using a fluorescence microplate reader ($\lambda_{\text{ex}} = 350 \text{ nm}$ and $\lambda_{\text{em}} = 460 \text{ nm}$).

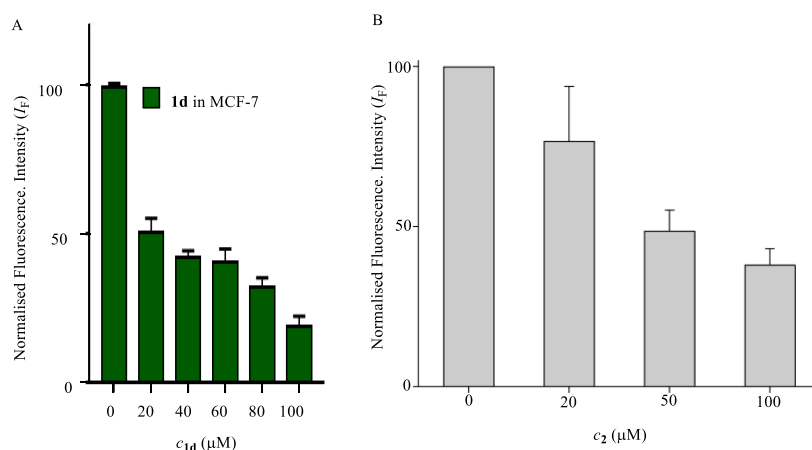


Figure 2.36. Normalized fluorescence intensity of MCF-7 cells incubated with MQAE (5 mM) for 3 h followed by treatment with **2** (0–100 μM).

To evaluate whether the compound **1d** can be an effective chloride carrier in the biological system, we monitored the chloride transport by **1d** across the cell membrane in MCF-7 cells using a cell-permeable chloride selective dye N-(ethoxycarbonylmethyl)-methoxyquinoliniumbromide (MQAE).⁴⁰ A significant amount of dose-dependent quenching of the fluorescence intensity of MQAE ($\lambda_{\text{ex}} = 350 \text{ nm}$ and $\lambda_{\text{em}} = 460 \text{ nm}$) was encountered upon

preincubation of MCF-7 cells with **1d** (Figure 2.36), which implies the enhanced chloride concentration in the intracellular matrix, transported by **1d**. A control experiment using the same dye for compound **2** under similar conditions gave similar results (Figure 2.36), indicating the function of **2** as a transporter of chloride ions across the cell membrane.

2.2.14.3. MTT-based cytotoxicity assay:⁴¹

Cells were dispersed in a 96-well flat bottom tissue culture treated plates (Corning) at density of 10⁴ cells/well (per 100 μ L) and incubated at 37 °C in a 5% CO₂ incubator for 24 h. Compounds were added to each well in different concentration by maintaining maximum amount of DMSO at 1.5 μ L and incubated for 24 h. DMEM solution containing compounds in each well were replaced with 110 μ L of MTT-DMEM mixture (0.5 mg MTT/mL of DMEM) and incubated for 4 h in identical condition. After 4 h, MTT solution was removed and 100 μ L of DMSO was added in each well to dissolve the formazan crystals. The absorbance was recorded in a microplate reader (Varioskan Flash) at the wavelength of 570 nm. All experiments were performed in triplicates, and the relative cell viability (%) was expressed as a percentage of untreated cells.

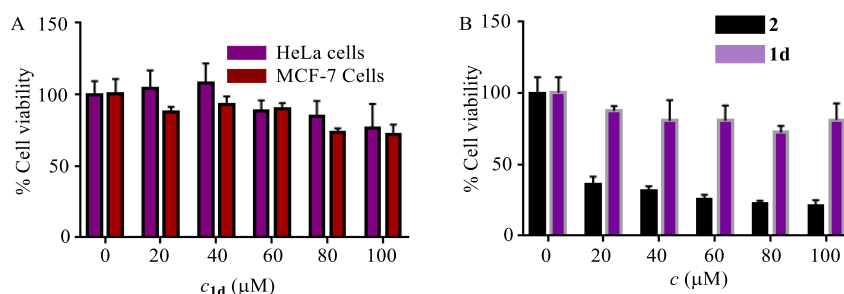


Figure 2.37. Cell viability assay of MCF-7 and HeLa cells incubated with compound **1d** at variable concentrations for 24 hours (A), and comparison of cell viability of MCF-7 cells incubated with compound **1d** and **2** at variable concentrations (B).

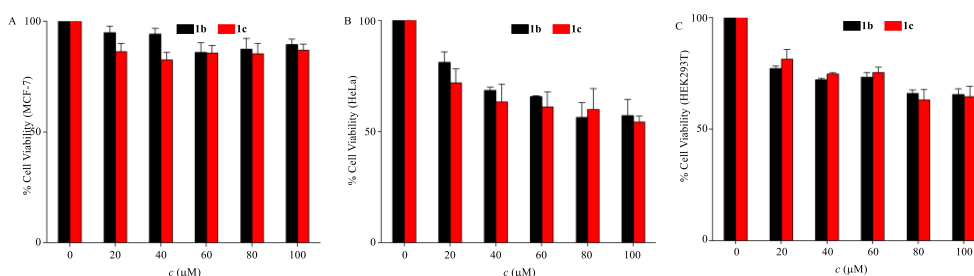


Figure 2.38. Cell viability of MCF-7 (A), HeLa (B), and HEK293T (C) cells incubated with either compound **1b**, or **1c** at variable concentrations for 24 hours.

Further, we investigated the effect of such Cl[−] transport on cell viability. The HEK293T, HeLa, and MCF-7 cells were incubated with **1b**, **1c**, and **1d** for 24 hours, followed by evaluation of cell viability using MTT assay.⁴¹ As a control experiment, MCF-7 cells were incubated with compound **2** for 24 hours. The results showed the less toxic behavior of **1b**, **1c**, and **1d**, while, when the viability of MCF-7 cells was evaluated in presence of the **2**, significant cell death was observed (Figure 2.37 and Figure 2.38). These results indicated that the substitution of one carboxamide moiety with its bioisostere 1,2,3-triazole, leads to the lesser toxic behavior of the 3-(1*H*-1,2,3-triazol-1-yl) benzamides.

Since compound **1d** was shown to have a good balance between its toxicity and chloride transport capabilities, further research on the effects of compound **1d** on actual CFTR channels is presently being conducted, and we anticipate learning more about these effects in the near future.

2.2.15. NMR spectra:

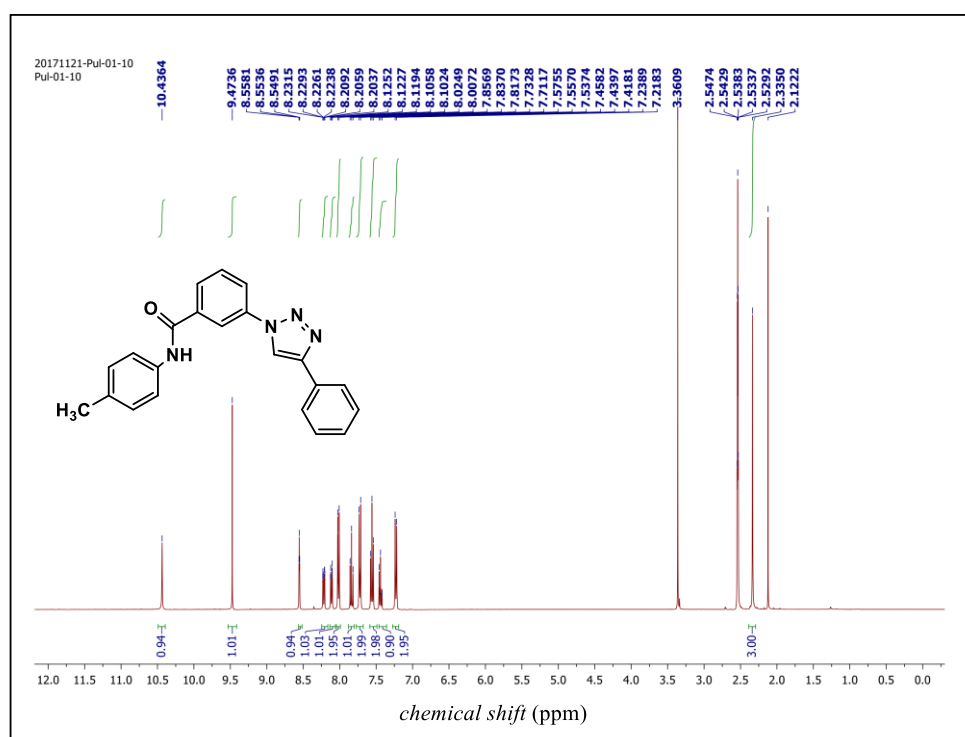


Figure 2.39. ¹H NMR spectrum of **1a** in DMSO-*d*₆.

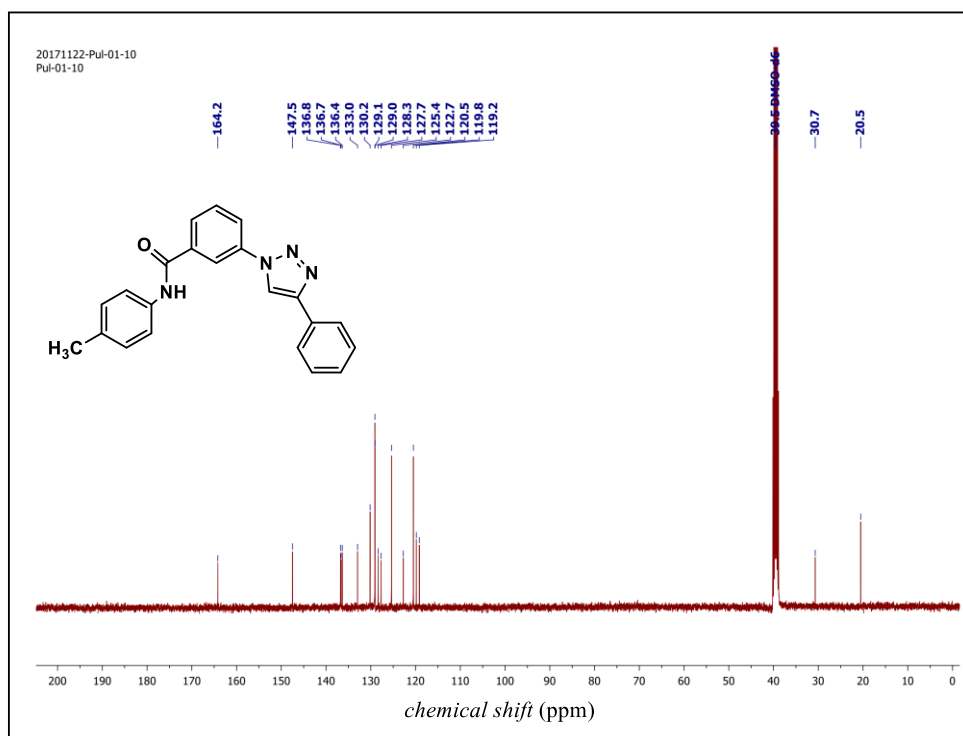


Figure 2.40. ¹³C NMR spectrum of **1a** in DMSO-*d*₆.

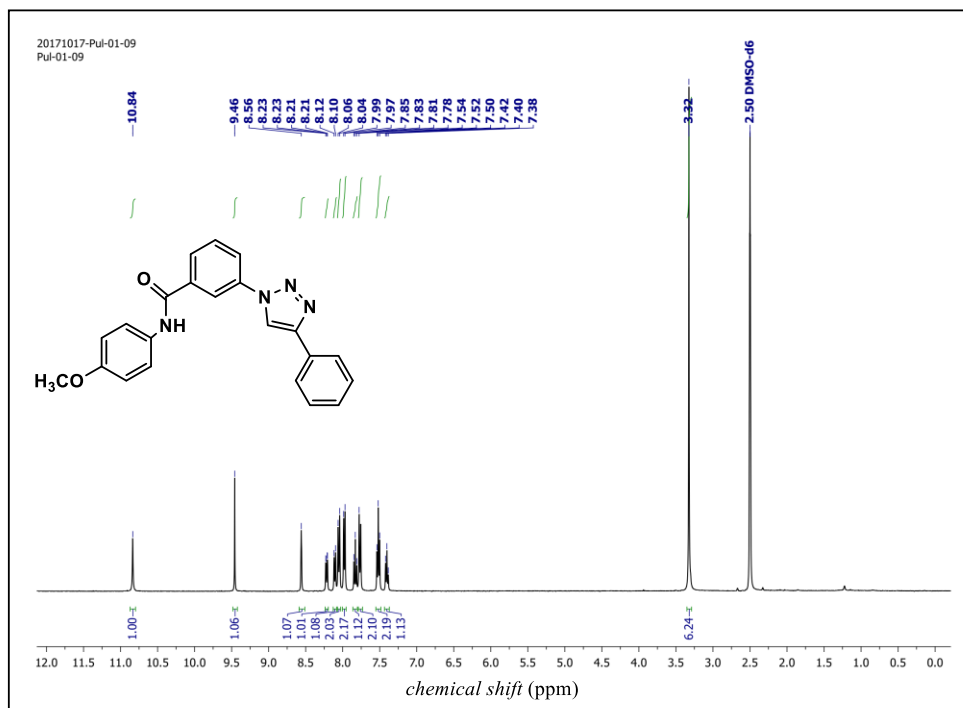


Figure 2.41. ¹H NMR spectrum of **1b** in DMSO-*d*₆.

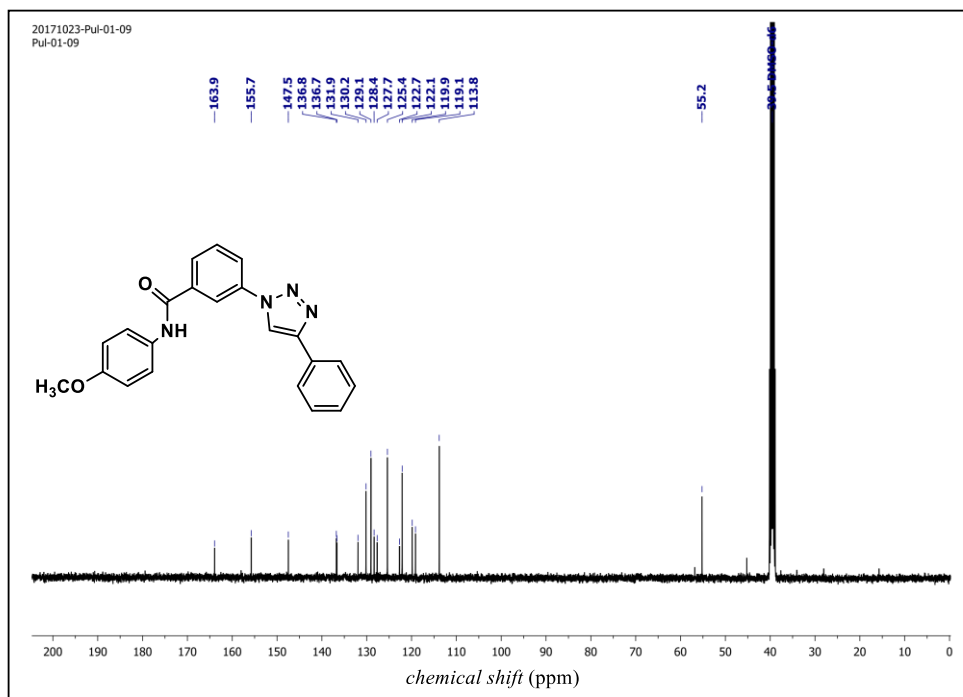


Figure 2.42. ¹³C NMR spectrum of **1b** in DMSO-*d*₆.

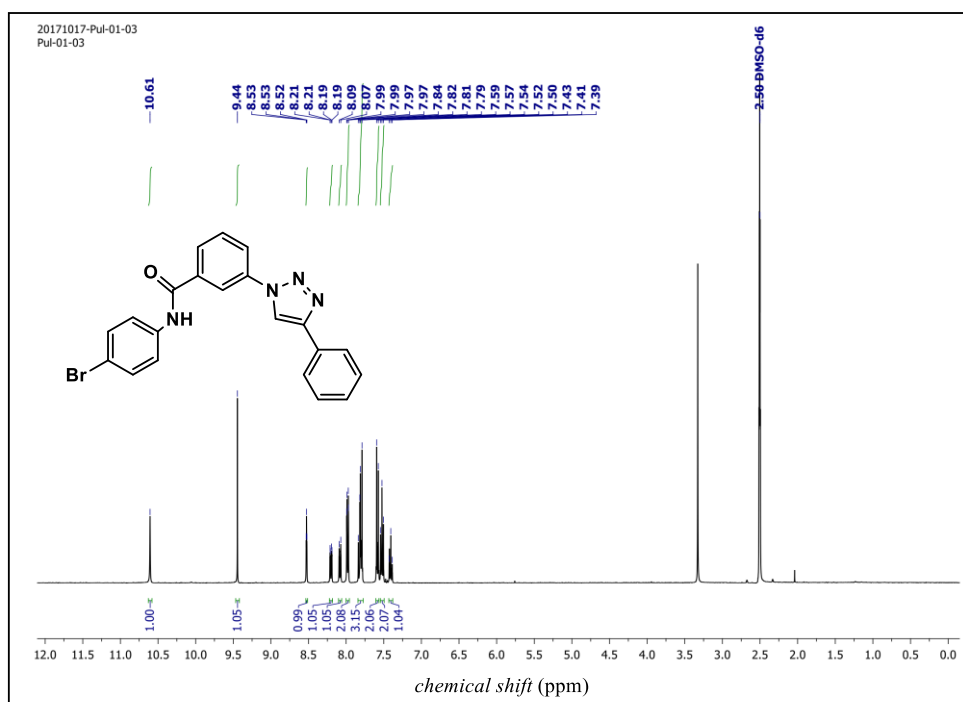


Figure 2.43. ¹H NMR spectrum of **1c** in DMSO-*d*₆.

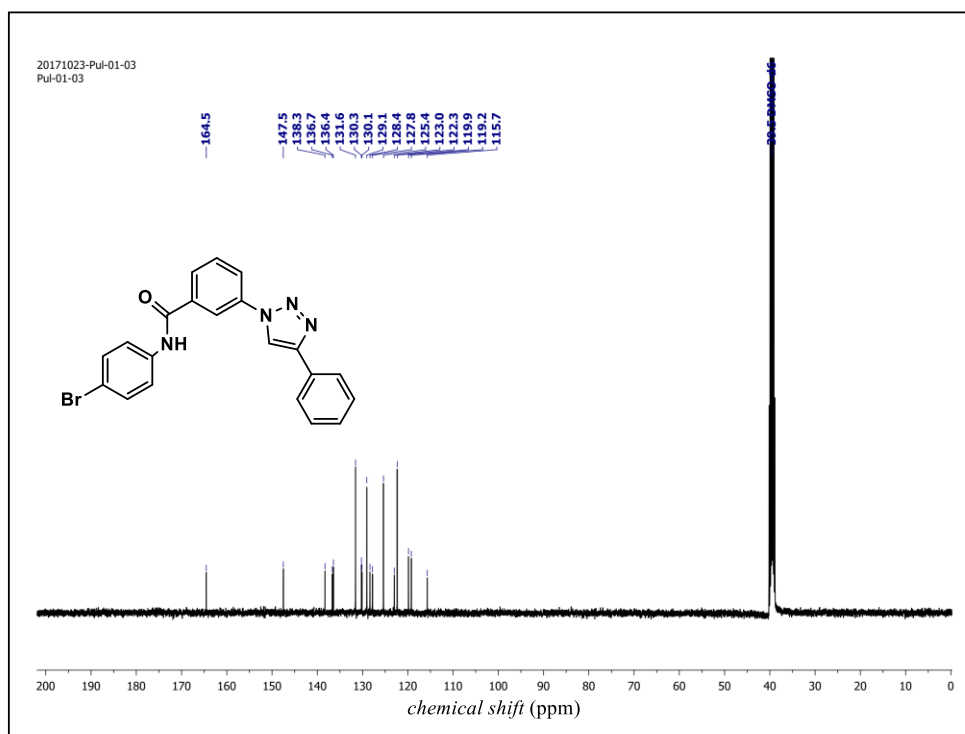


Figure 2.44. ¹³C NMR spectrum of **1c** in DMSO-*d*₆.

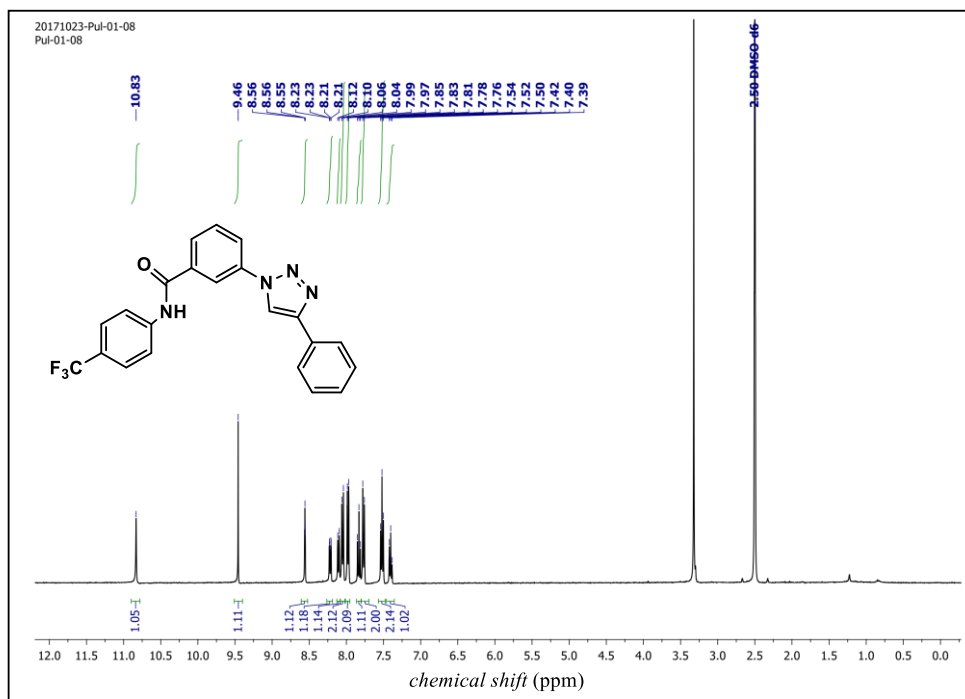


Figure 2.45. ¹H NMR spectrum of **1d** in DMSO-*d*₆.

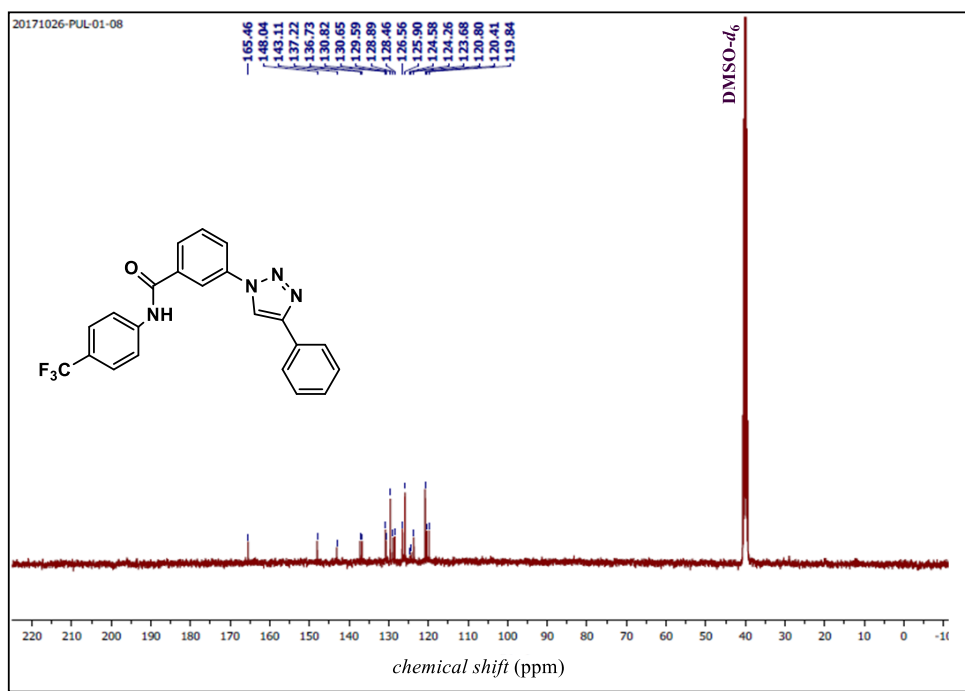


Figure 2.46. ¹³C NMR spectrum of **1d** in DMSO-*d*₆.

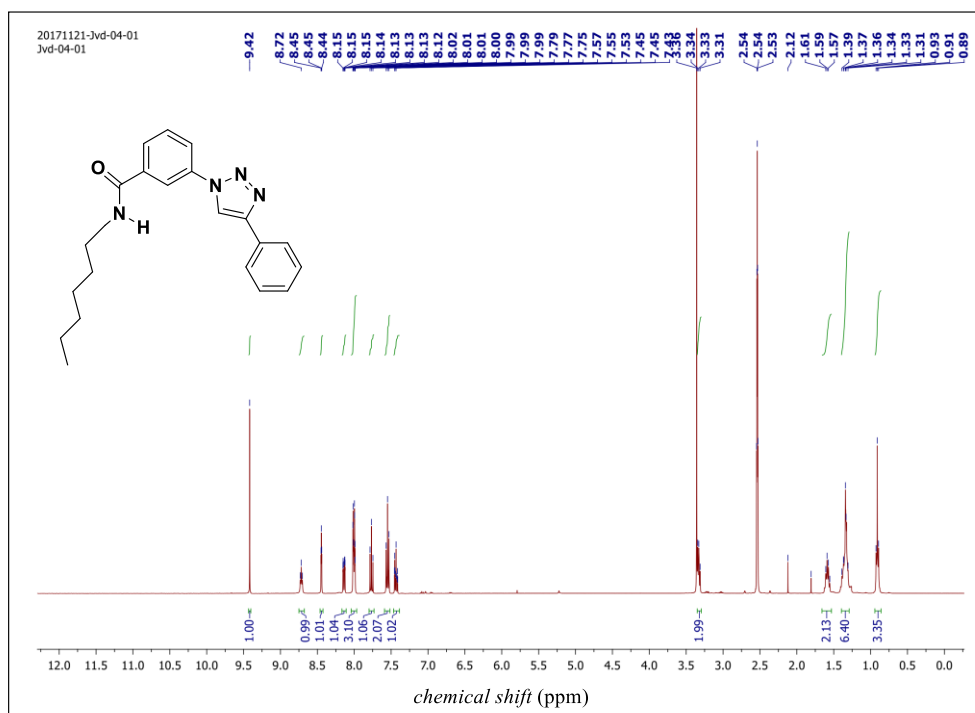


Figure 2.47. ¹H NMR spectrum of **1e** in DMSO-*d*₆.

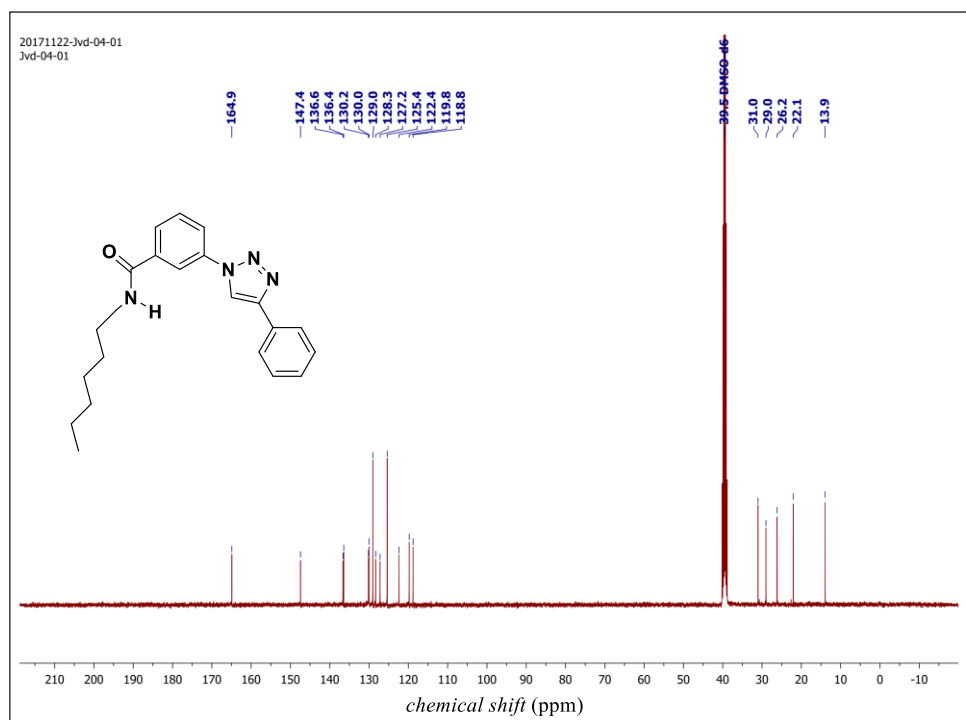


Figure 2.48. ¹³C NMR spectrum of **1e** in DMSO-*d*₆.

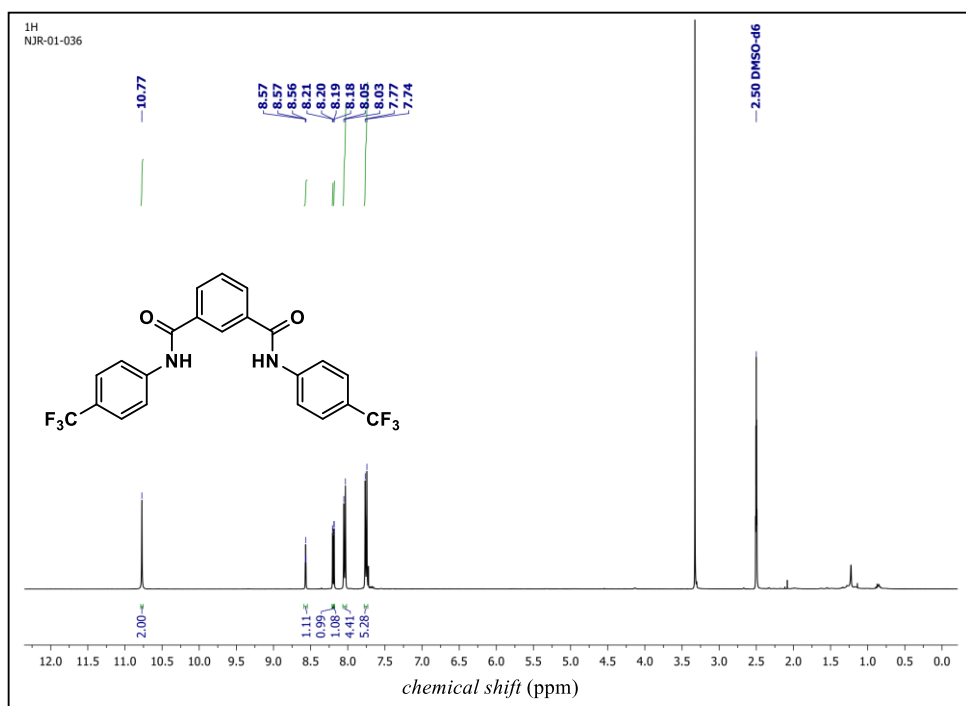


Figure 2.49. ¹H NMR spectrum of **2** in DMSO-*d*₆.

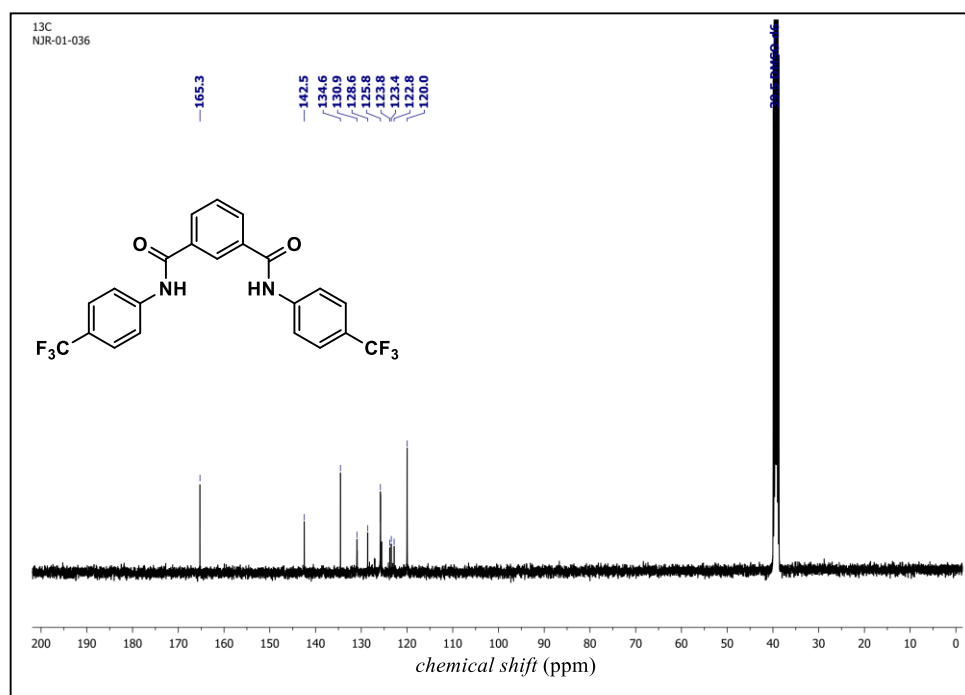


Figure 2.50. ¹³C NMR spectrum of 2 in DMSO- *d*₆.

2.2.16. References:

1. Davis, J. T.; Okunola, O.; Quesada, R., Recent advances in the transmembrane transport of anions. *Chem. Soc. Rev.* **2010**, 39 (10), 3843-3862.
2. Becchetti, A.; Munaron, L.; Arcangeli, A., The role of ion channels and transporters in cell proliferation and cancer. *Front. Physiol.* **2013**, 4 (312), 312.
3. Shinde, S. V.; Talukdar, P., An anion receptor that facilitates transmembrane proton–anion symport by deprotonating its sulfonamide N–H proton. *Chem. Commun.* **2018**, 54 (73), 10351-10354.
4. Dworakowska, B.; Dołowy, K., Ion channels-related diseases. *Acta Biochim. Pol.* **2000**, 47 (3), 685-703.
5. Niemeyer, B. A.; Mery, L.; Zawar, C.; Suckow, A.; Monje, F.; Pardo, L. A.; Stuhmer, W.; Flockerzi, V.; Hoth, M., Ion channels in health and disease. 83rd Boehringer Ingelheim Fonds International Titisee Conference. *EMBO Rep.* **2001**, 2 (7), 568-573.

6. Busschaert, N.; Park, S. H.; Baek, K. H.; Choi, Y. P.; Park, J.; Howe, E. N. W.; Hiscock, J. R.; Karagiannidis, L. E.; Marques, I.; Félix, V.; Namkung, W.; Sessler, J. L.; Gale, P. A.; Shin, I., A synthetic ion transporter that disrupts autophagy and induces apoptosis by perturbing cellular chloride concentrations. *Nat. Chem.* **2017**, 9 (7), 667-675.
7. Stoltz, D. A.; Meyerholz, D. K.; Welsh, M. J., Origins of cystic fibrosis lung disease. *N. Engl. J. Med.* **2015**, 372 (4), 351-362.
8. Hernando, E.; Capurro, V.; Cossu, C.; Fiore, M.; García-Valverde, M.; Soto-Cerrato, V.; Pérez-Tomás, R.; Moran, O.; Zegarra-Moran, O.; Quesada, R., Small molecule anionophores promote transmembrane anion permeation matching CFTR activity. *Sci. Rep.* **2018**, 8 (1), 2608.
9. Li, H.; Valkenier, H.; Judd, L. W.; Brotherhood, P. R.; Hussain, S.; Cooper, J. A.; Jurček, O.; Sparkes, H. A.; Sheppard, D. N.; Davis, A. P., Efficient, non-toxic anion transport by synthetic carriers in cells and epithelia. *Nat. Chem.* **2016**, 8 (1), 24-32.
10. Deriy, L. V.; Gomez, E. A.; Zhang, G.; Beacham, D. W.; Hopson, J. A.; Gallan, A. J.; Shevchenko, P. D.; Bindokas, V. P.; Nelson, D. J., Disease-causing mutations in the cystic fibrosis transmembrane conductance regulator determine the functional responses of alveolar macrophages. *J. Biol. Chem.* **2009**, 284 (51), 35926-35938.
11. Duran, C.; Thompson, C. H.; Xiao, Q.; Hartzell, H. C., Chloride channels: often enigmatic, rarely predictable. *Annu. Rev. Physiol.* **2010**, 72 (1), 95-121.
12. Somaraju, U. R. R.; Solis-Moya, A., Pancreatic enzyme replacement therapy for people with cystic fibrosis. *Cochrane Database Syst. Rev.* **2020**, (8), 10.1002/14651858.CD008227.pub4.
13. Shen, B.; Li, X.; Wang, F.; Yao, X.; Yang, D., A synthetic chloride channel restores chloride conductance in human cystic fibrosis epithelial cells. *PLoS One* **2012**, 7 (4), e34694.
14. Muraglia, K. A.; Chorghade, R. S.; Kim, B. R.; Tang, X. X.; Shah, V. S.; Grillo, A. S.; Daniels, P. N.; Cioffi, A. G.; Karp, P. H.; Zhu, L.; Welsh, M. J.; Burke, M. D., Small-molecule ion channels increase host defences in cystic fibrosis airway epithelia. *Nature*. **2019**, 567 (7748), 405-408.

15. Picci, G.; Carreira-Barral, I.; -Carrillo, D.; Sanz-González, D.; Fernández-López, P.; García-Valverde, M.; Caltagirone, C.; Quesada, R., Simple isophthalamides/dipicolineamides as active transmembrane anion transporters. *Supramol. Chem.* **2020**, 32 (2), 112-118.
16. Davis, J. T.; Gale, P. A.; Quesada, R., Advances in anion transport and supramolecular medicinal chemistry. *Chem. Soc. Rev.* **2020**, 49 (16), 6056-6086.
17. Yamnitz, C. R.; Negin, S.; Carasel, I. A.; Winter, R. K.; Gokel, G. W., Dianilides of dipicolinic acid function as synthetic chloride channels. *Chemical Communications* **2010**, 46 (16), 2838-2840.
18. Busschaert, N.; Caltagirone, C.; Van Rossom, W.; Gale, P. A., Applications of supramolecular anion recognition. *Chem. Rev.* **2015**, 115 (15), 8038-8155.
19. Macreadie, L. K.; Gilchrist, A. M.; McNaughton, D. A.; Ryder, W. G.; Fares, M.; Gale, P. A., Progress in anion receptor chemistry. *Chem* **2022**, 8 (1), 46-118.
20. Costa, A. V.; Oliveira, M. V. L. d.; Pinto, R. T.; Moreira, L. C.; Gomes, E. M. C.; Alves, T. d. A.; Pinheiro, P. F.; Queiroz, V. T. d.; Vieira, L. F. A.; Teixeira, R. R.; Júnior, W. C. d. J., Synthesis of novel glycerol-derived 1,2,3-Triazoles and evaluation of their fungicide, phytotoxic and cytotoxic activities. *Molecules* **2017**, 22 (10), 1666.
21. Chen, S.; Zhang, S.; Bao, C.; Wang, C.; Lin, Q.; Zhu, L., Oligo(aryl-triazole)s CH⁺...Cl⁻ interactions guide chloride efficient and selective transmembrane transport. *Chem. Commun.* **2016**, 52 (89), 13132-13135.
22. Etoc, F.; Vicario, C.; Lisse, D.; Siaugue, J.-M.; Piehler, J.; Coppey, M.; Dahan, M., Magnetogenetic control of protein gradients inside living cells with high spatial and temporal resolution. *Nano Lett.* **2015**, 15 (5), 3487-3494.
23. El-Shafey, H. W.; Gomaa, R. M.; El-Messery, S. M.; Goda, F. E., Synthetic approaches, anticancer potential, HSP90 inhibition, multitarget evaluation, molecular modeling and apoptosis mechanistic study of thioquinazolinone skeleton: Promising antibreast cancer agent. *Bioorg. Chem.* **2020**, 101, 103987.
24. Kinsella, M.; Duggan, P. G.; Muldoon, J.; Eccles, K. S.; Lawrence, S. E.; Lennon, C. M., Synthesis and NMR binding studies towards rational design of a series of electron-

withdrawing diamide receptors/organocatalysts. *Eur. J. Org. Chem.* **2011**, 2011 (6), 1125-1132.

25. BindFit, <http://app.supramolecular.org/bindfit/>.

26. Ahmad, M.; Metya, S.; Das, A.; Talukdar, P., A sandwich azobenzene–diamide dimer for photoregulated chloride transport. *Chem. Eur. J.* **2020**, 26 (40), 8703-8708.

27. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. *Gaussian 16 Rev. B.01*, Wallingford, CT, **2016**.

28. Frisch, M. J.; Pople, J. A.; Binkley, J. S., Self-consistent molecular orbital methods 25. Supplementary functions for Gaussian basis sets. *J. Chem. Phys* **1984**, 80, 3265-3269.

29. Goto, H.; Osawa, E., Corner flapping: a simple and fast algorithm for exhaustive generation of ring conformations. *J. Am. Chem. Soc.* **1989**, 111 (24), 8950-8951.

30. Goto, H. O., S.; Nakayama, N.; Ohta, K. CONFLEX 8; CONFLEX Corporation, T., Japan, **2017**.

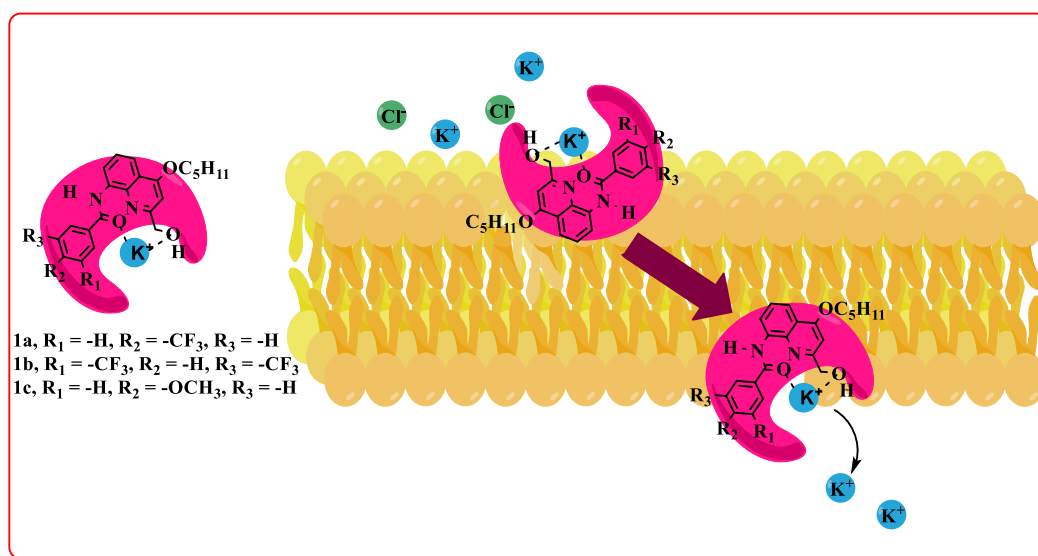
31. Roy, A.; Saha, D.; Mandal, P. S.; Mukherjee, A.; Talukdar, P., pH-Gated chloride transport by a triazine-based tripodal semicage. *Chem. Eur. J.* **2017**, 23 (6), 1241-1247.

32. Roy, A.; Saha, D.; Mukherjee, A.; Talukdar, P., One-pot synthesis and transmembrane chloride transport properties of C3-symmetric benzoxazine urea. *Org. Lett.* **2016**, 18 (22), 5864-5867.

33. Malla, J. A.; Roy, A.; Talukdar, P., Anion selective ion channel constructed from a self-assembly of bis(cholate)-substituted fumaramide. *Org. Lett.* **2018**, *20* (19), 5991-5994.
34. Shinde, S. V.; Talukdar, P., A dimeric bis(melamine)-substituted bispidine for efficient transmembrane H⁺/Cl⁻ cotransport. *Angew. Chem., Int. Ed.* **2017**, *56* (15), 4238-4242.
35. Li, Y. H.; Zheng, S.; Legrand, Y. M.; Gilles, A.; Van der Lee, A.; Barboiu, M., Structure-driven selection of adaptive transmembrane Na⁺ carriers or K⁻ channels. *Angew. Chem., Int. Ed.* **2018**, *57* (33), 10520-10524.
36. Mondal, D.; Ahmad, M.; Panwaria, P.; Upadhyay, A.; Talukdar, P., Anion recognition through multivalent C–H hydrogen bonds: Anion-induced foldamer formation and transport across phospholipid membranes. *J. Org. Chem.* **2022**, *87* (1), 10-17.
37. Roy, A.; Gautam, A.; Malla, J. A.; Sarkar, S.; Mukherjee, A.; Talukdar, P., Self-assembly of small-molecule fumaramides allows transmembrane chloride channel formation. *Chem. Commun.* **2018**, *54* (16), 2024-2027.
38. Wu, X.; Judd, Luke W.; Howe, Ethan N. W.; Withecombe, Anne M.; Soto-Cerrato, V.; Li, H.; Busschaert, N.; Valkenier, H.; Pérez-Tomás, R.; Sheppard, David N.; Jiang, Y.-B.; Davis, Anthony P.; Gale, Philip A., Nonprotonophoric electrogenic Cl⁻ transport mediated by Valinomycin-like carriers. *Chem* **2016**, *1* (1), 127-146.
39. Mondal, D.; Sathyan, A.; Shinde, S. V.; Mishra, K. K.; Talukdar, P., Tripodal cyanurates as selective transmembrane Cl⁻ transporters. *Org. Biomol. Chem.* **2018**, *16* (45), 8690-8694.
40. Saha, T.; Hossain, M. S.; Saha, D.; Lahiri, M.; Talukdar, P., Chloride-mediated apoptosis-inducing activity of bis(sulfonamide) anionophores. *J. Am. Chem. Soc.* **2016**, *138* (24), 7558-7567.
41. Saha, T.; Gautam, A.; Mukherjee, A.; Lahiri, M.; Talukdar, P., Chloride transport through supramolecular barrel-rosette ion channels: lipophilic control and apoptosis-inducing activity. *J. Am. Chem. Soc.* **2016**, *138* (50), 16443-16451.

Chapter 3

Quinoline-based Small Molecules as Transmembrane K^+/H^+ Transporters



3.1. Introduction:

For the maintenance of osmoregulatory functions, neural transmission for nerve coordination, muscle stimulation, the survival of cells, and cell differentiation, ion transport across cell membranes is crucial.¹⁻³ Ion carriers and channels are a specific class of membrane transport proteins assigned to cells by nature to prevent polar components from passing through the hydrophobic compartment of the cell membranes.^{4, 5} The transmembrane transport of cations is essential for several crucial biological processes, such as skeletal muscle contraction, transmission of nerve impulses, hormone production and so on.⁶⁻⁸ Additionally, a crucial step for preserving intracellular ion homeostasis in a variety of environments is the movement of cations and protons through cellular membranes.⁹ Among the biologically relevant cations in the living cell, K^+ is the most common, and its concentration in cells is approximately between 0.1 M and 0.2 M.⁹ Several essential biological activities, including the maintenance of the membrane potential, adjustment of the osmotic and the turgor pressure requires K^+ .^{10, 11} A large number of naturally occurring ion transporters control the transmembrane exchange of K^+ with H^+ . Among these, a major subgroup in the superfamily of ion transporters is the cation/proton antiporter (CPA) superfamily.^{9, 12} CPAs have a variety of localizations and transport directions, and they also have a variety of physiological functions.¹³⁻¹⁵

The scope of study of cation transporters utilizing small molecules is still in its budding stage and has not been investigated into, with the exception of a few rare instances of cation transporters employing small molecules.⁶ For cation transport, certain synthetic molecules have been used, including hybrid gramicidin channels^{16, 17}, crown ether-based transporters^{18, 19}, bola amphiphiles²⁰, cyclic peptides²¹, calixarenes²², hydrapiles²³, and cyclic depsipeptides²⁴. To develop new synthetic cation transporters with a pre-organized cavity to bind to the cations, we have utilized quinoline as the main scaffold. Baruah and his coworkers have developed very efficient quinoline-based receptors for the detection of metal cations like Zn^{2+} and Cu^{2+} by binding to them.²⁵ In another representative work, oxa-azamacrocycles reported by Beletskaya and co-workers incorporating quinoline moieties bearing crown-ether arms have shown binding to cations.²⁶ This kind of binding inspired us to investigate the effect of utilizing the oxygen atoms of the carbonyl bond and the hydroxyl bond of the target molecule to bind to the K^+ ion and consequently facilitate its transmembrane transport.

Herein, we report a series of small molecules possessing a quinoline core that selectively bind to and aid the transmembrane transport of K^+ ion. In order to achieve a better tunability in terms of the partition coefficient values, we varied the different substituents on the phenyl ring, to aid the transmembrane transport of these small molecules.

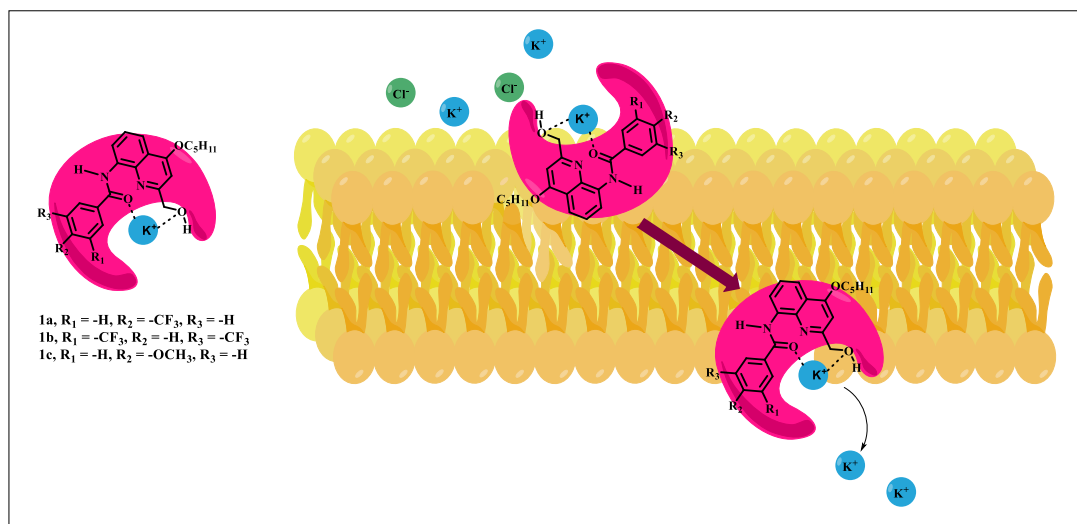


Figure 3.1. Structure of transporter molecules and their representative cation transport across the cell membrane.

Table 1. The pK_a and $\log P$ values of transporters 1a–1c:

Compound	pK_a of (N–H)	pK_a of (O–H)	$\log P$
1a	12.27	3.17	5.32
1b	11.89	3.17	6.15
1c	11.77	3.17	5.57

3.2. Results and discussion:

3.2.1. General methods:

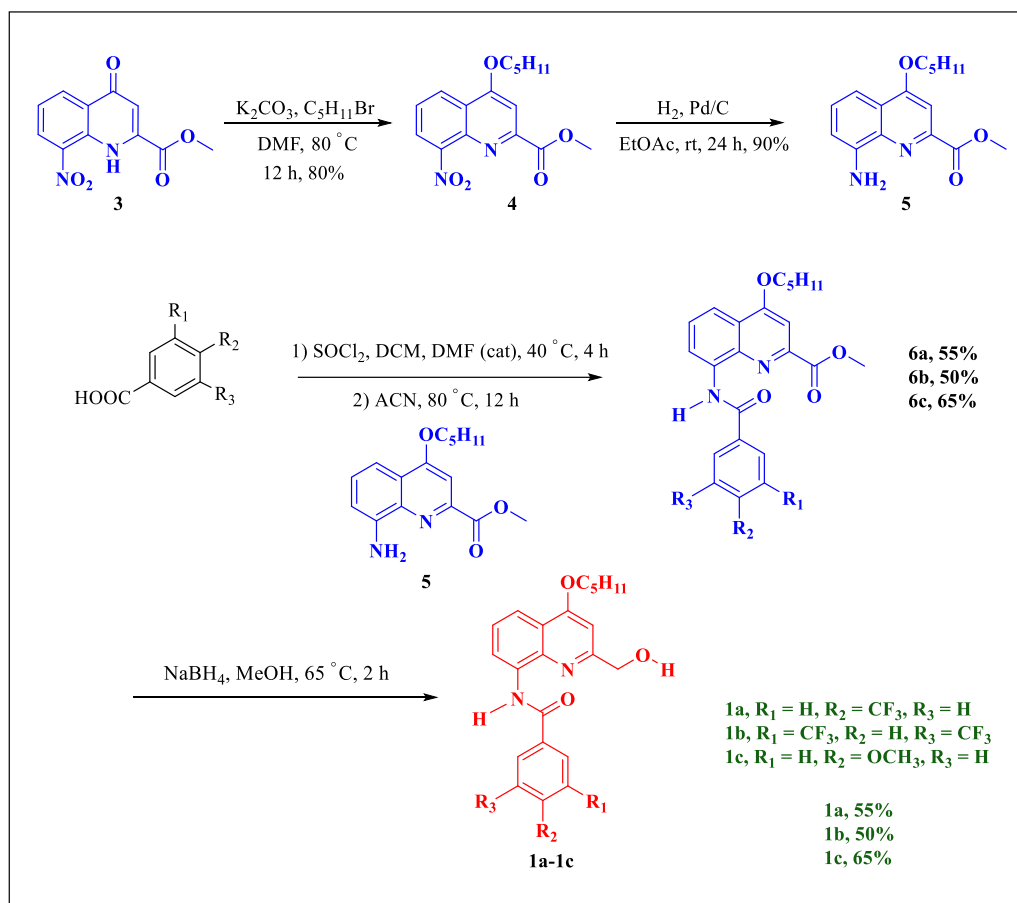
All chemical reactions were performed under a nitrogen atmosphere. All synthesized reagents and solvents were purchased from commercial sources (Sigma Aldrich, Spectrochem) and used further without purification. Potassium picrate used was purchased from a commercial source. (Avra chemicals). The column chromatography was carried out using Merck silica (100–200 or 230–400 mesh size). The thin-layer chromatography was performed on E. Merck silica gel 60–F254 plates. Egg yolk phosphatidylcholine (EYPC) as a solution of chloroform (25 mg/

mL), mini extruder, polycarbonate membrane of 100 nm and 200 nm were purchased from Avanti Polar Lipid. HEPES, HPTS, Lucigenin, NaOH, Triton X-100, FCCP, Valinomycin and all inorganic salts were obtained as molecular biology grade from Sigma Aldrich.

3.2.2. Physical measurements:

The ^1H NMR spectra were recorded at 400 MHz, ^{19}F NMR spectra were recorded at 377 MHz whereas ^{13}C NMR spectra were recorded at 101 MHz. The ^{39}K NMR spectra were recorded at 23.3 MHz. The residual solvent signals were considered as an internal reference (^{39}K NMR: 0.1 KCl in D_2O ; ^1H NMR CDCl_3 : δ 7.26 ppm; ^{13}C NMR CDCl_3 : δ 77.2 ppm; ^1H NMR CD_3OD : δ 3.31 ppm; ^{13}C NMR CD_3OD : δ 49.2 ppm; ^1H NMR $\text{DMSO}-d_6$: δ 2.5 ppm; ^{13}C NMR $\text{DMSO}-d_6$: δ 39.5 ppm) to calibrate spectra. The chemical shifts were reported in ppm. Following abbreviations were used to indicate multiplicity patterns m: multiplet, s: singlet, d: doublet, t: triplet, q: quartet, dd: doublet of doublet, ddd: doublet of doublet of doublet, td: triplet of doublet, dt: doublet of triplet. Coupling constants were measured in Hz. High-resolution mass spectra (HRMS) were recorded on electrospray ionization time-of-flight (ESI-TOF). Fluorescence experiments were recorded on Fluoromax-4 from Jobin Yvon Edison equipped with an injector port and magnetic stirrer in a microfluorescence cuvette. All buffer solutions were prepared from the autoclaved water. The Helmer pH meter was adjusted to the pH of buffer solutions. The extravesicular dye was removed by performing gel chromatography using Sephadex-G. The fluorescence studies were conducted using Origin 8.5. ChemBio Draw 15 Ultra software was used for drawing structures and processing figures. UV-vis spectra were recorded on a Varian Cary 5000 spectrophotometer.

3.2.3. Synthesis:

Scheme 3.1. Synthesis of compounds **1a-1c**.

Compound **3** was synthesized by a previously reported protocol and its NMR and HRMS data were matched with the reported compound.²⁷

Methyl 8-nitro-4-(pentyloxy) quinoline-2-carboxylate 4: Compound **3** (1 equiv, 0.56 mmol) was dissolved in dry dimethylformamide (5 mL), followed by the addition of potassium carbonate (1.5 equiv, 0.85 mmol), and 1-bromopentane (1.1 equiv, 1.13 mmol). The reaction mixture was allowed to stir at $80^\circ C$ for 4 hours. Then, water was added to the reaction mixture followed by extraction with EtOAc (10 mL $\times$ 3 times). The combined organic phases were washed with brine and dried over sodium sulfate and concentrated *in vacuo*. The compound was purified using silica gel chromatography over silica gel to get a yellow coloured solid (Yield: 80%) (Eluent: 5% EtOAc in petroleum ether). Compound **4** was characterized by 1H NMR, ^{13}C NMR, HRMS and IR data. 1H NMR (400 MHz, $CDCl_3$): δ 8.47 (dd, $J = 8.6, 1.4$ Hz, 1H), 8.11 (dd, $J = 7.6, 1.4$ Hz, 1H), 7.67 – 7.62 (m, 2H), 4.32 (t, $J = 6.5$ Hz, 2H), 4.04 (s, 3H), 1.99 (dq, $J = 13.2, 6.6$ Hz, 2H), 1.53 (ddd, $J = 14.0, 10.0, 6.3$ Hz, 2H), 1.49 – 1.40 (m,

2H), 0.98 (t, J = 7.2 Hz, 3H), ¹³C NMR (101 MHz, CDCl₃): δ 165.75, 162.80, 151.37, 148.40, 140.11, 126.52, 125.90, 125.09, 123.34, 102.19, 69.69, 53.39, 28.52, 28.15, 22.38, 13.99, IR (Neat, v/cm⁻¹): 3313.24, 2942.67, 1021.13. HRMS (ESI): Calc. for C₁₁H₈N₂O₅ [M+H]⁺: 319.1294; Found: 319.1289.

Methyl 8-amino-4-(pentyloxy) quinoline-2-carboxylate (C₁₆H₂₀N₂O₃) 5: In a 100 mL round bottomed flask, compound **4** was taken and dissolved in 20 mL of MeOH. The compound solution was then degassed for about 1 hour using a nitrogen gas balloon. Then into that degassed solution, a catalytic amount of 10% Pd/C was added. The reaction mixture was then stirred under a hydrogen gas balloon for 24 hours at room temperature. After completion of the reaction, the whole solution was passed through a celite bed while washing with MeOH. Then the solvent was evaporated to get the amine **5** as a yellow solid (Yield: 90%). ¹H NMR (400 MHz, CDCl₃): δ 7.51 (dd, J = 9.0, 1.9 Hz, 2H), 7.39 – 7.34 (m, 1H), 6.93 (dd, J = 7.5, 1.2 Hz, 1H), 5.10 (s, 2H), 4.24 (t, J = 6.5 Hz, 2H), 4.02 (s, 3H), 1.95 (dq, J = 13.3, 6.5 Hz, 2H), 1.54 (dt, J = 20.2, 7.1 Hz, 2H), 1.49 – 1.39 (m, 2H), 0.96 (t, J = 7.2 Hz, 3H), ¹³C NMR (101 MHz, CDCl₃): δ 166.50, 162.68, 145.90, 144.93, 138.44, 128.65, 123.06, 110.91, 109.72, 100.84, 68.89, 52.88, 28.67, 28.31, 22.49, 14.10, IR (Neat, v/cm⁻¹): 3473, 3367, 2953, 2927, 2869, 1718, 1615, 1580, 1514, 1473, 1425, 1357, 1269, 1229, 1127, 1105, 1064, 1022. HRMS (ESI): Calc. for C₁₆H₂₀N₂O₃ [M+H]⁺: 289.1552; Found: 289.1558.

General method for the synthesis of 6a–6c:

The corresponding acid derivatives were taken in a 25 mL round bottomed flask. To them, dry dichloromethane, 3 equiv of thionyl chloride and catalytic amount of dry dimethylformamide was added. The reaction mixture was stirred at 80 °C for 4 hours. The progress of the reaction was monitored by thin layer chromatography, after which it was concentrated under rotary evaporator and kept under high vacuum for half an hour. In another 25 mL round bottomed flask, compound **5** (1 equiv, 0.56 mmol) and N,N-diisopropylethylamine (2.5 equiv, 1.4 mmol) were dissolved in dry acetonitrile which was left to stir at room temperature for half an hour. The acid chloride was then dissolved again in dry acetonitrile and added dropwise to the second solution, which was then stirred at 80 °C for 4 hours. After completion of the reaction monitored by TLC, water was added to the reaction mixture followed by extraction with CHCl₃ (10 mL × 3 times). The combined organic phases were washed with brine and dried over sodium sulfate and concentrated *in vacuo*. The compound was purified using silica gel

chromatography over silica gel. The compounds were characterized by ^1H NMR, ^{13}C NMR, HRMS and IR data.

Methyl 4-(pentyloxy)-8-(4-(trifluoromethyl)benzamido)quinoline-2-carboxylate ($\text{C}_{24}\text{H}_{23}\text{F}_3\text{N}_2\text{O}_4$) 6a: The crude product was purified by column chromatography over silica gel (5% EtOAc in petroleum ether) to furnish **6a** as a white solid (Yield: 55%); ^1H NMR (400 MHz, CDCl_3): δ 10.92 (s, 1H), 8.93 (d, J = 7.1 Hz, 1H), 8.23 (d, J = 8.2 Hz, 2H), 8.04 – 7.91 (m, 1H), 7.83 (d, J = 8.2 Hz, 2H), 7.65 (s, 1H), 4.30 (s, 1H), 4.32 – 4.29 (m, 2H), 4.08 (s, 3H), 2.16 – 1.90 (m, 3H), 1.46 (dd, J = 15.0, 7.3 Hz, 3H), 0.98 (t, J = 7.2 Hz, 3H), ^{19}F NMR (377 MHz, CDCl_3): δ -62.92, ^{13}C NMR (101 MHz, CDCl_3): δ 165.80, 163.96, 163.38, 147.02, 139.08, 138.34, 134.86, 133.87, 133.54, 128.54, 127.92, 126.10, 126.06, 126.03, 125.99, 125.23, 122.52, 122.21, 117.71, 116.42, 101.51, 69.45, 53.20, 28.68, 28.36, 22.55, 14.15, IR (Neat, v/cm^{-1}): 2917, 2856, 2674, 2550, 1696, 1581, 1522, 1424, 1375, 1322, 1175, 1133, 1065. HRMS (ESI): Calc. for $\text{C}_{24}\text{H}_{23}\text{F}_3\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$: 461.1688; Found: 461.1689.

Methyl 8-(3,5-bis(trifluoromethyl)benzamido)-4-(pentyloxy)quinoline-2-carboxylate ($\text{C}_{25}\text{H}_{22}\text{F}_6\text{N}_2\text{O}_4$) 6b: crude product was purified by column chromatography over silica gel (5% EtOAc in petroleum ether) to furnish **6b** as a white solid (Yield : 65%) ^1H NMR (400 MHz, CDCl_3): δ 11.12 (s, 1H), 8.89 (dd, J = 7.7, 1.0 Hz, 1H), 8.61 (s, 2H), 8.11 (s, 1H), 7.98 (dd, J = 8.5, 1.1 Hz, 1H), 7.74 – 7.47 (m, 2H), 4.31 (t, J = 6.5 Hz, 2H), 4.08 (s, 3H), 2.12 – 1.89 (m, 2H), 1.58 (d, J = 2.9 Hz, 1H), 1.54 (d, J = 7.4 Hz, 1H), 1.51 – 1.41 (m, 2H), 0.98 (s, 3H), ^{19}F NMR (377 MHz, CDCl_3): δ -62.90, ^{13}C NMR (101 MHz, CDCl_3): δ , 165.98, 163.44, 161.63, 147.17, 138.87, 136.88, 134.25, 133.09, 132.75, 132.41, 132.08, 128.48, 127.77, 125.50, 124.53, 122.18, 121.81, 117.64, 116.85, 101.59, 69.54, 53.08, 28.67, 28.34, 22.56, 14.16, IR (Neat, v/cm^{-1}): 3326, 2958, 2875, 1724, 1683, 1624, 1540, 1469, 1441, 1422, 1364, 1334, 1279, 1241, 1183, 1130, 1036. HRMS (ESI): Calc. for $\text{C}_{25}\text{H}_{22}\text{F}_6\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$: 528.1484; Found: 528.1482.

Methyl 8-(4-methoxybenzamido)-4-(pentyloxy)quinoline-2-carboxylate ($\text{C}_{24}\text{H}_{26}\text{N}_2\text{O}_5$) 6c: The crude product was purified by column chromatography over silica gel (7.5% EtOAc in petroleum ether) to furnish **6c** as a greyish sticky solid (Yield : 75%) ^1H NMR (400 MHz, CDCl_3): δ 10.80 (s, 1H), 8.93 (d, J = 7.5 Hz, 1H), 8.09 (d, J = 8.5 Hz, 2H), 7.90 (d, J = 8.6 Hz, 1H), 7.62 (t, J = 8.3 Hz, 1H), 7.57 (s, 1H), 7.05 (d, J = 8.8 Hz, 2H), 4.29 (t, J = 6.4 Hz, 2H), 4.07 (s, 3H), 3.91 (s, 3H), 2.05 – 1.93 (m, 2H), 1.57 (d, J = 7.2 Hz, 2H), 1.45 (dt, J = 14.4, 7.1 Hz, 2H), 0.98 (t, J = 7.3 Hz, 3H), ^{13}C NMR (101 MHz, CDCl_3): δ , 164.66, 162.49, 162.29,

159.26, 138.01, 133.53, 128.71, 127.13, 125.62, 120.38, 117.01, 115.70, 113.88, 97.99, 76.84, 68.62, 65.17, 55.27, 28.37, 28.03, 22.24, 13.83, **HRMS (ESI)**: Calc. for $C_{25}H_{22}F_6N_2O_4$ $[M+H]^+$: 528.1484; Found: 528.1482.

General method for the synthesis of 1a–1c:

In a 25 mL round bottomed flask, a mixture of **6a–6c** (1 equiv, 0.32 mmol) and $NaBH_4$ (10 equiv, 3.22 mmol) in dry THF (10 mL) was stirred at 65 °C for 30 min. Then, dry MeOH (2.6 mL) was added dropwise during 30 min and effervescence was observed. Stirring was maintained for further 60 min. The reaction mixture was cooled to room temperature and then evaporated to give the crude solid. Then, water was added to the reaction mixture followed by extraction with $CHCl_3$ (10 mL $\times$ 3 times). The combined organic phases were washed with brine and dried over sodium sulfate and concentrated *in vacuo*.

N-(2-(hydroxymethyl)-4-(pentyloxy)quinolin-8-yl)-4-(trifluoromethyl)benzamide ($C_{23}H_{23}F_3N_2O_3$) **1a**: The crude product was purified by column chromatography over silica gel (5% EtOAc in petroleum ether) to furnish **7a** as a white crystalline solid (Yield: 55%); **1H NMR (400 MHz, $CDCl_3$)**: δ 10.51 (s, 1H), 8.89 (d, J = 7.7 Hz, 1H), 8.11 (d, J = 8.1 Hz, 2H), 8.01 – 7.89 (m, 1H), 7.80 (d, J = 8.2 Hz, 2H), 7.53 (t, J = 8.1 Hz, 1H), 6.79 (s, 1H), 4.91 (s, 2H), 4.21 (t, J = 6.4 Hz, 2H), 3.25 (s, 1H), 1.96 (dt, J = 14.4, 6.6 Hz, 2H), 1.55 (dd, J = 10.8, 4.9 Hz, 2H), 1.46 (dt, J = 15.1, 7.3 Hz, 2H), 0.98 (t, J = 7.2 Hz, 3H), **^{19}F NMR (377 MHz, $CDCl_3$)**: δ -62.95, **^{13}C NMR (101 MHz, $CDCl_3$)**: δ 164.73, 163.64, 160.50, 139.24, 139.07, 134.43, 134.00, 128.31, 126.81, 126.77, 126.73, 126.69, 126.64, 123.09, 121.46, 118.49, 117.51, 99.13, 69.73, 66.14, 29.32, 29.00, 23.18, 14.78, **IR (Neat, v/cm^{-1})**: 3424, 3316, 2956, 2936, 2871, 2316, 1651, 1551, 1462, 1426, 1365, 1327, 1250, 1165, 1123, 1069, 1048. **HRMS (ESI)**: Calc. for $C_{23}H_{23}F_3N_2O_3$ $[M+H]^+$: 433.1739; Found: 433.1743.

N-(2-(hydroxymethyl)-4-(pentyloxy)quinolin-8-yl)-4-methoxybenzamide ($C_{23}H_{26}N_2O_4$) **1b**: The crude product was purified by column chromatography over silica gel (5% EtOAc in petroleum ether) to furnish **7b** as a yellowish crystalline solid (Yield: 50%); **1H NMR (400 MHz, $CDCl_3$)**: δ 10.38 (s, 1H), 8.89 (d, J = 7.7 Hz, 1H), 8.48 (s, J = 8.61 Hz, 2H), 7.98 (d, J = 8.6 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.50 (t, J = 8.1 Hz, 1H), 6.74 (s, 1H), 4.90 (s, 2H), 4.19 (t, J = 6.4 Hz, 2H), 3.45 (s, 1H), 2.06 – 1.79 (m, 2H), 1.53 (dd, J = 9.2, 5.0 Hz, 2H), 1.46 (dt, J = 15.0, 7.4 Hz, 2H), 0.98 (t, J = 7.2 Hz, 3H), **^{19}F NMR (377 MHz, $CDCl_3$)**: δ -62.95, **^{13}C NMR (101 MHz, $CDCl_3$)**: δ 165.30, 163.13, 162.93, 159.90, 138.65, 134.17, 129.35, 127.77,

126.26, 121.02, 117.65, 116.34, 114.52, 98.63, 69.26, 65.81, 55.91, 29.01, 28.67, 22.88, 14.47, **IR (Neat, ν/cm^{-1}):** 3339, 2933, 2867, 1652, 1602, 1535, 1511, 1461, 1412, 1339, 1251, 1171, 1115, 1033. **HRMS (ESI):** Calc. for $\text{C}_{23}\text{H}_{26}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$: 395.1971; Found: 395.1968.

N-(2-(hydroxymethyl)-4-(pentyloxy)quinolin-8-yl)-3,5 bis(trifluoromethyl)benzamide ($\text{C}_{24}\text{H}_{22}\text{F}_6\text{N}_2\text{O}_3$) 1c: The crude product was purified by column chromatography over silica gel (5% EtOAc in petroleum ether) to furnish **7c** as a white crystalline solid (Yield: 65%); **^1H NMR (400 MHz, CDCl_3):** δ 10.67 (s, 1H), 8.85 (d, $J = 7.7$ Hz, 1H), 7.98 (d, $J = 8.6$ Hz, 2H), 7.86 (d, $J = 8.4$ Hz, 1H), 7.95 (d, $J = 8.4$ Hz, 1H), 7.55 (t, $J = 8.1$ Hz, 2H), 6.86 (s, 1H), 4.93 (s, 2H), 4.23 (t, $J = 6.4$ Hz, 2H), 3.89 (s, 3H), 3.45 (s, 1H), 2.09 – 1.84 (m, 2H), 1.59 (d, $J = 6.8$ Hz, 1H), 1.51 (d, $J = 7.5$ Hz, 1H), 1.45 (dt, $J = 14.4, 7.1$ Hz, 2H), 0.98 (t, $J = 7.2$ Hz, 3H), **^{13}C NMR (101 MHz, CDCl_3):** δ 165.30, 163.13, 162.93, 159.90, 138.65, 134.17, 129.35, 127.77, 126.26, 121.02, 117.65, 116.34, 114.52, 98.63, 69.26, 65.81, 55.91, 29.01, 28.67, 22.88, 14.47, **IR (Neat, ν/cm^{-1}):** 3339, 2961, 2872, 1667, 1623, 1599, 1551, 1469, 1424, 1362, 1279, 1248, 1184, 1134, 1058. **HRMS (ESI):** Calc. for $\text{C}_{24}\text{H}_{22}\text{F}_6\text{N}_2\text{O}_3$ $[\text{M}+\text{H}]^+$: 501.1613; Found: 501.1616.

3.2.4. Single crystal X-ray diffraction (SCXRD) studies:

The needle-shaped crystals of compound **1c** were grown in the solvent Acetonitrile at room temperature. The single-crystal X-ray data of compound **1c** was recorded at 296 K on a Bruker KAPPA APEX II CCD Duo diffractometer (operated at 1500 W power: 50 kV, 30 mA) using graphite-mono chromated Mo $\text{K}\alpha$ radiation ($\lambda = 0.71073$ Å). The crystal was kept on a nylon Cryo-Loops (Hampton Research) with Paraton-N (Hampton Research). The data reduction and integration were computed with SAINT software.²⁸ A correction on multi-scan absorption was employed on the collected reflections. The structure solving was achieved by the direct method using SHELXTL²⁹ and was further refined on F^2 by full-matrix least-squares technique using the SHELXL-97³⁰ program package within the WINGX³¹ program. All the non-hydrogen atoms were refined anisotropically. All hydrogen atoms were located in successive difference Fourier maps, and they were treated as riding atoms using SHELXL default parameters. The structures were examined using the *Adsym* subroutine of PLATON³² to ensure that no additional symmetry could be applied to the models.

Details of crystal of 1c:

Empirical formula	$C_{23}H_{26}N_2O_4$	
Formula weight	394.46	
Temperature	296 K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	$a = 8.370(2)$ Å	$\alpha = 107.354(9)^\circ$.
	$b = 10.022(3)$ Å	$\beta = 97.815(9)^\circ$.
	$c = 12.700(4)$ Å	$\gamma = 98.579(9)^\circ$.
Volume	987.0(5) Å ³	
Z	2	
Density (calculated)	1.327 Mg/m ³	
Absorption coefficient	0.091 mm ⁻¹	
F (000)	420	
Crystal size	0.140 x 0.120 x 0.110 mm ³	
Theta range for data collection	2.173 to 25.998°.	
Index ranges	$-9 \leq h \leq 10$, $-12 \leq k \leq 12$, $-15 \leq l \leq 15$	
Reflections collected	27617	
Independent reflections	3829 [R (int) = 0.0604]	
Completeness to theta = 25.242°	98.9 %	
Absorption correction	Semi-empirical from equivalents	

Max. and min. transmission	0.97 and 0.94
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3829 / 7 / 268
Goodness-of-fit on F^2	1.043
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0447$, $wR_2 = 0.0969$
R indices (all data)	$R_1 = 0.0682$, $wR_2 = 0.1082$
Extinction coefficient	n/a
largest diff. peak and hole	0.218 and $-0.221 \text{ e.}\text{\AA}^{-3}$

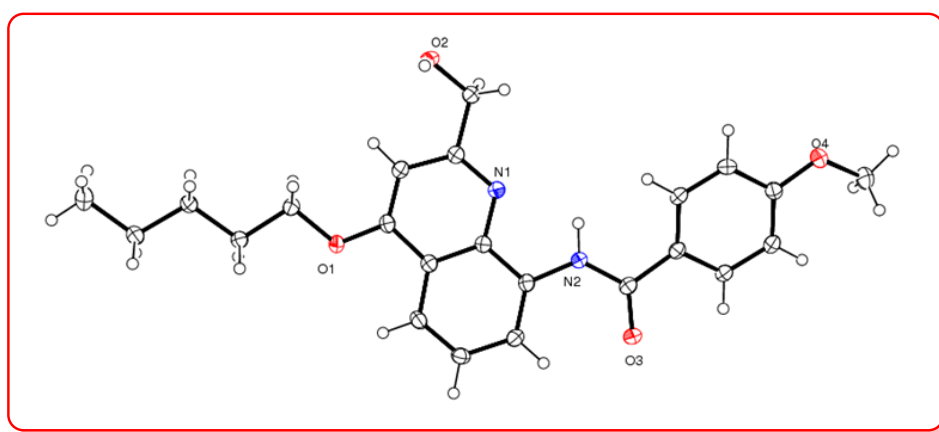


Figure 3.2. ORTEP image of **1c**.

Table 3. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **1c.**

$U(\text{eq})$ is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	$U(\text{eq})$
C(1)	-905(3)	9091(2)	11935(2)	42(1)

C(2)	-891(2)	7742(2)	10989(2)	29(1)
C(3)	-104(2)	8046(2)	10052(1)	25(1)
C(4)	-142(2)	6706(2)	9081(1)	24(1)
C(5)	619(2)	7064(2)	8165(1)	23(1)
C(6)	1436(2)	5813(2)	6472(1)	21(1)
C(7)	1691(2)	4480(2)	5790(1)	21(1)
C(8)	1259(2)	3190(2)	6019(1)	25(1)
C(9)	1577(2)	1962(2)	5342(2)	27(1)
C(10)	2307(2)	1940(2)	4404(2)	26(1)
C(11)	2745(2)	3179(2)	4162(1)	21(1)
C(12)	2467(2)	4495(2)	4871(1)	20(1)
C(13)	2776(2)	6927(2)	5313(1)	21(1)
C(14)	1990(2)	7030(2)	6242(1)	23(1)
C(15)	3403(2)	8271(2)	5052(2)	26(1)
C(16)	3859(2)	2236(2)	2424(1)	22(1)
C(17)	4566(2)	2672(2)	1534(1)	21(1)
C(18)	4465(2)	1627(2)	507(1)	24(1)
C(19)	5156(2)	1924(2)	-350(2)	25(1)
C(20)	6003(2)	3301(2)	-168(1)	23(1)
C(21)	6111(2)	4355(2)	858(1)	24(1)
C(22)	5392(2)	4052(2)	1692(1)	22(1)

C(23)	6527(3)	2728(2)	-2046(2)	39(1)
N(1)	3002(2)	5714(1)	4629(1)	21(1)
N(2)	3461(2)	3282(2)	3242(1)	23(1)
O(1)	658(1)	5740(1)	7326(1)	25(1)
O(2)	3810(2)	9520(1)	6012(1)	32(1)
O(3)	3649(2)	978(1)	2403(1)	31(1)
O(4)	6771(2)	3718(1)	-931(1)	31(1)

Table 4. Bond lengths [Å] and angles [°] for 1c:

C(1)-C(2)	1.520(3)
C(2)-C(3)	1.519(2)
C(3)-C(4)	1.521(2)
C(4)-C(5)	1.508(2)
C(5)-O(1)	1.441(2)
C(6)-O(1)	1.353(2)
C(6)-C(14)	1.367(2)
C(6)-C(7)	1.424(2)
C(7)-C(12)	1.414(2)
C(7)-C(8)	1.414(2)
C(8)-C(9)	1.362(2)
C(9)-C(10)	1.407(2)

C(10)-C(11)	1.376(2)
C(11)-N(2)	1.406(2)
C(11)-C(12)	1.429(2)
C(12)-N(1)	1.374(2)
C(13)-N(1)	1.323(2)
C(13)-C(14)	1.412(2)
C(13)-C(15)	1.517(2)
C(15)-O(2)	1.420(2)
C(16)-O(3)	1.238(2)
C(16)-N(2)	1.356(2)
C(16)-C(17)	1.490(2)
C(17)-C(18)	1.390(2)
C(17)-C(22)	1.394(2)
C(18)-C(19)	1.386(2)
C(19)-C(20)	1.390(2)
C(20)-O(4)	1.364(2)
C(20)-C(21)	1.393(2)
C(21)-C(22)	1.373(2)
C(23)-O(4)	1.432(2)
C(3)-C(2)-C(1)	112.84(16)

C(2)-C(3)-C(4)	113.22(15)
C(5)-C(4)-C(3)	111.22(14)
O(1)-C(5)-C(4)	107.64(14)
O(1)-C(6)-C(14)	125.75(15)
O(1)-C(6)-C(7)	114.67(14)
C(14)-C(6)-C(7)	119.58(15)
C(12)-C(7)-C(8)	120.06(15)
C(12)-C(7)-C(6)	116.85(15)
C(8)-C(7)-C(6)	123.05(16)
C(9)-C(8)-C(7)	119.65(16)
C(8)-C(9)-C(10)	121.42(16)
C(11)-C(10)-C(9)	120.22(16)
C(10)-C(11)-N(2)	125.19(15)
C(10)-C(11)-C(12)	119.90(16)
N(2)-C(11)-C(12)	114.91(14)
N(1)-C(12)-C(7)	123.52(15)
N(1)-C(12)-C(11)	117.76(15)
C(7)-C(12)-C(11)	118.70(15)
N(1)-C(13)-C(14)	124.17(15)
N(1)-C(13)-C(15)	116.47(15)
C(14)-C(13)-C(15)	119.36(15)

C(6)-C(14)-C(13)	118.93(16)
O(2)-C(15)-C(13)	113.45(14)
O(3)-C(16)-N(2)	122.55(16)
O(3)-C(16)-C(17)	120.95(15)
N(2)-C(16)-C(17)	116.51(15)
C(18)-C(17)-C(22)	118.23(16)
C(18)-C(17)-C(16)	118.05(15)
C(22)-C(17)-C(16)	123.64(15)
C(19)-C(18)-C(17)	121.94(16)
C(18)-C(19)-C(20)	118.94(16)
O(4)-C(20)-C(19)	124.54(15)
O(4)-C(20)-C(21)	115.89(15)
C(19)-C(20)-C(21)	119.56(16)
C(22)-C(21)-C(20)	120.86(16)
C(21)-C(22)-C(17)	120.45(16)
C(13)-N(1)-C(12)	116.90(14)
C(16)-N(2)-C(11)	128.92(15)
C(6)-O(1)-C(5)	117.51(13)
C(20)-O(4)-C(23)	118.09(14)

Symmetry transformations used to generate equivalent atoms:

Table 5. Anisotropic displacement parameters (Å² × 10³) for 1c:

The anisotropic displacement factor exponent takes the form: $-2\pi^2 [h^2 a^{*2} U^{11} + 2 h k a^* b^* U^{12}]$

	U ¹¹	U ²²	U ³³	U ²³	U ¹³	U ¹²
C(1)	54(1)	40(1)	29(1)	6(1)	17(1)	8(1)
C(2)	35(1)	29(1)	28(1)	13(1)	12(1)	8(1)
C(3)	26(1)	24(1)	26(1)	8(1)	9(1)	3(1)
C(4)	25(1)	22(1)	25(1)	8(1)	7(1)	5(1)
C(5)	24(1)	23(1)	23(1)	6(1)	6(1)	6(1)
C(6)	18(1)	26(1)	16(1)	7(1)	1(1)	4(1)
C(7)	19(1)	23(1)	18(1)	6(1)	0(1)	2(1)
C(8)	27(1)	28(1)	21(1)	9(1)	6(1)	1(1)
C(9)	33(1)	22(1)	27(1)	9(1)	6(1)	0(1)
C(10)	31(1)	21(1)	25(1)	5(1)	6(1)	6(1)
C(11)	21(1)	24(1)	18(1)	6(1)	2(1)	3(1)
C(12)	19(1)	22(1)	18(1)	6(1)	-1(1)	3(1)
C(13)	20(1)	24(1)	19(1)	7(1)	0(1)	5(1)
C(14)	24(1)	22(1)	21(1)	5(1)	3(1)	6(1)
C(15)	33(1)	24(1)	23(1)	9(1)	7(1)	6(1)

C(16)	22(1)	21(1)	21(1)	4(1)	0(1)	4(1)
C(17)	19(1)	22(1)	22(1)	6(1)	2(1)	7(1)
C(18)	27(1)	20(1)	26(1)	5(1)	6(1)	5(1)
C(19)	28(1)	24(1)	23(1)	4(1)	5(1)	8(1)
C(20)	23(1)	27(1)	24(1)	12(1)	5(1)	10(1)
C(21)	24(1)	19(1)	28(1)	9(1)	2(1)	3(1)
C(22)	25(1)	20(1)	21(1)	4(1)	2(1)	7(1)
C(23)	53(1)	41(1)	27(1)	12(1)	17(1)	13(1)
N(1)	21(1)	21(1)	20(1)	6(1)	2(1)	4(1)
N(2)	27(1)	19(1)	22(1)	6(1)	7(1)	5(1)
O(1)	30(1)	23(1)	20(1)	5(1)	9(1)	4(1)
O(2)	46(1)	20(1)	31(1)	6(1)	9(1)	7(1)
O(3)	44(1)	19(1)	31(1)	7(1)	14(1)	6(1)
O(4)	39(1)	30(1)	27(1)	12(1)	13(1)	7(1)

Table 6. Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for 1c:

	x	y	z	U(eq)
H(1A)	206	9582	12277	63
H(1B)	-1451	8843	12488	63

H(1C)	-1478	9700	11638	63
H(2A)	-294	7139	11293	35
H(2B)	-2014	7222	10678	35
H(3A)	1031	8535	10359	30
H(3B)	-675	8679	9769	30
H(4A)	453	6079	9354	28
H(4B)	-1273	6204	8777	28
H(5A)	-28	7617	7835	28
H(5B)	1727	7624	8470	28
H(8)	761	3181	6628	30
H(9)	1306	1119	5503	33
H(10)	2494	1085	3945	31
H(14)	1852	7911	6689	27
H(15A)	4371	8153	4720	31
H(15B)	2566	8396	4501	31
H(18)	3919	702	392	29
H(19)	5053	1214	-1036	30
H(21)	6678	5276	980	29
H(22)	5457	4773	2366	27
H(23A)	6964	1903	-2018	58
H(23B)	7082	3173	-2504	58

H(23C)	5371	2444	-2360	58
H(2)	4605	9470	6443	49
H(2N)	3630(20)	4188(17)	3217(16)	34(5)

3.2.5. Theoretical studies:³³⁻³⁶

Based on the Hill co-efficient value of $n \sim 1$ obtained from dose-response studies of compounds **1a-1c**, geometry optimization of the highest active compound **1a** and [**1a**+ K^+] were performed. During the geometry optimization instead of pentyl linker, a methyl linker was used in order to minimize the optimization timing. Initially, the most probable conformers of **1a** and [**1a**+ K^+] were obtained by using the CONFLEX-8 software program³⁴ and subsequently, their geometry optimization were carried out by using the Gaussian 09 program package.³³ The geometry optimized [**1a**+ K^+] and further calculations confirmed the formation of electrostatic interactions between the potassium cation, the nitrogen atom of the quinoline ring and the oxygen atom of the –OH group and the –CONH group. The binding energy of the geometrically optimized [**1a**+ K^+] complex was calculated to be –35.12 kcal/mol.

To visualize the different conformers of compound **1a** and [**1a**+ K^+] complex, several geometries of the free compounds and complex were obtained by using the CONFLEX-8 software package using MMFF94S force field.³⁴ The calculation provided the 14 possible conformers of compound **1a**. The Boltzmann populations of the two highest populated conformations are **Conf-1** with 49.51%, **Conf-2** with 48.71%. (Figure 3.3). For the [**1a**+ K^+] complex, 38 possible conformers were obtained. The Boltzmann populations of the four highest populated conformations are **Conf-1** with 44.09%, **Conf-2** with 42.55%, **Conf-3** with 4.83%, **Conf-4** with 4.64%. (Figure 3.4)

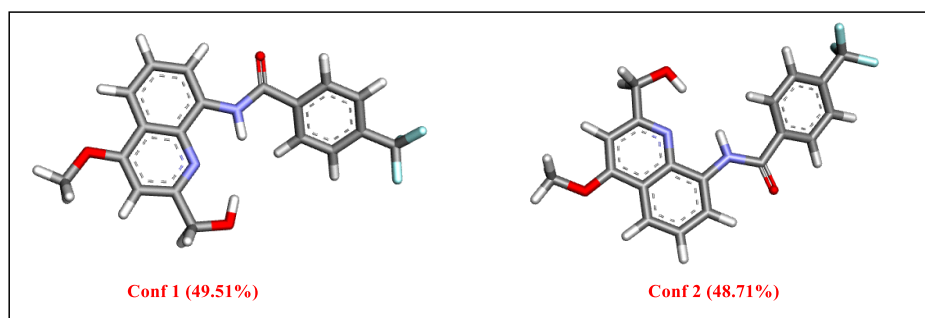


Figure 3.3. Initial geometries **Conf-1** and **Conf-2** for **1a**, optimized by CONFLEX 8 software using MMFF94s force field.

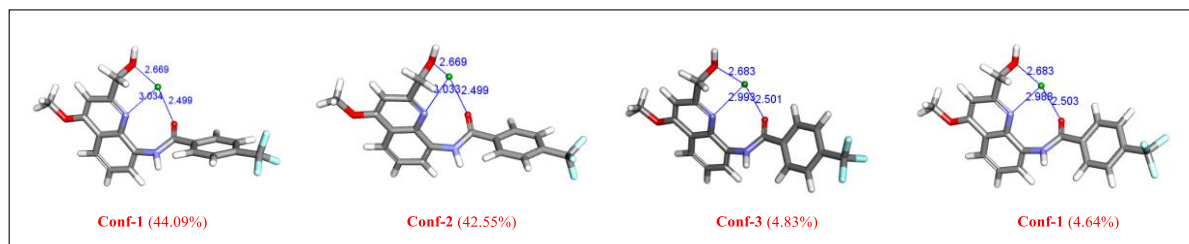


Figure 3.4. Initial geometries **Conf-1** to **Conf-4** for $[1a+K^+]$, optimized by CONFLEX 8 software using MMFF94s force field.

The green ball represents the K^+ cation which is seen to be bound to the nitrogen atom of the quinoline ring and the oxygen atoms of the hydroxyl and the amide functional groups

The Gaussian 09 program was used to calculate the zero-point energy (ZPE) and basis set superposition error (BSSE) corrected bonding energy of $[1a+K^+]$ which was used for the calculation of binding energy (BE) using the following equations. Geometry-optimized energetically more stable **Conf-1** of structure **1a** and **Conf-1** of $[1a+K^+]$ complex were used during the binding energy (BE) calculation.

$$BE = [HF_{[1a+K^+]} + ZPE_{[1a+K^+]} + BSSE_{[1a+K^+]}] - [HF_{1a} + ZPE_{1a}] - [HF_{K^+}] \quad (\text{Equation 1})$$

where, $HF_{[1a+K^+]}$ = electronic energy of $[1a+K^+]$ complex, $ZPE_{[1a+K^+]}$ = zero-point energy of $[1a+K^+]$ complex, $BSSE_{[1a+K^+]}$ = BSSE of $[1a+K^+]$ complex, HF_{1a} = electronic energy of the receptor **1a**, ZPE_{1a} = zero-point energy of the receptor **1a**, HF_{K^+} = electronic energy of cation K^+ .

Table 7. The electronic energy (HF), zero point energy (ZPE), basis set superposition error (BSSE) corrected energy (in Hartree unit) for all structures and complexes are calculated at the DFT B3LYP/6-311++G(d,p) level of theory.

Parameters	Energy
$HF_{[1a+K^+]}$ (in Hartree)	-1967.997917
$ZPE_{[1a+K^+]}$ (in Hartree)	0.313535

BSSE _[1a+K⁺] (in Hartree)	0.000863081
HF _{1a} (in Hartree)	-1368.178681
ZPE _{1a} (in Hartree)	0.312179
HF _{K⁺} (in Hartree)	-599.7610546
BE (in Hartree)	-0.055962919
BE (in kcal/mol)	-35.11689929

Table 8. Atomic coordinates of 1a after geometry optimization by Gaussian 09 program using B3LYP functional and 6-311++G(d,p) basis set.

Charge = 0

Multiplicity = 1

Atom number	Atom type	X	Y	Z
1	O	0.777067	-3.08794	-0.04915
2	O	-6.17531	0.173444	0.023131
3	N	-2.09248	0.924893	-0.06554
4	O	-0.95255	3.329559	-0.17865
5	N	-0.29445	-1.06483	0.029853
6	F	7.009534	-0.12349	0.161628
7	C	-2.57986	-0.35172	-0.0017
8	C	-3.9687	-0.64121	0.026576
9	F	6.132509	1.688934	0.986475
10	C	0.818001	-1.86849	0.006735
11	C	-2.94513	1.924578	-0.0953
12	C	2.134155	-1.13453	0.038455
13	C	-4.86258	0.477707	-0.00738
14	C	-1.64419	-1.43546	0.039522
15	C	-4.41439	-1.98059	0.086295
16	C	-2.10542	-2.74036	0.095112
17	C	3.248335	-1.80028	-0.48392

18	C	-3.48936	-2.99911	0.117528
19	C	3.549815	0.749511	0.601826
20	C	2.298353	0.140673	0.589975
21	C	-4.34881	1.754948	-0.06641
22	C	4.49727	-1.19419	-0.48278
23	C	4.647981	0.083338	0.060791
24	F	6.197821	1.392062	-1.16436
25	C	-7.13169	1.232574	-0.00822
26	C	-2.36127	3.323341	-0.17775
27	C	5.993537	0.75715	0.018017
28	H	-0.69027	2.398337	-0.2317
29	H	-0.16896	-0.06281	-0.024
30	H	-5.47613	-2.18471	0.105749
31	H	-1.3937	-3.55099	0.117938
32	H	3.115946	-2.79797	-0.88267
33	H	-3.82319	-4.02939	0.161466
34	H	3.671226	1.731121	1.041493
35	H	1.465552	0.661435	1.048643
36	H	-4.98583	2.628611	-0.09403
37	H	5.354688	-1.71386	-0.89189
38	H	-8.10589	0.749603	0.026239
39	H	-7.04304	1.813951	-0.93068
40	H	-7.01762	1.890177	0.858601
41	H	-2.72419	3.913336	0.673846
42	H	-2.73893	3.807362	-1.08976

Table 9. Atomic coordinates of [1a+K⁺] after geometry optimization by Gaussian 09 program using B3LYP functional and 6-311++G(d,p) basis set.

Charge = 1

Multiplicity = 1

Atom number	Atom type	X	Y	Z
1	C	3.078125	-3.40289	0.524107
2	C	1.724605	-3.01806	0.465186
3	C	1.359869	-1.73624	0.112247
4	C	2.359575	-0.76254	-0.19809

5	C	3.724809	-1.1776	-0.15191
6	C	4.066492	-2.4991	0.216869
7	N	1.985442	0.501453	-0.55527
8	C	2.93436	1.351458	-0.91102
9	C	4.312295	1.04826	-0.91682
10	C	4.717494	-0.21639	-0.52818
11	N	-0.02871	-1.44181	-0.04216
12	C	2.474076	2.737005	-1.30555
13	O	2.162571	3.473652	-0.09471
14	O	5.98588	-0.63853	-0.47897
15	C	7.043585	0.245447	-0.87747
16	C	-0.75047	-0.5687	0.70716
17	O	-0.2574	0.073971	1.638514
18	C	-2.19844	-0.44002	0.35235
19	C	-2.66272	-0.57682	-0.961
20	C	-4.01667	-0.42906	-1.24525
21	C	-4.91343	-0.15462	-0.21411
22	C	-4.45899	-0.01406	1.098205
23	C	-3.10508	-0.14739	1.377397
24	H	-0.54607	-2.05281	-0.65775
25	H	2.036124	4.40091	-0.3253
26	C	-6.37715	0.054731	-0.52283
27	F	-6.75965	-0.61025	-1.63063
28	F	-6.64579	1.365443	-0.73294
29	F	-7.16599	-0.35222	0.491402
30	H	3.331655	-4.41734	0.806106
31	H	0.950193	-3.7411	0.695663
32	H	5.109665	-2.78355	0.242052
33	H	5.02287	1.803057	-1.22216
34	H	1.579131	2.659726	-1.92796
35	H	3.259159	3.258079	-1.85828
36	H	7.959283	-0.3291	-0.76385
37	H	6.923292	0.545351	-1.9214
38	H	7.078094	1.125496	-0.23014
39	H	-1.97205	-0.76161	-1.77585
40	H	-4.37286	-0.52644	-2.26277

41	H	-5.16201	0.191067	1.895639
42	H	-2.74139	-0.04001	2.391455
43	K	1.262538	2.031097	1.963139

3.2.6. Ion transport studies:

3.2.6.1. Buffer and stock solution preparation: HEPES buffer (10 mM) was prepared by dissolving solid HEPES in autoclaved water, NaCl (100 mM) was added and followed by adjustment of pH = 7.0 by adding NaOH solution. Stock solutions of all compounds were prepared in either HPLC grade DMSO or ACN.

3.2.6.2. Preparation of EYPC-LUVs $\Rightarrow$ HPTS: Vesicles were prepared according to the reported protocol.^{37, 38}

3.2.6.3. Ion transport activity: 1975 μ L of HEPES buffer (10 mM HEPES, 100 mM NaCl, pH = 7.0) was taken in a clean fluorescence cuvette followed by the addition of 25 μ L of EYPC-LUVs $\Rightarrow$ HPTS in the same cuvette and was placed on the fluorescence instrument (at $t = 0$ s) equipped with a magnetic stirrer. The fluorescence emission intensity of HPTS dye, F_t was monitored at $\lambda_{em} = 510$ nm ($\lambda_{ex} = 450$ nm) with time. After that, a pH gradient between the intra and extra vesicular system was created by adding 20 μ L of 0.5 M NaOH to the same cuvette at $t = 20$ s. All compounds were added at $t = 100$ s in different concentrations and finally, at $t = 300$ s, 25 μ L of 10% Triton X-100 was added to destroy all vesicles which resulted in the destruction of pH gradient (Figure. 3.5), which resulted in saturation in fluorescence emission intensity. The fluorescence intensity was normalized according to Equation 2:

$$I_F = [(F_t - F_0) / (F_\infty - F_0)] \times 100\% \quad (\text{Equation 2})$$

where, F_0 = Fluorescence intensity just before the compound addition (at $t = 0$ s). F_∞ = Fluorescence intensity at saturation after complete leakage (at $t = 340$ s). F_t = Fluorescence intensity at time t . The time of compound addition can be normalized to $t = 0$ s and the time of Triton X-100 addition was normalized to $t = 200$ s.

Fluorescence intensities (F_t) were normalized to fractional emission intensity I_F using Equation 3:

$$Y = Y_{\infty} + (Y_0 - Y_{\infty}) / [1 + (c / EC_{50})^n] \quad (\text{Equation 3})$$

where, Y_0 = Fluorescence intensity just before the channel addition (at 0 s). Y_{∞} = Fluorescence intensity with excess channel concentration, c = Concentration of channel-forming molecule. (Figure 3.5)

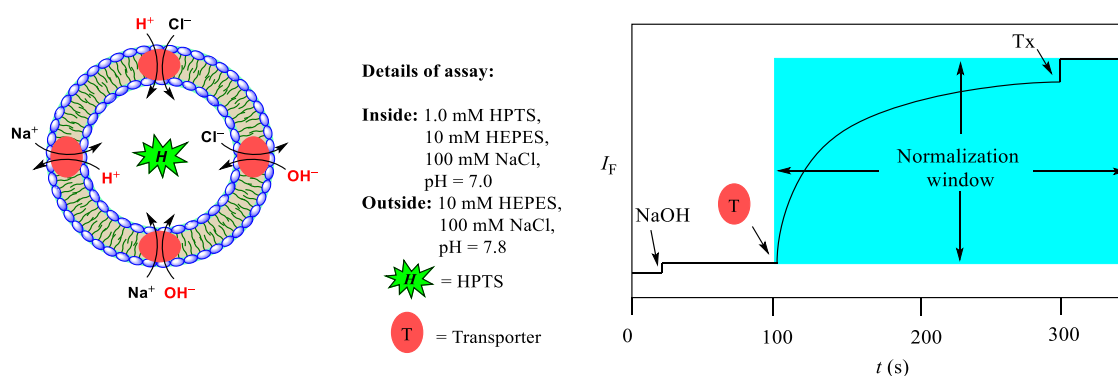


Figure 3.5. Representations and illustration of ion transport activity across EYPC-LUVs containing HPTS.

On successfully synthesizing the desired derivatives **1a-1c**, investigation of their ion transport activities at 40 μ M revealed that compound **1a** possessed the highest transport efficiency among the entire series, followed by **1c** and **1b**. (Figure 3.6)

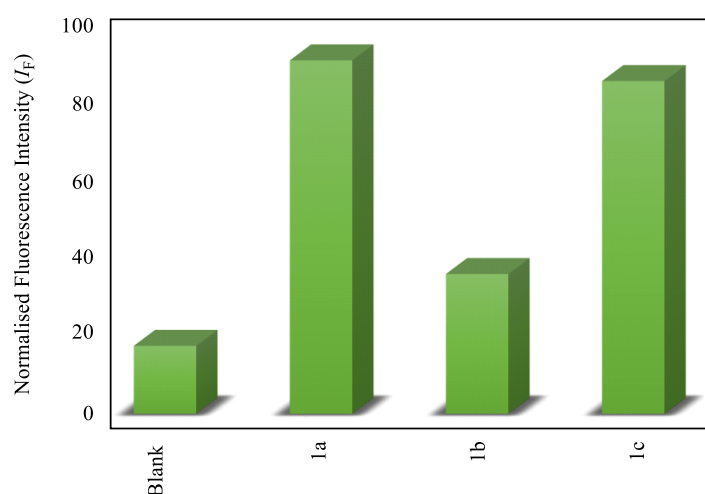


Figure 3.6. Bar diagram representing comparison of ion transport activities of **1a-1c** at 40 μ M across EYPC-LUVs containing HPTS.

Ion transport kinetics was studied at different concentrations for each compound. The change of HPTS emission intensity in this process was monitored with time. The concentration profile data were analyzed by the Hill Equation (Equation 3) to get the (EC_{50}) and Hill coefficient (n) values. EC_{50} value of $3.86 \mu\text{M}$ was calculated by the dose-dependent transport study of **1a**. The n value was found out to be 2.28, which implied that an active dimeric transport complex is formed by two molecules of transporter **1a**. (Figure 3.7).

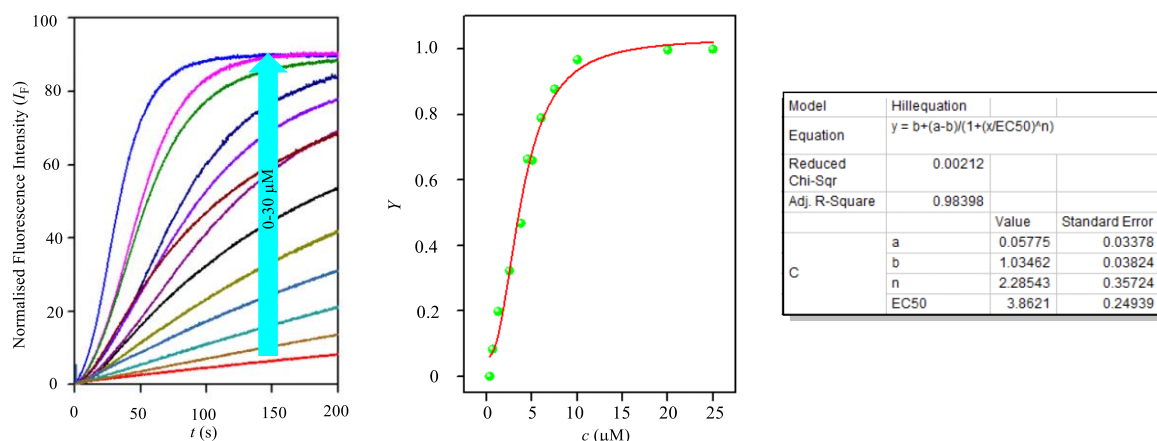


Figure 3.7. Dose-response curves and Hill analysis of compound **1a** across EYPC–LUVs→HPTS at $t=190$ s.

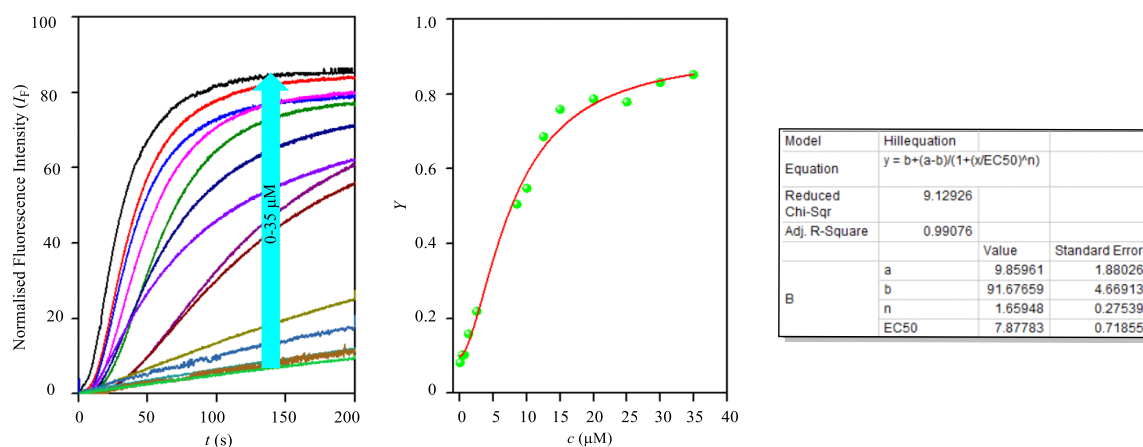


Figure 3.8. Dose-response curves and Hill analysis of compound **1b** across EYPC–LUVs→HPTS at $t=190$ s.

Unfortunately, the precipitation issue in case of higher concentrations of compound **1c** became a hindrance for investigating its dose-response activity across EYPC–LUVs→HPTS.

3.2.7. Determination of ion selectivity by HPTS assay:³⁹

3.2.7.1. Buffer and stock solution preparation: HEPES buffers with all salts were prepared by dissolving solid HEPES (10 mM) followed by the addition of appropriate salt (100

mM) in autoclaved water and adjustment of pH (pH = 7.0) was done by dropwise addition of NaOH solution.

3.2.7.2. Preparation of EYPC-LUVs $\supset$ HPTS for cation selectivity:

EYPC-LUVs $\supset$ HPTS (~ 5.0 mM EYPC, inside: 1 mM HPTS, 10 mM HEPES, 100 mM MCl, pH = 7.0 and outside: 10 mM HEPES, 100 mM MCl, pH = 7.0 where $M^+ = K^+, Na^+, Rb^+, Cs^+$ and Li^+) were prepared following reported protocol. (Figure 3.9)³⁷⁻³⁹

3.2.7.3. Preparation of EYPC-LUVs $\supset$ HPTS for anion selectivity:⁴⁰

EYPC-LUVs $\supset$ HPTS (~ 5.0 mM EYPC, inside: 1 mM HPTS, 10 mM HEPES, 100 mM NaX, pH = 7.0 and outside: 10 mM HEPES, 100 mM NaX, pH = 7.0; where, $X^- = Cl^-, Br^-, ClO_4^-$, NO_3^- and I^-) were prepared following the reported protocol. (Figure 3.9)

3.2.7.4. Anion and cation selectivity assays: In a clean fluorescence cuvette, 1975 μ L of buffer solution (100 mM NaX or MCl, 10 mM HEPES, pH 7.0;), 25 μ L of above-prepared vesicles solution were taken and fluorescence cuvette was placed in a fluorescence instrument equipped with a magnetic stirrer at $t = 0$ s. The fluorescence emission intensity (I_t) of the HPTS dye was measured at $\lambda_{em} = 510$ nm (where $\lambda_{ex} = 450$ nm) for the time course of 0 to 350 s. At $t = 50$ s, 20 μ L of 0.5 M NaOH solution was added to the same cuvette to generate a pH gradient (Δ pH = 0.8) between the intra and extra vesicular medium which leads to the increase in the fluorescence intensity. At 100 s, 5 μ M of transporter molecule **1a** was added. Finally, Triton X-100 (25 μ L of 10% solution in water) was added at $t = 300$ s to destroy all the vesicles for destructing the pH gradient. Fluorescence intensities (F_t) were normalized to fractional emission intensity I_F using Equation 2.

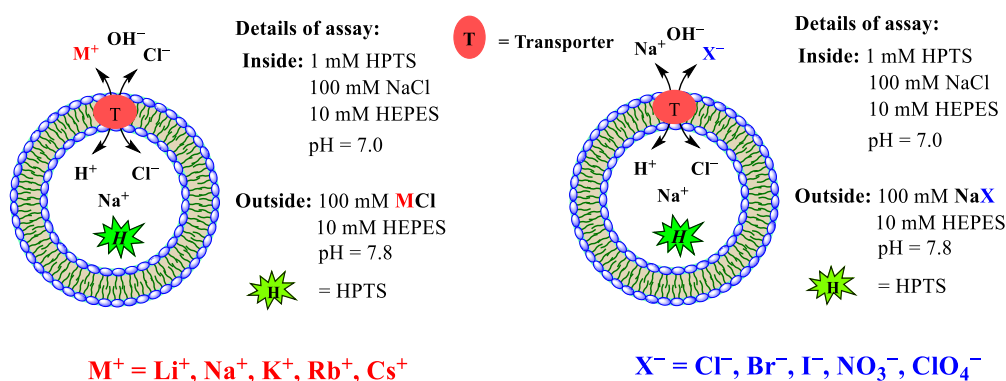


Figure 3.9. Representations of fluorescence-based cation selectivity (A) and anion selectivity (B) assays across EYPC–LUVs $\Rightarrow$ HPTS.

The commendable transport activity of **1a** encouraged us to assess its ion transport mechanism and ion selectivity. The transmembrane ion transport activity of **1a** was scrutinized by employing EYPC–LUVs $\Rightarrow$ HPTS by adjusting the extravesicular ionic buffers in order to determine which cations and anions were favored for transport. Neither the H^+/X^- symport nor the X^-/OH^- antiport is favored throughout the transport process, as seen by the small differences in the transport rate of **1a** when multiple exogenous anions are present. (Figure 3.10B) When the extra-vesicular cationic buffers were varied to determine the cation selectivity of **1a**, a considerable change in the transport rate of **1a** was observed for each MCl salt used (where, M = Li^+ , Cs^+ , Rb^+ , K^+ and Na^+) This gave a direct insight into the involvement of various alkali metal cations in the transport process exhibiting the sequence $K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$ (Figure 3.10A), which follows the Hofmeister series. Recent modeling studies have demonstrated that the underlying mechanism of the Hofmeister series is a difference in solvation energy between the ions and the surrounding water molecules. More recently, a quantum study in chemistry reveals that the Hofmeister series has an electrostatic basis.

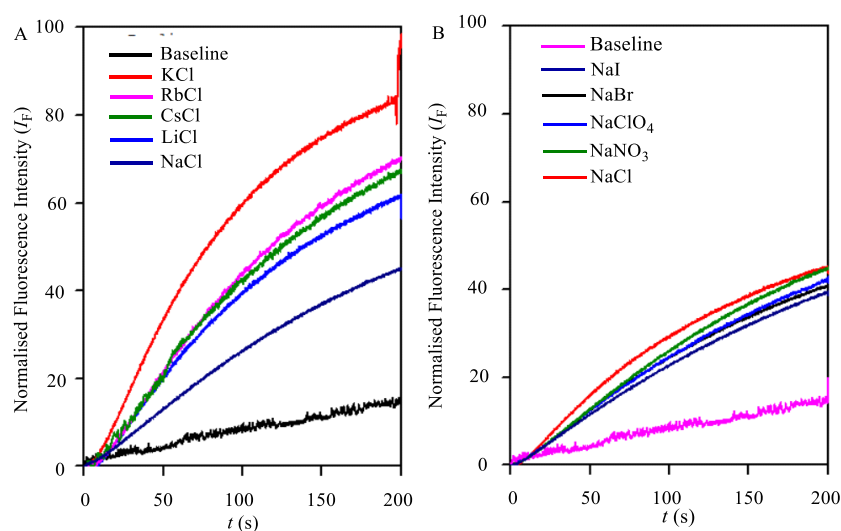


Figure 3.10. (A) Comparative ion transport activity of **1a** (5 μ M) across EYPC-LUVs $\Rightarrow$ HPTS by varying extravesicular cations. (B) Comparative ion transport activity of **1a** (5 μ M) across EYPC-LUVs $\Rightarrow$ HPTS by varying extravesicular anions.

3.2.8. Ion transport studies in the presence of KCl buffer:

3.2.8.1. Buffer and stock solution preparation: 10 mM HEPES buffer (10 mM) was prepared by dissolving solid HEPES in autoclaved water, 100 mM KCl was added and followed by adjustment of pH = 7.0 by adding NaOH solution. Stock solutions of all compounds were prepared in HPLC grade DMSO.

3.2.8.2. Preparation of EYPC–LUVs $\Rightarrow$ HPTS: Vesicles were prepared according to the reported protocol.⁴¹

3.2.8.3. Ion transport activity: Ion transport activity was checked using the same method as NaCl buffer, following the same equation (Equation 2) for normalisation of data.

Remarkable selectivity for K^+ ion inspired us to evaluate the ion transport activity of the compounds across the lipid bilayer membrane in the presence of KCl buffer. Interestingly, an EC_{50} value of 1.34 μ M, calculated by Equation 3, which was almost half of that in the presence of NaCl buffer, further confirmed the outcomes of the selectivity studies. However, a Hill-coefficient value (n) of approximately 1 for both **1a** and **1b** indicated that only one molecule is sufficient to form the active transport complex with the potassium cation. (Figure 3.11 and 3.12).

Dose-dependent studies were carried out for compound **1b** in KCl buffer too (Figure 3.12). Unfortunately, precipitation issues happened in case of compound **1c**, as had happened previously.

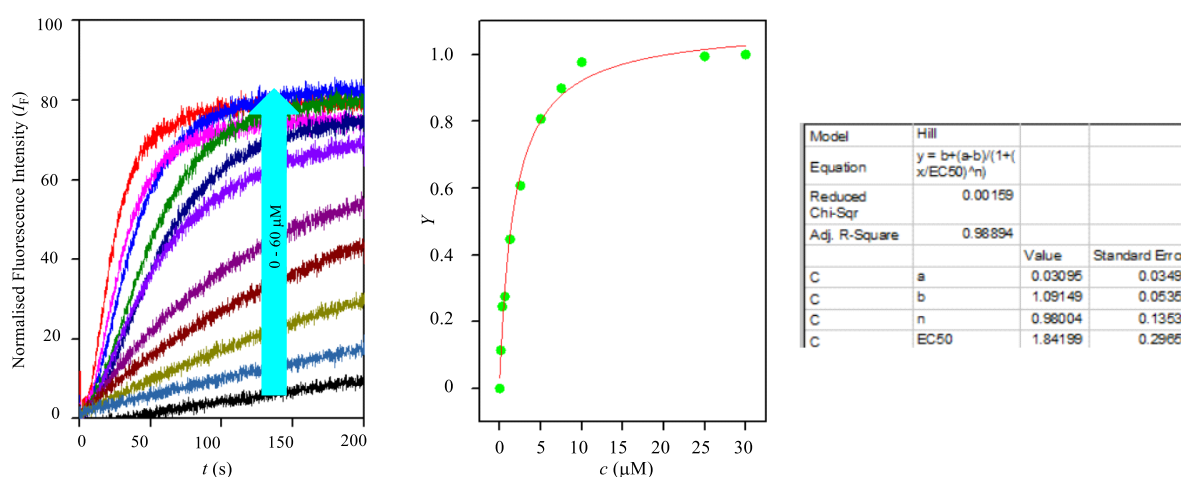


Figure 3.11. Dose–response curve and Hill analysis of compound **1a** across EYPC–LUVs $\Rightarrow$ HPTS in KCl buffer at $t = 190$ s.

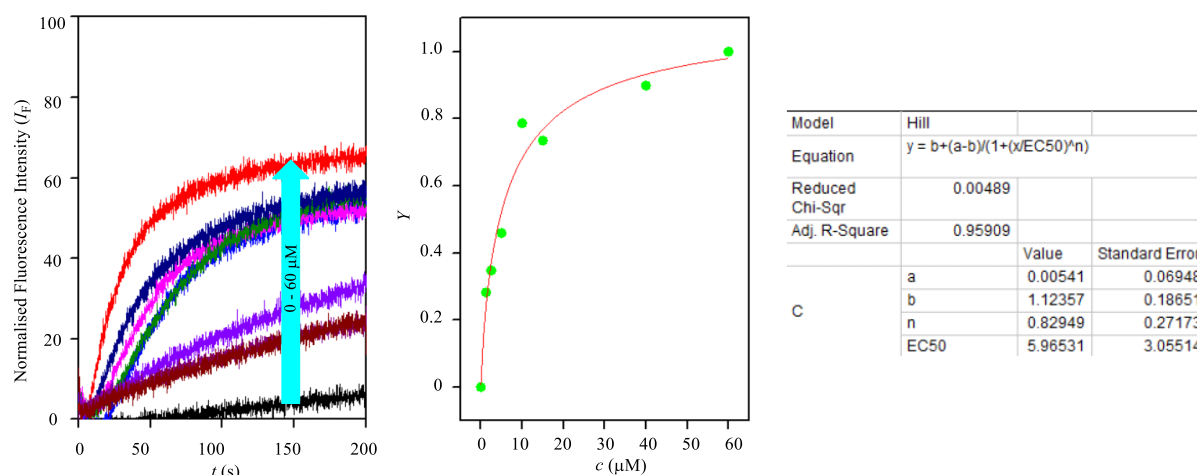


Figure 3.12. Dose–response curve and Hill analysis of compound **1b** across EYPC–LUVs $\Rightarrow$ HPTS in KCl buffer at $t=190$ s.

3.2.9. Chloride transport activity across EYPC–LUVs $\Rightarrow$ Lucigenin vesicles:

3.2.9.1. Preparation of EYPC–LUVs $\Rightarrow$ Lucigenin vesicles:

In a 10 mL clean and dry round bottomed flask, the thin transparent film of egg yolk phosphatidylcholine (EYPC) was formed by drying 1 mL egg yolk phosphatidylcholine (EYPC, 25 mg/mL in $CHCl_3$) with continuous rotation and purging nitrogen. The transparent thin film was kept on high vacuum for 4 hours to remove all trace of $CHCl_3$. Then the transparent thin film was hydrated with 1 mL aqueous $NaNO_3$ (200 mM, 1 mM Lucigenin) with occasional vertexing at 10 minute intervals for 1 hour. The resulting suspension was subjected for freeze and thaw cycles (≥ 15 , liquid nitrogen, 55 °C water bath) and 21 times extrusion through 200 nm pore size polycarbonate membrane. The size exclusion chromatography (using Sephadex G –50) was performed to remove extracellular dye using 200 mM $NaNO_3$ solution as eluent, finally collected vesicles were diluted to 4 mL. Final conditions: ~ 5 mM EYPC; inside: 200 mM $NaNO_3$, 1 mM lucigenin, pH 7.0; outside: 200 mM $NaNO_3$. (Figure 3.13)

3.2.9.2. Ion transport activity by Lucigenin assay:

In clean and dry fluorescence cuvette 1975 μ L 200 mM $NaNO_3$ solution and 25 μ L EYPC–LUVs $\Rightarrow$ Lucigenin was taken. This suspension was placed in a slowly stirring condition in fluorescence instrument equipped with a magnetic stirrer (at $t = 0$ s). The fluorescence intensity

of lucigenin was monitored at $\lambda_{em} = 535$ nm ($\lambda_{ex} = 450$ nm) as a course of time. The chloride gradient was created by addition of 33.3 μ L 2.0 M NaCl at $t = 20$ s between intra and extra vesicular system, followed by addition of 20 μ L transporter molecule **1a** at $t = 100$ s. Finally, vesicles were lysed by addition of Triton X-100 at $t = 300$ s to completely lyse the vesicles. The time-dependent data were normalized to percent change in fluorescence intensity using Equation 2:

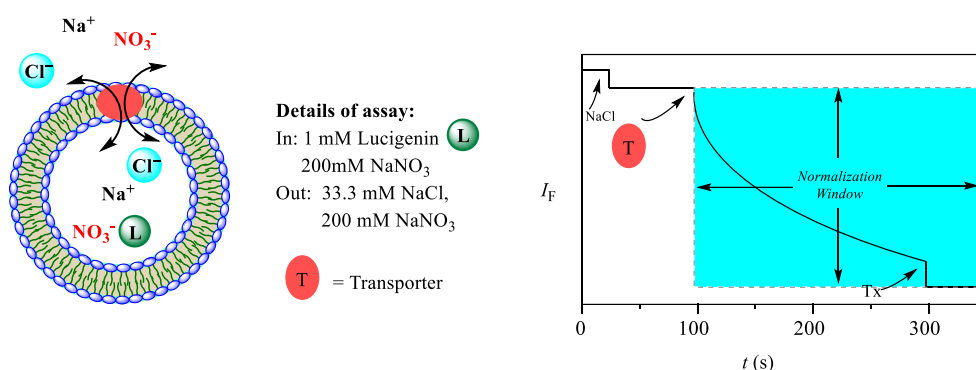


Figure 3.13. Representations of fluorescence-based ion transport activity assay using EYPC-LUVs⊃Lucigenin.

To examine the transport of Cl^- ion by **1a**, ion transport activity across the lipid bilayer membrane was evaluated using EYPC-LUV⊃Lucigenin where lucigenin is a Cl^- sensitive dye. In the presence of Cl^- ions, quenching of fluorescence of the lucigenin dye is observed.⁴² Compound **1a** did not cause any significant fluorescence quenching even at elevated concentrations (Figure 3.14), reinstating the fact that there was no Cl^- influx.

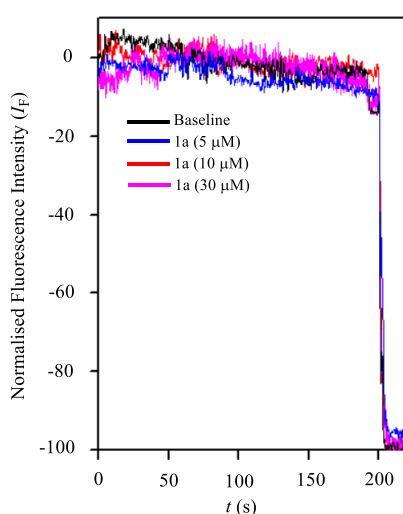


Figure 3.14. Comparison of activities of **1a** across EYPC-LUVs⊃Lucigenin showing no Cl^- influx even at higher concentrations.

3.2.10. Ion transport mechanism study by HPTS assay:¹⁰

3.2.10.1. Preparation of buffer and stock solution for FCCP and Valinomycin assays:

The buffer solution for FCCP assay was prepared using 100 mM of NaCl and 10 mM of HEPES. The pH of the solution was adjusted to 7.0 by the addition of 0.5 M NaOH solution. Then 1 mM of HPTS solution was prepared from the same buffer solution. Buffer solution for valinomycin assay was prepared using autoclaved water of strength 100 mM of KCl and 10 mM of HEPES and the pH was adjusted similarly. The stock solutions of **1a**, FCCP, and valinomycin were prepared using HPLC grade DMSO.

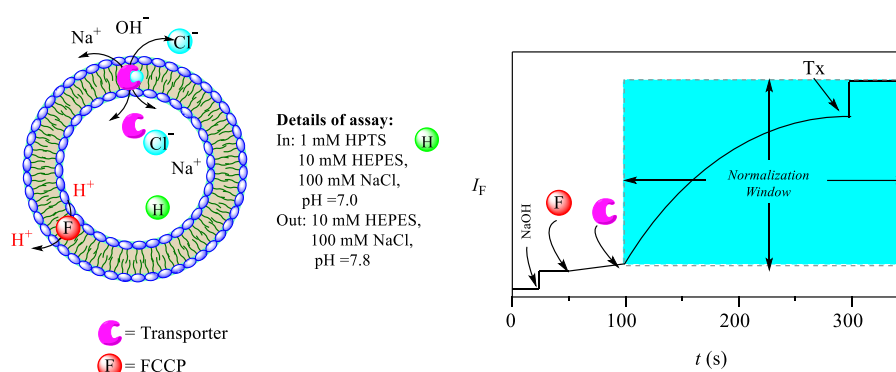


Figure 3.15. Representation of fluorescence-based FCCP assay across EYPC-LUVs $\Rightarrow$ HPTS.

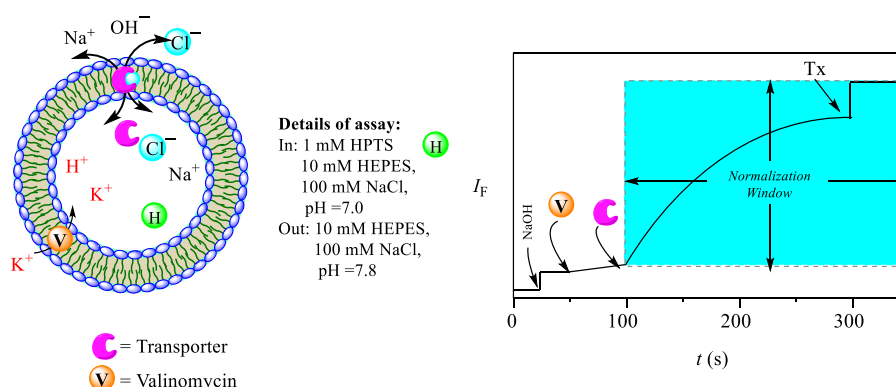


Figure 3.16. Representation of fluorescence-based Valinomycin assay across EYPC-LUVs $\Rightarrow$ HPTS.

3.2.10.2. Preparation of vesicles for FCCP and Valinomycin assay:

The vesicles for the FCCP assay were prepared following the same procedure as mentioned earlier.

3.2.10.3. Ion transport activity in the presence of FCCP: In a clean and dry fluorescence cuvette 1975 μL of HEPES buffer (10 mM HEPES, 100 mM NaCl, pH = 7.0) was taken followed by the addition of 25 μL of EYPC-LUVs $\Rightarrow$ HPTS in stirring condition by a magnetic stirrer equipped with the fluorescence instrument (at $t = 0$ s). HPTS fluorescence emission intensity was monitored with time and F_t was observed at $\lambda_{\text{em}} = 510$ nm ($\lambda_{\text{ex}} = 450$ nm). At $t = 20$ s, 20 μL of 0.5 M NaOH was added to the cuvette to make the pH gradient between the intra and extra vesicular solution. FCCP (0.625 μM) was added at $t = 50$ s (whenever necessary) and transporter molecule was added at $t = 100$ s and finally at $t = 300$ s, 25 μL of 10% Triton X-100 was added to that cuvette resulting destruction of pH gradient. Fluorescence intensities (F_t) were normalized to fractional emission intensity I_F using Equation 2. (Figure 3.15)

3.2.10.4. Ion transport activity in the presence of Valinomycin: In a clean and dry fluorescence cuvette 1975 μL of HEPES buffer (10 mM HEPES, 100 mM NaCl, pH = 7.0) was added followed by the addition of 25 μL of EYPC-LUVs $\Rightarrow$ HPTS in slowly stirring condition by a magnetic stirrer equipped with the fluorescence instrument (at $t = 0$ s). The time course of HPTS fluorescence emission intensity, F_t was observed at $\lambda_{\text{em}} = 510$ nm ($\lambda_{\text{ex}} = 450$ nm). 20 μL of 0.5 M NaOH was added to the cuvette at $t = 20$ s to make the pH gradient between the intra and extra vesicular system. Valinomycin (1 pM) was added at $t = 50$ s and transporter molecule was added at $t = 100$ s and finally at $t = 300$ s 25 μL of 10% Triton X-100 was added to lyse those vesicles destroying pH gradient. Fluorescence intensities (F_t) were normalized to fractional emission intensity I_F using Equation 2. (Figure 3.16)

To identify relative K^+/H^+ transport of compound **1a**, the HPTS assay was performed in the presence of Valinomycin, an electrogenic K^+ carrier and carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP), an electroneutral H^+ carrier, to balance out the ion that limits the rate. A significant increase in the fluorescence of HPTS by compound **1a** was observed in the presence of Valinomycin but not FCCP, suggesting that **1a** evinces a $H^+ > K^+$ transport rate. The data further corroborates to the fact that compound **1a** can facilitate the movement of both K^+ and H^+ down their electrochemical gradients independently (Figure 3.17).¹⁰

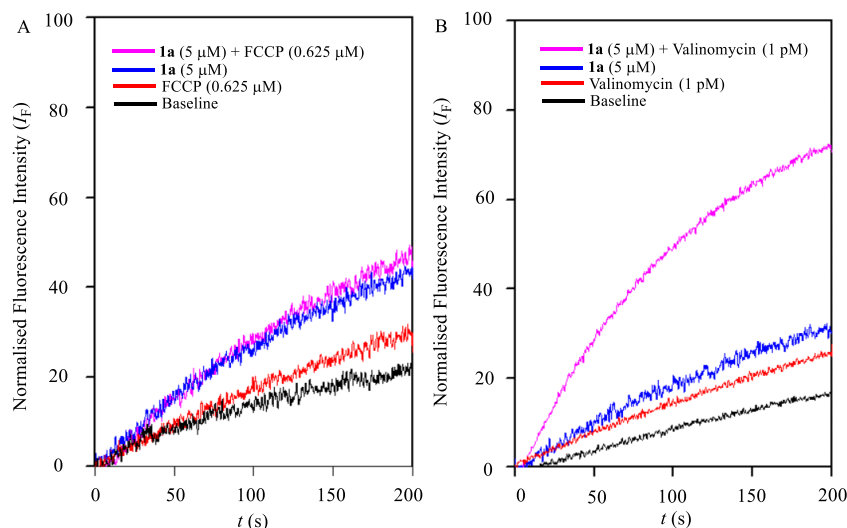


Figure 3.17. (A) Cooperative transport of **1a** (5 μ M) across EYPC-LUVs $\supset$ HPTS in the presence of FCCP (B) Cooperative transport of **1a** (5 μ M) across EYPC-LUVs $\supset$ HPTS in the presence of Valinomycin.

3.2.11. Evidence of membrane integrity using CF assay:

3.2.11.1. Preparation of EYPC-LUVs $\supset$ CF:⁴⁰ A thin lipid film was prepared by evaporating a solution of 12.5 mg EYPC in 0.5 ml CHCl_3 in vacuo for 4 h. After that lipid film was hydrated with 0.5 mL buffer (10 mM HEPES, 10 mM NaCl, 50 mM Carboxyfluorescein (CF), pH 7.0) for 1 h with occasional vortexing of 4–5 times and then subjected to freeze-thaw cycle (≥ 20 times). The vesicle solution was extruded through a polycarbonate membrane with 100 nm pores 19 times (has to be an odd number), to give vesicles with a mean diameter of ~ 100 nm. The extracellular dye was removed with size exclusion chromatography (Sephadex G-50) with 10 mM HEPES buffer (100 mM NaCl, pH 7.0. Final) Final concentration: ~ 2.5 mM EYPC lipid; intravesicular solution: 10 mM HEPES, 10 mM NaCl, 50 mM CF, pH 7.0; extravesicular solution: 10 mM HEPES, 100 mM NaCl, pH 7.0. (Figure 3.18)

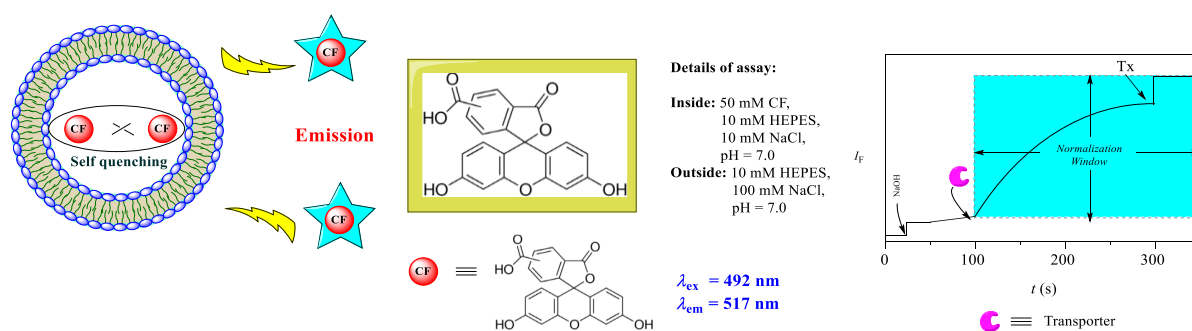


Figure 3.18. Representations of fluorescence-based assay across EYPC-LUVs $\supset$ CF.

3.2.11.2. CF leakage assay:

In a clean and dry fluorescence cuvette 25 μL of above lipid solution and 1975 μL of 10 mM HEPES buffer (100 mM NaCl, pH 7.0) was taken and kept in slowly stirring condition by a magnetic stirrer equipped with the fluorescence instrument (at $t = 0$ s). The time course of CF fluorescence emission intensity, F_t was observed at $\lambda_{\text{em}} = 517$ nm ($\lambda_{\text{ex}} = 492$ nm). Compound **1a** was added at $t = 100$ s and finally at $t = 300$ s, 25 μL of 10% Triton X-100 was added to lyse those vesicles for 100% chloride influx. Fluorescence intensities (F_t) were normalized to fractional emission intensity I_F according to Equation 2. This study confirmed that neither the bilayer membranes are defected nor large transmembrane pores are formed by **1a**, even at higher concentrations. (Figure 3.19)

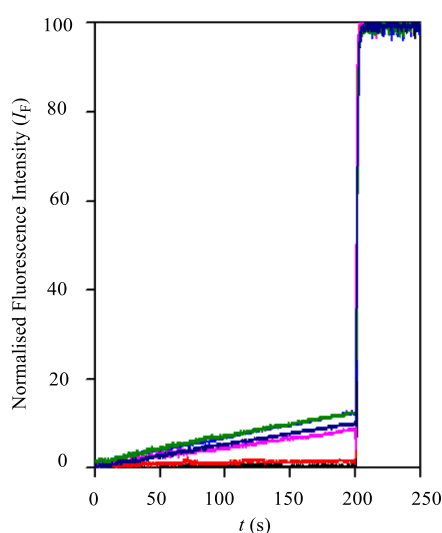


Figure 3.19. Evidence of membrane integrity at concentrations of 5 μM , 10 μM , 15 μM and 20 μM of **1a** using EYPC LUVs $\supset$ CF.

3.2.12. UV-vis titration studies:⁴³⁻⁴⁵

Initially, stock solution of the most active compound **1a** (2 mM in HPLC grade ACN) was prepared. Absorption spectra of compound **1a** was recorded in the presence of two different guests, KPF_6 or NaPF_6 .

The UV-vis experiments were performed in a 3 mL UV cuvette where 2 mL of ACN was added and to it 15 μL of this solution was taken out of the cuvette and 15 μL of the compound was added from the stock solution to make a final concentration of 30 μM in the cuvette. Next, we

recorded the change in the absorbance intensity upon addition of increasing concentration of the guest molecule, that is, KPF_6 or $NaPF_6$ in this case.

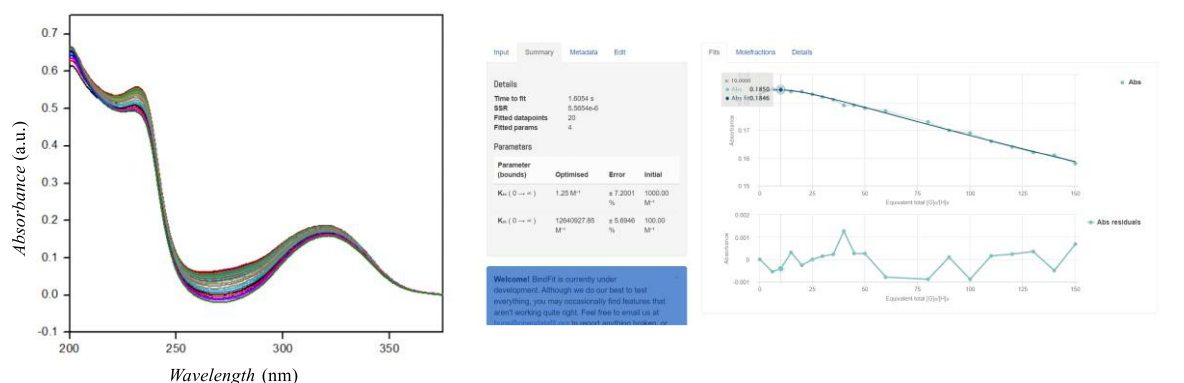


Figure 3.20. Screenshot of the fitted data plot from supramolecular.org. The binding constant was found to be $1.25 \text{ M}^{-1} \pm 7 \%$ in 1:1 receptor to cation (K^+) binding model and $1.26 \times 10^7 \text{ M}^{-2} \pm 6 \%$ in 2:1 receptor to cation (K^+) binding model.

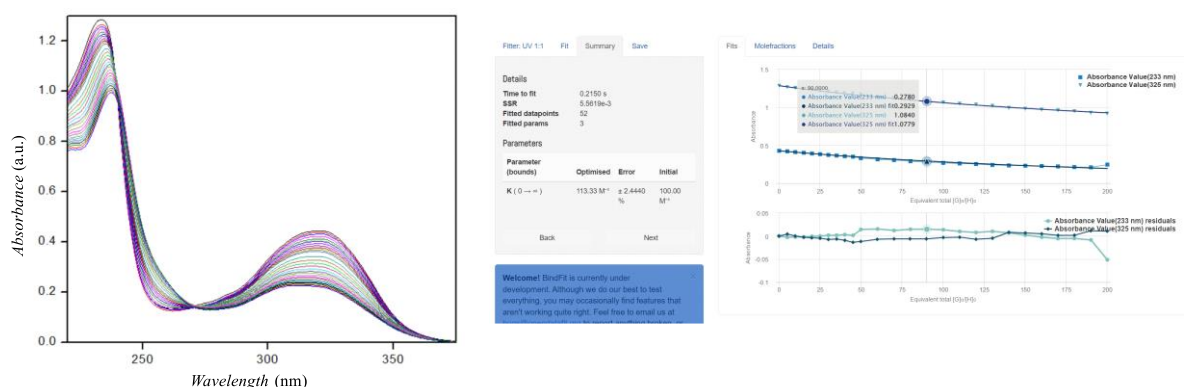


Figure 3.21. Screenshot of the fitted data plot from supramolecular.org. The binding constant was found to be $113.33 \text{ M}^{-1} \pm 2 \%$ in 1:1 receptor to cation (Na^+) binding model.

To ascertain the binding of the cations, K^+ and Na^+ to **1a**, UV-Vis titration studies were performed by gradually increasing the concentrations of KPF_6 and $NaPF_6$ where the counter anion, i.e., PF_6^- , was large and non-coordinating.⁴⁶ The gradual change in the absorption spectra proved that there was indeed some kind of binding of **1a** with both the cations K^+ and Na^+ . Binding constants of $1.25 \pm 7.2\% \text{ M}^{-1}$ (1:1 model) and $1.26 \times 10^7 \pm 5.7\% \text{ M}^{-2}$ (2:1 model) provided evidence supporting the binding of **1a** to K^+ . (Figure 3.20)⁴⁵ However, a binding constant of $113.33 \pm 2.4\% \text{ M}^{-1}$ relevant to a 1:1 binding model was obtained in case of binding with Na^+ . (Figure 3.21)⁴⁵ Moreover, an isosbestic point was obtained in case of binding with Na^+ , further corroborating to the fact that equivalent amounts of host and guest molecules were involved in the binding process.

3.2.13. Liquid-liquid extraction experiment to validate K⁺ transport:

3.2.13.1. UV-visible studies:

The complexation of the quinoline derivatives with the metal cation was determined by liquid-liquid extraction using Pederson's technique at 25 °C.^{47, 48} 1 mM stock solutions of **1a** and 10 mM stock solutions of K⁺-picrate were prepared in HPLC grade EtOAc and H₂O. 10 mL of each solution were vigorously mixed and left at room temperature for 30 min until each phase was completely separated. The final extracted organic phase was qualitatively collected and measured by the UV-vis spectrophotometer at $\lambda_{\text{max}} = 355 \text{ nm}$ ($\epsilon = 1.45 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$). The indication of complex formation between **1a** and potassium picrate was confirmed by ¹H NMR and ESI-MS.⁴⁹ Figure 3.22 shows the absorption spectra of K⁺-picrate, **1a**, and that of **1a** and K⁺-picrate in EtOAc. The peak shift implied the complex formation between **1a** and K⁺-picrate. The percentage extraction of potassium picrate was calculated by Equation 4.

$$\% \text{ efficiency}_{\text{extraction}} = [(A_0 - A)/A_0] \times 100 \quad (\text{Equation 4})$$

where A_0 = initial absorbance of the picrate solution and A = absorbance of the picrate solution after extraction with **1a**.⁵⁰

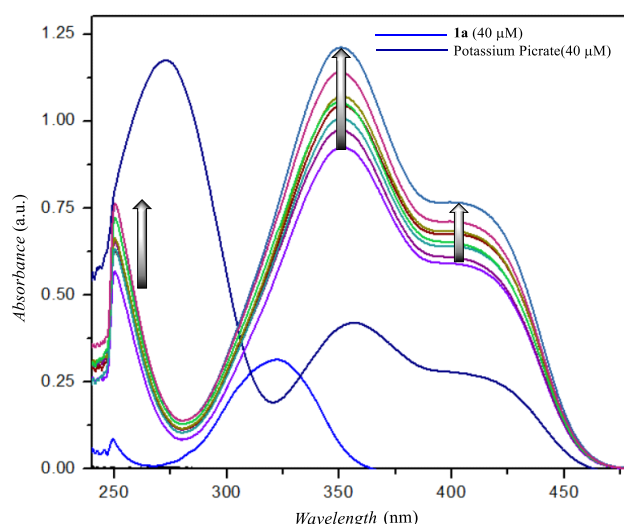


Figure 3.22. UV–Vis spectra of **1a** (blue), K⁺-picrate (navy blue), and the complex of **1a** and K⁺-picrate.

We have proceeded with the maximum wavelength $\lambda_{\text{max}} = 273 \text{ nm}$ in this case since it corresponds to the maximum absorbance for picrate solution and therefore, the maximum molar extinction coefficient ϵ values according to the report “*Asian Journal of Chemistry*, **2010**, 22(10), 7628-7640.” The A_0 and A values reported are 1.175 and 0.183 respectively where A_0 correspond

to the initial picrate absorbance value and A corresponds to the absorbance value after the final extraction. Thus, by applying equation 4, the percentage extraction is 84.4%.

3.2.13.2. ^1H -NMR and ESI-MS studies:

The extraction of the picrate from the aqueous to the organic layer was further confirmed by ESI-MS and ^1H NMR experiments.⁴⁹ From the ^1H NMR spectrum of the final organic layer, the appearance of the peak corresponding to the picrate ion in CDCl_3 confirms the extraction of potassium picrate from the aqueous to the organic layer by compound **1a**. Similarly, from the ESI-MS spectrum, the appearance of the peak corresponding to the picrate anion further supported the conclusion. (Figure 3.23 A and B)

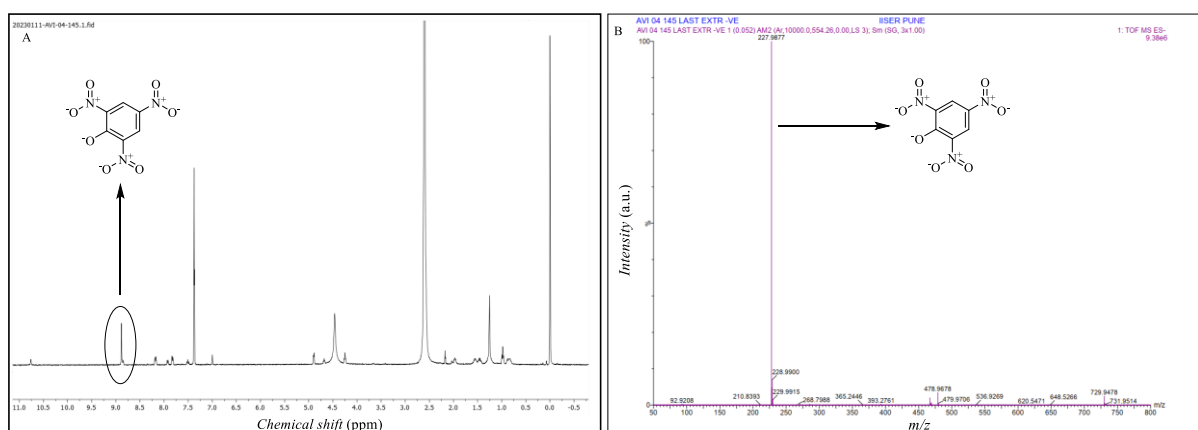


Figure 3.23. (A) ESI-MS Spectrum and (B) ^1H -NMR Spectrum of (c) the complex of **1a** and K^+ ion.

3.2.14. ^{39}K NMR experiment to validate K^+ transport:

In order to validate cation transport, a ^{39}K NMR experiment was performed following the well-established protocol of Riddell and co-workers.⁵¹ The technique depends on monitoring the rate of cation exchange between the chemical's intra- and extra-vesicular environment where the shift of the 'outside' cation is subject to a chemical shift reagent (e.g. Dy^{3+})⁵²⁻⁵⁴ (Figure 3.24).

3.2.14.1. Preparation of vesicles and shift reagent:

Vesicles were prepared in 200 mM KCl (1 mL) solution without HPTS dye. The procedure for vesicle preparation was similar to that described for the HPTS assay. The only difference is that the vesicle solution was extruded 15 times through a 400 nm membrane to give the large unilamellar vesicle (LUV) solution and then directly used for NMR experiment without any

further purification. The vesicle solution and sample prepared for NMR study were stored in 1.5 mL Eppendorf vials. The shift reagent was prepared by mixing 2 mL (0.2 M) sodium tri (poly) phosphate and 1 mL (0.1 M) dysprosium trichloride solution⁵⁵ (Figure 3.24).

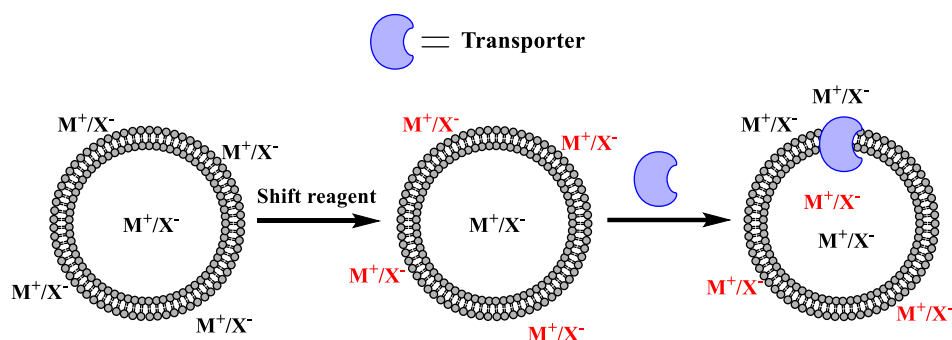


Figure 3.24. Schematic representation of vesicles prepared for ³⁹K NMR assay.

3.2.14.2. Experimental procedure for ³⁹K NMR experiment with **1a**:^{56, 57}

The lipid suspension (180 μ L) from the stock solution, conductivity water (120 μ L), D₂O (100 μ L), shift reagent (100 μ L) and an appropriate amount of **1a** in DMSO were mixed in a 1.5 mL Eppendorf vial and kept at 23 °C for 30 minutes. After every 30 minutes the ³⁹K NMR spectrum was recorded by gradually increasing the concentration of **1a**. The rate constant values (k_{obs}) were calculated by using Equation 5⁶ where, ν and ν_0 are the half line widths in the presence and absence of the **1a**.

$$k_{\text{obs}} = 1/\tau = \pi (\nu - \nu_0) \quad (\text{Equation 5})$$

Table 10. Calculation of rate constant of K⁺ transport with increase in concentration of **1a:**

Concentration of 1a (μ M)	Half line-width (Hz)	Rate Constant k_{obs} (Hz ⁻¹)
0	28.972	0
80	35.854	21.620
250	105.563	240.618
300	120.804	288.499

With increasing concentration of **1a** being added, sharpening of the external K^+ peaks was seen, indicating K^+ exchange across the lipid bilayer membrane. Transporter molecule **1a** confirmed compound-mediated K^+ transport as it resulted in an increase in the line width, i.e., broadening of the internal K^+ ion peaks (Figure 3.25). The increase in the rate of transport of the K^+ ion with enhancement in the concentration of **1a** is summarized in figure 3.25 and Table 10. The non-covalent interaction between the carbonyl oxygen, hydroxyl oxygen and the potassium cation is one of the plausible reasons for cation transport.

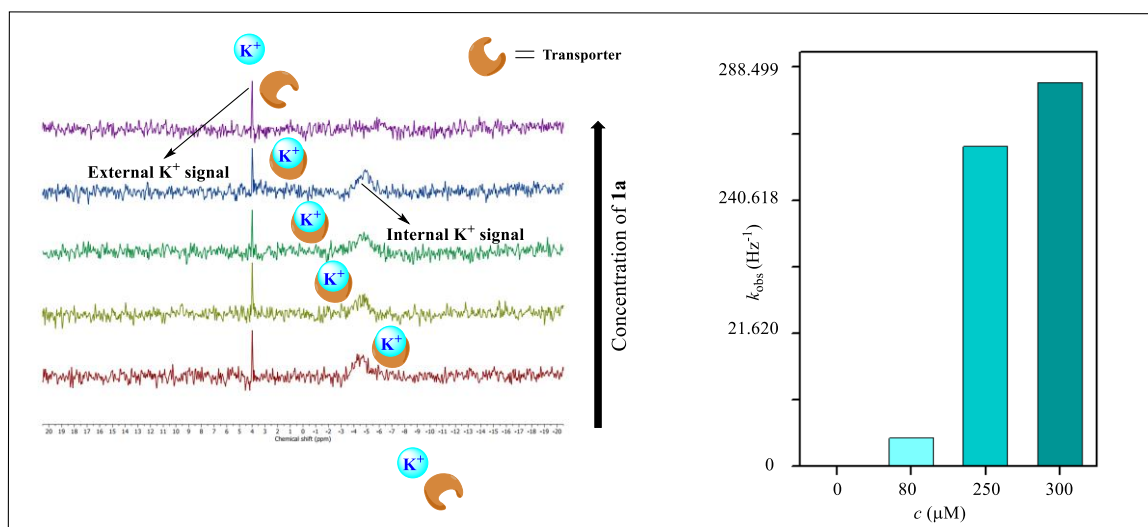


Figure 3.25. Sharpening of external K^+ ion signals and broadening of internal K^+ signals with increase in concentration of **1a** in ^{39}K NMR experiment.

3.2.15. NMR spectra:

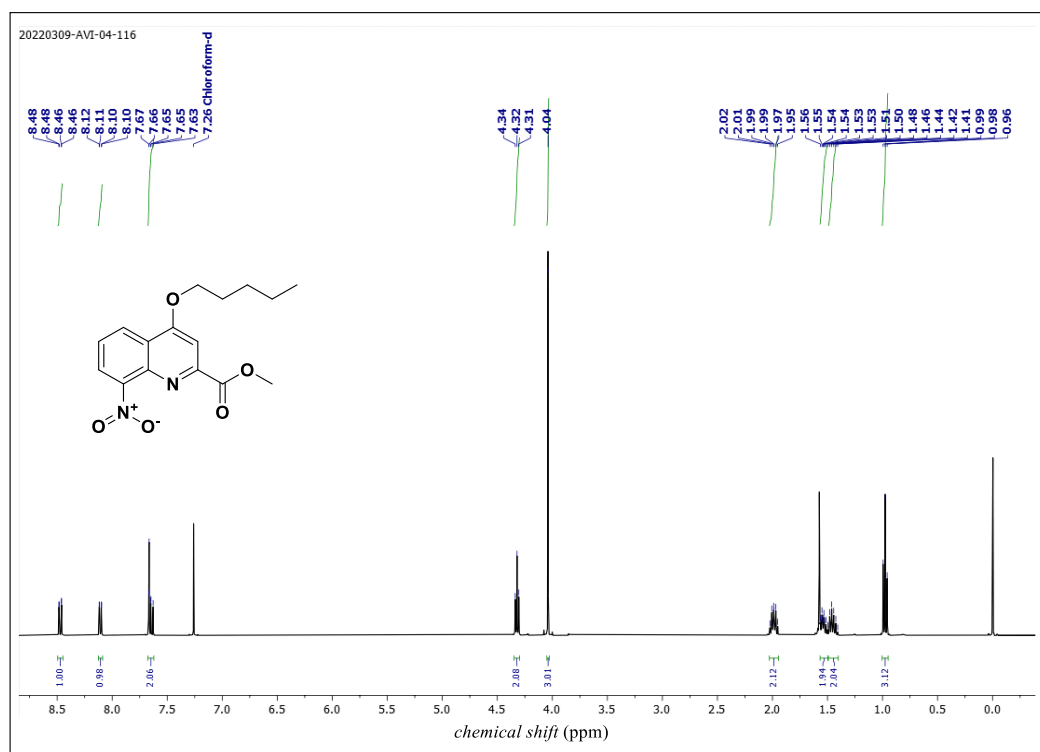


Figure 3.26. ¹H NMR spectrum of **4** in CDCl₃.

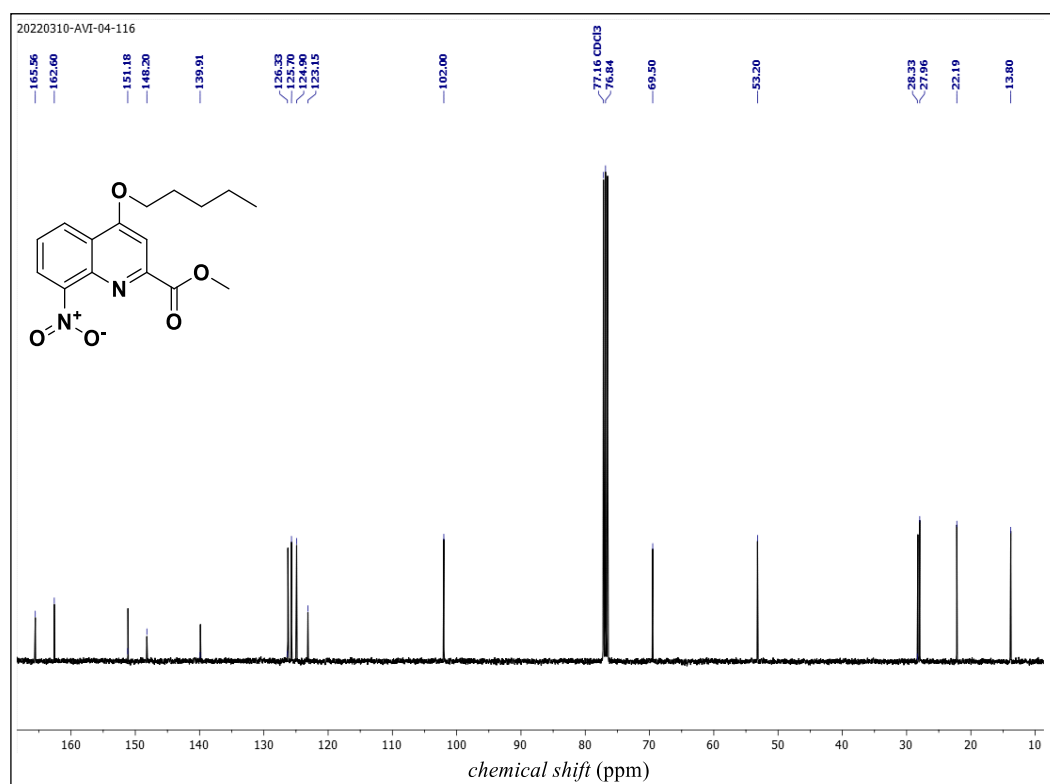


Figure 3.27. ¹³C NMR spectrum of **4** in CDCl₃.

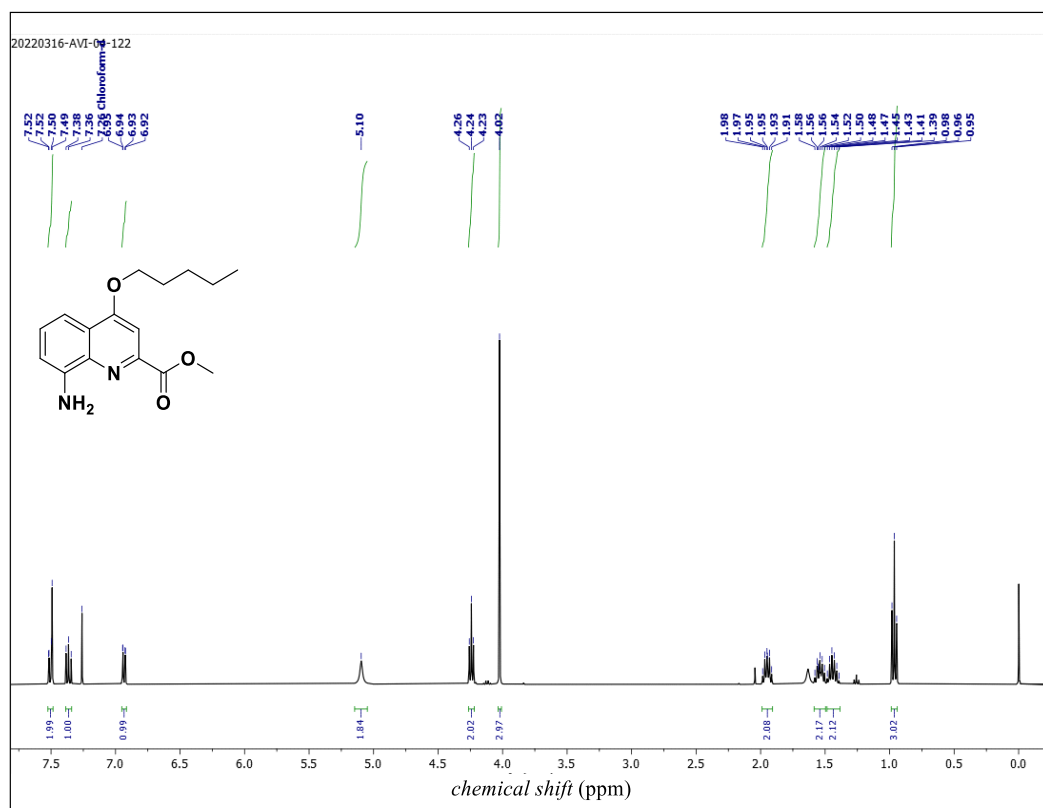


Figure 3.28. ^1H NMR spectrum of 5 in CDCl_3 .

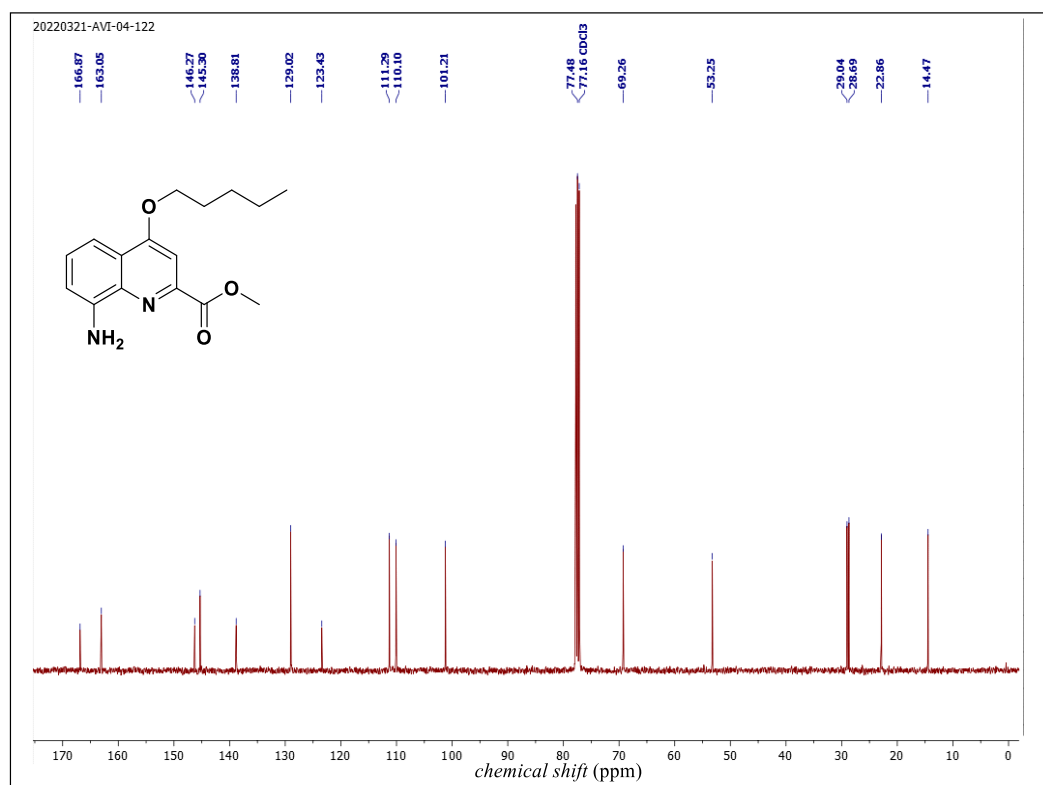


Figure 3.29. ^{13}C NMR spectrum of 5 in CDCl_3 .

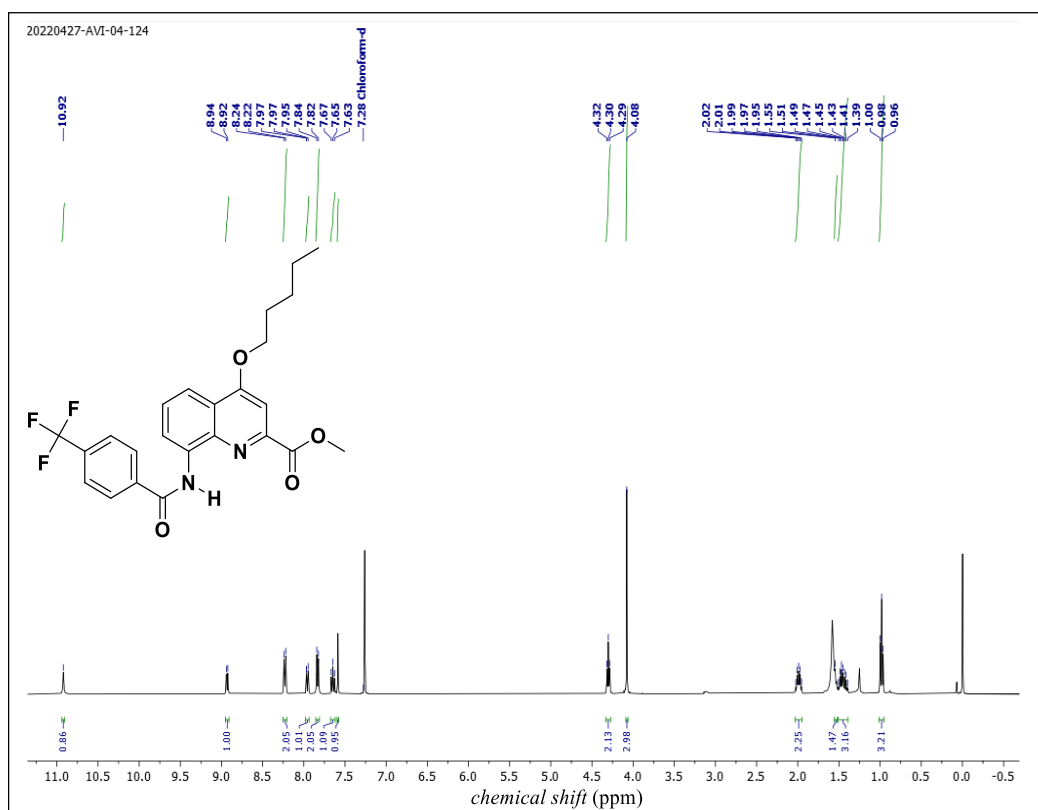


Figure 3.30. ^1H NMR spectrum of **6a** in CDCl_3 .

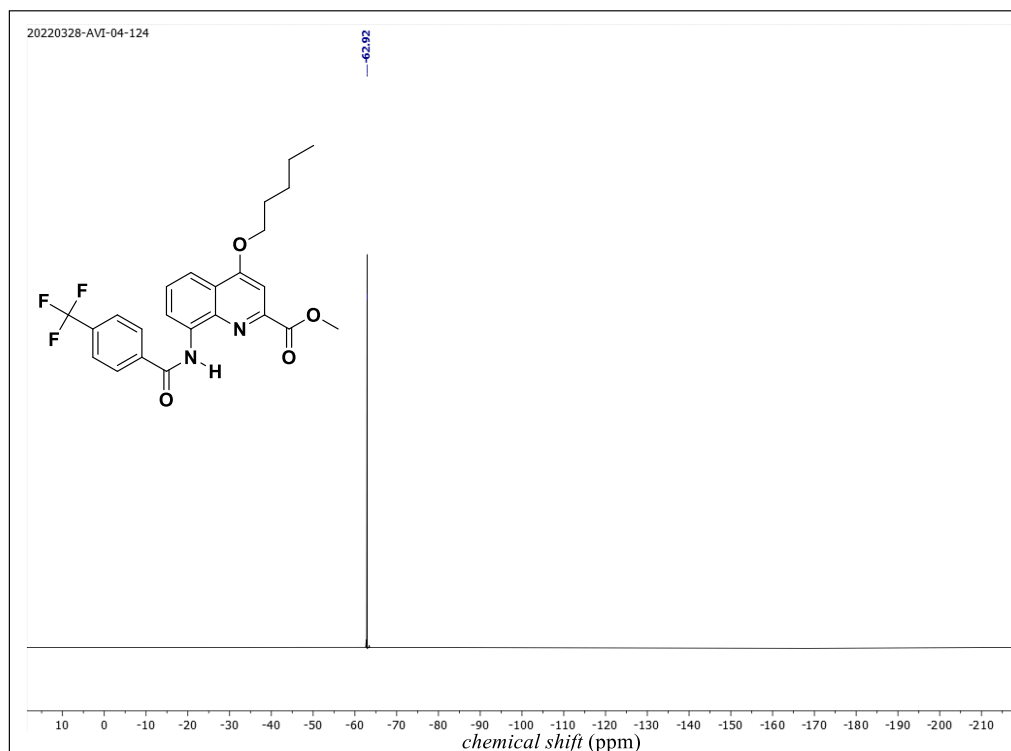


Figure 3.31. ^{19}F NMR spectrum of **6a** in CDCl_3 .

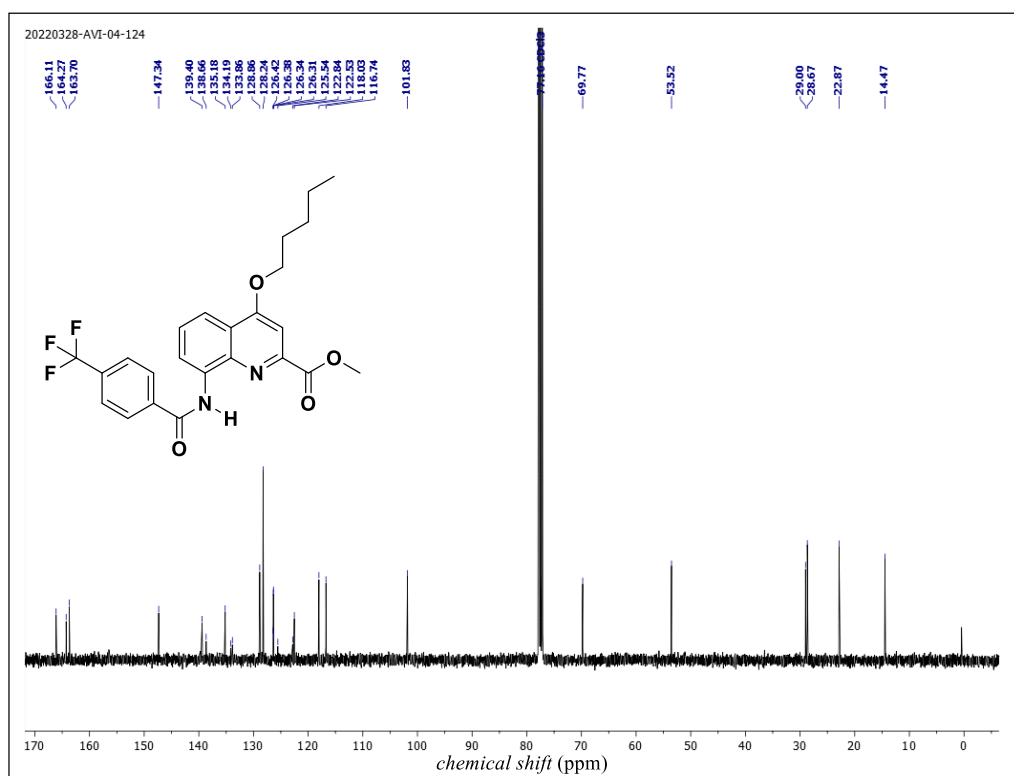


Figure 3.32. ¹³C NMR spectrum of **6a** in CDCl₃.

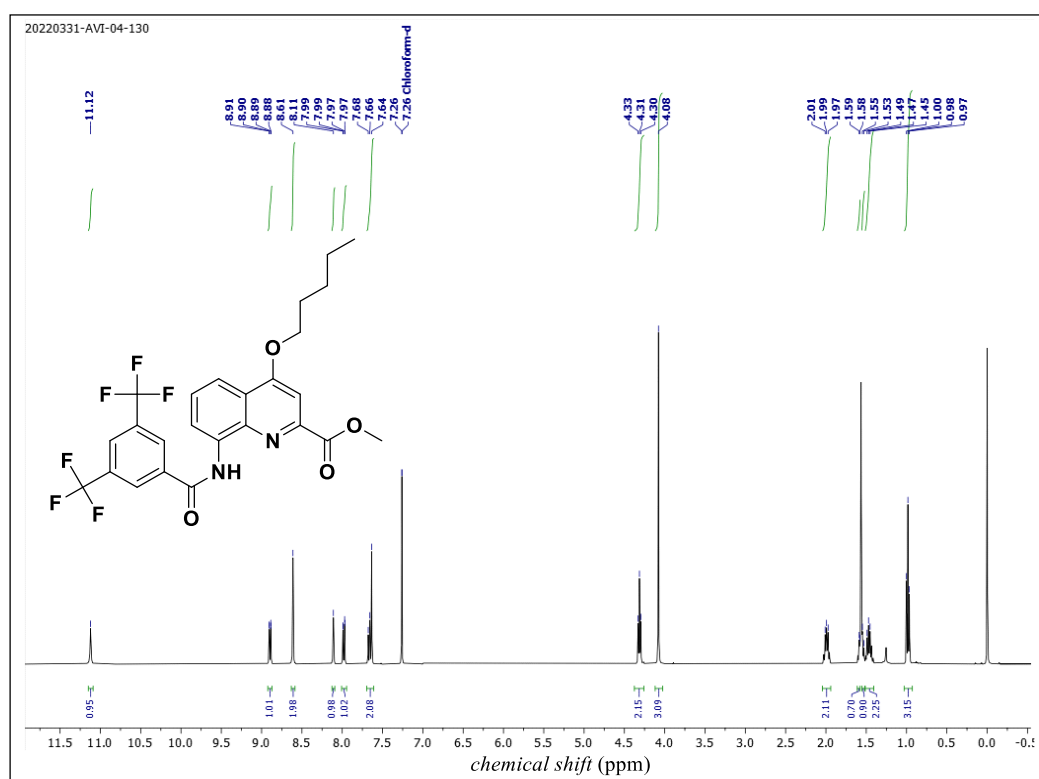


Figure 3.33. ¹H NMR spectrum of **6b** in CDCl₃.

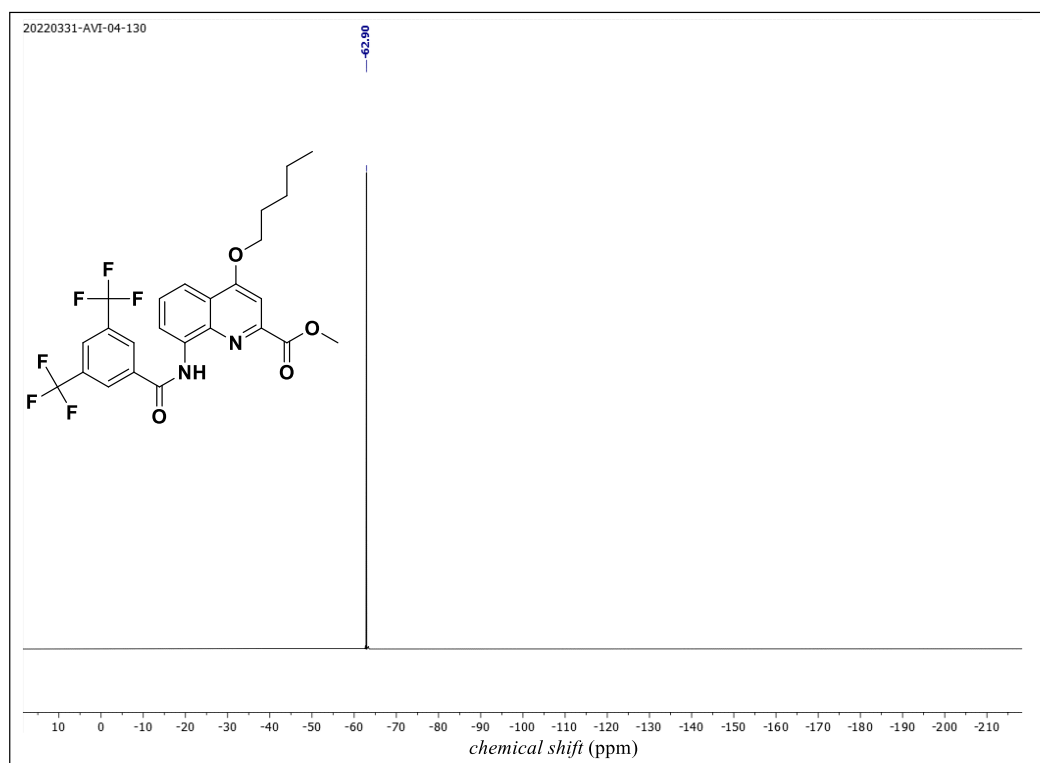


Figure 3.34. ^{19}F NMR spectrum of **6b** in CDCl_3 .

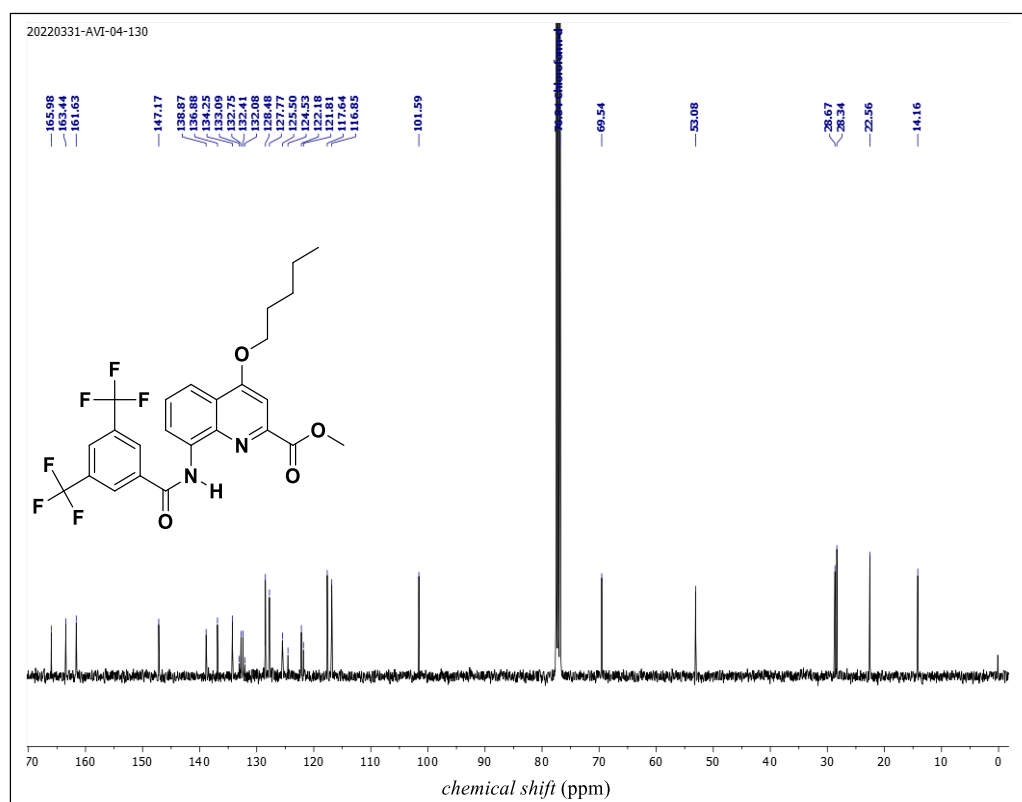


Figure 3.35. ^{13}C NMR spectrum of **6b** in CDCl_3 .

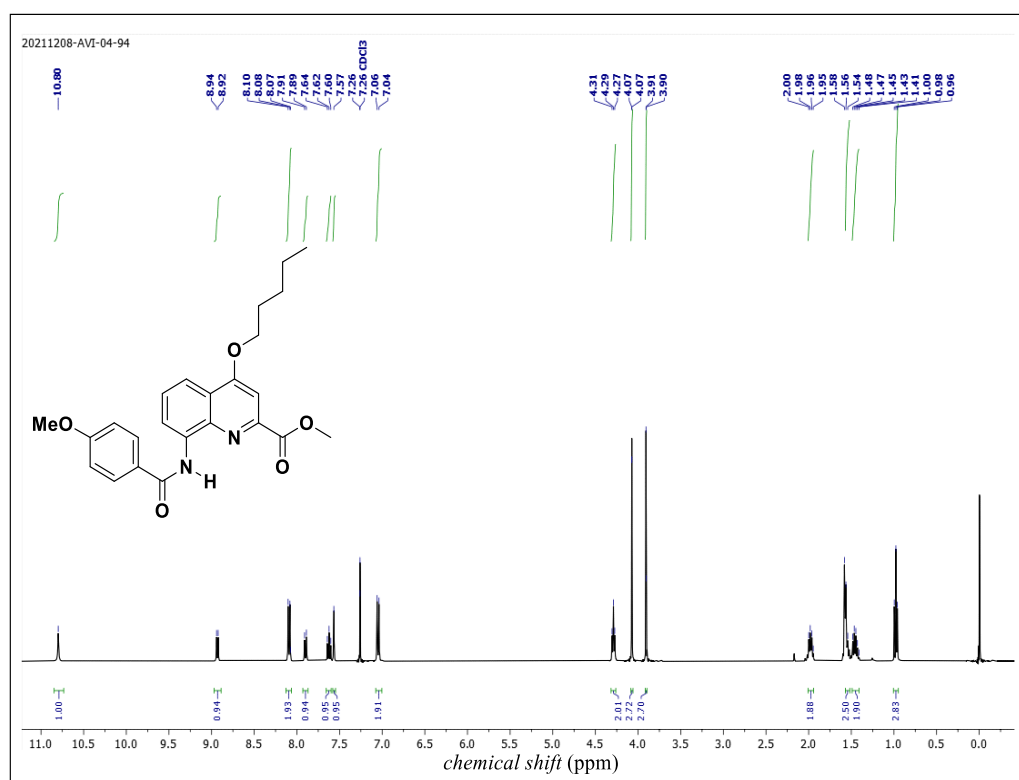


Figure 3.36. ¹H NMR spectrum of **6c** in CDCl₃.

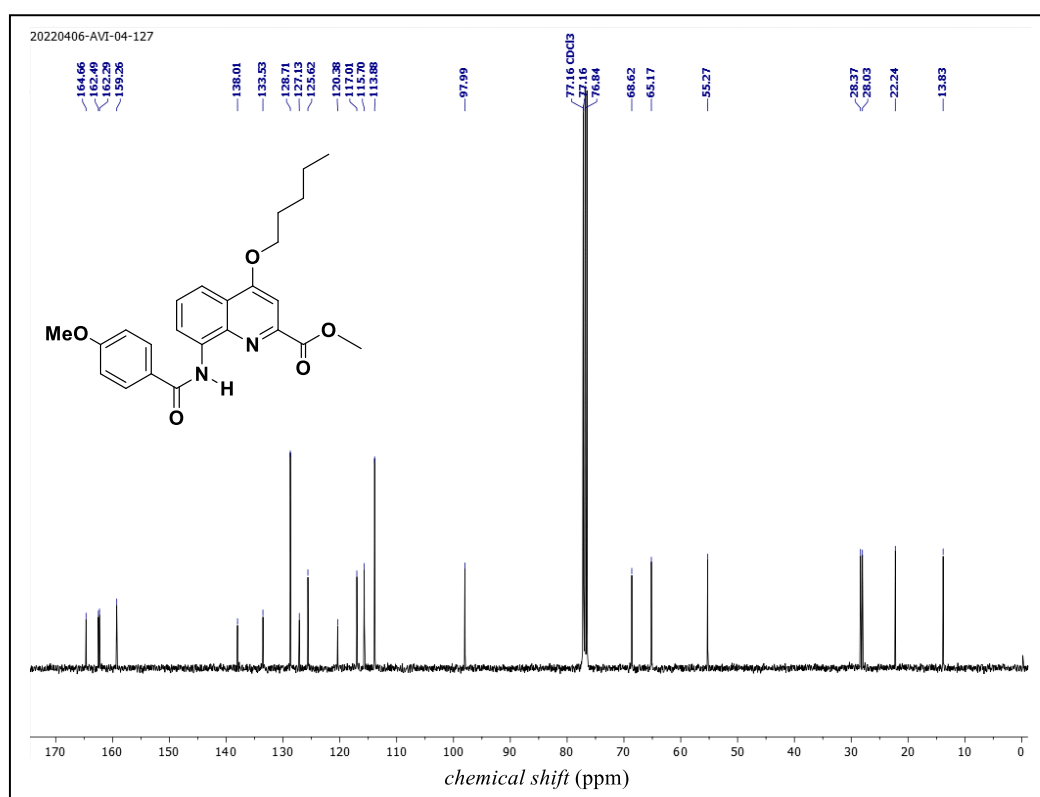


Figure 3.37. ¹³C NMR spectrum of **6c** in CDCl₃.

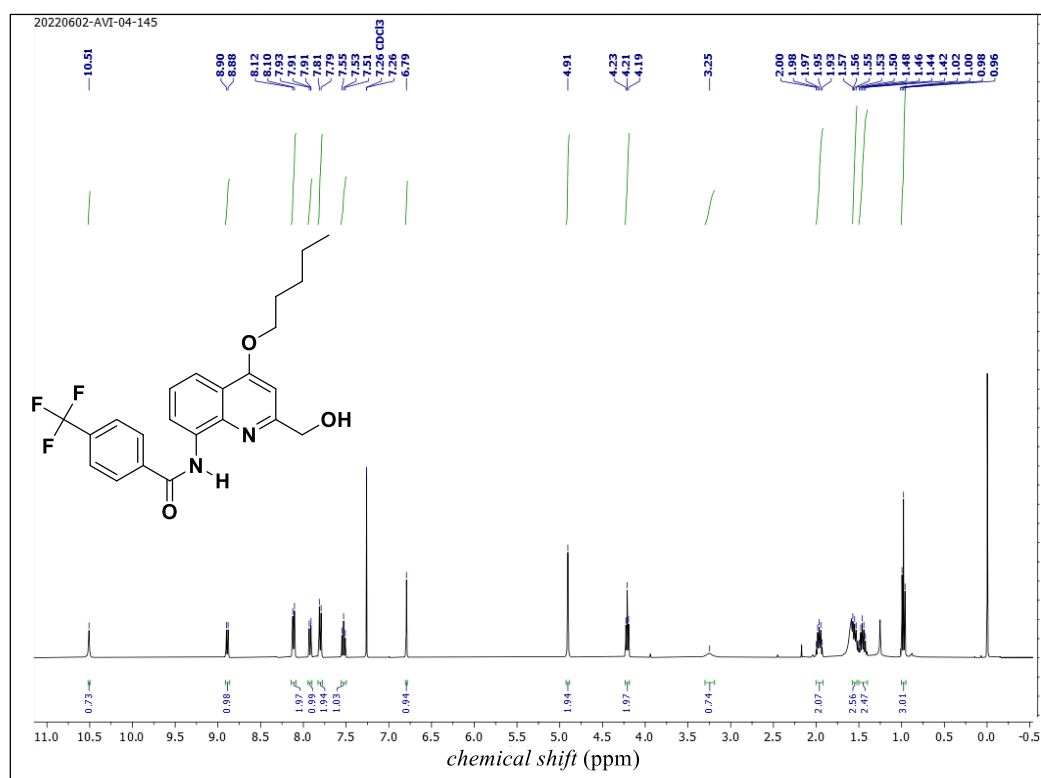


Figure 3.38. ¹H NMR spectrum of **1a** in CDCl₃.

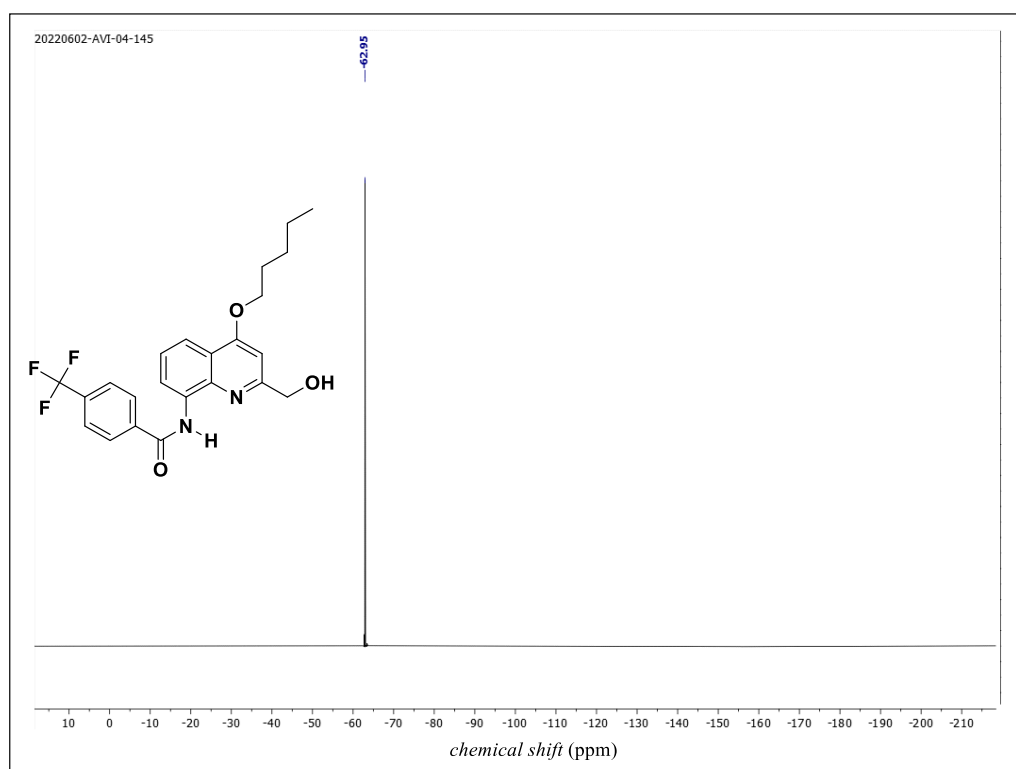


Figure 3.39. ¹⁹F NMR spectrum of **1a** in CDCl₃.

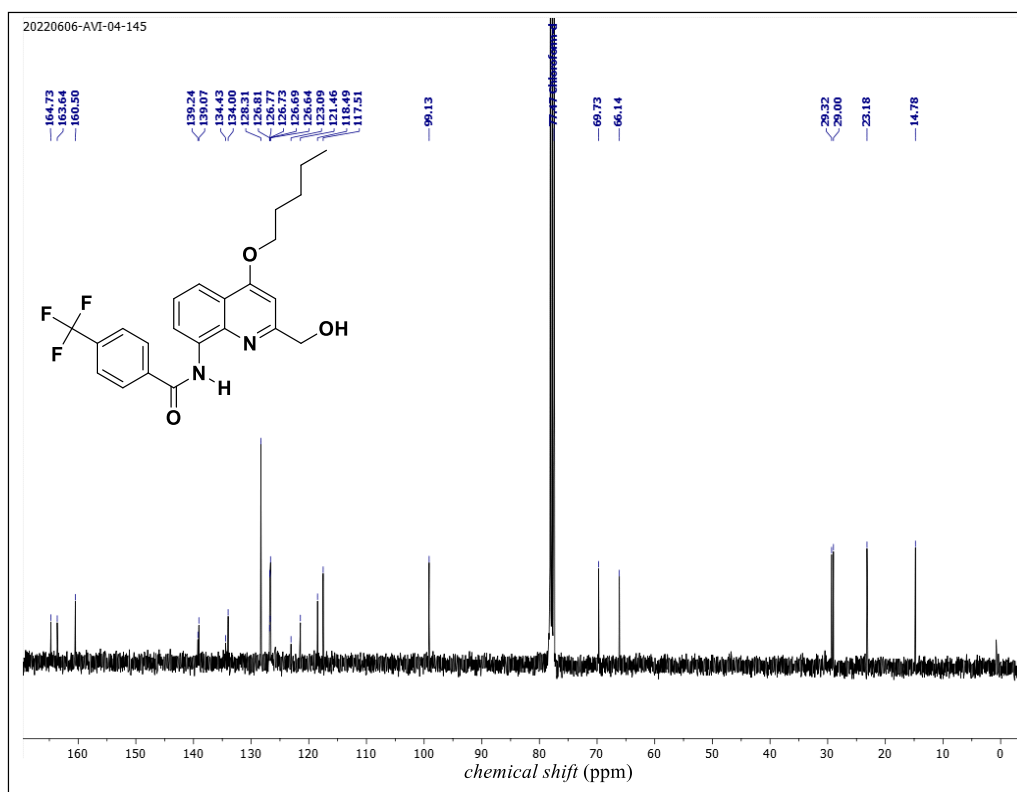


Figure 3.40. ¹³C NMR spectrum of **1a** in CDCl₃.

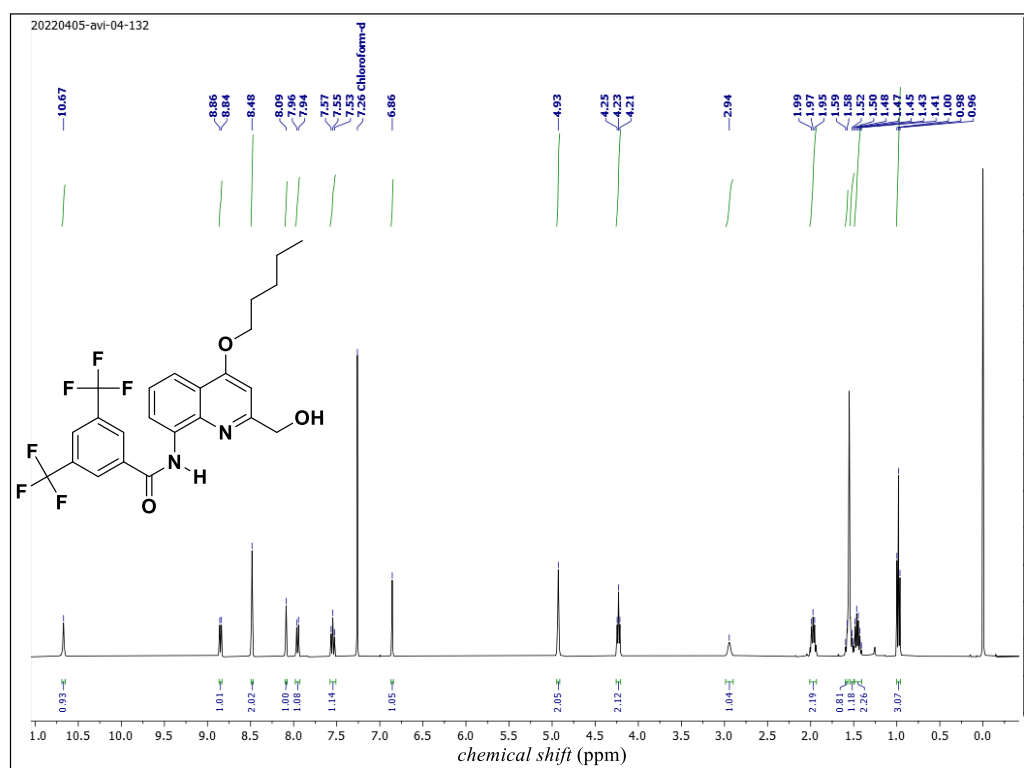


Figure 3.41. ¹H NMR spectrum of **1b** in CDCl₃.

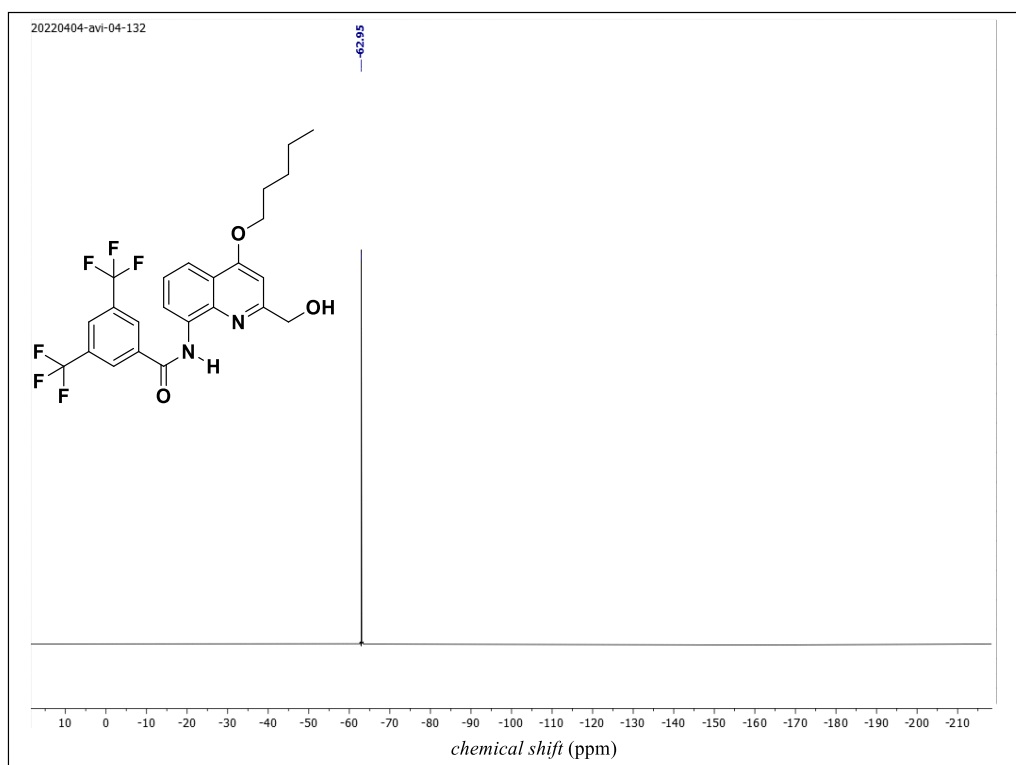


Figure 3.42. ^{19}F NMR spectrum of **1b** in CDCl_3 .

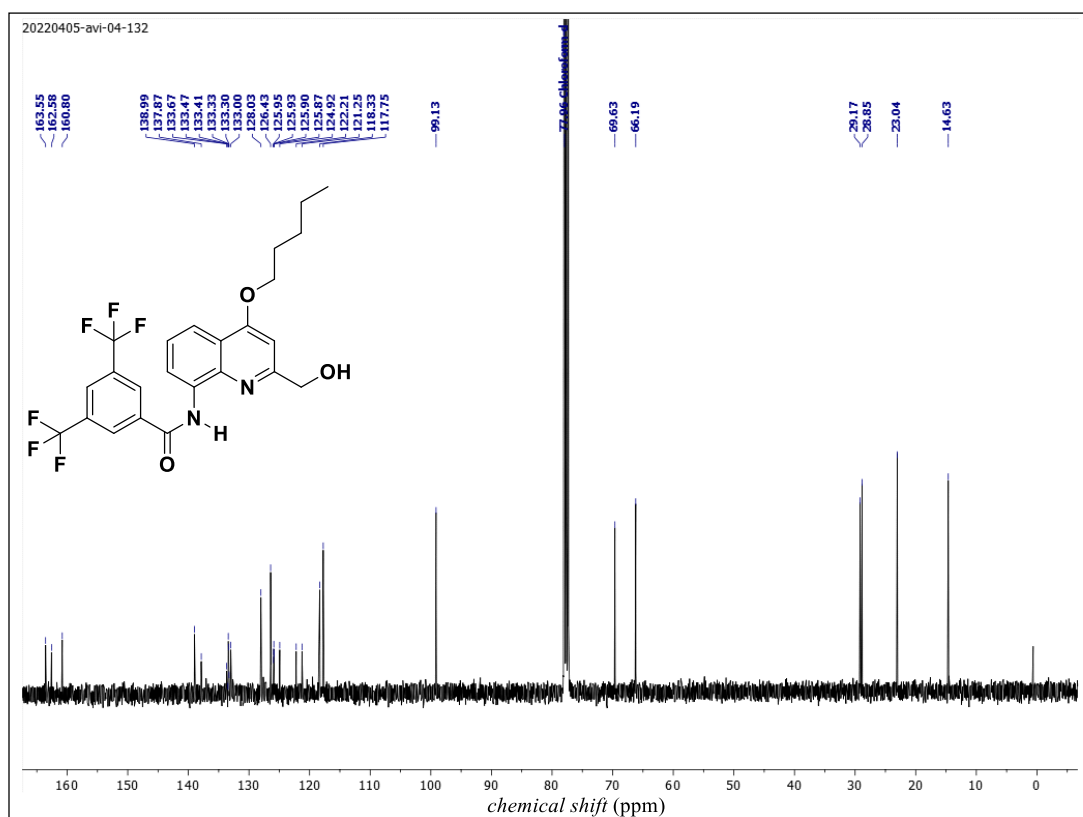


Figure 3.43. ^{13}C NMR spectrum of **1b** in CDCl_3 .

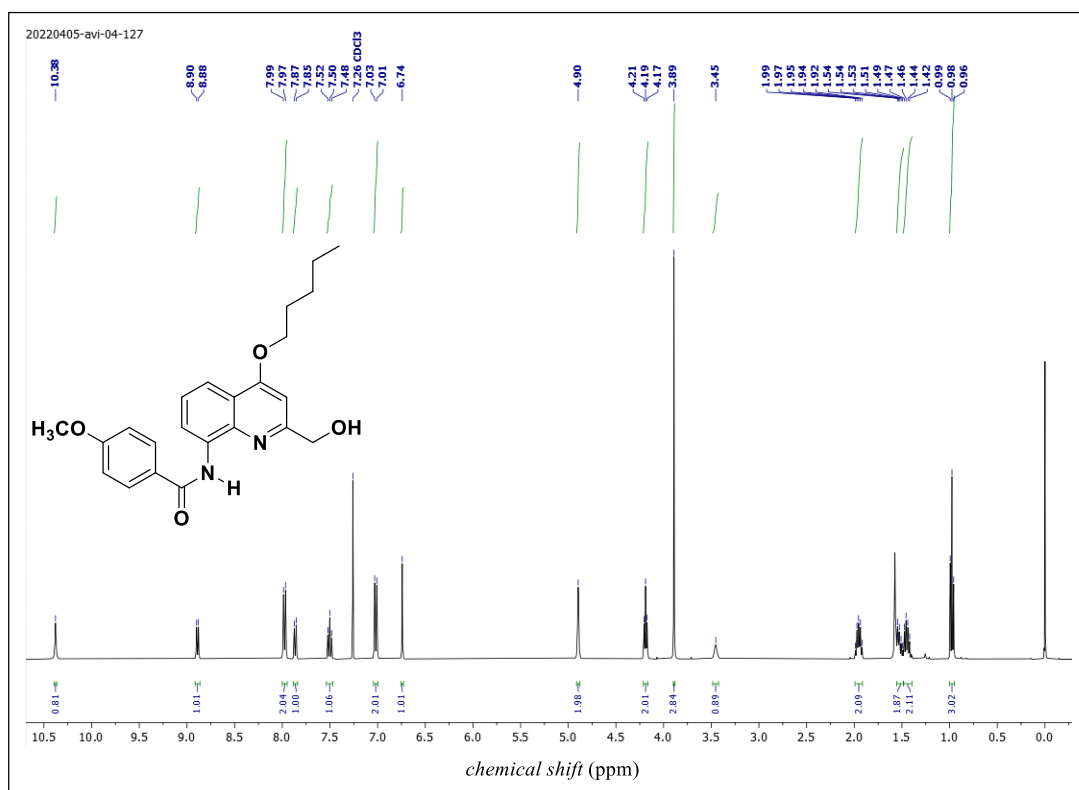


Figure 3.44. ¹H NMR spectrum of **1c** in CDCl₃.

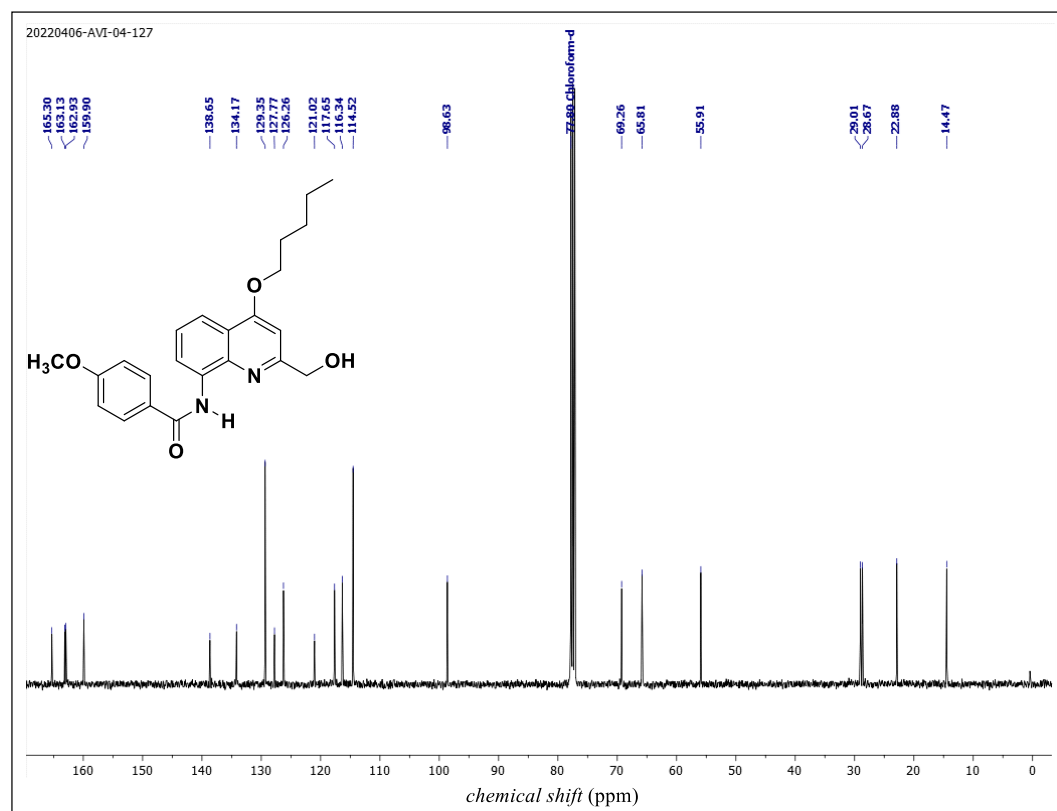


Figure 3.45. ¹³C NMR spectrum of **1c** in CDCl₃.

3.3. References:

1. Davis, J. T.; Okunola, O.; Quesada, R., Recent advances in the transmembrane transport of anions. *Chem. Soc. Rev.* **2010**, 39 (10), 3843-3862.
2. Becchetti, A.; Munaron, L.; Arcangeli, A., The role of ion channels and transporters in cell proliferation and cancer. *Front. Physiol.* **2013**, 4.
3. Shinde, S. V.; Talukdar, P., An anion receptor that facilitates transmembrane proton–anion symport by deprotonating its sulfonamide N–H proton. *Chem. Commun.* **2018**, 54 (73), 10351-10354.
4. Niemeyer, B. A.; Mery, L.; Zawar, C.; Suckow, A.; Monje, F.; Pardo, L. A.; Stühmer, W.; Flockerzi, V.; Hoth, M., Ion channels in health and disease. *EMBO reports* **2001**, 2 (7), 568-573.
5. Busschaert, N.; Park, S.-H.; Baek, K.-H.; Choi, Y. P.; Park, J.; Howe, E. N. W.; Hiscock, J. R.; Karagiannidis, L. E.; Marques, I.; Félix, V.; Namkung, W.; Sessler, J. L.; Gale, P. A.; Shin, I., A synthetic ion transporter that disrupts autophagy and induces apoptosis by perturbing cellular chloride concentrations. *Nat. Chem.* **2017**, 9 (7), 667-675.
6. Benke, B. P.; Behera, H.; Madhavan, N., Low molecular weight di- to tetrapeptide transmembrane cation transporters. *Eur. J. Org. Chem.* **2020**, 2020 (44), 6898-6902.
7. Gouaux, E.; MacKinnon, R., Principles of selective ion transport in channels and pumps. *Science*. **2005**, 310 (5753), 1461-1465.
8. Zaydman, M. A.; Silva, J. R.; Cui, J., Ion channel associated diseases: overview of molecular mechanisms. *Chem. Rev.* **2012**, 112 (12), 6319-6333.
9. Tsujii, M.; Tanudjaja, E.; Uozumi, N. Diverse physiological functions of cation proton antiporters across bacteria and plant cells. *Int. J. Mol. Sci.*, **2020**.
10. Shen, F.-F.; Dai, S.-Y.; Wong, N.-K.; Deng, S.; Wong, A. S.-T.; Yang, D., Mediating K^+/H^+ transport on organelle membranes to selectively eradicate cancer stem cells with a small molecule. *J. Am. Chem. Soc.* **2020**, 142 (24), 10769-10779.
11. Osakabe, Y.; Arinaga, N.; Umezawa, T.; Katsura, S.; Nagamachi, K.; Tanaka, H.; Ohiraki, H.; Yamada, K.; Seo, S.-U.; Abo, M.; Yoshimura, E.; Shinozaki, K.; Yamaguchi-Shinozaki, K., Osmotic stress responses and plant growth controlled by potassium transporters in arabidopsis. *The Plant Cell* **2013**, 25 (2), 609-624.
12. Brett, C. L.; Donowitz, M.; Rao, R., Evolutionary origins of eukaryotic sodium/proton exchangers. *Am J Physiol Cell Physiol.* **2005**, 288 (2), 223-239.

13. Wu, S. J.; Ding, L.; Zhu, J. K., SOS1, A genetic locus essential for salt tolerance and potassium acquisition. *The Plant Cell* **1996**, 8 (4), 617-627.
14. Beck, J. C.; Rosen, B. P., Cation/proton antiport systems in Escherichia coli: Properties of the sodium/proton antiporter. *Archives of Biochemistry and Biophysics* **1979**, 194 (1), 208-214.
15. Schuldiner, S.; Fishkes, H., Sodium-proton antiport in isolated membrane vesicles of Escherichia coli. *Biochemistry* **1978**, 17 (4), 706-711.
16. Reiß, P.; Koert, U., Ion-channels: Goals for function-oriented synthesis. *Acc. Chem. Res.* **2013**, 46 (12), 2773-2780.
17. Otis, F.; Auger, M.; Voyer, N., Exploiting peptide nanostructures to construct functional artificial ion channels. *Acc. Chem. Res.* **2013**, 46 (12), 2934-2943.
18. Feng, W.-X.; Sun, Z.; Zhang, Y.; Legrand, Y.-M.; Petit, E.; Su, C.-Y.; Barboiu, M., Bis-15-crown-5-ether-pillar[5]arene K^+ -responsive channels. *Org. Lett.* **2017**, 19 (6), 1438-1441.
19. Ye, R.; Ren, C.; Shen, J.; Li, N.; Chen, F.; Roy, A.; Zeng, H., Molecular ion fishers as highly active and exceptionally selective K^+ transporters. *J. Am. Chem. Soc.* **2019**, 141 (25), 9788-9792.
20. Goto, C.; Yamamura, M.; Satake, A.; Kobuke, Y., Artificial ion channels showing rectified current behavior. *J. Am. Chem. Soc.* **2001**, 123 (49), 12152-12159.
21. Montenegro, J.; Ghadiri, M. R.; Granja, J. R., Ion channel models based on self-assembling cyclic peptide nanotubes. *Acc. Chem. Res.* **2013**, 46 (12), 2955-2965.
22. De Riccardis, F.; Izzo, I.; Montesarchio, D.; Tecilla, P., Ion transport through lipid bilayers by synthetic ionophores: Modulation of activity and selectivity. *Acc. Chem. Res.* **2013**, 46 (12), 2781-2790.
23. Gokel, G. W.; Negin, S., Synthetic ion channels: From pores to biological applications. *Acc. Chem. Res.* **2013**, 46 (12), 2824-2833.
24. García-Calvo, J.; Torroba, T.; Brañas-Fresnillo, V.; Perdomo, G.; Cózar-Castellano, I.; Li, Y.-H.; Legrand, Y.-M.; Barboiu, M., Manipulation of transmembrane transport by synthetic K^+ ionophore depsipeptides and its implications in glucose-stimulated insulin secretion in β -cells. *Chem. Eur. J.* **2019**, 25 (39), 9287-9294.
25. Hakhlyar, P.; Baruah, J. B., Synthesis and characterization of tri-quinoline based receptors and study interactions with Zn^{2+} and Cu^{2+} ions. *Inorganica Chimica Acta* **2016**, 440, 53-61.

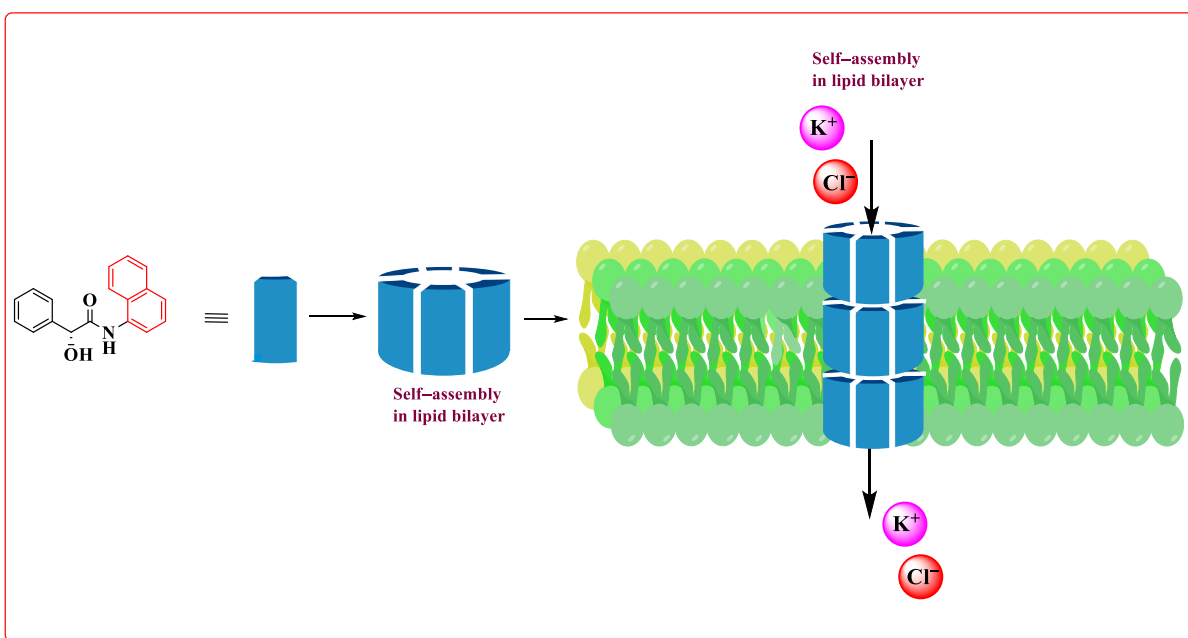
26. Abel, A. S.; Averin, A. D.; Beletskaya, I. P., Oxaazamacrocycles incorporating the quinoline moiety: Synthesis and the study of their binding properties towards metal cations. *New J. Chem.* **2016**, *40* (7), 5818-5828.
27. Peet, N. P.; Baugh, L. E.; Sunder, S.; Lewis, J. E., Synthesis and antiallergic activity of some quinolinones and imidazoquinolinones. *J. Med. Chem.* **1985**, *28* (3), 298-302.
28. Zhang, L.; Brett, C. M.; Giacomini, K. M., Role of organic cation transporters in drug absorption and elimination. *Annual Review of Pharmacology and Toxicology* **1998**, *38* (1), 431-460.
29. Schlesinger, P. H.; Gross, A.; Yin, X.-M.; Yamamoto, K.; Saito, M.; Waksman, G.; Korsmeyer, S. J., Comparison of the ion channel characteristics of proapoptotic BAX and antiapoptotic BCL-2. *Proc. Natl. Acad. Sci. U.S.A* **1997**, *94* (21), 11357-11362.
30. G. M. Sheldrick, *Acta Crystallogr. Sect. A*, **2008**, *112*.
31. *WINGX version 1.80.05* Louis Farrugia, University of Glasgow.
32. A. L. Spek, *A PLATON, Multipurpose Crystallographic Tool*, Utrecht University, Utrecht, The Netherlands, **2005**.
33. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. *Gaussian 16 Rev. B.01*, Wallingford, CT, **2016**.
34. Goto, H. O., S.; Nakayama, N.; Ohta, K. CONFLEX 8,, CONFLEX Corporation, T., Japan, **2017**.
35. Frisch, M. J.; Pople, J. A.; Binkley, J. S., Self-consistent molecular orbital methods 25. Supplementary functions for Gaussian basis sets. *J. Chem. Phys* **1984**, *80*, 3265-3269.
36. Goto, H.; Osawa, E., Corner flapping: A simple and fast algorithm for exhaustive generation of ring conformations. *J. Am. Chem. Soc.* **1989**, *111* (24), 8950-8951.

37. Malla, J. A.; Roy, A.; Talukdar, P., Anion selective ion channel constructed from a self-assembly of bis(cholate)-substituted fumaramide. *Org. Lett.* **2018**, *20* (19), 5991-5994.
38. Shinde, S. V.; Talukdar, P., A dimeric bis(melamine)-substituted bispidine for efficient transmembrane H^+/Cl^- cotransport. *Angew. Chem., Int. Ed.* **2017**, *56* (15), 4238-4242.
39. Roy, A.; Gautam, A.; Malla, J. A.; Sarkar, S.; Mukherjee, A.; Talukdar, P., Self-assembly of small-molecule fumaramides allows transmembrane chloride channel formation. *Chem. Commun.* **2018**, *54* (16), 2024-2027.
40. Malla, J. A.; Upadhyay, A.; Ghosh, P.; Mondal, D.; Mondal, A.; Sharma, S.; Talukdar, P., Chloride transport across liposomes and cells by nontoxic 3-(1*H*-1,2,3-triazol-1-yl)benzamides. *Org. Lett.* **2022**, *24* (23), 4124-4128.
41. Shinde, S. V.; Talukdar, P., A dimeric bis(melamine)-substituted bispidine for efficient transmembrane H^+/Cl^- cotransport. *Angew.Chem.Int.Ed.* **2017**, *56* (15), 4238-4242.
42. Legg, K. D.; Hercules, D. M., Quenching of lucigenin fluorescence. *J. Phys. Chem.* **1970**, *74* (10), 2114-2118.
43. Alorabi, A. Q.; Abdelbaset, M.; Zabin, S. A. Colorimetric detection of multiple metal ions using schiff base 1-(2-Thiophenylimino)-4-(N-dimethyl)benzene *Chemosensors*, **2020**.
44. Song, I.-H.; Torawane, P.; Lee, J.-S.; Warkad, S. D.; Borase, A.; Sahoo, S. K.; Nimse, S. B.; Kuwar, A., The detection of Al^{3+} and Cu^{2+} ions using isonicotinohydrazide-based chemosensors and their application to live-cell imaging. *Mater. Adv.* **2021**, *2* (19), 6306-6314.
45. BindFit, <http://app.supramolecular.org/bindfit/> (accessed June 2023).
46. Grzyska, P. K.; Hausinger, R. P.; Proshlyakov, D. A., Metal and substrate binding to an Fe(II) dioxygenase resolved by UV spectroscopy with global regression analysis. *Anal. Biochem.* **2010**, *399* (1), 64-71.
47. Pedersen, C. J., Cyclic polyethers and their complexes with metal salts. *J. Am. Chem. Soc.* **1967**, *89* (26), 7017-7036.
48. Pedersen, C. J.; Frensdorff, H. K., Macrocyclic polyethers and their complexes. *Angew. Chem. Int. Ed. Engl.* **1972**, *11* (1), 16-25.
49. Mondal, D.; Ahmad, M.; Dey, B.; Mondal, A.; Talukdar, P., Formation of supramolecular channels by reversible unwinding-rewinding of bis(indole) double helix via ion coordination. *Nat. Commun.* **2022**, *13* (1), 6507.
50. Rambo, B. M.; Kim, S. K.; Kim, J. S.; Bielawski, C. W.; Sessler, J. L., A benzocrown-6-calix[4]arene methacrylate copolymer: Selective extraction of caesium ions from a multi-component system. *Chem. Sci.* **2010**, *1* (6), 716-722.

51. Riddell, F. G.; Arumugam, S.; Brophy, P. J.; Cox, B. G.; Payne, M. C. H.; Southon, T. E., The nigericin-mediated transport of sodium and potassium ions through phospholipid bilayers studied by sodium-23 and potassium-39 NMR spectroscopy. *J. Am. Chem. Soc.* **1988**, *110* (3), 734-738.
52. L. Murray, C.; W. Gokel, G., Cation flux dependence on carbon chain length in hydrophile channels as assessed by dynamic ^{23}Na NMR methods in phospholipid bilayers. *Chem. Commun.* **1998**, 22, 2477-2478.
53. Shabany, H.; Gokel, G. W., Enhancement of cation transport in synthetic hydrophile channels having covalently-linked headgroups. *Chem. Commun.* **2000**, 23, 2373-2374.
54. Shabany, H.; Murray, C. L.; Gloeckner, C. A.; Grayson, M. A.; Gross, M. L.; Gokel, G. W., Evidence for multiple alkali metal cation complexation in membrane-spanning ion transporters. *Chem. Commun.* **2000**, 23, 2375-2376.
55. Madhavan, N.; Robert, E. C.; Gin, M. S., A highly active anion-selective aminocyclodextrin ion channel. *Angew. Chem. Int. Ed.* **2005**, *44* (46), 7584-7587.
56. De Cola, C.; Lichen, S.; Comegna, D.; Cafaro, E.; Bifulco, G.; Izzo, I.; Tecilla, P.; De Riccardis, F., Size-dependent cation transport by cyclic α -peptoid ion carriers. *Org. Biomol. Chem.* **2009**, *7* (14), 2851-2854.
57. Madhavan, N.; Gin, M. S., Increasing pH Causes faster anion- and cation-transport rates through a synthetic ion channel. *ChemBioChem.* **2007**, *8* (15), 1834-1840.

Chapter 4

Mandelic Acid-Based Small Molecules as K^+/Cl^- Symporters



4.1. Introduction:

The previous two chapters demonstrated the importance of selective anion (Cl^-) and cation (K^+) transport separately. However, designing a transporter that can selectively transport both Cl^- and K^+ is challenging because mimicking the selectivity filter already present in the naturally occurring KCl co-transporters is quite tedious.¹ It is crucial for controlling neurotransmission², heart rhythm³, cell homeostasis⁴, muscular contraction⁵, and the resting potential of the cell⁶. The potassium-chloride cotransporters KCC1 to KCC4 perform vital functions in controlling cell volume, the operation of the auditory system, and inhibitory neurotransmission mediated by glycine and aminobutyric acid (GABA). They facilitate the coupled export of potassium and chloride across the plasma membrane. However, it is more difficult and less studied upon, to co-transport cations and anions through biological membranes by a single synthetic molecule.

The supramolecular host-salt complex that forms in situations when the bonded anion and cation have the same charge will be neutral. This results in the complex having the best solubility in organic solvents, which is beneficial when using these systems as transmembrane carriers for charged species or in extraction operations. There are numerous cation-anion co-transporters that can simultaneously transport both type of ions, including K-Cl, Na-Cl and Na-K-Cl symporters.⁷⁻¹⁰ The malfunctioning of these symporters is closely related to certain lethal diseases such as Bartter syndrome, Gitelman syndrome, deafness, renal tubular acidosis, and thyroid disorders because of the critical roles they play in crucial processes like augmentation of neurons, synaptic plasticity, and cellular communication. Some symporters may stimulate the transport of an ion against its concentration gradient by combining the mobility of one ion with an upsurge in the electrochemical gradient.¹¹

Smith and coworkers reported the first synthetic KCl symporter where they employed a ditopic receptor for transporting both NaCl and KCl.¹² Kyu-Sung Jeong and coworkers followed close in line, using an azacrown as a binding moiety for K^+ and urea as a binding moiety for Cl^- .¹¹ Calix[4]pyrrole was developed by Gale and co-workers as RbCl and CsCl transporters where the binding to Cl^- happened via H-bonding whereas Cs^+ was strongly anchored to the pyrrole rings.¹³ A ditopic transport system engaging halogen bonding was also reported by Matile and coworkers.¹⁴ The mode of mechanism of these systems is the transport of anion after being activated by the cation. The most recent instance of a metal halide symporter was illustrated by

Madhavan and co-workers, employing a peptide connected to an aminopicolinic acid unit. This type of transport system was shown to prefer cations over anions.¹⁵

Mandelic acid (MA) is a popular AHA (α -hydroxy acid) that is frequently used as an antibacterial and antiseptic, for skin treatments.^{16, 17} Additionally, it has been used to treat urinary tract infections and as an antibacterial agent. Due to its promotion of collagen production, the primary protein present in skin and connective tissue, MA also improves the appearance of the skin.¹⁸⁻²⁰

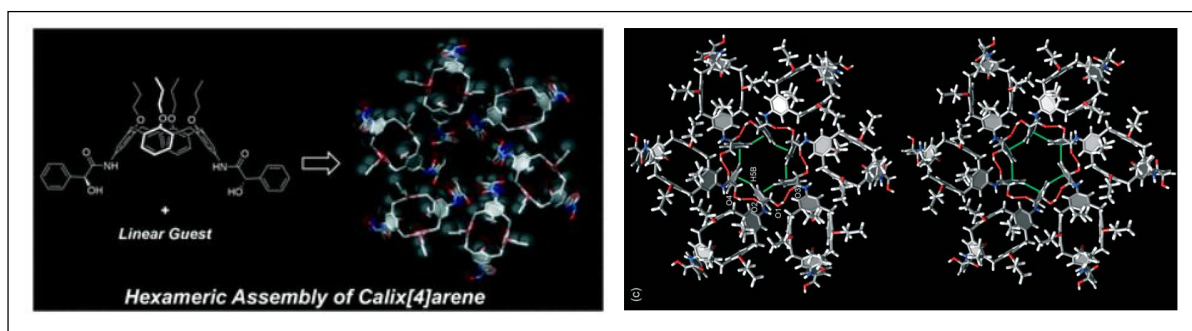


Figure 4.1. Hexameric self-assembly based on hydrogen bonding and C-H $\cdots\pi$ interactions.²¹

Intrigued by the hexameric crystal structures that were obtained using Mandelic acid as the core as seen in Figure 4.1, reported by Haino and co-workers²¹, we reflected on using the same moiety, to report a series of small molecules based on the same core, which were hypothesized to form self-assembled barrel-rosette channel systems. It was proposed that the intermolecular hydrogen bonding and the π - π interactions would provide a pre-organization to the individual structures that would form an active channel system in the bilayer membrane. (Figure 4.2).

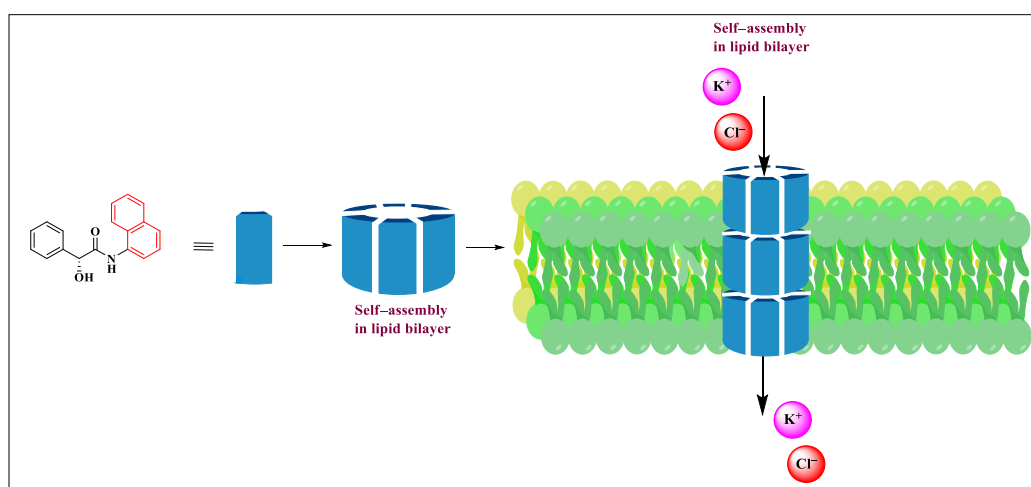


Figure 4.2. Representative mechanism of co-transport across the cell membrane by the channel molecule.

4.2. Results and discussion:

4.2.1. General methods:

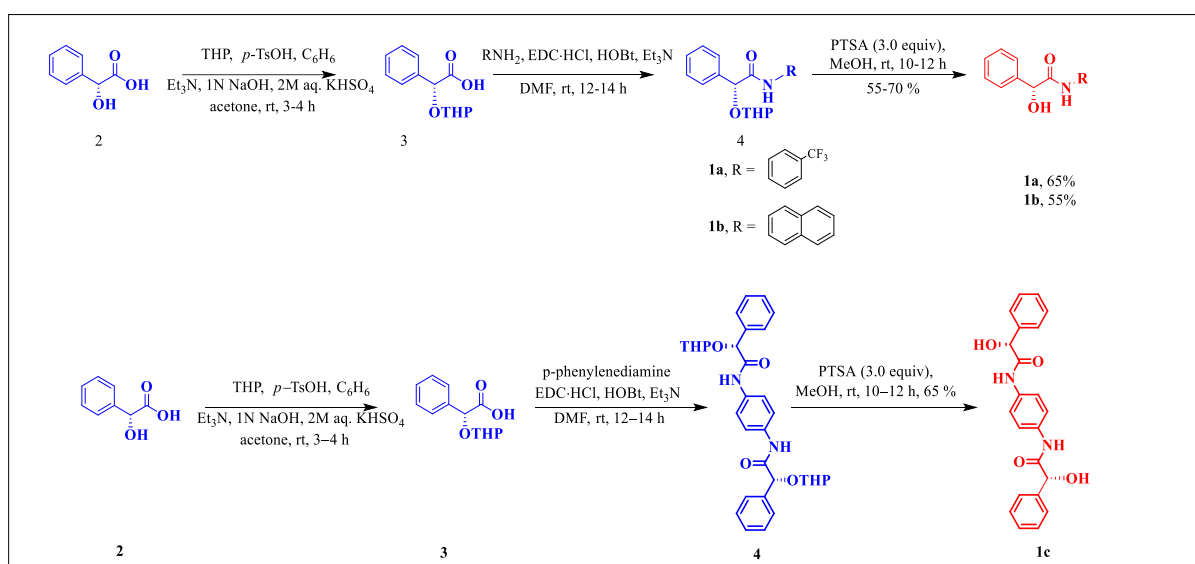
The general methods are similar as have been described in the previous chapters.

4.2.2. Physical measurements:

The physical measurements are similar as have been described in the previous chapters.

The three derivatives **1a-1c** were synthesized according to the scheme discussed below (Scheme 4.1) and they were characterized by their ^1H , ^{19}F , ^{13}C NMR, HRMS and IR data.

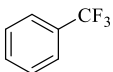
4.2.3. Synthesis:

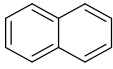
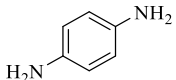


Scheme 4.1. Synthetic scheme of compounds **1a-1c**.

Estimation of pK_a and $\log P$ of **1a-1c**:

Table 1. The pK_a and $\log P$ values of **1a-1c**:

Compound	R (para position)	pK_a of (N-H)	$\log P$
1a		14.22	3.37

1b		11.96	3.13
1c		14.14	2.69

S(2R)-2-phenyl-2-((tetrahydro-2H-pyran-2-yl) oxy) acetic acid 2:

To benzene (100 mL) in a single-necked round bottomed flask (*R*)-(+)-mandelic acid (5.0 g, 32.86 mmol) was added followed by 3,4-dihydro-2H-pyran (9.4 mL, 103 mmol) and *p*-TsOH (10 mg). Mandelic acid was dissolved over 1 hour and stirring was continued at room temperature for 1 more hour. Sufficient amount of Et_3N was added and the volatile material was carefully removed under vacuum on a rotary evaporator. Thereafter, the oily residue was dissolved in acetone (100 mL) and 1N NaOH (40 mL) was added. After stirring at room temperature for 2 hours, acetone was evaporated, H_2O (50 mL) was added and the alkaline solution was extracted with $CHCl_3$ (3×50 mL). To the remaining water solution $CHCl_3$ (120 mL) was added. The flask was introduced in a water-bath at room temperature and 2 M aqueous $KHSO_4$ solution (45 mL) was added under vigorous stirring. The organic layer was separated and the aqueous solution was extracted with $CHCl_3$ (2×50 mL). The $CHCl_3$ extracts were combined and washed with H_2O (2×50 mL) and brine (50 mL). The extract was dried over anhydrous Na_2SO_4 , filtered, evaporated and the oily residue **2** was obtained as a mixture of diastereomers was confirmed by HRMS. **HRMS (ESI):** Calc. for $C_{24}H_{22}F_6N_2O_3$ $[M+H]^+$: 237.1021; Found: 237.1085.

General method for the synthesis of 3a-3c:

For the synthesis of **3a-3c**, in a 25 mL round bottomed flask, a mixture of compound **2** (500 mg, 2.12 mmol, 1.00 equiv) in dry DMF (10 mL) was stirred at room temperature for some time. Then, Et_3N (590 μ L, 4.23 mmol, 2.5 equiv) was added to the solution. To the mixture, EDC·HCl (608 mg, 3.17 mmol, 1.5 equiv) and HOBt (372 mg, 2.75 mmol, 1.25 equiv) were added. Stirring was maintained for further 30 min. The corresponding amines (2.12 mmol, 1.0 equiv) were added thereafter, as mentioned in Scheme 4.1. The reaction mixture was further stirred at room temperature for 12 hours.

For the synthesis of **3c**, in a 25 mL round bottomed flask, compound **2** (200 mg, 0.846 mmol, 3.00 equiv) in dry DMF (10 mL) was stirred at room temperature for some time. Then, Et_3N (275 μ L, 1.98 mmol, 7.0 equiv) was added to the solution. To the mixture, EDC·HCl (190 mg, 0.987 mmol, 3.5 equiv) and HOBt (133 mg, 0.987 mmol, 3.5 equiv) were added. Stirring was maintained for further 30 min. p-phenylene diamine (30.51 mg, 2.12 mmol, 1.0 equiv) was added thereafter, as mentioned in Scheme 4.1. This reaction mixture was stirred at room temperature for 12 hours. The progress of the reaction mixtures was monitored by thin layer chromatography. After completion of the reactions, brine solution was added to the reaction mixture followed by extraction with $CHCl_3$ (10 mL $\times$ 3 times). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo* to obtain diastereomeric mixtures of compounds **3a-3c** which were characterized by their HRMS data.

HRMS (ESI): Calc. for **3a**, $C_{20}H_{20}F_3NO_3$ $[M+Na]^+$: 470.1166; Found: 470.1166. **HRMS (ESI):** Calc. for **3b**, $C_{23}H_{23}NO_3$ $[M+Na]^+$: 384.1575; Found: 384.1575. **HRMS (ESI):** Calc. for **3c**, $C_{32}H_{36}N_2O_6$ $[M+Na]^+$: 567.2470; Found: 567.2474.

General method for the synthesis of **1a-1c**:

In a 25 mL round bottomed flask, compounds **3a-3c** (200 mg, 0.527 mmol, 1.0 equiv) and p-toluene sulfonic acid (PTSA) (273 mg, 1.58 mmol, 3.0 equiv) in dry MeOH (10 mL) was stirred at r.t. for 2-3 hours. The progress of the reaction mixture was monitored by thin layer chromatography. MeOH was then evaporated. Then, water was added to the reaction mixture followed by extraction with $CHCl_3$ (10 mL $\times$ 3 times). The combined organic phases were washed with brine and dried over Na_2SO_4 and concentrated *in vacuo* to obtain the final series of compounds **1a-1c** which were characterized by their 1H , ^{13}C and ^{19}F NMR, HRMS and IR data.

(R)-2-hydroxy-N-(naphthalen-1-yl)-2-phenylacetamide 1a ($C_{19}H_{19}NO_2$): The crude product was purified by column chromatography over silica gel (25% EtOAc in petroleum ether) to furnish **1a** as a white crystalline solid (Yield: 65%) **1H NMR (400 MHz, $CDCl_3$):** δ 8.40 (s, 1H), 7.69 (d, J = 8.6 Hz, 2H), 7.58 (d, J = 8.6 Hz, 2H), 7.50 (dd, J = 7.9, 1.5 Hz, 2H), 7.45 – 7.41 (m, 1H), 7.41 – 7.38 (m, 2H), 5.26 (d, J = 2.5 Hz, 1H), 3.16 (d, J = 3.0 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$):** δ 170.36, 138.98, 133.84, 131.23, 128.90, 128.78, 128.63, 126.72, 126.48, 126.25, 125.85, 125.66, 125.56, 119.89, 119.72, 74.78. **HRMS (ESI):** Calc. for $C_{15}H_{12}F_3NO_2$ $[M+Na]^+$: 318.0712; Found: 318.0713.

(R)-2-hydroxy-2-phenyl-N-(4-(trifluoromethyl) phenyl) acetamide 1b ($C_{15}H_{12}F_3NO_2$): 1H NMR (400 MHz, $CDCl_3$): The crude product was purified by column chromatography over silica gel (20% EtOAc in petroleum ether) to furnish **1b** as a light pink sticky solid (Yield: 55%) δ 8.55 (s, 1H), 8.02 (d, J = 7.5 Hz, 1H), 7.89 – 7.83 (m, 1H), 7.69 (d, J = 8.2 Hz, 1H), 7.65 – 7.56 (m, 3H), 7.52 – 7.39 (m, 6H), 5.37 (s, 1H), 3.55 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.56, 141.10, 140.09, 128.89, 128.65, 127.08, 126.49, 126.45, 119.66, 74.68, 40.74, 40.53, 40.32, 40.12, 39.91, 39.69. HRMS (ESI): Calc. for $C_{15}H_{12}F_3NO_2$ $[M+Na]^+$: 300.0995; Found: 300.0900.

(2R,2'R)-N, N'-(1,4-phenylene)bis(2-hydroxy-2-phenylacetamide) 1c ($C_{22}H_{20}N_2O_4$): The crude product was purified by column chromatography over silica gel (45% EtOAc in petroleum ether) to furnish **1c** as a greyish solid (Yield : 50%) 1H NMR (400 MHz, $DMSO-d_6$): δ 9.85 (s, 2H), 7.60 (s, 3H), 7.54 – 7.44 (m, 4H), 7.38 – 7.32 (m, 5H), 7.31 – 7.26 (m, 2H), 6.40 (d, J = 4.7 Hz, 2H), 5.08 (d, J = 4.7 Hz, 2H). ^{13}C NMR (101 MHz, $DMSO-d_6$): δ 170.21, 140.24, 133.57, 127.42, 126.93, 125.91, 119.28, 73.31. HRMS (ESI): Calc. for $C_{22}H_{20}N_2O_4$ $[M+H]^+$: 377.1496; Found: 377.1479.

4.2.4. Ion transport studies:

4.2.4.1. Buffer and stock solution preparation:

Buffer solutions were prepared as mentioned in the previous chapters. Stock solutions of all compounds were prepared in HPLC grade DMSO.

4.2.4.2. Preparation of EYPC-LUVs $\Rightarrow$ HPTS:

Vesicles were prepared according to the reported protocol.^{22, 23}

4.2.4.3. Comparison of ion transport activities:

Ion transport activity of the mandelic acid series was investigated using the reported protocol as mentioned in the previous chapters.

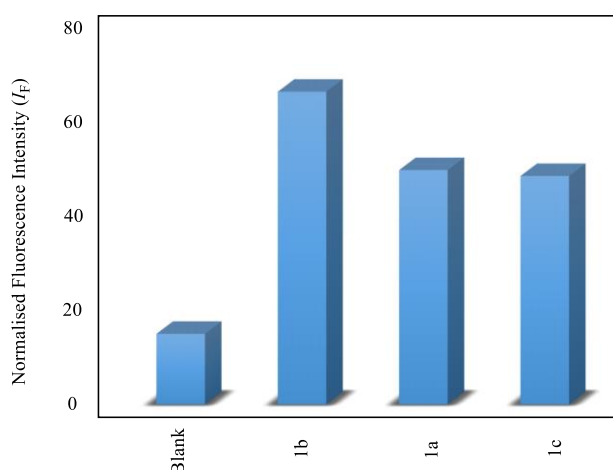


Figure 4.3. Bar diagram representing the comparison of activities of **1a-1c** at 40 μM .

On investigating the ion transport activity of the synthesized derivatives **1a-1c** at 40 μM each, across EYPC-LUV $\supset$ HPTS, it was seen that **1b** is the most active compound of the series. (Figure 4.3)

4.2.4.5. Calculation of EC_{50} and Hill-coefficient (n) values:

After determining the most efficient transporter, the dose-dependent ion transport kinetic studies were studied at gradually increasing concentrations of compound **1b**. The change of HPTS emission intensity in this process was monitored with time. The concentration profile data were analyzed by the Hill Equation mentioned in the previous chapters to get the effective activity at half-maximal concentration (EC_{50}) and the Hill coefficient value (n).

As evident from Figure 4.4, an EC_{50} value of 7.54 μM was obtained for the most active derivative **1b**. A Hill-coefficient (n) value of 1 indicated that one molecule of **1b** formed the active transport system.

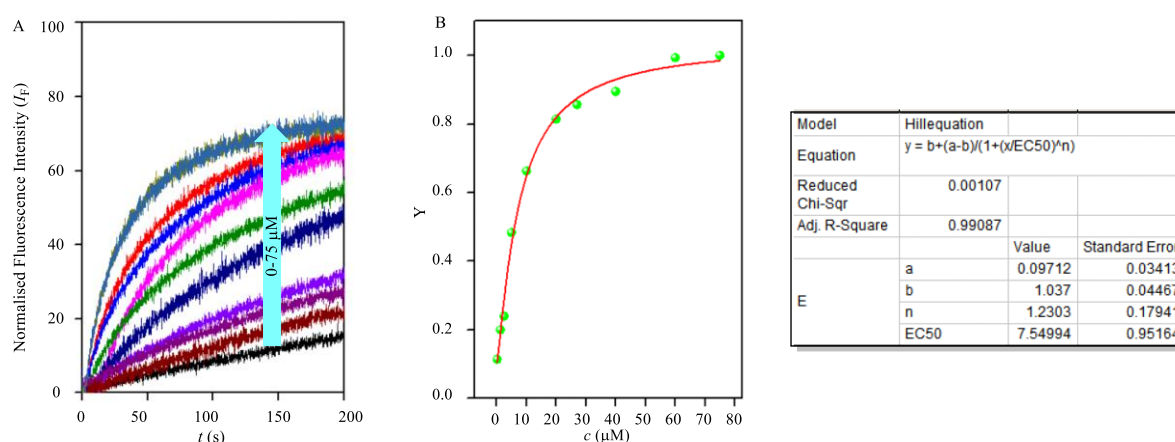


Figure 4.4. Dose-response curves and Hill analysis of compound **1b** across EYPC-LUVs $\supset$ HPTS at $t = 190$ s.

In this context, the significance of a Hill-coefficient value of n approximately equal to 1 is important. Exergonic self-assembly is the primary mechanism by which active, efficient supramolecular structures originate when $n = 1$. Since they already begin to self-assemble in the media into pre-pores with hydrophobic exterior surfaces that tend to precipitate before incorporating into the lipid bilayer membrane, these stable synthetic multifunctional structures create a challenge.^{24, 25} Linear supramolecular structures with $n \approx 1$ indicate monomeric pores. Moreover, stable oligomers are found in linear concentration profiles with $n \approx 1$ as the overall relevant populations.^{26, 27}

4.2.5. Determination of ion selectivity by HPTS assay:²⁸

To determine the favourable transport of anions and cations, the selectivity studies of **1b** were investigated using the HPTS assay.

4.2.5.1. Buffer and stock solution preparation: The buffer and stock solutions were prepared according to the protocol as mentioned in the previous chapters.

4.2.5.2. Preparation of EYPC-LUVs $\Rightarrow$ HPTS for cation and anion selectivities:²⁹

Vesicles were prepared as described in the preceding chapters.

4.2.5.3. Cation selectivity assay: Cation selectivity of **1b** ($8 \mu\text{M}$) was investigated and fluorescence intensities (F_t) were normalized to fractional emission intensity I_F as mentioned in the previous chapters.

4.2.5.4. Anion selectivity assay: Anion selectivity of **1b** ($8 \mu\text{M}$) was investigated and fluorescence intensities (F_t) were normalized to fractional emission intensity I_F as mentioned in the previous chapters.

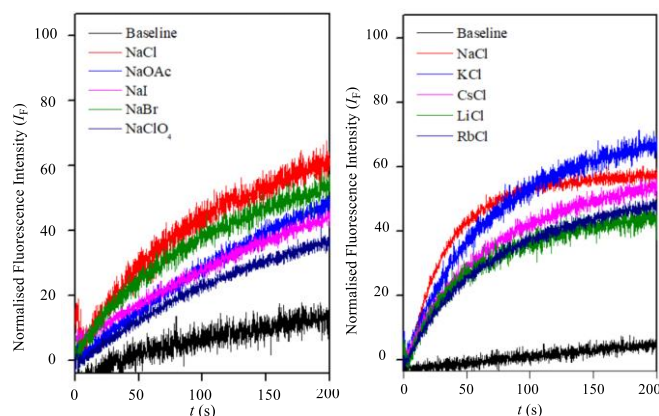


Figure 4.5. Determination of cation selectivity (A) and anion selectivity (B) of **1b** at 8 μ M across EYPC-LUVs $\Rightarrow$ HPTS.

On inspecting the ion selectivity studies of **1b**, it was concluded that for the compound **1b**, the selectivity towards transport of cations followed the order $K^+ > Na^+ > Cs^+ > Rb^+ > Li^+$ whereas that for the anions followed the order $Cl^- > Br^- > OAc^- > I^- > ClO_4^-$. This means that **1b** was selective towards both K^+ and Cl^- ions, that is, it indicated an interesting symporting efficiency. (Figure 4.5).

4.2.6. Determination of mechanism of ion transport by HPTS assay:

4.2.6.1. Preparation of vesicles, buffer and stocks for FCCP and Valinomycin assays:³⁰

The vesicles, buffer and stock solutions for the FCCP and Valinomycin assays were prepared following the same procedure as mentioned in the earlier chapters.

4.2.6.3. Ion transport mechanism study in presence of FCCP and Valinomycin:³¹

Since the ion selectivity studies pointed towards a possible co-transport of K^+ and Cl^- by compound **1b**, the HPTS assay was carried out in the presence of electroneutral and the electrogenic H^+ and the K^+ carriers, FCCP and Valinomycin, respectively, following the similar procedures as described in the previous chapters.

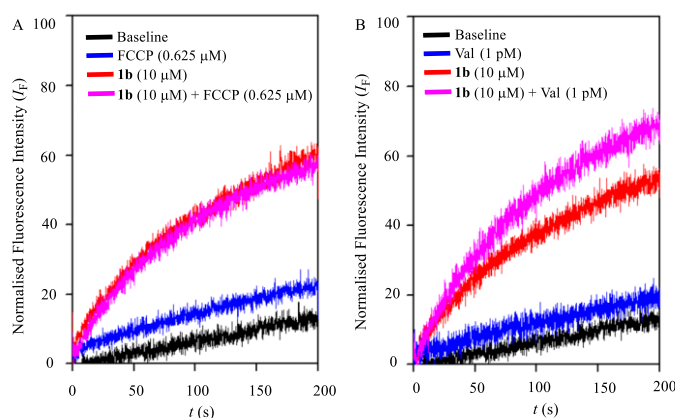


Figure 4.6. Ion transport activity of **1b** (10 μ M) in the presence of FCCP (0.625 μ M) (A) and valinomycin (1 pM) (B) across EYPC-LUVs $\Rightarrow$ HPTS.

There was no enhancement of ion transport activity in the presence of FCCP due to the coupling of transporter **1b** and FCCP, compared to **1b** alone, indicating that the M^+/Cl^- symport could

be an operating mechanism. However, enhanced transport activity in the presence of valinomycin further suggested the same. (Figure 4.6).

4.2.7. Chloride transport activity across EYPC-LUVs $\supset$ lucigenin vesicles:

4.2.7.1. Preparation of EYPC-LUVs $\supset$ lucigenin vesicles:³²

The vesicles for studying the ion transport activity by the lucigenin assay were prepared following the previously reported protocols.

4.2.7.2. Ion transport activity by Lucigenin assay:³³

Ion transport activity was investigated using the reported protocol as mentioned in the previous chapters.

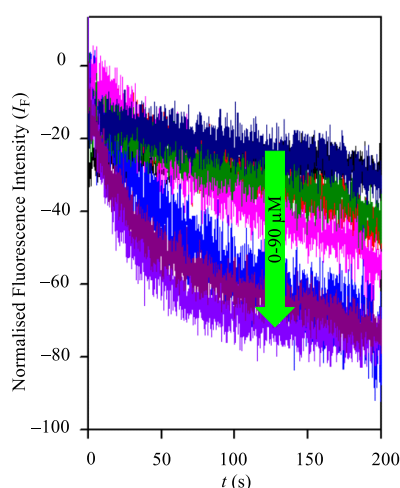


Figure 4.7. Efflux of Cl^- by **1b** across EYPC-LUVs $\supset$ Lucigenin in a concentration dependant manner.

It is evident from the quenching of the fluorescence of the lucigenin dye that chloride influx occurs by **1b** in a dose-dependent manner. (Figure 4.7)

4.2.8. Determination of mechanism of ion transport using the lucigenin assay:

4.2.8.1. Preparation of EYPC-LUVs $\supset$ lucigenin vesicles:³⁴

The vesicles were prepared using the procedures that were previously described in the preceding chapters. The lucigenin assay in the presence of valinomycin was used to experimentally establish the symport mechanism of **1b**, which is the simultaneous transport of two ions with different charges moving in the same direction, across the membrane. In vesicles entrapped with lucigenin (1 mM) and $NaNO_3$ (200 mM) suspended in KCl (2M) solution with and without valinomycin, the ion transport activity of **1b** was observed. Direct experimental

insight into the symporting mechanism of ion transport was provided by the identical rates of ion transport activity of **1b** in the presence and absence of valinomycin.

4.2.8.2. Determination of the symport mechanism:³⁵

In a clean and dry fluorescence cuvette 1975 μL 200 mM NaNO_3 solution and 25 μL EYPC–LUVs $\Rightarrow$ lucigenin vesicles were taken and slowly stirred in a fluorescence instrument equipped with a magnetic stirrer (at $t = 0$ s). The time-dependent fluorescence intensity of lucigenin was monitored at $\lambda_{\text{em}} = 535$ nm and $\lambda_{\text{ex}} = 455$ nm. A solution of 2M KCl (33.3 μL) was added at $t = 20$ s to create a chloride gradient between intra and extra vesicular systems, followed by the addition of valinomycin (1.25 μM) at $t = 50$ s and transporter **1b** (60 μM) at $t = 100$ s. Finally, the destruction of the chloride gradient and complete disintegration of the vesicles was done by the addition of 10% Triton X–100 (25 μL) at $t = 300$ s. The time-dependent data were normalized using previously mentioned equations.

When investigated at a concentration of 60 μM , valinomycin showed minimal impact on the ion transport activity of **1b**, providing significant experimental insight into the symport mechanism of transmembrane ion transport. (Figure 4.8)

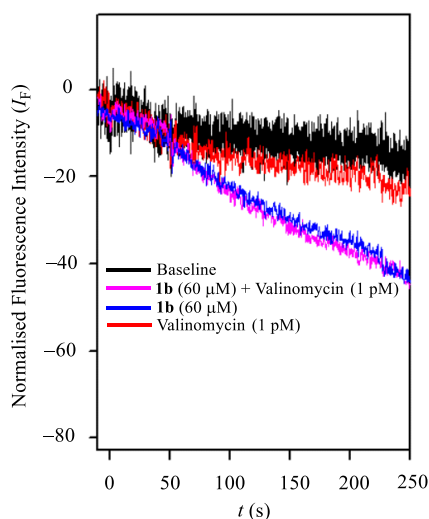


Figure 4.8. Estimation of symport mechanism of **1b** in the presence of valinomycin.

4.2.9. Estimation of integrity of bilayer membrane by CF leakage assay:²⁹

4.2.9.1. Preparation of EYPC–LUVs $\Rightarrow$ CF:

Vesicles were prepared following the protocol as mentioned in the previous chapters.

4.2.9.2. CF leakage assay:

As mentioned in the previous chapters, ion transport activity was investigated into in order to determine if the bilayer membrane retained its integrity.

From the graph below, it is evident that membrane integrity is maintained and **1b** is not causing the bilayer membrane to leak since there was no change in the fluorescence of CF even at elevated concentrations of **1b**. (Figure 4.9)

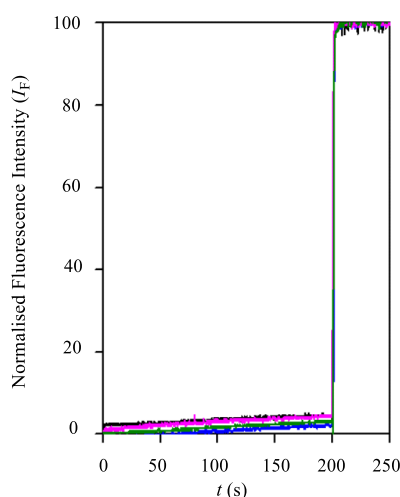


Figure 4.9. Evidence of membrane integrity at 8 μ M, 10 μ M, 15 μ M and 20 μ M of **1b** using EYPC LUVs $\rightarrow$ CF.

4.2.10. Single crystal X-ray diffraction (SCXRD) studies:

A solution of compounds **1a** and **1b** in HPLC grade ACN was made and the solution was left undisturbed in the closed vial at ambient temperature for seven days. Crystals for compound **1c** was grown in a mixture of HPLC grade $CHCl_3$ and Acetone (1:1) (v/v). The suitable colourless rectangular white crystals for each compound were obtained and analysed in single crystal XRD.

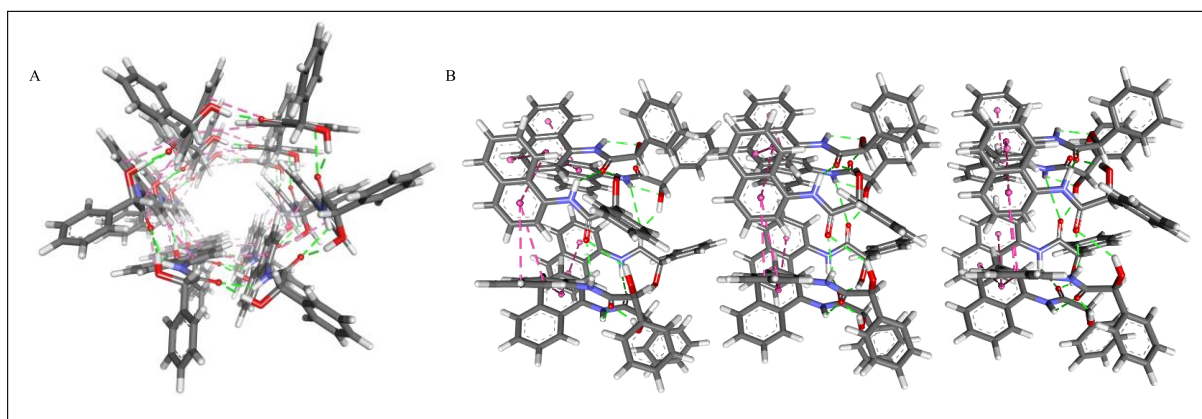


Figure 4.10. (A) Top view and (B) side view of **1b**.

The crystal structure of the most active molecule, **1b**, revealed a barrel rosette-like assembly. from the top and the side views (Figure 4.10A and B). A hexameric assembly could be seen in each layer, where six molecules arranged themselves, well in accordance with the observation reported by Haino and co-workers²¹. Based on the length, it was hypothesized that three layers would be needed to span the lipid bilayer membrane.

The calculated diameter from the geometry optimized structure was found to be 7.9 Å. It has been hypothesized that the possible mode of binding and transport of the cation and the anion could be through the cation- π interaction of the cation to the pi cloud of the aromatic benzene ring and the hydrogen bonding interactions of the anion with the hydroxyl –OH and the amide –NH.

However, further detailed computational molecular modelling studies will be done to validate the experimental findings and to ascertain the possible self-assembly of **1b**, the most active system.

Single crystal X-ray crystallographic analysis of **1a**, **1b** and **1c**:

The single-crystal X-ray data of compounds **1a**, **1b** and **1c** was recorded at respective temperatures mentioned below on a Bruker KAPPA APEX II CCD Duo diffractometer (operated at 1500 W power: 50 kV, 30 mA) using graphite-mono chromated Mo $K\alpha$ radiation ($\lambda = 0.71073$ Å). The crystal was kept on a nylon Cryo-Loops (Hampton Research) with Paratone-N (Hampton Research). The data reduction and integration were computed with SAINT software.³⁶ A correction on multi-scan absorption was employed on the collected reflections. The structure solving was achieved by the direct method using SHELXTL³⁷ and was further refined on F^2 by full-matrix least-squares technique using the SHELXL-97³⁸ program package within the WINGX³⁹ program. All the non-hydrogen atoms were refined anisotropically. All hydrogen atoms were located in successive difference Fourier maps, and they were treated as riding atoms using SHELXL default parameters. The structures were examined using the *Adsym* subroutine of PLATON⁴⁰ to ensure that no additional symmetry could be applied to the models.

Table 2. Crystal data and structure refinement for **1a**:

Empirical formula	C ₁₅ H ₁₂ F ₃ NO ₂
Formula weight	295.26

Temperature	273(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2 ₁ /c	
Unit cell dimensions	a = 14.766(6) Å	a = 90°.
	b = 11.262(5) Å	b = 92.842(13)°
	c = 8.354(4) Å	g = 90°.
Volume	1387.6(11) Å ³	
Z	4	
Density (calculated)	1.413 Mg/m ³	
Absorption coefficient	0.121 mm ⁻¹	
F(000)	608	
Crystal size	0.300 x 0.300 x 0.200 mm ³	
Theta range for data collection	2.275 to 24.995°.	
Index ranges	-16 ≤ h ≤ 17, -13 ≤ k ≤ 13, -9 ≤ l ≤ 9	
Reflections collected	15505	
Independent reflections	2440 [R(int) = 0.1093]	
Completeness to theta = 24.995°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.93 and 0.89	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	2440 / 271 / 223	
Goodness-of-fit on F ²	1.022	
Final R indices [I > 2σ(I)]	R1 = 0.0602, wR2 = 0.1298	
R indices (all data)	R1 = 0.1481, wR2 = 0.1664	
Extinction coefficient	0.015(3)	
Largest diff. peak and hole	0.243 and -0.217 e.Å ⁻³	

Table 3. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$)

For **1a**, $U(\text{eq})$ is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	$U(\text{eq})$
--	---	---	---	----------------

C(1)	3830(2)	1386(4)	7448(4)	50(1)
C(2)	3011(2)	1952(4)	7693(4)	60(1)
C(3)	2424(2)	1472(5)	8773(4)	68(1)
C(4)	2638(3)	446(5)	9565(4)	69(1)
C(5)	3449(2)	-120(4)	9345(4)	65(1)
C(6)	4047(2)	366(4)	8290(4)	58(1)
C(7)	4457(2)	1868(4)	6231(4)	50(1)
C(8)	5418(2)	1997(4)	6970(4)	49(1)
C(9)	7000(2)	1285(4)	6717(4)	46(1)
C(10)	7444(2)	2158(4)	7636(4)	53(1)
C(11)	8379(2)	2109(4)	7868(4)	57(1)
C(12)	8868(2)	1206(4)	7203(4)	56(1)
C(13)	8427(2)	353(4)	6277(4)	62(1)
C(14)	7494(2)	394(4)	6029(4)	58(1)
C(15)	9870(3)	1180(5)	7478(5)	69(1)
N(1)	6050(2)	1282(3)	6389(3)	48(1)
O(1)	4412(1)	1105(2)	4870(2)	52(1)
O(2)	5571(1)	2739(3)	8032(3)	56(1)
F(1)	10139(3)	1221(9)	9013(5)	84(2)
F(2)	10233(5)	96(7)	7092(10)	102(3)
F(3)	10299(6)	1886(9)	6653(13)	129(4)
F(1')	10189(9)	256(13)	8010(20)	132(5)
F(2')	10205(4)	2188(13)	8249(19)	109(5)
F(3')	10260(9)	1399(13)	6112(12)	91(4)

Table 4. Bond lengths [Å] and angles [°] for 1a:

C(1)-C(6)	1.376(5)
C(1)-C(2)	1.391(5)
C(1)-C(7)	1.510(4)
C(2)-C(3)	1.391(5)
C(2)-H(2)	0.9300

C(3)-C(4)	1.361(6)
C(3)-H(3)	0.9300
C(4)-C(5)	1.378(5)
C(4)-H(4)	0.9300
C(5)-C(6)	1.391(5)
C(5)-H(5)	0.9300
C(6)-H(6)	0.9300
C(7)-O(1)	1.425(4)
C(7)-C(8)	1.526(4)
C(7)-H(7)	0.9800
C(8)-O(2)	1.232(4)
C(8)-N(1)	1.340(4)
C(9)-C(14)	1.382(5)
C(9)-C(10)	1.391(5)
C(9)-N(1)	1.416(4)
C(10)-C(11)	1.386(5)
C(10)-H(10)	0.9300
C(11)-C(12)	1.380(5)
C(11)-H(11)	0.9300
C(12)-C(13)	1.376(5)
C(12)-C(15)	1.487(5)
C(13)-C(14)	1.384(4)
C(13)-H(13)	0.9300
C(14)-H(14)	0.9300
C(15)-F(1')	1.216(10)
C(15)-F(3)	1.247(7)
C(15)-F(1)	1.324(6)
C(15)-F(3')	1.326(10)
C(15)-F(2)	1.377(8)
C(15)-F(2')	1.385(8)
N(1)-H(1A)	0.926(18)
O(1)-H(1)	0.8200
C(6)-C(1)-C(2)	119.3(3)

C(6)-C(1)-C(7)	120.6(3)
C(2)-C(1)-C(7)	120.1(4)
C(1)-C(2)-C(3)	119.5(4)
C(1)-C(2)-H(2)	120.3
C(3)-C(2)-H(2)	120.3
C(4)-C(3)-C(2)	120.5(4)
C(4)-C(3)-H(3)	119.7
C(2)-C(3)-H(3)	119.7
C(3)-C(4)-C(5)	120.7(4)
C(3)-C(4)-H(4)	119.7
C(5)-C(4)-H(4)	119.7
C(4)-C(5)-C(6)	119.1(4)
C(4)-C(5)-H(5)	120.4
C(6)-C(5)-H(5)	120.4
C(1)-C(6)-C(5)	120.9(4)
C(1)-C(6)-H(6)	119.6
C(5)-C(6)-H(6)	119.6
O(1)-C(7)-C(1)	108.4(3)
O(1)-C(7)-C(8)	112.7(3)
C(1)-C(7)-C(8)	110.6(3)
O(1)-C(7)-H(7)	108.4
C(1)-C(7)-H(7)	108.4
C(8)-C(7)-H(7)	108.4
O(2)-C(8)-N(1)	124.2(3)
O(2)-C(8)-C(7)	119.3(3)
N(1)-C(8)-C(7)	116.5(3)
C(14)-C(9)-C(10)	119.9(3)
C(14)-C(9)-N(1)	117.1(3)
C(10)-C(9)-N(1)	122.9(3)
C(11)-C(10)-C(9)	119.2(4)
C(11)-C(10)-H(10)	120.4
C(9)-C(10)-H(10)	120.4
C(12)-C(11)-C(10)	120.7(4)
C(12)-C(11)-H(11)	119.7

C(10)-C(11)-H(11)	119.7
C(13)-C(12)-C(11)	119.9(3)
C(13)-C(12)-C(15)	120.9(4)
C(11)-C(12)-C(15)	119.3(4)
C(12)-C(13)-C(14)	120.1(4)
C(12)-C(13)-H(13)	120.0
C(14)-C(13)-H(13)	120.0
C(9)-C(14)-C(13)	120.2(3)
C(9)-C(14)-H(14)	119.9
C(13)-C(14)-H(14)	119.9
F(3)-C(15)-F(1)	112.1(6)
F(1')-C(15)-F(3')	107.2(10)
F(3)-C(15)-F(2)	102.8(6)
F(1)-C(15)-F(2)	99.0(6)
F(1')-C(15)-F(2')	114.1(8)
F(3')-C(15)-F(2')	95.0(8)
F(1')-C(15)-C(12)	116.0(8)
F(3)-C(15)-C(12)	115.6(6)
F(1)-C(15)-C(12)	113.4(4)
F(3')-C(15)-C(12)	109.5(7)
F(2)-C(15)-C(12)	112.1(5)
F(2')-C(15)-C(12)	112.7(5)
C(8)-N(1)-C(9)	129.0(3)
C(8)-N(1)-H(1A)	118.4(18)
C(9)-N(1)-H(1A)	112.6(18)
C(7)-O(1)-H(1)	109.5

Table 5. Anisotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for 1a:

The anisotropic displacement factor exponent takes the form: $-2p^2 [h^2 a^{*2} U^{11} + 2 h k a^* b^* U^{12}]$

U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
----------	----------	----------	----------	----------	----------

C(1)	25(2)	102(3)	23(2)	-7(2)	-2(1)	-8(2)
C(2)	24(2)	122(4)	33(2)	4(2)	1(2)	2(2)
C(3)	26(2)	138(4)	43(2)	-2(3)	6(2)	-5(2)
C(4)	42(2)	129(4)	36(2)	-3(3)	11(2)	-25(2)
C(5)	48(2)	109(4)	40(2)	0(2)	12(2)	-12(2)
C(6)	38(2)	101(3)	34(2)	-1(2)	10(2)	-5(2)
C(7)	24(2)	100(3)	25(2)	1(2)	3(1)	-2(2)
C(8)	27(2)	96(3)	26(2)	3(2)	7(2)	-7(2)
C(9)	23(2)	92(3)	24(2)	-2(2)	2(1)	-5(2)
C(10)	31(2)	93(3)	36(2)	-8(2)	1(2)	-3(2)
C(11)	32(2)	100(3)	38(2)	-11(2)	-1(2)	-9(2)
C(12)	26(2)	105(3)	36(2)	-12(2)	1(2)	-8(2)
C(13)	32(2)	105(3)	50(2)	-20(2)	9(2)	0(2)
C(14)	31(2)	103(3)	39(2)	-20(2)	2(2)	-6(2)
C(15)	38(2)	107(4)	62(3)	-18(3)	3(2)	-2(3)
N(1)	24(2)	92(3)	27(2)	-10(2)	2(1)	-4(2)
O(1)	26(1)	103(2)	25(1)	-2(1)	1(1)	-4(1)
O(2)	31(1)	104(2)	33(1)	-12(2)	6(1)	-6(1)
F(1)	32(2)	170(7)	49(3)	-9(3)	-11(2)	4(3)
F(2)	38(3)	146(6)	121(6)	-37(5)	-19(4)	9(3)
F(3)	44(3)	174(8)	168(8)	88(7)	-7(5)	-26(5)
F(1')	61(6)	169(11)	162(11)	78(9)	-40(9)	-6(7)
F(2')	32(3)	154(9)	138(9)	-99(7)	-15(5)	7(5)
F(3')	21(4)	153(9)	102(6)	-29(6)	28(4)	-12(5)

Table 6. Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for 1a:

	x	y	z	U(eq)
H(2)	2858	2645	7139	72
H(3)	1881	1856	8955	82
H(4)	2232	123	10262	83

H(5)	3595	-818	9895	78
H(6)	4601	-3	8151	69
H(7)	4240	2656	5895	60
H(10)	7117	2766	8089	64
H(11)	8680	2691	8478	68
H(13)	8756	-251	5818	75
H(14)	7198	-180	5396	69
H(1)	4707	1393	4160	77
H(1A)	5865(19)	690(20)	5670(30)	41(8)

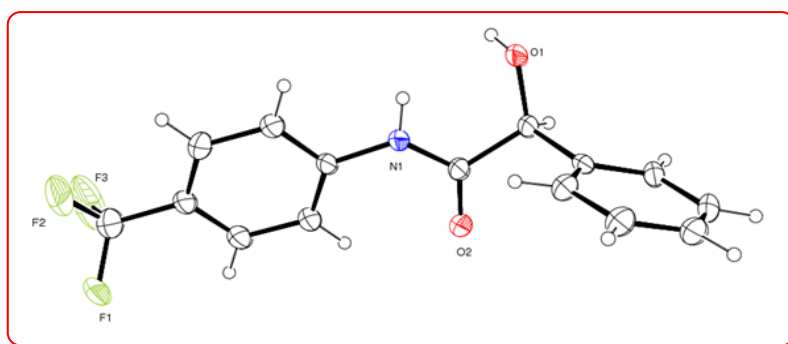


Figure 4.11. ORTEP Image of **1a**.

Table 7. Crystal data and structure refinement for 1b:

Empirical formula	$C_{18}H_{15}NO_2$	
Formula weight	277.31	
Temperature	273(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1/n$	
Unit cell dimensions	$a = 8.423(3)$ Å	$a = 90^\circ$.
	$b = 19.623(6)$ Å	$b = 109.934(9)^\circ$
	$c = 8.941(3)$ Å	$g = 90^\circ$.
Volume	$1389.3(7)$ Å ³	
Z	4	
Density (calculated)	1.326 Mg/m ³	

Absorption coefficient	0.087 mm ⁻¹
F(000)	584
Crystal size	0.350 x 0.200 x 0.200 mm ³
Theta range for data collection	2.076 to 25.997°.
Index ranges	-10<= <i>h</i> <=10, -24<= <i>k</i> <=24, -11<= <i>l</i> <=11
Reflections collected	14886
Independent reflections	2736 [R(int) = 0.0498]
Completeness to theta = 25.242°	100.0 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.97 and 0.93
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	2736 / 7 / 194
Goodness-of-fit on F ²	1.045
Final R indices [I>2sigma(I)]	R1 = 0.0394, wR2 = 0.0939
R indices (all data)	R1 = 0.0498, wR2 = 0.1017
Extinction coefficient	n/a
Largest diff. peak and hole	0.230 and -0.223 e.Å ⁻³

Table 8. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$)

For **1b**, U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	5082(2)	4247(1)	1277(2)	21(1)
C(2)	3616(2)	3830(1)	788(2)	25(1)
C(3)	2340(2)	3931(1)	1383(2)	32(1)
C(4)	2465(2)	4450(1)	2503(2)	33(1)
C(5)	3860(2)	4857(1)	3012(2)	30(1)
C(6)	5223(2)	4764(1)	2436(2)	22(1)
C(7)	6693(2)	5177(1)	2955(2)	25(1)
C(8)	7965(2)	5091(1)	2345(2)	25(1)
C(9)	7817(2)	4596(1)	1165(2)	23(1)

C(10)	6416(2)	4186(1)	647(2)	20(1)
C(11)	7351(2)	3239(1)	-646(2)	19(1)
C(12)	6914(2)	2852(1)	-2226(2)	22(1)
C(13)	8261(2)	2996(1)	-2976(2)	21(1)
C(14)	9854(2)	2704(1)	-2334(2)	26(1)
C(15)	11091(2)	2825(1)	-2999(2)	32(1)
C(16)	10733(2)	3235(1)	-4330(2)	35(1)
C(17)	9147(2)	3522(1)	-4990(2)	36(1)
C(18)	7908(2)	3406(1)	-4312(2)	28(1)
N(1)	6223(1)	3714(1)	-616(1)	22(1)
O(1)	8657(1)	3117(1)	481(1)	24(1)
O(2)	5272(1)	3042(1)	-3232(1)	29(1)

Table 9. Bond lengths [Å] and angles [°] for 1b:

C(1)-C(2)	1.4207(19)
C(1)-C(10)	1.4232(19)
C(1)-C(6)	1.4253(19)
C(2)-C(3)	1.367(2)
C(2)-H(2)	0.9300
C(3)-C(4)	1.406(2)
C(3)-H(3)	0.9300
C(4)-C(5)	1.365(2)
C(4)-H(4)	0.9300
C(5)-C(6)	1.4212(19)
C(5)-H(5)	0.9300
C(6)-C(7)	1.419(2)
C(7)-C(8)	1.368(2)
C(7)-H(7)	0.9300
C(8)-C(9)	1.4086(19)
C(8)-H(8)	0.9300
C(9)-C(10)	1.3709(19)
C(9)-H(9)	0.9300
C(10)-N(1)	1.4268(17)

C(11)-O(1)	1.2355(15)
C(11)-N(1)	1.3377(17)
C(11)-C(12)	1.5337(18)
C(12)-O(2)	1.4185(16)
C(12)-C(13)	1.5280(19)
C(12)-H(12)	0.9800
C(13)-C(18)	1.386(2)
C(13)-C(14)	1.3905(19)
C(14)-C(15)	1.384(2)
C(14)-H(14)	0.9300
C(15)-C(16)	1.382(2)
C(15)-H(15)	0.9300
C(16)-C(17)	1.384(2)
C(16)-H(16)	0.9300
C(17)-C(18)	1.393(2)
C(17)-H(17)	0.9300
C(18)-H(18)	0.9300
N(1)-H(1A)	0.906(12)
O(2)-H(2A)	0.8200
C(2)-C(1)-C(10)	123.38(12)
C(2)-C(1)-C(6)	118.85(12)
C(10)-C(1)-C(6)	117.76(12)
C(3)-C(2)-C(1)	120.83(13)
C(3)-C(2)-H(2)	119.6
C(1)-C(2)-H(2)	119.6
C(2)-C(3)-C(4)	120.30(14)
C(2)-C(3)-H(3)	119.8
C(4)-C(3)-H(3)	119.8
C(5)-C(4)-C(3)	120.55(14)
C(5)-C(4)-H(4)	119.7
C(3)-C(4)-H(4)	119.7
C(4)-C(5)-C(6)	120.91(14)
C(4)-C(5)-H(5)	119.5

C(6)-C(5)-H(5)	119.5
C(7)-C(6)-C(5)	122.04(13)
C(7)-C(6)-C(1)	119.43(12)
C(5)-C(6)-C(1)	118.52(12)
C(8)-C(7)-C(6)	120.90(12)
C(8)-C(7)-H(7)	119.6
C(6)-C(7)-H(7)	119.6
C(7)-C(8)-C(9)	120.18(13)
C(7)-C(8)-H(8)	119.9
C(9)-C(8)-H(8)	119.9
C(10)-C(9)-C(8)	120.19(13)
C(10)-C(9)-H(9)	119.9
C(8)-C(9)-H(9)	119.9
C(9)-C(10)-C(1)	121.49(12)
C(9)-C(10)-N(1)	120.00(12)
C(1)-C(10)-N(1)	118.40(11)
O(1)-C(11)-N(1)	124.01(12)
O(1)-C(11)-C(12)	121.53(11)
N(1)-C(11)-C(12)	114.46(11)
O(2)-C(12)-C(13)	112.52(11)
O(2)-C(12)-C(11)	109.28(10)
C(13)-C(12)-C(11)	109.43(10)
O(2)-C(12)-H(12)	108.5
C(13)-C(12)-H(12)	108.5
C(11)-C(12)-H(12)	108.5
C(18)-C(13)-C(14)	119.21(13)
C(18)-C(13)-C(12)	120.79(12)
C(14)-C(13)-C(12)	120.00(12)
C(15)-C(14)-C(13)	120.94(14)
C(15)-C(14)-H(14)	119.5
C(13)-C(14)-H(14)	119.5
C(16)-C(15)-C(14)	119.64(14)
C(16)-C(15)-H(15)	120.2
C(14)-C(15)-H(15)	120.2

C(15)-C(16)-C(17)	119.98(14)
C(15)-C(16)-H(16)	120.0
C(17)-C(16)-H(16)	120.0
C(16)-C(17)-C(18)	120.36(14)
C(16)-C(17)-H(17)	119.8
C(18)-C(17)-H(17)	119.8
C(13)-C(18)-C(17)	119.86(14)
C(13)-C(18)-H(18)	120.1
C(17)-C(18)-H(18)	120.1
C(11)-N(1)-C(10)	125.35(11)
C(11)-N(1)-H(1A)	115.9(10)
C(10)-N(1)-H(1A)	118.8(10)
C(12)-O(2)-H(2A)	109.5

Table 10. Anisotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for 1b:

The anisotropic displacement factor exponent takes the form: $-2\pi^2 [h^2 a^{*2} U^{11} + 2 h k a^* b^* U^{12}]$

	U ¹¹	U ²²	U ³³	U ²³	U ¹³	U ¹²
C(1)	22(1)	19(1)	19(1)	4(1)	4(1)	4(1)
C(2)	25(1)	22(1)	25(1)	2(1)	5(1)	0(1)
C(3)	24(1)	34(1)	35(1)	8(1)	8(1)	-3(1)
C(4)	27(1)	47(1)	30(1)	8(1)	14(1)	7(1)
C(5)	32(1)	36(1)	21(1)	1(1)	9(1)	9(1)
C(6)	25(1)	23(1)	17(1)	4(1)	5(1)	6(1)
C(7)	30(1)	22(1)	19(1)	-3(1)	3(1)	3(1)
C(8)	24(1)	22(1)	25(1)	-1(1)	1(1)	-2(1)
C(9)	23(1)	23(1)	23(1)	2(1)	6(1)	3(1)
C(10)	21(1)	17(1)	18(1)	1(1)	3(1)	4(1)
C(11)	19(1)	17(1)	22(1)	3(1)	9(1)	-2(1)
C(12)	20(1)	17(1)	25(1)	-2(1)	6(1)	0(1)
C(13)	24(1)	17(1)	21(1)	-6(1)	7(1)	-3(1)
C(14)	28(1)	28(1)	23(1)	-1(1)	9(1)	4(1)

C(15)	28(1)	37(1)	33(1)	-11(1)	13(1)	-1(1)
C(16)	43(1)	34(1)	39(1)	-13(1)	27(1)	-11(1)
C(17)	56(1)	27(1)	30(1)	1(1)	21(1)	-6(1)
C(18)	34(1)	23(1)	27(1)	-2(1)	7(1)	1(1)
N(1)	21(1)	22(1)	19(1)	-2(1)	2(1)	3(1)
O(1)	21(1)	25(1)	24(1)	2(1)	5(1)	3(1)
O(2)	20(1)	29(1)	32(1)	-11(1)	2(1)	-2(1)

Table 11. Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for 1b:

	x	y	z	U(eq)
H(2)	3524	3483	53	30
H(3)	1385	3655	1046	38
H(4)	1589	4516	2899	40
H(5)	3921	5200	3747	36
H(7)	6794	5511	3721	30
H(8)	8929	5360	2710	30
H(9)	8673	4546	736	28
H(12)	6914	2363	-2008	26
H(14)	10093	2424	-1445	32
H(15)	12156	2631	-2553	38
H(16)	11559	3317	-4782	42
H(17)	8907	3795	-5891	43
H(18)	6845	3602	-4756	34
H(2A)	4768	2704	-3696	43
H(1A)	5270(17)	3731(8)	-1482(17)	27(4)

Table 12. Torsion angles [$^\circ$] for 1b:

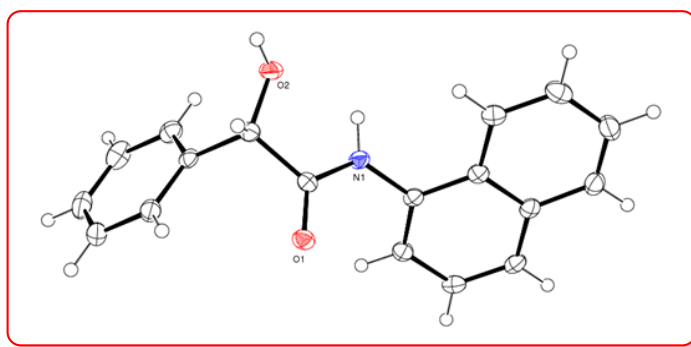
C(10)-C(1)-C(2)-C(3)	-177.11(12)
C(6)-C(1)-C(2)-C(3)	1.65(19)

C(1)-C(2)-C(3)-C(4)	-0.4(2)
C(2)-C(3)-C(4)-C(5)	-0.2(2)
C(3)-C(4)-C(5)-C(6)	-0.5(2)
C(4)-C(5)-C(6)-C(7)	-179.51(13)
C(4)-C(5)-C(6)-C(1)	1.7(2)
C(2)-C(1)-C(6)-C(7)	178.93(12)
C(10)-C(1)-C(6)-C(7)	-2.24(18)
C(2)-C(1)-C(6)-C(5)	-2.26(18)
C(10)-C(1)-C(6)-C(5)	176.57(12)
C(5)-C(6)-C(7)-C(8)	-177.96(13)
C(1)-C(6)-C(7)-C(8)	0.81(19)
C(6)-C(7)-C(8)-C(9)	1.1(2)
C(7)-C(8)-C(9)-C(10)	-1.5(2)
C(8)-C(9)-C(10)-C(1)	-0.04(19)
C(8)-C(9)-C(10)-N(1)	176.15(11)
C(2)-C(1)-C(10)-C(9)	-179.34(12)
C(6)-C(1)-C(10)-C(9)	1.88(18)
C(2)-C(1)-C(10)-N(1)	4.41(18)
C(6)-C(1)-C(10)-N(1)	-174.37(11)
O(1)-C(11)-C(12)-O(2)	173.70(11)
N(1)-C(11)-C(12)-O(2)	-7.26(15)
O(1)-C(11)-C(12)-C(13)	-62.68(16)
N(1)-C(11)-C(12)-C(13)	116.36(12)
O(2)-C(12)-C(13)-C(18)	13.69(17)
C(11)-C(12)-C(13)-C(18)	-108.00(14)
O(2)-C(12)-C(13)-C(14)	-165.25(11)
C(11)-C(12)-C(13)-C(14)	73.06(15)
C(18)-C(13)-C(14)-C(15)	0.8(2)
C(12)-C(13)-C(14)-C(15)	179.73(12)
C(13)-C(14)-C(15)-C(16)	-0.7(2)
C(14)-C(15)-C(16)-C(17)	0.0(2)
C(15)-C(16)-C(17)-C(18)	0.5(2)
C(14)-C(13)-C(18)-C(17)	-0.2(2)
C(12)-C(13)-C(18)-C(17)	-179.16(12)

C(16)-C(17)-C(18)-C(13)	-0.4(2)
O(1)-C(11)-N(1)-C(10)	5.5(2)
C(12)-C(11)-N(1)-C(10)	-173.50(12)
C(9)-C(10)-N(1)-C(11)	54.57(18)
C(1)-C(10)-N(1)-C(11)	-129.13(13)

Table 12. Hydrogen bonds for 1b [$\text{\AA}$ and $^\circ$]:

D-H...A	d(D-H)	d(H...A)	d(D...A)	$\angle$ (DHA)
O(2)-H(2A)...O(1)#1	0.82	1.88	2.6985(14)	173.1
N(1)-H(1A)...O(2)	0.906(12)	2.068(15)	2.5640(16)	113.2(12)
O(2)-H(2A)...O(1)#1	0.82	1.88	2.6985(14)	173.1
N(1)-H(1A)...O(2)	0.906(12)	2.068(15)	2.5640(16)	113.2(12)

**Figure 4.12.** ORTEP Image of **1b**.**Table 13. Crystal data and structure refinement for 1c:**

Empirical formula	$C_{22}H_{20}N_2O_4$	
Formula weight	376.40	
Temperature	296 K	
Wavelength	0.71073 $\text{\AA}$	
Crystal system	Monoclinic	
Space group	$P2_1/c$	
Unit cell dimensions	$a = 19.345(7) \text{ \AA}$	$a = 90^\circ$
	$b = 11.708(4) \text{ \AA}$	$b = 92.065(11)^\circ$
	$c = 7.965(3) \text{ \AA}$	$\gamma = 90^\circ$
Volume	$1802.9(11) \text{ \AA}^3$	

Z	4
Density (calculated)	1.387 Mg/m ³
Absorption coefficient	0.096 mm ⁻¹
F(000)	792
Crystal size	0.200 x 0.200 x 0.150 mm ³
Theta range for data collection	2.033 to 23.999°.
Index ranges	-22 ≤ h ≤ 22, -13 ≤ k ≤ 13, -9 ≤ l ≤ 9
Reflections collected	27251
Independent reflections	27251
Completeness to theta = 23.999°	100.0 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.91 and 0.85
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	27251 / 16 / 264
Goodness-of-fit on F ²	0.998
Final R indices [I > 2σ(I)]	R1 = 0.1795, wR2 = 0.3202
R indices (all data)	R1 = 0.4618, wR2 = 0.4706
Extinction coefficient	0.034(8)
Largest diff. peak and hole	0.327 and -0.386 e.Å ⁻³

Table 14. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for 1c:

U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	8060(17)	7902(19)	7590(40)	74(9)
C(2)	8727(19)	7750(20)	8250(40)	86(12)
C(3)	9089(16)	8550(30)	9080(40)	79(10)
C(4)	8746(16)	9570(20)	9340(30)	62(8)
C(5)	8097(15)	9767(18)	8710(30)	53(8)
C(6)	7709(14)	8934(18)	7810(30)	37(7)
C(7)	7018(14)	9190(16)	7150(30)	43(7)

C(8)	6449(13)	8773(19)	8260(30)	39(7)
C(9)	5374(12)	7662(19)	8340(30)	33(6)
C(10)	4917(13)	8394(16)	9110(30)	42(7)
C(11)	4329(13)	8044(16)	9820(30)	42(7)
C(12)	4187(13)	6830(17)	9850(30)	33(6)
C(13)	4626(13)	6119(17)	9070(30)	41(7)
C(14)	5219(13)	6459(17)	8330(30)	47(7)
C(15)	3136(13)	6890(20)	11580(30)	39(6)
C(16)	2665(14)	6095(15)	12570(30)	51(8)
C(17)	1933(13)	6209(17)	12050(30)	37(6)
C(18)	1541(16)	7118(17)	12650(30)	66(9)
C(19)	862(15)	7220(20)	12120(40)	67(9)
C(20)	544(15)	6470(20)	11090(40)	76(10)
C(21)	930(17)	5570(20)	10560(40)	84(11)
C(22)	1610(17)	5420(20)	10960(30)	63(9)
N(1)	5986(10)	8039(14)	7560(20)	42(6)
N(2)	3622(10)	6379(13)	10690(20)	36(5)
O(1)	6892(9)	8705(11)	5550(20)	71(6)
O(2)	6418(8)	9106(11)	9770(20)	52(5)
O(3)	3036(8)	7936(11)	11560(18)	56(5)
O(4)	2917(9)	4929(10)	12444(18)	50(5)

Table 15. Bond lengths [Å] and angles [°] for 1c:

C(1)-C(2)	1.39(3)
C(1)-C(6)	1.40(3)
C(1)-H(1)	0.9300
C(2)-C(3)	1.33(3)
C(2)-H(2)	0.9300
C(3)-C(4)	1.39(3)
C(3)-H(3)	0.9300
C(4)-C(5)	1.35(3)
C(4)-H(4)	0.9300

C(5)-C(6)	1.41(3)
C(5)-H(5)	0.9300
C(6)-C(7)	1.45(3)
C(7)-O(1)	1.41(2)
C(7)-C(8)	1.52(3)
C(7)-H(7)	0.9800
C(8)-O(2)	1.26(2)
C(8)-N(1)	1.35(3)
C(9)-C(10)	1.39(3)
C(9)-N(1)	1.43(3)
C(9)-C(14)	1.44(2)
C(10)-C(11)	1.35(3)
C(10)-H(10)	0.9300
C(11)-C(12)	1.45(2)
C(11)-H(11)	0.9300
C(12)-C(13)	1.36(3)
C(12)-N(2)	1.40(3)
C(13)-C(14)	1.37(3)
C(13)-H(13)	0.9300
C(14)-H(14)	0.9300
C(15)-O(3)	1.24(2)
C(15)-N(2)	1.34(3)
C(15)-C(16)	1.54(3)
C(16)-O(4)	1.455(19)
C(16)-C(17)	1.47(3)
C(16)-H(16)	0.9800
C(17)-C(22)	1.40(3)
C(17)-C(18)	1.40(3)
C(18)-C(19)	1.37(3)
C(18)-H(18)	0.9300
C(19)-C(20)	1.34(3)
C(19)-H(19)	0.9300
C(20)-C(21)	1.36(3)
C(20)-H(20)	0.9300

C(21)-C(22)	1.36(3)
C(21)-H(21)	0.9300
C(22)-H(22)	0.9300
N(1)-H(1A)	0.85(6)
N(2)-H(2A)	0.85(6)
O(1)-H(1B)	0.8200
O(4)-H(4A)	0.8200

C(2)-C(1)-C(6)	121(2)
C(2)-C(1)-H(1)	119.6
C(6)-C(1)-H(1)	119.6
C(3)-C(2)-C(1)	124(3)
C(3)-C(2)-H(2)	117.9
C(1)-C(2)-H(2)	117.9
C(2)-C(3)-C(4)	116(3)
C(2)-C(3)-H(3)	122.1
C(4)-C(3)-H(3)	122.1
C(5)-C(4)-C(3)	122(3)
C(5)-C(4)-H(4)	118.9
C(3)-C(4)-H(4)	118.9
C(4)-C(5)-C(6)	123(2)
C(4)-C(5)-H(5)	118.6
C(6)-C(5)-H(5)	118.6
C(1)-C(6)-C(5)	114(2)
C(1)-C(6)-C(7)	125(2)
C(5)-C(6)-C(7)	120(2)
O(1)-C(7)-C(6)	112(2)
O(1)-C(7)-C(8)	106.9(19)
C(6)-C(7)-C(8)	114(2)
O(1)-C(7)-H(7)	108.1
C(6)-C(7)-H(7)	108.1
C(8)-C(7)-H(7)	108.1
O(2)-C(8)-N(1)	122(2)
O(2)-C(8)-C(7)	121(2)

N(1)-C(8)-C(7)	117(2)
C(10)-C(9)-N(1)	124(2)
C(10)-C(9)-C(14)	118(2)
N(1)-C(9)-C(14)	118(2)
C(11)-C(10)-C(9)	124(2)
C(11)-C(10)-H(10)	118.1
C(9)-C(10)-H(10)	118.1
C(10)-C(11)-C(12)	118(2)
C(10)-C(11)-H(11)	121.1
C(12)-C(11)-H(11)	121.1
C(13)-C(12)-N(2)	120(2)
C(13)-C(12)-C(11)	118(2)
N(2)-C(12)-C(11)	122(2)
C(12)-C(13)-C(14)	125(2)
C(12)-C(13)-H(13)	117.7
C(14)-C(13)-H(13)	117.7
C(13)-C(14)-C(9)	117(2)
C(13)-C(14)-H(14)	121.3
C(9)-C(14)-H(14)	121.3
O(3)-C(15)-N(2)	123(2)
O(3)-C(15)-C(16)	121(2)
N(2)-C(15)-C(16)	116(2)
O(4)-C(16)-C(17)	112.8(18)
O(4)-C(16)-C(15)	109(2)
C(17)-C(16)-C(15)	112.4(19)
O(4)-C(16)-H(16)	107.5
C(17)-C(16)-H(16)	107.5
C(15)-C(16)-H(16)	107.5
C(22)-C(17)-C(18)	119(2)
C(22)-C(17)-C(16)	121(2)
C(18)-C(17)-C(16)	120(2)
C(19)-C(18)-C(17)	119(3)
C(19)-C(18)-H(18)	120.4
C(17)-C(18)-H(18)	120.4

C(20)-C(19)-C(18)	123(3)
C(20)-C(19)-H(19)	118.5
C(18)-C(19)-H(19)	118.5
C(19)-C(20)-C(21)	117(3)
C(19)-C(20)-H(20)	121.6
C(21)-C(20)-H(20)	121.6
C(22)-C(21)-C(20)	124(3)
C(22)-C(21)-H(21)	117.8
C(20)-C(21)-H(21)	117.8
C(21)-C(22)-C(17)	118(3)
C(21)-C(22)-H(22)	121.0
C(17)-C(22)-H(22)	121.0
C(8)-N(1)-C(9)	125(2)
C(8)-N(1)-H(1A)	118(8)
C(9)-N(1)-H(1A)	111(10)
C(15)-N(2)-C(12)	131.1(19)
C(15)-N(2)-H(2A)	117(8)
C(12)-N(2)-H(2A)	111(8)
C(7)-O(1)-H(1B)	109.5
C(16)-O(4)-H(4A)	109.5

Symmetry transformations used to generate equivalent atoms:

Table 16. Anisotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for 1c:

The anisotropic displacement factor exponent takes the form: $-2p^2 [h^2 a^{*2} U^{11} + 2 h k a^* b^* U^{12}]$

	U ¹¹	U ²²	U ³³	U ²³	U ¹³	U ¹²
C(1)	100(30)	26(16)	100(30)	-9(16)	20(20)	-4(17)
C(2)	110(30)	31(18)	120(30)	5(19)	20(30)	40(20)
C(3)	100(30)	70(20)	70(20)	12(19)	40(20)	30(20)
C(4)	60(20)	60(20)	70(20)	-20(16)	30(20)	-14(17)
C(5)	50(20)	39(16)	70(20)	-3(14)	14(19)	0(16)

C(6)	60(20)	25(14)	28(16)	13(12)	12(15)	-11(14)
C(7)	60(20)	25(13)	50(20)	-10(13)	1(19)	-10(13)
C(8)	60(20)	48(16)	14(16)	12(13)	-2(16)	9(15)
C(9)	38(18)	49(16)	13(14)	10(12)	8(13)	-11(14)
C(10)	51(19)	13(12)	61(19)	-7(12)	4(17)	1(13)
C(11)	60(20)	25(14)	42(18)	4(12)	-17(16)	0(13)
C(12)	46(19)	37(15)	14(14)	-2(12)	-11(14)	-6(14)
C(13)	50(20)	28(13)	43(17)	-5(13)	3(16)	-1(14)
C(14)	60(20)	38(16)	43(17)	-2(12)	25(16)	5(14)
C(15)	41(19)	52(16)	23(16)	-10(14)	2(14)	-15(15)
C(16)	90(20)	20(13)	46(18)	-3(12)	4(18)	-3(14)
C(17)	37(18)	29(14)	44(17)	3(12)	-13(15)	6(14)
C(18)	80(30)	26(15)	90(20)	2(15)	20(20)	11(15)
C(19)	50(20)	52(18)	100(30)	-4(17)	0(20)	14(16)
C(20)	80(30)	46(18)	100(30)	-9(18)	-20(20)	-12(18)
C(21)	60(30)	60(20)	130(30)	-30(20)	-10(20)	-28(18)
C(22)	60(20)	80(20)	50(20)	-12(16)	-12(18)	-29(18)
N(1)	64(17)	31(12)	30(14)	-6(10)	-8(13)	-11(11)
N(2)	43(14)	27(11)	39(13)	6(11)	18(12)	3(11)
O(1)	84(15)	85(12)	43(12)	-2(10)	4(11)	-41(11)
O(2)	65(14)	51(10)	40(12)	-4(9)	9(11)	-13(8)
O(3)	76(14)	27(9)	67(13)	7(9)	6(11)	1(9)
O(4)	77(14)	21(8)	52(12)	7(8)	-16(11)	3(8)

Table 17. Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for 1c:

	x	y	z	U(eq)
H(1)	7844	7311	6998	89
H(2)	8935	7042	8108	103
H(3)	9543	8432	9470	95
H(4)	8970	10145	9962	75

H(5)	7899	10480	8879	63
H(7)	6975	10021	7046	52
H(10)	5022	9169	9140	50
H(11)	4026	8566	10279	51
H(13)	4515	5346	9029	49
H(14)	5510	5934	7835	56
H(16)	2710	6318	13760	61
H(18)	1739	7645	13397	79
H(19)	610	7841	12502	81
H(20)	82	6552	10749	91
H(21)	711	5027	9878	101
H(22)	1855	4813	10525	76
H(1B)	6474	8691	5338	106
H(4A)	3100	4734	13345	76
H(2A)	3650(80)	5650(50)	10700(200)	40(30)
H(1A)	6130(60)	7540(110)	6900(190)	50(30)

Table 18. Hydrogen bonds for 1c [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
C(7)-H(7)...O(3)#1	0.98	2.64	3.52(2)	150.0
C(11)-H(11)...O(3)	0.93	2.32	2.90(3)	120.3
C(16)-H(16)...O(3)#2	0.98	2.46	3.42(3)	168.5
O(4)-H(4A)...O(2)#3	0.82	1.89	2.70(2)	171.3
N(2)-H(2A)...O(4)	0.85(6)	2.17(12)	2.61(2)	112(8)
N(1)-H(1A)...O(2)#4	0.85(6)	2.64(6)	3.48(2)	173(11)

Symmetry transformations used to generate equivalent atoms:

#1 -x+1, -y+2, -z+2 #2 x, -y+3/2, z+1/2 #3 -x+1, y-1/2, -z+5/2 #4 x, -y+3/2, z-1/2

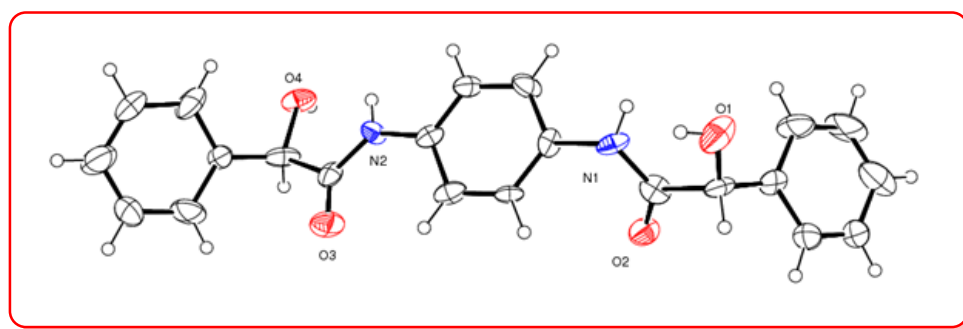


Figure 4.13. ORTEP image of **1c**.

4.2.11. Planar bilayer conductance measurements:⁴¹⁻⁴³

With lipid diphytanoylphosphatidylcholine (Avanti Polar Lipids), dissolved in n-decane (18 mg/mL), a bilayer membrane (BLM) was created over an aperture of 150 μ m diameter in a polystyrene cup (Warner Instrument, USA). One M of KCl was included in a symmetrical solution that was poured into both chambers (cis and trans). The cis chamber was coupled to the BC 535 head-stage (Warner Instrument, USA) via matched Ag–AgCl electrodes, while the trans compartment was maintained at virtual ground. The trans chamber was then filled with compound **1b** (10 μ M), and the solution was agitated with a magnetic stirrer for 30 min. The discrete channel opening and closing events after applying voltages served as confirmation of channel creation. Using pClamp9 software (Molecular Probes, USA) and an analog-to-digital converter, currents were low pass filtered at 1 kHz.

The complete data trace observed for 10 minutes contained a series of opening and closing events at some indefinite intervals. The average current of 5.36 pA was calculated from this trace and then other calculations were made accordingly. (Figure 4.14) This is a preliminary indication of the fact that the transporter is operating via a channel mechanism, based on which the molecular dynamic simulations studies were performed.

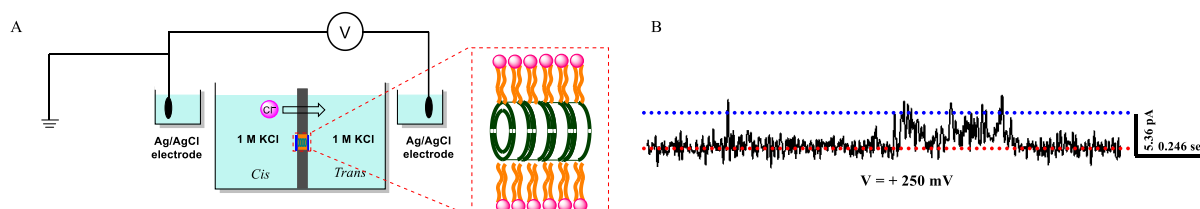


Figure 4.14. Systematic representation of black lipid membrane (BLM) experiment (A) and Single-channel conductance of **1b** (10 μ M) recorded at +250 mV (B).

4.2.11.1. Calculation of average ion channel diameter:

The diameter of the artificial ion channel **1b** was calculated according to Hille's equation.

$$1/g = (l + \pi d/4) \times (4\rho/\pi d^2) \quad (\text{Equation 5})$$

where, g = corrected conductance (obtained by multiplying measured conductance with the Sansom's correction factor) = 3.37×10^{-10} S, l = length of the ion channel = 34 Å, and ρ = resistivity of the 1 M KCl solution = 9.47 Ω·cm).

The calculated diameter was found out to be $4.38 \pm 0.5\%$ Å which is in good accordance with the diameters of the K⁺ and the Cl⁻ ions.

4.2.12. Biological data:

4.2.12.1. Cell culture and cell lines used:

A non-cancerous cell line from human source, namely, human embryonic kidney cells (HEK293T); and a cancerous cell line, namely, human breast cancer cells (MCF-7), were used for the study. Both the cell lines were cultured in the presence of high glucose Dulbecco's modified Eagle media (DMEM; Lonza) containing 2 mM L-glutamine. The media was further supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS; ThermoFisher Scientific), 100 units/ml penicillin, and 100 µg/ml streptomycin. The cells were maintained in tissue-culture-treated T25 flasks at 37 °C in a humidified 5% CO₂ incubator (Thermo Scientific) and were passaged every 2 days.

4.2.12.2. Assessment of cellular viability by MTT assay:

Cytotoxicity of the mandelic acid K⁺/Cl⁻ transporter library (**1a-1c**) was assessed in MCF-7 and HEK293T cells at concentrations ranging from 0-100 µM

Briefly, cells were seeded in tissue culture treated 96-well plates (Corning) at a density of 8 x 10⁴ cells/well/100 µL in complete DMEM medium and were allowed to recover at 37 °C in a humidified 5% CO₂ incubator for 24 h. Post-incubation, the cells were incubated with increasing concentrations of the compounds (concentration range 1-100 µM) for 24 h by maintaining the maximum amount of the solvent (DMSO) at 2 µL/well. Post incubation, the media was removed and the plates were incubated with 100 µL of MTT solution (0.5 mg/ml MTT in PBS) at 37 °C for a period of 4 h. The formazan crystals, thus formed, were dissolved using 100 µL solubilization buffer (10% SDS/0.1N HCl in PBS) by incubating the plates

overnight at RT. The absorbance was measured at 570 nm using a multi-mode plate reader (Hidex). All experiments were performed in triplicate ($N = 3$). The cellular viability was expressed as a percentage of viable cells for each treatment when compared with the untreated cells (control, set as 100%) and have been depicted for HEK293T (Figure 4.15A) and MCF-7 (Figure 4.15B) cells.

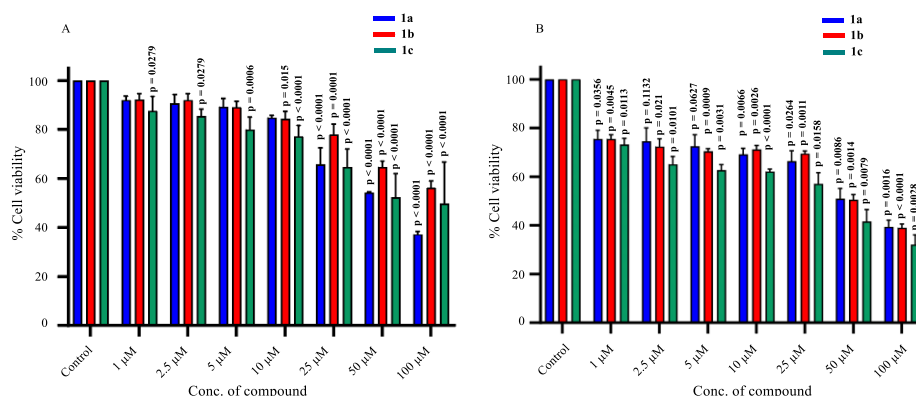


Figure 4.15. Cellular viability as assessed by the MTT assay for HEK293T (A) and MCF-7 cells (B) incubated with compounds **1a**, **1b** and **1c** at increasing concentrations for 24 hours. The p-values compare the treatment group with the control and $p < 0.05$ was considered to be statistically significant.

The three tested compounds (**1a-c**) were found to be cytotoxic to the breast cancer MCF-7 cells and to the non-cancerous HEK293T cells, as evident from Figure 4.15A and B, respectively. Thus, the compounds did not show selective toxicity in the cancerous cells. However, these studies are preliminary and further detailed biological studies need to be planned in the future to assess the mechanism of cell death in the MCF-7 cells.

4.2.13. NMR data:

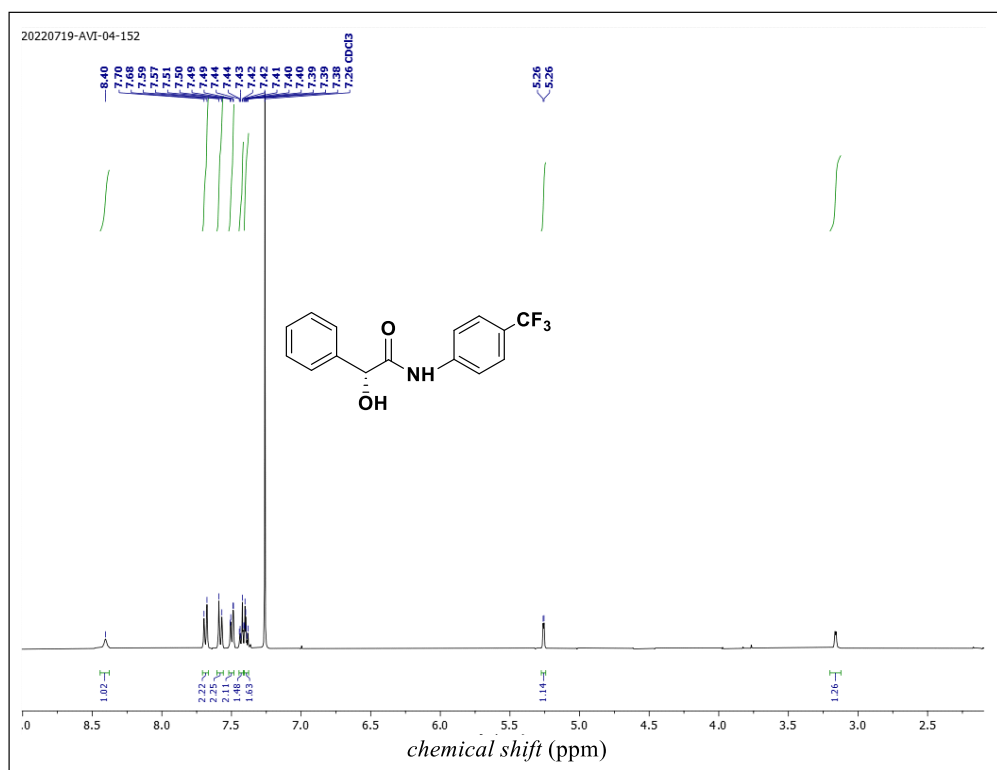


Figure 4.16. ^1H NMR spectrum of compound **1a** in CDCl₃.

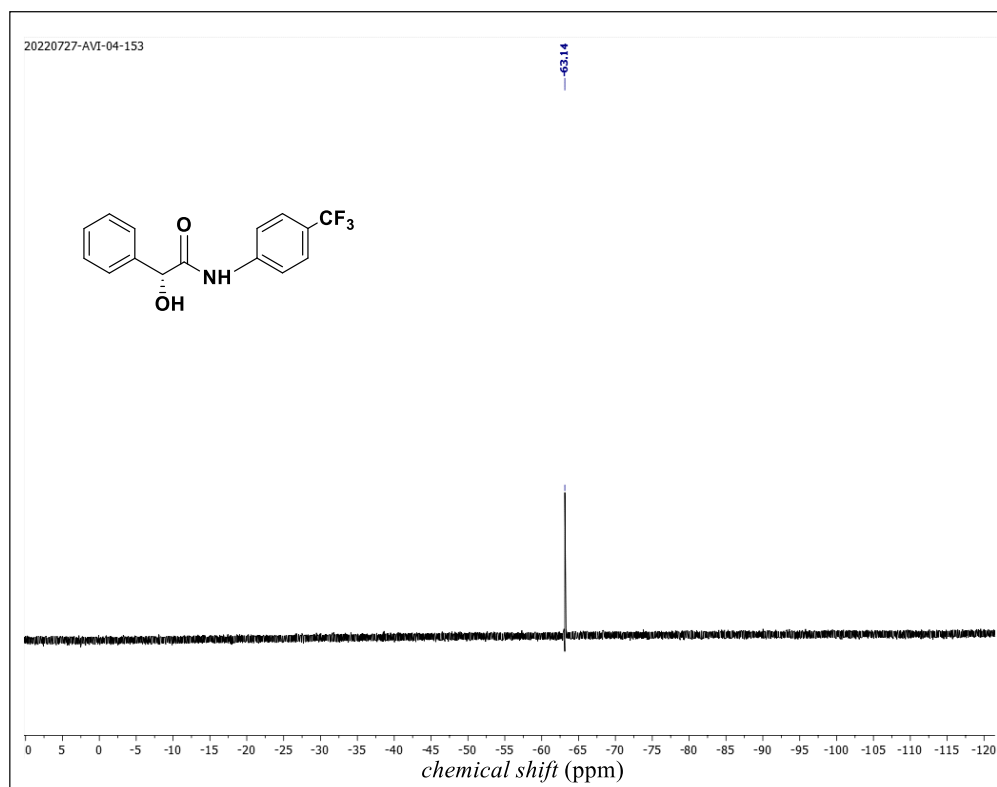
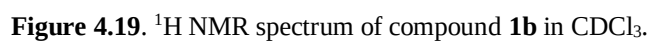
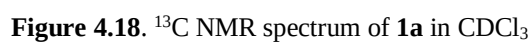


Figure 4.17. ^{19}F NMR spectrum of **6b** in CDCl₃.



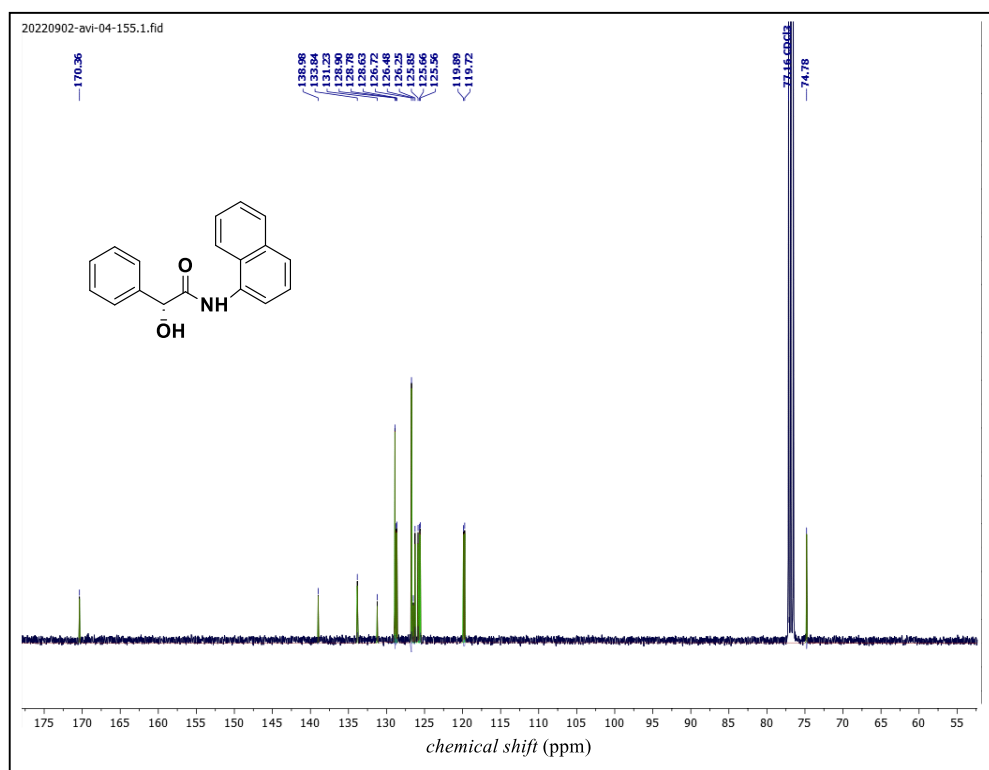


Figure 4.20. ¹³C NMR spectrum of compound **1b** in CDCl₃.

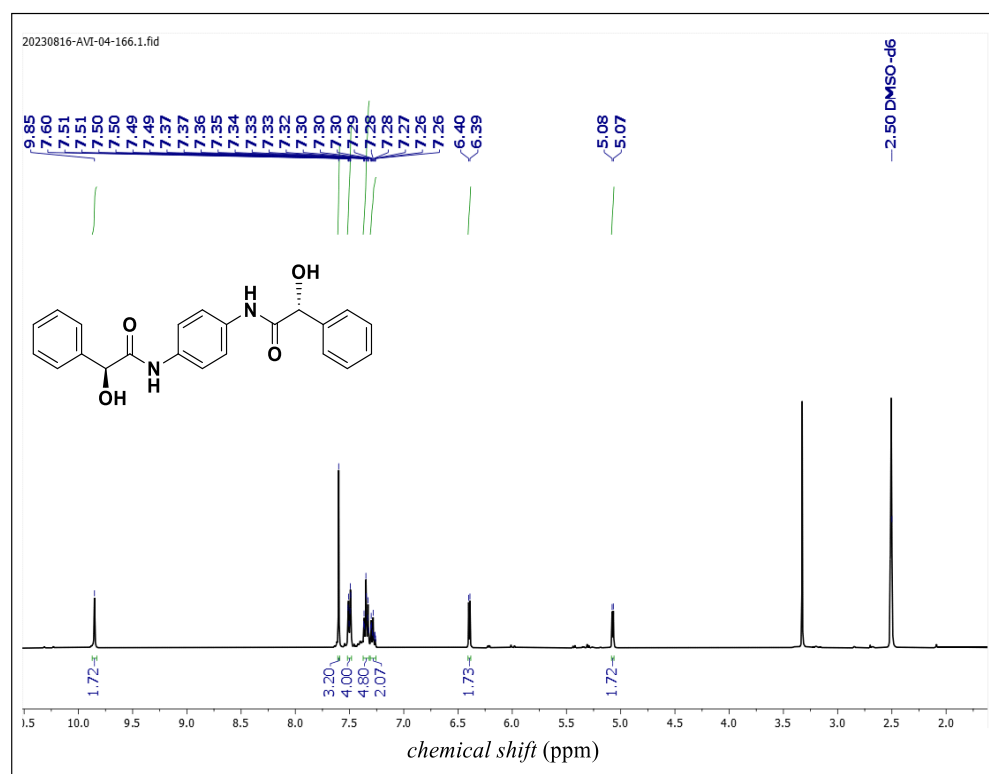


Figure 4.21. ¹H NMR spectrum of compound **1c** in DMSO-*d*₆.

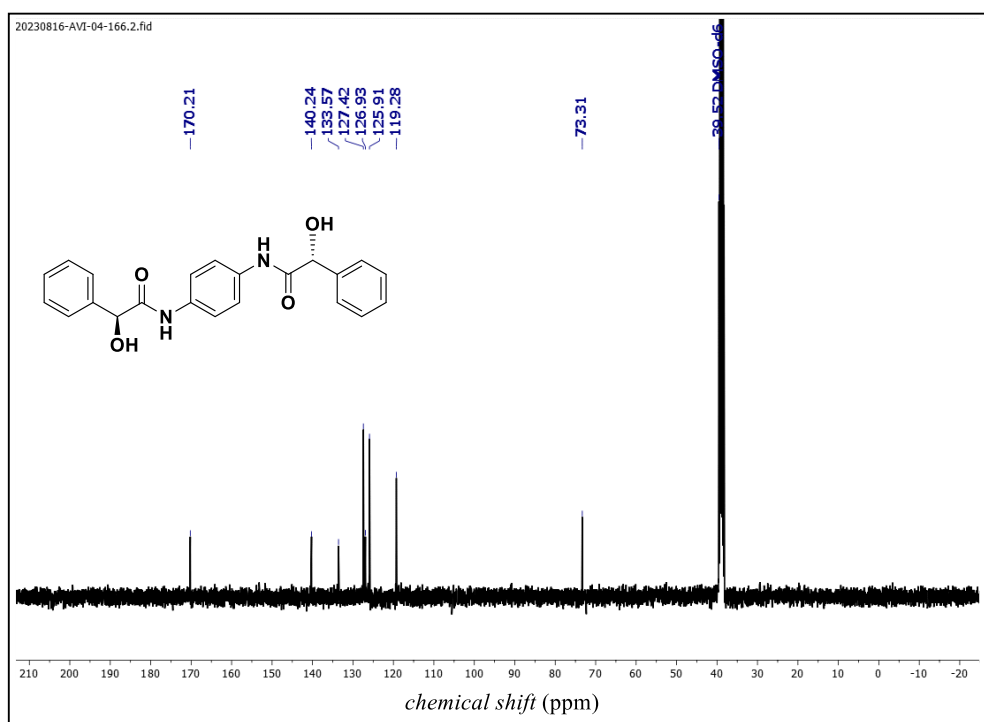


Figure 4.22. ¹³C NMR spectrum of compound **1c** in DMSO-*d*₆.

4.3. References:

1. Davison, S. P.; Irio, M.; Oh, C., Transconjunctival lower lid blepharoplasty with and without fat repositioning. *Clinics in Plastic Surgery* **2015**, 42 (1), 51-56.
2. Iversen, L., Neurotransmitter transporters and their impact on the development of psychopharmacology. *British Journal of Pharmacology*. **2006**, 147 (1), S82-S88.
3. Pandey, A. K.; Okaj, I.; Kaur, H.; Belley-Cote, E. P.; Wang, J.; Oraili, A.; Benz, A. P.; Johnson, L. S. B.; Young, J.; Wong, J. A.; Verma, S.; Conen, D.; Gerstein, H.; Healey, J. S.; McIntyre, W. F., Sodium-glucose co-transporter inhibitors and atrial fibrillation: A systematic review and meta-analysis of randomized controlled trials. *Journal of the American Heart Association* **2021**, 10 (17), e022222.
4. Kahle, K. T.; Khanna, A. R.; Alper, S. L.; Adragna, N. C.; Lauf, P. K.; Sun, D.; Delpire, E., K-Cl cotransporters, cell volume homeostasis, and neurological disease. *Trends in Molecular Medicine*. **2015**, 21 (8), 513-523.
5. Kravchenko, I. V.; Furalyov, V. A.; Popov, V. O., Potassium chloride released from contracting skeletal muscle may stimulate development of its hypertrophy. *Biochemistry and Biophysics Reports*. **2019**, 18, 100627.

6. Richardson, B. D.; Saha, K.; Krout, D.; Cabrera, E.; Felts, B.; Henry, L. K.; Swant, J.; Zou, M.-F.; Newman, A. H.; Khoshbouei, H., Membrane potential shapes regulation of dopamine transporter trafficking at the plasma membrane. *Nat Comm.* **2016**, 7 (1), 10423.
7. Russell, J. M., Sodium-potassium-chloride cotransport. *Physiol Rev* **2000**, 80 (1), 211-276.
8. Payne, J. A.; Rivera, C.; Voipio, J.; Kaila, K., Cation–chloride co-transporters in neuronal communication, development and trauma. *Trends Neurosci.* **2003**, 26 (4), 199-206.
9. Gu, Y.; Shrivastava, I. H.; Amara, S. G.; Bahar, I., Molecular simulations elucidate the substrate translocation pathway in a glutamate transporter. *Proc Natl Acad Sci U S A.* **2009**, 106 (8), 2589-2594.
10. Blaesse, P.; Airaksinen, M. S.; Rivera, C.; Kaila, K., Cation-chloride cotransporters and neuronal function. *Neuron.* **2009**, 61 (6), 820-838.
11. Lee, J. H.; Lee, J. H.; Choi, Y. R.; Kang, P.; Choi, M.-G.; Jeong, K.-S., Synthetic K^+/Cl^- -Selective symporter across a phospholipid membrane. *J. Org. Chem.* **2014**, 79 (14), 6403-6409.
12. Koulov, A. V.; Mahoney, J. M.; Smith, B. D., Facilitated transport of sodium or potassium chloride across vesicle membranes using a ditopic salt-binding macrobicycle. *Org. Biomol. Chem.* **2003**, 1 (1), 27-29.
13. Tong, C. C.; Quesada, R.; Sessler, J. L.; Gale, P. A., meso-Octamethylcalix[4]pyrrole: an old yet new transmembrane ion-pair transporter. *Chem. Commun.* **2008**, 47, 6321-6323.
14. Vargas Jentsch, A.; Emery, D.; Mareda, J.; Metrangolo, P.; Resnati, G.; Matile, S., Ditopic ion transport systems: Anion– π interactions and halogen bonds at work. *Angew. Chem., Int. Ed. Engl.* **2011**, 50 (49), 11675-11678.
15. Basak, D.; Sridhar, S.; Bera, A. K.; Madhavan, N., Cation–halide transport through peptide pores containing aminopicolinic acid. *Org. Biomol. Chem.* **2016**, 14 (20), 4712-4717.
16. Saha, K.; Agasti, S. S.; Kim, C.; Li, X.; Rotello, V. M., Gold nanoparticles in chemical and biological sensing. *Chem Rev.* **2012**, 112 (5), 2739-2779.
17. Reddy, K. L.; Mathew, J. P.; Maniappan, S.; Tom, C.; Shibby, E.; Pujala, R. K.; Kumar, J., Mandelic acid appended chiral gels as efficient templates for multicolour circularly polarized luminescence. *Nanoscale.* **2022**, 14 (13), 4946-4956.
18. Wójcik, A.; Kubiak, M.; Rotsztein, H., Original paper Influence of azelaic and mandelic acid peels on sebum secretion in ageing women. *Postep Derm Alergol* **2013**, 30 (3), 140-145.

19. Bishop, A. H., Potentiating effect of mandelate and lactate on chemically induced germination in members of *Bacillus cereus Sensu Lato*. *Appl. Environ. Microbiol.* **2017**, 83 (24), e01722-17.
20. Egner, P.; Pavlačková, J.; Sedlaříková, J.; Pleva, P.; Mokrejš, P.; Janalíková, M. Non-alcohol hand sanitiser gels with mandelic acid and essential oils *Int. J. Mol. Sci.*, **2023**.
21. Yamasaki, Y.; Sekiya, R.; Haino, T., Hexameric assembly of 5,17-di-substituted calix[4]arene in the solid state. *CrystEngComm.* **2017**, 19 (45), 6744-6751.
22. Malla, J. A.; Roy, A.; Talukdar, P., Anion selective ion channel constructed from a self-assembly of bis(cholate)-substituted fumaramide. *Org. Lett.* **2018**, 20 (19), 5991-5994.
23. Shinde, S. V.; Talukdar, P., A dimeric bis(melamine)-substituted bispidine for efficient transmembrane H^+/Cl^- cotransport. *Angew. Chem. Int. Ed.* **2017**, 56 (15), 4238-4242.
24. Litvinchuk, S.; Bollot, G.; Mareda, J.; Som, A.; Ronan, D.; Shah, M. R.; Perrottet, P.; Sakai, N.; Matile, S., Thermodynamic and kinetic stability of synthetic multifunctional rigid-rod β -barrel pores: Evidence for supramolecular catalysis. *J. Am. Chem. Soc.* **2004**, 126 (32), 10067-10075.
25. Sakai, N.; Kamikawa, Y.; Nishii, M.; Matsuoka, T.; Kato, T.; Matile, S., Dendritic folate rosettes as ion channels in lipid bilayers. *J. Am. Chem. Soc.* **2006**, 128 (7), 2218-2219.
26. Talukdar, P.; Sakai, N.; Sordé, N.; Gerard, D.; Cardona, V. M. F.; Matile, S., Outer surface modification of synthetic multifunctional pores. *Bioorg. Med. Chem.* **2004**, 12 (6), 1325-1336.
27. Sakai, N.; Matile, S., Synthetic multifunctional pores: lessons from rigid-rod β -barrels. *Chem. Commun.* **2003**, 20, 2514-2523.
28. Roy, A.; Gautam, A.; Malla, J. A.; Sarkar, S.; Mukherjee, A.; Talukdar, P., Self-assembly of small-molecule fumaramides allows transmembrane chloride channel formation. *Chem. Commun.* **2018**, 54 (16), 2024-2027.
29. Malla, J. A.; Upadhyay, A.; Ghosh, P.; Mondal, D.; Mondal, A.; Sharma, S.; Talukdar, P., Chloride transport across liposomes and cells by nontoxic 3-(1h-1,2,3-triazol-1-yl)benzamides. *Org. Lett.* **2022**, 24 (23), 4124-4128.
30. Shen, F.-F.; Dai, S.-Y.; Wong, N.-K.; Deng, S.; Wong, A. S.-T.; Yang, D., Mediating K^+/H^+ Transport on organelle membranes to selectively eradicate cancer stem cells with a small molecule. *J Am Chem Soc.* **2020**, 142 (24), 10769-10779.

31. Mondal, D.; Sathyan, A.; Shinde, S. V.; Mishra, K. K.; Talukdar, P., Tripodal cyanurates as selective transmembrane Cl^- transporters. *Org. Biomol. Chem.* **2018**, *16* (45), 8690-8694.
32. Sharma, R.; Vijay, A.; Mukherjee, A.; Talukdar, P., Bis(cholyl)-based chloride channels with oxalamide and hydrazide selectivity filters. *Org. Biomol. Chem.* **2022**, *20* (10), 2054-2058.
33. Sharma, R.; Vijay, A.; Chattopadhyay, S.; Mukherjee, A.; Talukdar, P., Self-assembled anion channel formation by bis(1,3-propanediol)-linked meta-dipropynylbenzene-based small molecules. *Chem. Commun.* **2023**, *59* (24), 3602-3605.
34. Malla, J. A.; Umesh, R. M.; Vijay, A.; Mukherjee, A.; Lahiri, M.; Talukdar, P., Apoptosis-inducing activity of a fluorescent barrel-rosette M^+/Cl^- channel. *Chem. Sci.* **2020**, *11* (9), 2420-2428.
35. Malla, J. A.; Sharma, V. K.; Lahiri, M.; Talukdar, P., Esterase-activatable synthetic M^+/Cl^- channel induces apoptosis and disrupts autophagy in cancer cells. *Chem. Eur. J.* **2020**, *26* (52), 11946-11949.
36. SAINT Plus, (Version 7.03); Bruker AXS Inc.: Madison, WI, **2004**.
37. G. M. Sheldrick, SHELXTL, Reference Manual: version 5.1: Bruker AXS; Madison, WI, **1997**.
38. G. M. Sheldrick, Acta Crystallogr. Sect. A, **2008**, *112*.
39. WINGX version 1.80.05 Louis Farrugia, University of Glasgow.
40. A. L. Spek, A PLATON, Multipurpose Crystallographic Tool, Utrecht University, Utrecht, The Netherlands, **2005**.
41. Qi, Z.; Sokabe, M.; Donowaki, K.; Ishida, H., Structure-function study on a de novo synthetic hydrophobic ion channel. *Biophys. J.* **1999**, *76* (2), 631-641.
42. Strilets, D.; Fa, S.; Hardiagon, A.; Baaden, M.; Ogoshi, T.; Barboiu, M., Biomimetic approach for highly selective artificial water channels based on tubular pillar[5]arene dimers. *Angew Chem Int Ed Engl.* **2020**, *59* (51), 23213-23219.
43. Hu, X.-B.; Chen, Z.; Tang, G.; Hou, J.-L.; Li, Z.-T., Single-molecular artificial transmembrane water channels. *J. Am. Chem. Soc.* **2012**, *134* (20), 8384-8387.

Overall Conclusion

The main goal of my doctoral dissertation was to establish the fundamental concepts underlying the non-covalent interactions that help in the synthesis of synthetic ion channels and carriers as well as to delve into the cation/anion selectivity or cation/anion co-transport across the lipid bilayer membrane. In conclusion, the research results are consistent with the primary hypothesis of the study. The current study focuses on understanding the basic principles that control the behaviour of low-molecular weight molecules that can self-assemble into transmembrane ion channels or carriers to facilitate ion transport through a number of supramolecular and non-covalent interactions. The main objectives of this dissertation are the chemical synthesis, characterization, and analysis of synthetic ion carriers and channels that mimic the structure and functions of the natural congeners.

The lack of selectivity of transporting biologically important cations and anions like K^+ and Cl^- in conventional transporters, however, can cause injury to both healthy tissues and cancerous cells. In an effort to facilitate such selective transport, the self-assembly of the individual monomeric units can be tweaked to involve multiple ion binding sites to enhance the transmembrane ion transport. Additional methods to modify the selectivity of the ion involve incorporating several ion-binding sites and introducing modification in the ion transport process. This thesis focuses on the binding and subsequent transmembrane transport of the biologically relevant K^+ and Cl^- by the synthetic molecules in order to combat the detrimental outcomes of the naturally dysfunctional channels.
