SYNTHESIS, BIOLOGICAL EVALUATION AND *IN SILICO* STUDIES OF AZO-BASED CALIX[4]ARENES

BY

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A thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy in Biosciences

Kulliyyah of Science
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AUGUST 2017

ABSTRACT

Calix[n]arenes are cyclic oligomers that were initially obtained from the condensation of phenols and formaldehyde. Calixarene modifications produce various useful derivatives with numerous applications. Azo calixarenes are obtained by incorporation of one or more azo units at the upper rim and/or lower rim of calixarene. Applications of azo calixarenes are observed in various fields such as agriculture, biotechnology and in the development of optical devices, chemical sensor devices, and luminescence probes. However, their potential use as pharmaceutical agents has not been fully explored. The present study aimed to synthesise and explore the biological potentials of azo calix[4]arenes. Both upper and lower rim-modified azo calix[4]arenes were synthesised, characterised and evaluated for their antibacterial, antifungal and antiproliferative activities. In order to keep the molecular weight in possible minimum range, selective modification of calix[4]arene scaffold was adopted for certain compounds and mono and di-substituted products were obtained in good yields. Compounds obtained from sulphanilamide, sulfaguanidine and 2-methyl-4-aminobenzoic acid showed good activity against bacterial strains with MIC values ranging from 0.97-62.5 μg/mL. In cytotoxicity assay, 4-phenylazobenzoyl chloride was the most active compounds against the examined four cancer cell lines (MCF-7, MDA-MB-231, HCT-116 and A549), with IC_{50} value ranging from 15.56 to 21.4 μM. Moderate activity was shown by azo calix[4]arenes containing sulfonamide group. *In silico* study (molecular docking) was performed to see the interaction of the designed compounds with targeted proteins (PDB IDs 4CJN, 1CEF, 1FVT, 3TI6 and 1ZXM). Some of the azo calix[4]arenes showed good interaction with the active site of microbial and/or human enzymes through hydrogen, hydrophobic and pi-pi interactions, and could inhibit the activity of these enzymes. Among the five receptors, the compounds showed more affinity towards penicillin-binding proteins (PDB IDs 4CJN and 1CEF) that suggested their prospective antibacterial activity.

خلاصة البحث

الكلاغزرين هي مركبات حلقية قليلة الوحدات يتم الحصول عليها من تكاثف مركبات الفينول والفورمالدهايد. يتم إنتاج مشتقات مفيدة من مركب الكلاغزرين بإستخدام تطبيقات متعددة. يتم الحصول على مركبات الايزوكلاغزرين من خلال دمج وحدة واحده أو أكثر من الجهة العلوية و\أو السفلية لمركب الكلاغزرين. تم ملاحظة تطبيقات مركبات الايزو كلاغزرين في جالات معددة مثل الزراعة والتقنيات الحيوية وفي تطويرالاجهزة البصرية وأجهزة الاستشعار الكيميائية ومجسات التلألؤ. علي أي حال لم يتم إلى الان الكشف عن مقدرة هذه المركبات كعوامل صيدلانية . تهدف هذه الدراسة لبناء وكشف المقدرة البيولوجية لمركبات الايزوكلاغزرين. تم بناء وتوصيف كلا الجهتين العلوية والسفلية من المركب لقياس مدى فاعليتها كمضادات للبكتيريا والفطريات. من اجل الحفاظ على الوزن الجزيئي للحد الادنى الممكن تم الاعتماد على تعديل إنتقائي لمركبات الكلاغزرين وتم الحصول على إحلال أحادي وثنائي كمنتج كمي. أظهرت المركبات التي تم الحصول عليها من سلفانيل امايد وسلفاغونايد نشاطية جيده ضد سلالات البكتيريا مع معامل تثبيط 0.97 - 62.5 مايكروغرام/مل. كان مركب فينل ازبنزين في فحص السمية الخلوية الاكثر نشاطا ضد الخلايا السرطانية التي تم فحصها (MCF-7, MDA-MB-231, HCT-116 and A549) بقيمة تعادل 15.56 إلى 21.4. ظهرت نشاطية معتدلة لمركبات الايزوكلاغزرين التي تحوي سلفامايد في دراسة السيليكو والتي تم إنجازها لاظهار التوافق بين المركبات التي تم تصميمها وبروتيينات مستهدفة (PDB IDs 4CJN, 1CEF, 1FVT, 3TI6 and 1ZXM) .أظهرت بعض هذه المركبات تفاعل مع الموقع النشط لانزيمات ميكروبية وانسانية من خلال الترابط الهيدروجين والترابط الكاره للماء مع إحتملية تثبيطها لهذه الانزيمات.من بين الخمس مستقبلات أظهرت المركبات ألفه باتجاه بروتينات البنسلين (PDB IDs 4CJN and 1CEF) والتي تقترح إمكانية نشاطيتها كمضادات للبكتيريا.

APPROVAL PAGE

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Yousaf Ali

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This thesis is dedicated to my late father Fazal Rabbi,

May Almighty ALLAH give him Jannatul-Firdaous,

&

My Grandfather, Baba.

ACKNOWLEDGMENTS

First, I would like to thank my supervisor Assoc. Prof. Dr Shafida Abdul Hamid for her professional and amicable guidance over the last three years. Her integrity and technical and moral support always resonated me to do the best that I could. I feel privileged to have had my education under her supervision. I thank You Dr for the trust you gave me and the freedom you let me.

I would like to offer my thanks to Dr. Noraslinda M. Bunnori, my co-supervisor, for helping in the computational part of the thesis. Thanks also go out to Dr Salizawati who supervised my duration in Universiti Sains Malaysia, and her student, sister Nagla for assisting me in the cytotoxicity assay. I am also thankful to Dr. Deny Susanti for the scientific discussion and useful advice on bioactivity. Thanks to Dr. Ibrahim Shogar for the amazing class of 'ethics in science and technology'. I am truly grateful to Prof. Ahmed Jalal, Dr Muzafar (KAHS), and Dr Umer Rashid (Pakistan, my supervisor in Master).

I am deeply and strongly obliged to my lab mate sister Akmar and brother Wafi, for their positive attitude and cooperative nature. I feel very lucky to have worked in lab in a friendly environment. I also sincerely thank the persons who helped me with the technical and administrative problems: Sister Norsa'adah Md Yunos, Ain, Hafiah, Muizzah, Hafizah (UMP) and brother Mizan, Shuhail. The MIS scholarship from MOHE Malaysia is gratefully acknowledged. Thank you **sci-hub**, yes, you ''remove all barriers in the way of science''

I owe my deepest gratitude to my hostel friends (Dr) Ali, Mahmood, Azizur-Rahman, Afeez, Sakib, Abdulllah, Hussam, Haris, Allhasan, Abdulrahman, Saleh, Tahir, Hamza, especially Hasan Sheikh (helping guy), the people with whom I shared the moments of excitements and disappointments. The tea-breaks and 'Jalan-Jalan' with Dr Ali and bro Mahmood will never be forgotten. Special thanks are extended to my 'online' friends M. Alam Khan, Mia Bahadur Zada, Anwar khan, Arshad Iqbal ,Ali Rahman and Dr Sdiq Ahmad who encouraged me over the past three years.

My cordial gratitude and appreciation to my family, whose prayers and emotional support gave me the ''wings to fly''. Thank you Haider Ali, Hazrat ali, and Muhammad Sajjad, you took care of my kids.

Above all, entire acclamation and appreciation are for **Almighty ALLAH**, who is the real source of knowledge and the only God of all the blessings.

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LIST OF ABBREVIATION

ACN	Acetonitrile
Ar	Aryl
br	Broad signal
dd	Double doublet
DCM	Dichloromethane
DMF	Dimethylformamide
DMSO	Dimethyl sulphoxide
Hz	Hertz
h	Hour
J	Coupling constant
NMR	Nuclear magnetic resonance
qn	Quintet
s	Singlet
t	Triplet
TLC	Thin layer chromatography

CHAPTER ONE

INTRODUCTION

1.1 CALIXARENES

The word 'calixarene' is generally used for a class of cyclic oligomers that was initially obtained from the condensation of phenols and formaldehyde (Gutsche et al., 1981; Vicens & Böhmer, 2012). This informal name was given by C. D. Gutsche due to the similarity in shape of this family to a type of Greek vase, known as calix. The word 'arene' was linked to express their aromatic nature. The number of aryl units (phenol rings) in calixarene vary from four to twenty (Gutsche, 2008). A calixarene with four phenolic rings is generally represented as calix[4]arene while that with five phenolic rings is represented as calix[5]arene and so on (Figure 1.1).

Calixarene has two sides, upper (wide) rim and lower (narrow) rim. Modification of calixarene at either one or both rims gives a variety of products of several interests (Mandolini & Ungaro, 2000). Various methods of functionalisation of calixarenes have been developed and numerous derivatives have been reported. One or more hydroxyl groups of the lower rim can easily be modified to get other useful derivatives like esters, amides, thioamides, ketones, etc. The introduction of an alkyl or aryl group gives the corresponding mono, di or poly alkoxy ethers, depending on the reaction conditions applied (Agrawal et al., 2009; Lynam, 2002). Functionalisation of the upper rim is achieved after partial or complete removal of the *tert*-butyl group(s) by Lewis acid catalysis. Different procedures are then employed to modify the basic core at one or more positions of the upper rim. These procedures include electrophilic substitution (halogenation, fonation, acylation, chlorosulfonation,

formylation, nitration, diazo coupling), Claisen rearrangement and Mannich-type reaction (Gutsche, 2008; Vicens & Böhmer, 2012). Diazo coupling is an example of electrophilic substitution reaction where a diazonium salt is added to calixarene at 0-5 ^{o}C that gives the corresponding azo compound (Deligöz & Ercan, 2002; Tang et al., 2015).

The structural modification of calixarene has an impact on the geometrical shape of the products and their applications. The modified calixarene may be in a cone shape or other configurations (Agrawal et al., 2009; Sliwa & Deska, 2011). The *p-tert*-butylcalix[4]arene is insoluble in water and possesses limited solubility in commonly used organic solvents. However, suitable functionalisation enhances its solubility in common solvents. Sulfonatocalix[4]arene is an example of water soluble calix[4]arene (Perret, Lazar, & Coleman, 2006).

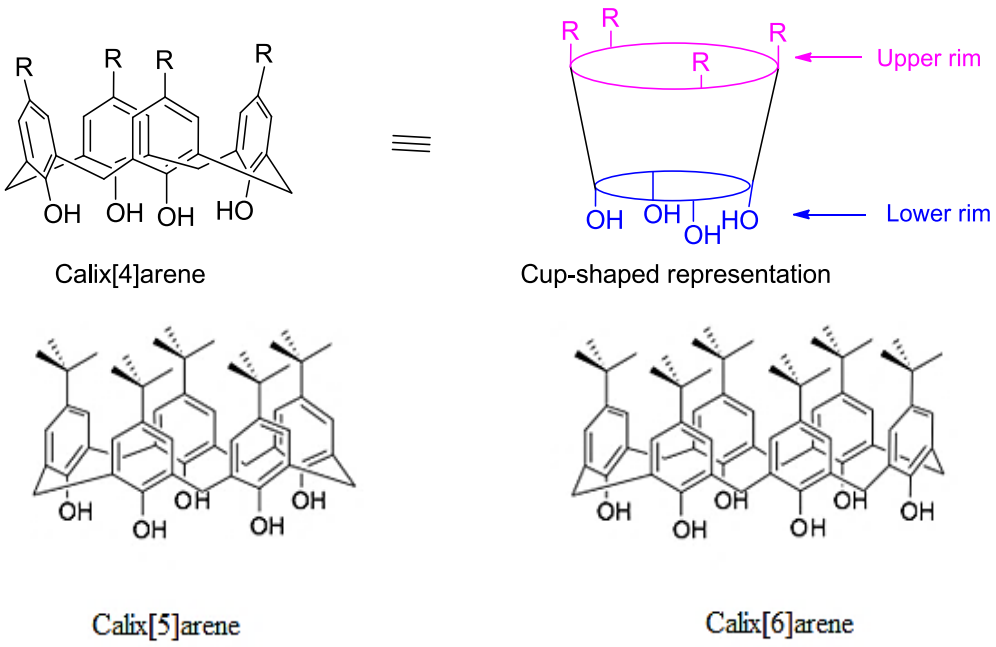

Figure 1.1 Structures of some representative calixarenes

1.2 APPLICATIONS OF CALIXARENES

The applications of calixarene derivatives are observed in the development of metal extractants, ions transporters, stationary phases, electrode ionophores, optical sensors and in biopharmaceutical research (Vicens & Böhmer, 2012). Cornforth reported the first medical application of a calixarene derivative, Macrocyclon (Cornforth et al., 1955). Later on, biomedical stand of various calixarenes was explored by different researchers. Some of the biological activities shown by this versatile class include antibacterial, antiviral, antifungal, antithrombotic, antituberculosis and anticancer activities. In addition, various calixarenes are acting as a 'cargo' for pharmaceutical agents and have the potential to be used in targeted drug delivery (Nimse & Kim, 2013; Tauran et al., 2015).

1.3 AZO CALIXARENES

Compounds containing one or more R^1-N=N-R^2 functional groups are called azo compounds. The R^1 and R^2 may be alkyl or aryl group that result in aliphatic and aromatic azo classes, respectively. They absorb light in the visible spectrum and usually appear in red, yellow or orange colour. Azo compounds do not occur in nature and they belong to the synthetic class of dyes (Christie, 2014; Hamon et al., 2009). The most common pathway for their synthesis is azo coupling reaction.

The structure of calixarene is convenient to incorporate azo moiety directly at the *p*-position of the upper rim or indirectly via OH group at lower rim. The direct incorporation is electrophilic substitution reaction where an aromatic amine of choice is converted into diazonium cation, which reacts with calixarene and produces the corresponding azo compound (Deligöz & Ercan, 2002). The incorporation of azo moiety at the lower rim can be obtained by stepwise procedure (Sliwa & Kozlowski,

2009; Sutariya et al., 2013a; Sutariya et al., 2013b). Either OH group(s) of calixarene is functionalised and connected to azo compounds or the azo compounds are modified first and then attached to the calixarene through OH group(s).

1.3.1 Applications of Azo Calixarenes

Azo chromophore (–N=N–) has wide applications in the development of chromogenic compounds which are widely used in textile, cosmetic, paint, leather, and printing industries. Many of them are utilised in the development of optical sensors molecular devices, metal electrodes and as indicators (Waring & Hallas, 2013). Azo compounds also show therapeutic applications. They are used as drugs and have the potential to be used as drug carriers. They show antibiotic, antifungal, antidiabetic, cytotoxic and antiproliferative properties. Examples of azo drugs include prontosil and sulfasalazine. The reduction of azo bond in the small intestine by the microbial azoreductase makes this class suitable platform for colon targeted drug delivery (Roldo et al., 2007).

Likewise, the insertion of azo moiety further multiplies the applications of calixarenes. Azo calixarenes are used for dying textile and as colourants for various materials (Tang et al., 2015). Other applications of azo calixarenes are observed in various fields such as microelectronics, catalyst recovery, agriculture, biotechnology processes and drinking water purification. They are also utilised in the development of optical devices, chemical sensor devices, ion transport membranes and luminescence probes (Yilmaz & Memon 2009; Deligoz & Memon, 2011). However, Azo calixarenes are mostly reported for their metals extraction potential (Chawla, Singh, & Upreti, 2006; Elçin et al., 2016). They are used as molecular receptors to recognise neutral molecules, cations and anions. The significance of ionophoric potential of azo calixarenes is observed in chemical, biological and environmental sciences.

As for biological applications of azo calixarenes are concerned, there is no comprehensive study addressing drug-like activities of azo calixarenes. There are only a few reports on their biological activities, mostly antibacterial activity. Lamartine's group reported slight activity of two azo calix[n]arene (n = 4,6) against *Corynebacterium* (bacteria) and *F. solani* (fungus) (Lamartine et al., 2002), while Jain, Mandalia, & Bhojak, (2010) evaluated azo calix[4]pyrrole against *Escherichia coli, S. aureus,* and *Aspergillus niger*. Recently, a pyridine-based azo calix[4]arene has been reported to show activity against *B. cereus.* (Rahnama et al., 2016).

1.4 COMPUTER-AIDED DRUG DESIGN

Drugs are usually small organic molecules or compounds that regulate the function of biomolecule either as stimulators or inhibitors. Drug designing is the process of development of new therapeutic agents on the basis of the biological target(s). Computer-Aided Drug Design (CADD) or *in silico* drug design deals with ligand-receptor (drug-protein) binding interactions. It has marvellous role in the introduction of new drugs and is often utilised in two ways; screening a library of compounds in search of suitable candidates for a targeted receptor or discovering the possible interaction of a potent molecule against a selected receptor (Gill et al., 2016; Macalino et al., 2015). Docking is a proficient tool for elucidating the three-dimensional (3D) structure of the ligand-receptor complex and estimates the constancy and reliability of the complex with respect to bioactivity. Molecular docking describes the "best-fit" orientation of a ligand that binds to the target receptor (protein) (Halperin et al., 2002; Kitchen et al., 2004).

Many drugs are enzyme inhibitors (Hopkins & Groom, 2002). The enzyme inhibitors are chemical compounds that reduce or completely stop the catalytic

activity of enzymes. Docking study is performed to predict the affinity of a potential inhibitor for an enzyme. Ligand (drug-like compound/the inhibitor) is placed inside the binding pocket of the enzyme in a suitable conformation that gives minimum energy for the complex. The *in silico* screening of enzyme-inhibitor complex gives insight which compounds are the probable potent inhibitors that may be considered for synthesis or bioassay**.** Molecular docking shows how a particular molecule behaves in the binding pocket of a receptor that makes the molecule more or less active drug (Ekins, Mestres, & Testa, 2007; Ferreira et al., 2015). On the basis of in vitro and *in silico* studies, some calixarene derivatives have been recommended as enzyme inhibitors (Chini et al., 2010; Trush et al., 2013).

1.5 PROBLEM STATEMENT

Azo calixarenes are an important class of organic compounds that act as dyes, indicators and complexing agents. However, the drug-like activity of azo calix[4]arene has not been fully explored yet. So far, only a few reports are available regarding their bioactivities. The data is insufficient to draw a clear conclusion and understand the medicinal stands of azo calixarenes. The addition of azo moiety into the basic core of calixarenes may grant them, or enhance their drug-like property. Research on this particular aspect of azo calixarenes might provide a good podium in the development of potent drugs for the treatment of chronic and infectious diseases. Computational study of calixarene derivatives has been done to explore and understand their bioactivities. However, investigation on the medicinal potentail of azo calix[4]arene via computational study has not been given much consideration. Further, due to the symmetry of calix[4]arene, it is quite a challenge to introduce functional group(s) in a selective fashion on the *para* position. For selective functionalisation, the reaction

conditions need to be optimised to produce the mono azo product in quantitative yield.

1.6 RESEARCH OBJECTIVES

The research work was carried out with the following main objectives:

1. To synthesise and characterise both upper and lower rim modified new azo-based calix[4]arenes.
2. To evaluate the biological properties of synthesised compounds using antiproliferative, antibacterial and antifungal assays.
3. To perform molecular docking study of the selected compounds based on the activity shown in the *in vitro* assay.

1.7 RESEARCH HYPOTHESIS

The research work is based on the assumption that some new azo calix[4]arenes can be synthesised and characterised that might show better solubility in common organic solvents. The insertion of azo moiety might enhance the biological stand of calix[4]arene. The docking study might be helpful in the exploration of biomedical aspect of azo calix[4]arene.

1.8 SCOPE OF THE STUDY

The aim of this project is to expand the applications of azo calix[4]arenes by investigating their biological potential as active drugs. The targeted compounds were synthesised by structural modification at upper or/and lower rims of calix[4]arene. Aromatic amines of different chemical nature were selected to observe the influence of diversity in their biological activity. Compounds with already known biological