SYNTHESIS AND COMPUTATIONAL STUDIES OF SYDNONE-BASED DERIVATIVES AS POTENTIAL ANTI-INFLAMMATORY AGENTS

BY

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ABSTRACT

Various literature sources have documented that sydnones are important bioactive molecules with a wide spectrum of activities involving the anti-proliferative and anti-inflammatory actions. Phenyl styrylketones and their derivatives as members of the chalcone family have also been reported as significant biological agents. The current study was initiated to evaluate *in vitro* cytotoxic and anti-inflammatory activity of sydnone-based compounds including some novel sydnone-styrylketone hybrids. The classical cyclodehydration of *N*-nitroso amino acids was applied in the preparation of the sydnone ring. Aldol condensation was utilized to join two sydnone rings by a styrylketone linker. Compounds identity was confirmed using FTIR, NMR and MS spectroscopy. MTT assay was used to evaluate the cytotoxicity of the synthesized compound. ELISA test was performed to investigate the COX-1/COX-2 inhibitory activity. The binding of sydnones with COX enzymes was examined by Glide docking and the Molecular Mechanics-Generalized Born Surface Area (MM-GBSA). The drug-likeness scores and membrane permeability of the compounds were also *in silico* predicted. Twenty-five sydnone-containing compounds were synthesized. Compounds **46-48** and **56-58** were reported as new sydnone derivatives. Compounds **61-63** were synthesized as novel structures containing two sydnone rings linked via α,β-unsaturated ketone. The structure of the synthesized compounds was confirmed by FTIR, ^{1}H NMR, ^{13}C NMR and ToF-MS analyses. All compounds exhibited low cytotoxicity especially against normal cell lines (IC_{50} in the range of mM). Only compound **45** had a significant antiproliferative activity against prostate (IC_{50} = 42 μM) and breast (IC_{50} = 63 μM) cancer cell lines. The *in vitro* COX inhibition assay showed varied activity. Compounds **47, 51, 58** and **63** showed the most potent COX inhibitory effect at a concentration of 200 μM. Selectivity index showed that only compound **63** was a selective COX-2 inhibitor. Acetylation of the sydnone ring at C4 was detrimental to the cytotoxic activity while prolific for the anti-inflammatory effects (COX inhibition). Docking analysis showed that COX-2 selectivity was due to a favorable positive charged interaction between the sydnone ring of **63** and Arg513 in the catalytic region of COX-2. Compound **51** was hydrogen bonded to the guanidinium group of Arg513. The low inhibitory effect of **63** against COX-1 was due to an unfavorable polar interaction with His513 in the binding pocket of COX-1. Drug-likeness prediction disclosed that the compounds comply with Lipinski's rule and CMC-like rule. Similarity search delineated that sydnone-styrylketone hybrids had common structural features with known anti-inflammatory agents. Prediction of permeability through the physiological membrane revealed a good pharmacokinetic profile with intestinal absorption more than 80% and a potential BBB penetration. In conclusion, the compounds were successfully synthesized and characterized. However, only two compounds **59** and **60** were not successfully prepared. The structure of 3-(4-chloro-3-nitrophenyl)sydnone **45** could be a lead molecule in designing potent chemotherapeutic agents. Compound **63** shared architecture and pharmacophoric characters with known selective COX-2 inhibitors (coxib family) making it a good candidate for designing selective and safe NSAID.

خلاصة البحث

أثبتت مصادر علمية عديدة الأهمية الحيوية لمركبات السيدنون في مجالات متنوعة وخاصة الفعالية المضادة للتكاثر الخلوي والمضادة للالتهاب. تعتبر مركبات الفنيل ستيريل كيتون من عائلة الشالكون وهي ذات فعالية علاجية قوية. الهدف من هذه الدراسة هو تقييم الفعالية الخلوية السمية والمضادة للالتهاب في الزجاج لمركبات ثناية السيدنون مرتبطة بالفنيل ستيريل كيتون. استخدمت الطريقة التقليدية في تحلق مركبات نتروزو الحموض الأمينية لتحضير حلقة السيدنون. استخدم التكاثف الألدولي لربط حلقتي سيدنون برابط الستيريل كيتون. وتم التأكد من بنية المركبات باستخدام طرائق المطيافية. تم تقييم الارتباط الجزيئي لمركبات السيدنون مع أنزيمات الكوكس باستخدام تقنية غلايد Glide والطاقة الحرة للارتباط MM-GBSA. بالإجمال تم اصطناع خمسة وعشرين مركباً. وكانت المركبات 46-48 و 56-58 مشتقات جديدة للسيدنون. المركبات 61-63 اعتبرت على أنها بنية جديدة تماماً. أظهرت كل المركبات فعالية ضعيفة سامة للخلايا وخاصة ضد الخلايا الطبيعية. فقط مركب 45 أظهر فعالية قوية كمضاد لسرطان البروستات (IC_{50}= 42 ميكرومولار) وسرطان الثدي (IC_{50}= 63 ميكرومولار). أظهرت مركبات 47و 51و 58 و 63 فعالية مثبطة لأنزيم الكوكس وكان المركب 63 أكثر المركبات انتقائية على الكوكس -2. أظهرت دراسة الارتباط االجزيئي أن المركب 63 يرتبط مع الكوكس -2 من خلال الحمض الأميني Arg513 من خلال تفاعل الشحن الموجبة. انخفاض الفعالية المثبطة لمركب 63 تجاه الكوكس -1 بسبب ارتباط غير قطبي غير ثابت مع الحمض الأميني His513. أثبتت الدراسة أن مركب 63 يمتلك تشابه بنيوي مع المركبات المعروفة المضادة للالتهاب. أظهرت دراسة النفاذ عبر الأغشية عن طريق الحاسب أن المركبات المصطنعة تمتلك حرائك دوائية جيدة من خلال امتصاصية عالية عبر الغشاء المعوي وحاجز الدماغي الوعائي. خلاضة البحث: تم اصطناع المركبات بنجاح باستثناء مركبين 59 و 60. يمكن اعتبار بنية المركب 45 دليلاً جيداً لتطوير مركبات قوية لعلاج السرطان. إن المركب 63 يمتلك خواص دوائية وبنيوية مشابهة للمركبات الانتقائية على كوكس-2 ولذلك يمكن اعتباره مركب جيد كدليل لتطوير وتحضير صيغ جديدة مضادة للالتهاب.

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.

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Special thanks to you dear great Mom, your selfless spiritual imparted moral support have always been with me helping me in achieving my goals. Tons of thanks to my beloved Dad, you've sacrificed both personally and professionally for me to chase down my dreams. I have to express my feelings of appreciations to you my dear wife, most especially if I remember your love towards me. Millions thanks to my brothers and sisters for their continuous support and motivation.

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LIST OF ABBREVIATIONS

^{1}H NMR	Proton nuclear magnetic resonance
^{13} C NMR	Carbon-13 nuclear magnetic resonance
AA	Arachidonic acid
AChE	Acetyl choline esterase
AD	Alzheimer's disease
ANOVA	Analysis of variance
BA	B-amyloid
BBB	Blood-brain barrier
BHA	Butyl hydroxy anisole
CADD	Computer-aided drug design
CGM	Complete growth media
CNS	Central nervous system
COX	Cyclooxygenase
DBH	1,3-Dibromo-5,5-dimethylhydantoin
DCM	Dichloromethane
DF	Dilution factor
DME	Dimethoxy ethane
DMSO	Dimethyl sulfoxide
ELISA	Enzyme-linked immunosorbent assay
EtOH	Ethanol
ER	Endothelial reticulum
ESI	Electrospray ionization
FTIR	Fourier transform infrared spectroscopy
GABA	Gamma-aminobutyric acid

GIT	Gastro-intestinal tract
GLDH	Glutamate dehydrogenase
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HOMO	High occupied molecular orbital
IA	Intestinal absorption
IAN	Iso-amyl nitrite
IR	Infrared
LUMO	Lowest unoccupied molecular orbital
MBD	Membrane binding domain
MM-GBSA	Molecular Mechanics-Generalized Born Surface Area
MO	Molecular orbital
MS	Mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NADPH	Nicotinamide adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance
NSAID	Non-steroidal anti-inflammatory drug
NSB	Non-specific binding
OD	Optical density
OPLS	Optimized Potential for Liquid Simulations
PBS	Phosphate buffer saline
PDB	Protein data bank
PG	Prostaglandin
PMA	Phorbol myristate acetate ester
RMSD	Root-mean-square deviation