Abstract

Alteration in dopaminergic and serotonergic neurotransmission influences various neurological and mental disorders such as depression, anxiety, bipolar disorder, schizophrenia and drug abuse. The naturally occurring aporphine alkaloids are well known for their activity at D_1, D_2 and 5-HT_{1A} receptors, but only a few have been shown to bind to the 5-HT_{2A} receptor. Aim of this study was to identify aporphines with significant activity at dopamine and serotonin receptors using both *in silico* and *in vitro* screening approaches. A 3D homology model of the rat 5-HT_{2A} receptor was generated using the crystal structure of the human β_2-adrenergic receptor (PDB ID: 2RH1) and validated with standard 5-HT_{2A} receptor ligands. A filtered set of aporphines obtained from the ZINC database using (*S*)-boldine as the backbone structure was docked into the generated 5-HT_{2A} receptor model. A set of 13 compounds were identified with higher or comparable activity to (*S*)-boldine for experimental testing across the D_1, D_2, 5-HT_{1A} and 5-HT_{2A} receptors using a medium throughput radioligand receptor binding assay. (*R*)-roemerine was found to have selective 5-HT_{2A} binding affinity with 20–400-fold higher affinity for the 5-HT_{2A} receptor versus the D_1, D_2, and 5-HT_{1A} receptors. Investigation into the structures of the selected compounds revealed that substitution at positions 1 and 2, particularly with a methylenedioxy group, non-substitution at positions 10 and 11 and a protonated amino group at position 6 may be responsible for the good affinity-selectivity profile of (*R*)-roemerine for the 5-HT_{2A} receptor compared to the other compounds. Further analysis of the binding modes of the selected compounds also showed that the combination of an electrostatic interaction and the hydrogen bonding between the protonated amino group of (*R*)-roemerine and Asp155 and a pi-cation interaction with Phe339 appears to explain its enhanced affinity and selectivity as compared to the other compounds. The results illustrate the usefulness of a

combined *in silico* and *in vitro* approach in the search for lead molecules for the development of new selective drugs acting at dopamine and serotonin receptors.

Abstrak

Perubahan pada neurotransmisi dopamin dan serotonin mempengaruhi pelbagai gangguan neurologi dan mental seperti kemurungan, keresahan, gangguan bipolar, skizofrenia dan penyalahgunaan dadah. Alkaloid aporphine semulajadi terkenal dengan aktivitinya pada reseptor D_1, D_2 dan 5-HT_{1A}, tetapi hanya sebilangan daripadanya menunjukkan ikatan pada reseptor 5-$HT_{2A.}$ Tujuan kajian ini adalah untuk mengenalpasti aporphine dengan aktiviti yang siknifikan pada reseptor dopamin dan serotonin menggunakan kedua-dua pendekatan *in silico* dan *in vitro*. Satu model homologi 3D bagi reseptor tikus 5-HT_{2A} telah dijana dengan menggunakan struktur kristal reseptor manusia β_2-adrenergik (PDB ID: 2RH1) dan divalidasi dengan ligan asas 5-HT_{2A}. Satu set aporphines yang ditapis diperoleh dari pangkalan data ZINC dengan menggunakan (*S*)-boldine sebagai struktur asas untuk didok dalam reseptor 5-HT_{2A} yang telah dijana. Sebanyak 13 kompaun telah dikenalpasti dengan aktiviti yang lebih tinggi atau setanding dengan (*S*)-boldine untuk diuji secara experimental pada reseptor D_1, D_2, 5-HT_{1A} dan 5-HT_{2A} dengan pengendalian asei reseptor radioligan. (*R*)-roemerine menunjukkan ikatan afiniti yang selektif pada reseptor 5-HT_{2A} dengan 20–400-kali ganda lebih tinggi untuk reseptor 5-HT_{2A} berbanding dengan reseptor D_1, D_2 dan 5-$HT_{1A.}$ Kajian menyeluruh bagi struktur kompaun yang terpilih menunjukkan bahawa (*R*)-roemerine dengan penggantian pada kedudukan 1 dan 2, khususnya dengan kumpulan metilenadioksi, tanpa penggantian pada kedudukan 10 dan 11 dan kumpulan amino berproton pada kedudukan 6 bertanggungjawab kepada profil afiniti-selektiviti yang baik oleh (*R*)-roemerine bagi reseptor 5-HT_{2A} berbanding kompaun lain. Analisis lanjutan bagi jenis interaksi oleh kompaun terpilih juga menunjukkan bahawa gabungan interaksi elektrostatik dan ikatan hidrogen antara kumpulan amino berproton dalam (*R)*-roemerine dan Asp155 dan interaksi pi-kation dengan Phe339 menjurus kepada

peningkatan afiniti dan selektiviti berbanding dengan kompaun lain. Keputusan ini menunjukkan kepentingan gabungan kedua-dua kaedah *in silico* dan *in vitro* dalam pencarian molekul baru bagi perkembangan ligan yang selektif yang bertindak pada reseptor dopamin dan serotonin.

Acknowledgements

First and foremost, I'm indebted to my supervisors, Prof. Dr. Chung Lip Yong and Dr. Michael James Christopher Buckle for their support, guidance and encouragement throughout this project. I'm thankful to Prof. Dr. Stephen Doughty for his comments and guidance, especially in molecular modelling.

I wish to express my special and sincere thanks to Dr. Azura and all the staff from the animal house for their assistance. I would also like to acknowledge the help of all the staff from Department of Pharmacy and Department of Molecular Medicine. I wish to thank my labmates and friends for their support and friendship.

I sincerely thank to Ministry of Science, Technology and Innovation, Malaysia for the National Science Fellowship award (Ref No: M/0138/02/2008/LAIN2) and University of Malaya for the research grants (PPP vote: PS192/2009C; UMRG: RG009/09BIO).

At last but not the least I would like to thank my beloved family for their undying support, love and blessings, and finally to God who made all the things possible.

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List of Symbols and Abbreviations

2D	2 Dimensional
3D	3 Dimensional
5-HT	5-hydroxytryptamine, serotonin
5-HT_{1A}	5-hydroxytryptamine$_{1A}$
5-HT_{2A}	5-hydroxytryptamine$_{2A}$
5-MeOT	5-methoxytryptamine
8-OH-DPAT	(±)-8-hydroxy-2-(di-*n*-propylamino)-tetralin
[^{3}H] 8-OH-DPAT	8-Hydroxy-2-[2,3-^{3}H]di-*n*-(propylamino)tetralin
[^{3}H] SCH 23390	[^{3}H] 7-chloro-8-hydroxy-3-methyl-5-phenyl-2,3,4,5-tetrahydro-1-*H*-3-benzazepine
aa	Amino acid
AMP	Adenosine monophosphate
APDs	Antipsychotic drugs
B_{max}	Maximal binding capacity
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
CV	Coefficient of variation
D_1	Dopamine$_1$
D_2	Dopamine$_2$
DOI	(±)-2, 5-dimethoxy-4-iodoamphetamine
DMSO	Dimethyl sulphoxide
ECL	Extracellular loop
EtOH	Ethanol

GF/B	Glass fibre type B
GF/C	Glass fibre type C
GPCR	G-protein coupled receptor
HCl	Hydrochloride
IC_{50}	Concentration at 50% inhibition
K_d	Equilibrium dissociation constant
K_i	Inhibition constant
[L]	concentration of radioligand
MDMA	Methylenedioxymethamphetamine
MeOH	Methanol
NSB	Nonspecific binding
PDB	Protein Data Bank
SAR	Structure activity relationship
SB	Specific binding
S/B	Signal to background ratio
SCH 23390	*R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine
SD	Standard deviation
SEM	Standard error mean
SBDD	Structure-based drug design
TB	Total binding
Tris	Tris(hydroxymethyl)aminomethane
UV	Ultraviolet
WHO	World Health Organization

Units of Measurement

%	Percent
Å	Angstrom
$^{\circ}$C	Degree celsius
C_i	Curie
ε	Epsilon
× g	Times gravity
µg	Microgram
cpm	Count per minute
M	Molar
mg	Milligram
min	Minute
ml	Milliliter
mM	Millimolar
nM	Nanomolar
min	Minute
Rpm	Revolutions per minute
v/v	Volume per volume
w/v	Weight per volume

List of Amino Acids

Ala (A)	Alanine
Arg (R)	Arginine
Asn (N)	Asparagine
Asp (D)	Aspartate
Cys (C)	Cysteine
Gln (Q)	Glutamine
Glu (E)	Glutamate
Gly (G)	Glycine
His (H)	Histidine
Ile (I)	Isoleucine
Leu (L)	Leucine
Lys (K)	Lysine
Met (M)	Methionine
Phe (F)	Phenylalanine
Pro (P)	Proline
Ser (S)	Serine
Thr (T)	Threonine
Trp (W)	Tryptophan
Tyr (Y)	Tyrosine
Val (V)	Valine