Three Dimensional Quantitative Structure Activity Relationship of a Series of Arylpiperazines: α_{1a}-Adrenoceptor Antagonists

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Agents having selectivity to α_{1a}, α_{1b} and α_{1d} adrenoceptors could be useful in generation of effective molecules for treatment of variety of diseases. In order to gain an insight into the structural principles governing subtype selectivity, three dimensional quantitative structural activity relationship studies have been performed on a set of arylpiperazines for their α_{1a}-adrenergic receptor antagonistic activity by using the logico-structural based approach for pharmacophore mapping. All compounds were superimposed on the identified biophores and three dimensional quantitative structurel activity relationship models were developed. The resulting models exhibited good r^2 (>0.80) values. Among several models, one model of comparable probability was selected on the basis of $r^2 > 0.80$, chance≤ 0.10, size≤ 3 and match>0.30, which might show optimum desired effects.

Quantitative structure activity relationship (QSAR) is an important tool in drug designing techniques. It is a technique that quantifies the relationship between structure and biological data and is useful for optimizing the groups that modulate the potency of the molecule[1,2]. Classical and three-dimensional (3D) QSAR techniques are used for optimization of drug molecules. The difference between two approaches depends on the type of the molecular descriptors used to correlate molecular structure to biological activity. Classical QSAR methods rely on the descriptors that are scalars and essentially measure one dimension in the parameter space (e.g. Log P, MR, Hansh-Fujita lipophilic constants, Hammett electronic constants and Verloop Sterimol parameter). Three-dimensional QSAR methods are based on the detailed description of the local properties of each chemical structure. They are sensitive to particular conformation adopted by a molecule as well as its orientation with respect to other molecules[3]. Proposed work includes development of a 3-D QSAR model of subtype-selective α_{1a}-adrenergic receptor

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(α_{1a}-ARs) antagonist for the treatment of benign prostatic hyperplasia (BPH).

α_{1a}-ARs, the membrane proteins and members of G-protein coupled receptor superfamily, which function primarily by increasing or decreasing the intracellular production of second messengers (cAMP or IP_3/DAG) can be ramified into at least three subtypes, $\alpha_{1a}(\alpha_{1A})$, $\alpha_{1b}(\alpha_{1B})$ and $\alpha_{1d}(\alpha_{1D})$ with upper and lower case subscripts being used to designate native or recombinant receptors, respectively[4]. So the drugs that interact selectively as antagonists with α_1-ARs, have been used in the treatment of prostatic hypertropy, which can reduce prostatic intraurethral pressure, by the complete blockade of sympathetic outflow to the lower urinary tract. Several α_1-ARs antagonists such as prazosin[5], terazosin[6], doxazosin[7] and tamsulosin[8] have been developed for the treatment of BPH. These agents block α_1 adrenoceptor thereby prompt smooth muscle relaxation in prostate and the neck of bladder[9] but all of them possess a side effect of orthostatic hypotension[10], presumably as a result of their lack of selectivity for any one of these α_{1a}-ARs subtypes[11,12]. These findings suggest that the development of a subtype-selec-

tive α_{1a}-ARs antagonist might result in a therapeutically effective agent with reduced side effects. On the basis of above findings, development of a subtype-selective α_{1a}-ARs antagonist with a potential to reduce risk of orthostatic hypotension, for the treatment of BPH, is of current interest.

In order to design selective antagonist, there is a need for the identification of biophores (pharmacophores) in terms of essential structural and electronic features important for the activity and development of 3D-QSAR models. Some 3D-QSAR studies of α_1-ARs antagonists have been reported[13] so far, however no such studies have been carried out on arylpiperazines[14]. Among the various approaches available, logico-structural based approach was used by means of Apex-3D software[15] of Molecular Simulations Incorporation (MSI) having INDY R-4000 workstation. Apex-3D expert sys-

tem, developed by Golender et al.[16], uses the data for classification and prediction of biological activity. It automatically identifies biophores that represent a certain structural and electronic pattern in a bioactive molecule, which is responsible for its activity through interaction with the receptors. A number of linear 3D-QSAR models have also been developed by Apex-3D expert system from a set of arylpiperazine derivatives that act as α_{1a}-ARs antagonists and the results are described in this paper.

MATERIALS AND METHODS

The molecular modeling software (particularly Apex 3D module) of Molecular Simulation Inc. was used to develop pharmacophore modules. The structures of all compounds were drawn in 2D using the sketch programme in the builder

TABLE 1: *IN VITRO* ACTIVITY (KI) DATA FOR ARYLPIPERAZINE SERIES AS α_{1a} ADRENOCEPTOR ANTAGONISTS
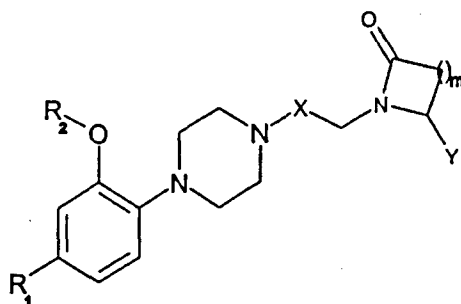
N-[1-(lactam)methyl] substituted arylpiperazine derivatives

Comp. No.	R$_1$	R$_2$	X	Y	KI	m	–logKi
la.	H	i-pr	$C_2H_4N(CH_3)CO$	H	53	3	–1.725
1b.	H	Me	$(CH_2)_3NHCO$	H	98	3	–1.991
1c.	H	i-pr	C_2H_4NHCO	H	8.7	3	–0.939
1d.	H	i-pr	C_2H_4NHCO	CO$_2$Et	20	2	–1.30
1e.	H	i-pr	C_2H_4NHCO	H	18	4	–1.225
1f.	F	i-pr	C_2H_4NHCO	H	83	3	–1.920
1g.	F	CH_2CF_3	C_2H_4NHCO	H	129	3	–2.11
1h.	H	i-pr	$(CH_2)_3NHCO$	H	0.66	3	0.180
8.	H	i-pr	$(CH_2)_2OCO$	H	38	3	–1.580
11.	H	i-pr	CH_2COCH_2CO	H	417	3	–2.638
14.	H	i-pr	$CH_2CH(OH)CH_2CO$	H	28	3	–1.447
15.	H	i-pr	$CH_2CHCHCO$	H	18	3	–1.255

Ki data expressed in nanomolar concentration (nm) are determined by radioligand binding assay which tested the binding affinity of these compounds to COS cell membranes expressing the human adrenoceptor subtype α_{1a}-ARs.

module of insight II software and then were converted to 3D for optimization of their geometry (net charge 0.0) by selecting the force field viz. potential action, partial charge action and formal charge action as fixed. The energies of molecular structures were finally minimized using the Steepest Descent, Conjugate Gradients and Newton-Raphson's algorithm in sequence followed by Quasi-Newton-Raphson (Va09a). Optimization techniques were implemented in the Discover module (Ver. 2.9) by using energy tolerance value of 0.001 Kcal/mol and minimum number of iterations set to 1000. This method of energy minimization is suitable because the minimum energy obtained by this method and the total energy obtained by molecular dynamics (MD) simulations using consistent-valence forcefield (CVFF) was comparable. The structures were stored in MDL format and then converted with MOPAC 6.0 (MNDO Hamiltonian) module for computational calculation of different physico-chemical properties like atomic charges, π-population, electron donor and acceptor indices, highest occupied moleclular orbital (HOMO) and lowest un-occupied orbital (LUMO) coefficients, hydrophobicity and molar refractivity based on atomic contributions. These data were exported into the insight II module of Apex-3D by (MSI) automated identification of biophore(s), superimposition of compounds and quantitative model building.

Robustness of each model was also evaluated on the basis of statistical reasons. For ideal robust model, the difference between root mean square approximation (RMSA) and root mean square prediction (RMSP) should be minimum, explained variance (r^2) > 0.50, chance (evaluated as the ratio of equivalent regression equation to the total number of randomized sets) should be close to zero, match value (quality of superimposition of biophore) should be close to one and size (number of descriptor center(s) in the biophore) as minimum as possible.

RESULTS AND DISCUSSION

In vitro activity data of a set of arylpiperazines for α_{1a}-antagonistic activity[14] are given in Table 1. Among several

3D-pharmacophore models with different sizes and arrangements, one model of comparable probability was selected on the basis on squared correlation coefficient (r^2) > 0.80, chance $\leq$ 0.10, size $\leq$ 3 and match value > 0.30. Statistical results of this model are given in Table 2.

In fig. 1, site A and site B represent the two biophoric sites corresponding to the oxygen atom of NHCO group and the nitrogen atom of the lactam ring, respectively. These biophoric sites are important for binding with the α_{1a}-ARs having physico-chemical properties in terms of π-electron density on atoms [π-population]: 0.963($\pm$0.033), point atomic charge in a.u. [charge_heteroatom]: -0.387 ($\pm$0.020), and mean electron donor reactivity of atoms with loan pair [Don_01]: 8.295($\pm$0.054) for site A and π-population: 0.429($\pm$0.014), and charge_heteroatom: -0.404 ($\pm$0.015) for site B. Fig. 2 indicates superimposition of all molecular structures on the identified biophores. All these properties of the biophoric sites are given in Table 3.

In addition to this, site A is electron rich site capable of donating electrons by the oxygen atom. So we can say that carbonyl oxygen should be involved in ionic bonding and/or electrostatic and π-π interactions. Site C, which is an electronic cloud on the benzene ring as indicated in fig. 1 contributes to the π-electrons.

The mean interatomic distance between the biophoric sites, A-B and the distances between the biophoric sites and the electronic cloud (A-C and B-C) indicated by C have been 3.357, 9.636 and 11.091Å, respectively. All these properties, distances and spatial arrangement of the biophoric sites are important for their interaction with the α_{1a}-receptor to show antagonistic activity.

After identification of the key structural features described above as biophoric centers for all the molecules, 3D-multiparameter equation was derived (Eqn. 1) using these biophores as a template for superimposition. The in vitro activity was related to three secondary parameters: LUMO, heteroatom and steric refractivity corresponding to S_1, S_2 and

TABLE 2: 3D-QSAR MODEL DESCRIBING CORRELATION AND STATISTICAL RELIABILITY OF α_{1a}-ANTAGONISTIC ACTIVITY.

RMSA	RMSP	r^2	Chance	Size	Match	Var.	Comp.
0.49	0.45	0.83	0.10	3	0.38	3	12

RMSA stands for root mean square approximation, RMSP is root mean square pridiction, r^2 is squared correlation coefficient, Chance is probability of correlation, Size is number of descriptor centers in the biophores, Match is quality of superimposition of biophore, Var. is number of variable in 3D-QSAR model and Comp. is number of compounds in 3D-QSAR model.

TABLE 3: PROPERTIES OF THE BIOPHORIC SITES IN SELECED MODEL.

Compound No.	Site A			Site B	
	π_pop	Charge_Het	Don_01	π_pop	Charge_Het
1a.	0.967	-0.384	8.269	0.410	-0.427
1b.	0.963	-0.384	8.405	0.430	-0.403
1c.	0.956	-0.400	8.248	0.408	-0.428
1d.	0.961	-0.393	8.228	0.441	-0.407
1e.	0.955	-0.401	8.268	0.398	-0.433
1f.	0.963	-0.387	8.405	0.430	-0.403
1g.	0.958	-0.397	8.219	0.408	-0.428
1h.	0.963	-0.387	8.295	0.429	-0.404
8.	0.961	-0.382	8.260	0.411	-0.422
11.	0.985	-0.386	8.410	0.441	-0.401
14.	1.040	-0.285	8.544	0.413	-0.426
15.	1.243	-0.251	8.618	0.418	-0.432

π_pop stands for π-electron density on atoms, Charge_Het is point atomic charge in a.u. and Don_01 is mean electron donor reactivity of atoms with loan pair.

TABLE 4: STRUCTURE-ACTIVITY DATA FOR THE SELECTED MODEL.

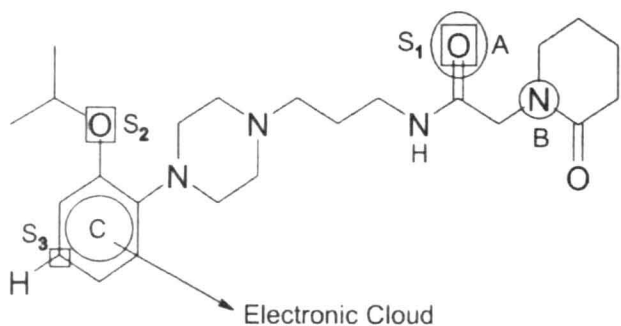| Compound No.	Experimental Activity	Calculated Activity	Calculated Error	Predicted Activity	Predicted Error
la.	-1.72	-1.90	0.18	-2.04	0.32
1b.	-1.99	-1.90	-0.09	-1.84	-0.15
1c.	-0.94	-1.42	0.49	-1.54	0.60
1d.	-1.30	-1.42	0.12	-1.45	0.15
1e.	-1.25	-1.42	0.17	-1.47	0.22
1f.	-1.92	-1.61	-0.31	-1.54	-0.38
1g.	-2.11	-1.61	-0.50	-1.49	-0.62
1h.	0.18	0.24	-0.06	-1.15	-0.97
8.	-1.58	-1.79	0.21	-1.85	0.27
11	-2.64	2.50	-0.14	2.46	-0.18
14.	-1.45	-1.47	0.02	-1.54	0.10
15.	-1.25	-1.22	0.03	-1.19	-0.07

Fig. 1: Representative example of the most active compound (1h).

Most active compound showing biophoric site [○] and secondary site [□].

S_3, respectively as indicated in fig. 1. -log Ki =-7.294(±1.731)LUMO+0.479(±0.261), Heteroatom - 0.621(±0.118), Steric refractivity + 0.239.. 1, n=10, r=0.91, r^2=0.83, $F_{(3,6)}$=9.882.

Secondary site S_1, which also acts as a biophoric site A and secondary site S_3 at the carbon atom of R_1 substituent negatively contributes for the activity. It indicates that energy of the LUMO at S_1 should be minimum and steric interactions at site S_3 are not necessary for the activity. So substitution of less bulky substituent at this site is favorable for the α_{1a}-antagonistic activity. This equation also suggests that the presence of heteroatom with more electronegativity at site S_2 will be favorable for the activity. Experimental, calculated and predicted (by Leave One Out Technique) structure activity data for the selected model are given in Table 4.

We have been able to develop statistically significant and highly predictive QSAR model together with the electronic and structural features in terms of physico-chemical properties π-population, charge_heteroatom, H-site DON_01) which are essential for binding very well to the α_{1a}-subtype. This has been achieved by the superimposition of molecular structures on the identified biophores. These biophores are extremely important for a compound to interact with the receptors. All features (physico-chemical properties, structural and secondary site parameters) should be considered for the design of new potent and selective antagonists. The field is further open for study of other drug design techniques.
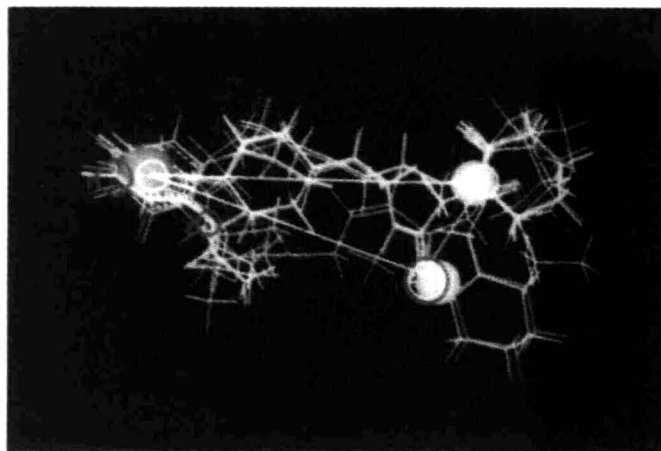
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Fig. 2: Photograph showing superimposition of the structure on the biophore.

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