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3-D QSAR Studies of Triazolinone Based Balanced AT_1/AT_2 Receptor Antagonists†

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Abstract—Essential structural and physicochemical requirements in terms of common biophoric sites (pharmacophore) and secondary sites for binding and interacting with AT_1 and AT_2 receptors have been identified using APEX-3-D expert system on 16 N^2-aryl triazolinone biphenyl sulphonamides. Among several biophoric 3-D QSAR models two models (Nos. 1 and 2) having $R^2 > 0.7$, chance < 0.05 and match > 0.5 and two models (Nos. 3 and 4) having $R^2 > 0.89$, chance < 0.03 and match > 0.5 with three biophoric sites and two secondary sites (except model No. 4 with three secondary sites) describe the variation in AT_1 and AT_2 antagonistic activities, respectively.

Introduction

Renin Angiotensin System (RAS) constitutes a proteolytic cascade that results in the formation of the endogenous octapeptide hormone angiotensin II (AII).[1] Two distinct types of AII receptors have been identified following the discovery of potent non-peptide AII antagonists Losartan[2] and PD-123,177,[3] which are selective for the two receptors respectively, and the area is of current interest.[4] Modifications in the structures of Losartan with various replacements ranging from fused rings containing the imidazole to a number of *N*- or *C*-linked nitrogen heterocycles have resulted in a large number of molecules which have strong affinity for the AT_1 receptor.[5] The AT_1 receptor is G-protein coupled[6] and is now established to mediate the known physiological effects of AII including regulation of blood pressure.[7] The AT_2 receptor may be involved in regulation of renal function[8] and may play a role in restenosis following vascular injury,[9] wound healing,[10] cardiac fibroblast collagen synthesis,[11] cell differentiation and cell proliferation process.[7,12] AT_2 receptor also has a seven transmembrane domain and is linked to phosphotyrosine phosphatase activity[13] but, unlike a large number of AT_1 receptor ligands, only some AT_2 selective ligands with high affinity have been described.[14,15] The plasma concentration of AII increases considerably in presence of AT_1 selective antagonists.[16] The physiological effect of prolonged stimulation of AT_2 receptor by increased levels of AII is not known. Nevertheless simultaneous inhibition of both the receptors might be beneficial.[17] Thus attempts have been made to obtain compounds with equal affinity for both the receptor subtypes. Most of the potent peptide ligands for the AII receptors, e.g. saralasin (Sar-1,Ala-8) AII, (Sar-1,Ile-8) AII and sarmesin (Sar-1,(Me)Tyr-4) AII bind equally to both AT_1 and AT_2 receptors with high affinity,[7,18] but their partial agonism and poor pharmacological properties have precluded their use beyond a pharmacological tool.[19] Several non-peptide AT_1/AT_2 balanced AII antagonists have been obtained either as hybrid structures of selective AT_1 and AT_2 receptor antagonists to give compounds with dual activity[14] or as AT_1 selective antagonists modified to enhance their AT_2 receptor binding potency.[20] The SARs of some balanced antagonists are summarized in Figure 1.

Most of the structures of compounds with balanced activity centre around a biphenyl moiety which has an acidic fragment (AF) and a lipophilic fragment (LF) attached to it at specific positions. The acidic fragment can be tetrazole or benzoyl or acyl sulphonamoyl group and the lipophilic fragment can be 6-(*N*-alkyl-*N*-acyl) amino quinazolinones[21–23] or substituted imidazoles[24–26] or imidazopyridines[27] with a 2-alkyl-*N*-5 acetamide, 3-*F* and 2′-carboxamido sulphonyl substituents or N^2-aryl triazolinones.[28–30] Structure–Activity Relationship

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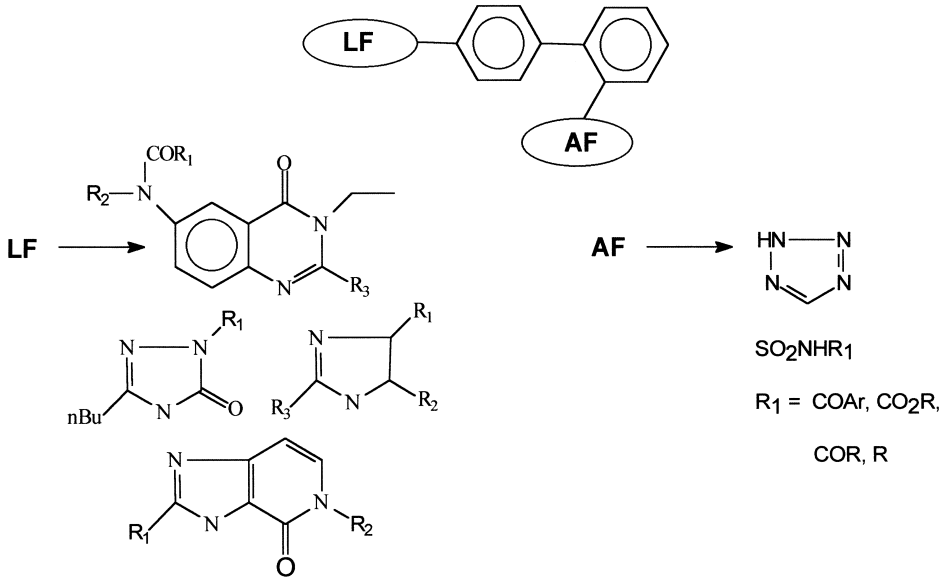


Figure 1.

(SAR) study of the triazolone based AII antagonists which are selective for AT_1 receptor has been performed [31] and the groups responsible for AT_1 activity have been identified. Using the approach of designing balanced antagonists by modification of AT_1 selective agents in order to improve their AT_2 binding potency, synthesis of a series of triazolinone based compounds was carried out and the SAR studies[28–30] reveal that, in the N^2-aryl triazolinone based antagonists, substitution at the 5 position of N^2-aryl preferably by acetylamino or valerylamino group with a halo or trifluoromethyl group at 2 position together with 3-fluoro and 5′-propyl groups on the biphenyl moiety and an alkyl group like ethyl or butyl at C5 of the triazolinone ring showed maximum contribution for the balanced AT_1/AT_2 antagonistic activity. Based on the simple SAR studies in 2′-substituted biphenyl-methyl, 2-propyl, 4-ethyl imidazole 5-carboxylates,[25] the critical features for dual binding at AT_1 and AT_2 receptors have been proposed; however, no such studies have been made on triazolinones. So in order to identify the necessary structural and physicochemical requirements for binding with AT_1 and AT_2 receptors in N^2-aryl triazolinone biphenyl sulphonamides, an APEX-3-D expert system has been used to derive the important biophores (pharmacophores) and 3-D QSAR models and the results of these studies are presented in this paper.

Materials and Methods

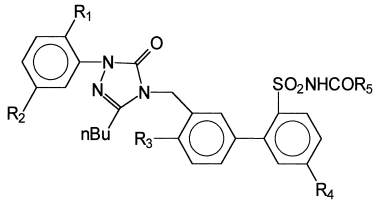
Molecular modelling

Quantitative structure–activity relationship (QSAR). The compounds were analysed by physicochemical based Quantitative structure–activity relationship (QSAR) (Hansch) approach using physicochemical parameters such as hydrophobic (π), steric (molar refractivity or MR) and electronic (field effect or $\mathcal{F}$, resonance or $\mathcal{R}$, Hammett's constant or σ) as independent and AT_1 and AT_2 binding affinities (log $1/IC_{50}$) as dependent variable. The values of the physicochemical parameters used were taken from the literature,[32] and multiparameter regression analysis was carried out on PC-486 using SYSTAT (version 7.0) software.[33]

All molecular modelling and 3-D QSAR studies were performed on a Silicon Graphics *INDY* R-4000 workstation employing Molecular Simulations Incorporation (MSI) software[34] (InsightII, Builder, Search_compare, Discover, APEX-3-D). The molecular structures of all sixteen (16) compounds (Table 1) were constructed in 2-D using the sketch program in the Builder module of InsightII software[35] and were then converted to 3-D for optimization of their geometry (net charge 0.0) by selecting the Potentials force field, viz. potential action, partial charge action and formal charge action, as fixed. The molecular structures were finally minimized using the steepest descent, conjugate gradients, and Newton–Raphson's algorithms in sequence followed by Quasi-Newton–Raphson (Va09a)[36] optimization techniques implemented in the Discover module by using energy tolerance value of 0.001 kcal/mol and maximum number of iteration set to 1000.

In order to check the validity of energy minimized method by above procedure vis a vis other low energy conformations near global minimum, the most active compound **13** for AT_2 activity was subjected to molecular dynamics (MD) simulations using CVFF force field.[37] In this procedure the optimized conformation of compound **13** was randomized by setting random velocities and carrying out MD simulations at 0.1 ps at temperature of $T = 1000$ K. The average conformation obtained from this calculation was used as starting point for another 5 ps of MD simulation at $T = 1000$ K. The purpose of high temperature MD was to explore conformational space extensively. In order to obtain low energy conformations an annealing procedure was

Table 1. In vitro activity (IC_{50}) data for AT_1 and AT_2 receptor antagonistic activities of the N^2-aryltriazolinone biphenyl sulphonamides[38]



Compound no.	R_1	R_2	R_3	R_4	R_5	Rabbit aorta/ rat midbrain[a] IC_{50} (nM) AT_1	AT_2
1	CF_3	H	H	H	(2-Cl)Ph	0.11	36
2	CF_3	H	H	H	O-*t*-Bu	0.45	17
3	CF_3	H	H	*n*-Pr	(2-Cl)Ph	1.3	14
4	CF_3	H	H	*n*-Pr	O-*t*-Bu	10	8.1
5	CF_3	H	F	H	(2-Cl)Ph	0.15	12
6	CF_3	H	F	H	O-*t*-Bu	0.31	4.2
7	CF_3	H	F	*n*-Pr	(2-Cl)Ph	3.9	3.5
8	Cl	NHCO-*n*-Bu	H	Et	(2-Cl)Ph	1.0	4.9
9	Cl	NHCOEt	H	Et	(2-Cl)Ph	0.25	3.15
10	Cl	NHCOMe	F	*n*-Pr	(2-Cl)Ph	0.90	0.15
11	Cl	NHCOMe	F	*n*-Pr	(2-F)Ph	0.62	0.15
12	Cl	NHCOMe	F	*n*-Pr	O-*t*-Bu	0.84	0.24
13	Cl	NHCOMe	F	*n*-Pr	O-*t*-Et	1.4	0.10
14	Cl	NHCO-*n*-Bu	H	H	(2-Cl)Ph	0.16	1.6
15	Cl	NHCOEt	H	H	(2-Cl)Ph	0.17	2.5
16	Cl	NHCOMe	H	H	(2-Cl)Ph	0.052	12

[a]Rabbit aorta and rat midbrain binding assays with no bovine serum albumin (BSA) in assay mixtures.

subsequently applied to each average conformation obtained in high temperature simulation. The annealing was carried out as a slow cooling down of the structure from 1000 to 300 K during a period of 0.1 ps followed by a 5 ps MD run at 300 K. The last step of annealing procedure was energy minimization. Using this approach 15 conformations for a given starting geometry which gives a total of 75–150 ps of simulation time were obtained. The total energy of these conformations ranged between 79.38 and 88.58 kcal which was not very different from the conformational energy (81.65 kcal) obtained from the standard energy minimization procedure described earlier. Hence the same energy minimized conformations were used in the 3-D QSAR model development. The molecular structures were stored in MDL format and were converted with MOPAC 6.0 (MNDO Hamiltonian)[38] version for computational calculation of different physicochemical properties including atomic charges, π-population, electron donor and acceptor indexes, HOMO and LUMO coefficients and hydrophobicity and molar refractivity based on atomic contributions.[39,40] The compounds for both activities AT_1 and AT_2 were classified into following four classes: (i) very active ($-\log IC_{50} \geq 0.477$), (ii) active ($-\log IC_{50} < 0.477 \geq 0.00$), (iii) less active ($-\log IC_{50} < 0.00$ and ≥ -0.47) and (iv) poor active ($-\log IC_{50} < -0.477$). The data were used by APEX-3-D programme for automated biophore (pharmacophore) identification and 3-D QSAR model building.[41,42] APEX-3-D expert system is based on logico structural approach to drug design developed by V. E. Golender et al. and is used for classification and prediction of biological activity. APEX-3-D automatically identifies biophores (pharmacophores) which represent a certain structural and electronic pattern in a bioactive molecule which is responsible for its activity through interaction with the receptor and can be regarded as the local arrays of descriptor centres (user defined atoms, pseudo atoms like ring centres, hydrophobic regions or hydrogen binding sites) which are common to a class of molecules having similar biological activity. The 3-D QSAR equations were derived with the site radius set at 0.80, occupancy at 10 and sensitivity at 0.80, and the randomization value at 100. The biophoric centre properties (π-population, charge, hydrogen donor, hydrogen acceptor, HOMO, LUMO, hydrophobicity and refractivity sites) and secondary site properties (hydrogen acceptor, presence; hydrogen donor, presence; heteroatom, presence; ring, presence; hydrophobic, hydrophobicity; steric, refractivity) combined to global properties (total hydrophobicity and total refractivity) were used to obtain equations describing the variations in AT_1 and AT_2 binding affinity ($-\log IC_{50}$ value).

Quality of each model was estimated from the observed R^2 (correlation coefficient), RMSA (calculated root mean square error based on all compounds with degrees of freedom correction), RMSP (root mean square error based on 'leave one out' with no degrees of freedom correction), chance statistics (evaluated as the ratio of the equivalent regression equations to the total number of randomized sets; a chance value of 0.01 corresponds to 1% chance of fortuitous correlation) and match parameter (evaluated for the quality of superimposition for molecules having common biophores; a value of 1 corresponding to the best possible fit).

Results and Discussion

Different physicochemical parameters; hydrophobic (π_s, π_3, π_4, π_5), steric (MR_s, MR_3, MR_4, MR_5) and electronic ($\mathcal{F}_s$, $\mathcal{F}_3$, $\mathcal{F}_4$, $\mathcal{F}_5$; $\mathcal{R}_s$, $\mathcal{R}_3$, $\mathcal{R}_4$, $\mathcal{R}_5$; σ_s, σ_3, σ_4, σ_5) where the subscript numbers 3, 4 and 5 correspond to the physicochemical parameters of the substituents R_3, R_4 and R_5 respectively, while s represents the sum of the physicochemical parameter values at R_1 and R_2 positions, were correlated as independent parameters with log $1/IC_{50}$ for AT_1 and AT_2 activities as dependent variable. The linear correlation ($r = > 0.80$) of high statistical significance $>99.9\%$ ($F_{1,14} = 24.5$; $F_{1,14\alpha:\ 0.001} = 20.2$) (eq (1)) and ($F_{1,14} = 31.10$; $F_{1,14\alpha:\ 0.01} = 20.2$) (eq (2)) was observed for AT_1 activity with steric MR_4 and electronic σ_4 parameters respectively.

$$-\log AT_1 = -0.074\ (\pm 0.015)MR_4 + 0.826\ (\pm 0.157)$$
$$n = 16,\ \ r = 0.800,\ \ s = 0.391,\ \ F = 24.498 \quad (1)$$

It appears from eq (1) that molar refractivity at R_4 negatively contributes for activity at AT_1 receptor and steric interactions in the vicinity of substituent at R_4 position are unfavourable for the activity.

$$-\log AT_1 = 6.800\ (\pm 1.219)\sigma_4 + 0.784\ (\pm 0.137)$$
$$n = 16,\ \ r = 0.830,\ \ s = 0.363,\ \ F = 31.103 \quad (2)$$

The eq (2) indicates electronic influence of substituents at R_4 position in terms of the positive contribution by the electron withdrawing groups. However in view of the high intercorrelation ($r = 0.975$) between steric MR_4 and electronic σ_4 parameters, it is difficult to decide between the influence of two parameters in present data set.

The linear correlation ($r = 0.908$) of $>99\%$ statistical significance ($F_{2,13} = 9.22$; $F_{2,13\alpha:\ 0.01} = 8.19$) (eq (3)) was observed for AT_2 activity with π_3 and $\mathcal{R}_s$ together, suggesting that hydrophobicity at R_3 and combined resonance effect of substituents R_1 and R_2 make positive and negative contributions respectively.

$$-\log AT_2 = 7.081\ (\pm 1.319)\pi_3 - 1.726\ (\pm 0.308)\mathcal{R}_s - 1.109\ (\pm 0.130)$$
$$n = 16,\ \ r = 0.908,\ \ s = 0.563, F = 9.224 \quad (3)$$

The eqs (1)–(3) well explain the variation in AT_1 and AT_2 activity data with change in substitutions in the compounds (**1**–**16**) as reflected by a good agreement between the observed and calculated biological activities ($-\log IC_{50}$) (Table 2).

Among several 3-D pharmacophore models with different size and arrangements (centre of aromatic rings, hydrophobicity, refractivity, hydrogen bond donor, hydrogen bond acceptor, hydrogen binding site etc.) two models (model No. 1 and model No. 2) of comparable probability were selected, based on correlation coefficient $R^2 > 0.7$, chance < 0.05, match value > 0.5 and the number of compounds 16. These models were found to describe most accurately the distribution of the pharmacophores for activity at AT_1 receptors. Statistical results of these two models are given in Table 3.

Three common biophoric features important for activity were identified in both the models (Fig. 2), one being an electron-rich site (A) capable of donating electrons provided by the oxygen of the triazolinone in model No. 1 and the chloro group at *ortho* position of the N^2-phenyl in model No. 2 and the other two π-rich sites (B and C) correspond to the two oxygen atoms of the sulphonamido group in model No. 1 and to the π-orbitals of the phenyl ring containing sulphonamido group in model No. 2.

Table 2. Physicochemical parameters and calculated activities with the 2-D QSARs, eqs (1)–(3)

Compound no.	MR_4	σ_4	R_s	π_3	Biological activity ($-\log IC_{50}$)				
					Observed		Calculated		
					AT_1	AT_2	eq (1)	eq (2)	eq (3)
1	1.03	0.0	0.19	0.0	0.96	−1.56	0.75	0.78	−1.35
2	1.03	0.0	0.19	0.0	0.35	−1.232	0.75	0.78	−1.35
3	14.96	−0.15	0.19	0.0	−0.14	−1.15	−0.29	−0.24	−1.35
4	14.96	−0.15	0.19	0.0	−1.00	−0.91	−0.29	−0.24	−1.35
5	1.03	0.0	0.19	0.14	0.82	−1.08	0.75	0.78	−0.20
6	1.03	0.0	0.19	0.14	0.51	−0.62	0.75	0.78	−0.20
7	14.96	−0.15	0.19	0.14	−0.59	0.54	−0.29	−0.24	−0.20
8	10.03	−0.15	−0.41	0.0	0.00	−0.69	0.06	−0.24	−0.47
9	10.03	−0.15	−0.41	0.0	−0.60	−0.54	0.06	−0.24	−0.47
10	14.96	−0.15	−0.41	0.14	0.05	0.82	−0.29	−0.24	0.68
11	14.96	−0.15	−0.41	0.14	0.21	0.82	−0.29	−0.24	0.68
12	14.96	−0.15	−0.41	0.14	0.08	0.62	−0.29	−0.24	0.68
13	14.96	−0.15	−0.41	0.14	−0.15	1.00	−0.29	−0.24	0.68
14	1.03	0.0	−0.41	0.0	0.80	−0.20	0.75	0.78	−0.47
15	1.03	0.0	−0.41	0.0	0.77	−0.40	0.75	0.78	−0.47
16	1.03	0.0	−0.41	0.0	1.28	−1.08	0.75	0.78	−0.47

Table 3. 3-D QSAR models describing correlation and statistical reliability for activity at AT_1 receptor

Model no.	RMSA	RMSP	R^2	Chance	Size	Match	Variable	No. of compounds
1	0.37	0.39	0.71	0.03	3	0.69	2	16
2	0.34	0.36	0.75	0.03	3	0.59	2	16

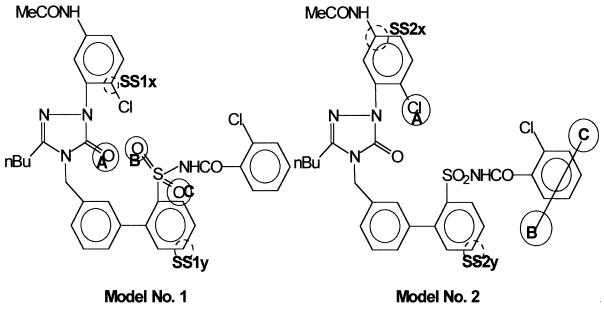


Figure 2. Biophoric ○ and ⟨ ⟩ secondary sites represented on the most active compound, no. **16**.

The spatial dispositions of the three biophoric sites in two pharmacophores which are important for binding at AT_1 receptor were determined to describe interactions with the specific receptor sites depending not only on the physicochemical properties of biophoric centres corresponding to sites A (π-population: 0.912±0.015; charge-heteroatom: −0.418±0.007; Don-01: 8.326±0.022), B (π-population: 0.252±0.004; charge-heteroatom: −0.647± 0.008; Don-01: 7.407±0.020) and C (π-population: 0.261±0.004; charge-heteroatom: −0.635±0.076; Don-01: 7.502±0.019) for model 1 and sites A (Don-01: 8.081± 0.862), B (Cycle-size: 6±0.0; π-electrons: 6.000±0.000) and C (Cycle-size: 6±0.0; π-electrons: 6.000±0.000) for model 2 but also on their spatial arrangements; the mean interatomic distances between the three biophoric descriptor centres A, B and C being A-B (8.05±0.201), B-C (2.519±0.013) and A-C (9.207±0.306) Å for model no. 1 and A-B (12.134±0.314), B-C (0.199±0.0008) and A-C (11.606±0.438) Å for model no. 2.

In addition to the identification of three common key structural features described above as biophoric centres for all the molecules, 3-D multi parameter equations were derived using these pharmacophores as a template for superimposition. The in vitro activity ($-\log IC_{50}$) at AT_1 receptor was related to two parameters: atomic hydrophobicity indexes at the hydrophobic sites in the vicinity of R_1 substituent (SS1x) (4.387±0.007, 9.662±0.124 and 10.628±0.226 Å from the biophoric centres A, B and C respectively) and of R_4 substituent (SS1y) (9.346±0.221, 5.429±0.021 and 5.111±0.344 Å from the biophoric centres A, B and C respectively) in the model No. 1 (eq (4)); atomic hydrophobic index at the hydrophobic site in the vicinity of R_4 substituent (SS2y) (12.866±0.241, 1.726±0.006 and 1.714±0.005 Å from the biophoric centres A, B and C respectively), atomic refractivity index at steric site in the vicinity of R_2 substituent (SS2x) (4.568±0.003, 12.275±0.264 and 11.285± 0.365 Å from the biophoric centres A, B and C respectively) in model No. 2 (eq (5)). One of these sites was common to both models. The statistical results of both the equations show good correlation coefficient values ($r = 0.841$ and 0.864 for model Nos. 1 and 2 respectively) of high statistical significance >99% ($F_{2,13\ \alpha:\ 0.001} = 14.4$; $F_{2,13} = 15.7$ and 19.2 for model Nos. 1 and 2 respectively).

$$-\log IC_{50} = -7.083\ (\text{hydrophobicity at SS1x}) - 3.004\ (\text{hydrophobicity at SS1y}) + 0.827 \quad (4)$$
$$n = 16, \quad r = 0.841, \quad F_{2,13} = 15.701$$

$$-\log IC_{50} = 0.181\ (\text{refractivity at SS2x}) - 7.355\ (\text{hydrophobicity at SS2y}) + 0.448 \quad (5)$$
$$n = 16, \quad r = 0.864, \quad F_{2,13} = 19.178$$

Hydrophobicity in the vicinity of R_4 negatively contributes to activity in both the models, suggesting that the hydrophobic interactions at these secondary sites are not favourable for activity. It also correlates with the results obtained by classical QSAR (eq (1)), where steric interactions were unfavourable for the activity; this may be due to the high intercorrelation ($r = 1.00$) between steric (MR_4) and hydrophobic (π_4) parameters. The hydrophobic interactions also do not contribute in the vicinity of R_1 in model No. 1 (eq (4)); however, the steric interactions in polar space parameterized as molar refractivity in the vicinity of R_2 positively contribute for activity as shown in model No. 2 (eq (5)) (Table 4). The importance of the biophoric centre A in the model No. 2 for binding and of corresponding secondary sites SS1x in model No. 1 and SS2x in model No. 2 corresponding to *ortho* position of the phenyl ring at N-4 position of the triazolinone system corroborates with the earlier visualized pocket on the receptor in the vicinity of the binding site which can accomodate phenyl group containing a bulky *ortho* substituent.[28] Both the models show good superimposition (Fig. 3) of all the molecules (match value >0.5) and also have a very low difference (0.02) between RMSA and RMSP as well as very low chance values (<0.04), which are also reflected in the comparison of experimental, calculated and predicted (leave one out) values of biological activities (Table 5). The compounds **5** and **7** have been the most outlying in both the models where the observed activity data are very much different than the predicted activity from the models which is difficult to explain. Removal of these two compounds improved the correlation to a great extent.

In view of the earlier published data of the same authors for the same activity parameter (rabbit aorta), the robustness of the above models was checked by comparing the observed AT_1 activity of the most active compound no. **31** ($IC_{50} = 0.74$ nm) of that series[28] with the predicted IC_{50} values of 10.23 and 1.10 nM by model

Table 4. Parameter values for the secondary sites (SS) in model Nos. 1 and 2

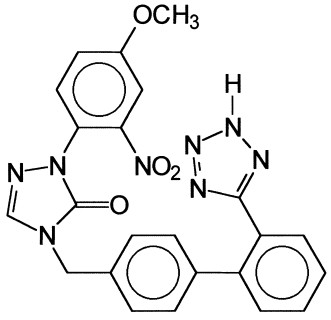
Compound no.	Model No. 1[a]		Model No. 2[a]	
	Hydrophobicity at SS1x	Hydrophobicity at SS1y	Refractivity at SS2x	Hydrophobicity at SS2y
1	0.000	—	0.000	3.450
2	0.000	0.150	0.000	—
3	0.150	—	0.150	3.450
4	0.150	0.150	0.150	—
5	0.150	—	0.150	3.450
6	0.150	0.150	0.000	—
7	0.150	—	0.150	3.450
8	0.150	−0.100	0.150	3.750
9	0.150	—	0.150	—
10	0.150	−0.100	0.150	3.750
11	0.150	−0.100	0.150	3.750
12	0.150	−0.100	0.150	3.750
13	0.150	−0.100	0.150	3.750
14	0.000	−0.100	0.000	3.750
15	0.000	—	0.000	—
16	0.000	−0.100	0.000	3.750

[a](—) indicates absence of property.

Figure 3. Superimposition of the compounds **1**–**16** with biophore sites (solid spheres) and secondary sites (red circles); a biophoric pattern for affinity at AT_1 receptor.

Nos. 1 and 2 respectively. A good agreement between the observed and predicted values by model No. 2 is suggestive of better robustness of statistically superior model No. 2 over model No. 1.

Similarly for the activity at AT_2 receptor, two biophore models for 16 compounds with $R^2 > 0.89$, chance < 0.03, match value > 0.5, size and variable < 4 were selected after screening of several models. These models (model Nos. 3 and 4) showing excellent superimposition (match value 0.64 and 0.68 respectively) of high statistical significance are described in terms of their statistical results in Table 6. Among the three common key structural features described as biophoric sites (D, E and F) identified for each of the two models, two sites were common to both pharmacophores (Fig. 4); one of these was the substituent R_1 and the carbonyl oxygen of the triazolinone ring. The third biophore site is the sulphonamido oxygen in model No. 3, and sulphonamido sulphur in model No. 4. The mean interatomic distances D-E, E-F and D-F between the three biophoric descriptor centres were 5.518±0.211, 8.05±0.192 and 9.461±0.569 Å for model No. 3 and 5.518±0.211, 7.893±0.264 and 9.632±0.394 Å for model No. 4.



Compound No. 31

Both biophoric sites D and E are common to both models also in terms of their physicochemical characteristics in terms of charge (−0.145±0.072 and −0.418±0.007) and electron donor reactivity (Don-01) (8.146±0.872 and 8.326±0.022); however, in addition to these properties π-population (0.909±0.007) at site E has been considered in model No. 4. The two models differ in terms of the third biophoric site F which is described by the oxygen

Table 5. Experimental, calculated and predicted activity data ($-\log IC_{50}$) for AT_1 receptor in model Nos. 1 and 2

Compound no.	Biological activity ($-\log IC_{50}$)				
	Experimentally observed	Model No. 1		Model No. 2	
		Calculated	Predicted	Calculated	Predicted
1	0.96	0.83	0.80	1.07	1.10
2	0.35	0.38	0.39	0.45	0.48
3	−0.11	−0.24	−0.25	−0.03	−0.02
4	−1.00	−0.69	−0.50	−0.66	−0.05
5	0.82	−0.24	−0.37	−0.03	−0.14
6	0.51	0.38	0.32	0.45	0.43
7	−0.59	−0.24	−0.19	−0.03	0.03
8	0.00	0.07	0.08	0.02	0.02
9	−0.60	−0.24	−0.19	−0.66	−0.68
10	0.05	0.07	0.07	0.02	0.02
11	0.21	0.07	0.04	0.02	0.00
12	0.08	0.07	0.06	0.02	0.01
13	−0.15	0.07	0.10	0.02	0.04
14	0.80	1.13	1.25	1.13	1.24
15	0.77	0.83	0.84	0.45	0.34
16	1.28	1.13	1.07	1.13	1.07

Table 6. 3-D QSAR models describing correlation and statistical reliability for activity at AT_2 receptor

Model no.	RMSA	RMSP	R^2	Chance	Size	Match	Variable	No. of compounds
3	0.27	0.31	0.91	0.00	3	0.64	3	16
4	0.29	0.32	0.89	0.00	3	0.68	2	16

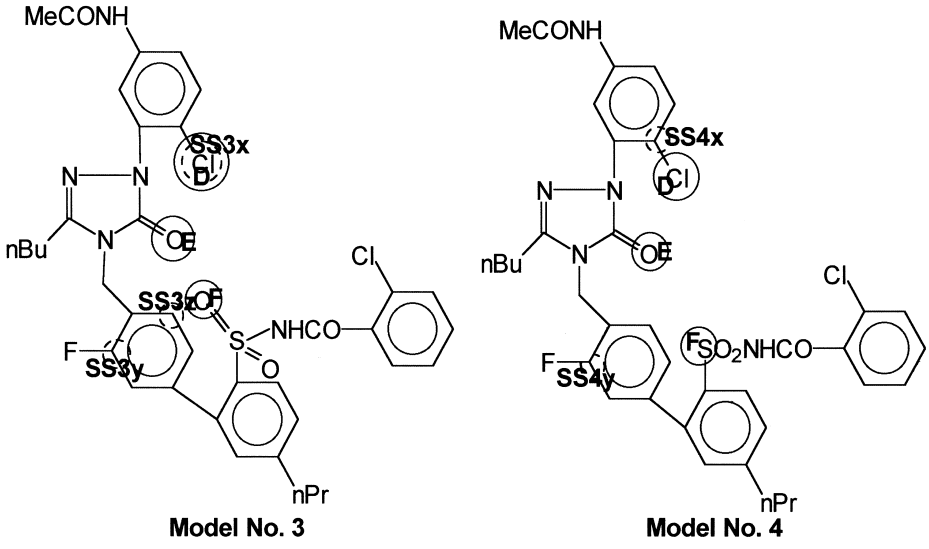


Figure 4. Biophoric ○ and ⟨ ⟩ secondary sites represented on the most active compound no. **13**.

of the sulphonamido group in terms of its charge (−0.648±0.004) and electron donor reactivity (7.407± 0.020) at this centre for model No. 3, while this site is described by the sulphur of sulphonamido in terms of charge (1.517±0.009) and π-population (0.450±0.006) for this centre in model No. 4. In addition to the nucleophilicity of the two centres (D and E) for interacting with the hydrogen atoms of the water molecules, the third biophoric site (F) in model No. 3 also plays a similar role as the other two biophoric sites, while this site in model No. 4 does not appear to act as nucleophilic centre for interaction with the hydrogen atoms of the water molecules. In addition to above three biophoric sites which are present in all the molecules, the variation in AT_2 binding affinity is also influenced by the secondary sites which are not common to all the molecules as shown by the 3-D multi parameter (eqs (6) and (7)) derived for the two pharmacophores 3 and 4 respectively taking AT_2 binding affinity ($-\log IC_{50}$) as dependent parameter and three independent parameters: atomic refractivity indexes at steric sites in the vicinity of R_1 substituent (SS3x) (0.000±0.000, 5.518±0.183 and

9.461±0.246 Å from biophoric centres D, E and F respectively), R_3 substituent (SS3y) (7.921±0.089, 3.699± 0.179 and 5.261±0.113 Å from biophoric centres D, E and F respectively) and at 5′ position biphenyl ring (SS3z) (8.079±0.066, 5.045±0.072 and 3.884±0.058 Å from biophoric centres D, E and F respectively) in model No. 3 for eq (6) and two independent parameters: atomic hydrophobicity index at the hydrophobic site in the vicinity of R_1 substituent (SS4x) (2.014±0.293, 4.451± 0.086 and 9.675±0.118 Å from biophoric centres D, E and F respectively) and atomic refractivity indexes at steric site in the vicinity of R_3 substituent (SS4y) (7.888±0.126, 3.698±0.168 and 4.969±0.078 Å from biophoric centres D, E and F respectively) in model No. 4 for eq (7). The positive contributions of all these parameters in eq (6) for AT_2 affinity suggest that steric interactions in polar space at these sites are favourable for this activity. The eq (7) describing the 3-D QSAR model 4 also has positive contribution of refractivity in the vicinity of R_3 similar to secondary sites in model No. 3, and describes the negative contribution of hydrophobicity in the vicinity of R_1 substituent (SS4x), indicating unfavourable hydrophobic interactions at this site (Table 7). The positive contribution of substituent R_3 by steric parameter in 3-D

Table 7. Parameter values for the secondary sites (SS) in model Nos. 3 and 4

Compound no.	Model No. 3		Model No. 4[a]		
	SS3x	SS3y	SS3z	SS4x	SS4y
1	1.150	3.450	3.450	0.150	3.450
2	1.150	3.450	3.450	0.150	3.450
3	1.150	3.450	3.450	0.150	3.450
4	1.150	3.450	3.450	0.150	3.450
5	1.150	3.450	3.750	0.150	3.450
6	1.150	3.450	3.750	—	3.450
7	1.150	3.450	3.750	0.150	3.450
8	5.400	3.450	3.450	−0.100	3.450
9	5.400	3.450	3.450	−0.100	3.450
10	5.400	3.750	3.450	−0.100	3.750
11	5.400	3.750	3.450	−0.100	3.750
12	5.400	3.750	3.450	−0.100	3.750
13	5.400	3.750	3.450	−0.100	3.750
14	5.400	3.450	3.450	−0.100	3.450
15	5.400	3.450	3.450	−0.100	3.450
16	5.400	3.450	3.450	−0.100	3.450

[a](—) indicates absence of property.

Figure 5. Superimposition of the compounds **1**–**16** with biophore sites (solid spheres) and secondary sites (red circles); a biophoric pattern for affinity at AT_2 receptor.

Table 8. Experimental, calculated and predicted activity data (−log IC_{50}) for AT_2 receptor in model No. 3 and 4

Compound no.	Biological activity (−log IC_{50})				
	Experimentally observed	Model No. 3		Model No. 4	
		Calculated	Predicted	Calculated	Predicted
1	−1.56	−1.21	−1.10	−1.07	−0.97
2	−1.23	−1.21	−1.20	−1.07	−1.04
3	−1.15	−1.21	−1.23	−1.07	−1.05
4	−0.91	−1.21	−1.31	−1.07	−1.10
5	−1.08	−0.75	−0.58	−1.07	−1.07
6	−0.62	−0.75	−0.81	−0.77	−0.78
7	−0.54	−0.75	−0.85	−1.07	−1.17
8	−0.69	−0.58	−0.56	−0.57	−0.54
9	−0.54	−0.58	−0.59	−0.57	−0.57
10	0.82	0.82	0.81	0.82	0.81
11	0.82	0.82	0.81	0.82	0.81
12	0.62	0.82	0.88	0.82	0.88
13	1.00	0.82	0.76	0.82	0.76
14	−0.20	−0.58	−0.68	−0.57	−0.65
15	−0.40	−0.58	−0.63	−0.57	−0.60
16	−1.08	−0.58	−0.46	−0.57	−0.45

model eqs (6) and (7) corresponds to the observed positive contribution by hydrophobic substituent parameter π_3 in classical QSAR derived eq (3), and possibly due to high intercorrelation ($r = 1.00$) between the two parameters. Both the 3-D QSAR models with good superimposition (Fig. 5) of all the compounds (match value > 0.6), low difference between the RMSA and RMSP values, 100% reliability (chance = 0.00) and excellent correlation ($r > 0.94$) with $> 99.9\%$ statistical significance ($F_{3,12\ \alpha:\ 0.001} = 12.7$; $F_{3,12} = 40.7$ for model No. 3 and $F_{2,13\ \alpha:\ 0.001} = 14.4$; $F_{2,13} = 52.3$ for model No. 4) describes a good agreement between the predicted and observed activity (Table 8).

$$-\log IC_{50} = 0.148\ (\text{refract. at SS3x}) + 4.667\ (\text{refract. at SS3y}) + 1.538\ (\text{refract. at SS3z}) - 22.787$$
$$n = 16, \quad r = 0.954, \quad F_{3,12} = 40.714 \tag{6}$$

$$-\log IC_{50} = -2.008\ (\text{hydrophobicity at SS4x}) + 4.609\ (\text{refractivity at SS4y}) - 16.669$$
$$n = 16,\ r = 0.943, \quad F_{2,13} = 52.263 \tag{7}$$

Conclusion

A comparison of the more robust model No. 2 as compared to model no. 1 for AT_1 and model No. 3/4 suggests that there is one common biophore site corresponding to the biophore centre A in model No. 2 and D in model No. 3/4, while there are differences in other biophoric and secondary sites for AT_1 and AT_2 receptors which correspond to the *ortho* substituted phenyl ring at N_4 position of the triazolinone ring. This electron rich centre for electrostatic interaction and/or capable of donating electrons, is important for dual AT_1/AT_2 receptor antagonistic activity.

Thus the 3-D QSAR models discussed above for the affinity at both AT_1 and AT_2 receptors have led to the identification of the essential structural features in terms of the physicochemical properties (Charge, Don-01 and π-population) and spatial disposition for specific interactions with the two receptors, suggesting that there is commonality at the interacting sites in both the receptors. As the biophoric centres represented by the oxygen of the triazolyl carbonyl and sulphonamido group and the substituent R_1 are common for interacting with these receptors, this may possibly be the reason that they have less selectivity, which is the requisite for balanced activity at AT_1/AT_2 receptors. Judicious substituent modulations keeping in view the secondary site contributions may lead to molecules for desired triazolinone based AT_1/AT_2 balanced angiotensin II antagonists.

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