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Pharmacophore Mapping of Tricyclic Isoxazoles for Their Affinity Towards Alpha–2 Adrenoreceptors

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Abstract

Motivation. Blockage of alpha–2 adrenoreceptors in brain enhances noradrenergic neurotransmission and increases extracellular dopamine as well as serotonin (5–HT) levels, which is beneficial for depressant patients. To identify pharmacophoric requirements, a quantitative structure activity relationship (QSAR) study was performed using electrotopological state atom (ETSA) indices and refractotopological state atom (RTSA) indices on tricyclic isoxazole derivatives for their affinity towards the alpha–2 adrenoreceptors.

Method. Correlation analysis and multiple linear regression analysis were employed to model the experimental activity.

Results. The QSAR models were obtained separately for alpha–2A and 2C adrenoreceptor binding affinity. It was found that some atoms played important roles to both the activities and some other atoms played different roles in selectivity of compound towards alpha–2A and 2C adrenoreceptor binding affinity.

Conclusions. Electrotopological state atom (ETSA) and refractotopological state atom (RTSA) indices have potentiality to determine or recognize the pharmacophoric atoms and combination of these two helps to map pharmacophore of trycyclic isoxazoles.

Keywords. Tricyclic isoxazoles; alpha–2 adrenoreceptors; QSAR; ETSA; RTSA; pharmacophore.

Abbreviations and notations

ETSA, electrotopological state atom	QSAR, quantitative structure–activity relationships
RTSA, refractotopological state atom	PLS, partial least squares

1 INTRODUCTION

Depression is often described as a stress–related disorder [1]. It results from functionally deficient monoaminergic (noradrenaline and/or 5–hydroxytryptamine) transmission [2] in the CNS. Noradrenaline and 5–hydroxytryptamine both are neurotransmitter. Alpha–2 adrenoreceptors have an important function in the regulation of the release of neurotransmitters. Alpha–2A and alpha–2C

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adrenoreceptors both function as autoreceptors on noradrenergic neurons and regulate the release of norepinephrine (noradrenaline). These are also act as postsynaptic receptors on neurons that receive noradrenergic innervation and regulate the release of other neurotransmitters (heteroreceptors) [3].

The large majority of people (~80%) suffering from depression show some improvement with several antidepressants [1]. The major classes of agents were found to be effective antidepressants are monoamine uptake inhibitors and monoamine oxidase inhibitors. Tricyclic antidepressants imipramine, amitriptyline are non–selective (or in some cases noradrenaline selective) inhibitors of monoamine uptake. Fluoxetine, fluvoxatine, paroxetine, sertraline are selective serotonin (5–hydroxytryptamine) uptake inhibitors and moclobemide is monoamine oxidase–A (MAO–A) selective inhibitor [2] Combinations of adrenoreceptor antagonists (*e.g.*, mianserin) with monoamine uptake inhibitors (*e.g.*, imipramine) [4] or serotonin uptake inhibitors (*e.g.*, fluoxetine) [5] improve recovery from depression compare to monoamine uptake inhibitors or serotonin uptake inhibitors alone. A new series of tricyclic isoxazoles was reported with serotonin uptake and alpha–2 adrenoreceptor blocking activity by Andres *et al*. [6].

A pharmacophore element is traditionally defined as an atom or a group (*e.g.*, a functional group) common for active compounds with respect to a receptor and essential for the activity of compounds. Pharmacophoric mapping is of great value in generating new chemical structures. For optimizing a lead structure, it is necessary to utilize the information from quantitative activity data and from the structural properties in a more efficient way to predict more active congeners [7].

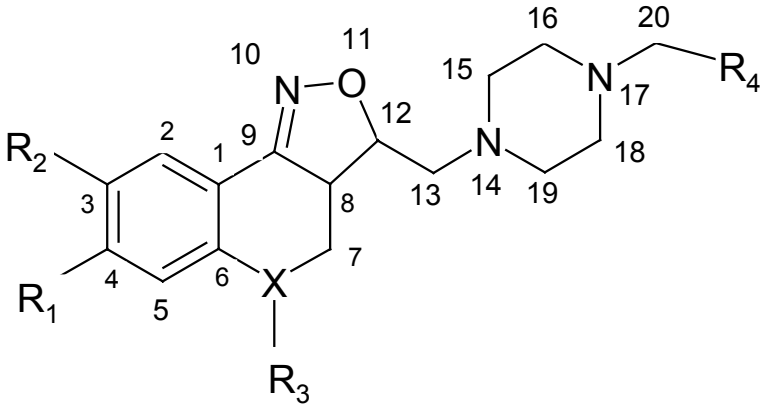


Figure 1. Common structure of tricyclic isoxazole derivatives.

In the present work, QSAR study has been performed on a new series of tricyclic isoxazoles derivatives using electrotopological state atom (ETSA) and refractotopological state atom (RTSA) indices to determine or recognize the atom/fragments of molecule (pharmacophoric atom) required for activity as a part of our composite program of rational drug design [8–13]. The general structure with arbitrary numbering used for QSAR analysis is shown in Figure 1. The structural details and activity data were collected from the work by Andres *et al*. [6].

Table 1. Biological Activity (Ref. [6]) Data of Tricyclic Isoxazole Derivatives

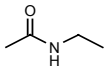
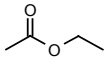
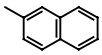
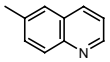
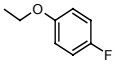
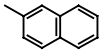
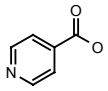
Cpd	X	R_1	R_2	R_3	R_4	C1/nM	C_2/nM	C_1/M	C_2/M	pC_1	pC_2
1	O	H	H	–		0.9	1.7	9.0×10^{-10}	1.7×10^{-09}	9.046	8.770
2	O	OMe	OMe	–		8.8	6.2	8.8×10^{-09}	6.2×10^{-09}	8.056	8.208
3	O	OMe	OMe	–		0.8	0.2	8.0×10^{-10}	2.0×10^{-10}	9.097	9.699
4	O	OMe	OMe	–		1.4	0.6	1.4×10^{-09}	6.0×10^{-10}	8.854	9.222
5	O	OMe	OMe	–		5	3.1	5.0×10^{-09}	3.1×10^{-09}	8.301	8.509
6	N	H	H	H		20	15	2.0×10^{-08}	1.5×10^{-08}	7.699	7.824
7	N	H	H	H		2.4	9.6	2.4×10^{-09}	9.6×10^{-09}	8.620	8.018
8	N	H	H	Me		1.6	4.5	1.6×10^{-09}	4.5×10^{-09}	8.796	8.347
9	N	H	H			20	2.7	2.0×10^{-08}	2.7×10^{-09}	7.699	8.569
10	N	H	H			33	9.8	3.3×10^{-08}	9.8×10^{-09}	7.481	8.009
11	N	H	H			47	11	4.7×10^{-08}	1.1×10^{-08}	7.328	7.959
12	N	H	H			26	6.6	2.6×10^{-08}	6.6×10^{-09}	7.585	8.180
13	N	OMe	OMe	H		7.9	5.4	7.9×10^{-09}	5.4×10^{-09}	8.102	8.268
14	N	OMe	OMe	H		2.4	0.1	2.4×10^{-09}	1.0×10^{-09}	8.620	10.000
15	N	OMe	OMe	H		6.5	3.6	6.5×10^{-09}	3.6×10^{-09}	8.187	8.444
16	N	OMe	OMe	H		1000	23	1.0×10^{-06}	2.3×10^{-08}	6.000	7.638
17	N	OMe	OMe	H		74	12	7.4×10^{-08}	1.2×10^{-08}	7.131	7.921
18	N	OMe	OMe	H		1000	1000	1.0×10^{-06}	1.0×10^{-06}	6.000	6.000
19	N	OMe	OMe	H		9.1	1.6	9.1×10^{-09}	1.6×10^{-09}	8.041	8.796
20	N	OMe	OMe	Me		11	4	1.1×10^{-08}	4.0×10^{-09}	7.959	8.398
21	CH	H	H	H		43	112	4.3×10^{-08}	1.12×10^{-07}	7.367	6.951
22	CH	OMe	H	H		1.8	3.9	1.8×10^{-09}	3.9×10^{-09}	8.745	8.409
23	CH	OMe	H	H		1.8	6.3	1.8×10^{-09}	6.3×10^{-09}	8.745	8.201
24	O	OMe	H	–		0.5	0.2	5.0×10^{-10}	2.0×10^{-10}	9.301	9.699

Table 1. (Continued)

Cpd	X	R_1	R_2	R_3	R_4	C1/nM	C_2/nM	C_1/M	C_2/M	pC_1	pC_2
25	O	OH	H	–		0.2	0.1	2.0×10^{-10}	1.0×10^{-10}	9.699	10.000
26	O	$CH_3O(CH_2)_2O$	H	–		0.1	0.03	1.0×10^{-10}	3.0×10^{-11}	10.000	10.523
27	O	$CH_3CH_2O(CH_2)_2O$–$(CH_2)_2O$	H	–		1.3	0.2	1.3×10^{-09}	2.0×10^{-10}	8.886	9.699
28	O		H	–		1.3	0.5	1.3×10^{-09}	5.0×10^{-10}	8.886	9.301
29	O	$(CH_3)_2N(CH_2)_2O$	H	–		0.1	0.1	1.0×10^{-10}	1.0×10^{-10}	10.000	10.000
30	O	$CH_3(C=O)O$	H	–		0.4	0.2	4.0×10^{-10}	2.0×10^{-10}	9.398	9.699
31	O	$CH_3CH_2(C=O)O$	H	–		0.6	0.2	6.0×10^{-10}	2.0×10^{-10}	9.222	9.699
32	O	$CH_3OCH_2(C=O)O$	H	–		0.7	0.2	7.0×10^{-10}	2.0×10^{-10}	9.155	9.699
33	O		H	–		0.9	0.3	9.0×10^{-10}	3.0×10^{-10}	9.046	9.523
34	O		H	–		0.9	0.5	9.0×10^{-10}	5.0×10^{-10}	9.046	9.301
35	O		H	–		4.7	1.6	4.7×10^{-09}	1.6×10^{-09}	8.328	8.796
36	O		H	–		1.0	0.5	1.0×10^{-09}	5.0×10^{-10}	9.000	9.301

2 MATERIALS AND METHODS

Alpha–2 adrenoreceptor binding affinity of tricyclic isoxazole derivatives reported by Andres *et al*. [6] was used for QSAR study and listed in Table 1. Alpha–2 adrenoreceptor binding affinities of tricyclic isoxazole derivatives was determined by radioligand binding assay using frozen membranes of CHO cells, stably transfected with either human adrenergic 2A or 2C receptors. Bound counts were measured in a Topcount Scintillation Counter in the presence of Microscint O. Alpha–2A adrenoreceptor binding affinity (C_1) and alpha–2C adrenoreceptor binding affinity (C_2) of tricyclic isoxazole derivatives were used for QSAR analysis. In order to get the linear relationship with independent variables, negative logarithms of the binding affinity (pC_1 and pC_2) were used. C_1 and C_2 represent the molar K_i value of the compound, *i.e.*, the concentration giving the half–maximal inhibition.

2.1 ETSA Index

Electrotopological state atom (ETSA) index [14–17] is an atom/sub–molecular descriptor encoding both electronic and topological information. Electronic factors include the concept of polarity, charge, and energy levels. Topological factors include the arrangement of atoms across the skeleton, concepts of steric relations and bulk as well as the relationships between various non–bonded parts of a molecule. The E–state index S_i of an atom i in a molecule is composed of an intrinsic state I_i and the perturbation effect ΔI_j. The E–state value for atom in a molecule is computed as follows

$$S_i = I_i + \Delta I_j \tag{1}$$

The atom intrinsic value includes both electronic and topological information. The count of pi and lone pair of electrons gives important electronic information. The important topological attribute is relative location of the atom within the molecule or relative degree of surface–atom or buried–atom status. The intrinsic state value of atom i is expressed as

$$I_i = [((2/N)^2\ \delta^v + 1)/\delta] \tag{2}$$

where N = principle quantum number of valence electrons, δ^v = number of valence electrons – number of hydrogen atom attached, and δ = number of sigma electrons – number of hydrogen atom attached. The perturbation effect ΔI_j stands for influence of information field on the intrinsic atom value I_i. It is the function of the difference in intrinsic values I_i (of atom i) and I_j (of atom j) and expressed as:

$$\Delta I_j = f(I_i – I_j) \tag{3}$$

The influence of atom j on atom i decreases with increase in the topological distance in the shortest path (graph separation) between atom i and j. To account for this Eq. (3) is modified with a function r_{ij}^2, which is the square of graph separation. The general expression for the perturbation effect is as follows:

$$\Delta I_j = \Sigma(I_i – I_j)/r_{ij}^2 \tag{4}$$

2.2 RTSA Index

The refractotopological state atom (RTSA) index [17] is a novel atomic index for QSAR defined by Carrasco *et al*. The R–state index is based on the influence of dispersive forces of each atom on the other atoms in the molecules, modified by molecular topology. The R–state index R_i of an atom i in a molecule is composed of an intrinsic refractivity AR_i and the perturbation effect ΔAR_i, as shown in Eq. (5)

$$R_i = AR_i + \Delta AR_i \tag{5}$$

The perturbation term is defined as:

$$\Delta AR_i = \Sigma(AR_i – AR_i)/r_{ij}^2 \tag{6}$$

where r_{ij}^2 = square of the topological distance between atoms *i* and *j*, and AR_i = intrinsic value of atom *i*. The RTSA index depends on the atomic refractivities and the topological environment of the atom and sum of the atomic refractivities, that is, molar refractivity is directly proportional to the polarizability of a substance that determines London force/dispersive force between nonpolar molecules [19,20]. ETSA and RTSA indices were calculated using the computer program 'mouse' [21]. In the programme molecular connection table in a specified format is given along with the intrinsic state values of different atoms as inputs. The atoms of molecules were numbered consecutively keeping the serial number of atoms same in all molecules. ETSA and RTSA indices are listed in Table 2.

Table 2. ETSA and RTSA indices of of tricyclic isoxazole derivatives

Cpd	S_3 [a]	S_{20} [a]	R_6 [b]	R_9 [b]
1	2.034	1.003	3.274	4.134
2	0.671	0.980	3.486	4.293
3	0.671	1.008	3.48	4.288
4	0.671	0.986	3.481	4.289
5	0.671	0.99	3.49	4.305
6	2.074	1.013	2.701	3.991
7	2.077	1.023	2.706	4.003
8	2.094	1.014	2.702	3.98
9	1.998	0.970	2.955	4.088
10	1.978	0.962	3.098	4.152
11	2.001	0.975	2.955	4.086
12	1.735	0.865	3.421	4.332
13	0.711	0.991	2.913	4.15
14	0.711	1.018	2.907	4.145
15	0.711	0.996	2.908	4.146
16	0.706	0.965	2.919	4.158
17	0.711	1.000	2.917	4.161
18	0.708	0.973	2.922	4.168
19	0.690	0.854	2.943	4.192
20	0.722	1.019	2.908	4.133
21	2.114	1.023	2.521	3.946
22	2.03	1.013	2.657	4.008
23	2.033	1.022	2.662	4.02
24	1.952	1.020	3.404	4.191
25	1.674	1.010	3.43	4.201
26	1.962	1.014	3.416	4.207
27	1.966	1.010	3.43	4.235
28	2.062	1.023	3.368	4.183
29	2.003	1.018	3.354	4.172
30	1.773	0.998	3.494	4.246
31	1.793	0.999	3.478	4.24
32	1.751	0.992	3.526	4.27
33	1.827	1.001	3.499	4.255
34	1.745	0.993	3.473	4.237
35	1.803	0.996	3.445	4.225
36	1.771	0.99	3.489	4.257

[a] S_3, S_{20} indicate ETSA indices of atom number 3 and 20 respectively
[b] R_6, R_9 indicate RTSA indices of atom number 6 and 9 respectively

2.3 Statistical Analysis

Correlation analysis [22] of biological activities was carried out withthe ETSA and RTSA indices. The intercorrelated parameters were eliminated stepwise. All possible combinations of parameters were considered for multiple regression analysis [22], which was carried out using the program 'Multi Regress' [23] developed in our laboratory. Statistical quality of these equations were justified by parameters like correlation coefficients (*R*), percentage of explained variance (*%EV*), adjusted R^2 (R_A^2), variance ratio (*F*), standard error of estimate (*SEE*). Significance of the regression coefficients was justified by t–test and p (probability factor) values. The predictive powers of equation were validated by leave–one–out (LOO) cross–validation method. R^2_{cv}, *SEE*, *PRESS*, *SSY*, *PSE*, S_{PRESS} are cross–validated R^2, standard error of estimate, predicted residual sum of squares, total sum of squares, uncertainty factor, standard error of *PRESS* respectively.

3 RESULTS AND DISCUSSION

QSAR study was performed on two biological activity data, alpha–2A adrenoreceptor binding affinity (C_1) and alpha–2C adrenoreceptor binding affinity (C_2) of tricyclic isoxazole derivatives. In order to get the linear relationship with independent variables, negative logarithms of the binding affinity (pC_1 and pC_2) were used. C_1 and C_2 is the molar K_i value of the compound, *i.e.*, the concentration giving the half–maximal inhibition. The calculated ETSA and RTSA indices are listed in Table 2. Correlation analysis of useful independent parameters and dependent variables was performed and the result is shown in Table 3. In developing QSAR equations, predictor variable with higher p–values and higher intercorrelation coefficient were removed to get more acceptable QSAR models.

3.1 QSAR for Alpha–2A Adrenoreceptor Binding Affinity

Multiple regression analysis using combination of ETSA indices S_3, S_{20} and RTSA indices R_6 developed the following QSAR equation as shown in below.

$$\begin{gathered} pC_1 = -6.536\ (\pm 3.413) + 0.459\ (\pm 0.198)\ S_3 + 9.250\ (\pm 3.220)\ S_{20} + 1.611\ (\pm 0.353)\ R_6 \\ n = 36;\ R = 0.731;\ \%EV = 53.426;\ R^2_A = 0.491;\ F_{(3,32)} = 12.236;\ p<0.0001;\ SEE = 0.677; \\ PRESS = 14.684;\ SSY = 31.528;\ R^2_{cv} = 0.534;\ S_{PRESS} = 0.677;\ PSE = 0.639 \end{gathered} \quad (7)$$

where *n* is the number of data points. The values within the parenthesis are confidence intervals of the corresponding parameters. Eq. (7) explains 53.426% of the variances in the activity data and also shows the importance of atoms numbered as 3, 6 and 20. The positive regression coefficient of S_3, S_{20} (ETSA index of atom 3 and 20 respectively) and R_6 (RTSA index of atom 6) suggests that the binding affinity will increase with increasing the value of S_3, S_{20} and R_6.

After deleting the outliers in a stepwise fashion (compound number **19**, **12**, **28**, **and 21**) we obtained the following equations:

$$pC_1 = -16.085\ (\pm 3.738) + 0.519\ (\pm 0.165)\ S_3 + 17.866\ (\pm 3.462)\ S_{20} + 1.872\ (\pm 0.301)\ R_6$$
$$n = 35;\ \mathbf{DC} = \mathbf{19};\ R = 0.829;\ \%EV = 68.709;\ R^2_A = 0.657;\ F_{(3,31)} = 22.690;\ p<0.0001; \quad (8)$$
$$SEE = 0.563;\ PRESS = 9.817;\ SSY = 31.374;\ R^2_{cv} = 0.687;\ S_{PRESS} = 0.563;\ PSE = 0.530$$

$$pC_1 = -29.552\ (\pm 5.287) + 0.3746\ (\pm 0.151)\ S_3 + 31.398\ (\pm 5.157)\ S_{20} + 1.911\ (\pm 0.264)\ R_6$$
$$n = 34;\ \mathbf{DC} = \mathbf{19}, \mathbf{12};\ R = 0.873;\ \%EV = 76.259;\ R^2_A = 0.739;\ F_{(3,30)} = 32.121;\ p<0.0001; \quad (9)$$
$$SEE = 0.492;\ PRESS = 7.270;\ SSY = 30.622;\ R^2_{cv} = 0.763;\ S_{PRESS} = 0.492;\ PSE = 0.462$$

$$pC_1 = -31.963\ (\pm 5.176) + 0.401(\pm 0.145)\ S_3 + 33.544\ (\pm 5.0261)\ S_{20} + 1.992\ (\pm 0.254)\ R_6$$
$$n = 33;\ \mathbf{DC} = \mathbf{19}, \mathbf{12}, \mathbf{28};\ R = 0.889;\ \%EV = 79.053;\ R^2_A = 0.769;\ F_{(3,29)} = 36.481;\ p<0.0001; \quad (10)$$
$$SEE = 0.469;\ PRESS = 6.376;\ SSY = 30.439;\ R^2_{cv} = 0.791;\ S_{PRESS} = 0.469;\ PSE = 0.440$$

$$pC_1 = -32.970\ (\pm 4.906) + 0.435\ (\pm 0.137)\ S_3 + 35.055\ (\pm 4.795)\ S_{20} + 1.826\ (\pm 0.252)\ R_6$$
$$n = 32;\ \mathbf{DC} = \mathbf{19}, \mathbf{12}, \mathbf{28}, \mathbf{21};\ R = 0.901;\ \%EV = 81.245;\ R^2_A = 0.792;\ F_{(3,28)} = 40.432; \quad (11)$$
$$SEE = 0.442;\ PRESS = 5.481;\ SSY = 29.224;\ R^2_{cv} = 0.812;\ S_{PRESS} = 0.442;\ PSE = 0.414$$

where DC is deleted compound, behaves as outliers and these may act through a different mechanism of action. After deletion, statistical quality of these models was improved accordingly. The final Eq. (11) has higher correlation coefficient (R = 0.901) and lower value of standard error of estimate (SEE = 0.442), thus, equation (11) is the best model that explains 81.245% of variance in activity. The predictive power of the final equations was evaluated by the Leave–One–Out cross–validation method. In this method, each compound was left out of the model and subsequently, prediction of activity of that compound was performed. Amongst compounds 24 to 36, compound 29 containing dimethyl aminoethyloxy group at 3 position of general structure is the most potent, this has higher S_3 value. It shows that substituents at 3 position which increase the value of S_3 is essential for improving the activity. On comparison between compound 1 and 6 (shown in Table 1), it was found that compound 1 has higher binding affinity towards alpha–2A adrenoreceptor. It may be due to presence of oxygen atom at X position which may increase the value of R_6. Similarly, it was found that amongst compound 13 to18, compound 14 is having higher S_{20} value and has greater binding affinity towards alpha–2A adrenoreceptor. It may be due to the presence of methyl cinnamyl group at position 20 (of the general structure) which may increase the value of S_{20}.

3.2 QSAR for Alpha–2C Adrenoreceptor Binding Affinity

In the same manner using combination of ETSA indices S_3, S_{20} and RTSA indices R_9 developed the following equation for alpha–2C binding affinity as shown below

$$pC_2 = -30.909\ (\pm 7.474) + 0.561\ (\pm 0.220)\ S_3 + 9.701\ (\pm 3.522)\ S_{20} + 7.003\ (\pm 1.353)\ R_9$$
$$n = 36\ ;\ R = 0.699;\ \%EV = 48.870;\ R^2_A = 0.441;\ F_{(3,32)} = 10.195;\ p< 0.0001;\ SEE = 0.715; \quad (12)$$
$$PRESS = 16.358;\ SSY = 31.994;\ R^2_{cv} = 0.489;\ S_{PRESS} = 0.715;\ PSE = 0.674$$

where R_9 is the RTSA index of atom numbered as 9. Eq. (12) explains 48.870% of variance in activity. The positive regression coefficient of S_3, S_{20} (ETSA index of atom 3 and 20 respectively) and R_9 suggests the positive contribution of atom 3, 9, 20 toward alpha–2C adrenoreceptor binding affinity.

On deletion of outliers in stepwise fashion (compound number **19**, **14**) yielded the following

equations as follows

$$pC_2 = -46.939\ (\pm 7.428) + 0.697\ (\pm 0.186)\ S_3 + 19.771\ (\pm 3.890)\ S_{20} + 8.380\ (\pm 1.177)\ R_9$$
$$n = 35;\ \mathbf{DC = 19};\ R = 0.811;\ \%EV = 65.848;\ R^2_A = 0.625;\ F_{(3,31)} = 19.924;\ p<0.0001\ ; \quad (13)$$
$$SEE = 0.594;\ PRESS = 10.926;\ SSY = 31.993;\ R^2_{cv} = 0.658;\ S_{PRESS} = 0.594;\ PSE = 0.559$$

$$pC_2 = -47.029\ (\pm 6.493) + 0.8523\ (\pm 0.170)\ S_3 + 18.341\ (\pm 3.429)\ S_{20} + 8.673\ (\pm 1.033)\ R_9$$
$$n = 34;\ \mathbf{DC = 19, 14};\ R = 0.857\ ;\ \%EV = 73.445;\ R^2_A = 0.708;\ F_{(3,30)} = 27.658;\ p<0.0001\ ; \quad (14)$$
$$SEE = 0.519;\ PRESS = 8.079;\ SSY = 30.423;\ R^2_{cv} = 0.734;\ S_{PRESS} = 0.519;\ PSE = 0.487$$

Exclusion of compound 19, 14 in stepwise fashion improved statistical significance of the model. Eq. (14) explains 73.445% of variance in activity. Equation (14) is the best QSAR model for alpha–2C adrenoreceptor binding affinity. It has higher correlation coefficient ($R = 0.857$) and lower value of standard error of estimate ($SEE = 0.519$). *t*–Values and associated p–values of all derived QSAR models are shown in Table 4. The observed (Obs), calculated (Calc), residual (Res), predicted residual (Pres) values of equation (14) are shown in Table 5. Amongst compounds 2–5, compound 3 is the most potent which has higher S_{20} value. This may be due to the presence of methyl cinnamyl group at atom numbered 20 of the general structure. This result shows that compounds having higher S_{20} value have greater alpha–2C adrenoreceptor binding affinity.

Table 3. Correlation matrix of the ETSA indices, RTSA indices and biological activity

	S_3	S_{20}	R_6	R_9	pC_1	pC_2
S_3	1.00	0.26	–0.02	–0.38	0.38	0.18
S_{20}		1.00	–0.09	–0.34	0.39	0.21
R_6			1.00	0.90	0.52	0.66
R_9				1.00	0.24	0.47
pC_1					1.00	0.87
pC_2						1.00

Table 4. *t*–values and *p*–values of equations

Eq	Intercept/Parameter	*t*–Value	*p*–Value	Eq	Intercept/Parameter	*t*–Value	*p*–Value
7	Intercept	–1.915	0.064 [a]	11	Intercept	–6.720	0.000
	S_3	2.318	0.027		S_3	3.165	0.004
	S_{20}	2.872	0.007		S_{20}	7.311	0.000
	R_6	4.556	0.000		R_6	7.237	0.000
8	Intercept	–4.303	0.000	12	Intercept	–4.136	0.000
	S_3	3.138	0.004		S_3	2.546	0.016
	S_{20}	5.160	0.000		S_{20}	2.754	0.010
	R6	6.215	0.000		R_9	5.174	0.000
9	Intercept	–5.590	0.000	13	Intercept	–6.319	0.000
	S_3	2.476	0.019		S_3	3.742	0.000
	S_{20}	6.088	0.003		S_{20}	5.082	0.000
	R_6	7.249	0.000		R_9	7.118	0.000
10	Intercept	–6.176	0.000	14	Intercept	–7.243	0.000
	S_3	2.772	0.010		S_3	5.024	0.000
	S_{20}	6.674	0.000		S_{20}	5.349	0.000
	R_6	7.832	0.000		R_9	8.396	0.000

[a] Confidence interval is less than 95%

Table 5. Observed, Calculated, Residual, LOO–Predicted (Pred), Predicted Residual (Pres) Values of Eqs. (11) and (14)

Cpd	Obs pC_1	Obs pC_2	Eq. (11)				Eq. (14)			
			Calc	Res	Pred	Pres	Calc	Res	Pred	Pres
1	9.046	8.770	9.053	–0.007	9.053	–0.007	8.958	–0.188	8.967	–0.198
2	8.056	8.208	8.040	0.015	8.038	0.018	8.753	–0.545	8.822	–0.615
3	9.097	9.699	9.011	0.086	8.995	0.102	9.223	0.476	9.143	0.556
4	8.854	9.222	8.241	0.612	8.154	0.700	8.828	0.394	8.778	0.444
5	8.301	8.509	8.398	–0.097	8.412	–0.111	9.040	–0.531	9.115	–0.607
6	7.699	7.824	8.374	–0.675	8.480	–0.781	7.935	–0.111	7.950	–0.126
7	8.620	8.018	8.735	–0.116	8.758	–0.138	8.225	–0.207	8.251	–0.233
8	8.796	8.347	8.420	0.376	8.360	0.436	7.875	0.472	7.802	0.545
9	7.699	8.569	7.298	0.401	7.194	0.505	7.923	0.646	7.843	0.726
10	7.481	8.009	7.270	0.212	7.199	0.283	8.314	–0.305	8.349	–0.340
11	7.328	7.959	7.474	–0.146	7.504	–0.176	8.000	–0.041	8.004	–0.045
12	7.585	8.180	–	–	–	–	7.889	0.292	7.246	0.935
13	8.102	8.268	7.397	0.705	7.309	0.793	7.748	0.519	7.679	0.589
14	8.620	10.000	8.333	0.287	8.272	0.348	–	–	–	–
15	8.187	8.444	7.563	0.624	7.486	0.701	7.805	0.639	7.719	0.725
16	6.000	7.638	6.494	–0.494	6.626	–0.626	7.336	0.302	7.281	0.357
17	7.131	7.921	7.720	–0.589	7.794	–0.664	8.009	–0.088	8.020	–0.099
18	6.000	6.000	6.781	–0.781	6.932	–0.932	7.572	–1.572	7.804	–1.804
19	8.041	8.796	–	–	–	–	–	–	–	–
20	7.959	8.398	8.374	–0.416	8.465	–0.506	8.124	0.274	8.078	0.320
21	7.367	6.951	–	–	–	–	7.762	–0.811	7.930	–0.979
22	8.745	8.409	8.275	0.470	8.195	0.549	8.045	0.364	8.002	0.407
23	8.745	8.201	8.601	0.144	8.572	0.173	8.316	–0.116	8.329	–0.128
24	9.301	9.699	9.850	–0.549	9.919	–0.618	9.694	0.005	9.693	0.006
25	9.699	10.000	9.426	0.273	9.405	0.294	9.360	0.640	9.327	0.673
26	10.000	10.523	9.666	0.334	9.633	0.367	9.731	0.792	9.663	0.860
27	8.886	9.699	9.553	–0.667	9.613	–0.727	9.904	–0.205	9.925	–0.226
28	8.886	9.301	–	–	–	–	9.773	–0.472	9.822	–0.521
29	10.000	10.000	9.711	0.289	9.680	0.320	9.536	0.464	9.500	0.500
30	9.398	9.699	9.165	0.233	9.147	0.251	9.615	0.084	9.609	0.090
31	9.222	9.699	9.180	0.042	9.177	0.045	9.598	0.101	9.591	0.108
32	9.155	9.699	9.004	0.151	8.990	0.165	9.694	0.005	9.694	0.005
33	9.046	9.523	9.303	–0.257	9.324	–0.279	9.794	–0.271	9.818	–0.295
34	9.046	9.301	8.940	0.106	8.932	0.114	9.421	–0.120	9.428	–0.127
35	8.328	8.796	9.019	–0.691	9.067	–0.739	9.421	–0.626	9.457	–0.661
36	9.000	9.301	8.875	0.125	8.864	0.136	9.562	–0.261	9.581	–0.280

4 CONCLUSIONS

In a molecule, all atoms may not be responsible for the activity, rather a part of the structure or some specific atoms, called pharmacophore, are required for the desired activity. ETSA and RTSA indices have potentiality to determine or recognize the pharmacophoric atoms and thus, used here. This QSAR study shows that atoms numbered as 3, 6, 9, and 20 may form pharmacophore for alpha–2 adrenoreceptors binding affinity. ETSA index of an atom combines both the electronic character and the topological environment of each skeleton atom in a molecule. This study shows that atoms numbered as 3 and 20 are of great importance as these are associated with the electronic interactions of tricyclic isoxazoles with alpha–2 adrenoreceptors. As the electronic and topological

influences of other atoms on 3 and 20 changes the value of S_3 and S_{20} for alpha–2A and alpha–2C adrenoreceptor, the surrounding atoms should be such that their electrotopological influence will increase the value of S_3 and S_{20}.

RTSA index of an atom encode the dispersive/Van der Waals force involved in interactions with active sites and also contains topological information. QSAR studies shows that atom 6 is important for alpha–2A adrenoreceptor binding affinity and atom 9 is important for 2–C adrenoreceptor binding affinity. Atoms numbered as 6 and 9 are important atoms as these are associated with dispersive/Van der Waals interactions of tricyclic isoxazoles with alpha–2 adrenoreceptors. Pharmacophoric mapping through atoms associated with electronic as well as dispersive/Van der Waals interactions for alpha–2 adrenoreceptors binding affinity are presented in Figure 2.

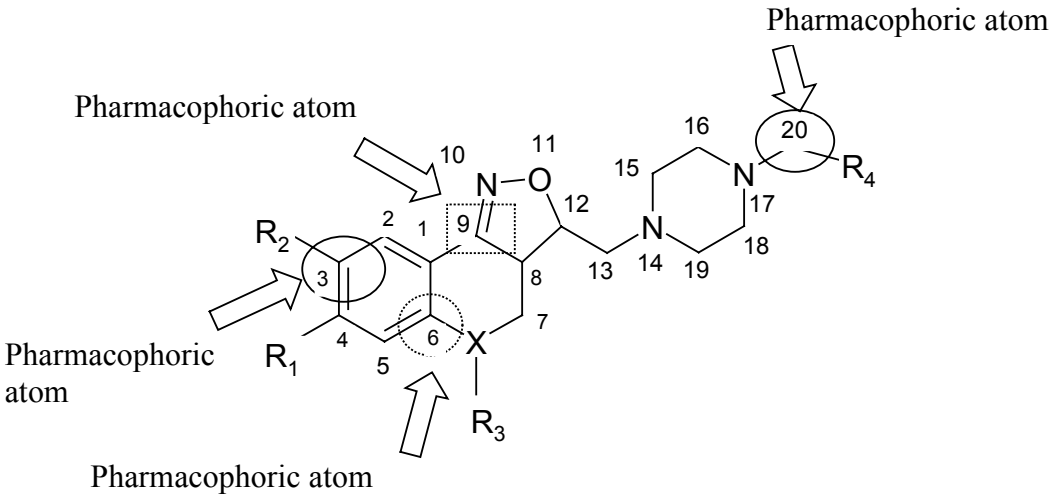


Figure 2. Pharmacophore mapping: atoms bound by solid line represent the pharmacophore required for both alpha–2A and –2C adrenoreceptors binding, atom bound by dashed circle represent the pharmacophore required for alpha–2A adrenoreceptor binding, atom bound by dashed rectangle represent the pharmacophore required alpha–2C adrenoreceptor binding.

These pharmacophore mapping of trycyclic isoxazole for their affinity towards alpha–2 Adrenoreceptors will be helpful in designing new compounds of this series to get useful leads.

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5 REFERENCES

[1] E. J. Nestler, M. Barrot, R. J. DiLeone, A. J. Eisch, S. J. Glod, and L. M. Monteggia, Neurobiology of depression, *Neuron* **2002**, *34*, 13–25.
[2] H. P. Rang, M. M. Dale, J. M. Ritter and P. K. Moore, *Pharmacology*, Churchill Livingstone, Edinburgh, 2003, pp. 186–189.
[3] P. Szot, M. Lester, M. L. Laughlin, R. D. Malmiter, L. C. Liles, and D. Weinshenker, The anticonvulsant and

proconvulsant effects of alpha–2 adrenoreceptor agonists are mediated by distinct populations of alpha–2A adrenoreceptors, *Neuroscience* **2004**, *126*, 795–803.

[4] L. Lauritzen, L. Clemmesen, R. Klysner, D. Loldrup, M. Lunde, E. Schaumburg, S. Waarst and P. Bech, Combined treatment with imipramine and mianserin. A controlled pilot study, *Pharmacopsychiatry* **1992**, *25*, 182–186.

[5] J. Dam, L. Ryde, J. Svejso, N. Lauge, B. Lauritsen and P. Bech, Morning fluoxetine plus evening mianserin versus morning fluoxetine plus evening placebo in the acute treatment of major depression, *Pharmacopsychiatry* **1998**, *31*, 48–54.

[6] J. I. Andres, J. Alcazar, J. M. Alonso, R. M. Alvarez, M. H. Bakker, I. Biesmans, J. M. Cid, A. I. D. Lucas, J. Fernandez, L. M. Font, K. A. Hens, L. Iturrino, I. Lenaerts, S. Martinez, A. A. Megens, J. Pastor, P. C. M. Vermote, and T. Steckler, Discovery of a New Series of Centrally Active Tricyclic Isoxazoles Combining Serotonin (5–HT) Reuptake Inhibition with alpha–2–Adrenoceptor Blocking Activity, *J. Med. Chem.***2005**, *48*, 2054–2071.

[7] P. K. Larsen, T. Liljefores, and U. Madsen, *Textbook of Drug Design and Discovery*, Taylor and Francis, New York, 2002, pp. 86–90.

[8] S. Gayen, B. Debnath, S. Samanta, and T. Jha, QSAR study on some anti–HIV HEPT analogues using physicochemical and topological parameters. *Bioorg. Med. Chem.* **2004**, *12*, 1493–1503.

[9] S. Samanta, K. Srikanth, S. Banerjee, B. Debnath, S. Gayen and T. Jha, 5–N–Substituted–2–(substituted benzenesulphonyl) glutamines as antitumor agents. Part II: Synthesis, biological activity and QSAR study. *Bioorg. Med. Chem.* **2004**, *12*, 1413–1423.

[10] S. Samanta, B. Debnath, S. Gayen, B. Ghosh, K. Srikanth, and T. Jha, QSAR modeling on dopamine D2 receptor binding affinity of 6–methoxy benzamides. *Farmaco*. **2005**, *10*, 818–825.

[11] B. Debnath, S. Samanta, S. Gayen, A. Basu, B. Ghosh, and T. Jha, QSAR Study on 5–*N*–Substituted–2–(Substituted Benzenesulphonyl) Glutamines as Antitumor Agents through Synthesis and Biological Evaluation: Part III, *Internet Electron. J. Mol. Des.* **2005**, *4*, 393–412, http://www.biochempress.com.

[12] B. Debnath, S. Gayen, S. Samanta, A. Basu, B. Ghosh, and T. Jha, QSAR Study on Some Synthesized and Biologically Evaluated Glutamine Analogs as Possible Anticancer Agents. *Ind. J. Chem A* **2006**, *45A*, 93–99.

[13] A. Basu, S. Gayen, S. Samanta, P. Panda, K. Srikanth, and T. Jha, QSAR modeling of neonicotinoid insecticides for their selective affinity towards Drosophila nicotinic receptors over mammalian $\alpha_4\beta_2$ receptors. *Canadian J. Chem*. **2006**, *84*, 458–463.

[14] L. H. Hall, B. Mohney and L. B. Kier, an Electrotopological–State: An Atom Index for QSAR. *Quant. Struct. Act. Relat*. **1991**, *10*, 43–51.

[15] C. de Gregorio, L. B. Kier and L. H. Hall, QSAR modeling with the electrotopological state indices: Corticosteroids, *J. Comput. Aid. Mol. Des*. **1998**, *12*, 557–561.

[16] K. Rose, L. H. Hall and L. B. Kier, Modeling blood brain barrier partitioning using the electrotopological state. *J. Chem. Inf. Comput. Sci*. **2002**, *42*, 651–666.

[17] L. B. Kier and L. H. Hall, *Molecular Structure Description*: *The Electrotopological State*, Academic Press, San Francisco, 1999

[18] R. Carrasco, J. A. Padron and J. Galvez, Definition of a novel atomic index for QSAR: The refractotopological state. *J. Pharm. Pharmaceut. Sci*. **2004**, *7*, 19–26.

[19] A. K. Ghose and G. M. Crippen, Atomic physicochemical parameters for three-dimensional-structure-directed quantitative structure-activity relationships. 2. Modeling dispersive and hydrophobic interactions. *J. Chem. Inf. Comput. Sci*. **1987**, *27*, 21–35.

[20] B. Debnath, S. Gayen, A. Basu, B. Ghosh, K. Srikanth, and T. Jha, Quantitative Structure-Activity Relationship Study Using Refractotopological State Atom Index on Some Neonicotinoid Insecticides, *Bioorg. Med. Chem.* **2004**, *12*, 6137–6145.

[21] Mouse, a computer program developed by Jadavpur University.

[22] Snedecor, G. W., Cochran, W. G., *Statistical Methods*, Oxford & IBH, New Delhi, 1967.

[23] Multi Regress, a computer program developed by Jadavpur University.