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Discovery and validation of potential impact of cannabinoid receptor agonists by insilico means

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Abstract

Endocannabinoid receptor agonists are highly recognized for its anti–inflammatory property. Their immunomodulatory effect can be utilized for the treatment of different types of immunomodulatory diseases. The present study comprised of 3 D QSAR analysis of a well aligned set cannabinoid receptor agonists. The model generated has revealed its statistical significance and ability for external test set prediction by producing better R^2 and Q^2 values. A five feature pharmacophore, AAADH generated from the cannabinoid receptor agonistic activity has produced valid information on the agonistic activity of the compounds. The systematic analysis of the QSAR model has furnished a clear output on how to improve the activity of the compounds through structural modifications. Since cannabinoid receptor agonists are having the property of reducing the expression of macrophages and T cells, especially interleukin 4, interleukin6, and interleukin 5, they are highly recommended for drug designing for those diseases managed by T–cell signaling pathways.

Keywords: Endocannabinoid receptors, 3D QSAR study, Pharmacophore, Regression analysis, Model validation

1. INTRODUCTION

Cannabinoids are a class of compounds showing activity towards cannabinoid receptors (CB). Cannabinoid receptors include CB 1 and CB2. CB1 is expressed in brain and CB2 is seen in the cells of immune system. Both the cannabinoid receptors play a vital role in the regulation of immune system. Several studies have been suggested for the therapeutic effect of cannabinoids and their endogenous ligands [1]. Arachidonic acid metabolites were found to have properties similar to the compounds seen in the plant cannabis sativa. These compounds are indicated as endocannabinoids. Cannabinoids are explored as potent anti–inflammatory agents and the actions include induction of apoptosis, inhibition of cell proliferation, suppression of cytokine production and induction of T–

regulatory cells. Cytokines are important cell signaling proteins works behind immunomodulatory diseases. Asthma is a chronic inflammatory disease of the airways characterized by eosinophilia, increased vascular permeability in the bronchial mucosa, mucus hyper secretion and airway hyper responsiveness [2]. Although there is a general consensus about the use of corticosteroids and bronchodilators as main therapeutic measures for the prevention and management of asthma, the identification and development of promising new substances with anti–asthmatic effects that can flank and co–operate with the above drugs is a fertile field for basic and clinical research [3] because of its primary medical interest. Arachoidonic acid metabolic pathway is a causative for asthma like autoimmune diseases [4]. Recently, claims have been made for the beneficial effects of cannabis and cannabinoids, the active components of Cannabis sativa. Many works have been reported for the antiasthmatic activity of cannabinoids. Δ 9–tetrahydrocannabinol (THC), the principal component of cannabis sativa has reported for reduction in the expression of interleukin 4 by creating inhibition in the IL4 signaling pathways. The THC is the first reported cannabinoid receptor ligand[1]. Asthma is one among those diseases, managed by IL4 signaling pathways. Δ 9–tetrahydrocannabinol (THC) is a well known cannabinoid receptor agonist. Many compounds including phytochemical compounds were reported to act as cannabinoid receptor agonists.

Quantitative structure activity relationship (QSAR) is a well accepted insilico technique utilized by leading pharmaceutical companies for effective, systematic and speedy method of drug designing process. The QSAR model is a statistically derived structure activity relationship which can make use of successful activity prediction of new set of compounds before going for its synthesis and can design compounds with better activity properties. The process can reduce the time of discovery of drugs and can do the whole process in a more economic way [5]. The present study include QSAR analysis of a set of cannabinoid receptor agonists to derive functional alert for the receptor agonistic property and the responsible functionality can be utilized for designing drugable compounds for asthma like immunomodulatory diseases.

2. EXPERIMENTAL

2.1. Insilico analysis – Data set

A bioassay of 82 cannabinoid receptor agonists was reported by Angela Berry et al, in their patent work US 7,928,123 B2, Apr. 19, 2011 [6]. The data set comprised of a diverse set of compounds having the scaffold of sulfonamides with activity (EC50) concentrations ranging from 0.04nM to 99nM. The present study has executed a three dimensional (3 D) QSAR analysis on the dataset for generating a successful QSAR model for the activity prediction of new series of compounds (Figure 1). The dataset was divided into training and test set in the ratio 75:25. The splitting were done in such a way that test set should have all the features of the training set, means, maximum structural and activity diversity were seen among training set compounds [7–8]. The EC50 values were converted into their negative logarithmic representations for statistical significance. The data set with activity values are included in supplementary information (Table SI. 1)

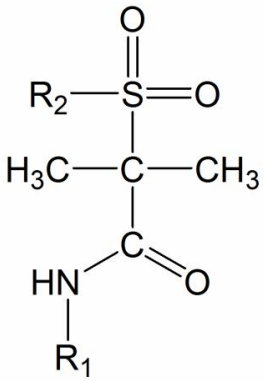


Fig. 1: General structure of bioassay compounds

2.2. Ligand preparation for QSAR study

All the 82 compounds were drawn in the 2D sketcher of maestro interface of the schrodinger software. Structure optimization and energy minimization were done using the ligprep module at a force field of OPLS_2005 and pH 7.4.

The structural alignment (Figure 2) is an essential feature for a meaningful QSAR modeling [8]. The present study has been aligned all the structures with the most active compound from the bioassay through flexible ligand based alignment using largest common Bemis–murcko scaffold alignment. A total of 68 compounds showing very good alignment with the active compound were obtained. Those 68 compounds were subjected to atom based 3D QSAR analysis after splitting them into training and test set in the ratio 75: 25.

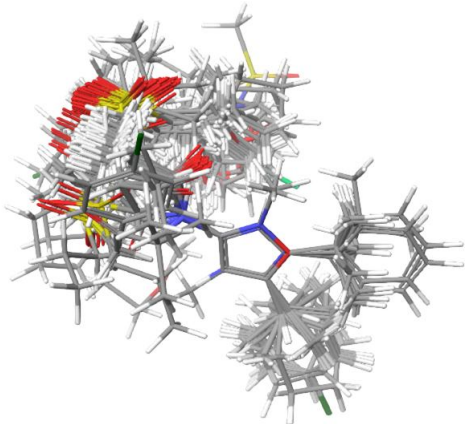


Fig. 2: Structural alignment with the active bioassay compound number 9 (EC50= 0.04nM)

2.3. Generation of pharmacophore

Pharmacophoric feature is a 3 dimensional feature of a set of compounds meant for biological activity [9]. Compounds were assigned as actives and inactives by assuming that compounds with activity (pEC50) below 7.25 as inactive and compounds with activity above 9.6 as actives. A total of 9 active and 5 inactive compounds were considered for pharmacophore generation. The activity threshold for dataset was generated on the basis of activity distribution (7.047–10.398). Pharmacophore feature generation was done by using the phase module of Schrodinger software. The module has offered some 6 pharmacophoric features including aromatic feature, hydrophobic, hydrogen bond acceptor, hydrogen bond donor, negatively charged and positively charged groups. A common pharmacophoric feature has derived from a set of 14 least and top active compounds using a tree–based partition algorithm which clusters similar pharmacophore based on their intersite distances and angles. The best pharmacophoric feature, the feature which is better aligned with the most active compounds, among a set of generated features was selected on the basis of survival active and survival inactive score. The survival score is the net result of volume and selectivity scores of aligned ligands, contributions from number of matches, relative energy of reference ligand and the activity of reference ligand. The pharmacophoric features were also mapped for least active compounds to discriminate between active and inactive compounds. A feature with very good active–inactive distinguishing ability should have high survival active score and less survival inactive scores. The scoring methodology include alignment of sites, vector and volume scores, selectivity, number of compounds matched, activity and relative conformational energy.

2.4. 3D QSAR analysis

A total of 68 better aligned sulfonyl amide compounds were chosen for atom based 3D QSAR modeling. 17 compounds were considered as test set while remaining 51training set compounds were utilized to develop a meaningful QSAR model using the 3D descriptor properties such as hydrogen

bond donors, hydrophobic effect, negative ionic effect, positive ionic effect and electron withdrawing effect. Partial least square analysis was executed to derive the model from the dataset [10]. The PLS factor should be 1/5 of the number of compounds in the training set. The PLS factor for the present study was 5. Leave one out cross validation technique was done for checking the validity and robustness of the model.

3. RESULTS AND DISCUSSIONS

3.1. Pharmacophore generation

All the 68 structurally aligned sulfonamide derivatives were subjected for pharmacophore generation using the phase module. After careful analysis of the scores and alignment of the active ligands to the generated hypothesis, the pharmacophore hypothesis AAADH was selected as the potential feature responsible for the biological activity. This five feature pharmacophore encompasses three hydrogen bond acceptors, one hydrogen bond donor and a hydrophobic group. Oxygen atom in the oxazole ring, carbonyl group of the amide linkage and S=O of the sulfonyl group contributed to the biological activity as hydrogen bond acceptors, the 3° carbon attached to the oxazole ring stands for hydrophobic effect and amino group of amide linkage could work behind hydrogen bond donor effect.

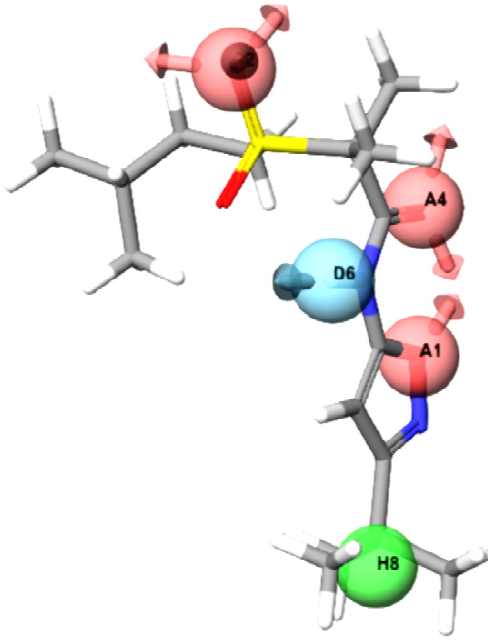


Fig. 3: (a) Pharmacophoric feature furnished by the cannabinoid receptor agonists, highest active compound aligned with the pharmacophore

The pharmacophoric feature should retain as such for an effective cannabinoid receptor agonistic activity. The highest active compound 3–tert–butyl–N–{3–methyl–3–[(3–methylbutane) sulfonyl] but–1–en–2–yl}–1, 2–oxazol–5–amine could align better with the feature with a finess score of 2.82 (Figure 3). Pharmacophoric feature with inter atomic distances and angles is included in supplementary information (Figure SI 1)

The feature AAADH has got highest survival score (Table 1), survival inactive score and lowest energy of conformation and all the top 9 active compounds could furnish high fitness score with the feature. These findings shed light on the potential effect of the five feature pharmacophore AAADH towards the cannabinoid receptor as very good agonists. This agonistic feature can be exploited for the treatment of immunomodulatory diseases like asthma, cancer etc.

Table 1: Pharmacophore scoring data

Pharmacophoric feature	Survival score	Survival inactive score	Matches	Activity	Energy
AAADH.1170	3.783	1. 811	9	9.699	0.000
AAADH.1509	3.742	1. 617	9	9.699	1.162
AAADR.90	3.780	1.329	9	10.000	1.767
AAADR.54	3.763	1.408	9	9.699	2.338
AAADR.371	3.771	1.473	9	10.000	1.767

3.2. 3D QSAR analysis

Atom based 3D QSAR model has been furnished with 68 cannabinoid receptor agonists by partial least square method keeping 51 compounds as training and 17 compounds as test. The PLS factor was chosen as 5. Five models were generated. The statistical data table is shown below (Table 2).

Table 2: Statistical analysis, F– variance, p– statistical significance, RMSE– deviation from reference ligand, SD– standard deviation

PLS factor	SD	R2	F	P	RMSE	Q2	Pearson–r
1	0.7354	0.2433	14.8	0.000367	0.75	0.2672	0.5333
2	0.571	0.5537	27.9	1.31E–08	0.56	0.5962	0.7866
3	0.491	0.6774	30.8	6.95E–11	0.55	0.6118	0.7869
4	0.3562	0.8341	54	3.19E–16	0.51	0.6623	0.8336
5	0.2683	0.9081	83	1.18E–20	0.39	0.8002	0.9042

Among the five models, model having PLS factor 5 has chosen as the stable better predictive model. The robustness and stability of the model has revealed through the regression coefficient, R^2= 0.9081, statistical significance of the model was expressed through high F value and low p value, here the values obtained were 83 for F and 1.18E–20 for p. external predictability of the model was assessed by cross correlation coefficient, Q^2 = 0.8002. The value of Q^2 >0.5 indicate external predictability of the model. Linearity of the model was checked by plotting experimental and predicted activity for training set and test set (Figure 4). Contour plots for the QSAR model could explain the structural requirement for a better cannabinoid receptor agonistic activity (Figure 5). Experimental and predicted activities are seen in supplementary information (Table SI. 2).

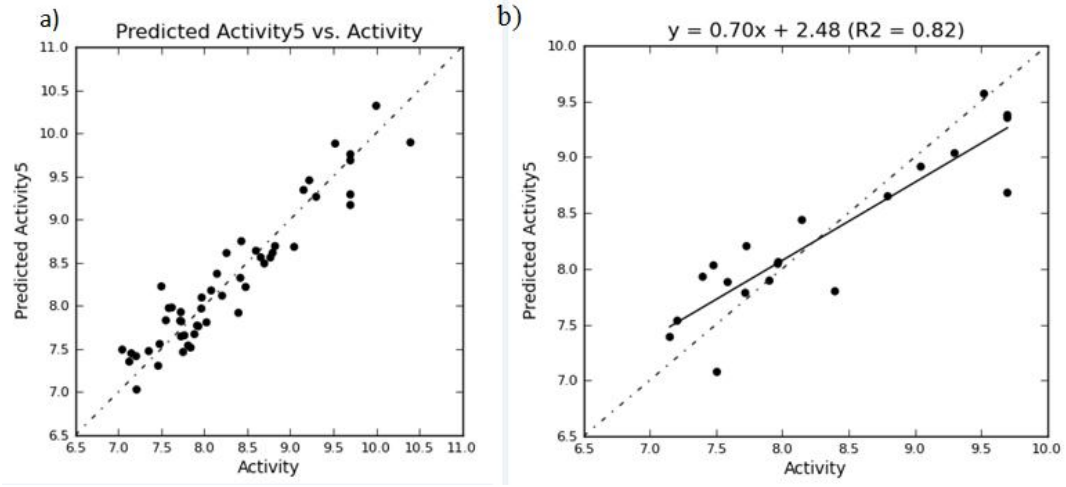


Fig. 4: (a) training set, (b) test set linear relationship

Red cubes in the contour map indicate negative contribution towards activity property and blue cubes indicate positive contribution towards receptor agonistic property. Hydrophobic effect, hydrogen bond donor effect, negative ionic effect and electron withdrawing effect were found to have contribution towards cannabinoid receptor agonist property. Hydrogen bond donor effect of oxazole ring and amide NH2 group had a positive contribution towards the agonistic property. The hydrophobic effect of isopropyl group has positive contribution towards the agonistic property. If a methyl group occupy the position of isopropyl group (compound 36), then the activity was found to be considerably decreases. The hydrophobic effect of tertiary methyl group near to the oxazole ring favors the receptor agonistic property. The negative ionic effect of oxygen atom of oxazole ring disfavors the activity. Electron withdrawing effect of the same group again degrades the agonistic property. This QSAR model of cannabinoid receptor agonists can make use of finding out of drug like compounds for immunomodulatory diseases like asthma.

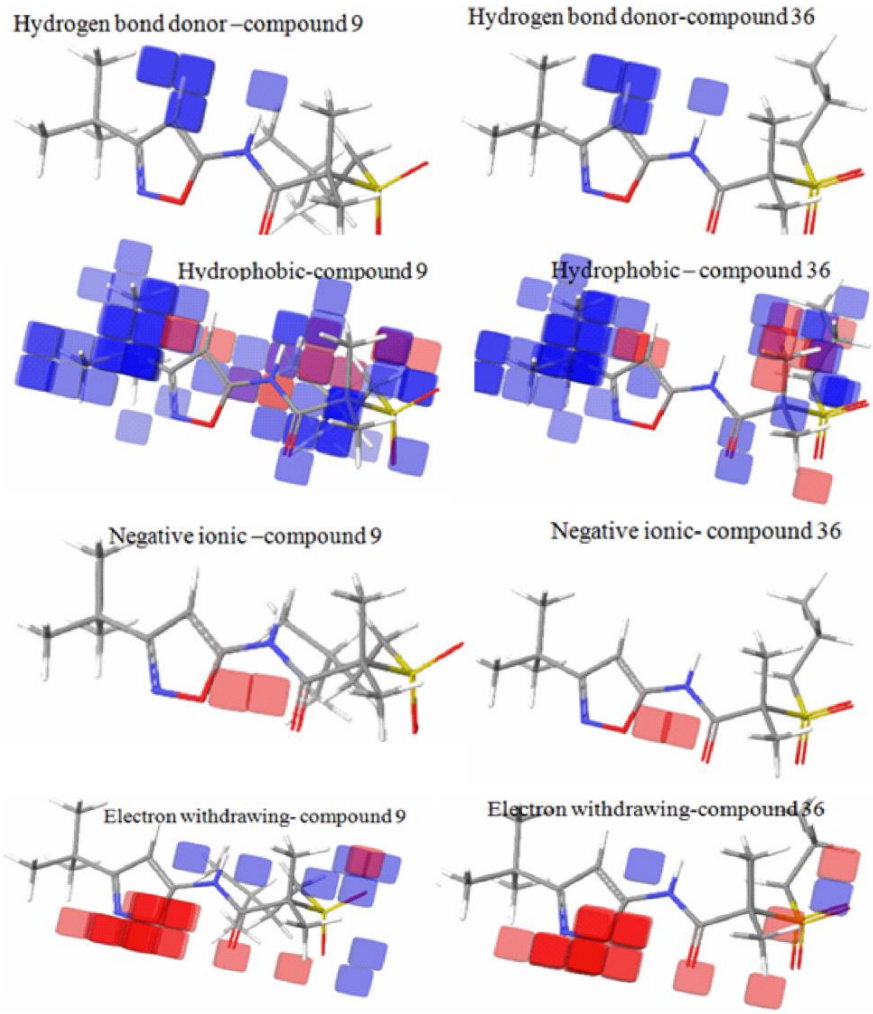


Fig. 5: QSAR contour plots, blue cubes– activate the system, red cubes– deactivate the system, compound 9– top active , compound 36– least active

Supplementary Information

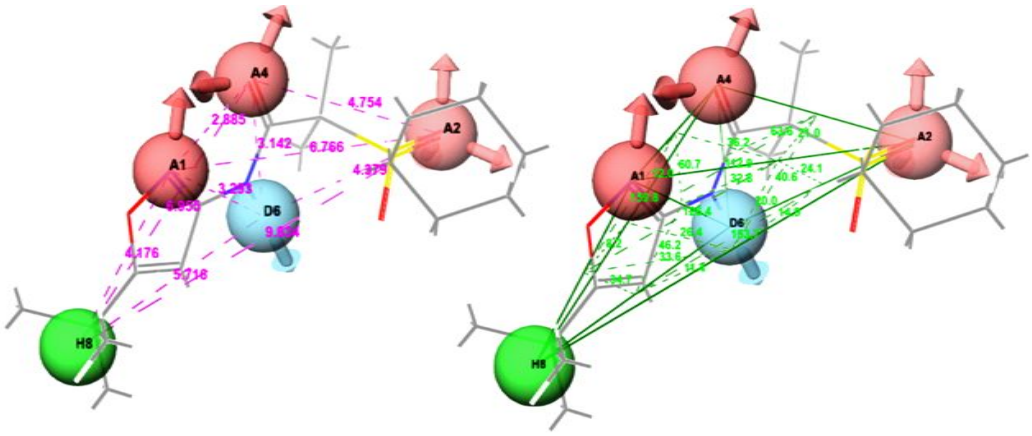
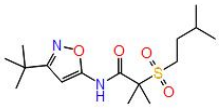
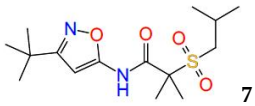
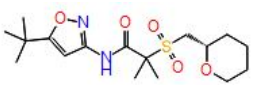
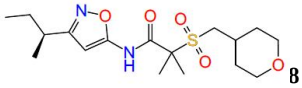
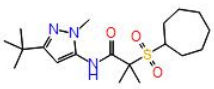
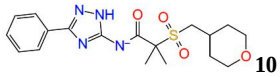
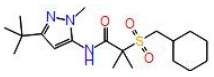
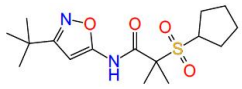
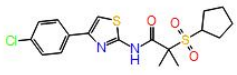
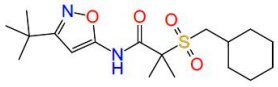
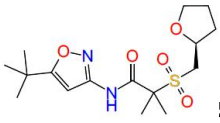
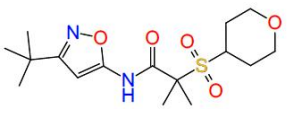
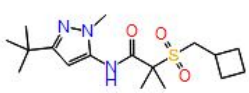
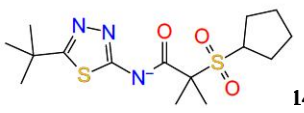
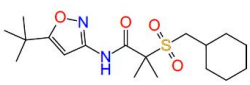
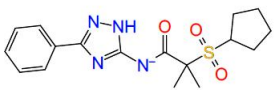
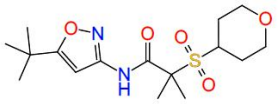
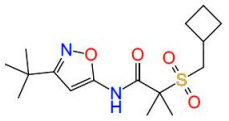
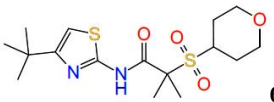
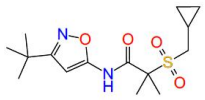
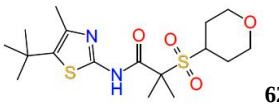
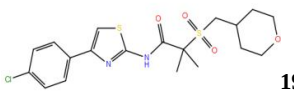
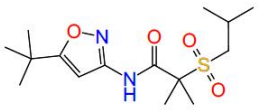
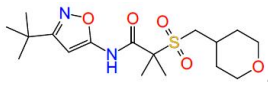
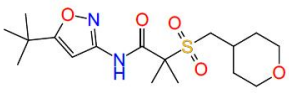
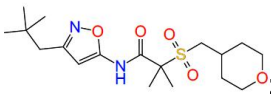
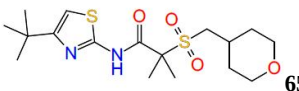
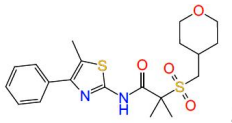
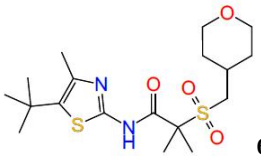
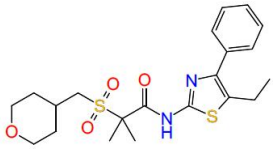
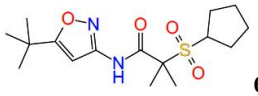
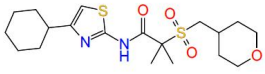
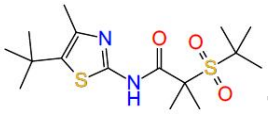
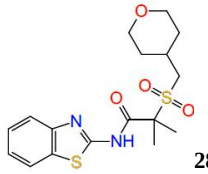
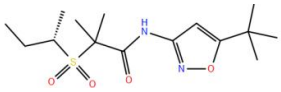
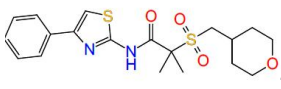
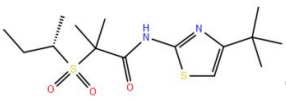
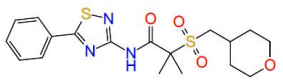
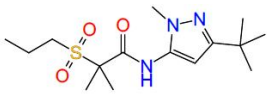
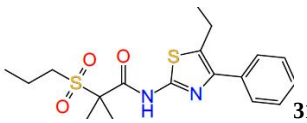
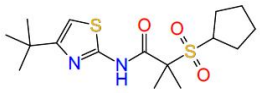
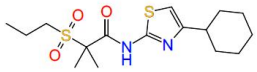
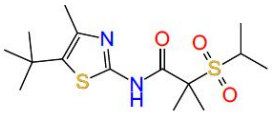
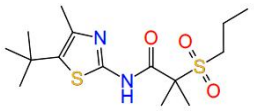
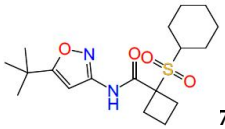
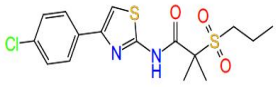
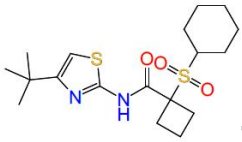
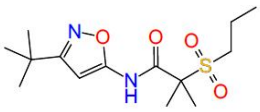
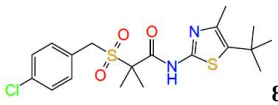
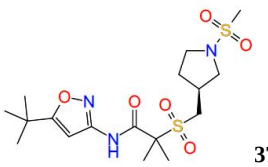
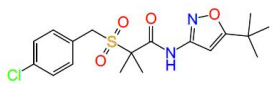
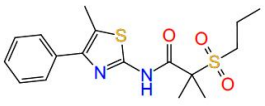
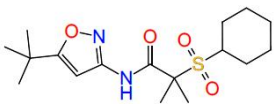
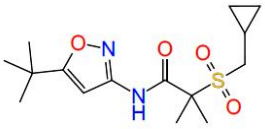
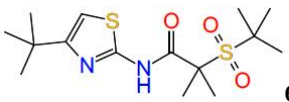
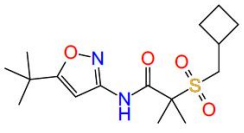
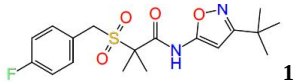
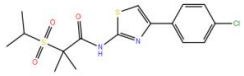


Fig. SI 1: Pharmacophore with (a) inter atomic distances and (b) angles

Table SI 1: Data set for QSAR analysis

Structure and Compound ID	EC50 (nM)	pEC50	Structure and Compound ID	EC50 (nM)	pEC50
 **9**	0.04	10.398	 **7**	1.6	8.796
 **51**	0.2	9.699	 **8**	6.2	8.208
 **52**	6.5	8.187	 **10**	1.7	8.77
 **53**	23.8	7.623	 **11**	0.9	9.046
 **55**	1.5	8.824	 **12**	0.1	10
 **56**	70.5	7.152	 **13**	0.1	10
 **57**	33	7.481	 **14**	18.6	7.73

 **58**	0.6	9.222	 **15**	3.8	8.42
 **59**	0.7	9.155	 **16**	3.7	8.432
 **60**	12.4	7.907	 **17**	12.5	7.903
 **62**	0.3	9.523	 **19**	2.2	8.658
 **63**	44.6	7.351	 **21**	4	8.398
 **64**	4.5	8.347	 **22**	74.3	7.129
 **65**	23	7.638	 **25**	2.5	8.602
 **66**	0.2	9.699	 **26**	2	8.699
 **68**	2.4	8.62	 **27**	8.3	8.081
 **70**	10.7	7.971	 **28**	27.9	7.554
 **71**	18.7	7.728	 **29**	7.1	8.149

 **72**	14.4	7.842	 **30**	17.6	7.754
 **73**	61.9	7.208	 **31**	10.8	7.967
 **75**	19	7.721	 **32**	31.1	7.507
 **77**	25.8	7.588	 **33**	7.2	8.143
 **78**	0.5	9.301	 **35**	13	7.886
 **79**	11.7	7.932	 **36**	89.7	7.047
 **80**	12.1	7.917	 **37**	44.1	7.356
 **81**	16.6	7.78	 **38**	52.2	7.282
 **82**	0.2	9.699	 **39**	34.3	7.465
 **69**	61.1	7.214	 **40**	3.4	8.469
 **1**	17	7.77	 **41**	44.9	7.348

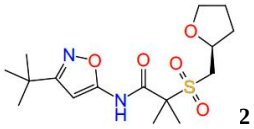
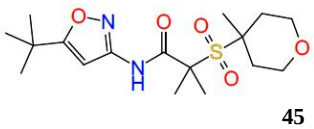
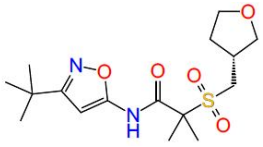
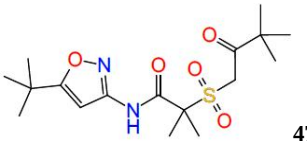
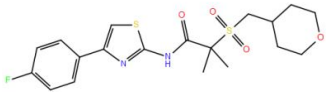
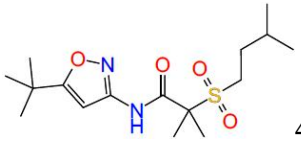
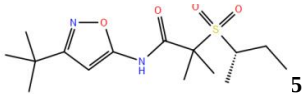
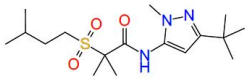
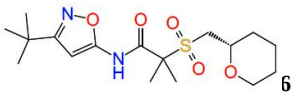
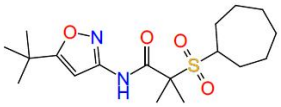
 **2**	31.5	7.502		 **45**	9.4	8.027
 **3**	18.5	7.733		 **47**	39.8	7.4
 **4**	5.5	8.26		 **48**	0.2	9.699
 **5**	3.3	8.481		 **49**	15.4	7.812
 **6**	0.2	9.699		 **50**	0.1	10

Table SI. 2: Data set experimental activity vs predicted activity

Comp. ID	QSAR set	pEC50	Predicted pEC50	Prediction error		Comp. ID	QSAR set	pEC50	Predicted pEC50	Prediction error
9	training	10.398	9.6801	-0.717842		7	training	8.796	8.77226	-0.02362
51	test	9.699	9.47192	-0.227052		8	training	8.208	8.35615	0.148539
52	test	8.187	8.77964	0.592551		10	training	8.77	8.67728	-0.0922718
53	training	7.623	7.89008	0.266656		11	training	9.046	9.07498	0.029225
55	training	8.824	8.61947	-0.204441		12	training	10	9.96743	-0.0325713
56	training	7.152	7.19752	0.0457043		13	test	10	9.85228	-0.147721
57	training	7.481	7.54609	0.0646071		14	training	7.73	7.69398	-0.036506
58	training	9.222	9.18004	-0.0418104		15	training	8.42	8.3951	-0.025117
59	training	9.155	9.34103	0.18613		16	training	8.432	8.53315	0.10135
60	test	7.907	8.12637	0.219788		17	test	7.903	8.02274	0.119648
62	training	9.523	9.60147	0.0785926		19	training	8.658	8.65333	-0.0042489
63	test	7.351	8.62973	1.27907		21	training	8.398	8.03332	-0.364619
64	test	8.347	8.94083	0.594042		22	training	7.129	7.10838	-0.0206345
65	test	7.638	8.12787	0.48959		25	training	8.602	8.63269	0.0306333
66	training	9.699	9.29249	-0.406476		26	training	8.699	8.73257	0.0336
68	test	8.62	8.57511	-0.044674		27	training	8.081	8.07824	-0.0026833
70	training	7.971	7.97145	0.00083213		28	training	7.554	8.11246	0.558066
71	training	7.728	7.73938	0.0112258		29	training	8.149	8.25114	0.102402
72	training	7.842	7.83926	-0.0023731		30	training	7.754	7.76457	0.0100847
73	training	7.208	7.19677	-0.0115426		31	training	7.967	7.87896	-0.0876144
75	training	7.721	7.64738	-0.0738618		32	test	7.507	7.63933	0.132094
77	training	7.588	7.86684	0.278461		33	test	8.143	8.10202	-0.0406441
78	training	9.301	9.20349	-0.0975403		35	training	7.886	7.67424	-0.211814
79	training	7.932	7.88681	-0.0450086		36	training	7.047	7.58875	0.541542
80	training	7.917	7.83818	-0.0790376		37	training	7.356	7.36949	0.0139261
81	training	7.78	7.80597	0.0260798		38	test	7.282	7.73844	0.456107
82	test	9.699	9.67298	-0.0259857		39	training	7.465	7.49711	0.0324086
69	training	7.214	7.26149	0.0475314		40	test	8.469	8.43126	-0.0372636
1	training	7.77	7.73025	-0.0392977		41	test	7.348	7.30492	-0.0428357
2	training	7.502	8.49557	0.993884		45	training	8.027	8.08945	0.0625777
3	training	7.733	7.95356	0.220728		47	test	7.4	7.85626	0.456146
4	training	8.26	8.24248	-0.017154		48	training	9.699	9.58391	-0.11506
5	training	8.481	8.54184	0.06035		49	training	7.812	7.59503	-0.217451
6	training	9.699	9.42945	-0.269517		50	test	10	9.18682	-0.81318

4. CONCLUSION

The pharmacophoric feature AAADH derived from the cannabinoid receptor agonists comprised of 5 potential features. The features include three hydrogen bond acceptors, one hydrogen bond donor and one hydrophobic feature. This feature gave a clear idea on the potential feature of the ligands responsible for the receptor agonistic property. The 3D qsar model derived from the aligned date set of 68 sulfonamide compounds revealed its statistical significance by the regression coefficient R^2= 0.9081, analysis of variance, F= 83, statistical significance, p = 1.18E–20 and cross correlation coefficient, Q^2= 0.8002. $Q^2 > 0.5$ stands for external test set predictability of the model. The model analysis has revealed the significance of electron withdrawing effect of alkyl substituents attached to the sulfonyl group, hydrophobic effect of oxazole ring substituents and electron withdrawing effect of sulfonyl group behind the receptor agonistic property. The observations suggested that the present QSAR model of cannabinoid receptor agonists can be utilized for rational drug designing for immuno modulatory diseases.

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