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Research article ***Analytical research***

Exploring the structural necessities of novel Indole-2-carboxamides in terms of QSAR modeling; a CB1 receptor antagonist

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ABSTRACT

Obesity and its connected co-morbidities, such as type 2 diabetes, hypertension, dyslipidemias and cancer masquerade a serious life threat to the community health. A novel class of indole- 2- carboxamide antagonist was demonstrating a signs of a potent CB1 activity along with an excellent CB1 selectivity. These CB1 receptor antagonists could considerably decrease fatness and obesity was suggested to have potential therapeutic usefulness as hunger suppressants for the management of obesity. To investigate the structural necessities for more energetic antiobesity agents, QSAR study was performed on twenty-four indole- 2- carboxamides. The QSAR study was performed here comprises few statistical analysis methods like MLR, FA-MLR; PCRA etc.. The substituted Indole- 2- carboxamide pharmacophore was number as per the international union of Pure and applied chemistry. QSAR study reveals that increase charge at atom numbers 2 and 7 of Indole- 2- carboxamide may be unfavorable for antiobesity activity. Increasing value of the electrostatic potential charges at atom numbers 8 and 9 may be detrimental but increasing electrostatic potential charge at the atom number 3 may be favorable for CB1 antagonistic activity. Presence of electron withdrawing group at atom numbers 8 and 9 and electron releasing group at atom numbers 3 may contribute positivity to the CB1 receptor antagonistic activity. The above structure activity relationship outcome will have been an appropriate target for further synthesis and biological activity determination with low cost and less time consuming promising approach.

Keywords: CB_1 antagonist, Obesity, QSAR study, Wang ford's charge, electrostatic potential charge, Indole-2- carboxamide.

INTRODUCTION

Obesity is a physiological condition with excess body mass as well adipose tissue that may have an terrible effect on health with various complications, particularly heart disease, type 2 diabetes, breathing difficulties during sleep, certain types of cancer, and osteoarthritis as per Krushnapriya et al. [1] Obese people required a large excess energy to maintain the normal body homeostasis but few people eat very little however; gain body weight due to their low basal metabolic rate (BMR) and at present obesity is a leading cause for death of people with various diseases with complications specially for children[2,3].

As per estimation by World Health Organization (WHO), it was predicted that 30% of world wide death will be due to lifestyle diseases by 2030 [4]. Obesity and Overweight is considered a principal health challenges for public health and it is the 5th foremost cause of natural death. The complication arises from obesity is quite difficult to identify or difficult to recognize the actual cause of disease [5, 6]. Thus, quick detection and diagnosing of obesity is important for obese people with simple health challenges. Moreover, it is important to improve the diet quality and anti-obesity drugs may be recommended as supplement to reduce appetite or to inhibit fat absorption [7]. In some cases, surgery is preferred or use intragastric balloon to reduced ability to absorb nutrients to control fat deposition [8].

A recent study highlight that, the loss of body weight accomplish by bariatric surgery was linked with a 32% lower risk of developing cancer and a 48% lower risk of cancer-related death; is one of the recorded benefit of surgical

removal of fat [9]. In the current research on obesity, the rapid deposition of fat is the cause of happening of lack of blood supply, oxygen to the underlying tissue and fat deposited cells unable to divide further. In additional to this; most of the obese patients will suffer from insulin resistance, inflammation, and arthritis [10].

PATHOPHYSIOLOGY OF OBESITY

Excess food intake; lack of Calorie utilization will cause deposition of fat on adipose tissue and as a outcome leptin and inflammatory cytokines will produces within the fatty tissue[11,12]. As a feedback inhibition, leptin reduces the tendency to intake of food as well as reduces body weight [13]. As a result; there will an elevated levels of leptin and large fat per mass ratio will alter leptin gene signaling and induce "leptin resistance" [14]. Furthermore, cellular label of leptin resistance induce by obesity will complicates pathology of adipose tissue [15]. Over nutrition of any specific nutrients has an effect to generate Leptin peripherally; which control appetite via central nervous system (CNS) [16]. Appetite-related hormones will come into action on hypothalamus, increase food intake and lack energy expenditure [17]. The central and peripheral leptin deficiency or leptin resistance leads to impede nutrients absorption, metabolism of foodstuff, and increase hungriness for feeding [18]. As a consequence; body will acquired stable forms of obesity with complications.

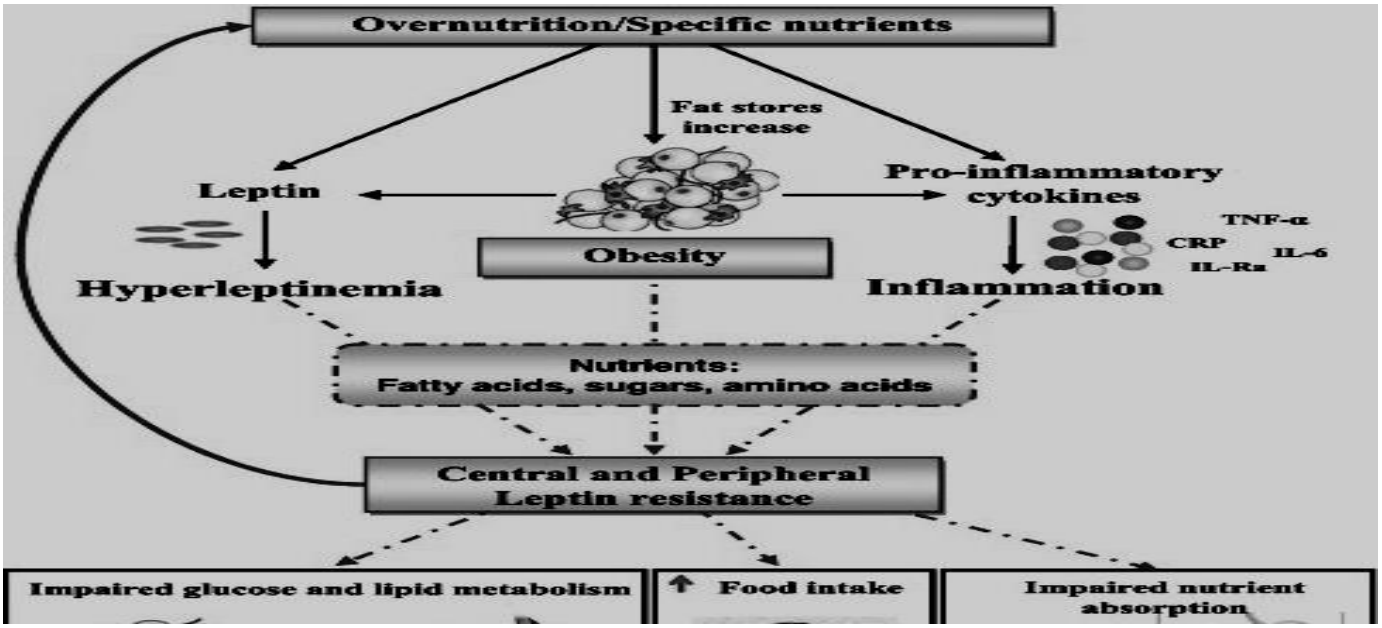


Fig 1: Pathophysiology for Leptin Resistance in Obesity

As per previous research, Pro-inflammatory cytokines like tumor necrosis factor α (TNF-α) and interleukin 6(IL-6) possibly release by adipose tissue as a response of excess of macronutrients of fatty tissue [19,20]. The lacks of adiponectin in association with elevated level of cytokines have an effect to produce low-grade chronic inflammation as well as oxidative stress. The elevated amount of IL-6 stimulates the release of protein C-reactive protein (CRP) by liver and where inflammation arises as a response to protect body's tissue with various allergic manifestations [21,22]. The underlying mechanism of inflammation developed cardiovascular diseases metabolic syndrome, insulin resistance, and diabetes mellitus, hypertension, stroke, and gallbladder disease, some forms of cancer, osteoarthritis, and psychosocial problems [23,24].

Effect of Cannabinoid Receptor Antagonist on Obesity

The Cannabinoid receptors (CB) are the part of endo-cannabinoid system, found all over the body, which is involved in a variety of physiological processes including appetite, pain-sensation, mood, and memory [25, 26]. The CB is a class of G- protein-coupled receptor (GPCR) family. The well known CB receptor Subtype are CB_1 and CB_2.The CB_1 receptor is expressed mainly in the central nervous system (CNS), and also in the lungs, liver and kidneys. The CB_2 receptor is expressed mainly on T-cells of the immune system, on macrophages and B cells, in hematopoietic cells, and in the brain and CNS [27,28].

One mechanism through which they function is endocannabinoid-mediated depolarization-induced suppression of inhibition, a very common form of retrograde signaling, in which the depolarization of a single neuron induces a reduction in GABA-mediated neurotransmission and also cause a reduction in GABA release due to limited presynaptic calcium ions entry [29,30]. CB1 receptor is thought to inhibit adenylcyclase enzyme the activation of Mitogen-activated protein (MAP) kinase, inhibit presynaptic calcium channel and activate the potassium ion channel and for instance, in the liver, activation of the CB_1 receptor is known to increase de novo lipogenesis. CB2 receptors are found largely on immune cells where they modulate proinflammatory cytokine suppression [31,32].

Therefore, Endo-cannabinoids act at the CB_1 receptors to increase hunger and promote feeding and it is speculated that

they decrease intestinal peristalsis and gastric emptying. Thus, antagonism at these receptors can inverse these effects. Also, in peripheral tissues, antagonism of CB_1 receptors increases insulin sensitivity and oxidation of fatty acids in muscles and the liver [33,33]. The appetite regulating effects of plant-derived Cannabinoid have been known for centuries. Rimonabant; a specific CB1 receptor inverse agonist (functional antagonist) have been marketed as an antiobesity agent which acts centrally to suppress food intake and regulate body-weight gain [34, 35]. CB_1 antagonists produce inverse Cannabinoid mimetic effects that are opposite in direction from those produced by agonists for these receptors. However, this first line CB1 receptor antagonist/inverse agonist, demonstrating effectiveness for obesity treatment and smoking cessation, displays serious psychiatric side effects, including anxiety, depression and even suicidal ideation, resulting in its eventual withdrawal from the European market [36, 37, 38, 39, 40]. For our present study; we have an intention for searching of best effective antiobesity drug with fewer side effects by QSAR study.

QSAR STUDY

To find the structural necessities for more bioactive antiobesity agents, QSAR study was performed on twenty-four indole -2-carboxamide derivatives as a part of a research program of rational drug design, detection, development and progress [41-50]. The general structure or of these compounds with random numbering is shown in Figure 1 and structure of other compounds given in table 1.

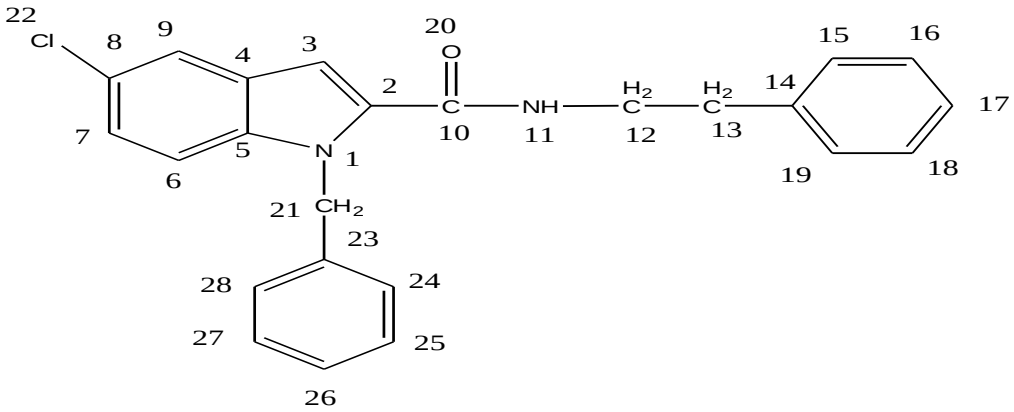
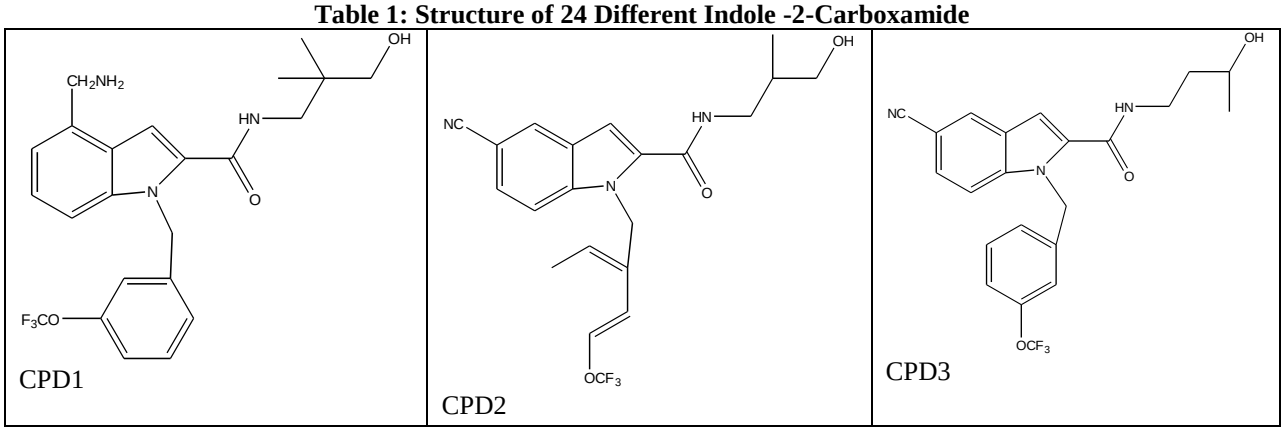


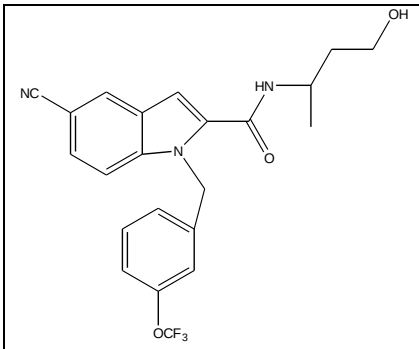
Fig 2: General Structure of Indole -2-Carboxamide With Numbering

MATERIAL METHODS

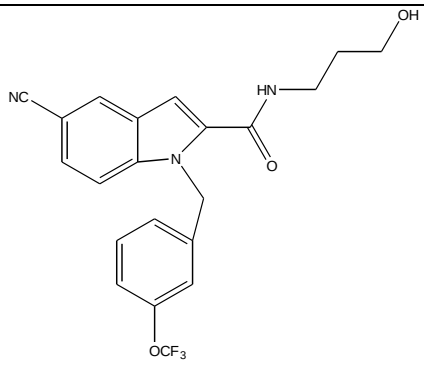
QSAR study was performed on twenty-four (24) compounds that were synthesized and biologically evaluated previously by Cowley et al [51]. The functional activities were determined by inhibition of human CB1 receptors in a CB1 reporter gene assay as well as radioligand binding assay. These activities were expressed as pIC_{50}, and considered as the biological activity parameters for the QSAR study. Here, the negative logarithm of inhibitory activity of these compounds (pIC_{50}) ware used to develop the QSAR models to obtain a linear relationship as well as improve the robustness of collected data's. The pIC_{50} values along with structures of all these compounds are shown in Table 2.

Table 1: Structure of 24 Different Indole -2-Carboxamide

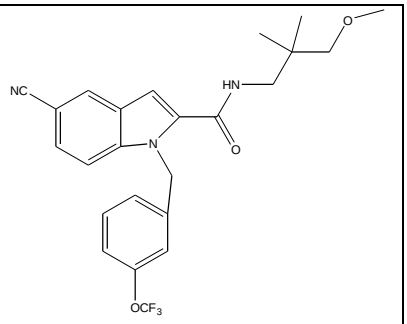




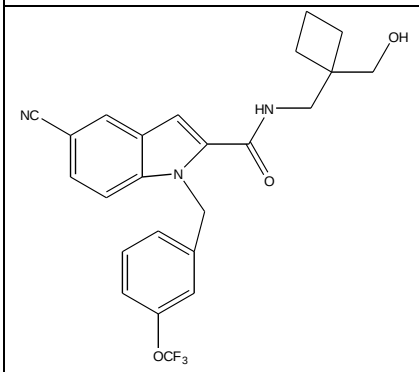
CPD4



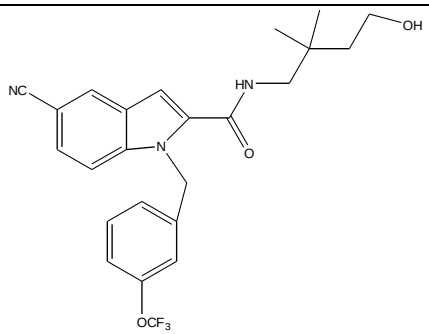
CPD5



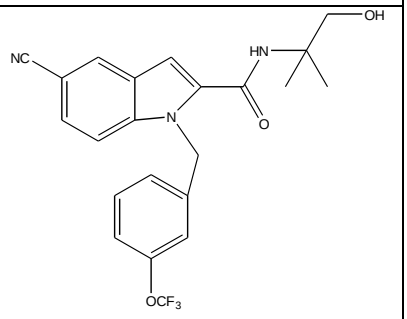
CPD6



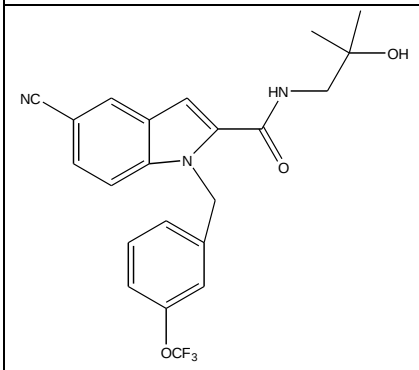
CPD7



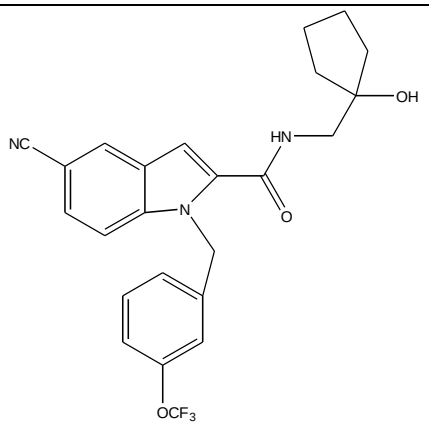
CPD8



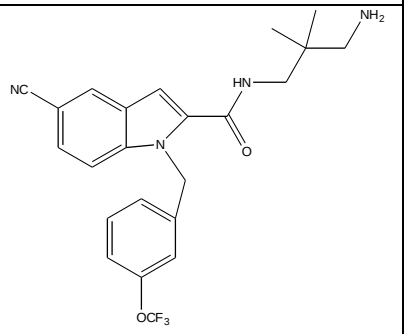
CPD9



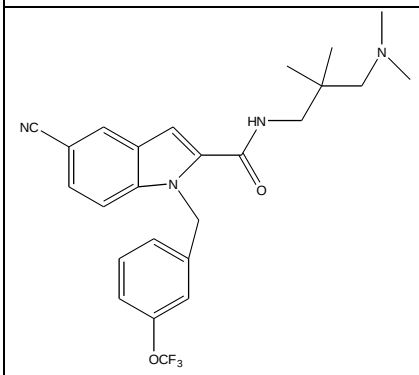
CPD10



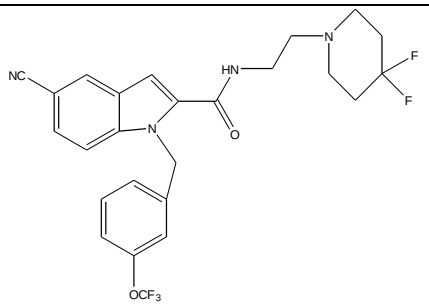
CPD11



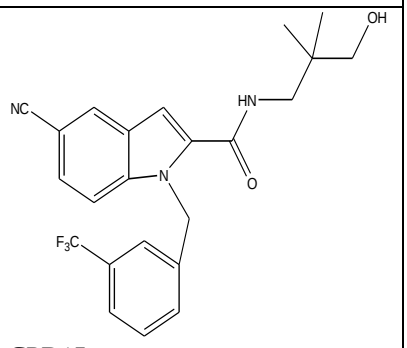
CPD12



CPD13



CPD14



CPD15

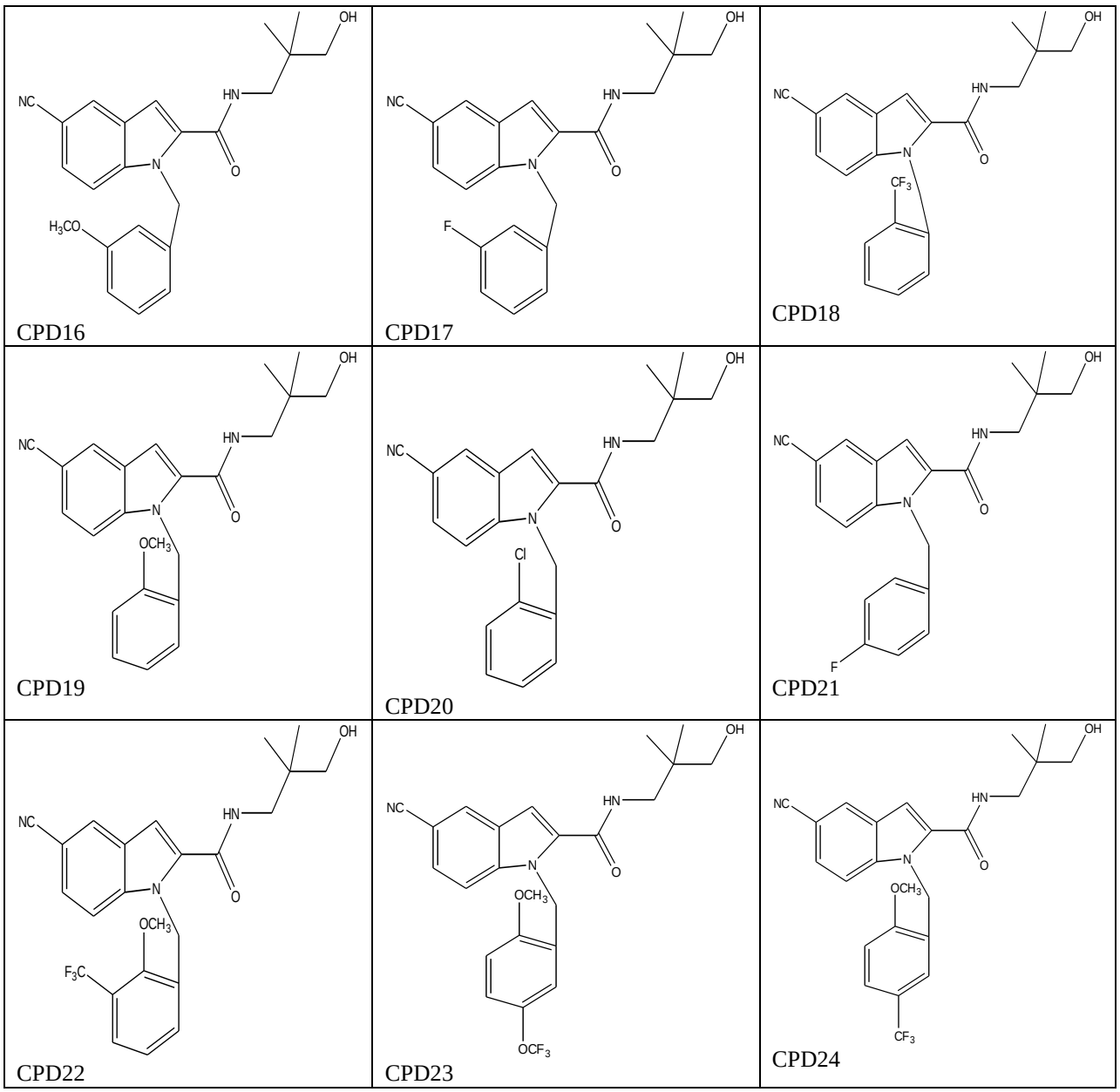


Table2. Anti-Obesity Activity Of Indole -2-Carboxamide Derivatives

Cpd[a]	pIC_{50} (M)	Cpd[a]	pIC_{50} (M)	Cpd[a]	pIC_{50} (M)	Cpd[a]	pIC_{50} (M)
CPD1	6.37	**CPD7**	5.67	**CPD13**	6.80	**CPD19**	7.64
CPD2	5.93	**CPD8**	5.37	**CPD14**	7.21	**CPD20**	6.32
CPD3	6.57	**CPD9**	5.55	**CPD15**	7.42	**CPD21**	7.82
CPD4	5.83	**CPD10**	6.57	**CPD16**	7.64	**CPD22**	8.74
CPD5	5.35	**CPD11**	5.80	**CPD17**	7.23	**CPD23**	6.67
CPD6	5.65	**CPD12**	7.71	**CPD18**	7.81	**CPD24**	6.29

Cpd[a] Compound number

QSAR study was performed using electronic descriptors like atomic charges (Q), electrostatic potential charges (EP), constitutional descriptors as well as all dragon descriptors were calculated to quantify the variation on targeted biological effect of the structural variation on $pMIC_{50}$. Apart from these descriptors, *indicator parameters* were also considered on the data set but not used to highlight a structural feature present in some of these molecules in the dataset that confers unusual activity or lack of it to these particular members. Prior to calculation, atoms of these molecules were numbered arbitrarily keeping the serial number of atoms identical for all molecules.

A large number of dissimilar descriptors were calculated by using special type well recognize QSAR software's in order to develop mathematical models. Different atom based and whole molecular quantum chemical molecular descriptors like total electronic energy of molecule (E), absolute electronegativity (χ), chemical hardness (η), electrophilicity index (ω), etc. were calculated by using *Chem. 3D Pro package*. The *2D* structure of all molecules was drawn separately in *Chem draw ultra* ver 5.0 [52] . To create the 3-

D models, all these structures were transformed to *Chem 3D* ver 5.0. Energy minimization of individual structure was done under MOPAC module using RHF (restricted Hartee-Fock: closed shell) wave function [53]. These energy minimized geometry were subjected to calculate Wang-Ford charges. Calculation of different quantum chemical descriptors were done using computer program *Hyperchem Release 7.0 Pro. Package* [54]. *Dragon* software [55] was utilized for the calculation of different topological, geometrical and constitutional descriptors.

Various statistical analysis methods were used for the searching the correlation of biological activity with obesity, and development of QSAR models are correlation analysis[60], factor analysis (FA)[61], multiple linear regression analysis (MLR)[56], Principal component regression analysis (PCRA) [58,59] as well as factor analysis multiple linear regression analysis ((FA-MLR)[57]. The statistical robustness of the models were justified by parameters like correlation coefficient (R), adjusted R^2 (R^2_A), variance ratio (F) at specified degrees of freedom, probability factor related to F-ratio (p) and standard error of estimate (SEE). The Leave-one-out (LOO) cross validation method [58] was applied to validate QSAR models.

Descriptor assortment is intended to get rid of those calculated descriptors that are redundant, inappropriate, noisy, or irrelevant for this data matrix. These were done in such a way that the input data points can be reduced without loss of significant information and correlations. The descriptors that were used as independent variables include different informational content indices, electronic descriptors like the highest-energy occupied orbital or lowest-energy unoccupied orbital in a molecule, Wang-Ford charges, frontier electron density, and constitutional descriptors. The above mention orbital's, and electron density have a large influence on chemical properties. Furthermore, the different atom based and whole molecular quantum chemical descriptors were also used to describe the properties of the molecules mathematically generated by different algorithm like FA, MLR, PCRA, FA-MLR as stated above[39-42] .

RESULTS

CORRELATION ANALYSIS

Correlation analysis [60] was carried out with the response variable and independent parameters of the training set. Intercorrelated independent parameters were not considered for multiple linear regression analysis and eliminated stepwise depending on their individual correlation with the biological activity. The calculated values of selected independent parameters to develop QSAR models are listed in Table 3.

Table 2: Calculated Values Of Selected Independent Parameters

CPD	pIC50	BEHv2	JGI8	MATS6V	MATS8P	MATS6P	RDF070u	MATS8v	MATS6v
1	6.3700	3.8070	0.0100	0.0250	0.0150	0.0300	25.1330	-0.0100	0.0250
2	5.9300	3.7970	0.0100	0.0310	-0.0080	0.0340	21.0850	-0.0380	0.0310
3	6.5700	3.8110	0.0090	0.0040	-0.0010	0.0150	22.9770	-0.0160	0.0040
4	5.8300	3.7780	0.0100	0.0660	-0.0240	0.0710	24.5330	-0.0580	0.0660
5	5.3500	3.8070	0.0100	0.0250	0.0150	0.0300	24.8160	-0.0100	0.0250
6	5.6500	3.7900	0.0190	0.1360	-0.1470	0.1070	28.8470	-0.1470	0.1360
7	5.6700	3.7780	0.0100	0.0160	-0.0550	0.0360	26.9520	-0.0750	0.0160
8	5.3700	3.7780	0.0100	0.0650	-0.0200	0.0680	26.2550	-0.0520	0.0650
9	5.5500	3.7780	0.0090	0.0390	-0.0830	0.0610	20.1100	-0.1080	0.0390
10	6.5700	3.7780	0.0150	-0.0050	-0.1440	0.0180	25.0280	-0.1670	-0.0050
11	5.8000	3.8070	0.0090	-0.0280	0.0380	-0.0120	21.7900	0.0240	-0.0280
12	7.7100	3.8120	0.0170	-0.0130	-0.1390	-0.0270	22.0020	-0.1710	-0.0130
13	6.8000	3.7890	0.0170	-0.0010	-0.1660	0.0070	23.4460	-0.2030	-0.0010
14	7.2100	3.7890	0.0260	0.0170	-0.2820	0.0330	22.6900	-0.2620	0.0170
15	7.4200	3.8100	0.0190	0.0490	-0.2920	0.0380	27.1490	-0.2980	0.0490
16	7.6400	3.7880	0.0190	-0.0430	-0.2280	-0.0430	22.3150	-0.2290	-0.0430
17	7.2300	3.8440	0.0210	-0.0900	-0.1830	-0.1070	23.8630	-0.1900	-0.0900
18	7.8100	3.7880	0.0230	-0.1000	-0.2080	-0.1190	28.0470	-0.2120	-0.1000
19	7.6400	3.7870	0.0200	-0.0680	-0.2010	-0.0740	30.0020	-0.1950	-0.0680
20	6.3200	3.7880	0.0200	-0.0380	-0.1490	-0.0320	28.6680	-0.1590	-0.0380
21	7.8200	3.7900	0.0230	-0.1040	-0.2310	-0.1170	34.6630	-0.2330	-0.1040
22	8.7400	3.7910	0.0220	-0.0980	-0.2100	-0.1090	26.5470	-0.2070	-0.0980
23	6.6700	3.7980	0.0230	-0.0800	-0.1340	-0.1030	38.6250	-0.1450	-0.0800
24	6.2900	3.7950	0.0220	-0.1170	-0.2180	-0.1290	40.1920	-0.2150	-0.1170

The correlation matrix among the response variable and those selected descriptors is shown in Table 4

Table 3: Correlation Analysis Of Variables Used To Develop QSAR Models.

	pIC50	MATS6v	MATS8v	MATS6p	MATS8p	RDF070u	RDF135m	RDF020e	Mor04e	H6m
pIC50	1.00	-0.63	-0.72	-0.67	-0.72	0.13	-0.41	-0.17	-0.43	0.45
MATS6v		1.00	0.40	0.98	0.42	-0.47	0.60	0.10	0.17	-0.58
MATS8v			1.00	0.45	0.99	-0.32	0.34	-0.25	0.15	-0.43
MATS6p				1.00	0.48	-0.55	0.52	0.04	0.26	-0.66
MATS8p					1.00	-0.35	0.32	-0.25	0.14	-0.46
RDF070u						1.00	-0.05	0.32	0.18	0.69
RDF135m							1.00	0.23	0.01	-0.10
RDF020e								1.00	0.05	0.14
Mor04e									1.00	-0.10
H6m										1.00

Validation of QSAR Models

Leave-one-out (LOO) cross validation method [58] was applied to validate the QSAR models developed. Predictive powers of these equations were justified by this method. Predicted residual sum of square (PRESS), total sum of squares (SSY), cross-validated R^2 (R^2_{CV}), standard deviation of error of prediction (SDEP) and standard error of PRESS (S_{PRESS}) were considered for validation of these models.

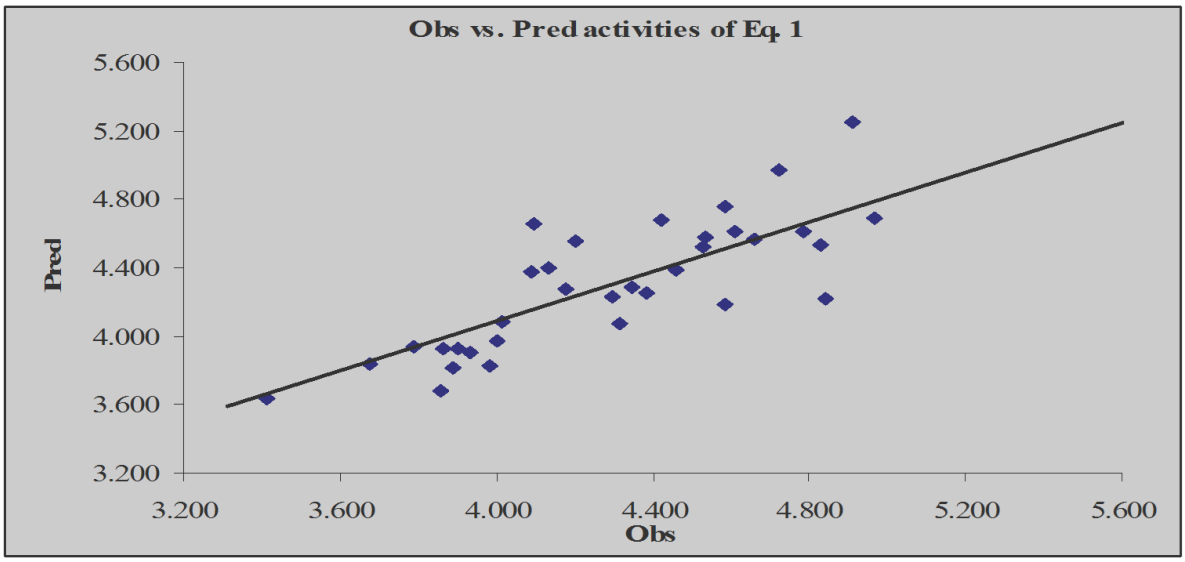
Stepwise Regression [56]

Attempts were made to develop QSAR models for the antiobesity activity of indole- 2- carboxamide antagonist derivatives. By forward stepwise selection method on the basis of F value (F=1.0 for inclusion; F=0.2 for exclusion), Eq. 1 was derived.

pIC_{50} = 8.217(±0.7886) –8.438(±1.406) MATS8v – 6.778(±1.826) MATS6v – 1.372(±0.516) Mor04e – 0.375 (±0.116) RDF020e + 1.250(±0.568) Mor04u Eq. (1)

n= 24; R= 0.923; R^2= 0.852; R^2_A = 0.810; F (5, 18) =20.665; p<0.00000; S.E.E. = 0.408; SSY= 20.169; PRESS= 5.209; R^2_{cv}= 0.741; SDEP= 0.217; S_{PRESS}= 0.289.

Where n is the number of data points i.e., the number of indole- 2- carboxamide antagonist compounds present in the data sets. The 95% confidence intervals of the regression coefficient are shown in parentheses. Eq. (1) explains 85.20% and predicts 74.10% variances of the inhibition of human CB1 receptors in a CB1 reporter gene assay as well as radioligand binding assay. Values within the parenthesis are the standard error of corresponding parameters. Eq. 1 suggests importance's of the dragon descriptors like MATS8v (Moran autocorrelation of lag 8 weighted by vander Waals volume), MATS6v (Moran autocorrelation of lag 6 weighted by vander Waals volume), Mor04e (3DMoRSE dragon descriptors with signal 04 / weighted by Sanderson electronegativity), RDF020e (Radial Distribution Function - 020 / weighted by Sanderson electronegativity) and Mor04u(3DMoRSE dragon descriptors with signal 04 / unweighted) of whole molecules in the biological activity. The positive coefficients of those the above mentioned parameters imply that higher values of these parameters may correspond to the higher binding affinity towards the CB-1 receptor as far as the better antiobesity activity is concerned. The increase of vander Waals volume of all the molecules may likely decrease antagonistic activity as the coefficients of these descriptors are negative. Eq. 1 also suggests that the increases of electronegativity of whole molecule of all the compounds present in the data set are not favorable for improving the biological activity. **i.e.,** lower the electronegativity of whole molecule may be conducive to the CB-1 receptor antagonistic activity. The Observed (Obs) vs. LOO-predicted (Pred) activities of Eq. 1 is shown in Fig 2.



Attempts were made to develop another QSAR models for the antiobesity activity of indole- 2- carboxamide antagonist derivatives for whole data set. By forward stepwise selection method on the basis of F value (F=1.2for inclusion; F=0.1 for exclusion), Eq. 2 was derived.

pIC_{50} = 11.118(±0.936) – 7.980(±1.136) *MATS8p* – 6.951(±1.493) *MATS6p* – 0.146(±0.025) *RDF070u* – 22.335 (±5.759) *HATS6m* + 4.163 (±1.430) *H6m* Eq. (2)
n= 24; R= 0.940; R^2= 0.884; R^2_A =0.852; F (5, 18) =27.445; p<0.0000; S.E.E. = 0.360; SSY= 20.168; PRESS= 4.202; R^2_{cv}= 0.792; SDEP= 0.175; S_{PRESS}= 0.233.

Where n is the number of data points i.e., the number of indole- 2- carboxamide moiety containing compounds present in the data sets. The Eq. (2) explains 88.40% and predicts 79.20% variances of the inhibition of human CB1 receptors in a CB1 reporter gene as well as radioligand binding activity. In Eq. (2) positive coefficients of the H autocorrelation of lag 6 / weighted by mass, a GETAWAY H-indices (*H6m*) be a sign of that with the increase of *H6m* increase antagonistic activity. Importance's of other descriptors towards CB-1 receptor antagonistic activity be fond of Moran autocorrelation of lag 8 weighted by polarizability (MATS8p); Moran autocorrelation of lag 6 weighted by polarizability (MATS6p); Radial Distribution Function - 070 / unweighted (RDF070u); and leverage-weighted autocorrelation of lag 6 / weighted by mass (HATS6m); are also observed. The negative coefficients all of these descriptors except *H6m* are implies that increaser the values of these descriptors are detrimental for biological activity. Eq. 2 also suggests that the increases of electronegativity of whole molecule of all the compounds present in the data set are not encouraging for improving the biological activity. **i.e.,** inferior the electronegativity of whole molecule may be encouraging to the CB-1 receptor antagonistic activity.

FACTOR ANALYSIS- MULTIPLE LINEAR REGRESSION (FA-MLR) [57]

Factor analysis was performed after VARIMAX rotation and different combinations of parameters having factor loading of more than 0.6(excluding very poorly loaded factors) were subjected to multiple linear regression. Intercorrelation less than 0.5 in between variables were taken in consideration for the development of equations. The results evolved with Eq. (3) with five descriptors after regression analysis:

pIC_{50} = 115.782(±27.778) – 318.027(±33.844) *JGI8* + 0.765(±0.112) *mor03m* – 11.040(±2.144) *E2u* – 28.132 (±7.280) *BEHv2* + 0.566 (±0.212) *RDF135m* Eq. (3)
n= 24; R= 0.946; R^2= 0.894; R^2_A =0.865; F (5, 18) =30.419; p<0.0000; S.E.E. = 0.344; SSY= 20.168; PRESS= 3.604; R^2_{cv}= 0.820; SDEP= 0.150; S_{PRESS}= 0.200.

It was observed that 8 factors can explain the data matrix to the extent of 89.40% and predicts 82.00%. The pIC_{50} is highly loaded with factor 6 and 8, moderately loaded with factor 1, 2 and 5, and poorly loaded with factor 3, 4 and 7. The factor loading of these parameters are shown in Table 3. The newly developed descriptor in that equation is the2nd component accessibility or 2nd component symmetry directional WHIM index unweighted (*E2u).* The negative coefficient of *E2u* emphasizes that the BC1 receptors binding activity may increase with the increase of polarizibilies of any compounds present in this data set that are supportive for above mention equations. JGI8 is the mean topological charge index of order 8, a dragon charge descriptor. From this various topology associated matrix concept, the topological charge index (GG_k) and mean topological charge index (JG_k) are appear as descriptor in QSAR and QSPR study.

The *Topological Charge Index* 'GG_k' and *Mean Topological Charge Index* 'JG_k' is defined as:

$$GG_k = ½ \sum \sum | CT_{ij} | \delta (k.d_{ij})$$
$$JG_k = GG_k / n\text{-}1$$

Where, n is the total number of nonhydrogen atom in the molecule, δ (k, d $_{ij}$) is Kronecker delta. Numerically δ (k, d $_{ij}$) is 1 if d $_{ij}$ is equal to k and otherwise it is 0 (zero). The negative coefficient associated with the mean topological charge index of order 8 (JGI8) suggests that it may be disadvantageous for the binding activity. Importance's of other descriptors towards CB-1 receptor antagonistic activity be tender of a 3D-MoRSE descriptors with signal 03 weighted by mass (Mor03m); highest eigenvalue n.2 of Burden matrix that is weighted by atomic vander waal,s volume (BEHv2) and Radial Distribution Function - 135 weighted by mass (RDF135m); are also observed. The negative coefficients all of these descriptors except *RDF135m* are implies that increaser the values of these descriptors are detrimental for biological activity.

Different combinations of parameters having factor loading of more than 0.60 on whole 24 compounds were further subjected to multiple linear regressions excluding the Intercorrelated parameters. The following equations are obtained on the basis of the factor analysis:

pIC_{50} = 9.930(±1.033) – 10.286(±1.240) MATS8p – 0.398(±0.084) *Mor04e* – 0.487(±0.133) *RDF020u*+ 0.089(±0.026) *RDF115u* – 0.089(±0.029) *RDF050m* Eq. (4)

n= 24; R= 0.930; R^2= 0.865; R^2_A = 0.827; F (5, 18) =23.052; p<0.0000; S.E.E. = 0.389; SSY= 20.168; PRESS= 4.981; R^2_{cv}= 0.753; SDEP=0.207; S_{PRESS}= 0.277.

Where n is the number of compounds present in the training set after regression analysis. Eq. 4 explains 86.50% and predicts 75.30% of variances of antagonistic activity. The RDF115u is the Radial Distribution Function - 115 / unweighted (RDF115u) and positive coefficient of *it* emphasizes that the BC1 receptors binding activity may increase with the increase of its value of atoms of any compounds present in this data set. Moran autocorrelation of lag 8 weighted by polarizability (MATS8p); a 3D-MoRSE descriptor with signal 04 weighted by Sanderson electronegativity (Mor04e) are appear as descriptor in QSAR and QSPR study. The negative coefficients all of these descriptors except *RDF115u* are implies that increaser the values of these descriptors as well as increase polarizability and are electronegativity detrimental for biological activity.

Table 4: T- And P- Values of Equations all Above Developed Equations

Eq.1	**Intercept/Parameter**	***t*-value**	***p*-value**	**Eq. 2**	**Intercept/Parameter**	***t*-value**	***p*-value**
	Intercept	10.420	0.000		Intercept	11.878	0.000
	MATS8v	-6.003	0.000		MATS8p	-7.025	0.000
	MATS6v	-3.712	0.002		MATS6p	-4.656	0.000
	Mor04e	-2.659	0.016		RDF070u	-5.769	0.000
	RDF020e	-3.238	0.005		HATS6m	-3.878	0.001
	Mor04u	2.201	0.041		H6m	2.912	0.009
Eq.3	**Intercept/Parameter**	**t-value**	**p-value**	**Eq. 4**	**Intercept/Parameter**	**t-value**	**p-value**
	Intercept	4.168	0.001		Intercept	10.420	0.000
	JGI8	9.397	0.000		MATS8v	-6.003	0.000
	Mor03m	6.811	0.000		MATS6v	-3.712	0.002
	E2u	-5.150	0.000		Mor04e	-2.659	0.016
	BEHv2	-3.864	0.001		RDF020e	-3.238	0.005
	RDF135m	2.664	0.016		Mor04u	2.201	0.041

3.5. Principal Component Regression Analysis (PCRA)

In PCRA [58, 59] factor scores were obtained from factor analysis on the data matrix containing all independent variables. Regression analysis was performed considering factor scores as predictor variables. Here, eight factor scores were extracted by principle component method and then rotated by VARIMAX rotation. These factor scores were used as independent parameters for developing QSAR equations. As factor score contains information for the different descriptors, the chance for loss of information is less. Using forward selection technique the following equation was developed:

pIC_{50}= *6.665 (±0.143) –0.401 (±0.146) f_6 + 0.396 (±0. 0.146) f_8 – 0.361(±0.146) f_2* Eq (5)

n = 24; R= 0.715; R^2= 0.511; R^2_A= 0.438; F (3, 20) = 6.977; p<0.0021; S.E.E = 0.702; R^2_{cv}= 0.279; SSY = 20.169; PRESS = 14.54; SDEP=0.606; S_{PRESS}= 0.808.

Eq. (7) explains 51.10% and predicts 27.90% variances of the biological activity and shows the importance of factor 2, 6and 8. This equation also shows that the factor 6 (*f_6)* is highly significant for that data set and factor loading is maximum among eight factors.

Table 5: Supportive Regression Analysis Data for Structural Requirements of Indole- 2- Carboxamide Derivatives.

	Beta	Standard Error of Beta	t(16)	p-level
Intercept	6.116	1.279	4.780	0.000
C4	17.795	3.392	5.247	0.000
EP3	9.930	2.236	4.442	0.000
EP9	-3.936	1.335	-2.949	0.009
C2	-4.405	1.811	-2.432	0.027
EP8	-13.493	2.630	-5.129	0.000
C7	-11.317	2.622	-4.317	0.001
C8	10.213	2.636	3.874	0.001

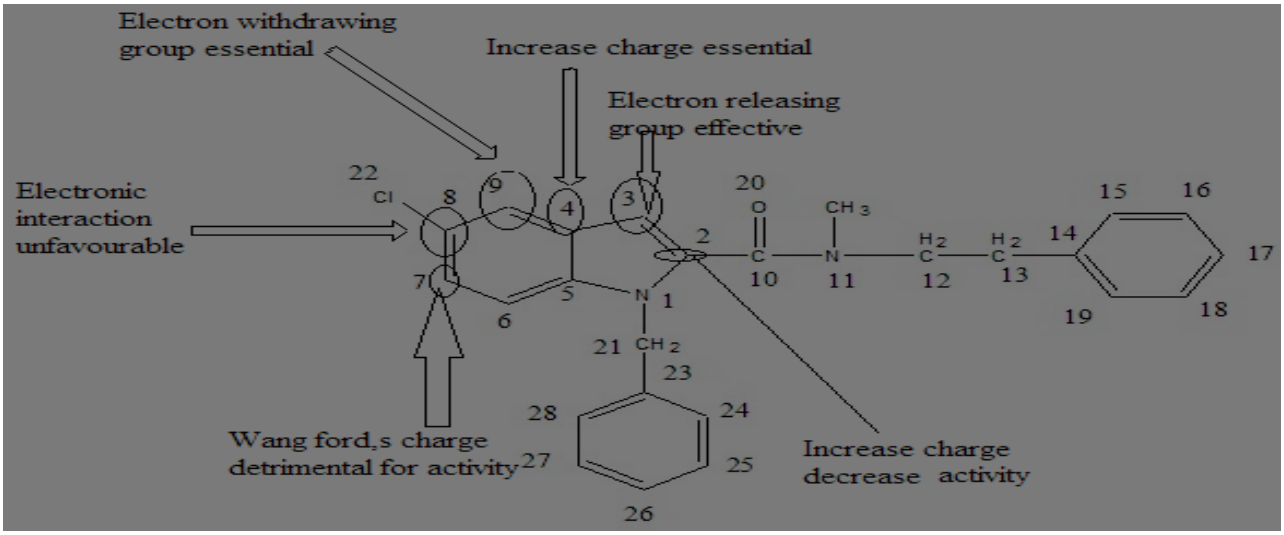


Fig 3: Structural Requirements of Indole- 2- Carboxamide Derivatives

The result was supported by the energy minimized geometry (shown in Figure 9) as well as the 3D isosurface electrostatic potential map of the most active compound (shown in Figure 10) obtained during the energy minimizations by AM1 calculations (Compound 5).

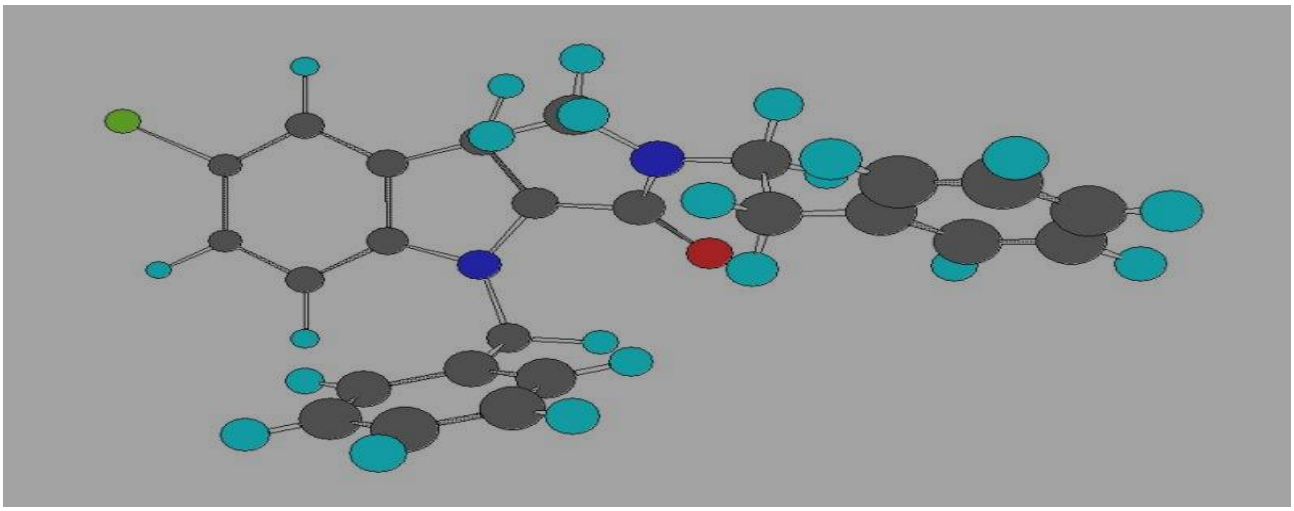


Fig 4: Energy Minimized Geometry of The Most Active Compound (Compound 5)

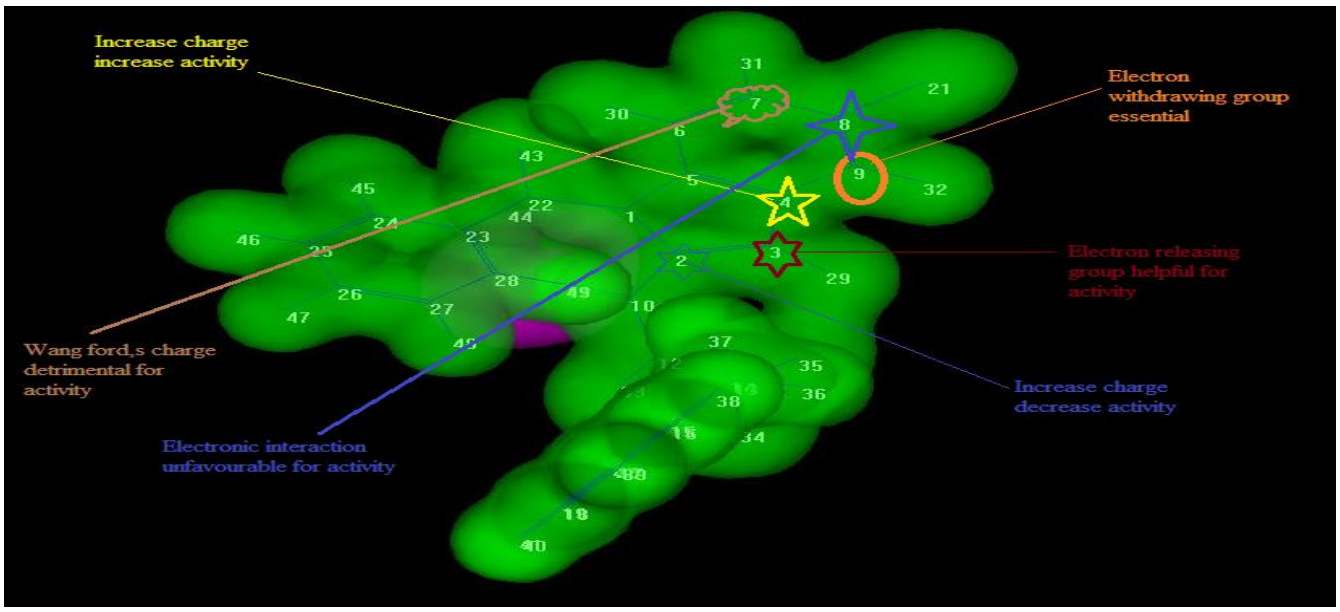


Fig 5: 3d Isosurface Electrostatic Potential Map of The Most Active Compound (Compound 5)

CONCLUSION

A novel class of Indole- 2- carboxamide antagonist was exhibited a potent CB1 activity and good CB1/2 selectivity. CB1 receptor antagonists could significantly reduce obesity and overweight were suggested to have potential therapeutic utility as appetite suppressants for the treatment of obesity.
QSAR study reveals that increase charge at atom number 2 and 7 may be disadvantageous for antiobesity activity. Increasing value of the electrostatic potential charges at atom numbers 8 and 9 may be detrimental but increasing electrostatic potential charge at the atom number 3 may be favorable for CB1 antagonistic activity. Presence of electron withdrawing group at atom numbers 8 and 9 and electron releasing group at atom numbers 3 may contribute positivity to the CB1 receptor antagonistic activity. It also emphasizes that the decrease in the value Polarizibilities and electronegativity (whole molecular properties) are essential for biological activity i.e., lower the electronegativity and Polarizibilities of whole molecule may be conducive to the CB-1 receptor antagonistic activity. The important atoms and substituents of these derivatives for obesity activity are shown in Fig 8, was supported by following data obtained after regression analysis are shown in Table 6. The above structure activity relationship outcome will have been an appropriate target for further synthesis and biological activity determination with low cost and less time consuming promising approach.

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