DESIGNING AND SYNTHESIS OF SOME MEDICINALLY ACTIVE NOVEL HETEROCYCLIC COMPOUNDS

A THESIS SUBMITED TO

THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA

FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

PHARMACY

BY

MAYANK KUMAR SHARMA

UNDER THE GUIDANCE OF

PROF. M. R. YADAV

Pharmacy Department,
Faculty of Pharmacy, Kalabhavan Campus,
The Maharaja Sayajirao University of Baroda
Vadodara-390001

October 2017

SUMMARY

According to World Health Organization (WHO), overweight and obesity are defined as abnormal or excessive fat accumulation in body that may impair health. Obesity is continuously increasing day by day in the whole world. A report from WHO revealed that more than 1.9 billion adults were overweight, out of which 600 million were obese. A very surprising fact is that more than 41 million children under the age of 5 were overweight or obese. A number of noncommunicable diseases are associated with obesity i.e. diabetes mellitus, hypertension, dyslipidemia, sleep apnea, stroke, osteoarthritis and certain types of cancers. Thus, obesity is the leading epidemic health problem these days for the entire world.

Very few drugs are available in the market for the treatment of obesity with significant side effects. So, there is an urgent need to develop a "magic bullet" which could significantly reduce body weight with lesser side effects. Rimonabant, a CB1 receptor antagonist came into the European market for the treatment of obesity in 2006 but due to its CNS side effects such as anxiety, depression and even suicidal tendency, the drug was withdrawn from the market in 2008. Although, rimonabant was withdrawn from the market but CB1 receptor as a therapeutic target still remains to be fully explored. Thus, researchers shifted their attention for designing of peripherally acting CB1 receptor antagonists so that CNS side effects could be avoided. This can be achieved by making the compounds more polar and less lipophillic so that they do not cross the blood brain barrier (BBB) and thus CNS side effects could be avoided.

Designing of peripherally acting CB1 receptor antagonists is possible by using molecular modeling techniques. Both ligand based and structure based drug designing could prove beneficial for the designing and development of novel peripherally acting CB1 receptor antagonists. The main aim of the present work was to design and synthesize some novel peripherally acting selective CB1 receptor antagonists for the treatment of obesity with the help of molecular modeling. Modeling studies could suggest the essential structural features required for the activity as well as ligand-receptor interactions which might prove very informative or helpful for the designing purposes. So, the broader aims and objectives of the study were as given below:

1. To determine structural requirements for the peripherally acting 1,5-diaryl pyrazole containing CB1 receptor antagonists used for the treatment of obesity by 3D-QSAR (CoMFA/CoMSIA) studies. The best developed 3D-QSAR model

was to be used for the designing of some newer compounds having better activity and properties.

2. Development of pharmacophore models and 3D-QSAR (atom-based) models using different scaffolds for the identification of the essential features responsible for CB1 receptor antagonistic activity.
3. To perform virtual screening for the identification of novel scaffolds by using different filters like pharmacophore model, 3D-QSAR, molecular docking, Lipinski's rule of five, CNS score, receptor-ligand interactions etc.
4. Optimization of the hits obtained through virtual screening by using molecular modeling techniques and synthesis of the designed compounds.
5. Pharmacological evaluation/screening of the synthesized compounds as peripherally acting CB1 receptor antagonists for the potential treatment of obesity.

1. To determine structural requirements for the peripherally acting 1,5-diaryl pyrazole containing CB1 receptor antagonists to be used for the treatment of obesity by 3D-QSAR (CoMFA/CoMSIA) studies

A dataset of 72 compounds containing 1,5-diaryl pyrazole scaffold having peripheral CB1 receptor antagonistic activity was selected for 3D-QSAR studies. The dataset was divided into training set and test set in an appropriate ratio (3:1) offering 54 compounds in training set and 18 compounds in test set. All computational studies were performed using Linux based SYBYL 7.0 molecular modeling software from Tripos, Inc., USA. Conjugate gradient and steepest descent methods were used for energy minimization. Thus, the lowest energy conformers of structures were used for the alignments. Different alignment methods such as atom-based, data-based, centroid-based, centroid/atom-based were used.

The best results of CoMFA analysis were obtained with database alignment (VI). Alignment (VI) afforded r^2_{cv} = 0.552 with six components, r^2_{ncv} = 0.973, F-value = 281.239 and r^2_{pred} = 0.528. Contributions of steric and electrostatic fields for the best developed model were 43.4 and 56.6 % respectively. The same alignment was further carried out for CoMSIA analysis. The best CoMSIA model developed with a combination of steric (S), hydrophobic (H) and hydrogen bond acceptor (A) fields yielded cross-validated r^2 = 0.571 with six component, non-cross validated r^2 = 0.960, F-value = 188.701 and predictive r^2 = 0.679 and was found to be

the best and highly predictive 3D-QSAR model. The contributions of SHA fields were 19.9, 39.9 and 40.3 % respectively. Thus, SHA were found to be the governing parameters for the peripherally acting CB1 receptor antagonistic activity of 1,5-diaryl pyrazole compounds.

Validation of the best developed CoMSIA (SHA) model

The best developed CoMSIA (SHA) model was validated using various statistical parameters as discussed below:

1. Internal validation of the best developed model offered (r^2_{cv} = 0.571 and r^2_{ncv} = 0.960).
2. F-value was 188.701 [F_{05} (6,71) = 3.06 (Tab)] at 99% confidence level.
3. Y-randomization test was performed for 10 random trials giving poor values of non-cross-validated and cross-validated r^2 to indicate that the original model was not a case of chance correlation.
4. The bootstrapped variance value (r^2_{bs} = 0.977) and bootstrapped standard deviation (SD_{bs} = 0.008) suggested a good internal consistency within the dataset.
5. The predicted r^2 (r^2_{pred}) value of the best CoMSIA (SHA) model of 0.679 indicated good external predictive power.
6. The r^2_m value of 0.575 for test set indicated that the developed model was highly predictive and reliable one.
7. Validation of the developed model was also carried out by using an external test set compounds. The predictive activities of these external compounds were observed very near to the experimental values which validated the predictive capability of the best developed SHA model.
8. Tropsha's Validation tests
   i) r^2_{cv} = 0.571.
   ii) r^2_{pred} = 0.679.
   iii) $[(r^2 - r^2_0)/r^2]$ and $[(r^2 - r'^2_0)/r^2]$ were observed to be 0.0534 and 0.0197 respectively.
   iv) The k and k' were observed to be 1.103 and 0.984 respectively.
9. Applicability domain was determined by both similarity measurement and leverage method. All the test set compounds fell within the applicability domain and were considered reliable one.

Graphical interpretation of contour maps

The CoMFA and CoMSIA model contour maps signify the area in space where the aligned compounds would interact favourably or unfavourably with the receptor. Contour maps with the template molecule (**20**) are shown in the Fig. 1 which justify its high level of activity. The contour maps generated through the best CoMSIA model were utilized to modify the template molecule to design more potent CB1 receptor antagonists.

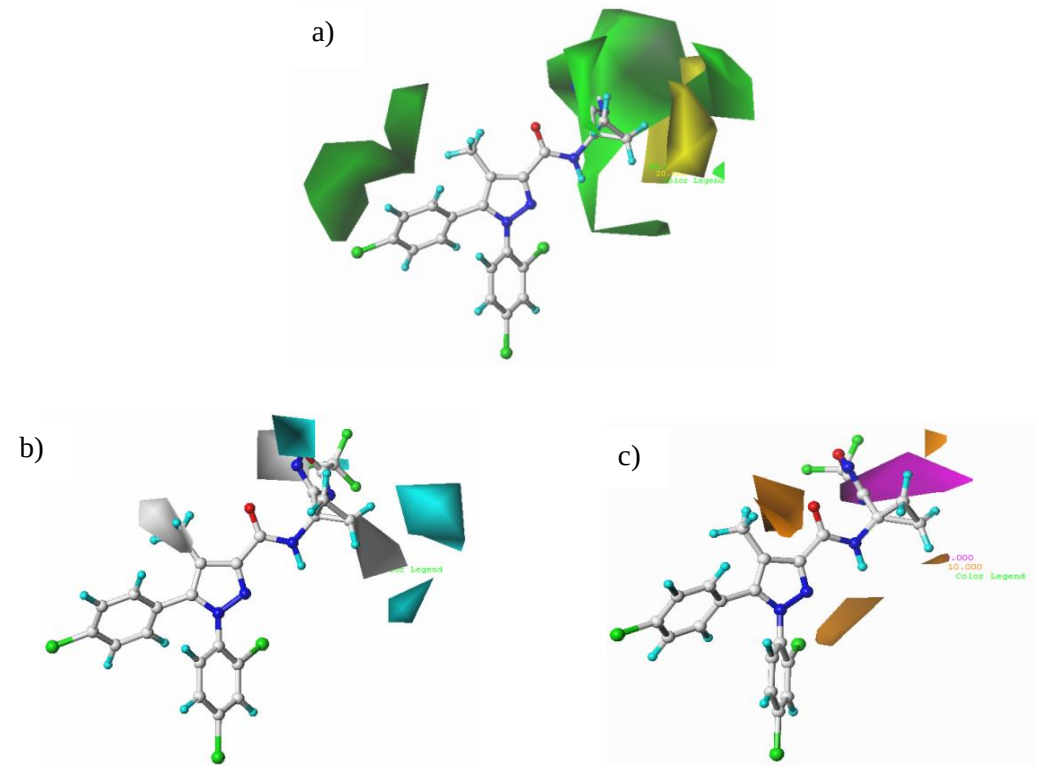


Fig. 1. a) CoMSIA steric contour map, b) CoMSIA hydrophobic contour map and c) CoMSIA hydrogen bond acceptor contour map.

Designing of some potent and novel compounds based on 3D-QSAR model

On the basis of contour maps obtained through 3D-QSAR method, ten (10) novel and potent peripherally acting CB1 receptor antagonists were designed. A lot of modifications in the template molecule were carried out with combinations of different groups at different positions to increase the biological activity with the aim to decrease the lipophilicity and increase the PSA. On the basis of steric, hydrophobic and hydrogen acceptor contours maps, the template molecule was modified. The most promising compound was found to possess higher activity (pIC_{50} = 10.60), with much higher PSA (136.12), lowered logP (4.46) and absence of CNS activity.

2. Development of pharmacophore models and 3D-QSAR (atom-based) models using different scaffolds for the identification of the essential features responsible for CB1 receptor antagonistic activity

A set of 190 compounds having six different scaffolds such as imidazole, purine, pyrazine, piperazine, pyrazole and pyrazolines were selected from the reported literature. Finally a set of 25 compounds were selected for model building. The threshold value of active and inactive compounds was fixed at 7.5 and 6.6 respectively to get 8 active, 9 moderately active and 8 inactive compounds for model-building set.

The chemical structures of all the compounds were built in 'building tools' which were further minimized by 'ligprep' option in Maestro 9.4 molecular modeling software. For creating pharmacophore sites, a default setting having acceptor, donor, hydrophobic, positive and aromatic features were used. Based on 8 active compounds in the model-building set, several 3-point, 4-point and 5-point pharmacophore hypotheses were generated. The survival score generated from all the hypotheses ranged from 2.860 to 5.606. Among all the hypotheses the best hypothesis obtained from AHRR showed the highest survival score of 5.606. The 4-point hypothesis (AHRR.6) containing one hydrogen bond acceptor, one hydrophobic group, and two aromatic rings is shown in Fig. 2.

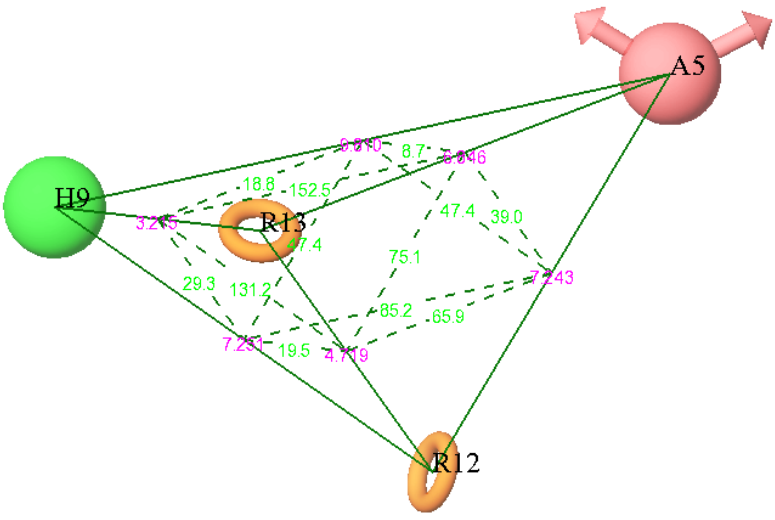


Fig. 2. Distances and angels of the best developed pharmacophore model AHRR.6. Orange coloured torus represents aromatic rings (R12 and R13), green coloured sphere represents hydrophobic group (H9), and light red coloured vector represents hydrogen bond acceptor (A5). Purple colour represents distances and green colour represents the angles between the pharmacophoric features.

Each generated common hypotheses yielded an alignment of all the compounds which were further used for the development of 3D-QSAR (atom-based) model. Atom-based 3D-QSAR models were developed in this study using Partial least square (PLS) analysis. Several 3D-QSAR models were generated and validated by the internal test set compounds as well as external test set compounds. The best 3D-QSAR model was developed with 4-point pharmacophore hypotheses (AHRR.6) having survival score 5.606, r^2_{ncv} = 0.990, r^2_{cv} = 0.938, SD = 0.164, F-value = 389, RMSE = 0.144 and Pearson-R = 0.982. The best model was validated by various validation parameters and was found reliable one. Graphical interpretation of contour maps was also done to identify the area for active and inactive compounds.

3. To perform virtual screening for the identification of novel scaffolds by using different filters like pharmacophore model, 3D-QSAR, molecular docking, Lipinski's rule of five, CNS score and receptor-ligand interactions

The main aim of this study was identification of novel hits and development of novel peripherally acting selective CB1 receptor antagonists. Virtual screening of Asinex database containing 4,35,214 diverse chemical compounds were performed. The different filters used in virtual screening were pharmacophore features, molecular docking, Lipinski's rule of five, minimum predicted potency using 3D-QSAR (atom-based) model, ligand-receptor interactions and CNS scoring as shown in Fig. 3.

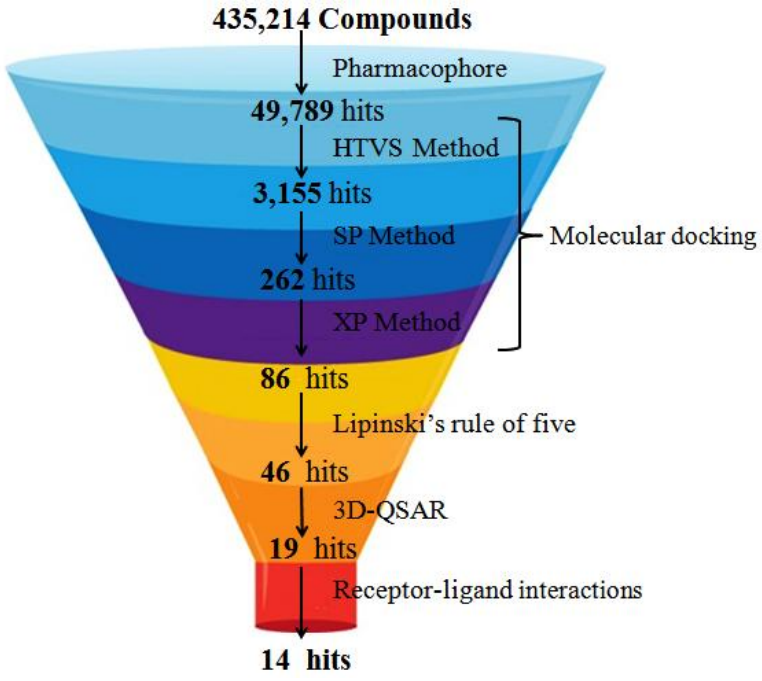
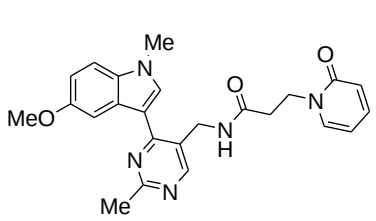
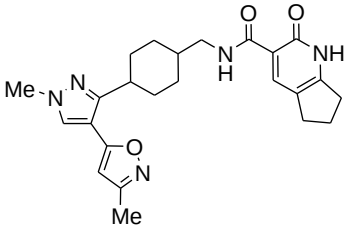


Fig. 3. Flowchart of virtual screening using Asinex database.

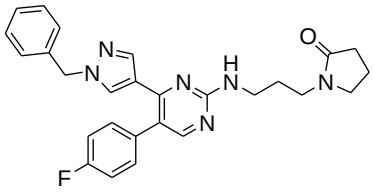
Finally, 14 hits were identified through virtual screening as shown in Fig. 4. Virtual screening studies of the Asinex database offered very interesting hits and we were able to achieve the targeted objective of discovering entirely new scaffolds which were never known to be present in the existing CB1 receptor antagonists prior to this study. The most promising hits were **V1**, **V4**, **V7**, **V8**, **V11**, **V12** and **V14**.



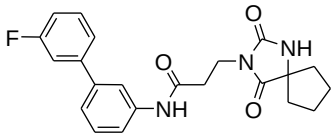
(V1, pK_i = 7.55)



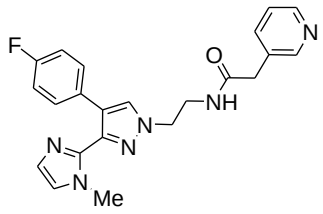
(V2, pK_i = 8.53)



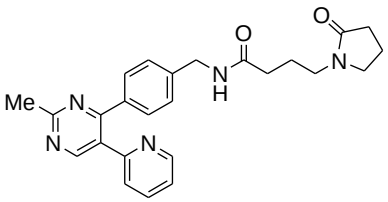
(V3, pK_i = 8.41)



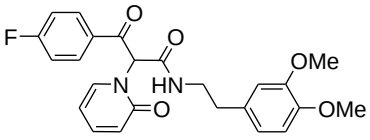
(V4, pK_i = 8.08)



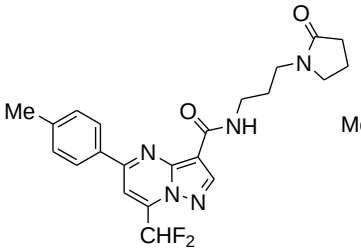
(V5, pK_i = 7.71)



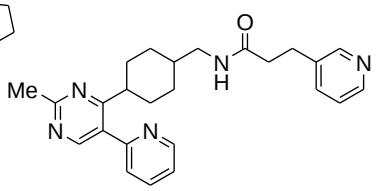
(V6, pK_i = 8.49)



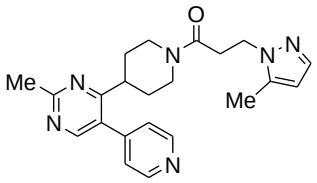
(V7, pK_i = 7.67)



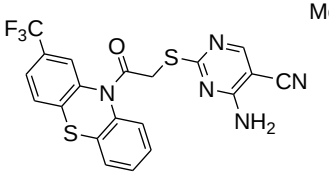
(V8, pK_i = 7.99)



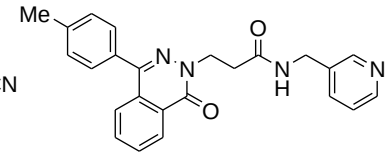
(V9, pK_i = 8.55)



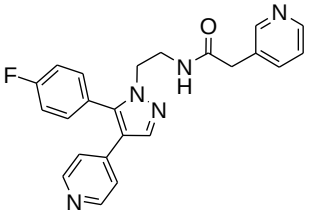
(V10, pK_i = 8.50)



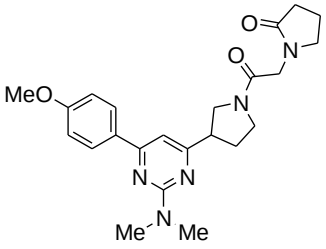
(V11, pK_i = 7.55)



(V12, pK_i = 7.92)



(V13, pK_i = 7.79)

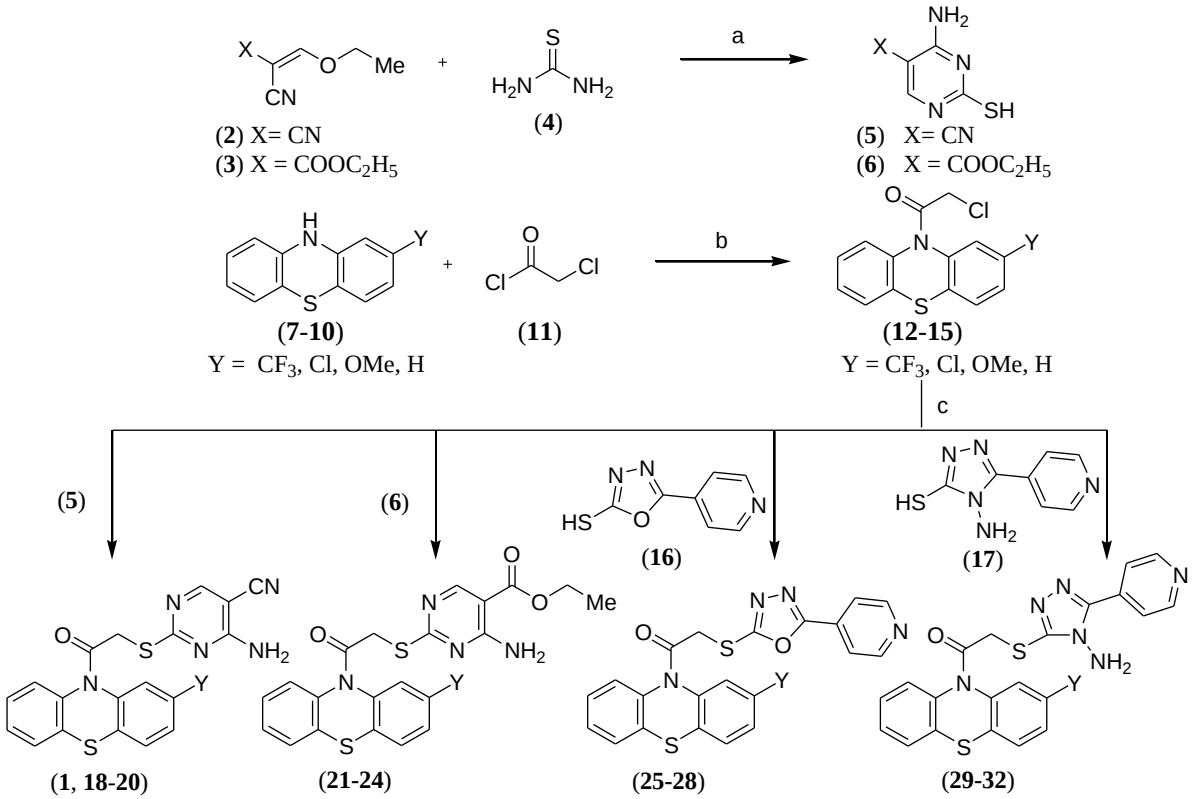


(V14, pK_i = 7.80)

Fig. 4. Chemical structures and predicted activity of 14 hits obtained through virtual screening.

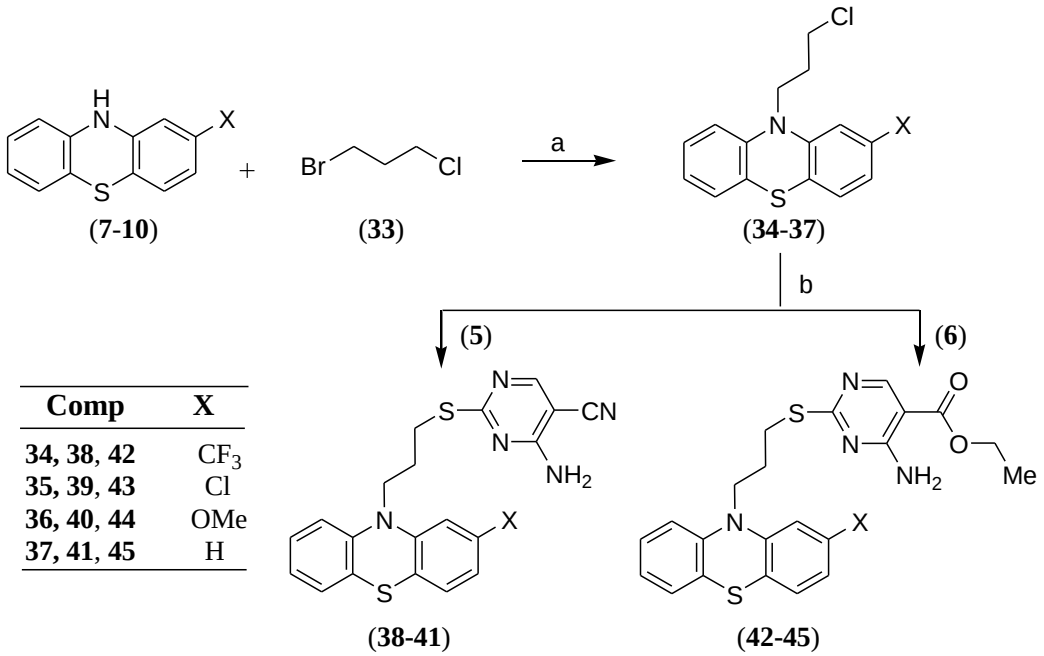
4. Optimization of hits obtained through virtual screening by using molecular modeling techniques and synthesis of the designed compounds

Out of these 14 hits, seven hits (**V1**, **V4**, **V7**, **V8**, **V11**, **V12** and **V14**) were found having totally new scaffolds which were never reported as CB1 receptor antagonists. Out of these 7 hits, all were found to be having quite similar diaryl containing scaffold except for hit **V11**, which contained a phenothiazine scaffold which was never reported in the literature as CB1 receptor antagonists. Thus, hit **V11** was selected for the optimization and synthesis, so that the domain of newer CB1 receptor antagonists could be expanded. The designed compounds were synthesized by the given scheme 1 and 2. All of the synthesized compounds were characterized on the basis of their spectral data. Methods of synthesis for these new compounds, their spectral (IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectrometry) data have been discussed in detail in the thesis.



Comp	Y
7, 12, 1, 21, 25, 29	CF_3
8, 13, 18, 22, 26, 30	Cl
9, 14, 19, 23, 27, 31	OMe
10, 15, 20, 24, 28, 32	H

Scheme 1. Reagents and conditions: a) $NaOC_2H_5$, EtOH, rt; b) triethylamine, DCM, reflux; c) K_2CO_3, DMF, rt.



Scheme 2. Reagents and conditions: a) NaH, DMSO, THF, 0 ^{0}C; b) K_2CO_3, DMF, rt.

5. Pharmacological evaluation/screening of the synthesized compounds as peripherally acting CB1 receptor antagonists for the treatment of obesity

The *in vitro* model PAMPA-BBB assay was performed to assess the ability of the test compounds to cross the BBB (Table 1). It was found that none of the synthesized compound would cross the BBB by passive diffusion indicating that the synthesized compound would act peripherally having minimal CNS side effects.

Table 1 Permeability (P_e) results from the PAMPA-BBB assay with their predicted penetration in CNS. (Data expressed as mean±SEM of three independent experiments. CNS- indicates low passive CNS permeation)

Compound	P_e (10^{-6} cm s^{-1})	Prediction
1	2.78±0.14	CNS-
18	2.28±0.11	CNS-
19	3.08±0.16	CNS-
21	2.72±0.19	CNS-
22	3.66±0.67	CNS-
23	2.80±0.58	CNS-
28	3.22±0.63	CNS-
29	1.05±0.22	CNS-
30	2.09±0.82	CNS-
38	2.60±0.18	CNS-
39	3.47±0.66	CNS-
40	3.58±0.73	CNS-
42	2.84±0.28	CNS-
43	3.28±0.38	CNS-

Further, acute hypophagia experiments were carried out in which compounds (**1, 18, 22, 39**, **40**, **42** and **43**) were found weak short acting inhibitors of food uptake after 2 hr of treatment showing non-significant effects (Fig. 5). On the other side, compounds (**19**, **21**, **23** and **30**) showed significant hypophagic activity compared to the control animals ($p < 0.01$). In another experiment, the test compounds (**19**, **21**, **23** and **30**) were further evaluated in presence of WIN-55212-2, a potent cannabinoid receptor agonist to assess their *in vivo* CB1 receptor antagonistic activity. It was observed that compounds (**19**, **21**, **23** and **30**) showed significant decrease in food intake indicating CB1 receptor antagonist activity of the test compounds.

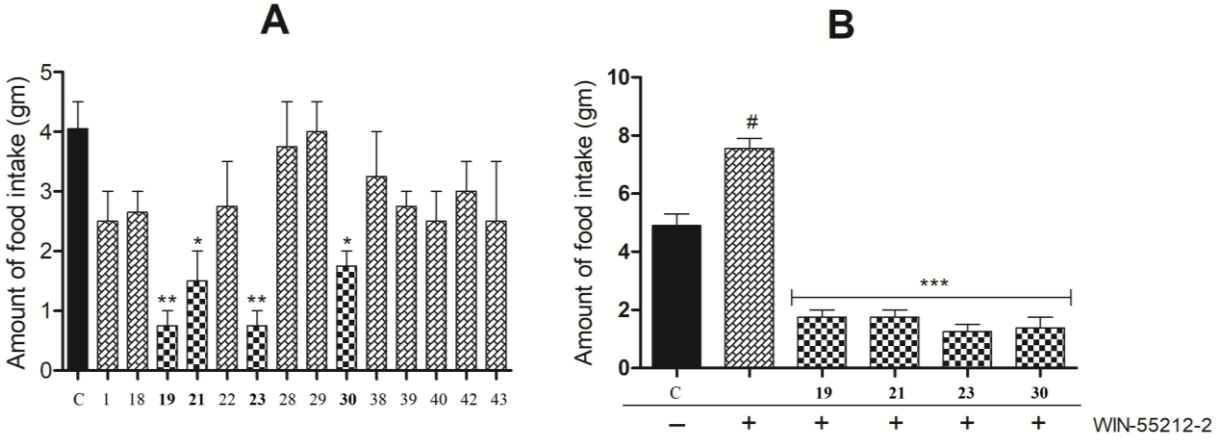


Fig. 5. Hypophagic response of the test compounds alone (A) and in presence of WIN-55212-2 (B). Data expressed as mean ± SEM (n=6). ** $p < 0.01$, * $p < 0.05$ *vs.* vehicle-treated control group (A). $^{\#}p < 0.01$ *vs.* vehicle-treated control group. ***$p < 0.001$ *vs.* WIN-55212-2-treated group (B).

In nutshell, some novel phenothiazine derivatives were designed using various molecular modeling techniques, synthesized and evaluated as peripherally acting CB1 receptor antagonists having the potential to be used as anti-obesity agents.