6. Conclusion

Obesity is a leading health problem for the entire world these days. More than two billion children and adults (nearly one third population of the world) are suffering from obesity problem worldwide and the number of obese population is increasing day by day with a high alarming rate. Recent data revealed that four million deaths were attributed to overweight and obesity in 2015. A very surprising fact is that India is the third most obese country in the world and it is found that 14.4 million children in India are obese which is the second highest number of obese children in the world. A serious concern with the obesity is that it is linked with a number of other diseases such as diabetes mellitus, hypertension, dyslipidemia, sleep apnea, stroke, and some types of cancers which increase the overall mortality. So, overweight and obesity have become a major health epidemic for the entire human race.

It was envisaged that blocking of CB1 receptors by peripherally acting CB1 receptor antagonists would be effective for the treatment of obesity without CNS side effects. So, designing of peripherally acting CB1 receptor antagonists could offer a very big advantage over the existing CB1 receptor antagonists.

1,5-Diaryl pyrazole containing compounds have been evaluated in the literature as peripherally acting CB1 receptor antagonists. So, identification of essential features responsible for peripheral CB1 receptor antagonistic activity were carried out. Efforts were made to develop reliable 3D-QSAR (CoMFA and CoMSIA) models using 1,5-diaryl pyrazole containing peripherally acting CB1 receptor antagonists. Different alignments (I-X) were performed for the development of CoMFA model. Out of these alignments, data-based alignment (VI) proved to be the best for CoMFA studies. The same alignment (VI) was further considered for the CoMSIA studies also. The best statistics was obtained by CoMSIA model having a combination of steric, hydrophobic and hydrogen bond acceptor (SHA) fields. The CoMSIA (SHA) model was found to be a highly predictive 3D-QSAR model. Thus, this study revealed that SHA are the governing field parameters for the peripheral CB1 receptor antagonistic activity for 1,5-diaryl pyrazole derivatives. The generated contour maps of the best CoMFA and CoMSIA models have illustrated the structural requirements for the purpose of designing of more potent and novel peripherally active CB1 receptor antagonists with better properties. This study was used for the designing of ten novel compounds with the help of generated counter maps of the best CoMSIA model. The most promising

designed compound (**M10**) showed higher activity (pIC_{50} = 10.61) than the template molecule (pIC_{50} = 10.00), much higher PSA (136.13 $Å^2$) than the template molecule (PSA = 86.67 $Å^2$), lowered logP (4.47) than the template molecule (logP = 6.61), and good percentage of human oral absorption. Along with this, the designed compound (**M10**) showed absence of CNS activity which was one of the essential requirements, indicating its peripheral activity. Thus, the properties of the designed compound (**M10**) were found favourable and thus it could be considered as a lead molecule for further development of peripherally acting selective CB1 receptor antagonists.

Further, it was necessary to identify the common essential features responsible for the peripherally acting CB1 receptor antagonistic activity having different scaffolds. So, by using compounds having different scaffolds such as imidazoles, purines, pyrazines, piperazines, pyrazoles and pyrazolines, various three-featured, four-featured and five-featured ligand-based pharmacophore models were developed. Out of them, a pharmacophore model with four pharmacophoric features was developed followed by the development of a 3D-QSAR (atom-based) model to identify the essential structural features required for the peripherally acting selective CB1 receptor antagonists. The best developed pharmacophore model (AHRR.6) was having four features i.e. one H-bond acceptor, one hydrophobic center and two aromatic rings. The training and test set compounds were aligned on the best pharmacophore model (AHRR.6), and the obtained atom based alignment was used for the development of a highly predictive 3D-QSAR model. The predictive power of the developed 3D-QSAR model was validated by different validation parameters as well as using an external test set compounds. Biological activity of the external set of test compounds was correctly predicted by the best developed 3D-QSAR model indicating that the developed 3D-QSAR model was a reliable one.

Newer scaffolds were identified by using virtual screening in order to extend the chemical domain of peripherally acting CB1 receptor antagonists. The pharmacophore model and 3D-QSAR (atom-based) model developed by using six different scaffolds were used for screening of Asinex database having 435,214 compounds to search for diverse chemical entities possessing peripherally acting CB1 receptor antagonist activity. Different filters used in virtual screening were pharmacophore features (one H-bond acceptor, one hydrophobic center and two aromatic rings), molecular docking (HTVS, SP and XP method), Lipinski's rule of five, minimum predicted potency using 3D-QSAR model, ligand-receptor interactions and CNS scoring. Only those

compounds were carried forward in the next step which followed the above criteria successfully one by one. Finally the most favourable 14 hits were found out having potential peripherally restricted CB1 receptor antagonistic activity. The obtained hits were well oriented in the active site of CB1 receptor indicating favourable interactions of the ligands with the CB1 receptor. The obtained hits formed H-bonds with Lys192, Asp266, Ile267, Phen278 and Ser383 residues which were part of the active site. The obtained hits showed their absence or restricted presence in CNS indicating their peripheral activity. Thus, virtual screening studies of the Asinex database offered very interesting hits and we were able to achieve the laid objective of discovering entirely new scaffolds which were never known to be present in the existing CB1 receptor antagonists prior to this study, especially structures **V1**, **V4**, **V7**, **V8**, **V11**, **V12** and **V14**.

The obtained hits were utilized to expand the chemical domain of the existing peripherally acting CB1 antagonists. The virtual screening hit **V11** was selected for the synthesis and biological evaluation. Thus, a novel series of phenothiazine derivatives were synthesized having higher PSA and lower hydrophobicity as compared to rimonabant. The PAMPA assay was carried out for the synthesized compounds and they were found out to be not crossing the blood brain barrier, indicating their peripheral activity that could render them devoid of unwanted psychiatric side effects. In the preliminary evaluation, it was found that the hit V11 (**1**) possessed weak and short acting food inhibitory property. Optimization of the hit V11 (**1**) was carried out by substituting different hydrophobic and hydrogen bond acceptor groups in the molecule. The hit V11 (**1**) was modified by attaching hydrophobic groups such as chloro and methoxy groups at 2^{nd} position of the phenothiazine ring which was found beneficial for the activity in computational studies as well as in the *in vivo* studies. Substituted pyrimidine and triazole rings acted as hydrogen bond acceptors that formed hydrogen bonds with Lys192 residue in the active site of CB1 receptor. The designed compounds (**19, 21, 23** and **30**) exhibited a significant decrease in food intake in the *in vivo* studies. Thus, the phenothiazine scaffold exhibited promising potential in producing peripherally acting CB1 receptor antagonists for the treatment of obesity. To the best of our knowledge this is the first study in which phenothiazine scaffold has been found to show peripherally acting CB1 receptor antagonistic activity.