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Computation-based experimentation: Identification of piperazine containing antidepressants

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Abstract: Depression, a common mental disorder, is one of the major contributors to the overall global burden with more than 264 million individuals affected worldwide. Monoamine oxidase inhibitors (MAOIs) have well-known efficacy for treating depression and other related disorders. Herein we report the implementation of extensive *in-silico* calculations to predict the mono-amine inhibitory potential of an in-house library of piperazine-based compounds. In this connection, a multistep virtual screening protocol based on pharmacophore modeling, molecular docking and Quantitative Structure-Activity Relationship (QSAR) was carried out by MOE. Further, to assess its ability to cross the blood brain barrier, ADME properties of the compounds were predicted. Compounds predicted the highest enzyme inhibition by QSAR was synthesized for experimental validation. Both the synthesized compounds (**I**15 and **I**21) presented good strength against Monoamine Oxidase in *in vitro* enzyme inhibitory activity.

Keywords: MAO-A, antidepressants, piperazine derivatives, *in-silico*, molecular docking, pharmacophore screening, 2D-QSAR, MOE

INTRODUCTION

Depression is a multi-factorial and multi-targeted human disorder ranging from mild to severe and life-threatening complications with a variety of medicines available in the market. Almost the entire population in the world is experiencing varying conditions with different social situations even with no cause (Miller *et al*., 2013).Target-based diversity in antidepressants is available; however, a limited number of patients respond to the medicine, indicating the need for more effective discovery to cure the disease or idiopathic disorder (Blackburn, 2019). Out of all the targets, monoamine oxidase (MAO)-A is the most focused antidepressant target with the ability to regulate the concentration of neuroamines to control brain chemistry (Pereira and Hiroaki-Sato, 2018). In depression, the levels of enzymes are very high that increase the neuroamines clearance, thus, depressing the nerves. MAO-A inhibitors block the active geometry of MAO-A metabolizing enzyme and allow the neurotransmitters to stay in the cleft longer to neutralize the depressive effects. Initially, non-selective, irreversible MAO inhibitors were designed associated with a variety of side effects, followed by restricted development of selective and reversible MAO-A inhibitors. Moclobemide is the most common example of recent successful development as a therapeutic of depression (Pathak *et al*., 2015). The irreversible drugs phenelzine, clorgyline and others block the enzyme permanently, switching the normal state to hyperactive neurons, thereby leading to death. Thus, an adequate effect with reversibly bound molecule is required to treat a depressive condition to normal (Bet *et al*., 2013). The main hurdle to be resolved while identifying an effective antidepressant is the brain penetration properties that is, needed for therapeutics causing least toxicity, thus indicating the importance of adequate lipophilicity in the molecule to reach the target site along with its therapeutic affinity (Sacher *et al*., 2011). The drugs before the discovery of selective moclobemide were shown to cause hepatotoxicity along with hypertensive crises and other side effects as major clinic problems (Chang *et al*., 2020).

In different studies, piperazine; a heterocyclic moiety, due to its magical therapeutic sparks in medicinal chemistry was selected to be derivatized for discovering a new selective reversible neuro-active molecule (Al-Ghorbani *et al*., 2015, Singh *et al*., 2021). Therapeutic ability enclosed in a small two nitrogen-associated lead has been studied for decades and still shows unseen therapeutic potential with slight chemical changes (Lemke *et al*., 1995). Small to large substitutions across the two nitrogen resulted in a variety of chemical structures with diverse pharmacological effects. Thus, literature reported their bio-activities in various disease conditions (Asif, 2015, Sharma *et al*., 2020).

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In-silico based studies are important nowadays for optimizing and developing new drug candidates in a manageable time and finance. With the purpose of identifying appropriate molecular chemistry in piperazine for the treatment of depression at the level of neurotransmitters, computational tools were applied. Thus, almost 1200 compounds-based library was constructed for identifying their therapeutic potential as antidepressants, computationally. Recruited compounds were synthesized and experimentally evaluated for the confirmation of their therapeutic potential. Computation supplements bulk purchasing, laborious procedures and experimentations effectively. This amalgamated piece of work has hypothesized that substitutions in fluorobenzyl piperazine impart MAO-A binding affinity in order to significantly inhibit the target enzyme.

MATERIALS AND METHODS

Selection of biological target and construction of compound library

Chemical structures with piperazine nucleus were retrieved from Sigma Aldrich (https://www.sigma aldrich.com/european-export.html) through smile-based screening. The collected moieties with varied chemistry were labeled as A-T, and these molecules were cross-linked with available reagents (1-47) along with some drug-like piperazine containing compounds from sigma to produce a library of almost 1200 compounds, virtually. Reported structures of medicines, available in the clinic, were also included in the library for referencing the molecular behavior. MOE builder helped us to sketch the chemistry and supplement it to the human physiology through protonation, structure correction, charge application and minimization to the least energy state. On the other hand, RCSB protein data bank (Berman *et al*., 2000) screening with reference to the resolution, information collected and release date leads to the selection of 2Z5X as a therapeutic target for depression (presented in table 1). For computational use, the structure was prepared along with the application of modified MMFF94s (Halgren, 1999) for clinical compatibility using the MOE protein preparation tool. The docking grid was generated using hot spot residues along with the co-crystal ligand to limit the computational time and efforts (Geha *et al*., 2002, Khan *et al*., 2018).

Software validation and protocol selection

Software validation reduces the chance of false-positive and error-based results. Here, in this study, Molecular Operating Environment (MOE) was selected as the tool of screening that was first evaluated for its working ability through re-docking and correlation potential. Inhibitory concentrations of known inhibitors were collected from the database and correlated with the docking fit of unknown compounds to check the software discriminating ability. Among all, PT-AdG was found to be the most robust model to distinguish actives from inactives to antidepressant target.

Structure-based screening and affinity analysis

The therapeutic potential and interacting capability of the generated piperazine-based library within the antidepressant target were evaluated through free binding energy, orientation obtained and the interaction they made with hotspot residues. Active site water molecules were reserved while docking to assess the role of water bridging in drug binding. Proxy triangle in combination with Affinity dG was applied as a validated working combination along with GBVI-WSA dG as a rescoring tool in an induced fit environment. The top ranked 100 compounds with least binding energy were collected for in-depth affinity analysis and therapeutic potential.

Pharmacophore-based screening and compound's ranking

Multiple ligand-protein docked poses were aligned to obtain the best chemical features-based sieve for compound screening. The model was validated using internal and external test sets for reliability of pocket compatibility. Therapeutically essential features-based pharmacophore model was used to screen a constructed library of piperazine containing compounds for the retrieval of chemically similar molecules. Three models with moderate reproducibility were selected for stepwise molecular screening for the reliable recruitment of candidate compounds. While sieving through generated models, compounds obtained screening scores that were used to select top 100 fitted compounds for pharmacophore mapping and feature analysis.

2D-QSAR based screening and feature matching

Activity prediction of compounds in the library for a particular target is possible by computational QSAR. Here, 2D- QSAR, a fast and reliable compound screening method, was applied to predict molecular bio-activity based on molecular indices, connectivity and geometry (Geldenhuys *et al*., 2006). Different chemical parameters and structural properties, in MOEQuaSAR contingency, were combined to produce a reliable model using a correlation matrix. The leave one out (LOO) method-based correlation among the descriptors should be the least and with IC_{50} should be high to obtain the best model. However, training and test sets, containing reported compounds with a huge range of bioactivity, were used to check the model reliability. The 196 reported compounds from the binding database were used to model the valid query that was then validated through 32 active compounds. Thus, a set of 25 descriptors was found to be most appropriate with regression analysis and principal component analysis, RMSE, r^2 and predicted r^2 values for best structure-activity correlation. However, the predictive potential of external test sets (0.62 predictive r^2) *via* generated models showed predictive robustness. The top 100 compounds with the least predicted inhibitory

concentrations were collected for the next step of cross screening.

Cross screening and compounds mining

Results from computational analysis were evaluated statistically to collect compounds that had passed all screenings efficiently. Target-based calculations helped to mine the data for potential compounds showing selection eligibility for in-depth visualization. Thus, ligand-protein interactions, molecular geometry, chemical features, pocket complementary and activity predictions were examined that further reduced the number to more valuable true candidates. PreADMET (http://preadmet.bmdrc.org/), an online server for computational predictions of kinetics, defined the lipophilicity of shortlisted compounds as therapeutically enough to be a neuro-active drug (Balachandar *et al*., 2017). Thus, with all parameters, two compounds from one core were elected as more eligible antidepressant contenders.

Synthetic protocols and procedures

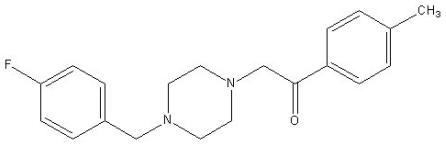
With reference to the intensive *in-silico* screening and analysis, two compounds were selected for synthesis and experimentation. For that purpose, all chemicals, reagents, precursors and kits were purchased from Sigma Aldrich and solvents were distilled before use. HP-UVIS Desaga (Heidelberg, Germany) at 254 and 365 nm wavelengths, melting point apparatus of STUART, USA with open capillary tube, UV spectroscopy from Shimadzu UV-1800 Germany and Perkin-Elmer KBr based FTIR spectrophotometer, mass spectroscopy (FAB-positive) on JEOL 600H-2, USA and proton NMR in deuterated methanol and DMSO on Burker Advance AV-400 and AV-500 MHz, France, were utilized for final product confirmation and structural elucidation.

Alkaline acetone solution of fluorobenzyl piperazine (**I**) with 2-bromo-4'-methylacetophenone (15) and 3,5-dinitrobenzoyl chloride (21) in equimolar concentrations was stirred at room temperature for about 24 hours, followed by refluxing at 50°C till reaction completion. TLC in a mixture of ethanol and hexane (6:4) along with a few drops of ethyl acetate is frequently used to find the path of reaction completion. Vacuum based filtration helped to separate the solid product that was then purified by several washings with cold acetone and recrystallization.

Instrumental verification of synthesized compounds

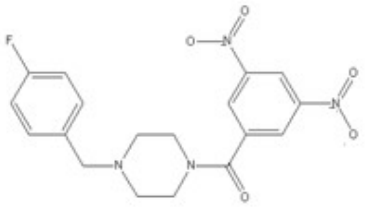
2-(4-(4-fluorobenzyl)piperazin-1-yl)-1-p-tolylethanone (I15)

White powder, yield: 14.92%, Melting Point: d266.1°C, UV (MeOH): 257nm, IR (ν_{max}) cm^{-1}: Amine (-C-N) 1110; Aromatic (-C-H, -C=C) 3030, 1430; Carbonyl (-C=O) 1700; Halide (-C-F) 1210; Alkane (-C-H) 2890, ^{1}H NMR (MeOD, 500 MHz) δ: 2.5 (3H, s, methyl protons), 3.5-4.0 (8H, s, piperazinyl protons), 4.5-5.2 (4H, s, methylene protons), 7.2-7.5 (4H, d, *J*=8 fluorobenzene protons), 7.6-8.2 (4H, d, *J*=8.5 methyl benzene protons), MS: FAB+ m/z: 327a.m.u.



(4-(4-fluorobenzyl)piperazin-1-yl)(3,5-dinitrophenyl) methanone (I21)

White powder, yield: 63.49%, Melting Point: d284-289°C, UV (MeOH): 214.5 nm, IR (ν_{max}) cm^{-1}: Amine (-C-N) 1060; Aromatic (-C-H, -C=C) 3070, 1450; Carbonyl (-C=O) 1670; Halide (-C-F) 1290; Nitro (-N-O, -N=O) 1540, 1340; Alkane (-C-H) 2990, ^{1}H NMR (DMSO, 500 MHz) δ: 3.1-3.7 (8H, m, piperazinyl protons), 4.2-4.6 (2H, s, methylene protons), 7.2-7.6 (4H, t, s, *J*=7.5, fluorobenzene), 8.6-8.9 (3H, d, *J*=8.9, nitrobenzene), MS: FAB+ m/z: 389 a.m.u.



Experimental validation of bioactivity

Correlation of animal-based target selection for therapeutic efficacy in humans is possible due to high structural similarity in species-specific proteins (http://www.bioinformatics.org/sms/ident-sim.html). A simple fluorometric method was used (Badavath *et al*., 2016), with monoamine oxidase enzyme that was isolated from rat's brain through their cervical dislocation using sucrose solution and homogenization. Amplex red MAO kit (Molecular Probes, Eugene, OR, USA) (Yoon *et al*., 2014) was used for antidepressant activity estimations of synthesized compounds. A solution of enzyme, sample and clorgyline was prepared in a phosphate buffer and incubated in a 96-well plate at 37°C for 15minutes. After incubation, the test samples were allowed to react with H_2O_2 produced by horseradish peroxidase and substrate *p*-tyramine to form fluorescent resorufin. Thus, the inhibitory potential of sample compounds was measured in terms of absorbance at 570nm wavelength that would increase if the compound had inhibitory ability. Clorgyline as a standard, in parallel, is important for comparative study; however, the absorbance of compounds was excluded from the results for real estimations.

STATISTICAL ANALYSIS

In-vitro inhibitory percentages were calculated using MS Excel 2007.

RESULTS

Computational studies and enzyme inhibition results are presented in table 2 and 3. Molecules (**I**15 and **I**21) were selected after comprehensive *in-silico* screening predicting their good antidepressant potential. They revealed better energy scores than standards and interacted with all the key amino acid residues along with cofactor FAD in the active pocket of MAO-A enzyme. Selected molecules were synthesized by targeting the free amine of fluorobenzyl piperazine. Synthesized molecules showed enzyme inhibition potential in *in-vitro* assay where **I**15 exhibited profound potential (83.33%) to block the enzyme as compared to **I**21 (59.9%). **I**15 demonstrated better inhibitory strength than standard phenelzine (66.18%). The results of *in-vitro* enzyme inhibition assay presenting the good correlation of experimental values with computational screening and predicting IC_{50} values of **I**15 (7.7nM) and **I**21 (71.9nM).

DISCUSSION

The compounds were filtered after extensive computational search that specified their therapeutic ability in terms of binding affinity, structural compatibility and inhibitory predictions. For that, a virtual library was constructed without restriction of leads with available reagents which resulted in almost 1200 compounds with chemical diversity. Moclobemide, clorgyline and phenelzine were also included in the chemical library with their reversible and irreversible potentials to correlate the biological activity of selected candidates. From the variety of antidepressant targets, MAO-A enzyme was selected as it is the most frequently studied direct regulator of neuroamines, a functional unit of neural activity. 2Z5X (Son *et al*., 2008), on the basis of its completely resolved structure providing valuable target information was retrieved from the protein databank. Tyr69, Gly110, Ala111,Ile180, Asn181, Ile207, Phe208,

Table 1: The basis on which protein is selected is important to specify the structural value in computational studies. Here, 2Z5X was selected because of its geometrical values and reporting in recent literature.

Disease	Target	Code	Resolution	Sequence Length	Chain	Release Year	Recent Publication Year
Antidepressant	MAO-A	2Z5X	2.2 A	513	A	2008	2021 (Aggarwal *et al.,* 2021)

Table 2: Percentage inhibitions in *in-vitro* MAO-A inhibition assay and computational screenings of reported drugs and newly identified compounds through different tools resulted in authentic candidates selection.

S No	Compound Codes	%age inhibition (*in vitro* MAO-A inhibition assay)	Pharmacophore RMSDs	2D-QSAR predictions	preIC_{50} Value	Docking Scores	BBB crossing ability	Drug likeness	Toxicity (hERG)
Reference Compounds									
1	Clorgyline	-	0.5287	11.3098	0.0049 nM	-7.2674	6.73708	Yes	Medium risk
2	Moclobemide	-	0.6152	6.4425	360nM	-7.6958	0.228224	Yes	Medium risk
3	Phenelzine	66.18	0.5628	7.1739	67nM	-5.7615	0.983	Yes	Medium risk
New Hits									
1	**I**21	59.9	0.5463	7.1431	71.9nM	-8.4547	0.158182	Yes	Medium risk
2	**I**15	83.33	0.466	8.1185	7.7nM	-8.3626	0.31956	Yes	Medium risk

Table 3: Interacting hotspot residues of MAO-A with different reference compounds and with new drugs showing their comparable therapeutic potential.

S No.	Compounds	Chemical Names	Residues involved in Hydrogen bonding	Water Bridges	Residues involved in Van Der Wall interactions
Reference Compounds					
1.	Clorgyline	*N*-[3-(2,4-dichlorophenoxy)propyl]-*N*-methylprop-2-yn-1-amine	FAD	Asn181	-
2.	Moclebamide	4-chloro-N-(2-morpholin-4-ylethyl)benzamide	-	Asn181	-
3.	Phenelzine	2-phenylethylhydrazine	-	Tyr69	Phe208
Lead Compounds					
1.	**I**21	(4-(4-fluorobenzyl)piperazin-1-yl)(3,5-dinitrophenyl)methanone	Tyr69, Gly110, Tyr407	-	Phe208, Phe352, Ile335
2.	**I**15	2-(4-(4-fluorobenzyl)piperazin-1-yl)-1-p-tolylethanone	Gly110	Asn181	Phe208, Tyr407, Ile335

Phe209, Val210, Gln215, Met324, Ile325, Ile335, Leu337, Phe352, and Tyr407 collectively form a flexible pocket assuring the binding of substrate or an inhibitor (R Ramsay, 2013, Dhiman *et al*., 2018). From the pocket site, Tyr407 and Tyr444 are the crucial catchers in either case. However, additional binding of Ile335 confirms the inhibitory potential of tested molecules over the substrate (Geha *et al*., 2002). Moreover, a number of water molecules among these residues and FAD also facilitate water bridging and specific binding, thus, included as a part of the system.

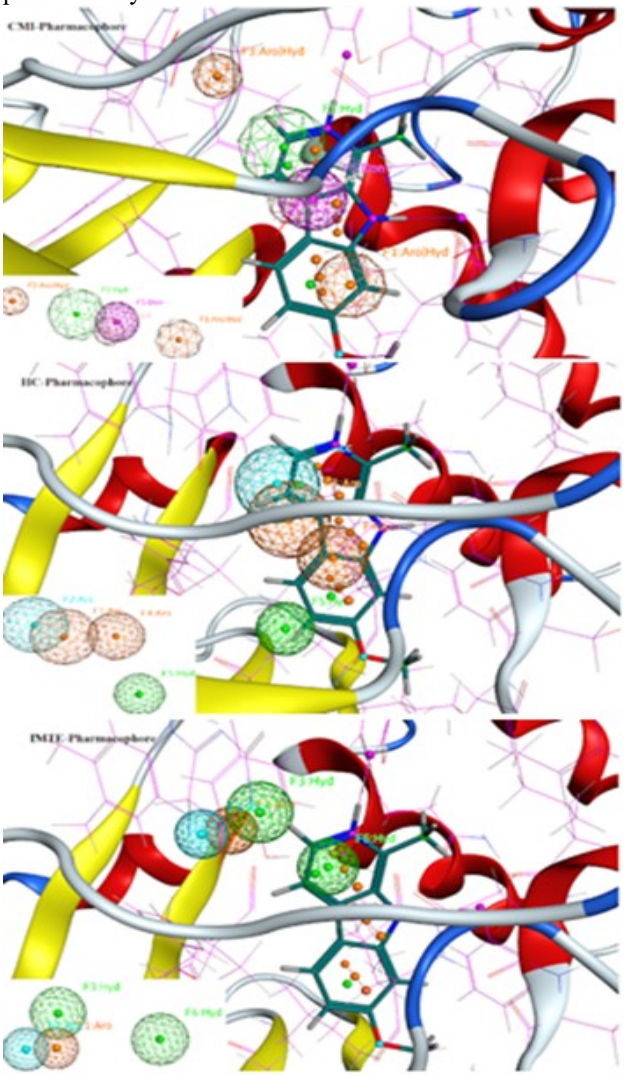


Fig. 1: In order to maintain the screening integrity, three validated sets of relevant features with variable relative distances were collected. Stepwise compound's screenings through them indicate the selection compatibility with all essential features.

Physiologically equilibrated biological systems were generated using MOE incorporated modules; however, cross correlation matrix selected protocols were preferred. Triangle Matcher with ASE, Alpha PMI with ASE and Proxy Triangle with Affinity dG were found to be optimal statics for particular target. Depending upon their ability to discriminate antidepressants from non-antidepressants, these statistical combinations appropriately reduced the chances of false selection. 32 reported compounds with their known MAO-A inhibitory bioactivity in terms of IC_{50} preferred Triangle Matcher with ASE, Alpha PMI with ASE and Proxy Triangle with Affinity dG showing 0.808, 0.838 and 0.904 correlation. To maintain the selection integrity, three screenings were followed by Proxy Triangle with Affinity dG based in-depth analysis assuring the candidate's ability to be a valuable therapeutic agent.

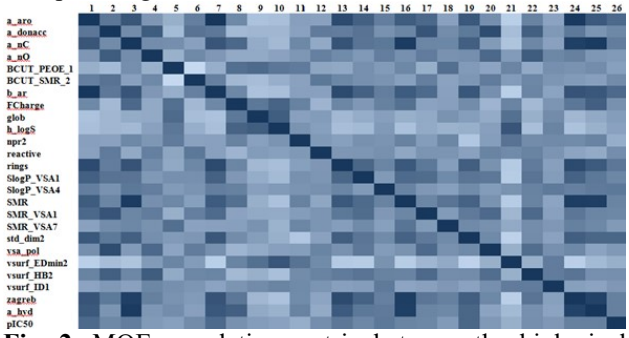


Fig. 2: MOE correlation matrix between the biological activity of known MAO-A inhibitors and the appropriate set of physicochemical descriptors presented their high correlation with IC_{50} values with no or low association among them. Lighter to darker shades presented the least to high correlation of selected parameters, indicating the value of selected model.

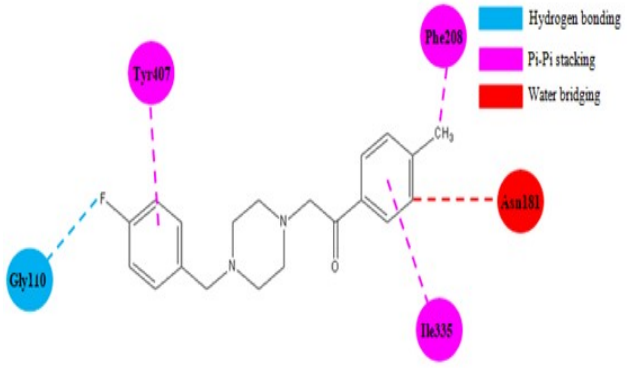


Fig. 3: Interaction pattern of **I**15 within binding pocket of MAO-A assured feature complementarity with essential bindings indicating its therapeutic worth.

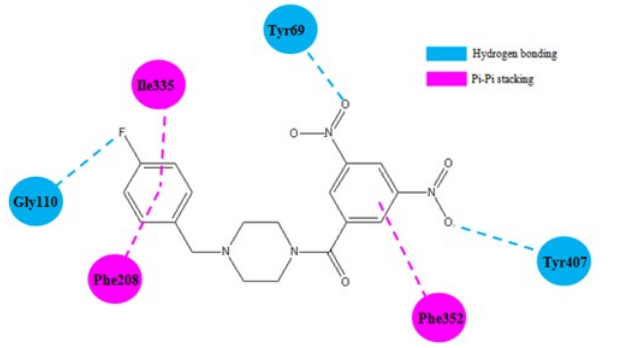


Fig. 4: Di-nitro substituted compound (**I**21) with more polar features favor polar interactions, however, the

chemical combination across the piperazine balances its geometry within the active site to interact and inhibit the enzyme for antidepressant activity.

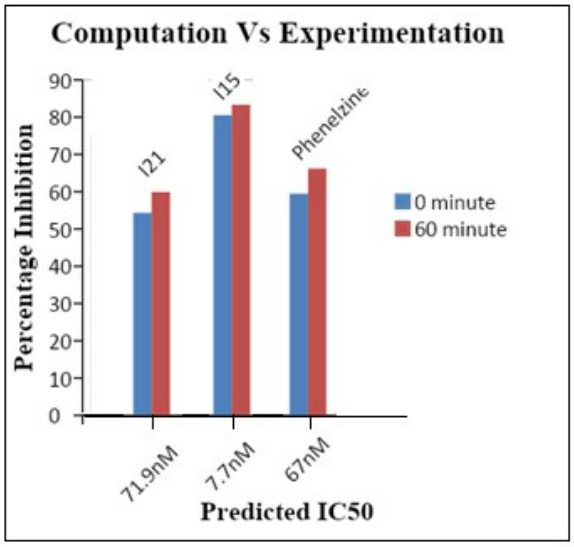


Fig. 5: The best correlation of computational predictions with experimental results was observed in a bar graph indicating the rational selection of drug candidates.

Structure-based screening of a virtual database within selected MAO-A enzyme using preferred protocol resulted in short listing of compounds with highest affinity and lowest ranks. The compounds were also visualized inside the pocket for their orientation and the geometry they obtained during docking. Moreover, interactive capacities of moclobemide, clorgyline and phenelzine were used as reference for the examination of new binding patterns. However, at this stage, in-depth analysis was kept on hold and the study was preceded forward for the next protocol. MAO-A, a specific antidepressant target is a hydrophobic pocket with a huge size. The chemistry has a functional correlation with the therapeutics through the nature of chemical features that help in drug discovery. This scaffold hopping provides another means of virtual screening *via* pharmacophore defined by the features-based ensembles. Crystallographic co-structures were collected; however, generated models with broad size showed limited specificity. Over the 100 generated pharmacophore, the collected features indicated pocket complimentary requirement as an average of hydrophobic with a partial of hydrophilic prerequisites to accommodate the molecule appropriately. Statistically validated models comprise three aromatic/hydrophobic and a polar feature, presented in fig. 1. Three pharmacophores were collected and applied to the virtual library. However, all of the three resulted in appropriate selection with compounds that showed valuable pocket complimentary as they fully mapped onto the generated pharmacophores. However, only top ranked compounds with maximum fitting and least feature RMSD were collected for further study.

The bioactivity prediction is another important tool of screening. However, for the construction of a valid 2D QSAR model, an authentic correlation between the molecular descriptors and the activity predictive ability should be developed. A set of known compounds (196) was used to train the model and verified by internal and external test sets. The multiple linear regression with the leave one out method was used to generate the model showing appropriate q^2, r^2, predicted r^2 and RMSE values. Out of the 378 molecular descriptors, a combination of 25 was selected using a correlation matrix showing high association with bioactivity and lowest connection among the multiple descriptors. The matrix map is depicted in fig. 2, presenting the color correlation graph of their relation among parameters. a_aro, a_donacc, a_nC, a_nO, BCUT_PEOE_1, BCUT_SMR_2, b_ar, FCharge, glob, h_logS, npr2, reactive, rings, SlogP_VSA1, SlogP_VSA4, SMR, SMR_VSA1, SMR_VSA7, std_dim2, vsa_pol, vsurf_EDmin2, vsurf_HB2, vsurf_ID1, Zagreb & a_hyd resulted in an appropriate model of q^2= 0.5165, r^2= 0.7187, RMSE= 0.815 and predicted r^2= 0.626 ensuring the good predictive ability for virtual screening. The bundle of features was incorporated in the model assuring the hydrophobic and surface properties as a dominant parameter for antidepressant evaluation. Thus, compounds with least predictive bioactivity were collected as more prominent candidates.

The top sieved compounds that passed through docking, pharmacophoric maps and also showed the least bio-inhibitory values were collected for cross screening. The compounds that were persistently present in the top of all three screening were shortlisted as more potential antidepressant candidates. However, in-depth mapping of selected compounds from one core (**I**) within an active site, onto generated feature maps and fitted onto the QSAR model reduces the number to two. Thus, the selection proceeded further for synthesis and experimentation.

Moreover, the next elimination phase is totally based on calculations that favor the lipophilicity of selected compounds as they have to cross the physiological barrier; blood brain barrier. The crossing potential along with the best chemistry complementary to the pocket side reduces the number to the selection of two most favorable candidates for synthesis and therapeutic evaluation. Clorgyline, moclobemide and phenelzine are the activity range of antidepressants, thus using their potential as reference; detailed examination of new drug candidates was conducted. Tables 2 and 3 presented the correlated features of new compounds with reference molecules with interaction potential and the capturing residues. An irreversible clorgyline holds covalently the FAD to block the pocket side for monoamines; this is the only experimental reference interaction in it. However, reversible moclobemide and non-selective phenelzine

prefer water bridging and hydrophobic interactions. Moreover, they appropriately mapped onto the features generated in valid pharmacophoric and QSAR queries ensuring the more hydrophobic prerequisites in new candidates. However, predicted bioactivity ensured authenticity of computational models.

Thus, taking the information from reference compounds, new identified molecules were evaluated from all aspects; the interaction patterns, energies, essential features and biological activities. The fluorobenzyl piperazine substituted with methylacetophenone and dinitrobenzoyl passed all parameters to be an effective antidepressant with very low and effective binding energies (-8.3626Kcal/mol and -8.4547 Kcal/mol, respectively). The least inhibitory concentration among all tested molecules, after clorgyline, marked **I**15 as a therapeutic nominee with predicted IC_{50} of 7.7nM showing valuable hydrophobic interactions with Phe208, Ile335 and Tyr407. The interaction with Ile335 ensured its inhibitory potential as Geha reported the importance of Ile335 involvement in ligand binding in his study (Geha *et al*., 2002). Moreover, a hydrogen bond with Gly110 and water bridging with Asn181 also facilitate the binding of **I**15 within the binding side of MAO-A enzyme (fig. 3). On the other hand, the next selected compound **I**21 is more focused on hydrogen bonding and pi-pi interactions for binding, as depicted in fig. 4. Tyr69 (ONO-HN) at a distance of 2.40Å, Gly110 (F-OC) at a distance of 2.47Å and Tyr407 (CO-HO) at a distance of 2.50Å generated hydrogen bonds to strengthen ligand binding, however, the predicted IC_{50}, in comparison to **I**15 was much greater (72nM) unexpectedly. Moreover, along with other hydrophobic interactions, **I**21 also follows the restriction of Ile335 to be an effective antidepressant. Thus, based on all results, **I**15 and **I**21 were selected for synthesis using defined protocols.

Computation helped rationally to select more valuable compounds of a series with inhibitory potential and subjected to synthesis. Substitutions were placed using the simplest synthetic protocols to the free N of piperazine directly. Finally, synthesized 2-(4-(4-fluorobenzyl) piperazin-1-yl)-1-p-tolylethanone and (4-(4-fluorobenzyl) piperazin-1-yl) (3,5-dinitrophenyl) methanone were subjected to experimental validation using ELISA. In the strength of 50mM, an hour-long observation revealed better therapeutic potential of **I**15 than phenelzine. **I**15 showed 83.3% inhibition at the used dose; however, **I**21 is equivalent to the reference in bioactivity indicating the comparative therapeutic potential as computational tools predicted displayed in fig. 5. Thus, the molecular potential to block the monoamine oxidase enzyme and to elevate the depressive mood is confirmed. **I**15 having methyl acetophenone in combination with highly electronegative fluorobenzene on two sides of piperazine satisfied the enzyme pocket appropriately. On the other hand, di-nitro benzoyl moiety of **I**21 along with chemically unique fluorobenzene attached across the piperazine also facilitated pocket requirement effectively. The bulk and hydrophobicity at both terminals with a slight polarity in the middle compliment appropriately for effective binding and marked the selected molecules to be valuable antidepressants.

CONCLUSION

The piperazine with diverse therapeutic potential was explored here as a sparkling antidepressant moiety. Compounds with their ability to capture Ile335 in addition to the binding with other hotspot residues are exclusive among the series for antidepressant potential. The computational strategy sequentially shortlisted two compounds from a series, proving effective MAO-A inhibitor from all aspects, thus, experimental correlation seconds the *in-silico* results. Fluorobenzyl piperazine substituted with more lipophilic substitution at terminal aromatic ring (**I**15) revealed its potential as a profound MAO-A inhibitor in both computational and experimental screening.

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