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Identification of Benzoxazolinone Derivatives Based Inhibitors for Depression and Pain Related Disorders Using Human Serotonin and Norepinephrine Transporter as Dual Therapeutic Target: A Computational Approach

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Abstract

Pain is commonly associated with depression. Both pain and depression share common biological pathways and neurotransmitters, which has implications for the treatment of both disorders. A drug that could ameliorate both pain and depression could be beneficial in the development of new therapeutics in the management of disorders associated with pain/depression dyad. Alterations in the neurotransmitters namely, serotonin and norepinephrine in the central nervous system (CNS) have been implicated in the pathophysiology of pain and depression. Serotonin and norepinephrine reuptake inhibitors (SNRIs) have been implicated as a novel therapeutic target for a wide range of biological functions, including pain, anxiety and depression. 2-benzoxazolinone (2-BOA) from the mangrove *Acanthus ilicifolius* and its derivatives have been reported for its analgesic and antidepressant activities. In the present work, docking studies were done on the crystal structure of human transporters of serotonin (hSERT) and on homology modeled human transporters of norepinephrine (hNET) as therapeutic targets of depression and pain related disorders using 2-BOA and its derivatives as potential candidates. A homology model for hNET was constructed using MODELLER and validated. Further docking studies were done on hSERT and hNET using 2-BOA and its structural analogs. The result of the study proposes the possible potential candidate among 2-BOA derivatives that may be further developed as a therapeutic lead compound for use in disorders associated with depression and pain.

Keywords: 2-Benzoxazolinone, Serotonin transporter, Norepinephrine transporter, Acanthus ilicifolius, Genetic Optimization for Ligand Docking, hNET/hSERT.

Introduction

Norepinephrine and Serotonin are strongly associated with depression [1-6] and also modulate pain sensitivity via the descending pain pathway [7-10]. Serotonergic and noradrenergic neurons are localized in the Pons and medulla (raphe nuclei), and their axons project to brain regions such as the limbic system, the cerebral cortex and hypothalamus [11]. Norepinephrine transporter (NET) and Serotonin transporter (SERT) are integral membrane proteins belong to the large neurotransmitter: sodium symporter (NSS) family of transporters and they regulate monoamine concentrations at neuronal synapses by carrying monoamines across neuronal membranes into presynaptic nerve cells, using an inwardly directed sodium gradient as an energy source [4, 11-12]. Selective serotonin and norepinephrine reuptake transporter inhibitors (SNRIs) are the pharmacological targets in clinical conditions associated with pain/depression dyad [12]. A number of antidepressant medications have demonstrated efficacy in treating chronic pain disorders [13-14]. Recent studies reported that compounds with dual activity at both NET and SERT are effective analgesics [15]. SNRIs such as duloxetine [16] and milnacipran [3, 17] are approved for the treatment of chronic pain syndromes such as painful diabetic peripheral neuropathy, chronic musculoskeletal pain and/or fibromyalgia [17-20]. According to various medical literatures, several adverse reactions are known to be associated with these conventional SNRIs, thereby limiting the widespread application of these agents.

2-Benzoxazolinone (2-BOA) is a bioactive compound isolated from a mangrove plant Acanthus ilicifolius [21]. 2-BOA and its structural analogs have been investigated widely for their analgesic, anticonvulsant, hypnotic, skeletal muscle relaxant and CNS depressant activities [22-24]. The present study investigated the interactions of 2-BOA and its structural analogs with active site residues of human serotonin transporter (hSERT) and norepinephrine transporter (hNET) proteins. Molecular docking is basically a conformational sampling procedure in which hundreds

of molecules can be screened to identify plausible binder by docking them into the predicted binding pocket on the target protein. This paper reports the docking studies and the binding properties of BOA and its analogs towards hSERT and hNET membrane receptor proteins.

Materials and Methods

Homology modeling and structure validation

The protein crystal structure of hNET was unavailable and therefore homology modeling is the alternative choice to construct a reasonable three dimensional (3D) model of the target. The protein sequence of hNET was obtained from the Uniprot database [25] [Accession no: P23975] using the Gapped-BLAST [26]. Through PDB BLAST the crystal structure of dopamine transporter (PDB code: 4M48) with 2.95Å resolution with 60% sequence identity with hNET was identified as a template. Thus, Dopamine transporter chosen as a suitable template to construct a 3D model of the target protein hNET using MODELLER 9.11 [27].

Based on the template structure and target sequence alignment, 10 structural models were constructed using MODELLER. The initial models were assessed using Z-DOPE, a normalized atomic distance-dependent statistical potential based on known protein structures [28]. The constructed 3D model of hNET was refined and the model quality was validated using PROCHECK server [29]. PROCHECK analysis, which includes checks on chirality, dihedral angles, planarity, disulphide bonds, covalent geometry, non-bonded interactions, stereo chemical parameters, main-chain hydrogen bonds, parameter comparisons, and residue-by-residue analysis. The backbone conformation of Phi and Psi angles for polypeptide was predicted using Ramachandran plot.

Molecular docking protocol

Ligand Preparation

The 3D structure of 2-BOA, 6-Methyl-2-BOA, 6-hydroxy- 2-BOA, 6-chloro-2-BOA, and 6-Bromo-2-BOA were obtained from NCBI PubChem compound [30]. Further the 3D structural conformations were optimized using Marvin Sketch (MarvinSketch V 5.2.6, ChemAxon Ltd, copyright 1998-2009.) Energy minimization of ligands was done using chimera software [31].

Docking studies with hSERT and hNET

The hSERT and hNET proteins were selected as depression and pain modulation drug target in the present study. The hSERT protein structure was obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank (PDB code: 4IB4) with 2.7Å resolution [28, 32]. The crystallographic water molecules and co-crystallized ligands were identified and removed from the 3-dimensional (3D) atomic coordinate file and hydrogen atoms were added, and partial charges were assigned.

Similarly, the optimized homology model of the human norepinephrine transporter (hNET) was considered for docking studies. Molecular docking studies were done using GOLD (Genetic Optimization for Ligand Docking) program version 5.1. GOLD is an automated docking program that employs a genetic algorithm to search the ligand conformational flexibility and partial protein's active site flexibility [33]. Four different scoring functions, namely ChemPLP, GoldScore, ChemScore, and ASP (the Astex Statistical Potential) were employed, but among them GoldScore was identified as the suitable scoring function for both hSERT and hNET. In the case of hSERT, the ergotamine binding site was defined as the active site with 12 Å radius for docking, while in the case of docking the hNET homology model, the nortriptyline binding site as in the template structure (PDB code: 4M48) was considered as the active site within 12 Å for docking all the five BOA derivatives with 100% genetic algorithm (GA) using the Goldscore fitness function.

The ranking of the compounds was based on the firstly ranked solution as well as the lowest energy conformation of the most populated cluster of the docking procedure. During the analysis, it was prioritized that the binding mode of BOA derivatives was compared with the binding mode of Ergotamine as reference for hSERT inhibitor. While in the case of hNET the binding of the inhibitor were based on the binding mode of nortriptyline as reference. As a validation step to determine the plausibility binding mode of the five BOA derivatives, docking study was also performed in AutoDock [34-35]. The automated molecular docking simulations were performed by Genetic Algorithm- Local Search (GA-LS) [33] with standard parameters. The compounds were ranked based on the docking energy. The compound with the highest affinity for the target proteins active site pattern with lowest docking energy was selected. Here, we examined the performance of this docking software to select the best compound without any bias. The interaction energy includes Vander Waals energy, electrostatic energy, as well as intermolecular hydrogen bonding was also considered for each binding mode and visualized and analyzed using PyMol software [36]. Finally, the five BOA derivatives top ranked binding modes in agreement with GOLD upon superimposition were considered as the reliable binding mode.

Results and Discussion

Homology based model of hNET was accomplished by MODELLER 9.11 [34] using the dopamine transporter homolog (PDB code: 4M48), with a resolution of 2.95 Å as a template. The sequence identity between the hNET and the template dopamine transporter was about 60%. The 3D structural model of hNET generated by homology modeling has been examined by their stereo-chemical quality using PROCHECK shows that in the Ramachandran plot phi/psi angles of 95.2% residues were in the most favored regions, 4.2% residues were in the additional allowed regions and 0.6% fell in the generously allowed regions; only 0.0% of residues were in the disallowed conformations (Figure 1a). This analysis confirmed that the quality of the modeled hNET (Figure 1b) was almost in good state and reliable.

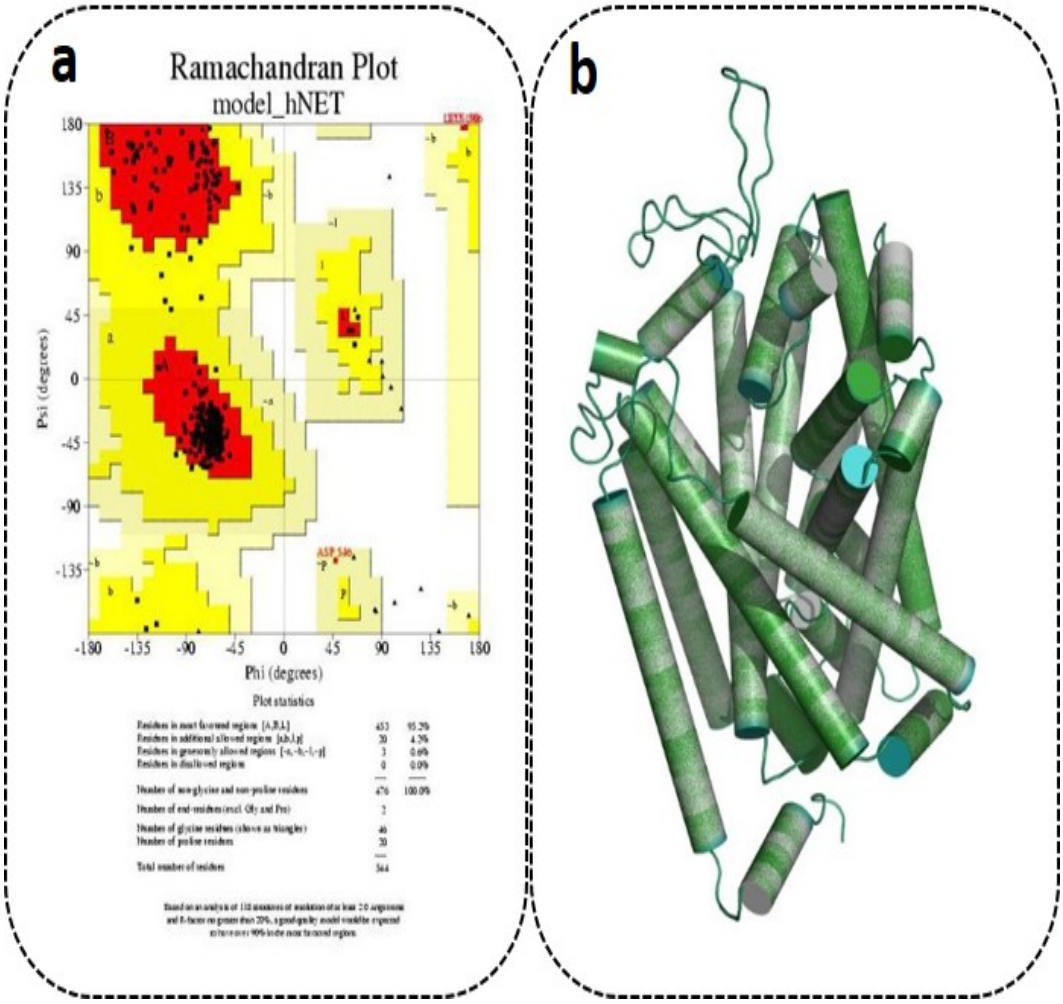

Figure 1 - Ramachandran plot and homology model of hNET

(a) Ramachandran plot of hNET model depicts the red, yellow, pale yellow and white color shaded regions correspond to residues in most favored regions, residues in additional allowed regions, residues in generously allowed regions and residues in disallowed regions. (b) Shows the homology modeled structure of hNET in green and gray color cartoon.

Binding mode of BOA derivatives in hSERT

The BOA derivatives 1-5 were docked on the GOLD to reveal their inhibitory potential on hSERT. Further the docked binding mode of GOLD was validated through AutoDock (Figure 2a). The binding mode of BOA shows that the oxygen in the ring forms hydrogen bond with the NH of the Asn153 side chain, while the benzene ring forms hydrophobic interaction with Val148, Met424 and Ala145 (Figure 2b). Likewise, in the binding mode of 6-Methyl-2-BOA the oxygen attached to the ring forms hydrogen bond with NH of Asn153.

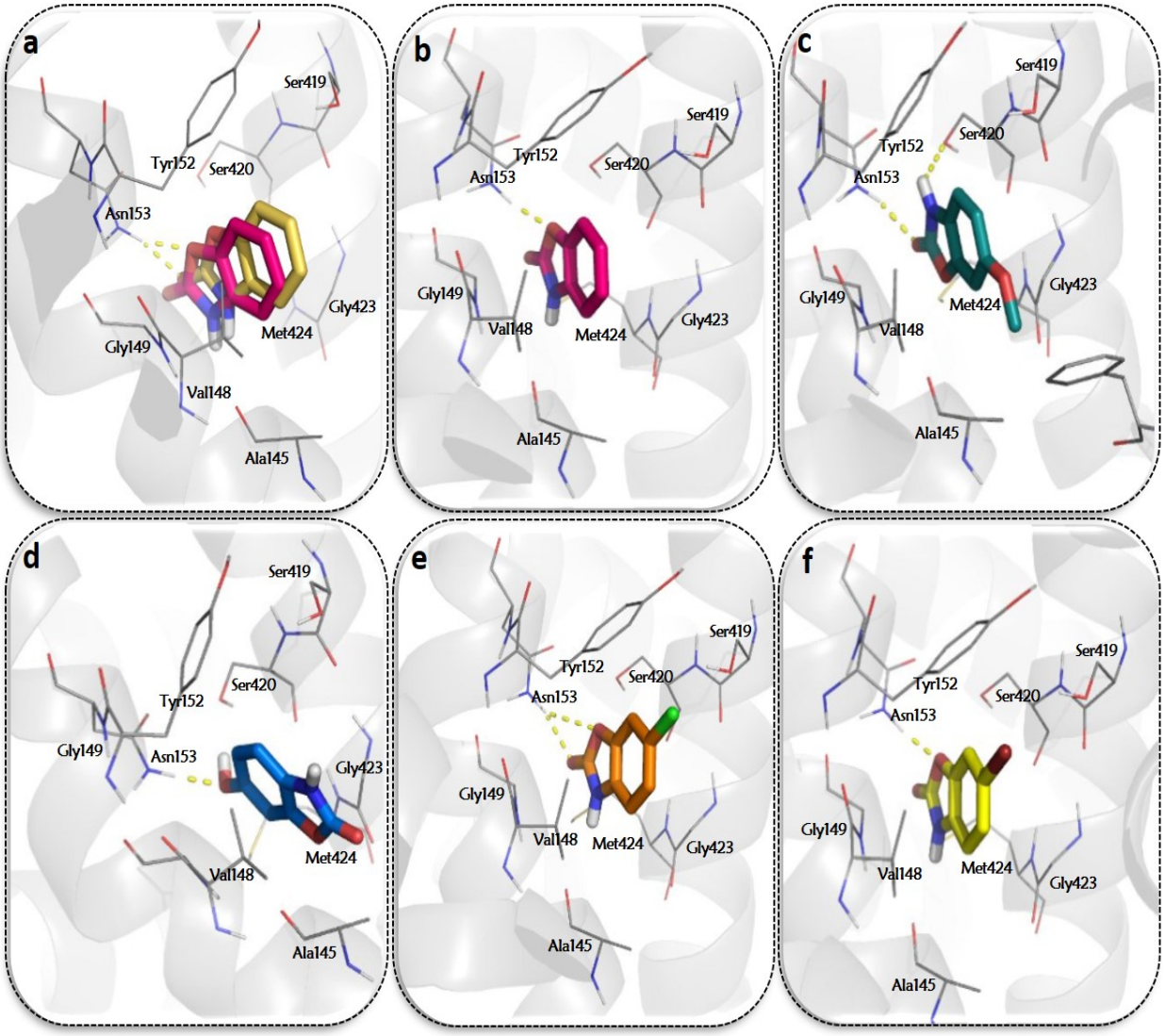

Figure 2 - The 2-BOA derivatives binding mode in hSERT

Binding mode a. Comparison of GOLD and AutoDock in yellow and magenta color respectively. b. 2-BOA (magenta color) c. 6-Methoxy-2-BOA (green color), d. 6-hydroxy-2-BOA (blue color), e. 6-chloro-2-BOA (orange color), f. 6-Bromo-2-BOA (yellow color). Key residues are only shown and the hydrogen bond interactions are represented by yellow dashed lines. The BOA derivatives are shown as sticks and key amino acids as lines.

While the NH on the ring of the compound forms hydrogen bond with Ser420, while the benzene ring and the methyl group form hydrophobic interaction with Val148, Met424 and Ala145 as in Figure 2c. The plausible binding mode of 6-hydroxy- 2-BOA shows that the hydroxyl oxygen forms hydrogen bond with NH of Asn153, while rings forms hydrophobic interaction with Tyr152,Val148, Met424 and Ala145 (Figure 2d). While in 6-chloro-2-BOA the ring oxygen and oxygen group forms hydrogen bond with Asn153 NH and the compound ring forms hydrophobic interaction with Ala145, Val148 and Met424 as in Figure 2e. In the case of 6-Bromo-2-BOA,
the Val148, Met424 and Ala145 forms hydrophobic interaction with the hydrophobic part of the ring system, while the oxygen at the ring forms hydrogen bond with Asn153 (Figure 2f).

Binding mode of BOA derivatives in hNET

Docking study of the BOA derivatives with the hNET are reported below. The binding mode agreement between GOLD and AutoDock binding mode is shown in Figure 3a.

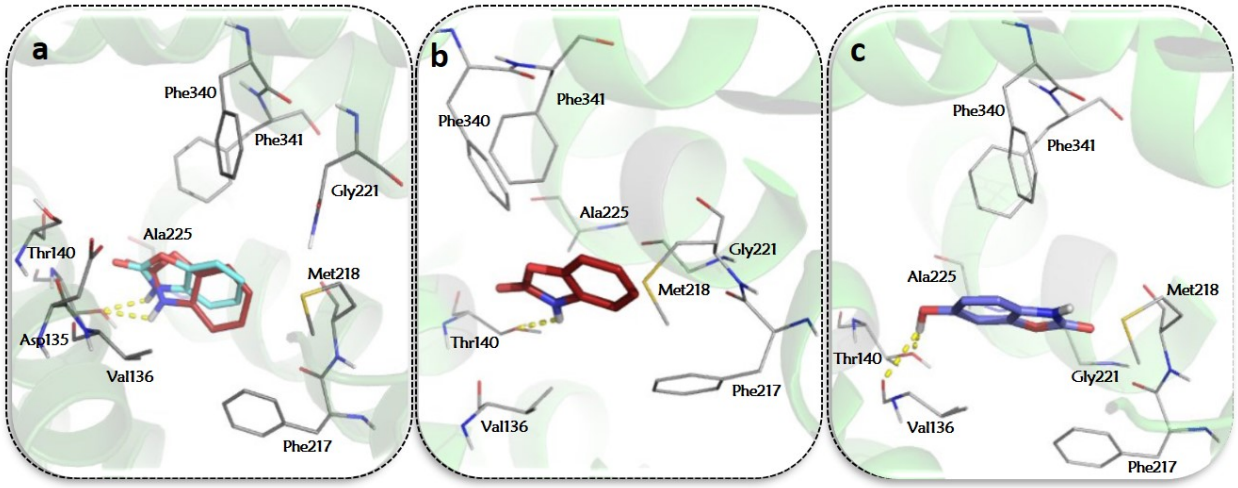

Figure 3 - The 2-BOA derivatives binding mode in hNET

Binding mode a. Comparison of GOLD and AutoDock in brown and blue color respectively. b. 2-BOA (brown color) c. 6-Hydroxy-2-BOA (violet color). Key residues are only shown and the hydrogen bond interactions are represented by yellow dashed lines. The BOA derivatives are shown as sticks and key amino acids as lines.

Among the five derivatives of BOA and 6-Hydroxy-2-BOA forms stable binding mode while 6-Methoxy-2-BOA, 6-Bromo-2-BOA and 6-chloro-2-BOA were not able to produce stable binding conformation in both GOLD and AutoDock docking studies. Therefore the later derivatives were not considered as potential binders of hNET. The binding mode of benzoxazolinone shows that the NH group form hydrogen bond interaction with Thr140 and the aromatic part of the ring forms hydrophobic interaction with hydrophobic residues such as Val136, Phe217, Met218, Ala225, Phe340 and Phe341(Figure 3b). While in the case of 6-Hydroxy-2-BOA the hydroxyl group forms hydrogen bond with Val136 main chain oxygen and with OH group of Thr140 side chain. While the compound ring is also stabilized by hydrophobic interaction with Val136, Phe217, Met218, Ala225, Phe340 and Phe341 (Figure 3c). The putative binding orientation of the most potent BOA derivatives in the active sites of hSERT and hNET has been demonstrated. The analysis of docking results allowed us to postulate their theoretical capability of being potential compounds.

Conclusion

Finally, the molecular docking strategy reveals the binding mode of the 2-BOA derivatives and the key moieties responsible for the activity. Both active and inactive compounds adopt a diverse interaction pattern. The binding pose is clearly demonstrated that both the hydrophilic moieties and hydrophobic groups of the derivatives were crucial for the interaction which may determine the biological activity profile. These results suggest that 2-BOA derivatives have great potential to be further developed as a therapeutic lead molecule for use in disorders associated with depression and pain. Finally, we expect that these results will contribute to the development of newer analgesic with fewer adverse side effects.

Authors' contributions

MS performed *in silico* work, analysis of *in silico* data and drafted the manuscript. SAR participated in collecting the relevant literatures required for the study and manuscript. KKS and RT contributed to experiment design. KKS involved in manuscript correction, formatting the manuscript and references as per author's guidelines. TLK and SMZ provided the software analysis tools for *in silico* studies and interpretation of data. All the authors read and corrected the manuscript.

Conflict of Interest

The authors declare that there are no conflicts of interests.

References

[1]. Hanna MM, Eid NM, George RF and Safwat HM. Synthesis of some tropane derivatives of anticipated activity on the reuptake of norepinephrine and/or serotonin. Bioorg Med Chem. 2007; 15(24): 7765-7772.

[2]. Andersen J, Stuhr-Hansen N, Zachariassen L, Toubro S, Hansen SM, Eildal JN, Bond AD, Bogeso KP, Bang-Andersen B, Kristensen AS and Stromgaard K. Molecular determinants for selective recognition of

antidepressants in the human serotonin and norepinephrine transporters. Proc Natl Acad Sci U S A. 2011; 108(29): 12137-12142.

[3]. Vickers T, Dyck B, Tamiya J, Zhang M, Jovic F, Grey J, Fleck BA, Aparicio A, Johns M, Jin L, Tang H, Foster AC and Chen C. Studies on a series of milnacipran analogs containing a heteroaromatic group as potent norepinephrine and serotonin transporter inhibitors. Bioorg Med Chem Lett. 2008; 18 (11): 3230-3235.

[4]. Nencetti S, Mazzoni MR, Ortore G, Lapucci A, Giuntini J, Orlandini E, Banti I, Nuti E, Lucacchini A, Giannaccini G and Rossello A. Synthesis, molecular docking and binding studies of selective serotonin transporter inhibitors. Eur J Med Chem. 2011; 46(3): 825-834.

[5]. Kharkar PS, Reith ME and Dutta AK. Three-dimensional quantitative structure-activity relationship (3D QSAR) and pharmacophore elucidation of tetrahydropyran derivatives as serotonin and norepinephrine transporter inhibitors. J Comput Aided Mol Des. 2008; 22 (1): 1-17.

[6]. Manepalli S, Geffert LM, Surratt CK and Madura JD. Discovery of novel selective serotonin reuptake inhibitors through development of a protein-based pharmacophore. J Chem Inf Model. 2011; 51(9): 2417-2426.

[7]. Hall FS, Schwarzbaum JM, Perona MT, Templin JS, Caron MG, Lesch KP, Murphy DL and Uhl GR. A greater role for the norepinephrine transporter than the serotonin transporter in murine nociception. Neuroscience. 2011; 175: 315-327.

[8]. Games G and Hutchison A. Tapentadol-ER for the treatment of diabetic peripheral neuropathy. Consult Pharm. 2013; 28 (10): 672-675.

[9]. Attal N. Pharmacological treatment of neuropathic pain in primary care. Rev Prat. 2013; 63 (6): 795-802.

[10]. Fitzgerald KT and Bronstein AC. Selective serotonin reuptake inhibitor exposure. Top Companion Anim Med. 2013; 28 (1): 13-17.

[11]. Gabrielsen M, Sylte I, Dahl SG and Ravna AW. A short update on the structure of drug binding sites on neurotransmitter transporters. BMC Res Notes. 2011; 4: 559.

[12]. Whiteside GT, Dwyer JM, Harrison JE, Beyer CE, Cummons T, Manzino L, Mark L, Johnston GH, Strassle BW, Adedoyin A, Lu P, Piesla MJ, Pulicicchio CM, Erve JC, Platt BJ, Hughes ZA, Rogers KE, Deecher DC, Trybulski EJ, Kennedy JD, Zhang P and Leventhal L. WAY-318068: a novel, potent and selective noradrenaline re-uptake inhibitor with activity in rodent models of pain and depression. Br J Pharmacol. 2010; 160 (5): 1105-1118.

[13]. Van Orden LJ, Van Dyke PM, Saito DR, Church TJ, Chang R, Smith JA, Martin WJ, Jaw-Tsai S and Stangeland EL. A novel class of 3-(phenoxy-phenyl-methyl)-pyrrolidines as potent and balanced norepinephrine and serotonin reuptake inhibitors: synthesis and structure-activity relationships. Bioorg Med Chem Lett. 2013; 23 (5): 1456-1461.

[14]. Marks DM, Shah MJ, Patkar AA, Masand PS, Park GY and Pae CU. Serotonin-norepinephrine reuptake inhibitors for pain control: premise and promise. Curr Neuropharmacol. 2009; 7 (4): 331-336.

[15]. Bannister K, Bee LA and Dickenson AH. Preclinical and early clinical investigations related to monoaminergic pain modulation. Neurotherapeutics. 2009; 6 (4): 703-712.

[16]. Mostert JP, Koch MW, Heerings M, Heersema DJ and De Keyser J. Therapeutic potential of fluoxetine in neurological disorders. CNS Neurosci Ther. 2008; 14 (2): 153-164.

[17]. Tamiya J, Dyck B, Zhang M, Phan K, Fleck BA, Aparicio A, Jovic F, Tran JA, Vickers T, Grey J, Foster AC and Chen C. Identification of 1S, 2R-milnacipran analogs as potent norepinephrine and serotonin transporter inhibitors. Bioorganic & Medicinal Chemistry Letters. 2008; 18 (11): 3328-3332.

[18]. Chappell AS, Ossanna MJ, Liu-Seifert H, Iyengar S, Skljarevski V, Li LC, Bennett RM and Collins H. Duloxetine, a centrally acting analgesic, in the treatment of patients with osteoarthritis knee pain: a 13-week, randomized, placebo-controlled trial. Pain. 2009; 146 (3): 253-260.

[19]. Collins SL, Moore RA, McQuayHj and Wiffen P. Antidepressants and anticonvulsants for diabetic neuropathy and postherpetic neuralgia: a quantitative systematic review. J Pain Symptom Manage. 2000; 20 (6): 449-458.

[20]. Solitar BM. Fibromyalgia: knowns, unknowns, and current treatment. Bull NYU Hosp Jt Dis. 2010; 68 (3): 157-161.

[21]. Murty MSR, Solimabi and Kamat SY. Isolation of 2-benzoxazolinone from *Acanthus ilicifolius*. Indian J. Pharm. Sci. 1984; 46: 218-219.

[22]. Sam J and Plampin JN. Benzoxazoles: Potent Skeletal Muscle Relaxants. J Pharm Sci. 1964; 53: 538-544.

[23]. Goekhan N, Aktay G and Erdogan H. Synthesis of some new pyridylethylated benzoxa(thia)zolinones with analgesic activity. Turk. J. Chem. 2004; 28: 123-132.

[24]. Dogruer DS, Unlu S, Sahin MF and Yesilada E. Anti-nociceptive and anti-inflammatory activity of some (2-benzoxazolone-3-yl and 2-benzothiazolone-3-yl) acetic acid derivatives. Farmaco. 1998; 53 (1): 80-84.

[25]. Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J and Wheeler DL. GenBank. Nucleic Acids Res. 2007; 35: D21-D25.

[26]. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W and Lipman DJ. Gapped BLAST and PSI-BLAST: a

new generation of protein database search programs. Nucleic Acids Res. 1997; 25 (17): 3389-3402.

[27]. Sali A and Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. J Mol Biol. 1993; 234 (3): 779-815.

[28]. Wacker D, Wang C, Katritch V, Han GW, Huang XP, Vardy E, McCorvy JD, Jiang Y, Chu M, F. Y. Siu, W. Liu, H. E. Xu, V. Cherezov, B. L. Roth and R. C. Stevens. Structural features for functional selectivity at serotonin receptors. Science. 2013; 340 (6132): 615-619.

[29]. R. A. Laskowski, M. W. MacArthur, D. S. Moss and J. M. Thornton. PROCHECK: a program to check the stereochemical quality of protein structures. J. Appl. Crystallogr. 1993; 26: 283-291.

[30]. Bolton EE, Wang Y, Thiessen PA and Bryant SH. PubChem: Integrated platform of small molecules and biological activities. Annu. Rep. Comput. Chem. 2008; 4: 217-241.

[31]. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC and Ferrin TE. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. 2004; 25 (13): 1605-1612.

[32]. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN and Bourne PE. The Protein Data Bank. Nucleic Acids Res. 2000; 28: 235-242.

[33]. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK and Olson AJ. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J. Comput. Chem. 1998; 19: 1639-1662.

[34]. Seeliger D and de Groot BL. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. J Comput Aided Mol Des. 2010; 24 (5): 417-422.

[35]. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS and Olson AJ. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem. 2009; 30 (16): 2785-2791.

[36]. Lill MA and Danielson ML. Computer-aided drug design platform using PyMOL. J Comput Aided Mol Des. 2011; 25 (1): 13-19.