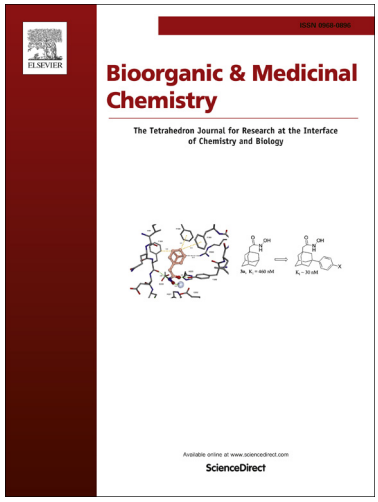
Accepted Manuscript



Synthesis, receptor affinity and effect on pentylenetetrazole-induced seizure threshold of novel benzodiazepine analogues: 3-Substituted 5-(2-phenoxybenzyl)-4*H*-1,2,4-triazoles and 2-amino-5-(phenoxybenzyl)-1,3,4-oxadiazoles

Siavash Mashayekh, Narges Rahmanipour, Behnaz Mahmoodi, Fatemeh Ahmadi, Dina Motaharian, Soraya Shahhosseini, Hamed Shafaroodi, Hamid R. Banafshe, Abbas Shafiee, Latifeh Navidpour

PII: S0968-0896(14)00069-8
DOI: http://dx.doi.org/10.1016/j.bmc.2014.01.041
Reference: BMC 11372

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 11 December 2013
Revised Date: 21 January 2014
Accepted Date: 22 January 2014

Please cite this article as: Mashayekh, S., Rahmanipour, N., Mahmoodi, B., Ahmadi, F., Motaharian, D., Shahhosseini, S., Shafaroodi, H., Banafshe, H.R., Shafiee, A., Navidpour, L., Synthesis, receptor affinity and effect on pentylenetetrazole-induced seizure threshold of novel benzodiazepine analogues: 3-Substituted 5-(2-phenoxybenzyl)-4*H*-1,2,4-triazoles and 2-amino-5-(phenoxybenzyl)-1,3,4-oxadiazoles, *Bioorganic & Medicinal Chemistry* (2014), doi: http://dx.doi.org/10.1016/j.bmc.2014.01.041

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Synthesis, receptor affinity and effect on pentylenetetrazole-induced seizure threshold of novel benzodiazepine analogues: 3-Substituted 5-(2-phenoxybenzyl)-4*H*-1,2,4-triazoles and 2-amino-5-(phenoxybenzyl)-1,3,4-oxadiazoles

Siavash Mashayekh,[a] Narges Rahmanipour,[a] Behnaz Mahmoodi,[b] Fatemeh Ahmadi,[c] Dina Motaharian,[b] Soraya Shahhosseini,[c] Hamed Shafaroodi,[b] Hamid R. Banafshe,[d] Abbas Shafiee[a] and Latifeh Navidpour[a,e*]

[a] *Department of Medicinal Chemistry, Faculty of Pharmacy and Drug Design & Development Research Center, Tehran University of Medical Sciences, Tehran 14176, Iran*

[b] *Pharmacology and Toxicology Department, Pharmaceutical Sciences Branch and Pharmaceutical Sciences Research Center, Islamic Azad University, Tehran, Iran*

[c] *Department of Pharmaceutical Chemistry, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[d] *Department of Physiology & Pharmacology, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran*

[e] *Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Iran*

Correspondence: Latifeh Navidpour, Department of Medicinal Chemistry, Faculty of Pharmacy, Drug Design & Development Research Center and Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Tehran 14176, Iran.
E-mail: navidpur@sina.tums.ac.ir; **Fax:** +98 21 66461178.

Abstract

The new series of 5-(2-phenoxybenzyl)-4*H*-1,2,4-triazoles, possessing C-3 thio, alkylthio and ethoxy substituents, and 2-amino-5-(2-phenoxybenzyl)-1,3,4-oxadiazoles were designed and synthesized as novel benzodiazepine analogues. Most of them revealed similar to superior binding affinity to the $GABA_A$/Benzodiazepine receptor complex, relative to diazepam as the

ACCEPTED MANUSCRIPT

reference drug. Among them, 5-(4-chloro-2-(2-fluorophenoxy)benzyl)-3-benzylthio-4*H*-1,2,4-triazole (**8l**) showed the highest affinity (IC_{50} = 0.892 nM) relative to diazepam (IC_{50} = 2.857 nM) and also showed the most increase in pentylenetetrazole-induced seizure threshold relative to diazepam as the reference drug.

Keywords: 1,2,4-triazole; 1,3,4-oxadiazole; flexible benzodiazepines; receptor binding affinity; PTZ-induced clonic seizure threshold.

1. Introduction

Benzodiazepines (BZDs) are amongst therapeutic agents which have widespread use in treatment of central nervous system disorders and act as anxiolytic, anticonvulsant, sedative-hypnotic and muscle relaxant.[1] BZDs exert their pharmacological effects by allosteric modulation of $GABA_A$ receptors through a specific binding domain, known as BZD receptor (BZRP); this interaction increases the frequency of opening of chloride ion channel and results in an enhancement of γ-aminobutyric acid (GABA) inhibitory action.[2,3] Despite their widespread clinical use, the classical BZDs such as diazepam are also known to produce unwanted side effects including sedation, development of tolerance and dependence, rebound symptoms at withdrawal and amnesic effects.[4] Thus, the search continues for new BZRP ligands with enhanced selectivity, safety and efficacy.

Several studies exploring essential pharmacophore model and structure activity relationship for BZDs have been done through past decades and various models have been proposed.[5-9] An aromatic ring and a coplanar proton-accepting group in appropriate distance are two common features among these models. It is also stated that an out-of-plane aromatic ring could potentiate binding of ligands to the receptor. [10-13]

Moreover, an impressive number of structurally novel agents, which do not contain the BZD nucleus, have been shown to interact with the BZRP.[14,15] In previous studies, it has been shown that simple non-rigid structures of 2-substituted-5-(2-phenoxyphenyl)-4*H*-1,2,4-triazoles and 1,3,4-oxadiazoles could mimic the structure of BZD agonists and possess considerable anticonvulsant activity (Figure 1).[16,17]

ACCEPTED MANUSCRIPT

In the present study, new series of 5-(2-phenoxybenzyl)-4*H*-1,2,4-triazoles possessing C-3 thio, alkylthio and ethoxy substituents, and 2-amino-5-(2-phenoxybenzyl)-1,3,4-oxadiazoles have been synthesized and evaluated as novel BZD analogues. On the contrary to the accepted requirements for the BZD agonists, in these newly designed compounds, due to the presence of the methylene bridge with sp^3 hybridization, the proton-accepting moiety diverges from the plane of aromatic ring. Thus, the high affinity of these newly synthesized compounds for the BZRP opens new insights to the pharmacophore requirements of non-rigid BZD analogues.

2. Results and Discussion

2.1. Chemistry

The synthetic reactions leading to the substituted 3-(2-phenoxybenzyl)-4*H*-1,2,4-triazoles and 2-amino-5-(2-phenoxybenzyl)-1,3,4-oxadiazoles (**7**, **8**, **11** and **12**) are outlined in Scheme 1.

2-Phenoxyphenylacetic acids (**6**) are the key intermediates for the production of the title compounds **7**, **8**, **11** and **12**. The starting materials, 2-phenoxybenzoic acids (**1b-c**) were prepared by the refluxing of sodium salts of 2,4-dichlorobenzoic acid and 2-substiuted phenols in DMF in the presence of catalytic amount of copper.[18] Esterification of the resulted benzoic acid derivatives **1** using thionyl chloride in ethanol afforded the ethyl esters **2**. The reduction of ethyl esters **2** with lithium aluminum hydride in THF yielded alcohol derivatives **3**.[19] To substitute the hydroxyl moiety with cyano group, first the hydroxyl group was converted to chlorine as a suitable leaving group using thionyl chloride in benzene in the presence of pyridine, then the substitution was approached by using sodium cyanide in DMSO.[19] Subsequent hydrolysis of the cyano compound **5** gave the key intermediates, 2-(2-phenoxyphenyl)acetic acids **6**.[19]

Reaction of the acetic acids (**6**) with thionyl chloride followed by the treatment of the resulted acid chlorides with thiosemicarbazide and subsequent cyclization in basic media afforded the 2*H*-1,2,4-triazole-3(4*H*)-thiones (**7**).[18,20] Sonicating compounds **7** in the presence of suitable alkyl halides in alkaline media afforded 3-alkylthio-5-(2-phenoxybenzyl)-4*H*-1,2,4-triazoles (**8**) in 5 min.[17]

ACCEPTED MANUSCRIPT

2-(2-Phenoxyphenyl)acetic acid hydrazides (**10**) were readily prepared via esterification of corresponding acetic acid derivatives (**9**), followed by treatment with hydrazine hydrate in methanol.[20] The hydrazides were reacted with cyanogen bromide to produce 2-amino-5-(2-phenoxybenzyl)-1,3,4-oxadiazoles (**11**) which rearranged to 3-ethoxy-5-(2-phenoxybenzyl)-4*H*-1,2,4-triazoles (**12**) upon treatment with ethanolic potassium hydroxide.[17]

2.2. Relative Binding Affinity

The $GABA_A$/BZD Receptor complex binding affinity of the title compounds was evaluated by their ability to displace [^{3}H]-flumazenil (Ro15-1788) from its specific binding in rat cortical membrane tissue.[21-29] The concentration of the tested compounds (non-radioactive ligands) that inhibits the binding of [^{3}H]-flumazenil by 50% is called as IC_{50} values and is displayed in table 1.

In general, most analogues revealed respectable in vitro activity, showing similar to superior affinity to BZD receptors relative to diazepam as the reference drug.

In 5-(2-phenoxybenzyl)-2*H*-1,2,4-triazole-3(4*H*)-thione subgroup (**7a-c**), ortho substitution of the phenol moiety as well as chloro-substitution of the central phenyl ring improved the affinity, while slightly superior affinity was observed when phenoxy moiety is substituted with fluorine than chlorine.

The effect of alkylthio substitution on the five-memebered triazole ring on the receptor binding affinity was next investigated. Alkylation of thio group produced compounds with slightly less to improved activities. In alkylated analogues of unsubstituted phenol compound **7a** (**8a-d**), increasing the size of the alkyl group slightly decreased the affinity. In 2-chlorophenol subgroup (**8e-h**), increasing the size of this side chain, first increased the affinity (SMe < SEt < SPr), then decreased with bulky benzyl moiety. In 2-fluorophenol subgroup (**8i-l**), increasing the size of the alkyl group even with bulky benzyl group improved the affinity (except for **8k**), presenting the most active compound among the thioalkylated analogues, 5-(4-chloro-2-(2-fluorophenoxy)benzyl)-3-benzylthio-4*H*-1,2,4-triazole (**8l**) (IC_{50} = 0.892 nM) relative to diazepam reference drug (IC_{50} = 2.857 nM).

Regarding the other two subgroups (**11** and **12**), generally 2-amino-1,3,4-oxadiazoles (**11a-c**) have shown slightly more improved affinity than 3-ethoxy-1,2,4-triazoles (**12a-c**), but in both

ACCEPTED MANUSCRIPT

subgroups, chlorine substitution of the central phenyl ring as well as chlorine and fluorine substitution of 2-phenoxy moiety improved the afftinity, respectively.

2.3. The effect on clonic pentylenetetrazole-induced seizure threshold

The effect of *i.p.* administration of different doses (5, 10 and 20 mg/kg) of **7**, **8**, **11** and **12**, 60 min before *i.v.* administration of pentylenetetrazole (PTZ) on clonic induced seizure threshold in mice was investigated and the results are displayed in table 1.[30-31]

The analysis of the results showed that alkylation of 2*H*-1,2,4-triazole-3(4*H*)-thiones (**7a-c**) resulted in compounds with slightly less to improved activities. In unsubstituted (**8a-d**) and 2-chlorophenol subgroups (**8e-h**), the activity was improved while the size of the side chain was increased from SMe to SEt, then decreased with SPr and bulky benzylthio groups (SMe < SEt > SPr > SBn). In 2-fluorophenol subgroup (**8i-l**), similar to their binding affinities, increasing the size of the alkyl group(except for **8k**) improved the activity. Among all the 3-thio and alkylthio analogues, 5-(4-chloro-2-(2-fluorophenoxy)benzyl)-3-benzylthio-4*H*-1,2,4-triazole (**8l**) exhibited the most increase in pentylenetetrazole-induced seizure threshold relative to diazepam as the reference drug.

2-Amino-1,3,4-oxadiazole subgroup (**11a-c**) exhibited improved activity in comparison with the 2*H*-1,2,4-traizole-3(4*H*)-thions (**7a-c**), whereas 3-ethoxy analogues (**12a-c**) were less active than the 3-thione derivatives (**7a-c**).

It is well understood that the onset and duration of action of a single bolus administration of a BZD depend on the lipid solubility of the drug. BZDs with higher lipid solubility have a more rapid onset of action. Also, the more rapid redistribution of the more lipophilic ones accounts for the shorter duration of their actions.[32]

Due to having different substituents, the lipophilicity of the synthesized analogues and so their cLogP (shown in table 1, calculated using MOE software version 2012.10) are highly different. Therefore, each one achieves its peak blood-brain barrier concentration, and maximum anti-convulsant effect at a different time, while in studying the *in vivo* activity of the compounds, the seizure threshold of all are determined after 60 min of *i.p.* administration (similar time) of the

ACCEPTED MANUSCRIPT

compound. It probably explains the poor correlation between the receptor binding affinity of the synthesized analogues and their effect on PTZ-induced clonic seizure threshold.

2.4. Molecular modeling (docking) studies

The orientation of the two potent BZD analogues, 5-(2-phenoxybenzyl)-3-methylthio-4*H*-1,2,4-triazole (**8a**) along with 2-amino-5-(2-phenoxybenzyl)-1,3,4-oxadiazole (**11a**), in the BZD-binding site of $GABA_A$ receptor were examined by a flexible docking experiment using MOE software version 2012.10 (CCG Inc.).[34] For validation purpose, the ligand (diazepam) was also docked.

For the diazepam ligand, the program finds three conformations with interaction energies -5.14, -4.94 and -4.86 kcal/mol. The second conformation matches exactly (RMSD: 0.12 Å) and the third one was very similar (RMSD: 1.81 Å) with the position of the Diazepam in the homology model. In the docking studies of the other two compounds, the second conformation of **8a** with the interaction energy of -13.59 kcal/mol and the third conformation of **11a** with the interaction energy of -13.79 kcal/mol showed similar binding modes in the diazepam pocket of $GABA_A$ receptor.

Figure 2 shows the superimposed poses of structures **8a**, **11a** and the ligand diazepam and Figure 3 displays the important amino acids interacting with the compounds **8a** and **11a**. Since the phenyl group of **8a** and **11a** diverges about 60 degrees from the aromatic-A ring of diazepam, their positioning into the binding pocket should accommodate the nitrogen moiety of 1,2,4-triazole and 1,3,4-oxadiazole near the carbonyl group of diazepam which is an important pharmacophore in BZDs, thus providing the hydrogen bond with A-Thr^{206} (2.65 and 2.84 Å in **8a** and **11a**, respectively).

Moreover, there are two arene-cation interactions between the phenyl moiety in phenoxy side chain and central phenyl ring with A-His^{101}. Other two arene π-π interactions have been revealed between the central phenyl ring and five-membered rings, 1,2,4-triazole or 1,3,4-oxadiazole, with A-Tyr^{209}.

ACCEPTED MANUSCRIPT

3. Conclusion

The new series of 5-(2-phenoxybenzyl)-4*H*-1,2,4-triazoles possessing C-3 thio, alkylthio and ethoxy substituents, and 2-amino-5-(2-phenoxybenzyl)-1,3,4-oxadiazoles were designed to develop further structure-activity relationship data. The results of this investigation show: (i) 3-thio or ethoxy substituted 5-(-2-phenoxybenzyl)-2*H*-1,2,4-triazoles and 3-amino substituted 5-(-2-phenoxybenzyl)-1,3,4-oxadiazoles provide high receptor binding affinity on $GABA_A$/BZD Receptor complex, (ii) these analogues significantly increase pentylenetetrazole-induced seizure threshold, (iii) molecular modeling studies indicate that the central phenyl moiety of studied analogues diverges about 60 degrees from the aromatic-A ring of diazepam, so their positioning into the binding pocket should accommodate the nitrogen moiety of 1,2,4-triazole and 1,3,4-oxadiazole near the carbonyl group of diazepam, providing the hydrogen bond with A-Thr^{206}.

4. Experimental

4.1. Chemistry

Melting points were determined with a Reichert-Jung hot-stage microscope (Reichert-Jung, Germany) and are uncorrected. Infrared spectra were recorded on a Nicolet Magna 550-FT spectrometer (Nicolet, Madison, WI, USA). ^{1}H NMR spectra were measured on a Bruker FT-500 MHz spectrometer (Bruker Bioscience, USA) in $CDCl_3$ or DMSO-d_6 with TMS as the internal standard. Elemental microanalyses were carried out with a Perkin-Elmer 240-C apparatus (Perkin-Elmer, Beaconsfield, UK) and were within ± 0.4% of the theoretical values for C, H and N. Male NMRI mice and male Wistar rats, used in the analgesic and anti-inflammatory screens, respectively, were purchased from Pasteur Institute (Karaj, Iran), and experiments were carried out using protocols approved by the ethics committee of Tehran University of Medical Sciences.

4.1.1. General procedure for preparation of ethyl 2-aryloxybenzoates (2a-c)

To a stirred solution of 2-phenoxybenzoic acid derivative (**1a-c**, 181 mmol) in ethanol (150 mL) was added dropwise thionyl chloride (60 mL, 825 mmol) and the reaction mixture was refluxed for 4 h. Then, the volatiles were evaporated under reduced pressure, water was added to the

ACCEPTED MANUSCRIPT

residue (250 mL) and the aqueous layer was extracted with ethyl acetate (3×100 mL). The organic layer was washed with saturated sodium hydrogen carbonate solution (100 mL), dried over anhydrous sodium sulfate and evaporated to furnish **2a-c**. The spectral data of **2a** was similar with those reported.[34]

4.1.1.1. Ethyl 2-(2-chlorophenoxy)-4-chlorobenzoate (2b)

Yield, 98%; IR (KBr): 1729 (C=O), 1239 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24 (t, J = 7.2 Hz, 3H, CH_3), 4.28 (q, J = 7.2 Hz, 2H, CH_2), 6.85 (d, J = 2.0 Hz, 1H, H_3), 6.86 (dd, J = 2.0, 7.5 Hz, 1H, $H_{6'}$), 7.09 (dt, J = 1.6, 7.5 Hz, 1H, $H_{4'}$), 7.16 (dd, J = 2.0, 8.4 Hz, 1H, H_5), 7.21 (dt, J = 1.6, 7.5 Hz, 1H, $H_{5'}$), 7.47 (dd, J = 1.6, 8.4 Hz, 1H, $H_{3'}$), 7.90 (d, J = 8.4 Hz, 1H, H_6).

Anal. Calcd for $C_{15}H_{12}Cl_2O_3$: C, 57.90; H, 3.89. Found: C, 58.14; H, 3.66.

4.1.1.2. Ethyl 4-chloro-2-(2-fluorophenoxy)benzoate (2c)

Yield, 98%; IR (KBr): 1728 (C=O), 1269 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28 (t, J = 7.0 Hz, 3H, CH_3), 4.08 (q, J = 7.0 Hz, 2H, CH_2), 6.88 (d, J = 1.2 Hz, 1H, H_3), 6.99 (dt, J = 1.2, 8.0 Hz, 1H, $H_{6'}$), 7.08-7.29 (m, 4H, aromatic), 7.89 (d, J = 8.0 Hz, 1H, H_6).

Anal. Calcd for $C_{15}H_{12}ClFO_3$: C, 61.13; H, 4.10. Found: C, 61.28; H, 3.92.

4.1.2. General procedure for preparation of 2-aryloxyphenylmethanols (3a-c)

To an ice-cold suspension of lithium aluminum hydride (9.52 g, 250 mmol) in dry THF (40 mL) was added dropwise a solution of ethyl ester (**2a-c**, 158 mmol) in THF (20 mL) under an inert atmosphere of argon and the reaction mixture was stirred at room temperature for 24 h. Then, water (100 mL) was added to the reaction mixture and the aqueous layer was extracted with diethyl ether (3×100 mL). The organic layer was dried over anhydrous sodium sulfate, evaporated and recrystallized from ethyl acetate/hexane to obtain the required alcohol (**3a-c**). The spectral data of **3a** was similar with those reported.[19,35]

4.1.2.1. 2-(2-Chlorophenoxy)-4-chlorophenylmethanol (3b)

Yield, 51%; mp 74-75 °C; IR (KBr): 3334 (OH), 1238 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.18 (t, J = 6.0 Hz, 1H, OH), 4.80 (d, J = 6.0 Hz, 2H, CH_2), 6.62 (d, J = 1.6 Hz, 1H, H_3), 7.02-7.05 (m, 2H, $H_{5,6'}$), 7.18 (dt, J = 1.6, 7.4 Hz, 1H, $H_{4'}$), 7.29 (dt, J = 1.6, 7.4 Hz, 1H, $H_{5'}$), 7.39 (d, J = 8.0

ACCEPTED MANUSCRIPT

Hz, 1H, H_6), 7.49 (dd, J = 1.6, 8.0 Hz, 1H, $H_{3'}$).

Anal. Calcd for $C_{13}H_{10}Cl_2O_2$: C, 58.02; H, 3.75. Found: C, 57.88; H, 3.57.

4.1.2.2. 2-(2-Fluorophenoxy)-4-chlorophenylmethanol (3c)

Yield, 86%; mp 77-79 °C; IR (KBr): 3368 (OH), 1274 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.81 (s, 2H, CH_2), 6.68 (s, 1H, H_3), 7.05-7.35 (m, 5H, aromatic), 7.39 (d, J = 7.5 Hz, 1H, H_6).

Anal. Calcd for $C_{13}H_{10}ClFO_2$: C, 61.80; H, 3.99. Found: C, 61.98; H, 4.07.

4.1.3. General procedure for preparation of 1-chloromethyl-2-aryloxybenzenes (4a-c)

To a solution of 2-(2-phenoxyphenyl)methanol derivative (**3a-c**, 150 mmol) in benzene (650 mL) and pyridine (1 mL) was added dropewise thionyl chloride (18.5 g, 155 mmol) in benzene (90 mL) and the reaction mixture was refluxed under an inert atmosphere of argon for 2 h. The volatiles were evaporated under reduced pressure and water was added to the residue (250 mL). The aqueous layer was extracted with diethyl ether (3×100 mL). The organic layer was washed by saturated sodium chloride solution, dried over anhydrous sodium sulfate and evaporated to obtain chloromethyl compound (**4a-c**). The spectral data of **4a** was similar with those reported.[19]

4.1.3.1. 1-Chloromethyl-4-chloro-2-(2-chlorophenoxy)benzene (4b)

Yield, 94%; IR (KBr): 1267 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.74 (s, 2H, CH_2), 6.62 (d, J = 1.8 Hz, 1H, H_3), 7.06-7.10 (m, 2H, $H_{5,6'}$), 7.18 (dt, J = 1.6, 7.8 Hz, 1H, $H_{4'}$), 7.29 (dt, J = 1.6, 7.8 Hz, 1H, $H_{5'}$), 7.41 (d, J = 8.2 Hz, 1H, H_6), 7.49 (dd, J = 1.6, 7.8 Hz, 1H, $H_{3'}$).

Anal. Calcd for $C_{13}H_9Cl_3O$: C, 54.30; H, 3.15. Found: C, 54.08; H, 3.27.

4.1.3.2. 1-Chloromethyl-4-chloro-2-(2-fluorophenoxy)benzene (4c)

Yield, 91%; mp 43-44 °C; IR (KBr): 1267 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.72 (s, 2H, CH_2), 6.83 (s, 1H, H_3), 6.95-7.55 (m, 6H).

Anal. Calcd for $C_{13}H_9Cl_2FO$: C, 57.59; H, 3.35. Found: C, 57.82; H, 3.52.

4.1.4. General procedure for preparation of 2-(2-aryloxyphenyl)acetonitriles (5a-c)

To a solution of chloromethyl derivative (**4a-c**, 144 mmol) in dry dimethylsulfoxide (500 mL) was added sodium cyanide (10.8g, 220 mmol) and the reaction mixture was stirred at room

ACCEPTED MANUSCRIPT

temperature for 2 h. After addition of water (500 mL), the reaction mixture was extracted by ethyl acetate (3×100 mL). The resulted organic layer was washed by saturated sodium chloride solution, dried over anhydrous sodium sulfate and evaporated to obtain **5a-c** as oily compounds. The spectral data of **5a** was similar with those reported.[19]

4.1.4.1. 2-(4-Chloro-2-(2-chlorophenoxy)phenyl)acetonitrile (5b)

Yield, 80%; IR (KBr): 2253 (CN), 1239 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.84 (s, 2H, CH_2), 6.59 (d, J = 2.0 Hz, 1H, H_3), 7.05-7.14 (m, 2H, $H_{5,6'}$), 7.19 (dt, J = 1.6, 8.0 Hz, 1H, $H_{4'}$), 7.31 (dt, J = 1.6, 8.0 Hz, 1H, $H_{5'}$), 7.41 (d, J = 8.0 Hz, 1H, H_6), 7.48 (dd, J = 1.6, 8.0 Hz, 1H, $H_{3'}$).

Anal. Calcd for $C_{14}H_9Cl_2NO$: C, 60.46; H, 3.26; N, 5.04. Found: C, 60.52; H, 3.07; N, 5.29.

4.1.4.2. 2-(4-Chloro-2-(2-fluorophenoxy)phenyl)acetonitrile (5c)

Yield, 98%; IR (KBr): 2253 (CN), 1225 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.93 (s, 2H, CH_2), 6.66 (s, 1H, H_3), 7.04-7.28 (m, 5H, aromatic), 7.39 (d, J = 8.0 Hz, 1H, H_6).

Anal. Calcd for $C_{14}H_9ClFNO$: C, 64.26; H, 3.47; N, 5.35. Found: C, 64.49; H, 3.25; N, 5.23.

4.1.5. General procedure for preparation of 2-(2-aryloxyphenyl)acetic acids (6a-c)

To a solution of acetonitrile derivative (**5a-c**, 139 mmol) in *n*-butanol (or ethanol for **5c**) (400 mL) was added potassium hydroxide (235 mmol) and the reaction mixture was refluxed for 24 h. Then, the solvent was evaporated under reduced pressure, the residue was dissolved in water (200 mL) and the mixture was washed by diethyl ether (200 mL). The aqueous layer was acidified by hydrochloric acid and extracted by diethyl ether (3×100 mL). The organic layer was washed by brine, dried over anhydrous sodium sulfate, evaporated and the residue was recrystallized from benzene/hexane to give **6a-c**. The spectral data of **6a** was similar with those reported.[19,35]

4.1.5.1. 2-(4-Chloro-2-(2-chlorophenoxy)phenyl)acetic acid (6b)

Yield, 91%; mp 130-132 °C; IR (KBr): 2820-3210 (broad, OH), 1693 (C=O), 1243 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.78 (s, 2H, CH_2), 6.61 (d, J = 2.0 Hz, 1H, H_3), 7.01-7.09 (m, 2H, $H_{5,6'}$), 7.14 (dt, J = 1.6, 8.0 Hz, 1H, $H_{4'}$), 7.20-7.29 (m, 2H, $H_{6,5'}$), 7.45 (dd, J = 1.6, 8.0 Hz, 1H, $H_{3'}$).

Anal. Calcd for $C_{14}H_{10}Cl_2O_3$: C, 56.59; H, 3.39. Found: C, 59.73; H, 3.17.

ACCEPTED MANUSCRIPT

4.1.5.2. 2-(4-Chloro-2-(2-fluorophenoxy)phenyl)acetic acid (6c)

Yield, 80%; mp 110-112 °C; IR (KBr): 2810-3250 (broad, OH), 1695 (C=O), 1267 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.66 (s, 2H, CH_2), 6.71 (s, 1H, H_3), 7.08-7.45 (m, 6H, aromatic), 12.50 (bs, 1H, OH).

Anal. Calcd for $C_{14}H_{10}ClFO_3$: C, 59.91; H, 3.59. Found: C, 59.70; H, 3.47.

4.1.6. General procedure for preparation of 5-(2-aryloxybenzyl)-2*H*-1,2,4-triazole-3(4*H*)-thiones (7a-c)

A solution of acetic acid (**6a-c**, 67 mmol) in thionyl chloride (30 mL) was stirred at 50-55 °C for 1 h. Then, the solvent was evaporated under reduced pressure. The resulted acetyl chloride was dissolved in dry benzene (50 mL) and added dropwise to the ice-cold suspension of thiosemicarbazide (5.8 g, 64 mmol) in dry pyridine (50 mL). The mixture was stirred for half an hour at -5 °C and then overnight at room temperature. The solvent was evaporated under reduced pressure and water (100 mL) was added to the residue. The resulted crude yellow precipitate was filtered, washed with water and suspended in aqueous solution of sodium hydroxide (5%, 200 mL) and refluxed for 4 h. After cooling, the solution was acidified with hydrochloric acid, the precipitate was filtered and recrystallized from ethanol to give **7a-c**.

4.1.6.1. 5-(2-Phenoxybenzyl)-2*H*-1,2,4-triazole-3(4*H*)-thione (7a)

Yield, 60%; mp 217-219 °C; IR (KBr): 3498 (NH), 1235 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.86 (s, 2H, CH_2), 7.27-6.85 (m, 9H, aromatic).

Anal. Calcd for $C_{15}H_{13}N_3OS$: C, 63.58; H, 4.62; N, 14.83. Found: C, 63.72; H, 4.87; N, 14.62.

4.1.6.2. 5-(4-Chloro-2-(2-chlorophenoxy)benzyl)-2*H*-1,2,4-triazole-3(4*H*)-thione (7b)

Yield, 90%; mp 90-92 °C; IR (KBr): 3450 (NH), 1236 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.70 (s, 2H, CH_2), 6.58 (d, J = 2.0 Hz, 1H, H_3), 6.95-7.40 (m, 6H, aromatic).

Anal. Calcd for $C_{15}H_{11}Cl_2N_3OS$: C, 51.15; H, 3.15; N, 11.93. Found: C, 51.08; H, 3.37; N, 12.11.

4.1.6.3. 5-(4-Chloro-2-(2-fluorophenoxy)benzyl)-2*H*-1,2,4-triazole-3(4*H*)-thione (7c)

Yield, 89%; mp 212-215 °C; IR (KBr): 3497 (NH), 1220 (C-O) cm^{-1}, ^1H NMR ($CDCl_3$) δ 3.72

ACCEPTED MANUSCRIPT

(s, 2H, CH_2), 6.65 (s, 1H, H_3), 7.0-7.55 (m, 6H, aromatic).

Anal. Calcd for $C_{15}H_{11}ClFN_3OS$: C, 53.65; H, 3.30; N, 12.51. Found: C, 53.48; H, 3.47; N, 12.62.

4.1.7. General procedure for alkylation of 5-(2-aryloxybenzyl)-2*H*-1,2,4-triazole-3(4*H*)-thiones

To a solution of 5-(2-aryloxybenzyl)-2*H*-1,2,4-triazole-3(4*H*)-thione (**7a-c**, 1.41 mmol) in ethanol (5 mL) and 10% aqueous solution of sodium hydroxide (0.75 mL) was added alkyl iodide or alkyl bromide (1.60 mmol) and the mixture was sonicated for 5 min. The volatiles were evaporated under reduced pressure and the residue was purified by preparative TLC (hexane/ EtOAc, 60:40) to give **8a-l**.

4.1.7.1. 5-(2-Phenoxybenzyl)-3-methylthio-4*H*-1,2,4-triazole (8a)

Yield, 80%; mp 102-104 °C; IR (KBr): 3424 (NH), 1234 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.56 (s, 3H, CH_3), 4.15 (s, 2H, CH_2), 6.89 (d, J = 8.2 Hz, 1H, H_3), 6.95 (d, J = 8.0 Hz, 2H, $H_{2',6'}$), 7.08-7.14 (m, 2H, $H_{5,4'}$), 7.24 (t, J = 8.2 Hz, 1H, H_4), 7.34 (t, J = 8.0 Hz, 2H, $H_{3',5'}$), 7.37 (d, J = 8.2 Hz, 1H, H_6).

Anal. Calcd for $C_{16}H_{15}N_3OS$: C, 64.62; H, 5.08; N, 14.13. Found: C, 64.46; H, 5.17; N, 14.34.

4.1.7.2. 5-(2-Phenoxybenzyl)-3-ethylthio-4*H*-1,2,4-triazole (8b)

Yield, 63%; mp 90-92 °C; IR (KBr): 3415 (NH), 1244 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (t, J = 7.5 Hz, 3H, CH_3), 3.08 (q, J = 7.5 Hz, 2H, CH_2), 4.14 (s, 2H, CH_2), 6.88 (d, J = 8.0 Hz, 1H, H_3), 6.94 (d, J = 8.0 Hz, 2H, $H_{2',6'}$), 7.08-7.13 (m, 2H, $H_{5,4'}$), 7.23 (t, J = 8.0 Hz, 1H, H_4), 7.31 (t, J = 8.0 Hz, 2H, $H_{3',5'}$), 7.37 (d, J = 8.0 Hz, 1H, H_6).

Anal. Calcd for $C_{17}H_{17}N_3OS$: C, 65.57; H, 5.50; N, 13.49. Found: C, 65.38; H, 5.41; N, 13.27.

4.1.7.3. 5-(2-Phenoxybenzyl)-3-propylthio-4*H*-1,2,4-triazole (8c)

Yield, 60%; mp 98-100 °C; IR (KBr): 3266 (NH) , 1241 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (t, J = 7.0 Hz, 3H, CH_3), 1.51 (m, 2H, CH_2), 3.23 (t, J = 7.0 Hz, 2H, SCH_2), 4.30 (s, 2H, CH_2), 6.90 (d, J = 8.2 Hz, 1H, H_3), 6.92 (d, J = 8.0 Hz, 2H, $H_{2',6'}$), 7.12-7.08 (m, 2H, $H_{5,4'}$), 7.21-7.08 (t, J = 8.0 Hz, 1H, H_4), 7.32 (t, J = 8.0 Hz, 2H, $H_{3',5'}$), 7.34 (d, J = 8.0 Hz, 1H, H_6).

ACCEPTED MANUSCRIPT

Anal. Calcd for $C_{18}H_{19}N_3OS$: C, 66.43; H, 5.88; N, 12.91. Found: C, 66.58; H, 5.70; N, 13.08.

4.1.7.4. 5-(2-Phenoxybenzyl)-3-benzylthio-4*H*-1,2,4-triazole (8d)

Yield, 88%; mp 80-82 °C; IR (KBr): 3224 (NH), 1214 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.14 (s, 2H, CH_2), 4.29 (s, 2H, SCH_2), 6.88 (dd, J = 1.5, 8.5 Hz, 1H, H_3), 6.92 (dd, J = 1.0, 8.5 Hz, 2H, $H_{2',6'}$), 7.08-7.12 (m, 2H, $H_{5,4'}$), 7.20-7.36 (m, 9H, aromatic).

Anal. Calcd for $C_{22}H_{19}N_3OS$: C, 70.75; H, 5.13; N, 11.25. Found: C, 70.68; H, 5.32; N, 11.10.

4.1.7.5. 5-(4-Chloro-2-(2-chlorophenoxy)benzyl)-3-methylthio-4*H*-1,2,4-triazole (8e)

Yield, 15%; mp 129-133 °C; IR (KBr): 3134 (NH), 1238 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.54 (s, 3H, SCH_3), 4.17 (s, 2H, CH_2), 6.62 (s, 1H, H_3), 6.96 (d, J = 8.0 Hz, 1H, $H_{6'}$), 7.01 (d, J = 8.0 Hz, 1H, H_5), 7.14 (t, J = 7.6 Hz, 1H, $H_{4'}$), 7.15-7.25 (m, 2H, $H_{6,5'}$), 7.45 (d, J = 7.6 Hz, 1H, $H_{3'}$).

Anal. Calcd for $C_{16}H_{13}Cl_2N_3OS$: C, 52.47; H, 3.58; N, 11.47. Found: C, 52.68; H, 3.69; N, 11.32.

4.1.7.6. 5-(4-Chloro-2-(2-chlorophenoxy)benzyl)-3-ethylthio-4*H*-1,2,4-triazole (8f)

Yield, 14%; IR (KBr): 3132 (NH), 1239 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.32 (t, J = 7.4 Hz, 3H, CH_3), 3.06 (q, J = 7.4 Hz, 2H, SCH_2), 4.18 (s, 2H, CH_2), 6.61 (d, J = 1.2 Hz, 1H, H_3), 6.95 (d, J = 7.6 Hz, 1H, $H_{6'}$), 6.99 (dd, J = 1.6, 8.0 Hz, 1H, H_5), 7.12 (t, J = 7.6 Hz, 1H, $H_{4'}$), 7.17-7.28 (m, 2H, $H_{6,5'}$), 7.43 (d, J = 7.6 Hz, 1H, $H_{3'}$).

Anal. Calcd for $C_{17}H_{15}Cl_2N_3OS$: C, 53.69; H, 3.98; N, 11.05. Found: C, 53.88; H, 4.11; N, 10.92.

4.1.7.7. 5-(4-Chloro-2-(2-chlorophenoxy)benzyl)-3-propylthio-4*H*-1,2,4-triazole (8g)

Yield, 15%; IR (KBr): 3275 (NH), 1238 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (t, J = 7.4 Hz, 3H, CH_3), 1.68 (m, 2H, CH_2), 3.04 (t, J = 7.4 Hz, 2H, SCH_2), 4.18 (s, 2H, CH_2), 6.63 (s, 1H, H_3), 6.95 (d, J = 7.6 Hz, 1H, $H_{6'}$), 6.99 (d, J = 8.0 Hz, 1H, H_5), 7.13 (t, J = 7.5 Hz, 1H, $H_{4'}$), 7.17-7.28 (m, 2H, $H_{6,5'}$), 7.43 (d, J = 7.5 Hz, 1H, $H_{3'}$).

Anal. Calcd for $C_{18}H_{17}Cl_2N_3OS$: C, 54.83; H, 4.35; N, 10.66. Found: C, 54.98; H, 4.19; N, 10.82.

4.1.7.8. 5-(4-Chloro-2-(2-chlorophenoxy)benzyl)-3-benzylthio-4*H*-1,2,4-triazole (8h)

ACCEPTED MANUSCRIPT

Yield, 18%; IR (KBr): 3215 (NH), 1240 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.20 (s, 2H, CH_2), 4.32 (s, 2H, SCH_2), 6.65 (s, 1H, H_3), 6.95 (d, J = 7.6 Hz, 1H, $H_{6'}$), 6.99 (d, J = 8.0 Hz, 1H, H_5), 7.10-7.35 (m, 8H, $H_{4',5',6}$ & Ph), 7.48 (d, J = 7.6 Hz, 1H, $H_{3'}$).

Anal. Calcd for $C_{22}H_{17}Cl_2N_3OS$: C, 59.73; H, 3.87; N, 9.50. Found: C, 59.98; H, 3.62; N, 9.42.

4.1.7.9. 5-(4-Chloro-2-(2-fluorophenoxy)benzyl)-3-methylthio-4*H*-1,2,4-triazole (8i)

Yield, 60%; IR (KBr): 3140 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.54 (s, 3H, CH_3), 4.18 (s, 2H, CH_2), 6.66 (s, 1H, H_3), 6.95-7.35 (m, 6H, aromatic).

Anal. Calcd for $C_{16}H_{13}ClFN_3OS$: C, 54.94; H, 3.75; N, 12.01. Found: C, 54.88; H, 3.59; N, 12.22.

4.1.7.10. 5-(4-Chloro-2-(2-fluorophenoxy)benzyl)-3-ethylthio-4*H*-1,2,4-triazole (8j)

Yield, 44%; IR (KBr): 3116 (NH), 1265 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (t, J = 7.5 Hz, 3H, CH_3), 3.14 (q, J = 7.5 Hz, 2H, CH_2), 4.32 (s, 2H, CH_2), 6.61 (s, 1H, H_3), 6.95-7.30 (m, 6H, aromatic), 11.42 (bs, 1H, NH).

Anal. Calcd for $C_{17}H_{15}ClFN_3OS$: C, 56.12; H, 4.16; N, 11.55. Found: C, 55.98; H, 4.29; N, 11.33.

4.1.7.11. 5-(4-Chloro-2-(2-fluorophenoxy)benzyl)-3-propylthio-4*H*-1,2,4-triazole (8k)

Yield, 41%; IR (KBr): 3242 (NH), 1267 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.97 (t, J = 7.0 Hz, 3H, CH_3), 1.69 (m, 2H, CH_2), 3.03 (t, J = 7.0 Hz, 2H, SCH_2), 4.19 (s, 2H, CH_2), 6.66 (s, 1H, H_3), 6.95-7.25 (m, 6H, aromatic).

Anal. Calcd for $C_{18}H_{17}ClFN_3OS$: C, 57.21; H, 4.53; N, 11.12. Found: C, 57.45; H, 4.39; N, 11.04.

4.1.7.12. 5-(4-Chloro-2-(2-fluorophenoxy)benzyl)-3-benzylthio-4*H*-1,2,4-triazole (8l)

Yield, 20%; IR (KBr): 3120 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.20 (s, 2H, CH_2), 4.30 (s, 2H, SCH_2), 6.67 (s, 1H, H_3), 6.95-7.38 (m, 11H, aromatic).

Anal. Calcd for $C_{22}H_{17}ClFN_3OS$: C, 62.04; H, 4.02; N, 9.87. Found: C, 61.85; H, 4.19; N, 10.02.

4.1.8. General procedure for preparation of ethyl 2-(2-aryloxyphenyl)acetates (9a-c)

ACCEPTED MANUSCRIPT

To a stirred solution of acetic acid (**6a-c**, 13 mmol) in ethanol (100 mL) was added dropwise thionyl chloride (4.77 mL, 65 mmol). The reaction mixture was refluxed for 4 h. Then, the volatiles were evaporated under reduced pressure and water (250 mL) was added to the residue. The aqueous layer was extracted with ethyl acetate (3×200 mL). The ethyl acetate layer was washed by saturated sodium hydrogen carbonate solution (100 mL), dried over anhydrous sodium sulfate and evaporated to furnish **9a-c**.

4.1.8.1. Ethyl 2-(2-phenoxyphenyl)acetate (9a)

Yield, 98%; IR (KBr): 1740 (C=O), 1236 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.18 (t, J = 7.0 Hz, 3H, CH_3), 3.69 (s, 2H, CH_2), 4.08 (q, J = 7.0 Hz, 2H, CH_2), 6.88 (d, J = 7.5 Hz, 1H, H_3), 6.97 (d, J = 7.5 Hz, 2H, $H_{2',6'}$), 7.08 (t, J = 7.5 Hz, 1H, $H_{4'}$), 7.11 (dt, J = 1.5, 7.5 Hz, 1H, H_5), 7.24 (dt, J = 1.5, 7.5 Hz, 1H, H_4), 7.29-7.34 (m, 3H, $H_{3',5',6}$).

Anal. Calcd for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found: C, 74.78; H, 6.15.

4.1.8.2. Ethyl 2-(4-chloro-2-(2-chlorophenoxy)phenyl)acetate (9b)

Yield, 95%; IR (KBr): 1729 (C=O), 1239 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.21 (t, J = 7.2 Hz, 3H, CH_3), 3.72 (s, 2H, CH_2), 4.12 (q, J = 7.2 Hz, 2H, OCH_2), 6.65 (s, 1H, H_3), 7.00 (d, J = 7.2 Hz, 1H, $H_{6'}$), 7.06 (d, J = 8.0 Hz, 1H, H_5), 7.14 (t, J = 7.2 Hz, 1H, $H_{4'}$), 7.20-7.29 (m, 2H, $H_{6,5'}$), 7.45 (d, J = 7.2 Hz, 1H, $H_{3'}$).

Anal. Calcd for $C_{16}H_{14}Cl_2O_3$: C, 59.10; H, 4.34. Found: C, 58.98; H, 4.52.

4.1.8.3. Ethyl 2-(4-chloro-2-(2-fluorophenoxy)phenyl)acetate (9c)

Yield, 82%; IR (KBr): 1728 (C=O), 1244 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.21 (t, J = 7.2 Hz, 3H, CH_3), 3.72 (s, 2H, CH_2), 4.12 (q, J = 7.2 Hz, 2H, CH_2), 6.69 (d, J = 1.6 Hz, 1H, H_3), 7.02-7.25 (m, 6H, aromatic).

Anal. Calcd for $C_{16}H_{14}ClFO_3$: C, 62.25; H, 4.57. Found: C, 62.08; H, 4.40.

4.1.9. General procedure for preparation of 2-(2-aryloxyphenyl)acetic acid hydrazides (10a-c)

ACCEPTED MANUSCRIPT

A solution of ethyl 2-(2-phenoxyphenyl)acetate derivative (**9a-c**, 12 mmol) and hydrazine hydrate (3g, 60 mmol) in ethanol (20 mL) was stirred at room temperature for 24 h. The reaction mixture poured into a mixture of crushed ice-water giving a crude precipitate, which was filtered and recrystallized from ethanol/water to give **10a-c**.

4.1.9.1. 2-(2-Phenoxyphenyl)acetic acid hydrazide (10a)

Yield, 94%; mp 62-64 °C; IR (KBr): 3308 (NH), 1654 (C=O), 1236 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.7 (bs, 2H, NH_2), 3.57 (s, 2H, CH_2), 6.87 (dd, J = 1.5, 7.5 Hz, 1H, H_3), 6.93 (dd, J = 7.5 Hz, 2H, $H_{2',6'}$), 7.07-7.13 (m, 2H, $H_{5,4'}$), 7.23 (dt, J = 1.5, 7.5 Hz, 1H, H_4), 7.32 (t, J = 1.5, 7.5 Hz, 2H, $H_{3',5'}$), 7.35 (dd, J = 1.5, 7.5 Hz, 1H, H_6).

Anal. Calcd for $C_{14}H_{14}N_2O_2$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.28; H, 5.99; N, 11.74.

4.1.9.2. 2-(4-Chloro-2-(2-chlorophenoxy)phenyl)acetic acid hydrazide (10b)

Yield, 91%; mp 139-142 °C; IR (KBr): 3301 (NH), 1647 (C=O), 1240 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.62 (s, 2H, CH_2), 3.85 (bs, 2H, NH_2), 6.65 (d, J = 1.7 Hz, 1H, H_3), 7.03 (d, J = 8.0 Hz, 1H, $H_{6'}$), 7.06 (dd, J = 1.7, 8.2 Hz, 1H, H_5), 7.19 (t, J = 8.0 Hz, 1H, $H_{4'}$), 7.28-7.35 (m, 2H, $H_{6,5'}$), 7.50 (d, J = 8.0 Hz, 1H, $H_{3'}$).

Anal. Calcd for $C_{14}H_{12}Cl_2N_2O_2$: C, 54.04; H, 3.89; N, 9.00. Found: C, 54.25; H, 3.72; N, 8.88.

4.1.9.3. 2-(4-Chloro-2-(2-fluorophenoxy)phenyl)acetic acid hydrazide (10c)

Yield, 54%; mp 157-159 °C; IR (KBr): 3425, 3272 (NH), 1685 (C=O), 1248 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.6 (bs, 2H, NH_2), 3.64 (s, 2H, CH_2), 6.68 (d, J = 1.6 Hz, 1H, H_3), 6.95-7.20 (m, 6H, aromatic).

Anal. Calcd for $C_{14}H_{12}ClFN_2O_2$: C, 57.06; H, 4.10; N, 9.51. Found: C, 56.91; H, 3.88; N, 9.38.

4.1.10. General procedure for preparation of 2-amino-5-(2-aryloxybenzyl)-1,3,4-oxadiazoles (11a-c)

To a stirring solution of acetohydrazide (**10a-c**, 8.26 mmol) in dioxane (20 mL) was added sodium bicarbonate (0.69 g, 8.26 mmol) in water (7 mL) and the mixture was stirred at room temperature for 5 min. Subsequently, cyanogen bromide (0.91 g, 8.56 mmol) was added and it

ACCEPTED MANUSCRIPT

was stirred for further 30 min. Then water (60 mL) was added to the mixture and the resulted precipitate was filtered and recrystallized from ethanol to give **11a-c**.

4.1.10.1. 2-Amino-5-(2-phenoxybenzyl)-1,3,4-oxadiazole (11a)

Yield, 72%; mp 164-166 °C; IR (KBr): 3267-3114 (NH_2), 1659 (C=N), 1234 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.12 (s, 2H, CH_2), 4.82 (bs, 2H, NH_2), 6.91 (dd, J = 1.0, 8.0 Hz, 1H, H_3), 6.95 (dd, J = 1.5, 8.0 Hz, 2H, $H_{2',6'}$), 7.07-7.12 (m, 2H, $H_{5,4'}$), 7.24 (dt, J = 1.5, 7.5 Hz, 2H, H_4), 7.30-7.33 (m, 3H, $H_{3',5',6}$).

Anal. Calcd for $C_{15}H_{13}N_3O_2$: C, 67.40; H, 4.90; N, 15.72. Found: C, 67.18; H, 5.09; N, 15.88.

4.1.10.2. 2-Amino-5-(4-chloro-2-(2-chlorophenoxybenzyl))-1,3,4-oxadiazole (11b)

Yield, 91%; mp 168-171 °C; IR (KBr): 3310, 3290 (NH_2), 1660 (C=N), 1238 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.15 (s, 2H, CH_2), 5.50 (bs, 2H, NH_2), 6.63 (s, 1H, H_3), 7.01 (d, J = 8.0 Hz, 1H, $H_{6'}$), 7.05 (d, J = 7.6 Hz, 1H, H_5), 7.16 (t, J = 7.6 Hz, 1H, $H_{4'}$), 7.17-7.28 (m, 2H, $H_{6,5'}$), 7.47 (d, J = 7.6 Hz, $H_{3'}$).

Anal. Calcd for $C_{15}H_{11}Cl_2N_3O_2$: C, 53.59; H, 3.30; N, 12.50. Found: C, 53.78; H, 3.19; N, 12.73.

4.1.10.3. 2-Amino-5-(4-chloro-2-(2-fluorophenoxybenzyl))-1,3,4-oxadiazole (11c)

Yield, 56%; mp 159-162 °C; IR (KBr): 3471, 3341 (NH_2), 1655 (C=N), 1266 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.09 (s, 2H, CH_2), 6.65 (s, 1H, H_3), 6.90-7.45 (m, 5H, aromatic).

Anal. Calcd for $C_{15}H_{11}ClFN_3O_2$: C, 56.35; H, 3.47; N, 13.14. Found: C, 56.52; H, 3.29; N, 13.22.

4.1.11. General procedure for preparation of 3-(2-aryloxybenzyl)-5-ethoxy-4*H*-1,2,4-triazoles (12a-c)

To 2-amino-5-(2-aryloxybenzyl)-1,3,4-oxadiazole (**11a-c**, 4.5 mmol) in ethanol (10 mL) was added potassium hydroxide (1.26 g, 22.5 mmol) and the solution was refluxed for 6 h. After cooling the solvent was evaporated under reduced pressure. Then water was added to the residue and the mixture was neutralized with acetic acid. The resulted white precipitate was filtered, washed with water, dried and the residue was purified by preparative TLC (hexane/ EtOAc, 65:35) to provide **12a-c**.

ACCEPTED MANUSCRIPT

4.1.11.1. 5-Ethoxy-3-(2-phenoxybenzyl)-4*H*-1,2,4-triazole (12a)

Yield, 76%; mp 132-133 °C; IR (KBr): 3441 (NH), 1583 (C=N), 1235 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38 (t, J = 7.0 Hz, 3H, CH_3), 4.06 (s, 2H, CH_2), 4.28 (q, J = 7.0 Hz, 2H, CH_2), 6.90 (d, J = 8.0 Hz, 1H, H_3), 6.94 (d, J = 7.5 Hz, 2H, $H_{2',6'}$), 7.08-7.13 (m, 2H, $H_{5,4'}$), 7.24 (dt, J = 1.5, 7.5 Hz, 1H, H_4), 7.33 (dt, J = 1.5, 7.5 Hz, 2H, $H_{3',5'}$), 7.38 (dd, J = 1.5, 8.0 Hz, 1H, H_6).

Anal. Calcd for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found: C, 69.02; H, 5.98; N, 14.10.

4.1.11.2. 5-Ethoxy-3-(4-chloro-2-(2-chlorophenoxy)benzyl)-4*H*-1,2,4-triazole (12b)

Yield, 37%; mp 90-92 °C; IR (KBr): 3249 (NH), 1238 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38 (t, J = 7.0 Hz, 3H, CH_3), 4.11 (s, 2H, CH_2), 4.28 (q, J = 7.0 Hz, 2H, OCH_2), 6.63 (d, J = 2.0 Hz, 1H, H_3), 6.98 (dd, J = 1.2, 7.8 Hz, 1H, $H_{6'}$), 7.02 (dd, J = 2.0, 8.15 Hz, 1H, H_5), 7.14 (dt, J = 1.2, 7.8 Hz, 1H, $H_{4'}$), 7.23 (dt, J = 1.2, 7.8 Hz, 1H, $H_{5'}$), 7.29 (d, J = 8.15 Hz, 1H, H_6), 7.45 (dd, J = 1.2, 7.8 Hz, 1H, $H_{3'}$).

Anal. Calcd for $C_{17}H_{15}Cl_2N_3O_2$: C, 56.06; H, 4.15; N, 11.54. Found: C, 55.88; H, 4.29; N, 11.72.

4.1.11.3. 5-Ethoxy-3-(4-chloro-2-(2-fluorophenoxy)benzyl)-4*H*-1,2,4-triazole (12c)

Yield, 37%; mp 129-131 °C; IR (KBr): 3272 (NH), 1626 (C=N), 1263 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38 (t, J = 7.0 Hz, 3H, CH_3), 4.11 (s, 2H, CH_2), 4.31 (q, J = 7.0 Hz, 2H, CH_2), 6.71 (d, J = 1.8 Hz, 1H, H_3), 7.05 (dd, J = 1.8, 8.15 Hz, 1H, H_5), 7.07 (dt, J = 1.2, 8.0 Hz, 1H, $H_{4'}$), 7.12-7.25 (m, 3H, $H_{3',5',6'}$), 7.30 (d, J = 8.15 Hz, 1H, H_6).

Anal. Calcd for $C_{17}H_{15}ClFN_3O_2$: C, 58.71; H, 4.35; N, 12.08. Found: C, 58.90; H, 4.19; N, 12.22.

4.2. Radioligand Receptor Binding Assays[21]

4.2.1. Membrane preparation: Male Sprague-Dawley rats with weights of 300-350 g (Pasteur Institute, Tehran, Iran) were anesthetized with CO_2 and then sacrificed. The cortical membrane tissue was immediately removed and homogenized for 30 s in 20 mL ice-cold Tris-HCl buffer (30 mM, pH 7.4) using a Silent S homogenizer (Heidolph, Germany) at medium speed. The homogenates were centrifuged at 600 g for 10 min using a Beckman Coulter L90K centrifuge. The resulting supernatant was centrifuged at 27000 g for 15 min. The pellet was washed 3 times

ACCEPTED MANUSCRIPT

with ice-cold buffer by re-suspension and re-centrifugation. The washed pellet was suspended in 20 mL buffer, incubated at 37°C for 30 min and then centrifuged for 10 min at 27000 g. The pellet was washed once, and the final pellet was re-suspended in 30 mL Tris-HCl buffer (50 mM, pH 7.4). All of the centrifugation was performed at 4°C.[22-24] The amount of protein was estimated in the membrane preparation by the Bradford method (1976) using bovine serum albumin (BSA) as a standard.[25] The membrane preparation was stored at -20°C until it was used 1-15 days later.

4.2.2. Saturation studies: For the saturation binding studies of [^{3}H]-flumazenil, seven different concentrations of [^{3}H]-flumazenil (ranging from 0.05 nM to 0.97 nM) were used. The amount of radioligand required to saturate the receptors was used to determine the receptor binding affinity of [^{3}H]-flumazenil (K_d) and the BZD receptor density (B_{max}) based on non-linear regression analysis of the saturation curve data.[26]

4.2.3. Competition binding studies: 100 µg of membrane protein in Tris-HCl buffer (50 mM, pH 7.4) was incubated with 8.6×10^{-5} nmole [^{3}H]-Flumazenil and increasing amount of newly synthesized ligands in a final volume 0.5mL at 30°C for 35 min.[23] After incubation, the assay was terminated by centrifugation (1500 g, 4C, 5 min). The concentration of non-radioactive ligand that inhibits the binding of [^{3}H]-flumazenil by 50% is IC_{50} value.[27-29]

4.2.4. Data Analysis: All of experiments were done in triplicates. The binding parameters (K_d and B_{max} of [^{3}H]-flumazenil) were calculated from non-linear regression analysis of the saturation curve data by using the activity base software package (Program Prism, Graph Pad, San Diego, CA). The amount of SB was calculated by subtracting NSB from total binding (TB). TB is the amount of binding of the radioligand in the absence of diazepam. A large excess of diazepam was used in the control experiments to saturate the receptor sites to determine NSB of the radioligand.[26]

4.3. Determination of anticonvulsant activity[30-31]

4.3.1. Chemicals. Pentylenetetrazole (PTZ) was purchased from Sigma (UK). It was dissolved in physiological saline solution and all compounds were dispersed in carboxymethyl cellulose (CMC, 0.5%) to such concentrations that requisite doses were administered in a volume of 10

ACCEPTED MANUSCRIPT

ml/kg. In all experiments PTZ was administered intravenously (*i.v.*) and all other drugs were administered intraperitoneally (*i.p.*) 60 min before PTZ testing.

4.3.2. Subjects. Male NMRI mice (24-30 g, Pasteur Institute of Iran) were used throughout this study. The animals were housed in temperature-controlled room (24 ± 1 °C) on a 12 h light/dark cycle with free access to food and water. All procedures were carried out in accordance with institutional guidelines for animal care and use. Each mouse was used only once and each treatment group consisted of at least eight animals.

4.3.3. Determination of seizure threshold. Threshold of PTZ-induced seizures was determined by inserting a 30-gauge butterfly needle into the tail vein of mice and the infusion of PTZ (0.5%) at a constant rate of 0.5 ml/min to unrestrained animals. Infusion was halted when forelimb clonus followed by full clonus of the body was observed. Minimal dose of PTZ (mg/kg of mice weight) needed to induce clonic seizure was measured as an index of seizure threshold.

4.3.4. Statistics. The results are expressed as the mean ± SEM of 8 animals per group. The data were statistically analyzed by one way analysis of Variance (ANOVA) followed by Tukey multicomparison test. Differences with $P < 0.05$ between experiments group were considered statistically significant.

4.4. Molecular modeling (Docking) Studies

The docking studies were performed using MOE software version 2012.10 (CCG Inc.).[32] The homology model of the Diazepam-bound $GABA_A$ receptor developed by Ernest et al. was retrieved from the supplementary material of their published paper.[37] The automated docking program of MOE 2012.10 was used to dock diazepam along with **8a** and **11a** into the diazepam binding site. The complexes were energy-minimized with the MMFF94 force field till the gradient convergence to less than 0.01 kcal/mol/Å.

A molecular database (mdb) file containing ligand conformers generated by stochastic search was used as ligand; placement was made by use of triangle matcher, rescoring and refinement were done by use of London dG and forcefield, respectively.

ACCEPTED MANUSCRIPT

Acknowledgments

This work was supported by grants from Research Council of Tehran University of Medical Sciences and Drug Design & Development Research Center.

References and Notes:

1. Smith, G. B.; Olsen, R. W. *Trends Pharmacol. Sci.* **1995**, *16*, 162.

2. Macdonal, R. I.; Olsen, R. W. *Annu. Rev. Neurosci.* **1994**, *17*, 569.

3. Sieghart, W. *Pharmacol. Rev.* **1995**, *47*, 181.

4. DeSimone, R. W.; Blum, C. A. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 2723.

5. Haefely, W.; Kyburz, E.; Gerecke, M.; Mohler, H. *Adv. Drug Res.* **1985**, *14*, 165.

6. Villar, H. O.; Davies, M. F.; Loew, G. H.; Maguire, P. A. *Life Sci.* **1991**, *48*, 593.

7. Ghose, A. K.; Crippen, G. M. *Mol. Pharmacol.* **1990**, *37*, 725.

8. Zhang, W.; Koehler, K. F.; Harris, B.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1994**, *37*, 745.

9. Zhang, P.; Zhang, W.; Liu, R.; Harris, B.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1995**, *38*, 1679.

10. Codding, P. W.; Muir, A. K. *Mol. Pharmacol.* **1985**, *28*, 178.

11. Fryer, R. I.; Cook, C.; Gilman, N. W.; Wasler, A. *Life Sci.* **1986**, *39*, 1974.

12. He, X.; Zhang, J. M.; Cook, J. M. *Med. Chem. Res.* **2001**, *10*, 269.

13. Diaz-Arauzo, H.; Koehler, K. F.; Hagan, T. J.; Cook, J. M. *Life Sci.* **1991**, *49*, 207.

14. Dumoulin, H.; Boulouard, M.; Daoust, M.; Rault, S. *Eur. J. Med. Chem*. **1998**, *33*, 201.

15. Trapani, G.; Franco, M.; Latrofa, A.; Carotti, A.; Gerichi, G.; Serra, M.; Biggio, G.; Liso, G. *Eur. J. Med. Chem*. **1996**, *31*, 575.

16. Almasirad, A.; Tabatabai, S. A.; Faizi, M.; Kebriaeezadeh, A.; Mehrabi, N.; Dalvandi, A.; Shafiee, A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 6057.

17. Akbarzadeh, T.; Tabatabai, S. A.; Khoshnoud, M. J.; Shafaghi, B.; Shafiee, A. *Bioorg. Med. Chem.* **2003**, *11*, 769.

18. Hawkins, A.F.; Terence, W.; Bracknel, L.; Jones, I. U.S. Patent 4,242,121, 1980.

19. Paduraru, M. P.; Wilson, P. D. *Org. Lett.* **2003**, *5*, 4911.

20. Shafiee, A.; Naimi, E.; Mansobi, P.; Foroumadi, A.; Shekari, M. *J. Heterocycl. Chem.* **1995**, *32*, 1235.

ACCEPTED MANUSCRIPT

21. Ahmadi, F.; Faizi, M.; Tabatabai S. A.; Beiki, D.; Shahhosseini, S. *Nucl. Med. Biol.* **2013**, *40*, 896.

22. Guerrini, G.; Ciciani, G.; Cambi, G.; Bruni, F.; Selleri, S.; Melani, F.; Montali, M.; Martini, C.; Ghelardini, C.; Norcini, M.; Costanzo, A. *Bioorg. Med. Chem.* **2008**, *16*, 4471.

23. Kahnberg, P.; Lager, E.; Rosenberg, C.; Schougaard, J.; Camet, L.; Sterner, O.; Nielsen, E. Ø.; Nilsen, M.; Liljefors, T. *J. Med. Chem.* **2002**, *45*, 4188.

24. Marini, C.; Lucacchini, A.; Ronca, G.; Hrelia, S.; Rossi, C. A. *J. Neurochem.* **1982**, *38*, 15.

25. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.

26. Motulsky, H. The GraphPad guide to analysing radioligand binding data. 1995-1996 by GraphPad software, Inc. 1-19.

27. Bigott-Hennkens, H. M.; Dannoon, S.; Lewis, M. R.; Jurisson. S. S. *Q. J. Nucl. Med. Mol. Im.* **2008**, *52*, 245.

28. Bennet, J. P. Methods in binding studies. In: Yamamura, H. l.; Enna, S. J.; Kuhar, M. J., Eds.; Neurotransmitter Receptor Binding. Raven Press: New York, 1978; pp 57-90.

29. Motulsky, H. The GraphPad guide to analysing radioligand binding data. San Diego, California: GraphPad software Inc., 1995, p 1-19.

30. Shafaroodi, H.; Asadi, S.; Sadeghipour, H.; Ghasemi, M.; Ebrahimi, F.; Tavakoli, S.; Hajrasouliha, A. R.; Dehpour, A. R. *Epilepsy Res.* **2007**, *75*, 63.

31. Navidpour, L.; Shafaroodi, H.; Miri, R.; Dehpour, A. R.; Shafiee, A. *Il Farmaco* **2004**, *59*, 261.

32. Miller, R. D.; Eriksson, L. I.; Fleisher, L.; Wiener-Kronish, J. P.; Young, W. L. Eds.; Miller's Anesthesia. Churchill Livingstone: Philadelphia, 2000.

33. Molecular Operating Environment 2012, Chemical Computing Group, Montreal, Canada.

34. Murphy, J. A.; Zhou, S.; Thomson, D. W.; Schoenebeck, F.; Mahesh, M.; Park, S. R.; Tuttle, T.; Berlouis, L. E. A. *Angew. Chem. Int. Ed.* **2007**, *46*, 5178.

35. Maiti, D.; Modak, A.; Naveen, T. *Chem. Commun.* **2013**, *49*, 252.

36. Harris, T. W.; Smith, H. E.; Mobley, P. L.; Manier, D. H.; Sulser, F. *J. Med. Chem.* **1982**, *25*, 855.

ACCEPTED MANUSCRIPT

37. Richter, L.; de Graaf, C.; Sieghart, W.; Varagic, Z.; Mörzinger, M.; Pde Esch, I. J.; Ecker, G. F.; Ernst, M. *Nat. Chem. Biol.* **2012**, *8*, 455.

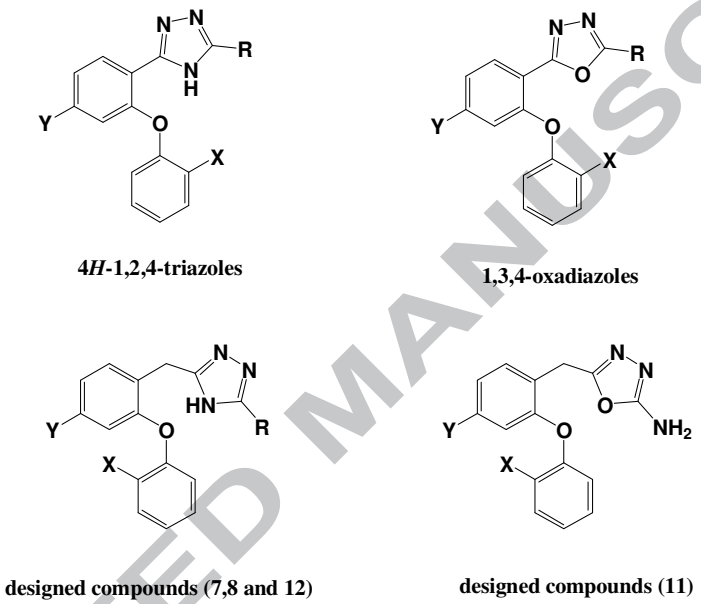


Figure 1. Representative examples of non-rigid benzodiazepine agonists and designed structures.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

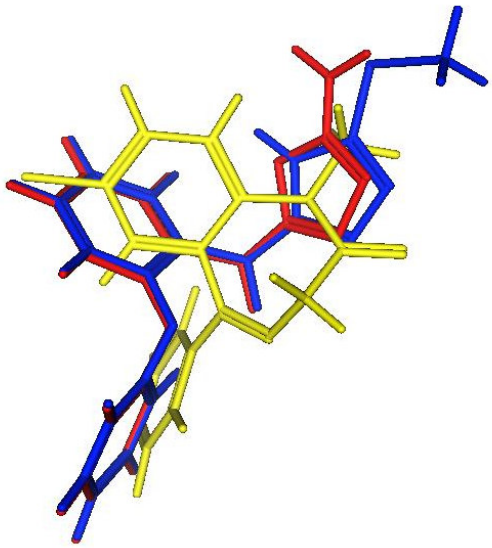


Figure 2. Superposed structures of diazepam (yellow), **8a** (blue), and **11a** (red) inside the diazepam-binding pocket of $GABA_A$ receptor.

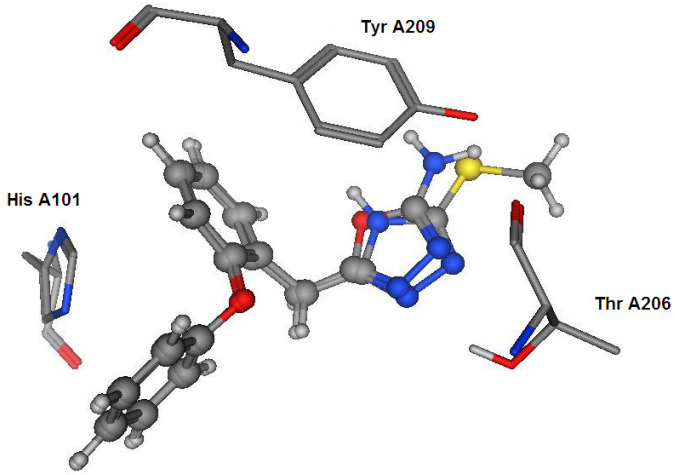
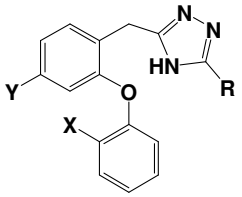


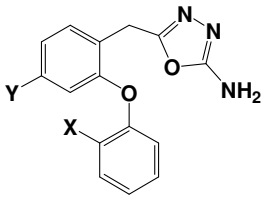
Figure 3. Interaction of **8a** and **11a** with amino acids of diazepam-binding pocket of $GABA_A$ receptor.

ACCEPTED MANUSCRIPT

Table 1. cLogP, receptor binding affinity (RBA) and clonic seizure threshold of compounds **7**, **8**, **11** and **12**.



7,8 and 12



11

Compound	X	Y	R	cLogP	RBA IC_{50} (nM)	K_d nM	Clonic seizure threshold (%)		
							5 mg/kg	10 mg/kg	20 mg/kg
7a	H	H	SH	2.81/3.47[a]	3.095	1.90	126.20±4.55*	141.88±7.85**	160.47±9.22***
7b	Cl	Cl	SH	3.60/4.27[a]	1.783	1.09	124.08±6.34*	138.87±2.65**	158.77±2.76***
7c	F	Cl	SH	4.12/4.78[a]	1.360	0.836	n.t.	n.t.	n.t.
8a	H	H	SMe	3.91	1.38	0.84	120.26±8.82	132.83±4.33*	147.06±9.46**
8b	H	H	SEt	4.30	n.t.	n.t.	132.47±4.53*	141.90±7.74**	168.55±9.75***
8c	H	H	SPr	4.60	1.238	0.75	127.77±4.76*	139.38±5.18**	157.50±16.91**
8d	H	H	SBn	5.74	2.069	1.27	129.27±3.67*	139.21±8.79**	143.40±13.33**
8e	Cl	Cl	SMe	5.21	2.52	1.54	125.02±11.27	131.61±11.20*	169.89±9.66***
8f	Cl	Cl	SEt	5.61	1.63	1.002	133.58±1.55**	139.99±1.39**	173.98±11.33***
8g	Cl	Cl	SPr	5.99	1.35	0.82	117.68±1.21	125.29±4.92*	134.09±3.36**
8h	Cl	Cl	SBn	7.05	2.23	1.36	112.52±6.37	122.18±3.84*	128.77±6.7**
8i	F	Cl	SMe	4.70	3.779	2.320	110.43±10.52	121.41±7.36	135.53±8.22**
8j	F	Cl	SEt	5.09	1.32	0.8	125.52±11.14*	144.44±5.59***	141.40±6.29***
8k	F	Cl	SPr	5.48	1.968	1.213	119.45±2.43	127.32±5.47*	133.41±6.01**
8l	F	Cl	SBn	6.54	0.892	0.544	165.66±5.17***	168.60±6.67***	178.53±16.62***
11a	H	H	-	3.03	1.901	1.171	131.63±6.56*	142.52±5.60**	174.36±9.66***
11b	Cl	Cl	-	3.82	1.753	1.098	125.22±7.77*	132.44±8.43**	170.25±4.63***
11c	F	Cl	-	4.34	1.885	1.156	111.61±4.96	128.95±5.62	154.23±12.27***
12a	H	H	OEt	3.59	3.494	2.144	122.56±3.10*	125.79±4.55*	143.52±7.30**
12b	Cl	Cl	OEt	4.38	2.13	1.3	120.06±2.36*	122.29±2.83*	134.63±4.02**
12c	F	Cl	OEt	4.89	2.07	1.26	110.82±9.73	115.67±10.85	138.25±3.99**
Diazepam					2.857	1.75	155.79±9.9***[b]	159.47±15.26***[c]	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to CMC group

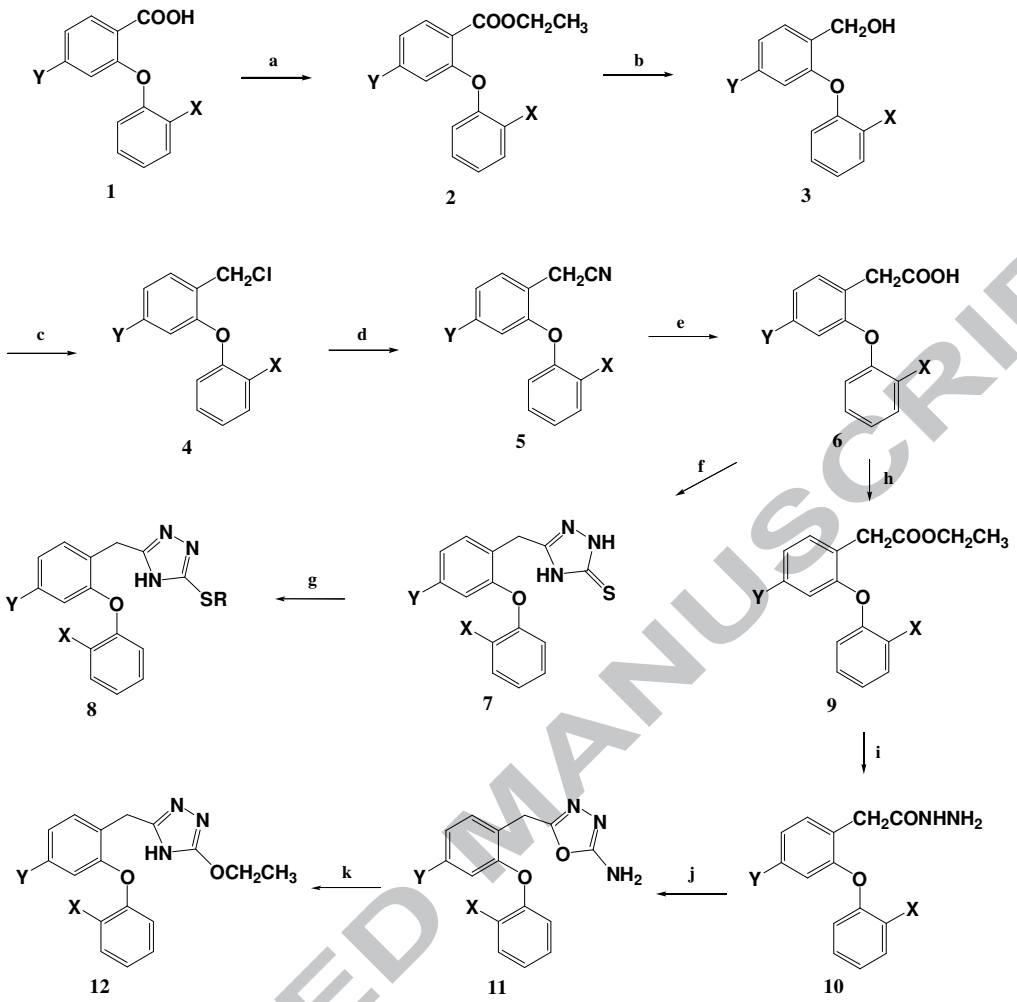
a. Two different cLogP, calculated for two tautomers.

b. Diazepam was administered at 0.05 mg/kg

c. Diazepam was administered at 0.1 mg/kg

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

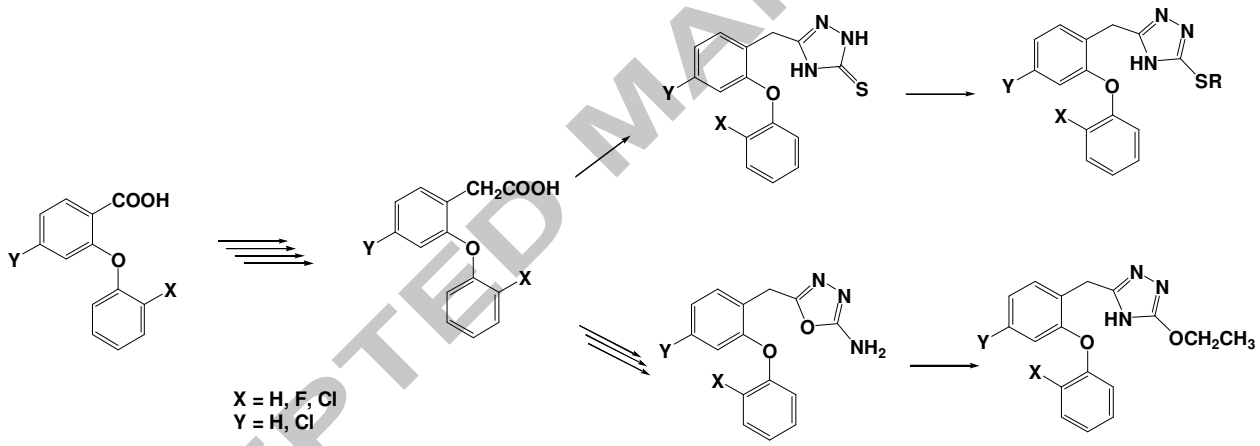


Scheme 1. Reagents and conditions: (a) $SOCl_2$, EtOH, reflux, 4 h; (b) $LiAlH_4$, THF, r.t., 24 h; (c) $SOCl_2$, benzene, pyridine, r.t., 2 h; (d) NaCN, DMSO, r.t., 24 h; (e) KOH, *n*-BuOH (or EtOH for **5b**), reflux, 24 h; (f) $SOCl_2$, 50-55 °C, 1 h then $H_2NCSNHNH_2$, pyridine, benzene, -5 °C to r.t., overnight; (g) NaOH, reflux, 4 h; (h) RI, NaOH, sonication, 5 min; (i) $SOCl_2$, EtOH, reflux, 4 h; (j) $NH_2NH_2.H_2O$, EtOH, r.t. 24 h; (k) dioxane, Na_2CO_3, H_2O, BrCN, r.t., 30 min; (l) KOH, EtOH, reflux, 6 h.

ACCEPTED MANUSCRIPT

Synthesis, receptor affinity and effect on pentylenetetrazole-induced seizure threshold of novel benzodiazepine analogues: 3-substituted 5-(2-phenoxybenzyl)-4*H*-1,2,4-triazoles and 2-amino-5-(phenoxybenzyl)-1,3,4-oxadiazoles

Siavash Mashayekh, Narges Rahmanipour, Behnaz Mahmoodi, Fatemeh Ahmadi, Dina Motaharian, Soraya Shahhosseini, Hamed Shafaroodi, Hamid R. Banafshe, Abbas Shafiee and Latifeh Navidpour*



ACCEPTED MANUSCRIPT