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Synthesis and Docking Study on Thiadiazolo[3,2-a][1,3]diazepin-8(5H)-one Derivatives as Selective GABAA agonists

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Abstract

HIE-124 is the new member of ultra-short acting hypnotics drug family. In this research, thiadiazole can act as the bio-isosteric replacement of thiazole in synthesized compounds as HIE-124 derivatives. HIE-124 drug, in which the heterocyclic thiazole ring replaced to thiadiazole, will be presented. Thiadiazolodiazepines were synthesized by a two-step reaction starting from the amino thiadiazole resulted from-various derivatives of benzoic acid and thiosemi-carbazide. In the first step, the reaction of synthetic raw material 2-amino thiadiazole and 4-chlorobutyrilchloride in toluene give the 4-chloro-N-(5-(methyl/aryl)-1,3,4-thiadiazol-2-yl)butanamide intermediate. In the next step, from the cyclization reaction of this intermediate ring in the presence of base under reflux, the target products are synthesized. Structure of products were identified based on IR, 1HNMR and 13CNMR spectroscopy analysis. The docking study of ligands were performed on the active site of GABAA that the common residues involved in allosteric modulators such as benzodiazepines and HIE-124 include ASN82, ASN81, PHE79, MET1, TYR106, ALA38 and AlA168. In the compounds 7a, 7c, 7d and 7e there is no any hydrogen bonding interaction, any π-π interactions and any π-cation interactions. According to the results the Ki's (inhibition-constant) of all compounds 7a-7e with amount 67.59, 21.45, 43.7, 83.83, and 82.85 can inhibit the enzyme more efficiently compare to HIE-124 in which has 693.3 inhibition-constant. Based on the Docking calculations the new compounds might show better interaction between receptor (GABAA) than the HIE-124.

Keywords: Thiopental Sodium; 4-Chloro-butyryl chloride; Thiosemi-carbazide; GABA A.

Introduction

Ultra-short acting benzodiazepines have an important place in the practice of anesthesiology. Benzodiazepines (BZDs) are the chemicals having the versatile medicinal values as tranquillizers and were used therapeutically as anxiolytics and anticonvulsants in epilepsy. Benzodiazepines (BZDs) are the type of psychotropic drug, that is, they concern the mind and can amend frame of mind [1]. Benzodiazepines have showed a

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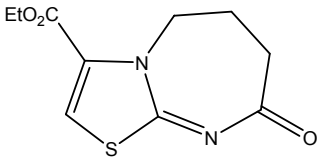
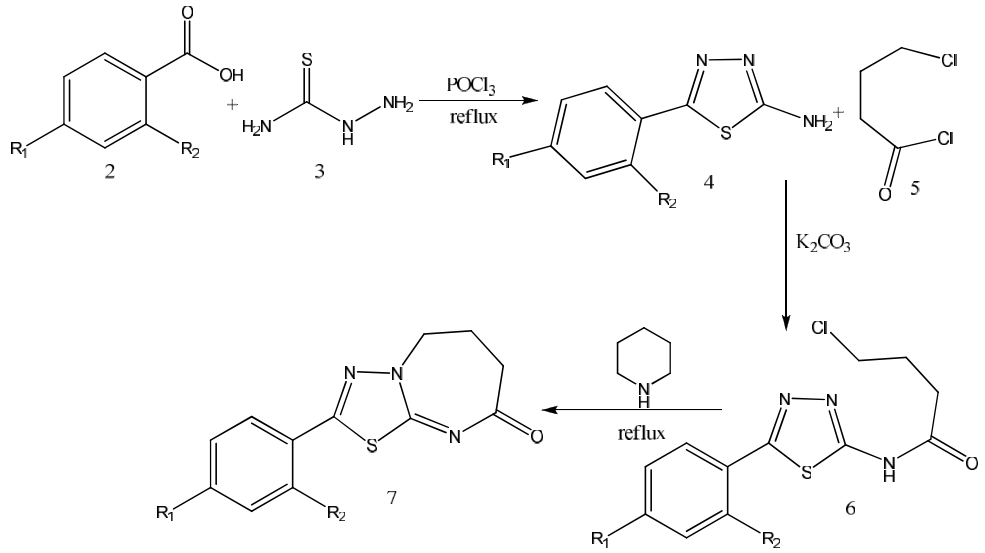
large spectrum of biological activities, for example, Thiazolo-[3,2-a][1,3] Diazepine and their derivatives show a wide range of ultra-short acting hypnotics [2-3]. Several range of chemical compounds such as benzodiazepines and some new thiazolodiazepine analogs as CNS active agents [4]. BZDs bind with specific receptors in the nervous system that are the part of GABA neurotransmitter system. GABA (gamma-amino-butyric acid) is the major neurotransmitter for the maintenance of chloride channel which controls the anxiolytic activity [5]. BZDs are source for sedation, striated (skeletal) muscle relaxation, and have anxiolytic (antianxiety) and anticonvulsant properties along with some anti-HIV activities [5-8]. In terms of chemical structure, benzodiazepines exhibit the similar mechanism of biological action like flunitrazepam, temazepam, triazolam and diazepam [7] In reference to the psychotropic activity, after entering in the brain, benzodiazepines sprayed rapidly and work after the binding to a specific type of protein (GABAAreceptor) that is also widely disseminate in the groups of nerve cells involved in anxiety, memory, sedation and coordination [9]. Benzodiazepines bind tightly to a specific part of the GABA receptor, imaginatively called the benzodiazepine site, which is different from the GABA binding site. Binding of benzodiazepine derivatives to that particular site, enhance the effect of GABA to shut down brain activity more effectively [10-11]. In the present investigation, we would like to report the synthesis of 6,7-dihydro-[1,3,4]thiadiazolo-[3,2-A][1,3]diazepin derivatives (HIE-124 1 analogous), a member of a novel class which might overcomes many of the disadvantages and problems that are usually appereae using the thiopental or benzodiazepines as intravenous anesthetic agents. HIE-124 has been shown to not only induce anesthesia but also maintain the anesthetic state during the surgical procedure. HIE-124 (Scheme 1) exhibited a very rapid onset of action and a shorter duration of action with no acute tolerance or noticeableside effects when compared with thiopental sodium. This new finding granted the issuance of a patent [12]. The thiadiazolo[3,2-a][1,3]diazepine nucleus, can be obtained by procedure of published methods [13]. 6,7-dihydro-[1,3,4]thiadiazolo-[3,2-A][1,3]diazepin derivatives (HIE-124 analogous), was synthesized according to an inventive method (Scheme 2).



Scheme 1. HIE-124



Scheme 2. Synthesis of 6,7-dihydro-[1,3,4]thiadiazolo-[3,2-A][1,3]diazepin derivatives

Materials and Methods

Molcular docking study's procedure for the synthesized compounds

In the present work, all the ligands used were made using GaussSum program (these synthesized ligands were optimized via GaussSum program softwar). Before the docking calculation of the ligands, the structures were fully optimized. Docking calculations were calculated by using Autodock 4.2.6. Crystal structure of the GABA(A) receptor associated were retrieved from Protein Databank (PDB) (http://www.pdb.org/), PDB id 1KJT, having resolution of 2.0 Å. All heteroatoms were removed from the PDB files and water molecules removed in the Notepad++ software. Kollman partial charges were assigned to all protein atoms. Autogrid was carried out for the preparation of the grid map using grid boxes of 28-28-28 A°points of 1.00A° spacing. We studied docking calculations in some grid boxes, finally we had found the best grid box for calculations it was grid boxes of 28-28-28 A°points of 1.00A°. A Lamarckian genetic algorithm (Amber force field) was used. A population of 150 individuals and 2,500,000 function evaluations were applied. Numbers of GA Runs 100 were applied. At the end of calculations, the best superimposing poses were chosen for the analysis.

Experimental

Melting points were determined with an electrothermal digital melting point instrument. Infrared (IR) spectra were recorded on a mattson 1000 FTIR. NMR spectra, were obtained in $CDCl_3$ witha Bruker DRX-300 MHz spectrometer. Chemical shifts (d) areexpressed in ppm and the coupling constants (J) in Hertz. Mass spectra were recorded on a Finnigan MAT-8430 mass spectrometer operating at an electron energy of 70 eV. Thiosemicarbazide and benzoic acid derivatives, were obtained from Fluka (Buchs, Switzerland) and were used without further purification, and amino thiadiazole derivatives were obtained via synthesized.

General procedure for synthesis of 5-aryle-1,3,4-thiadiazol-2-amine (4)

A mixture of benzoic acid derivatives (**2**, 0.02 mol) and thiosemicarbazide (**3**, 0.02 mol) in phosphoryl chloride (150 mL) was heated under reflux for 3 h. The phosphoryl chloride was then evaporated under reduced pressure. Then, the residue was quenched with water, stirred, and filtered. The solid obtained was washed, dried, and recrystallized from water to give the required product 4.

General procedure for synthesis of 4-chloro-N-(5-aryle -1,3,4-thiadiazol-2-yl)butanamide (6)

A mixture of 5-aryle-1,3,4-thiadiazol-2-amine (4, 0.02 mol) and 4-chloro-butyryl chloride (5, 0.03 mol) in toluene (100 mL) was heated under reflux for 4 h. The toluene was then evaporated under reduced pressure. Then, the residue was mixed with water, stirred, and filtered. The solid obtained was washed, dried, and recrystallized from water to give the required product 6.

4-chloro-N-(5-(nitrophenyl)-1,3,4-thiadiazol-2-yl)butanamide(6a): M.p. 218–220 C; IR(KBr)(ῡ $_{max}$, cm^{-1}): 1698 (C=O), 3137 (NH). ^{1}HNMR ($CDCl_3$) 2.09 (m, 2H, CH_2), 2.69 (t, 2H, J=7.5, CH_2), 3.71 (t, 2H, J=7.5 Hz, CH_2), 7.66 (d, 2H, J=7.5 Hz, Ar-H), 7.98 (d, 2H, J=7.5 Hz, Ar-H), 12.20 (brs, 1H, NH). ^{13}CNMR 27.8, 32.6, 45.3, 129.2, 129.8, 130.9, 135.9, 159.8, 161.2, 171.3.

4-chloro-N-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)butanamide(6b): M.p. 224–226 C; IR(KBr)(ῡ $_{max}$, cm^{-1}): 1696 (C=O), 3159 (NH). ^{1}HNMR ($CDCl_3$) 2.09 (m, 2H, CH_2), 2.68 (t, 2H, J=7.5, CH_2), 3.71 (t, 2H, J=7.5 Hz, CH_2), 7.59 (d, 2H, J=7.5 Hz, Ar-H), 7.96 (d, 2H, J=7.5 Hz, Ar-H), 12.4 (brs, 1H, NH). ^{13}CNMR 27.8, 32.6, 45.2, 129.0, 129.5, 129.9, 135.6, 159.0, 161.2, 171.2.

4-chloro-N-(5-(phenyl)-1,3,4-thiadiazol-2-yl)butanamide (6c): M.p. 198–200 C; IR(KBr)(ῡ $_{max}$, cm^{-1}): 1690 (C=O), 3180 (NH). ^{1}HNMR ($CDCl_3$) 2.07 (m, 2H, CH_2), 2.68 (t, 2H, J=7.5, CH_2), 3.70 (t, 2H, J=7.5 Hz, CH_2), 7.53, 7.93 (m, 5H, Ar-H). ^{13}CNMR 27.8, 32.6, 45.2, 127.4, 129.9, 130.6, 131.1, 159.1, 161.2, 171.2.

4-chloro-N-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)butanamide(6d): M.p. 171–172 C; IR(KBr)(ῡ $_{max}$, cm^{-1}): 1666 (C=O), 3129 (NH). ^{1}HNMR ($CDCl_3$) 2.06 (m, 2H, CH_2), 2.67 (t, 2H, J=7.5, CH_2), 3.71 (t, 2H, J=7.5 Hz, CH_2), 7.57 (d, 2H, J=7.5 Hz, Ar-H), 7.91 (d, 2H, J=7.5 Hz, Ar-H), 12.4 (brs, 1H, NH). ^{13}CNMR 27.6, 32.6, 45.2, 127.4, 129.9, 130.6, 131.2, 159.1, 161.2, 171.2.

4-chloro-N-(5-(2-fluorophenyl)-1,3,4-thiadiazol-2-yl)butanamide(6e): M.p. 188–190 C; IR(KBr)(ῡ $_{max}$, cm^{-1}): 1686 (C=O), 3159 (NH). ^{1}HNMR ($CDCl_3$) 2.06 (m, 2H, CH_2), 2.67 (t, 2H, J=7.5, CH_2), 3.71 (t, 2H, J=7.5 Hz, CH_2), 7.31 (m, 2H, Ar-H), 7.45 (d, 1H, Ar-H), 8.35 (t, 1H, Ar-H), 12.4 (brs, 1H, NH). ^{13}CNMR 27.6, 32.6, 45.2, 116.0, 116.4, 124.7, 124.8, 128.7, 128.8, 132.0, 132.1, 159.1, 161.2, 171.2.

General procedure for synthesis of 4-chloro-N-(5-aryle -1,3,4-thiadiazol-2-yl)butanamide (7)

A mixture of 4-chloro-N-(5- aryle -1,3,4-thiadiazol-2-yl)butanamide (6, 0.004 mol) and piperidine (0.8 mL, 0.008 mol) in toluene (50 mL) was heated under reflux for 3 h. The reaction mixture was cooled, poured into water, and stirred. Toluene was separated, dried, and evaporated to give a crude product, which was purified by repeated silica gel column chromatography eluting with CH_2Cl_2/hexane (80 : 20 v/v) to give 7.

2-(4-nitrophenyl)-6,7-dihydro-[1,3,4]thiadiazolo[3,2-a][1,3]diazepin-8(5H)-one (7a): M.p. 188–190 C; IR(KBr)(ῡ $_{max}$, cm^{-1}): 1725 (C=O), 1526 (C=N). ^{1}HNMR ($CDCl_3$) 2.25 (m, 2H, CH_2), 2.69 (t, 2H, J=7.5, CH_2), 4.14 (t, 2H, J=7.5 Hz, CH_2), 8.24 (d, 2H, J=8.3 Hz, Ar-H), 8.35 (d, 2H, J=8.3 Hz, Ar-H). ^{13}CNMR 27.8, 32.6, 45.3, 129.2, 129.8, 130.9, 135.9, 159.8, 161.2, 173.3.

2-(4-chlorophenyl)-6,7-dihydro-[1,3,4]thiadiazolo[3,2-a][1,3]diazepin-8(5H)-one (7b): M.p. 215–217 C; IR(KBr)(ῡ $_{max}$, cm^{-1}): 1711 (C=O), 1516 (C=N). ^{1}HNMR ($CDCl_3$) 2.22 (m, 2H, CH_2), 2.68 (t, 2H, J=7.5, CH_2), 4.13 (t, 2H, J=7.5 Hz, CH_2), 7.60 (d, 2H, J=7.5 Hz, Ar-H), 7.95 (d, 2H, J=7.5 Hz, Ar-H). ^{13}CNMR 18.2, 31.2, 45.2, 129.0, 129.4, 129.9, 135.8, 157.4, 163.9, 174.8.

2-phenyl-6,7-dihydro-[1,3,4]thiadiazolo[3,2-a][1,3]diazepin-8(5H)-one(7c): M.p. 224–227 C; IR(KBr)(ῡ $_{max}$, cm^{-1}): 1722 (C=O), 1520 (C=N). ^{1}HNMR ($CDCl_3$) 2.31 (m, 2H, CH_2), 2.71 (t, 2H, J=7.5, CH_2), 4.25 (t, 2H, J=7.5 Hz, CH_2), 7.46, 7.95 (m, 5H, Ar-H). ^{13}CNMR 18.2, 31.3, 47.9, 127.4, 128.8, 129.1, 130.6, 157.4, 163.9, 173.7.

2-(4-methoxyphenyl)-6,7-dihydro-[1,3,4]thiadiazolo[3,2-a][1,3]diazepin-8(5H)-one(7d): M.p. 210–213 C; IR(KBr)(ῡ $_{max}$, cm^{-1}): 1716 (C=O), 1509 (C=N). ^{1}HNMR ($CDCl_3$) 2.32 (m, 2H, CH_2), 2.72 (t, 2H, J=7.5, CH_2), 4.21 (t, 2H, J=7.5 Hz, CH_2), 7.44 (d, 2H, J=7.5 Hz, Ar-H), 7.95 (d, 2H, J=7.5 Hz, Ar-H). ^{13}CNMR 18.6, 32.2, 47.2, 129.4, 129.9, 130.6, 131.2, 159.1, 161.4, 172.2.

2-(2-fluorophenyl)-6,7-dihydro-[1,3,4]thiadiazolo[3,2-a][1,3]diazepin-8(5H)-one(7e): M.p. 202–204 C; IR(KBr)(ῡ $_{max}$, cm^{-1}): 1719 (C=O), 1511 (C=N). ^{1}HNMR ($CDCl_3$) 2.35 (m, 2H, CH_2), 2.75 (t, 2H, J=7.5, CH_2), 4.29 (t, 2H, J=7.5 Hz, CH_2), 7.31 (m, 2H, Ar-H), 7.45 (d, 1H, Ar-H), 8.35 (t, 1H, Ar-H). ^{13}CNMR 18.4, 31.3, 47.9, 116.0, 116.4, 124.7, 124.8, 128.7, 128.8, 132.0, 132.1, 159.1, 161.2, 173.7.

Results and Discussion

For the synthesis of HIE-124 analogues 7 (Table 1) in the first step, from reaction of benzoic acid derivatives 2 with thiosemicarbazide in the solvent of phosphoryl chloride, 5-aryle-1,3,4-thiadiazol-2-amine 4 were prepared [14]. Then, 5-aryle-1,3,4-thiadiazol-2-amine with 4-chlorobutyryl chloride 5 and potassium carbonate in toluene was heated under reflux for 4 hr. The toluene was then evaporated under reduced pressure. The solid obtained 6 was washed and dried. In the next step, Compound 6 was cyclized using piperidine as a base to produce HIE-124 analogues 7. The reaction mixture was cooled, poured into water and stirred. The toluene was then evaporated under reduced pressure to give a crude product which was purified by column chromatography. Structure elucidation of compounds 6 and 7 was obtained based on analysis of the IR, ^{1}H- and ^{13}C-NMR spectra spectrometry for each compound. Thus, the IR spectrum of 7b showed single absorption at 1711 cm^{-1} indicating the presence of carbonyl group. The ^{1}HNMR spectrum of 6b exhibits two triplet signal at δ = 2.69 ppm, δ = 3.71 ppm and a multiplet at δ = 2.09 ppm with $^3J_{HH}$ of about 7.5 Hz which are related tree methylene group which confirmed unambiguously the formation of a amide group. The off resonance decoupled ^{13}C-NMR spectra of 6b exhibited characteristic peaks for the carbonyl group and methylene group attached to the carbonyl at 171.3 and 32.6 ppm respectively. The ^{1}H-NMR spectrum of 7b exhibits two triplet signal at δ = 2.68 ppm, δ =4.13 ppm and a multiplet at δ = 2.22 ppm with $^3J_{HH}$ of about 7.5 Hz which are related to methylene group which confirmed the cyclization reaction of the intermediate compounds. The ^{13}C-NMR spectra of 7b exhibited characteristic peaks for the carbonyl group and methylene group attached to the carbonyl at 174.8 and 31.2 ppm respectively.

For the docking study, the procedure of docking of ligands were performed on the active site of GABAA that the common residues involved in allosteric modulators such as Benzodiazepines and HIE-124 include ASN82, ASN81, PHE79, MET1, TYR106, ALA38 and AlA168 (Figure 1). The analysis of the docking parameter file in this software (AutoDock) have showed that every docking contains some useful knowledge which includes Binding Energy (Eb) which is the sum of the Intermolecular Energy, the Torsional Energy and the Internal Energy which are reported in Table 2. The study and compare of all dockings indicates that HIE-124 and thiadiazolo[3,2-

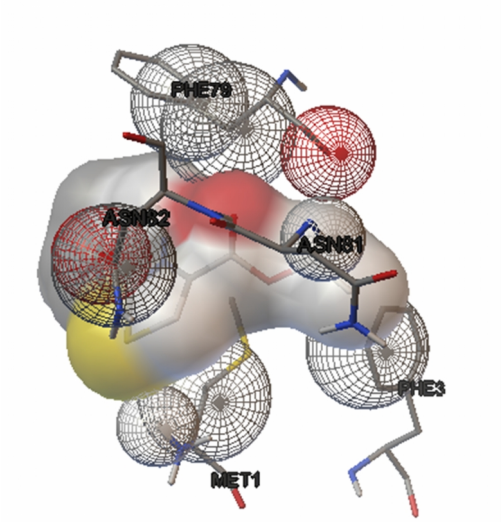


Figure 1. Docked structures of HIE-124 in GABA with pdb code 1KJT are represented

Table 1. Synthesis of 6,7-dihydro-[1,3,4]thiadiazolo-[3,2-A][1,3]diazepin derivatives

Entry	R_1	R_2	Products		
			4	6	7
a	NO_2	H	93	93	67
b	Cl	H	91	85	64
c	H	H	88	89	61
d	OMe	H	85	61	55
e	H	F	76	84	56

Table 2. Docking results Thiadiazolo[3,2-a][1,3]diazepin-8(5H)-one Derivatives by Autodock 4 software

Compound	Intermol ecular energy	Vdwhb desolve energy	Inhib- constant	Electrostatic energ	Total internal energy	Torsional energy	Unbound energy	Lgand efficienc y	Ref RMS	Building energy
HIE-124	-5.2	-5.13	693.3	-0.07	-0.4	0.89	-0.4	-0.27	8.99	-4.31
7a	-5.99	-5.96	67.59	-0.03	-0.25	0.3	-0.25	0.32	12.33	-5.69
7b	-6.97	-5.92	21.45	-1.05	-0.32	0.6	-0.32	-0,32	8.8	-6.37
7c	-6.54	-6.47	43.7	-0.07	-0.29	0.6	-0.29	-0.31	9.29	-5.95
7d	-5.86	-5.94	83.83	0.08	-0.24	0.3	-0.24	-0.33	12.81	-5.56
7e	-5.87	-5.8	82.85	-0.07	-0.22	0.3	-0.22	-0.31	13.2	-5.57

a][1,3]diazepin-8(5H)-one derivatives with the receptor protein, GABAA, According to Table 2, the complex of HIE-124 has the lowest amount of Binding Energy (-4.35) in comparing the binding energy (Eb)of other compounds.

Our docking results show that HIE-124 forms only one hydrogen bonding interactions with ASN82 (distance = 2.033) (energy = -4.021) while don't form any π-cation or π-π interactions (Figure 2). The Compound 7b has a hydrogen bonding interaction with MET1 (distance = 2.156) (energy = -1.16) while don't form any π-cation or π-π interactions (Figure 3). In the compounds 7a, 7c, 7d and 7e don't show any hydrogen bonding interaction, any π-π interactions and any π-cation interactions. According to the of Ki's amounts, all of compounds 7a-7e with 67.59, 21.45, 43.7, 83.83, and 82.85 can inhibit the enzyme more efficiently than the HIE-124 with 693.3 inhibition-constant. Docking studies have suggested that the thiadiazolo[3,2-a][1,3]diazepin-8(5H)-one Derivatives have important role in their high binding energies than HEI-124. Furthermore, our findings suggest that interaction results between receptor (GABAA) than HIE-124.

In conclusion, we synthesized a new series of 1,3-benzodiazepine derivatives 7 during two-step reaction starting from the amino thiadiazole resulted from-

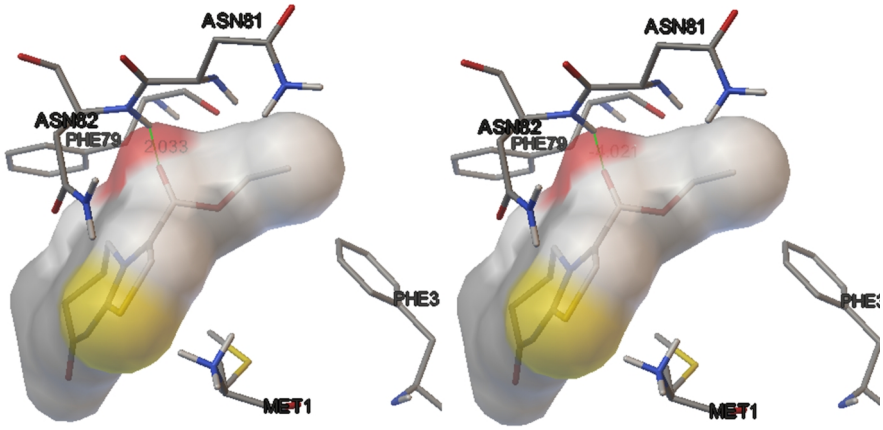


Figure 2. Docked structures of HIE-124 in GABAA with pdb code 1KJT as hydrogen bond distance and hydrogen bond energy are represented

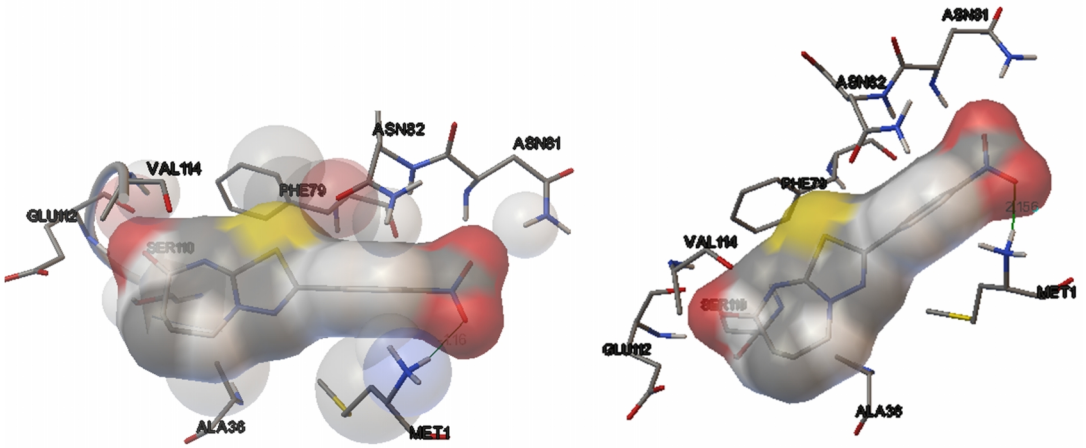


Figure 3. Docked structures of **7b** in GABAA with pdb code 1KJT as hydrogen bond distance and hydrogen bond energy are represented

various derivatives of benzoic acid and thiosemicarbazide were synthesized. The structures of the products were elucidated using IR, ^{1}H NMR and ^{13}C NMR spectral data. The docking study of ligands were performed on the active site of GABAA that the common residues involved in allosteric modulators such as Benzodiazepines and HIE-124 include ASN82, ASN81, PHE79, MET1, TYR106, ALA38 and AlA168. Consequently, These Docking calculations suggest that these new compounds can show better interaction between receptor (GABAA) than HIE-124 (besides, we had studied the AutoDock Vina calculations of these synthesized compound with this receptor (1KJT) and we observed the same results as the Docking calculations

Table 3. AutoDock Vina results Thiadiazolo[3,2-a][1,3]diazepin-8(5H)-one Derivatives by Autodock 4 software

	AutoDock Vina	**AutoDock**	
Mode	**Affinity(kcal/mol)**	**Intermolecular energy**	**Building energy**
HIE 124	-5.4	-5.2	**-4.31**
3a	-7.6	-5.99	**-5.69**
3b	-7.6	-6.97	**-6.37**
3c	-7.7	-6.54	**-5.95**
3d	-7.4	-5.86	**-5.56**
3e	-7.1	-5.87	**-5.57**

suggest (Table 3).

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