Synthesis, biological evaluation and molecular modeling study of thiadiazolo[3,2-a][1,3]diazepine analogues of HIE-124 as a new class of short acting hypnotics


Abstract

A new series of 6,7-dihydro-[1,3,4]thiadiazolo[3,2-a][1,3]diazepine analogues were synthesized, and biological evaluated. Compound GS-62 (33) exhibited potent in vivo short acting hypnotic activity with onset time, duration of sleep and therapeutic index of 6.4 ± 0.2, 94.8 ± 5.3 min, 6.62, respectively, in comparison to thiopental sodium (6). Compounds 33 did not show any sign of acute tolerance reported with the maintenance dose of 6. Meanwhile 33 potentiated the in vivo hypnotic effect of 6 in an equimolar amounts (0.06 mmol) combination showing an onset and duration of 7.5 ± 1.3, 62.5 ± 5.9 min, respectively. This combination allowed the use of lower doses of both drugs to avoid the undesirable side effects. Docking studies revealed favorable interactions and binding to BDZ binding site of the GABAA receptor especially with Arg87, Arg149, and Thr151 amino acid residues.

Keywords:
Thiadiazolodiazepine analogues
Short acting hypnotic agents
Molecular modeling study

1. Introduction

Episodes of sleep disorders such as insomnia and deterioration of sleep related to ageing processes resulted in poor day time performance and reduced quality of life. Medical treatment of insomnia started with the introduction of the first generation such as barbital, and phenobarbitone followed by chlordiazepoxide as the second generation of hypnotics [1,2]. Those two generations suffered from numerous side effects; barbiturates showed respiratory, renal and cardiovascular depression whereas benzodiazepines caused anterograde amnesia and menstrual disorders. The common disadvantages of those two generations included impaired day psychomotor performance, appearance of depressant residual actions; the morning-after effects e.g. headache, drowsiness; the precipitation of tolerance, addiction and rebound insomnia [3–6]. It was thought that ideal hypnotic was probably reached upon the discovery of Gaboxadol (1, Fig. 1) which was introduced as a new non-benzodiazepine hypnotic [7]. This is followed by the introduction of the cyclopyrrolone “Zopiclone” (2), the imidazopyridine “Zolpidem” (3) and the pyrazolopyrimidine “Zaleplon” (4) [8]. This third generation (2–4) was initially thought to be devoid of rebound insomnia yet later experience revealed their inherent capabilities to exert tolerance, dependence and rebound insomnia [3–5,9,10]. However, Gaboxadol (1) possessed a serious side effect by increasing the duration of spontaneous Petitmal seizures [11–13].

GABAA receptors regulate behavioral functions, including memory, and anxiety, and considered to be therapeutic targets for the treatment of insomnia, mood disorders, memory dysfunction, and pain. These receptors are responsive to sedative, hypnotic and...
stirred in FeCl₃ solution to get the required 2-amino-5-substituted-
the required thiosemicarbazones (and thiosemicarbazide in alcohol were mixed with stirring to get
an inventive method showed in Scheme 1. The proper aldehyde
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ultra-short acting hypnotics
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lower doses of both to avoid the undesirable side effects. The ob-
shorter than that of
potentiating effect toward the known
were then heated under reflux with piperidine in toluene to
the disadvantages and problems that usually associated with the
2.1. Chemistry
pounds of the present study (and thiosemicarbazide in alcohol were mixed with stirring to get
adverse reactions [18–20].
In the present investigation, 6,7-dihydro-[1,3,4]thiadiazolo[3,2-
diazepine analogues were synthesized as a new structure
modification derivatives of the previously patented agent “HIE 124, 5”. The new compounds proved to possess short acting hypnotic
activity in addition to in vivo potentiating effect toward the known
ultra-short acting hypnotics; this combination allowed the use of
lower doses of both to avoid the undesirable side effects. The obtained
results contributed in part to the issuance of European and
US patents [21,22].

2. Results and discussion
2.1. Chemistry
1,3,4-Thiadiazolo[3,2-a][1,3]diazepine analogues usually obtained by the use of reported literature procedures [23,24]. Com-
pounds of the present study (30–36) were synthesized according to an inventive method showed in Scheme 1. The proper aldehyde and thiosemicarbazide in alcohol were mixed with stirring to get the required thiosemicarbazones (9–15). Compounds 9–15 were stirred in FeCl₃ solution to get the required 2-amino-5-substituted-1,3,4-thiadiazoles (16–22) [25]. Compounds 16–22 were then acylated with 4-chlorobutyril chloride in toluene at room temperature to afford 4-Chloro-N-(5-substituted-1,3,4-thiadiazol-2-yl) butanamide analogues (23–29), which were purified by silica gel and neutral alumina chromatography. The butanamide analogues 23–29 were then heated under reflux with piperidine in toluene to give the cyclized derivatives 2-substituted-6,7-dihydro-thiazolo [3,2-a][1,3]diazepin-8(5H)-ones (30–36), Scheme 1, Table 1. Structure elucidation of the new synthesized intermediates and final products was attained by the aid of elemental analysis, ¹H, ¹³C NMR, and Mass spectrometry.

Fig. 1. Structures of some literature hypnotic lead compounds.

2.2. Evaluation of the hypnotic activity of compounds 30–36
The hypnotic activity of test compounds 30–36 in mice was measured using the standard righting reflex method [26–29]. Intraperitoneal administration of compounds 30, 31, 33 (GS-62), 34 (GS-53), 35 and thiopental sodium (6) but not 32 or 36 at doses of 0.2–2 mmol/kg into mice induced hypnosis and the loss of the righting reflex albeit with wide variations. The obtained minimal effective dose, onset time and duration of sleep for each compound are shown in Table 2. The onset (6.4 ± 0.2 min) and duration of sleep (94.8 ± 5.3 min) for compound 33 (GS-62) were significantly greater than the values obtained for the reference drug (6) (2 ± 0.1 min, 45 ± 3.6 min, 6.1 respectively; P < 0.05; n = 6). Compounds 32 and 36 showed no hypnotic activity; while 34 and 35 showed respiratory depression and thus the duration was not calculated. The onset and duration times of sleep induced by 30 and 31 were significantly different compared with those of GS-62 (33) and 6, with delayed onsets and shorter durations. Compound GS-63 (33) seemed to possess a delay in regard to the induction of the onset of sleep but twice as potent regarding the duration (P < 0.05, N = 6). The LD₅₀ values and the therapeutic indices of the tested compounds were calculated. Compounds were given intraperitoneally in doses ranging from 0.1 to 5 mmol/kg [30]. The percentage of death at each dose level was then calculated, and the LD₅₀ values were obtained, Table 2. It can be clearly observed, that the Therapeutic Index of 33 (GS-62) is similar to that of 6. The Therapeutic Indices of compounds 30 and 31 were 53–57% of 33. Compounds GS-53 (34) and 35 seemed to be toxic causing respiratory failure to the tested animals. Thus, compound GS-62 (33) emerged as the most effective and the safest among the tested compounds. Its safety is comparable to that of thiopental sodium (6), but its duration is significantly longer. Compounds 33 and 34 did not show any sign of acute tolerance reported with the second (maintenance) dose of 6.

2.3. Mode of action of the test compounds induced hypnosis
In order to study the mode(s) of action of the hypnotic activity of compounds GS-62 (33) or GS-53 (34), animals were injected with the minimal hypnotic doses (0.4 mmol/kg, i.p.). Following induction of sleep, each animal was injected with caffeine as adenosine A₁ receptor blocker at doses up to 800 mg/kg [31]; ketanserin as serotonin S₂ (5-HT₂) receptor blocker at 3 mg/kg [32]; picrotoxin as

general anesthetic drugs [14]. Several drugs are currently in the pipeline for treating insomnia, high development costs limit the further development of new drugs [15–17]. The ultra-short acting hypnotic activity of ethyl 8-oxo-5,6,7,8-tetrahydro-thiazolo[3,2-a][1,3]diazepin-3-carboxylate (HIE-124, 5, Fig. 1), overcame many of the disadvantages and problems that usually associated with the use of thiopental sodium (6) as intravenous anesthetic agents. Compound 5 proved to be a very short acting hypnotic with onset of action less than 60 s, and its duration of action is significantly shorter than that of 6. Compound 5 did not show any sign of acute tolerance [18–20].

In order to study the mode(s) of action of the hypnotic activity of compounds GS-62 (33) or GS-53 (34), animals were injected with the minimal hypnotic doses (0.4 mmol/kg, i.p.). Following induction of sleep, each animal was injected with caffeine as adenosine A₁ receptor blocker at doses up to 800 mg/kg [31]; ketanserin as serotonin S₂ (5-HT₂) receptor blocker at 3 mg/kg [32]; picrotoxin as
GABAA receptor blocker at doses up to 40 mg/kg [33]; and flumazenil as non-selective but specific benzodiazepine receptor blocker at 3 mg/kg [34–36]; or a combination of flumazenil and picrotoxin in the above indicated doses. The animals were then carefully observed for arousal from sleep and regaining of the righting reflex. Regaining of the righting reflex following injection of any of the above treatments was considered as antagonism of the mechanism(s) involved in the induced sleep. The onset time for reversal was noted. The results of the experiments revealed the failures of caffeine, ketanserin, and flumazenil to reverse compounds GS-62 (33) and GS-53 (34) induced hypnosis. This proves the dis-involvement of the brain adenosinergic, serotoninergic and benzodiazepinergic systems or receptors in the induced sleep. However, the tendency of reversal of the induced sleep following the administration of picrotoxin alone or the full reversal of sleep and regaining of the righting reflex following the administration of combination of picrotoxin and flumazenil clearly proved the involvement of the combined activation of GABAA and benzodiazepine 1 (Bnz-1) types of receptors either directly or indirectly.

2.4. Evaluation of the test compounds synergistic effect on thiopental sodium sleep induction

Combined Intraperitoneal administration of one tenth of the hypnotic dose of 33 and 34 (0.03–0.06 mmol/kg) and one third of the hypnotic dose of 6 (0.06 mmol/kg, P < 0.05, N = 6) synergized
each other and produced a stable sleep of rapid onset and significantly longer duration than that induced by 6 alone. Combination of an equimolar amounts (0.06 mmol) of 33 and 6 produced an onset of 7.5 ± 1.3 min and duration of 62.5 ± 5.9 min; combination of 34 (0.03 mol) and 6 (0.06 mol) produced an onset of 3.1 ± 1.1 min and duration of 75 ± 4.3 min; while thiopental sodium alone (6, 0.2 mol) showed an onset of 2 ± 0.3 min and duration of 45 ± 3.6 min (Table 3).

3. Structure activity correlations

The type of substituents at position 2- of the 1,3,4-thiadiazolo[3,2-α][1,3]diazepine analogues affected the onset and duration times of the hypnotic activity. Aliphatic or aromatic substituents at 2- position produced compounds with variable onsets (6.4–43.4 min). The presence of 2-methyl function produced compound 30 with onset time and duration of 25 ± 4.1, 6 ± 1.3 min, respectively. Replacement of the 2-methyl group by 2-trifluoromethyl moiety produced 31 with shorter onset and longer duration. The introduction of 2-ethyl group with one carbon atom longer afforded 32 with the loss of hypnotic activity. The presence of phenyl ring at position 2- produced the most active compound in the present study (33) with onset and duration of 6.4 ± 0.2, 94.8 ± 5.3 min; respectively. Introduction of 4-bromo or 4-methyl function to the phenyl ring of 33 produced 34 and 35 with respiratory depression, while the presence of 4-nitro group produced 36 with complete loss of hypnotic activity.

4. Molecular modeling study

4.1. Docking study

In order to further explore whether the test compounds could fit into the benzodiazepine (BZD)-binding site of the GABA<sub>A</sub> receptor and hence possibly rationalize the results of the in vivo experiments, the most active short acting hypnotic derivative 33, the least active derivatives 32, 36 as well as 34 which caused respiratory depression were docked against the appropriate standard GABA<sub>A</sub> agonist “diazepam”, into the extracellular domain of the receptor. Although its crystal structure has not been available yet, there are many homology models which are in a suitable matching with the experimental findings [37,38]. A recently reported unified model of GABA<sub>A</sub> receptor complex based on the glutamate-gated chloride ion channel was selected. The BDZ binding site is located at the interface of GABA<sub>A</sub> receptors, taking into account that the most reliable docking parameters for the evaluation of the whole extracellular domain of the receptor was explored [39]. Conformational analysis of the selected hypnotic compounds by AM1 calculation led to the best conformers suited for the docking study (Figs. 2 and 3). The favorable calculated pose for diazepam (binding energy 10.55 kcal/mol) was assessed.

Docking of diazepam identified a triad of amino acid residues, Arg87 and Arg149 which bind with diazepam via cationic-arene bonding through its aromatic ring substituent, in addition to Thr150 with a hydrogen bonding interaction of its carbonyl oxygen with a percentage of 15% which showed pronounced influence to higher diazepam affinity (Fig. 4a). Providing a considerable attention to the tested biologically active hypnotic derivatives of this study, compound 33 binds to the BDZ site via Arg87 and Arg149 by cationic-arene interaction; the same as diazepam but both amino acids binds with its aromatic ring substituent (Fig. 4b) while compound 34 binds with Arg87 residue by cationic-arene and with Thr151 by hydrogen bonding through its diazepine ring carbonyl oxygen by 59%, which probably explain its different behavior and the respiratory depression effect (Fig. 4c). Thus compound 33 could exert its action via BDZ binding site agonism at GABA<sub>A</sub> receptor. When compared to diazepam, it is clearly seen that 32 and 36 did not occupy any regions of the model that could lead to any binding (Fig. 5a,b), therefore, not expected to act as full agonists like diazepam, as they do not have the necessary required interactions into the model under study for positive allosteric modulation of the BDZ receptor.

According to the docking study results, the difference in the biological activity of the tested compounds despite of their similar chemical structure could be explained. It could be noted that the presence of aromatic substituent directly attached to the thiazole

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Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Effective dose mmol/kg (i.p.)</th>
<th>Onset of sleep (minutes)</th>
<th>Duration of sleep (minutes)</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; mmol/kg (i.p.)</th>
<th>Therapeutic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>1.0</td>
<td>25 ± 4.1**</td>
<td>6 ± 1.3**</td>
<td>3.55</td>
<td>3.55</td>
</tr>
<tr>
<td>31</td>
<td>0.8</td>
<td>14 ± 2.6**</td>
<td>14 ± 2.9**</td>
<td>2.74</td>
<td>3.32</td>
</tr>
<tr>
<td>32</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>33 (GS-62)</td>
<td>0.4</td>
<td>6.4 ± 0.2**</td>
<td>94.8 ± 5.3**</td>
<td>2.65</td>
<td>6.62</td>
</tr>
<tr>
<td>34 (GS-53)</td>
<td>0.4</td>
<td>43.4 ± 8.3</td>
<td>N.A.</td>
<td>0.6</td>
<td>1.5</td>
</tr>
<tr>
<td>35</td>
<td>1.6</td>
<td>20 ± 2.8</td>
<td>N.A.</td>
<td>1.0</td>
<td>1.25</td>
</tr>
<tr>
<td>36</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Thiopental Na</td>
<td>0.2</td>
<td>2 ± 0.1*</td>
<td>45 ± 3.6*</td>
<td>1.22</td>
<td>6.1</td>
</tr>
</tbody>
</table>

*: Significantly longer compared with that of thiopental Na (P < 0.05, N = 6).
N.A.: Values are not available due to Respiratory depression.
**: Significantly different compared with 33 (GS-62) and Thiopental Na.

Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mmol/kg, i.p.)</th>
<th>Onset time of sleep (minutes)</th>
<th>Duration of sleep (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>0.05</td>
<td>15 ± 1.5</td>
<td>5 ± 0.8*</td>
</tr>
<tr>
<td>Thiopental</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>0.06</td>
<td>7.5 ± 1.3*</td>
<td>62.5 ± 5.9*</td>
</tr>
<tr>
<td>Thiopental</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>0.03</td>
<td>3.1 ± 1.1</td>
<td>75 ± 4.3*</td>
</tr>
<tr>
<td>Thiopental</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>0.2</td>
<td>2 ± 0.3</td>
<td>45 ± 3.6</td>
</tr>
</tbody>
</table>

*P < 0.05, compared with thiopental alone (N = 6).
core without a spacer, seems to be crucial for the interaction with the BZD-binding pocket. One of the explanations (within the “aromatic” series) could be illustrated in conformational freedom property to the molecule. Thus, important structural characteristics of the active molecules may have favorable, diazepam like spatial arrangements. The inactive molecules such as 32 and 36 were much more constrained and possibly the absence of aromatic core in 32 or having certain structural attributes (such as highly electron withdrawing group) attached to the aromatic ring as in 36 cannot avoid the unfavorable interactions with “forbidden” regions of the receptor.

4.2. Flexible alignment and superposition

Ligand-based active site alignment is a well-known technique for structural analysis of ligand complexes. In the present study, flexible alignment comparative modeling experiment among the most active 33 and the least active 32 against diazepam was performed. An alignment is considered to be successful when the molecule strain energy is small, possessing similar shapes and their aromatic atoms overlap [40]. The goal of computing a multiple ligand alignment of a set of input ligands is to maximize the similarity between these ligands, while placed in sound
conformations. In order to test the similarity between the 3D structures of the most active 33 and diazepam, flexible alignment was performed using MOE/MMFF94 to generate superposition with minimal user bias [41]. It was interesting to note the similarity in the direction of flipping mode of both 33 and diazepam (Fig. 6a) while 32 seems to have different alignment angle which prevent its proper far reach into the receptor active site (Fig. 6b).

Superposition of 3D putative ligands could help to deduce specific structural requirements for biological activity [42,43]. Structure superposition of 33 and 34 (Fig. 7a) with mark points such as, the \( \pi \)-systems represented by aryl groups of the thiazolodizepine ring system core, phenyl ring substituent, and both N and S center atoms, have a fairly well alignment. This superposition implies the possible existence of region(s) on the receptor suitable for the specific binding and recognition of the previously mentioned features. This assumption is in accordance with the obtained experimental data. Moreover, these selected mark points could be used as template model for further optimization. In addition, upon conducting the flexible alignment experiments it was noticed that there is a similarity in the alignment profiles among the inactive derivatives 32 and 36 (Fig. 7b). On the other hand, applying the same conditions on the active 33 versus the inactive 36 showed a different pattern with the minimal alignment profile (Fig. 7c) which explains their different biological activity behavior.

4.3. Surface mapping

In a valuable extension, surface mapping comparing study among the most active 33, and the least active counterpart 32 against diazepam was performed. Compound 33 showed hydrophilic region (in red), hydrogen bond acceptor–donor region located on the 4-carbonyl, N-1, non-polar area located on the thiazole ring (in green), in addition to the mildly non polar center on the aryl moiety which are generally having a similar structural features with the surface mapping distribution of diazepam (Fig. 8a,b). On the contrary there is a clear difference in surface mapping distribution between 32 and diazepam (Fig. 8a,c). These results provided structure mapping similarity and hence explained the biological resemblance of 33 and diazepam. These findings supported the hypothesis that the tested compounds might exert their hypnotic action via GABA\(_A\) agonism.

4.4. Molecular properties and drug-likeness

Molecular properties are mixture of structural balanced features which determine the feasibility of comparing particular molecule to known drugs. Acceptable drug bioavailability could be achieved upon reaching an appropriate balance between solubility and partitioning properties. The most active compounds in this study were subjected to solubility prediction analysis, their conformity with Lipinski’s rule of five, in addition to other properties shown in
Table 4. The Lipinski’s rule of five depends on the statistical calculations stating that the most biologically active drugs have number of hydrogen bond donor not higher than 5, hydrogen bond acceptor sites not higher than 10, molecular weight of not more than 500, and logP values not higher than 5 [44]. Topological polar surface area (TPSA) and number of rotatable bonds are linked to drug bioavailability. Molecules violating more than one of Lipinski’s rule of five, and molecules with more than 10 rotatable bonds may have problems with bioavailability [45–47]. TPSA is a feasible descriptor for drug absorption, including intestinal bioavailability and blood–brain barrier penetration. Molecules with TPSA values around 140 Å or less are expected to exhibit high intestinal absorption [45]. All descriptors were calculated by the MOE package for the active compounds. Results shown in Table 4 indicated that compounds 33 and 34 have TPSA values < 140 Å, and all compounds have zero violation of Lipinski’s rule; therefore, they are not expected to have problems with bioavailability. Based on these data, it could be suggested that 33 and 34 have a lipinski drug likeness of 1 which make them suitable drug candidates in addition to their expected orally absorbed tendency as short acting hypnotics.

5. Conclusion

The obtained results clearly point to the discovery of compounds GS-62 (33) and GS-53 (34) as a new group of short acting hypnotics.

Table 4

<table>
<thead>
<tr>
<th>Compound</th>
<th>b_rotN</th>
<th>Lip_acc</th>
<th>Lip_don</th>
<th>Lip_druglike</th>
<th>Lip_viol</th>
<th>LogP</th>
<th>TPSA</th>
<th>Mwt</th>
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</thead>
<tbody>
<tr>
<td>33</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3.77</td>
<td>45</td>
<td>245.30</td>
</tr>
<tr>
<td>34</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4.57</td>
<td>48</td>
<td>324.20</td>
</tr>
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</table>

hypsnotics that induce their actions via interaction with GABA<sub>A</sub> and benzodiazepine ω<sub>1</sub> receptors as proven experimentally. Thus, a new means for treatment of insomnia with all of its sleep disorders seemed to be at hand. The safety of the new compounds is comparable to that of thiopental sodium (6) and its duration is significantly longer. In addition, 33 and 34 did not show any sign of acute tolerance reported with the second (maintenance) dose of 6. Therefore, the new compounds have the potential use as a preanesthetic medication, induction of anesthesia, and treatment of insomnia. Combined administration of one tenth of the hypnotic dose of 33 and 34 together with one third of 6, both in doses lower than the effective dose, attained the same hypnotic potency avoiding the drawbacks and side effects associated with 6 full dose administrations. The durations were significantly longer than that induced by the hypnotic dose of 6 alone. Fig. 9 showed the structures of the most active short acting hypnotics GS-62 (33) and GS-53 (34). Molecular docking interactions between the active ligands 33, 34 and diazepam at interface residues in GABA<sub>A</sub> receptor extracellular domain homology model have been calculated showing two types of interactions. A cationic-arene interaction with Arg 87 and Arg149 in addition to hydrogen bonding interactions showing two types of interactions. A cationic-arene interaction with the proposed structures within 6.1.1. General procedure for the preparation of 4-Chloro-N-(5-substituted-1,3,4-thiadiazol-2-yl)butanamide (23 – 29)

A mixture of 5-substituted-1,3,4-thiadiazol-2-amine (16 – 22, 0.04 mol), 4-chloro-butyl chloride (11.3 g, 0.10 mol) and potassium carbonate (5.5 g, 0.04 mol) in toluene (100 ml) was stirred at room temperature for 4 h. The toluene was then evaporated under reduced pressure. The residue was then quenched with water, stirred, and filtered then purified by silica gel and neutral alumina chromatography. The obtained solid was washed, dried and recrystallized to give the required products 23 – 29 (Table 1).

6.1.1.1. 4-Chloro-N-(5-(methyl)-1,3,4-thiadiazol-2-yl)butanamide (23).

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 1.88 – 2.04 (m, 2H, –CH<sub>2</sub>), 2.43 – 2.72 (m, 2H, –CH<sub>2</sub>), 3.69 (s, 3H, –CH<sub>3</sub>), 3.69 – 3.72 (m, 2H, –CH2), 7.04 (7.5 Hz, 2H, –CH<sub>2</sub>), 10.74 (br s, 1H, NH). <sup>13</sup>C NMR: δ 20.3, 26.3, 33.1, 44.6, 142.3, 153.1, 171.7 MS m/z (%): 219 (25.7, M<sup>+</sup>).

6.1.1.2. 4-Chloro-N-(5-trifluoromethyl)-1,3,4-thiazolid-2-yl)butanamide (24).

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 1.91 – 2.02 (m, 2H, –CH<sub>2</sub>), 2.47 (t, J = 7.0 Hz, 2H, –CH<sub>2</sub>), 3.69 (t, J = 7.0 Hz, 2H, –CH<sub>2</sub>), 10.65 (br s, 1H, NH). <sup>13</sup>C NMR: δ 14.3, 25.2, 26.9, 33.3, 43.4, 153.4, 164.5, 171.8 MS m/z (%): 273 (14, M<sup>+</sup>).

6.1.1.3. 4-Chloro-N-(5-ethyl-1,3,4-thiadiazol-2-yl)butanamide (25).

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 1.21 (t, J = 7.5 Hz, 3H, CH<sub>3</sub>), 1.89 – 2.06 (m, 2H, –CH<sub>2</sub>), 2.41 (t, J = 7.0 Hz, 2H, –CH<sub>2</sub>), 3.02 – 3.31 (q, J = 7.5 Hz, 2H, –CH<sub>2</sub>), 3.64 (t, J = 7.0 Hz, 2H, –CH<sub>2</sub>), 10.35 (br s, 1H, NH). <sup>13</sup>C NMR: δ 14.3, 25.2, 26.9, 33.3, 43.4, 153.4, 164.5, 171.8 MS m/z (%): 273 (14, M<sup>+</sup>).

6.1.1.4. 4-Chloro-N-(5-phenyl-1,3,4-thiadiazol-2-yl)butanamide (26).

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 2.07 – 2.10 (m, 2H, –CH<sub>2</sub>), 2.67 – 2.70 (m, 2H, –CH<sub>2</sub>), 7.30 – 7.32 (m, 2H, –CH<sub>2</sub>), 7.48 – 7.54 (m, 3H, ArH), 7.93 – 7.94 (m, 2H, ArH), 12.65 (br s, 1H, NH). <sup>13</sup>C NMR: δ 27.9, 32.7, 45.1, 127.4, 129.8, 130.7, 131.0, 158.5, 162.4, 171.2 MS m/z (%): 281 (15.7, M<sup>+</sup>).

6.1.1.5. N-(5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl)-4-chlorobutanamide (27).

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 1.96 – 2.22 (m, 2H, –CH<sub>2</sub>), 2.69 – 2.81 (m, 2H, –CH<sub>2</sub>), 3.71 – 3.72 (m, 2H, –CH<sub>2</sub>), 7.49 – 7.88 (dd, J = 7.0 Hz, 4H, ArH), 12.76 (br s, 1H, NH). <sup>13</sup>C NMR: δ 27.3, 32.1, 44.6, 123.8, 128.7, 129.4, 132.3, 158.5, 160.7, 170.7 MS m/z (%): 360 (15.7, M<sup>+</sup>).

6.1.1.6. 4-Chloro-N-(5-(4-methylphenyl)-1,3,4-thiadiazol-2-yl)butanamide (28).

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 2.07 – 2.09 (m, 2H, –CH<sub>2</sub>), 2.38 (s, 3H, CH<sub>3</sub>), 2.68 (t, 2H, J = 14.5 Hz, CH<sub>2</sub>), 3.71 (t, 2H, J = 14.5 Hz, CH<sub>2</sub>), 7.34 (d, 2H, J = 7.5 Hz, Ar-H), 7.83 (d, 2H, J = 7.5 Hz, Ar-H), 12.68 (s, 1H, NH). <sup>13</sup>C NMR: δ 20.9, 27.3, 32.1, 44.7, 126.8, 127.5, 129.9, 140.5, 157.9, 161.8, 170.6 MS m/z (%): 295 (3.5, M<sup>+</sup>).

6.1.1.7. 4-Chloro-N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)butanamide (29).

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 2.35 – 2.40 (m, 2H, –CH<sub>2</sub>), 2.78 (t, 2H, J = 7.5 Hz, Ar-H), 7.34 – 7.36 (m, 2H, –CH<sub>2</sub>), 7.48 – 7.61 (m, 3H, Ar-H), 12.68 (br s, 1H, NH). <sup>13</sup>C NMR: δ 26.8, 32.9, 44.7, 126.8, 127.5, 129.9, 140.1, 157.9, 161.8, 170.5 MS m/z (%): 354 (15.7, M<sup>+</sup>).
2H, J = 12.0 Hz, CH₂), 4.31 (t, 2H, J = 12.0 Hz, CH₂). 7.26 (s, 1H, NH), 8.15 (d, 2H, J = 8.5 Hz, Ar-H), 8.35 (d, 2H, J = 8.5 Hz, Ar-H). ¹³C NMR: δ 18.3, 31.2, 47.9, 125.0, 128.7, 129.5, 132.3, 157.5, 162.9, 173.9. MS m/z (%): 324 (70.0 M⁺).


The hypnotic activity of the test compounds in mice was measured using the standard righting reflex method as described [26–29,48,49] with some modifications. Mice were initially tested for the presence of the righting reflex by placing each mouse on its back and observing the rapid correction to the normal position i.e. the righting reflex. Then groups of mice were injected (i.p) with various doses of the test compound and placed each separately under a 30–cm glass funnel. The animals were then observed carefully for any change in behavior such as unsteady movements, drowsiness, ataxia and loss of the righting reflex. Failure of any treated mouse to correct its posture to the normal condition of standing on its feet within one minute was considered as loss of the righting reflex and hence onset of sleep. The onset of sleep was carefully noted and recorded and the mice were continuously monitored visually and by videotaping and the duration of the sleep was noted. The end of the duration of sleep was noted by the regaining of the righting reflex 3 times within one minute albeit some drowsiness is still observed. Intraperitoneal administration of compounds 30–36 and thiopental sodium in doses of 0.2–2 mmol/kg were performed and the minimal effective doses, the onset times and the durations of sleep were recorded, Table 2.

6.3. Study of the hypnotic effect mode(s) of action of compounds 30–36

6.3.1. 2-Methyl-6,7-dihydro-1H,3H-thiadiazol[3,2-a][1,3]diazepin-8(5H)-one (30).

6.3.2. 2-(Trifluoromethyl)-6,7-dihydro-1H,3H-thiadiazol[3,2-a][1,3]diazepin-8(5H)-one (31).

6.3.3. 2-Ethyl-6,7-dihydro-1H,3H-thiadiazol[3,2-a][1,3]diazepin-8(5H)-one (32).

6.3.4. 2-Phenyl-6,7-dihydro-1H,3H-thiadiazol[3,2-a][1,3]diazepin-8(5H)-one (33).

6.3.5. 2-(4-Bromophenyl)-6,7-dihydro-1H,3H-thiadiazol[3,2-a][1,3]diazepin-8(5H)-one (34).

6.3.6. 2-(4-Methylphenyl)-6,7-dihydro-1H,3H-thiadiazol[3,2-a][1,3]diazepin-8(5H)-one (35).

6.3.7. 2-(4-Nitrophenyl)-6,7-dihydro-1H,3H-thiadiazol[3,2-a][1,3]diazepin-8(5H)-one (36).

6.4. Potentiating effect of compounds 33 (GS-62) or 34 (G-53) to the non-hypnotic dose of thiopental sodium in mice

Combined intraperitoneal administration of GS-62 (33) or G-53 (34) and thiopental sodium in doses of 0.03–0.06 mmol/kg into mice induced hypnosis. The minimal effective doses, the onset times and the durations of sleep were recorded (Table 3).

6.5. Determination of the lethal dose (LD₅₀) and the therapeutic indices of compounds 30–36

Male mice were divided into various groups and test compounds were administered in various doses ranging from 0.1 to 5 mmol/kg, (i.p). Following treatments, the animals were observed for up to 6 h continuously and were then kept under observation for 72 h. All behavioral changes and death during the observation periods were recorded. The percentage of death at each dose level was then calculated, converted to probits and the LD₅₀ values were calculated as reported [30]. The Therapeutic Index of test compounds were calculated following the determination of the minimal effective hypnotic and the LD₅₀ values (Table 2) by the formula:

\[ \text{TI} = \frac{\text{LD₅₀ of the non-effective hypnotic dose}}{\text{Minimal effective hypnotic dose}} \]
6.6. Docking and molecular modeling study

Conformational analysis of some selected compounds represent the active short hypnotic derivatives (33 and 34), and the least active counterparts 32 and 36, along with diazepam as a standard were performed. Basically implemented using MMX force field method followed by AM1 calculation performed in MOE 2009.10. The model of the GABA<sub>A</sub> receptor was used for docking [39]. Three-dimensional structures of the selected thiaziolodiazepine representatives, in their neutral forms were built using the MOE of Chemical Computing Group Inc software. Lowest energy conformer of every new analogue ‘global-minima’ was docked into the homology model used. The energy-minimized structure was used for molecular modeling studies. Ligand structures were built with MOE and minimized using the MMFF94x force field until an RMSD gradient of 0.05 kcal/mol·Å was reached. For each ligand, energy minimizations (EM) were performed using 1000 steps of steepest descent, followed by conjugate gradient minimization to an RMSD energy gradient of 0.01 kcal/mol·Å. The docking experiments were done using the alpha triangle placement method and the London dG scoring method. 300 results for each ligand were generated, excluding the results having RMSD value > 3 [36]. The best scored result of the remaining conformations for each ligand was further analyzed. The investigated compounds were subjected to flexible alignment and superposition. Their geometry was optimized using the MMFF94 forcefield followed by a flexible alignment using systematic conformational search [40,50]. Surface mapping and molecular properties calculations using ‘Molecular Operating Environment’ software (MOE of Chemical Computing Group Inc., on a Core i7, 2.7 GHz workstation) were performed.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.08.038.

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