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Synthesis, pharmacological studies and molecular modeling of some tetracyclic 1,3-diazepinium chlorides

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ABSTRACT

Seven new 1,3-diazepinium chlorides exhibiting some structural similarities to the 1,4-benzodiazepines were synthesized. In a Hippocratic screen using mice, three of these salts, 3-methoxy-6-oxo-7,13-dihydro-6*H*-benzofuro[2,3-*e*]pyrido[1,2-*a*][1,3]diazepin-12-ium chloride (**8a**), 3-methoxy-9-methyl-6-oxo-7,13-dihydro-6*H*-benzofuro[2,3-*e*]pyrido[1,2-*a*][1,3]diazepin-12-ium chloride (**8c**) and 3-methoxy-11-methyl-6-oxo-7,13-dihydro-6*H*-benzofuro[2,3-*e*]pyrido[1,2-*a*][1,3]diazepin-12-ium chloride (**8e**) were examined for their effect on the central nervous system, and their activities compared to that of diazepam. On their own, salts **8a**, 8c and **8e** solicited no sedative effects on the behaviour of the animals. However, they elicited significant effects in combination with diazepam on diazepam-induced activities such as decreased motor activity, ataxia and loss of righting reflex. Compounds **8a** and **8c** were fitted into the pharmacophore/receptor model developed by Cook et al. with interaction at the L_1, H_1 and A_2 sites indicating that they are potential inverse agonists of the Bz receptor. The compounds displayed some affinity for the α1 isoform of the $GABA_A$/BzR (L_{Di} interaction) but are non-selective for α5 (no L_2 interaction). Results of binding affinity studies showed that compound **8a** is mildly selective for the α1 receptor although not very potent (K_i = 746.5 nM). The significant potentiation of diazepam-induced ataxia and decreased motor activity by compounds **8a** and **8c** in the Hippocratic screen may be associated with α1 selectivity.

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1. Introduction

The 1,4-benzodiazepines are an important group of anxiolytics and hypnotics that are commonly used for treating both anxiety states and insomnia.[1] They bind to specific regulatory sites on the $GABA_A$ benzodiazepine receptor ($GABA_A$/BzR) and enhance the inhibitory effect of GABA by increasing its frequency of binding, thus intensifying the conductance of chloride ions into the cells.[2] This action slows the activity of the central nervous system (CNS) and causes reduction of anxiety and aggression, sedation, induction of sleep, muscle relaxation, loss of motor coordination and suppression of convulsions.[3]

The $GABA_A$/BzRs are pentameric proteins composed mainly of α, β and γ subunits. These subunits are essential for the construction of recombinant $GABA_A$/BzR which mimic biological functions of the native $GABA_A$/BzR in the mammalian brain.[4,5] The various subunits have distinct distribution and regions that overlap in the brain.[6] Experimental evidence has shown that the Bz binding site is formed by contribution from both α and γ subunits.[7–9] Numerous structurally diverse compounds (1,4-Bzs, β-carbolines,[10–12] etc.) which bind to the Bz site have been found to induce a wide range of biological activities, classified as agonists, antagonists or inverse agonists.

A comprehensive pharmacophore/receptor model for $GABA_A$/BzR subtypes α1-6β3γ2 was developed using techniques of chemical synthesis, radioligand binding and receptor mapping (Fig. 1).[13–15] The model unified the previous models for inverse agonist, antagonist and agonist activity as well as diazepam-insensitive (DI) sites.[16] Four basic anchor points, H_1, H_2, A_2 and L_1, were assigned and three additional lipophilic regions were defined as L_2, L_3, and L_{Di} (see captions in Fig. 1 for details). Regions S_1, S_2 and S_3 represent areas of negative steric repulsion.

This unified pharmacophore/receptor model was employed to numerous ligands encompassing 12 families of structurally diverse compounds.[13–16] The synthesis of both partial agonists and partial inverse agonists has been achieved by using parts of this model.[17,18] The studies resulted in the design of novel selective BzR ligands at the α5-containing receptor subtype. Further studies are anticipated to lead to more selective ligands for specific receptor

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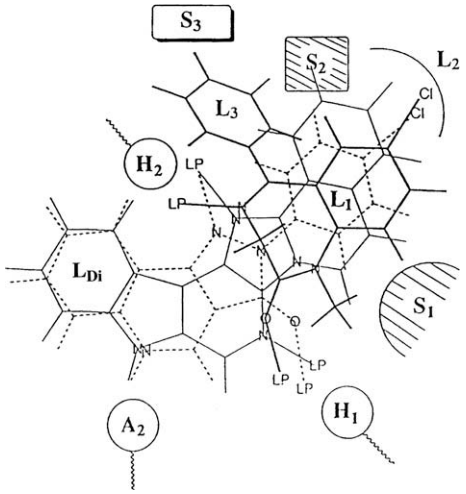


Figure 1. The pyrazolo[3,4-*c*]quinolin-3-one CGS-9896 (**1**) (dotted line), a diazadiindole (**2**) (thin line), and diazepam (**3**) (thick line) fitted to the inclusive pharmacophore model for the BzR. Sites H_1 and H_2 represent hydrogen bond donor sites on the receptor protein complex while A_2 represents a hydrogen bond acceptor site necessary for potent inverse activity in vivo. L_1, L_2, L_3 and L_{Di} are four lipophilic regions in the binding pharmacophore. Descriptors S_1, S_2, and S_3 are regions of negative steric repulsion. LP = lone pair of electrons on the ligands.[15,16]

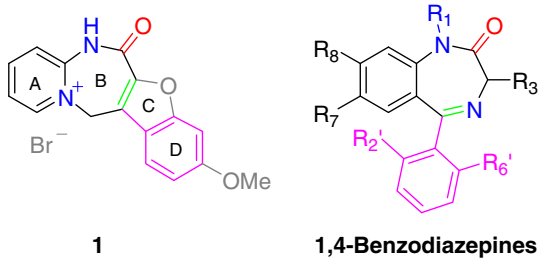
subtypes and subsequently new therapeutic agents with fewer side effects.

Other benzodiazepine analogues such as, the 1,3- 2,3- and 1,5-Bzs are known but have received much less attention in comparison to the 1,4-Bzs. Several of the 2,3- and 1,5-Bz analogues have displayed pharmacological activities similar to those of the 1,4-Bzs, while a few 1,3-Bzs, which were synthesized as part of a drug development program for novel psychotropic agents, have exhibited antidepressant activity comparable to that of the classic tricyclic antidepressant amitriptyline.[19]

In 1997, Jackson and Williams[20] reported the synthesis of the pyridinium bromide **1**—a 1,3-diazepinium salt- from the readily available 7-hydroxy-4-methylcoumarin. Structural comparison of compound **1** with the well known 1,4-Bzs is shown in Figure 2.

Both have a seven-membered heterocyclic ring fused to an aromatic ring, but the nitrogen atoms of the heterocyclic rings are in different relative positions. The 4,5- double bond of the 1,4-Bz is β- to the amide functionality and has an aryl moiety attached whereas in salt **1**, the double bond is part of an α,β-unsaturated amide and the aryl substituent is rigidly fixed by a fused furan ring.

Since tetracyclic 1,3-diazepinium bromide (**1**) displays some resemblance to the 1,4-Bzs, we proposed to synthesize analogues of this type with a view to conducting pharmacological studies to evaluate these compounds as potential therapeutic agents.



1 **1,4-Benzodiazepines**

Figure 2. Comparison of the structure of 1,3-diazepinium bromide (**1**) to 1,4-benzodiazepines.

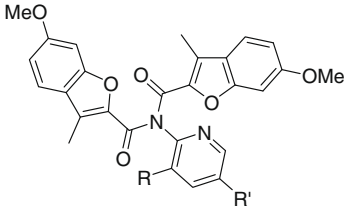
Bromides are not ideal for pharmacological studies as they are toxic to the body.[21–26] Iodides also have chronic effects and are unsuitable here.[27–30] Chlorides, on the other hand, are non-toxic and found naturally in the body and were therefore used in our studies.

Outlined in this paper are reports on the synthesis, pharmacological studies (Hippocratic screen) and molecular modeling of some tetracyclic 1,3-diazepinium chlorides of type **8**. The 1,3-diazepinum compounds were fitted into the pharmacophore/receptor model for $GABA_A$/BzR subtypes and observed for selectivity towards any of the BzR subtypes. The binding affinity of compound **8a** for the α1-6β3γ2 BzR isoforms was also tested. These results were then correlated with those of the pharmacological screen.

2. Chemistry

In the synthetic pathway outlined by Jackson and Williams,[20] tetracyclic 1,3-diazepinium bromide **1** was prepared by intramolecular cyclization of bromomethyl amide **6a** which readily occurred in refluxing acetone. Amide **6a** was obtained from 7-hydroxy-4-methylcoumarin (**2**) as shown in Scheme 1. It was proposed that this synthetic route could be modified to produce the required 1,3-diazepinium chlorides by cyclization of the respective chloromethyl amides (Scheme 1) or by ion exchange of the 1,3-diazepinium bromides (Scheme 2).

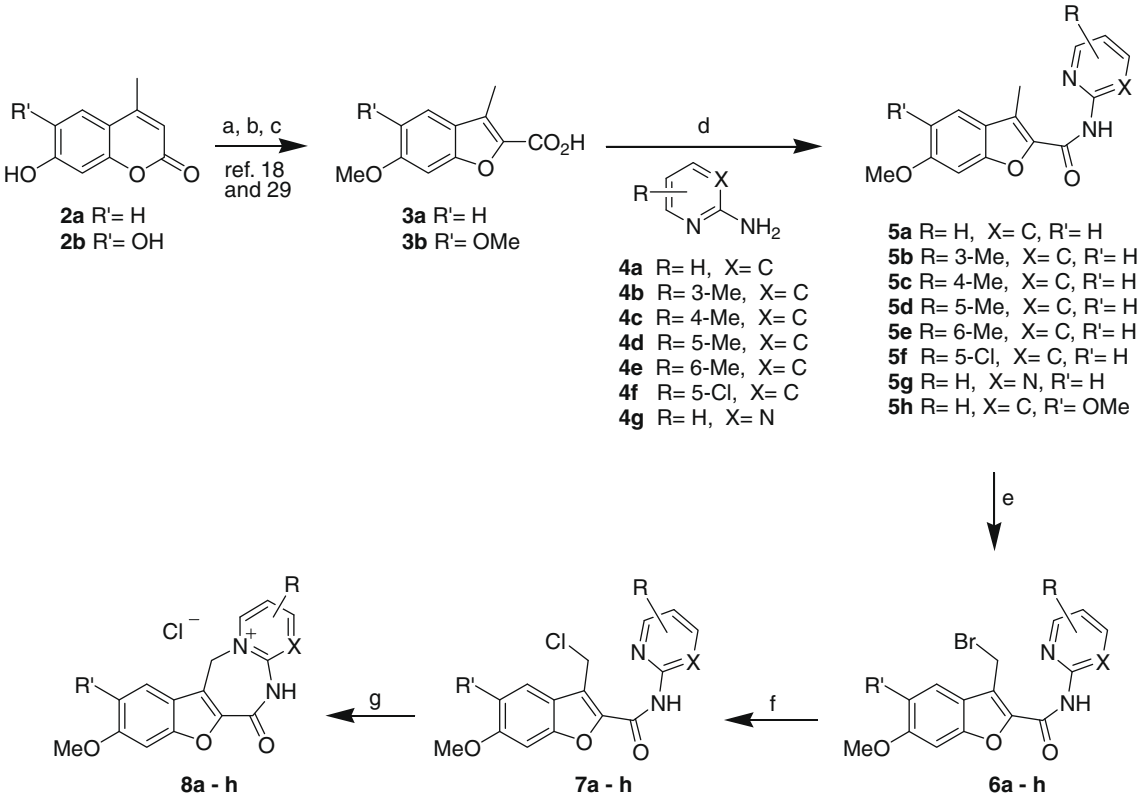
Methylation of 7-hydroxy-4-methylcoumarin (**2a**) with Me_2SO_4/K_2CO_3 followed by bromination with $Br_2/K_2CO_3/CHCl_3$, then hydrolysis and subsequent rearrangement of the 3-bromocoumarin in ethanolic NaOH, produced acid **3a** in 85% overall yield. 5,6-Dimethoxy-3-methylbenzofuran-2-carboxylic acid (**3b**) was also prepared, in similar fashion, from 6,7-dihydroxy-4-methylcoumarin (**2b**) in 72% overall yield.[31] Treatment of acid **3a** with $SOCl_2$ followed by substituted pyridines and pyrimidine **4a–g** produced amides **5a–g** and acid **3b** reacted, under these conditions, with amine **4a** to give amide **5h**. It was found that when the reaction was carried out in the typical way that is, addition of a solution of the amine (1.1 M equiv) in pyridine to the acid chloride, 2-amino-3-picoline (**4b**) reacted with **3a** to give imide **9** (96%). Under these conditions, 2-amino-5-picoline (**4d**) also reacted to produce imide **10** (26%) along with desired amide **5d** (56%).



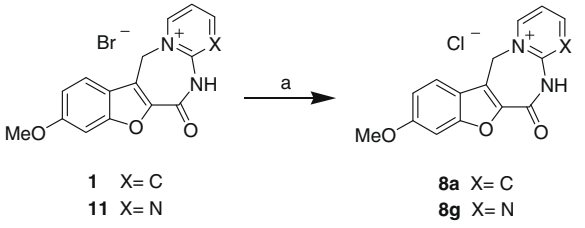
9. R= Me, R'= H
10. R= H, R'= Me

The experimental data confirmed the double acylation of **4b** and **4d** which was perhaps due to the effect of the electron donating methyl group in *ortho* and *para* positions, respectively, to the reacting amino group. Synthesis of amide **5b** was achieved by slowly adding the acid chloride mixture to a solution of **4b** (3 M equiv) in pyridine then heating the mixture at reflux for 3 h.

Attempts at allylic chlorination of the amides using *t*-butyl hypochlorite in methyl formate, dichloromethane or carbon tetrachloride to give chloromethyl amides **7a–h** were futile. Hence, amides **5a–h** were treated with NBS in CCl_4 and irradiated, in a darkened box, with a 150 W tungsten lamp for 2–2.5 h to give the respective bromomethyl amides **6a–h** in modest yields. Forma-



Scheme 1. Reagents and conditions: (ai) **2a**, K_2CO_3, Me_2SO_4, acetone, reflux, 13 h, 97%; (aii) **2b**, K_2CO_3, MeI, acetone, reflux, 8 h, 89%; (b) K_2CO_3, Br_2, $CHCl_3$, 70 °C, 3 h, 74% (for **2a**), 90% (for **2b**); (c) NaOH, EtOH, reflux, 3 h, 98% (**3a**), 90% (**3b**); (d) (i) $SOCl_2$, pyridine, CH_2Cl_2, rt, 30 min; (ii) **4a–g**, pyridine, toluene, reflux, 5 h, 51–77%; (e) NBS, CCl_4, *hv*, 2–3 h, 44–76%; (f) NaCl, $[CH_3(CH_2)_3]_4N^+ I^-$, $CHCl_3$, H_2O, reflux, 3–12 h, 68–88%; (g) acetone, reflux, 3–5 days, 12–50%, (**8b**, 2%).



Scheme 2. Reagents and condition: (a) AgCl, MeOH, rt.

tion of the allyl bromide was readily confirmed by both ^1H and ^{13}C NMR spectra. The methyl group attached to the benzofuran system of the respective amides appears at about δ 2.6 in the ^1H NMR spectrum and at δ 9.5 in ^{13}C NMR spectrum, while the CH_2–Br of the same system resonates further downfield at about δ 5.2 in the ^1H NMR spectrum and δ 21.20 in the ^{13}C NMR spectrum. It should be noted that irradiation of the amides with NBS for longer than 3 h caused cyclization of the bromomethyl amides to their respective tetracyclic bromide salts. This was not surprising as Jackson and Williams had reported this in their reaction with **5a**.

Allylic bromination of **5b** and **5g** proved less straightforward than for the other analogues. When amide **5b** was treated with NBS in CCl_4 and light, 3-bromomethyl-6-methoxy-*N*-(3-methylpyridin-2-yl)benzofuran-2-carboxamide (**6b**) was obtained as a mixture with 6-methoxy-*N*-(3-bromomethylpyridin-2-yl)-3-methylbenzofuran-2-carboxamide, in a crude yield of 89%. Analysis of the crude mixture confirmed that ratio of the desired **6b** to the other product was 3:2 and this crude mixture was used in the next step that is, halogen transfer. Treatment of amide **5g** with NBS in CCl_4 and light for 1–3 h produced the desired bromomethyl amide **6g** as a mixture with the 1,3-diazepinium bromide **11** in a ratio of 1:2. When the reaction was extended beyond 3 h, only the bromide salt **11** was obtained**,** indicating that bromomethyl amide **6g** is very susceptible to ring closure, and reacts to produce the salt **11** as soon as it is formed. Since intermediate **6g** consists of a pyrimidine ring with not one, but two ring nitrogen atoms in positions which are available for interaction with the $-CH_2-Br$ moiety of the molecule to effect cyclization, this process occurs readily.

Bromomethyl amides **6a–f** and **6h** were then converted to the respective chloromethyl amides **7a–f** and **7h** under phase transfer conditions,[32,33] using a NaCl solution (25% aqueous) and tetra-*n*-butylammonium iodide as the phase transfer catalyst. The chloromethyl amides were easily distinguished from the corresponding bromomethyl compounds by their ^{13}C NMR spectra, wherein the carbon atom of the $-CH_2-Cl$ group resonated at approximately δ 35.0, as compared to δ 21.2 for the analogous $-CH_2-Br$. Chloromethyl amides **7b** and **7d** were obtained in crude yields of 58% and 96%, respectively. Analysis of NMR data confirmed the presence of the products and the crude products were used in the cyclization reaction.

Jackson and Williams accomplished cyclization of a 0.7% solution of bromomethyl amide **6a** in refluxing acetone in 70% yield[20] however, when the corresponding chloro analogue **7a** was treated under similar conditions for 28 h, 1,3-diazepinium chloride **8a** was obtained in only 6% yield. Heating 10–20% solutions of chloromethyl amides **7a–f** and **7h** at reflux in the more polar and higher boiling 1,4-dioxane for several days, however, furnished the corresponding 1,3-diazepinium chlorides in 12–50% yield, except in the case of **7b** where the tetracycle was obtained in only 2% yield. In the ^1H and ^{13}C NMR spectra, the methylene protons resonate at about δ 6.0 and δ 50.0, respectively.

Since the tetracyclic 1,3-diazepinium bromides were so readily formed, especially for compound **11**, we also considered converting these bromides to their respective chlorides. Yamazaki et al.[34] and later Cook and co-workers[35] reported the conversion of quaternary ammonium iodides to chlorides using AgCl in anhydrous MeOH. However, compounds **1** and **11** were found to be insoluble in MeOH and even when the reaction was heated at reflux, only the starting bromides were obtained.

3. Pharmacology

Pharmacological assessment of the 1,3-diazepinium chlorides was done using the Hippocratic screen.[36] Of the seven salts prepared, only salts **8a**, **8c** and **8e** were tested since they were more soluble in the sample vehicle. The observed activities of the chlorides were compared with those of diazepam.

3.1. Materials and methods

3.1.1. Animals

Albino male and female mice, aged 7–8 weeks with a weight of 16–25 g were obtained from the Animal House of the University of the West Indies, Mona. The animals were provided with food and water ad libitum. The studies were performed according to protocols approved by the Faculty of Medicinal Sciences/University Hospital of the West Indies Ethics Committee.

3.1.2. Preparation of drug samples

Diazepam was dissolved in corn oil (5 mg/mL) and compounds **8a**, **8c** and **8e** were dissolved in distilled water (2 mg/ mL).

3.1.3. Hippocratic Screen 1

Mice were randomly assigned to six groups with six animals in each group. Animals in groups one (C1) and two (C2) were administered distilled water and corn oil, respectively. Group three (DZ1) was administered the diazepam solution and groups four to six administered solutions of **8a**, **8c** and **8e**, respectively. All administrations were done by intraperitoneal (IP) injection at a dosage volume of 0.2 mL/10 g of body weight. The grouping and dosing of the animals was done independent of the observer, hence all observations were made without prejudice. Activities measured in the Hippocratic screen include: decreased motor activity, ataxia, loss of righting reflex, analgesia, anesthesia, pinnal reflex, loss of screen grip, paralysis, respiratory rate, tremor and startle reaction.

All observations were made at +5, 10, 15, 30, 45, 60, 90 and 120 min, then 24 and 48 h from the time of injection and the activities were rated as –, ±, +, ++, +++ or ++++, in all cases, – represented no change compared to the control animals.

3.1.4. Hippocratic Screen 2

In a second set of experiments, the animals were divided into four groups of six. Distilled water was given 15 min prior to administering diazepam to animals in group one (DZ2) and the remaining groups two to four (**8a**+DZ, **8c**+DZ and **8e**+DZ) were administered salts **8a**, **8c** and **8e**, respectively, 15 min before diazepam. All administrations were done by IP injection at a dosage volume of 0.2 mL/10 g of body weight. All observations are unbiased. Activities measured and assigned ratings were the same as in Hippocratic Screen 1. All observations are made at +5, 10, 15, 30, 45, 60, 90 and 120 min, then 24 and 48 h from the time of diazepam injection.

3.1.5. Statistical analysis

The ratings: –, ±, +, ++, +++ or ++++ were quantified to 5, 4, 3, 2, 1 or 0, respectively. Statistical analysis of the data was performed using SPSS version 12.0 and consisted of General Linear Model (Repeated Measures) with Dunnett *t* (two-sided) Post Hoc test and Kruskal–Wallis test followed Mann-Whitney test. Differences were considered statistically significant when associated with a probability level of less than 0.05.

3.2. Results and discussion

3.2.1. Hippocratic Screen 1

Diazepam significantly induced decreased motor activity, ataxia, loss of righting reflex and screen grip within 10 min of administration to the animals (p <0.01). The effects of diazepam persisted for more than 2 h, but activities were restored within 24 h. These results confirm the CNS sedative activity of diazepam in the Hippocratic screen. Diazepam, however, had no influence on the animals' pinnal reflex, respiratory rate or analgesic and anesthetic response. It also produced no paralysis or tremor and did not significantly affect the startle reaction of the animals.

Compounds **8a**, **8c** and **8e** were found to have no significant effect on the behaviour of the animals. Since all the compounds are quaternary salts, it is highly probable that they are unable to penetrate the blood–brain barrier (BBB) and have on the CNS.

3.2.2. Hippocratic Screen 2

The 1,3-diazepinium chlorides (**8a, 8b** and **8c**) had some significant effects on the animals' response to diazepam. Activities affected include decreased motor activity, ataxia, righting and pinnal reflex.

3.2.2.1. Decreased motor activity. Salts **8a** and **8c** potentiated the decrease in motor activity produced by diazepam by shortening the time taken to reach maximum efficacy for 10–15 min (p <0.01 and 0.05, respectively) (Fig. 3). **8e** attenuated the animals' response to diazepam-induced decreased motor activity (p <0.05) which persisted for over 2 h (p <0.01). Compound **8c** potentiated diazepam-induced decreased motor activity (p <0.01) and caused it to persist for more than 24 h (p <0.01) but less than 48 h. **8c** also significantly increased the maximum efficacy induced by diazepam (p <0.05).

3.2.2.2. Ataxia. Salt **8a** enhanced the onset of ataxia induced by diazepam to within 5 min (p <0.05) and augmented the maximum of diazepam-induced ataxic effect (p <0.05). However, **8e** retards

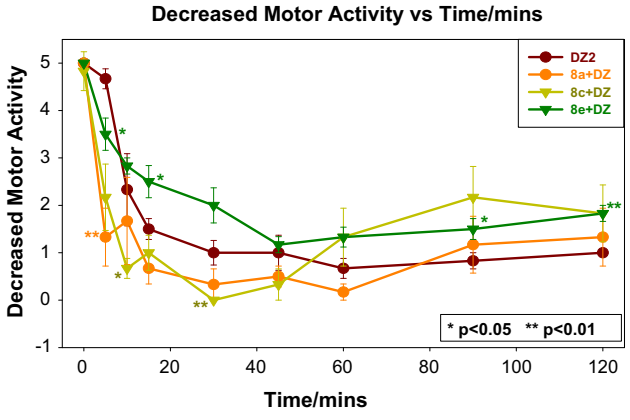


Figure 3. Effects of **8a**, **8c** and **8e** on diazepam-induced decreased motor activity in mice.

the onset of the ataxia to greater than 15 min (p <0.05) but does not interfere with the maximum induced ataxic effect of diazepam (Fig. 4). Compound **8c** also produced a significant increase in the maximum of the diazepam-induced ataxic effect (p <0.001) (Fig. 4). Both **8a** and **8c** potentiated the efficacy of diazepam (p <0.01 and p <0.05, respectively).

3.2.2.3. Loss of righting reflex. Salts **8a**, **8c** and **8e** did not significantly affect the onset nor the overall efficacy of diazepam-induced loss of righting reflex however, **8c** attenuated the effect caused by diazepam at times greater than 1 h after diazepam was administered (p <0.01) (Fig. 5). None of the compounds prolonged the loss of righting reflex induced by diazepam for more than 2 h.

3.2.2.4. Pinnal reflex. The dose of diazepam did not affect the pinnal reflex in mice (Fig. 6). However, **8a** produced an overall reduction in the efficacy of diazepam on pinnal reflex in mice (p <0.01). **8c** and **8e** had no effect on the activity of diazepam towards pinnal reflex (Fig. 6).

The effect of diazepam on the animals with respect to screen grip, respiratory rate, analgesia, anesthesia, paralysis, tremor and startle reaction was not significantly affected by 1,3-diazepinium chlorides **8a, 8c** and **8e**.

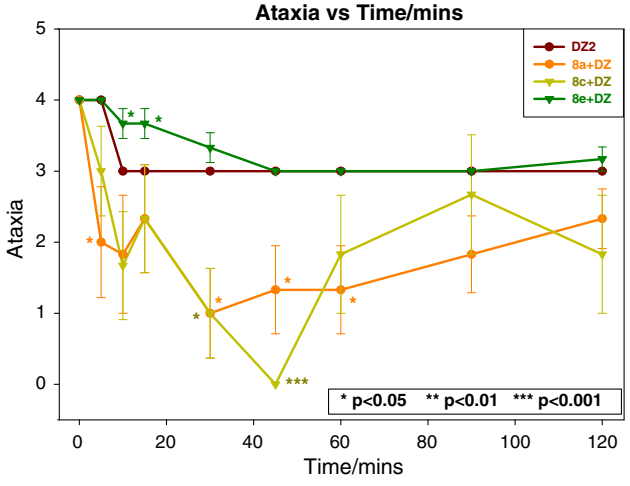


Figure 4. Effects of **8a**, **8c** and **8e** on diazepam-induced ataxia in mice.

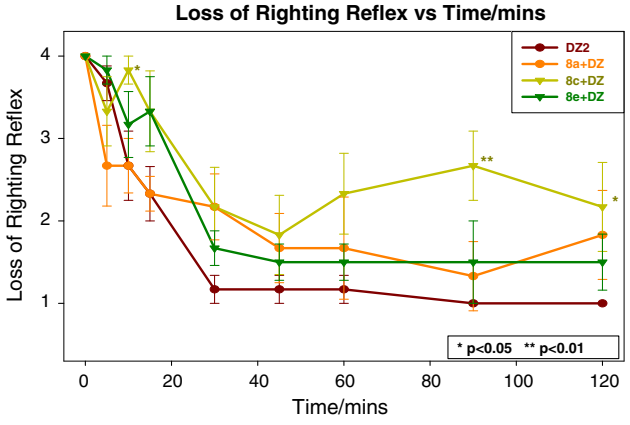


Figure 5. Effects of **8a**, **8c** and **8e** on diazepam-induced loss of righting reflex in mice.

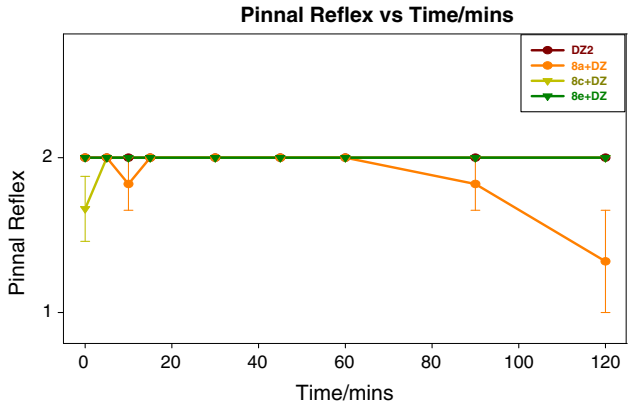


Figure 6. Effects of **8a**, **8c** and **8e** on pinnal reflex in mice when treated with diazepam.

The 1,3-diazepinium chlorides (**8a, 8c** and **8e**), on their own, had no effect on the behaviour of the animals but elicited significant effects on diazepam-induced decreased motor activity, ataxia and loss of righting reflex. This suggests that the chlorides may either be binding to specific sites on the $GABA_A$/BzR and influencing the CNS depressant or sedative activity of diazepam or be undergoing a synergistic effect with the diazepam.

Chlorides **8a** and **8c** potentiated the maximum decreased motor activity and disrupted coordinated movement (ataxia) caused by diazepam. This suggests that they are BzR agonists. In particular, chloride **8a** enhanced the onset of these activities which means that it promotes the binding of diazepam more readily. However, chloride **8c** inhibited the loss of righting reflex, caused by diazepam, after 1 h which suggests antagonist or inverse agonist effect over time. The chlorides did not significantly alter loss of screen grip, thus indicating no effect on the muscle skeletal activity of diazepam on the animals. Chloride **8c** also decreased the efficacy of pinnal reflex in the animals thereby indicating some CNS depressant activity.

Chloride **8e** attenuated the diazepam-induced decreased motor activity and ataxia. This suggests that **8e** either blocks the diazepam binding site or binds at another site and inhibits the binding of diazepam to its receptor site. Chloride **8e** neither affects loss of righting reflex caused by diazepam nor pinnal reflex in the animals.

Based on these results, it is obvious that there is drug interaction between diazepam and the 1,3-diazepinium chlorides. The activities of compounds **8a** and **8c** resemble those of the CNS depressants which potentiate the actions of diazepam, while, **8e** acts like antidepressants or anticonvulsants such as carbamazepine and phenytoin, which decrease the action of diazepam.[37,38]

4. Molecular modeling

The cloning, expression and anatomical localization of multiple $GABA_A$ subunits has facilitated both the identification and design of subtype selective compounds. With the availability of binding data from different recombinant receptor subtypes, affinities of ligands from a number of different structural classes of ligands[16] have been evaluated. Based on SAR data obtained for these ligands at 6 recombinant BzR subtypes, an effort has been made to establish different pharmacophore/receptor models for BzR subtypes. This approach is similar to the previous one which resulted in the unified pharmacophore/receptor model.[13,14]

4.1. Computer modeling methods

The alignment of the 12 different structural classes of benzodiazepine receptor ligands is based on the least squares fitting of at least three points. The coordinates of the four anchor points (A_2, H_1, H_2 and L_1) employed in the alignment are outlined in Figure 7. Some of the differences and similarities among these subtypes can be gleaned from this study and serve as a guide for future drug design.

The core structures of the ligands were taken from available X-ray crystallographic coordinates or generated using the SYBYL fragment library. The structures which resulted were energy minimized using MM2 (molecular mechanics program 2) or MMFF (Merck molecular force field) force fields and the subsequent Monte Carlo conformational searches were carried out on MacroModel 4.5 or MacroModel 6.0[39] on a Silicon Graphics Personal Iris 4D/35 workstation or a Silicon Graphics Octane SI 2P 175 R10000 workstation, respectively. The low energy conformations were then fully optimized via molecular orbital calculations at the 3-21G basis set with torsional angles fixed. The structures which resulted were further calibrated with 6-31G* single point calculations at an 'SCF = TIGHT' convergence criteria via GAUSSIAN 92[40] on a Silicon Graphics Indigo[14] R4400 workstation, or GAUSSIAN 94[41] on a Silicon Graphics Octane SI2P175R10000 workstation. With ligands containing heavy atoms (bromine or iodine), the necessary 6-31G* basis sets were not included in the commercially available GAUSSIAN 92 and GAUSSIAN 94 programs. These basis sets were taken from splitting the MP4 basis set reported by Andzelm et al. and then addition of d functions.[42]

The ab initio 6-31G* geometries of the ligands were used for the included volume analysis. Ligands which possessed BzR affinities of <20 nM at one recombinant receptor subtype were considered as 'active' and were used for the included volume analysis for that subtype. Zolpidem, CL 218872 and ligands reported to be more selective for one subtype[43,44] were included even though the binding affinities were somewhat higher than 20 nM. Molecular graphics, root-mean-squares (RMS) fit, calculations of centroids of substructures and included volume analyses were carried out by means of SYBYL 5.5 on a Silicon Graphics Personal Iris 4D/35 workstation or SYBYL 6.5[45] on a Silicon Graphics Octane SI 2P 175 R10000 workstation. The lengths of hydrogen bond extension vectors (HBV) were set to 1.84 Å, while the C–N–HBV and C═O–HBV valence angles used were chosen to mimic the geometry of an ideal hydrogen bond.

4.2. Competition binding assays

Competition binding assays were performed in a total volume of 0.5 mL at 4 °C for 1 h using [^{3}H] flunitrazepam as the radiolabel. For these binding assays, 20–50 μg of membrane protein harvested with hypotonic buffer (50 mM Tris–acetate pH 7.4 at 4°) was incubated with the radiolabel as previously described.[46] Nonspecific binding was defined as radioactivity bound in the presence of 100 μM diazepam and represented less than 20% of total binding. Membranes were harvested with a Brandel cell harvester followed by three ice-cold washes onto polyethyleneimine-pretreated (0.3%) Whatman GF/C filters. Filters were dried overnight and then soaked in Ecoscint A liquid scintillation cocktail (National Diagnostics; Atlanta, GA). Bound radioactivity was quantified by liquid scintillation counting. Membrane protein concentrations were determined using an assay kit from Bio-Rad (Hercules, CA) with bovine serum albumin as the standard.

4.3. Results and discussion

The αβγ2 $GABA_A$ receptors are highly influenced by the type of α subunit present.[6,47,48] Ligands with affinities (K_i values) of <1000 nM show activity at a receptor subtype but those with K_i values ⩽20 nM are considered significantly active for the respective subtype. Generally, the 1,4-Bzs are non-selective ligands[49] and bind to all BzR isoforms which are diazepam sensitive (DS) namely, α1-3 and α5. These receptor subtypes are located in distinct regions of the brain and exhibit significant roles in specific biological activities.[6,50–54] For example, when benzodiazepines bind to the α1 subtype the associated effects include; sedation, ataxia and some anticonvulsant action, while at the α5 subtype they affect temporal and spatial memory.[55]

Studies of the pharmacophore/receptor model indicated that occupation of the L_3 region of the receptor would lead to agonist activity.[13,56] Many 1,4-Bzs are full agonists since the 5-aryl moiety fully occupies the L_3 region of the receptor site. It was also observed that the 5-phenyl-substituted 1,4-Bz did not bind to the α6-containing receptor isoform which is one of the diazepam-insensitive (DI) subtypes.[13,14,57] Studies showed that the α6 sub-

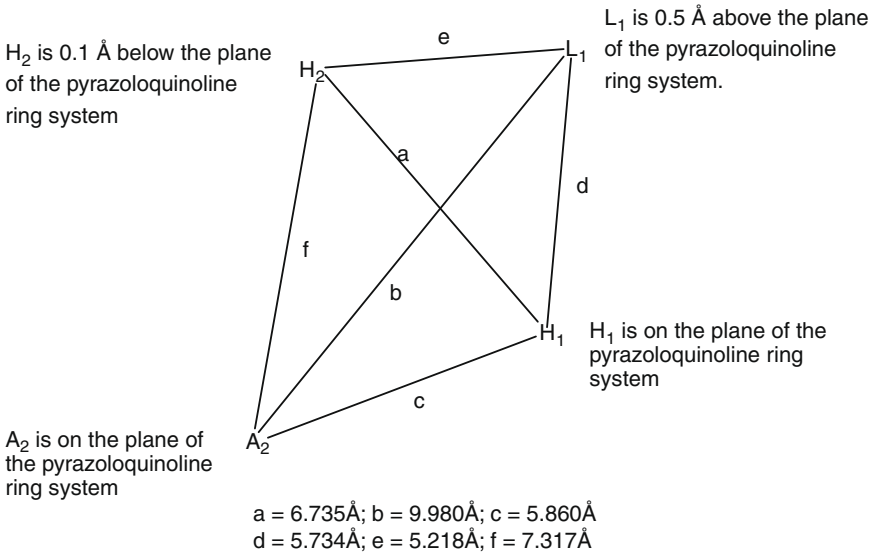


Figure 7. The schematic representation of the descriptors for the inclusive BzR pharmacophore.

type may lack the L_3 region which would accommodate the aryl moiety at position 5.

In the unified pharmacophore/receptor model, it was observed that occupation of the L_2 region with lipophilic groups is important for α5 selectivity and that α1 selectivity was enhanced by occupation of the L_{Di} region. The β-carbolines, which generally display higher selectivity for α1-containing receptor subtype, can produce agonist, antagonist, inverse agonist, partial agonist and partial inverse agonist activities. Positive allosteric modulators (agonists) of the Bz binding site must interact with H_1, H_2 and L_1 as well as occupy the L_2 and/or L_3 regions, while, inverse agonists only require interactions at H_1, A_2 and L_1 for in vivo activity.[13,58–61]

Alignment of compounds **8a** and **8c** within the pharmacophore/receptor for α1β3γ2 subtype is shown in Figures 8 and 9, respectively. The centroid of ring D in **8a** and **8c** is overlapped with the lipophilic region L_1. The lone pair of electrons of the C6 amide carbonyl oxygen is hydrogen-bonded to H_1 of the receptor (bond length of 1.84 Å and bond angle of C=O–H_1 is 135°). There is also potential hydrogen bonding between the lone pair of electrons of the furan oxygen (O5) and the H_1 site giving rise to a 3-centered hydrogen bond interaction with the C6 carbonyl oxygen, H_1 and O5 (C=O–H_1–O5). The N7 proton interacts with the A_2 site (bond length of 1.84 Å and bond angle of N7–H–A_2 is 180°). Although **8a** and **8c** are aligned similarly into the model, **8a** fits better as the methyl substituent at position 9 on ring A of **8c** sticks out into the extracellular region (Fig. 9b).

When compared to diazepam, it is clearly seen that **8a** and **8c** did not occupy the L_2 nor L_3 regions of the pharmacophore/receptor model (Fig. 10). Also, there was no lone pair of electrons available on the iminium ion at the junction of rings A and B for interaction with the A_2 descriptor. Compounds **8a** and **8c** are, therefore, not expected to be full agonists like diazepam, since they do not have the required interactions in the model for positive allosteric modulation of the Bz receptor (Fig. 10).

On the other hand, **8a** and **8c** are potential Bz receptor inverse agonists since they have interactions at the L_1, H_1 and A_2 sites. 3-Methoxy-11-methyl-6-oxo-7,13-dihydro-6*H*-benzofuro[2,3-*e*]pyrido[1,2-*a*][1,3]diazepin-12-ium chloride (**8e**) exhibited some inverse agonist activities in the Hippocratic screen and although it was not fitted into the pharmacophore model, it is expected to align in similar fashion to **8a** and **8c**.

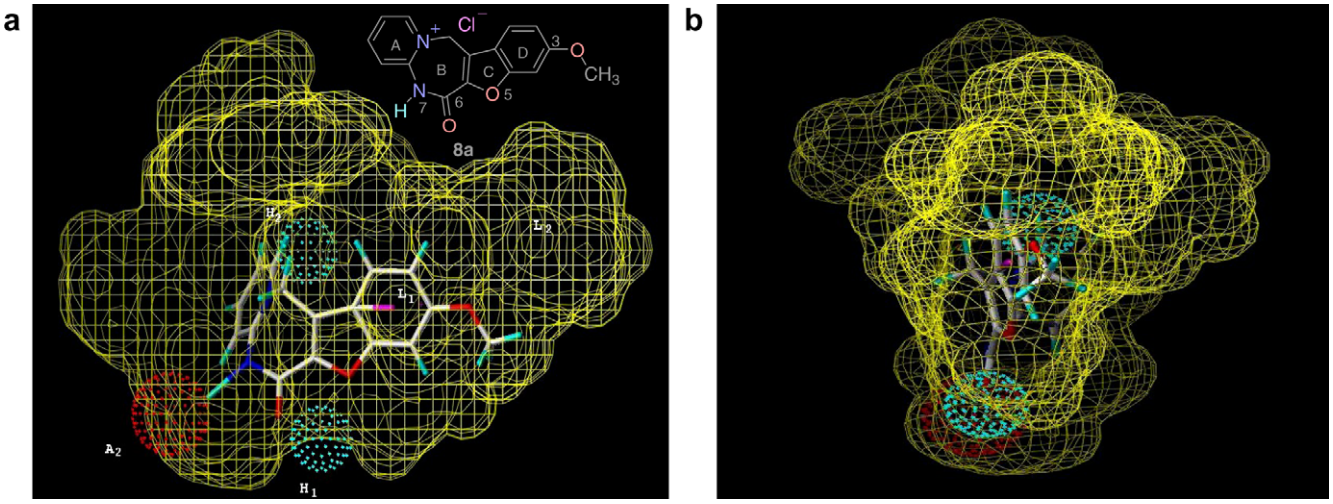


Figure 8. (a) Alignment of 1,3-diazepinium chloride **8a** within the pharmacophore/receptor model for the α1β3γ2 $GABA_A$/BzR subtype; (b) Alignment of **8a** in the model rotated 90°.

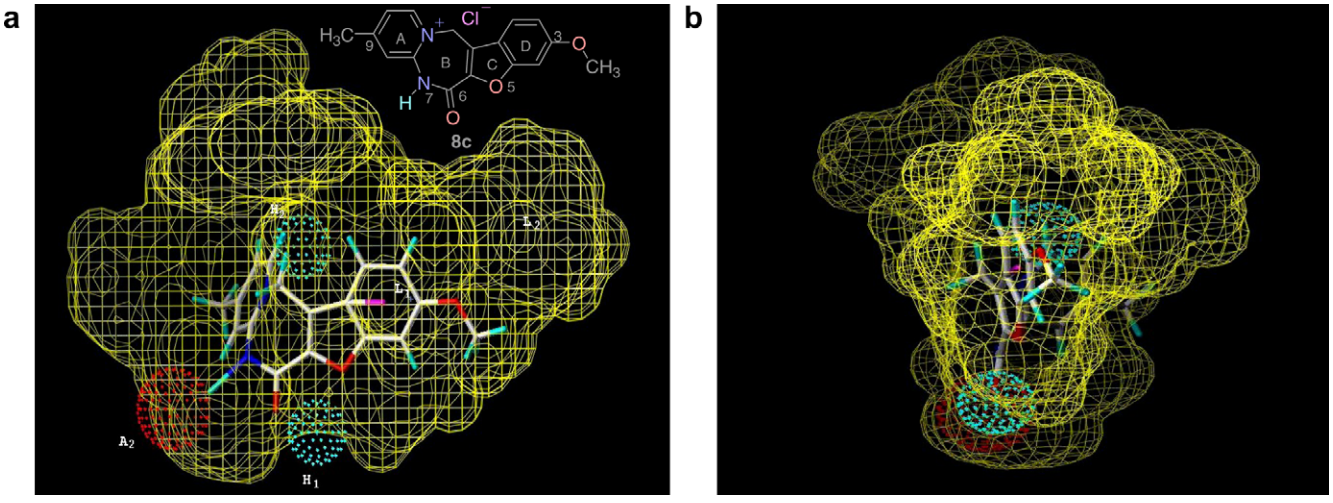


Figure 9. (a) Alignment of 1,3-diazepinium chloride **8c** within the pharmacophore/receptor model for the α1β3γ2 $GABA_A$/BzR subtype; (b) Alignment of **8c** in the model rotated to 90° showing methyl group on ring A sticking out into the extracellular region.

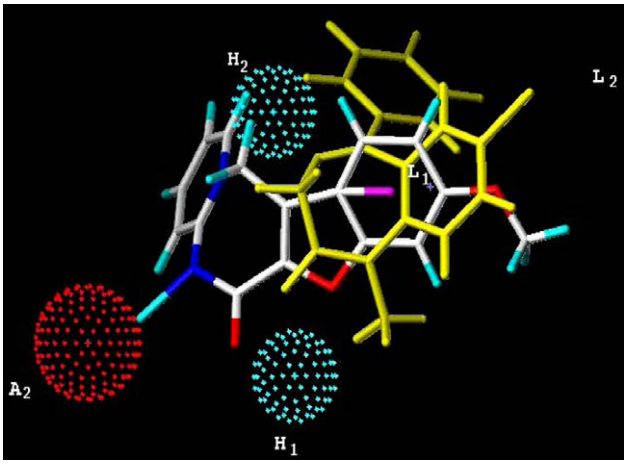


Figure 10. 1,3-Diazepinium chloride **8a** overlaid with diazepam (yellow) in the pharmacophore/receptor model.

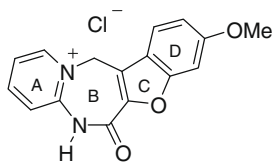
The lack of occupation of the L_2 lipophilic region by **8a** and **8c** implies that they have no affinity for the α5 isoform.[13,14,56,62] However, there may be some α1 selectivity as there appears to be some occupation of the L_{Di} region of the receptor by ring A of **8a** and **8c**.

The β-carbolines generally display some selectivity at the α1-containing receptor subtypes which, based on experimental evidence, is due to occupation of the L_{Di} site of the molecules in the receptor model. Inverse agonist methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) occupies the L_{Di} region of the pharmacophore model and has a high affinity (K_i = 5.7 nM) for the α1β3γ2-subtype.[16]

In the Hippocratic screen, both **8a** and **8c** produced significant potentiation of diazepam-induced ataxia and decreased motor activity. These effects could be associated with α1 selectivity. Binding affinity studies showed that compound **8a** exhibit poor affinity at the α1, α2, α3 and α5 receptor subtypes (Table 1). Compound **8a**, however, shows very slight selectivity at the α1-containing receptor subtype (K_i = 746.5 nM) and therefore, could be used for the design of a next generation ligand.

Compound **8a** was also tested in the NIMH (National Institute of Mental Health) Psychoactive Drugs Screening Program (PDSP). In this program novel psychoactive compounds are screened for pharmacological and functional activity at cloned human or rodent CNS receptors, channels, and transporters. Binding experiments in the PDSP revealed that compound **8a** inhibited dopamine, norepinephrine and serotonin transporters with a mean % inhibition of >10,000 at all three transporters (significant inhibition is considered >50%). By inhibiting these transporters compound **8a** would prevent the reuptake of the neurotransmitters and cause prolonged neuronal activity. This is a characteristic feature of many antidepressant drugs. Some of these antidepressants also exhibit anticholinergic activity which can lead to ataxia.[63]

Table 1
Affinity of 3-methoxy-6-oxo-7,13-dihydro-6*H*-benzofuro[2,3-*e*]pyrido[1,2-*a*][1,3]diazepin-12-ium chloride (**8a**) for αxβ3γ2 (x = 1–6) BzR isoforms, using [^{3}H] flunitrazepam as the radiolabel



8a

Ligand	K_i (nM)					
	α1	α2	α3	α4	α5	α6
8a	746.5	8081	1543	ND	2364	ND

ND = Not determined.

5. Conclusion

Seven new tetracyclic 1,3-diazepinium chlorides, with some structural similarities to the 1,4-Bzs, were synthesized via intramolecular cyclization of the respective allyl chlorides. Three of these chlorides (**8a**, **8c** and **8e**) were evaluated in a Hippocratic screen and their activities compared to diazepam. On their own, compounds **8a**, **8c** and **8e** had no notable effect on the behaviour of the animals (mice) but, they significantly affected diazepam-induced ataxia, decreased motor activity, righting and pinnal reflex. Compounds **8a** and **8c** exhibited CNS depressant effects since they enhanced these diazepam-induced activities, while **8e** attenuated these activities and therefore displayed antidepressant effects. Compounds **8a** and **8c** were aligned into the pharmacophore/receptor with interactions at the L_1, H_1 and A_2 sites and some occupation the L_{Di} region of the model. These results indicate that the compounds are potential inverse agonists with possible α1 selectivity. Binding affinity studies confirmed that there is very slight selectivity of compound **8a** for the α1 subtype but the compound is not very potent (K_i = 746.5 nM). Hence, the potentiation of diazepam-induced ataxia and decreased motor activity by compounds **8a** and **8c** in the Hippocratic screen may have resulted from α1 associated effects. Compound **8a** also display antidepressant activity since it significantly inhibited the dopamine, norepinephrine and serotonin transporters (>10,000%).

It is clearly shown that these ligands fit into the pharmacophore for BzR and may exert their effects on diazepam via these receptors, however, more work is needed to verify this.

6. Experimental

6.1. Chemistry

Unless otherwise stated, the following generalizations apply. Evaporation means evaporation under reduced pressure. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained in deuteriochloroform solution on ACE 200 Bruker spectrophotometers (200 MHz or 500 MHz) instruments. Chemical shifts are reported in ppm. The abbreviations are as follows: s, singlet; d, doublet; dd, doublet of doublets; dt doublet of triplets; m, multiplet. Infrared spectra were obtained on a Bruker Vector FT-IR spectrophotometer and are for NaCl discs. Elemental analyses were carried out at MEDAC Ltd, Brunel Science Centre, United Kingdom. Column chromatography was performed using silica gel (40–63 μm). The solvent systems used were: A = ethyl acetate/hexane (1:1); B = ethyl acetate/hexane (1:2); C = ethyl acetate/dichloromethane (1:1) and D = ethyl acetate/dichloromethane (1:2).

6.1.1. General preparation of 6-methoxy-3-methylbenzofuran-2-carboxamides (5a–h)

To a 100 mL round-bottomed flask fitted with a reflux condenser and a calcium chloride guard tube was added a 10% mixture (wt/vol) of acid **3** (1.00 g, 4.85 mmol) in dichloromethane (10 mL). $SOCl_2$ (2 M equiv, 0.70 mL) and pyridine (2 mL) were then added to the mixture which was stirred at room temperature for 30 min. The amine (**4a–g**) (1.1 M equiv) in pyridine (4 mL) and toluene (3 mL) was then added and the mixture heated at reflux for 5 h. The mixture was concentrated then pyridine was removed by

azeotroping with toluene and evaporating under vacuum. The crude was purified by flash-column chromatography on silica which had been treated with 5% triethylamine in eluting solvent, to give corresponding amides **5a–h**.

6.1.1.1. 6-Methoxy-3-methyl-*N*-(pyridin-2-yl)benzofuran-2-carboxamide (5a). Acid **3a** was reacted with 2-aminopyridine (**4a**) and column eluted with solvent system D to give amide **5a**, (0.92 g, 67%) as off-white crystals; mp 168–170 °C (MeOH), [lit.[20] 167–169 °C]; IR ν_{max} 1688, 3408 cm^{-1}; ^1H NMR δ = 2.57 (s, 3H, –CH_3), 3.81 (s, 3H, –OCH_3), 6.89 (m, 2H, H-5 and H-7), 7.00 (m, 1H, H-5′), 7.42 (m, 1H, H-4), 7.68 (m, 1H, H-4′), 8.28 (m, 2H, H-3′, 6′), 9.00 ppm (1H, s, –NH); ^{13}C NMR δ = 9.6, 56.2, 95.8, 113.5, 114.4, 120.2, 121.7, 123.5, 125.4, 138.8, 141.8, 148.4, 151.6, 155.1, 158.6, 161.1 ppm.

6.1.1.2. Di-(6-methoxy-3-methylbenzofuran-2-carbonyl)(3-methylpyridin-2-yl)amine (9). Acid **3a** was reacted with 2-amino-3-picoline (**4b**) and column eluted with system B to give imide **9**, (1.13 g, 96%) as off-white crystals; mp >300 °C (MeOH); IR ν_{max} 1612, 1620 cm^{-1}; ^1H NMR δ = 2.43 (s, 3H, py-CH_3), 2.57 (s, 6H, furan-CH_3), 3.80 (s, 6H, –OCH_3), 6.73 (d, J = 2 Hz, 2H, H-7′ and H-7″), 6.87 (dd, J = 2 and 9 Hz, 2H, H-5′ and H-5″), 7.18 (dt, J = 5 and 8 Hz, 1H, H-5), 7.43 (d, J = 9 Hz, 2H, H-4′ and H-4″), 7.67 (d, J = 8 Hz, 1H, H-4), 8.92 ppm (d, J = 5 Hz, 1H, H-6); ^{13}C NMR δ = 9.9, 18.2, 56.1, 95.6, 113.7, 121.8, 123.0, 123.5, 128.1, 131.2, 140.0, 145.4, 147.1, 151.9, 155.7, 161.3, 163.0 ppm. Anal. Calcd for $C_{28}H_{24}N_2O_6$: C, 69.41; H, 4.99; N, 5.78. Found: C, 69.33; H, 4.84; N, 5.59.

6.1.1.3. 6-Methoxy-3-methyl-*N*-(4-methylpyridin-2-yl)benzofuran-2-carboxamide (5c). Acid **3a** was reacted with 2-amino-4-picoline (**4c**) and column eluted with system B to give amide **5c**, (0.73 g, 51%) as off-white crystals; mp 184.5–185.5 °C (MeOH); IR ν_{max} 1672, 3409 cm^{-1}; ^1H NMR δ = 2.40 (s, 3H, py-CH_3), 2.66 (s, 3H, furan-CH_3), 3.89 (s, 3H, –OCH_3), 6.94 (m, 3H, H-4, H-5 and H-7), 7.50 (dd, J = 3 and 5 Hz, 1H, H-5′), 8.20 (m, 2H, H-3′ and H-6′), 8.92 ppm (s, 1H, –NH); ^{13}C NMR δ = 9.6, 21.8, 56.2, 95.9, 113.5, 114.9, 121.4, 121.8, 123.5, 125.4, 141.8, 148.1, 150.3, 151.6, 155.1, 158.7, 161.1 ppm. Anal. Calcd for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45. Found: C, 68.60; H, 5.54; N, 9.00.

6.1.1.4. 6-Methoxy-3-methyl-*N*-(5-methylpyridin-2-yl)benzofuran-2-carboxamide (5d). Acid **3a** was reacted with 2-amino-5-picoline (**4d**) and column eluted with system B to give amide **5d**, (0.81 g, 56%) as cream needle-like crystals; mp 150–151 °C (MeOH); IR ν_{max} 1670, 3408 cm^{-1}; ^1H NMR δ = 2.35 (s, 3H, py-CH_3), 2.68 (s, 3H, furan-CH_3), 3.91 (s, 3H, –OCH_3), 6.96 (dd, J = 2 and 9 Hz, 1H, H-5), 6.98 (d, J = 2 Hz, 1H, H-7), 7.52 (d, J = 9 Hz, 1H, H-4), 7.58 (dd, J = 2 and 9 Hz, 1H, H-4′), 8.18 (d, J = 2 Hz, 1H, H-6′), 8.29 (d, J = 9 Hz, 1H, H-3′), 8.94 ppm (s, 1H, –NH); ^{13}C NMR δ = 9.6, 18.2, 56.2, 96.0, 113.5, 113.9, 121.7, 123.6, 129.6, 139.3, 142.0, 148.4, 149.5, 155.1, 158.5, 161.1 ppm. Anal. Calcd for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45. Found: C, 68.62; H, 5.31; N, 9.31.

6.1.1.5. Di-(6-methoxy-3-methylbenzofuran-2-carbonyl)(5-methylpyridin-2-yl)amine (10). Acid **3a** was reacted with 2-amino-5-picoline (**4d**) and column eluted with system B to give imide **10**, (0.31 g, 26%) as off-white crystals; mp >300 °C (MeOH); IR ν_{max} 1634, 1628 cm^{-1}; ^1H NMR δ = 2.37 (s, 3H, py-CH_3), 2.59 (s, 6H, furan-CH_3), 3.82 (s, 6H, –OCH_3), 6.80 (d, J = 2 Hz, 2H, H-7′ and H-7″), 6.91 (dd, J = 2 and 9 Hz, 2H, H-5′ and H-5″), 7.31 (d, J = 8 Hz, 1H, H-4), 7.47 (d, J = 9 Hz, 2H, H-4′ and H-4″), 7.60 (d, J = 8 Hz, 1H, H-3), 8.23 ppm (s, 1H, H-6); ^{13}C NMR δ = 9.5, 18.0, 55.7, 95.4, 113.4, 120.8, 121.5, 122.6, 127.7, 131.6, 138.6, 143.2, 149.2, 150.6, 155.5, 161.0, 163.0 ppm. Anal. Calcd for $C_{28}H_{24}N_2O_6$: C, 69.41; H, 4.99; N, 5.78. Found: C, 69.06; H, 5.15; N, 5.63.

6.1.1.6. 6-Methoxy-3-methyl-*N*-(6-methylpyridin-2-yl)benzofuran-2-carboxamide (5e). Acid **3a** was reacted with 2-amino-6-picoline (**4e**) and column eluted with system B to give amide **5e**, (1.09 g, 76%) as pale yellow crystals; mp 130–132 °C (MeOH); IR ν_{max} 1675, 3412 cm^{-1}; ^1H NMR δ = 2.51 (s, 3H, py-CH_3), 2.65 (s, 3H, furan-CH_3), 3.89 (s, 3H, –OCH_3), 6.94 (m, 3H, H-4, H-5 and H-7), 7.50 (d, J = 8 Hz, 1H, H-5′), 7.63 (t, J = 8 Hz, 1H, H-4′), 8.17 (d, J = 8 Hz, 1H, H-3′), 8.89 ppm (s, 1H, –NH); ^{13}C NMR δ = 9.6, 24.5, 56.2, 95.9, 111.2, 113.5, 119.7, 121.7, 123.6, 125.2, 139.0, 141.9, 150.9, 155.1, 157.4, 158.6, 161.0 ppm. Anal. Calcd for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45. Found: C, 68.49; H, 5.45; N, 9.25.

6.1.1.7. *N*-(5-Chloropyridin-2-yl)-6-methoxy-3-methylbenzofuran-2-carboxamide (5f). Acid **3a** was reacted with 2-amino-5-chloropyridine (**4f**) and column eluted with system B to give amide **5f**, (1.19 g, 77%) as pale yellow crystals; mp 147–149 °C (MeOH); IR ν_{max} 1680, 3396 cm^{-1}; ^1H NMR δ = 2.63 (s, 3H, –CH_3), 3.88 (s, 3H, –OCH_3), 6.94 (m, 2H, H-5 and H-7), 7.48 (d, J = 9 Hz, 1H, H-4), 7.70 (dd, J = 3 and 9 Hz, 1H, H-4′), 8.29 (d, J = 3 Hz, 1H, H-6′), 8.36 (d, J = 9 Hz, 1H, H-3′), 9.01 ppm (s, 1H, –NH); ^{13}C NMR δ = 9.2, 55.8, 95.4, 113.5, 113.3, 114.6, 121.4, 123.0, 125.5, 126.7, 138.0, 141.1, 146.7, 149.6, 154.7, 158.0, 160.8 ppm. Anal. Calcd for $C_{16}H_{13}N_2O_3Cl$: C, 60.67; H, 4.14; N, 8.84. Found: C, 60.25; H, 4.10; N, 8.46.

6.1.1.8. 6-Methoxy-3-methyl-*N*-(pyrimidin-2-yl)benzofuran-2-carboxamide (5g). Acid **3a** was reacted with 2-aminopyrimidine (**4g**) and column eluted with system C to give amide **5g**, (0.98 g, 69%) as a cream crystals; mp 169–171 °C, [lit.[64] 156–158 °C]; IR ν_{max} 1704, 3421 cm^{-1}; ^1H NMR δ = 2.58 (s, 3H, –CH_3), 3.80 (s, 3H, –OCH_3), 6.86 (m, 2H, H-5 and H-7), 7.00 (t, J = 5 Hz, 1H, H-5′), 7.42 (d, J = 8 Hz, 1H, H-4), 8.6 (d, J = 5 Hz, 2H, H-4′ and H-6′), 9.04 ppm (s, 1H, –NH); ^{13}C NMR δ = 9.6, 53.9, 56.2, 95.9, 113.7, 117.2, 122.0, 123.5, 126.7, 141.7, 155.1, 157.4,157.9, 158.9, 161.3 ppm.

6.1.1.9. 6-Methoxy-3-methyl-*N*-(3-methylpyridin-2-yl)benzofuran-2-carboxamide (5b). To a 50 mL round-bottomed flask fitted with a calcium chloride guard tube was added a 10% mixture (wt/vol) of acid **3a** (1.02 g, 4.97 mmol) in dichloromethane (10 mL). Pyridine (5 mL) and $SOCl_2$ (2 M equiv) were then added and the solution stirred at room temperature for 40 min. A 20% solution of amine **4b** (3 M equiv) in pyridine/toluene (4:3) was made up in a 100 mL two-neck round-bottomed flask fitted with a constant pressure dropping funnel, reflux condenser and calcium chloride guard tube. The acid chloride mixture was transferred to the constant pressure dropping funnel and added dropwise over a period of 90 min then the whole heated at reflux for 3 h. The mixture was concentrated and pyridine removed by azeotroping with toluene and evaporating under vacuum. The crude was purified by flash-column chromatography on silica which had been treated with 5% triethylamine, and eluted with solvent system B, to give amide **5b** (1.03 g, 70%) as pale yellow crystals; mp 171–172 °C, IR ν_{max} 1603, 3273 cm^{-1}; ^1H NMR δ = 2.41 (s, 3H, py-CH_3), 2.64 (s, 3H, furan-CH_3), 3.90 (s, 3H, –OCH_3), 6.97 (dd, J = 2 and 9 Hz, 1H, H-5), 6.99 (d, J = 2 Hz, 1H, H-7), 7.16 (dd, J = 5 Hz and 8 Hz, 1H, H-5′), 7.51 (d, J = 9 Hz, 1H, H-4), 7.64 (d, J = 8 Hz, 1H, H-4′), 8.35 (d, J = 5 Hz, 1H, H-6′), 8.64 ppm (s, 1H, –NH); ^{13}C NMR δ = 9.1, 18.4, 55.8, 95.5, 113.0, 121.3, 121.7, 123.1, 124.7, 128.5, 139.8, 141.8, 145.9, 149.0, 154.7, 158.2, 160.5 ppm; Anal. Calcd for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45. Found: C, 68.86; H, 5.47; N, 9.29.

6.1.1.10. 5,6-Dimethoxy-3-methyl-*N*-(pyridin-2-yl)benzofuran-2-carboxamide (5h). 5,6-Dimethoxy-3-methylbenzofuran-2-carboxylic acid (**3b**) (0.60 g, 2.53 mmol) was reacted with 2-aminopyridine (**4a**) (0.29 g, 3.03 mmol). Column was eluted with solvent system D to give amide **5h**, (0.49 g, 62%) as off-white crystals; mp 230–232 °C (EtOH); IR ν_{max} 1695, 3417 cm^{-1}; ^1H NMR δ = 2.68 (s, 3H, $-CH_3$), 3.97 (s, 3H, $-OCH_3$), 3.98 (s, 3H, $-OCH_3$), 6.98 (s, 1H, H-7), 6.99 (s, 1H, H-4), 7.08 (m, 1H, H-4′), 7.76 (m, 1H, H-5′), 8.37 (m, 2H, H-3′, 6′), 8.98 ppm (s, 1H, –NH); ^{13}C NMR δ = 9.7, 56.7, 56.8, 95.3, 101.5, 114.4, 120.1, 122.0, 125.5, 138.7, 141.8, 147.7, 148.5, 149.0, 151.5, 151.7, 158.5 ppm. Anal. Calcd for $C_{17}H_{16}N_2O_4$: C, 66.04; H, 5.85; N, 8.55. Found: C, 65.97; H, 5.81; N, 8.25.

6.1.2. General preparation of bromomethyl amides (6a–h)

A solution of amide in carbon tetrachloride was made up in a round-bottomed flask fitted with reflux condenser and calcium chloride guard tube. *N*-Bromosuccinimide (1.1 M equiv) was added with stirring and the mixture irradiated, in a darkened box, with a 150 W tungsten lamp for 2–3 h. The suspension was filtered and the filter cake washed with hot CCl_4. The filtrate was then concentrated and recrystallized to give the respective bromomethyl amide. No elemental analysis was carried out on these allyl bromides.

6.1.2.1. 3-(Bromomethyl)-6-methoxy-*N*-(pyridin-2-yl)benzofuran-2-carboxamide (6a). Amide **5a** (0.40 g, 1.42 mmol) reacted in CCl_4 (24 mL) to give **6a** (0.39 g, 76%) as an off-white solid, mp 158–160 °C (MeOH); [lit.[20] 123–124 °C]; IR ν_{max} 1578, 1665, 3401 cm^{-1}; ^1H NMR δ = 3.90 (s, 3H, $-OCH_3$), 5.14 (s, 2H, $-CH_2Br$), 7.00 (m, 3H, H-4, H-5 and H-7), 7.73 (m, 2H, H-4′ and H-5′), 8.36 (m, 2H, H-3′ and H-6′), 9.01 ppm (s, 1H, –NH); ^{13}C NMR δ = 21.2, 56.2, 96.1, 114.3, 114.6, 120.6, 121.0, 122.1, 125.0, 138.8, 141.9, 148.5, 151.1, 155.3, 157.7, 161.4 ppm.

6.1.2.2. 3-(Bromomethyl)-6-methoxy-*N*-(4-methylpyridin-2-yl) benzofuran-2-carboxamide (6c). Amide **5c** (1.12 g, 3.78 mmol) reacted in CCl_4 (95 mL) to give **6c** (0.73 g, 51%) as a pale yellow solid, mp 191–193 °C (EtOH); IR ν_{max} 1558, 1619, 3393 cm^{-1}; IR ν_{max} 1578, 1665, 3401 cm^{-1}; ^1H NMR δ = 2.41 (s, 3H, $-CH_3$) 3.90 (s, 3H, $-OCH_3$), 5.14 (s, 2H, $-CH_2Br$), 6.93 (dd, *J* = 1 and 5 Hz, 1H, H-5), 7.03 (m, 2H, H-4 and H-7), 7.67 (dd, *J* = 1 and 8 Hz, 1H, H-5′), 8.20 (m, 2H, H-3′ and H-6′), 8.97 ppm (s, 1H, –NH); ^{13}C NMR δ = 21.2, 21.8, 56.2, 96.1, 114.3, 115.2, 121.0, 121.8, 122.1, 125.0, 141.9, 147.9, 150.6, 151.1, 155.3, 157.7, 161.4 ppm.

6.1.2.3. 3-(Bromomethyl)-6-methoxy-*N*-(5-methylpyridin-2-yl) benzofuran-2-carboxamide (6d). Amide **5d** (0.80 g, 2.70 mmol) reacted in CCl_4 (80 mL) to give **6d** (0.45 g, 44%) as a pale yellow solid, mp 178–180 °C (EtOH); IR ν_{max} 1599, 1673, 3430 cm^{-1}; ^1H NMR δ = 2.36 (s, 3H, $-CH_3$), 3.91 (s, 3H, $-OCH_3$), 5.12 (s, 3H, $-CH_2Br$), 6.99 (dd, *J* = 2 and 9 Hz, 1H, H-5), 6.98 (d, *J* = 2 Hz, 1H, H-7), 7.52 (d, *J* = 9 Hz, 1H, H-4), 7.58 (dd, *J* = 2 and 9 Hz, 1H, H-4′), 8.18 (d, *J* = 2 Hz, 1H, H-6′), 8.29 (d, *J* = 9 Hz, 1H, H-3′), 8.98 ppm (s, 1H, –NH); ^{13}C NMR δ = 18.3, 21.3, 56.3, 96.1, 114.2, 114.4, 121.1, 122.2, 125.4, 139.4, 139.7, 148.5, 149.0, 151.0, 155.3, 158.6, 161.4 ppm.

6.1.2.4. 3-(Bromomethyl)-6-methoxy-*N*-(6-methylpyridin-2-yl) benzofuran-2-carboxamide (6e). Amide **5e** (0.85 g, 2.87 mmol) reacted in CCl_4 (60 mL) to give **6e** (0.74 g, 69%) as a pale yellow solid, mp 150–152 °C (EtOH); IR ν_{max} 1597, 1670, 3414 cm^{-1}; ^1H NMR δ = 2.79 (s, 3H, $-CH_3$) 3.92 (s, 3H, $-OCH_3$), 5.07 (s, 2H, $-CH_2Br$), 7.02 (m, 3H, H-4, H-5 and H-7), 7.67 (m, 2H, H-4′ and H-5′), 8.20 (d, *J* = 8 Hz, 1H, H-3′), 9.06 ppm (s, 1H, –NH); ^{13}C NMR δ = 21.2, 24.2, 56.2, 96.1, 112.0, 114.3, 120.1, 122.1, 123.9, 125.7, 139.9, 142.2, 151.0, 155.6, 157.8, 158.9, 161.6 ppm.

6.1.2.5. 3-(Bromomethyl)-*N*-(5-chloropyridyl)-6-methoxybenzofuran-2-carboxamide (6f). Amide **5f** (1.17 g, 3.70 mmol) reacted in CCl_4 (100 mL) to give **6f** (0.95 g, 65%) as a pale yellow solid, mp 218–220 °C (EtOH); IR ν_{max} 1577, 1678, 3394 cm^{-1}; ^1H NMR δ = 3.90 (s, 3H, $-OCH_3$), 5.12 (s, 2H, $-CH_2Br$) 7.01 (m, 2H, H-5 and H-7) 7.68 (d, *J* = 9 Hz, 1H, H-4), 7.73 (dd, *J* = 3 and 9 Hz, 1H, H-4′), 8.31 (d, *J* = 9 Hz, 1H, H-3′), 8.37 (d, *J* = 3 Hz, 1H, H-6′), 9.00 ppm (s, 1H, –NH); ^{13}C NMR δ = 20.6, 55.8, 95.7, 114.0, 114.9, 120.6, 121.8, 125.1, 127.3, 138.1, 141.2, 146.7, 149.1, 155.0, 157.2, 161.2 ppm.

6.1.2.6. 3-Methoxy-6-oxo-7,13-dihydro-6*H*-benzofuro[2,3-*e*]pyrimido[1,2-*a*][1,3]diazepin-12-ium bromide (11). Amide **5g** (0.40 g, 1.41 mmol) reacted in CCl_4 (24 mL) to give **11** (0.11 g, 28%) as a light brown solid; mp. 283–285 °C (decomp.); IR ν_{max} (KBr) 1708, 3404 cm^{-1}; ^1H NMR (DMSO-d_6) δ = 3.86 (s, 3H, $-OCH_3$), 6.03 (s, 2H, $-CH_2N^+$), 7.18 (dd, *J* = 2 and 9 Hz, 1H, H-2), 7.41 (d, *J* = 2 Hz, 1H, H-4), 7.92 (m, 1H, H-10), 8.00 (d, *J* = 9, 1H, H-1), 9.30 (dd, *J* = 2 and 5 Hz, 1H, H-9), 9.38 ppm (dd, *J* = 2 and 5 Hz, 1H, H-11); ^{13}C NMR (DMSO-d_6) δ = 51.8, 56.4, 96.4, 113.68, 115.1, 118.1, 119.0, 122.8, 123.3, 152.4, 152.7, 157.0, 157.2, 161.7, 166.2 ppm; Anal. Calcd for $C_{15}H_{12}N_3O_3Br$: C, 49.74; H, 3.34; N, 11.60. Found: C, 49.46; H, 3.40; N, 11.67.

6.1.2.7. 3-(Bromomethyl)-5,6-dimethoxy-*N*-(pyridin-2-yl)benzofuran-2-carboxamide (6h). Amide **5h** (0.38 g, 1.20 mmol) reacted in CCl_4 (40 mL) to give **6h** (0.11 g, 23%) as an off-white solid, mp 216–218 °C (EtOH); IR ν_{max} 1574, 1643, 3379 cm^{-1}; ^1H NMR δ = 4.01 (s, 3H, $-OCH_3$), 4.02 (s, 3H, $-OCH_3$), 5.18 (s, 2H, $-CH_2Br$), 7.05 (s, 1H, H-7), 7.14 (dd, *J* = 2 and 5 Hz, 1H, H-4′), 7.16 (s, 1H, H-4), 7.80 (dt, *J* = 2 and 8 Hz, 1H, H-5′), 8.38 (d, , *J* = 5 Hz, 1H, H-3′), 8.41 (d, *J* = 8 Hz, 1H, H-6′), 9.05 ppm (s, 1H, –NH); ^{13}C NMR δ = 21.6, 56.8, 56.9, 95.4, 101.6, 114.7, 119.8, 120.6, 125.2, 139.0, 141.9, 148.2, 148.5, 149.3, 151.2, 151.9, 157.7 ppm.

6.1.3. Preparation of chloromethyl amides (7a–f and 7h)

A solution of bromomethyl amide in chloroform was placed in a round-bottomed flask fitted with a reflux condenser. Tetra-*n*-butylammonium iodide (0.3 M equiv) was added to the solution with stirring, followed by an equal volume of 25% aqueous sodium chloride solution. The mixture was then heated at 90 °C for 3–12 h. The layers were separated and the organic layer washed with 2% HCl (2 × 20 mL), then 2% NaOH (2 × 20 ml) and finally water (2 × 20 ml). The resultant organic layer was dried with anhydrous sodium sulfate, filtered, concentrated and recrystallized to give the corresponding chloromethyl amide. No elemental analysis was carried out on these allyl chlorides.

6.1.3.1. 3-(Chloromethyl)-6-methoxy-*N*-(pyridin-2-yl)benzofuran-2-carboxamide (7a). Bromomethyl amide **6a** (0.43 g, 1.19 mmol) reacted in chloroform (20 mL) for 3 h to give **7a** (0.26 g, 68%) as light brown needle-like crystals, mp 163–165 °C (MeOH); IR ν_{max} 1565, 1672, 3411 cm^{-1}; ^1H NMR δ = 3.90 (s, 3H, $-OCH_3$), 5.28 (s, 2H, $-CH_2Cl$), 7.07 (m, 3H, H-4, H-5 and H-7), 7.74 (m, 2H, H-4′ and H-5′), 8.36 (m, 2H, H-3′ and H-6′), 9.03 ppm (s, 1H, –NH); ^{13}C NMR δ = 35.0, 55.8, 95.7, 114.0, 114.3, 120.3, 120.7, 122.0, 124.3, 138.5, 141.8, 148.2, 150.8, 155.0, 157.3, 161.0 ppm.

6.1.3.2. 3-(Chloromethyl)-6-methoxy-*N*-(4-methylpyridin-2-yl) benzofuran-2-carboxamide (7c). Bromomethyl amide **6c** (0.29 g, 0.77 mmol) reacted in chloroform (25 mL) for 6 h to give **7c** (0.20 g, 81%) as beige crystals, mp 179–181 °C (EtOH); IR ν_{max} 1524, 1602,

1675, 3427 cm^{-1}; ^1H NMR δ = 2.41 (s, 3H, $-CH_3$) 3.90 (s, 3H, $-OCH_3$), 5.28 (s, 2H, $-CH_2Cl$), 6.93 (dd, J = 2 and 5 Hz, 1H, H-5), 6.99 (d, J = 2 Hz, 1H, H-7) 7.03 (d, J = 5 Hz, 1H, H-4), 7.71 (dd, J = 2 and 7 Hz, 1H, H-5′), 8.19 (m, 2H, H-3′ and H-6′), 8.97 ppm (s, 1H, –NH); ^{13}C NMR δ = 21.8, 35.3, 56.2, 96.0, 114.3, 115.1, 121.1, 121.8, 122.3, 124.5, 142.3, 148.1, 150.4, 151.1, 153.3, 155.3, 161.3 ppm.

6.1.3.3. 3-(Chloromethyl)-6-methoxy-*N*-(6-methylpyridin-2-yl) benzofuran-2-carboxamide (7e). Bromomethyl amide **6e** (0.55 g, 1.47 mmol) reacted in chloroform (40 mL) for 12 h to give **7e** (0.41 g, 88%) as beige crystals, mp 143–144 °C (EtOH); IR ν_{max} 1544, 1664, 3413 cm^{-1}; ^1H NMR δ = 2.51 (s, 3H, $-CH_3$) 3.90 (s, 3H, $-OCH_3$), 5.28 (s, 2H, $-CH_2Cl$), 7.00 (m, 3H, H-4, H-5 and H-7), 7.69 (m, 2H, H-4′ and H-5′), 8.17 (d, J = 8 Hz, 1H, H-3′), 8.94 ppm (s, 1H, –NH); ^{13}C NMR δ = 24.5, 35.4, 56.2, 96.0, 111.4, 114.3, 120.2, 122.4, 123.9, 125.5, 139.1, 142.1, 151.8, 155.3, 157.7, 158.7, 161.3 ppm.

6.1.3.4. 3-Chloromethyl-*N*-(5-chloropyridin-2-yl)-6-methoxybe nzofuran-2-carboxamide (7f). Bromomethyl amide **6f** (0.55 g, 1.47 mmol) reacted in chloroform (40 mL) for 4 h to give **7f** (0.41 g, 88%) as beige crystals, mp 207–208 °C (EtOH); IR ν_{max} 1549, 1666, 3398 cm^{-1}; ^1H NMR δ = 3.90 (s, 3H, $-OCH_3$), 5.26 (s, 2H, $-CH_2Cl$), 7.01 (m, 2H, H-5 and H-7), 7.73 (m, 2H, H-4 and H-4′), 8.30 (d, J = 6 Hz, 1H, H-6′), 8.36 (d, J = 9 Hz, 1H, H-3′), 9.01 ppm (s, 1H, –NH); ^{13}C NMR δ = 34.8, 55.8, 95.6, 114.0, 114.9, 120.6, 122.0, 125.1, 127.3, 138.1, 141.4, 146.7, 149.1, 155.8, 157.5, 161.4 ppm.

6.1.3.5. 3-(Chloromomethyl)-5,6-dimethoxy-*N*-(pyridin-2-yl)be nzofuran-2-carboxamide (7h). Bromomethyl amide **6h** (0.13 g, 0.33 mmol) reacted in chloroform (12 mL) to give **7h** (0.41 g, 68%) as beige crystals, mp 207–208 °C (EtOH); IR ν_{max} 1557, 1669, 3403 cm^{-1}; ^1H NMR δ = 4.00 (s, 3H, $-OCH_3$), 4.01 (s, 3H, $-OCH_3$), 5.14 (s, 3H, $-CH_2Cl$), 6.99 (s, 1H, H-7), 7.00 (s, 1H, H-4), 7.13 (m, 1H, H-4′), 7.80 (m, 1H, H-5′), 8.40 (m, 2H, H-3′ and H-6′), 8.98 ppm (s, 1H, –NH), ^{13}C NMR δ = 35.6, 56.8, 56.9, 95.4, 101.9, 114.6, 119.9, 120.6, 125.2, 139.1, 142.0, 147.9, 148.0, 149.0, 151.5, 151.9, 157.8 ppm.

6.1.4. Preparation of tetracyclic salts (8a–f, 8h)

In a round-bottomed flask fitted with a reflux condenser and calcium chloride guard tube, was placed a solution (10–20%) of chloromethyl amide in 1,4-dioxane. This was heated at reflux for several days. The resultant precipitate was collected by filtration and was washed with hot acetone to give the respective tetracyclic chloride salt.

6.1.4.1. 3-Methoxy-6-oxo-7,13-dihydro-6*H*-benzofuro[2,3-*e*]py rido[1,2-*a*][1,3]diazepin-12-ium chloride (8a). Chloromethyl amide **7a** (0.35 g, 1.11 mmol) in 1,4-dioxane (2 mL) for 3 days gave **8a** (0.15 g, 43%) as a pale gray solid, mp 285–287 °C; IR ν_{max} 1721, 3430 cm^{-1}, ^1H NMR (DMSO-d_6) δ = 3.88 (s, 3H, $-OCH_3$), 6.10 (s, 2H, $-CH_2N^+$), 7.17 (dd, J = 1 and 9 Hz, 1H, H-2), 7.43 (d, J = 1 Hz, 1H, H-4), 7.83 (dd, J = 2 and 8 Hz, 2H, H-8 and H-9), 8.07 (d, J = 9 Hz, 1H, H-1), 8.49 (dt, J = 2 and 6 Hz, 1H, H-10), 9.1 (d, J = 6 Hz, 1H, H-11), 12.21 ppm (s, 1H, –NH); ^{13}C NMR (DMSO-d_6) δ = 50.2, 56.4, 96.5, 115.0, 118.1, 121.9, 122.9, 123.1, 123.6, 143.6, 143.7, 146.8, 148.4, 157.2, 157.8, 161.6 ppm; Anal. Calcd for $C_{16}H_{13}N_2O_3Cl$: C, 60.67; H, 4.14; N, 8.84. Found: C, 60.49; H, 4.21; N, 8.82.

6.1.4.2. 3-Methoxy-8-methyl-6-oxo-7,13-dihydro-6*H*-benzofuro [2,3-*e*]pyrido[1,2-*a*][1,3]diazepin-12-ium chloride (8b). Crude chloromethyl amide **7b** (0.44 g) in 1,4-dioxane (4 mL) for 3 days gave **8b** (0.019 g, 2% from amide) as a brown solid, mp >300 °C; IR ν_{max} 1708, 3467 cm^{-1}, ^1H NMR (DMSO-d_6) δ = 2.42 (s, 3H, $-CH_3$), 3.90 (s, 3H, $-OCH_3$), 6.12 (s, 3H, $-CH_2N^+$), 6.97 (dd, J = 2 and 9 Hz, 1H, H-2), 7.00 (d, J = 2 Hz, 1H, H-4), 7.23 (dd, J = 5 and 8 Hz, 1H, H-10), 7.58 (d, J = 9 Hz, 1H, H-1), 7.76 (d, J = 8 Hz, 1H, H-9), 8.64 (d, J = 5 Hz, 1H, H-11), 11.94 (s, 1H, –NH), ^{13}C NMR (DMSO-d_6) δ = 18.6, 50.0, 55.9, 95.8, 113.0, 121.3, 121.7, 123.3, 124.8, 128.5, 139.8, 141.8, 146.2, 149.0, 155.2, 158.0, 160.9 ppm.

6.1.4.3. 3-Methoxy-9-methyl-6-oxo-7,13-dihydro-6*H*-benzofuro [2,3-*e*]pyrido[1,2-*a*][1,3]diazepin-12-ium chloride (8c). Chloromethyl amide **7c** (0.074 g, 0.23 mmol) in 1,4-dioxane (4 mL) for 3 days gave **8c** (0.036 g, 49%) as a cream solid, mp 283–285 °C; IR ν_{max} 1711, 3485 cm^{-1}, ^1H NMR (DMSO-d_6) δ = 2.52 (s, 3H, $-CH_3$), 3.85 (s, 3H, $-OCH_3$), 5.90 (s, 2H, $-CH_2N^+$), 7.13 (dd, J = 2 and 9 Hz, 1H, H-2), 7.38 (d, J = 2 Hz, 1H, H-4), 7.54 (s, 1H, H-8), 7.62 (d, J = 6, 1H, H-10), 8.01 (d, J = 9 Hz, 1H, H-1), 8.85 (d, J = 6 Hz, 1H, H-11), 12.13 ppm (s, 1H, –NH); ^{13}C NMR (DMSO-d_6) δ = 22.2, 49.9, 56.9, 80.0, 97.0, 115.4, 118.6, 122.0, 123.4, 123.9, 143.0, 144.5, 148.5, 157.5, 158.6, 159.7, 161.9 ppm; Anal. Calcd for $C_{17}H_{15}N_2O_3Cl$: C, 61.73; H, 4.57; N, 8.47. Found: C, 61.56; H, 4.50; N, 8.25.

6.1.4.4. 3-Methoxy-10-methyl-6-oxo-7,13-dihydro-6*H*-benzof uro[2,3-*e*]pyrido[1,2-*a*][1,3]diazepin-12-ium chloride (8d). Crude chloromethyl amide **7d** (0.20 g) in 1,4-dioxane (2 mL) for 3 days gave **8d** (0.05 g, 24%) as a pale gray solid, mp >300 °C; IR ν_{max} 1716, 3465 cm^{-1}, ^1H NMR (DMSO-d_6) δ = 2.41 (s, 3H, $-CH_3$), 3.88 (s, 3H, $-OCH_3$), 6.05 (s, 2H, $-CH_2N^+$), 7.17 (dd, J = 2 and 9 Hz, 1H, H-2), 7.42 (d, J = 2 Hz, 1H, H-4), 7.76 (d, J = 9 Hz, 1H, H-1), 8.05 (d, J = 9 Hz, 1H, H-9), 8.37 (d, J = 9, 1H, H-8), 9.03 (s, 1H, H-11), 12.12 ppm (s, 1H, –NH); ^{13}C NMR (DMSO-d_6) δ = 17.9, 50.6, 56.9, 97.0, 115.5, 118.6, 121.8, 123.6, 124.2, 133.8, 142.9, 144.1, 146.6, 148.2, 157.7, 158.4, 162.1; Anal. Calcd for $C_{17}H_{15}N_2O_3Cl$: C, 61.73; H, 4.57; N, 8.47. Found: C, 61.56; H, 4.58; N, 8.41.

6.1.4.5. 3-Methoxy-11-methyl-6-oxo-7,13-dihydro-6*H*-benzof uro[2,3-*e*]pyrido[1,2-*a*][1,3]diazepin-12-ium chloride (8e). Chloromethyl amide **7e** (0.38 g, 1.20 mmol) in 1,4-dioxane (2 mL) for 5 days gave **8e** (45 mg, 12%) as a cream solid, mp 260–261 °C; IR ν_{max} 1707, 3458 cm^{-1}, ^1H NMR (DMSO-d_6) δ = 2.82 (s, 3H, $-CH_3$), 3.76 (s, 3H, $-OCH_3$), 5.81 (s, 2H, $-CH_2N^+$), 7.05 (dd, J = 2 and 9 Hz, 1H, H-2), 7.32 (d, J = 2 Hz, 1H, H-4), 7.63 (dd, J = 2 and 8 Hz, 2H, H-8 and H-10), 8.04 (d, J = 9 Hz, 1H, H-1), 8.56 (t, J = 8 Hz, 1H, H-9), 12.07 ppm (s, 1H, –NH); ^{13}C NMR (DMSO-d_6) δ = 21.7, 45.0, 56.4, 96.6, 115.2, 117.9, 119.5, 122.9, 123.8, 124.6, 144.8, 145.8, 149.0, 152.9, 157.3, 158.0, 161.5 ppm; Anal. Calcd for $C_{17}H_{15}N_2O_3Cl$: C, 61.73; H, 4.57; N, 8.47. Found: C, 61.37; H, 4.51; N, 7.91.

6.1.4.6. 10-Chloro-3-methoxy-6-oxo-7,13-dihydro-6*H*-benzof uro[2,3-*e*]pyrido[1,2-*a*][1,3]diazepin-12-ium chloride (8f). Chloromethyl amide **7f** (0.10 g) in 1,4-dioxane (0.5 mL) for 3 days gave **8f** (0.052 g, 50%) as a pale gray solid, mp 204–206 °C; IR ν_{max} 1720, 3431 cm^{-1}, ^1H NMR (DMSO-d_6) δ = 3.88 (s, 3H, $-OCH_3$), 5.75 (s, 2H, $-CH_2N^+$), 7.08 (dd, J = 2 and 9 Hz, 1H, H-2), 7.28 (d, J = 2 Hz, 1H, H-4), 7.80 (d, J = 9 Hz, 1H, H-1), 8.00 (dd, J = 2 and 9 Hz, 1H, H-9), 8.19 (d, J = 9 Hz, 1H, H-8), 8.48 (d, J = 2 Hz, 1H, H-11), 10.61 ppm (s, 1H, –NH); ^{13}C NMR (DMSO-d_6) δ = 55.4, 56.3, 96.5, 114.4, 116.3, 120.4, 122.5, 124.0, 126.7, 138.5, 142.6, 147.1, 149.1, 150.1, 155.0, 157.9, 161.0 ppm; Anal. Calcd for $C_{16}H_{12}N_2O_3Cl_2$: C, 54.72; H, 3.44; N, 7.98. Found: C, 54.40; H, 3.48; N, 8.01.

6.1.4.7. 2,3-Dimethoxy-6-oxo-7,13-dihydro-6*H*-benzofuro[2,3-*e*]pyrido[1,2-*a*][1,3]diazepin-12-ium chloride (8h). Chloro-

methyl amide **7h** (0.35 g, 1.11 mmol) in 1,4-dioxane (2 mL) for 3 days gave **8h** (0.15 g, 34%) as a pale gray solid, mp 256–258 °C; IR ν_{max} 1701, 3461 cm^{-1}, ^1H NMR (DMSO-d_6) δ = 3.88 (s, 3H, $-OCH_3$), 3.90 (s, 3H, $-OCH_3$), 6.17 (s, 2H, $-CH_2N^+$), 7.45 (s, 2H, H-1 and H-7), 7.84 (m, 2H, H-8 and H-10), 8.50 (t, J = 8 Hz, 1H, H-9), 9.11 (d, J = 6 Hz, 1H, H-11), 12.15 ppm (s, 1H, –NH); ^{13}C NMR (DMSO-d_6) δ = 50.2, 56.8, 56.9, 95.5, 102.0, 114.5, 120.0, 120.8, 125.1, 139.1, 141.6, 147.9, 148.1, 149.0, 151.4, 152.3, 158.2 ppm; Anal. Calcd for $C_{17}H_{15}N_2O_4Cl$: C, 58.88; H, 4.36; N, 8.07. Found: C, 58.74; H, 4.30; N, 7.96.

6.2. Pharmacology

6.2.1. Extraction of diazepam from Valium tablets (5 mg)

Valium® tablets (5 × 5 mg), contributed by the University Health Centre, U.W.I., Mona, were crushed with a mortar and pestle then placed in a 50 ml round-bottomed flask fitted with reflux condenser and calcium chloride guard tube. Chloroform (25 mL) was added and the mixture heated at reflux for 2 h with stirring. The mixture was then filtered and the chloroform evaporated under vacuum to give diazepam (23 mg, 92%) as pale yellow crystals; mp 129–132 °C, [lit.[65] 125–126 °C (acetone/petroleum ether)]; ^1H NMR δ = 3.42 (s, 3H, $-NCH_3$), 3.80 (d, J = 11 Hz, 1H, H-3), 4.86 (d, J = 11 Hz, 1H, H-3), 7.33 (m, 2H, H-3′ and H-5′), 7.45 (m, 2H, H-2′ and H-6′), 7.51 (dd, J = 1 and 7 Hz, 1H, H-8), 7.55 (m, 1H, H-4′), 7.62 (d, J = 1 Hz 1H, H-6), 7.64 ppm (d, J = 7 Hz 1H, H-9); ^{13}C NMR δ = 35.2, 57.4, 122.9, 128.9, 129.7, 129.9, 130.3, 130.5, 131.1, 131.8, 138.6, 143.0, 169.3, 170.3 ppm.

6.2.2. Hippocratic screen activities

The following activities were measured in the Hippocratic screen:

(a) *Decreased motor activity*—spontaneous movement of the animals: ±, is quiet but moves spontaneously; +, does not move spontaneously but moves rapidly when handled; ++, moves slowly when handled; +++, moves very sluggishly when handled; ++++, does not move at all when handled.

(b) *Ataxia*—coordinated movement of the animals: +, shows slight incoordination; ++, has difficulty walking in a straight line but its course is true; +++, cannot walk in a straight line and its course is erratic; ++++, cannot walk on any course.

(c) *Loss of righting reflex*—the animals' response when placed on their sides or back: +, can be placed on one side; ++, can be placed be placed on either side equally well; +++, can be placed on back as well as both sides; ++++, cannot get up from back position on its own.

(d) *Analgesia*—animals' response to pain inflicted across the instep and toes: ±, sluggish response with vocalization and or attempts to bite or escape; +, no vocalization nor attempts to bite or escape but does attempt to calmly withdraw foot; ++, no response.

(e) *Anesthesia*—animals' response when a hypodermic needle is pressed to foot pad: ±, a sluggish response; +, no response to needle pressure; ++, no response to needle penetration.

(f) *Pinnal reflex*—the animals' response to a gentle touch with a probe (brush bristle) into the ear canal: ±, sluggish response; +, no response.

(g) *Loss of screen grip*—animals' ability to remain on a wire screen when tilted at 45–180° or gently shaken in a horizontal plane when inverted: ±, unequivocal loss; +, falls at first shake when screen is inverted; ++, falls when screen is inverted; +++, falls at 90°, ++++, falls at 45°.

(h) *Paralysis*—animals were inspected for signs of paralysis in forelegs, hind legs and neck: ±, unequivocal; +, complete.

(i) *Respiratory rate*—animals' rate of breathing per minute compared to the period before the animal is tested: +, 10% change; ++, 20% change; +++, 40% change; ++++, 80% change.

(j) *Tremor*—animals were observed for any signs of abnormal or excessive shaking: ±, unequivocal presence; +, definite but periodically; ++, continuous; +++, convulsions.

(k) *Startle reaction*—the animals' response when the observation rink was sharply slapped with a flat metal object: ±, a mild startle; +, animal visibly jerks; ++, animal jerks, jumps and attempts to escape; +++, animal goes into convulsions.

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References and notes

1. Craig, C. R.; Stitzel, R. E. *Modern Pharmacology*, 4th ed.; Little and Brown: Boston, 1994. p 369.
2. Katzung, B. G. *Basic and Clinical Pharmacology*, 10th ed.; McGraw-Hill Medical: New York, 2007. p 344.
3. Rang, H. P.; Dale, M. M.; Ritter, J. M.; Gardner, P. *Pharmacology*, 5th ed.; Churchill and Livingstone: New York, 2003. p 518.
4. Verdoorn, T. A.; Draguhn, A.; Ymer, S.; Seeburg, P. H.; Sakmann, B. *Neuron* **1990**, *4*, 919.
5. Sigel, E.; Baur, R.; Trube, G.; Möhler, H.; Malherbe, P. *Neuron* **1990**, *5*, 703.
6. Seighart, W. *Pharm. Rev.* **1995**, *47*, 181.
7. Stephenson, F. A.; Duggan, M. J.; Pollard, S. *J. Biol. Chem.* **1990**, *265*, 21160.
8. McKernan, R. M.; Wafford, K.; Quirk, K.; Hadingham, K. L.; Harley, E. A.; Ragan, C. I.; Whiting, P. J. *J. Recept. Signal Transduct. Res.* **1995**, *15*, 173.
9. Wingrove, P. B.; Thompson, S. A.; Wafford, K. A.; Whiting, P. J. *Mol. Pharm.* **1997**, *52*, 874.
10. Lippke, K. P.; Schunack, W. G.; Wenning, W.; Müeller, W. E. *J. Med. Chem.* **1983**, *26*, 499.
11. Hagen, T. J.; Guzman, F.; Schultz, C.; Cook, J. M. *Heterocycles* **1986**, *24*, 2845.
12. Allen, M. S.; Tan, Y. C.; Trudell, M. L.; Narayanan, K.; Schindler, L. R.; Martin, M. J.; Schultz, C.; Hagen, T. J.; Koehler, K. F.; Codding, P. W.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1990**, *33*, 2343.
13. Zhang, W.; Koehler, K. F.; Zhang, P.; Cook, J. M. *Drug Des. Disc.* **1995**, *12*, 193.
14. Zhang, W.; Diaz-Arauzo, H.; Allen, M. S.; Koehler, K. F.; Cook, J. M. In *Studies in Medicinal Chemistry*; Choudhary, M. I., Ed.; Harwood Academic Publishers: New York, 1996; p 303.
15. Huang, Q.; He, X.; Ma, C.; Liu, R.; Yu, S.; Dayer, C. A.; Wenger, G. R.; McKernan, R.; Cook, J. M. *J. Med. Chem.* **2000**, *43*, 71.
16. Clayton, T.; Chen, J. L.; Ernst, M.; Richter, L.; Cromer, B. A.; Morton, C. J.; Ng, H.; Kaczorowski, C. C.; Helmstetter, F. J.; Furtmüller, R.; Ecker, G.; Parker, M. W.; Sieghart, W.; Cook, J. M. *Curr. Med. Chem.* **2007**, *14*, 2755.
17. Cox, E.; Diaz-Arauzo, H.; Huang, Q.; Reddy, M.; Ma, C.; Harris, B.; McKernan, R.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1998**, *41*, 2537.
18. Trullas, R.; Ginter, H.; Jackson, B.; Skolnick, P.; Allen, M.; Hagen, T.; Cook, J. *Life Sci.* **1988**, *43*, 1189.
19. Geyer, H. M., III; Martin, L. L.; Crichlow, C. A.; Dekow, F. W.; Ellis, D. B.; Kruse, H.; Setescak, L. L.; Worm, M. *J. Med. Chem.* **1982**, *25*, 340.
20. Jackson, Y. A.; Williams, M. F. *Heterocycles* **1997**, *45*, 787.
21. Bagajewicz, M. J.; Levin, R. R.; Welsh, C. T.; Schartz-Bloom, R. D. *Levine's Pharmacology: Drug Action and Reactions*, 7th ed.; Taylor and Francis: Oxon, 2005. p 252.
22. Van Leewen, F. X. R.; Den Tonkelaar, E. M.; Van Logten, M. J. *Food Chem. Toxicol.* **1983**, *21*, 383.
23. Van Leeuwen, F. X. R.; Sangster, B. C. R. C. *Crit. Rev. Toxicol.* **1987**, *18*, 189.
24. Ewing, J. A.; Grant, W. J. *South Med. J.* **1965**, *58*, 148.
25. Pavelka, S. *Physiol. Res.* **2004**, *53*, S81.
26. Loeber, J. G.; Franken, M. A. M.; Van Leeuwen, F. X. R. *Food Chem. Toxicol.* **1983**, *21*, 391.
27. Cameron, A. T.; Hollenberg, M. S. *J. Gen. Physiol.* **1922**, 411.
28. Andes, E. *Science* **1962**, *138*, 431.
29. Sadhu, D. P. *Am. J. Physiol.* **1948**, *152*, 263.
30. Markou, K. B.; Paraskevopoulou, P.; Karaiskos, K. S.; Makri, M.; Georgopoulos, N. A.; Iconomou, G.; Mengreli, C.; Vagenakis, A. G. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 617.

31. Jackson, Y. A.; Marriott, K. C. *Heterocycles* **2002**, *57*, 1897.
32. Yonovich-Weiss, M.; Sasson, Y. *Synthesis* **1984**, 34.
33. Sasson, Y.; Weiss, M.; Loupy, A.; Bram, G.; Pardo, C. *J. Chem. Soc., Chem. Commun.* **1986**, 1250.
34. Yamazaki, H.; Dokoshi, W.; Kibayashi, C. *Org. Lett.* **2001**, *3*, 193.
35. Yu, J.; Wang, T.; Liu, X.; Dechamps, J.; Flippen-Anderson, J.; Liao, X.; Cook, J. M. *J. Org. Chem.* **2003**, *68*, 7565.
36. Malone, M. H.; Robichaud, R. C. *Lloydia* **1962**, *25*, 320.
37. Moody, D. In *Handbook of Drug Interactions: a Clinical and Forensic Guide*; Raymon, L. P., Mozayani, A., Eds.; Humana Press Inc.: New Jersey, 2004; p 3.
38. Norman, T. R.; Ellen, S. R.; Burrows, G. D. *Med. J. Aust.* **1997**, *167*, 490.
39. Mohamadi, F.; Richards, N.; Guida, W.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. *J. Comput. Chem.* **1990**, *11*, 440.
40. GAUSSIAN *92*; Gaussian: Carnegie Office Park, Bldg.6: Pittsburgh, PA.
41. GAUSSIAN 94; Gaussian: Carnegie Office Park, Bldg. 6 15106: Pittsburgh, PA.
42. Andzelm, J.; Klobukowski, M.; Radzio-Andzelm, E. *J. Comput. Chem.* **1984**, *5*, 146.
43. Wafford, K.; Whiting, P.; Kemp, J. *Mol. Pharmacol.* **1993**, *43*, 240.
44. Benavides, J.; Peny, B.; Dubois, A.; Perrault, G.; Morel, E.; Zivkovic, B.; Scatton, B. *J. Pharmacol. Exp. Ther.* **1988**, *245*, 1033–1041.
45. SYBYL *6.5*; Tripos: 1699 S. Hanley Road: St. Louis, MO, 63144.
46. Choudhary, M. S.; Craigo, S.; Roth, B. L. *Mol. Pharmacol.* **1992**, *42*, 627.
47. Hevers, W.; Luddens, H. *Mol. Neurobiol.* **1998**, *18*, 35.
48. Sieghart, W.; Ernst, M. *Curr. Med. Chem. Cent. Nervous Syst. Agents* **2005**, *5*, 217.
49. Atack, J. R.; Smith, A. J.; Emms, F.; McKernan, R. M. *Neuropsychopharmacology* **1999**, *20*, 255.
50. Sieghart, W.; Sperk, G. *Curr. Top. Med. Chem.* **2002**, *2*, 795.
51. Doble, A.; Martin, I. L. *Trends Pharmacol. Sci.* **1992**, *13*, 76.
52. Collinson, N.; Cothliff, R.; Rosahl, T.; Sur, C.; Kuenzi, F. M.; Howell, O.; Seabrook, G.; Atack, J.; McKernan, R.; Dawson, G. R.; Whiting, P. *Eur. J. Neurosci.* **2000**, *12*, 171.
53. Bailey, D. J.; Tetzlaff, J. E.; Cook, J. M.; He, X. H.; Helmstetter, F. J. *J. Neurobiol. Learn Mem.* **2002**, *78*, 1.
54. Delorey, T. M.; Lin, L. R.; McBrad, B.; He, X. H.; Cook, J. M.; Lameh, J.; Loew, G. H. *Eur. J. Pharmacol.* **2001**, *426*, 45.
55. Möhler, H.; Rudolph, U.; McKernan, R. Presented at the 43rd Annual Meeting of the American College of Neuropsychopharmacology, San Juan, Puerto Rico, 2004.
56. Diaz-Arauzo, H.; Evoniuk, G. E.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1991**, *34*, 1754.
57. Huang, Q.; Liu, R.; Zhang, P.; He, X.; McKernan, R. M.; Gan, T.; Bennett, D. W.; Cook, J. M. *J. Med. Chem.* **1998**, *41*, 4130.
58. Allen, M. S.; Tan, Y. C.; Trudell, M. L.; Narayanan, K.; Schindler, L.; Martin, M. J.; Schultz, C. A.; Hagen, T. J.; Koehler, K. F.; Codding, P.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1995**, *38*, 1679.
59. Zhang, P.; Zhang, W.; Liu, R.; Harris, B.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1995**, *38*, 1679.
60. Allen, M. S.; Laloggia, A. J.; Dorn, L. J.; Martin, M. L.; Constantino, G.; Hagen, T. J.; Koehler, K. F.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1992**, *35*, 4001.
61. Wong, G.; Koehler, K. F.; Skolnick, P.; Gu, Z. Q.; Ananthan, S.; Schönholzer, P.; Hunkeler, W.; Zhang, W.; Cook, J. M. *J. Med. Chem.* **1993**, *36*, 1820.
62. Huang, Q.; Cox, E. D.; Gan, T.; Ma, C.; Bennett, D. W.; McKernan, R. M.; Cook, J. M. *Drug Des. Disc.* **1999**, *16*, 55.
63. Huang, Q.; Cox, E. D.; Gan, T.; Ma, C.; Bennett, D. W.; McKernan, R. M.; Cook, J. M. *Drug Des. Disc.* **1999**, *3*, 539.
64. Williams, M. F. Approaches to the Synthesis of Azarotenoids and Novel Heterocyclic Salts. M. Phil Thesis, University of the West Indies, Mona, Jamaica, 1996, p 39.
65. 5th ed.*Dictionary of Organic Compounds*; Buckingham, J., Donaghy, S. M., Eds.; Chapman and Hall: New York, 1982; 2, p 1585.