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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Benzoxepin derivatives: design, synthesis, and pharmacological evaluation with sedative–hypnotic effect

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Abstract A series of novel benzoxepin-derived compounds were synthesized and evaluated for their sedative–hypnotic effect using Phenobarbital-induced sleep test in mice. Compound **6** in which the Phenobarbital moiety was incorporated into the benzoxepin nucleus was the most active one. Molecular modeling, including fitting to a 3D-pharmacophore model using Discovery Studio 2.1 programs into optimized benzodiazepine receptor (hypothesis) showed high fit values. The experimental studies for the in vivo sedative–hypnotic effect of compounds **2–6** and **11a–c** were consistent with the molecular modeling.

Keywords Benzoxepin · Mannich reaction · Bromination · Phenobarbital · Sedative and hypnotic

Introduction

According to the US National Institute of Mental Health, anxiety disorders affect annually about 40 million adults ranging in age from 18 years and older who represents 18.1% of people in this age (PHRMA, 2010) making this an important social problem. The BZ (benzodiazepine) classes of drugs are used clinically for their anxiolytic (Mombereau *et al.*, 2010; Jiménez-Velázquez *et al.*, 2010; Benedetti *et al.*, 2004; Westra and Stewart, 2002), hypnotic (Nishino *et al.*, 2008; Westra and Stewart, 2002), muscle-relaxant (Delgado *et al.*, 2010; Kazanietz and Elgoyhen, 1990), and anticonvulsant actions (Shah *et al.*, 2010; Sommer *et al.*, 2007). Recently, BZ derivatives significantly showed anti-neuroinflammatory effects by suppressing iNOS (inducible NO synthase enzyme) activity (Ha *et al.*, 2010).

Benzodiazepine receptors can be subdivided into two types: CBR (central benzodiazepine receptors) and PBR (peripheral benzodiazepine receptors). The CBR is found only on neurons in the central nervous system, coupled with the GABA a (γ-aminobutyric acid) receptor (Peterson, 1987). Ligands acting at the benzodiazepine binding site of the GABA a receptor allosterically modulate the action of GABA on neuronal chloride ion flux. They show a wide variety of pharmacological actions ranging in a continuum from full agonists (sedative/hypnotic, anxiolytic, and anticonvulsant activities) to inverse agonists (anxiogenic, and proconvulsant activities). Antagonists do not exhibit any direct pharmacological effects but can antagonize the actions of both agonists and inverse agonists (Bell *et al.*, 2003; Barnard *et al.*, 1998; Bloom *et al.*, 1996; McKernan and Whiting, 1996; Sieghart and Sieghart, 1995; Smith and Olsen, 1995; Yeh and Grigorenko, 1995, Bellantuono *et al.*, 1980).

From another point of view, considerable interest has been focused on the benzoxepin structures, which have been reported to possess central nervous system depressants (Porter and Prus, 2009; Freedman, 1975). A compound disclosed is 6,7,8,9,10,11-hexahydro-6,6-dimethyldibenz [b,d]oxepin-3-ol **I** (Freedman, 1975). However, benzoxepin derivatives have documented consistent advances in the design of novel atypical antipsychotic (Porter and Prus, 2009). On the other hand, pharmacological evaluation of a structurally related compound to benzoxepin,

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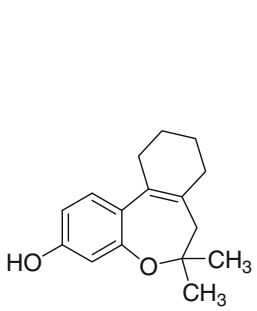
2-(4-chlorophenyl)-5,6-dihydrobenzothiepino[5,4-c]pyridazin-(2*H*)-on-7-oxide **II** had revealed that, it is highly active as an anxioselective anxiolytic agent (Yasumatsu et al., 1994); (Nakao *et al.*, 1992); (Nakao *et al.*, 1991). The selectivity of this interesting compound was attributed to its partial agonist effect on BZR (benzodiazepine receptors) (Yasumatsu *et al.*, 1994); (Nakao *et al.*, 1992); (Nakao *et al.*, 1991). BZR partial agonists were reported as candidates that could maintain the therapeutic potential with fewer side effects than BZR full agonists in the treatment of anxiety (Tanaka *et al.*, 1995). Consequently, in the light of these facts, in the scope of a research program aimed at the development of new isosteric analogues to compound **II**, we described in this study, the synthesis and the pharmacological evaluation of some new benzoxepine derivatives **11a–c** as sedative–hypnotic agents. In our research, new ligands acting on BZR among benzoxepin derivatives were synthesized and investigated for their sedative–hypnotic activity. Moreover, in view of the biological activity of Phenobarbital that acts as sedative–hypnotic through enhancing GABA binding (Gillon *et al.*, 2010), we planned to synthesize a hybridized benzoxepin derivative **6** by incorporating the Phenobarbital moiety at the 4-position of the benzoxepin nucleus aiming to obtain a highly sedative–hypnotic agent. Furthermore, other new substituted aminomethyl-benzoxepin **3**, and benzylideno-benzoxepin **4** were also synthesized and evaluated for their sedative–hypnotic effect.



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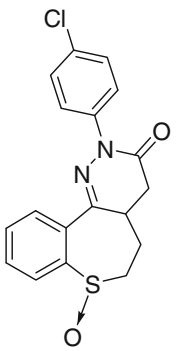
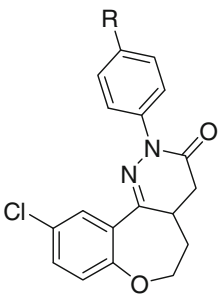
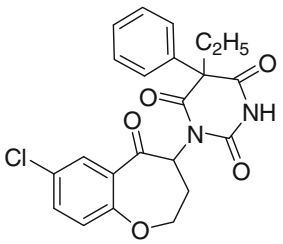


II



11a-c



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In addition, the design was also based on the molecular modeling simulation study using Discovery Studio 2.1 programs, which was performed in order to predict sedative–hypnotic effect of the proposed compounds. A new BDZ receptor agonist pharmacophore model (hypothesis) was generated in order prioritize the activity of the proposed new molecules. Comparing the fit studies between such hypothesis and the planned compounds indicated that they have promising sedative–hypnotic activity and could be considered hit molecules.

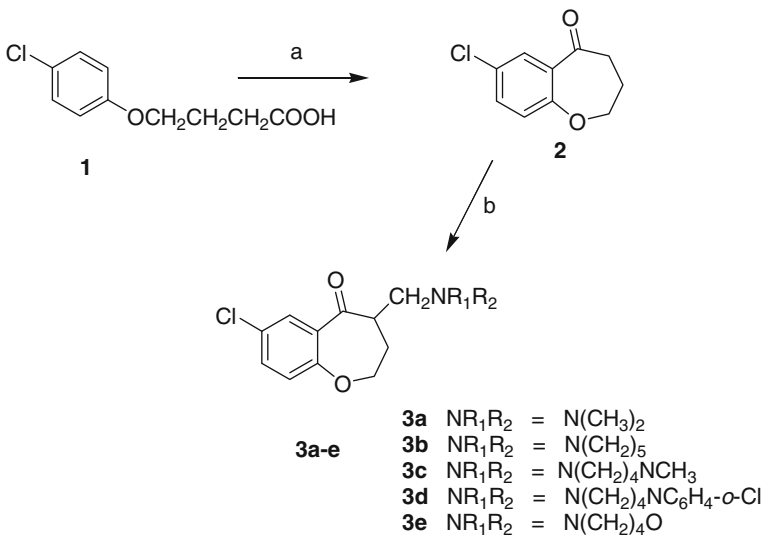
Experimental

Chemistry

Melting points are uncorrected and determined in one end open capillary tubes using Gallen Kamp melting point apparatus MFB-595-010 M (Gallen Kamp, London, England). Microanalysis was carried out at Micro analytical Unit, Faculty of Science, Cairo University. IR spectra were determined using KBr discs (cm^{-1}) on Shimadzu Infrared Spectrometer IR-435 (Shimadzu, Kyoto, Japan), Perkin-Elmer FT-IR 1650 (Perkin-Elmer, Waltham, Massachusetts 02451, USA) and Mattson Genesis II FTIR^{TM} Spectrometer (Mattson, Madison, WI, USA). ^{1}H-NMR (DMSO-d_6, D_2O) δ ppm spectra were determined using Joel NMR Varian Gemini 200 MHz Spectrometer (Joel, Tokyo, Japan) and Varian Mercury VX-300 MHz NMR Spectrometer (Varian, Oxford, England). ^{13}C-NMR spectra were obtained on a Bruker APX400 spectrometer in the specified solvent (Varian, Darmstadt, Germany). Mass spectra were recorded using Hewlett Packard Varian (Varian, Polo, USA) and Shimadzu Gas Chromatograph Mass spectrometer-QP 1000 EX (Shimadzu, Kyoto, Japan). TLC were carried out using Art.DC-Plastikfolien, Kieselgel 60 F254 sheets (Merck, Darmstadt, Germany), the developing solvents were benzene/methanol (4:1) and the spots were visualized at 366, 254 nm by UV Vilber Lourmat 77202 (Vilber, Marne La Vallee, France). Log P were calculated using ChemDraw Ultra V 8.0.

7-Chloro-3,4-dihydrobenzo[b]oxepin-5(2H)-one ***2*** *(Scheme 1)*

To a solution of 4-(4-chlorophenoxy)butanoic acid **1** (4.29 g, 20 mmol) in dry toluene (20 mL), was added with stirring Celite (5 g) and phosphorus pentaoxide (5.68 g, 40 mmol). The mixture was refluxed for 4 h and then



Scheme 1 Reagents and reaction conditions: *a* P_2O_5/Celite/dry toluene, reflux. *b 1* $NH.R_1R_2.HCl/HCHO$/acetic anhydride, reflux. *2* NH_4OH

filtered while hot. The filtrate was washed with 1% $NaHCO_3$, dried over Na_2SO_4, and concentrated in *vacuum* to give compound **2**. The residue was recrystallized from methanol gave yellowish-white crystals, m.p. 72–74°C, yield 85%. ^{1}H-NMR ($CDCl_3$-D_2O) δ: 2.29 (2H, m, CH_2), 2.94 (2H, t, CH_2CO, $J = 7.4$ Hz), 4.16 (2H, t, OCH_2, $J = 11.7$ Hz), 6.84 (1H, d, ArH, $J = 9.0$ Hz), 7.17 (1H, d, ArH, $J = 11.0$ Hz), 7.45 (1H, d, ArH, $J = 2.7$ Hz). IR (KBr) cm^{-1}: 3097, 3076, 3034 (CH aromatic), 2970, 2951 (CH aliphatic), 1718 (C=O), 740 (C–Cl). MS (*m/z*): 196 (M^+). Anal. $C_{10}H_9ClO_2$ (196.63): C, 61.08; H, 4.61. Found: C, 61.20; H, 4.30.

*General method for preparation of compounds **3a–e** (Scheme 1)*

A solution of the appropriate secondary amine hydrochloride (15 mmol) in 37% HCHO (0.6 g, 1.6 ml, 20 mmol) was stirred at room temperature for 0.5 h. Acetic anhydride (5 ml) was added dropwise and the mixture was stirred at 70–75°C for further 0.5 h. Compound **2** (1.96 g, 10 mmol) was added and the mixture was stirred at 70–75°C for 3 h. After cooling, the reaction mixture was concentrated in *vacuum*. The residue was dissolved in chilled water, neutralized with 28% NH_4OH and extracted with $CHCl_3$. The extract was washed with water, dried over Na_2SO_4, and evaporated below 40°C. The residue was recrystallized from $CHCl_3$/ether.

*7-Chloro-4-(dimethyamino)methyl-3,4-dihydrobenzo[b]oxepin-5(2H)-one (**3a**)* Yield 45%, m.p. 79–82°C. ^{1}H-NMR ($CDCl_3$-D_2O) δ: 2.25 (2H, m, CH_2), 2.34–2,57 (8H, m, CH_2–$N(CH_3)_2$), 2.94 (1H, m, CHCO), 4.16 (2H, t, OCH_2, $J = 11.7$ Hz), 6.85 (1H, d, ArH, $J = 8.7$ Hz), 7.17 (1H, d, ArH, $J = 11.4$ Hz), 7.46 (1H, d, ArH, $J = 2.4$ Hz). IR (KBr) cm^{-1}: 3050 (CH aromatic), 2924, 2850 (CH aliphatic), 1762 (C=O), 760 (C–Cl). MS (*m/z*): 255 (M^++2), 253 (M^+). Anal. $C_{13}H_{16}ClNO_2$ (253.72): C, 61.54; H, 6.36; N, 5.52. Found: C, 61.04; H, 6.10; N, 5.20.

*7-Chloro-4-((piperdin-1-yl)methyl)-3,4-dihydrobenzo[b]oxepin-5(2H)-one (**3b**)* Yield 54%, m.p. 67–69°C. ^{1}H-NMR (DMSO-d6) δ: 2.09-2.20 (6H, m, $(CH_2)_3$), 2.31 (2H, m, CH_2), 2.89–2.94 (6H, m, –CH_2–N–$(CH_2)_2$), 4.16 (2H, t, OCH_2, $J = 11.4$ Hz), 4.73 (1H, s, CHCO), 6.88 (1H, d, ArH, $J = 8.7$ Hz), 7.19 (1H, d, ArH, $J = 12.6$ Hz), 7.46 (1H, d, ArH, $J = 2.7$ Hz). IR (KBr) cm^{-1}: 3078, 3062 (CH aromatic), 2966, 2941 (CH aliphatic), 1762 (C=O), 765 (C–Cl). MS (*m/z*): 291 (M^+- 2). Anal. $C_{16}H_{20}ClNO_2$ (293.79): C, 65.41; H, 6.86; N, 4.77. Found: C, 65.80; H, 6.60; N, 4.90.

*7-Chloro-4-((4-methylpiperazin-1-yl)methyl)-3,4-dihydrobenzo[b]oxepin-5(2H)-one (**3c**)* Yield 50%, m.p. 81–84°C. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, s, NCH_3), 2.25–2.34 (6H, m, $(CH_2)_2$ and CH_2), 2.89–2.94 (6H, m, –CH_2–N–$(CH_2)_2$), 4.12-4.20 (3H, m, OCH_2 and CHCO), 6.85 (1H, d, ArH, $J = 9.0$ Hz), 7.17 (1H, d, ArH, $J = 11.4$ Hz), 7.46 (1H, d, ArH, $J = 2.7$ Hz). ^{13}C-NMR ($CDCl_3$) δ: 24.3 (C-3), 25.5 (CH_3), 29.7 (C-4), 30.2 (C-2′, C-3′), 67.9 (CH_2N), 114.3 (C-2), 123.9 (C-9), 127.6 (C–Cl), 131.9 (C-6), 133.0 (C-8), 145.6 (C-5a), 153.1 (C-9a), 170.4 (C=O). IR (KBr) cm^{-1}: 3095, 3076 (CH aromatic), 2921, 2852 (CH aliphatic), 1764 (C=O), 759 (C–Cl). MS (*m/z*): 310 (M^++2), 308 (M^+). Anal. $C_{16}H_{21}ClN_2O_2$ (308.80): C, 62.23; H, 6.85; N, 9.07. Found: C, 62.40; H, 6.50; N, 8.70.

*7-Chloro-4-((4-(2-chlorophenyl)piperazin-1-yl)methyl)-3,4-dihydrobenzo[b]oxepin-5(2H)-one (**3d**)* Yield 55%, m.p. 78–80°C. ^{1}H-NMR ($CDCl_3$) δ: 2.00–2.20 (6H, broad, –$N(CH_2)_2$ and CH_2), 2.58–2.78 (6H, broad, $CH_2N(CH_2)_2$), 4.01–4.20 (3H, broad, OCH_2, CHCO), 6.71–6.85 (2H, broad, ArH), 7.10–7.40 (5H, m, ArH). IR (KBr) cm^{-1}:

3095, 3078 (CH aromatic), 2972, 2889 (CH aliphatic), 1762 (C=O), 759 (C–Cl). MS (*m/z*): 405 (M^+), 407 (M^++2). Anal. $C_{21}H_{22}Cl_2N_2O_2$ (405.32): C, 62.23; H, 5.47; N, 6.91. Found: C, 61.90; H, 5.20; N, 6.70.

*7-Chloro-4-((morphlino-1-yl)methyl)-3,4-dihydrobenzo [b]oxepin-5(2H)-one (**3e**)* Yield 50%, m.p. 91–93°C. ^{1}H-NMR ($CDCl_3$) δ: 2.25–2.34 (6H, m, CH_2 and $N(CH_2)_2$), 2.91 (2H, t, $-CH_2N$, $J = 14.1$ Hz), 4.12–4.16 (7H, m, OCH_2, CHCO and $O(CH_2)_2$), 6.88 (1H, d, ArH, $J = 8.7$ Hz), .20 (1H, d, ArH, $J = 11.1$ Hz), 7.46 (1H, d, ArH, $J = 2.4$ Hz). IR (KBr) cm^{-1}: 3095, 3075 (CH aromatic), 2966, 2885 (CH aliphatic), 1762 (C=O), 758 (C–Cl). MS (*m/z*): 296 (M^++1). Anal. $C_{15}H_{18}ClNO_3$ (295.76): C, 60.91; H, 6.13; N, 4.74. Found: C, 60.70; H, 5.90; N, 4.30.

*General method for preparation of compounds **4a–d** (Scheme 2)*

A mixture of benzoxepin derivative **2** (1.96 g, 10 mmol), the appropriate aromatic aldehyde (10 mmol) and anhydrous sodium acetate (1.64 g, 20 mmol) in glacial acetic acid (10 mL) was refluxed for 6 h, cooled, and poured into chilled water. The separated solid was filtered, washed with water, dried and recrystallized from methanol.

*4-Benzylidene-7-chloro-3,4-dihydrobenzo[b]oxepin-5(2H)-one (**4a**)* Yield 65%, m.p. 96–98°C. ^{1}H-NMR ($CDCl_3$) δ: 2.17 (2H, t, CH_2, $J = 13.2$ Hz), 4.14 (2H, t, OCH_2, $J = 12$ Hz), 6.82 (1H, d, ArH, $J = 8.7$ Hz), 7.17 (1H, d, ArH, $J = 6.6$ Hz), 7.27 (1H, s, =CH–Ar), 7.36–7.40 (5H, m, ArH), 8.11 (1H, d, ArH, $J = 2.5$ Hz). IR (KBr) cm^{-1}: 3093, 3034 (CH aromatic), 2968, 2887 (CH aliphatic), 1766 (C=O), 760 (C–Cl). MS (*m/z*): 285 (M^++1), 283 (M^+-1). Anal. $C_{17}H_{13}ClO_2$ (284.74): C, 71.71; H, 4.60. Found: C, 71.50; H, 4.40.

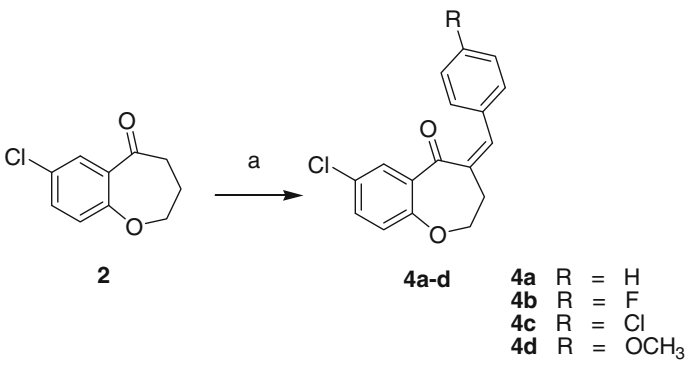
*4-(Fluorobenzylidene)-7-chloro-3,4-dihydro-benzo[b]oxepin-5(2H)-one (**4b**)* Yield 60%, m.p. 178–180°C. ^{1}H-NMR (DMSO-d_6) δ: 2.19 (2H, t, CH_2, $J = 13.8$ Hz), 4.09 (2H, t, OCH_2, $J = 12$ Hz), 6.85 (3H, d, ArH, $J = 8.7$ Hz), 7.15 (3H, d, ArH, $J = 11.1$ Hz), 7.27 (1H, s, =CH–Ar), 7.36 (1H, d, ArH, $J = 2.4$ Hz). IR (KBr) cm^{-1}: 3095, 3078, 3034 (CH aromatic), 2970, 2891 (CH aliphatic), 1766 (C=O), 760 (C–Cl). MS (*m/z*): 302 (M^+), 267 (M^+-35). Anal. $C_{17}H_{12}ClFO_2$ (302.72): C, 67.45; H, 4.00. Found: C, 67.70; H, 4.30.

*4-(Chlorobenzylidene)-7-chloro-3,4-dihydrobenzo[b] oxepin-5(2H)-one (**4c**)* Yield 63%, m.p. 112–114°C. ^{1}HNMR (DMSO-d_6) δ: 2.30 (2H, t, CH_2), 4.14 (2H, t, OCH_2), 6.89 (3H, d, ArH), 7.08–7.39 (4H, m, ArH, and =CH–Ar), 7.46 (1H, d, ArH). IR (KBr) cm^{-1}: 3097, 3038 (CH aromatic), 2970, 2889 (CH aliphatic), 1716 (C=O), 740 (C–Cl). MS (*m/z*): 320 (M^++2), 318 (M^+). Anal. $C_{17}H_{12}Cl_2O_2$ (319.18): C, 63.97; H, 3.79. Found: C, 63.70; H, 3.90.

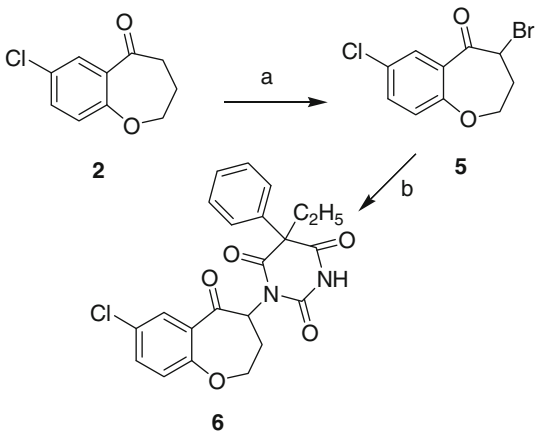
*7-Chloro-4-(methoxybenzylidene)-3,4-dihydrobenzo[b] oxepin-5(2H)-one (**4d**)* Yield 54%, m.p. 128–130°C. ^{1}H-NMR ($CDCl_3$) δ: 2.27 (2H, t, CH_2, $J = 12.2$ Hz), 3.90 (3H, s, OCH_3), 4.16 (2H, t, OCH_2, $J = 12$ Hz), 6.85 (1H, d, ArH, $J = 8.7$ Hz), 7.07–7.28 (5H, m, ArH), 7.38 (1H, s, =CH–Ar), 7.46 (1H, d, ArH, $J = 2.3$ Hz). ^{13}C-NMR ($CDCl_3$) δ: 24.3 (C-3), 59.0 (OCH_3), 67.9 (C-2), 114.3 (C-3'), 114.5 (C-9), 123.9 (C-5a), 126.0 (C-7), 127.5 (C-2′), 127.9 (C-1′), 130.0 (C-6), 130.1 (C-8), 131.9 (C-4), 145.6 (-CH=Ar), 153.0 (C-9a), 154.0 (C-4'), 170.4 (C=O). IR (KBr) cm^{-1}: 3095, 3035 (CH aromatic), 2950, 2889 (CH aliphatic), 1764 (C=O), 760 (C–Cl). MS (*m/z*): 302 [(M^++2)-CH_3]. Anal. $C_{18}H_{15}ClO_3$ (314.76): C, 68.68; H, 4.80. Found: C, 68.50; H, 4.60

*4-Bromo-7-chloro-3,4-dihydrobenzo[b]oxepin-5(2H)-one **5** (Scheme 3)*

To a suspension of copper (II) bromide (4.46 g, 20 mmol) in chloroform (20 mL) and ethyl acetate (20 mL), compound **2** (1.96 g, 10 mmol) was added. The mixture was refluxed for 30-60 min. Completion of the reaction is indicated by disappearance of all the black copper (II) bromide solid and appearance of white copper (I) bromide. The mixture was filtered while hot and the residue was washed with hot chloroform. The combined filtrate and washing were concentrated in *vacuum* to give compound **5**, which were recrystallized from benzene.



Scheme 2 Reagents and reaction conditions: *a* ArCHO/anh.NaOAc/glacial acetic acid, reflux



Scheme 3 Reagents and reaction conditions: *a* $CuBr_2$/chloroform/ethyl acetate, reflux. *b* Phenobarbital Sodium/anh.K_2CO_3/DMF, reflux

Yield 90%, m.p. 175–177°C. ^{1}H-NMR ($CDCl_3$) δ: 2.31 (2H, m, CH_2), 4.16 (2H, t, OCH_2, *J*= 12 Hz), 6.83 (1H, d, ArH, $J = 8.7$ Hz), 7.10 (1H, t, -CH-Br), 7.38 (1H, d, ArH, $J = 11.1$ Hz), 7.46 (1H, d, ArH, $J = 2.3$ Hz). IR (KBr) cm^{-1}: 3097, 3078 (CH aromatic), 2970, 2951, 2889 (CH aliphatic), 1762 (C=O), 740 (C–Cl). MS (*m/z*): 275 (M^+), 277 (M^++2). Anal. $C_{10}H_8BrClO_2$ (275.53): C, 43.59; H, 2.93. Found: C, 43.70; H, 3.20.

1-(7-Chloro-2,3,4,5-tetrahydro-5-oxobenzo[b]oxepin-4-yl)-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione 6 (Scheme 3)

A mixture of compound **5** (2.75 g, 10 mmol), Phenobarbital mono sodium salt (2.76 g, 10 mol), anhydrous potassium carbonate (2 g) and dimethyl formamide (20 mL) was heated at 100°C for 8 h. The mixture was filtered while hot and the filtrate was concentrated in *vacuum* to give compound **6**. The residue was extracted with chloroform and washed several times with water. The organic solution was concentrated in *vacuum* and the solid was recrystallized from methanol. Yield 65%, m.p. 135–137°C. ^{1}H-NMR ($CDCl_3$, D_2O) δ: 1.29 (3H, t, $CH_2\underline{CH_3}$), 2.59 (2H, m, CH_2), 4.03 (2H, m, $C\underline{H_2}CH_3$), 4.19 (2H, t, OCH_2, $J = 12$ Hz), 5.01 (1H, broad, CH) 6.82–7.60 (8H, m, ArH), 8.84 (1H, s, NH exch. D_2O). IR (KBr) cm^{-1}: 3308 (NH), 3087 (CH aromatic), 2970, 2933 (CH aliphatic), 1736, 1716 (2 C=O), 728 (C–Cl).MS (*m/z*): 426 (M^+). Anal. $C_{22}H_{19}ClN_2O_5$ (426.85): C, 61.90; H, 4.40; N, 6.56. Found: C, 62.00; H, 4.10; N, 6.80.

7-Chloro-4-trimethylammoniummethyl-3,4-dihydrobenzo[b]oxepin-5(2H)-one 7 (Scheme 4)

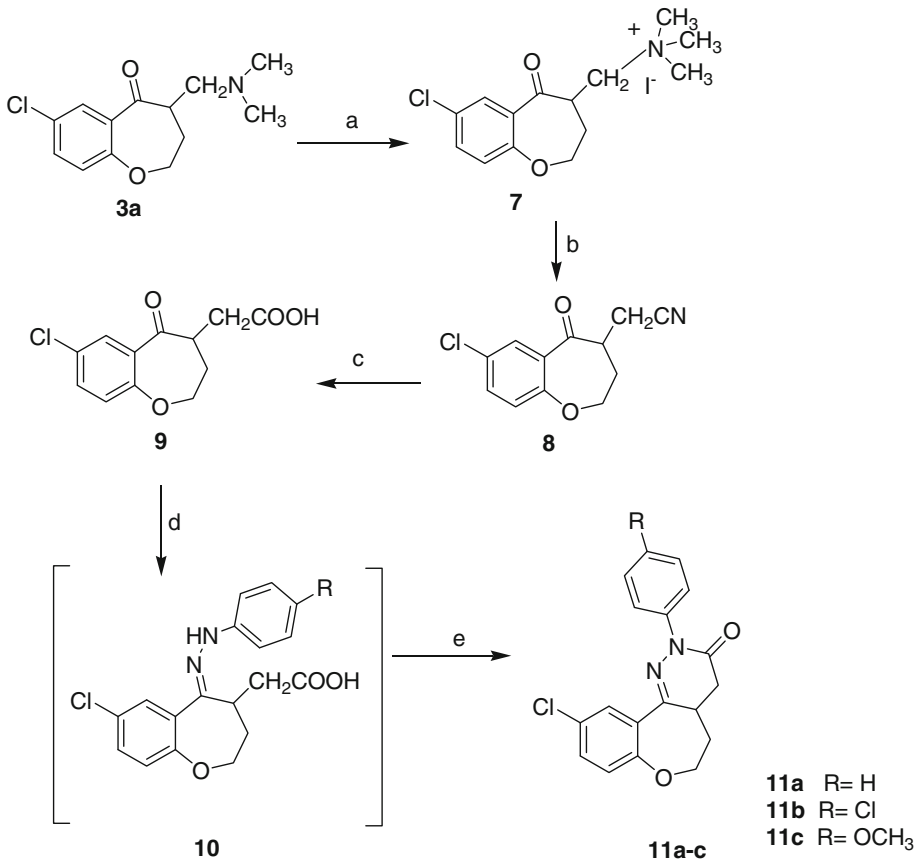
Compound (**3a**) (2.53 g, 10 mmol) was dissolved in dry acetone (50 mL), and iodomethane (2.8 g, 1.3 mL, 20 mmol) was added to the resulting solution below 5°C in an ice bath. The mixture was allowed to stand at room temperature for 24 h., and filtered. The solid was washed with acetone and recrystallized with methanol/water. Yield 55%, m.p. >300°C. ^{1}H-NMR ($CDCl_3$) δ: 2.30 (2H, t, CH_2, *J*= 12.0 Hz), 2.46–2.52 (9H, m, $N(CH_3)_3$), 3.61–4.12 (5H, m, OCH_2 CHCO, and CH_2N), 7.13 (1H, d, ArH, *J*= 8.7 Hz), 7.32 (1H, d, ArH, *J*= 11.1 Hz), 7.49 (1H, d, ArH, *J*= 2.3 Hz). IR (KBr) cm^{-1}: 3075 (CH aromatic), 2970, 2942 (CH aliphatic), 1736 (C=O), 736 (C–Cl). MS (*m/z*): 397 (M^++2), 395 (M^+). Anal. $C_{14}H_{19}ClINO_5$ (395.66): C, 42.50; H, 4.84; N, 3.54. Found: C, 42.90; H, 4.60; N, 3.80.

2-(7-Chloro-2,3,4,5-tetrahydro-5-oxobenzo[b]oxepin-4-yl) acetonitrile 8 (Scheme 4)

To a solution of compound **7** (3.95 g, 10 mmol) in methanol (50 mL) was added a solution of KCN (1.95 g, 30 mmol) in water (10 mL) dropwise at room temperature. The solution was stirred at room temperature for 1 h and poured into ice-water. The resulting mixture was extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and concentrated in *vacuum*. After the addition of diethyl ether to the residue, the crystals formed were collected by filtration. The solid was recrystallized with ethanol/water. Yield 50%, m.p. 151–153°C. ^{1}H-NMR ($CDCl_3$) δ: 2.50 (2H, t, CH_2, $J = 11.0$ Hz), 3.56 (2H, d, CH_2CN, $J = 1$ Hz), 3.95 (2H, d, OCH_2, $J = 11.5$ Hz), 4.55 (1H, broad, C4-H), 6.70 (1H, d, ArH, *J*= 8.7 Hz), 7.15 (1H, d, ArH, $J = 11.1$ Hz), 7.42 (1H, d, ArH, $J = 2.4$ Hz). IR (KBr) cm^{-1}: 3075 (CH aromatic), 2930 (CH aliphatic), 2261 (C≡N), 1736 (C=O), 728 (C–Cl). MS (*m/z*): 235 (M^+), 233 (M^+-2). Anal. $C_{12}H_{10}ClNO_2$ (235.67): C, 61.16; H, 4.28; N, 5.94. Found: C, 61.40; H, 4.50; N, 5.80.

2-(7-chloro-2,3,4,5-tetrahydro-5-oxobenzo[b]oxepin-4-yl) acetic acid 9 (Scheme 4)

To a solution of conc. HCl (10 mL) and acetic acid (10 mL) was added compound **8** (2.35 g, 10 mmol). The solution was refluxed for 4 h, and poured into ice-water. The precipitate was collected by filtration, washed with water, and recrystallized from methanol. Yield 57%, m.p. 193–195°C. ^{1}H-NMR ($CDCl_3$-D_2O) δ: 2.50 (2H, t, CH_2, $J = 11.0$ Hz), 2.73 (2H, d, CH_2COO, $J = 1$ Hz), 4.09 (3H, m, OCH_2 and C4-H), 6.80 (1H, d, ArH, $J = 8.6$ Hz), 7.16 (1H, d, ArH, $J = 11.4$ Hz), 7.36 (1H, d, ArH, $J = 2.4$ Hz), 7.95 (1H, s, COOH, exch. D_2O). IR (KBr) cm^{-1}: 3097, 3076 (CH aromatic), 2927 (CH aliphatic), 1716, 1693 (2 C=O), 742 (C–Cl). MS (*m/z*): 252 (M^+-2). Anal. $C_{12}H_{11}ClO_4$ (254.67): C, 56.59; H, 4.35. Found: C, 56.90; H, 4.20.



Scheme 4 Reagents and reaction conditions: *a* CH_3I, dry actone, reflux. *b* KCN, stirr at room temperature. *c* HCl, acetic acid, reflux. *d* *p*-$RC_6H_4NHNH_2$·HCl, sodium acetate, ethanol, reflux. *e* Acetic acid, reflux

General method for preparation of compounds ***11a–c*** *(Scheme 4)*

A mixture of compound **9** (2.54 g, 10 mmol), sodium acetate (0.82 g, 10 mmol), and the appropriate phenyl hydrazine hydrochloride (10 mmol) in ethanol (20 mL) was refluxed for 15 h. After the evaporation of the solvent, the residue was dissolved in acetic acid (10 mL). The mixture was refluxed for 3 h, cooled, poured into ice-water, and extracted with chloroform. The extract was washed with water, dried over Na_2SO_4, and concentrated in *vacuum*. The solid residue was recrystallized with chloroform/ethanol.

2-Phenyl-4,4′,5,6-tetrahydro-1′-chlorobenzo[4′,3′:2,3] oxepino[4,5-c]pyrdiazin-3(2H)one (**11a**). Yield 67%, m.p. 120–122°C. ^{1}H-NMR ($CDCl_3$) δ: 1.90 (2H, t, CH_2, $J = 10.5$ Hz), 2.40 (2H, d, $CH_2C{=}O$), 3.40 (2H, d, OCH_2, $J = 11.0$ Hz), 4.20 (1H, m, CH), 6.65–7.80 (8H, m, ArH). IR (KBr) cm^{-1}: 3097, 3076 (CH aromatic), 2924, 2854 (CH aliphatic), 1680 (C=O), 760 (C–Cl). MS (*m/z*): 328 (M^++2). Anal. $C_{18}H_{15}ClN_2O_2$ (326.78): C, 66.16; H, 4.35; N, 8.57. Found: C, 65.90; H, 4.20; N, 8.50.

2-(4-Chlorophenyl)-4,4′,5,6-tetrahydro-1′-chlorobenzo [4′,3′:2,3]oxepino[4,5-c]pyrdiazin-3(2H)one (**11b**). Yield 60%, m.p. 136–138°C. ^{1}H-NMR ($CDCl_3$) δ: 2.10 (2H, t, CH_2, $J = 10.5$ Hz), 2.65 (2H, d, $CH_2C{=}O$), 4.08 (3H, m, OCH_2 and CH), 6.81–7.40 (7H, m, ArH). ^{13}C-NMR ($CDCl_3$) δ: 24.1 (C-4a), 30.3 (C-5), 31.0 (C-4), 68.2 (C-6), 114.0 (C-5′), 118.0 (C-9), 123.8 (C-2′), 125.8 (C–Cl), 129.0 (C-2′ & C-3′), 129.8 (C–Cl), 131.0 (C-6′), 139.0 (C-4′), 153.0 (C-8), 179.0 (C=O). IR (KBr) cm^{-1}: 3097, 3076 (CH aromatic), 2924, 2854 (CH aliphatic), 1716 (C=O), 740 (C–Cl). MS (*m/z*): 360 (M^+), 362 (M^++2). Anal. $C_{18}H_{14}Cl_2N_2O_2$ (361.22): C, 59.85; H, 3.91; N, 7.76. Found: C, 59.80; H, 3.70; N, 7.50.

2-(4-Methoxyphenyl)-4,4′,5,6-tetrahydro-1′-chlorobenzo [4′,3′:2,3]oxepino[4,5-c]pyrdiazin-3(2H)one (**11c**). Yield 70%, oil. ^{1}H-NMR ($CDCl_3$) δ: 2.40 (2H, t, CH_2, $J = 10.5$ Hz), 2.85 (2H, d, $CH_2C{=}O$), 3.85 (3H, s, OCH_3), 4.17 (3H, m, OCH_2 and CH), 6.81–7.40 (7H, m, ArH). IR (KBr) cm^{-1}: 3097, 3076 (CH aromatic), 2924, 2854 (CH aliphatic), 1685 (C=O), 740 (C–Cl). MS (*m/z*): 356 (M^+). Anal. $C_{19}H_{17}ClN_2O_3$ (356.8): C, 63.96; H, 4.80; N, 7.85. Found: C, 64.00; H, 4.50; N, 7.60.

Pharmacology

Animals

Male mice (20–25 g) were randomly divided into groups of 8, housed in a breeding room with 12 h light–dark cycle at

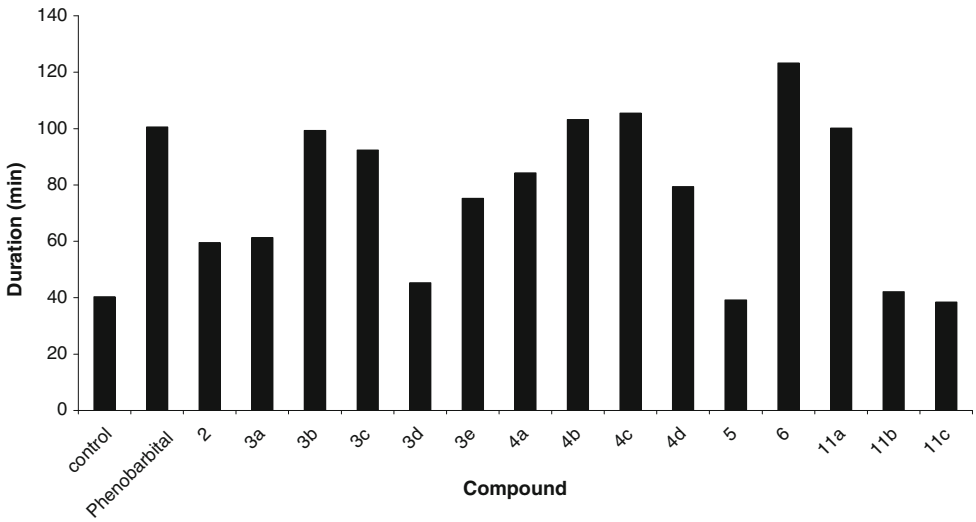


Fig. 1 Effects of the new heterocyclic compounds according to the Phenobarbital-induced sleep test

24 ± 1°C and, fed with standard rodent diet and water. Animals were allowed to adapt to the laboratory environment for 1 week before experimentation. The experimental tests on animals have been performed in accordance with the Institutional Ethical Committee approval, Faculty of Pharmacy, Cairo University.

Preparation of the tested chemical compounds

All the tested chemicals and Phenobarbital (as reference drug) were dissolved in DMSO.

Phenobarbital-induced sleep test in mice (Mora et al., 2005; Tsuji et al., 1996; Fig. 1; Table 2)

The mice were divided into seventeen groups of 8 animals each. The first group (control) was i.p. treated with DMSO. Another group (reference) was i.p. injected Phenobarbital (25 mg kg^{-1}, 0.1 mmol.). The remaining groups received equimolar dose i.p. (19.6–42.6 mg kg^{-1}, 0.1 mmol) of one of the test compounds (**2**–**11**). After 30 min, each animal in all groups was injected with 25 mg kg^{-1} i.p. Phenobarbital. The onset and duration of sleep were noted by recording the difference between the time of loss and recovery of the righting reflex.

Molecular modeling

Pharmacophore was produced using the Discovery Studio 2.1 (Accelrys Inc., San Diego, CA, USA).

Generation of GABA agonist hypothesis

The pharmacophore modeling method has been widely used in lead discovery and optimization as a key tool of computer aided drug design. A hypothesis was formulated using generation common feature pharmacophore model in Discovery studio 2.1. The lead compounds, which were reported to have GABA agonist activity (Fig. 2), were used to generate common feature hypotheses for the GABA agonist (Georgey, 2007; Krogsgaard-Larsen *et al.*, 2002; Tanaka *et al.*, 1995). The set of conformational models of each structure of the lead compounds was performed and used to generate the common feature hypotheses, where ten hypotheses were generated (Girgis *et al.*, 2010; Ismail *et al.*, 2006). The assessment of the preferred hypothesis among the generated ones indicated that hypothesis ranked number 2 was the most appropriate (Fig. 3). This is because the simulated fitting values of such a hypothesis with the training sedative–hypnotic test set compounds **2**–**6** and **11a**–**c** were more consistent with the experimental results than the other hypotheses.

Such hypothesis encompassed four features consisting of two hydrophobic (A_1 and A_2) appeared as a spherical mesh and two hydrogen bond acceptor receptors (H_1 and H_2) appeared as vector and its complementary site on the receptor appeared as spherical mesh. Furthermore, the constraint distance between the different features of the hypothesis were recorded, Figs. 3 and 4 (Table 1). Then, the mapping of the lead compounds as well as the test set compounds with the above hypothesis were carried out using best fit algorithm, during the compare/fit process. The data collected included the fit value of the best conformer for each compound (Table 2).

Result and discussion

Chemistry

The synthesis of 7-chlorobenzoxepin-derived compounds was illustrated in Schemes 1, 2, 3, and 4. The starting

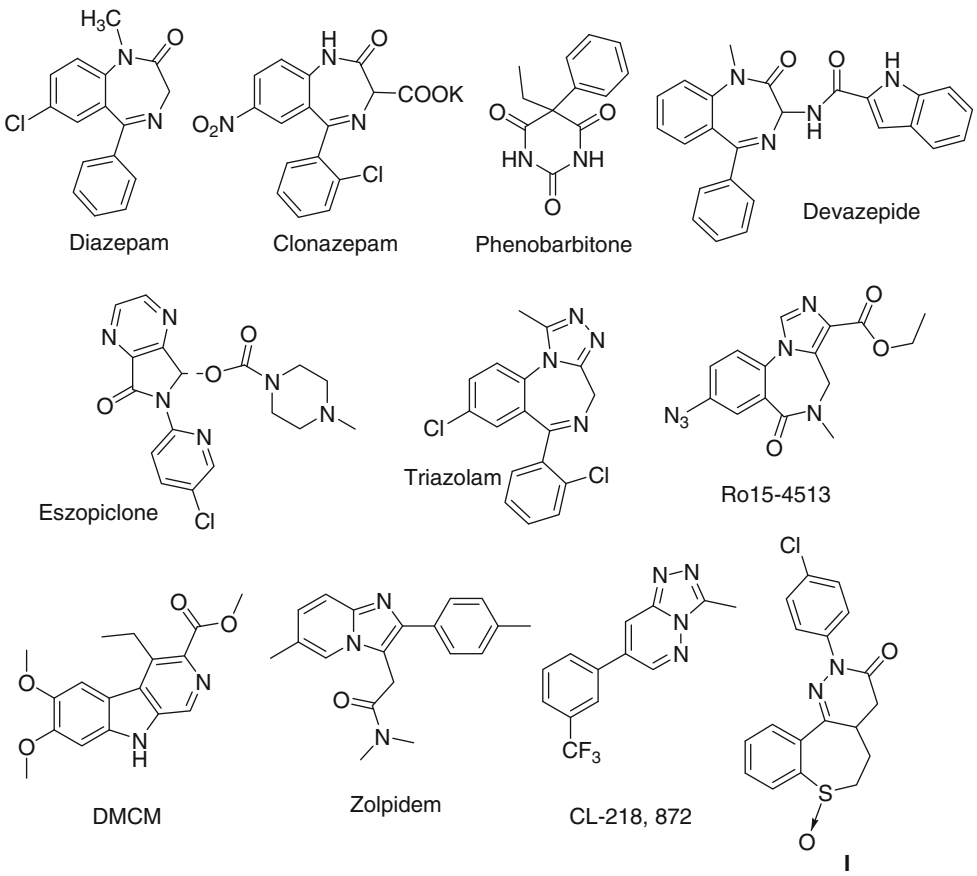


Fig. 2 Structures of some ligands for the benzodiazepine site

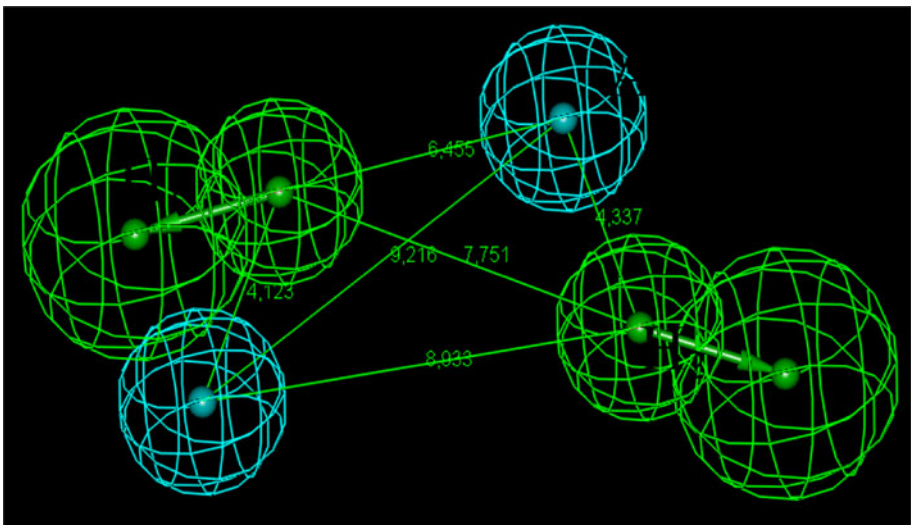


Fig. 3 Constraint distances of Benzodiazepine agonist hypothesis

compound, 7-Chloro-3,4-dihydrobenzo[*b*]oxepin-5(2*H*)-one **2** was obtained via the intramolecular cyclization of 4-chorophenoxy butyric acid **1** using phosphorous pentaoxide in the presence of Celite and toluene. This method gave a higher reproducible yield than the reported one that used PPA (polyphosphoric acid) as a dehydrating agent (Nioche and Decerprit, 1995); (Thuillier and Bessin, 1975). Introduction of a variable substituent at position-4 of the benzoxepin system was achieved as shown in compounds **3–6**. So, compounds **3a–e** was prepared from **2** by the Mannich reaction (Scheme 1). In another pathway, aldol condensation of the benzoxepin **2** with the appropriate aryl aldehydes in the presence of acidic medium afforded the corresponding 4-benzylidenbenzoxapin-5-ones **4a–d**, (Barrett *et al.*, 2008) (Scheme 2). Moreover, selective bromination of the α ketone with copper (II) bromide in

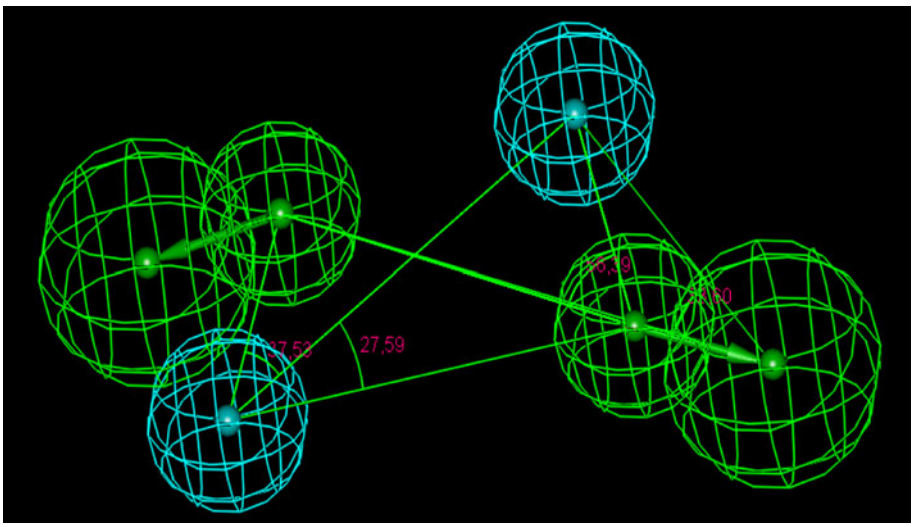


Fig. 4 Constraint angel of Benzodiazepine agonist hypothesis

Table 1 Constraint distances and angles between the features of Benzodiazepine agonist hypothesis

Dimensions	Features of benzodiazepine agonist hypothesis
Constraint distances (Å) between features	H_1–H_2, 7.751; H_1–A_1, 4.123; H_1–A_2, 6.455; A_1–A_2, 9.218; A_1–H_2, 8.033; A_2–H_2, 4.337
Constraint angles (Å) between features	H_1–A_1–A_2, 27.50; H_1–H_2–A_1, 33.60; H_2–A_1–A_2, 27.59; A_1–H_2–A_2, 56.39.

Table 2 Effects of the new heterocyclic compounds according to the Phenobarbital-induced sleep test

Compound	Latency (min) (mean ± SE)	Duration (min) (mean ± SE)	Log *P*	Fit values
Control	44.6 ± 0.54	40.30 ± 2.29		
Phenobarbital	46.8 ± 0.73	100.5 ± 1.34	1.41	3.28
2	34.5 ± 0.53	59.50* ± 2.25	1.92	2.00
3a	34.0 ± 0.51	61.30* ± 1.94	1.94	1.97
3b	24.5 ± 0.38	99.30* ± 2.35	2.68	2.65
3c	38.8 ± 0.60	92.35*± 2.43	1.7	2.75
3d	45.5 ± 0.23	45.25 ± 2.18	4.33	3.00
3e	40.8 ± 0.82	75.25* ± 1.72	1.54	2.99
4a	18.0 ± 0.26	84.30* ± 2.76	3.86	3.02
4b	16.5 ± 0.25	103.15* ± 2.11	4.02	2.88
4c	15.0 ± 0.32	105.50* ± 2.56	4.42	2.99
4d	17.6 ± 0.27	79.40* ± 1.83	3.73	3.12
5	47.0 ± 0.42	39.15 ± 1.13	2.44	2.65
6	21.5 ± 0.33	123.25* ± 3.12	3.12	3.81
11a	20.0 ± 0.30	100.15* ± 2.16	3.39	2.97
11b	49.5 ± 0.26	42.15 ± 1.19	3.95	2.99
11c	42.5 ± 0.27	38.45 ± 1.33	3.27	2.32

Derivatives were injected i.p. 30 min before i.p. injection of 25 mg kg^{-1} of Phenobarbital sodium. Data represent means of time between loss and recovery of the righting reflex. * $P < 0.05$ relative to Phenobarbital-treated group. Log *P* (Partition coefficients) were calculated using ChemDraw Ultra V 8.0. Fit value mapped with BDZ receptor agonist pharmacophore model (hypothesis) using Discovery Studio 2.1 programs

chloroform–ethyl acetate led to the formation of the bromo derivative **5** in which it was reacted with Phenobarbital sodium giving the adduct **6** (Scheme 3).

On the other hand, the benzoxazepins containing a condensed-ring system of pyridazinone **11a–c** were prepared through multistep reactions. In the first step, methylation of the Mannich base (**3a**) with iodomethane to afford the corresponding quaternary ammonium salt **7**. Cyanation of **7** and consequently hydrolysis of the product gave the carboxylic acid derivative **9**. This carboxylic acid was then cyclocondensed with un/substituted phenyl hydrazine's hydrochloride to yield **11a–c** via intermediate hydrazones **10** (Scheme 4).

The structures of the prepared compounds **3a–e**, **4a–d**, **5**, **6**, **7**, **8**, **9**, and **11a–c** were confirmed by spectroscopic methods (see "Experimental" section) and their microanalytical results for C, H, and N which they were within ±0.4 of the calculated values.

Pharmacological evaluation

Statistical analysis

It was performed using SPSS 14.0 statistical software. Differences among groups were examined using ANOVA (parametric one-way analysis of variance). Results are expressed as the mean ± SEM. The minimal level of significance was identified at $P < 0.05$.

Phenobarbital-induced sleep test in mice

The hypnotic effect of the synthesized derivatives was initially investigated by using the Phenobarbital-induced sleep test (Mora *et al.*, 2005; Tsuji *et al.*, 1996). With the exception of **3d**, **5**, **11b**, and **11c**, all the derivatives decreased the onset of Phenobarbital-induced sleep as well as prolonged the duration of hypnosis in an equimolar dose (Fig. 1; Table 1).

Concerning the reduction in sleep onset, compound **4c** was the most potent followed by compound **4b**, then compounds **4d**, and **4a**. The reduction in sleep onset was found to be proportional with the Log P.

Considering the duration of hypnosis, 7-chloro-3,4-dihydrobenzo[*b*]oxepin-5(2*H*)-one **2** increased the Phenobarbital-induced sleep from 40.30 ± 2.29 min (DMSO) to 59.50 ± 2.25. Addition of dimethylamino substituent with a methylene bridge in position 4 as shown in compound **3a** retained the activity of **2** and the duration of sleeping was 61.30 ± 1.94, while replacement of the dimethylamino by piperdino, 4-methylpiperazino, morpholino **3b**, **3c**, and **3e** led to significant increase in the Phenobarbital-induced sleep to 99.30 ± 2.35, 92.35 ± 2.43, and 75.25 ± 1.72, respectively, while substitution with 2-chlorophenylpiperzino (**3d**) gave inactive derivative.

Furthermore, substitution in position 4 with un/substituted benzylidene derivatives (**4a**, **4b**, **4c**, **4d**) afforded increase in the activity with different comportment and the sleeping duration were 84.30 ± 2.76, 103.15 ± 2.11, 105.50 ± 2.56, and 79.40 ± 1.83, respectively.

Moreover, bromo substitution in position 4 yield inactive derivative while hybridization with Phenobarbital afforded the most active derivative and it increased in the Phenobarbital-induced sleep to 123.25 ± 3.12.

On the other hand, incorporation of the condensed pyridazinone ring system into the benzoxepin nucleus **2** was unfruitful as it led to inactive derivatives except with the unsubstituted derivative (**11a**) that increased the sleeping time to 100.15 ± 2.16.

We confirmed that substitution in position 4 with un/substituted benzylidene derivatives, **4a–d** afforded reduction in the sleep onset and the order of activity is **4c**, **4b**, **4d**, and **4a**. On the other hand, 7-chloro-3,4-dihydrobenzo[b]oxepin-5(2H)-one **2** showed increase in the duration of hypnosis induced by Phenobarbital. Substitution with dimethylaminomethyl (**3a**) retained the activity and they had nearly the same Log P. Substitution with piperdin-1-yl methyl, 4-methylpiperazin-1-ylmethyl, morpholino-1-yl methyl (**3b**, **3c**, **3e**) led to significant increase in the activity while substitution with 2-chlorophenylpiperzin-1-ylmethyl (**3d**) abolish the activity with no correlation with Log P (Partition coefficients).

Considering the 4-un/substituted benzylidene derivatives **4a–d**, it was found that they increased the activity especially in the Presence of electronegative atom (Garg *et al.*, 2010) such as the 4-choro and 4-fluoro analogues and the activity was directly proportional with the Log P. Moreover, the hybridized analogue **6** between the benzoxepin **2** and Phenobarbital increased the sleeping duration three times and gave the most active derivative. Unfortunately, only condensed-ring system of pyridazinone (**11a**) showed activity.

Molecular modeling

The hypothetical study suggested that the basic nucleus binds with one hydrophobic center through the benzene ring and one hydrogen bond acceptor through the carbonyl group as shown in compound **2** (Fig. 5). Addition of a hydrophobic group in position 4 led to extra binding with the second hydrophobic center, and consequently the fit value was increased as shown in compounds **4a–d** (Fig. 6).

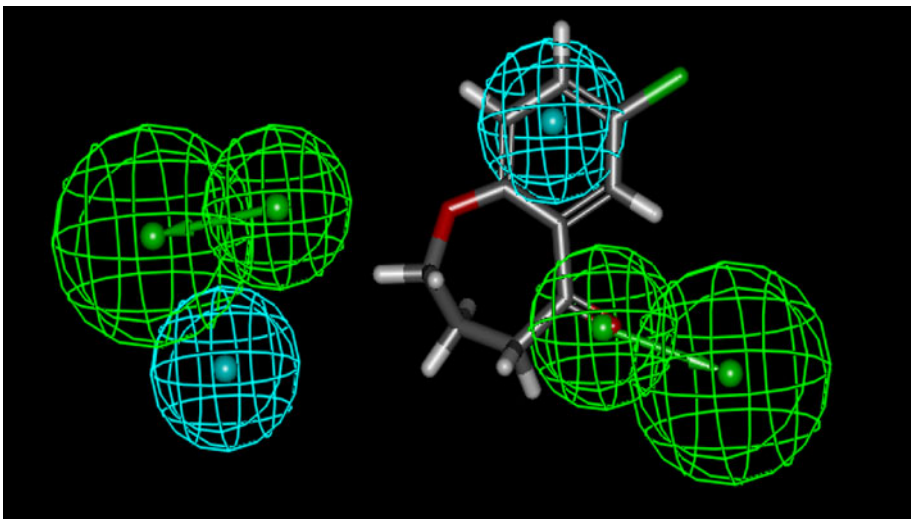


Fig. 5 Mapping of BZR agonist pharmacophore with compound **2**, fit value = 2

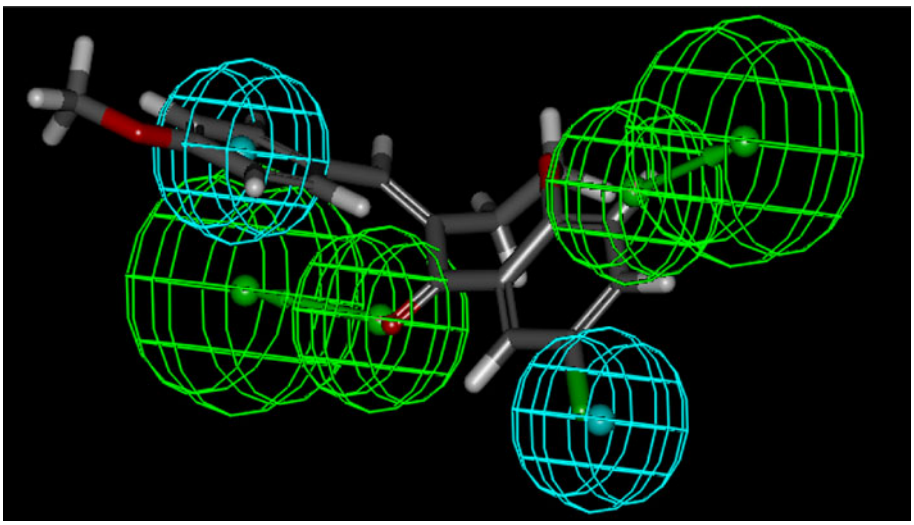


Fig. 6 Mapping of BZR agonist pharmacophore with compound **4d**, fit value = 3.12

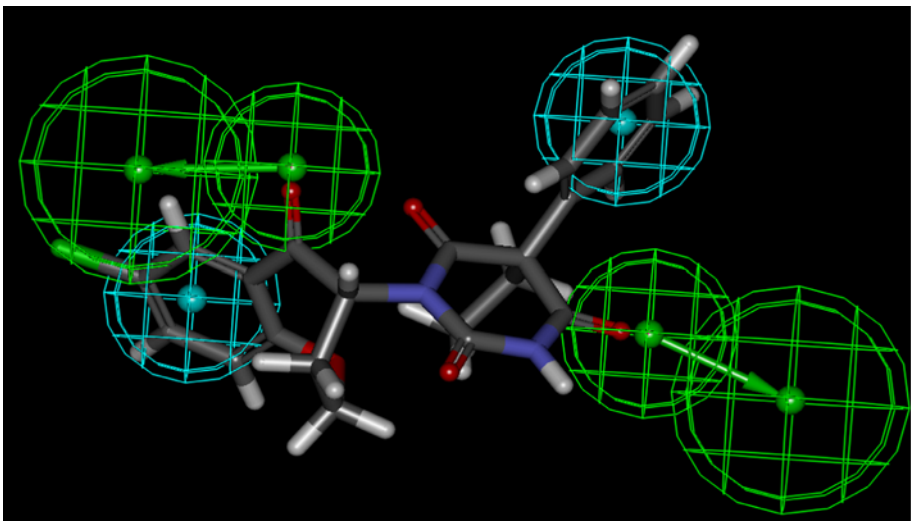


Fig. 7 Mapping of BZR agonist pharmacophore with compound **6**, fit value = 3.81

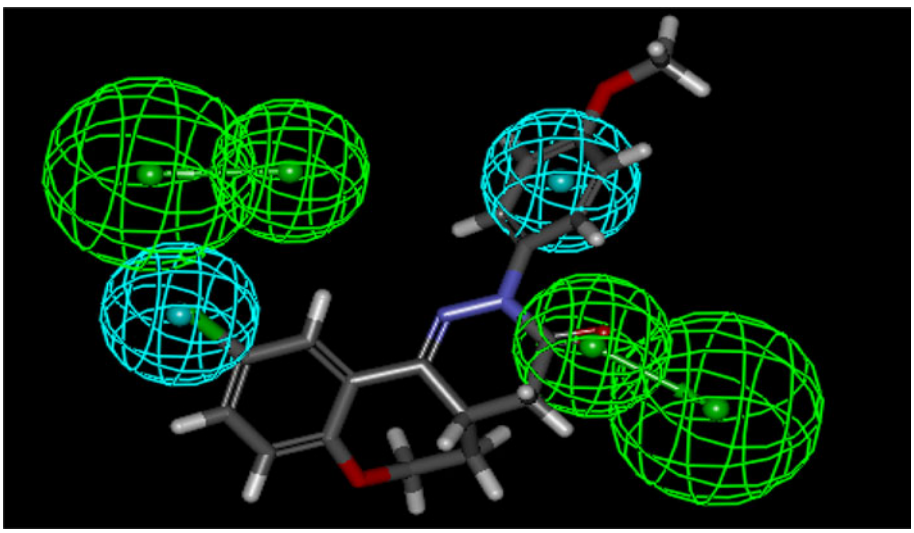


Fig. 8 Mapping of BZR agonist pharmacophore with compound **11b**, fit value = 2.99

Moreover, addition of carbonyl group and hydrophobic group at position 4 exemplified by compound **6** (Fig. 7) led to the best fitted derivative with the four features in which the two hydrophobic centers interact with the two benzene rings and the two hydrogen bond acceptor centers interact with the two carbonyl groups in position 5 and 4 of the benzoxepin and Phenobarbital, respectively. Comparing the fit value of **6** and **11a–c** (Fig. 8) suggested that rigidification of the structure afforded less fit properties.

Conclusion

In conclusion, we described the synthesis of novel benzoxepin derivatives **3–11**. The structure of the newly synthesized compounds was established by microanalytical and spectral (IR, 1H-NMR, mass) data. Moreover, the hybridized analogue **6** between the benzoxepin **2** and Phenobarbital increased the sleeping duration three times and gave the most active derivative. From the hypothetical study and the pharmacological screening, it can be concluded that, maximum activity was achieved for non rigid derivative bearing hydrophobic center and hydrogen bond as compound **6**.

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