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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, spectroscopic characterization, X-ray structure, and in vivo neurotropic activity of new 1,5-benzodiazepin-2-ones

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Abstract The paper reports the synthesis and in vivo pharmacological studies of a series of *N*-alkyl-1,5-benzodiazepine-2-ones. In this work, 19 novel benzodiazepine derivatives have been prepared and characterized by spectroscopic methods including 2D nuclear magnetic resonance techniques. Crystal structure of 1-benzyl-8-methyl-4-phenyl-1*H*-benzo[*b*][1,4]diazepin-2(3*H*)-one has also been determined by X-ray diffraction. Prediction of activity spectra for substances prediction and docking studies onto human serum albumin were conducted. Two compounds under these investigation showed high antihypoxic, tranquilizing, and anticonvulsant activity in vivo.

Keywords Synthesis · 1,5-Benzodiazepin-2-one · Neurotropic activity · Crystal structure · Biological activity spectrum

Introduction

Many biologically active compounds, both natural and synthetic in origin, incorporate azaheterocycles as substructural units. Benzodiazepines (BDZs) represent an important class of pharmaceuticals and possess sedative, hypnotic, anxiolytic, anticonvulsant, miorelaxant, amnesic, antimicrobial, and antitumor activities (Borges et al., 2016; Wang et al., 2015; Hammer et al., 2015; Gavai et al., 2015; Olkkola and Ahonen, 2008; Page et al., 2002). They are also used to treat a variety of conditions such as alcohol addiction, seizures, anxiety, panic, agitation, and insomnia. BDZ-based sedatives and hypnotics are among the most prescribed drugs in the community (Drummer, 2009).

There are some differences in activity between 1,5- and 1,4-BDZs. A greater therapeutic potential and lower incidence of side effects were noted for 1,5-BDZs compared to 1,4-BDZs (Pandeya and Rajput, 2012). 1,5-Benzodiazepin-2-ones have been used in clinics as antisecretory, anxiolytic, and anticonvulsant agents. Examples thereof include, telenzepine (Eltze et al., 1985), arfendazam (Hofmann et al., 1982), lofendazam (Müller et al., 1986), triflubazam (Nicholson et al., 1977), clobazam (Kruse, 1982), and CP-1414S (Carli et al., 1981; Caccia et al., 1982). Members of this family also exhibit a wide range of other activities such as interleukin-1β-converting enzyme inhibition, antiarrhythmic properties (Claremon et al., 1996), delayed rectifier potassium current blocking (Herpin et al., 2000) and are used for adjuvant therapy of drug-resistant epilepsy (Fisher and Blum, 1995). Furthermore, 2,3-dihydro-1*H*-1,5-benzodiazepin-2-ones are viewed as potential imaging agents for the metabotropic glutamate receptor subtype 2 (Gilfillan et al., 2013). As a result, benzodiazepin-2-ones are considered as privileged scaffolds in drug discovery and in drug development (Viviano et al., 2015; Welsch et al., 2010).

Starting from the 1960s, the important biological properties of these compounds have led to active synthetic work and extensive pharmacological studies.

We analyzed structures of 50 FDA approved drugs containing 1,4- and 1,5-BDZ subunits and found that many

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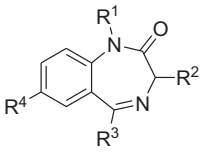
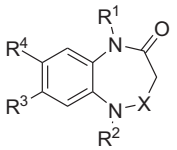


1,4-BDZ core drugs

Diazepam: R^1 = Me, R^2 = H, R^3 = Ph, R^4 = Cl; **Prazepam**: R^1 = cyclopropylmethyl, R^2 = H, R^3 = Ph, R^4 = Cl; **Pinazepam**: R^1 = propargyl, R^2 = H, R^3 = Ph, R^4 = Cl; **Halazepam**: R^1 = 2,2,2-trifluoroethyl, R^2 = H, R^3 = Ph, R^4 = Cl; **Diclazepam**: R^1 = Me, R^2 = H, R^3 = 2-ClC_6H_4, R^4 = Cl; **Flurazepam**: R^1 = $Et_2NCH_2CH_2$, R^2 = H, R^3 = 2-FC_6H_4, R^4 = Cl; **Flutoprazepam**: R^1 = cyclopropylmethyl, R^2 = H, R^3 = 2-FC_6H_4, R^4 = Cl; **Nimetazepam**: R^1 = Me, R^2 = H, R^3 = Ph, R^4 = NO_2; **Flunitrazepam**: R^1 = Me, R^2 = H, R^3 = 2-FC_6H_4, R^4 = NO_2; **Tetrazepam**: R^1 = Me, R^2 = H, R^3 = cyclohex-1-en-1-yl, R^4 = Cl; **Temazepam**: R^1 = Me, R^2 = OH, R^3 = Ph, R^4 = Cl; **Lormetazepam**: R^1 = Me, R^2 = OH, R^3 = 2-ClC_6H_4, R^4 = Cl; **Cinolazepam**: R^1 = $NCCH_2CH_2$, R^2 = OH, R^3 = 2-FC_6H_4, R^4 = Cl



1,5-BDZ core drugs

Arfendazam: R^1 = Ph, R^2 = EtOC(O), R^3 = H, R^4 = Cl, X = CH_2; **Lofendazam**: R^1 = Ph, R^2 = H, R^3 = H, R^4 = Cl, X = CH_2; **Triflubazam**: R^1 = Me, R^2 = Ph, R^3 = CF_3, R^4 = H, X = C=O; **Clobazam**: R^1 = Me, R^2 = Ph, R^3 = Cl, R^4 = H, X = C=O

Fig. 1 General structures of some commercial drugs containing 1,4- or 1,5-BDZ core

compounds include *N*-alkylamide fragments (Fig. 1). We therefore synthesized a family of 25 *N*-alkyl-1,5-benzodiazepine-2-ones and evaluated their biological potential in silico using prediction of activity spectra for substances (PASS, Goel et al., 2011) and molecular docking approaches. The compounds were characterized using infrared radiation (IR), ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, X-ray diffraction, and elemental analysis. Two compounds were tested for neurotropic (antihypoxic, tranquilizing, and anticonvulsant) properties. We envision that synthetic and biological studies of the novel 1,5-BDZs can be further expanded toward a greater scope and utility.

Experimental methods

Chemistry

Solvents were dried and distilled immediately prior to use. Melting points were determined in open capillary tubes and reported uncorrected. IR spectra were recorded on a UR-20 or Specord 75IR spectrometer using KBr pellets. NMR spectra were measured on Varian VXR, Bruker Spectrospin DPX-400 (only for 2D NMR spectra of compound **27**), or Bruker Avance DRX-500 spectrometer at room temperature in appropriate solvents. Chemical shifts are reported in parts per million (ppm) with respect to the solvent residual signal ($CDCl_3$ ^{1}H: $\delta = 7.26$ ppm, ^{13}C: $\delta = 77.16$ ppm; dimethyl sulfoxide (DMSO)-d_6 ^{1}H: $\delta = 2.50$ ppm, ^{13}C: $\delta = 39.52$ ppm). Coupling constants (J) are reported in Hertz (Hz). Low resolution mass-spectra of compounds **34, 35** were recorded on a Varian 1200L spectrometer in electron impact ionization mode at the energy of 70 eV. The elemental analysis (C, H, N) was performed using Carlo Erba analyzer. The analytical results were within ± 0.4 % of the theoretical values. Thin-layer chromatography (TLC) was performed on Silufol UV-254 plates using diethyl ether and hexane as eluents; the plates were visualized with iodine vapor.

X-ray diffraction study

The colourless crystals of compound **29** ($C_{23}H_{20}N_2O$) are monoclinic. At 293 K, $a = 9.540(3)$, $b = 12.832(1)$, $c = 14.928(3)$ Å, $\beta = 94.47(2)°$, $V = 1821.9(7)$ Å^3, $M_r = 340.41$, $Z = 4$, space group $P2_1/n$, $d_{calc} = 1.241$ g/cm^3, $\mu(MoK_\alpha) = 0.077$ mm^{-1}, $F(000) = 720$. Intensities of 8854 reflections (3164 independent, $R_{int} = 0.020$) were measured on an Xcalibur-3 diffractometer (graphite-monochromated MoK_α radiation, CCD detector, ω-scanning, $2\Theta_{max} = 50°$). The structure was solved by direct methods using SHELXTL package (Sheldrick, 2008). Positions of the hydrogen atoms were located from electron density difference maps and refined by "riding" the model with $U_{iso} = nU_{eq}$ ($n = 1.5$ for methyl group and $n = 1.2$ for other hydrogen atoms) of the carrier atom. Full-matrix least-squares refinement against F^2 in anisotropic approximation for nonhydrogen atoms using 3127 reflections was converged to $wR_2 = 0.096$ ($R_1 = 0.036$ for 1875 reflections with $F > 4\sigma(F)$, $S = 0.862$). The final atomic coordinates and crystallographic data for the molecule **29** have been deposited to the Cambridge Crystallographic Data Centre, 12 Union Road, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk) and are available on request quoting the deposition number CCDC 1044106.

The starting BDZs **1–8** were prepared according to the literature procedures (Vernin et al., 1980; El-Shafei et al., 1982). Their physical and spectral data were in correspondence with those reported (Vernin et al., 1980; El-Shafei et al., 1982). Compounds **10**, **17**, **22**, **24**, and **28** were described by Solomko et al. (1990) and El-Shafei et al. (1982) without spectral data. The NMR spectra of compounds **18**, **19**, and **20** in $CDCl_3$ were described in Chen et al. (2011) and Essassi et al. (1987) without IR data.

*General procedures for the synthesis of substituted benzo [b][1,4]diazepin-2(3H)-ones (**9–35**)*

Method A: The appropriate benzodiazepin-2-one (5 mmol, see Table 1), tetrabutylammonium bromide (TBAB, 0.097 g, 0.3 mmol), and 50 % aqueous NaOH (3 ml) were mixed in benzene (30 mL) at room temperature. The alkyl halide (10 mmol, see Table 1) was added dropwise to the stirred mixture. The stirring was continued at 60 °C for 1.5 h. After cooling, the benzene layer was separated and washed with brine (2 × 10 mL), then dried over Na_2SO_4. The solvent was removed in vacuo to afford the crude product. Pure compounds **9–12, 14–20, 22–35** were obtained after recrystallization from hexane (or hexane–benzene mixture) or by flash chromatography on silica (hexane–ethyl acetate, 3:1 v/v).

Method B: The appropriate alkyl halide (10 mmol, see Table 1) was added dropwise to the stirred mixture of the benzodiazepin-2-one (5 mmol, see Table 1) and potassium hydroxide (1.40 g, 25 mmol) in acetone (30 mL). The reaction was heated at reflux for 3 h. After cooling, the solid was filtered off and the solvent was removed in vacuo. The residue was then dissolved in 10 mL of chloroform and washed with brine (2 × 10 mL), dried over Na_2SO_4, and concentrated in vacuo to afford the crude product. Pure compounds **10, 12, 14, 16–19, 22–24, 27–29, 33** were obtained after recrystallization from hexane (or hexane–benzene mixture) or by flash chromatography on silica (hexane–ethyl acetate, 3:1 v/v).

Method C: A stirred mixture of the benzodiazepin-2-one **1** or **4** (5 mmol) and sodium hydride (60 % in oil, 0.40 g, 10 mmol) in N,N-Dimethylformamide (20 mL) was heated to 100 °C over 0.5 h. 2-(Chloromethyl)oxirane (0.93 g, 10 mmol) was then added dropwise, and the reaction mixture was heated for additional 3 h. Upon cooling to room temperature, the solid was filtered off and the filtrate was poured into brine. The aqueous phase was extracted with chloroform (3 × 30 mL). The combined organic layers were washed with brine (25 mL) and dried over Na_2SO_4. The solvent was removed in vacuo to afford the crude product. Pure compounds **13** and **21** were obtained after recrystallization from hexane (or hexane–benzene mixture).

Table 1 Synthesized *N*-alkyl-BDZs **9-33**

Starting compound	R^1	R^2	R^3	R^4	R^1-X (alkylating agent)	Product	Method (A/B/C), yield (%)
1	Me	Me	H	H	MeI	**9**	A, 93
1	Et	Me	H	H	EtBr	**10**	A, 90; B, 82
1	*n*-Pr	Me	H	H	*n*-PrCl	**11**	A, 72
1	Bn	Me	H	H	BnCl	**12**	A, 74; B, 78
1	2,3-Epoxypropyl	Me	H	H	2-(Chloromethyl) oxirane	**13**	C, 79
2	Me	Me	H	Cl	MeI	**14**	A, 88; B, 81
3	Me	Me	H	Me	MeI	**15**	A, 76
3	Bn	Me	H	Me	BnCl	**16**	A, 78; B, 75
4	Me	Ph	H	H	MeI	**17**	A, 92; B, 89
4	Et	Ph	H	H	EtBr	**18**	A, 88; B, 91
4	Bn	Ph	H	H	BnCl	**19**	A, 94; B, 90
4	Allyl	Ph	H	H	Allyl-Br	**20**	A, 86
4	2,3-Epoxypropyl	Ph	H	H	2-(Chloromethyl) oxirane	**21**	C, 77
5	Me	Ph	H	Cl	MeI	**22**	A, 77; B, 81
5	Bn	Ph	H	Cl	BnCl	**23**	A, 80; B, 74
6	Me	Ph	H	OMe	MeI	**24**	A, 85; B, 77
6	Et	Ph	H	OMe	EtBr	**25**	A, 88
6	Bn	Ph	H	OMe	BnCl	**26**	A, 91
6	Allyl	Ph	H	OMe	Allyl-Br	**27**	A, 83; B, 85
7	Me	Ph	H	Me	MeI	**28**	A, 87; B, 79
7	Bn	Ph	H	Me	BnCl	**29**	A, 90; B, 94
7	Allyl	Ph	H	Me	Allyl-Br	**30**	A, 88
8	Me	Ph	NO_2	H	MeI	**31**	A, 82
8	Et	Ph	NO_2	H	EtBr	**32**	A, 79
8	Allyl	Ph	NO_2	H	Allyl-Br	**33**	A, 80; B, 75

The yield, melting point, and spectral data for each compound are given below.

1,4-Dimethyl-1H-benzo[b][1,4]diazepin-2(3H)-one (***9***) Yield: method A, 0.88 g (93 %); m.p. 28–29 °C (28 °C (El-Shafei et al., 1982)); IR (KBr) cm^{-1}: 1670, 1610, 1585; ^1H NMR (500 MHz, DMSO-d_6): 2.30 (s, 3H, C–CH_3), 2.76 (d, 1H, $J = 11.4$, CH_2), 3.28 (d, 1H, $J = 11.8$, CH_2), 3.30 (s, 3H, N–CH_3), 7.15 (m, 3H, H_{Ar}), 7.32 (d, 1H, $J = 7.6$, H_{Ar}); anal. calcd. for $C_{11}H_{12}N_2O$ (188.23): C, 70.19; H, 6.43; N, 14.88; found: C, 70.02; H, 6.46; N, 15.07.

1-Ethyl-4-methyl-1H-benzo[b][1,4]diazepin-2(3H)-one (***10***) Yield: method A, 0.91 g (90 %); method B, 0.83 g (82 %); m.p. 129–130 °C (130 °C (Vernin et al., 1980)), 130 °C (El-Shafei et al., 1982); IR (KBr) cm^{-1}: 1675, 1605, 1590; ^1H NMR (500 MHz, DMSO-d_6): 0.98 (t, 3H, $J = 7.1$, $CH_2\underline{CH_3}$), 2.24 (s, 3H, C–CH_3), 2.79 (d, 1H, $J = 11.3$, C(O)CH_2), 3.30 (d, 1H, $J = 11.3$, C(O)CH_2), 3.73 (m, 1H, $\underline{CH_2}CH_3$), 4.00 (m, 1H, $\underline{CH_2}CH_3$), 7.23 (m, 3H, H_{Ar}), 7.49 (d, 1H, $J = 7.7$, H_{Ar}); ^{13}C NMR (125 MHz, DMSO-d_6): 13.29 ($CH_2\underline{CH_3}$), 27.00 (C–$\underline{CH_3}$), 41.88 ($\underline{CH_2}CH_3$), 43.61 (C(O)$\underline{CH_2}$), 122.59 (C_{Ar}), 125.10 (C_{Ar}), 125.78 (C_{Ar}), 126.19 (C_{Ar}), 133.23 (C_{Ar}), 141.89 (C_{Ar}), 164.07 (C=N), 165.30 (C=O); anal. calcd. for $C_{12}H_{14}N_2O$ (202.26): C, 71.26; H, 6.98; N, 13.85; found: C, 71.05; H, 6.80; N, 13.99.

4-Methyl-1-propyl-1H-benzo[b][1,4]diazepin-2(3H)-one (***11***) Yield: method A, 0.91 g (72 %); m.p. 68–69 °C (70 °C (Vernin et al., 1980)); IR (KBr) cm^{-1}: 1670, 1605, 1580; ^1H NMR (400 MHz, DMSO-d_6): 0.74 (t, 3H, $CH_2CH_2\underline{CH_3}$), 1.36 (m, 1H, $CH_2\underline{CH_2}CH_3$), 1.48 (m, 1H, $CH_2\underline{CH_2}CH_3$), 2.28 (s, 3H, C–CH_3), 2.73 (d, 1H, $J = 10.8$, C(O)CH_2), 3.27 (d, 1H, $J = 11.4$, C(O)CH_2), 3.55 (m, 1H, $\underline{CH_2}CH_2CH_3$), 4.10 (m, 1H, $\underline{CH_2}CH_2CH_3$), 7.14 (m, 3H, H_{Ar}), 7.30 (d, 1H, $J = 7.9$, H_{Ar}); anal. calcd. for $C_{13}H_{16}N_2O$ (216.28): C, 72.19; H, 7.46; N, 12.95; found: C, 71.98; H, 7.50; N, 13.07.

1-Benzyl-4-methyl-1H-benzo[b][1,4]diazepin-2(3H)-one (***12***) Yield: method A, 0.98 g (74 %); method B, 1.03 g (78 %); m.p. 124–125 °C (124 °C (Vernin et al., 1980)); IR (KBr) cm^{-1}: 1685, 1610, 1590; ^1H NMR (400 MHz, DMSO-d_6): 2.33 (s, 3H, CH_3), 2.94 (d, 1H, $J = 11.0$, C(O)CH_2), 3.40 (d, 1H, $J = 11.5$, C(O)CH_2), 5.03 (dd, 2H, $J = 7.0, 2.5$, $\underline{CH_2}C_6H_5$), 7.02 (d, 2H, $J = 7.3$, H_{Ar}), 7.15 (m, 4H, H_{Ar}), 7.24 (m, 2H, H_{Ar}), 7.31 (d, 1H, $J = 7.4$, H_{Ar}); anal. calcd. for $C_{17}H_{16}N_2O$ (264.33): C, 77.25; H, 6.10; N, 10.60; found: C, 77.12; H, 5.97; N, 10.44.

1-(2,3-Epoxypropyl)-4-methyl-1H-benzo[b][1,4]diazepin-2(3H)-one (***13***) Yield: method C, 0.91 g (79 %); m.p. 128–130 °C; IR (KBr) cm^{-1}: 1670, 1610, 1580, 1260, 925, 860; ^1H NMR (400 MHz, DMSO-d_6): 2.30 (s, 3H, CH_3), 3.02 (m, 2H, CH_2), 3.81 (m, 2H, CH_2), 4.15 (m, 1H, CH), 5.35 (d, 1H, $J = 11.0$, C(O)$C\underline{H_2}$), 5.87 (d, 1H, $J = 11.0$, C(O)$C\underline{H_2}$), 7.18 (m, 3H, H_{Ar}), 7.48 (s, 1H, H_{Ar}); anal. calcd. for $C_{13}H_{14}N_2O_2$ (230.27): C, 67.81; H, 6.13; N, 12.17; found: C, 67.52; H, 5.87; N, 12.40.

8-Chloro-1,4-dimethyl-1H-benzo[b][1,4]diazepin-2(3H)-one (***14***) Yield: method A, 0.98 g (88 %); method B, 0.90 g (81 %); m.p. 107–108 °C; IR (KBr) cm^{-1}: 1690, 1600, 1560; ^1H NMR (400 MHz, DMSO-d_6): 2.32 (s, 3H, C–CH_3), 2.78 (d, 1H, $J = 11.1$, CH_2), 3.30 (s, 3H, N–CH_3), 3.35 (d, 1H, $J = 11.7$, CH_2), 7.13 (dd, 2H, $J = 7.1, 2.6$, H_{Ar}), 7.32 (d, 1H, $J = 2.6$, H_{Ar}); anal. calcd. for $C_{11}H_{11}ClN_2O$ (222.67): C, 59.33; H, 4.98; N, 12.58; found: C, 59.27; H, 5.04; N, 12.36.

1,4,8-Trimethyl-1H-benzo[b][1,4]diazepin-2(3H)-one (***15***) Yield: method A, 0.77 g (76 %); m.p. 103–104 °C; IR (KBr) cm^{-1}: 1680, 1605, 1535; ^1H NMR (400 MHz, DMSO-d_6): 2.28 (s, 3H, N = C–CH_3), 2.37 (s, 3H, C–CH_3), 2.74 (d, 1H, $J = 11.2$, CH_2), 3.25 (d, 1H, $J = 11.6$, CH_2), 3.30 (s, 3H, N–CH_3), 6.96 (d, 1H, $J = 8.3$, H_{Ar}), 7.05 (d, 1H, $J = 8.3$, H_{Ar}), 7.10 (s, 1H, H_{Ar}); anal. calcd. for $C_{12}H_{14}N_2O$ (202.26): C, 71.26; H, 6.98; N, 13.85; found: C, 71.02; H, 7.10; N, 13.98.

1-Benzyl-4,8-dimethyl-1H-benzo[b][1,4]diazepin-2(3H)-one (***16***) Yield: method A, 1.09 g (78 %); method B, 1.04 g (75 %); m.p. 119–120 °C; IR (KBr) cm^{-1}: 1675, 1600, 1560; ^1H NMR (500 MHz, DMSO-d_6): 2.25 (s, 3H, N=C–CH_3), 2.28 (s, 3H, C–CH_3), 2.97 (d, 1H, $J = 10.6$, C(O)CH_2), 3.39 (d, 1H, $J = 10.6$, C(O)CH_2), 4.99 (d, 1H, $J = 15.9$, $\underline{CH_2}C_6H_5$), 5.26 (d, 1H, $J = 15.9$, $\underline{CH_2}C_6H_5$), 6.98 (d, 1H, $J = 6.7$, H_{Ar}), 7.07 (d, 2H, $J = 8.1$, H_{Ar}), 7.14 (m, 1H, H_{Ar}), 7.24 (m, 3H, H_{Ar}), 7.28 (s, 1H, H_{Ar}); ^{13}C NMR (125 MHz, DMSO-d_6): 20.62 (C–$\underline{CH_3}$), 26.86 (N=C–$\underline{CH_3}$), 43.35 (C(O)$\underline{CH_2}$), 49.18 ($\underline{CH_2}C_6H_5$), 122.48 (C_{Ar}), 125.98 (C_{Ar}), 126.09 (C_{Ar}), 126.26 (C_{Ar}), 126.84 (C_{Ar}), 128.39 (C_{Ar}), 128.76 (C_{Ar}), 132.98 (C_{Ar}), 135.02 (C_{Ar}), 137.60 ($\underline{C}$–CH_3), 139.52 (C_{Ar}), 164.35 (C=N), 164.62 (C=O); anal. calcd. for $C_{18}H_{18}N_2O$ (278.36): C, 77.67; H, 6.52; N, 10.06; found: C, 77.48; H, 6.45; N, 9.94.

1-Methyl-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one (***17***) Yield: method A, 1.15 g (92 %); method B, 1.12 g (89 %); m.p. 66–67 °C; IR (KBr) cm^{-1}: 1680, 1640, 1615, 1580. ^1H NMR (500 MHz, DMSO-d_6): 2.98 (d, 1H, $J =$ 12.2, CH_2), 3.30 (s, 3H, CH_3), 4.14 (d, 1H, $J = 12.4$, CH_2), 7.33 (m, 2H, H_{Ar}), 7.40 (dd, 1H, $J = 7.7, 1.7$, H_{Ar}), 7.55 (m, 4H, H_{Ar}), 8.09 (dd, 2H, $J = 8.0, 1.5$, H_{Ar}); ^{13}C NMR (125 MHz, DMSO-d_6): 34.69 (CH_3), 39.68 (C(O)$\underline{CH_2}$), 122.30 (C_{Ar}), 125.07 (C_{Ar}), 126.30 (C_{Ar}), 127.67 (C_{Ar}), 128.83 (C_{Ar}), 131.30 (C_{Ar}), 134.94 (C_{Ar}), 136.81 (C_{Ar}), 140.98 (C_{Ar}), 160.32 (C=N), 165.67 (C=O); anal. calcd. for

$C_{16}H_{14}N_2O$ (250.30): C, 76.78; H, 5.64; N, 11.19; found: C, 76.61; H, 5.54; N, 11.04.

1-Ethyl-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one ***(18)*** Yield: method A, 1.16 g (88 %); method B, 1.20 g (91 %); m.p. 75–76 °C (72–74 °C (Vernin et al., 1980); 78–80 °C (Essassi et al., 1987)); IR (KBr) cm^{-1}: 1675, 1635, 1615, 1580; ^1H NMR (500 MHz, DMSO-d_6): 0.98 (t, 3H, $J = 7.1$, CH_3), 2.96 (d, 1H, $J = 12.1$, C(O)CH_2), 3.76 (q, 1H, $J = 7.1$, $\underline{CH_2}CH_3$), 4.03 (q, 1H, $J = 7.1$, $\underline{CH_2}CH_3$), 4.11 (d, 1H, $J = 12.1$, C(O)CH_2), 7.33 (m, 2H, H_{Ar}), 7.39 (dd, 1H, $J = 7.4$, 2.1, H_{Ar}), 7.55 (m, 4H, H_{Ar}), 8.09 (dd, 2H, $J = 7.9$, 1.5, H_{Ar}); ^{13}C NMR (125 MHz, DMSO-d_6): 13.33 (CH_3), 39.69 (C(O)$\underline{CH_2}$), 41.90 ($\underline{CH_2}CH_3$), 122.61 (C_{Ar}), 125.31 (C_{Ar}), 126.34 (C_{Ar}), 126.88 (C_{Ar}), 127.70 (C_{Ar}), 128.81 (C_{Ar}), 131.27 (C_{Ar}), 133.36 (C_{Ar}), 136.84 (C_{Ar}), 141.97 (C_{Ar}), 160.71 (C=N), 164.50 (C=O); anal. calcd. for $C_{17}H_{16}N_2O$ (264.33): C, 77.25; H, 6.10; N, 10.60; found: C, 76.96; H, 5.94; N, 10.79.

1-Benzyl-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one ***(19)*** Yield: method A, 1.52 g (94 %); method B, 1.46 g (90 %); m.p. 128–129 °C (128 °C (Vernin et al., 1980)); IR (KBr) cm^{-1}: 1670, 1630, 1610, 1575; ^1H NMR (500 MHz, DMSO-d_6): 3.17 (d, 1H, J = 11.8, C(O)CH_2), 4.24 (d, 1H, $J = 11.9$, C(O)CH_2), 5.05 (d, 1H, $J = 16.1$, $\underline{CH_2}C_6H_5$), 5.28 (d, 1H, $J = 16.0$, $\underline{CH_2}C_6H_5$), 6.97 (d, 2H, $J = 7.1$, H_{Ar}), 7.14 (m, 1H, H_{Ar}), 7.20 (m, 2H, H_{Ar}), 7.25 (s, 2H, H_{Ar}), 7.36 (s, 1H, H_{Ar}), 7.56 (m, 4H, H_{Ar}), 8.12 (d, 2H, $J = 7.3$, H_{Ar}); ^{13}C NMR (125 MHz, DMSO-d_6): 39.69 (C(O)$\underline{C}$H2), 49.27 ($\underline{CH_2}C_6H_5$), 122.50 (C_{Ar}), 125.35 (C_{Ar}), 126.17 (C_{Ar}), 126.29 (C_{Ar}), 126.81 (C_{Ar}), 126.89 (C_{Ar}), 127.74 (C_{Ar}), 128.40 (C_{Ar}), 128.81 (C_{Ar}), 131.29 (C_{Ar}), 133.29 (C_{Ar}), 136.79 (C_{Ar}), 137.41 (C_{Ar}), 141.81 (C_{Ar}), 160.70 (C=N), 165.16 (C=O); anal. calcd. for $C_{22}H_{18}N_2O$ (326.40): C, 80.96; H, 5.56; N, 8.58; found: C, 81.21; H, 5.39; N, 8.48.

1-Allyl-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one ***(20)*** Yield: method A, 1.19 g (86 %); m.p. 106–108 °C (108 °C (Vernin et al., 1980)); IR (KBr) cm^{-1}: 1675, 1620, 1605, 1575; ^1H NMR (500 MHz, DMSO-d_6): 3.06 (d, 1H, $J = 12.1$, C(O)CH_2), 4.17 (d, 1H, $J = 12.1$, C(O)CH_2), 4.49 (q, 2H, $J = 15.0$, $\underline{CH_2}$–CH=CH_2), 4.95 (d, 1H, $J = 16.9$, CH_2–CH=$\underline{CH_2}$), 5.03 (d, 1H, $J = 10.4$, CH_2–CH=$\underline{CH_2}$), 5.73 (m, 1H, CH_2–$\underline{CH}$=CH_2), 7.31 (dd, 2H, $J = 9.3$, 2.1, H_{Ar}), 7.40 (d, 1H, $J = 9.3$, H_{Ar}), 7.55 (d, 4H, $J = 7.2$, H_{Ar}), 8.10 (d, 2H, $J = 7.5$, H_{Ar}); ^{13}C NMR (125 MHz, DMSO-d_6): 39.69 (C(O)$\underline{CH_2}$), 49.01 ($\underline{CH_2}$–CH=CH_2), 115.93 (CH_2–CH=$\underline{CH_2}$), 122.36 (C_{Ar}), 125.25 (C_{Ar}), 126.20 (C_{Ar}), 126.81 (C_{Ar}), 127.65 (C_{Ar}), 128.76 (C_{Ar}), 131.25 (C_{Ar}), 133.58 (C_{Ar}), 133.64 (CH_2–$\underline{CH}$=CH_2), 136.71 (C_{Ar}), 141.54 (C_{Ar}), 160.58 (C=N), 164.68 (C=O); anal. calcd. for $C_{18}H_{16}N_2O$ (276.34): C, 78.24; H, 5.84; N, 10.14; found: C, 78.52; H, 5.90; N, 10.08.

1-(2,3-Epoxypropyl)-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one ***(21)*** Yield: method C, 1.13 g (77 %); m.p. 74–76 °C; IR (KBr) cm^{-1}: 1680, 1620, 1580, 1255, 925, 855; ^1H NMR (400 MHz, DMSO-d_6): 2.78 (m, 2H, CH_2), 3.98 (m, 2H, CH_2), 4.31 (m, 1H, CH), 5.58 (d, 1H, $J = 11.5$, C(O)$C\underline{H_2}$), 6.16 (d, 1H, $J = 11.5$, C(O)$C\underline{H_2}$), 6.70 (m, 1H, H_{Ar}), 7.20 (m, 8H, H_{Ar}); anal. calcd. for $C_{18}H_{16}N_2O_2$ (292.34): C, 73.95; H, 5.52; N, 9.58; found: C, 74.22; H, 5.47; N, 9.85.

8-Chloro-1-methyl-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one ***(22)*** Yield: method A, 1.10 g (77 %); method B, 1.16 g (81 %); m.p. 85–86 °C; IR (KBr) cm^{-1}: 1690, 1630, 1605, 1575; ^1H NMR (500 MHz, DMSO-d_6): 3.05 (d, 1H, $J = 11.3$, C(O)CH_2), 3.30 (s, 3H, CH_3), 4.17 (d, 1H, $J = 10.9$, C(O)CH_2), 7.35 (d, 1H, $J = 9.6$, H_{Ar}), 7.41 (d, 1H, $J = 8.6$, H_{Ar}), 7.55 (m, 3H, H_{Ar}), 7.64 (s, 1H, H_{Ar}), 8.09 (d, 2H, $J = 7.0$, H_{Ar}); ^{13}C NMR (125 MHz, DMSO-d_6): 34.63 (NCH_3), 39.69 (C(O)$\underline{CH_2}$), 122.05 (C_{Ar}), 125.01 (C_{Ar}), 127.70 (C_{Ar}), 128.39 (C_{Ar}), 128.81 (C_{Ar}), 130.01 (C_{Ar}), 131.45 (C_{Ar}), 135.93 (C_{Ar}), 136.49 ($\underline{C_{Ar}}$–Cl), 139.84 (C_{Ar}), 160.80 (C=N), 165.46 (C=O); anal. calcd. for $C_{16}H_{13}ClN_2O$ (284.74): C, 67.49; H, 4.60; N, 9.84; found: C, 67.68; H, 4.53; N, 9.98.

1-Benzyl-8-chloro-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one ***(23)*** Yield: method A, 1.44 g (80 %); method B, 1.34 g (74 %); m.p. 131–132 °C; IR (KBr) cm^{-1}: 1680, 1635, 1610, 1580; ^1H NMR (500 MHz, DMSO-d_6): 3.17 (d, 1H, $J = 11.8$, C(O)CH_2), 4.24 (d, 1H, $J = 11.9$, C(O)CH_2), 5.08 (d, 1H, $J = 16.0$, $\underline{CH_2}C_6H_5$), 5.28 (d, 1H, $J = 15.9$, $\underline{CH_2}C_6H_5$), 6.95 (d, 2H, $J = 7.1$, H_{Ar}), 7.18 (m, 4H, H_{Ar}), 7.31 (d, 1H, $J = 11.9$, H_{Ar}), 7.52 (m, 4H, H_{Ar}), 8.14 (d, 2H, $J = 7.2$, H_{Ar}); anal. calcd. for $C_{22}H_{17}ClN_2O$ (360.84): C, 73.23; H, 4.75; N, 7.76; found: C, 73.50; H, 4.79; N, 7.54.

8-Methoxy-1-methyl-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one ***(24)*** Yield: method A, 1.19 g (85 %); method B, 1.08 g (77 %); m.p. 92–93 °C; IR (KBr) cm^{-1}: 1675, 1640, 1600, 1580; ^1H NMR (500 MHz, DMSO-d_6): 2.98 (d, 1H, $J = 12.1$, C(O)CH_2), 3.32 (s, 3H, N–CH_3), 3.84 (s, 3H, CH_3), 4.12 (d, 1H, $J = 12.1$, C(O)CH_2), 6.94 (dd, 1H, $J = 8.6$, 2.2, H_{Ar}), 7.03 (d, 1H, $J = 2.2$, H_{Ar}), 7.33 (d, 1H, $J = 8.8$, H_{Ar}), 7.52 (d, 3H, $J = 6.6$, H_{Ar}), 8.06 (dd, 2H, $J = 7.8$, 1.6, H_{Ar}); ^{13}C NMR (125 MHz, DMSO-d_6): 34.76 (NCH_3), 39.69 (C(O)$\underline{CH_2}$), 55.57 (OCH_3), 106.49 (C_{Ar}), 112.02 (C_{Ar}), 127.40 (C_{Ar}), 128.17 (C_{Ar}), 128.71 (C_{Ar}), 130.88 (C_{Ar}), 134.93 (C_{Ar}), 135.84 (C_{Ar}), 136.94 (C_{Ar}), 157.34 (C=N), 158.24 ($\underline{C}$–OCH_3), 165.30 (C=O); anal. calcd. for $C_{17}H_{16}N_2O_2$ (280.33): C, 72.84; H, 5.75; N, 9.99; found: C, 72.94; H, 5.79; N, 10.05.

1-Ethyl-8-methoxy-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one ***(25)*** Yield: method A, 1.29 g (88 %); m.p. 154–155 °C; IR (KBr) cm^{-1}: 1680, 1630, 1605, 1570;

^{1}H NMR (500 MHz, DMSO-d_6): 0.97 (t, 3H, $J = 7.0$, $CH_2\underline{CH_3}$), 2.97 (d, 1H, $J = 11.1$, C(O)CH_2), 3.78 (d, 1H, $J = 11.2$, C(O)CH_2), 3.84 (s, 3H, CH_3), 4.09 (q, 2H, $J = 7.0$, $\underline{CH_2}CH_3$), 6.95 (dd, 1H, $J = 8.5$, 1.7, H_{Ar}), 7.05 (s, 1H, H_{Ar}), 7.33 (d, 1H, $J = 8.8$, H_{Ar}), 7.52 (d, 3H, $J = 6.1$, H_{Ar}), 8.06 (d, 2H, $J = 6.4$, H_{Ar}); ^{13}C NMR (125 MHz, DMSO-d_6): 13.19 ($CH_2\underline{C}H_3$), 39.69 (C(O)$\underline{C}H_2$), 41.84 ($\underline{C}H_2CH_3$), 55.54 (OCH_3), 106.76 (C_{Ar}), 112.27 (C_{Ar}), 127.43 (C_{Ar}), 128.19 (C_{Ar}), 128.69 (C_{Ar}), 130.86 (C_{Ar}), 134.15 (C_{Ar}), 136.01 (C_{Ar}), 136.96 (C_{Ar}), 157.35 (C=N), 158.72 ($\underline{C}$–OCH_3), 164.17 (C=O); anal. calcd. for $C_{18}H_{18}N_2O_2$ (294.35): C, 73.45; H, 6.16; N, 9.52; found: C, 73.40; H, 6.02; N, 9.70.

*1-Benzyl-8-methoxy-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one (**26**)* Yield: method A, 1.62 g (91 %); m.p. 99–100 °C; IR (KBr) cm^{-1}: 1675, 1625, 1600; ^{1}H NMR (500 MHz, DMSO-d_6): 3.08 (d, 1H, $J = 11.8$, C(O)CH_2), 3.71 (s, 3H, CH_3), 4.13 (d, 1H, $J = 11.9$, C(O)CH_2), 5.11 (s, 2H, $\underline{CH_2}C_6H_5$), 6.79 (dd, 1H, $J = 7.2$, 2.4, H_{Ar}), 6.87 (d, 1H, $J = 6.7$, H_{Ar}), 7.07 (d, 2H, $J = 8.6$, H_{Ar}), 7.19 (m, 4H, H_{Ar}), 7.49 (d, 3H, $J = 6.3$, H_{Ar}), 8.10 (d, 2H, $J = 7.2$, H_{Ar}); anal. calcd. for $C_{23}H_{20}N_2O_2$ (356.43): C, 77.51; H, 5.66; N, 7.86; found: C, 77.38; H, 5.51; N, 7.92.

*1-Allyl-8-methoxy-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one (**27**)* Yield: method A, 1.27 g (83 %); method B, 1.31 g (85 %); m.p. 106–108 °C; IR (KBr) cm^{-1}: 1668, 1606, 1563; ^{1}H NMR (400 MHz, DMSO-d_6): 3.06 (d, 1H, $J = 11.9$, C(O)CH_2), 3.79 (s, 3H, CH_3), 4.14 (d, 1H, $J = 11.9$, C(O)CH_2), 4.49 (m, 2H, $\underline{CH_2}$–CH=CH_2), 4.97 (d, 1H, $J = 17.3$, CH_2–CH=$\underline{CH_2}$), 5.05 (d, 1H, $J = 10.5$, CH_2–CH=$\underline{CH_2}$), 5.74 (m, 1H, CH_2–$\underline{CH}$=CH_2), 6.94 (dd, 1H, $J = 8.8$, 2.2, H_{Ar}), 7.05 (s, 1H, H_{Ar}), 7.33 (d, 1H, $J = 8.8$, H_{Ar}), 7.53 (m, 3H, H_{Ar}), 8.06 (d, 2H, $J = 7.6$, H_{Ar}); ^{1}H NMR (400 MHz, $CDCl_3$): 3.01 (d, 1H, $J = 11.7$, C(O)CH_2), 3.75 (s, 3H, CH_3), 4.09 (d, 1H, $J = 11.7$, C(O)CH_2), 4.26 (d, 1H, $J = 15.2$, $\underline{CH_2}$–CH=CH_2), 4.50 (d, 1H, $J = 15.2$, $\underline{CH_2}$–CH=CH_2), 5.09 (m, 2H, CH_2–CH=$\underline{CH_2}$), 5.83 (m, 1H, CH_2–$\underline{CH}$=CH_2), 6.80 (d, 1H, $J = 8.9$, H_{Ar}), 6.89 (s, 1H, H_{Ar}), 7.36 (m, 4H, H_{Ar}), 8.06 (m, 2H, H_{Ar}); ^{13}C NMR (100 MHz, DMSO-d_6): 39.68 (C(O)$\underline{C}H_2$), 49.28 ($\underline{C}H_2$–CH=CH_2), 55.55 (OCH_3), 106.71 (C_{Ar}), 112.30 (C_{Ar}), 116.05 (CH_2–CH=$\underline{C}H_2$), 127.55 (C_{Ar}), 128.32 (C_{Ar}), 128.81 (C_{Ar}), 131.02 (C_{Ar}), 133.79 (CH_2–$\underline{C}$H=CH_2), 134.69 (C_{Ar}), 135.63 (C_{Ar}), 136.98 (C_{Ar}), 157.33 (C=N), 158.71 ($\underline{C}$–OCH_3), 164.56 (C=O); ^{13}C NMR (100 MHz, $CDCl_3$): 39.96 (C(O)$\underline{C}H_2$), 50.95 ($\underline{C}H_2$–CH=CH_2), 55.53 (OCH_3), 106.42 (C_{Ar}), 112.11 (C_{Ar}), 116.70 (CH_2–CH=$\underline{C}H_2$), 127.69 (C_{Ar}), 128.55 (C_{Ar}), 128.66 (C_{Ar}), 131.08 (C_{Ar}), 133.32 (CH_2–$\underline{C}$H=CH_2), 135.45 (C_{Ar}), 136.93 (C_{Ar}), 157.53 (C=N), 163.14 ($\underline{C}$–OCH_3), 164.81 (C=O); anal. calcd. for $C_{19}H_{18}N_2O_2$ (306.36): C, 74.49; H, 5.92; N, 9.14; found: C, 74.68; H, 5.97; N, 9.03.

*1,8-Dimethyl-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one (**28**)* Yield: method A, 1.15 g (87 %); method B, 1.05 g (79 %); m.p. 91–92 °C; IR (KBr) cm^{-1}: 1690, 1620, 1605; ^{1}H NMR (500 MHz, DMSO-d_6): 2.39 (s, 3H, C–CH_3), 2.97 (d, 1H, $J = 11.7$, C(O)CH_2), 3.29 (s, 3H, N–CH_3), 4.11 (d, 1H, $J = 11.7$, C(O)CH_2), 7.12 (d, 1H, $J = 8.0$, H_{Ar}), 7.28 (d, 1H, $J = 8.0$, H_{Ar}), 7.34 (s, 1H, H_{Ar}), 7.53 (d, 3H, $J = 6.9$, H_{Ar}), 8.07 (d, 2H, $J = 7.2$, H_{Ar}); ^{13}C NMR (125 MHz, DMSO-d_6): 20.72 (C–$\underline{C}H_3$), 34.60 (N–CH_3), 39.68 (C(O)$\underline{C}H_2$), 122.23 (C_{Ar}), 125.95 (C_{Ar}), 126.66 (C_{Ar}), 127.49 (C_{Ar}), 128.72 (C_{Ar}), 131.05 (C_{Ar}), 134.66 (C_{Ar}), 135.83 (C_{Ar}), 136.85 ($\underline{C}$–CH_3), 138.70 (C_{Ar}), 159.44 (C=N), 165.44 (C=O); anal. calcd. for $C_{17}H_{16}N_2O$ (264.33): C, 77.25; H, 6.10; N, 10.60; found: C, 77.39; H, 5.97; N, 10.78.

*1-Benzyl-8-methyl-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one (**29**)* Yield: method A, 1.53 g (90 %); method B, 1.60 g (94 %); m.p. 145–146 °C; IR (KBr) cm^{-1}: 1680, 1615, 1575; ^{1}H NMR (500 MHz, DMSO-d_6): 2.29 (s, 3H, CH_3), 3.14 (d, 1H, $J = 11.7$, C(O)CH_2), 4.20 (d, 1H, $J = 11.7$, C(O)CH_2), 5.03 (d, 1H, $J = 16.1$, $\underline{CH_2}C_6H_5$), 5.30 (d, 1H, $J = 16.1$, $\underline{CH_2}C_6H_5$), 6.95 (d, 2H, $J = 7.3$, H_{Ar}), 7.07 (d, 1H, $J = 8.0$, H_{Ar}), 7.19 (m, 4H, H_{Ar}), 7.37 (s, 1H, H_{Ar}), 7.55 (d, 3H, $J = 6.1$, H_{Ar}), 8.10 (d, 2H, $J = 6.4$, H_{Ar}); ^{13}C NMR (125 MHz, DMSO-d_6): 20.70 (C–$\underline{C}H_3$), 39.69 (C(O)$\underline{C}H_2$), 49.17 ($\underline{C}H_2C_6H_5$), 122.58 (C_{Ar}), 126.22 (C_{Ar}), 126.35 (C_{Ar}), 126.69 (C_{Ar}), 126.85 (C_{Ar}), 127.64 (C_{Ar}), 128.36 (C_{Ar}), 128.76 (C_{Ar}), 131.11 (C_{Ar}), 133.05 (C_{Ar}), 135.72 (C_{Ar}), 136.91 (C_{Ar}), 137.52 ($\underline{C}$–CH_3), 139.69 (C_{Ar}), 159.97 (C=N), 165.00 (C=O); anal. calcd. for $C_{23}H_{20}N_2O$ (340.43): C, 81.15; H, 5.92; N, 8.23; found: C, 81.09; H, 5.80; N, 8.29.

*1-Allyl-8-methyl-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one (**30**)* Yield: method A, 1.28 g (88 %); m.p. 138–139 °C; IR (KBr) cm^{-1}: 1670, 1620, 1600; ^{1}H NMR (500 MHz, DMSO-d_6): 2.36 (s, 3H, CH_3), 3.04 (d, 1H, $J = 11.9$, C(O)CH_2), 4.14 (d, 1H, $J = 11.9$, C(O)CH_2), 4.49 (q, 2H, $J = 16.1$, $\underline{CH_2}$–CH=CH_2), 4.93 (d, 1H, $J = 13.4$, CH_2–CH=$\underline{CH_2}$), 5.02 (d, 1H, $J = 13.8$, CH_2–CH=$\underline{CH_2}$), 5.73 (m, 1H, CH_2–$\underline{CH}$=CH_2), 7.12 (d, 1H, $J = 7.9$, H_{Ar}), 7.29 (d, 1H, $J = 7.8$, H_{Ar}), 7.36 (s, 1H, H_{Ar}), 7.54 (d, 3H, $J = 6.7$, H_{Ar}), 8.08 (d, 2H, $J = 6.7$, H_{Ar}); ^{13}C NMR (125 MHz, DMSO-d_6): 20.76 (C–$\underline{C}H_3$), 39.68 (C(O)$\underline{C}H_2$), 48.93 ($\underline{C}H_2$–CH=CH_2), 115.79 (CH_2–CH=$\underline{C}H_2$), 122.37 (C_{Ar}), 126.24 (C_{Ar}), 126.72 (C_{Ar}), 127.55 (C_{Ar}), 128.73 (C_{Ar}), 131.08 (C_{Ar}), 133.42 (C_{Ar}), 133.63 (CH_2–$\underline{C}$H=CH_2), 135.75 (C_{Ar}), 136.83 ($\underline{C}$–CH_3), 139.37 (C_{Ar}), 159.82 (C=N), 164.53 (C=O); anal. calcd. for $C_{19}H_{18}N_2O$ (290.37): C, 78.59; H, 6.25; N, 9.65; found: C, 78.82; H, 6.10; N, 9.58.

*1-Methyl-7-nitro-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one (**31**)* Yield: method A, 1.21 g (82 %); m.p. 151–153 °C; IR (KBr) cm^{-1}: 1685, 1635, 1600; ^{1}H NMR

(500 MHz, DMSO-d_6): 3.06 (d, 1H, $J = 11.6$, C(O)CH_2), 3.37 (s, 3H, CH_3), 4.21 (d, 1H, $J = 11.8$, C(O)CH_2), 7.55 (m, 3H, H_{Ar}), 7.81 (d, 1H, $J = 7.9$, H_{Ar}), 8.15 (m, 4H, H_{Ar}); anal. calcd. for $C_{16}H_{13}N_3O_3$ (295.30): C, 65.08; H, 4.44; N, 14.23; found: C, 65.21; H, 4.48; N, 14.28.

1-Ethyl-7-nitro-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one (***32***) Yield: method A, 1.22 g (79 %); m.p. 119–121 °C; IR (KBr) cm^{-1}: 1670, 1625, 1600; ^{1}H NMR (300 MHz, DMSO-d_6): 1.06 (t, 3H, $J = 6.8$, CH_2CH_3), 3.13 (d, 1H, $J =$ 11.0, C(O)CH_2), 3.90 (d, 1H, $J = 7.0$, CH_2CH_3), 4.06 (d, 1H, $J = 7.1$, CH_2CH_3), 4.25 (d, 1H, $J = 11.1$, C(O)CH_2), 7.60 (m, 3H, H_{Ar}), 7.83 (d, 1H, $J = 7.8$, H_{Ar}), 8.17 (m, 4H, H_{Ar}); anal. calcd. for $C_{17}H_{15}N_3O_3$ (309.32): C, 66.01; H, 4.89; N, 13.58; found: C, 66.09; H, 4.98; N, 13.73.

1-Allyl-7-nitro-4-phenyl-1H-benzo[b][1,4]diazepin-2(3H)-one (***33***) Yield: method A, 1.29 g (80 %); method B, 1.21 g (75 %); m.p. 141–143 °C; IR (KBr) cm^{-1}: 1680, 1630, 1600; ^{1}H NMR (500 MHz, DMSO-d_6): 3.23 (d, 1H, $J =$ 11.0, C(O)CH_2), 4.29 (d, 1H, $J = 11.0$, C(O)CH_2), 4.57 (s, 2H, CH_2–CH=CH_2), 5.00 (d, 1H, $J = 17.3$, CH_2–CH=CH_2), 5.09 (d, 1H, $J = 10.4$, CH_2–CH=CH_2), 5.78 (m, 1H, CH_2–CH=CH_2), 7.59 (m, 3H, H_{Ar}), 7.76 (d, 1H, $J = 9.1$, H_{Ar}), 8.16 (m, 4H, H_{Ar}); ^{13}C NMR (125 MHz, DMSO-d_6): 39.68 (C(O)CH_2), 49.21 (CH_2–CH=CH_2), 116.49 (CH_2–CH=CH_2), 120.47 (C_{Ar}), 122.21 (C_{Ar}), 123.85 (C_{Ar}), 128.00 (C_{Ar}), 128.89 (C_{Ar}), 131.93 (C_{Ar}), 133.08 (C_{Ar}), 136.05 (CH_2–CH=CH_2), 139.03 (C_{Ar}), 141.50 (C_{Ar}), 143.72 (C–NO_2), 162.92 (C=N), 164.46 (C=O); anal. calcd. for $C_{18}H_{15}N_3O_3$ (321.34): C, 67.28; H, 4.71; N, 13.08; found: C, 67.48; H, 4.80; N, 12.91.

4-Methyl-2-oxo-1,5-di(prop-2-yn-1-yl)-2,3-dihydro-1H-benzo[b][1,4]diazepin-5-ium bromide (***34***) Yield: method A, 0.91 g (55 %); semicrystalline solid; IR (KBr) cm^{-1}: 3310, 2120, 1720, 1635; ^{1}H NMR (500 MHz, DMSO-d_6): 2.20 (s, 3H, CH_3), 2.26 (m, 1H, N–CH_2C≡CH), 2.87 (m, 4H, $2 \times CH_2$C≡CH), 3.19 (m, 1H, N^+–CH_2C≡CH), 4.66 (m, 2H, C(O)CH_2), 7.30 (m, 3H, H_{Ar}), 7.66 (m, 1H, H_{Ar}); ^{13}C NMR (125 MHz, DMSO-d_6): 15.77 (CH_3), 22.07 (N–CH_2C≡CH), 36.17 (C(O)CH_2), 48.84 (N^+–CH_2C≡CH), 72.59 (N^+–CH_2C≡CH), 74.69 (N–CH_2C≡CH), 79.43 (N–CH_2C≡CH), 82.02 (N^+–CH_2C≡CH), 122.10 (C_{Ar}), 125.68 (C_{Ar}), 125.78 (C_{Ar}), 125.91 (C_{Ar}), 132.32 (C_{Ar}), 140.62 (C_{Ar}), 164.28 (C=N), 165.49 (C=O); anal. calcd. for $C_{16}H_{15}BrN_2O$ (331.21): C, 58.02; H, 4.57; N, 8.46; found: C, 58.35; H, 4.29; N, 8.59. MS *m/z*: 251 (M^+, cationic part of the salt).

2-Oxo-4-phenyl-1,5-di(prop-2-yn-1-yl)-2,3-dihydro-1H-benzo[b][1,4]diazepin-5-ium bromide (***35***) Yield: method A, 1.28 g (65 %); m.p. 134–136 °C; IR (KBr) cm^{-1}: 3300, 2120, 1720, 1630; ^{1}H NMR (500 MHz, DMSO-d_6): 2.12 (m, 1H, N–CH_2C≡CH), 2.68 (m, 1H, N–CH_2C≡CH), 2.75 (dd, 1H, $J = 17.0$, 7.4, N^+–CH_2C≡CH), 2.92 (dd, 1H, $J =$ 17.0, 7.4, N^+–CH_2C≡CH), 3.21 (m, 1H, N^+–CH_2C≡CH), 3.28 (m, 1H, N–CH_2C≡CH), 4.74 (d, 1H, $J = 17.8$, C(O)CH_2), 4.80 (d, 1H, $J = 17.8$, C(O)CH_2), 7.40–7.74 (m, 9H, H_{Ar}); ^{13}C NMR (125 MHz, DMSO-d_6): 16.70 (N–CH_2C≡CH), 36.39 (C(O)CH_2), 48.14 (N^+–CH_2C≡CH), 72.53 (N^+–CH_2C≡CH), 74.82 (N–CH_2C≡CH), 79.31 (N–CH_2C≡CH), 81.46 (N^+–CH_2C≡CH), 122.39 (C_{Ar}), 126.04 (C_{Ar}), 126.39 (C_{Ar}), 126.55 (C_{Ar}), 126.74 (C_{Ar}), 127.77 (C_{Ar}), 128.33 (C_{Ar}), 128.74 (C_{Ar}), 129.82 (C_{Ar}), 132.50 (C_{Ar}), 137.26 (C_{Ar}), 140.65 (C_{Ar}), 163.46 (C=N), 165.68 (C=O); anal. calcd. for $C_{21}H_{17}BrN_2O$ (393.28): C, 64.13; H, 4.36; N, 7.12; found: C, 64.35; H, 4.10; N, 7.45. MS *m/z*: 313 (M^+, cationic part of the salt).

Docking studies

Compounds **9–33** were subjected to docking studies against human serum albumin (HSA). *Ligand preparation*: substance structures were drawn using MarvinSketch 6.3.0 and saved in *mol* format (MarvinSketch, http://www.chemaxon.com)] Geometries were then optimized with HyperChem 8.0.8 using molecular mechanics algorithm MM+ over 1000 cycles using Polak-Ribiere (Conjugate Gradient) algorithm. The pre-optimized structures were further refined using semi-empirical (PM3) molecular modeling and saved as *pdb* files. The *pdb* files were converted to PDBQT using AutoDockTools-1.5.6, leaving the number of active torsions at the default setting (Trott and Olson, 2010). *Protein preparation*: the PDB file (2BXF) was downloaded from the protein data bank (http://www.pdb.org). Discovery Studio 4.0 was used to delete water molecules and the ligand from the crystal structure. The obtained protein structure was then saved as a *pdb* file. Polar hydrogens were added using AutoDockTools-1.5.6 and the structure was saved as a *PDBQT* file. Grid box was set as the following: center_$x = 53.967$, center_$y =$ 35.000, center_$z = 74.155$, size_$x = 18$, size_$y = 18$, size_$z =$ 16. The docking was performed using Vina 1.1.2 (Sanner, 1999). The results were visualized using Discovery Studio 4.0.

In silico PASS screening

PASS has been employed as a strong potential tool to predict the biological activity spectrum of synthetic substances for the discovery of new drugs. PASS is based on the SAR analysis of a training set containing more than 205,000 compounds exhibiting more than 3750 kinds of biological activities (Goel et al., 2011). We performed virtual screening of BDZs **9–33** using PASS online tool (http://www.way2drug.com/PASSOnline) in order to select the most promising anticonvulsant (main activity for Diazepam according to PASS results) compounds for further in vivo tests. The PASS software predicts the activities of a given

compound as probable activity (Pa) and probable inactivity (Pi). Being probabilities, the Pa and Pi vary from 0.000 to 1.000. In general, $Pa + Pi \neq 1$, since these values are calculated independently. Only activities with $Pa > Pi$ are considered promising for a particular compound.

Pharmacology

An approval of the Institutional Ethical Committee for Animal Experiments was obtained prior to performing the tests below. The experiments were carried out in compliance with the International Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985) as well as the Law of Ukraine "On Protection of Animals from Inhumane Treatment".

In *vivo tests*: the neurotropic (antihypoxic, tranquilizing and anticonvulsant) activity and acute toxicity (LD_{50}) of the newly synthesized compounds **22** and **24** were studied using white mice of both sexes, weighing 20–30 g. The animals were housed in plastic cages with the stainless steel top, had access to food and water ad libitum and were contained at 21±2 °C with a 12-h light/dark cycle. Humidity and ventilation were controlled according to international standards.

Aqueous suspensions of the tested compounds **22** and **24** were prepared with the addition of TWEEN-80 and introduced intraperitoneally 30 min prior to the testing. The same volume of 0.8 % sodium chloride isotonic solution was injected into the animals of the control group. The acute toxicity was determined following intraperitoneal injection of an investigated substance by establishing the lethal dose (LD_{50}) for 12 observations, according to the Prozorovskii's rapid method (Prozorovskii, 2007). The effect of a substance injected in a dose of 1/10 LD_{50} was compared in groups of 12 animals. Neurotropic activity was estimated from: (a) the influence on the lifetime of mice under hypoxic hypoxia caused by placing the animals in a separate 125-ml chamber without absorption of CO_2; (b) the influence on the duration of hexobarbital-induced anesthesia caused by a dose of 60 mg/kg; (c) the pentetrazol-induced spasms caused by an intraperitoneal injection of 10 % aqueous pentetrazol solution in the dose of 150 mg/kg. Activity of the tested compounds **22** and **24** was compared to that of a reference drug Diazepam (oral LD_{50} for a mouse is 48 mg/kg (Peterson and Talcott, 2013)) used in its standard dose of 1 mg/kg.

The antihypoxic, tranquilizing and anticonvulsant activities (NA) were estimated in the group of experimental animals with respect to the control group and expressed in percentage. The normalized values were calculated as $NA=(N_e/N_c)\cdot 100$ %, where N_c and N_e are the mean values in the control and experimental groups, respectively. The experimental data were treated statistically by Student's test. Differences at $p < 0.05$ were considered significant.

Results and Discussion

Chemistry

A series of the starting benzodiazepine-2-ones **1–8** (**1**: R^2=Me, R^3=R^4=H; **2**: R^2=Me, R^3=H, R^4=Cl; **3**: R^2=Me, R^3=H, R^4=Me; **4**: R^2=Ph, R^3=R^4=H; **5**: R^2=Ph, R^3=H, R^4=Cl; **6**: R^2=Ph, R^3=H, R^4=OMe; **7**: R^2=Ph, R^3=H, R^4=Me; **8**: R^2=Ph, R^3=NO_2, R^4=H) was synthesized using *one-pot* cyclocondensation of benzoylacetic and acetoacetic esters with appropriate *o*-phenylenediamines (*caution*: *o*-phenylenediamines are toxic and environmentally hazardous). Three methods for the direct alkylation of compounds **1–8** were then examined:

Method A: Reaction in benzene at 60 °C with 6 mol % of a phase-transfer catalyst, TBAB, and an excess of aqueous 50 % NaOH.

Method B: Reaction in acetone with 5 equivalents of solid KOH under reflux conditions.

Method C: Reaction in dry DMF at 100 °C using 2 equivalents of NaH.

In all cases, good yields were obtained, ranging from 70 to 95 % (Scheme 1, Table 1). This allowed to produce various *N*-alkyl-BDZs for subsequent in vivo tests in an efficient manner under mild conditions. The reactions were clean and complete within 1.5–3 h.

Our attempts to monoalkylate the substrates **1** and **4** with propargyl bromide failed. Treatment of the starting materials with excess of the alkylating agent (method A) gave products identified as salts **34** and **35** (Scheme 2).

The structures of compounds **9–33** have been confirmed using IR, ^{1}H, and ^{13}C NMR spectroscopy as well as elemental analysis. In the IR spectra, a characteristic band at around 1600–1620 cm^{-1} indicates the presence of the C=N bond of the seven-membered diazepine ring; a sharp band at 1668–1690 cm^{-1} represents carbonyl group stretching vibrations. The ^{1}H NMR spectra of the BDZs **9–33** show characteristic signals for the non-equivalent methylene protons of the seven-membered diazepine ring as two doublets at 2.79–3.23 and 3.30–4.29 ppm (^2J 10.6–12.3 Hz), respectively. All the benzyl derivatives **12**, **16**, **19**, **23**, **26**, and **29** also display two characteristic doublets at 4.99–5.05 and 5.26–5.30 ppm (^2J 15.9–16.1 Hz). The structures of compounds **34** and **35** are consistent with their NMR spectra and were further confirmed using mass spectrometry.

In addition, the structure of the BDZ **27** possessing an *N*-allyl substituent was studied in more detail using Correlation Spectroscopy (COSY), Nuclear Overhauser Effect Spectroscopy (NOESY), Heteronuclear Single-Quantum Correlation spectroscopy (HSQC), and Heteronuclear Multiple-Bond Correlation spectroscopy (HMBC) experiments in DMSO-d_6. The results are summarized in Fig. 2.

Assignments of the ^{1}H and ^{13}C signals for all other products were performed by analogy.

Single crystals of the compound **29** were grown from hexane solution and studied by X-ray diffraction (Fig. 3).

The seven-membered diazepine ring adopts a boat-like conformation. The N(2), C(7), C(9), and N(1) atoms are coplanar within 0.005 Å, whereas deviations of the C(1), C(6), and C(8) atoms from the plane are − 0.73, − 0.72, and − 0.77 Å, respectively. As the result, the H(5)…C(16) and H(5)…C(17) come in close intramolecular contact (corresponding distances are 2.56 and 2.59 Å as compared to the sum of van der Waals radii being 2.87 Å (Zefirov, 1997)). In the crystal phase, the C–H···π intermolecular hydrogen bonds (C(18)–H(18)…C(10)′ (π) (0.5 − *x*, − 0.5 + *y*, 0.5 − *z*) H…C 2.82 Å C–H…C 151° and C(8)–H(8a)…C(13)′ (π) (−0.5 − *x*, − 0.5 + *y*, 0.5 − *z*) H…C 2.83 Å C–H…C 142°) are observed.

Molecular docking

HSA is an abundant plasma protein and one of the main endogenous vehicles for biodistribution of molecules by blood plasma. It has various physiological functions, including the maintenance of osmotic pressure, transport, distribution, and participation in the metabolism of many endogenous and exogenous ligands (e.g., drugs, metabolites, fatty acids, amino acids, and hormones). HSA increases the solubility of ligands in blood plasma, which can reduce their toxicity, and/or protect them against oxidation or other reactions (Chaves et al., 2015; Taguchi et al., 2012; Zsila et al., 2011). On the other hand, binding of drugs by HSA restricts their free, active concentration. The problem of overcoming the binding affinity for HSA represents a major challenge in drug development.

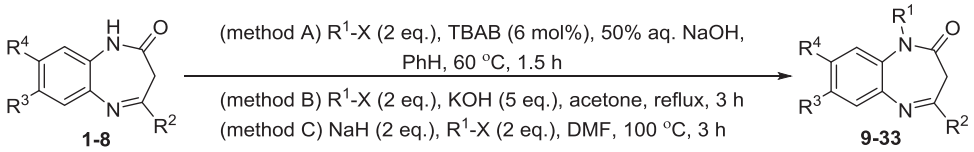
Among all diversity of various BDZs represented in the literature, we were able to find only one work devoted to binding studies with HSA (Ghuman et al., 2005). The authors isolated a corresponding complex with Diazepam. We have extracted X-ray diffraction structure of this complex from Protein Data Bank (http://www.pdb.org) and used it to study the binding of BDZs **9–33** to HSA.

The highest binding affinity was observed for compound **33** with binding energy of − 9.8 kcal/mol (Table 2), which is comparable to that of the reference drug, diazepam (−9.7 kcal/mol). Visualization of interactions for compound **33** is presented in Fig. 4. Four major hydrogen bonds with HSA were identified: between LEU430 and the CO group (3.29 Å), ASN391 and the unsubstituted nitrogen atom of BDZ (3.25 Å), GLU450 and O of the nitro group (3.32 Å), and PRO384 and O of the nitro group (3.77 Å) (Bissantz et al., 2010; Pierce et al., 2002).

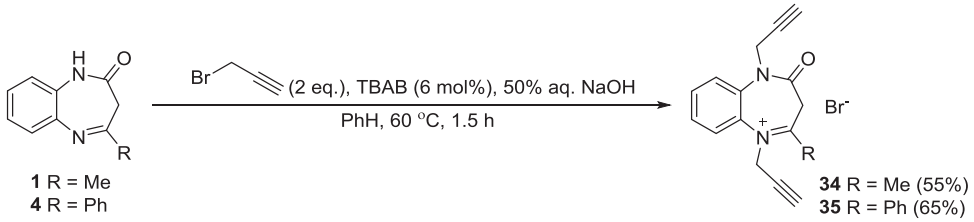
In silico PASS screening

PASS-predicted results for diazepam (reference drug) and BDZs **9–33** are summarized in Table 2. The results for basic neurotropic activities such as anticonvulsant, antihypoxic, tranquilizing, and analgesic are given. In addition, we have also identified other important biological activities with Pa (probability of activity) > 0.7. The tranquilizing potential of compounds **9–16** (R^2=Me) is significantly higher than that of their analogs **17–33** (R^2=Ph). Compound **9** has the most promising predicted activity (Pa 0.829). In general, antihypoxic action is moderate for the two groups of BDZs **9–16** and **17–33**, and close to the value for diazepam (Pa 0.432). Analgesic activity probabilities are equally very low for all compounds, **9–33**, and are characterized by Pa value within 0.208–0.300, which is significantly lower than that for diazepam (Pa 0.748). The anticonvulsant activities of BDZs **9–16** and **17–33** have similar predicted probabilities. The best result was found for compound **22**. In silico screening of the synthesized BDZs also showed high probability levels for the inhibition of amine/taurine dehydrogenase, gluconate 2-dehydrogenase, and glycosylphosphatidylinositol phospholipase D.

Scheme 1 The synthesis of target compounds **9–33**



Scheme 2 The synthesis of BDZ salts **34** and **35**



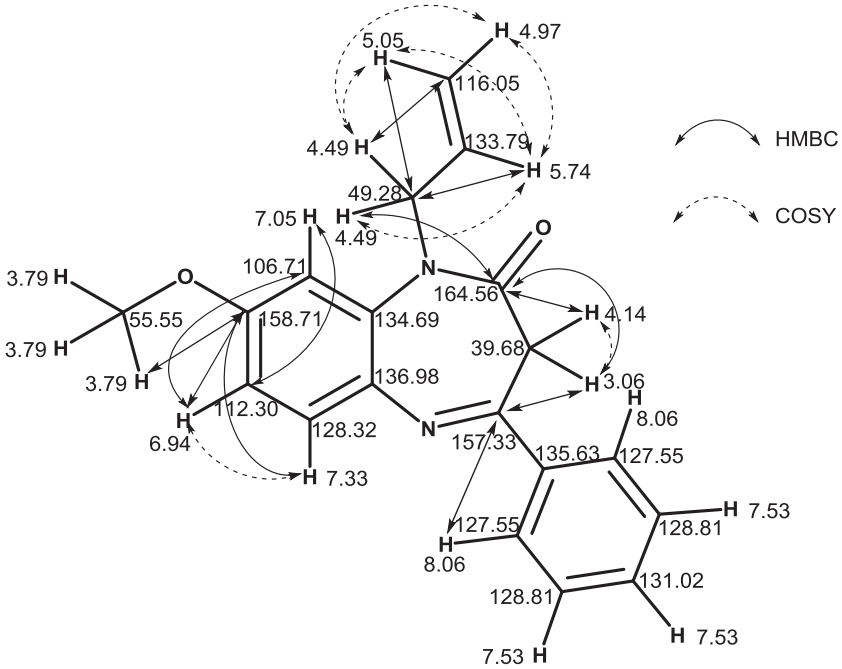


Fig. 2 NMR chemical shifts and important 2D correlations for the compound **27** (DMSO-d_6, ppm)

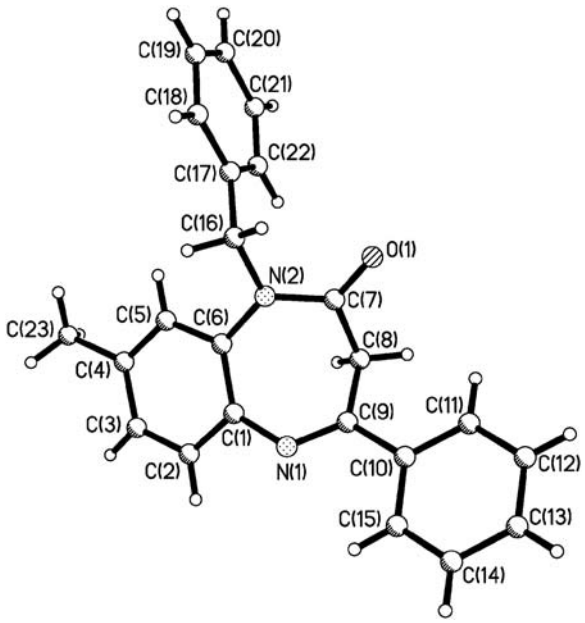


Fig. 3 Molecular structure of 1-benzyl-8-methyl-4-phenyl-1*H*-benzo[*b*][1,4]diazepin-2(3*H*)-one **29** according to X-ray diffraction

Pharmacology

Based on the results of in silico screening, compound **22** was subjected to further in vivo testing. Our additional interest to the derivative **22** was caused by its structural isomerism to diazepam. For comparison with compound **22**, we also studied its analog **24**. The BDZs **22** and **24** were tested for in vivo toxicity, antihypoxic, tranquilizing, and anticonvulsant properties. The intraperitoneal LD_{50} for compounds **22** and **24** in white mice was found to be 687.5 $\pm$ 88.6 and 658.0 $\pm$ 76.0 mg/kg, respectively. Both *N*-methyl BDZs showed significant tranquilizing activity of $>$ 400 % with respect to the control experiments. This value is comparable to the activity of diazepam. The antihypoxic activity of the new BDZs is higher than that of diazepam by up to 1.34 times (for the chlorinated BDZ **22**). The anticonvulsant activity of the new BDZs is at the level of 337–398 % with respect to the control experiments, but is significantly lower than that of diazepam. This result is generally consistent with PASS predictions. To our surprise, the nature of the substituent at C-8 of the 1,5-BDZ core (Cl for **22** and OMe for **24**) does not affect the tranquilizing and anticonvulsant activity, as was expected based on the PASS data (Table 2). Thus, of the two compounds, the chlorine-containing BDZ **22** is a better antihypoxic agent and the BDZ **24** exhibits a stronger anticonvulsant effect. We believe that the neurotropic agents we have discovered could be possible drug candidates in the future. The obtained experimental results are summarized in Table 3.

Conclusions

In this work, we have tested tree different *one-pot* methods for the synthesis of *N*-alkyl-1,5-benzodiazepine-2-ones. We found that all three are effective for the appropriate cases and provide desired products within 70–95 % yield. The products **13** and **21** are the first examples of *N*-(oxirane-2-ylmethyl)-containing 1,5-BDZs. These compounds have great synthetic potential with respect to further

Table 2 Affinity to human serum albumin (protein code 2BXF) and PASS predicted results (Pa and Pi) BDZs **9–33** and diazepam standard

Compound	Affinity kcal/mol	Antihypoxic activity		Tranquilizing activity		Anticonvulsant activity		Analgesic activity		Selected important activities with Pa > 0.7 (Pa;Pi)
		Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	
Diazepam	–9.7	0.432	0.062	0.802	0.005	0.933	0.004	0.748	0.005	Skeletal muscle relaxant (0.937; 0.002), antineurotic (0.913; 0.004), GABA receptor agonist (0.882; 0.003), antiparkinsonian (0.776; 0.003)
9	–7.1	0.517	0.033	0.829	0.005	0.441	0.050	0.274	0.065	Nicotinic alpha2beta2 receptor antagonist (0.787; 0.012), taurine dehydrogenase inhibitor (0.746; 0.022)
10	–7.7	0.460	0.050	0.767	0.005	0.423	0.055	–	–	Amine dehydrogenase inhibitor (0.842; 0.004), proteasome ATPase inhibitor (0.834; 0.004), insulin promoter (0.774; 0.004)
11	–7.3	0.485	0.042	0.692	0.009	0.370	0.078	–	–	Insulin promoter (0.765; 0.004), amine dehydrogenase inhibitor (0.732; 0.006), taurine dehydrogenase inhibitor (0.718; 0.028)
12	–8.1	0.482	0.043	0.674	0.010	0.272	0.139	–	–	Taurine dehydrogenase inhibitor (0.707; 0.030)
13	–7.5	0.402	0.078	0.692	0.009	0.213	0.182	–	–	Mood disorders treatment (0.737; 0.006), antidepressant (0.736; 0.006)
14	–6.9	0.435	0.061	0.788	0.005	0.552	0.026	0.274	0.064	Taurine dehydrogenase inhibitor (0.778; 0.017), glycosylphosphatidylinositol phospholipase D inhibitor (0.724; 0.029)
15	–7.1	0.488	0.041	0.769	0.005	0.384	0.071	0.233	0.130	CYP2A8 substrate (0.813; 0.003)
16	–6.8	0.452	0.053	0.636	0.012	0.223	0.171	–	–	–
17	–7.9	0.468	0.047	0.221	0.108	0.446	0.049	0.300	0.039	CYP2A8 substrate (0.848; 0.003), nicotinic alpha2beta2 receptor antagonist (0.831; 0.006)
18	–8.0	0.422	0.067	0.172	0.150	0.438	0.051	0.208	0.183	Glutathione thiolesterase inhibitor (0.853, 0.004), amine dehydrogenase inhibitor (0.840; 0.004), insulin promoter (0.785; 0.004)
19	–7.8	0.433	0.061	–	–	0.272	0.139	0.230	0.135	–
20	–8.9	0.311	0.147	–	–	0.469	0.043	–	–	Gluconate 2-dehydrogenase (acceptor) inhibitor (0.703; 0.054)
21	–7.3	0.362	0.104	0.191	0.130	0.216	0.179	–	–	–
22	–8.1	0.398	0.080	0.246	0.093	0.569	0.023	0.229	0.193	CYP2A8 substrate (0.894; 0.002), glycosylphosphatidylinositol phospholipase D inhibitor (0.823; 0.009), taurine dehydrogenase inhibitor (0.771; 0.018), phobic disorders treatment (0.713; 0.007)
23	–6.9	0.368	0.100	0.163	0.160	0.375	0.075	0.230	0.136	Glycosylphosphatidylinositol phospholipase D inhibitor (0.773; 0.018), CYP2A8 substrate (0.746; 0.005), gluconate 2-dehydrogenase (acceptor) inhibitor (0.730; 0.041), taurine dehydrogenase inhibitor (0.722; 0.027)
24	–7.5	0.331	0.129	0.177	0.144	0.243	0.158	0.232	0.132	Gluconate 2-dehydrogenase (acceptor) inhibitor (0.761; 0.028)
25	–7.6	0.291	0.165	–	–	0.236	0.162	–	–	Amine dehydrogenase inhibitor (0.811; 0.004), gluconate 2-dehydrogenase (acceptor) inhibitor (0.776; 0.023), spermidine dehydrogenase inhibitor (0.713; 0.008)
26	–7.8	0.301	0.155	–	–	–	–	–	–	Gluconate 2-dehydrogenase (acceptor) inhibitor (0.747; 0.033)
27	–7.9	–	–	–	–	0.267	0.143	–	–	Gluconate 2-dehydrogenase (acceptor) inhibitor (0.797; 0.017)
28	–8.2	0.458	0.051	0.195	0.126	0.405	0.062	0.253	0.094	CYP2A8 substrate (0.773; 0.004)
29	–7.0	0.419	0.068	–	–	0.236	0.162	–	–	–
30	–8.0	0.302	0.155	–	–	0.426	0.055	–	–	Antieczematic (0.755; 0.029)
31	–8.8	0.479	0.043	–	–	0.452	0.047	0.214	0.169	(*R*)-6-hydroxynicotine oxidase inhibitor (0.793; 0.004), superoxide dismutase inhibitor (0.719; 0.012)
32	–7.8	0.432	0.062	–	–	0.444	0.049	–	–	Arylalkyl acylamidase inhibitor (0.866; 0.003), spermidine dehydrogenase inhibitor (0.827; 0.004)
33	–9.8	0.320	0.138	–	–	0.475	0.041	–	–	–

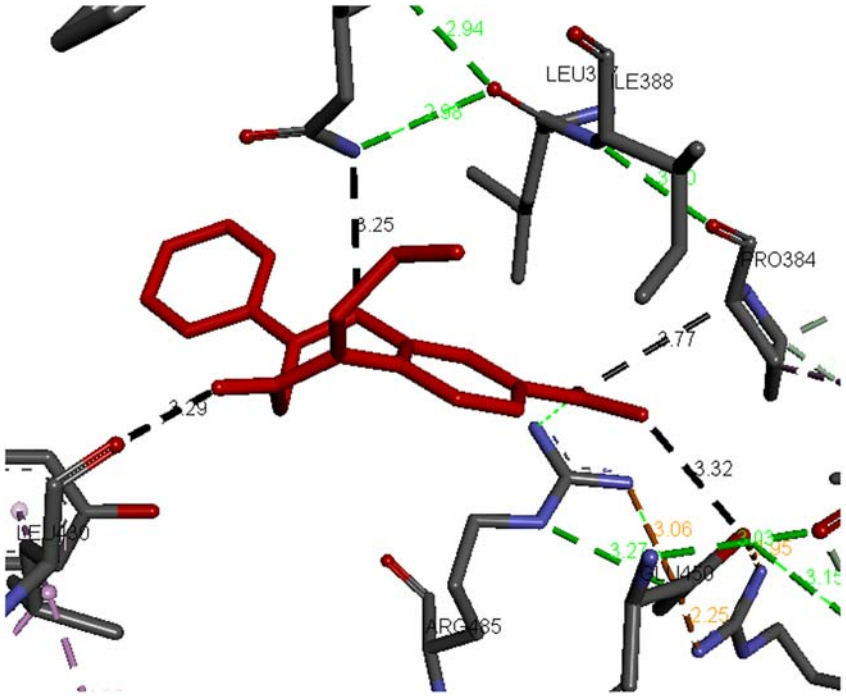


Fig. 4 Interaction between compound **33** (shown in red) and HSA. Hydrogen bonds are shown in black

Table 3 Neurotropic activity of BDZs **22** and **24**

Compound	Antihypoxic activity		Tranquilizing activity		Anticonvulsant activity	
	Life span, min	% to the control group of animals	The duration of sleep, min	% to the control group of animals	Life span, min	% to the control group of animals
Control	16.4 ± 0.9	148.8	19.9 ± 3.1	418.1	4.3 ± 1.5	2325.6
Diazepam	24.4 ± 1.7		83.2 ± 11.8		100.0 ± 10.6	
Control	16.2 ± 0.6	199.4	19.9 ± 3.1	411.6	4.3 ± 1.5	337.2
Compound **22**	32.3 ± 0.3		81.9 ± 10.2		14.5 ± 3.3	
Control	16.0 ± 0.7	156.3	15.0 ± 1.9	412.0	5.8 ± 2.1	398.3
Compound **24**	25.0 ± 1.2		61.8 ± 17.7		23.1 ± 7.8	

transformations into various oxazaheterocycles (Kas'yan et al., 2011a, b; Pal'chikov, 2013). In silico screening of the synthesized compounds showed their high tranquilizing and anticonvulsant potential coupled with strong probability levels of inhibition of amine dehydrogenase, taurine dehydrogenase, gluconate 2-dehydrogenase, and glycosylphosphatidylinositol phospholipase D. According to the molecular docking studies, the highest binding affinity to HSA was observed for compound **33** (−9.8 kcal/mol), which is slightly better than for diazepam (−9.7 kcal/mol). The two in vivo tested compounds **22** and **24** showed high levels of antihypoxic, tranquilizing, and anticonvulsant activity compared to the reference drug diazepam. The new compounds were characterized using IR, ^{1}H NMR, ^{13}C NMR, COSY, NOESY, HSQC, HMBC spectroscopy, X-ray diffractometry, and elemental analysis techniques.

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Compliance with ethical standards

Competing interest The author declares that they have no competing interests.

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