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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis and preliminary evaluation of some novel [1,4]diazepino [5,6-*b*]pyrrolizine and 6-(2-oxopyrrolidino)-*1H*-pyrrolizine derivatives as anticonvulsant agents

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Abstract New series of diazepino[5,6-*b*]pyrrolizines **7a–c** and **8a–c** and 6-(2-oxopyrrolidino)-1*H*-pyrrolizines **10a–c** were synthesized through acylation of the key aminonitrile derivatives **5a–c** (Scheme 1) with the appropriate acid chlorides. Subsequent cyclization reaction yielded the target compounds (Schemes 2, 3). The chemical structure of the synthesized compounds was elucidated by spectral and elemental analyses. The synthesized compounds were evaluated for their ability to protect mice against PTZ-induced seizures, the most active compounds were **10a–c** where compound **10b** exhibited 67.9% relative potency compared to phenobarbitone and compound **10a** provided the maximum protection % of all compounds (60%) at dose of 50 mg/kg comparable to phenobarbitone at a dose of 20 mg/kg.

Keywords [1,4]Diazepino[5,6-*b*]pyrrolizines · 6-(2-Oxopyrrolidino)-1*H*-pyrrolizines · Anticonvulsant activity

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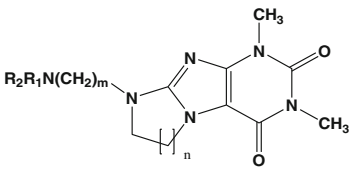
Introduction

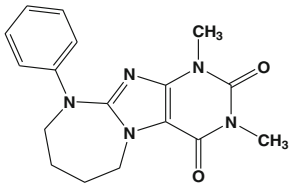
Epilepsy is a common neurological condition, affecting 0.5–1% of the population worldwide. Till now, the known anticonvulsant drugs are effective in only 60–70% of patients and therefore there is a continuous need to develop improved agents with higher activity profile (Rang *et al.*, 2003).

Review of several potent anticonvulsant agents revealed that they possess a tricyclic nitrogen bridgehead heterocyclic skeleton as exemplified by compounds **I–III** (Bruno-Blanch *et al.*, 2003; Drabczyńska *et al.* 2006; Estrada and Pena, 2000; Pawlowski *et al.*, 2002). Also, the fused [1,4]diazepine derivatives **IV** (Vega' *et al.*, 1994) and **V** (Narayana *et al.*, 2006) exhibited both anticonvulsant and sedative activities.

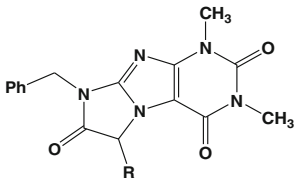


I

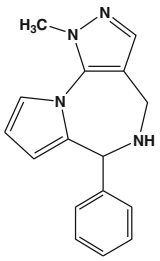
n = 1,2,3
R_1R_2=alkyl,cycloalkyl, substituted piperazine or piperidine
m=2-4



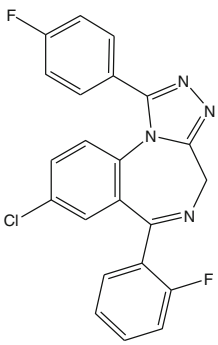
II



III R = H, CH_3

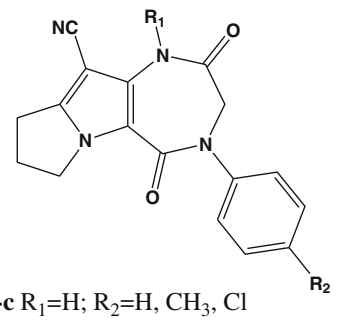


IV



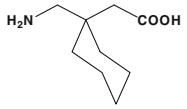
V

Depending on these findings the authors aimed to synthesize novel tricyclic diazepinopyrrolizine derivatives **7a–c** and **8a–c** featuring a 2,5-dioxodiazepine nucleus fused to a pyrrolizine ring, in addition to an (un)substituted phenyl group attached to the diazepine ring at position 4. The influence of the electronic nature of the substituent present on the phenyl ring on biological activity was studied by introducing an electron donating group (R_2=CH_3) or an electron withdrawing group (R_2=Cl) in comparison to the unsubstituted derivative (R_2=H).

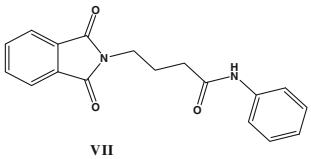


7a-c R_1=H; R_2=H, CH_3, Cl
8a-c R_1=CH_3; R_2=H, CH_3, Cl

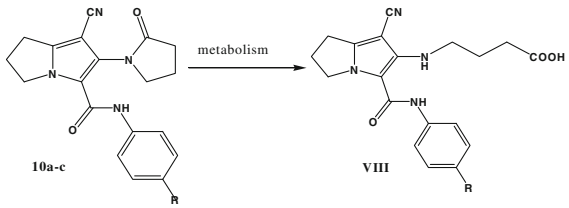
Compounds bearing γ-aminobutyric acid moiety such as gabapentin **VI** or a substituted γ-aminobutyric acid such as the phthalimido compound **VII** (Ragavendran *et al.*, 2007) were found to be potent anticonvulsant agents. Moreover, compounds carrying a 2-oxopyrrolidino function were considered as prodrugs for N-substituted γ-aminobutyric acids and were also found to possess good anticonvulsant activity (Fasolato *et al.*, 1988). In the light of these facts, a second group of compounds, the 2-oxopyrrolidinopyrrolizine derivatives **10a–c**, were designed as prodrugs that were expected to afford N-substituted γ-aminobutyric acid derivatives **VIII** in vivo.



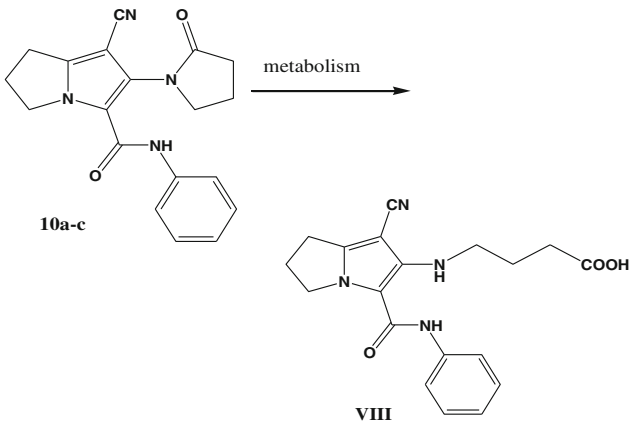
VI



VII



10a-c **VIII**

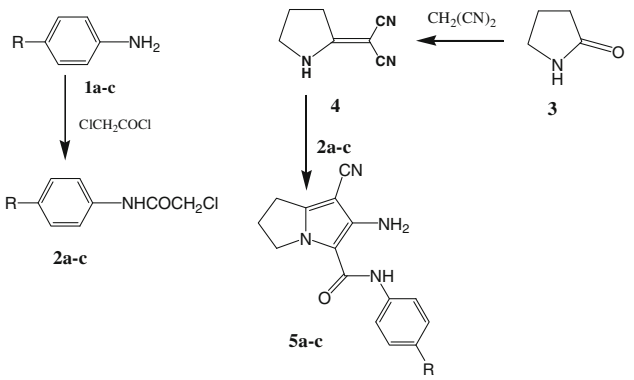


10a-c **VIII**

Results and discussion

Chemistry

Previously reported procedures were used in the preparation of the substituted acetanilides **2a–c** (El-Moghazy, 1992) and 2-pyrrolidin-2-ylidene-malononitrile **4** (Etienne and Correia, 1969; Ebeid and Bitter, 1978; Schlack and Rieker, 1971). The required starting compounds, 6-amino-7-cyano-*N*-[4-(un)substitutedphenyl]-2,3-dihydro-1*H*-pyrrolizine-5-carboxamides **5a–c**, were prepared following the reported method (Ebeid *et al.*, 1997) (Scheme 1).



Scheme 1 Preparation of compounds **5a–c**

6-Chloroacetamido-7-cyano-*N*-(4-(un)substitutedphenyl)-2,3-dihydro-1*H*-pyrrolizine-5-carboxamides **6a–c** were synthesized from the reaction of compounds **5a–c** with chloroacetyl chloride according to the reported procedure (Schlack and Rieker, 1971). The target [1,4]diazepino[5,6-*b*]pyrrolizine-10-carbonitrile compounds **7a–c** were obtained through intramolecular cyclization of **6a–c** in DMF in the presence of anhydrous potassium carbonate. The proposed structures of compounds **7a–c** were confirmed by spectral data. The obtained products **7a–c** underwent methylation using methyl iodide in DMF in the presence of potassium carbonate, yielding the 1-methyl analogues **8a–c**. Methylation was confirmed by the IR spectra which revealed the disappearance of the NH stretching vibration bands, in addition to the ^{1}H-NMR spectra which showed the characteristic singlet signal of the CH_3 group (Scheme 2).

The target 2-oxopyrrolidinopyrrolizine derivatives **10a–c** were prepared as depicted in Scheme 3. The aminonitrile compounds **5a–c** were reacted with chlorobutyryl chloride to furnish the intermediate chlorobutyrylamino derivatives **9a–c**. Evidence for acylation of the amino group was drawn from the ^{1}H-NMR spectra of compounds **9a–c** which revealed additional signals characteristic to the side chain methylene groups. Intramolecular cyclization of compounds **9a–c** in refluxing acetone in the presence of potassium carbonate gave derivatives **10a–c**. The proposed structures for compounds **10a–c** was confirmed by spectral analyses in addition to the single X-ray crystallographic data for compound **10a** (Fig. 1).

All newly synthesized compounds were characterized by spectral and elemental analyses.

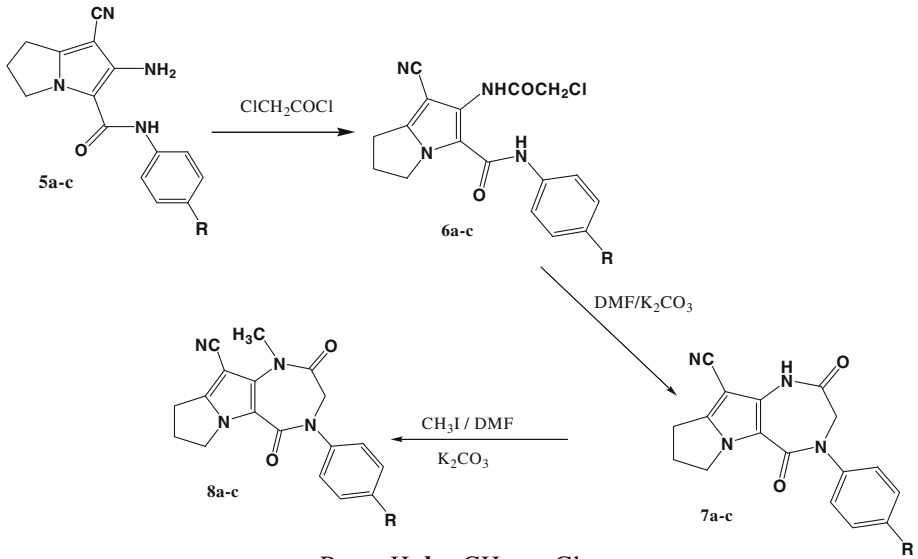
Anticonvulsant activity

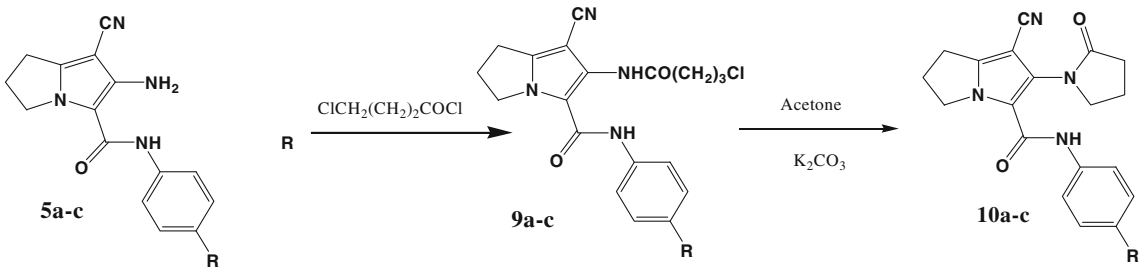
All new compounds were tested for their anticonvulsant activity against PTZ-induced convulsion (Turner, 1964). The intermediate compounds **6**, **9a–c** were also evaluated for their anticonvulsant activity. The relationship between the drug concentrations and the percentage of protection as a dependent variable was used for prediction of the concentrations of the drug that cause protection of 50% of the animals [PD_{50}] (Table 1). Relative potency % of tested compounds relative to phenobarbitone were calculated and included in Table 1 and illustrated in Fig. 2.

The results of anticonvulsant screening showed that all tested compounds possessed weak to moderate activity compared to phenobarbitone.

The nor-pyrrolizinodiazepine derivatives **7a–c** exhibited weak activity that slightly increased with the N-methyl derivatives **8a–c**. The uncyclized chloroacetylamino derivatives **6a–c** and the chlorobutyryl amino compounds **9a–c** showed activity higher than the pyrrolizinodiazepine

Scheme 2 Preparation of compounds **7a–c** and **8a–c**





R: a= H, b=CH_3, c= Cl

Scheme 3 Preparation of compounds **10a–c**

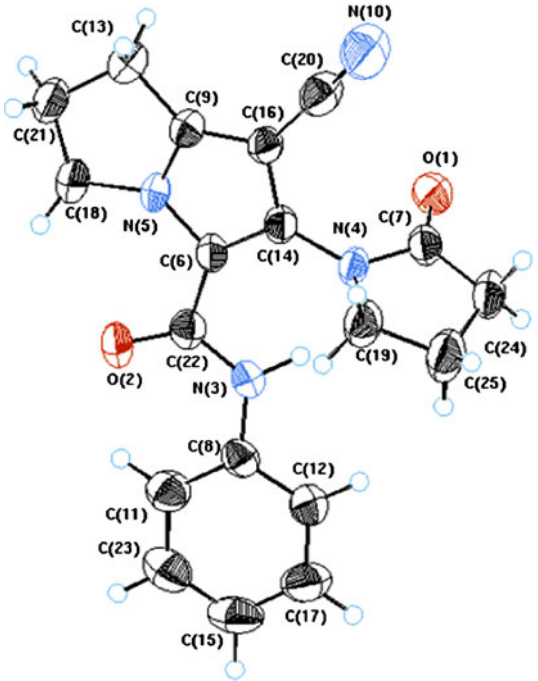


Fig. 1 ORTEP preview of compound **10a**

derivatives **7a–c** and **8a–c**. The most active compounds were the 2-oxopyrrolidinopyrrolizines **10a–c** especially compound **10b** which exhibited 67.9% relative potency and compound **10c** which showed 55.8% relative potency compared to phenobarbitone. It could also be observed that among all compounds, **10a** produced the highest protection (60%) at a dose of 50 mg/kg, which is equipotent to phenobarbitone at a dose of 20 mg/kg (Table 1). The puzzling inverse relation between dose and percentage protection for compounds **10a–c** is to be noted (Table 1). LD_{50} for compound **10b** is 1011.58 mg/kg compared to 251.19 mg/kg for phenobarbitone indicating a higher safety margin (Tables 2, 3).

In conclusion, it was found that pyrrolizine derivatives having an open chain substituent at position 6 as in compounds **6a–c** and **9a–c** or having a group that might be metabolized into an open chain as in compounds **10a–c** are more potent than the cyclized pyrrolizinodiazepines.

Experimental

Chemistry

Melting points were uncorrected and were carried out by open capillary tube method using IA 9100MK-Digital Melting Point Apparatus. Elemental microanalyses were carried out at the microanalytical Center, Faculty of Science, Cairo University. Infrared spectra were made on BRUKER Vector 22 (Japan) infrared spectrophotometers and were expressed in wavenumber (cm^{-1}) using potassium bromide disc. The proton magnetic resonance ^{1}H-NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer at 300 MHz and BRUKER APX400 spectrometer at 400 MHz in the specified solvent using tetramethylsilane (TMS) as an internal standard. Chemical shifts were reported on the δ scale and were related to that of the solvent and J values are given in Hz. ^{13}C NMR spectra were obtained on a Bruker APX400 at 100 MHz. Mass spectra were recorded on Fennigan MAT, SSQ 7000, Mass spectrometer, at 70 eV (EI).

*2,5-Dioxo-4-(un)substituted-phenyl-1,2,3,4,5,7,8,9-octahydro[1,4]diazepino[5,6-b]pyrrolizine-10-carbonitrile (**7a–c**)*

A mixture of the appropriate chloroacetamido derivative **6a–c** (3.75 mmol), and powdered anhydrous potassium carbonate (0.52 g, 3.75 mmol) in dry DMF (10 ml) was stirred at room temperature for 48 h. The reaction mixture was poured over ice-cooled water; the precipitate was filtered, washed with water and recrystallized from ethanol–acetone where glassy prismatic crystals were separated.

*2,5-Dioxo-4-phenyl-1,2,3,4,5,7,8,9-octahydro[1,4]diazepino[5,6-b]pyrrolizine-10-carbonitrile (**7a**)* Glassy prismatic crystals, yield 64%; m.p. 255–258°C; IR (KBr, cm^{-1}): 3281 (NH), 3090 (C–H aromatic), 3006, 2958 (CH_2), 2230 (CN), 1677, 1652 (COs), 1606, 1566, 1530 (C=C, NH). ^{1}H-NMR (300 MHz, DMSO-d_6: δ, ppm): 2.50

Table 1 % Protection, PD_{50} and relative potency % of tested compounds

Comp.	Dose (mg/kg)	Protection (%)	PD_{50} mg(mmol)/kg	Relative potency %
6a	50	0	112.5 (328.2)	17.3
	75	20		
	100	40		
6b	50	20	116.67 (326.98)	17.4
	75	40		
	100	40		
6c	50	0	88.89 (235.65)	24.1
	75	40		
	100	60		
7a	50	20	116.67 (380.88)	14.9
	75	40		
	100	40		
7b	50	0	166.68 (520.31)	10.9
	75	20		
	100	20		
7c	50	0	183.33 (538)	10.6
	75	0		
	100	20		
8a	50	0	112.5 (351.18)	16.2
	75	20		
	100	40		
8b	50	0	166.67 (498.46)	11.4
	75	20		
	100	20		
8c	50	20	133.33 (375.79)	15.1
	75	20		
	100	40		
9a	50	0	88.89 (239.71)	23.7
	75	40		
	100	60		
9b	50	0	112.5 (292.31)	19.4
	75	20		
	100	40		
9c	50	20	116.67 (287.88)	19.7
	75	40		
	100	40		
10a	50	60	55.56 (166.16)	34.2
	75	20		
	100	0		
10b	50	40	29.17 (83.72)	67.9
	75	0		
	100	0		
10c	50	40	37.5 (101.78)	55.8
	75	20		
	100	0		
Phenob.	10	40	14.44 (56.8)	100
	20	60		
	30	100		

Relative potency % = [PD_{50} of Phenob./PD_{50} of tested compounds (mmol)] × 100

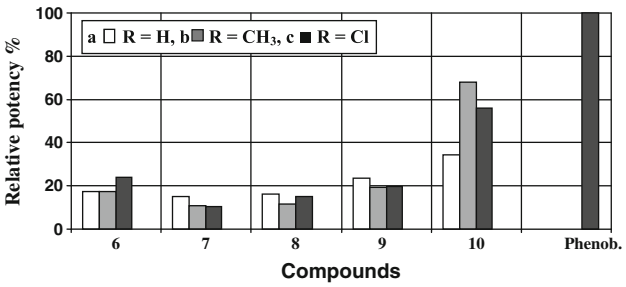


Fig. 2 Relative potency % of tested compounds to phenobarbitone. *White bar* R=H, *grey bar* R=CH_3, *black bar* R=Cl

Table 2 LD_{50} of compound **10b**

Dose (mg/kg)	Log dose	Number of dead animals
300	2.47	0
600	2.77	1
1200	3.07	5
2400	3.38	8

Table 3 LD_{50} of phenobarbitone

Dose (mg/kg)	Log dose	Number of dead animals
100	2.0	0
160	2.2	1
250	2.4	3
400	2.6	8

(m, 2H, CH_2-8), 3.00 (t, 2H, $J = 7.5$ Hz, CH_2-9), 4.24 (t, 2H, $J = 7.2$ Hz, CH_2-7), 4.31 (s, 2H, CH_2-3), 7.25–7.45 (m, 5H, 5 aromatic protons) and 10.99 (s, H, NH, which disappeared on deuteration), ^{13}C-NMR (DMSO-d_6): 24.86 (C-9), 25.61 (C-8), 49.40 (C-7), 54.98 (C-3), 78.01 (C-10), 113.19 (CN), 114.58 (C-5a), 125.87 (C-2′, -6′), 126.84 (C-4′), 129.38 (C-3′, -5′), 132.96 (C-9a), 142.77 (C-10a), 148.93 (C-1′), 159.97 (O=C–N), 167.96 (CONH). MS: *m/z* (%) 306 (M^+, 52). Anal. Calc. for $C_{17}H_{14}N_4O_2$ (306.32): C, 66.66; H, 4.61, N, 18.29; found: C, 66.48; H, 4.57; N, 18.55%.

*2,5-Dioxo-4-(4-tolyl)-1,2,3,4,5,7,8,9-octahydro[1,4]diazepino[5,6-b]pyrrolizine-10-carbonitrile (**7b**)* Glassy prismatic crystals, yield 71%; m.p. 282–284°C; IR (KBr, cm^{-1}): 3273 (NH), 3072 (C–H aromatic), 2998, 2949, 2868 (CH_3 CH_2), 2221 (CN), 1675, 1647 (COs), 1606, 1566, 1513 (C=C, NH). ^{1}H-NMR (400 MHz, DMSO-d_6: δ, ppm): 2.31 (s, 3H, CH_3), 2.47 (m, 2H, CH_2-8), 3.00 (t, 2H, $J = 7.5$ Hz, CH_2-9), 4.23 (t, 2H, $J = 7.2$ Hz, CH_2-7), 4.27 (s, 2H, CH_2-3), 7.22 and 7.28 (2 d, 4H, $J = 8.4$, 4 aromatic protons of the para substituted phenyl ring) and 10.98 (s, 1H, NH, which disappeared on deuteration), Anal. Calc. for $C_{18}H_{16}N_4O_2$ (320.35): C, 67.49; H, 5.03; N, 17.49; found: C, 67.38; H, 4.89; N, 17.21%.

*2,5-Dioxo-4-(4-chlorophenyl)-1,2,3,4,5,7,8,9-octahydro[1,4]diazepino[5,6-b]pyrrolizine-10-carbonitrile (**7c**)* Glassy crystals, yield 62%; m.p. 296–298°C; IR (KBr, cm^{-1}): 3270 (NH), 3065 (C–H aromatic), 2998, 2950 (CH_2), 2226 (CN), 1675, 1651 (COs), 1606, 1570, 1547 (C=C, NH). ^{1}H-NMR (300 MHz, DMSO-d_6: δ, ppm): 2.48 (m, 2H, CH_2-8), 2.99 (t, 2H, $J = 7.5$ Hz, CH_2-9), 4.28 (t, 2H, $J =$ 7.2 Hz, CH_2-7), 4.35 (s, 2H, CH_2-3), 7.14 and 7.51 (2d, 4H, $J = 8.4$, 4 aromatic protons) and 10.99 (s, 1H, NH, which disappeared on deuteration), Anal. Calc. for $C_{17}H_{13}ClN_4O_2$ (340.76): C, 59.92; H, 3.85; N, 16.44; found: C, 60.13; H, 4.34; N, 16.72%.

*1-Methyl-2,5-dioxo-4-(un)substituted-phenyl-1,2,3,4,5,7,8,9-octahydro[1,4]diazepino[5,6-b]pyrrolizine-10-carbonitrile (**8a–c**)*

A mixture of the appropriate compound **7a–c** (3.75 mmol), methyl iodide (1.07 g, 7.5 mmol) and powdered anhydrous potassium carbonate (0.52 g, 3.75 mmol), in dry DMF (10 ml), was stirred for 48 h at room temperature. The reaction mixture was poured over ice-cooled water, filtered, washed with water, dried and recrystallized from ethanol–acetone.

*1-Methyl-2,5-dioxo-4-phenyl-1,2,3,4,5,7,8,9-octahydro[1,4]diazepino[5,6-b]pyrrolizine-10-carbonitrile (**8a**)* White needle crystals, yield 82%; m.p. 209–211°C; IR (KBr, cm^{-1}): 3068 (C–H aromatic), 3004, 2942, 2849 (CH_3, CH_2), 2221 (CN), 1682, 1651 (COs), 1594, 1551 (C=C, NH). ^{1}H-NMR (400 MHz, DMSO-d_6: δ, ppm): 2.47 (m, 2H, CH_2-8), 3.03 (t, 2H, $J = 7.5$ Hz, CH_2-9), 3.44 (s, 3H, CH_3N), 4.24-4.40 (broad m, 4H, CH_2-3 + CH_2-7), 7.27–7.42 (m, 5H, 5 aromatic protons), ^{13}C-NMR (DMSO-d_6): 25.09 (C-9), 25.45 (C-8), 34.40 (NCH_3), 49.38 (C-7), 54.85 (C-3), 79.39 (C-10), 113.93 (CN), 114.87 (C-5), 125.33 (C-2′, -6′), 127.08 (C-4′), 129.15 (C-3′, -5′), 135.76 (C-9a), 141.59 (C-10a), 148.46 (C-1′), 159.63 (C-5), 166.27 (C-2). MS: *m/z* (%) 320 (M^+, 23). Anal. Calc. for $C_{18}H_{16}N_4O_2$ (320.35): C, 67.49; H, 5.03, N, 17.49; found: C, 67.53; H, 5.58; N, 17.29%.

*1-Methyl-2,5-dioxo-4-(4-tolyl)-1,2,3,4,5,7,8,9-octahydro[1,4]diazepino[5,6-b] pyrrolizine-10-carbonitrile (**8b**)* White needle crystals, yield 81%; m.p. 211–214°C; IR (KBr, cm^{-1}): 2988, 2940 (CH_3, CH_2), 2216 (CN), 1678, 1650 (COs), 1548, 1514 (C=C, NH). ^{1}H-NMR (400 MHz, DMSO-d_6: δ, ppm): 2.30 (s, 3H, CH_3-Ph), 2.46 (m, 2H, CH_2-8), 3.00 (t, 2H, $J = 7.5$ Hz, CH_2-9), 3.43 (s, 3H, $\underline{CH_3}$N), 4.23 (broad m, 4H, CH_2-3 + CH_2-7), 7.20-7.28 (2d, 4H, 4 aromatic protons). Anal. Calc. for $C_{19}H_{18}N_4O_2$ (334.37): C, 68.25; H, 5.43, N, 16.76; found: C, 68.53; H, 5.32; N, 17.00%.

*1-Methyl-2,5-dioxo-4-(4-chlorophenyl)-1,2,3,4,5,7,8,9-octahydro-[1,4]diazepino [5,6-b]pyrrolizine-10-carbonitrile (**8c**)* White crystals, yield 72%; m.p. 229–231°C; IR (KBr, cm^{-1}): 3076, 3048 (C–H aromatic) 2950, 2902 (CH_3, CH_2), 2221 (CN), 1687, 1652 (COs), 1593, 1548 (C=C, NH). ^{1}H-NMR (300 MHz, DMSO-d_6: δ, ppm): 2.50 (m, 2H, CH_2-8), 3.04 (t, 2H, $J = 7.5$ Hz, CH_2-9), 3.44 (s, 3H, $\underline{CH_3}$N), 4.25 (s, 2H, CH_2-3), 4.41 (t, 2H, CH_2-7), 7.31-7.49 (m, 4H, aromatic protons). Anal. Calc. for $C_{18}H_{15}ClN_4O_2$ (354.79): C, 60.94; H, 4.26; N, 15.79; found: C, 60.31; H, 4.61; N, 15.46%.

*6-(4-Chlorobutyrylamino)-7-cyano-N-(un)substituted-phenyl-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**9a–c**)*

A mixture of the appropriate carboxamide **5a–c** (3.75 mmol) and chlorobutyryl chloride (1.06 g, 7.5 mmol) in dry benzene (20 ml) was stirred for 2 h and left to stand for 48 h at room temperature. The separated product was filtered, washed with water and hot ethanol to give analytically pure product.

*6-(4-Chlorobutyrylamino)-7-cyano-N-phenyl-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**9a**)* White crystals, yield 82%; m.p. 196–199°C; IR (KBr, cm^{-1}): 3279, 3244 (NHs), 3081, 3035 (C–H aromatic), 2961 (CH_2), 2219 (CN), 1661, 1643 (COs), 1601, 1552 (C=C, NH). ^{1}H-NMR (300 MHz, DMSO-d_6: δ, ppm): 2.04 (m, 2H, $COCH_2\underline{CH_2}$-), 2.43 (t, 2H, $J = 7.2$ Hz, $CO\underline{CH_2}$), 2.52 (m, 2H, CH_2-2), 2.99 (t, 2H, $J = 7.5$ Hz, CH_2-1), 3.68 (t, 2H, $J = 6.6$ Hz, $\underline{CH_2}Cl$), 4.27 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.07–7.61 (m, 5H, 5 aromatic protons), 9.48 (s, 1H, $\underline{NH}COCH_2$) and 10.05 (s, 1H, $\underline{NH}$-Ar) which disappeared on deuteration. MS: *m/z* (%) 370 (M^+, 8). Anal. Calc. for $C_{19}H_{19}ClN_4O_2$ (370.83): C, 61.54; H, 5.16; N, 15.11; found: C, 61.72; H, 4.99; N, 15.35%.

*6-(4-Chlorobutyrylamino)-7-cyano-N-(4-tolyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**9b**)* White crystals, yield 84%; m.p. 203–205°C; IR (KBr, cm^{-1}): 3415, 3247 (NH), 2962, 2921, 2867 (CH_3, CH_2), 2223 (CN), 1666 (COs), 1596, 1564, 1518 (C=C, NH). ^{1}H-NMR (300 MHz, C*D*Cl_3: δ, ppm): 2.24 (m, 2H, $COCH_2\underline{CH_2}$-), 2.33 (s, 3H, CH_3), 2.55 (m, 2H, CH_2-2), 2.68 (t, 2H, $J = 7.2$ Hz, $\underline{COCH_2}$), 3.01 (t, 2H, $J = 7.5$ Hz, CH_2-1), 3.63 (t, 2H, $J = 6.6$ Hz, CH_2Cl), 4.37 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.12–7.42 (2d, 4H, 4 aromatic protons), 7.57 (s, 1H, $\underline{NH}COCH_2$) and 9.34 (s, 1H, $\underline{NH}$-Ar). Anal. Calc. for $C_{20}H_{21}ClN_4O_2$ (384.86): C, 62.42; H, 5.50; N, 14.56; found: C, 62.85; H, 5.17; N, 14.86%.

*6-(4-Chlorobutyrylamino)-7-cyano-N-(4-chlorophenyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**9c**)* White crystals, yield 76%; m.p. 225–226°C; IR (KBr, cm^{-1}): 3410, 3257 (NH), 3065 (C–H aromatic), 2960 (CH_2), 2222 (CN), 1665 (COs), 1595, 1562, 1539 (C=C, NH). ^{1}H-NMR (300 MHz, C*D*Cl_3: δ, ppm): 2.05 (m, 2H, $COCH_2\underline{CH_2}$-), 2.38 (m, 2H, CH_2-2), 2.46 (t, 2H, $J = 7.2$ Hz, $CO\underline{CH_2}$), 2.83 (t, 2H, $J = 7.5$ Hz, CH_2-1), 3.46 (t, 2H, $J = 6.6$ Hz, $\underline{CH_2}Cl$), 4.18 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.08-7.38 (2d, 4H, $J = 8.7$ Hz, aromatic protons), 9.48 and 9.91 (2 s, 2H, two NHCO which disappeared on deuteration. Anal. Calc. for $C_{19}H_{18}Cl_2N_4O_2$ (405.28): C, 56.31; H, 4.48; N, 13.82; found: C, 56.52; H, 4.34; N, 13.89%.

*7-Cyano-6-(2-oxopyrrolidino)-N-(un)substituted-phenyl-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**10a–c**)*

A mixture of the appropriate compound **9a–c** (3.75 mmol) and anhydrous potassium carbonate (0.52 g, 3.75 mmol) in dry acetone (30 ml) was refluxed for 4 h, filtered while hot and set a side to cool where a white crystalline product was formed, collected, dried and recrystallized from ethanol–acetone.

*7-Cyano-6-(2-oxopyrrolidino)-N-phenyl-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**10a**)* Glassy cubic crystals, yield 63%; m.p. 217–220°C; IR (KBr, cm^{-1}): 3319 (NH), 3057 (C–H aromatic), 2921, 2879 (CH_2), 2223 (CN), 1701, 1661 (COs), 1598, 1536 (C=C, NH). ^{1}H-NMR (300 MHz, DMSO-d_6: δ, ppm): 2.15 (m, 2H, CH_2-4″), 2.50 (m, 4H, CH_2-3″ + CH_2-2), 3.00 (t, 2H, $J = 7.5$ Hz, CH_2-1), 3.85 (t, 2H, $J = 6.9$ Hz, CH-5″), 4.27 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.07–7.59 (m, 5H, 5 aromatic protons), 9.59 (s, 1H, NH, which disappeared on deuteration), ^{13}C-NMR (DMSO-d_6: δ, ppm): 18.93 (C-4″), 25.03 (C-1), 25.65 (C-2), 31.38 (C-3″), 49.35 (C-5″), 51.52 (C-3), 83.75 (C-7), 114.40 (CN), 119.47 (C-2′, -6′), 120.94 (C-5), 124.32 (C-4′), 127.00 (C-7a), 129.08 (C-3′, -5′), 138.14 (C-6), 146.41 (C-1′), 157.38 (CONH), 177.55 (O=C–N). MS: *m*/*z* (%) 334 (M^+, 100). Anal. Calc. for $C_{19}H_{18}N_4O_2$ (334.37): C, 68.25; H, 5.43; N, 16.76; found: C, 68.50; H, 5.20; N, 16.58%.

*7-Cyano-6-(2-oxopyrrolidino)-N-(4-tolyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**10b**)* Glassy cubic crystals, yield 68%; m.p. 225–228°C; IR (KBr, cm^{-1}): 3229 (NH), 3050 (C–H aromatic), 3003, 2974, 2910 (CH_3, CH_2), 2217 (CN), 1687, 1656 (COs), 1606, 1548, 1510 (C=C, NH). ^{1}H-NMR (400 MHz, DMSO-d_6: δ, ppm): 2.13 (m, 2H, CH_2-4″), 2.27 (s, 3H, CH_3) 2.48 (m, 4H, CH_2-3″ + CH_2-2), 3.00 (t, 2H, $J = 7.5$ Hz, CH_2-1), 3.84 (t, 2H, $J = 6.9$ Hz, CH-5″), 4.26 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.14 and 7.46 (2d, 4H, $J = 8.4$, 4 aromatic protons), 9.50 (s, 1H, NH, which disappeared on deuteration). Anal. Calc. for $C_{20}H_{20}N_4O_2$ (348.40): C, 68.95; H, 5.79, N, 16.08; found: C, 69.15; H, 5.50; N, 16.20%.

*7-Cyano-6-(2-oxopyrrolidino)-N-(4-chlorophenyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**10c**)* Glassy crystals, yield 65%; m.p. 239–241°C; IR (KBr, cm^{-1}): 3226 (NH), 3099 (C–H aromatic), 2974, 2906 (CH_2), 2222 (CN), 1689, 1656 (COs), 1598, 1532 (C=C, NH). ^{1}H-NMR (400 MHz, DMSO-d_6: δ, ppm): 2.13 (m, 2H, CH_2-4″), 2.45 (m, 4H, CH_2-3″ + CH_2-2), 3.00 (t, 2H, $J = 7.5$ Hz, CH_2-1), 3.85 (t, 2H, $J = 6.9$ Hz, CH-5″), 4.26 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.39 and 7.61 (2d, 4H, $J = 8.7$, 4 aromatic protons), 9.73 (s, 1H, NH, disappeared on deuteration). Anal. Calc. for $C_{19}H_{17}ClN_4O_2$ (368.82): C, 61.87; H, 4.65, N, 15.19; found: C, 62.09; H, 4.79; N, 14.89%.

Single X-ray crystallography

Compound **10a** was recrystallized as colourless cubic crystals from acetone. The crystallographic data were collected at $T = 298$ K on a Kappa CCD Enraf–Nonius FR 590 diffractometer using a graphite monochromator with Mo K_α radiation ($\lambda = 0.71073$ Å). The crystal structure was determined by SIR92 (Altomare *et al.*, 1994) and refined by maXus (Bruker Nonius, Delft and MacScience, Japan). Chemical formula $C_{19}H_{18}N_4O_2$, $M_r = 334.379$, triclinic, crystallizes in space group $P2_1/C$, Cell lengths "$a = 8.1313$ (6), $b = 10.7242$ (7), $c = 10.7657$ (7) Å", Cell angles "$\alpha = 116.893$ (7)°, $\beta = 92.060$ (4)°, $\gamma = 90.296$ (3)°", $V = 836.47$ (10) Å^3, $Z = 2$, $D_c = 1.328$ mg/m^3, θ values 2.910°–27.485°, absorption coefficient μ (Mo K_α) =0.09 mm^{-1}, $F(000) = 720$. The unique reflections measured 3899 of which 1202 reflections with threshold expression $I > 3\sigma(I)$ were used in the structural analysis. Convergence for 226 variable parameters by least-squares refinement on F^2 with $w = 1/[\sigma^2(F_o^2) + 0.10000\ F_o^2]$. The final agreement factors were

$R = 0.051$ and $wR = 0.118$ with a goodness-of-fit of 1.248.

Anticonvulsant activity

Fifteen compounds, **6a–c, 7a–c**, **8a–c**, **9a–c** and **10a–c**, were tested for their anticonvulsant activity by measuring their protective effect against PTZ-induced convulsions in mice (Turner, 1964).

Procedure

Swiss albino male mice weighing 20–25 g, were randomly arranged in groups of 5. The animals were acclimated to their environment for at least 2 days before the experiments and were allowed for free access to food and water before being tested. Room temperature was kept at 23 ± 2°C and animals were fed standard laboratory chow and tap water ad libitum. The test compounds were suspended in Tween 80 (0.2%) (Sigma, USA) and were given i.p. in doses of 50, 75 and 100 mg kg^{-1} body weight. Dosing volume was 0.2 ml per 20 g and phenobarbitone sodium (sigma) was dissolved in 2% Tween 80 and used as a reference standard, and was given i.p. in doses of 10, 20 and 30 mg kg^{-1}. Pentylenetetrazole (PTZ) 2% in water was given i.p. in a dose of 100 mg kg^{-1} 1 h after the test compound or phenobarbitone sodium. Animals were observed for PTZ threshold convulsion (protection) which is defined as one episode of clonic spasms which persists for at least 5 s duration during a 1 h period following administration of PTZ. Absence of this threshold convulsion over 1 h indicated that the tested substances had the ability to elevate pentylenetetrazole seizure threshold.

Data were collected, checked, revised and fed in the computer. Excel computer program was used to tabulate the results.

Determination of LD_{50}

Male albino mice weighing 25–30 g were divided into groups each of 8 animals. Preliminary experiments were done to determine the minimal dose that kills all animals (LD_{100}) and the maximal dose that fails to kill any animal. Several doses at equal logarithmic intervals were chosen in between these two doses, each dose was injected into a group of eight animals, the number of dead animals in each group after 24 h was recorded and the LD_{50} was calculated according to *Spearman Karber method* (Finney, 1946; Amin *et al.*, 2008) applying the following formula:

$$M = X_k + {}^1/_2 d - dr/N$$

where $M = \log LD_{50}$, $X_k = \log$ dose causing 100% mortality, $d =$ logarithmic interval of doses, $r =$ sum of the number of dead animals at each of the individual dose levels, and $N =$ number of animals at each of the dose levels.

Data for LD_{50} of compound **10b** and phenobarbitone are presented in Tables 2 and 3.

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