Full Paper

Synthesis and Biological Evaluation of Some Pyrazoline Derivatives Bearing a Dithiocarbamate Moiety as New Cholinesterase Inhibitors

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In the present study, new pyrazoline derivatives were synthesized via the reaction of 1-(chloroacetyl)-3-(2-furyl)-5-aryl-2-pyrazolines with sodium salts of *N*,*N*-disubstituted dithiocarbamic acids. Each derivative was evaluated for its ability to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) using a modification of Ellman's spectrophotometric method. The compounds were also investigated for their cytotoxic properties using the MTT assay. The most potent AChE inhibitor was found as compound **7** followed by compounds **27** and **17**, when compared with eserine. Compounds effective on AChE carry the 2-dimethylaminoethyl moiety, which resembles the trimethylammonium group and the ethylene bridge of acetylcholine. Among all compounds, compound **7** bearing 2-dimethylaminoethyl and 3,4-methylenedioxyphenyl moieties was also found to be the most effective inhibitor of BuChE. The MTT assay indicated that the effective concentration of compound **7** was lower than its cytotoxic concentration.

Keywords: Acetylcholinesterase / Anticholinesterase activity / Butyrylcholinesterase / Dithiocarbamate / Pyrazoline

Received: October 8, 2012; Revised: December 5, 2012; Accepted: December 21, 2012

DOI 10.1002/ardp.201200384

Introduction

Enzymes have attracted a great deal of interest as targets for drug discovery due to their essential roles in life processes and pathophysiology. Inhibition of disease-associated enzymes is a promising approach for pharmacologic intervention in human diseases. Among oral therapeutic agents in clinical use today, nearly half of them exert their therapeutic action by inhibiting specific enzymes. Likewise, current drug discovery efforts in pharmaceutical industry are focused on the identification and optimization of drug candidates that act as specific enzyme inhibitors [1–3].

Cholinesterases have been the subject of intense investigation in medicinal chemistry as important drug targets. Two cholinesterases are present in humans: acetylcholinesterase (AChE), which selectively hydrolyses acetylcholine, and butyrylcholinesterase (BuChE), which hydrolyses acetylcholine and other choline esters as a non-specific cholinesterase. The difference between the two types of cholinesterase is the respective preference for substrates: the former hydrolyses acetylcholine more quickly; whereas the latter hydrolyses butyrylcholine more quickly. The main function of AChE is the termination of cholinergic neurotransmission, but the function of BuChE is not so clear [4–7].

The role of the cholinergic system in cognition and the identification of cholinergic deficits in Alzheimer's disease has led to the development of cholinesterase inhibitors as the first-line treatment for symptoms of this disease [4–11].

Carbamates are the most widely studied class of anticholinesterase agents and considerable research on them in relation to Alzheimer's disease has been accomplished. Rivastigmine possesses a carbamate moiety that resembles the ester linkage of acetylcholine. It is one of the most widely used anticholinesterase agents for the treatment of Alzheimer's disease [5–12].

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Dithiocarbamates are important pharmacophores owing to their lipophilicity, which is crucial for the delivery of central nervous system drugs to their site of action through the blood-brain barrier. Medicinal chemists have studied dithiocarbamates extensively due to the fact that new effective compounds can be obtained by the bioisosteric replacement of a carbamate moiety with a dithiocarbamate moiety [13–20].

Pyrazolines have also received considerable attention as privileged scaffolds due to their synthetic and biological importance in medicinal chemistry. Pyrazoline derivatives have been reported to exhibit a wide spectrum of biological effects including anticholinesterase activity [21–26]. Ucar *et al*. [26] synthesized *N*-substituted pyrazoline derivatives and evaluated these compounds as MAO-B and cholinesterase inhibitors which may have promising features in the treatment of Alzheimer's and Parkinson's diseases.

On the basis of these findings and in the continuation of our ongoing research program in the field of synthesis and biological evaluation of heterocyclic compounds as cholinesterase inhibitors [27, 28], herein we report the synthesis and biological evaluation of some pyrazoline derivatives bearing a dithiocarbamate moiety as new anticholinesterase agents. The compounds were also investigated for their cytotoxic effects.

Results and discussion

The synthesis of pyrazoline derivatives (**1–30**) was carried out according to the steps shown in Scheme 1. In the initial step, 1-(2-furyl)-3-aryl-2-propen-1-ones were synthesized via the base-catalyzed Claisen-Schmidt condensation of 2-acetylfuran with appropriate aldehydes. The ring closure reaction of chalcones with hydrazine hydrate afforded 5-aryl-3-(2-furyl)-2-pyrazolines. 1-(Chloroacetyl)-3-(2-furyl)-5-aryl-2-pyrazolines were obtained by the reaction of 5-aryl-3-(2-furyl)-2-pyrazolines with chloroacetyl chloride in the presence of triethylamine. Sodium salts of *N*,*N*-disubstituted dithiocarbamic acids were prepared by the reaction of a secondary amine with carbon disulfide in the presence of sodium hydroxide.

The reaction of 1-(chloroacetyl)-3-(2-furyl)-5-aryl-2-pyrazolines with sodium salts of *N*,*N*-disubstituted dithiocarbamic acids afforded 1-[(*N*,*N*-disubstituted thiocarbamoylthio)-acetyl]-3-(2-furyl)-5-aryl-2-pyrazoline derivatives (**1–30**). Some properties of the compounds were given in Table 1.

The structures of all compounds (**1–30**) were confirmed by IR, ^{1}H NMR, mass spectral data, and elemental analyses. In the IR spectra of all compounds (**1–30**), all derivatives have a strong, characteristic band in the region 1684–1654 cm^{-1} due to C=O stretching vibration.

In the 500 MHz ^{1}H NMR spectra of the compounds (**1–30**), the CH_2 protons of the pyrazoline ring resonated as a pair of doublets of doublets at δ 3.00–3.25 ppm and 3.76–3.82 ppm. The CH proton appeared as doublet of doublets at δ 5.45–5.49 ppm due to the vicinal coupling with two magnetically non-equivalent protons of the methylene group at position 4 of the pyrazoline ring. The CH_2 protons of the acetyl group were observed at 4.54–4.76 ppm as double doublets. This geminal coupling resulted from the steric structure of the compound. These geminal protons were observed as double doublet due to two different possible conformations since rigid protons occurred. All the other aromatic and aliphatic protons were observed in the expected regions.

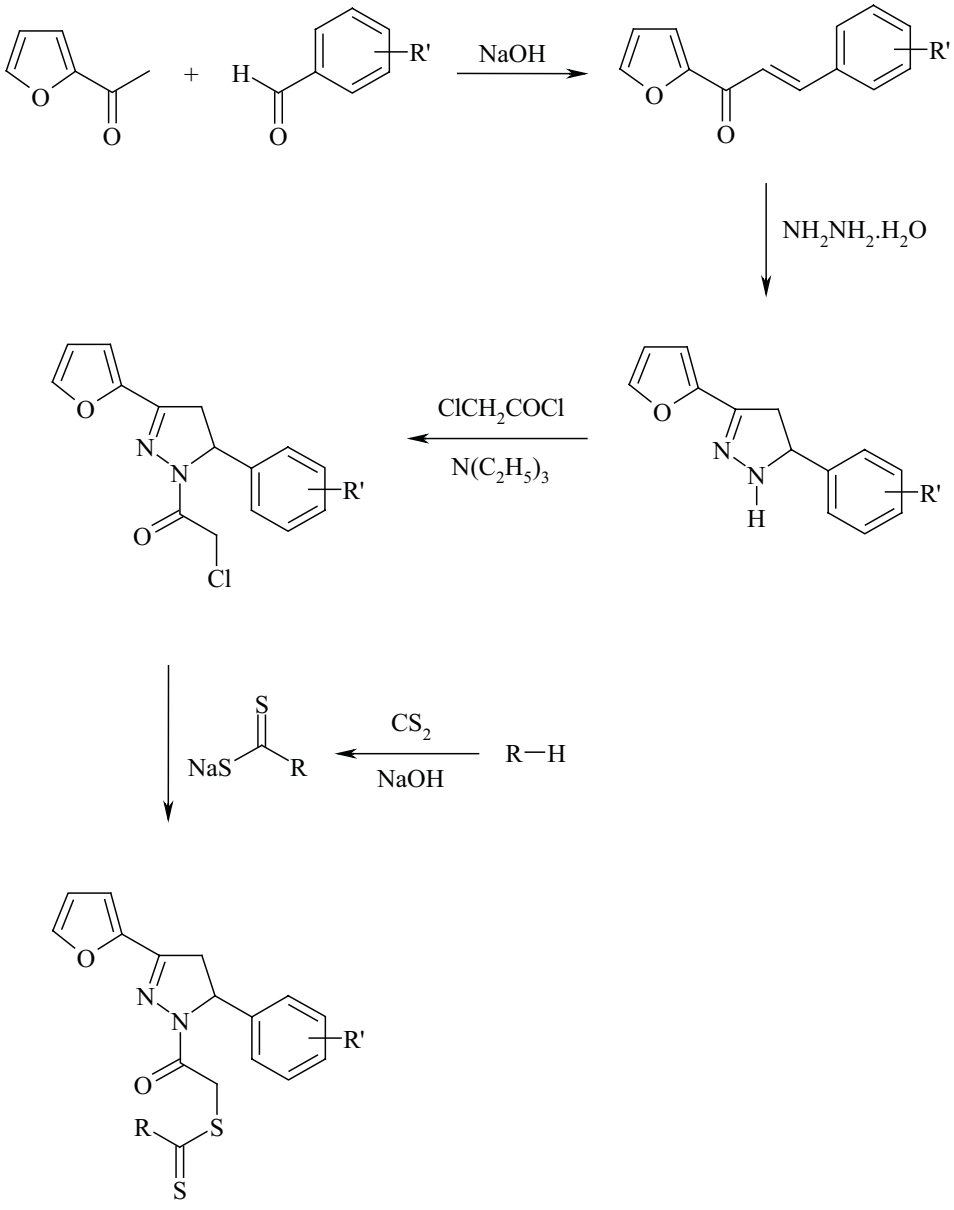
In the mass spectra of the compounds (**1–30**), the M+1 peak was observed. All compounds gave satisfactory elemental analysis results.

The anticholinesterase effects of the compounds (**1–30**) on AChE and BuChE were determined by a modification of Ellman's spectrophotometric method (Table 2).

Among these compounds (**1–30**), compound **7** can be identified as the most promising anticholinesterase agent due to its inhibitory effect on AChE with an IC_{50} value of 0.72 ± 0.06 μg/mL when compared with eserine ($IC_{50} = 0.0013 \pm 0.0001$ μg/mL) (Fig. 1). Compounds **27** and **17** exhibited AChE inhibitory activity with IC_{50} values of 2.32 ± 0.76 μg/mL and 7.2 ± 1.04 μg/mL, respectively. Effective compounds on AChE carry the 2-dimethylaminoethyl moiety. It is apparent that there is a positive correlation between AChE inhibitory activity and the 2-dimethylaminoethyl moiety. It can be attributed to the similarity of the 2-dimethylaminoethyl group and the trimethylammonium and the ethylene groups of acetylcholine (Scheme 2). Compounds **5**, **1**, and **6** exhibited AChE inhibitory activity with IC_{50} values of 48 ± 5.64 μg/mL, 50.68 ± 2.72 μg/mL, and 62 ± 8.48 μg/mL, respectively. Compounds **14** and **25** were inactive against AChE, whereas other derivatives showed weak inhibition on AChE ($IC_{50} > 80$ μg/mL).

Compound **7** also exhibited the highest inhibitory effect on BuChE with an IC_{50} value of 7.46 ± 0.83 μg/mL when compared with eserine ($IC_{50} = 0.012 \pm 0.005$ μg/mL; Fig. 2). Compound **27** exhibited BuChE inhibitory activity with an IC_{50} value of 26.93 ± 2.54 μg/mL. Although compound **17** carries the 2-dimethylaminoethyl group, it exhibits weak inhibition on BuChE ($IC_{50} > 80$ μg/mL). This outcome confirms that functional groups at the fifth position of the pyrazoline ring may have a considerable influence on BuChE inhibitory activity. Compounds **1**, **6**, and **5** exhibited BuChE inhibitory activity with IC_{50} values of 36 ± 5.66 μg/mL, 57.33 ± 2.31 μg/mL, and 65.33 ± 2.31 μg/mL, respectively. Compounds **3**, **4**, **10**, **13**, **14**, **18**, **19**, **21**, **22**, and **28** were inactive against BuChE, whereas other derivatives showed weak inhibition on BuChE ($IC_{50} > 80$ μg/mL).

The compounds were also evaluated for their cytotoxic properties using the MTT assay (Table 3). The biological study indicated that compounds **4**, **8**, and **9** possessed the highest



Scheme 1. The synthesis of the compounds (**1–30**).

cytotoxicity with an IC_{50} value of <7.8 μg/mL, whereas compound **22** exhibited the lowest cytotoxicity with an IC_{50} value of 206.7 ± 47.3 μg/mL against mouse fibroblast (NIH/3T3) cell lines.

Conclusion

Cholinesterase inhibitors (ChEIs) have attracted a great deal of interest among researchers due to their importance in the first-line treatment for symptoms of Alzheimer's disease.

In the present paper, we synthesized a series of pyrazoline derivatives bearing a dithiocarbamate moiety and evaluated their anticholinesterase effects and cytotoxicity.

The biological results indicate that compound **7** is the most potent inhibitor of AChE and BuChE with IC_{50} values of 0.72 ± 0.06 μg/mL and 7.46 ± 0.83 μg/mL, respectively. This outcome confirms that the 2-dimethylaminoethyl and 3,4-methylenedioxyphenyl moieties may have a considerable influence on the anticholinesterase activity. In addition, the cytotoxic concentration (IC_{50} = 9.2 ±

Table 1. Some properties of the compounds (**1**–**30**).

Compound	R	R′	Yield (%)	m.p. (°C)	Molecular formula	Molecular weight
1	4-Ethylpiperazin-1-yl	3,4-Methylenedioxy	78	82	$C_{23}H_{26}N_4O_4S_2$	486.61
2	Morpholin-4-yl	3,4-Methylenedioxy	77	165	$C_{21}H_{21}N_3O_5S_2$	459.54
3	4-(4-Methoxyphenyl)piperazin-1-yl	3,4-Methylenedioxy	48	47	$C_{28}H_{28}N_4O_5S_2$	564.68
4	4-Benzylpiperazin-1-yl	3,4-Methylenedioxy	75	98	$C_{28}H_{28}N_4O_4S_2$	548.68
5	4-Methylpiperazin-1-yl	3,4-Methylenedioxy	86	118	$C_{22}H_{24}N_4O_4S_2$	472.58
6	Thiomorpholin-4-yl	3,4-Methylenedioxy	88	85	$C_{21}H_{21}N_3O_4S_3$	475.60
7	4-(2-Dimethylaminoethyl)piperazin-1-yl	3,4-Methylenedioxy	69	63	$C_{25}H_{31}N_5O_4S_2$	529.67
8	4-Phenylpiperazin-1-yl	3,4-Methylenedioxy	73	95	$C_{27}H_{26}N_4O_4S_2$	534.65
9	Pyrrolidin-1-yl	3,4-Methylenedioxy	91	81	$C_{21}H_{21}N_3O_4S_2$	443.54
10	Piperidin-1-yl	3,4-Methylenedioxy	93	89	$C_{22}H_{23}N_3O_4S_2$	457.57
11	4-Ethylpiperazin-1-yl	4-Methyl	84	86	$C_{23}H_{28}N_4O_2S_2$	456.62
12	Morpholin-4-yl	4-Methyl	77	91	$C_{21}H_{23}N_3O_3S_2$	429.56
13	4-(4-Methoxyphenyl)piperazin-1-yl	4-Methyl	80	67	$C_{28}H_{30}N_4O_3S_2$	534.69
14	4-Benzylpiperazin-1-yl	4-Methyl	87	94	$C_{28}H_{30}N_4O_2S_2$	518.69
15	4-Methylpiperazin-1-yl	4-Methyl	84	106	$C_{22}H_{26}N_4O_2S_2$	442.60
16	Thiomorpholin-4-yl	4-Methyl	92	101	$C_{21}H_{23}N_3O_2S_3$	445.62
17	4-(2-Dimethylaminoethyl)piperazin-1-yl	4-Methyl	90	98	$C_{25}H_{33}N_5O_2S_2$	499.69
18	4-Phenylpiperazin-1-yl	4-Methyl	92	108	$C_{27}H_{28}N_4O_2S_2$	504.67
19	Pyrrolidin-1-yl	4-Methyl	94	102	$C_{21}H_{23}N_3O_2S_2$	413.56
20	Piperidin-1-yl	4-Methyl	93	121	$C_{22}H_{25}N_3O_2S_2$	427.58
21	4-Ethylpiperazin-1-yl	4-Methoxy	52	75	$C_{23}H_{28}N_4O_3S_2$	472.62
22	Morpholin-4-yl	4-Methoxy	78	87	$C_{21}H_{23}N_3O_4S_2$	445.56
23	4-(4-Methoxyphenyl)piperazin-1-yl	4-Methoxy	68	125	$C_{28}H_{30}N_4O_4S_2$	550.69
24	4-Benzylpiperazin-1-yl	4-Methoxy	78	120	$C_{28}H_{30}N_4O_3S_2$	534.69
25	4-Methylpiperazin-1-yl	4-Methoxy	72	132	$C_{22}H_{26}N_4O_3S_2$	458.60
26	Thiomorpholin-4-yl	4-Methoxy	67	55	$C_{21}H_{23}N_3O_3S_3$	461.62
27	4-(2-Dimethylaminoethyl)piperazin-1-yl	4-Methoxy	71	137	$C_{25}H_{33}N_5O_3S_2$	515.69
28	4-Phenylpiperazin-1-yl	4-Methoxy	69	65	$C_{27}H_{28}N_4O_3S_2$	520.67
29	Pyrrolidin-1-yl	4-Methoxy	57	59	$C_{21}H_{23}N_3O_3S_2$	429.56
30	Piperidin-1-yl	4-Methoxy	64	67	$C_{22}H_{25}N_3O_3S_2$	443.58

1.6 μg/mL) of compound **7** is higher than its effective concentration. In the view of this study, further research can be carried out on the development of new effective anticholinesterase agents by the modification of compound **7**.

Experimental

Chemistry

All reagents were purchased from commercial suppliers and were used without further purification. Melting points (m.p.) were determined on a Electrothermal 9100 melting point apparatus (Weiss-Gallenkamp, Loughborough, UK) and were uncorrected. Infrared spectra (IR) were recorded on a Shimadzu 8400 Fourier transform (FT)-IR spectrophotometer (Shimadzu, Tokyo, Japan). Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker a 500 MHz spectrometer (Bruker, Billerica, MA, USA). Chemical shifts were expressed in parts per million (ppm) and tetramethylsilane was used as an internal standard. Mass spectra were recorded on a VG Quattro Mass spectrometer (Agilent, Minnesota, USA). Elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyzer (Perkin-Elmer, Norwalk, CT, USA).

General procedure for the synthesis of the compounds

1-(2-Furyl)-3-aryl-2-propen-1-ones

A mixture of 2-acetylfuran (0.06 mol), aromatic aldehyde (0.06 mol) and 10% aqueous sodium hydroxide (10 mL) in ethanol (30 mL) was stirred at room temperature for about 5 h. The resulting solid was washed, dried, and crystallized from ethanol [29, 30].

5-Aryl-3-(2-furyl)-2-pyrazolines

A mixture of appropriate furyl chalcone (0.03 mol) and 80% hydrazine hydrate (0.06 mol) in ethanol (30 mL) was refluxed for 3 h. The reaction mixture was cooled and kept at 0°C overnight. The resulting solid was recrystallized from ethanol [29, 30].

1-(Chloroacetyl)-3-(2-furyl)-5-aryl-2-pyrazolines

5-Aryl-3-(2-furyl)-2-pyrazolines (0.02 mol) and triethylamine (0.02 mol) were dissolved in dry acetone (30 mL) with constant stirring. Later, the mixture was cooled in an ice bath, and chloroacetyl chloride (0.02 mol) was added dropwise with stirring. The reaction mixture thus obtained was further agitated for 2 h at room temperature. The precipitate was filtered, the solvent was evaporated to dryness under reduced pressure, and the products were recrystallized from ethanol [29].

Table 2. The anticholinesterase activities of the compounds (**1**–**30**) as IC_{50} values (μg/mL).

Compound	AChE % Inhibition (80 μg/mL)	IC_{50} (μg/mL)	BuChE % Inhibition (80 μg/mL)	IC_{50} (μg/mL)
1	96.92 ± 0.92	50.68 ± 2.72	86.39 ± 7.91	36 ± 5.66
2	47.66 ± 2.65	>80	46.04 ± 0.96	>80
3	21.51 ± 3.70	>80	na	nt
4	9.44 ± 1.84	>80	na	nt
5	68.78 ± 3.93	48 ± 5.64	69.97 ± 3.49	65.33 ± 2.31
6	57.69 ± 4.80	62 ± 8.48	69.74 ± 2.49	57.33 ± 2.31
7	96.22 ± 1.65	0.72 ± 0.06	92.41 ± 0.37	7.46 ± 0.83
8	25.45 ± 2.40	>80	43.15 ± 5.91	>80
9	45.30 ± 2.40	>80	23.07 ± 0.63	>80
10	16.04 ± 3.73	>80	na	nt
11	28.01 ± 2.74	>80	9.35 ± 1.84	>80
12	27.30 ± 2.69	>80	19.18 ± 2.25	>80
13	5.60 ± 1.26	>80	na	nt
14	na	nt	na	nt
15	19.49 ± 2.93	>80	25.15 ± 0.16	>80
16	15.76 ± 4.23	>80	33.64 ± 8.17	>80
17	89.74 ± 3.62	7.2 ± 1.04	41.43 ± 2.46	>80
18	14.51 ± 3.05	>80	na	nt
19	15.18 ± 0.32	>80	na	nt
20	29.14 ± 0.83	>80	35.68 ± 5.92	>80
21	31.02 ± 3.49	>80	na	nt
22	9.24 ± 1.05	>80	na	nt
23	15.92 ± 1.69	>80	10.89 ± 1.93	>80
24	20.01 ± 2.74	>80	16.58 ± 3.94	>80
25	na	nt	10.91 ± 1.03	>80
26	28.44 ± 1.79	>80	13.85 ± 0.21	>80
27	90.25 ± 0.78	2.32 ± 0.76	59.76 ± 4.95	26.93 ± 2.54
28	13.36 ± 3.58	>80	na	nt
29	27.30 ± 2.24	>80	13.7 ± 2.23	>80
30	35.15 ± 3.52	>80	36.86 ± 4.50	>80
Eserine	nt	0.0013 ± 0.0001	nt	0.012 ± 0.005

na, not active; nt, not tested.

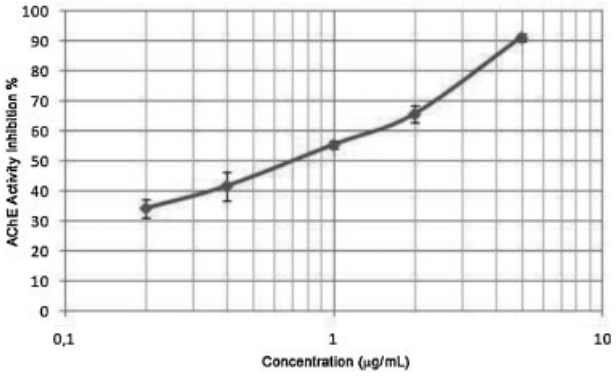


Figure 1. AChE inhibitory activity of compound **7**.

Sodium salts of N,N-disubstituted dithiocarbamic acids

Sodium hydroxide (10 mmol) was dissolved in ethanol (80 mL) with constant stirring. After addition of the secondary amine (10 mmol) the mixture was cooled in an ice bath and carbon disulfide (100 mmol) was added dropwise with stirring. The reaction mixture was stirred for 1 h at room temperature. The solvent was evaporated under reduced pressure and then dry ether was added until precipitation. The products were afforded by filtration and recrystallized from ethanol [20].

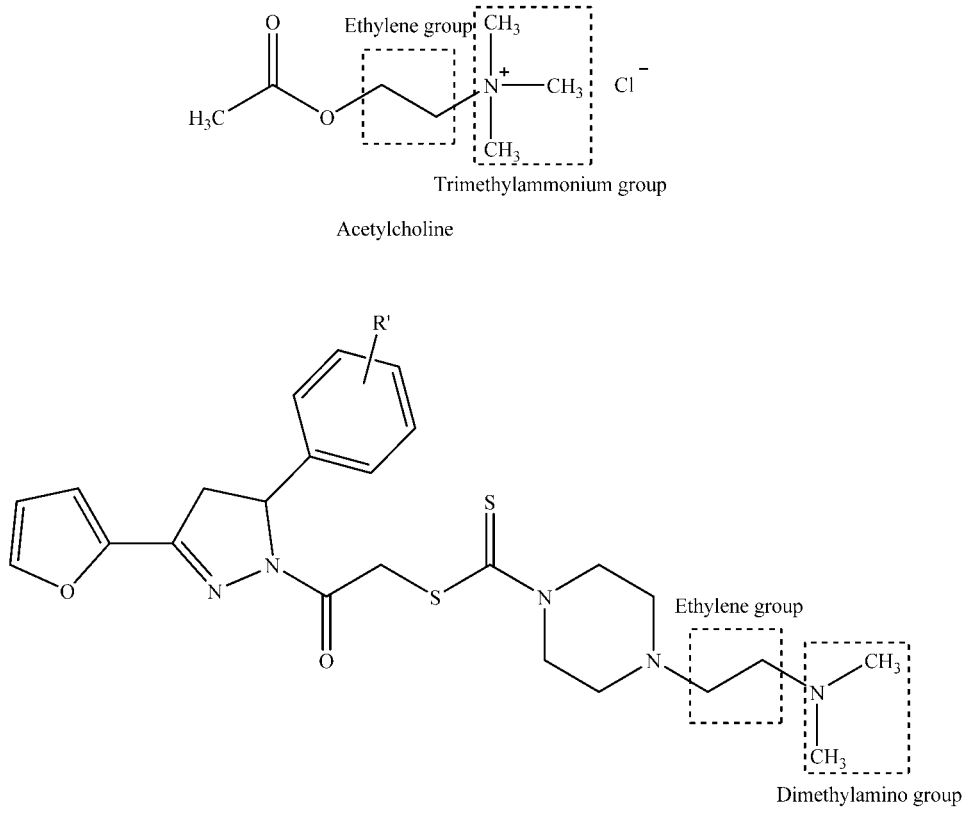
*1-[(N,N-Disubstituted thiocarbamoylthio)acetyl]-3-(2-furyl)-5-aryl-2-pyrazolines (**1**–**30**)*

A mixture of 1-(chloroacetyl)-3-(2-furyl)-5-aryl-2-pyrazolines (0.01 mol) and appropriate sodium salts of *N*,*N*-disubstituted dithiocarbamic acid (0.01 mol) was treated in acetone at room temperature for 3 h. The solvent was evaporated, the resulting solid was washed with water and recrystallized from ethanol.

*1-[(4-Ethylpiperazin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline (**1**)*

IR (KBr) ν_{max} (cm^{-1}): 3112.89 (aromatic C–H), 2902.67 (aliphatic C–H asymmetric), 2810.09 (aliphatic C–H symmetric), 1658.67 (C=O), 1487.01, 1425.30 (C=N and C=C), 1382.87, 1236.29, 1035.70, 1018.34 (C–N, C–O, and C=S).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.01 (3H, s), 2.35 (2H, m), 2.43 (4H, m), 3.02 (1H, dd, J = 18.0, 4.5 Hz), 3.77 (1H, dd, J = 18.0, 11.5 Hz), 3.92–4.19 (4H, two br. s), 4.56 (1H, d, J = 14.0 Hz), 4.73 (1H, d, J = 13.5 Hz), 5.45 (1H, dd, J = 11.5, 4.5 Hz), 5.97 (2H, s), 6.66–7.01 (5H, m), 7.91 (1H, s).



Scheme 2. The similarity of acetylcholine and compounds **7**, **17**, and **27**.

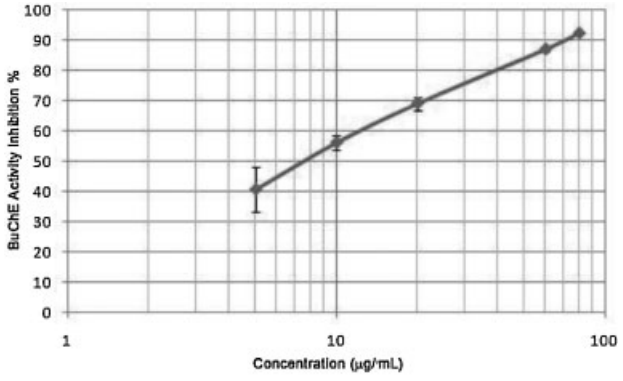


Figure 2. BuChE inhibitory activity of compound **7**.

For $C_{23}H_{26}N_4O_4S_2$·, calculated: C, 56.77; H, 5.39; N, 11.51; found: C, 56.78; H, 5.38; N, 11.49; MS (FAB) $[M+1]^+$: m/z 487.

*1-[((Morpholin-4-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline (**2**)*

IR (KBr) ν_{max} (cm^{-1}): 3109.04 (aromatic C–H), 2920.03 (aliphatic C–H asymmetric), 2850.59 (aliphatic C–H symmetric), 1666.38 (C=O), 1485.09, 1429.15 (C=N and C=C), 1388.65, 1267.14, 1234.36, 1224.71, 1031.85 (C–N, C–O and C=S), 991.34, 867.91 (C–H out of plane deformation).

^{1}H NMR (500 MHz, δ ppm, DMSO-d_6): 3.18 (1H, dd, J = 18.0, 4.5 Hz), 3.67 (4H, m), 3.78 (1H, dd, J = 18.0, 12.0 Hz), 3.95–4.20 (4H, two br), 4.58 (1H, d, J = 14.0 Hz), 4.75 (1H, d, J = 13.5 Hz), 5.45 (1H, dd, J = 12.0, 4.5 Hz), 5.99 (2H, s), 6.67–6.87 (4H, m), 7.01 (1H, d, J = 3.5 Hz), 7.92 (1H, s).

For $C_{21}H_{21}N_3O_5S_2$, calculated: C, 54.89; H, 4.61; N, 9.14; found: C, 54.90; H, 4.59; N, 9.17; MS (FAB) $[M+1]^+$: m/z 460.

*1-[[(4-(4-Methoxyphenyl)piperazin-1-yl)-thiocarbamoylthio]acetyl]-3-(2-furyl)-5-(3,4-methylene-dioxyphenyl)-2-pyrazoline (**3**)*

IR (KBr) ν_{max} (cm^{-1}): 3114.82 (aromatic C–H), 2902.67 (aliphatic C–H asymmetric), 2831.31 (aliphatic C–H symmetric), 1654.81 (C=O), 1647.10, 1512.09, 1487.01, 1425.30 (C=N and C=C), 1386.72, 1240.14, 1220.86, 1035.70, 1004.84 (C–N, C–O and C=S), 808.12 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.12 (4H, m), 3.25 (1H, dd, J = 18.0, 4.5 Hz), 3.69 (3H, s), 3.78 (1H, dd, J = 18.0, 11.5 Hz), 3.96–4.22 (4H, m), 4.59 (1H, d, J = 14.0 Hz), 4.76 (1H, d, J = 13.5 Hz), 5.46 (1H, m), 5.98 (2H, m), 6.64–7.03 (9H, m), 7.91 (1H, m).

For $C_{28}H_{28}N_4O_5S_2$, calculated: C, 59.56; H, 5.00; N, 9.92; found: C, 59.54; H, 5.01; N, 9.90; MS (FAB) $[M+1]^+$: m/z 565.

Table 3. *In vitro* cytotoxicity of the compounds (**1–30**) against NIH/3T3 cells for 24 h.

Compound	IC_{50} value (μg/mL)[a)]
1	12.2 ± 2.0
2	51.3 ± 3.2
3	191.0 ± 18.9
4	<7.8
5	8.3 ± 0.3
6	13.3 ± 1.5
7	9.2 ± 1.6
8	<7.8
9	<7.8
10	16.7 ± 4.2
11	18.7 ± 2.1
12	12.3 ± 1.5
13	61.0 ± 8.5
14	17.7 ± 2.5
15	22.3 ± 1.5
16	16.3 ± 1.2
17	63.7 ± 9.1
18	31.3 ± 1.2
19	13.7 ± 1.5
20	67.7 ± 2.5
21	26.7 ± 4.2
22	206.7 ± 47.3
23	24.0 ± 1.0
24	22.0 ± 1.0
25	28.3 ± 0.6
26	13.0 ± 1.0
27	9.3 ± 0.3
28	22.7 ± 2.1
29	17.0 ± 1.0
30	51.0 ± 7.9

[a)] Cytotoxicity of compounds to mouse fibroblast (NIH/3T3) cell line. Incubation for 24 h. IC_{50} is the drug concentration required to inhibit 50% of the cell proliferation. The values represent mean ± standard deviation of triplicate determinations.

*1-[((4-Benzylpiperazin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline (**4**)*

IR (KBr) ν_{max} (cm^{-1}): 3025.20 (aromatic C–H), 2916.17 (aliphatic C–H asymmetric), 2810.09 (aliphatic C–H symmetric), 1662.50 (C=O), 1647.10, 1454.03, 1423.37 (C=N and C=C), 1249.79, 1178.43, 1029.92 (C–N, C–O and C=S), 814.90 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.45 (4H, m), 3.02 (1H, dd, J = 18.0, 4.5 Hz), 3.52 (2H, s), 3.77 (1H, dd, J = 18.0, 11.5 Hz), 3.94–4.21 (4H, two br. s), 4.56 (1H, d, J = 14.0 Hz), 4.72 (1H, d, J = 13.5 Hz), 5.45 (1H, dd, J = 11.5, 4.5 Hz), 5.97 (2H, s), 6.65–7.01 (5H, m), 7.24–7.35 (5H, m), 7.91 (1H, s).

For $C_{28}H_{28}N_4O_4S_2$, calculated: C, 61.29; H, 5.14; N, 10.21; found: C, 61.30; H, 5.12; N, 10.19; MS (FAB) $[M+1]^+$: m/z 549.

*1-[((4-Methylpiperazin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline (**5**)*

IR (KBr) ν_{max} (cm^{-1}): 3112.89 (aromatic C–H), 2906.53 (aliphatic C–H asymmetric), 2790.80 (aliphatic C–H symmetric), 1672.17 (C=O), 1485.09, 1427.23 (C=N and C=C), 1380.94, 1290.29, 1242.07, 1143.71, 1041.49 (C–N, C–O and C=S), 810.05 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.20 (3H, s), 2.39 (4H, m), 3.03 (1H, dd, J = 18.0, 4.5 Hz), 3.78 (1H, dd, J = 18.0, 11.5 Hz), 3.92–4.19 (4H, two br. s), 4.57 (1H, d, J = 14.0 Hz), 4.73 (1H, d, J = 13.5 Hz), 5.45 (1H, dd, J = 11.5, 4.5 Hz), 5.96 (2H, s), 6.65–7.03 (5H, m), 7.91 (1H, s).

For $C_{22}H_{24}N_4O_4S_2$, calculated: C, 55.91; H, 5.12; N, 11.86; found: C, 55.89; H, 5.10; N, 11.88; MS (FAB) $[M+1]^+$: m/z 473.

*1-[((Thiomorpholin-4-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline (**6**)*

IR (KBr) ν_{max} (cm^{-1}): 3112.89 (aromatic C–H), 2910.38 (aliphatic C–H), 1666.38 (C=O), 1485.09, 1431.08 (C=N and C=C), 1388.65, 1282.57, 1242.07, 1191.93, 1037.63 (C–N, C–O and C=S), 935.41, 811.98 (C–H out of plane deformation).

^{1}H NMR (500 MHz, δ ppm, DMSO-d_6): 2.73 (4H, m), 3.04 (1H, dd, J = 18.0, 4.5 Hz), 3.77 (1H, m), 4.20–4.52 (4H, two br), 4.59 (1H, d, J = 14.0 Hz), 4.74 (1H, d, J = 13.5 Hz), 5.46 (1H, m), 5.98 (2H, s), 6.67–7.03 (5H, m), 7.91 (1H, s).

For $C_{21}H_{21}N_3O_4S_3$, calculated: C, 53.03; H, 4.45; N, 8.84; found: C, 53.05; H, 4.44; N, 8.87; MS (FAB) $[M+1]^+$: m/z 476.

*1-[[(4-(2-Dimethylaminoethyl)piperazin-1-yl)-thiocarbamoylthio]acetyl]-3-(2-furyl)-5-(3,4-methylene-dioxyphenyl)-2-pyrazoline (**7**)*

IR (KBr) ν_{max} (cm^{-1}): 3112.89 (aromatic C–H), 2939.31 (aliphatic C–H asymmetric), 2815.88 (aliphatic C–H symmetric), 1666.38 (C=O), 1487.01, 1425.30 (C=N and C=C), 1236.29, 1130.21, 1035.70 (C–N, C–O and C=S), 997.13, 808.12 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.12 (6H, s), 2.34 (2H, m), 2.41 (2H, m), 2.47 (4H, m), 3.02 (1H, dd, J = 18.0, 4.5 Hz), 3.76 (1H, dd, J = 18.0, 11.5 Hz), 3.90–4.19 (4H, two br. s), 4.56 (1H, d, J = 14.0 Hz), 4.72 (1H, d, J = 13.5 Hz), 5.45 (1H, dd, J = 11.5, 4.5 Hz), 5.97 (2H, s), 6.66–7.01 (5H, m), 7.91 (1H, s).

For $C_{25}H_{31}N_5O_4S_2$, calculated: C, 56.69; H, 5.90; N, 13.22; found: C, 56.69; H, 5.90; N, 13.22; MS (FAB) $[M+1]^+$: m/z 530.

*1-[(4-Phenylpiperazin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline (**8**)*

IR (KBr) ν_{max} (cm^{-1}): 3116.75 (aromatic C–H), 2935.46 (aliphatic C–H asymmetric), 2854.45 (aliphatic C–H symmetric), 1658.67 (C=O), 1500.52, 1485.09, 1425.30 (C=N and C=C), 1380.94, 1244.00, 1226.64, 1114.78, 1035.70 (C–N, C–O and C=S), 819.69 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.03 (1H, dd, J = 18.0, 4.5 Hz), 3.28 (4H, m), 3.79 (1H, dd, J = 18.0, 11.5 Hz), 3.92–4.19 (4H, two br. s), 4.56 (1H, d, J = 14.0 Hz), 4.71 (1H, d, J = 13.5 Hz), 5.45 (1H, dd, J = 11.5, 4.5 Hz), 5.98 (2H, s), 6.67–7.02 (5H, m), 7.23–7.34 (5H, m), 7.91 (1H, s).

For $C_{27}H_{26}N_4O_4S_2$, calculated: C, 60.65; H, 4.90; N, 10.48; found: C, 60.63; H, 4.88; N, 10.47; MS (FAB) $[M+1]^+$: m/z 535.

*1-[(Pyrrolidin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline (**9**)*

IR (KBr) ν_{max} (cm^{-1}): 3112.89 (aromatic C–H), 2968.24 (aliphatic C–H asymmetric), 2894.95 (aliphatic C–H symmetric), 1666.38 (C=O), 1488.94, 1436.87 (C=N and C=C), 1326.93, 1244.00, 1159.14, 1035.70, 1006.77 (C–N, C–O, and C=S), 808.12 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.88–2.06 (4H, m), 3.03 (1H, dd, J = 18.0, 4.5 Hz), 3.63–3.75 (4H, m), 3.78 (1H, dd, J = 18.0, 11.5 Hz), 4.54 (1H, d, J = 14.0 Hz), 4.72 (1H, d, J = 13.5 Hz), 5.45 (1H, dd, J = 11.5, 4.5 Hz), 5.98 (2H, s), 6.67–7.01 (5H, m), 7.91 (1H, s).

For $C_{21}H_{21}N_3O_4S_2$, calculated: C, 56.87; H, 4.77; N, 9.47; found: C, 56.85; H, 4.77; N, 9.49; MS (FAB) $[M+1]^+$: m/z 444.

*1-[(Piperidin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline (**10**)*

IR (KBr) ν_{max} (cm^{-1}): 3112.89 (aromatic C–H), 2902.67 (aliphatic C–H asymmetric), 2821.66 (aliphatic C–H symmetric), 1666.38 (C=O), 1598.88, 1488.94, 1425.30 (C=N and C=C), 1386.72, 1240.14, 1151.42, 1035.70, 1004.84 (C–N, C–O, and C=S), 808.12 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.53 (6H, m), 3.02 (1H, dd, J = 18.0, 4.5 Hz), 3.78 (1H, dd, J = 18.0, 11.5 Hz), 4.10–4.34 (4H, m), 4.60 (1H, d, J = 14.0 Hz), 4.76 (1H, d, J = 13.5 Hz), 5.47 (1H, dd, J = 11.5, 4.5 Hz), 5.97 (2H, m), 6.65–7.25 (5H, m), 7.91 (1H, s).

For $C_{22}H_{23}N_3O_4S_2$, calculated: C, 57.75; H, 5.07; N, 9.18; found: C, 57.74; H, 5.07; N, 9.21; MS (FAB) $[M+1]^+$: m/z 458.

*1-[((4-Ethylpiperazin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(4-methylphenyl)-2-pyrazoline (**11**)*

IR (KBr) ν_{max} (cm^{-1}): 3114.82 (aromatic C–H), 2968.24 (aliphatic C–H), 2918.10 (aliphatic C–H), 1666.38 (C=O), 1467.73, 1445.62 (C=N and C=C), 1269.07, 1236.29, 1157.21, 1018.34 (C–N, C–O, and C=S), 813.90 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.01 (3H, s), 2.25 (3H, s), 2.35 (2H, m), 2.44 (4H, m), 3.01 (1H, dd, J = 18.0, 4.5 Hz), 3.80 (1H, dd, J = 18.0, 12.0 Hz), 3.92–4.19 (4H, two br s), 4.57 (1H, d, J = 14.0 Hz), 4.72 (1H, d, J = 13.5 Hz), 5.48 (1H, dd, J = 12.0, 4.5 Hz), 6.67 (1H, m), 7.01 (1H, s), 7.08–7.12 (4H, m), 7.91 (1H, s).

For $C_{23}H_{28}N_4O_2S_2$, calculated: C, 60.50; H, 6.18; N, 12.27; found: C, 60.52; H, 6.17; N, 12.29; MS (FAB) $[M+1]^+$: m/z 457.

*1-[((Morpholin-4-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(4-methylphenyl)-2-pyrazoline (**12**)*

IR (KBr) ν_{max} (cm^{-1}): 3114.82 (aromatic C–H), 2918.10 (aliphatic C–H asymmetric), 2854.45 (aliphatic C–H symmetric), 1666.38 (C=O), 1514.02, 1423.37 (C=N and C=C), 1386.72, 1269.07, 1230.50, 1112.85, 1027.99, 1000.99 (C–N, C–O, and C=S), 813.90 (C–H out of plane deformation).

^{1}H NMR (500 MHz, δ ppm, DMSO-d_6): 2.25 (3H, s), 3.01 (1H, dd, J = 18.0, 4.5 Hz), 3.70 (4H, m), 3.80 (1H, dd, J = 18.0, 12.0 Hz), 3.95–4.20 (4H, two br.), 4.59 (1H, d, J = 14.0 Hz), 4.74 (1H, d, J = 13.5 Hz), 5.49 (1H, dd, J = 12.0, 4.5 Hz), 6.67 (1H, t, J = 3.5, 2.0 Hz), 7.01 (1H, d, J = 3.5 Hz), 7.08–7.20 (4H, m), 7.91 (1H, s).

For $C_{21}H_{23}N_3O_3S_2$, calculated: C, 58.72; H, 5.40; N, 9.78; found: C, 58.70; H, 5.41; N, 9.80; MS (FAB) $[M+1]^+$: m/z 430.

*1-[[(4-(4-Methoxyphenyl)piperazin-1-yl)-thiocarbamoylthio]acetyl]-3-(2-furyl)-5-(4-methylphenyl)-2-pyrazoline (**13**)*

IR (KBr) ν_{max} (cm^{-1}): 3116.75 (aromatic C–H), 2918.10 (aliphatic C–H asymmetric), 2829.38 (aliphatic C–H symmetric), 1666.38 (C=O), 1512.09, 1425.30 (C=N and C=C), 1274.86, 1222.79, 1004.84 (C–N, C–O, and C=S), 813.90 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.24 (3H, s), 3.12 (4H, m), 3.25 (1H, dd, J = 18.0, 4.5 Hz), 3.69 (3H, s), 3.81 (1H, dd, J = 18.0, 11.5 Hz), 4.09–4.35 (4H, two br.), 4.60 (1H, d, J = 14.0 Hz), 4.75 (1H, d, J = 13.5 Hz), 5.49 (1H, dd, J = 11.5, 4.5 Hz), 6.67 (1H, m), 6.84 (2H, d, J = 8.6 Hz), 6.92 (2H, d, J = 8.5 Hz), 6.99–7.13 (5H, m), 7.90 (1H, m).

For $C_{28}H_{30}N_4O_3S_2$, calculated: C, 62.90; H, 5.66; N, 10.48; found: C, 62.89; H, 5.65; N, 10.48; MS (FAB) $[M+1]^+$: m/z 535.

*1-[((4-Benzylpiperazin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(4-methylphenyl)-2-pyrazoline (**14**)*

IR (KBr) ν_{max} (cm^{-1}): 3024.18 (aromatic C–H), 2916.17 (aliphatic C–H asymmetric), 2810.09 (aliphatic C–H symmetric), 1662.52 (C=O), 1454.03, 1423.37 (C=N and C=C), 1228.57, 1139.85 (C–N, C–O, and C=S), 995.20, 813.90 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.26 (3H, s), 2.45 (4H, m), 3.00 (1H, dd, J = 18.0, 4.5 Hz), 3.52 (2H, s), 3.80 (1H, dd, J = 18.0, 11.5 Hz), 3.93–4.21 (4H, two br. s), 4.57 (1H, d, J = 14.0 Hz), 4.71 (1H, d, J = 13.5 Hz), 5.48 (1H, dd, J = 11.5, 4.5 Hz), 6.67 (1H, m), 7.00 (1H, d, J = 3.5 Hz), 7.08–7.32 (9H, m), 7.91 (1H, s).

For $C_{28}H_{30}N_4O_2S_2$, calculated: C, 64.84; H, 5.83; N, 10.80; found: C, 64.84; H, 5.80; N, 10.82; MS (FAB) $[M+1]^+$: m/z 519.

*1-[((4-Methylpiperazin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(4-methylphenyl)-2-pyrazoline (**15**)*

IR (KBr) ν_{max} (cm^{-1}): 3116.75 (aromatic C–H), 2937.38 (aliphatic C–H asymmetric), 2792.73 (aliphatic C–H symmetric), 1662.52 (C=O), 1461.94, 1425.30 (C=N and C=C), 1288.36, 1141.78 (C–N, C–O, and C=S), 995.20, 813.90 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.20 (3H, s), 2.25 (3H, s), 2.39 (4H, m), 3.01 (1H, dd, J = 18.0, 4.5 Hz), 3.80 (1H, dd, J = 18.0, 11.5 Hz), 3.92–4.19 (4H, two br. s), 4.58 (1H, d, J = 14.0 Hz), 4.72 (1H, d, J = 13.5 Hz), 5.48 (1H, dd, J = 11.5, 4.5 Hz), 6.66 (1H, m), 7.01 (1H, s), 7.08–7.18 (4H, m), 7.91 (1H, s).

For $C_{22}H_{26}N_4O_2S_2$, calculated: C, 59.70; H, 5.92; N, 12.66; found: C, 59.71; H, 5.90; N, 12.65; MS (FAB) $[M+1]^+$: m/z 443.

*1-[((Thiomorpholin-4-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(4-methylphenyl)-2-pyrazoline (**16**)*

IR (KBr) ν_{max} (cm^{-1}): 3116.75 (aromatic C–H), 2916.17 (aliphatic C–H), 1666.38 (C=O), 1467.73, 1421.44 (C=N and C=C), 1282.57, 1215.07, 1143.71 (C–N, C–O, and C=S), 813.90 (C–H out of plane deformation), 667.32 (C–S).

^{1}H NMR (500 MHz, δ ppm, DMSO-d_6): 2.25 (3H, s), 2.72 (4H, m), 3.01 (1H, dd, J = 18.0, 4.5 Hz), 3.81 (1H, dd, J = 18.0, 4.5 Hz), 4.22–4.51 (4H, two br), 4.60 (1H, d, J = 14.0 Hz), 4.73 (1H, d, J = 13.5 Hz), 5.49 (1H, dd, J = 12.0, 4.5 Hz), 6.66 (1H, m), 7.01 (1H, s), 7.06–7.12 (4H, m), 7.91 (1H, s).

For $C_{21}H_{23}N_3O_2S_3$, calculated: C, 56.60; H, 5.20; N, 9.43; found: C, 56.59; H, 5.19; N, 9.45; MS (FAB) $[M+1]^+$: m/z 446.

*1-[[(4-(2-Dimethylaminoethyl)piperazin-1-yl)-thiocarbamoylthio]acetyl]-3-(2-furyl)-5-(4-methylphenyl)-2-pyrazoline (**17**)*

IR (KBr) ν_{max} (cm^{-1}): 3097.47 (aromatic C–H), 2939.31 (aliphatic C–H asymmetric), 2815.88 (aliphatic C–H symmetric), 1672.17 (C=O), 1465.54, 1425.30 (C=N and C=C), 1232.43, 1132.14, 1013.25 (C–N, C–O, and C=S), 813.90 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.14 (6H, s), 2.25 (3H, s), 2.35 (2H, m), 2.42 (2H, m), 2.48 (4H, m), 3.01 (1H, dd, J = 18.0, 4.5 Hz), 3.80 (1H, dd, J = 18.0, 11.5 Hz), 3.91–4.18 (4H, two br. s), 4.56 (1H, d, J = 14.0 Hz), 4.72 (1H, d, J = 13.5 Hz), 5.48 (1H, dd, J = 11.5, 4.5 Hz), 6.66 (1H, m), 7.01 (1H, s), 7.06–7.12 (4H, m), 7.91 (1H, s).

For $C_{25}H_{33}N_5O_2S_2$, calculated: C, 60.09; H, 6.66; N, 14.02; found: C, 60.11; H, 6.65; N, 14.03; MS (FAB) $[M+1]^+$: m/z 500.

*1-[((4-Phenylpiperazin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(4-methylphenyl)-2-pyrazoline (**18**)*

IR (KBr) ν_{max} (cm^{-1}): 3022.25 (aromatic C–H), 2914.24 (aliphatic C–H asymmetric), 2821.66 (aliphatic C–H symmetric), 1658.67 (C=O), 1598.88, 1502.44, 1425.30 (C=N and C=C), 1384.79, 1272.93, 1222.79, 1151.42, 1004.84 (C–N, C–O, and C=S), 813.90 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.25 (3H, s), 3.00 (1H, dd, J = 18.0, 4.5 Hz), 3.29 (4H, m), 3.81 (1H, dd, J = 18.0, 11.5 Hz), 3.09–4.34 (4H, two br. s), 4.61 (1H, d, J = 14.0 Hz), 4.76 (1H, d, J = 13.5 Hz), 5.49 (1H, dd, J = 11.5, 4.5 Hz), 6.67 (1H, m), 6.75–6.95 (4H, m), 7.01 (1H, s), 7.11–7.24 (5H, m), 7.92 (1H, m).

For $C_{27}H_{28}N_4O_2S_2$, calculated: C, 64.26; H, 5.59; N, 11.10; found: C, 64.26; H, 5.61; N, 11.09; MS (FAB) $[M+1]^+$: m/z 505.

*1-[((Pyrrolidin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(4-methylphenyl)-2-pyrazoline (**19**)*

IR (KBr) ν_{max} (cm^{-1}): 3116.75 (aromatic C–H), 2947.03 (aliphatic C–H asymmetric), 2869.88 (aliphatic C–H symmetric), 1662.52 (C=O), 1450.15, 1433.01 (C=N and C=C), 1328.86, 1159.14, 1006.77 (C–N, C–O, and C=S), 813.90 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.88–2.05 (4H, m), 2.26 (3H, s), 3.01 (1H, dd, J = 18.0, 4.5 Hz), 3.63–3.76 (4H, m), 3.80 (1H, dd, J = 18.0, 11.5 Hz), 4.56 (1H, d, J = 14.0 Hz), 4.71 (1H, d, J = 13.5 Hz), 5.48 (1H, dd, J = 11.5, 4.5 Hz), 6.67 (1H, m), 7.01 (1H, m), 7.08–7.18 (4H, m), 7.91 (1H, s).

For $C_{21}H_{23}N_3O_2S_2$, calculated: C, 60.99; H, 5.61; N, 10.16; found: C, 60.98; H, 5.59; N, 10.17; MS (FAB) $[M+1]^+$: m/z 414.

*1-[((Piperidin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(4-methylphenyl)-2-pyrazoline (**20**)*

IR (KBr) ν_{max} (cm^{-1}): 3116.75 (aromatic C–H), 2937.38 (aliphatic C–H asymmetric), 2856.38 (aliphatic C–H symmetric), 1666.38 (C=O), 1481.23, 1427.23 (C=N and C=C), 1244.00, 1132.14, 1006.77 (C–N, C–O, and C=S), 813.90 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.58 (6H, m), 2.26 (3H, s), 3.01 (1H, dd, J = 18.0, 4.5 Hz), 3.80 (1H, dd, J = 18.0, 11.5 Hz), 3.91–4.19 (4H, m), 4.57 (1H, d, J = 14.0 Hz), 4.70 (1H, d, J = 13.5 Hz), 5.48 (1H, dd, J = 11.5, 4.5 Hz), 6.67 (1H, m), 7.01 (1H, m), 7.06–7.20 (4H, m), 7.91 (1H, s).

For $C_{22}H_{25}N_3O_2S_2$, calculated: C, 61.80; H, 5.89; N, 9.83; found: C, 61.79; H, 5.88; N, 9.84; MS (FAB) $[M+1]^+$: m/z 428.

*1-[((4-Ethylpiperazin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(4-methoxyphenyl)-2-pyrazoline (**21**)*

IR (KBr) ν_{max} (cm^{-1}): 3124.47 (aromatic C–H), 2931.60 (aliphatic C–H asymmetric), 2833.24 (aliphatic C–H symmetric), 1664.45 (C=O), 1610.45, 1512.09, 1427.23 (C=N and C=C), 1247.86, 1178.43, 1029.92 (C–N, C–O, and C=S), 829.33 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.01 (3H, s), 2.36 (2H, m), 2.44 (4H, m), 3.02 (1H, dd, J = 18.0, 4.5 Hz), 3.70 (3H, s), 3.78 (1H, dd, J = 18.0, 11.5 Hz), 3.92–4.20 (4H, two br. s), 4.58 (1H, d, J = 14.0 Hz), 4.71 (1H, d, J = 13.5 Hz), 5.47 (1H, dd, J = 11.5, 4.5 Hz), 6.61–7.14 (6H, m), 7.91 (1H, s).

For $C_{23}H_{28}N_4O_3S_2$, calculated: C, 58.45; H, 5.97; N, 11.85; found: C, 58.44; H, 5.97; N, 11.87; MS (FAB) $[M+1]^+$: m/z 473.

*1-[((Morpholin-4-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(4-methoxyphenyl)-2-pyrazoline (**22**)*

IR (KBr) ν_{max} (cm^{-1}): 3136.04 (aromatic C–H), 2958.60 (aliphatic C–H asymmetric), 2852.52 (aliphatic C–H symmetric), 1664.45 (C=O), 1610.45, 1512.09, 1463.87, 1421.44 (C=N and C=C), 1247.86, 1178.43, 1029.92 (C–N, C–O, and C=S), 829.33 (C–H out of plane deformation).

^{1}H NMR (500 MHz, δ ppm, DMSO-d_6): 3.02 (1H, dd, J = 18.0, 4.5 Hz), 3.70 (4H, m), 3.72 (3H, s), 3.82 (1H, dd, J = 18.0, 12.0 Hz), 3.96–4.19 (4H, two br.), 4.59 (1H, d, J = 14.0 Hz), 4.72 (1H, d, J = 13.5 Hz), 5.47 (1H, dd, J = 12.0, 4.5 Hz), 6.65–7.29 (6H, m), 7.91 (1H, s).

For $C_{21}H_{23}N_3O_4S_2$, calculated: C, 56.61; H, 5.20; N, 9.43; found: C, 56.59; H, 5.21; N, 9.44; MS (FAB) $[M+1]^+$: m/z 446.

*1-[[(4-(4-Methoxyphenyl)piperazin-1-yl)-thiocarbamoylthio]acetyl]-3-(2-furyl)-5-(4-methoxyphenyl)-2-pyrazoline (**23**)*

IR (KBr) ν_{max} (cm^{-1}): 3122.54 (aromatic C–H), 2931.60 (aliphatic C–H asymmetric), 2833.24 (aliphatic C–H symmetric), 1658.67 (C=O), 1610.45, 1512.09, 1423.37 (C=N and C=C), 1247.86, 1029.92 (C–N, C–O, and C=S), 827.41 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.12 (4H, m), 3.25 (1H, dd, J = 18.0, 4.5 Hz), 3.70 (6H, s), 3.79 (1H, m), 4.10–4.34 (4H, two br), 4.60 (1H, d, J = 14.0 Hz), 4.73 (1H, d, J = 13.5 Hz), 5.48 (1H, dd, J = 11.5, 4.5 Hz), 6.61–7.15 (10H, m), 7.91 (1H, s).

For $C_{28}H_{30}N_4O_4S_2$, calculated: C, 61.07; H, 5.49; N, 10.17; found: C, 61.05; H, 5.50; N, 10.16; MS (FAB) $[M+1]^+$: m/z 551.

*1-[((4-Benzylpiperazin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(4-methoxyphenyl)-2-pyrazoline (**24**)*

IR (KBr) ν_{max} (cm^{-1}): 2916.17 (aliphatic C–H asymmetric), 2833.24 (aliphatic C–H symmetric), 1662.52 (C=O), 1512.09, 1463.87, 1423.37 (C=N and C=C), 1249.79, 1178.43, 1029.92 (C–N, C–O, and C=S), 829.33 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.46 (4H, m), 3.02 (1H, dd, J = 18.0, 4.5 Hz), 3.52 (2H, s), 3.71 (3H, s), 3.79 (1H, dd, J = 18.0, 11.5 Hz), 3.94–4.21 (4H, two br. s), 4.57 (1H, d, J = 14.0 Hz), 4.70 (1H, d, J = 13.5 Hz), 5.47 (1H, dd, J = 11.5, 4.5 Hz), 6.66–7.32 (11H, m), 7.91 (1H, s).

For $C_{28}H_{30}N_4O_3S_2$, calculated: C, 62.90; H, 5.66; N, 10.48; found: C, 62.92; H, 5.65; N, 10.48; MS (FAB) $[M+1]^+$: m/z 535.

*1-[((4-Methylpiperazin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(4-methoxyphenyl)-2-pyrazoline (**25**)*

IR (KBr) ν_{max} (cm^{-1}): 3122.54 (aromatic C–H), 2933.53 (aliphatic C–H asymmetric), 2835.16 (aliphatic C–H symmetric), 1662.52 (C=O), 1512.09, 1463.87, 1425.30 (C=N and C=C), 1247.86, 1230.50, 1178.43, 1029.92 (C–N, C–O, and C=S), 829.33 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.20 (3H, s), 2.39 (4H, m), 3.02 (1H, dd, J = 18.0, 4.5 Hz), 3.74 (3H, s), 3.80 (1H, dd, J = 18.0, 11.5 Hz), 3.92–4.19 (4H, two br.), 4.58 (1H, d, J = 14.0 Hz), 4.70 (1H, d, J = 13.5 Hz), 5.47 (1H, dd, J = 11.5, 4.5 Hz), 6.68–7.26 (6H, m), 7.91 (1H, m).

For $C_{22}H_{26}N_4O_3S_2$, calculated: C, 57.62; H, 5.71; N, 12.22; found: C, 57.61; H, 5.69; N, 12.23; MS (FAB) $[M+1]^+$: m/z 459.

*1-[((Thiomorpholin-4-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(4-methoxyphenyl)-2-pyrazoline (**26**)*

IR (KBr) ν_{max} (cm^{-1}): 3114.82 (aromatic C–H), 2910.38 (aliphatic C–H asymmetric), 2833.24 (aliphatic C–H symmetric), 1662.52 (C=O), 1512.09, 1463.87, 1436.87 (C=N and C=C), 1247.86, 1178.43, 1029.92 (C–N, C–O, and C=S), 829.33 (C–H out of plane deformation).

^{1}H NMR (500 MHz, δ ppm, DMSO-d_6): 2.72 (4H, m), 3.02 (1H, dd, J = 18.0, 4.5 Hz), 3.71 (3H, s), 3.79 (1H, dd, J = 18.0, 4.5 Hz), 4.26–4.49 (4H, two br), 4.60 (1H, d, J = 14.0 Hz), 4.71 (1H, d, J = 13.5 Hz), 5.48 (1H, dd, J = 12.0, 4.5 Hz), 6.67–7.28 (6H, m), 7.92 (1H, s).

For $C_{21}H_{23}N_3O_3S_3$, calculated: C, 54.64; H, 5.02; N, 9.10; found: C, 54.63; H, 5.04; N, 9.09; MS (FAB) $[M+1]^+$: m/z 462.

*1-[[(4-(2-Dimethylaminoethyl)piperazin-1-yl)-thiocarbamoylthio]acetyl]-3-(2-furyl)-5-(4-methoxyphenyl)-2-pyrazoline (**27**)*

IR (KBr) ν_{max} (cm^{-1}): 3122.54 (aromatic C–H), 2933.53 (aliphatic C–H asymmetric), 2819.73 (aliphatic C–H symmetric), 1662.52 (C=O), 1512.09, 1471.59 (C=N and C=C), 1247.86, 1178.43, 1031.85 (C–N, C–O, and C=S), 829.33 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.16 (6H, s), 2.39 (2H, m), 2.42 (2H, m), 2.48 (4H, m), 3.02 (1H, dd, J = 18.0, 4.5 Hz), 3.71 (3H, s), 3.80 (1H, dd, J = 18.0, 11.5 Hz), 3.91–4.18 (4H, two br. s), 4.57 (1H, d, J = 14.0 Hz), 4.70 (1H, d, J = 13.5 Hz), 5.47 (1H, dd, J = 11.5, 4.5 Hz), 6.66–7.14 (6H, m), 7.91 (1H, s).

For $C_{25}H_{33}N_5O_3S_2$, calculated: C, 58.23; H, 6.45; N, 13.58; found: C, 58.22; H, 6.47; N, 13.55; MS (FAB) $[M+1]^+$: m/z 516.

*1-[((4-Phenylpiperazin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(4-methoxyphenyl)-2-pyrazoline (**28**)*

IR (KBr) ν_{max} (cm^{-1}): 3114.82 (aromatic C–H), 2927.74 (aliphatic C–H asymmetric), 2833.24 (aliphatic C–H symmetric), 1674.10 (C=O), 1512.09, 1423.37 (C=N and C=C), 1386.72, 1247.86, 1176.50, 1027.99 (C–N, C–O, and C=S), 829.33 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.03 (1H, dd, J = 18.0, 4.5 Hz), 3.29 (4H, m), 3.71 (3H, s), 3.80 (1H, dd, J = 18.0, 11.5 Hz), 4.10–4.34 (4H, two br. s), 4.61 (1H, d, J = 14.0 Hz), 4.74 (1H, d, J = 13.5 Hz), 5.48 (1H, dd, J = 11.5, 4.5 Hz), 6.67–7.25 (11H, m), 7.95 (1H, s).

For $C_{27}H_{28}N_4O_3S_2$, calculated: C, 62.28; H, 5.42; N, 10.76; found: C, 62.30; H, 5.39; N, 10.76; MS (FAB) $[M+1]^+$: m/z 521.

*1-[((Pyrrolidin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(4-methoxyphenyl)-2-pyrazoline (**29**)*

IR (KBr) ν_{max} (cm^{-1}): 3116.75 (aromatic C–H), 2952.81 (aliphatic C–H asymmetric), 2871.81 (aliphatic C–H symmetric), 1683.74 (C=O), 1564.16, 1512.09, 1436.87 (C=N and C=C), 1247.86, 1178.43, 1029.92 (C–N, C–O and C=S), 829.33 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.88–2.04 (4H, m), 3.03 (1H, dd, J = 18.0, 4.5 Hz), 3.63–3.69 (4H, m), 3.71 (3H, s), 3.79 (1H, dd, J = 18.0, 11.5 Hz), 4.56 (1H, d, J = 14.0 Hz), 4.70 (1H, d, J = 13.5 Hz), 5.47 (1H, dd, J = 11.5, 4.5 Hz), 6.66–7.14 (6H, m), 7.91 (1H, s).

For $C_{21}H_{23}N_3O_3S_2$, calculated: C, 58.72; H, 5.40; N, 9.78; found: C, 58.71; H, 5.39; N, 9.80; MS (FAB) $[M+1]^+$: m/z 430.

*1-[((Piperidin-1-yl)thiocarbamoylthio)acetyl]-3-(2-furyl)-5-(4-methoxyphenyl)-2-pyrazoline (**30**)*

IR (KBr) ν_{max} (cm^{-1}): 3120.61 (aromatic C–H), 2933.53 (aliphatic C–H asymmetric), 2854.45 (aliphatic C–H symmetric), 1666.38 (C=O), 1610.45, 1512.09, 1431.08 (C=N and C=C), 1245.93, 1178.43, 1027.99 (C–N, C–O and C=S), 829.33 (C–H out of plane deformation).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.61 (6H, m), 3.02 (1H, dd, J = 18.0, 4.5 Hz), 3.71 (3H, s), 3.79 (1H, dd, J = 18.0, 11.5 Hz), 3.91–4.19 (4H, m), 4.57 (1H, d, J = 14.0 Hz), 4.69 (1H, d, J = 13.5 Hz), 5.48 (1H, dd, J = 11.5, 4.5 Hz), 6.66–7.14 (6H, m), 7.91 (1H, s).

For $C_{22}H_{25}N_3O_3S_2$, calculated: C, 59.57; H, 5.68; N, 9.47; found: C, 59.57; H, 5.66; N, 9.48; MS (FAB) $[M+1]^+$: m/z 444.

AChE and BuChE inhibitory activity

AChE and BuChE inhibitory activity was determined by Ellman's method with minor modifications (electric eel AChE enzyme was used instead of bovine AChE enzyme and buffer was added at 2.4 mL instead of 3 mL) [31]. Compounds **1**–**30** were dissolved in DMSO and tested in the final concentration range of 5–80 μg/mL. Twenty microliter of enzyme (AChE or BuChE, 1 U/mL), 10 μL sample added to 2.4 mL buffer, the mixture was incubated at 37°C for 15 min. After 15 min incubation, 50 μL of 0.01 M 5,5′-dithio-*bis*(2-nitrobenzoic acid (DTNB) and 20 μL of 75 mM acetylthiocholine iodide (ATCI) or 25 mM butyrylthiocholine iodide (BTCI) were added, and the final mixture was incubated at room temperature for 30 min. Blank was prepared using 10 μL of DMSO instead of the test sample, with all other procedures similar to those used in the case of the sample mixture. Absorbances were measured at 412 nm and 37°C using polystyrol cuvets with a spectrophotometer (Shimadzu, UV-1700). Experiments were done in triplicate. Data are expressed as mean ± standard deviation (SD).

The inhibition (percent) of AChE or BuChE was calculated using the following equation.

$$I(\%) = 100\text{-}\left(\frac{\text{OD sample}}{\text{OD control}}\right)\times 100$$

Toxicity

The level of cellular MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma) reduction was quantified as previously described in the literature with small modifications as represented below in detail [32, 33].

Cell culture and drug treatment

NIH/3T3 cells were obtained from the American Type Culture Collection (ATCC, USA). The cells were incubated in Dulbecco's

modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Life Technologies, UK), 100 IU/mL penicillin (Gibco, Paisley, Scotland) and 100 mg/mL streptomycin (Gibco) at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Exponentially growing cells were plated at 2×10^4 cells/mL into 96-well microtiter tissue culture plates (Nunc, Denmark) and incubated for 24 h before the addition of the drugs (the optimum cell number for cytotoxicity assays was determined in preliminary experiments). Stock solutions of compounds were prepared in DMSO (Sigma–Aldrich, Poole, UK) and further dilutions were made with fresh culture medium (the concentration of DMSO in the final culture medium was <0.1%, which had no effect on the cell viability).

MTT assay for cytotoxicity of the compounds

The MTT assay is widely used as a measure of cytotoxicity. After 24 h of preincubation, the tested compounds were added to give final concentrations in the range of 7.8–500 μg/mL and the cells were incubated for 24 h. At the end of this period, MTT was dissolved in phosphate buffered saline (PBS) at 5 mg/mL and filtered to sterilize. At the time indicated above, stock MTT solution (20 μL/200 μL medium) was added to all assay wells, and the plates were incubated for 4 h at 37°C. After the medium was removed, the formazan crystals formed by MTT metabolism were solubilized by addition of 200 μL DMSO (instead of acid-isopropanol) to each well and after a few minutes at room temperature to ensure that all crystals were dissolved, absorbance was read at 540 nm with a microtitre plate spectrophotometer (Bio-Tek plate reader). Each concentration was repeated in three wells and IC_{50} values were defined as the drug concentrations that reduced absorbance to 50% of control values.

The authors have declared no conflict of interest.

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