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Synthesis, characterization, and antioxidant and anticholinesterase activities of pyrazolo derivatives

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Abstract

Twenty-four pyrazolo derivatives **(1–4)(a-f)** were synthesized and characterized by FTIR, ^{1}H, and ^{13}C NMR (Nuclear Magnetic Resonance), and elemental analysis. The synthesized compounds were also investigated for their antioxidant and anticholinesterase activities. The compounds **(3–4)(a-f)** carrying morpholine ring were more active than the piperidinyl containing compounds **(1–2)(a-f)** in both activities. The compound **4f** showed higher activity in both assays as compared with the others. Additionally, the anticholinesterase activity test provided higher values than the galantamine in the BChE assay. Therefore, compound **4f** can be used as anticholinesterase agent and/or anticholinesterase assay standard.

1 | INTRODUCTION

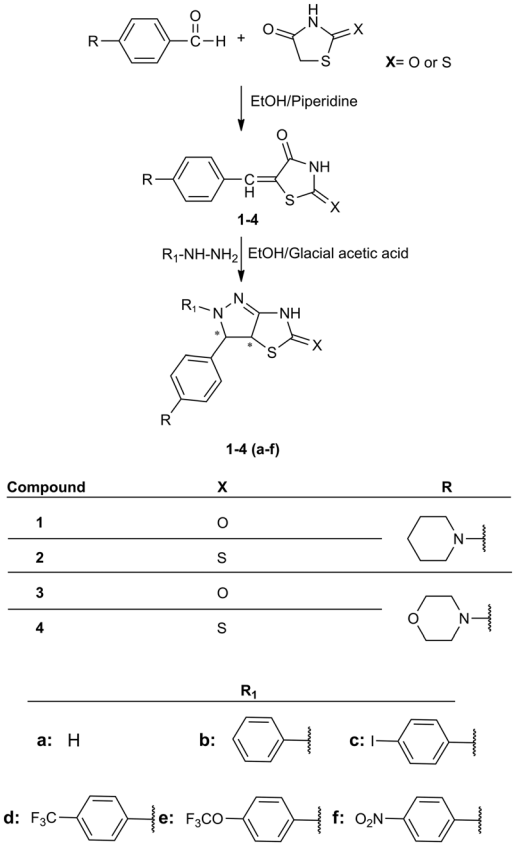
Pyrazole is an important family in the synthetic organic chemistry; played their roles in various branches of human life. There are numerous studies reported related to the synthesis of various functionalized pyrazoles, for example, 4,6-substituted pyrazolo[3,4-*d*]pyrimidines[1], microwave assisted solvent-free synthesis of pyrazolo[3,4-*b*]quinolines, pyrazolo[3,4-*c*]pyrazoles using *p*-TsOH,[2] etc. Birault and his coworkers synthesized novel 3-amino pyrazolo-[1,5-*a*]-pyridines for dyeing keratinous fibers containing 3-amino-pyrazolo-[1,5-*a*]-pyridines.

Quinoline-based pyrazoles have been reported as potential antibacterial and antifungal agents.[3] Akbaş and Berber (2005) reported the synthesis of several new pyrazolo-[3,4-*d*]-pyridazines by reacting 1*H*-pyrazole-3-carboxylic acid with various hydrazines. The synthesized compounds showed antimicrobial activities against gram-negative, gram-positive bacteria and fungi.[4] In another study, a series of 3-phenylpyrazolo [1,5-*a*] pyrimidines were prepared and found to have great affinity toward human corticotropin-releasing factor (CRF-1) receptor.[5] In other studies, pyrazolo derivatives were found as kinase inhibitors, for example, pyrazolo-[1,5-*a*]-pyridines inhibited rearranged during transfection (RET) kinase[6], pyrazolo-pyrimidine derivatives inhibited Bruton's tyrosine kinase,[7] etc. Senga[8] et al has reported the synthesis and antischistosomal activity of certain pyrazolo[1,5-*a*]-pyrimidines. Pyrazolo-[3,4-*d*]-pyrimidines are reported by Hubbard et al as potent inhibitors of the insulin-like growth factor receptor.[9]

Morpholine is a unique heterocyclic compound having oxygen and nitrogen atoms. Being able to make three and four bonds, nitrogen atom of the morpholine can be used to derivative it or bond to other organic compounds. Additionally, morpholine also plays as a ligand to make complexes with the transition metals. Morpholine itself has been used as a template in the synthesis of small pore SAPO-34 molecular sieve.[10] Morpholine derivatives have been reported as biologically active against various organisms. Penneerselvam et al reported the synthesis of 4-(4-aminophenyl)-morpholine-based Schiff bases as potential antimicrobial agents.[11] Daniel and his team developed a method for crosslinking hydrogels using morpholine-2,3-diones.[12] In another interesting study, Knapp and Gally degraded morpholine microbially.[13] Morpholines[14] and thiomorpholines[15] have also been reported as prodrugs; useful as tachykinin receptor antagonists. Chrysselis et al reported the hypocholesterolemic and hypolipidemic activities of certain novel 2-biphenylyl morpholine derivatives with antioxidant activity.[16] Hale and coworkers studied the phosphorylated morpholine acetal as human neurokinin-1 receptor antagonists and described it as water-soluble prodrug.[17]

Piperidine is a basic organic solvent, sometimes also used as a cosolvent and a catalyst. Literature shows that piperidine is mainly used in the synthesis of alkaloids,[18] although there are certain piperidine-based natural alkaloids presents in plants.[19] Piperidine derivatives have been used in various biological activities. Fur and Uzan observed a release of noradrenaline, dopamine, and 5-hydroxytryptamine when they applied 4-(3-indolylalkyl)piperidine derivatives to the rat brain synaptosomes, rat heart, and human blood platelets.[20] Orjales et al synthesized and evaluated their binding studies of [(Aryl)(aryloxy)methyl]piperidine derivatives and found them as potential antidepressant drugs having high affinity toward serotonin (5-HT) and norepinephrine (NE) transporters.[21] Cain and coworkers obtained a patent about the synthesis of piperidine ether derivatives and their usage as psychotropic drugs as well as plant fungicides.[22] Aridos and his team also reported the synthesis, antibacterial, and antitubercular studies of piperidin-4-one and tetrahydropyridine derivatives.[23]

The earlier discussion reflects that morpholines, pyrazoles, and piperidines all are very versatile and can be used in wide range of fields. Herein, we report the synthesis, antioxidant and anticholinesterase activities of



SCHEME 1 Schematic diagram for the preparation of target molecules

TABLE 1 Antioxidant activities of the synthesized compounds[a]

Compound	β-Carotene/linoleic acid assay IC_{50} (μM)	DPPH assay IC_{50} (μM)	ABTS + Assay IC_{50} (μM)	CUPRAC $A_{0.5}$ (μM)
1a	66.41 ± 0.51	73.46 ± 1.26	75.77 ± 1.19	76.36 ± 0.02
1b	63.19 ± 0.46	71.14 ± 0.38	75.01 ± 1.43	70.74 ± 0.02
1c	60.97 ± 1.44	70.07 ± 0.49	74.16 ± 0.60	66.64 ± 0.00
1d	59.13 ± 0.61	69.13 ± 1.14	70.18 ± 0.72	62.62 ± 0.00
1e	54.77 ± 0.22	66.27 ± 0.19	68.19 ± 0.49	60.21 ± 0.00
1f	50.01 ± 0.15	64.18 ± 0.16	63.72 ± 0.45	57.12 ± 0.00
2a	57.64 ± 0.27	76.16 ± 0.27	59.60 ± 0.41	69.40 ± 0.00
2b	53.71 ± 0.77	73.75 ± 1.51	53.57 ± 0.38	66.91 ± 0.00
2c	49.12 ± 0.32	70.06 ± 0.56	51.53 ± 0.31	64.40 ± 0.00
2d	42.66 ± 0.63	68.44 ± 0.43	48.70 ± 0.27	59.88 ± 0.00
2e	39.27 ± 0.93	65.21 ± 0.47	43.55 ± 0.09	56.29 ± 0.00
2f	32.11 ± 0.19	63.63 ± 0.18	40.42 ± 0.15	54.41 ± 0.00
3a	50.08 ± 0.28	72.64 ± 0.73	56.26 ± 0.28	72.74 ± 0.00
3b	48.48 ± 0.42	70.14 ± 0.50	54.29 ± 0.34	69.30 ± 0.04
3c	41.44 ± 0.73	66.19 ± 0.12	51.50 ± 0.36	60.25 ± 0.01
3d	39.41 ± 0.22	64.15 ± 0.18	46.91 ± 0.43	58.19 ± 0.01
3e	36.00 ± 0.36	65.03 ± 0.06	43.44 ± 1.77	53.13 ± 0.04
3f	35.69 ± 0.47	62.65 ± 0.78	41.45 ± 0.79	51.54 ± 0.02
4a	45.19 ± 0.55	73.46 ± 0.65	54.73 ± 0.36	61.42 ± 0.00
4b	41.48 ± 0.33	69.81 ± 0.61	51.10 ± 0.46	55.60 ± 0.00
4c	33.47 ± 0.16	67.52 ± 0.63	49.28 ± 0.16	50.11 ± 0.00
4d	28.11 ± 0.67	65.13 ± 0.55	46.40 ± 0.06	48.16 ± 0.01
4e	27.57 ± 0.50	62.42 ± 1.29	40.74 ± 0.10	47.06 ± 0.02
4f	21.42 ± 0.94	60.66 ± 1.57	36.35 ± 0.99	44.12 ± 0.00
BHT[b]	2.40 ± 0.15	54.72 ± 0.61	2.93 ± 0.67	3.98 ± 0.01
α-TOC[b]	4.55 ± 0.23	12.24 ± 0.18	4.98 ± 0.42	40.46 ± 0.02

Abbreviation: BHT, butylated hydroxyl toluene.

[a]Values expressed are means ± SD of three parallel measurements. $p < .05$, significantly different with student's *t*-test.

[b]Reference compounds.

piperidin-morpholin-based pyrazoles. The aim of this study was to obtain new chemically and biologically active pyrazoles, piperidines and morpholines derivatives in the one structure. The synthesized pyrazolo derivatives are expected to be utilized in the future in the development of new drugs

2 | RESULTS AND DISCUSSION

2.1 | Synthesis

The synthetic route to prepare the target molecules is given in Scheme 1. The materials 2,4-thiazolidinedione and rhodanine were selected as starting materials. (4-piperidin-1-yl)benzaldehyde or (4-morpholin-1-yl)benzaldehyde were reacted with 5-(4-piperidine-1-yl)benzylidene) thiazolidine-2,4-dione **(1–2)** and 5-(4-morpholin-1-yl)benzylidene)-2-thioxothiazolidin-4-one **(3–4)** in piperidine and ethanol *via* Knoevenegal Condensation. After that, 3-(4-substitutedphenyl)-2-(4-(substituted)-3,3a-dihydro-2*H*-pyrazolo [3,4-*d*]thiazol-5(6*H*)-one **(1–2)(a-f)** and 3-(4-substitutedphenyl)-2-(4-(substituted)-3,3a-dihydro-2*H*-pyrazolo [3,4-*d*]thiazol-5(6*H*)-thione **(3–4)(a-f)** were obtained from reaction 5-(4-piperidin-1-yl)benzylidene)thiazolidin-2,4-dione **(1–2)** and 5-(4-morpholin-1-yl)benzylidene)-2-thioxothiazolidin-4-one **(3–4)** with different hydrazines.

In the FTIR, stretching bands of N—H and C═O groups related to **(1–3)(a-f)** and stretching bands of N—H and C═S groups **(2–4)(a-f)** were observed at

TABLE 2 Anticholinesterase activities of the synthesized compounds[a]

Compound	AChE (IC_{50} μM)	BChE (IC_{50} μM)
1a	72.90 ± 0.73	66.72 ± 0.92
1b	70.29 ± 0.91	60.19 ± 0.96
1c	67.03 ± 0.44	58.14 ± 0.38
1d	64.66 ± 0.33	55.53 ± 0.25
1e	62.97 ± 0.17	53.04 ± 0.24
1f	60.61 ± 0.11	49.51 ± 0.61
2a	69.25 ± 1.23	60.63 ± 0.76
2b	67.83 ± 0.46	56.41 ± 0.55
2c	65.13 ± 0.19	53.19 ± 0.52
2d	62.42 ± 0.49	51.54 ± 0.19
2e	59.89 ± 0.61	47.78 ± 0.38
2f	56.65 ± 0.48	46.42 ± 0.27
3a	57.12 ± 0.46	65.27 ± 0.26
3b	54.50 ± 0.31	63.19 ± 1.16
3c	49.51 ± 0.63	61.06 ± 0.57
3d	46.79 ± 0.20	52.58 ± 0.64
3e	42.46 ± 0.06	49.49 ± 0.63
3f	39.45 ± 0.78	45.48 ± 0.46
4a	49.47 ± 0.43	60.61 ± 0.91
4b	43.64 ± 0.42	56.22 ± 0.48
4c	39.28 ± 0.34	54.18 ± 0.67
4d	37.98 ± 0.18	50.26 ± 0.35
4e	32.18 ± 0.67	44.80 ± 0.44
4f	30.54 ± 0.45	40.12 ± 0.25
Galantamine[b]	4.48 ± 0.78	46.03 ± 0.14

[a]Values expressed are means ± SD of three parallel measurements. $p < 0.05$, significantly different with student's *t*-test.
[b]Reference compounds.

3278–2945, 1686–1670, and 3242–3105, 1391–1331 cm^{-1}, respectively. In pyrazolo derivatives **(1–4)(a-f)**, the stretching bands of C═S in compound **(3–4)** with one C═O group in the 2,4-thiazolidinedione ring in compound **(1–2)** and the presence of the C═N stretching bands observed in 1599–1570 cm^{-1} proved the synthesis of pyrazolo **(1–4)(a-f)**.

In the ^{1}H NMR spectrum, the proton peak of the —CH═C— group after Knoevenagel condensation was found at 7.75–7.82 ppm. The presence of proton peak of —CH═C— in compound **(1–4)** and the proton peak of the pyrazolo (—CH—CH—) at 4.07–4.44 ppm also supported the synthesis of the desired products. In the ^{13}C NMR spectrum of the target products, the disappearance of the C═O group in the starting material by pyrazole formation was another proof that the target products were obtained.

2.2 | Antioxidant activities of the synthesized compounds

The synthesized compounds were screened for their antioxidant activity using four different assays (Table 1) where α-tocopherol (α-TOC) and butylated hydroxyl toluene (BHT) were used as the positive standards. As the results showed, compounds **4f**, **4e**, **4d**, **2f**, **4c**, and **3f** were most active in the lipid peroxidation inhibitory activity, while in DPPH free scavenging activity assay, compounds **4f**, **4e**, **3f**, **2f**, **3d**, **1f**, **3e**, **4d**, and **2e** were most active. On the other hand, compounds **4f**, **2f**, and **4e** exhibited higher cation radical scavenging activity in $ABTS^{\cdot+}$, while in CUPRAC reducing assay, compounds **4f**, **4e**, **4d**, and **4c** were found on the top.

2.3 | Anticholinesterase activities of the synthesized compounds

All 24 pyrazolo derivatives were tested for their *in vitro* anticholinesterase inhibitory activity against AChE and BChE enzymes whereas the results were compared with those of galantamine as a standard (Table 2). The IC_{50} values of all of the tested compounds were lower than 75 μM in the inhibition of both enzymes. Compound **4f** (IC_{50}: 30.54 ± 0.45 μM) exhibited the highest activity followed by **4e**, **4d**, **4c**, and **3f**. In the BChE inhibitory assay, **4f** (IC_{50}: 40.12 ± 0.25 μM), **4e** (IC_{50}: 44.80 ± 0.44 μM), and **3f** (IC_{50}: 45.48 ± 0.46 μM) displayed higher activity than galantamine (IC_{50}: 46.03 ± 0.14 μM).

3 | CONCLUSIONS

Twenty-four pyrazolo compounds (1–4)(a-f) were synthesized in this study and subjected to *in vitro* antioxidant and anticholinesterase inhibitory activities. The pyrazolo derivatives, which were condensed with the 2-thioxothiazolidine (2–4), were more active than the ones condensed with the thiazolidin-2-on (1–3). Heterocyclic pyrazolo derivatives (3–4) containing the morpholino group were found more active than the pyrazolos containing piperidinyl group (1–2). The —CF_3, —OCF_3, and —NO_2 containing pyrazolo derivatives were more effective than other functional groups. Compound 4f was most active in the β-carotene-linoleic acid, DPPH free-radical scavenging, ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) cation radical scavenging, and CUPRAC (Copper (II) Ion

Reduction Antioxidant Capacity) capacity activity assays; still less active than the standards.

In the AChE enzyme inhibition activity assay of the pyrazolo derivatives, 4f (IC_{50}: 30.54 ± 0.45 μM) was the most active, but lower than galantamine while both 4f (IC_{50}: 40.11 ± 0.25 μM) and 4e (IC_{50}: 44.80 ± 0.44 μM) were most active in BChE inhibition; even higher than galantamine (IC_{50}: 46.03 ± 0.14 μM). As compounds 4f and 4e were both more active than the standards in antioxidant and anticholinesterase activity, we suggest them as new standards for these activities

4 | EXPERIMENTAL SECTION

4.1 | Chemicals and instruments

Ethanol (EtOH), piperidine, hydrazine monohydrate, acetic acid, sodium hydrogen phosphate, sodium dihydrogen phosphate, sodium hydroxide, and sodium acetate were obtained from E. Merck (Darmstadt, Germany); 4-iodophenylhydrazine, 4-(trifluoromethyl)phenylhydrazine, 4-nitrophenylhydrazine, phenylhdyrazine, thiazolidine-2,4-dione, and 4-(trifluoromethoxy)phenylhydrazine were obtained from Alfa Aesar Co., Inc.; 2-thioxothiazolidin-4-one and 4-(1-pyrrolidinyl)benzaldehyde were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany).

The progress of the reactions and the product formations was monitored using thin layer chromatography. Products were purified by crystallization (trial and error method) using various solvents. The FTIR spectra of the synthesized compounds were recorded on Perkin Elmer 1620 and Shimadzu IR-8400 spectrophotometer. Elemental analyses (C, H, N, and S) were performed on a VarioMICRO, Elementar Analysen Systeme, GmbH, Hanau, Germany. ^{1}H NMR spectra were recorded on a Bruker Avance-DPX-400 spectrometer (BioSpin, Billerca, USA) in DMSO-d6, while ^{13}C NMR spectra were recorded on 150 MHz NMR Agilent Technologies. Bioactivity assays were carried out on a 96-well microplate reader, SpectraMax 340PC384, Molecular Devices (USA).

4.2 | Synthesis

4.2.1 | General synthetic procedure of compounds (1–4)

A mixture of thiazolidine-2,4-dione (20 mmol) or 2-thioxothiazolidin-4-one (20 mmol), 4-(1-substitued) benzaldehyde (20 mmol), piperidine (16 mmol) was refluxed in EtOH (50 mL) for 24 hours. The reaction mixture was poured into H_2O and acidified with glacial AcOH that provided compounds **1–4** as solids, which were recrystallized from ethanol.[24]

4.3 | 5-(4-(piperidin-1-yl)benzylidene) thiazolidine-2,4-dione (1)

Yellow crystals; yield 72%; mp. 143.1°C; IR (ν_{max}, cm^{-1}): 3284, 3242 (N—H), 2928 (C—H of the piperidinyl ring), 1672, 1635 (C═O), 1504, 1450, 1406 (C═C); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.51 (m, 4H, —CH_2—C**H**$_2$—CH_2—N—), 1.60 (m, 2H, —C**H**$_2$—CH_2—CH_2—N—), 3.47 (t, 4H, —CH_2—CH_2—C**H**$_2$—N—), 6.72 (d, J_1 = *12 Hz*, 2H, *ortho* position of pyrrolidinyl —C**H**—), 7.76 (d, J_1 = *12 Hz*, 2H, *meta* position of pyrrolidinyl —C**H**—), 7.78 (s, 1H, —C**H**—), 12.45 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 24.7 (C_1), 25.8 (C_2), 55.8 (C_3), 112.1 (C_5), 116.4 (C_9), 125.2 (C_7), 130.0 (C_6), 144.6 (C_8), 149.9 (C_4), 168.1 (C_{10}), 170.2 (C_{11}); Anal. Calcd. for molecular formula $C_{15}H_{16}N_2O_2S$: C, 62.48; H, 5.59; N, 9.71; S, 11.12%. Found: C, 62.39; H, 5.62; N, 9.68; S, 11.16%.

4.4 | 5-(4-(piperidin-1-yl)benzylidene)-2-thioxothiazolidin-4-one (2)

Yellow crystals; yield 77%; mp. 152.1°C; IR (ν_{max}, cm^{-1}): 3270, 3228 (N—H), 2928 (C—H of the piperidinyl ring), 1654 (C═O), 1512, 1444, 1400 (C═C), 1380 (C═S); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.60 (m, 4H, —CH_2—C**H**$_2$—CH_2—N—), 1.68 (m, 2H, —C**H**$_2$—CH_2—CH_2—N—), 3.50 (t, 4H, —CH_2—CH_2—C**H**$_2$—N—), 6.77 (d, J_1 = *12 Hz*, 2H, *ortho* position of pyrrolidinyl —C**H**—), 7.76 (d, J_1 = *12 Hz*, 2H, *meta* position of pyrrolidinyl —C**H**—), 7.82 (s, 1H, —C**H**—), 12.49 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 24.7 (C_1), 25.8 (C_2), 55.2 (C_3), 112.0 (C_5), 116.8 (C_9), 124.4 (C_7), 129.0 (C_6), 144.7 (C_8), 149.2 (C_4), 168.9 (C_{10}), 194.2 (C_{11}); Anal. Calcd. for molecular formula $C_{15}H_{16}N_2OS_2$: C 59.18; H, 5.30; N, 9.20; S, 21.07%. Found: C, 59.20; H, 5.32; N, 9.28; S, 21.06%.

4.5 | 5-(4-morpholinobenzyliden) thiazolidin-2,4-dione (3)

Yellow crystals; yield 66%; mp. 150.9°C; IR (ν_{max}, cm^{-1}): 3225 (N—H), 2962 (C—H of the morpholinyl ring), 1670, 1635 (C═O), 1533, 1500, 1450 (C═C); ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.26 (t, 4H, —O—CH_2—C**H**$_2$—N—), 3.71 (t, 4H, —O—C**H**$_2$—CH_2—N—), 7.04 (d, 2H, *J = 12 Hz*, *ortho* position of morpholinyl —C**H**—), 7.44 (d, 2H, *J = 12 Hz*, *meta* position of morpholinyl —C**H**—),

7.67 (s, 1H, —C**H**—), 12.37 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 55.7 (C2), 66.8 (C1), 112.4 (C4), 116.5 (C8), 126.4 (C6), 130.0 (C5), 144.2 (C7), 150.2 (C3), 167.2 (C9), 167.8 (C10); Anal. Calcd. for molecular formula $C_{14}H_{14}N_2O_3S$: C 57.92; H, 4.86; N, 9.65; S, 11.04%. Found: C, 57.97; H, 4.81; N, 9.72; S, 11.09%.

4.6 | 5-(4-morpholinobenzyliden)-2-thioxothiazolidin-4-one (4)

Yellow crystals; yield 61%; mp. 155.6°C; IR (ν_{max}, cm^{-1}): 3212 (N—H), 2928 (C—H of the morpholinyl ring), 1652 (C═O), 1538, 1490, 1442 (C═C); ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.20 (t, 4H, —O—CH$_2$—C**H$_2$**—N—), 3.70 (t, 4H, —O—C**H$_2$**—CH$_2$—N—), 6.74 (d, 2H, *J = 12 Hz*, *ortho* position of morpholinyl —C**H**—), 7.44 (d, 2H, *J = 12 Hz*, *meta* position of morpholinyl —C**H**—), 7.80 (s, 1H, —C**H**—), 12.57 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 55.0 (C2), 66.5 (C1), 112.1 (C4), 116.8 (C8), 125.2 (C6), 129.1 (C5), 144.8 (C7), 149.5 (C3), 168.9 (C9), 194.0 (C10); Anal. Calcd. for molecular formula $C_{14}H_{14}N_2O_2S_2$: C 54.88; H, 4.61; N, 9.14; S, 20.93%. Found: C, 54.77; H, 4.71; N, 9.12; S, 20.79%.

4.6.1 | General synthesis of pyrazolo derivatives (1–4)(a-f)

A mixture of 5 mmol 5-(4-(pyrrolidin-1-yl)benzylidene) thiazolidin-2,4-dione (**1**) or 5-(4-(pyrrolidin-1-yl) benzylidene)-2-thioxothiazolidin-4-one (**2**) with different hydrazines (5 mmol) was refluxed with 2.5 mmol sodium acetate in absolute ethanol (20 mL). The obtained product was separated, washed, and crystallized from glacial AcOH.[25]

4.7 | 3-(4-(piperidin-1-yl)phenyl)-3,3a-dihydro-2H-pyrazolo[3,4-d] thiazol-5(6H)-one (1a)

Yellow solid; yield 33%; mp. 153.8°C; IR (ν_{max}, cm^{-1}): 3278, 3236 (N—H), 2922 (C—H of the piperidinyl ring), 1670 (C═O), 1590 (C═N); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.65 (m, 4H, —CH$_2$—C**H$_2$**—CH$_2$—N—), 1.88 (m, 2H, —C**H$_2$**—CH$_2$—CH$_2$—N—), 3.51 (t, 4H, —CH$_2$—CH$_2$—C**H$_2$**—N—), 4.21 (dd, 1H, *J$_1$ = 4 Hz*, *J$_2$ = 10 Hz*, —C**H**—CH—S—), 4.38 (d, 1H, *J$_1$ = 4 Hz*, —CH—C**H**—S—), 6.77 (d, 2H, *J$_1$ = 12 Hz*, *ortho* position of piperidinyl —C**H**—), 7.20 (d, *J$_1$ = 12 Hz*, 2H, *meta* position of piperidinyl —C**H**—), 11.65 (d, 1H, *J$_1$ = 10 Hz*, —CH—N**H**—), 13.01 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 26.6 (C$_1$), 27.8 (C$_2$), 51.4 (C$_8$), 56.7 (C$_3$), 58.9 (C$_9$), 114.9 (C$_5$), 132.5 (C$_6$), 138.1 (C$_7$), 154.6 (C$_4$), 160.1 (C$_{10}$), 201.2 (C$_{11}$); Anal. Calcd. for molecular formula $C_{15}H_{18}N_4OS$: C, 59.58; H, 6.00; N, 18.53; S, 10.60%. Found: C 60.33; H, 6.13; N, 18.52; S, 11.11%.

4.8 | 2-phenyl-3-(4-(piperidin-1-yl) phenyl)-3,3a-dihydro-2H-pyrazolo[3,4-d] thiazol-5(6H)-one (1b)

Yellow solid; yield 27%; mp. 158.6°C; IR (ν_{max}, cm^{-1}): 3278, 3230 (N—H), 2927 (C—H of the piperidinyl ring), 1674 (C═O), 1595 (C═N); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.60 (m, 4H, —CH$_2$—C**H$_2$**—CH$_2$—N—), 1.66 (m, 2H, —C**H$_2$**—CH$_2$—CH$_2$—N—), 3.72 (t, 4H, —CH$_2$—CH$_2$—C**H$_2$**—N—), 3.90 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 4.16 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 6.78 (d, 2H, *J = 7.2 Hz*, *ortho* position of piperidinyl —C**H**—), 6.88 (dd, 1H, *J$_1$ = 12 Hz*, *J$_2$ = 12 Hz*, *para* position of *N*-phenyl —C**H**—), 6.99 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 7.32 (d, 2H, *J = 7.2 Hz*, *meta* position of piperidinyl —C**H**—), 7.40 (dd, 2H, *J$_1$ = 8 Hz*, *J$_2$ = 12 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.60 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 26.0 (C$_1$), 27.5 (C$_2$), 55.6 (C$_9$), 59.5 (C$_3$), 64.4 (C$_8$), 114.3 (C$_5$), 120.8 (C$_{13}$), 124.8 (C$_{15}$), 133.6 (C$_6$), 136.6 (C$_{14}$), 138.1 (C$_7$), 146.6 (C$_{12}$), 154.2 (C$_4$), 166.3 (C$_{10}$), 175.0 (C$_{11}$); Anal. Calcd. for molecular formula $C_{21}H_{22}N_4OS$: C, 66.64; H, 5.86; N, 14.80; S, 8.47%. Found: C, 67.72; H, 5.92; N, 15.00; S, 8.40%.

4.9 | 2-(4-iodophenyl)-3-(4-(piperidin-1-yl)phenyl)-3,3a-dihydro-2H-pyrazolo [3,4-d]thiazol-5(6H)-one (1c)

Yellow solid; yield 38%; mp. 166.1°C; IR (ν_{max}, cm^{-1}): 3272, 3241 (N—H), 2927 (C—H of the piperidinyl ring), 1671 (C═O), 1593 (C═N); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.92 (m, 4H, —CH$_2$—C**H$_2$**—CH$_2$—N—), 1.98 (m, 2H, —C**H$_2$**—CH$_2$—CH$_2$—N—), 3.76 (t, 4H, —CH$_2$—CH$_2$—C**H$_2$**—N—), 4.18 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.26 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.78 (d, 2H, *J = 12 Hz*, *ortho* position of piperidinyl —C**H**—), 7.00 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 7.24 (d, 2H, *J = 12 Hz*, *meta* position of piperidinyl —C**H**—), 7.37 (d, 2H, *J = 8 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.79 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 26.4 (C$_1$), 28.8 (C$_2$), 56.1 (C$_9$), 59.4 (C$_3$), 62.2 (C$_8$), 80.8 (C$_{14}$), 115.8 (C$_5$), 119.6 (C$_{13}$), 133.1 (C$_6$), 139.8 (C$_7$), 142.6 (C$_{12}$), 152.8 (C$_4$), 163.2 (C$_{10}$), 179.4 (C$_{11}$); Anal. Calcd. for molecular formula

$C_{21}H_{21}IN_4OS$: C, 50.01; H, 4.20; N, 11.11; S, 6.36%. Found: C, 50.17; H, 3.99; N, 11.18; S, 6.44%.

4.10 | 3-(4-(piperidin-1-yl)phenyl)-2-(4-(trifluoromethyl)phenyl)-3,3a-dihydro-2H-pyrazolo [3,4-d] thiazole-5(6H)-one (1d)

Yellow solid; yield 30%; mp. 157.4°C; IR (ν_{max}, cm^{-1}): 3263, 3230 (N—H), 2930 (C—H of the piperidinyl ring), 1680 (C═O), 1580 (C═N); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.56 (m, 4H, —CH_2—C**H**$_2$—CH_2—N—), 1.64 (m, 2H, —C**H**$_2$—CH_2—CH_2—N—), 3.56 (t, 4H, —CH_2—CH_2—C**H**$_2$—N—), 4.08 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.16 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.66 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 6.82 (d, 2H, *J = 12 Hz*, *ortho* position of piperidinyl —C**H**—), 7.24 (d, 2H, *J = 12 Hz*, *meta* position of piperidinyl —C**H**—), 7.55 (d, 2H, *J = 8 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.72 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 26.4 (C_1), 28.8 (C_2), 55.6 (C_9), 57.1 (C_3), 61.0 (C_8), 113.6 (C_5), 115.7 (C_{13}), 129.4 (C_{16}), 130.9 (C_{15}), 131.8 (C_{14}), 134.3 (C_6), 139.8 (C_7), 149.4 (C_{12}), 156.6 (C_4), 160.0 (C_{10}), 175.4 (C_{11}); Anal. Calcd. for molecular formula $C_{22}H_{21}F_3N_4OS$: C, 59.18; H, 4.73; N, 12.55; S, 7.18%. Found: C, 58.88; H, 4.85; N, 13.08; S, 7.33%.

4.11 | 3-(4-(piperidin-1-yl)phenyl)-2-(4-(trifluoromethoxy)phenyl)-3,3a-dihydro-2H-pyrazolo [3,4-d] thiazole-5(6H)-one (1e)

Yellow solid; yield 36%; mp. 155.0°C; IR (ν_{max}, cm^{-1}): 3269, 3236 (N—H), 2936 (C—H of the piperidinyl ring), 1686 (C═O), 1586 (C═N); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.60 (m, 4H, —CH_2—C**H**$_2$—CH_2—N—), 1.68 (m, 2H, —C**H**$_2$—CH_2—CH_2—N—), 3.66 (t, 4H, —CH_2—CH_2—C**H**$_2$—N—), 4.12 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.28 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.88 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 6.94 (d, 2H, *J = 12 Hz*, *ortho* position of piperidinyl —C**H**—), 7.12 (d, 2H, *J = 12 Hz*, *meta* position of piperidinyl —C**H**—), 7.30 (d, 2H, *J = 8 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.89 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 26.0 (C_1), 27.2 (C_2), 54.5 (C_3), 56.7 (C_9), 63.4 (C_8), 114.4 (C_5), 116.9 (C_{13}), 117.5 (C_{14}), 130.5 (C_6), 130.2 (C_{16}), 135.6 (C_7), 140.4 (C_{12}), 144.4 (C_{15}), 153.1 (C_4), 160.2 (C_{10}), 188.4 (C_{11}); Anal. Calcd. for molecular formula $C_{22}H_{21}F_3N_4O_2S$: C, 57.13; H, 4.58; N, 12.11; S, 6.93%. Found: C, 57.22; H, 4.66; N, 12.44; S, 7.01%.

4.12 | 2-(4-nitrophenyl)-3-(4-(piperidin-1-yl)phenyl)-3,3a-dihydro-2H-pyrazolo [3,4-d] thiazol-5(6H)-one (1f)

Yellow solid; yield 24%; mp. 161.1°C; IR (ν_{max}, cm^{-1}): 3272, 3233 (N—H), 2931 (C—H of the piperidinyl ring), 1682 (C═O), 1581 (C═N); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.58 (m, 4H, —CH_2—C**H**$_2$—CH_2—N—), 1.63 (m, 2H, —C**H**$_2$—CH_2—CH_2—N—), 3.58 (t, 4H, —CH_2—CH_2—C**H**$_2$—N—), 4.20 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.28 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.70 (d, 2H, *J = 12 Hz*, *ortho* position of piperidinyl —C**H**—), 7.16 (d, 2H, *J = 12 Hz*, *meta* position of piperidinyl —C**H**—), 7.28 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 8.00 (d, 2H, *J = 8 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.70 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 27.0 (C_1), 29.4 (C_2), 55.4 (C_9), 57.0 (C_3), 62.0 (C_8), 110.8 (C_{13}), 114.7 (C_5), 129.2 (C_{14}), 131.7 (C_6), 138.5 (C_7), 140.0 (C_{15}), 150.6 (C_{12}), 152.5 (C_4), 158.4 (C_{10}), 176.0 (C_{11}); Anal. Calcd. for molecular formula $C_{21}H_{21}N_5O_3S$: C, 59.56; H, 5.00; N, 16.54; S, 7.57%. Found: C, 60.08; H, 5.14; N, 16.95; S, 7.88%.

4.13 | 3-(4-(piperidin-1-yl)phenyl)-3,3a-dihydro-2H-pyrazolo[3,4-d] thiazol-5(6H)-thione (2a)

Yellow solid; yield 34%; mp. 149.8°C; IR (ν_{max}, cm^{-1}): 3262, 3234 (N—H), 2928 (C—H of the piperidinyl ring), 1580 (C═N), 1387 (C═S); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.65 (m, 4H, —CH_2—C**H**$_2$—CH_2—N—), 1.88 (m, 2H, —C**H**$_2$—CH_2—CH_2—N—), 3.51 (t, 4H, —CH_2—CH_2—C**H**$_2$—N—), 4.21 (dd, 1H, J_1 = *4 Hz*, J_2 = *10 Hz*, —C**H**—CH—S—), 4.38 (d, 1H, J_1 = *4 Hz*, —CH—C**H**—S—), 6.77 (d, 2H, J_1 = *12 Hz*, *ortho* position of piperidinyl —C**H**—), 7.20 (d, J_1 = *12 Hz*, 2H, *meta* position of piperidinyl —C**H**—), 11.65 (d, 1H, J_1 = *10 Hz*, —CH—N**H**—), 13.01 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 25.4 (C_1), 26.6 (C_2), 52.8 (C_8), 56.6 (C_3), 61.2 (C_9), 114.1 (C_5), 131.2 (C_6), 136.4 (C_7), 152.2 (C_4), 161.5 (C_{10}), 204.8 (C_{11}); Anal. Calcd. for molecular formula $C_{15}H_{18}N_4S_2$: C, 56.57; H, 5.70; N, 17.59; S, 20.04%. Found: C, 57.01; H, 5.73; N, 18.08; S, 21.03%.

4.14 | 2-phenyl-3-(4-(piperidin-1-yl) phenyl)-3,3a-dihydro-2H-pyrazolo [3,4-d] thiazol-5(6H)-thione (2b)

Yellow solid; yield 39%; mp. 151.4°C; IR (ν_{max}, cm^{-1}): 3251, 3240 (N—H), 2977 (C—H of the piperidinyl ring),

1591 (C═N), 1391 (C═S); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.64 (m, 4H, —CH_2—C**H$_2$**—CH_2—N—), 1.72 (m, 2H, —C**H$_2$**—CH_2—CH_2—N—), 3.86 (t, 4H, —CH_2—CH_2—C**H$_2$**—N—), 4.25 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.29 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.80 (d, 2H, *J = 7.2 Hz*, *ortho* position of piperidinyl —C**H**—), 6.92 (dd, 1H, J_1 *= 12 Hz*, J_2 *= 12 Hz*, *para* position of *N*-phenyl —C**H**—), 7.12 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 7.40 (d, 2H, *J = 7.2 Hz*, *meta* position of piperidinyl —C**H**—), 7.46 (dd, 2H, J_1 *= 8 Hz*, J_2 *= 12 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.64 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 28.2 (C_1), 29.6 (C_2), 58.4 (C_9), 60.4 (C_3), 64.4 (C_8), 116.0 (C_5), 124.1 (C_{13}), 126.6 (C_{15}), 136.3 (C_6), 139.8 (C_{14}), 142.3 (C_7), 149.0 (C_{12}), 155.3 (C_4), 168.0 (C_{10}), 177.7 (C_{11}); Anal. Calcd. for molecular formula $C_{21}H_{22}N_4S_2$: C, 63.93; H, 5.62; N, 14.20; S, 16.25%. Found: C, 64.82; H, 5.77; N, 14.01; S, 16.33%.

4.15 | 2-(4-iodophenyl)-3-(4-(piperidin-1-yl)phenyl)-3,3a-dihydro-2H-pyrazolo[3,4-d]thiazole-5(6H)-thione (2c)

Yellow solid; yield 31%; mp. 157.6°C; IR (ν_{max}, cm^{-1}): 3244, 3221 (N—H), 2936 (C—H of the piperidinyl ring), 1590 (C═N), 1391 (C═S); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.66 (m, 4H, —CH_2—C**H$_2$**—CH_2—N—), 1.78 (m, 2H, —C**H$_2$**—CH_2—CH_2—N—), 3.62 (t, 4H, —CH_2—CH_2—C**H$_2$**—N—), 3.88 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.24 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.60 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 6.80 (d, 2H, *J = 12 Hz*, *ortho* position of piperidinyl —C**H**—), 7.32 (d, 2H, *J = 12 Hz*, *meta* position of piperidinyl —C**H**—), 7.48 (d, 2H, *J = 8 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.85 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 27.5 (C_1), 29.4 (C_2), 55.9 (C_3), 58.4 (C_9), 63.6 (C_8), 79.8 (C_{14}), 114.4 (C_5), 118.1 (C_{13}), 136.4 (C_6), 140.4 (C_7), 143.2 (C_{12}), 155.8 (C_4), 161.4 (C_{10}), 201.6 (C_{11}); Anal. Calcd. for molecular formula $C_{21}H_{21}IN_4S_2$: C, 48.46; H, 4.07; N, 10.77; S, 12.32%. Found: C, 49.06; H, 3.95; N, 10.89; S, 12.21%.

4.16 | 3-(4-(piperidin-1-yl)phenyl)-2-(4-trifluoromethyl)phenyl)-3,3a-dihydro-2H-pyrazolo[3,4-d]thiazole-5(6H)-thione (2d)

Yellow solid; yield 28%; mp. 152.4°C; IR (ν_{max}, cm^{-1}): 3250, 3242 (N—H), 2981 (C—H of the piperidinyl ring), 1595 (C═N), 1391 (C═S); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.56 (m, 4H, —CH_2—C**H$_2$**—CH_2—N—), 1.64 (m, 2H, —C**H$_2$**—CH_2—CH_2—N—), 3.56 (t, 4H, —CH_2—CH_2—C**H$_2$**—N—), 4.08 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.16 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.66 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 6.82 (d, 2H, *J = 12 Hz*, *ortho* position of piperidinyl —C**H**—), 7.24 (d, 2H, *J = 12 Hz*, *meta* position of piperidinyl —C**H**—), 7.55 (d, 2H, *J = 8 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.72 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ27.2 (C_1), 29.4 (C_2), 56.2 (C_3), 60.2 (C_9), 64.5 (C_8), 114.0 (C_5), 116.3 (C_{13}), 126.7 (C_{16}), 129.1 (C_{15}), 133.9 (C_{14}), 135.0 (C_6), 138.7 (C_7), 150.1 (C_{12}), 157.5 (C_4), 162.1 (C_{10}), 202.2 (C_{11}); Anal. Calcd. for molecular formula $C_{22}H_{21}F_3N_4S_2$: C, 57.13; H, 4.58; N, 12.11; S, 13.86%. Found: C, 57.26; H, 4.67; N, 12.47; S, 13.93%.

4.17 | 3-(4-(piperidin-1-yl)phenyl)-2-(4-(trifluoromethoxy)phenyl)-3,3a-dihydro-2H-pyrazolo[3,4-d]thiazole-5(6H)-thione (2e)

Yellow solid; yield 26%; mp. 157.3°C; IR (ν_{max}, cm^{-1}): 3252, 3233 (N—H), 2989 (C—H of the piperidinyl ring), 1599 (C═N), 1390 (C═S); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.62 (m, 4H, —CH_2—C**H$_2$**—CH_2—N—), 1.69 (m, 2H, —C**H$_2$**—CH_2—CH_2—N—), 3.60 (t, 4H, —CH_2—CH_2—C**H$_2$**—N—), 4.22 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.36 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.60 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 6.75 (d, 2H, *J = 12 Hz*, *ortho* position of piperidinyl —C**H**—), 7.00 (d, 2H, *J = 12 Hz*, *meta* position of piperidinyl —C**H**—), 7.22 (d, 2H, *J = 8 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.72 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 26.1 (C_1), 28.3 (C_2), 56.8 (C_3), 59.1 (C_9), 63.4 (C_8), 113.9 (C_5), 115.8 (C_{13}), 118.9 (C_{14}), 132.6 (C_6), 135.8 (C_{16}), 139.0 (C_7), 144.4 (C_{12}), 148.7 (C_{15}), 158.0 (C_4), 160.0 (C_{10}), 202.0 (C_{11}); Anal. Calcd. for molecular formula $C_{22}H_{21}F_3N_4OS_2$: C, 55.22; H, 4.42; N, 11.71; S, 13.40%. Found: C, 56.26; H, 4.50; N, 11.80; S, 13.55%.

4.18 | 2-(4-nitrophenyl)-3-(4-(piperidin-1-yl)phenyl)-3,3a-dihydro-2H-pyrazolo[3,4-d]thiazole-5(6H)-thione (2f)

Yellow solid; yield 21%; mp. 164.7°C; IR (ν_{max}, cm^{-1}): 3249, 3231 (N—H), 2977 (C—H of the piperidinyl ring), 1586 (C═N), 1391 (C═S); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.55 (m, 4H, —CH_2—C**H$_2$**—CH_2—N—), 1.62 (m, 2H, —C**H$_2$**—CH_2—CH_2—N—), 3.55 (t, 4H, —CH_2—CH_2—C**H$_2$**—N—), 4.11 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.23 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.75 (d, 2H, *J = 12 Hz*, *ortho* position of piperidinyl —C**H**—), 7.13 (d,

2H, *J = 12 Hz*, *meta* position of piperidinyl —C**H**—), 7.32 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 8.11 (d, 2H, *J = 8 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.74 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 25.9 (C_1), 27.8 (C_2), 56.1 (C_3), 58.7 (C_9), 63.6 (C_8), 112.8 (C_{13}), 115.1 (C_5), 126.4 (C_{14}), 130.0 (C_6), 135.2 (C_7), 139.2 (C_{15}), 150.4 (C_{12}), 154.1 (C_4), 158.4 (C_{10}), 204.1 (C_{11}); Anal. Calcd. for molecular formula $C_{21}H_{21}N_5O_2S_2$: C, 57.38; H, 4.82; N, 15.93; S, 14.59%. Found: C, 58.03; H, 4.94; N, 16.08; S, 14.44%.

4.19 | 3-(4-(morpholinophenyl)-3,3a, dihydro-2H-pyrazolo[3,4-d]thiazole-5(6H)-one (3a)

Yellow solid; yield 30%; mp. 154.7°C; IR (ν_{max}, cm^{-1}): 3234, 2975 (N—H), 2977 (C—H of the morpholinyl ring), 1672 (C═O), 1574 (C═N); ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.33 (d, 4H, —O—CH_2—C**H$_2$**—N—), 3.83 (d, 4H, —O—C**H$_2$**—CH_2—N—), 4.18 (dd, 1H, *J_1 = 4 Hz*, *J_2 = 10 Hz*, —C**H**—CH—S—), 4.21 (d, 1H, *J_1 = 4 Hz*, —CH—C**H**—S—), 6.78 (d, 2H, *J_1 = 12 Hz*, *ortho* position of morpholinyl —C**H**—), 7.24 (d, *J_1 = 12 Hz*, 2H, *meta* position of morpholinyl —C**H**—), 11.72 (d, 1H, *J_1 = 10 Hz*, —CH—N**H**—), 13.12 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 52.8 (C_7), 55.0 (C_8), 56.3 (C_2), 65.8 (C_1), 114.5 (C_4), 132.2 (C_5), 135.1 (C_6), 149.3 (C_3), 158.9 (C_9), 177.5 (C_{10}); Anal. Calcd. for molecular formula $C_{14}H_{16}N_4O_2S$: C, 55.25; H, 5.30; N, 18.41; S, 10.53%. Found: C, 56.08; H, 5.43; N, 18.58; S, 10.43%.

4.20 | 3-(4-morpholinophenyl)-2-phenyl-3,3a-dihydro-2H-pyrazolo[3,4-d]thiazole-5(6H)-one (3b)

Yellow solid; yield 36%; mp. 163.4°C; IR (ν_{max}, cm^{-1}): 3242, 2980 (N—H), 2945 (C—H of the morpholinyl ring), 1676 (C═O), 1571 (C═N); ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.25 (t, 4H, —O—CH_2—C**H$_2$**—N—), 3.80 (t, 4H, —O—C**H$_2$**—CH_2—N—), 4.24 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.30 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.85 (d, 2H, *J = 7.2 Hz*, *ortho* position of morpholinyl —C**H**—), 6.90 (dd, 1H, *J_1 = 12 Hz*, *J_2 = 12 Hz*, *para* position of *N*-phenyl —C**H**—), 7.02 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 7.24 (d, 2H, *J = 7.2 Hz*, *meta* position of morpholinyl —C**H**—), 7.40 (dd, 2H, *J_1 = 8 Hz*, *J_2 = 12 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.20 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 55.5 (C_8), 58.6 (C_2), 60.0 (C_7), 68.9 (C_1), 118.6 (C_4), 120.0 (C_{12}), 124.8 (C_{14}), 130.7 (C_5), 133.3 (C_{13}), 137.7 (C_6), 144.5 (C_{11}), 155.3 (C_3), 160.1 (C_9), 172.0 (C_{10}); Anal. Calcd. for molecular formula $C_{20}H_{20}N_4O_2S$: C, 63.14; H, 5.30; N, 14.73; S, 8.43%. Found: C, 63.22; H, 5.47; N, 14.65; S, 8.52%.

4.21 | 2-(4-iodophenyl)-3-(4-morpholinophenyl)-3,3a-diydro-2H-pyrazolo[3,4-d]thiazole-5(6H)-one (3c)

Yellow solid; yield 25%; mp. 162.0°C; IR (ν_{max}, cm^{-1}): 3230, 2983 (N—H), 2954 (C—H of the morpholinyl ring), 1676 (C═O), 1578 (C═N); ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.21 (t, 4H, —O—CH_2—C**H$_2$**—N—), 3.71 (t, 4H, —O—C**H$_2$**—CH_2—N—), 4.14 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.20 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.75 (d, 2H, *J = 12 Hz*, *ortho* position of morpholinyl —C**H**—), 7.08 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 7.17 (d, 2H, *J = 12 Hz*, *meta* position of morpholinyl —C**H**—), 7.42 (d, 2H, *J = 8 Hz*, *N- meta* position of *N*-phenyl —C**H**—), 12.65 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 54.8 (C_2), 55.5 (C_8), 60.7 (C_7), 67.9 (C_1), 81.1 (C_{14}), 114.2 (C_4), 116.0 (C_{12}), 130.3 (C_5), 133.2 (C_6), 134.6 (C_{13}), 140.2 (C_{11}), 149.1 (C_3), 158.4 (C_9), 176.8 (C_{10}); Anal. Calcd. for molecular formula $C_{20}H_{19}IN_4O_2S$: C, 47.44; H, 3.78; N, 11.06; S, 6.33%. Found: C, 47.52; H, 3.88; N, 11.09; S, 6.27%.

4.22 | 3-(4-morpholinophenyl)-2-(4-(trifluoromethyl)phenyl)-3,3a-dihydro-2H-pyrazolo[3,4-d]thiazole-5(6H)-one (3d)

Yellow solid; yield 19%; mp. 167.1°C; IR (ν_{max}, cm^{-1}): 3237, 2957 (N—H), 2972 (C—H of the morpholinyl ring), 1678 (C═O), 1578 (C═N); ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.19 (t, 4H, —O—CH_2—C**H$_2$**—N—), 3.60 (t, 4H, —O—C**H$_2$**—CH_2—N—), 4.11 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.23 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.50 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 6.77 (d, 2H, *J = 12 Hz*, *ortho* position of morpholinyl —C**H**—), 7.45 (d, 2H, *J = 12 Hz*, *meta* position of morpholinyl —C**H**—), 7.48 (d, 2H, *J = 8 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.77 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 57.9 (C_2), 59.0 (C_8), 60.8 (C_7), 69.7 (C_1), 115.7 (C_4), 116.9 (C_{12}), 124.8 (C_{15}), 125.1 (C_{14}), 128.4 (C_{13}), 130.1 (C_5), 136.9 (C_6), 148.5 (C_{11}), 150.0 (C_3), 156.4 (C_9), 178.1 (C_{10}); Anal. Calcd. for molecular formula $C_{21}H_{19}F_3N_4O_2S$: C, 56.24; H, 4.27; N, 12.49; S, 7.15%. Found: C, 56.17; H, 4.39; N, 12.27; S, 7.11%.

4.23 | 3-(4-morpholinophenyl)-2-(4-(trifluoromethoxy)phenyl)-3,3a-dihydro-2H-pyrazolo[3,4-d]thiazole-5(6H)-one (3e)

Yellow solid; yield 28%; mp. 160.6°C; IR (ν_{max}, cm^{-1}): 3231, 2984 (N—H), 2966 (C—H of the morpholinyl ring), 1673 (C═O), 1579 (C═N); ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.17 (t, 4H, —O—CH_2—C**H**$_2$—N—), 3.72 (t, 4H, —O—C**H**$_2$—CH_2—N—), 4.18 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.26 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.55 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 6.70 (d, 2H, *J = 8 Hz*, *meta* position of *N*-phenyl —C**H**—), 6.78 (d, 2H, *J = 12 Hz*, *ortho* position of morpholinyl —C**H**—), 7.30 (d, 2H, *J = 12 Hz*, *meta* position of morpholinyl —C**H**—), 12.72 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 53.5 (C_2), 56.8 (C_8), 60.1 (C_7), 66.9 (C_1), 113.0 (C_4), 114.2 (C_{12}), 116.3 (C_{13}), 128.8 (C_{15}), 130.0 (C_5), 133.5 (C_6), 138.2 (C_{11}), 142.2 (C_{14}), 148.6 (C_3), 159.2 (C_9), 178.2 (C_{10}); Anal. Calcd. for molecular formula $C_{21}H_{19}F_3N_4O_3S$: C, 54.30; H, 4.12; N, 12.06; S, 6.90%. Found: C, 54.27; H, 4.20; N, 12.07; S, 6.77%.

4.24 | 3-(4-morpholinophenyl)-2-(4-nitrophenyl)-3,3a-dihydro-2H-pyrazolo[3,4-d]thiazole-5(6H)-one (3f)

Yellow solid; yield 39%; mp. 163.9°C; IR (ν_{max}, cm^{-1}): 3241, 3003 (N—H), 2960 (C—H of the morpholinyl ring), 1677 (C═O), 1579 (C═N); ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.24 (t, 4H, —O—CH_2—C**H**$_2$—N—), 3.72 (t, 4H, —O—C**H**$_2$—CH_2—N—), 4.19 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.34 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.76 (d, 2H, *J = 12 Hz*, *ortho* position of morpholinyl —C**H**—), 7.16 (d, 2H, *J = 12 Hz*, *meta* position of morpholinyl —C**H**—), 7.31 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 8.00 (d, 2H, *J = 8 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.84 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 53.6 (C_2), 56.1 (C_8), 60.2 (C_7), 63.3 (C_1), 111.0 (C_{12}), 113.7 (C_4), 124.4 (C_{13}), 129.7 (C_5), 135.7 (C_6), 139.8 (C_{14}), 147.9 (C_3), 150.2 (C_{11}), 158.1 (C_9), 175.5 (C_{10}); Anal. Calcd. for molecular formula $C_{20}H_{19}N_5O_4S$: C, 56.46; H, 4.50; N, 16.46; S, 7.54%. Found: C, 56.52; H, 4.52; N, 16.78; S, 7.42%.

4.25 | 3-(4-(morpholinophenyl)-3,3a-dihydro-2H-pyrazolo[3,4-d]thiazole-5(6H)-thione (4a)

Yellow solid; yield 29%; mp. 154.4°C; IR (ν_{max}, cm^{-1}): 3216, 3105 (N—H), 2930 (C—H of the morpholinyl ring), 1590 (C═N); 1350 (C═S); ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.23 (d, 4H, —O—CH_2—C**H**$_2$—N—), 3.77 (d, 4H, —O—C**H**$_2$—CH_2—N—), 4.01 (d, 1H, *J$_1$ = 4 Hz*, —CH—C**H**—S—), 4.29 (dd, 1H, *J$_1$ = 4 Hz*, *J$_2$ = 10 Hz*, —C**H**—CH—S—), 6.71 (d, 2H, *J$_1$ = 12 Hz*, *ortho* position of morpholinyl —C**H**—), 7.18 (d, *J$_1$ = 12 Hz*, 2H, *meta* position of morpholinyl —C**H**—), 11.66 (d, 1H, *J$_1$ = 10 Hz*, —CH—N**H**—), 13.04 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 54.6 (C_7), 57.1 (C_8), 59.5 (C_2), 68.6 (C_1), 113.6 (C_4), 136.2 (C_5), 139.4 (C_6), 151.3 (C_3), 160.9 (C_9), 204.8 (C_{10}); Anal. Calcd. for molecular formula $C_{14}H_{16}N_4OS_2$: C, 52.48; H, 5.03; N, 17.48; S, 20.01%. Found: C, 52.44; H, 5.12; N, 17.40; S, 21.14%.

4.26 | 3-(4-morpholinophenyl)-2-phenyl-3,3a-dihydro-2H-pyrazolo[3,4-d]thiazole-5(6H)-thione (4b)

Yellow solid; yield 37%; mp. 158.7°C; IR (ν_{max}, cm^{-1}): 3205, 3111 (N—H), 2918 (C—H of the morpholinyl ring), 1577 (C═N), 1338 (C═S); ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.20 (t, 4H, —O—CH_2—C**H**$_2$—N—), 3.77 (t, 4H, —O—C**H**$_2$—CH_2—N—), 4.18 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.32 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.70 (d, 2H, *J = 7.2 Hz*, *ortho* position of morpholinyl —C**H**—), 6.82 (dd, 1H, *J$_1$ = 12 Hz*, *J$_2$ = 12 Hz*, *para* position of *N*-phenyl —C**H**—), 6.92 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 7.18 (d, 2H, *J = 7.2 Hz*, *meta* position of morpholinyl —C**H**—), 7.29 (dd, 2H, *J$_1$ = 8 Hz*, *J$_2$ = 12 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.11 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 55.1 (C_2), 58.2 (C_8), 62.1 (C_7), 68.3 (C_1), 115.9 (C_4), 119.2 (C_{12}), 122.7 (C_{14}), 131.4 (C_5), 132.7 (C_{13}), 133.2 (C_6), 144.4 (C_{11}), 150.0 (C_3), 159.8 (C_9), 201.1 (C_{10}); Anal. Calcd. for molecular formula $C_{20}H_{20}N_4OS_2$: C, 60.58; H, 5.08; N, 14.13; S, 16.17%. Found: C, 61.02; H, 5.19; N, 14.15; S, 17.00%.

4.27 | 2-(4-iodophenyl)-3-(4-morpholinophenyl)-3,3a-dihydro-2H-pyrazolo[3,4-d]thiazole-5(6H)-thione (4c)

Yellow solid; yield 24%; mp. 155.6°C; IR (ν_{max}, cm^{-1}): 3211, 3119 (N—H), 2919 (C—H of the morpholinyl ring), 1578 (C═N), 1334 (C═S); ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.29 (t, 4H, —O—CH_2—C**H**$_2$—N—), 3.66 (t, 4H, —O—C**H**$_2$—CH_2—N—), 4.00 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.34 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.48 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 6.70 (d, 2H, *J = 12 Hz*, *ortho* position of morpholinyl —C**H**—), 7.20 (d, 2H, *J = 12 Hz*, *meta* position of

morpholinyl —C**H**—), 7.41 (d, 2H, *J = 8 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.61 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 53.0 (C_2), 56.5 (C_8), 62.2 (C_7), 66.3 (C_1), 79.8 (C_{14}), 113.1 (C_4), 115.7 (C_{12}), 130.8 (C_5), 134.7 (C_6), 140.1 (C_{13}), 144.6 (C_{11}), 148.4 (C_3), 160.5 (C_9), 200.8 (C_{10}); Anal. Calcd. for molecular formula $C_{20}H_{19}IN_4OS_2$: C, 45.98; H, 3.67; N, 10.72; S, 12.28%. Found: C, 46.14; H, 3.58; N, 11.00; S, 12.37%.

4.28 | 3-(4-morpholinophenyl)-2-(4-(trifluoromethyl)phenyl)-3,3a-dihydro-2H-pyrazolo[3,4-d]thiazole-5(6H)-thione (4d)

Yellow solid; yield 28%; mp. 165.5°C; IR (ν_{max}, cm^{-1}): 3217, 3114 (N—H), 2923 (C—H of the morpholinyl ring), 1582 (C═N), 1331 (C═S); ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.16 (t, 4H, —O—CH_2—C**H$_2$**—N—), 3.65 (t, 4H, —O—C**H$_2$**—CH_2—N—), 4.07 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.20 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.58 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 6.70 (d, 2H, *J = 12 Hz*, *ortho* position of morpholinyl —C**H**—), 7.21 (d, 2H, *J = 12 Hz*, *meta* position of morpholinyl —C**H**—), 7.40 (d, 2H, *J = 8 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.81 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 54.0 (C_2), 57.2 (C_8), 62.6 (C_7), 66.7 (C_1), 113.4 (C_4), 115.2 (C_{12}), 124.0 (C_{15}), 125.2 (C_{14}), 126.3 (C_{13}), 130.5 (C_5), 135.5 (C_6), 148.0 (C_{11}), 149.9 (C_3), 160.0 (C_9), 201.4 (C_{10}); Anal. Calcd. for molecular formula $C_{21}H_{19}F_3N_4OS_2$: C, 54.30; H, 4.12; N, 12.06; S, 13.81%. Found: C, 54.27; H, 4.28; N, 11.87; S, 13.80%.

4.29 | 3-(4-morpholinophenyl)-2-(4-(trifluoromethoxy)phenyl)-3,3a-dihydro-2H-pyrazolo[3,4-d]thiazole-5(6H)-thione (4e)

Yellow solid; yield 25%; mp. 162.7°C; IR (ν_{max}, cm^{-1}): 3210, 3116 (N—H), 2927 (C—H of the morpholinyl ring), 1583 (C═N), 1337 (C═S); ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.21 (t, 4H, —O—CH_2—C**H$_2$**—N—), 3.69 (t, 4H, —O—C**H$_2$**—CH_2—N—), 4.11 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.29 (d, 1H, *J = 5.6 Hz*, —CH—C**H**—S—), 6.59 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 6.73 (d, 2H, *J = 12 Hz*, *ortho* position of morpholinyl —C**H**—), 6.80 (d, 2H, *J = 8 Hz*, *meta* position of *N*-phenyl —C**H**—), 7.22 (d, 2H, *J = 12 Hz*, *meta* position of morpholinyl —C**H**—), 12.70 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 53.0 (C_2), 56.9 (C_8), 63.3 (C_7), 68.0 (C_1), 112.2 (C_4), 113.5 (C_{12}), 114.4 (C_{13}), 130.8 (C_{15}), 131.4 (C_5), 135.5 (C_6), 139.2 (C_{11}), 143.3 (C_{14}), 147.6 (C_3), 160.2 (C_9), 176.0 (C_{10}); Anal. Calcd. for molecular formula $C_{21}H_{19}F_3N_4O_2S_2$: C, 52.49; H, 3.99; N, 11.66; S, 13.35%. Found: C, 52.47; H, 4.00; N, 11.97; S, 13.27%.

4.30 | 3-(4-morpholinophenyl)-2-(4-nitrophenyl)-3,3a-dihydro-2H-pyrazolo[3,4-d]thiazole-5(6H)-thione (4f)

Yellow solid; yield 23%; mp. 148.6°C; IR (ν_{max}, cm^{-1}): 3217, 3117 (N—H), 2924 (C—H of the morpholinyl ring), 1570 (C═N), 1331 (C═S); ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.28 (t, 4H, —O—CH_2—C**H$_2$**—N—), 3.64 (t, 4H, —O—C**H$_2$**—CH_2—N—), 4.14 (d, 1H, *J = 5.6 Hz*, —C**H**—CH—S—), 4.22 (d, 1H, *J = 5.6 Hz*, —C**H**—C**H**—S—), 6.70 (d, 2H, *J = 12 Hz*, *ortho* position of morpholinyl —C**H**—), 7.11 (d, 2H, *J = 12 Hz*, *meta* position of morpholinyl —C**H**—), 7.22 (d, 2H, *J = 8 Hz*, *ortho* position of *N*-phenyl —C**H**—), 8.10 (d, 2H, *J = 8 Hz*, *meta* position of *N*-phenyl —C**H**—), 12.79 (s, 1H, —N**H**—); ^{13}C NMR (150 MHz, DMSO-d_6): δ 54.1 (C_2), 55.5 (C_8), 61.3 (C_7), 67.0 (C_1), 111.6 (C_{12}), 112.9 (C_4), 125.6 (C_{13}), 129.1 (C_5), 134.4 (C_6), 139.2 (C_{14}), 148.2 (C_3), 151.3 (C_{11}), 158.7 (C_9), 201.2 (C_{10}); Anal. Calcd. for molecular formula $C_{20}H_{19}N_5O_3S_2$: C, 54.41; H, 4.34; N, 15.86; S, 14.72%. Found: C, 55.00; H, 4.42; N, 15.77; S, 14.80%.

4.31 | Antioxidant activity

Solutions of synthesized compounds with various concentrations, that is, 200, 100, 50, and 25 μM were prepared. DMSO was used as a control while α-tocopherol and BHT were used as antioxidant standards. The obtained results were given as 50% concentration (IC_{50}). For β-carotene-linoleic acid, $DPPH^{\cdot}$ scavenging, and $ABTS^{\cdot+}$ scavenging activity assays, the sample concentration was calculated from the graph of antioxidant activity percentage against sample concentration. Results of CUPRAC capacity activity assay were given as absorbance.

The total antioxidant activity was evaluated using β-carotene-linoleic acid.[26,27] Free-radical scavenging activity was determined by spectrophotometer using DPPH assay as described by Blois.[27,28] $ABTS^{\cdot+}$ scavenging activity was determined according to the method of Re et al,[29] while the cupric reducing capacity was determined according to Apak's method[30].

4.32 | Anticholinesterase activity

AChE (electric eel) and BChE (horse serum) inhibitions were measured spectrophotometrically. Solutions of the

synthesized compounds of various concentrations, that is, 200, 100, 50, and 25 ppm were prepared. Acetylthiocholine iodide and butyrylthiocholine chloride were employed as substrates. DTNB (5,50-dithiobis(2-nitrobenzoic)acid was used for the measurement of the anticholinesterase activity.[31] The results were given as 50% concentration (IC_{50}).

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