

SYNTHESIS AND ANTIOXIDANT EVALUATION OF NOVEL PHENOTHIAZINE LINKED SUBSTITUTED BENZYLIDENEAMINO-1,2,4-TRIAZOLE DERIVATIVES

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ABSTRACT

A series of novel 5-((10*H*-phenothiazin-10yl)methyl)-4-(substitutedbenzylideneamino)-4*H*-1,2,4-triazole-3-thiol derivatives (**6a-i**) have been synthesized from compound (**1**) through a multi-step reaction. The key intermediate (**5**) afforded a series of title compounds (**6a-i**) on condensation with various suitable aldehydes in the presence of H₂SO₄. The structures of novel compounds were characterized based on their elemental analysis, IR, ¹H-NMR, ¹³C-NMR and MS spectral data. All these novel compounds were screened for their *in vitro* antioxidant activity by employing nitric oxide, hydrogen peroxide, and DPPH radical scavenging assays. The compounds **6d**, **6e** and **6i** demonstrated potent antioxidant activity as these contain the electron-releasing groups.

Keywords: Synthesis, Anti-oxidant activity, 1,2,4-Triazole, Phenothiazines.

INTRODUCTION

The heterocyclic compounds chemistry continues to be an emerging field in the organic and pharmaceutical chemistry¹. Heterocyclic compounds are present in various drugs, several natural products, some vitamins, biomolecules, and biologically active compounds such as anti-inflammatory, antitumor, antimalarial, antidepressant, anti-HIV, antibiotic and antimicrobial agents etc^{2,3}. Nowadays, antioxidants provoke researcher's interest in both medicinal plants and synthetic compounds. Antioxidants (natural or synthetic) are the molecules, which are able to neutralize the free radicals by acting at various stages like interception, prevention and repair^{4,5}. It is therefore necessary to develop therapeutic agents with improved potential for treating broad-spectrum of oxidant infections. Vast data suggests that the free radical-reactive nitrogen and oxygen species influence on the damage of biomolecules, which will further lead to etiology of many human diseases⁶. The high resistance acquired by microbes against the antioxidant drugs existing in the market is of a great challenge to the scientific organizations, involved in the development of novel and effective drugs against human diseases⁷. Synthesis of nitrogen and sulfur bearing heterocyclic derivatives has been the great interest of researchers for the past few decades, due to their potential use in the pharmaceutical and medicinal applications^{8,9}.

Phenothiazines are heterocyclic molecules with two benzene rings associated to the tricyclic system via nitrogen, sulfur atoms. Phenothiazine occurs in various antipsychotic and antihistaminic drugs^{10, 11}. These phenothiazine moieties are widely employed as antibacterial¹², antiviral¹³, anti-inflammatory^{14,15}, anticancer^{16,17}, tranquilizers agents^{18,19}, antitubercular²⁰, anticonvulsant²¹, antiproliferative²² and as anti-HIV agents²³. Triazoles and their derivatives established a main class of organic molecules in a broad spectrum of biological activities, various industrial, agricultural and pharmaceutical fields²⁴⁻²⁶. Triazole ring derivatization lies on the 'bioisosterism phenomenon' in which oxygen of oxadiazole nucleus is replaced with the nitrogen of triazole analogue²⁷. Triazole derivatives are the therapeutically fascinating drug candidates including antifungal²⁸, antioxidant²⁹, antibacterial³⁰, insecticidal³¹, anti-inflammatory¹⁵, antineoplastic³², antiviral³³, sedatives³⁴, anti-convulsant^{35,36}, anti-histaminic^{27,32}, antitumor³⁷, and CNS stimulants³⁸ etc.

In continuation of our research in the domain of biologically active molecules^{15,27-31, 39-43}, the novel phenothiazine and triazole derivatives, in combination, were synthesized with the hope that these derivatives may exhibit a synergistic effect for the antioxidant activity.

RESULTS AND DISCUSSION

Chemistry

A few procedures have been reported in the literature for the synthesis of triazole bearing phenothiazines derivatives and their antimicrobial activity. Literature survey shows quite many reaction methods were reported for

synthesis of various triazole bearing phenothiazines derivatives. To mention a few, such protocols employed microwave irradiation [44] and microwave and catalyst [45]. These methods have some limitations and drawbacks, such as use of toxic reagents, strong acidic or basic conditions, costly reagents and catalysts, strict reaction conditions, tedious steps, low product yields and/or long reaction times, which restrict their scope in practical applications. Therefore, a novel protocol with good and inexpensive catalyst demanding less reaction times is sought after. The method for the synthesis of triazole bearing phenothiazines is described in Scheme 1. By adapting our previously described methods¹⁵, the compound 10*H*-phenothiazine was preserved with chloroacetate **2** with K₂CO₃ catalyst present in acetone solvent medium to give ethyl 2-(10*H*-phenothiazin-10-yl)acetate (**3**). Further, the compound was reacted with KOH as a basic media to afford 2-(10*H*-phenothiazin-10-yl) acetic acid (**4**). The compound **4** was treated under solvent and catalyst free conditions with thiocarbonylhydrazide to obtain the cyclic triazole compound (**5**). Next, substituted aldehydes were treated with compound **5** in the presence of concentrated H₂SO₄ as a catalyst in DMF solvent media to attain the proposed title 5-((10*H*-phenothiazin-10yl)methyl)-4-(substitutedbenzylideneamino)-4*H*-1,2,4-triazole-3-thiol (**6a-i**) derivatives in good to high yields (**Scheme 1**). The characterization of the synthesized title compounds was executed by FT-IR, ¹H-NMR, ¹³C-NMR and LC-MS spectral analysis.

All the analysed compounds gave satisfactory analyses for the anticipated structures, which were established by their spectral data. The ¹H-NMR spectra of the compound (**3**) demonstrated distinctive peaks in the range of triplet at δ 1.32 ppm for CH₂CH₃, quartet at δ 4.24 ppm for CH₂CH₃ and singlet at δ 4.84 ppm due to CH₂CO. The IR spectra of the compound (**3**) exhibited absorption bands at 1576, 1609, 1672, 2832 and 3378 cm⁻¹ corresponding to the stretching frequencies of, C=N, C=C, C=O, CH and COOH groups, respectively. The ¹H-NMR spectra of the compound (**4**) exhibited a singlet for the two protons of the CH₂CO group at δ = 4.35 ppm and a broad singlet at δ 11.49 ppm due to COOH proton. IR spectra of compound (**5**) showed characteristic absorption bands at 1571, 1658, 2762, 2894, and 3458 cm⁻¹ for the C=N, C=O, SH, CH and NH₂ stretching vibrations, respectively. Its ¹H-NMR spectra showed a broad singlet for the one proton of the SH group at δ 12.53 ppm, while the NH₂ group appeared as a singlet for the two protons at δ 5.67 ppm and singlet at 5.30 ppm due to the NCH₂ group respectively.

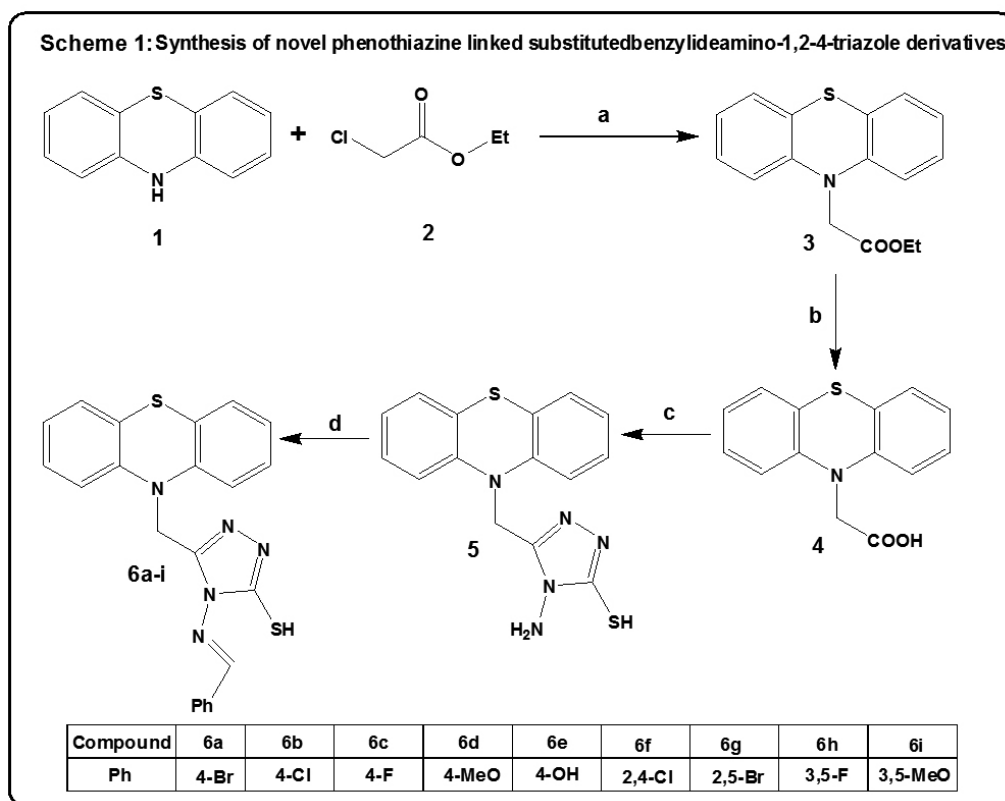
The title derivatives (**6a-i**) were assigned on the basis of spectral analysis. The IR spectra of the title compounds exhibited very similar features and showed the absorption bands range at 2888–2898 cm⁻¹, 2758–2770 cm⁻¹, 1650–1658 cm⁻¹ and 1570–1578 cm⁻¹ for the CH, SH, C=O and C=N stretching vibrations. The physical data, ¹H-NMR, ¹³C-NMR, LC-MS and elemental spectral data for all the synthesized compounds are reported in experimental protocols.

Antioxidant testing

The synthesized novel compounds **6a-i** were evaluated for *in vitro* antioxidant activity by NO, H₂O₂, DPPH methods and the results are tabulated

in Table 1, 2 and 3 respectively. Among all the compounds screened for antioxidant activity, the compounds **6d**, **6e**, **6i** showed potential antioxidant activity as compared to the ascorbic acid (Reference drug) in all three methods employed. This potential activity by compounds **6d**, **6e**, **6i** can be ascribed to the presence of mild electron releasing groups (methoxy, dimethoxy and hydroxyl) appended to the aromatic benzene rings. However, the compounds **6a**, **6b**, **6c**, **6f**, **6g** and **6h** revealed moderate antioxidant activity. Results suggest that methoxy, dimethoxy and hydroxyl substituted compounds (**6d**, **6e**,

6i) offered better antioxidant activity when compared to that of the compounds **6a**, **6b**, **6c**, **6f**, **6g** and **6h** with electron-withdrawing groups (halo). The IC_{50} value of the standard ascorbic acid using the NO method was found to be 15.70 $\mu\text{g/mL}$ at 25 $\mu\text{g/mL}$ whereas the IC_{50} values of the compounds **6e**, **6i** and **6d** were found to be 16.98, 17.54 and 17.91 $\mu\text{g/mL}$, respectively. More, the tables 1, 2 and 3 designates that the antioxidant activity in NO, DPPH and H_2O_2 methods which increased with concentration.



Reagents & Conditions: (a) K_2CO_3 , 78 °C, reflux, 3-4 h; (b) KOH, R.T., 12 h; (c) thiocarbohydrazide, heat, 4-5 h; (d) substituted aldehydes, Con H_2SO_4 , DMF, 25 °C, 5-6 h.

Table 1: *In vitro* antioxidant activity of compounds (**6a-i**) determined by NO method.

Compound	Concentration ($\mu\text{g/mL}$)				IC_{50}
	25	50	75	100	
6a	68.41 \pm 0.90	72.59 \pm 1.37	74.18 \pm 0.94	82.40 \pm 1.23	19.52 \pm 1.04
6b	60.97 \pm 1.41	63.37 \pm 1.30	67.65 \pm 1.24	72.35 \pm 0.80	20.49 \pm 0.95
6c	62.73 \pm 1.17	67.48 \pm 1.24	69.94 \pm 0.88	73.16 \pm 0.95	21.01 \pm 0.70
6d	75.02 \pm 0.22	78.14 \pm 0.45	85.45 \pm 0.61	84.88 \pm 0.76	17.91 \pm 0.86
6e	78.18 \pm 0.30	83.25 \pm 0.49	86.60 \pm 0.71	86.15 \pm 0.78	16.98 \pm 0.69
6f	65.37 \pm 1.17	70.22 \pm 1.57	75.69 \pm 1.41	80.53 \pm 0.71	20.41 \pm 1.24
6g	73.90 \pm 0.86	75.06 \pm 0.94	79.25 \pm 1.06	83.36 \pm 1.41	18.14 \pm 0.57
6h	54.64 \pm 1.38	57.49 \pm 1.24	61.03 \pm 0.71	65.28 \pm 1.08	24.30 \pm 1.08
6i	71.26 \pm 0.84	74.84 \pm 1.05	79.14 \pm 1.40	84.93 \pm 1.26	17.54 \pm 0.93
Ascorbic acid*	85.76 \pm 0.13	84.92 \pm 0.38	87.32 \pm 0.55	91.42 \pm 0.68	15.70 \pm 0.54
Control	—	—	—	—	—

(—) Showed no scavenging activity. Values were the means of three replicates \pm SD.

* = Standard

Table 2: *In vitro* antioxidant activity of compounds (6a-i) determined by H2O2 method.

Compound	Concentration (µg/mL)				IC ₅₀
	25	50	75	100	
6a	61.27 ± 1.07	64.49 ± 1.28	68.84 ± 1.58	71.93 ± 0.83	21.74 ± 0.53
6b	52.36 ± 1.17	55.03 ± 0.87	59.12 ± 0.96	64.72 ± 0.63	25.33 ± 1.05
6c	55.51 ± 1.37	58.31 ± 1.22	61.11 ± 0.71	64.27 ± 1.08	26.10 ± 0.97
6d	64.88 ± 0.31	66.50 ± 0.47	69.97 ± 0.66	73.52 ± 0.77	20.57 ± 1.25
6e	67.29 ± 1.14	70.52 ± 1.30	72.68 ± 0.61	75.19 ± 0.71	19.85 ± 0.61
6f	54.09 ± 0.89	57.60 ± 1.38	60.95 ± 0.79	65.20 ± 1.05	24.54 ± 0.41
6g	57.68 ± 1.14	61.48 ± 1.19	68.12 ± 1.47	70.93 ± 1.54	21.33 ± 1.07
6h	47.08 ± 0.87	49.37 ± 1.16	52.44 ± 1.26	55.62 ± 1.38	27.76 ± 0.64
6i	63.07 ± 0.81	66.84 ± 1.58	70.22 ± 1.06	72.59 ± 1.37	20.47 ± 1.24
Ascorbic acid*	77.42 ± 0.17	79.61 ± 0.34	83.39 ± 0.63	87.59 ± 0.70	17.35 ± 0.28
Control	–	–	–	–	–

(–) Showed no scavenging activity. Values were the means of three replicates ± SD.

* = Standard

Table 3: *In vitro* antioxidant activity of compounds (6a-i) determined by DPPH method.

Compound	Concentration (µg/mL)				IC ₅₀
	25	50	75	100	
6a	64.87 ± 0.71	68.93 ± 1.32	72.48 ± 1.29	77.64 ± 1.34	20.57 ± 1.23
6b	61.27 ± 1.07	64.49 ± 1.28	68.84 ± 1.58	71.93 ± 0.83	21.74 ± 0.53
6c	54.75 ± 1.74	60.25 ± 1.05	63.37 ± 0.92	67.81 ± 1.58	25.70 ± 1.47
6d	71.30 ± 1.06	75.56 ± 0.88	75.87 ± 1.30	82.28 ± 1.00	18.48 ± 1.04
6e	78.43 ± 0.28	81.64 ± 0.48	85.28 ± 0.62	84.76 ± 0.78	17.88 ± 0.28
6f	63.04 ± 1.40	67.83 ± 1.56	71.92 ± 0.78	74.89 ± 1.07	21.14 ± 0.96
6g	65.70 ± 1.44	69.41 ± 1.21	74.84 ± 1.56	78.53 ± 0.96	20.30 ± 1.12
6h	49.65 ± 0.60	53.86 ± 1.24	57.63 ± 0.55	62.93 ± 0.82	26.69 ± 1.10
6i	76.46 ± 0.30	73.83 ± 0.66	74.36 ± 0.76	89.13 ± 0.80	18.08 ± 0.51
Ascorbic acid*	84.68 ± 0.18	83.52 ± 0.32	88.52 ± 0.43	86.22 ± 0.54	16.10 ± 0.45
Control	–	–	–	–	–

(–) Showed no scavenging activity. Values were the means of three replicates ± SD.

* = Standard

CONCLUSION

In the current study, we have developed the synthesis of 9 new derivatives of novel phenothiazine linked substitutedbenzylideneamino-1,2,4-triazole derivatives (6a-i). All have been characterized by spectral studies. *In vitro*, their antioxidant activity was also investigated by employing nitric oxide, hydrogen peroxide, and DPPH radical scavenging assays. The evaluation of antioxidant screening data reveals that among the 9 compounds screened, compounds 6d, 6e & 6i showed potent antioxidant activity almost equivalent to that of standards.

EXPERIMENTAL

All chemicals and reagents required for the reaction were of analytical grade and were used without any further purification. Melting points were recorded on a Buchi Melting Point B-545 apparatus. Bruker AMX 400 MHz NMR spectrometer was used to record the ¹H NMR and ¹³C NMR (400 MHz) spectral values. The CDCl₃/DMSO-d₆ solution was utilized for this while TMS served as the internal standard. TMS was further used as an internal standard for reporting the all chemical shifts in δ (ppm). The FT-IR spectrum for the samples was established using a Perkin Elmer Precisely 100 FT-

IR spectrometer at the 400-4000 cm⁻¹ area. LCMS spectral data was recorded on a MASPEC low resolution mass spectrometer operated at 70 eV. Purity of all the reaction products was confirmed by TLC using aluminum plates coated with silica gel (Merck Kieselgel 60 F254).

Synthesis of ethyl 2-(10H-phenothiazin-10-yl)acetate (3)

To a solution of 10H-phenothiazine **1** (1 mmol) in acetone (20 mL) was added K₂CO₃ (3 mmol) at 25 °C followed by the addition of ethyl chloroacetate **2** (1.2 mmol) with reflux at 78 °C for 3-4 h. The completion of reaction was monitored by TLC. Next, the reaction mixture was cooled, poured into ice-water and was extracted using ethyl acetate solution. Further, the organic layer was evaporated and recrystallized to obtain ethyl 2-(10H-phenothiazin-10-yl)acetate as solid.

Synthesis of 2-(10H-phenothiazin-10-yl)acetic acid (4)

The solutions of compound **3** (1 mmol), dissolved in ethanol (10 mL) and potassium hydroxide (3 mmol) in water (5 mL) were combined and the mixture was left for stirring for 12 h at 25 °C. The reaction was monitored by TLC for completion. The reaction mixture was then transferred into cold water and acidified with HCl to obtain a solid product by filtration. Finally, the crude was recrystallized with ethyl acetate to afford a pure compound 2-(10H-phenothiazin-10-yl)acetic acid.

General procedure for the synthesis of 5-((10H-phenothiazin-10-yl)

methyl)-4-amino-4H-1,2,4-triazole-3-thiol (5)

A mixture of compound **4** (1 mmol) and thiocarbonylhydrazide (1 mmol) was heated under solvent-free conditions for 4-5 h and the reaction end-point was examined by TLC. The reaction mixture was left to cool for 1 h and subsequently excess acid compound was removed using sodium bicarbonate solution. Thereafter, the above mixture was filtered, dried and finally recrystallized with DMF to achieve compound **5**.

Synthesis of 5-((10H-Phenothiazin-10yl)methyl)-4-(substitutedbenzylideneamino)-4H-1,2,4-triazole-3-thiol (6a-i)

An equimolar ratio of compound **5** and substituted aldehydes (1x1), a few drops of concentrated H₂SO₄ in DMF (10 mL) solvent were stirred at 25 °C for 5-6 h. After this, the reaction mixture was poured into ice-cold water, filtered to collect the solid product and was dried. The resultant crude was then recrystallized with ethyl acetate to accomplish the title compounds as excellent yields (**6a-i**).

5-((10H-Phenothiazin-10yl)methyl)-4-(4-bromobenzylideneamino)-4H-1,2,4-triazole-3-thiol (6a)

Yield 80%; m.p. 222-224 °C; IR (KBr)v (cm⁻¹): 2892 (CH), 2761 (SH), 1654 (C=O), 1570 (C=N) 720 (C-Br); ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 12.50 (s, 1H, SH), 9.30 (s, 1H), 7.71 (dd, 2H, Ar-H, *J* = 8.01, 2.51 Hz), 7.59 (dd, 2H, *J* = 8.05, 2.49, Ar-H), 7.28 (m, 4H, Ar-H), 7.11 (dd, 2H, Ar-H, *J* = 7.95, 2.36 Hz), 7.05 (dd, 2H, *J* = 7.80, 2.10 Hz), 5.20 (s, 2H, NCH₂); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ: 165.10, 158.18, 154.20, 144.12 (2C), 131.20 (2C), 130.30, 128.90 (2C), 128.10 (2), 127.62 (2C), 125.32, 123.24 (2C), 122 (2C), 115.67 (2C), 55.14; LC-MS (70 eV): *m/z* = 495. Anal. Calcd. for C₂₂H₁₆BrN₅S₂: C, 53.44; H, 3.26; N, 14.16. Found: C, 53.47; H, 3.29; N, 14.19.

5-((10H-Phenothiazin-10yl)methyl)-4-(4-chlorobenzylideneamino)-4H-1,2,4-triazole-3-thiol (6b)

Yield 70%; m.p. 208-210 °C; IR (KBr)v (cm⁻¹): 2893 (CH), 2768 (SH), 1650 (C=O), 1574 (C=N) 680 (C-Cl); ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 12.40 (s, 1H, SH), 9.35 (s, 1H), 7.77 (dd, 2H, Ar-H, *J* = 8.05, 2.55 Hz), 7.54 (dd, 2H, *J* = 8.10, 2.48, Ar-H), 7.22 (m, 4H, Ar-H), 7.15 (dd, 2H, Ar-H, *J* = 7.95, 2.36 Hz), 7.01 (dd, 2H, *J* = 7.85, 2.10 Hz), 5.10 (s, 2H, NCH₂); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ: 165.15, 158.22, 154.28, 144.25 (2C), 135.40, 131.20, 130.80 (2C), 128.70 (2C), 128.12 (2), 127.23 (2C), 123.22 (2C), 122.50 (2C), 115.60 (2C), 55.14; LC-MS (70 eV): *m/z* = 449.05. Anal. Calcd. for C₂₂H₁₆ClN₅S₂: C, 58.72; H, 3.58; N, 15.56. Found: C, 58.75; H, 3.56; N, 15.59.

5-((10H-Phenothiazin-10yl)methyl)-4-(4-fluorobenzylideneamino)-4H-1,2,4-triazole-3-thiol (6c)

Yield 72%; m.p. 160-162 °C; IR (KBr)v (cm⁻¹): 2890 (CH), 2760 (SH), 1658 (C=O), 1572 (C=N) 1195 (C-F); ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 12.42 (s, 1H, SH), 9.10 (s, 1H), 7.67 (dd, 2H, Ar-H, *J* = 8.0, 2.40 Hz), 7.23 (dd, 2H, *J* = 8.06, 2.40, Ar-H), 7.20 (dd, 2H, *J* = 8.0, 2.40, Ar-H), 7.14 (dd, 2H, Ar-H, *J* = 7.94, 2.38 Hz), 7.08 (t, 2H, *J* = 8.50, Ar-H), 7.04 (dd, 2H, *J* = 7.84, 2.12 Hz), 5.12 (s, 2H, NCH₂); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ: 165.50 (d, *J* = 248.20), 161.20, 154.20, 147.28, 144.30 (2C), 130.70 (2C), 128.10 (2C), 127.90, 127.15 (2C), 123.30 (2C), 121.60 (2C), 116.90 (2C), 115.50 (d, *J* = 18 Hz, 2C), 54.14; LC-MS (70 eV): *m/z* = 433.08. Anal. Calcd. for C₂₂H₁₆FN₅S₂: C, 60.95; H, 3.72; N, 16.15. Found: C, 60.98; H, 3.75; N, 16.17.

5-((10H-Phenothiazin-10yl)methyl)-4-(4-methoxybenzylideneamino)-4H-1,2,4-triazole-3-thiol (6d)

Yield 68%; m.p. 218-220 °C; IR (KBr)v (cm⁻¹): 2893 (CH), 2768 (SH), 1650 (C=O), 1574 (C=N); ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 13.10 (s, 1H, SH), 9.16 (s, 1H), 7.90 (dd, 2H, Ar-H, *J* = 7.98, 2.45 Hz), 7.51 (dd, 2H, *J* = 8.00, 2.44, Ar-H), 7.24 (m, 4H, Ar-H), 7.14 (dd, 2H, Ar-H, *J* = 7.96, 2.42 Hz), 7.00 (dd, 2H, *J* = 7.84, 2.10 Hz), 5.24 (s, 2H, NCH₂), 3.82 (s, 3H, CH₃); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ: 165.15, 160.10, 158.20, 154.24, 144.38 (2C), 130.40 (2C), 127.70 (2C), 127.23 (2C), 125.12, 123.18 (2C), 122.46 (2C), 115.40 (2C), 114.20 (2C), 55.20 (CH₃), 55.14; LC-MS (70 eV): *m/z* = 445.10. Anal. Calcd. for C₂₂H₁₆ClN₅S₂: C, 62.00; H, 4.30; N, 15.72. Found: C, 61.98; H, 4.33; N, 15.70.

5-((10H-Phenothiazin-10yl)methyl)-4-(4-hydroxybenzylideneamino)-4H-1,2,4-triazole-3-thiol (6e)

Yield 75%; m.p. 214-216 °C; IR (KBr)v (cm⁻¹): 3320 (O-H), 2888 (CH), 2758 (SH), 1656 (C=O), 1578 (C=N); ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 13.12 (s, 1H, SH), 9.90 (s, O-H), 9.16 (s, 1H), 7.80 (dd, *J* = 7.98, 2.45 Hz, 2H, Ar-H), 7.22 (dd, 2H, Ar-H, *J* = 8.10, 2.44 Hz), 7.20 (dd, 2H, Ar-H, *J* = 7.94, 2.46 Hz), 7.10 (dd, 2H, *J* = 7.82, 2.20 Hz), 6.90 (m, 4H, Ar-H), 5.24 (s, 2H, NCH₂); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ: 165.00, 161.10, 158.20, 154.24, 144.98 (2C), 130.30 (2C), 127.80 (2C), 126.90 (2C), 124.92, 123.16 (2C), 122.26 (2C), 116.10 (2C), 115.00 (2C), 53.80; LC-MS (70 eV): *m/z* = 431.01. Anal. Calcd. for C₂₂H₁₆ClN₅S₂: C, 61.23; H, 3.97; N, 16.23. Found: C, 61.21; H, 4.00; N, 16.25.

5-((10H-Phenothiazin-10yl)methyl)-4-(2,4-dichlorobenzylideneamino)-4H-1,2,4-triazole-3-thiol (6f)

Yield 75%; m.p. 230-232 °C; IR (KBr)v (cm⁻¹): 2893 (CH), 2768 (SH), 1650 (C=O), 1574 (C=N) 682 (C-Cl); ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 13.12 (s, 1H, SH), 9.18 (s, 1H), 7.92 (d, Ar-H, *J* = 7.98 Hz), 7.72 (s, Ar-H), 7.38 (d, Ar-H, *J* = 8.10 Hz), 7.20 (m, 4H, Ar-H), 7.10 (dd, 2H, Ar-H, *J* = 7.96, 2.38 Hz), 6.95 (dd, 2H, *J* = 7.88, 2.15 Hz), 5.24 (s, 2H, NCH₂); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ: 163.15, 158.30, 154.24, 144.88 (2C), 131.40, 131.10, 129.00, 129.60, 128.50 (2C), 128.10, 127.50 (2C), 127.10, 123.14 (2C), 122.40 (2C), 115.20 (2C), 55.18; LC-MS (70 eV): *m/z* = 483.01. Anal. Calcd. for C₂₂H₁₅Cl₂N₅S₂: C, 54.55; H, 3.12; N, 14.46. Found: C, 54.57; H, 3.15; N, 14.48.

5-((10H-Phenothiazin-10yl)methyl)-4-(2,5-dibromobenzylideneamino)-4H-1,2,4-triazole-3-thiol (6g)

Yield 81%; m.p. 228-230 °C; IR (KBr)v (cm⁻¹): 2892 (CH), 2761 (SH), 1654 (C=O), 1570 (C=N) 726 (C-Br); ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 12.50 (s, 1H, SH), 9.30 (s, 1H), 7.92 (s, Ar-H), 7.60 (d, *J* = 8.05, Ar-H), 7.55 (d, *J* = 8.10, Ar-H), 7.22 (dd, *J* = 8.20, 2.40, 2H, Ar-H), 7.20 (d, *J* = 8.10, 2H, Ar-H), 7.12 (dd, 2H, Ar-H, *J* = 7.98, 2.36 Hz), 7.00 (dd, 2H, *J* = 7.80, 2.30 Hz), 5.22 (s, 2H, NCH₂); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ: 165.10, 158.18, 154.20, 144.12 (2C), 134.00, 133.20, 132.90, 130.30, 128.80 (2C), 127.60 (2C), 125.10, 124.16, 123.22 (2C), 122.10 (2C), 115.66 (2C), 55.14; LC-MS (70 eV): *m/z* = 572.91. Anal. Calcd. for C₂₂H₁₆Br₂N₅S₂: C, 46.09; H, 2.64; N, 12.22. Found: C, 46.07; H, 2.61; N, 12.25.

5-((10H-Phenothiazin-10yl)methyl)-4-(3,5-difluorobenzylideneamino)-4H-1,2,4-triazole-3-thiol (6h)

Yield 68%; m.p. 150-152 °C; IR (KBr)v (cm⁻¹): 2892 (CH), 2766 (SH), 1656 (C=O), 1572 (C=N) 1190 (C-F); ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 12.80 (s, 1H, SH), 9.08 (s, 1H), 7.50 (d, 2H, Ar-H, *J* = 7.50 Hz), 7.20 (m, 4H, Ar-H), 7.10 (dd, 2H, Ar-H, *J* = 7.98, 2.30 Hz), 7.00 (dd, 2H, *J* = 7.88, 2.22 Hz), 6.95 (t, *J* = 7.5, Ar-H), 5.14 (s, 2H, NCH₂); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ: 163.80 (d, *J* = 246.80, 2C-F), 161.10, 154.40, 147.28, 144.60 (2C), 134.70, 128.20 (2C), 127.15 (2C), 123.10 (2C), 122.60 (2C), 115.90 (2C), 110.50 (d, 2C), 104.50, 54.16; LC-MS (70 eV): *m/z* = 451.07. Anal. Calcd. for C₂₂H₁₆F₂N₅S₂: C, 58.52; H, 3.35; N, 15.51. Found: C, 58.54; H, 3.37; N, 15.54.

5-((10H-Phenothiazin-10yl)methyl)-4-(3,5-dimethoxybenzylideneamino)-4H-1,2,4-triazole-3-thiol (6i)

Yield 70%; m.p. 239-240 °C; IR (KBr)v (cm⁻¹): 2898 (CH), 2770 (SH), 1658 (C=O), 1578 (C=N); ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 13.48 (s, 1H, SH), 9.26 (s, 1H), 7.22 (dd, 2H, Ar-H, *J* = 7.90, 2.40 Hz), 7.20 (dd, 2H, *J* = 8.10, 2.44, Ar-H), 7.14 (d, *J* = 8.00, 2H, Ar-H), 7.00 (dd, 2H, Ar-H, *J* = 8.18, 2.46 Hz), 7.00 (dd, 2H, *J* = 7.84, 2.10 Hz), 6.90 (s, Ar-H), 5.20 (s, 2H, NCH₂), 3.84 (s, 6H, CH₃); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ: 164.15 (2C), 160.15, 154.24, 148.50, 144.20 (2C), 134.40, 127.80 (2C), 127.40 (2C), 123.22 (2C), 122.44 (2C), 115.48 (2C), 104.20 (2C), 103.50, 55.40 (2CH₃), 55.14; LC-MS (70 eV): *m/z* = 475.11. Anal. Calcd. for C₂₄H₂₁N₅O₂S₂: C, 60.61; H, 4.45; N, 14.73. Found: C, 60.63; H, 4.42; N, 14.75.

PHARMACOLOGICAL SCREENING**Antioxidant screening in vitro**

The compounds **6a-i** were evaluated for antioxidant activity using the Nitric oxide (NO), Hydrogen peroxide (H₂O₂) and 1,1-Diphenyl-1-picrylhydrazyl (DPPH) methods.

NO scavenging activity

NO biological activity was determined using the methods by Green et al. and Marcocci et al. with slight modification^{46,47}. Briefly, NO radicals were first created with the sodium nitroprusside. Sodium nitroprusside (10 mmol, 1 mL), phosphate buffer saline (PBS) (0.25 mmol, 1.5 mL, pH 7.5) were added to the various amounts of experimental compounds (25, 50, 75 and 100 µg/mL) and incubated at 25 °C for 2.5 h. Next, the reaction mixtures (1 mL) were preserved with 1 mL of Griess reagent. All experiments were performed in triplicates. The absorbance of the samples was measured at 546 nm using the chromatophore and NO activity was calculated by the following formula

$$\text{Scavenging activity (\%)} = \left[\frac{\text{A control} - \text{A sample}}{\text{A control}} \right] \times 100$$

A control = Absorbance of the control reaction (all reagents without the test compounds)

$$\text{A sample} = \text{Absorbance of the test compound.}$$

H₂O₂ scavenging activity

The compounds H₂O₂ biological activity was determined using the previous method⁴⁸. A solution of H₂O₂ (10 mmol) in PBS (0.25 mmol) was prepared and various amounts of test compounds as described in above section were added to the PBS containing H₂O₂ (4 mL). The absorbance of the compounds was recorded at 230 nm, percent of H₂O₂ scavenging activity was calculated by using the above mentioned formula. All the tests were carried out in triplicate.

DPPH scavenging activity

The compounds ability to donate electrons or hydrogen atoms was evaluated by the DPPH colorimetric method based on the decolorization of purple methanol solution⁴⁹. This assay produces the DPPH stable radicals. Various concentrations of compounds (refer to above sections) in methanol (1 mL) were added to the 4 mL of DPPH methanol solution (0.004% (%)) and incubated for 0.5 h at 25 °C. After this incubation period, the absorbance was measured at 517 nm. The percentage of DPPH scavenging activity was calculated using the above equation.

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