The Reactivity of *Gem* Cyanoester Ketene Dithiolates towards the Development of Potent Antioxidant Heterocycles

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The reactivity of *gem* cyanoester ketene dithiolates towards the development of a variety of heterocycles was studied and tested for antioxidant property. The compounds bis benzoxazolylmethylthiomethylene pyrazoles and isoxazoles displayed excellent radical scavenging activity when compared with the standard ascorbic acid.

Key words *gem* cyanoester ketene dithiolate; cyclocondensation; antioxidant activity; bis benzoxazolyl; benzothiazolyl; benzoimidazolyl–pyrazole

Pyrazole and isoxazole derivatives are interesting heterocyclic compounds since they show a wide range of pharmacological properties including antiinflammatory,[1] antibacterial[2] and antifungal.[3] In addition pyrazoles and isoxazoles are present in leading pharmaceuticals *viz.*, Celebrex, Viagra, Valdecoxib.[4,5] Pyrimidine derivatives have been very well known in medicinal chemistry for their therapeutic applications.[6,7] One possible reason for their activity is the presence of a pyrimidine base in thymine, cytosine and uracil, which are essential building blocks of nucleic acids, DNA and RNA. Benzoxazole ring is one of the most common heterocycle in medicinal chemistry. Substituted benzoxazoles possess diverse chemotherapeutic activities including antibiotic,[8] antimicrobial[9–13] and antitumor.[14,15] Benzothiazole derivatives are important members of fused heterocycles as they exhibit antimicrobial properties[16] and have found applications in industry as antioxidants.[17] In fact, 2-arylbenzothiazoles are confirmed as a novel class of potent and selective antitumor agents.[18,19] The benzimidazole moiety is present in many biologically active natural products and synthetic compounds. Some of them exhibit clinical value towards breast cancer,[20–23] leukemia,[24,25] tumor cells[26,27] *etc*. With this background we designed the present work to synthesize bis benzoxazolyl/benzothiazolyl/benzimidazolyl–pyrazoles/isoxazoles/pyrimidines from ketene dithiolates and to study the antioxidant property of these compounds.

Chemistry

In continuation of our interest to study the reactivity of ketene dithiolates towards the development of a new class of heterocycles and their bioassay,[28–30] the following work has been taken up. The synthetic intermediates 2-(chloromethyl) benzoxazole (**1**) and 2-(chloromethyl)benzothiazole (**2**) were prepared by the irradiation of 2-aminophenol/2-aminothiophenol and chloroacetyl chloride for 10–15 min at a power of 500 W.[31,32] However, 2-(chloromethyl)-1*H*-benzimidazole (**3**) was obtained by treating *o*-phenylenediamine with chloroacetic acid in the presence of 5 N HCl.[33]

A one pot reaction of ethyl cyanoacetate, carbon disulfide and **1**/**2**/**3** in the presence of K_2CO_3 in *N*,*N*-dimethylformamide (DMF) resulted in *gem* difunctionalized compounds, ethyl 3,3-bis((benzo[*d*]oxazol-2-yl)methylthio)-2-cyanoacrylate (**4**), ethyl 3,3-bis((benzo[*d*]thiazol-2-yl)methylthio)-2-cyanoacrylate (**5**) and ethyl 3,3-bis((1*H*-benzo[*d*]imidazol-2-yl)methylthio)-2-cyanoacrylate (**6**) (Chart 1).

In order to develop a new class of tris heterocyclic compounds, the *gem* cyanoester functionality in **4** was made to react with hydrazine hydrate in the presence of 1 eq of K_2CO_3 in DMF. Instead of the expected tris heterocyclic compound, intramolecular cyclization of **4** occurred with the formation of a mixture of tetrasubstituted thiophene, ethyl 2-((benzo[*d*]-oxazol-2′-yl)methylthio)-4-amino-5-(benzo[*d*]oxazol-2-yl)thiophene-3-carboxylate (**7**) and 4-amino-2,5-di(benzo[*d*]oxazol-2′-yl)thieno[2,3-*b*]thiophen-3(2*H*)-one (**10**) in a ratio of 3 : 1. However, the reaction was repeated in the absence of hydrazine hydrate wherein the products **7** and **10** were obtained in the same ratio (Chart 2, Method A). The ^{1}H-NMR spectrum of **7** displayed a singlet at δ 4.37 for methylene protons, a quartet and a triplet at 4.22, 1.36 for carbethoxy protons and a broad singlet at 6.88 ppm for NH_2. The latter signal disappeared on deuteration. The ^{1}H-NMR spectrum of **10** showed a singlet at δ 4.51 ppm for methine proton

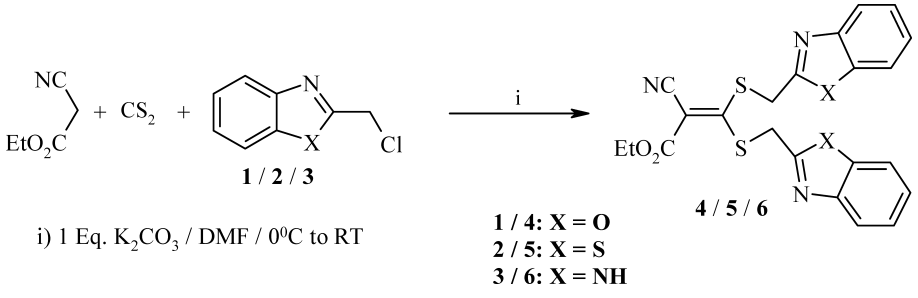

Chart 1

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and a broad singlet at 6.69 ppm for NH_2 which disappeared on deuteration. Then, the reaction was carried out with **5** and **6** in the presence of 1 eq of K_2CO_3 in DMF, a mixture of products ethyl 2-((benzo[*d*]thiazol-2′-yl)methylthio)-4-amino-5-(benzo[*d*]thiazol-2-yl)thiophene-3-carboxylate (**8**), ethyl 2-((1′*H*-benzo[*d*]imidazol-2′-yl)methylthio)-4-amino-5-(1′*H*-benzo[*d*]imidazol-2′-yl)-thiophene-3-carboxylate (**9**) and 4-amino-2,5-di(benzo[*d*]thiazol-2′-yl)thieno[2,3-*b*]thiophen-3(2*H*)-one (**11**), 4-amino-2,5-di(1′*H*-benzo[*d*]imidazol-2′-yl)thieno[2,3-*b*]thiophen-3(2*H*)-one (**12**) were obtained almost in the same ratio.

On the other hand, the intramolecular cyclization reaction of **4**/**5**/**6** with 2 eq of K_2CO_3 afforded thienothiophene derivatives **10**, **11** and **12** only (Chart 3, Method B). The latter compounds were also prepared in a one pot reaction of ethyl cyanoacetate, carbon disulfide and **1**/**2**/**3** in the presence of 3 eq of K_2CO_3 (Chart 3, Method C).

However, when the reaction was repeated by adding ethanolic solution of **4**/**5**/**6** dropwise into a solution of hydrazine hydrate and piperidine in ethanol, the tris heterocyclic compounds, 3-amino-4-(bis((benzo[*d*]oxazol-2′-yl)methylthio)methylene)-1*H*-pyrazol-5(4*H*)-one (**13**), 3-amino-4-(bis((benzo[*d*]thiazol-2′-yl)methylthio)methylene)-1*H*-pyrazol-5(4*H*)-one (**14**) and 3-amino-4-(bis((1′*H*-benzo[*d*]-imidazol-2′-yl)methylthio)methylene)-1*H*-pyrazol-5(4*H*)-one (**15**) were obtained. Similar reaction with hydroxylamine hydrochloride produced 3-amino-4-(bis((benzo[*d*]oxazol-2′-yl)methylthio)methylene)isoxazol-5(4*H*)-one (**16**), 3-amino-4-(bis-((benzo[*d*]thiazol-2′-yl)methylthio)methylene)isoxazol-5(4*H*)-one (**17**) and 3-amino-4-(bis((1′*H*-benzo[*d*]imidazol-2′-yl)methylthio)methylene)isoxazol-5(4*H*)-one (**18**). The ^{1}H-NMR spectra of **13**–**18** displayed two singlets in the region δ 4.28–4.40 which were accounted for two methylene protons and a broad singlet in the region 5.59–5.75 due to NH_2. In addition **13**–**15** showed another broad singlet at 9.67–9.86 ppm for NH. The compounds **15** and **18** also exhibited a broad singlet at 12.41, 12.35 ppm due to NH of benzimidazole. The signals of NH_2 and NH disappeared on deuteration. The cyclocondensation of **4**/**5**/**6** with urea in the presence of piperidine in ethanol gave 6-amino-5-(bis((benzo[*d*]oxazol-2′-yl)methylthio)methylene)-2-hydroxypyrimidin-4(5*H*)-one (**19**), 6-amino-5-(bis((benzo[*d*]thiazol-2′-yl)methylthio)methylene)-2-hydroxypyrimidin-4(5*H*)-one (**20**) and 6-amino-5-(bis((1′*H*-benzo[*d*]imidazol-2′-yl)methylthio)methylene)-2-hydroxypyrimidin-4(5*H*)-one (**21**).

Similar reaction of **4**/**5**/**6** with thiourea furnished 6-amino-5-(bis((benzo[*d*]oxazol-2′-yl)methylthio)methylene)-2-mercaptopyrimidin-4(5*H*)-one (**22**), 6-amino-5-(bis((benzo[*d*]-thiazol-2′-yl)methylthio)methylene)-2-mercaptopyrimidin-4(5*H*)-one (**23**) and 6-amino-5-(bis((1′*H*-benzo[*d*]imidazol-2′-yl)-methylthio)methylene)-2-mercaptopyrimidin-4(5*H*)-one (**24**). Likewise, 5-(bis((benzo[*d*]oxazol-2′-yl)methylthio)methylene)-dihydro-6-imino-1,3-dimethyl-pyrimidine-2,4(1*H*,3*H*)-dione (**25**), 5-(bis((benzo[*d*]thiazol-2′-yl)methylthio)methylene)-dihydro-6-imino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**26**) and 5-(bis((1′*H*-benzo[*d*]imidazol-2′-yl)methylthio)methylene)-dihydro-6-imino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**27**) were prepared by treating **4**/**5**/**6** with *N*,*N*′-dimethyl urea in the presence of piperidine in ethanol (Chart 4). The ^{1}H-NMR spectra of **19**–**27** presented two singlets at δ 4.29–4.43 for two methylene protons. The compounds **19**–**24** showed a broad singlet at 5.53–5.66 ppm for NH_2. In addition, **19**–**21** exhibited a broad singlet at 8.31–8.36 and **22**–**24** at 1.46–1.53 ppm due to OH and SH, respectively. The compounds **25**–**27** displayed a singlet at 2.62–2.79 ppm for N–Me protons and a broad singlet at 9.29–9.35 ppm for =NH. The compounds **21**, **24** and **27** showed a broad singlet at around 12.48 ppm for NH of benzimidazole. The signals due to highly acidic protons disappeared on deuteration. The structures of all the new compounds were further established by IR and ^{13}C-NMR spectral parameters and elemental analyses.

In-Vitro **Antioxidant Study** The compounds **4**–**27** were tested for antioxidant property by 2,2-diphenyl-1-picrylhydrazyl radical (DPPH),[34,35] nitric oxide[36,37] and 2,2′-azino-bis(3-

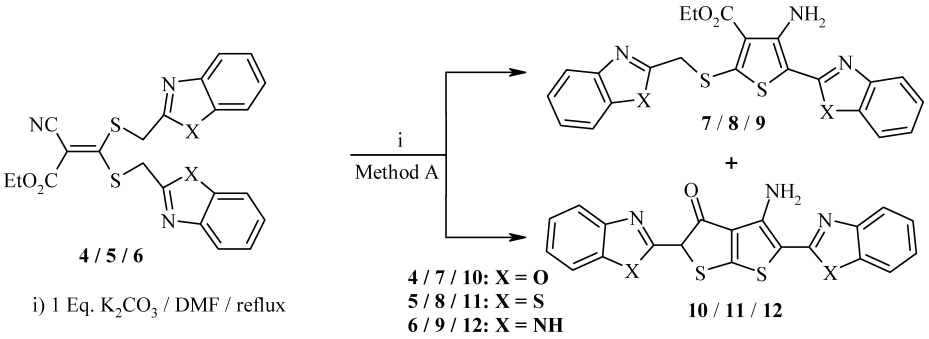

Chart 2

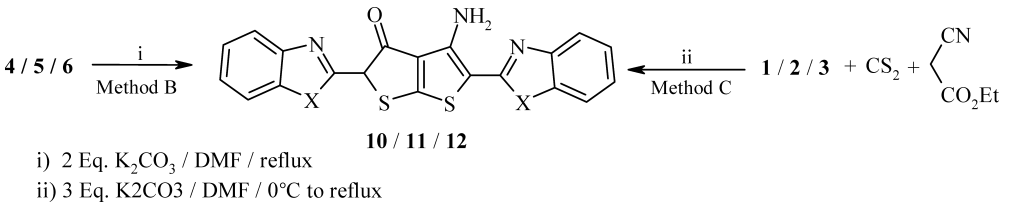

Chart 3

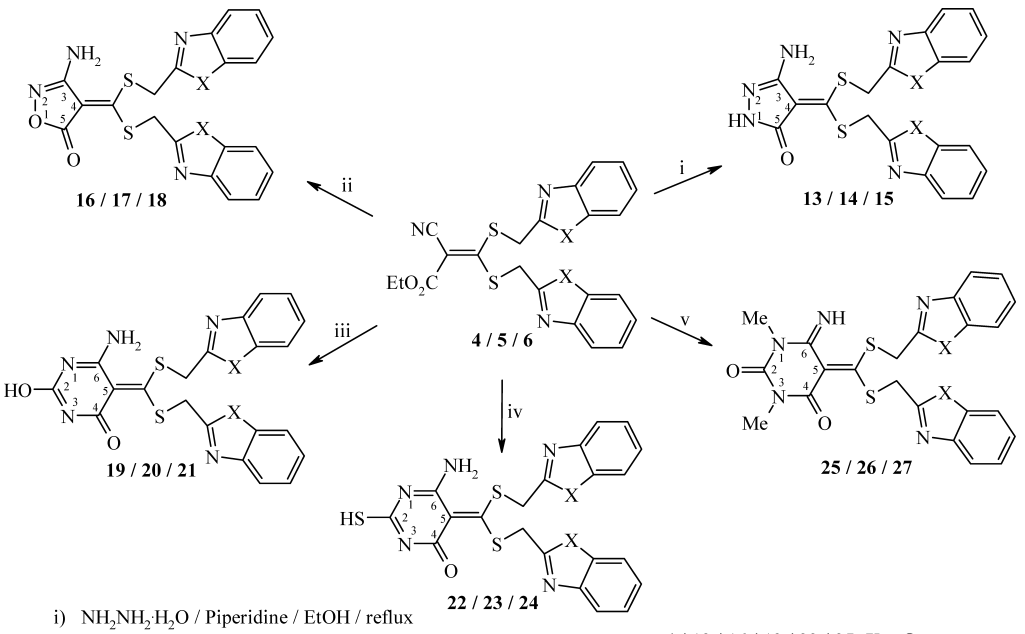

Chart 4

Table 1. Antioxidant Activities of the Test Compounds **4**–**27** and Standard Using DPPH Scavenging Method—% DPPH Radical Scavenging Activity

Compound	Concentration			
	50 μg/mL (%)	100 μg/mL (%)	150 μg/mL (%)	IC_{50} μg/mL
4	50.74±1.24	53.94±1.41	57.13±1.24	49.28±1.20
5	—	—	—	—
6	—	—	—	—
7	52.15±1.11	55.02±1.05	58.44±1.34	47.94±1.02
8	—	—	—	—
9	—	—	—	—
10	—	—	—	—
11	—	—	—	—
12	—	—	—	—
13	75.24±0.66	80.28±0.87	83.18±0.54	33.22±0.29
14	59.62±1.31	62.98±1.04	67.30±1.35	41.94±0.92
15	17.37±1.75	20.24±1.28	22.20±1.04	247.03±0.68
16	77.03±0.89	82.97±0.77	85.80±0.84	32.18±0.80
17	59.89±0.87	63.54±0.67	68.60±1.10	41.74±0.91
18	16.58±1.54	20.14±1.67	24.25±1.48	248.26±1.28
19	58.74±0.96	63.05±0.68	66.40±0.54	42.56±0.69
20	14.36±0.87	17.98±0.98	20.20±1.04	278.08±1.12
21	—	—	—	—
22	17.46±1.54	20.94±1.59	24.81±1.48	238.77±0.67
23	—	—	—	—
24	—	—	—	—
25	60.06±0.58	63.98±0.78	68.82±0.97	41.62±0.40
26	18.34±1.02	20.78±1.23	23.10±1.41	240.61±1.09
27	—	—	—	—
Ascorbic acid	77.15±0.43	83.82±0.64	86.30±0.57	32.40±0.18
Blank	—	—	—	—

(—) Showed no scavenging activity. Values were the means of three replicates±S.D.

Table 2. Antioxidant Activities of the Test Compounds **4**–**27** and Standard Using NO Scavenging Method—% NO Radical Scavenging Activity

Compound	Concentration			
	50 μg/mL (%)	100 μg/mL (%)	150 μg/mL (%)	IC_{50} μg/mL
4	60.36±0.98	62.78±0.87	65.48±1.12	41.64±0.39
5	—	—	—	—
6	—	—	—	—
7	61.26±0.87	64.08±084	67.38±0.94	40.80±0.58
8	—	—	—	—
9	—	—	—	—
10	—	—	—	—
11	—	—	—	—
12	—	—	—	—
13	83.81±0.54	88.69±0.49	89.42±0.78	29.82±0.19
14	66.53±1.24	69.24±1.34	72.42±1.62	37.58±0.87
15	24.24±1.12	26.75±1.23	30.45±1.20	193.24±1.05
16	85.49±0.87	90.05±0.64	94.30±0.59	29.24±0.30
17	68.14±0.81	71.83±0.67	75.28±0.88	36.68±0.44
18	22.92±1.31	25.45±1.36	28.50±1.18	212.35±1.21
19	64.24±1.54	68.14±1.64	70.13±1.52	38.92±0.93
20	20.91±0.87	24.03±0.77	28.34±0.86	224.56±0.69
21	—	—	—	—
22	25.14±0.58	27.89±0.97	29.92±0.74	186.44±0.78
23	—	—	—	—
24	—	—	—	—
25	70.23±0.87	75.18±1.05	78.20±0.96	35.44±0.24
26	27.56±1.34	31.18±1.54	35.70±1.28	174.71±1.12
27	—	—	—	—
Ascorbic acid	86.02±0.57	91.53±0.64	95.62±0.75	29.06±0.19
Blank	—	—	—	—

(—) Showed no scavenging activity. Values were the means of three replicates±S.D.

ethylbenzothiazoline-6-sulphonic acid) (ABTS)[38] methods and the results are shown in Tables 1–3 and Figs. 1–3. Amongst the *gem* cyanoester heterocycles, the compound **4** displayed moderate radical scavenging activity in all the three methods while **5** and **6** were inactive. In tetrasubstituted thiophene derivatives, the compound **7** exhibited good activity when compared with **8** and **9**. It was observed that there was no marked difference in activity between *gem* cyanoester heterocycles (**4**–**6**) and thiophene derivatives (**7**–**9**). On the other hand, the thienothiophene derivatives **10**, **11** and **12** were inactive. However, amongst tris heterocyclic systems, the compounds having pyrazolyl and isoxazolyl units in combination with bis benzoxazole ring **13** and **16** showed excellent radical scavenging activity when compared with the standard ascorbic acid. Besides, the compounds having pyrazolyl and isoxazolyl rings in combination with bis benzothiazole unit **14** and **17** exhibited good activity while **15** and **18** displayed least activity. Further the results indicated that the compounds having pyrimidine derivatives **19** and **25** showed good activity while the compounds **20**, **22** and **26** exhibited least activity. The compounds **21**, **23**, **24** and **27** were inactive. In general, the compounds with benzoxazole units exhibited promising activity than those having benzothiazole and benzimidazole units. Further the results exemplified that the compounds having benzoxazolyl unit in combination with pyrazole (**13**) and isoxazole (**16**) were the most potent antioxidant agents. Furthermore the free radical scavenging activity of compounds **7**, **13**, **14**, **16** and **17** was measured at different concentrations and monitored the change in absorbance at 10, 20 and 30 min in DPPH method. At these 10 min intervals the values are very close and the results showed that the antioxidant activity of these compounds is independent of time (Table 4).

Conclusion

The intramolecular and intermolecular cyclocondensation of *gem* cyanoester ketene dithiolates led to a variety of heteorocycles, tetrasubstituted thiophene derivatives, thieno-thiophene derivatives and tris heterocyclic compounds. The antioxidant property of the synthesized compounds showed that the bis benzoxazolylmethylthiomethylene pyrazole and isoxazole exhibited excellent radical scavenging activity.

Experimental

General Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The purity of the compounds was checked by TLC (silica gel H, BDH, ethyl acetate/hexane, 1 : 3). The IR spectra were recorded on a Thermo Nicolet IR 200 FT-IR spectrometer as KBr pellets and the wave numbers were given in cm^{-1}. The ^1H- and ^{13}C-NMR spectra were recorded on a Brucker-400 spectrometer at 400 MHz and 100 MHz, respectively. Chemical shifts were reported in parts per million from tetramethylsilane (TMS) as internal standard in DMSO-d_6 solution. The mass spectra were recorded on Jeol JMS-D 300 and Finnigan Mat 1210 B at 70 eV with an emission current of 100 μA. The microanalyses were performed on a Perkin-Elmer 240C elemental analyzer. The progress of the reaction was monitored by thin layer chromatography (TLC) using silica gel plates (silica gel

Table 3. Antioxidant Activities of the Test Compounds **4**–**27** and Standard Using ABTS Scavenging Method—% ABTS Radical Scavenging Activity

Compound	Concentration			
	50 μg/mL (%)	100 μg/mL (%)	150 μg/mL (%)	IC_{50} μg/mL
4	12.62±1.57	13.25±1.49	15.23±1.68	865.69±1.32
5	—	—	—	—
6	—	—	—	—
7	12.94±1.34	14.42±1.44	18.30±1.38	826.69±1.21
8	—	—	—	—
9	—	—	—	—
10	—	—	—	—
11	—	—	—	—
12	—	—	—	—
13	26.48±0.57	27.54±0.64	29.24±0.57	713.12±0.48
14	13.38±0.98	14.76±1.04	16.92±1.23	800.54±1.11
15	—	—	—	—
16	28.32±0.67	29.64±0.75	31.24±0.55	674.27±0.63
17	14.42±1.01	15.05±1.12	18.32±1.03	768.38±1.05
18	—	—	—	—
19	12.76±1.34	13.86±1.43	16.43±1.20	858.69±1.24
20	8.34±1.57	9.15±1.54	11.10±1.46	>1000
21	—	—	—	—
22	9.02±0.87	9.85±0.95	12.00±0.81	>1000
23	—	—	—	—
24	—	—	—	—
25	13.84±0.37	14.78±0.62	17.30±0.41	786.46±0.51
26	9.64±1.68	10.28±1.53	12.82±1.37	>1000
27	—	—	—	—
Ascorbic acid	28.80±0.54	29.70±0.34	31.30±0.75	650.05±0.58
Blank	—	—	—	—

(—) Showed no scavenging activity. Values were the means of three replicates±S.D.

60 F_{254} 0.25 mm) and components were visualized by observation under UV light (254, 365 nm). The compounds 2-(chloromethyl)benzoxazole (**1**), 2-(chloromethyl)benzothiazole (**2**) and 2-(chloro-methyl)-1*H*-benzimidazole (**3**) were prepared by the literature procedure.[31–33)]

General Procedure for the Synthesis of Ethyl 3,3-Bis((benzo[*d*]oxazol-2-yl)methyl-thio)-2-cyanoacrylate (4)/Ethyl 3,3-Bis((benzo[*d*]thiazol-2-yl)methylthio)-2-cyanoacrylate (5)/Ethyl 3,3-Bis((1*H*-benzo[*d*]imidazol-2-yl)-methylthio)-2-cyanoacrylate (6) To dried potassium carbonate (10 mmol) in DMF (2 mL), ethyl cyanoacetate (10 mmol) in DMF (1 mL) followed by carbon disulfide (15 mmol) were added dropwise under vigorous stirring. After 30 min, the reaction mixture was cooled to 0°C and to this **1**/**2**/**3** (20 mmol) in DMF (5 mL) was added in 20–30 min. The reaction mixture was further stirred for 1 h at room temperature and poured into cold water. The precipitate was collected, dried and recrystallized from aqueous methanol.

Ethyl 3,3-Bis((benzo[*d*]oxazol-2-yl)methylthio)-2-cyanoacrylate (**4**): Yellow solid, yield 82%, mp 160–162°C. IR (KBr) cm^{-1}: 1612 (C=N), 1638 (C=C), 1730 (CO), 2220 (C≡N). ^{1}H-NMR (DMSO-d_6) δ: 1.32 (t, 3H, $CH_2C\underline{H}_3$), 4.21 (q, 2H, $C\underline{H}_2CH_3$), 4.31 (s, 2H, CH_2), 4.35 (s, 2H, CH_2), 7.17–7.56 (m, 8H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 14.0 ($CH_2\underline{C}H_3$), 32.2, 32.7 (CH_2–S), 61.1 ($\underline{C}H_2CH_3$), 104.9 (=$\underline{C}$–CO), 115.3 (CN), 160.8, 161.1 (C-2′), 163.8 (CO), 174.9 (C=$\underline{C}$(S)(S)), 119.5, 120.4, 122.7, 124.2, 125.1, 138.4, 138.9, 148.3, 149.2 (aromatic carbons). MS *m*/*z*: 451.43 (M^+). *Anal.* Calcd for $C_{22}H_{17}N_3O_4S_2$: C, 58.52; H, 3.79; N, 9.31. Found: C, 58.46; H, 3.83; N, 9.27.

Table 4. Antioxidant Activity of the Compounds **7**, **13**, **14**, **16** and **17** at 10 min Time Intervals by DPPH Scavenging Method

Compound	10 min	20 min	30 min
7	51.56	51.92	52.15
13	74.86	75.07	75.24
14	58.93	59.34	59.62
16	76.59	76.85	77.03
17	59.12	59.45	59.89

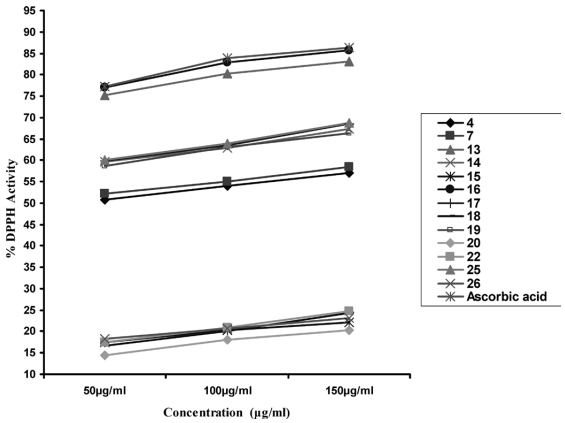

Fig. 1. Free Radical Scavenging Activity of Compounds **4**–**27** by DPPH Method

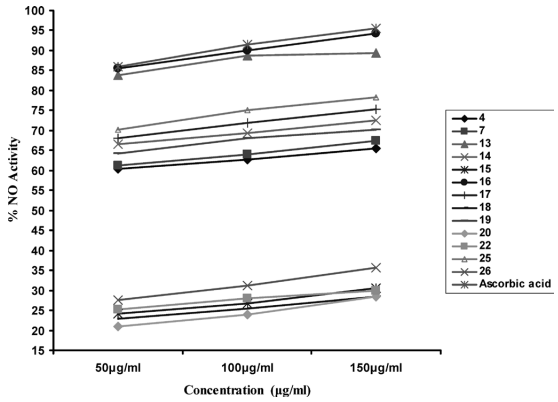

Fig. 2. Free Radical Scavenging Activity of Compounds **4**–**27** by Nitric Oxide Method

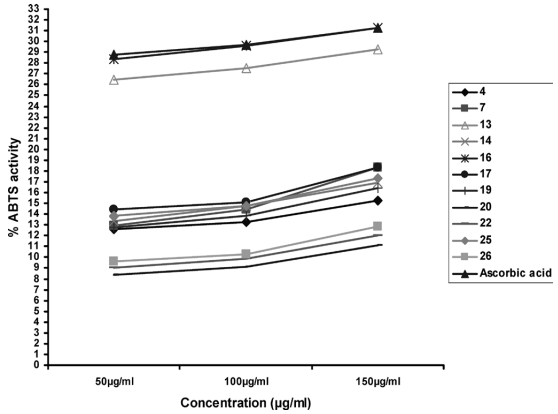

Fig. 3. Free Radical Scavenging Activity of Compounds **4**–**27** in ABTS Method

Ethyl 3,3-Bis((benzo[*d*]thiazol-2-yl)methylthio)-2-cyanoacrylate (**5**): Yellow solid, yield 80%, mp 169–171°C. IR (KBr) cm^{-1}: 1608 (C=N), 1634 (C=C), 1725 (CO), 2229 (C≡N). ^{1}H-NMR (DMSO-d_6) δ: 1.27 (t, 3H, $CH_2C\underline{H}_3$), 4.25 (q, 2H, $C\underline{H}_2CH_3$), 4.28 (s, 2H, CH_2), 4.38 (s, 2H, CH_2), 7.46–8.01 (m, 8H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 14.2 ($CH_2\underline{C}H_3$), 31.9, 32.6 (CH_2–S), 61.7 ($\underline{C}H_2CH_3$), 105.2 (=$\underline{C}$–CO), 114.9 (CN), 163.1 (CO), 169.4, 170.2 (C-2′), 174.5 (C=$\underline{C}$(S)(S)), 120.1, 122.4, 125.6, 127.2, 127.7, 135.2, 135.4, 148.1, 149.6 (aromatic carbons). MS (*m*/*z*): 483.51 (M^+). *Anal.* Calcd for $C_{22}H_{17}N_3O_2S_4$: C, 54.63; H, 3.54; N, 8.69. Found: C, 54.61; H, 3.57; N, 8.78.

Ethyl 3,3-Bis((1*H*-benzo[*d*]imidazol-2-yl)methylthio)-2-cyanoacrylate (**6**): Yellow solid, yield 85%, mp 177–179°C. IR (KBr) cm^{-1}: 1605 (C=N), 1626 (C=C), 1735 (CO), 2215 (C≡N), 3307 (NH). ^{1}H-NMR (DMSO-d_6) δ: 1.30 (t, 3H, $CH_2C\underline{H}_3$), 4.20 (q, 2H, $C\underline{H}_2CH_3$), 4.30 (s, 2H, CH_2), 4.39 (s, 2H, CH_2), 7.28–7.53 (m, 8H, Ar-H), 12.68 (br s, 2H, NH). ^{13}C-NMR (DMSO-d_6) δ: 14.5 ($CH_2\underline{C}H_3$), 32.5, 32.9 (CH_2–S), 62.2 ($\underline{C}H_2CH_3$), 104.7 (=$\underline{C}$–CO), 114.5 (CN), 150.7, 151.2 (C-2′), 163.4 (CO), 175.1 (C=$\underline{C}$(S)(S)), 118.4, 121.7, 122.4, 123.1, 128.5, 128.9, 132.3, 132.9, 138.5 (aromatic carbons). MS (*m*/*z*): 449.47 (M^+). *Anal.* Calcd for $C_{22}H_{19}N_5O_2S_2$: C, 58.78; H, 4.26; N, 15.58. Found: C, 58.82; H, 4.28; N, 15.64.

General Procedure for the Synthesis of Ethyl 2-((Benzo[*d*]oxazol-2′-yl)methylthio)-4-amino-5-(benzo[*d*]oxazol-2-yl)thiophene-3-carboxylate (7)/Ethyl 2-((Benzo[*d*]thiazol-2′-yl)-methylthio)-4-amino-5-(benzo[*d*]thiazol-2-yl)thiophene-3-carboxylate (8)/Ethyl 2-((1′*H*-Benzo[*d*]imidazol-2′-yl)methylthio)-4-amino-5-(1′*H*-benzo[*d*]imidazol-2′-yl)-thiophene-3-carboxylate (9) and 4-Amino-2,5-di(benzo[*d*]-oxazol-2′-yl)thieno[2,3-*b*]thiophen-3(2*H*)-one (10)/4-Amino-2,5-di(benzo[*d*]thiazol-2′-yl)thieno[2,3-*b*]thiophen-3(2*H*)-one (11)/4-Amino-2,5-di(1′*H*-benzo[*d*]imidazol-2′-yl)-thieno[2,3-*b*]thiophen-3(2*H*)-one (12) (Method A) To a mixture of dried potassium carbonate (10 mmol) and DMF (2 mL), **4**/**5**/**6** (10 mmol) was added and refluxed for 2 h and poured into cold water. The precipitate obtained was collected, dried and purified by column chromatography and identified as **7**/**8**/**9** (major) and **10**/**11**/**12** (minor).

Ethyl 2-((Benzo[*d*]oxazol-2′-yl)methylthio)-4-amino-5-(benzo[*d*]oxazol-2-yl)thiophene-3-carboxylate (**7**): Red solid, yield 57%, mp 165–167°C. IR (KBr) cm^{-1}: 1602 (C=N), 1620 (C=C), 1716 (CO), 3322, 3431 (NH_2). ^{1}H-NMR (DMSO-d_6) δ: 1.36 (t, 3H, $CH_2C\underline{H}_3$), 4.22 (q, 2H, $C\underline{H}_2CH_3$), 4.37 (s, 2H, CH_2), 6.88 (br s, 2H, NH_2), 7.18–7.48 (m, 8H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 14.3 ($CH_2\underline{C}H_3$), 32.1 (S–CH_2), 62.3 ($\underline{C}H_2CH_3$), 117.5 (C-3), 123.5 (C-5), 153.9 (C-4), 154.6 (C-2), 161.3, 162.1 (C-2′), 165.8 (CO), 118.9, 121.5, 122.2, 125.7, 126.4, 137.2, 137.9, 138.5, 148.6, 149.4 (aromatic carbons). *Anal.* Calcd for $C_{22}H_{17}N_3O_4S_2$: C, 58.52; H, 3.79; N, 9.31. Found: C, 58.58; H, 3.78; N, 9.28. MS (*m*/*z*): 451.47 (M^+).

Ethyl 2-((Benzo[*d*]thiazol-2′-yl)methylthio)-4-amino-5-(benzo[*d*]thiazol-2-yl)thiophene-3-carboxylate (**8**): Red solid, yield 61%, mp 174–176°C. IR (KBr) cm^{-1}: 1608 (C=N), 1624 (C=C), 1720 (CO), 3338, 3425 (NH_2). ^{1}H-NMR (DMSO-d_6) δ: 1.31 (t, 3H, $CH_2C\underline{H}_3$), 4.16 (q, 2H, $C\underline{H}_2CH_3$), 4.32 (s, 2H, CH_2), 6.81 (br s, 2H, NH_2), 7.44–7.98 (m, 8H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 14.1 ($CH_2\underline{C}H_3$), 32.9 (S–CH_2), 61.5 ($\underline{C}H_2CH_3$), 117.8 (C-3), 122.8 (C-5), 153.2 (C-4), 154.8 (C-2), 164.6 (CO), 169.4, 170.1 (C-2′), 119.2, 121.3, 124.6, 125.3, 127.4, 128.2, 134.8, 135.2, 149.7, 151.2 (aromatic carbons). MS (*m*/*z*): 483.60 (M^+). *Anal.* Calcd for $C_{22}H_{17}N_3O_2S_4$: C, 54.63; H, 3.54; N, 8.69. Found: C, 54.66; H, 3.57; N, 8.74.

Ethyl 2-((1′*H*-Benzo[*d*]imidazol-2′-yl)methylthio)-4-amino-5-(1′*H*-benzo[*d*]imidazol-2′-yl)thiophene-3-carboxylate (**9**): Red solid, yield 58%, mp 182–184°C. IR (KBr) cm^{-1}: 1601 (C=N), 1616 (C=C), 1725 (CO), 3291 (NH), 3315, 3438 (NH_2). ^{1}H-NMR (DMSO-d_6) δ: 1.33 (t, 3H, $CH_2C\underline{H}_3$), 4.18 (q, 2H, $C\underline{H}_2CH_3$), 4.34 (s, 2H, CH_2), 6.90 (br s, 2H, NH_2), 7.25–7.62 (m, 8H, Ar-H), 12.59 (bs, 2H, NH). ^{13}C-NMR (DMSO-d_6) δ: 14.5, ($CH_2\underline{C}H_3$), 32.6 (S–CH_2), 61.9 ($\underline{C}H_2CH_3$), 117.1 (C-3), 123.0 (C-5), 150.1, 151.4 (C-2′), 153.6 (C-4), 154.2 (C-2), 165.2 (CO), 118.5, 119.8, 121.6, 122.2, 126.4, 127.7, 132.3, 133.4, 137.4, 138.1 (aromatic carbons). MS (*m*/*z*): 449.49 (M^+). *Anal.* Calcd for $C_{22}H_{19}N_5O_2S_2$: C, 58.78; H, 4.26; N, 15.58. Found: C, 58.65; H, 4.30; N, 15.65.

General Procedure for the Synthesis of 4-Amino-2,5-di(benzo[*d*]oxazol-2′-yl)-thieno[2,3-*b*]thiophen-3(2*H*)-one (10)/4-Amino-2,5-di(benzo[*d*]thiazol-2′-yl)thieno[2,3-*b*]-thiophen-3(2*H*)-one (11)/4-Amino-2,5-di(1′*H*-benzo[*d*]-imidazol-2′-yl)thieno[2,3-*b*]thiophen-3(2*H*)-one (12) Method B To a mixture of dried potassium carbonate (10 mmol) and DMF (4 mL), **4**/**5**/**6** (5 mmol) was added, refluxed for 1 h and poured into cold water. The precipitate obtained was collected, dried and recrystallized from 2-propanol.

Method C A solution of ethyl cyanoacetate (10 mmol) in DMF (3 mL) followed by carbon disulfide (15 mmol) were

added dropwise to a solution of dried potassium carbonate (30 mmol) in DMF (2 mL) while stirring. After 30 min, the contents were cooled to 0°C and to this, compound **1**/**2**/**3** (20 mmol) in DMF (5 mL) was added in 20–30 min. The reaction mixture was then refluxed for 2 h and poured into cold water. The precipitate was collected, dried and recrystallized from 2-propanol.

4-Amino-2,5-di(benzo[*d*]oxazol-2′-yl)thieno[2,3-*b*]thiophen-3(2*H*)-one (**10**): Brown solid, yield 16 (Method A), 62 (Method B), 68% (Method C), mp 263–265°C. IR (KBr) cm^{-1}: 1595 (C=N), 1623 (C=C), 1738 (CO), 3321, 3427 (NH_2). ^{1}H-NMR (DMSO-d_6) δ: 4.45 (s, 1H, CH), 6.69 (br s, 2H, NH_2), 7.20–7.56 (m, 8H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 60.2 (C-2), 114.1 (C=C(S)(S)), 128.8 (C-5), 154.1 (C=C(S)(S)), 155.9 (C-4), 161.1 and 161.8 (C-2′), 182.5 (CO), 118.8, 121.5, 123.2, 125.1, 126.3, 136.4, 137.8, 148.7, 149.6 (aromatic carbons). MS (*m*/*z*): 405.38 (M^+). *Anal*. Calcd for $C_{20}H_{11}N_3O_3S_2$: C, 59.25; H, 2.73; N, 10.36. Found: C, 59.31; H, 2.71; N, 10.41.

4-Amino-2,5-di(benzo[*d*]thiazol-2′-yl)thieno[2,3-*b*]thiophen-3(2*H*)-one (**11**): Brown solid, yield 14 (Method A), 60 (Method B), 73% (Method C), mp 275–277°C. IR (KBr) cm^{-1}: 1602 (C=N), 1628 (C=C), 1733 (CO), 3317, 3420 (NH_2). ^{1}H-NMR (DMSO-d_6) δ: 4.51 (s, 1H, CH), 6.58 (br s, 2H, NH_2), 7.43–8.02 (m, 8H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 61.1 (C-2), 115.2 (C=C(S)(S)), 129.7 (C-5), 153.8 (C=C(S)(S)), 155.4 (C-4), 169.8 and 170.3 (C-2′), 183.2 (CO), 119.6, 120.4, 123.1, 125.2, 128.3, 134.8, 135.7, 147.4, 148.9 (aromatic carbons). MS (*m*/*z*): 437.52 (M^+). *Anal*. Calcd for $C_{20}H_{11}N_3OS_4$: C, 54.90; H, 2.53; N, 9.60. Found: C, 54.94; H, 2.56; N, 9.56.

4-Amino-2,5-di(1′*H*-benzo[*d*]imidazol-2′-yl)thieno[2,3-*b*]-thiophen-3(2*H*)-one (**12**): Brown solid, yield 18 (Method A), 65 (Method B), 79% (Method C), mp 280–282°C. IR (KBr) cm^{-1}: 1606 (C=N), 1625 (C=C), 1735 (CO), 3287 (NH), 3310, 3437 (NH_2). ^{1}H-NMR (DMSO-d_6) δ: 4.53 (s, 1H, CH), 6.61 (br s, 2H, NH_2), 7.29–7.75 (m, 8H, Ar-H), 12.65 (br s, 2H, NH). ^{13}C-NMR (DMSO-d_6) δ: 60.7 (C-2), 114.6 (C=C(S)(S)), 129.3 (C-5), 150.5 and 151.3 (C-2′), 154.5 (C=C(S)(S)), 156.3 (C-4), 182.8 (CO), 118.6, 122.8, 123.1, 128.5, 128.9, 131.6, 132.4, 137.2, 138.5 (aromatic carbons). MS (*m*/*z*): 403.41 (M^+). *Anal*. Calcd for $C_{20}H_{13}N_5OS_2$: C, 59.54; H, 3.25; N, 17.36. Found: C, 59.56; H, 3.24; N, 17.46.

General Procedure for the Synthesis of 3-Amino-4-(bis((benzo[*d*]oxazol-2′-yl)methyl-thio)methylene)-1*H*-pyrazol-5(4*H*)-one (13)/3-Amino-4-(bis((benzo[*d*]-thiazol-2′-yl)methyl-thio)methylene)-1*H*-pyrazol-5(4*H*)-one (14)/3-Amino-4-(bis((1′*H*-benzo[*d*]imidazol-2′-yl)-methylthio)methylene)-1*H*-pyrazol-5(4*H*)-one (15) The compound **4**/**5**/**6** (1 mmol), hydrazine hydrate (1.2 mmol) and piperidine (3 mL) in ethanol (10 mL) were refluxed for 6–8 h. After completion of the reaction, it was cooled and poured into ice-cold water containing conc. HCl. The solid obtained was filtered, dried and recrystallized from 2-propanol.

3-Amino-4-(bis((benzo[*d*]oxazol-2′-yl)methylthio)-methylene)-1*H*-pyrazol-5(4*H*)-one (**13**): Yellow solid, yield 74%, mp 189–191°C. IR (KBr) cm^{-1}: 1604 (C=N), 1622 (C=C), 1661 (CO), 3209 (NH), 3323, 3418 (NH_2). ^{1}H-NMR (DMSO-d_6) δ: 4.30 (s, 2H, CH_2), 4.37 (s, 2H, CH_2), 5.75 (br s, 2H, NH_2), 7.17–7.56 (m, 8H, Ar-H), 9.74 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 32.2, 33.1 (CH_2–S), 103.5 (C-4), 157.3 (C-3), 160.4, 161.3 (C-2′), 171.8 (C-5), 175.7 (C=C(S)(S)), 119.1, 120.6, 122.2, 124.6, 124.9, 138.7, 139.4, 148.3 (aromatic carbons). MS (*m*/*z*): 437.43 (M^+). *Anal*. Calcd for $C_{20}H_{15}N_5O_3S_2$: C, 54.91; H, 3.46; N, 16.01. Found: C, 54.88; H, 3.50; N, 16.12.

3-Amino-4-(bis((benzo[*d*]thiazol-2′-yl)methylthio)-methylene)-1*H*-pyrazol-5(4*H*)-one (**14**): Yellow solid, yield 70%, mp 211–213°C. IR (KBr) cm^{-1}: 1610 (C=N), 1628 (C=C), 1656 (CO), 3215 (NH), 3334, 3427 (NH_2). ^{1}H-NMR (DMSO-d_6) δ: 4.28 (s, 2H, CH_2), 4.39 (s, 2H, CH_2), 5.68 (br s, 2H, NH_2), 7.36–7.92 (m, 8H, Ar-H), 9.86 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 31.7, 32.6 (CH_2–S), 104.2 (C-4), 158.1 (C-3), 169.6, 170.2 (C-2′), 172.5 (C-5), 174.5 (C=C(S)(S)), 119.2, 122.7, 124.2, 126.4, 135.3, 135.7, 149.3, 150.4 (aromatic carbons). MS (*m*/*z*): 469.58 (M^+). *Anal*. Calcd for $C_{20}H_{15}N_5OS_4$: C, 51.15; H, 3.22; N, 14.91. Found: C, 51.20; H, 3.20; N, 14.98.

3-Amino-4-(bis((1′*H*-benzo[*d*]imidazol-2′-yl)methylthio)-methylene)-1*H*-pyrazol-5(4*H*)-one (**15**): Yellow solid, yield 79%, mp 194–196°C. IR (KBr) cm^{-1}: 1606 (C=N), 1619 (C=C), 1659 (CO), 3226 (NH), 3328, 3430 (NH, NH_2). ^{1}H-NMR (DMSO-d_6) δ: 4.31 (s, 2H, CH_2), 4.40 (s, 2H, CH_2), 5.60 (br s, 2H, NH_2), 7.31–7.80 (m, 8H, Ar-H), 9.67 (br s, 1H, NH), 12.41 (br s, 2H, NH). ^{13}C-NMR (DMSO-d_6) δ: 32.4, 32.8 (CH_2–S), 103.5 (C-4), 150.3, 151.6 (C-2′), 158.4 (C-3), 171.6 (C-5), 175.3 (C=C(S)(S)), 120.5, 122.2, 125.6, 126.4, 127.1, 131.5, 131.8, 137.9 (aromatic carbons). MS (*m*/*z*): 435.46 (M^+). *Anal*. Calcd for $C_{20}H_{17}N_7OS_2$: C, 55.16; H, 3.93; N, 22.51. Found: C, 55.12; H, 3.96; N, 22.62.

General Procedure for the Synthesis of 3-Amino-4-(bis((benzo[*d*]oxazol-2′-yl)methyl-thio)methylene)isoxazol-5(4*H*)-one (16)/3-Amino-4-(bis((benzo[*d*]thiazol-2′-yl)-methylthio)-methylene)isoxazol-5(4*H*)-one (17)/3-Amino-4-(bis((1′*H*-benzo[*d*]imidazol-2′-yl)methyl-thio)methylene)-isoxazol-5(4*H*)-one (18) A mixture of **4**/**5**/**6** (1 mmol), hydroxylamine hydrochloride (1.1 mmol), piperidine (3 mL) and ethanol (10 mL) was refluxed for 7–9 h. It was cooled and poured into ice-cold water containing conc. HCl. The solid separated was collected, dried and recrystallized from 2-propanol.

3-Amino-4-(bis((benzo[*d*]oxazol-2′-yl)methylthio)methylene)isoxazol-5(4*H*)-one (**16**): Yellow solid, yield 75%, mp 181–183°C. IR (KBr) cm^{-1}: 1598 (C=N), 1623 (C=C), 1732 (CO), 3325, 3442 (NH_2). ^{1}H-NMR (DMSO-d_6) δ: 4.29 (s, 2H, CH_2), 4.34 (s, 2H, CH_2), 5.59 (br s, 2H, NH_2), 7.14–7.64 (m, 8H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 32.7, 33.4 (CH_2-S), 103.9 (C-4), 158.9 (C-3), 160.5, 161.8 (C-2′), 173.2 (C-5), 174.9 (C=C(S)(S)), 120.6, 123.2, 126.7, 127.1, 128.2, 128.9, 139.8, 141.0, 149.4 (aromatic carbons). MS (*m*/*z*): 438.40 (M^+). *Anal*. Calcd for $C_{20}H_{14}N_4O_4S_2$: C, 54.78; H, 3.22; N, 12.78. Found: C, 54.84; H, 3.20; N, 12.84.

3-Amino-4-(bis((benzo[*d*]thiazol-2′-yl)methylthio)methylene)isoxazol-5(4*H*)-one (**17**): Yellow solid, yield 77%, mp 202–204°C. IR (KBr) cm^{-1}: 1607 (C=N), 1619 (C=C), 1741 (CO), 3320, 3438 (NH_2). ^{1}H-NMR (DMSO-d_6) δ: 4.32 (s, 2H, CH_2), 4.38 (s, 2H, CH_2), 5.62 (br s, 2H, NH_2), 7.28–7.82 (m, 8H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 31.9, 32.7 (CH_2–S), 105.1 (C-4), 158.2 (C-3), 169.8, 170.7 (C-2′), 174.1 (C-5), 175.7 (C=C(S)(S)), 121.3, 123.9, 126.5, 127.2, 135.6, 136.2, 149.7, 150.1 (aromatic carbons). MS (*m*/*z*): 470.54 (M^+). *Anal*. Calcd for $C_{20}H_{14}N_4O_2S_4$: C, 51.04; H, 3.00; N, 11.91. Found: C, 51.07; H, 3.03; N, 12.00.

3-Amino-4-(bis((1′*H*-benzo[*d*]imidazol-2′-yl)methylthio)-methylene)isoxazol-5(4*H*)-one (**18**): Yellow solid, yield 80%,

mp 214–215°C. IR (KBr) cm^{-1}: 1610 (C=N), 1628 (C=C), 1746 (CO), 3298 (NH), 3326, 3452 (NH_2). ^{1}H-NMR (DMSO-d_6) δ: 4.34 (s, 2H, CH_2), 4.39 (s, 2H, CH_2), 5.66 (br s, 2H, NH_2), 7.34–7.73 (m, 8H, Ar-H), 12.35 (br s, 2H, NH). ^{13}C-NMR (DMSO-d_6) δ: 32.1, 33.0 (CH_2–S), 104.3 (C-4), 150.2 (C-2′), 159.3 (C-3), 173.6 (C-5), 175.1 (C=C(S)(S)), 118.9, 121.5, 125.2, 128.9, 129.2, 130.2, 136.4, 137.3 (aromatic carbons). MS (*m*/*z*): 436.44 (M^+). *Anal.* Calcd for $C_{20}H_{16}N_6O_2S_2$: C, 55.03; H, 3.69; N, 19.25. Found: C, 55.08; H, 3.65; N, 19.31.

General Procedure for the Synthesis of 6-Amino-5-(bis((benzo[*d*]oxazol-2′-yl)methyl-thio)methylene)-2-hydroxypyrimidin-4(5*H*)-one (19)/6-Amino-5-(bis((benzo[*d*]-thiazol-2′-yl)methylthio)methylene)-2-hydroxypyrimidin-4(5*H*)-one (20)/6-Amino-5-(bis((1′*H*-benzo[*d*]imidazol-2′-yl)methylthio)methylene)-2-hydroxypyrimidin-4(5*H*)-one (21) To an equimolar (1 mmol) mixture of compound **4**/**5**/**6** and urea, piperidine (3 mL) and ethanol (10 mL) were added and refluxed for 8–10 h. The contents of the flask were cooled and poured into ice-cold water containing conc. HCl. The solid separated was filtered, dried and purified by recrystallization from 2-propanol.

6-Amino-5-(bis((benzo[*d*]oxazol-2′-yl)methylthio)-methylene)-2-hydroxypyrimidin-4(5*H*)-one (**19**): Pale yellow solid, yield 74%, mp 208–210°C. IR (KBr) cm^{-1}: 1603 (C=N), 1622 (C=C), 1670 (CO), 3328 (OH), 3342, 3457 (NH_2). ^{1}H-NMR (DMSO-d_6) δ: 4.31 (s, 2H, CH_2), 4.40 (s, 2H, CH_2), 5.61 (br s, 2H, NH_2), 7.23–7.59 (m, 8H, Ar-H), 8.31 (br s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ: 32.3, 33.1 (CH_2–S), 105.2 (C-5), 158.9 (C-6), 160.2, 161.5 (C-2′), 162.8 (C-2), 173.2 (C-4), 175.3 (C=C(S)(S)), 121.5, 125.3, 126.8, 128.7, 129.2, 138.5, 140.2, 148.3 (aromatic carbons). MS (*m*/*z*): 465.42 (M^+). *Anal.* Calcd for $C_{21}H_{15}N_5O_4S_2$: C, 54.18; H, 3.25; N, 15.04. Found: C, 54.15; H, 3.27; N, 15.11.

6-Amino-5-(bis((benzo[*d*]thiazol-2′-yl)methylthio)-methylene)-2-hydroxypyrimidin-4(5*H*)-one (**20**): Pale yellow solid, yield 71%, mp 225–227°C. IR (KBr) cm^{-1}: 1605 (C=N), 1626 (C=C), 1672 (CO), 3315 (OH), 3351, 3449 (NH_2). ^{1}H-NMR (DMSO-d_6) δ: 4.33 (s, 2H, CH_2), 4.42 (s, 2H, CH_2), 5.58 (br s, 2H, NH_2), 7.42–7.95 (m, 8H, Ar-H), 8.36 (br s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ: 31.6, 32.8 (CH_2–S), 104.8 (C-5), 159.5 (C-6), 163.7 (C-2), 169.1, 170.2 (C-2′), 174.5 (C-4), 175.8 (C=C(S)(S)), 120.3, 124.2, 124.6, 125.3, 127.6, 137.5, 148.3, 149.8 (aromatic carbons). MS (*m*/*z*): 497.57 (M^+). *Anal.* Calcd for $C_{21}H_{15}N_5O_2S_4$: C, 50.68; H, 3.04; N, 14.07. Found: C, 50.70; H, 3.03; N, 14.16.

6-Amino-5-(bis((1′*H*-benzo[*d*]imidazol-2′-yl)methylthio)-methylene)-2-hydroxy-pyrimidin-4(5*H*)-one (**21**): Pale yellow solid, yield 75%, mp 210–212°C. IR (KBr) cm^{-1}: 1596 (C=N), 1630 (C=C), 1675 (CO), 3324 (OH), 3348, 3452 (NH and NH_2). ^{1}H-NMR (DMSO-d_6) δ: 4.30 (s, 2H, CH_2), 4.38 (s, 2H, CH_2), 5.54 (br s, 2H, NH_2), 7.26–7.86 (m, 8H, Ar-H), 8.34 (br s, 1H, OH), 12.45 (br s, 2H, NH). ^{13}C-NMR (DMSO-d_6) δ: 32.1, 33.5 (CH_2–S), 104.5 (C-5), 150.2 (C-2′), 158.4 (C-6), 163.3 (C-2), 173.7 (C-4), 175.5 (C=C(S)(S)), 119.4, 122.2, 124.8, 125.6, 127.1, 132.5, 138.5, 139.3 (aromatic carbons). MS (*m*/*z*): 463.49 (M^+). *Anal.* Calcd for $C_{21}H_{17}N_7O_2S_2$: C, 54.41; H, 3.70; N, 21.15. Found: C, 54.45; H, 3.72; N, 21.26.

General Procedure for the Synthesis of 6-Amino-5-(bis((benzo[*d*]oxazol-2′-yl)methyl-thio)methylene)-2-mercaptopyrimidin-4(5*H*)-one (22)/6-Amino-5-(bis((benzo[*d*]-thiazol-2′-yl)methylthio)methylene)-2-mercaptopyrimidin-4(5*H*)-one (23)/6-Amino-5-(bis((1′*H*-benzo[*d*]imidazol-2′-yl)methylthio)methylene)-2-mercaptopyrimidin-4(5*H*)-one (24) The compound **4**/**5**/**6** (2 mmol), thiourea (2 mmol), piperidine (5 mL) and ethanol (20 mL) were refluxed for 12–15 h. The reaction mixture was cooled and poured into ice-cold water containing conc. HCl. The solid separated was filtered on a Buchner funnel, dried and recrystallized from 2-propanol.

6-Amino-5-(bis((benzo[*d*]oxazol-2′-yl)methylthio)-methylene)-2-mercaptopyrimidin-4(5*H*)-one (**22**): Pale yellow solid, yield 69%, mp 218–220°C. IR (KBr) cm^{-1}: 1609 (C=N), 1626 (C=C), 1673 (CO), 2564 (SH), 3347, 3451 (NH_2). ^{1}H-NMR (DMSO-d_6) δ: 1.48 (br s, 1H, SH), 4.35 (s, 2H, CH_2), 4.43 (s, 2H, CH_2), 5.66 (br s, 2H, NH_2), 7.15–7.48 (m, 8H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 31.4, 32.6 (CH_2–S), 105.8 (C-5), 160.4, 161.1 (C-2′), 163.2 (C-6), 174.8 (C=C(S)(S)), 176.6 (C-4), 185.5 (C-2), 121.5, 125.3, 126.8, 128.7, 129.5, 138.4, 140.2, 148.3 (aromatic carbons). MS (*m*/*z*): 481.50 (M^+). *Anal.* Calcd for $C_{21}H_{15}N_5O_3S_3$: C, 52.38; H, 3.14; N, 14.54. Found: C, 52.41; H, 3.13; N, 14.60.

6-Amino-5-(bis((benzo[*d*]thiazol-2′-yl)methylthio)-methylene)-2-mercaptopyrimidin-4(5*H*)-one (**23**): Pale yellow solid, yield 73% mp 233–235°C. IR (KBr) cm^{-1}: 1604 (C=N), 1624 (C=C), 1677 (CO), 2556 (SH), 3342, 3446 (NH_2). ^{1}H-NMR (DMSO-d_6) δ: 1.53 (br s, 1H, SH), 4.32 (s, 2H, CH_2), 4.39 (s, 2H, CH_2), 5.58 (br s, 2H, NH_2), 7.51–7.98 (m, 8H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 31.8, 33.1 (CH_2–S), 104.6 (C-5), 162.3 (C-6), 170.5, 171.3 (C-2′), 175.6 (C=C(S)(S)), 177.4 (C-4), 184.6 (C-2), 119.5, 123.8, 125.2, 125.8, 127.3, 136.9, 148.9, 149.4 (aromatic carbons). MS (*m*/*z*): 513.64 (M^+). *Anal.* Calcd for $C_{21}H_{15}N_5OS_5$: C, 49.10; H, 2.94; N, 13.63. Found: C, 49.08; H, 2.96; N, 13.71.

6-Amino-5-(bis((1′*H*-benzo[*d*]imidazol-2′-yl)methylthio)-methylene)-2-mercapto-pyrimidin-4(5*H*)-one (**24**): Pale yellow solid, yield 74%, mp 246–248°C. IR (KBr) cm^{-1}: 1601 (C=N), 1622 (C=C), 1679 (CO), 2552 (SH), 3298 (NH), 3341, 3460 (NH_2). ^{1}H-NMR (DMSO-d_6) δ: 1.46 (br s, 1H, SH), 4.31 (s, 2H, CH_2), 4.41 (s, 2H, CH_2), 5.53 (br s, 2H, NH_2), 7.28–7.74 (m, 8H, Ar-H), 12.53 (br s, 2H, NH). ^{13}C-NMR (DMSO-d_6) δ: 32.5, 33.7 (CH_2–S), 105.1 (C-5), 150.2, 151.5 (C-2′), 162.8 (C-6), 175.2 (C=C(S)(S)), 177.1 (C-4), 185.1 (C-2), 118.6, 120.4, 123.1, 124.5, 126.8, 127.6, 131.8, 139.7 (aromatic carbons). MS (*m*/*z*): 479.53 (M^+). *Anal.* Calcd for $C_{21}H_{17}N_7OS_3$: C, 52.59; H, 3.57; N, 20.44. Found: C, 52.64; H, 3.60; N, 20.54.

General Procedure for the Synthesis of 5-(Bis((benzo[*d*]oxazol-2′-yl)methylthio)-methylene)-dihydro-6-imino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (25)/5-(Bis((benzo-[*d*]thiazol-2′-yl)methylthio)methylene)-dihydro-6-imino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (26)/5-(Bis-((1′*H*-benzo[*d*]imidazol-2′-yl)methylthio)methylene)-dihydro-6-imino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (27) A mixture of **4**/**5**/**6** (1 mmol), *N*,*N*′-dimethylurea (1 mmol), piperidine (3 mL) and ethanol (10 mL) were refluxed for 9–11 h. The contents of the flask were cooled and poured into ice-cold water containing conc. HCl. The solid separated was filtered, dried and purified by recrystallization from 2-propanol.

5-(Bis((benzo[*d*]oxazol-2′-yl)methylthio)methylene)-dihydro-6-imino-1,3-dimethyl-pyrimidine-2,4(1*H*,3*H*)-dione (**25**): Yellow solid, yield 67%, mp 197–199°C. IR (KBr) cm^{-1}: 1601 (C=N), 1622 (C=C), 1679 (CO), 3298 (NH). ^{1}H-NMR (DMSO-d_6) δ: 2.69, 2.75 (s, 6H, N–CH_3), 4.32 (s, 2H, CH_2),

4.39 (s, 2H, CH_2), 7.19–7.52 (m, 8H, Ar-H), 9.31 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 27.2, 28.3 (N–CH_3), 30.5, 32.2 (CH_2–S), 103.8 (C-5), 158.2 (C-2), 160.3, 161.1 (C-2′), 163.5 (C-6), 173.2 (C-4), 174.3 (C=C(S)(S)), 119.2, 124.1, 124.5, 125.9, 138.5, 139.7, 148.4, 149.2 (aromatic carbons). MS (*m/z*): 493.48 (M^+). *Anal.* Calcd for $C_{23}H_{19}N_5O_4S_2$: C, 55.97; H, 3.88; N, 14.19. Found: C, 56.00; H, 3.86; N, 14.13.

5-(Bis((benzo[*d*]thiazol-2′-yl)methylthio)methylene)-dihydro-6-imino-1,3-dimethyl-pyrimidine-2,4(1*H*,3*H*)-dione (**26**): Yellow solid, yield 71%, mp 212–213°C. IR (KBr) cm^{-1}: 1599 (C=N), 1625 (C=C), 1674 (CO), 3226 (NH). ^{1}H-NMR (DMSO-d_6) δ: 2.62, 2.70 (s, 6H, N–CH_3), 4.30 (s, 2H, CH_2), 4.35 (s, 2H, CH_2), 7.19–7.52 (m, 8H, Ar-H), 9.29 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 27.6, 28.7 (N–CH_3), 31.7, 32.9 (CH_2–S), 104.2 (C-5), 159.3 (C-2), 162.8 (C-6), 169.7, 170.4 (C-2′), 173.9 (C-4), 175.1 (C=*C*(S)(S)), 119.4, 122.6, 123.2, 125.4, 126.8, 134.6, 135.7, 149.6 (aromatic carbons). MS (*m/z*): 525.61 (M^+). *Anal.* Calcd for $C_{23}H_{19}N_5O_2S_4$: C, 52.55; H, 3.64; N, 13.32. Found: C, 52.60; H, 3.63; N, 13.38.

5-(Bis((1′*H*-benzo[d]imidazol-2′-yl)methylthio)methylene)-dihydro-6-imino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**27**): Yellow solid, yield 68%, mp 222–224°C. IR (KBr) cm^{-1}: 1607 (C=N), 1623 (C=C), 1678 (CO), 3211 (NH), 3318 (NH). ^{1}H-NMR (DMSO-d_6) δ: 2.74, 2.79 (s, 6H, N–CH_3), 4.29 (s, 2H, CH_2), 4.40 (s, 2H, CH_2), 7.38–7.76 (m, 8H, Ar-H), 9.35 (br s, 1H, NH), 12.48 (br s, 2H, NH). ^{13}C-NMR (DMSO-d_6) δ: 27.8, 28.9 (N–CH_3), 31.5, 32.6 (CH_2–S), 104.9 (C-5), 150.3, 151.1 (C-2′), 158.7 (C-2), 163.2 (C-6), 173.6 (C-4), 174.7 (C=C(S)(S)), 118.9, 120.3, 123.2, 125.6, 128.5, 131.9, 136.2, 137.4 (aromatic carbons). MS (*m/z*): 491.52 (M^+). *Anal.* Calcd for $C_{23}H_{21}N_7O_2S_2$: C, 56.19; H, 4.31; N, 19.94. Found: C, 56.25; H, 4.34; N, 20.00.

Antioxidant Activity The compounds **4**–**27** are evaluated for antioxidant property by DPPH, nitric oxide and ABTS methods.

DPPH Radical Scavenging Activity The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple colored methanol solution of DPPH. The spectrophotometric assay uses the stable radical DPPH as a reagent. To 4 mL of 0.004% (w/v) methanol solution of DPPH, 1 mL of various concentrations of the test compounds (50, 75, 100 μg/mL) in methanol were added. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. Ascorbic acid was used as the standard. The percent of inhibition (*I*%) of free radical production from DPPH was calculated by the following equation,

$$I\% = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

Where A_{control} is the absorbance of the control reaction (containing methanolic DPPH and Ascorbic acid), A_{sample} is the absorbance of the test compound (containing methanolic DPPH and test compound) and A_{blank} is the absorbance of the blank (containing only methanolic DPPH). Tests were carried out in triplicate.

Nitric Oxide (NO) Scavenging Activity NO scavenging activity was measured by slightly modified methods of Green[36] and Marcocci[37] *et al.* NO radicals were generated from sodium nitroprusside. 1 mL of Sodium nitroprusside (10 mM) and 1.5 mL of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (50, 75, 100 μg/mL) of the test compounds and incubated for 150 min at 25°C. After incubation 1 mL of the reaction mixture was treated with 1 mL of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromatophore was measured at 546 nm. Ascorbic acid was used as standard. NO scavenging activity was calculated by the following equation,

$$\% \text{ of scavening} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

Where A_{control} is the absorbance of the control reaction (containing all reagents and Ascorbic acid), A_{sample} is the absorbance of the test compound (containing all reagents and test compound) and A_{blank} is the absorbance of the blank (containing only reagents). Tests were carried out in triplicate.

ABTS Radical Scavenging Activity The antioxidant activity of the test compounds and standard (ascorbic acid) were assessed on the basis of the radical scavenging effect of the stable ABTS free radical. The $ABTS^{\cdot+}$ solution was prepared by mixing 0.02 mol of ABTS salt with 0.01 mol of potassium persulfate in 25 mL of distilled water. The solution was kept at room temperature in the dark for 16 h before use. Then the $ABTS^{\cdot+}$ solution was diluted with methanol in order to obtain an absorbance between 0.7 and 0.9 at 734 nm using the spectrophotometer. Fresh $ABTS^{\cdot+}$ solutions were prepared for each assay. To a 50, 75 and 100 μg/mL of each test compounds and standard, 1 mL of $ABTS^{\cdot+}$ solution was added and allowed to react for 2 h in dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer. The corresponding blank reading was also taken and the results in percentage were expressed as the ratio of absorbance decrease at 734 nm and the absorbance of $ABTS^{\cdot+}$ solution in the absence of test compounds.

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