Full Paper

Synthesis, Antioxidant, and Cytotoxic Activities of *N*-Azole Substituted Thiomorpholine Derivatives

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A new class of *N*-azole substituted thiomorpholine derivatives were prepared and their antioxidant and cytotoxic activities were studied. The methyl substituted oxazolyl thiomorpholine dioxide **9b** exhibited radical scavenging activity greater than the standard ascorbic acid. On the other hand, the thiazolyl thiomorpholine **10c** having a chloro substituent on the aromatic ring was identified as a remarkable lead molecule for cytotoxic activity against A549 and HeLa cells, with IC_{50} values of 10.1 and 30.0 μM, respectively.

Keywords: Antioxidant activity / Cytotoxicity / Imidazoles / Oxazoles / Thiazoles / Thiomorpholines

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Introduction

Thiomorpholinone is a pharmacophore of great interest because of its unique biological properties. Thiomorpholine, and its *S*-oxide and *S*,*S*-dioxide derivatives are associated with a variety of pharmacological activities including antibacterial [1], antimicrobial [2], and anti-inflammatory [3]. This nucleus is present in 1,4-benzothiazine calcium antagonist semotiadil [4] as well as in pyrimidothiazine derivatives [5] designed as an inhibitor of the glycinamide ribonucleotide transformylase with potent cell growth inhibition. Thiomorpholines were synthesized by base-promoted aminoethylation of ethyl thioglycolate with 2-oxazolidinones [6] and from glycidic esters [7] besides the other methods like Ugi reactions [8], microwave-assisted Smiles rearrangement [9], and copper-catalyzed cascade reactions [10].

The azole derivatives are the prominent players in the pharmaceutical research as they possess several biological properties. In fact, simple oxazoles are common units in a wide variety of polyoxazole marine natural products possessing biological activity [11], while aminoalkyl oxazoles are constituents of peptide-based alkaloids and antibiotics with remarkable cytotoxic and antitumor properties [12, 13]. Imidazoles are common scaffolds in highly significant biomolecules including biotin, histidine, and histamine. Medicinal properties of imidazole containing compounds include anticancer [14], antimicrobial [15–18], and antioxidant [19]. Thiazoles are among the most frequently encountered heterocycles in compounds of biological interest. For example, the aminothiazole ring system is a useful structural element in medicinal chemistry and has found broad application in drug development for the treatment of allergies [20], hypertension [21], inflammation [22], bacterial infection [23], and HIV [24]. In addition, these compounds exhibited significant antimicrobial and anticancer activities [25–27]. Realizing the importance of thiomorpholines and azoles, it is planned to conjugate these two ligands under one construct and to study the cytotoxic activity.

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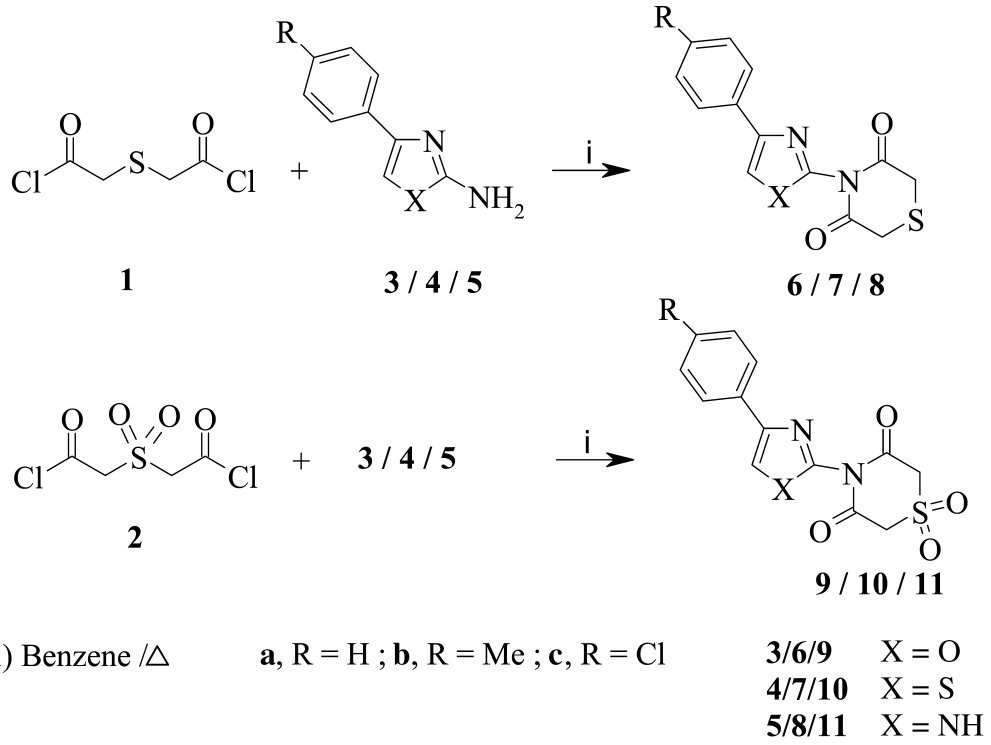
Results and discussion

Chemistry

The *N*-azole substituted thiomorpholine derivatives were prepared as outlined in Scheme 1. The compounds thiodiglycolic acid chloride (**1**), sulfonyldiglycolic acid chloride (**2**) [28], and the five-membered heterocyclic amines, 4-aryloxazol-2-amine (**3**), 4-arylthiazol-2-amine (**4**) [29], and 4-aryl-1*H*-imidazol-2-amine (**5**) [30], were prepared adopting the literature precedent. The reaction of **1** with **3** in benzene



Scheme 1. Synthesis of *N*-azole substituted thiomorpholines.

afforded 4-(4-aryloxazol-2-yl)thiomorpholine-3,5-dione (**6**) in quantitative yield. Aiming at the construction of another heterocyclic system, similar reaction of **1** was performed with **4**, which resulted in 4-(4-arylthiazol-2-yl)thiomorpholine-3,5-dione (**7**). Likewise, the compound 4-(4-aryl-1*H*-imidazol-2-yl)-thiomorpholine-3,5-dione (**8**) was prepared by the reaction of **1** with **5** (Scheme 1). The ^{1}H NMR spectrum of **6a** displayed a singlet for methylene protons of thiomorpholine at δ 3.41 and another singlet at 7.30 due to C_5-H of oxazole. Similarly, the compounds **7a** and **8a** exhibited two singlets at δ 3.36, 7.24 and 3.38, 7.27 due to methylene protons and C_5-H of thiazole/imidazole rings. The compound **8a** displayed a broad singlet at 11.13 ppm due to NH, which disappeared on deuteration. The compounds 1,1-dioxo-4-(4-aryloxazol-2-yl)thiomorpholine-3,5-dione (**9**), 1,1-dioxo-4-(4-arylthiazol-2-yl)thiomorpholine-3,5-dione (**10**), and 1,1-dioxo-4-(4-aryl-1*H*-imidazol-2-yl)-thiomorpholine-3,5-dione (**11**) were obtained by the reaction of **2** with **3**, **4**, and **5**, respectively. The ^{1}H NMR spectra of **9a**, **10a**, and **11a** showed a singlet for methylene protons at slightly downfield region than the thio derivatives at δ 4.41, 4.35, and 4.37. In addition, another singlet was observed at 7.32, 7.23, and 7.30 due to C_5-H of oxazole/thiazole/imidazole rings. The compound **11a** exhibited a broad singlet at 11.20 ppm for NH, which disappeared when D_2O was added. The structures of all the lead compounds were further established by IR, ^{13}C NMR, and elemental analyses.

Biological results

Antioxidant activity

The compounds **6**–**11** were tested for antioxidant activity by 2,2′-diphenyl-1-picrylhydrazyl (DPPH) [31, 32] and nitric oxide (NO) [33, 34] methods. The results are presented in Tables 1 and 2 and Figs. 1 and 2. Among all the tested compounds, the bis-heterocycles having thiomorpholinedioxides (**9**–**11**) displayed greater activity than the respective bis-heterocycles having thiomorpholines (**6**–**8**). In fact, the compound **9b** displayed excellent radical scavenging activity greater than the standard ascorbic acid. The compounds **9a**, **10b**, and **11b** also showed good radical scavenging activity. However, the compounds oxazolyl thiomorpholines **6** and imidazolyl thiomorpholines **8** displayed least activity. On the other hand, the thiazolyl thiomorpholines showed no activity. Thus, the results exemplified that thiomorpholinedioxide in combination with oxazole **9** showed excellent activity, which may be due to the presence of more oxygen atoms. The presence of electron-donating methyl substituent on the aromatic ring increases the activity when compared with those having chloro substituent. It was also observed that imidazolyl thiomorpholinedioxides **11** exhibited greater activity than thiazolyl thiomorpholinedioxides **10**. The free radical scavenging activity of the compounds **9a**, **9b**, and **11b** was measured at different concentrations and mentioned the change in absorbance at 10, 20, and 30 min in DPPH method

Table 1. The *in vitro* antioxidant activity of **6–11** in the DPPH method.

	Concentration (μg/mL)		
Compound	**50**	**75**	**100**
6a	21.70 ± 0.23	20.12 ± 0.63	26.10 ± 0.45
6b	27.57 ± 1.15	30.17 ± 0.85	32.75 ± 1.32
6c	–	–	–
7a	–	–	–
7b	–	–	–
7c	–	–	–
8a	–	–	–
8b	22.37 ± 0.35	25.93 ± 0.48	27.56 ± 0.61
8c	–	–	–
9a	62.34 ± 0.99	65.27 ± 0.75	66.41 ± 0.09
9b	79.92 ± 0.76	82.79 ± 1.21	85.56 ± 0.55
9c	55.27 ± 1.14	56.98 ± 0.16	58.36 ± 1.09
10a	39.46 ± 1.23	40.71 ± 0.45	43.38 ± 0.36
10b	50.17 ± 0.76	52.34 ± 0.37	54.95 ± 0.83
10c	31.37 ± 0.89	32.98 ± 0.19	35.37 ± 0.67
11a	49.49 ± 0.95	51.70 ± 0.68	53.35 ± 0.41
11b	62.37 ± 0.33	65.96 ± 0.51	67.65 ± 0.52
11c	39.73 ± 0.98	41.89 ± 0.23	43.37 ± 0.51
Ascorbic acid	78.16 ± 0.36	80.98 ± 1.12	83.82 ± 0.83
Blank	–	–	–

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

Table 2. The *in vitro* antioxidant activity of **6–11** in the nitric oxide method.

	Concentration (μg/mL)		
Compound	**50**	**75**	**100**
6a	20.25 ± 0.25	22.27 ± 0.49	25.11 ± 0.65
6b	29.95 ± 1.23	31.81 ± 0.96	34.87 ± 1.10
6c	–	–	–
7a	–	–	–
7b	–	–	–
7c	–	–	–
8a	–	–	–
8b	30.61 ± 0.24	33.27 ± 0.71	35.68 ± 1.02
8c	–	–	–
9a	65.53 ± 0.15	66.98 ± 0.78	67.24 ± 0.36
9b	88.75 ± 0.57	89.96 ± 0.76	92.79 ± 0.86
9c	58.54 ± 0.92	60.20 ± 0.35	61.39 ± 1.08
10a	47.75 ± 1.20	49.12 ± 0.82	50.65 ± 0.87
10b	54.59 ± 1.04	56.90 ± 0.16	57.37 ± 1.25
10c	40.73 ± 0.84	43.75 ± 0.30	45.21 ± 1.13
11a	50.57 ± 0.78	51.21 ± 0.28	53.56 ± 0.45
11b	64.61 ± 0.56	66.33 ± 0.51	67.75 ± 1.37
11c	44.37 ± 0.48	46.58 ± 0.40	48.12 ± 1.31
Ascorbic acid	86.02 ± 0.73	89.46 ± 0.80	91.53 ± 0.71
Blank	–	–	–

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

(Table 3). It was observed that at these 10-min intervals the values are very close and the results indicated that the antioxidant activity is independent of time.

Cytotoxicity

The newly synthesized compounds **6–11** are subjected to MTT assay to determine growth-inhibitory/cytotoxic capability. Only compound **10c** showed noticeable cytotoxic activity on A549 cells ($IC_{50} = 10.1\,\mu M$) and HeLa cells ($IC_{50} = 30\,\mu M$). However, all the other compounds did not show any cytotoxicity when used up to 200 μM concentration. Figures 3 and 4 show the results of cytotoxicity of **10c** using MTT assay on A549 lung carcinoma cells and HeLa cells, respectively. The cytotoxic activity observed with compound **10c** is concentration dependent. Compound **10c** at concentrations 12.5–200 μM showed lowest viability, while viability more than 50% is observed when this compound is used at a concentration below 12.50 μM on A549 cells and 75% cell viability on HeLa cells at 12.50 μM concentration. This suggests compound **10c** as a noticeable lead molecule for cytotoxic activity against tumor cells. It is of interest to note that compound **10c** is more potent on A549 cells compared to HeLa cells. HeLa cells are cervical carcinoma cells that are oncogenically driven by human papilloma viruses. On the other hand, A549 cells have a mutated K-ras oncogene. Further modifications to **10c** may provide a more potent molecule that could be developed as therapeutic drug. Efforts are in progress toward this goal.

Conclusion

In conclusion, a new class of *N*-azole substituted thiomorpholine derivatives were prepared and their antioxidant and cytotoxic activities were studied. The methyl substituted oxazolyl thiomorpholine dioxide **9b** exhibited radical scavenging activity greater than the standard ascorbic acid. On the other hand, the thiazolyl thiomorpholine **10c** having chloro substituent on the aromatic ring was identified as a noticeable lead molecule for cytotoxic activity against A549 cells and HeLa cells with IC_{50} values of 10.1 and 30.0 μM, respectively, which can be used as a lead compound in the future studies.

Experimental

Chemistry

Melting points were determined in open capillaries on a Mel Temp apparatus and are uncorrected. The progress of the reactions and the purity of the compounds were monitered by thin layer chromatography (TLC; silica gel H, BDH, ethyl acetate/hexane 0.5:2). 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 NMR spectra were recorded in DMSO-d_6 on a Jeol JNM λ-400 MHz. The ^{13}C NMR spectra

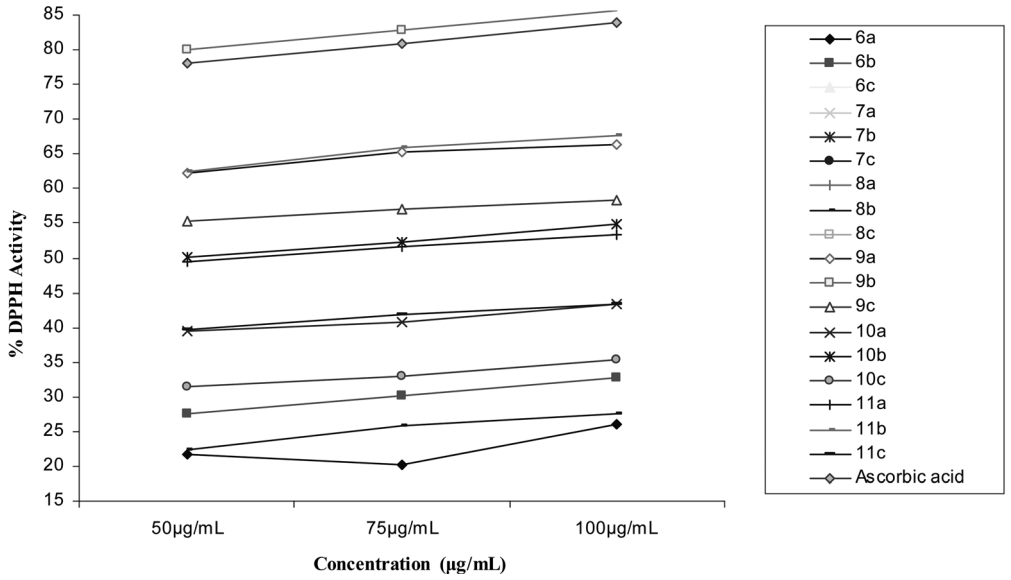


Figure 1. The *in vitro* antioxidant activities of **6**–**11** in the DPPH method.

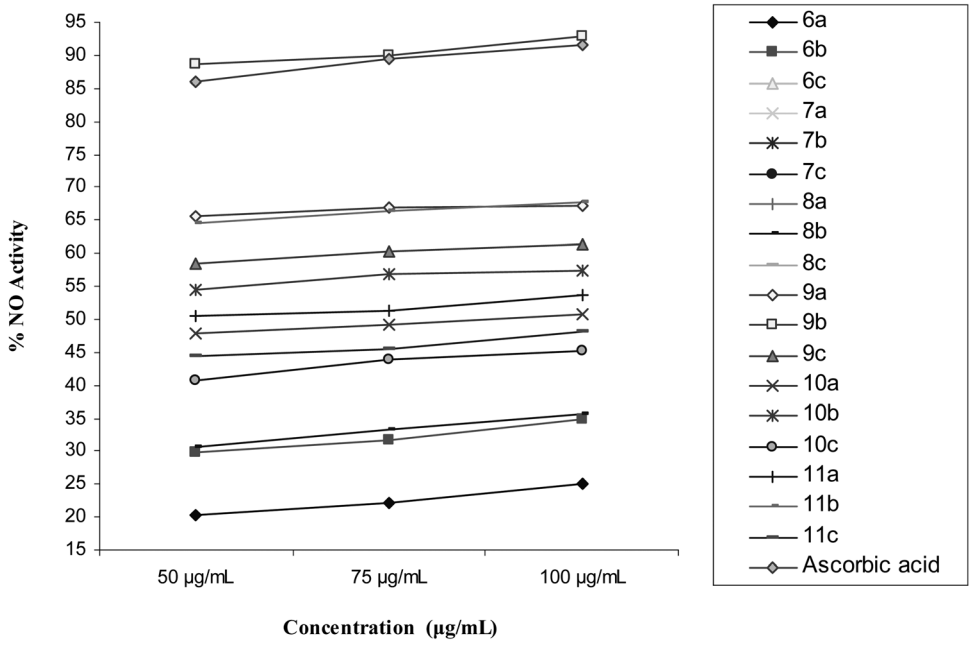


Figure 2. The *in vitro* antioxidant activities of **6**–**11** in the nitric oxide method.

Table 3. Antioxidant activities of the compounds **9a**, **9b**, and **11b** at 10-min intervals by the DPPH scavenging method.

Compound	10 min	20 min	30 min
9a	66.24	66.35	66.48
9b	85.40	85.61	85.95
11b	67.38	67.50	67.67

were recorded in DMSO-d_6 on a Jeol JNM spectrometer operating at 100 MHz. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. The microanalyses were performed on Perkin-Elmer 240C elemental analyzer. High-resolution mass spectra were recorded on Micromass Q-TOF micromass spectrometer using electrospray ionization. The compounds thiodiglycolic acid chloride (**1**), sulfonyldiglycolic acid chloride (**2**) [28], 4-aryloxazol-2-amine (**3**), 4-arylthiazol-2-amine (**4**) [29], and 4-aryl-1*H*-imidazol-2-amine (**5**) [30] were prepared as per the literature

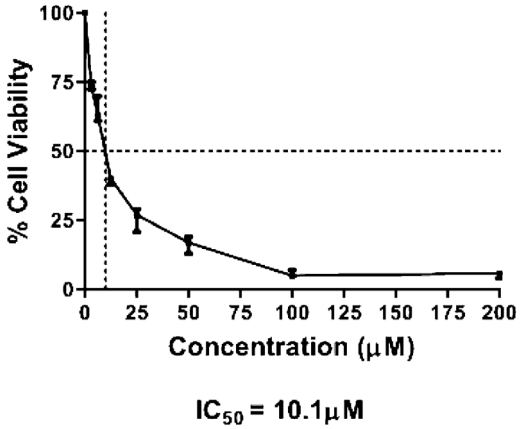


Figure 3. The dose–response curve of **10c** measured by MTT assay on A549 lung carcinoma cells. *X*-axis shows the concentration of the compound, and *Y*-axis, the cell viability.

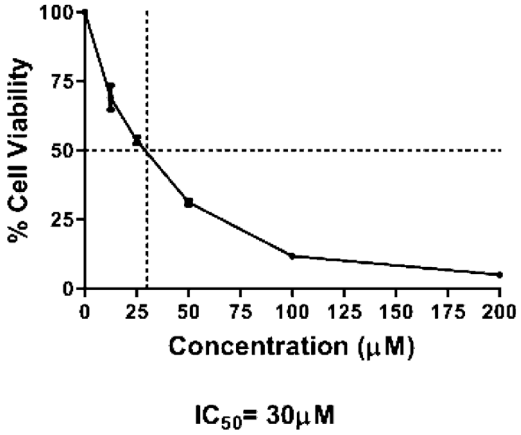


Figure 4. The dose–response curve of **10c** measured by MTT assay on HeLa cervical carcinoma cells. *X*-axis shows the concentration of the compound, and *Y*-axis, the cell viability.

procedure. All chemicals and solvents were purchased from Merck and used without further purification.

*General procedure for the synthesis of 4-(4-aryloxazol-2-yl)thiomorpholine-3,5-dione (**6a–c**)/4-(4-arylthiazol-2-yl)-thiomorpholine-3,5-dione (**7a–c**)/4-(4-aryl-1H-imidazol-2-yl)thiomorpholine-3,5-dione (**8a–c**)*

A mixture of **3**/**4**/**5** (1 mmol), thiodiglycolic acid chloride (1.1 mmol) and benzene (10 mL) was heated to reflux for 3–4 h and the solvent was removed under reduced pressure. The brown colored solid obtained was recrystallized from aqueous methanol (1:1).

*4-(4-Phenyloxazol-2-yl)thiomorpholine-3,5-dione (**6a**)*

Brown solid in 72% (0.28 g) yield; m.p.: 145–147°C; IR (KBr) ν_{max} (cm^{-1}): 1572 (C=N), 1625 (C=C), 1631 (C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.41 (s, 4H, CH_2), 7.30 (s, 1H, C_5-H), 7.45–7.67 (m, 5H, Ar–H); ^{13}C NMR (DMSO-d_6) δ (ppm): 38.9 (CH_2), 127.5, 127.9, 128.7, 133.8 (Ar–C), 142.1 (C-5), 143.2 (C-4), 153.2 (C-2), 164.0 (CO); HRMS [M+Na] (*m*/*z*): 297.2840. Anal. calcd. for $C_{13}H_{10}N_2O_3S$: C 56.92, H 3.67, N 10.21; Found: C 56.83; H 3.61; N 10.29%.

*4-(4-p-Tolyloxazol-2-yl)thiomorpholine-3,5-dione (**6b**)*

Brown solid in 75% (0.22 g) yield; m.p.: 162–164°C; IR (KBr) ν_{max} (cm^{-1}): 1568 (C=N), 1620 (C=C), 1627 (C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.23 (s, 3H, Ar–CH_3), 3.38 (s, 4H, CH_2), 7.26 (s, 1H, C_5-H), 7.40–7.58 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ (ppm): 25.5 (Ar–CH_3), 38.4 (CH_2), 127.1, 127.4, 128.2, 133.2 (Ar–C), 141.7 (C-5), 142.9 (C-4), 152.8 (C-2), 163.6 (CO); HRMS [M+Na] (*m*/*z*): 311.3118; Anal. calcd. for $C_{14}H_{12}N_2O_3S$: C 58.32, H 4.19, N 9.71; Found: C 58.45, H 4.14, N 9.82%.

*4-(4-(4-Chlorophenyl)oxazol-2-yl)thiomorpholine-3,5-dione (**6c**)*

Brown solid in 74% (0.23 g) yield; m.p.: 177–179°C; IR (KBr) ν_{max} (cm^{-1}): 1576 (C=N), 1629 (C=C), 1635 (C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.45 (s, 4H, CH_2), 7.34 (s, 1H, C_5-H), 7.51–7.71 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ (ppm): 39.2 (CH_2), 127.9, 128.3, 129.1, 134.1 (Ar–C), 142.5 (C-5), 143.7 (C-4), 153.6 (C-2), 164.8 (CO); HRMS [M+Na] (*m*/*z*): 331.7293. Anal. calcd. for $C_{13}H_9ClN_2O_3S$: C 50.57, H 2.93, N 9.07; Found: C 50.65, H 2.88, N 8.96%.

*4-(4-Phenylthiazol-2-yl)thiomorpholine-3,5-dione (**7a**)*

Brown solid in 69% (0.20 g) yield; m.p.: 157–159°C; IR (KBr) ν_{max} (cm^{-1}): 1570 (C=N), 1623 (C=C), 1630 (C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.36 (s, 4H, CH_2), 7.24 (s, 1H, C_5-H), 7.38–7.60 (m, 5H, Ar–H); ^{13}C NMR (DMSO-d_6) δ (ppm): 36.7 (CH_2), 103.2 (C-5), 127.0, 127.6, 128.4, 133.5 (Ar–C), 138.5 (C-4), 163.8 (CO), 169.7 (C-2); HRMS [M+Na] (*m*/*z*): 313.3502. Anal. calcd. for $C_{13}H_{10}N_2O_2S_2$: C 53.77, H 3.47, N 9.64; Found: C 53.87, H 3.39, N 9.78%.

*4-(4-p-Tolylthiazol-2-yl)thiomorpholine-3,5-dione (**7b**)*

Brown solid in 74% (0.22 g) yield; m.p.: 143–145°C; IR (KBr) ν_{max} (cm^{-1}): 1565 (C=N), 1619 (C=C), 1628 (C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.20 (s, 3H, Ar–CH_3), 3.33 (s, 4H, CH_2), 7.18 (s, 1H, C_5-H), 7.32–7.54 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ (ppm): 24.8 (Ar–CH_3), 36.2 (CH_2), 102.8 (C-5), 126.6, 127.1, 128.0, 132.9 (Ar–C), 138.1 (C-4), 163.4 (CO), 169.1 (C-2); HRMS [M+Na] (*m*/*z*): 327.3766. Anal. calcd. for $C_{14}H_{12}N_2O_2S_2$: C 55.24, H 3.97, N 9.20; Found: C 55.17, H 4.03, N 9.32%.

*4-(4-(4-Chlorophenyl)thiazol-2-yl)thiomorpholine-3,5-dione (**7c**)*

Brown solid in 72% (0.23 g) yield; m.p.: 169–171°C; IR (KBr) ν_{max} (cm^{-1}): 1574 (C=N), 1627 (C=C), 1634 (C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.45 (s, 4H, CH_2), 7.29 (s, 1H, C_5-H), 7.42–7.66 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ (ppm): 37.1 (CH_2), 103.7 (C-5), 127.3, 128.1, 128.9, 133.9 (Ar–C), 139.0 (C-4), 164.1 (CO), 170.0 (C-2); HRMS [M+Na] (*m*/*z*): 347.7959. Anal. calcd. for $C_{13}H_9ClN_2O_2S_2$: C 48.07, H 2.79, N 8.62; Found: C 47.95, H 2.64, N 8.73%.

*4-(4-Phenyl-1H-imidazol-2-yl)thiomorpholine-3,5-dione (**8a**)*

Brown solid in 73% (0.20 g) yield; m.p.: 235–237°C; IR (KBr) ν_{max} (cm^{-1}): 1567 (C=N), 1624 (C=C), 1632 (C=O), 3227 (NH); ^{1}H NMR

(DMSO-d_6) δ (ppm): 3.38 (s, 4H, CH_2), 7.27 (s, 1H, C_5-H), 7.43–7.62 (m, 5H, Ar–H), 11.13 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 37.6 (CH_2), 122.7 (C-5), 127.7, 128.4, 129.5, 134.6 (Ar–C), 140.3 (C-2), 142.7 (C-4), 163.7 (CO); HRMS [M+Na] (*m*/*z*): 296.3008. Anal. calcd. for $C_{13}H_{11}N_3O_2S$: C 57.12, H 4.05, N 15.37; Found: C 57.23, H 4.13, N 15.50%.

*4-(4-p-Tolyl-1H-imidazol-2-yl)thiomorpholine-3,5-dione (**8b**)*

Brown solid in 68% (0.19 g) yield; m.p.: 217–219°C; IR (KBr) ν_{max} (cm^{-1}): 1570 (C=N), 1621 (C=C), 1629 (C=O), 3221 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.18 (s, 3H, Ar–CH_3), 3.34 (s, 4H, CH_2), 7.23 (s, 1H, C_5-H), 7.39–7.57 (m, 4H, Ar–H), 11.11 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 24.3 (Ar–CH_3), 37.1 (CH_2), 122.3 (C-5), 127.1, 128.0, 129.2, 134.1 (Ar–C), 139.8 (C-2), 142.2 (C-4), 163.1 (CO); HRMS [M+Na] (*m*/*z*): 310.3264. Anal. calcd. for $C_{14}H_{13}N_3O_2S$: C 58.52, H 4.56, N 14.62; Found: C58.64, H 4.50, N 14.49%.

*4-(4-(4-Chlorophenyl)-1H-imidazol-2-yl)thiomorpholine-3,5-dione (**8c**)*

Brown solid in 67% (0.20 g) yield; m.p.: 262–264°C; IR (KBr) ν_{max} (cm^{-1}): 1578 (C=N), 1628 (C=C), 1636 (C=O), 3230 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.41 (s, 4H, CH_2), 7.31 (s, 1H, C_5-H), 7.46–7.66 (m, 4H, Ar–H), 11.15 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 38.2 (CH_2), 123.4 (C-5), 128.2, 128.9, 129.9, 135.1 (Ar–C), 140.8 (C-2), 143.2 (C-4), 164.2 (CO); HRMS [M+Na] (*m*/*z*): 330.7449. Anal. calcd. for $C_{13}H_{10}ClN_3O_2S$: C 50.73, H 3.27, N 13.65; Found: C 50.61, H 3.19, N 13.77%.

*General procedure for the synthesis of 1,1-dioxo-4-(4-aryloxazol-2-yl)-thiomorpholine-3,5-dione (**9a–c**)/1,1-dioxo-4-(4-arylthiazol-2-yl)thiomorpholine-3,5-dione (**10a–c**)/1,1-dioxo-4-(4-aryl-1H-imidazol-2-yl)-thiomorpholine-3,5-dione (**11a–c**)*

A mixture of **3**/**4**/**5** (1 mmol), sulfonyldiglycolic acid chloride (1.1 mmol) and benzene (10 mL) was heated to reflux for 3–4 h and solvent was removed under vacuum. The yellow colored solid obtained was recrystallized from aqueous methanol (1:1).

*1,1-Dioxo-4-(4-phenyl-oxazol-2-yl)thiomorpholine-3,5-dione (**9a**)*

Yellow solid in 85% (0.26 g) yield; m.p.: 157–159°C; IR (KBr) ν_{max} (cm^{-1}): 1150, 1350 (SO_2), 1576 (C=N), 1626 (C=C), 1633 (C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.41 (s, 4H, CH_2), 7.32 (s, 1H, C_5-H), 7.52–7.81 (m, 5H, Ar–H); ^{13}C NMR (DMSO-d_6) δ (ppm): 58.3 (CH_2), 127.9, 128.3, 129.2, 134.1 (Ar–C), 142.5 (C-5), 143.6 (C-4), 153.7 (C-2), 164.5 (CO); HRMS [M+Na] (*m*/*z*): 329.2840. Anal. calcd. for $C_{13}H_{10}N_2O_5S$: C 50.98, H 3.29, N 9.14; Found: C 50.86, H 3.22, N 9.03%.

*1,1-Dioxo-4-(4-p-tolyl-oxazol-2-yl)thiomorpholine-3,5-dione (**9b**)*

Yellow solid in 82% (0.26 g) yield; m.p.: 186–188°C; IR (KBr) ν_{max} (cm^{-1}): 1145, 1345 (SO_2), 1569 (C=N), 1622 (C=C), 1631 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ (ppm): 2.28 (s, 3H, Ar–CH_3), 4.38 (s, 4H, CH_2), 7.30 (s, 1H, C_5-H), 7.50–7.76 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ (ppm): 25.8 (Ar–CH_3), 57.8 (CH_2), 127.4, 128.0, 128.7, 133.7 (Ar–C), 142.1 (C-5), 143.3 (C-4), 153.3 (C-2), 163.7 (CO); HRMS [M+Na] (*m*/*z*): 343.3107. Anal. calcd. for $C_{14}H_{12}N_2O_5S$: C 52.49, H 3.77, N 8.74; Found: C 52.61, H 3.85, N 8.88%.

*4-(4-(4-Chlorophenyl)oxazol-2-yl)-1,1-dioxothiomorpholine-3,5-dione (**9c**)*

Yellow solid in 86% (0.29 g) yield; m.p.: 197–199°C; IR (KBr) ν_{max} (cm^{-1}): 1153, 1354 (SO_2), 1580 (C=N), 1629 (C=C), 1637 (C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.45 (s, 4H, CH_2), 7.35 (s, 1H, C_5-H), 7.57–7.85 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ (ppm): 58.7 (CH_2), 128.2, 128.7, 129.7, 134.6 (Ar–C), 143.0 (C-5), 144.1 (C-4), 154.0 (C-2), 165.2 (CO); HRMS [M+Na] (*m*/*z*): 363.7287; Anal. calcd. for $C_{13}H_9ClN_2O_5S$: C 45.82, H 2.61, N 8.22; Found: C 45.89, H 2.61, N 8.33%.

*1,1-Dioxo-4-(4-phenylthiazol-2-yl)thiomorpholine-3,5-dione (**10a**)*

Yellow solid in 83% (0.27 g) yield; m.p.: 163–165°C; IR (KBr) ν_{max} (cm^{-1}): 1148, 1342 (SO_2), 1574 (C=N), 1627 (C=C), 1632 (C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.35 (s, 4H, CH_2), 7.23 (s, 1H, C_5-H), 7.50–7.72 (m, 5H, Ar–H); ^{13}C NMR (DMSO-d_6) δ (ppm): 57.7 (CH_2), 103.6 (C-5), 127.6, 128.1, 128.9, 133.9 (Ar–C), 139.1 (C-4), 163.8 (CO), 170.2 (C-2); HRMS [M+Na] (*m*/*z*): 345.3499. Anal. calcd. for $C_{13}H_{10}N_2O_4S_2$: C 48.44, H 3.13, N 8.69; Found: C 48.54, H 3.19, N 8.24%.

*1,1-Dioxo-4-(4-p-tolylthiazol-2-yl)thiomorpholine-3,5-dione (**10b**)*

Yellow solid in 80% (0.27 g) yield; m.p.: 154–156°C; IR (KBr) ν_{max} (cm^{-1}): 1145, 1338 (SO_2), 1567 (C=N), 1624 (C=C), 1628 (C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.24 (s, 3H, Ar–CH_3), 4.33 (s, 4H, CH_2), 7.20 (s, 1H, C_5-H), 7.48–7.69 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ (ppm): 25.2 (Ar–CH_3), 57.1 (CH_2), 103.3 (C-5), 127.4, 127.7, 128.3, 133.4 (Ar–C), 138.8 (C-4), 164.0 (CO), 169.9 (C-2); HRMS [M+Na] (*m*/*z*): 359.3758. Anal. calcd. for $C_{14}H_{12}N_2O_4S_2$: C 49.99, H 3.59, N 8.33, Found: C 50.10, H 3.67, N 8.24%.

*4-(4-(4-Chlorophenyl)thiazol-2-yl)-1,1-dioxothiomorpholine-3,5-dione (**10c**)*

Yellow solid in 87% (0.31 g) yield; m.p.: 171–174°C; IR (KBr) ν_{max} (cm^{-1}): 1156, 1346 (SO_2), 1579 (C=N), 1630 (C=C), 1638 (C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.38 (s, 4H, CH_2), 7.27 (s, 1H, C_5-H), 7.54–7.76 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ (ppm): 58.1 (CH_2), 104.0 (C-5), 128.0, 128.6, 129.4, 134.1 (Ar–C), 139.4 (C-4), 164.7 (CO), 170.5 (C-2); HRMS [M+Na] (*m*/*z*): 379.7941. Anal. calcd. for $C_{13}H_9ClN_2O_4S_2$: C 43.76, H 2.54, N 7.85; Found: C 43.88, H 2.62, N 7.98%.

*1,1-Dioxo-4-(4-phenyl-1H-imidazol-2-yl)thiomorpholine-3,5-dione (**11a**)*

Yellow solid in 79% (0.24 g) yield; m.p.: 242–244°C; IR (KBr) ν_{max} (cm^{-1}): 1150, 1349 (SO_2), 1577 (C=N), 1628 (C=C), 1633 (C=O), 3230 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.37 (s, 4H, CH_2), 7.30 (s, 1H, C_5-H), 7.50–7.79 (m, 5H, Ar–H), 11.20 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 57.9 (CH_2), 123.6 (C-5), 128.5, 129.1, 129.9, 135.2 (Ar–C), 140.9 (C-2), 143.3 (C-4), 164.1 (CO); HRMS [M+Na] (*m*/*z*): 305.3096; Anal. calcd. for $C_{13}H_{11}N_3O_4S$: C 51.14, H 3.63, N 13.76; Found: C 51.07, H 3.58, N 13.85%.

*1,1-Dioxo-4-(4-p-tolyl-1H-imidazol-2-yl)thiomorpholine-3,5-dione (**11b**)*

Yellow solid in 80% (0.25 g); m.p.: 234–236°C; IR (KBr) ν_{max} (cm^{-1}): 1146, 1344 (SO_2), 1573 (C=N), 1622 (C=C), 1630 (C=O), 3225 (NH);

^{1}H NMR (DMSO-d_6) δ (ppm): 2.67 (s, 3H, Ar–CH_3), 4.35 (s, 4H, CH_2), 7.27 (s, 1H, C_5-H), 7.48–7.74 (m, 4H, Ar–H), 11.17 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 24.7 (Ar–CH_3), 57.4 (CH_2), 123.2 (C-5), 128.1, 128.9, 129.4, 134.8 (Ar–C), 140.5 (C-2), 143.0 (C-4), 163.8 (CO); HRMS [M+Na] (*m*/*z*): 319.3351. Anal. calcd. for $C_{14}H_{13}N_3O_4S$: C 52.65, H 4.10, N 13.16; Found: C 52.52, H 3.99, N 13.06%.

4-(4-(4-Chlorophenyl)-1H-imidazol-2-yl)-1,1-dioxothiomorpholine-3,5-dione (**11c**)

Yellow solid in 76% (0.26 g) yield; m.p.: 273–275°C; IR (KBr) ν_{max} (cm^{-1}): 1154, 1356 (SO_2), 1582 (C=N), 1630 (C=C), 1640 (C=O), 3236 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.40 (s, 4H, CH_2), 7.33 (s, 1H, C_5-H), 7.53–7.83 (m, 4H, Ar–H), 11.24 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 58.1 (CH_2), 124.1 (C-5), 129.0, 129.7, 130.1, 135.7 (Ar–C), 141.2 (C-2), 143.8 (C-4), 164.7 (CO); HRMS [M+Na] (*m*/*z*): 339.7547. Anal. calcd. for $C_{13}H_{10}ClN_3O_4S$: C 45.95, H 2.97, N 12.37; Found: C 45.87, H 2.92, N 12.49%.

Biological assays

Antioxidant activity

The compounds **6–11** are tested for antioxidant activity by DPPH and NO methods.

DPPH radical scavenging activity

The hydrogen atom or electron-donating 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, and 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

$$I\% = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{blank}}}\right] \times 100$$

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-scavenging activity

NO-scavenging activity was measured by slightly modified methods of Green et al. [33] and Marcocci et al. [34]. NO radicals were generated from sodium nitroprusside. One milliliter 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, and 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

$$\%\ \text{of scavenging} = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{black}}}\right] \times 100$$

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.

Cytotoxic activity

Compounds **6–11** were dissolved in DMSO at different concentrations of 12.5–200 μM.

Cells

A549 lung adenocarcinoma cells and HeLa cervical carcinoma cells were maintained in Dulbecco's modified Eagle's medium (DMEM) medium substituted with 10% fetal bovine serum and 1% penicillin and streptomycin. The cells were plated in T-25 tissue culture flask and incubated at 37°C in a 5% CO_2 atmosphere with 90% humidity.

MTT assay for cell viability

The cytotoxicity of the compounds was tested using A549 lung carcinoma cells and HeLa cervical carcinoma cells. 5×10^4 cells were plated in each well of a 96-well tissue culture cluster (Nunc, Inc., Germany) and incubated at 37°C in a medium containing DMEM, 10% fetal bovine serum, and antibiotics (Invitrogen, USA), in 5% CO_2 atmosphere [35, 36].Compounds were dissolved in DMSO. Serial dilutions were made for the active compound **10c** from stock solution 10 mg/mL (43800 μM) in DMSO. Compound **10c** stock solution 6.39 μL was added to 1393.61 μL (final volume 1400 μL) of DMEM medium substituted with 10% fetal bovine serum and 1% penicillin and streptomycin to make concentration 200 μM, which was further used for serial dilutions. After attachment of the cells (usually 3–4 h), different concentrations of dilutions were added to cells in 96-well plate and incubated for 20 h. MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) solution (20 μL of 5 mg/mL) was added to each well and the incubation continued for additional 3 h. The dark blue formazan crystals formed within the healthy cells were solubilized with DMSO, and the absorbance was estimated in ELISA plate reader (7520 Micro plate reader, Cambridge Technologies, Inc.) at 550 nm and the absorbance was correlated with the cell number. Experiments were performed in triplicates, and the values are the average of three ($n = 3$) independent experiments. The inhibitory concentration (IC_{50}) of the compound was assessed by Graph Pad Prism software.

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