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Original article

Synthesis, antimicrobial and antioxidant activities of substituted pyrazoles, isoxazoles, pyrimidine and thioxopyrimidine derivatives

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ABSTRACT

A new class of heterocycles, substituted pyrazoles, isoxazoles, pyrimidines, thioxopyrimidines were prepared from Michael adducts, 2-(1,2-diaroylethyl)malononitrile and 2-(1,2-diarylsulfonylethyl)malononitrile by cyclocondensation with appropriate nucleophiles. The lead compounds were screened for the antimicrobial and antioxidant activities.

1. Introduction

Over the years pyrazoles, isoxazoles and pyrimidines have emerged as an interesting class of five and six membered heterocycles with an astonishingly wide range of applications in pharmaceutical chemistry. Barbituric acid and its derivatives are known as sedatives, hypnotics since a long time. Substituted barbituric and thiobarbituric acids show analgesic, antipyretic and anti-inflammatory activities [1–3]. Apart from these, pyrazolidine derivatives are effective in the treatment of rheumatoid arthritis and allied conditions [4,5]. Isoxazoline derivatives possess potent antithrombic effect and improved pharmaco-kinetic properties [6]. In fact, a variety of pyrazole and isoxazole derivatives exhibit COX-I/COX-II inhibiting activity. It is found that Celecoxib, a pyrazole derivative and Valdecoxib, an isoxazole derivative have no effect on platelet aggregation and does not reduce increased PG levels in cerebrospinal fluid [7]. In addition, pyrazole and isoxazole derivatives exhibit various biological properties *viz.*, bacteriostatic, antidiabetic, analgesic, antiarrhythmic, anti-inflammatory, antifungal and antiviral [4,8–12]. Many synthetic procedures exist for the synthesis of substituted pyrazoles, isoxazoles and pyrimidines. However, the development of simple, facile and efficient methodologies to get five and six membered heterocycles is one of the major aspects in organic synthesis. In fact, the Michael acceptors are valuable intermediates in a variety of synthetic transformations and useful as building blocks in the synthesis of biologically active heterocycles. The present communication deals with the synthesis of diaminopyrazole, isoxazole, pyrimidine and thioxopyrimidine derivatives from Michael adducts by cyclocondensation with different nucleophiles.

2. Chemistry

The synthetic scheme involves the Michael addition of malononitrile to **1** and **8** in the presence of K_2CO_3 in methyl ethyl ketone to get **2** and **9**, respectively (Scheme 1). The *gem*-dicyano functionality in **2** and **9** was exploited to get the desired heterocycles. The cyclocondensation of **2** and **9** with hydrazine hydrate in the presence of NaOMe in methanol produced **10**. Likewise, **4** and **11** were prepared by the reaction of **2** and **9** with hydroxylamine hydrochloride (Schemes 1 and 2). Similarly, six membered heterocycles, **5**, **7**, **12**, and **14** were produced by cyclocondensation reaction of **2** and **9** with urea and *N,N′*-dimethyl urea. In addition, **6** and **13** were prepared by refluxing compounds **2** and **9** with thiourea (Schemes 1 and 2). The

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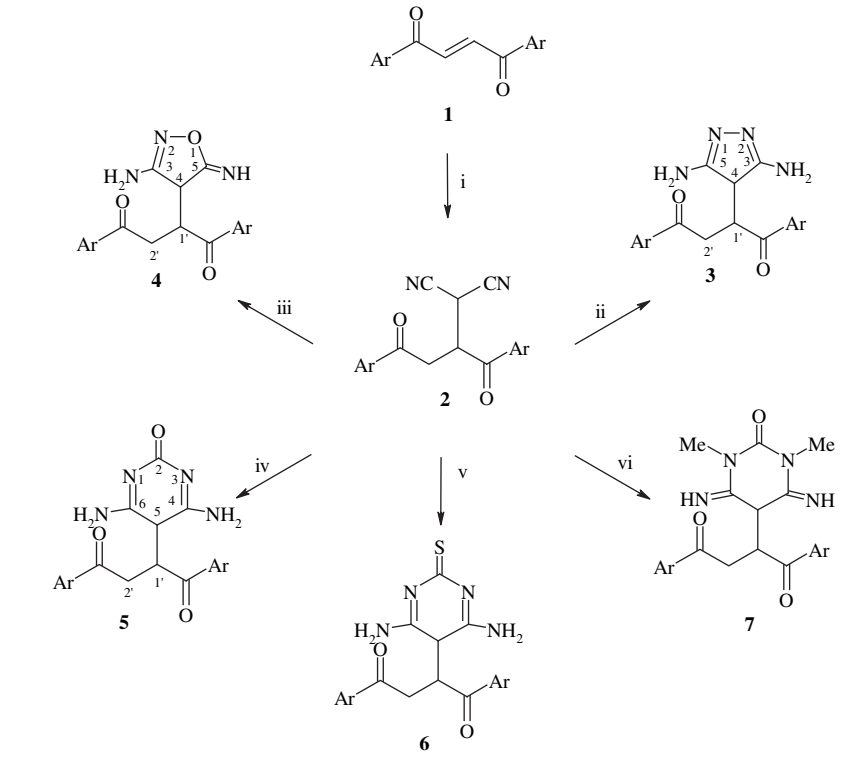


i. $CH_2(CN)_2$ / K_2CO_3 / MEK (60-65 %)
ii. $NH_2NH_2.H_2O$ / NaOMe / MeOH (68 -71%)
iii. $NH_2OH.HCl$ / NaOMe / MeOH (70-74 %)
iv. NH_2CONH_2 / NaOMe / MeOH (60-66%)
v. NH_2CSNH_2 / NaOMe / MeOH (63-72%)
vi. MeNHCONHMe / NaOMe / MeOH (61-66%)

a: Ar = Ph
b: Ar = 4-Me.Ph
c: Ar = 4-Cl.Ph

Scheme 1.

spectral data IR, ^{1}H NMR, ^{13}C NMR and microanalyses were used to acertain the structures of all the compounds.

3. Biology

3.1. Antimicrobial activity

Compounds **3**–**7** and **10**–**14** were tested for *in vitro* antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus* (NCIM No. 5021), *Bacillus subtilis* (NCIM No. 2063), the Gram-negative bacteria *Klebsiella pneumoniae* (NCIM No. 2957), *Proteus vulgaris* (NCIM No. 2027) and fungi *Fusarium solani* (NCIM No. 1330), *Curvularia lunata* (NCIM No. 716) and *Aspergillus niger* (NCIM No. 596). The primary screen was carried out by agar disc-diffusion method [13] using nutrient agar medium. The minimum inhibitory concentration for the most active compounds **3c**, **4c**, **10c** and **11c** against the same microorganisms used in the preliminary screening was carried out using microdilution susceptibility method [14]. Chloramphenicol and Ketoconazole were used as control drugs. The observed data on the antimicrobial activity of the compounds and control drugs are given in Tables 1–5.

3.2. Antioxidant testing

Compounds **3**–**7** and **10**–**14** were tested for antioxidant property by nitric oxide [15,16] and DPPH [17] methods. The observed data on the antioxidant activity is given in Table 6.

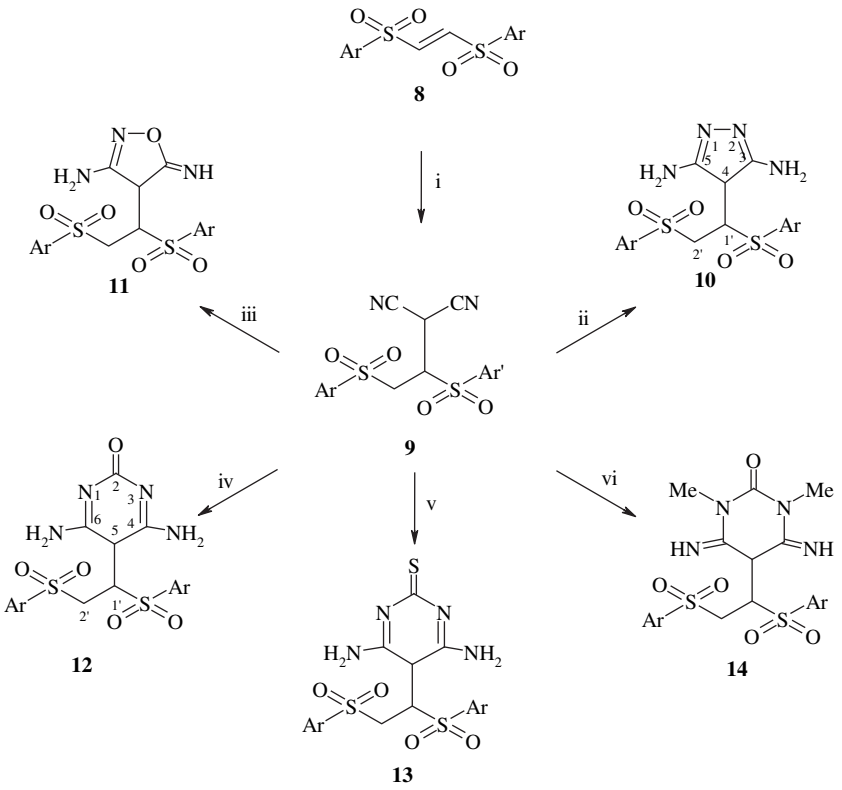
4. Results and discussion

We have synthesized a series of heterocycles, **3a**–**c** to **7a**–**c** and **10a**–**c** to **14a**–**c** by the reaction of **2a**–**c** and **9a**–**c** with appropriate nucleophiles in the presence of sodium methoxide in methanol as presented in Schemes 1 and 2.

4.1. Biological results

The results of preliminary antibacterial testing of compounds **3**–**7** are shown in Table 1. The results revealed that, compounds **3c** and **4c** displayed excellent activity against Gram-positive bacteria (inhibitory zone >28 mm) and good activity against Gram-negative bacteria (inhibitory zone >24 mm). Compound **6** displayed moderate to high activity towards gram (+ve) bacteria (16–25 mm) and moderate activity (16–22 mm) towards gram (−ve) bacteria. Compounds **5** and **7** exhibited least activity against both bacteria. All the test compounds except **5** and **7** showed moderate to high inhibitory effect towards tested fungi (Table 2).

On the other hand, the results of preliminary antibacterial testing of compounds **10**–**14** as shown in Table 3 revealed that, compounds **10c** and **11c** exhibited maximum activity against Gram-positive bacteria (inhibitory zone >30 mm) and good activity against Gram-negative bacteria (inhibitory zone >25 mm). Compound **13** showed moderate to high activity towards gram (+ve) bacteria (18–25 mm) and moderate activity (17–22 mm) towards gram (−ve) bacteria. Compounds **12** and **14** displayed low



i. $CH_2(CN)_2$ / K_2CO_3 / MEK (61-66%)
ii $NH_2NH_2.H_2O$ / NaOMe / MeOH (62-68%)
iii. $NH_2OH.HCl$ / NaOMe / MeOH (67-74%)
iv. NH_2CONH_2 / NaOMe / MeOH (64-73%)
v. NH_2CSNH_2 / NaOMe / MeOH (69-72%)
vi. MeNHCONHMe / NaOMe / MeOH (61-65%)

a: Ar = Ph
b: Ar = 4-Me.Ph
c: Ar = 4-Cl.Ph

Scheme 2.

activity against both bacteria. All the test compounds inhibited the spore germination of tested fungi. All the test compounds exhibited relatively high inhibitory activity on *F. solani*, *C. lunata*, than on *A. niger* (Table 4).

Further, the compounds having diaminopyrazole, amino-iminoisoxazole units displayed pronounced activity. Besides, compounds having diarylsulfonyl units (**10**–**14**) showed comparatively high antimicrobial activity than the compounds with diaroyl units (**3**–**7**).

The MIC values were determined as the lowest concentration that completely inhibited visible growth of the microorganisms (Table 5). The structure–antimicrobial activity relationship of the synthesized compounds revealed that diamino substituted pyrazoles and amino-imino substituted isoxazoles displayed high activity. The compounds having *N*,*N*′-dimethyl-imino substituted pyrimidine derivatives exhibited low activity. The maximum activity was attained with the compounds having chloro substituent in the aryl moiety *viz.*, **3c**, **4c**, **10c** and **11c**.

4.2. Antioxidant testing

Compounds **3**–**7** and **10**–**14** were tested for antioxidant property by nitric oxide and DPPH methods. Compounds **3c**, **4c**, **10a** and **11c** exhibited high antioxidant property in both nitric oxide and DPPH methods at 100 μM concentration (Table 6).

5. Conclusion

A new class of heterocycles diaminopyrazoles, amino-iminoisoxazoles, diaminopyrimidines, diaminothioxopyrimidines and *N*,*N*′-dimethyldiiminopyrimidines were prepared from simple substrates 2-(1′,2′-diaroylethyl)malononitrile (**2**) and 2-(1,2-diarylsulfonylethyl)malononitrile (**9**) by cyclocondensation with appropriate nucleophiles. The antimicrobial testing showed that compounds having diaminopyrazoles and aminoiminoisoxazole units possess excellent activity. Further, the compounds with sulfone moieties displayed pronounced antimicrobial and antioxidant activities than with diaroyl units. The maximum activity was observed with compounds having chloro substituent in the aryl moiety.

6. Experimental

6.1. Chemistry

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, 1:2). 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 $CDCl_3$/

Table 1
Antibacterial activity of **3–7**.

Compound	Concentration (μg/disc)	Zone of inhibition (mm)			
		Gram-positive bacteria		Gram-negative bacteria	
		Staphylococcus aureus	*Bacillus subtilis*	*Klebsiella pneumoniae*	*Proteus vulgaris*
3a	100	27	29	27	23
	200	30	31	29	26
3b	100	25	27	25	24
	200	28	29	27	26
3c	100	31	28	28	25
	200	33	32	30	28
4a	100	23	24	23	21
	200	26	27	25	24
4b	100	22	26	22	21
	200	25	28	24	23
4c	100	29	28	25	25
	200	32	30	27	26
5a	100	11	16	11	12
	200	14	15	12	14
5b	100	12	11	10	13
	200	15	12	12	14
5c	100	13	12	11	10
	200	15	14	13	12
6a	100	22	23	19	17
	200	24	24	21	19
6b	100	20	21	18	16
	200	21	24	20	18
6c	100	25	23	19	20
	200	26	25	22	22
7a	100	10	09	07	06
	200	13	11	09	08
7b	100	10	09	07	07
	200	12	10	08	09
7c	100	12	11	09	09
	200	14	13	10	11
Chloramphenicol	100	35	38	37	42
	200	41	44	42	45
Control (DMSO)		–	–	–	–

Table 2
Antifungal activity of **3–7**.

Compound	Concentration (μg/disc)	Zone of inhibition (mm)		
		Fusarium solani	*Curvularia lunata*	*Aspergillus niger*
3a	100	31	30	29
	200	33	32	30
3b	100	32	31	30
	200	35	33	31
3c	100	33	34	32
	200	37	36	33
4a	100	30	30	27
	200	31	32	29
4b	100	32	31	24
	200	34	33	27
4c	100	33	32	26
	200	35	34	28
5a	100	13	16	14
	200	16	20	17
5b	100	14	15	13
	200	18	17	15
5c	100	17	14	16
	200	19	18	20
6a	100	16	19	14
	200	20	22	19
6b	100	15	15	14
	200	19	18	16
6c	100	19	17	15
	200	22	21	20
7a	100	11	13	12
	200	13	15	16
7b	100	12	14	10
	200	15	16	12
7c	100	14	13	11
	200	16	15	13
Ketoconazole	100	38	41	36
	200	42	44	39
Control (DMSO)		–	–	–

DMSO-d_6 on a Jeol JNM λ-300 MHz. The ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Jeol JNM spectrometer operating at 75.5 MHz. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. The microanalyses were performed on a Perkin–Elmer 240C elemental analyzer. The starting compounds (*E*)-1,4-diarylbut-2-ene-1,4-dione (**1**) and 1,2-diarylsulfonylethene (**8**) were prepared as per the literature procedure [18,19].

*6.1.1. General procedure for the preparation of 2-(1,2-diaroylethyl)malononitrile **2a–c**/2-(1,2-diarylsulfonylethyl)malononitrile (**9a–c**)*

A mixture of malononitrile (15 mmol), methyl ethyl ketone (5 ml) and potassium carbonate (10 mmol) was cooled to 5–10 °C. To this, compound **1**/**8** (10 mmol) was added and stirred for 3–5 h maintaining the same temperature. The contents of the flask were diluted with water and extracted with dichloromethane. The organic layer was washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo*. The resultant solid was recrystallized from 2-propanol.

*6.1.1.1. 2-(1,2-Dibenzoylethyl)malononitrile (**2a**).* White solid (1.813 g, 60%); m.p. 122–124 °C; IR (KBr): 1703 (C═O), 2241 (C≡N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.21 (dd, 1H, C_4-H, J = 8.2, 14.1 Hz), 3.29 (dd, 1H, C_4-H, J = 4.0, 14.3 Hz), 4.07–4.09 (m, 1H, C_3-H), 4.32 (d, 1H, C_2-H, J = 8.3 Hz), 7.11–7.52 (m, 10H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 36.8 (C-3), 41.1 (C-2), 52.2 (C-4), 113.5 and 116.8 (CN), 204.6, 207.9 (Ar-CO), 129.6, 130.2, 131.8, 132.9, 133.6, 134.9, 135.4, 136.2 (aromatic carbons). Anal. Calcd. For $C_{19}H_{14}N_2O_2$: C, 75.48; H, 4.67; N, 9.27; Found: C, 75.60; H, 4.65; N, 9.41%.

*6.1.1.2. 2-(1,2-Di(4-methylbenzoyl)ethyl)malononitrile (**2b**).* White solid (2.048 g, 62%); m.p. 135–137 °C; IR (KBr): 1708 (C═O), 2239 (C≡N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.21, 2.28 (s, 6H, Ar-CH_3), 3.27 (dd, 1H, C_4-H, J = 8.9, 14.1 Hz), 3.39 (dd, 1H, C_4-H, J = 4.6, 14.5 Hz), 3.92–3.95 (m, 1H, C_3-H), 4.43 (d, 1H, C_2-H, J = 8.7 Hz), 7.15–7.49 (m, 8H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 22.4, 22.7 (Ar-CH_3), 35.2 (C-3), 42.6 (C-2), 52.6 (C-4), 114.6 and 115.2 (CN), 204.7, 205.8 (Ar-CO), 126.2, 128.4, 129.3, 132.0, 134.1, 134.8, 135.1 (aromatic carbons). Anal. Calcd. For $C_{21}H_{18}N_2O_2$: C, 76.34; H, 5.49; N, 8.48; Found: C, 76.40; H, 5.89; N, 8.60%.

*6.1.1.3. 2-(1,2-Di(4-chlorobenzoyl)ethyl)malononitrile (**2c**).* White solid (2.143 g, 65%); m.p. 150–152 °C; IR (KBr): 1694 (C═O), 2231 (C≡N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.24 (dd, 1H, C_4-H, J = 8.5, 14.0 Hz), 3.32 (dd, 1H, C_4-H, J = 4.3, 14.2 Hz), 4.01–4.06 (m, 1H, C_3-H), 4.47 (d, 1H, C_2-H, J = 8.6 Hz), 7.21–7.64 (m, 8H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 36.2 (C-3), 41.8 (C-2), 51.8 (C-4), 113.2 and 114.8 (CN), 203.9, 204.9 (Ar-CO), 126.1, 127.6, 129.0, 132.2, 133.4, 134.9, 136.4 (aromatic carbons). Anal. Calcd. For $C_{19}H_{12}Cl_2N_2O_2$: C, 61.47; H, 3.26; N, 7.55; Found: C, 61.41; H, 3.28; N, 7.50%.

*6.1.1.4. 2-(1,2-Diphenylsulfonylethyl)malononitrile (**9a**).* White solid (2.284 g, 61%); m.p. 136–138 °C; IR (KBr): 1136, 1336 (SO_2), 2245 (C≡N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.04 (dd, 1H, C_4-H, J = 8.0, 14.0 Hz), 3.27 (dd, 1H, C_4-H, J = 4.0, 14.1 Hz), 4.05–4.11 (m, 1H, C_3-H), 4.49 (d, 1H, C_2-H, J = 8.1 Hz), 7.13–7.64 (m, 10H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 37.1 (C-3), 40.7 (C-2), 52.9 (C-4), 115.3 and 116.2 (CN),

Table 3
Antibacterial activity of **10**–**14**.

Compound	Concentration (μg/disc)	Zone of inhibition (mm)			
		Gram-positive bacteria		Gram-positive bacteria	
		Staphylococcus aureus	*Bacillus subtilis*	*Klebsiella pneumoniae*	*Proteus vulgaris*
10a	100	30	31	28	24
	200	32	33	30	25
10b	100	27	26	23	22
	200	29	28	26	25
10c	100	34	35	30	31
	200	36	37	32	33
11a	100	29	30	26	26
	200	31	32	29	28
11b	100	26	26	25	24
	200	27	29	27	25
11c	100	32	31	26	28
	200	33	34	29	30
12a	100	18	16	16	14
	200	20	19	18	16
12b	100	21	22	17	16
	200	23	24	19	18
12c	100	22	23	18	17
	200	24	25	20	19
13a	100	22	23	21	19
	200	25	26	23	21
13b	100	21	20	19	20
	200	24	22	21	20
13c	100	25	23	20	21
	200	27	25	22	23
14a	100	13	14	09	10
	200	15	16	12	12
14b	100	11	12	09	08
	200	13	14	11	10
14c	100	16	15	12	11
	200	18	17	14	13
Chloramphenicol	100	35	38	37	42
	200	41	44	42	45
Control (DMSO)		–	–	–	–

Table 4
Antifungal activity of **10**–**14**.

Compound	Concentration (μg/ml)	Zone of inhibition (mm)		
		Fusarium solani	*Curvularia lunata*	*Aspergillus niger*
10a	100	34	33	31
	200	37	36	34
10b	100	30	31	28
	200	35	33	31
10c	100	35	34	32
	200	39	38	35
11a	100	31	29	28
	200	34	32	31
11b	100	28	28	26
	200	31	30	29
11c	100	33	31	29
	200	35	33	32
12a	100	14	18	15
	200	19	21	18
12b	100	13	16	11
	200	16	18	15
12c	100	17	19	16
	200	20	23	19
13a	100	18	19	16
	200	20	22	19
13b	100	19	21	17
	200	21	23	20
13c	100	22	25	20
	200	25	28	23
14a	100	13	16	14
	200	16	20	16
14b	100	11	13	10
	200	14	16	13
14c	100	16	17	15
	200	19	21	17
Ketoconazole	100	38	41	36
	200	42	44	39
Control (DMSO)		–	–	–

129.2, 130.6, 131.3, 132.3, 133.9, 134.2, 135.1, 135.9 (aromatic carbons). Anal. Calcd. For $C_{17}H_{14}N_2O_4S_2$: C, 54.53; H, 3.77; N, 7.48; Found: C, 54.56; H, 3.76; N, 7.57%.

*6.1.1.5. 2-(1,2-Di(*p*-methylphenylsulfonylethyl)malononitrile (**9b**).* White solid (2.576 g, 64%); m.p. 136–138 °C; IR (KBr): 1130, 1331 (SO_2), 2240 (C≡N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.24, 2.28 (s, 6H, Ar-CH_3), 3.09 (dd, 1H, C_4-H, $J = 8.2$, 14.2 Hz), 3.25 (dd, 1H, C_4-H, $J = 4.1$, 14.0 Hz), 4.09–4.15 (m, 1H, C_3-H), 4.37 (d, 1H, C_2-H, $J = 8.3$ Hz), 7.21–7.72 (m, 8H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 21.7, 22.3 (Ar-CH_3), 37.6 (C-3), 41.2 (C-2), 52.2 (C-4), 114.9 and 115.8 (CN), 128.4, 129.7, 131.4, 132.7, 133.4, 134.8, 135.0, 136.4 (aromatic carbons). Anal. Calcd. For $C_{19}H_{18}N_2O_4S_2$: C, 56.70; H, 4.51; N, 6.96; Found: C, 56.77; H, 4.55; N, 6.92%.

*6.1.1.6. 2-(1,2-Di(4-chlorophenylsulfonylethyl)malononitrile (**9c**).* White solid (2.926 g, 66%); m.p. 159–161 °C; IR (KBr): 1125, 1337 (SO_2), 2238 (C≡N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.11 (dd, 1H, C_4-H, $J = 8.3$, 14.1 Hz), 3.28 (dd, 1H, C_4-H, $J = 4.0$, 14.2 Hz), 4.11–4.17 (m, 1H, C_3-H), 4.36 (d, 1H, C_2-H, $J = 8.1$ Hz), 7.25–7.81 (m, 8H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 36.9 (C-3), 42.7 (C-2), 52.9 (C-4), 113.4 and 115.2 (CN), 128.0, 129.4, 131.9, 132.2, 134.9, 135.3, 135.9, 137.8 (aromatic carbons). Anal. Calcd. for $C_{17}H_{12}Cl_2N_2O_4S_2$: C, 46.06; H, 2.73; N, 6.32; Found: C, 46.13; H, 2.71; N, 6.37%.

*6.1.2. General procedure for the preparation of 3,5-diamino-4-(1′,2′-diaroylethyl)-4H-pyrazole (**3a**–**c**)/3,5-diamino-4-(1′,2′-diarylsulfonylethyl)-4H-pyrazole (**10a**–**c**)*

To a solution of **2**/**9** (10 mmol) in methanol (20 ml), hydrazine hydrate (15 mmol) and 10% NaOMe (5 ml) were added and refluxed for 4–6 h. The contents were cooled and poured onto crushed ice containing HCl. The product obtained was recrystallized from methanol.

Table 5
Minimum inhibitory concentration (MIC), μg/ml of **3c**, **4c**, **10c** and **11c**.

Compound	Minimum inhibitory concentration, MIC (μg/ml)						
	S. aureus	*B. subtilis*	*K. pneumoniae*	*P. vulgaris*	*F. solani*	*C. lunata*	*A. niger*
3c	100	100	100	100	200	200	200
4c	50	50	100	200	100	100	100
10c	25	25	50	50	200	100	100
11c	50	50	100	100	100	50	50
Chloramphenicol	6.25	6.25	12.5	12.5	–	–	–
Ketoconazole	–	–	–	–	12.5	6.25	6.25

Table 6
Antioxidant property of **3–7** and **10–14**.

Compound	% Inhibition at 100 μM	
	Nitric oxide method	DPPH method
3a	71.65	73.72
3b	68.15	66.52
3c	90.74	91.68
4a	43.52	42.27
4b	54.33	58.10
4c	90.26	89.61
5a	26.78	24.71
5b	27.19	28.64
5c	39.39	40.98
6a	35.66	37.80
6b	29.39	32.28
6c	43.44	41.58
7a	22.64	23.82
7b	20.26	21.74
7c	28.19	29.93
10a	59.96	58.49
10b	60.48	59.37
10c	92.42	92.37
11a	84.92	85.81
11b	81.49	80.84
11c	95.38	94.46
12a	29.68	28.76
12b	27.94	28.15
12c	35.93	35.84
13a	45.76	46.87
13b	39.86	38.74
13c	48.45	47.92
14a	26.92	27.74
14b	24.59	24.82
14c	30.21	31.64

*6.1.2.1. 3,5-Diamino-4-(1′,2′-dibenzoylethyl)-4*H*-pyrazole (**3a**).* White solid (2.039 g, 61%); m.p. 162–164 °C; IR (KBr): 1611 (C═N), 1699 (C═O), 3240, 3292 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.06 (dd, 1H, C_2'-H, $J = 4.1$, 14.3 Hz), 3.62 (dd, 1H, C_2'-H, $J = 8.9$, 14.1 Hz), 4.12–4.18 (m, 1H, C_1'-H), 4.29 (d, 1H, C_4-H, $J = 5.5$ Hz), 5.78 (bs, 4H, NH_2), 7.11–7.78 (m, 10H, Ar-H), ppm; ^{13}C NMR ($CDCl_3$) δ 52.8 (C-2′), 53.7 (C-1′), 61.4 (C-4), 172.6, 173.2 (C-3 and C-5), 204.6, 205.4 (Ar-CO), 129.6, 130.2, 131.8, 132.9, 133.6, 134.9, 135.4, 136.2 (aromatic carbons). Anal. Calcd. for $C_{19}H_{18}N_4O_2$: C, 68.25; H, 5.43; N, 16.76; Found: C, 68.32; H, 5.47; N, 16.90%.

*6.1.2.2. 3,5-Diamino-4-(1′,2′-di(*p*-methylbenzoyl)ethyl)-4*H*-pyrazole (**3b**).* White solid (2.319 g, 64%); m.p. 173–175 °C; IR (KBr): 1623 (C═N), 1701 (C═O), 3245, 3396 (NH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.21 and 2.26 (s, 6H, Ar-CH_3), 3.09 (dd, 1H, C_2'-H, $J = 4.0$, 14.1 Hz), 3.64 (dd, 1H, C_2'-H, $J = 8.5$, 14.0 Hz), 4.11–4.16 (m, 1H, C_1'-H), 4.36 (d, 1H, C_4-H, $J = 5.3$ Hz), 5.72 (bs, 4H, NH_2), 7.21–7.72 (m, 8H, Ar-H), ppm; ^{13}C NMR (DMSO-d_6) δ 22.6, 22.8 (Ar-CH_3), 51.6 (C-2′), 52.9 (C-1′), 60.8 (C-4), 171.4, 172.9 (C-3 and C-5), 205.2, 206.1 (Ar-CO), 128.5, 129.1, 130.6, 131.5, 132.2, 133.5, 134.1, 135.4 (aromatic carbons). Anal. Calcd. For $C_{21}H_{22}N_4O_2$: C, 69.59; H, 6.12; N, 15.46; Found: C, 69.64; H, 6.13; N, 15.57%.

*6.1.2.3. 3,5-Diamino-4-(1′,2′-di(*p*-chlorobenzoyl)ethyl)-4*H*-pyrazole (**3c**).* White solid (2.661 g, 66%); m.p. 178–180 °C; IR (KBr): 1616 (C═N), 1705 (C═O), 3250, 3300 (NH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.13 (dd, 1H, C_2'-H, $J = 4.3$, 14.2 Hz), 3.58 (dd, 1H, C_2'-H, $J = 8.3$, 14.1 Hz), 4.13–4.18 (m, 1H, C_1'-H), 4.32 (d, 1H, C_4-H, $J = 5.2$ Hz), 5.81 (bs, 4H, NH_2), 7.26–7.84 (m, 8H, Ar-H), ppm; ^{13}C NMR (DMSO-d_6) δ 52.7 (C-2′), 53.4 (C-1′), 61.3 (C-4), 172.7, 173.4 (C-3 and C-5), 203.6, 205.5 (Ar-CO), 128.9, 129.7, 130.2, 131.2, 132.9, 133.9, 134.9, 136.4 (aromatic carbons). Anal. Calcd. For $C_{19}H_{16}Cl_2N_4O_2$: C, 56.59; H, 4.00; N, 13.89; Found: C, 56.54; H, 4.05; N, 13.98%.

*6.1.2.4. 3,5-Diamino-4-(1′,2′-diphenylsulfonylethyl)-4*H*-pyrazole (**10a**).* White solid (2.520 g, 62%); m.p. 163–165 °C; IR (KBr): 1134, 1333 (SO_2), 1614 (C═N), 3233, 3300 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.15 (dd, 1H, C_2'-H, $J = 4.3$, 14.3 Hz), 3.68 (dd, 1H, C_2'-H, $J = 8.3$, 14.2 Hz), 4.15–4.21 (m, 1H, C_1'-H), 4.31 (d, 1H, C_4-H, $J = 5.6$ Hz), 5.74 (bs, 4H, NH_2), 7.14–7.69 (m, 10H, Ar-H), ppm; ^{13}C NMR (DMSO-d_6) δ 51.4 (C-2′), 52.5 (C-1′), 62.7 (C-4), 173.8, 174.7 (C-3 and C-5), 128.4, 129.6, 130.4, 131.6, 132.9, 133.5, 134.3, 135.1 (aromatic carbons). Anal. Calcd. For $C_{17}H_{18}N_4O_4S_2$: C, 50.23; H, 4.46; N, 13.78; Found: C, 50.28; H, 4.42; N, 13.86%.

*6.1.2.5. 3,5-Diamino-4-(1′,2′-di(*p*-methylphenyl)sulfonylethyl)-4*H*-pyrazole (**10b**).* White solid (2.824 g, 65%); m.p. 171–173 °C; IR (KBr): 1131, 1339 (SO_2), 1611 (C═N), 3245, 3311 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.21, 2.26 (s, 6H, Ar-CH_3), 3.13 (dd, 1H, C_2'-H, $J = 4.0$, 14.1 Hz), 3.59 (dd, 1H, C_2'-H, $J = 8.1$, 14.0 Hz), 4.10–4.17 (m, 1H, C_1'-H), 4.32 (d, 1H, C_4-H, $J = 5.7$ Hz), 5.76 (bs, 4H, NH_2), 7.12–7.71 (m, 8H, Ar-H), ppm; ^{13}C NMR (DMSO-d_6) δ 21.8, 22.4 (Ar-CH_3), 52.7 (C-2′), 53.4 (C-1′), 61.9 (C-4), 172.4, 173.8 (C-3 and C-5), 127.8, 129.1, 130.9, 131.2, 132.4, 133.9 134.6, 135.9 (aromatic carbons). Anal. Calcd. For $C_{19}H_{22}N_4O_4S_2$: C, 52.52; H, 5.10; N, 12.89; Found: C, 52.60; H, 5.13; N, 12.00%.

*6.1.2.6. 3,5-Diamino-4-(1′,2′-di(*p*-chlorophenyl)sulfonylethyl)-4*H*-pyrazole (**10c**).* White solid (3.232 g, 68%); m.p. 167–168 °C; IR (KBr): 1128, 1336 (SO_2), 1617 (C═N), 3232, 3285 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.19 (dd, 1H, C_2'-H, $J = 4.2$, 14.3 Hz), 3.61 (dd, 1H, C_2'-H, $J = 8.0$, 14.1 Hz), 4.16–4.23 (m, 1H, C_1'-H), 4.38 (d, 1H, C_4-H, $J = 5.9$ Hz), 7.21–7.85 (m, 8H, Ar-H), 5.84 (bs, 4H, NH_2) ppm; ^{13}C NMR (DMSO-d_6) δ 53.1 (C-2′), 54.6 (C-1′), 62.3 (C-4), 173.6, 174.2 (C-3 and C-5), 128.4, 129.7, 131.4, 132.6, 133.8, 134.5 135.7, 137.3 (aromatic carbons). Anal. Calcd. For $C_{17}H_{16}Cl_2N_4O_4S_2$: C, 42.95; H, 3.39; N, 11.79; Found: C, 43.00; H, 3.44; N, 11.85%.

*6.1.3. General procedure for the preparation of 3-amino-5-imino-4-(1′,2′-diaroylethyl)-4*H*-isoxazole (**4a–c**)/3-amino-5-imino-4-(1′,2′-diarylsulfonylethyl)-4*H*-isoxazole (**11a–c**)*

A mixture of compound **2**/**9** (10 mmol), hydroxylamine hydrochloride (15 mmol), methanol (20 ml) and 10% NaOMe (5 ml) was refluxed for 4–6 h. The solution was cooled and poured onto crushed ice containing HCl. The solid obtained was recrystallized from methanol.

*6.1.3.1. 3-Amino-5-imino-4-(1′,2′-dibenzoylethyl)-4*H*-isoxazole (**4a**).* White solid (2.347 g, 70%); m.p. 161–163 °C; IR (KBr): 1602 (C═N), 1708 (C═O), 3242, 3280 (NH_2), 3319 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.08 (dd, 1H, C_2'-H, $J = 4.2$, 14.3 Hz), 3.64 (dd, 1H, C_2'-H, $J = 8.8$, 14.1 Hz), 4.10–4.26 (m, 1H, C_1'-H), 4.34 (d, 1H, C_4-H, $J = 5.2$ Hz), 5.83 (bs, 2H, NH_2), 7.22–7.74 (m, 10H, Ar-H), 9.91 (bs, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 53.4 (C-2′), 55.7 (C-1′), 62.9 (C-4), 176.7, 178.4 (C-3 and C-5), 205.6, 206.2 (Ar-CO), 128.1, 129.5, 130.1, 131.2, 132.6, 133.2, 134.0, 135.2 (aromatic carbons). Anal. Calcd. For $C_{19}H_{17}N_3O_3$: C, 68.05; H, 5.11; N, 12.53; Found: C, 68.16; H, 5.08; N, 12.48%.

*6.1.3.2. 3-Amino-5-imino-4-(1′,2′-di-(*p*-methylbenzoyl)ethyl)-4*H*-isoxazole (**4b**).* White solid (2.471 g, 68%); m.p. 176–178 °C; IR (KBr): 1605 (C═N), 1710 (C═O), 3237, 3285 (NH_2), 3324 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.27, 2.29 (s, 6H, Ar-CH_3), 3.11 (dd, 1H, C_2'-H, $J = 4.3$, 14.4 Hz), 3.67 (dd, 1H, C_2'-H, $J = 8.6$, 14.2 Hz), 4.14–4.19 (m, 1H, C_1'-H), 4.31 (d, 1H, C_4-H, $J = 5.1$ Hz), 5.76 (bs, 2H, NH_2), 7.18–7.70 (m, 8H, Ar-H), 9.89 (bs, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 21.7, 22.4 (Ar-CH_3), 52.9 (C-2′), 54.9 (C-1′), 63.2 (C-4), 177.3, 178.9 (C-3 and C-5), 206.2, 207.3 (Ar-CO), 128.7, 129.3, 130.9, 131.9, 132.2, 133.9, 134.8, 135.1 (aromatic carbons). Anal. Calcd. For $C_{21}H_{21}N_3O_3$: C, 69.41; H, 5.82; N, 11.56; Found: C, 69.47; H, 5.86; N, 11.65%.

*6.1.3.3. 3-Amino-5-imino-4-(1′,2′-di-(*p*-chlorobenzoyl)ethyl)-4*H*-isoxazole (**4c**).* White solid (2.870 g, 71%); m.p. 181–183 °C; IR (KBr): 1610 (C═N), 1698 (C═O), 3245, 3282 (NH_2), 3311 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.07 (dd, 1H, C_2'-H, $J = 4.2, 14.2$ Hz), 3.65 (dd, 1H, C_2'-H, $J = 8.4$, 14.1 Hz), 4.11–4.16 (m, 1H, C_1'-H), 4.27 (d, 1H, C_4-H, $J = 5.0$ Hz), 5.79 (bs, 2H, NH_2), 7.21–7.82 (m, 8H, Ar-H), 9.82 (bs, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 51.5 (C-2′), 53.4 (C-1′), 62.8 (C-4), 176.8, 177.7 (C-3 and C-5), 205.9, 206.4 (Ar-CO), 128.1, 129.8, 131.4, 132.3, 133.7, 134.8, 135.2, 136.9 (aromatic carbons). Anal. Calcd. For $C_{19}H_{15}Cl_2N_3O_3$: C, 56.45; H, 3.74; N, 10.39; Found: C, 56.53; H, 3.71; N, 10.50%.

*6.1.3.4. 3-Amino-5-imino-4-(1′,2′-diphenylsulfonylethyl)-4*H*-isoxazole (**11a**).* White solid (2.934 g, 72%); m.p. 198–200 °C; IR (KBr): 1130, 1338 (SO_2), 1612 (C═N), 3240, 3296 (NH_2), 3320 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.17 (dd, 1H, C_2'-H, $J = 4.0$, 14.1 Hz), 3.72 (dd, 1H, C_2'-H, $J = 8.4$, 14.0 Hz), 4.11–4.19 (m, 1H, C_1'-H), 4.35 (d, 1H, C_4-H, $J = 5.0$ Hz), 5.77 (bs, 2H, NH_2), 7.12–7.68 (m, 10H, Ar-H), 9.86 (bs, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 52.6 (C-2′), 55.1 (C-1′), 61.7 (C-4), 175.3, 177.2 (C-3 and C-5), 127.6, 128.4, 129.9, 130.7, 131.5, 132.8, 133.6, 134.8 (aromatic carbons). Anal. Calcd. For $C_{17}H_{17}N_3O_5S_2$: C, 50.11; H, 4.21; N, 10.31; Found: C, 50.16; H, 4.25; N, 10.35%.

*6.1.3.5. 3-Amino-5-imino-4-(1′,2′-di-(*p*-methylphenyl)sulfonylethyl)-4*H*-isoxazole (**11b**).* White solid (2.918 g, 67%); m.p. 205–207 °C; IR (KBr): 1126, 1341 (SO_2), 1608 (C═N), 3238, 3298 (NH_2), 3315 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.27, 2.29 (s, 6H, Ar-CH_3), 3.19 (dd, 1H, C_2'-H, $J = 4.2$, 14.3 Hz), 3.47 (dd, 1H, C_2'-H, $J = 8.0$, 14.1 Hz), 4.18–4.25 (m, 1H, C_1'-H), 4.37 (d, 1H, C_4-H, $J = 5.2$ Hz), 5.85 (bs, 2H, NH_2), 7.10–7.64 (m, 8H, Ar-H), 9.84 (bs, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 21.4, 22.5 (Ar-CH_3), 51.9 (C-2′), 54.8 (C-1′), 62.5 (C-4), 174.8, 176.9 (C-3 and C-5), 128.9, 129.1, 129.7, 130.3, 132.4, 133.1, 134.4, 135.6 (aromatic carbons). Anal. Calcd. For $C_{19}H_{21}N_3O_5S_2$: C, 52.40;H, 4.86;N, 9.65; Found: C, 52.46; H, 4.87; N, 9.70%.

*6.1.3.6. 3-Amino-5-imino-4-(1′,2′-di-(*p*-methylphenyl)sulfonylethyl)-4*H*-isoxazole (**11c**).* White solid (3.525 g, 74%); m.p. 221–223 °C; IR (KBr): 1121, 1339 (SO_2), 1603 (C═N), 3242, 3294 (NH_2), 3325 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.21 (dd, 1H, C_2'-H, $J = 4.3$, 14.1 Hz), 3.59 (dd, 1H, C_2'-H, $J = 8.2$, 14.3 Hz), 4.22–4.28 (m, 1H, C_1'-H), 4.41 (d, 1H, C_4-H, $J = 5.4$ Hz), 5.81 (bs, 2H, NH_2), 7.24–7.84 (m, 8H, Ar-H), 9.79 (bs, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 52.4 (C-2′), 55.7 (C-1′), 61.9 (C-4), 173.9, 175.2 (C-3 and C-5), 129.1, 130.6, 131.7, 132.4, 133.7, 134.3, 135.6, 136.8 (aromatic carbons). Anal. Calcd. For $C_{17}H_{15}Cl_2N_3O_5S_2$: C, 42.86; H, 3.17; N, 8.82; Found: C, 42.83; H, 3.15; N, 8.80%.

*6.1.4. General procedure for the preparation of 4,6-diamino-5-(1′,2′-diaroylethyl)-pyrimidine-2(5*H*)-one (**5a–c**)/4,6-diamino-5-(1′,2′-diarylsulfonylethyl)-pyrimidine-2(5*H*)-one (**12a–c**)*

To a solution of 2/9 (10 mmol) in methanol (20 ml), urea (10 mmol) and 10% NaOMe (5 ml) were added and refluxed for 6–10 h. The contents were cooled, poured onto crushed ice containing conc. HCl and extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. Removal of the solvent under vacuum gave crude product which was recrystallized from methanol.

*6.1.4.1. 4,6-Diamino-5-(1′,2′-dibenzoylethyl)-pyrimidine-2(5*H*)-one (**5a**).* White solid (2.682 g, 74%); m.p. 194–196 °C; IR (KBr): 1613 (C═N), 1650 (N–C═O), 1700 (C═O), 3233, 3297 (NH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.09 (dd, 1H, C_2'-H, $J = 4.1$, 14.1 Hz), 3.69 (dd, 1H, C_2'-H, $J = 9.1$, 14.2 Hz), 4.16–4.25 (m, 1H, C_1'-H), 4.30 (d, 1H, C_5-H, $J = 5.1$ Hz), 5.64 (bs, 4H, NH_2), 7.20–7.71 (m, 10H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 52.9 (C-2′), 57.7 (C-1′), 64.3 (C-5), 159.8 (C-2), 168.8, 169.7 (C-4 and C-6), 204.6, 205.7 (Ar-CO), 126.4, 127.2, 128.2, 130.1, 131.5, 132.0, 134.5, 135.7 (aromatic carbons). Anal. Calcd. For $C_{20}H_{18}N_4O_3$: C, 66.29; H, 5.01; N, 15.46; Found: C, 66.36; H, 5.06; N, 15.59%.

*6.1.4.2. 4,6-Diamino-5-(1′,2′-di-(*p*-methylbenzoyl)ethyl)-pyrimidine-2(5*H*)-one (**5b**).* White solid (2.733 g, 70%); m.p. 199–201 °C; IR (KBr): 1611 (C═N), 1658 (N–C═O), 1707 (C═O), 3209, 3288 (NH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.22, 2.27 (s, 6H, Ar-CH_3), 3.10 (dd, 1H, C_2'-H, $J = 4.0$, 14.2 Hz), 3.64 (dd, 1H, C_2'-H, $J = 8.8$, 14.0 Hz), 4.10–4.16 (m, 1H, C_1'-H), 4.36 (d, 1H, C_5-H, $J = 5.3$ Hz), 5.67 (bs, 4H, NH_2), 7.12–7.69 (m, 8H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 22.6, 23.4 (Ar-CH_3), 51.4 (C-2′), 56.3 (C-1′), 65.9 (C-5), 158.6 (C-2), 167.6, 168.5 (C-4 and C-6), 205.2, 206.4 (Ar-CO), 126.9, 128.4, 128.9, 130.4, 131.4, 132.7, 133.8, 134.1 (aromatic carbons). Anal. Calcd. For $C_{22}H_{22}N_4O_3$: C, 67.68; H, 5.68; N, 14.35; Found: C, 67.62; H, 5.65; N, 14.43%.

*6.1.4.3. 4,6-Diamino-5-(1′,2′-di-(*p*-chlorobenzoyl)ethyl)-pyrimidine-2(5*H*)-one (**5c**).* White solid (3.105 g, 72%); m.p. 220–222 °C; IR (KBr): 1609 (C═N), 1650 (N–C═O), 1696 (C═O), 3220, 3284 (NH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.16 (dd, 1H, C_2'-H, $J = 4.3$, 14.6 Hz), 3.61 (dd, 1H, C_2'-H, $J = 8.2$, 14.3 Hz), 4.14–4.21 (m, 1H, C_1'-H), 4.32 (d, 1H, C_5-H, $J = 5.2$ Hz), 5.61 (bs, 4H, NH_2), 7.23–7.81 (m, 8H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 52.7 (C-2′), 57.1 (C-1′), 64.4 (C-5), 159.5 (C-2), 168.2, 169.3 (C-4 and C-6), 206.4, 207.3 (Ar-CO), 128.9, 129.4, 130.9, 131.7, 132.3, 133.6, 135.8, 139.4 (aromatic carbons). Anal. Calcd. For $C_{20}H_{16}Cl_2N_4O_3$: C, 55.70; H, 3.74; N, 12.99; Found: C, 55.64; H, 3.75; N, 13.06%.

*6.1.4.4. 4,6-Diamino-5-(1′,2′-diphenylsulfonylethyl)-pyrimidine-2(5*H*)-one (**12a**).* White solid (3.172 g, 73%); m.p. 192–194 °C; IR (KBr): 1119, 1340 (SO_2), 1618 (C═N), 1675 (C═O), 3242, 3295 (NH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.10 (dd, 1H, C_2'-H, $J = 4.0$, 14.0 Hz), 3.70 (dd, 1H, C_2'-H, $J = 9.0$, 14.1 Hz), 4.17–4.24 (m, 1H, C_1'-H), 4.37 (d, 1H, C_5-H, $J = 5.3$ Hz), 5.59 (bs, 4H, NH_2), 7.19–7.76 (m, 10H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 51.5 (C-2′), 56.5 (C-1′), 63.2 (C-5), 157.8 (C-2), 167.4, 168.2 (C-4 and C-6), 127.8, 128.7, 129.9, 130.6, 131.9, 132.8, 133.4, 134.6 (aromatic carbons). Anal. Calcd. For $C_{18}H_{18}N_4O_5S_2$: C, 49.76; H, 4.18; N, 12.89; Found: C, 49.80; H, 4.20; N, 12.98%.

*6.1.4.5. 4,6-Diamino-5-(1′,2′-di(*p*-methylphenyl)sulfonylethyl)-pyrimidine-2(5*H*)-one (**12b**).* White solid (3.238 g, 70%); m.p. 205–207 °C; IR (KBr): 1126, 1332 (SO_2), 1613 (C═N), 1664 (C═O), 3235, 3290 (NH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.22, 2.27 (s, 6H, Ar-CH_3), 3.12 (dd, 1H, C_2'-H, $J = 4.1$, 14.2 Hz), 3.68 (dd, 1H, C_2'-H, $J = 9.2$, 14.0 Hz), 4.14–4.22 (m, 1H, C_1'-H), 4.31 (d, 1H, C_5-H, $J = 5.1$ Hz), 5.63 (bs, 4H, NH_2), 7.21–7.72 (m, 8H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 22.6, 22.9 (Ar-CH_3), 52.8 (C-2′), 57.2 (C-1′), 61.7 (C-5), 158.4 (C-2), 166.9, 167.6 (C-4 and C-6), 128.1, 129.4, 130.2, 131.5, 132.3, 133.6, 134.8, 135.3 (aromatic carbons). Anal. Calcd. For $C_{20}H_{22}N_4O_5S_2$: C, 51.93; H, 4.79; N, 12.11; Found: C, 52.00; H, 4.77; N, 12.19%.

*6.1.4.6. 4,6-Diamino-5-(1′,2′-di(*p*-chlorophenyl)sulfonylethyl)-pyrimidine-2(5*H*)-one (**12c**).* White solid (3.221 g, 64%); m.p. 230–232 °C; IR (KBr): 1122, 1330 (SO_2), 1610 (C═N), 1668 (C═O), 3230, 3285 (NH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.09 (dd, 1H, C_2'-H, $J = 4.0$, 14.0 Hz), 3.62 (dd, 1H, C_2'-H, $J = 9.0$, 14.1 Hz), 4.16–4.25 (m, 1H, C_1'-H), 4.36 (d, 1H, C_5-H, $J = 5.4$ Hz), 5.67 (bs, 4H, NH_2), 7.25–7.86 (m, 8H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 51.4 (C-2′), 56.7 (C-1′), 62.2 (C-5), 159.5 (C-2), 167.3, 168.3 (C-4 and C-6), 127.9, 128.4, 131.7, 132.9, 133.8, 134.2, 135.2, 136.8 (aromatic carbons). Anal. Calcd. For $C_{18}H_{16}Cl_2N_4O_5S_2$: C, 42.95; H, 3.20; N, 11.13; Found: C, 42.93; H, 3.24; N, 11.02%.

*6.1.5. General procedure for the preparation of 4,6-diamino-5-(1′,2′-diaroylethyl)-pyrimidine-2-(5*H*)-thione (**6a–c**)/4,6-diamino-5-(1′,2′-diarylsulfonylethyl)-pyrimidine-2-(5*H*)-thione (**13a–c**)*

To an equimolar mixture (10 mmol) of **2**/**9** and thiourea, methanol (20 ml) and 10% NaOMe (5 ml) were added and refluxed for

10–15 h. The reaction mixture was cooled, poured onto crushed ice containing conc. HCl and extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The resultant solid was recrystallized from methanol.

*6.1.5.1. 4,6-Diamino-5-(1′,2′-dibenzoylethyl)-pyrimidine-2-(5H)-thione (**6a**).* White solid (2.785 g, 72%); m.p. 200–202 °C; IR (KBr): 1485 (C═S), 1611 (C═N), 1711 (C═O), 3207, 3285 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.13 (dd, 1H, C_2'-H, $J = 4.0$, 14.0 Hz), 3.71 (dd, 1H, C_2'-H, $J = 8.9$, 14.1 Hz), 4.15–4.22 (m, 1H, C_1'-H), 4.36 (d, 1H, C_5-H, $J = 5.0$ Hz), 5.67 (bs, 4H, NH_2), 7.24–7.78 (m, 10H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 52.6 (C-2′), 56.4 (C-1′), 64.9 (C-5), 162.8, 163.7 (C-4 and C-6), 172.8 (C-2), 205.9, 206.4 (Ar-CO), 127.6, 129.2, 130.6, 131.4, 132.6, 133.2, 134.2, 135.4 (aromatic carbons). Anal. Calcd. For $C_{20}H_{18}N_4O_2S$: C, 63.47; H, 4.79; N, 14.80; Found: C, 63.51; H, 4.80; N, 14.85%.

*6.1.5.2. 4,6-Diamino-5-(1′,2′-di-(p-methylbenzoyl)ethyl)-pyrimidine-2-(5H)-thione (**6b**).* White solid (2.642 g, 65%); m.p. 218–220 °C; IR (KBr): 1490 (C═S), 1618 (C═N), 1702 (C═O), 3210, 3275 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.26, 2.38 (s, 6H, Ar-CH_3), 3.11 (dd, 1H, C_2'-H, $J = 4.2$, 14.3 Hz), 3.59 (dd, 1H, C_2'-H, $J = 8.6$, 14.0 Hz), 4.17–4.25 (m, 1H, C_1'-H), 4.42 (d, 1H, C_5-H, $J = 5.0$ Hz), 5.71 (bs, 4H, NH_2), 7.27–7.82 (m, 8H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 21.9, 22.3 (Ar-CH_3), 51.9 (C-2′), 55.8 (C-1′), 64.2 (C-5), 163.6, 165.4 (C-4 and C-6), 171.3 (C-2), 204.6, 205.8 (Ar-CO), 127.1, 129.8, 130.9, 131.1, 132.3, 133.5, 134.8, 136.2 (aromatic carbons). Anal. Calcd. For $C_{22}H_{22}N_4O_2S$: C, 65.00; H, 5.46; N, 13.78; Found: C, 65.07; H, 5.51; N, 13.72%.

*6.1.5.3. 4,6-Diamino-5-(1′,2′-di-(p-chlorobenzoyl)ethyl)-pyrimidine-2-(5H)-thione (**6c**).* White solid (2.818 g, 63%); m.p. 228–230 °C; IR (KBr): 1505 (C═S), 1606 (C═N), 1700 (C═O), 3208, 3280 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.15 (dd, 1H, C_2'-H, $J = 4.4$, 14.5 Hz), 3.65 (dd, 1H, C_2'-H, $J = 8.7$, 14.3 Hz), 4.19–4.27 (m, 1H, C_1'-H), 4.39 (d, 1H, C_5-H, $J = 5.1$ Hz), 5.64 (bs, 4H, NH_2), 7.25–7.91 (m, 8H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 52.4 (C-2′), 56.1 (C-1′), 65.2 (C-5), 162.8, 164.8 (C-4 and C-6), 173.1 (C-2), 205.6, 206.9 (Ar-CO), 127.8, 129.2, 130.1, 131.8, 132.4, 133.9, 134.1, 136.8 (aromatic carbons). Anal. Calcd. For $C_{20}H_{16}Cl_2N_4O_2S$: C, 53.70; H, 3.61; N, 12.52; Found: C, 53.75; H, 3.59; N, 12.58%.

*6.1.5.4. 4,6-Diamino-5-(1′,2′-diphenylsulfonylethyl)-pyrimidine-2-(5H)-thione (**13a**).* White solid (2.818 g, 65%); m.p. 206–208 °C; IR (KBr): 1134, 1336 (SO_2), 1487 (C═S), 1614 (C═N), 3250, 3300 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.12 (dd, 1H, C_2'-H, $J = 4.3$, 14.2 Hz), 3.73 (dd, 1H, C_2'-H, $J = 8.8$, 14.0 Hz), 4.19–4.27 (m, 1H, C_1'-H), 4.41 (d, 1H, C_5-H, $J = 5.4$ Hz), 5.72 (bs, 4H, NH_2), 7.17–7.71 (m, 10H, Ar-H), ppm; ^{13}C NMR (DMSO-d_6) δ 51.8 (C-2′), 56.9 (C-1′), 63.4 (C-5), 161.4, 164.3 (C-4 and C-6), 171.4 (C-2), 128.1, 129.8, 130.2, 131.2, 132.4, 133.0, 134.9, 135.8 (aromatic carbons). Anal. Calcd. For $C_{18}H_{18}N_4O_4S_3$: C, 47.98; H, 4.03; N, 12.44; Found: C, 47.96; H, 4.01; N, 12.51%.

*6.1.5.5. 4,6-Diamino-5-(1′,2′-di-(p-methylphenyl)sulfonylethyl)-pyrimidine-2-(5H)-thione (**13b**).* White solid (3.015 g, 63%); m.p. 215–217 °C; IR (KBr): 1129, 1332 (SO_2), 1490 (C═S), 1621 (C═N), 3242, 3296 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) 2.21, 2.25 (s, 6H, Ar-CH_3), 3.10 (dd, 1H, C_2'-H, $J = 4.0$, 14.0 Hz), 3.59 (dd, 1H, C_2'-H, $J = 8.2$, 14.1 Hz), 4.20–4.29 (m, 1H, C_1'-H), 4.39 (d, 1H, C_5-H, $J = 5.3$ Hz), 5.69 (bs, 4H, NH_2), 7.21–7.79 (m, 8H, Ar-H), ppm; ^{13}C NMR (DMSO-d_6) δ 22.8, 22.9 (Ar-CH_3), 52.6 (C-2′), 57.1 (C-1′), 61.9 (C-5), 162.9, 165.6 (C-4 and C-6), 173.6 (C-2), 127.9, 128.2, 129.8, 130.6, 131.5, 132.4, 133.3, 134.9 (aromatic carbons). Anal. Calcd. For $C_{20}H_{22}N_4O_4S_3$: C, 50.19;H, 4.63;N, 11.71; Found: C, 50.26; H, 4.65; N, 11.66%.

*6.1.5.6. 4,6-Diamino-5-(1′,2′-di-(p-chlorophenyl)sulfonylethyl)-pyrimidine-2-(5H)-thione (**13c**).* White solid (3.015 g, 61%); m.p. 233–235 °C; IR (KBr): 1127, 1335 (SO_2), 1495 (C═S), 1617 (C═N), 3254, 3294 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) 3.17 (dd, 1H, C_2'-H, $J = 4.2$, 14.3 Hz), 3.64 (dd, 1H, C_2'-H, $J = 8.3$, 14.4 Hz), 4.16–4.25 (m, 1H, C_1'-H), 4.45 (d, 1H, C_5-H, $J = 5.5$ Hz), 5.65 (bs, 4H, NH_2), 7.27–7.82 (m, 8H, Ar-H), ppm; ^{13}C NMR (DMSO-d_6) δ 51.8 (C-2′), 56.9 (C-1′), 62.3 (C-5), 163.4, 164.8 (C-4 and C-6), 172.6 (C-2), 128.2, 129.9, 130.8, 131.3, 133.8, 133.7, 135.4, 136.8 (aromatic carbons). Anal. Calcd. For $C_{18}H_{16}Cl_2N_4O_4S_3$: C, 41.62; H, 3.10; N, 10.79; Found: C, 41.57; H, 3.11; N, 10.85%.

*6.1.6. General procedure for the preparation of 4,6-diimino-5-(1′,2′-diaroylethyl)-1,3-dimethylpyrimidine-2-(5H)-one (**7a–c**)/4,6-diimino-5-(1′,2′-diarylsulfonylethyl)-1,3-dimethylpyrimidine-2-(5H)-one (**14a–c**)*

A mixture of 2/9 (10 mmol), *N*,*N*′-dimethyl urea (10 mmol), methanol (10 ml) and 10% NaOMe (5 ml) was refluxed for 8–12 h. The contents were diluted with ice-cold water, acidified with conc. HCl and extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. Removal of the solvent with rotary evaporator afforded crude product which was purified by recrystallization from methanol.

*6.1.6.1. 4,6-Diimino-5-(1′,2′-dibenzoylethyl)-1,3-dimethylpyrimidine-2-(5H)-one (**7a**).* White solid (2.577 g, 66%); m.p. 191–193 °C; IR (KBr): 1651 (C═O), 1695 (Ar-CO), 3314 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.68 and 2.71 (s, 6H, N-CH_3), 3.15 (dd, 1H, C_2'-H, $J = 4.1$, 14.1 Hz), 3.74 (dd, 1H, C_2'-H, $J = 8.4$, 14.3 Hz), 4.13–4.21 (m, 1H, C_1'-H), 4.32 (d, 1H, C_5-H, $J = 5.1$ Hz), 7.09–7.49 (m, 10H, Ar-H), 9.94 (bs, 2H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 26.2, 27.0 (N-CH_3), 51.9 (C-2′), 54.0 (C-1′), 63.8 (C-5), 159.9 (C-2), 163.7, 167.2 (C-4 and C-6), 205.8, 206.9 (Ar-CO), 127.2, 129.4, 130.2, 131.6, 132.4, 133.1, 134.9, 135.6 (aromatic carbons). Anal. Calcd. For $C_{22}H_{22}N_4O_3$: C, 67.68; H, 5.68; N, 14.35; Found: C, 67.78; H, 5.70; N, 14.48%.

*6.1.6.2. 4,6-Diimino-5-(1′,2′-di-(p-methylbenzoyl)ethyl)-1,3-dimethylpyrimidine-2-(5H)-one (**7b**).* White solid (2.511 g, 60%); m.p. 208–210 °C; IR (KBr): 1658 (C═O), 1704 (Ar-CO), 3320 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.25, 2.31 (s, 6H, Ar-CH_3), 2.60 and 2.69 (s, 6H, N–CH_3), 3.13 (dd, 1H, C_2'-H, $J = 4.3$, 14.3 Hz), 3.68 (dd, 1H, C_2'-H, $J = 8.1$, 14.1 Hz), 4.19–4.26 (m, 1H, C_1'-H), 4.31 (d, 1H, C_5-H, $J = 5.0$ Hz), 7.14–7.51 (m, 8H, Ar-H), 9.98 (bs, 2H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 21.4, 22.6 (Ar-CH_3), 25.6, 26.7 (N-CH_3), 52.4 (C-2′), 54.6 (C-1′), 64.5 (C-5), 158.4 (C-2), 161.7, 165.9 (C-4 and C-6), 204.2, 205.6 (Ar-CO), 127.2, 129.9, 130.1, 131.8, 132.2, 133.9, 135.9, 136.3 (aromatic carbons). Anal. Calcd. For $C_{24}H_{26}N_4O_3$: C, 68.88; H, 6.26; N, 13.39; Found: C, 68.97; H, 6.31; N, 13.50%.

*6.1.6.3. 4,6-Diimino-5-(1′,2′-di-(p-chlorobenzoyl)ethyl)-1,3-dimethylpyrimidine-2-(5H)-one (**7c**).* White solid (2.848 g, 62%); m.p. 225–227 °C; IR (KBr): 1654 (C═O), 1712 (Ar-CO), 3317 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.57 and 2.61 (s, 6H, N-CH_3), 3.10 (dd, 1H, C_2'-H, $J = 4.1$, 14.2 Hz), 3.62 (dd, 1H, C_2'-H, $J = 8.0$, 14.0 Hz), 4.11–4.24 (m, 1H, C_1'-H), 4.34 (d, 1H, C_5-H, $J = 5.2$ Hz), 7.21–7.84 (m, 8H, Ar-H), 9.89 (bs, 2H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 25.9, 26.1 (N-CH_3), 53.6 (C-2′), 55.2 (C-1′), 63.9 (C-5), 157.6 (C-2), 162.4, 164.6 (C-4 and C-6), 205.5, 206.9 (Ar-CO), 128.6, 129.2, 130.8, 132.7, 133.5, 134.2, 135.3, 137.2 (aromatic carbons). Anal. Calcd. For $C_{22}H_{20}Cl_2N_4O_3$: C, 57.53; H, 4.39; N, 12.20; Found: C, 57.60; H, 4.42; N, 12.27%.

*6.1.6.4. 4,6-Diimino-5-(1′,2′-diphenylsulfonylethyl)-1,3-dimethylpyrimidine-2-(5H)-one (**14a**).* White solid (3.284 g, 71%); m.p. 189–190 °C; IR (KBr): 1125, 1331 (SO_2), 1665 (C═O), 3314 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.63 and 2.74 (s, 6H, N-CH_3), 3.14 (dd, 1H, C_2'-H,

$J = 4.3, 14.2$ Hz), 3.75 (dd, 1H, C_2'-H, $J = 8.8, 14.1$ Hz), 4.20–4.26 (m, 1H, C_1'-H), 4.45 (d, 1H, C_5-H, $J = 5.5$ Hz), 7.11–7.58 (m, 10H, Ar-H), 9.78 (bs, 2H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 26.9, 27.2 (N-CH_3), 52.3 (C-2′), 55.6 (C-1′), 61.5 (C-5), 158.4 (C-2), 164.6, 166.8 (C-4 and C-6), 128.6, 129.1, 130.6, 131.2, 132.9, 133.4, 134.0, 135.3 (aromatic carbons). Anal. Calcd. For $C_{20}H_{22}N_4O_5S_2$: C, 51.93; H, 4.79; N, 12.11; Found: C, 51.96; H, 4.84; N, 12.17%.

*6.1.6.5. 4,6-Diimino-5-(1′,2′-di-(*p*-methylphenyl)sulfonylethyl)-1,3-dimethylpyrimidine-2-(5*H*)-one (**14b**).* White solid (3.532 g, 72%); m.p. 212–214 °C; IR (KBr): 1131, 1339 (SO_2), 1662 (C═O), 3317 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.24, 2.29 (s, 6H, Ar-CH_3), 2.59 and 2.75 (s, 6H, N-CH_3), 3.08 (dd, 1H, C_2'-H, $J = 4.1, 14.0$ Hz), 3.64 (dd, 1H, C_2'-H, $J = 8.9$, 14.2 Hz), 4.21–4.26 (m, 1H, C_1'-H), 4.39 (d, 1H, C_5-H, $J = 5.6$ Hz), 7.14–7.63 (m, 8H, Ar-H), 9.16 (bs, 2H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 21.9, 22.8 (Ar-CH_3), 26.3, 28.4 (N-CH_3), 52.9 (C-2′), 54.4 (C-1′), 62.8 (C-5), 157.6 (C-2), 165.8, 167.4 (C-4 and C-6), 127.4, 128.5, 129.6, 130.5, 133.8, 134.8, 135.2, 135.9 (aromatic carbons). Anal. Calcd. For $C_{22}H_{26}N_4O_5S_2$: C, 53.86; H, 5.34; N, 11.42; Found: C, 53.84; H, 5.37; N, 11.48%.

*6.1.6.6. 4,6-Diimino-5-(1′,2′-di-(*p*-chlorophenyl)sulfonylethyl)-1,3-dimethylpyrimidine-2-(5*H*)-one (**14c**).* White solid (3.667 g, 69%); m.p. 228–230 °C; IR (KBr): 1126, 1341 (SO_2), 1667 (C═O), 3322 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.51 and 2.68 (s, 6H, N–CH_3), 3.10 (dd, 1H, C_2'-H, $J = 4.2, 14.1$ Hz), 3.68 (dd, 1H, C_2'-H, $J = 8.8, 14.3$ Hz), 4.24–4.28 (m, 1H, C_1'-H), 4.31 (d, 1H, C_5-H, $J = 5.7$ Hz), 7.25–7.83 (m, 8H, Ar-H), 9.77 (bs, 2H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 25.7, 27.3 (N-CH_3), 53.4 (C-2′), 55.7 (C-1′), 64.2 (C-5), 159.2 (C-2), 167.1, 168.6 (C-4 and C-6), 128.3, 129.2, 130.5, 131.8, 132.7, 133.5, 135.6, 137.2 (aromatic carbons). Anal. Calcd. For $C_{20}H_{20}Cl_2N_4O_5S_2$: C, 45.20; H, 3.79; N, 10.54; Found: C, 42.25; H, 3.76; N, 10.58%.

6.2. Biological assays

6.2.1. Compounds

Compounds **3**–**7** and **10**–**14** were dissolved in DMSO at different concentrations of 100, 200 and 800 μg/ml.

6.2.2. Cells

Bacterial strains *S. aureus*, *B. subtilis*, *E. coli*, *K. pneumoniae* and fungi *F. solani*, *C. lunata* and *A. niger* were obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India.

6.2.3. Antibacterial and antifungal assays

Preliminary antimicrobial activities of compounds **3**–**7** and **10**–**14** were tested by Agar disc-diffusion method. Sterile filter paper discs (6 mm diameter) moistened with the test compound solution in DMSO of specific concentration 100 μg and 200 μg/disc were carefully placed on the agar culture plates that had been previously inoculated separately with the microorganisms. The plates were incubated at 37 °C and the diameter of the growth inhibition zones were measured after 24 h in case of bacteria and after 48 h in case of fungi. All determinations were made in triplicate for each compound. Average of three independent readings for each organism was recorded.

The MICs of the compound assays were carried out using microdilution susceptibility method. Chloramphenicol was used as reference antibacterial agent. Ketoconazole was used as reference antifungal agent. The test compounds, chloramphenicol and ketoconazole were dissolved in DMSO at concentration of 800 μg/ml and two-fold dilution of the solution was prepared (400, 200, 100, 6.25 μg/ml). The microorganism suspensions were inoculated to the corresponding wells. The plates were incubated at 36 °C for 24 and 48 h for bacteria and fungi, respectively. The minimum inhibitory concentrations of the compounds were recorded as the lowest concentration of each chemical compounds in the tubes with no turbidity (i.e. no growth) of inoculated bacteria/fungi.

6.2.4. Antioxidant testing

Compounds **3**–**7** and **10**–**14** were tested for antioxidant property by nitric oxide and DPPH methods.

6.2.5. Assay for nitric oxide (NO) scavenging activity

Sodium nitroprusside (5 μM) in phosphate buffer pH 7.4 was incubated with 100 μM concentration of test compounds dissolved in a suitable solvent (dioxane/methanol) and tubes were incubated at 25 °C for 120 min. Control experiment was conducted with equal amount of solvent in an identical manner. At intervals, 0.5 ml of incubation solution was taken and diluted with 0.5 ml of Griess reagent (1% sulfanilamide, 0.1% *N*-naphthylethylenediamine dihydrochloride and 2% *o*-phosphoric acid dissolved in distilled water). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent *N*-naphthylethylenediamine dihydrochloride was read at λ 546 nm. The experiment was repeated in triplicate.

6.2.6. Reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (DPPH method)

The nitrogen centered stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) has often been used to characterize antioxidants. It is reversibly reduced and the odd electron in the DPPH free radical gives a strong absorption maximum at λ 517 nm, which is purple in color. This property makes it suitable for spectrophotometric studies. A radical scavenging antioxidant reacts with DPPH stable free radical and converts into 1,1-diphenyl-2-picrylhydrazine. The resulting decolorization is stoichiometric with respect to the number of electrons captured. The change in the absorbance produced in this reaction has been used to measure antioxidant properties.

The solutions of test compounds (100 μM) were added to DPPH (100 μM) in dioxane/ethanol. The tubes were kept at an ambient temperature for 20 min and the absorbance was measured at λ 517 nm. The difference between the test and the control experiments was taken and expressed as the per cent scavenging of the DPPH radical.

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