European Journal of Medicinal Chemistry 45 (2010) 698–704

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Anti-oxidant and anti-bacterial activities of novel *N*′-arylmethylidene-2-(3, 4-dimethyl-5, 5-dioxidopyrazolo[4,3-*c*][1,2]benzothiazin-2(4*H*)-yl) acetohydrazides

Matloob Ahmad [a,b], Hamid Latif Siddiqui [b,*], Muhammad Zia-ur-Rehman [a], Masood Parvez [c]

[a] *Applied Chemistry Research Centre, PCSIR Laboratories Complex, Ferozpur Road, Lahore-54600, Pakistan*
[b] *Institute of Chemistry, University of the Punjab, Lahore-54590, Pakistan*
[c] *Department of Chemistry, The University of Calgary, 2500 University Drive, NW, Calgary, Alberta, Canada T2N 1N4*

ARTICLE INFO

Article history:
Received 8 September 2009
Received in revised form
2 November 2009
Accepted 4 November 2009
Available online 11 November 2009

Keywords:
Pyrazolo[4,3-c][1,2]benzothiazine
5,5-dioxide
1,2-Benzothiazine 1,1-dioxides
Anti-oxidant activity
Anti-bacterial activity

ABSTRACT

A series of potential anti-oxidant and anti-bacterial *N*′-arylmethylidene-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-*c*][1,2]benzothiazin-2(4*H*)-yl)acetohydrazides was synthesized in a facile way starting from commercially available saccharine. 3,4-Dimethyl-2,4-dihydropyrazolo[4,3-*c*][1,2]benzothiazine 5,5-dioxide was obtained by ring expansion of 2-(2-oxopropyl)-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide followed by N-methylation and cyclization with hydrazine using ultrasonic irradiation. N-alkylation of the cyclized product with methyl chloroacetate followed by its reaction with hydrazine and subsequent ultrasonic mediated condensations with aromatic aldehydes afforded the title compounds. All the synthesized compounds were subjected to the preliminary evaluation for their anti-oxidant and anti-bacterial activities.

© 2009 Elsevier Masson SAS. All rights reserved.

1. Introduction

Pharmacologically, pyrazole and its derivatives represent one of the most important class of organic heterocyclic compounds, possessing anti-bacterial, anti-fungal [1], herbicidal [2] and anti-viral activities [3]. Some of its derivatives have been reported to exhibit significant anti-arrhythmic & sedative [4], hypoglycemic [5] and anti-inflammatory activities [6]. On the other hand, 1,2-benzothiazine 1,1-dioxides are also known as potentially biologically active molecules e.g., 1,2-benzothiazine-3-carboxamide 1,1-dioxide derivatives belonging to oxicams (Fig. 1) are well known as analgesic and anti-inflammatory compounds. In addition, benzothiazines are known as potent calpain I inhibitors [7], while its 3-aryl-quinazolin-4-one derivatives showed marked activity against *Bacillus subtilis* [8]. They have also been found as anti-oxidants [9] and for the treatment of rheumatoid arthritis, ankylosing spondylitis, osteoarthrosis and other inflammatory rheumatic and non-rheumatic processes, including onsets and traumatologic lesions [10]. Keeping in view the potential biological activities of 1,2-benzothiazine-1,1-dioxides and various pyrazoles, it was perceived that synergism of both the heterocyclic moieties in a single nucleus may result in the formation of some worthwhile molecules from the biological point of view. In the present paper, we report the synthesis of novel *N*′-arylmethylidene-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-*c*][1,2]benzothiazin-2(4*H*)-yl)acetohydrazides **8a–q** from commercially available saccharine along with their anti-oxidant and anti-bacterial activities.

2. Results and discussion

2.1. Chemistry

1-(4-Hydroxy-1,1-dioxido-2*H*-1,2-benzothiazin-3-yl)ethanone **3** was synthesized by literature procedure [11] from commercially available sodium saccharin [Scheme 1]. N-Methylation of **3** was carried out using dimethyl sulphate in acetone to yield 1-(4-hydroxy-2-methyl-1,1-dioxido-2*H*-1,2-benzothiazin-3-yl)ethanone **4**, which was further reacted with hydrazine hydrate in ultrasonic bath to get 3,4-dimethyl-2,4-dihydropyrazolo[4,3-*c*][1,2]benzothiazine 5,5-dioxide **5**. Reaction of 3,4-dimethyl-2,4-dihydropyrazolo[4,3-*c*][1,2]-benzothiazine 5,5-dioxide **5** with methyl chloroacetate in acetonitrile afforded methyl (3,4-dimethyl-5,5-dioxidopyrazolo[4,3-*c*][1,2]-benzothiazin-2(4*H*)-yl)acetate **6** which was then reacted with hydrazine hydrate to get 2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-

* Corresponding author. Tel.: +92 425830503; fax: +92 429231169.
E-mail address: drhamidlatif@yahoo.com (H.L. Siddiqui).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.11.016

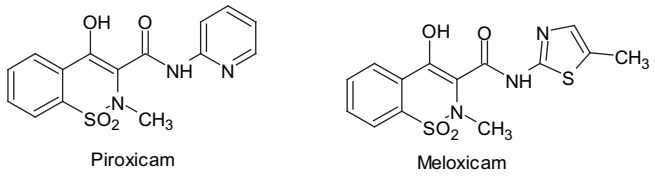


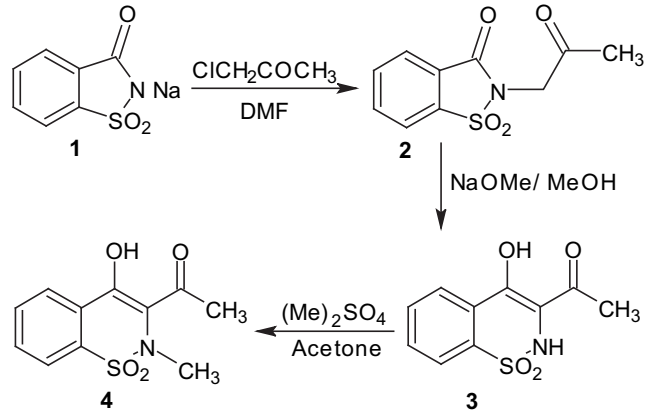
Fig. 1. Structure of two oxicams.

c][1,2]benzothiazin-2(4*H*)-yl)acetohydrazide **7** [Scheme 2]. Same reaction was attempted in ultrasonic mediated conditions and the reaction was completed in very good yield (86%) and reduction of reaction time (13 min instead of 180 min). Product **7** was then reacted with different aldehydes in ultrasonic bath to get the *N*′-arylmethylidene-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-*c*][1,2]benzothiazin-2(4*H*)-yl)acetohydrazides **8a–q** in excellent yields (See Table 1). All of the newly synthesized compounds were characterized through spectroscopic techniques along with their elemental analyses which were found in accordance with the calculated values [Table 1].

2.2. Stereochemistry

Configuration of C=N bond of *N*′-arylmethylidene-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-*c*][1,2]benzothiazin-2(4*H*)-yl) acetohydrazides **8a–q** was accomplished as ***E*** on the basis of single crystal X-ray analysis of 2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-*c*][1,2]benzothiazin-1(4*H*)-yl)-*N*′-[phenylmethylidene]acetohydrazide **8a**, as a representative compound of the series. However, it was interesting to know that on the basis of the structure confirmed by single crystal X-ray diffractometer of compound **8a**, compound **5**, previously reported as 3,4-dimethyl-1,4-dihydropyrazolo[4,3-*c*][1,2]benzothiazine 5,5-dioxide [12] was in fact 3,4-dimethyl-2,4-dihydropyrazolo[4,3-*c*][1,2]benzothiazine 5,5-dioxide, an isomer of the former. Crystal data and structure refinement parameters for compound **8a** have been provided in Table 2 while selected molecular dimensions have been given in Table 3.

The heterocyclic thiazine ring in **8a** (Fig. 2) adopts a half-chair conformation wherein S1 is displaced by 0.699(2) Å from the plane defined by the remaining atoms in the ring. The acetohydrazide moiety composed of atoms N4/N5/C13/C14/O3 is almost planar and the mean planes of pyrazole and phenyl rings are inclined at 87.19(9) and 25.54(12)°, respectively, with the mean plane of the acetohydrazide moiety. Crystallographic data for **8a** have been deposited with the Cambridge Crystallographic Data Center (CCDC deposition Number: 745411).



Scheme 1. Synthesis of 1-(4-hydroxy-2-methyl-1,1-dioxido-2*H*-1,2-benzothiazin-3-yl)-ethanone from commercially available saccharine.

2.3. Biological activities

2.3.1. Anti-oxidant studies

Generation of reactive oxygen species (ROS) and free radicals *in-vivo* is involved in a wide range of human diseases [13]. ROS, including super oxide anion, hydrogen peroxide and hydroxyl radical are byproducts of a variety of pathways of aerobic metabolism [14]. These are unstable and react readily with a wide range of biological substrates, such as lipids, DNA and protein molecules, consequently resulting in the cell damage [15]. All the synthesized compounds were assessed for their superoxide anion radical scavenging activity.

Anti-oxidant (super oxide) activities of the synthesized compounds along with the standard (n-propyl gallate) are presented in Table 4. It was found that almost all the newly synthesized compounds have exhibited moderate to good activity. An insight to the structure–activity relationship gives an idea that activity generally increases with number and strength of oxygen containing functional groups. Compounds having hydroxy and alkoxy groups in the benzene ring of the imines are found relatively more active e.g., compound **8k** (dihydroxy derivative; 98.34%) is more active than **8j** (monomethoxy derivative; 44.90%) which itself is more active than **8a** (bearing no substituent). Among the compounds bearing alkoxy groups, 3-ethoxy-4-hydroxy derivative **8l** is more active (71.08%) than its 2,4-dimethoxy analogue **8m** indicating that the substitution of an alkoxy group with hydroxyl group on the ring increases the activity and vice-versa perhaps due to the steric reasons. For the nitro and chloro derivatives, compounds show percentage radical scavenging activity in the following order: m > o > p.

2.3.2. Antibacterial studies

The MICs of the active compounds against the susceptible pathogenic organisms are presented in Table 5. It was found that compounds bearing hydroxyl and methoxy groups at the benzene ring of the *N*′-arylmethylidene-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-2(4*H*)-yl)acetohydrazides possess anti-bacterial activities against *B. subtillis* and *Escherichia coli*; the maximum activity was shown by dihydroxy derivative **8k** (MIC = 5.0 μg ml^{-1}). These are perhaps attributed to the presence of lone pairs on oxygen atoms of hydroxyl and methoxy groups. Besides, Compounds possessing hetero atoms (nitrogen & sulfur) in the benzene ring were found moderately to highly active against all the bacterial strains. Rest of the compounds were found either inactive or presented a MIC value more than 200 μg ml^{-1}.

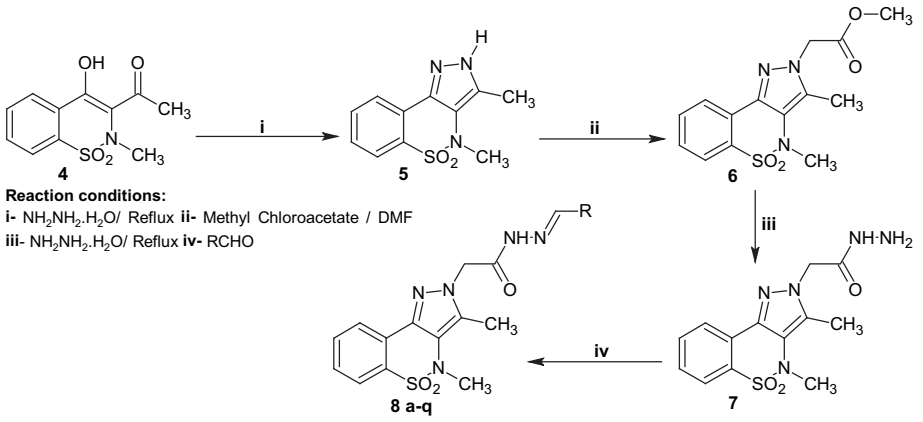
3. Conclusion

The present study revealed that the compounds obtained by synergism of the dihydropyrazolo[4,3-*c*][1,2]benzothiazine 5,5-dioxide moiety with different carbohydrazides were found to possess anti-oxidant & anti-bacterial activities and could be useful as a template for future development through modification or derivatization to design more potent biologically active compounds. The new skeleton may also possess other biological activities of the parent ring systems.

4. Experimental

4.1. Chemistry

All the chemicals were purchased from E. Merck, BDH or Fluka and used without purification. However, solvents were purified through distillation. ^{1}H NMR spectra were recorded on a Bruker DPX-400 instrument at 400 MHz. Chemical shifts are reported in ppm referenced to the residual solvent signal. FT-IR spectra were



Scheme 2. Conversion of 1-(4-hydroxy-1,1-dioxido-2*H*-1,2-benzothiazin-3-yl)ethanone 4 to *N*′-arylmethylidene-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4, 3-*c*][1,2]benzothiazin-2(4*H*)-yl) acetohydrazides **8a**–**q**.

recorded on a Thermo Nicolet IR 200 spectrometer. Mass spectra were recorded on Agilent 5973N instrument using EI mode. Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. Ultrasonic mediated reactions were carried out in Clifton Ultrasonic Bath (2 x T2A, 300W, DU-4) made by Nickel Electro Ltd, Weston-S-Mare Somerset, England. X-ray crystallography was carried out on Bruker Nonius Kappa CCD diffractometer with graphite monochromated Mo-K_α radiation and the data were corrected for Lorentz and polarization effects and for absorption using multi-scan method [16,17].

4.1.1. 1-(4-Hydroxy-1,1-dioxido-2 H-1,2-benzothiazin-3-yl)-ethanone (**3**)

The compound was synthesized according to the literature procedure [11]. A mixture of *N*-acetonyl saccharine (5.0 g, 20.9 mmol), sodium methoxide (7.89 g, 146.1 mmol) and dry methanol (50 ml) was refluxed for a period of 25 min till the formation of red colored suspension which was poured over ice cold solution of hydrochloric acid (50 ml; 10% v/v). Yellowish brown precipitates thus obtained were washed with excess water and dried. Yield: 62%; m.p 155–156 °C. IR (KBr) cm^{-1}: 3467, 3210, 1598, 1586, 1345, 1187. ^{1}H NMR ($CDCl_3$) (400 MHz) δ: 2.22 (3H, s, C*H*$_3$), 7.55 (1H, t, $J = 7.4$ Hz, Ar*H*), 7.67 (1H, t, $J = 7.2$ Hz, Ar*H*), 7.98 (2H, t, $J = 7.8$ Hz, Ar*H*), 8.43 (1H,br s, N*H*), 12.29 (1H, s, O*H* enolic). ^{13}C NMR: 21.5, 69.2, 122.5, 123.4, 124.4, 127.2, 129.2, 133.4, 136.7, 167.2. MS *m*/*z*: 239.0 [M^+].

4.1.2. 1-(4-Hydroxy-2-methyl-1,1-dioxido-2H-1,2-benzothiazin-3-yl)-ethanone (**4**)

A mixture of 1-(4-hydroxy-1,1-dioxido-2*H*-1,2-benzothiazin-3-yl)ethanone (**3**) (5.0 g; 20.9 mmol), 20% aqueous sodium hydroxide (8.4 ml) and acetone (50 ml) was stirred at room temperature for 5 min. Dimethyl sulfate (5.9 ml) was added to the mixture drop wise over a period of 5 min. The mixture was stirred for further half an hour followed by the careful addition of dilute HCl (20 ml; 5%) to get the white precipitates which were filtered, washed with water and dried. Yield: 88%.; mp 151–152 °C. IR (KBr) cm^{-1}: 3470, 1598, 1586, 1345, 1187. ^{1}H NMR ($CDCl_3$) (400 MHz) δ: 2.23 (3H, s, C*H*$_3$), 3.09 (3H, s, NC*H*$_3$), 7.55 (1H, t, $J = 7.3$ Hz, Ar*H*), 7.66 (1H, t, $J = 7.2$ Hz, Ar*H*), 7.96 (2H, t, $J = 7.7$ Hz, Ar*H*), 12.22 (1H, s, O*H*). ^{13}C NMR: 21.5, 38.7, 117.9, 122.5, 123.4, 124.4, 127.2, 129.2, 133.4, 136.7, 167.2. MS *m*/*z*: 253.0 [M^+].

4.1.3. Synthesis of 3,4-dimethyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**5**)

A mixture of 1-(4-hydroxy-2-methyl-1,1-dioxido-2*H*-1,2-benzothiazin-3-yl)ethanone (**4**) (5.0 g, 19.8 mmol) and hydrazine

Table 1
Reaction parameters and CHN analysis of *N*′-arylmethylidene-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-*c*][1,2]benzothiazin-2(4*H*)-yl)acetohydrazides (**8a**–**q**).

S. No.	Product	R	Reaction time (minutes)	Yield %[a]	Analysis %		
					Calculated (Found)		
					C	H	N
1	**8a**	Phenyl	3	67	58.67 (58.68)	4.68 (4.67)	17.10 (17.11)
2	**8b**	2-Nitrophenyl	2	79	52.86 (52.87)	3.99 (4.01)	18.49 (18.51)
3	**8c**	3-Nitrophenyl	2.5	84	52.86 (52.86)	3.99 (3.98)	18.49 (18.47)
4	**8d**	4-Nitrophenyl	2	88	52.86 (52.87)	3.99 (4.00)	18.49 (18.50)
5	**8e**	2-Chlorophenyl	2.5	81	54.11 (54.10)	4.09 (4.07)	15.78 (15.78)
6	**8f**	4-Chlorophenyl	2	90	54.11 (54.09)	4.09 (4.08)	15.78 (15.80)
7	**8g**	2,4-Dichlorophenyl	2.5	85	50.22 (50.20)	3.58 (3.60)	14.64 (14.65)
8	**8h**	3,4-Dichlorophenyl	3	83	50.22 (50.19)	3.58 (3.57)	14.64 (14.63)
9	**8i**	4-Fluorophenyl	2	89	56.20 (56.18)	4.24 (4.22)	16.38 (16.40)
10	**8j**	3-Methoxyphenyl	3.5	87	57.39 (57.41)	4.82 (4.80)	15.94 (15.92)
11	**8k**	2,4-Dihydroxyphenyl	3.5	78	54.41 (54.43)	4.34 (4.32)	15.86 (15.84)
12	**8l**	3-Ethoxy-4-hydroxyphenyl	3.0	82	56.28 (56.30)	4.94 (4.94)	14.92 (14.90)
13	**8m**	2,4-Dimethoxyphenyl	2.5	83	56.28 (56.27)	4.94 (4.96)	14.92 (14.94)
14	**8n**	Thiophen-2-yl	3.5	76	52.03 (51.99)	4.12 (4.11)	16.86 (16.88)
15	**8o**	Thiophen-3-yl	3.5	76	52.03 (52.01)	4.12 (4.13)	16.86 (16.85)
16	**8p**	Pyridin-2-yl	4.0	75	55.60 (55.58)	4.42 (4.41)	20.48 (20.50)
17	**8q**	Pyridin-3-yl	3.0	76	55.60 (55.62)	4.42 (4.39)	20.48 (20.49)

[a] Isolated yields based on 2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-*c*][1,2]benzothiazin-2(4*H*)-yl)acetohydrazide.

Table 2
Crystal data and structure refinement for compound **6a**.

Structural formula	$C_{20}\ H_{19}\ N_5\ O_3\ S$	Cell volume	1941.9(13) Å^3
Formula weight	409.46	Z	4
Crystal system	Orthorhombic	Calculated density	1.401 Mg/m^3
Space group	P 2_1 2_1 2_1	Crystal size (mm^3)	0.20 × 0.12 × 0.04
T (K)	173(2) K	Reflections collected	4291
a (Å)	8.101(2)	σ min; σ max	3.7; 27.5
b (Å)	9.221(4)	Goodness-of-fit on F^2	1.08
c (Å)	25.996(12)	F(000)	856
Final *R* indices [I > 2sigma(I)]	*R*1 = 0.042, w*R*2 = 0.080	Absolute structure parameter	−0.02(8)

monohydrate (4.8 ml, 99.0 mmol) was irradiated with ultrasonic waves for 10 min at 65 °C. Un-reacted hydrazine was removed under vacuum and the residue obtained was poured over hydrochloric acid (20 ml, 10%). Precipitates obtained were filtered, washed with excess water and cold ethanol. Light yellow solid; mp 230 °C. IR (KBr) cm^{-1}: 3359, 1599, 1322, 1142. ^{1}H NMR ($CDCl_3$) (400 MHz) δ: 2.39 (3H, s, C*H*$_3$), 3.06 (3H, s, NC*H*$_3$), 7.53 (1H, t, *J* = 7.4 Hz, Ar*H*), 7.66 (1H, t, *J* = 7.2 Hz, Ar*H*), 7.94 (2H, t, *J* = 7.8 Hz, Ar*H*), 10.09 (1H, br s, N*H*). ^{13}C NMR: 8.6, 38.8, 121.8, 123.3, 124.2, 128.8, 129.3, 131.1, 132.9, 133.2, 134.5. MS *m*/*z*: 249.1(M$^+$).

*4.1.4. Methyl (3,4-dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-2(4 H)-yl)acetate (**6**)*

A mixture of 3,4-dimethyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**5**) (5.0 g, 20.0 mmol), anhydrous potassium carbonate (3.31 g, 24.0 mmol), methyl chloroacetate (2.60 g, 24.0 mmol) and acetonitrile (30 ml) was refluxed for a period of 10 h followed by the removal of solvent under vacuum. Residue obtained was washed with cold water to get the white crystalline product. Yield: 80%; mp 180 °C. ^{1}H NMR ($CDCl_3$) (400 MHz) δ: 2.32 (3H, s, C*H*$_3$), 3.04 (3H, s, NC*H*$_3$), 3.79 (3H, s, OC*H*$_3$), 4.91 (2H, s, NC*H*$_2$), 7.51 (1H, t, *J* = 7.7 Hz, Ar*H*), 7.63 (1H, t, *J* = 7.7 Hz, Ar*H*), 7.90 (1H, d, *J* = 7.7 Hz, Ar*H*), 7.97 (1H, d, *J* = 7.7 Hz, Ar*H*). ^{13}C NMR: 8.6, 38.7, 51.3, 52.4, 122.5, 123.4, 124.4, 127.7, 129.2, 131.5, 133.4, 134.4, 136.8, 167.6. MS *m*/*z*: 321.2(M$^+$).

*4.1.5. 2-(3,4-Dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-2(4 H)-yl)acetohydrazide (**7**)*

A mixture of methyl (3,4-dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-2(4*H*)-yl)acetate (**6**) (16.07 g; 50 mmol), hydrazine hydrate (15 ml) and ethanol (200 ml) was refluxed for a period of 5 h followed by the removal of unreacted hydrazine and ethanol under vacuum. The crude product obtained was washed with water and dried. White powder; mp 187 °C. IR (KBr) cm^{-1}: 3451, 1678, 1346, 1150. ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 2.26 (3H, s, C*H*$_3$), 2.95 (3H, s, NC*H*$_3$), 4.35 (2H, s, N*H*$_2$), 4.83 (2H, s, NC*H*$_2$), 7.62 (1H, t, *J* = 7.5 Hz, Ar*H*), 7.77 (1H, t, *J* = 7.5 Hz, Ar*H*), 7.85–7.92 (2H, dd, *J* = 13.8, 7.78 Hz, Ar*H*), 9.45 (1H, br s, N*H*). ^{13}C NMR: 8.7, 38.9, 51.3, 122.3, 123.3, 124.3, 127.9, 128.8, 129.0, 131.4, 133.3, 136.4, 165.4. MS *m*/*z*: 321.0(M$^+$).

*4.1.6. General procedure for the synthesis of N′-arylmethylidene-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-2(4H)-yl)acetohydrazides (**8a**–**q**)*

A mixture of of 2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-2(4*H*)-yl)acetohydrazide (**7**) (20.0 mmol), corresponding aromatic aldehydes 20.0 mmol, methanol (50 ml) and glacial acetic acid (2 drops) was subjected to ultrasonic irradiation for 3–5 min. The precipitates formed were collected and washed with methanol.

*4.1.6.1. 2-(3,4-Dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)-N′-[phenylmethylidene]acetohydrazide (**8a**).* White powder; mp 287–288 °C. IR (KBr) cm^{-1}: 3208, 1698, 1599, 1376, 1124. ^{1}H NMR (DMSO-d_6) (500 MHz) δ: 2.32 (3H, s, C*H*$_3$), 2.98 (3H, s, NC*H*$_3$), 5.51 (2H, s, NC*H*$_2$), 7.44 (4H, d, *J* = 6.5 Hz, Ar*H*), 7.63 (1H, t, *J* = 7.6 Hz, Ar*H*), 7.70–7.79 (2H, m, Ar*H*), 7.87 (1H, d, *J* = 7.8 Hz, Ar*H*), 7.93 (1H, d, *J* = 7.7 Hz, Ar*H*), 8.07 (1H, s, NC*H*), 11.78 (1H, br s, N*H*). ^{13}C NMR: 8.7, 38.8, 51.9, 122.3, 122.7, 123.0, 123.4, 123.6, 123.8, 124.1, 125.3, 126.9, 127.4, 128.1, 128.9, 130.7, 133.3, 135.6, 135.9, 165.8. MS *m*/*z*: 409.0(M$^+$).

*4.1.6.2. 2-(3,4-Dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)-N′-[(2-nitrophenyl)methylidene]acetohydrazide (**8b**).* Off-white powder; mp 205–207 °C. IR (KBr) cm^{-1}: 3203, 1681, 1565, 1325, 1152. ^{1}H NMR (DMSO-d_6) (400 MHz) δ 2.32 (3H, s, C*H*$_3$), 2.98 (3H, s, NC*H*$_3$), 5.50 (2H, s, NC*H*$_2$), 7.62–7.69 (2H, m, Ar*H*), 7.76–7.81 (2H, q, *J* = 17.9, 7.6 Hz, Ar*H*), 7.87 (1H, d, *J* = 7.80 Hz, Ar*H*), 7.93 (1H, d, *J* = 7.7 Hz, Ar*H*), 8.06 (1H, d, *J* = 8.2 Hz, Ar*H*), 8.16 (1H, d, *J* = 8.0 Hz, Ar*H*), 8.44 (1H, s, N═C*H*), 12.06 (1H, br s, N*H*). ^{13}C NMR: 8.6, 39.0, 51.9, 121.9, 122.7, 123.4, 124.1, 124.5, 126.3, 126.9, 128.1, 128.5, 128.9, 130.1, 130.7, 133.3, 135.9, 136.7, 143.1, 165.8. MS *m*/*z*: 454.0(M$^+$).

*4.1.6.3. 2-(3,4-Dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)-N′-[(3-nitrophenyl)methylidene]acetohydrazide (**8c**).* White powder; mp 211–212 °C. IR (KBr) cm^{-1}: 3448, 3088, 1697, 1613, 1327, 1151. ^{1}H NMR (DMSO-d_6) (400 MHz) δ 2.33 (3H, s, C*H*$_3$), 2.98 (3H, s, NC*H*$_3$), 5.57 (2H, s, NC*H*$_2$), 7.62 (1H, t, *J* = 7.64 Hz, Ar*H*), 7.72–7.80 (2H, m, Ar*H*), 7.87 (1H, d, *J* = 7.80 Hz, Ar*H*), 7.92 (1H, d, *J* = 7.7 Hz, Ar*H*), 8.19 (1H, s, Ar*H*), 8.21 (1H, d, *J* = 7.8 Hz, Ar*H*), 8.25 (1H, d, *J* = 8.2 Hz, Ar*H*), 8.55 (1H, s, NC*H*), 12.05 (1H, br s, N*H*). ^{13}C NMR: 8.7, 39.0, 51.7, 118.1, 121.4, 123.1, 123.6, 124.0, 124.7, 126.4, 127.9, 128.9, 129.3, 130.1, 130.8, 133.4, 136.1, 137.3, 142.9, 166.5. MS *m*/*z*: 454.0(M$^+$).

*4.1.6.4. 2-(3,4-Dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)-N′-[(4-nitrophenyl)methylidene]acetohydrazide (**8d**).* White crystalline solid; mp 241–242 °C. IR (KBr) cm^{-1}: 3428, 1702, 1587, 1342, 1158. ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 2.32 (3H, s, C*H*$_3$), 2.98 (3H, s, NC*H*$_3$), 5.57 (2H, s, NC*H*$_2$), 7.64 (1H, t, *J* = 7.58 Hz, Ar*H*), 7.82 (1H, t, *J* = 7.5 Hz, Ar*H*), 7.87 (1H, d, *J* = 7.82 Hz, Ar*H*), 7.92 (1H, d, *J* = 7.8 Hz, Ar*H*), 7.97 (1H, d, *J* = 8.7 Hz, Ar*H*), 8.02 (1H, d, *J* = 8.7 Hz, Ar*H*), 8.14 (1H, d, *J* = 8.8 Hz, Ar*H*), 8.28 (1H, d, *J* = 8.7 Hz, Ar*H*), 8.35 (1H, s, NC*H*), 12.11 (1H, br s, N*H*). ^{13}C NMR: 8.7, 39.1, 51.4, 121.3, 121.4, 123.1, 123.6, 124.0, 125.6, 125.7, 126.4, 127.9, 128.9, 130.8, 133.4, 134.3, 136.1, 136.9, 145.7, 166.1. MS *m*/*z*: 454.0(M$^+$).

Table 3
Selected molecular dimensions for $C_{20}H_{19}N_5O_3S$.

a. Bond lengths [Å].			
S(1)–O(2)	1.4291(18)	N(2)–N(3)	1.360(2)
S(1)–O(1)	1.4346(17)	N(3)–C(10)	1.359(3)
S(1)–N(1)	1.648(2)	N(3)–C(12)	1.450(3)
S(1)–C(1)	1.766(2)	N(4)–C(13)	1.341(3)
O(3)–C(13)	1.225(3)	N(4)–N(5)	1.400(3)
N(1)–C(8)	1.434(3)	N(5)–C(14)	1.274(3)
N(1)–C(9)	1.466(3)	N(2)–C(7)	1.337(3)
b. Bond angles [°]			
O(2)–S(1)–O(1)	119.32(10)	O(2)–S(1)–N(1)	106.94(10)
O(1)–S(1)–N(1)	107.83(10)	O(2)–S(1)–C(1)	107.67(10)
O(1)–S(1)–C(1)	109.46(10)	N(1)–S(1)–C(1)	104.65(10)
C(8)–N(1)–C(9)	115.8(2)	C(8)–N(1)–S(1)	110.11(15)
C(9)–N(1)–S(1)	117.10(17)	C(7)–N(2)–N(3)	103.64(16)
C(10)–N(3)–N(2)	113.87(17)	C(10)–N(3)–C(12)	125.89(18)
N(2)–N(3)–C(12)	120.24(17)	C(13)–N(4)–N(5)	120.69(18)
c. Torsion angles [°].			
C(1)–S(1)–N(1)–C(8)	−49.3(2)	N(1)–S(1)–C(1)–C(6)	39.2(2)
S(1)–C(1)–C(6)–C(7)	−7.9(3)	C(1)–C(6)–C(7)–C(8)	−15.8(3)
C(6)–C(7)–C(8)–N(1)	1.3(3)	S(1)–N(1)–C(8)–C(7)	34.8(3)

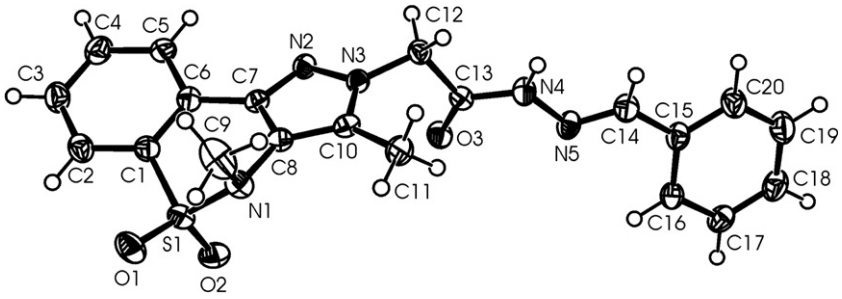


Fig. 2. ORTEP II diagram of compound **8a** with the numbering scheme. Displacement ellipsoids are drawn at the 50% probability level; H atoms are represented by circles of arbitrary radii.

*4.1.6.5. N′-[(2-Chlorophenyl)methylidene]-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)acetohydrazide (**8e**).* White crystals; mp 209–211 °C. IR (KBr) cm^{-1}: 3478, 1699, 1595, 1340, 1155. ^{1}H NMR (DMSO-d_6) (400 MHz) δ 2.32 (3H, s, CH_3), 2.98 (3H, s, NCH_3), 5.52 (2H, s, NCH_2), 7.63–7.69 (2H, m, Ar*H*), 7.76–7.80 (2H, q, J = 17.9, 7.6 Hz, Ar*H*), 7.86 (1H, d, J = 7.80 Hz, Ar*H*), 7.93 (1H, d, J = 7.7 Hz, Ar*H*), 8.08 (1H, d, J = 8.2 Hz, Ar*H*), 8.16 (1H, d, J = 8.0 Hz, Ar*H*), 8.46 (1H, s, NC*H*), 12.09 (1H, br s, N*H*). ^{13}C NMR: 8.5, 38.9, 51.3, 121.4, 122.4, 123.1, 124.6, 124.7, 124.9, 126.2, 126.4, 127.5, 127.7, 127.8, 128.1, 130.6, 132.9, 136.2, 136.8, 166.3. MS m/z: 444.0(M^+).

*4.1.6.6. N′-[(4-Chlorophenyl)methylidene]-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)acetohydrazide (**8f**).* White crystals; mp 226–227 °C. IR (KBr) cm^{-1}: 3445, 1690, 1598, 1332. ^{1}H NMR (DMSO-d_6) (300 MHz) δ 2.31 (3H, s, CH_3), 2.97 (3H, s, NCH_3), 5.52 (2H, s, NCH_2), 7.49 (2H, d, J = 8.4 Hz, Ar*H*), 7.63 (1H, t, J = 7.6 Hz, Ar*H*), 7.76 (3H, m, Ar*H*), 7.86–7.94 (2H, dd, J = 16.5, 7.7 Hz, Ar*H*), 8.05 (1H, s, NC*H*), 11.85 (1H, br s, N*H*). ^{13}C NMR: 8.6, 38.9, 51.2, 123.3, 124.1, 124.9, 125.2, 125.3, 126.3, 127.5, 127.7, 127.9, 128.4, 128.6, 129.6, 133.1, 133.3, 136.1, 139.1, 165.5. MS m/z: 444.0(M^+).

*4.1.6.7. N′-[(2,4-Dichlorophenyl)methylidene]-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)acetohydrazide (**8g**).* White crystals; mp 218–219 °C. IR (KBr) cm^{-1}: 3429, 1700, 1590, 1333, 1155. ^{1}H NMR (DMSO-d_6) (300 MHz) δ 2.31 (3H, s, CH_3), 2.97 (3H, s, NC*H*3), 5.54 (2H, s, NCH_2), 7.51–7.53 (1H, dd, J = 8.56, 1.92 Hz, Ar*H*), 7.64 (1H, t, J = 7.6 Hz, Ar*H*), 7.73 (1H, d, J = 1.98 Hz, Ar*H*), 7.78 (1H, t, J = 7.7 Hz, Ar*H*), 7.87 (1H, d, J = 7.8 Hz, Ar*H*), 7.92 (1H, d, J = 7.7 Hz, Ar*H*), 8.08 (1H, d, J = 8.6 Hz, Ar*H*), 8.38 (1H, s, NC*H*), 12.02 (1H, br s, N*H*). ^{13}C NMR: 8.6, 38.8, 51.7, 122.0, 123.3, 124.2, 124.6, 125.2, 126.1, 126.4, 127.3, 127.5, 128.1, 130.1, 130.6, 133.3, 133.5, 136.5, 137.4, 165.7. MS m/z: 478.0(M^+)

*4.1.6.8. N′-[(3,4-Dichlorophenyl)methylidene]-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)acetohydrazide (**8h**).* White amorphous powder; mp 249–250 °C. IR (KBr) cm^{-1}: 3412, 1682, 1597, 1325, 1147. ^{1}H NMR (DMSO-d_6) (400 MHz) δ 2.28 (3H, s, CH_3), 2.97 (3H, s, NCH_3), 5.55 (2H, s, NCH_2), 7.63 (1H, t, J = 7.6 Hz, Ar*H*), 7.72 (1H, s, Ar*H*), 7.74 (1H, d, J = 1.48 Hz, Ar*H*), 7.78 (1H, t, J = 7.65 Hz, Ar*H*), 7.87 (1H, d, J = 7.83 Hz, Ar*H*), 7.94 (1H, d, J = 7.8 Hz, Ar*H*), 8.03 (1H, s, NC*H*), 8.06 (1H, d, J = 1.41 Hz, Ar*H*), 11.97 (1H, br s, N*H*). ^{13}C NMR: 8.7, 38.8, 51.7, 123.3, 123.7, 124.1, 124.6, 125.4, 125.7, 126.3, 127.3, 128.1, 128.4, 130.5, 130.7, 133.3, 134.3, 136.8, 137.9, 165.7. MS m/z: 479.0(M^+).

*4.1.6.9. -(3,4-Dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)-N′-[(4-fluorophenyl)methylidene]acetohydrazide (**8i**).* White powder; mp 256–257 °C. IR (KBr) cm^{-1}: 3416, 3194, 3045, 1699, 1599, 1333, 1154. ^{1}H NMR (DMSO-d_6) (500 MHz) δ 2.32 (3H, s, CH_3), 2.98 (3H, s, NCH_3), 5.51 (2H, s, NCH_2), 7.26–7.30 (2H, m, Ar*H*), 7.63 (1H, t, J = 7.7 Hz, Ar*H*), 7.76–7.83 (3H, m, Ar*H*), 7.87 (1H, d, J = 7.8 Hz, Ar*H*), 7.92 (1H, d, J = 7.8 Hz, Ar*H*), 8.06 (1H, s, NC*H*), 11.78 (1H, br s, N*H*). ^{13}C NMR: 8.7, 38.9, 51.7, 111.6, 111.7, 123.1, 124.1, 124.5, 125.1, 125.4, 126.4, 127.3, 128.3, 130.2, 133.3, 134.1, 136.8, 138.7, 160.1, 165.6. MS m/z: 427.0(M^+).

*4.1.6.10. N′-[(3-Methoxyphenyl)methylidene]-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)acetohydrazide (**8j**).* White powder; mp 222–223 °C. IR (KBr) cm^{-1}: 3449, 3364, 3033, 1692, 1616, 1310, 1164. ^{1}H NMR (DMSO-d_6) (500 MHz) δ: 2.32 (3H, s, CH_3), 2.78 (3H, s, OCH_3), 2.98 (3H, s, NCH_3), 5.52 (2H, s, NCH_2), 6.99–7.02 (1H, dd, J = 8.2, 2.0 Hz, Ar*H*), 7.26–7.38 (3H, m, Ar*H*), 7.63

Table 4
Percent radical scavenging activity.

S. No.	Compound	% RSA
1	**6a**	33.9
2	**6b**	28.8
3	**6c**	52.2
4	**6d**	23.7
5	**6e**	44.2
6	**6f**	23.3
7	**6g**	48.7
8	**6h**	51.5
9	**6i**	17.4
10	**6j**	44.9
11	**6k**	98.34
12	**6l**	71.08
13	**6m**	53.09
14	**6n**	14.6
15	**6o**	16.5
16	**6p**	24.01
17	**6q**	25.1
18	*n*-Propyl gallate	91.3

Table 5
Anti-bacterial activity (MIC,μg ml^{-1}).

Sr. No	Compound	Susceptible micro organism		
		B. subtillis	*E. coli*	*S. aureus*
1	**6a**	69	–	–
2	**6b**	–	–	–
3	**6c**	–	–	–
4	**6d**	–	–	–
5	**6e**	–	–	–
6	**6f**	–	–	–
7	**6g**	–	–	–
8	**6h**	–	–	–
9	**6i**	–	–	–
10	**6j**	23	69	–
11	**6k**	5	39	–
12	**6l**	11	47	–
13	**6m**	17	–	–
14	**6n**	23	77	55
15	**6o**	24	82	61
16	**6p**	28	88	–
17	**6q**	27	95	–
18	Tetracycline	0.25	0.35	0.78

(1H, t, $J = 7.8$ Hz, ArH), 7.76 (1H, t, $J = 7.6$ Hz, ArH), 7.87 (1H, d, $J = 7.8$ Hz, ArH), 7.93 (1H, d, $J = 7.7$ Hz, ArH), 8.03 (1H, s, NCH), 11.79 (1H, br s, NH). ^{13}C NMR: 8.5, 38.9, 47.3, 51.6, 110.5, 113.6, 117.8, 123.1, 124.1, 124.5, 126.2, 126.7, 127.5, 128.3, 130.1, 131.8, 133.4, 136.9, 139.3, 157.6, 165.7. MS *m*/*z*: 439.0(M^+).

*4.1.6.11. N′-[(2,4-Dihydroxyphenyl)methylidene]-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)acetohydrazide (**8k**).* Light orange crystals; mp 190–191 °C. IR (KBr) cm^{-1}: 3562, 3226, 2988, 1680, 1621, 1362, 1127. ^{1}H NMR (DMSO-d_6) (300 MHz) δ 2.31 (3H, s, CH_3), 2.97 (3H, s, NCH_3), 5.02 (2H, s, OH), 5.42 (2H, s, NCH_2), 7.31 (1H, d, $J = 8.5$ Hz, ArH), 7.54 (1H, d, $J = 8.3$ Hz, ArH), 7.63 (1H, t, $J = 7.6$ Hz, ArH), 7.78 (1H, t, $J = 7.35$ Hz, ArH), 7.86–7.94 (2H, dd, $J = 17.0$, 7.8 Hz, ArH), 8.23 (1H, s, ArH). 8.33 (1H, s, NCH), 11.90 (1H, br s, NH). ^{13}C NMR: 8.6, 38.7, 51.6, 103.2, 107.8, 111.1, 123.1, 124.2, 124.7, 126.2, 127.4, 128.4, 130.1, 131.9, 133.5, 136.6, 139.6, 158.7, 158.9, 165.8. MS *m*/*z*: 441.0(M^+).

*4.1.6.12. N′-[(3-Ethoxy-4-hydroxy)methylidene]-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)acetohydrazide (**8l**).* White powder; mp 180–181 °C. IR (KBr) cm^{-1}: 3405, 1677, 1598, 1339, 1166. ^{1}H NMR (DMSO-d_6) (400 MHz) δ: 1.32 (3H, t, $J = 2.64$ Hz, CH_3), 2.32 (3H, s, CH_3), 2.97 (3H, s, NCH_3), 4.05 (2H, m, OCH_2), 5.01 (1H, s, OH), 5.48 (2H, s, NCH_2), 6.82 (1H, d, $J = 8.2$ Hz, ArH), 7.08–7.10 (1H, dd, $J = 8.2$, 1.6 Hz, ArH), 7.33 (1H, d, $J = 1.52$ Hz, ArH), 7.63 (1H, t, $J = 7.6$ Hz, ArH), 7.78 (1H, t, $J = 7.6$ Hz, ArH), 7.87 (1H, d, $J = 7.8$ Hz, ArH), 7.92 (1H, d, $J = 8.2$ Hz, ArH), 9.43 (1H, s, NCH), 11.62 (1H, br s, NH). ^{13}C NMR: 8.7, 12.3, 38.9, 51.2, 61.8, 113.1, 114.5, 118.5, 123.0, 123.4, 124.4, 124.8, 126.3, 127.5, 128.5, 130.2, 133.3, 136.8, 139.2, 145.7, 145.9, 166.0. MS *m*/*z*: 469.0(M^+).

*4.1.6.13. N′-[(2,4-Dimethoxyphenyl)methylidene]-2-(3,4-dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)acetohydrazide (**8m**).* White crystals; mp 207–208 °C. IR (KBr) cm^{-1}: 3422, 1699, 1614, 1325, 1157. ^{1}H NMR (DMSO-d_6) (300 MHz) δ 2.31 (3H, s, CH_3), 2.97 (3H, s, NCH_3), 3.81 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 5.46 (2H, s, NCH_2), 6.59 (1H, d, $J = 2.0$ Hz, ArH), 6.63 (1H, s, ArH), 7.62 (1H, t, $J = 7.6$ Hz, ArH), 7.70–7.95 (4H, m, ArH), 8.30 (1H, s, NCH). 11.70 (1H, br s, NH). ^{13}C NMR: 8.6, 38.8, 51.1, 54.7, 54.8, 101.5, 104.7, 108.3, 123.4, 124.5, 124.9, 126.4, 127.4, 127.9, 128.5, 130.1, 133.1, 136.7, 139.0, 159.8, 160.3, 166.1. MS *m*/*z*: 469.0(M^+).

*4.1.6.14. 2-(3,4-Dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)-N′-[thiophen-2-ylmethylidene]acetohydrazide (**8n**).* Light brown powder; mp 283–284 °C. IR (KBr) cm^{-1}: 3464, 1678, 1571, 1331, 1152. ^{1}H NMR (DMSO-d_6) (400 MHz) δ 2.32 (3H, s, CH_3), 2.97 (3H, s, NCH_3), 5.40 (2H, s, NCH_2), 7.13 (1H, t, $J = 4.28$ Hz, ArH), 7.47 (1H, d, $J = 3.8$ Hz, ArH), 7.61 (1H, d, $J = 7.70$ Hz, ArH), 7.66 (1H, t, $J = 4.2$ Hz, ArH), 7.78 (1H, t, $J = 7.6$ Hz, ArH), 7.86 (1H, d, $J = 7.8$ Hz, ArH), 7.92 (1H, d, $J = 7.8$ Hz, ArH), 8.24 (1H, s, NCH), 11.77 (1H, s, NH). ^{13}C NMR: 8.7, 38.8, 51.2, 121.4, 122.4, 123.3, 123.4, 123.5, 124.1, 124.6, 126.2, 127.4, 128.4, 130.2, 133.3, 136.6, 138.1, 165.8. MS *m*/*z*: 415.0(M^+).

*4.1.6.15. 2-(3,4-Dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)-N′-[thiophen-3-ylmethylidene]acetohydrazide (**8o**).* White powder; mp 265–266 °C. IR (KBr) cm^{-1}: 3198, 1696, 1599, 1341, 1158. ^{1}H NMR (DMSO-d_6) (400 MHz) δ 2.31 (3H, s, CH_3), 2.97 (3H, s, NCH_3), 5.46 (2H, s, NCH_2), 7.54 (1H, d, $J = 4.62$ Hz, ArH), 7.63 (2H, m, ArH), 7.78 (1H, m, ArH), 7.87 (1H, d, $J = 7.8$ Hz, ArH), 7.92 (2H, d, $J = 7.6$ Hz, ArH), 8.09 (1H, s, NCH), 11.70 (1H, br s, NH). ^{13}C NMR: 8.8, 38.9, 51.2, 121.3, 121.7, 122.0, 123.4, 123.7, 124.2, 124.6, 126.1, 127.3, 128.1, 130.2, 133.2, 136.6, 137.3, 165.7. MS *m*/*z*: 415.0(M^+).

*4.1.6.16. 2-(3,4-Dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)-N′-[pyridin-2-ylmethylidene]acetohydrazide (**8p**).* Off-white powder; mp 247–248 °C. IR (KBr) cm^{-1}: 3467, 3015, 1697, 1612, 1353, 1159. ^{1}H NMR (DMSO-d_6) (300 MHz) δ 2.33 (3H, s, CH_3), 2.98 (3H, s, NCH_3), 5.55 (2H, s, NCH_2), 7.42 (1H, t, $J = 6.47$ Hz, ArH), 7.64 (1H, t, $J = 7.6$ Hz, ArH), 7.82 (1H, t, $J = 7.6$ Hz, ArH), 7.87–7.95 (3H, m, ArH), 8.03 (1H, d, $J = 7.9$ Hz, ArH), 8.10 (1H, s, NCH), 8.51 (1H, d, $J = 4.5$ Hz, ArH), 11.99 (1H, s, NH). ^{13}C NMR: 8.6, 38.9, 51.3, 121.5, 123.1, 123.4, 124.2, 124.7, 126.3, 127.6, 128.5, 130.3, 133.2, 133.9, 135.6, 136.7, 144.5, 147.3, 165.6. MS *m*/*z*: 410.0(M^+).

*4.1.6.17. 2-(3,4-Dimethyl-5,5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-1(4H)-yl)-N′-[pyridin-3-ylmethylidene]acetohydrazide (**8q**).* White powder; mp 259–260 °C. IR (KBr) cm^{-1}: 3467, 3015, 1697, 1612, 1353, 1159. ^{1}H NMR (DMSO-d_6) (300 MHz) δ 2.32 (3H, s, CH_3), 2.98 (3H, s, NCH_3), 5.54 (2H, s, NCH_2), 7.45–7.50 (1H, dd, $J = 7.76$, 4.89 Hz, ArH), 7.63 (1H, t, $J = 7.6$ Hz, ArH), 7.82 (1H, t, $J = 7.6$ Hz, ArH), 7.87–7.95 (2H, dd, $J = 16.8$, 7.6 Hz, ArH), 8.1 (1H, s, NCH). 8.16 (1H, d, $J = 8.0$ Hz, ArH), 8.60 (1H, d, $J = 4.7$ Hz, ArH), 8.91 (1H, d, $J = 1.4$ Hz, ArH), 11.95 (1H, br s, NH). ^{13}C NMR: 8.7, 38.9, 51.2, 119.3, 123.5, 124.1, 124.7, 126.2, 127.4, 127.6, 128.4, 130.2, 133.1, 133.6, 136.6, 137.4, 147.5, 147.7, 165.5. MS *m*/*z*: 410.0(M^+).

4.2. Superoxide scavenging assay

Compounds were assessed by the method used by literature method [18]. The reaction mixture comprises 40 μl of 280 μM β-nicotinamide adenine dinucleotide reduced form (NADH), 40 μl of 80 μM nitro blue tetrazolium (NBT), 20 μl of 8 μM phenazine methosulphate (PMS) 10 μl of 1 mM sample and 90 μl of 0.1 M phosphate buffer (pH 7.4). The reagents were prepared in buffer and sample in DMSO. The reaction was performed in 96-well microtitre plate at room temperature and absorbance was measured at 560 nm. The formation of superoxide was monitered by measuring the formation of water soluble blue formazan dye. A lower absorbance of reaction mixture indicates a higher scavenging activity of sample. Percent Radical Scavenging Activity was determined in comparison with control using the formula given below and are presented in Table 4.

$$\%\text{RSA} = 100 - (\text{OD test compound}/\text{OD control}) \times 100$$

4.3. Anti-bacterial testing

All the newly synthesized compounds (dissolved in dimethylformamide) were subjected to antimicrobial screening by determining the minimum inhibitory concentration (MIC) using the agar dilution technique [19]. The *in vitro* antimicrobial activity of the prepared compounds (**8a**–**q**) against three strains of bacteria i.e., *E. coli*, *B. subtilis* and *Staphylococcus aureus* was determined by preparing suspensions of each microorganism to contain approximately 10^5–10^6 CFU (colony forming units)/well. The test compounds were applied to the wells at concentrations ranging from 200 to about 3.0 μg ml^{-1} in dimethyl formamide solution, in addition to the 0 (control) and the standard, tetracycline. The plates were incubated for 24 h at 37 °C and growth assessed by visual inspection. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of inhibitor at which microbial growth was not apparent disregarding a single colony or a faint haze caused by the inoculums.

Acknowledgements

The authors are grateful to the Higher Education Commission, Pakistan and Institute of Chemistry, University of the Punjab, Lahore, for financial assistance. We are also thankful to International Centre for Chemical and Biological Sciences, HEJ Research

Institute of Chemistry, University of Karachi, Karachi and School of Chemistry, University of Manchester for spectral measurements and anti-oxidant studies.

References

[1] M. Iovu, C. Zalaru, F. Dumitrascu, C. Draghici, M. Moraru, E. Criste, Farmaco 58 (2003) 301–307.
[2] R.N. Mahajan, F.H. Havaldar, P.S. Fernandes, J. Indian Chem. Soc. 68 (1991) 245–249.
[3] P.G. Baraldi, S. Manfredini, R. Romagnoli, L. Stevanato, A.N. Zaid, R. Manservigi, Nucleos. Nucleot. Nucleic Acids 17 (1998) 2165–2171.
[4] O. Bruno, F. Bondavalli, A. Ranise, P. Schenone, C. Losasso, L. Cilenti, C. Matera, E. Marmo, Farmaco 45 (1990) 147–166.
[5] B. Cottineau, P. Toto, C. Marot, A. Pipaud, J. Chenault, Bioorg. Med. Chem. Lett. 12 (2002) 2105–2108.
[6] S.R. Smith, G. Denhardt, C. Terminelli, Eur. J. Pharmacol. 432 (2001) 107–119.
[7] R. Bihovsky, M. Tao, J.P. Mallamo, G.J. Wells, J. Med. Chem. 44 (2001) 3488–3503.
[8] M. Zia-ur-Rehman, J.A. Choudary, S. Ahmad, H.L. Siddiqui, Chem. Pharm. Bull. 54 (2006) 1175–1178.
[9] M. Zia-ur-Rehman, J.A. Choudary, M.R.J. Elsegood, H.L. Siddiqui, K.M. Khan, Eur. J. Med. Chem. 44 (2009) 1311–1316.
[10] C. Gennari, B. Salom, D. Potenza, A. Williams, Angew. Chem. Int. Ed. Engl. 33 (1994) 2067–2069.
[11] H. Zinnes, R.A. Comes, F.R. Zuleski, A.N. Caro, J. Shavel Jr., J. Org. Chem. 30 (1965) 2241–2246.
[12] J. Shavel Jr., Mendham, H. Zinnes, U.S.Pat., 3,346,572, (1967).
[13] J.M.C. Gutteridge, Free Radic. Res. Commun. 19 (1993) 141–158.
[14] A. Foroumadi, A. Samzadeh-Kermani, S. Emami, G. Dehghan, M. Sorkhi, F. Arabsorkhi, M.R. Heidari, M. Abdollahi, A. Shafiee, Bioorg. Med. Chem. Lett. 17 (2007) 6764–6769.
[15] J.M. Braughler, L.A. Duncan, R.L. Chase, J. Biol. Chem. 261 (1986) 10282–10289.
[16] Z. Otwinowski, W. Minor, Methods Enzymol. 276 (1997) 307–326.
[17] R. Hooft, COLLECT. Nonius BV, Delft, The Netherlands, 1998.
[18] N.S.C. Gaulejac, Y. Glories, N. Vivas, Food Res. Int. 32 (1999) 327–333.
[19] J.G. Colle, J.P. Duguid, A.G. Fraser, B.P. Marmion, in: T.J. Mackie, J.E. McCartney (Eds.), Practical Medical Biology, thirteenth ed. Churchill Livingstone, London, UK, 1989.