Med Chem Res (2012) 21:3541–3548
DOI 10.1007/s00044-011-9859-y

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antimicrobial activities of some isoxazolyl thiazolyl pyrazoles

Satbir Mor · Rajni Mohil · Devinder Kumar · Munish Ahuja

Received: 30 April 2011 / Accepted: 31 October 2011 / Published online: 19 November 2011

Abstract A series of isoxazolyl thiazolyl pyrazoles **5a–d** was synthesized by multi-step process, starting from 3-acetyl-4-hydroxy-6-methyl-2*H*-pyran-2-one (dehydroacetic acid, DHAA) **1**. DHAA **1** was easily converted to thiosemicarbazone **2** which on reaction with α-bromoketones yielded thiazolyl hydrazones **3**. Refluxing **3** in ethanol-acetic acid furnished 1-(5-hydroxy-3-methyl-1-substituted pyrazol-4-yl)-1,3-butanediones **4**. Finally, the title compounds **5a–d** were synthesized from **4** on treatment with hydroxylamine. The in vitro antimicrobial activity of compounds **3a–d**, **4a–d** and **5a–d** were tested. Most of the synthesized compounds exhibited significant antibacterial and antifungal activities.

Keywords Isoxazole · Thiazole · Pyrazole · Antibacterial · Antifungal

Introduction

The designing of new types of polyheterocyclic compounds along with the refining of procedures for synthesis is an urgent target of the modern heterocyclic chemistry. Amongst the five-membered heterocyclic compounds, much interest has been focused on the pyrazole nucleus which is known to possess a broad spectrum of biological properties such as antipyretic, analgesic and anti-inflammatory (Menozzi *et al.*, 2000; Turan-Zitouni *et al.*, 2001; Palomer *et al.*, 2002; Bekhit *et al.*, 2008), antiparasitic (Rathelot *et al.*, 2002; Kumar *et al.*, 2006), antitubercular (Kaymakci oglu and Rollas 2002; Dixit *et al.*, 2006), anticonvulsant (Kucukguzel *et al.*, 2000), antimicrobial (El-Gaby *et al.*, 2000; Baraldi *et al.*, 2002; Akbas *et al.*, 2005), antiviral (Larsen *et al.*,1999), anticancer (Poreba *et al.*, 2001; Ishida *et al.*, 2002; Witherington *et al.*, 2003; Rostom *et al.*, 2003; Park *et al.*, 2005), herbicidal (Liu *et al.*, 2007), etc. Likewise, thiazole nucleus present in a number of different types of compounds has been found to be associated with different types of pharmacological properties like anti-inflammatory (Holla *et al.*, 2003; Venugopala and Jayashree, 2003; Pattan *et al.*, 2007; Rostom *et al.*, 2009), antitumor (El-Subbagh *et al.*, 1999; Ramla *et al.*, 2006), antifungal (Chimenti *et al.*, 2011), antihypertensive (William *et al.*, 1992), anti-HIV (Frank *et al.*, 1995), etc. Antimicrobial activities of some substituted thiazoles are well established because it possess (S–C=N) toxophoric unit in the ring. Further, isoxazoles possess analgesic, anti-inflammatory, antimicrobial and other pharmacological activities (Habeeb *et al.*, 2001; Velikorodov and Sukhenkol, 2003; Panda *et al.*, 2009; Madhavi *et al.*, 2010), etc. Numerous biological properties are possessed by the molecules in which both pyrazole and thiazole moieties are present together (Bekhit *et al.*, 2003; Rostom, 2006). Similarly, the compounds containing pyrazole and isoxazole moieties have been shown to exhibit antihyperglycemic, analgesic, anti-inflammatory, antipyretic antibacterial, antiviral, antitumor, antifungal and antidepressant activities (Sugiura *et al.*, 1977; Jungheim, 1989; Xue *et al.*, 1998; Kang *et al.*, 2000). Furthermore, various types of pharmacological properties are exhibited by the molecules containing isoxazole and thiazole

S. Mor (✉) · R. Mohil · D. Kumar
Department of Chemistry, Guru Jambheshwar University of Science and Technology, Hisar 125001, Haryana, India
e-mail: satbir_mor@yahoo.co.in

M. Ahuja
Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar 125001, Haryana, India

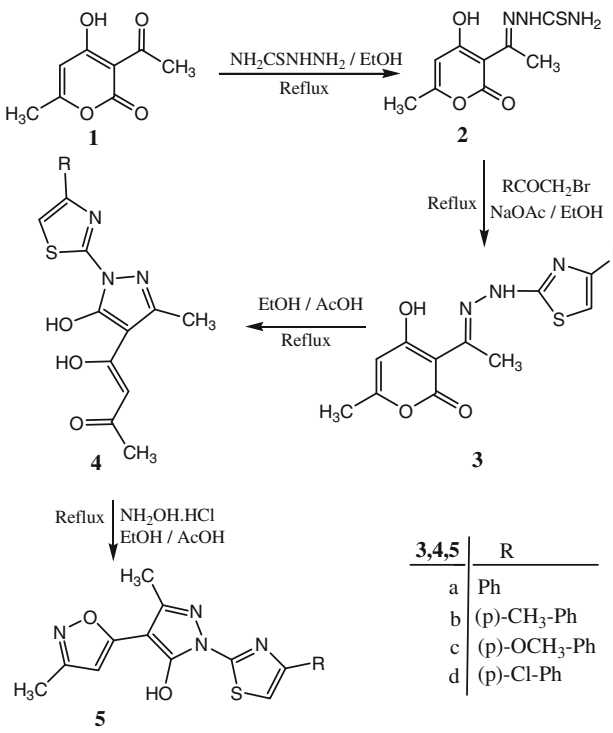
pharmacores existing in the same molecule (Rajanarendar *et al.*, 2010). It was, therefore, thought worthwhile to undertake the synthesis of some pyrazole derivatives possessing a thiazole moiety at postion-1 and isoxazole moiety at position-4 to frame potential biological molecules. Keeping in view of growing interest in the reactions of significance available in literature and numerous biological properties possessed by them, we herein report a detailed account of results of our investigations on the multistep synthesis, characterization and antimicrobial activities of **3a–d**, **4a–d** and the title compounds, 3-methyl-4-(3-methylisoxazol-5-yl)-1-(4-substituted phenylthiazol-2-yl)-1*H*-pyrazol-5-ol derivatives **5a–d**.

Results and discussion

Chemistry

The title compounds 1-(4-(phenyl/4-substituted phenyl) thiazol-2-yl)-3-methyl-4-(3-methylisoxazol-5-yl)-1H-pyrazol-5-ol **5a–d** were synthesized as illustrated in Scheme 1. The thiosemicarbazones **2** were prepared in high yields upon treatment of 2-acetyl-4-hydroxy-6-methyl-2*H*-pyran-2-one (dehydroacetic acid, DHAA) **1** with thiosemicarbazide in equimolar quantities which were smoothly converted into the corresponding hydrazones **3** upon reaction with different 4-substituted phenacyl bromides in the presence of sodium acetate/ethanol. The hydrazones **3** thus obtained underwent rearrangement in ethanol-acetic acid mixture to afford the key intermediate 1-(5-hydroxy-3-methyl-1-substituted pyrazol-4-yl)-1,3-butanediones **4** which on subsequent treatment with hydroxylamine hydrochloride furnished 1-(4-(phenyl/4-substitutedphenyl) thiazol-2-yl)-3-methyl-4-(3-methylisoxazol-5-yl)-1*H*-pyrazol-5-ol **5a–d**.

The structures of all the products were established on the basis of elemental analyses, IR, NMR (^{1}H and ^{13}C) and mass spectral data. The hydrazones **3** exhibited medium intensity NH, OH broad stretching bands in the region 3,600–3,100 cm^{-1} and strong C=O stretching bands in the region 1,699–1,684 cm^{-1} in their IR spectra. The ^{1}H NMR (400 MHz) spectra of these hydrazones displayed singlet in the region at δ 5.89–6.05 due to pyrone ring-H and singlet in the region at δ 6.62–6.85 due to thiazole ring-H which are in agreement with the results as reported in the literature [Singh *et al.*, 1993]. The IR spectra of **4** exhibited absorption bands in the regions 3,600–3,100 and 1,718–1,706 and 1,646–1,633 cm^{-1} assigned to OH and CO functionalities, respectively. The ^{1}H NMR (400 MHz) spectra of **4** displayed four signals due to each of the CH_3 groups present in pyrazolyl moiety in enol and keto forms, in the regions at δ 2.04–2.07 and 2.54–2.66 and δ



Scheme 1 Synthesis of 1-(4-(4-substituted-phenyl)thiazol-2-yl)-3-methyl-4-(3-methylisoxazol-5-yl)-1*H*-pyrazol-5-ols **5**

2.20–2.29 and δ 2.50–2.62, respectively, and one signal in each case due to CH_2 and oleifinic-H in the region at δ 4.04–4.06 and 6.67–6.69 (exchangeable with D_2O), respectively. The singlet observed in the region at δ 7.29–7.62 was assigned to thiazole ring-H. The ratio of aromatic to aliphatic protons was found satisfactory. In ^{13}C NMR spectra of **4** displayed peaks in the regions at δ 188.48–188.87, 186.94–187.44 and 183.02–183.24 due to carbonyl carbons. All these characteristic features corroborate existence of keto-enol tautomerism in **4** (Fig. 1). Further, the % ratio of keto/enol forms of **4 (4A**:**4B)** were calculated from ^{1}H NMR (400 MHz) spectral data, and were found as **4a** (35:65), **4b** (25:75), **4c** (50:50) and **4d**

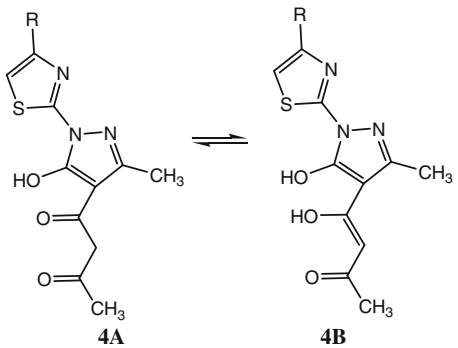


Fig. 1 Keto-enol tautomeric forms of 1-(1-(4-(4-substituted-phenyl)thiazol-2-yl)-5-hydroxy-3-methyl-1*H*-pyrazol-4-yl)butane-1, 3-diones **4**

(37:63). A careful analysis of spectral data shows an interesting trend that **4a**, **4b** and **4d** exist predominantly in enolic form but in **4c** keto-enol forms are in equal amounts.

The title compounds 1-(4-(phenyl/4-substituted phenyl) thiazol-2-yl)-3-methyl-4-(3-methylisoxazol-5-yl)-1*H*-pyrazol-5-ol **5a–d** exhibited the characteristics broad absorption bands of medium intensity in the region 3,432–3,204 cm^{-1} due to OH of pyrazole moiety in their IR spectra. The ^1H NMR (400 MHz) spectra of **5a–d** showed a singlet in the region δ 6.51–6.55 due to isoxazole-H and another singlet in the region δ 7.25–7.71 assigned to thiazole-H. The mass spectral studies and elemental analysis results were found satisfactory. Physical and chemical data of the compounds **3a–d**, **4a–d** and **5a–d** are detailed in Table 1.

Antimicrobial activity

All the newly synthesized compounds has been assayed for their in vitro antibacterial activity against Gram-positive *Bacillus subtilis*, *Staphylococcus aureus* and Gram-negative *Escherichia coli* and in vitro antifungal activity against *Candida albicans* and *Aspergillus niger*. The bacterial and fungal strains selected for this study are most common and easily available. *B. subtilis* may contaminate food and produces the proteolytic enzyme subtilisin and is responsible for causing ropiness. *S. aureus* is a common cause of boils, sties and skin infections and may cause serious infections in debilitated persons. *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. The fungus *C. albicans* is often a benign member of the mucosal flora; however, it commonly causes mucosal disease with substantial morbidity and in vulnerable patients it causes life-threatening bloodstream infections. *A. niger* is not only a xerophilic fungi, but is also a thermo-tolerant organism. Double strength nutrient broth and Sabouraud dextrose broth were employed for bacterial and fungal growth, respectively. The pMICs (negative logarithm of minimum inhibitory concentrations) were recorded as the minimum concentration of a compound that inhibits the growth of tested microorganisms. pMICs were determined by means of standard serial dilution method. The pMIC values are presented in Table 2. Most of the synthesized compounds showed good antibacterial activity with pMICs ranging from 1.435 to 1.778. The new title compounds isoxazolyl thiazolyl pyrazoles **5a–d** and their precursors **3a–d** and **4a–d** were successfully synthesized. The synthesized compounds were found to exhibit moderate

Table 1 Physical and analytical data of compounds **3a–d**, **4a–d** and **5a–d**

Compds	R	Reaction time (min)	Yield %	Melting point °C	Molecular formula	Found % (Calculated) C	H	N
3a	H	30	76	188–190 Lit. mp 190–191	$C_{17}H_{15}N_3O_3S$	60.20 (59.81)	4.22 (4.43)	12.69 (12.31)
3b	CH_3	25	71	204–206	$C_{18}H_{17}N_3O_3S$	60.34 (60.83)	4.71 (4.82)	12.08 (11.82)
3c	OCH_3	30	73	189–190	$C_{18}H_{17}N_3O_4S$	57.94 (58.21)	4.96 (4.61)	11.59 (11.31)
3d	Cl	35	67	178–180	$C_{17}H_{14}ClN_3O_3S$	54.72 (54.33)	3.83 (3.75)	11.26 (11.18)
4a	H	120	57	142–144 Lit. mp 145	$C_{17}H_{15}N_3O_3S$	60.15 (59.81)	4.27 (4.43)	12.43 (12.31)
4b	CH_3	120	59	110–112	$C_{18}H_{17}N_3O_3S$	61.14 (60.83)	5.08 (4.82)	12.23 (11.82)
4c	OCH_3	125	64	158–160	$C_{18}H_{17}N_3O_4S$	58.53 (58.21)	4.93 (4.61)	10.96 (11.31)
4d	Cl	125	61	120–122	$C_{17}H_{14}ClN_3O_3S$	54.56 (54.33)	3.61 (3.75)	11.47 (11.18)
5a	H	115	68	208–210	$C_{17}H_{14}N_4O_2S$	60.28 (60.34)	4.37 (4.17)	16.58 (16.56)
5b	CH_3	125	63	220–222	$C_{18}H_{16}N_4O_2S$	61.72 (61.35)	4.81 (4.58)	15.59 (15.90)
5c	OCH_3	110	74	232–234	$C_{18}H_{16}N_4O_3S$	59.01 (58.68)	4.10 (4.38)	15.07 (15.21)
5d	Cl	125	69	258–260	$C_{17}H_{13}ClN_4O_2S$	55.13 (54.77)	3.16 (3.51)	15.16 (15.03)

Table 2 In vitro antimicrobial activity (pMIC) of compounds **3a–d**, **4a–d** and **5a–d**

Compounds	Antibacterial activity			Antifungal activity	
	B. subtilis	*S. aureus*	*E. coli*	*C. albicans*	*A. niger*
3a	1.435	1.737	1.435	1.435	1.435
3b	1.453	1.453	1.453	1.152	1.435
3c	1.171	1.472	1.472	1.171	1.472
3d	1.478	1.478	1.478	1.478	1.478
4a	1.435	1.435	1.435	1.435	1.435
4b	1.453	1.754	1.754	1.453	1.453
4c	1.472	1.472	1.472	1.472	1.472
4d	1.477	1.778	1.477	1.477	1.477
5a	1.733	1.733	1.733	1.432	1.432
5b	1.750	1.750	1.750	1.449	1.750
5c	1.771	1.771	1.470	1.470	1.771
5d	1.775	2.077	1.775	1.477	1.474
Norfloxacin	2.698	2.698	2.698	–	–
Fluconazole	–	–	–	3.000	3.000

antibacterial activity, e.g. **5a–d** against *B. subtilis*; **3a**, **4b**, **4d**, **5a–c** against *S. aureus* and **4b**, **5a**, **5b**, **5d** against *E. coli,* whereas the compounds **5b**, **5c** were found to be active fungicidal agent against *A. niger*. However, amongst the newly synthesized title compounds, 1-(4-(4-chlorophenyl)thiazol-2-yl)-3-methyl-4-(3-methylisoxazol-5-yl)-1*H*-pyrazol-5-ol **5d** was found to exhibit the promising antibacterial activity against *S. aureus* (pMIC = 2.077, in comparison to the standard reference norfloxacin, pMIC = 2.698). Almost all the compounds showed moderate level of antifungal activity with pMICs ranging from 1.435 to 1.478 in comparison to the standard reference fluconazole (pMIC = 3.000). However, **5b** (pMIC = 1.750) and **5c** (pMIC = 1.771) exhibited good fungicidal activity. The results of antimicrobial activity illustrate that the presence of electron donating groups, CH_3 and OCH_3 on the phenyl ring, in compounds **3–5** increases the antibacterial as well as antifungal activities. Further, the presence of electron withdrawing group, Cl on the phenyl ring, in compounds **5d** enhances the antibacterial activity to a greater extent. The graphical data of antimicrobial activities of all the synthesized compounds is depicted in Fig. 2.

Conclusion

The synthesized compounds were found to exhibit moderate antibacterial activity, e.g. **5a–d** against *B. subtilis*; **3a**, **4b**, **4d**, **5a–c** against *S. aureus* and **4b**, **5a**, **5b**, **5d** against *E. coli,* whereas the compounds **5b**, **5c** were found to be active fungicidal agent against *A. niger*. However, amongst the newly synthesized title compounds, 1-(4-(4-chlorophenyl)thiazol-2-yl)-3-methyl-4-(3-methylisoxazol-5-yl)-1*H*-pyrazol-5-ol **5d** was found to exhibit the promising antibacterial activity against *S. aureus*.

Experimental

Melting points were determined in open capillaries and are uncorrected. The IR absorption spectra were scanned on Perkin Elmer Spectrum, BX II FTIR spectrometer using potassium bromide (KBr) pellets and the wave numbers were given in cm^{-1}. The ^1H NMR and ^{13}C NMR spectra were recorded on Bruker Avance II 400 spectrometer at 400 and 100 MHz, respectively, in deuteriochloroform or deuteriochloroform + dimethyl sulfoxide-d_6 or dimethyl sulfoxide-d_6. The chemical shifts are reported in parts per million (δ ppm) using tetramethylsilane (TMS) as an internal standard. Coupling constants J are valued in Hertz (Hz). Mass spectra were recorded on Waters Micromass Q-Tof Micro (ESI) spectrometer. Elemental analysis was carried out using Vario Micro Cube Elementar CHNS analyser. Analytical results for C, H and N were within ±0.4% of the theoretical values. The purities of the compounds were checked by thin layer chromatography (TLC) using readymade silica gel (SIL G/UV_{254}, ALUGRAM) plates. The spots were visualized under ultraviolet (UV) lamp. Solvents were dried using standard literature procedures. The thiosemicarbazones **2** were prepared by the condensation of equimolar quantities of 2-acetyl-4-

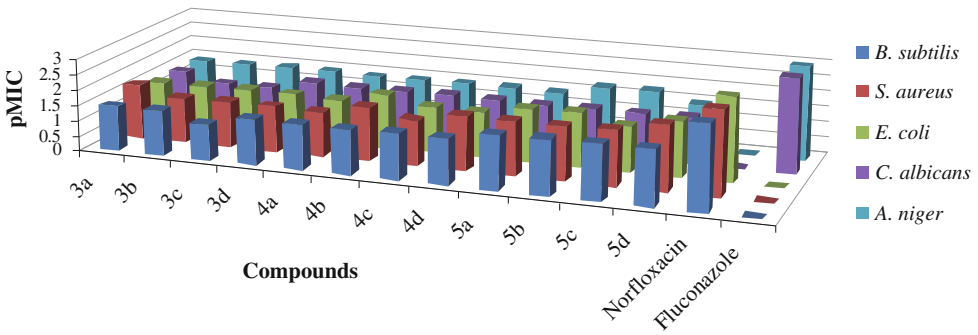


Fig. 2 In vitro antimicrobial activity (pMIC) of compounds **3a–d**, **4a–d** and **5a–d**

hydroxy-6-methyl-2*H*-pyran-2-one and thiosemicarbazide in absolute alcohol (Singh *et al.*, 1993).

General procedure for the synthesis of 4-hydroxy-6-methyl-3-(1-(2-(4-substituted-phenylthiazol-2-yl)hydrazono)ethyl)-2*H*-pyran-2-ones (**3a**–**3d**)

To a solution of **2** (2.41 g, 0.01 mol) and anhydrous sodium acetate (0.82 g, 0.01 mol) in absolute ethanol, the appropriate *p*-substituted phenacyl bromide (0.01 mol) was added slowly with stirring. The reaction mixture was refluxed gently for 25–35 min. The yellowish crystalline solid thus obtained was filtered, washed with cold ethanol, and recrystallized from dimethyl formamide and water. Physical and analytical data are recorded in Table 1.

4-Hydroxy-6-methyl-3-(1-(2-(4-phenylthiazol-2-yl)hydrazono)ethyl)-2H-pyran-2-one ***(3a)***

Yield 76%; mp 188–190°C (Lit. mp 190–191°C; Singh *et al.*, 1993); IR (KBr): 3,400–3,100 (NH, OH), 1,698 cm^{-1}(CO); MS: (TOF MS ES^+) *m/z* 342.2 $[M + H]^+$.

4-Hydroxy-6-methyl-3-(1-(2-(4-tolylthiazol-2-yl)hydrazono)ethyl)-2H-pyran-2-one ***(3b)***

Yield 71%; mp 204–206°C; IR (KBr) 3,550–3,200 (NH, OH), 1,699 cm^{-1}(CO); ^{1}H NMR (DMSO-d_6) δ 2.16 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 5.89 (s, 1H, pyrone-H), 6.62 (s, 1H, thiazole-H), 7.23 (d, 2H, $J = 8.0$ Hz, H-3″,5″), 7.61 (d, 2H, $J = 8.08$ Hz, H-2″,6″); MS: (TOF MS ES^+) *m/z* 356.1 $[M + H]^+$; $C_{18}H_{17}N_3O_3S$; Calc. C 60.83, H 4.82, N 11.82; Found C 60.34, H 4.71, N 12.08.

4-Hydroxy-3-(1-(2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazono)ethyl-6-methyl-2H-pyran-2-one ***(3c)***

Yield 73%; mp 189–190°C; IR (KBr): 3,600–3,220 (NH, OH), 1,687 cm^{-1} (CO); ^{1}H NMR (DMSO-d_6): δ 2.08 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 6.05 (s, 1H, pyrone-H), 6.69 (s, 1H, thiazole-H), 6.94 (d, 2H, $J = 8.72$ Hz, H-3″,5″), 7.88 (d, 2H, $J = 8.72$ Hz, H-2″,6″); MS: (TOF MS ES^+) *m/z* 372.2 $[M + H]^+$; $C_{18}H_{17}N_3O_4S$; Calc. C 58.21, H 4.61, N 11.31; Found C 57.94, H 4.96, N 11.59.

3-(1-(2-(4-(4-Chlorophenyl)thiazol-2-yl)hydrazono)ethy)-4-hydroxy-6-methyl-2H-pyran-2-one ***(3d)***

Yield 67%; mp 178–180°C; IR (cm^{-1}): 3,500–3,200 (NH, OH), 1,684 cm^{-1} (CO); ^{1}H NMR (DMSO-d_6) δ 2.20 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 5.89 (s, 1H, pyrone-H), 6.85 (s, 1H, thiazole-H), 7.38–7.76 (m, 4H, Ar–H) MS: (TOF MS ES^+) *m/z* 376.2 $[M + H]^+$; $C_{17}H_{14}ClN_3O_3S$; Calc. C 54.33, H 3.75, N 11.18; Found C 54.72, H 3.83, N 11.26.

General procedure for the synthesis of 1-(1-(4-(4-substituted-phenyl)thiazol-2-yl)-5-hydroxy -3-methyl-1*H*-pyrazol-4-yl)butane-1,3-diones **(4a**–**4d)**

The hydrazone **3** (0.01 mol) was added in one lot to a hot solution of acetic acid and refluxed for approximately 2 h. The solid thus obtained, on cooling the reaction mixture overnight, was filtered, washed with little cold ethanol and crystallised from acetonitrile. Physical and analytical data are recorded in Table 1.

1-(5-Hydroxy-3-methyl-1-(4-phenylthiazol-2-yl)-1H-pyrazol-4-yl)butane-1,3-dione ***(4a)***

Yield 57%; mp 142–144°C (Lit. mp 145°C; Singh *et al.*, 1993); IR (KBr) 3,550–3,200 (OH), 1,706, 1,633 cm^{-1} (CO); ^{1}H NMR (DMSO-d_6) δ 2.04, 2.27 (two s, 3H, CH_3), 2.59, 2.62 (two s, 3H, CH_3), 4.04 (s, 2H, CH_2), 6.68 (s, 1H, olefinic-H), 7.29–7.84 (m, 6H, ArH and thiazole-H); ^{13}C NMR (DMSO-d_6) (enol + keto forms) δ 13.49, 13.71, 24.17, 30.97, 56.01, 97.76, 100.22, 103.84, 108.77, 126.41, 128.31, 128.68, 133.96, 134.05, 149.80, 149.84, 152.19, 152.70, 153.52, 159.05, 160.08, 183.13, 187.28, 188.5; MS: (TOF MS ES^+) *m/z* 342.2 $[M + H]^+$.

Hydroxy-3-methyl-1-(4-tolylthiazol-2-yl)-1H-pyrazol-4-yl)butane-1,3-dione ***(4b)***

Yield 59%; mp 110–112°C; IR (KBr) 3,600–3,200 (OH), 1,707, 1,646 cm^{-1}(CO); ^{1}H NMR (DMSO-d_6) δ 2.06, 2.27 (two s, 3H, CH_3), 2.37, (s, 3H, CH_3), 2.60, 2.64 (two s, 3H, CH_3), 4.04 (s, 2H, CH_2), 6.69 (s, 1H, olefinic-H), 7.21 (d, 2H, $J = 7.9$ Hz, H-3″, 5″), 7.39 (s, 1H, thiazole-H), 7.84 (d, 2H, $J = 7.9$ Hz, H-2″,6″); ^{13}C NMR (DMSO-d_6) (enol + keto forms) δ 13.49, 13.79, 21.34, 24.15, 30.95, 56.00, 97.73, 100.19, 103.81, 108.01, 126.33, 129.22, 129.36, 131.60, 137.49, 149.84, 149.88, 152.12, 152.71, 153.52, 159.08, 160.12, 183.24, 187.17, 188.48; MS: (TOF MS ES^+) *m/z* 356.1 $[M + H]^+$; $C_{18}H_{17}N_3O_3S$; Calc. C 60.83, H 4.82, N 11.82; Found C 61.14, H 5.08, N 12.23.

1-(5-Hydroxy -1-(4-(4-methoxyphenyl)thiazol-2-yl) -3-methyl -1H-pyrazol-4-yl)butane-1,3-dione ***(4c)***

Yield 64%; mp 158–160°C; IR (KBr) 3,600–3,300 (OH), 1,718, 1,646 cm^{-1}(CO); ^{1}H NMR (DMSO-d_6) δ 2.05, 2.20 (two s, 3H, CH_3), 2.50, 2.54 (two s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 4.04 (s, 2H, CH_2), 6.67 (s, 1H, olefinic-H), 7.02 (d, $J = 8.7$ Hz, H-2″, 6″), 7.62 (s, 1H, thiazole-H), 7.93 (d, $J = 8.7$ Hz, H-3″,5″); ^{13}C NMR (DMSO-d_6) (enol + keto

forms) δ 13.39, 13.76, 24.14, 30.91, 55.40, 56.41, 98.61, 100.10, 103.78, 108.88, 114.28, 123.90, 126.89, 136.71, 149.81, 149.93, 152.17, 152.87, 153.18, 159.47, 159.70, 161.51, 183.22, 186.94, 188.87; MS: (TOF MS ES$^+$) *m/z* 372.04 [M + H] $^+$; $C_{18}H_{17}N_3O_4S$; Calc. C 58.21, H 4.61, N 11.31; Found C 58.53, H 4.93, N 10.96.

1-(1-(4-(4-Chlorophenyl)thiazol-2-yl)-5-hydroxy-3-methyl-1H-pyrazol-4-yl)butane-1,3-dione ***(4d)***

Yield 61%; mp 120–122°C; IR (KBr) 3,550–3,100 (OH), 1,717, 1,636 cm^{-1}(CO); ^{1}H NMR (DMSO-d_6) δ 2.07, 2.29 (two s, 3H, CH_3), 2.62, 2.66 (two s, 3H, CH_3), 4.06 (s, 2H, CH_2), 6.68 (s, 1H, olefinic-H), 7.40 (d, 2H, J = 8.4 Hz, H-2″,6″), 7.45 (s, 1H, thiazole-H), 7.933 (d, J = 8.4 Hz, H-3″,5″); ^{13}C NMR (DMSO-d_6) (enol + keto forms) δ 13.49, 13.69, 24.17, 30.94, 55.94, 97.75, 100.25, 103.86, 109.28, 127.80, 128.76, 132.64, 132.70, 133.58, 148.66, 152.32, 152.79, 153.64, 159.13, 160.15, 183.02, 187.44, 188.51; MS: (TOF MS ES$^+$) *m/z* 376 [M + H] $^+$; $C_{17}H_{14}ClN_3O_3S$; Calc. C 54.33, H 3.75, N 11.18; Found C 54.56, H 3.61, N 11.47.

General procedure for the synthesis of 1-(4-(4-substituted-phenyl)thiazol-2-yl)-3-methyl-4-(3-methylisoxazol-5-yl)-1*H*-pyrazol-5-ols **(5a–d)**

The solution of **4** (0.01 mol) and hydroxylamine hydrochloride (0.69 g, 0.01 mol) in 50 ml of ethanol and 50 ml of acetic acid was heated at reflux for approximately 2 h. The reaction mixture was cooled and then allowed to stand overnight. The solid thus obtained, was collected and recrystallised from acetonitrile. Physical and analytical data are recorded in Table 1.

*3-Methyl-4-(3-methylisoxazol-5-yl)-1-(4-phenylthiazol-2-yl)-1H-pyrazol-5-ol (****5a****)*

Yield 68%; mp 208–210°C; IR (KBr) 3,204 cm^{-1} (OH); ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 6.51 (s, 1H, isoxazole-H), 7.33–7.46 (m, 3H, Ar–H)), 7.71 (s, 1H, thiazole-H), 8.01 (d, 2H, J = 7.36 Hz, H-2″,6″); ^{13}C NMR (DMSO-d_6) δ 11.37, 12.92, 93.91, 99.32, 109.4, 126.45, 128.44, 128.96, 134.18, 148.09, 149.63, 152.51, 158.07, 159.42, 163.49; MS: (TOF MS ES$^+$) *m/z* 339.3 [M + H]$^+$; $C_{17}H_{14}N_4O_2S$; Calc. C 60.34, H 4.17, N 16.56; Found C 60.28, H 4.37, N 16.58.

3-Methyl-4-(3-methylisoxazol-5-yl)-1-(4-p-tolylthiazol-2-yl)-1H-pyrazol-5-ol ***(5b)***

Yield 63%; mp 220–222°C; IR (KBr) 3,431 cm^{-1} (OH); ^{1}H NMR (DMSO-d_6) δ 2.23 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 6.52 (s, 1H, isoxazole-H), 6.91–7.84 (m, 5H, Ar–H, thiazole-H); ^{13}C NMR (DMSO-d_6) δ 12.84, 11.41, 12.84, 21.36, 94.26, 99.31, 108.79, 126.30, 129.25, 129.39, 137.90, 147.72, 150.0, 151.37, 158.11, 159.41, 163.49; MS (TOF MS ES$^+$) *m/z* 353.4 [M + H]$^+$; $C_{18}H_{16}N_4O_2S$; Calc. C 61.35, H 4.58, N 15.90; Found C 61.72, H 4.81, N 15.59.

1-(4-(4-Methoxyphenyl)thiazol-2-yl)-3-methyl-4-(3-methylisoxazol-5-yl)-1H-pyrazol-5ol ***(5c)***

Yield 74%; mp 232–234°C; IR (cm^{-1}) 3,432 cm^{-1} (OH); ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 6.54 (s, 1H, isoxazole-H), 6.93 (d, 2H, J = 2.84 Hz, H-3″,5″), 7.25 (s, 1H, thiazole-H), 7.86 (d, 2H, J = 2.84 Hz, H-2″,6″); ^{13}C NMR (DMSO-d_6) δ 11.39, 12.83, 55.35, 94.37, 99.32, 106.71, 114.04, 126.94, 127.22, 127.62, 127.70, 147.69, 149.87, 159.46, 159.63, 163.44; MS (TOF MS ES$^+$) *m/z* 369.2 [M + H]$^+$; $C_{18}H_{16}N_4O_3S$; Calc.C 58.68, H 4.38, N 15.21; Found C 59.01, H 4.10, N 15.07.

1-(4-(4-Chlorophenyl)thiazol-2-yl)-3-methyl-4-(3-methylisoxazol-5-yl)-1H-pyrazol-5-ol ***(5d)***

Yield 69%; mp 258–260°C; IR (KBr) 3,431 cm^{-1} (OH); ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 6.55 (s, 1H, isoxazole-H), 7.41 (d, 2H, J = 8.4 Hz, H-2″,6″)), 7.44 (s, 1H, thiazole-H), 7.94 (d, 2H, J = 8.4 Hz, H-3″, 5″); ^{13}C NMR (DMSO-d_6) δ 11.38, 12.84, 94.43, 99.40, 109.1, 127.76, 128.76, 132.75, 133.63, 138.9, 147.92, 148.81, 155.4, 159.51, 163.34; MS (TOF MS ES$^+$) *m/z* 373.3 [M + H]$^+$; $C_{17}H_{13}ClN_4O_2S$; Calc. C 54.77, H 3.51, N 15.03; Found C 55.13, H 3.16, N 15.16.

Antimicrobial activity

All the twelve newly synthesized compounds **3a–d**, **4a–d** and **5a–d** were screened for their in vitro antimicrobial activity against five microorganisms, two Gram-positive bacteria *B. subtilis* (MTCC 441) and *S. aureus* (MTCC 7443) and one Gram-negative bacteria *E. coli* (MTCC 42) and two fungi *C. albicans* (MTCC 183) and *A. niger* (MTCC 282) by serial tube dilution technique using two solid media Double strength nutrient broth and Sabouraud dextrose broth for bacterial and fungal growth, respectively. The stock solutions (100 μg/ml) of all the test compounds were prepared by dissolving 1 mg of the test compound in 10 ml of dimethylsulphoxide. Norfloxacin and fluconazole were used as reference against bacteria and fungi, respectively. The fresh cultures were obtained by inoculation of respective microorganism in suitable medium (Double strength nutrient broth in case of bacteria and

Sabouraud dextrose broth in case of fungi) followed by incubation at 37 ± 1°C. The stock solutions of the test compounds were serially diluted in test tubes containing 1 ml of sterile medium to get the concentration of 50–3.12 μg/ml and then inoculated with 100 μl of suspension of respective microorganism in sterile saline. The inoculated test tubes were incubated at 37 ± 1°C for 24 h in case of *B. subtilis*, *S. aureus* and *E. coli*, at 37 ± 1°C for 48 h in case of *Candida albicans* and at 37 ± 1°C for 120 h in case of *A. niger* and their pMICs(-log of Minimum inhibitory concentrations) were determined. Minimum inhibitory concentration (MIC), in microbiology, is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after incubation.

The reference compounds Norfloxacin and fluconazole were also tested under similar conditions to compare the results of tested compounds. The data for the antibacterial and antifungal studies are listed in Table 2 and expressed in Fig. 2.

Acknowledgments We gratefully acknowledge the financial support from CSIR New Delhi. Thanks to SAIF, Panjab University, Chandigarh for providing NMR spectra of the compounds.

References

Akbas E, Berber I, Sener A, Hasanov B (2005) Synthesis and antibacterial activity of 4-benzoyl-1-methyl-5-phenyl-1*H*-pyrazole-3-carboxylic acid and derivatives. Il Farmaco 60:23–26

Baraldi PG, Pavani MG, Nunez MDC, Brigidi P, Vitali B, Gambari R, Romagnoli R (2002) Antimicrobial and antitumor activity of N-heteroimmine-1,2,3-dithiazoles and their transformation in triazolo-, imidazo-, and pyrazolopirimidines. Bioorg Med Chem 10:449–456

Bekhit AA, Fahmy HT, Rostom SAF, Baraka AM (2003) Design and Synthesis of some substituted 1H-pyrazolyl-thiazolo[4,5-d]pyrimidines as anti-inflammatory-antimicrobial agents. Eur J Med Chem 38:27–36

Bekhit AA, Ashour HMA, Ghany YSA, Bekhit AEA, Baraka A (2008) Synthesis and biological evaluation of some thiazolyl and thiadiazolyl derivatives of 1H-pyrazole as anti-inflammatory antimicrobial agents. Eur J Med Chem 43:456–463

Chimenti F, Bizzarri B, Bolasco A, Secci D, Chimenti P, Granese A, Carradori S, Ascenzio MD, Lilli D, Rivanera D (2011) Synthesis and biological evaluation of novel 2,4-disubstituted-1,3-thiazoles as anti-Candida spp agents. Eur J Med Chem 46:378–382

Dixit PP, Patil VJ, Nair PS, Jain S, Sinha N, Arora SK (2006) Synthesis of 1-[3-(4-benzotriazol-1/2-yl-3-fluorophenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-substituted-thiourea derivatives as antituberculosis agents. Eur J Med Chem 41:423–428

El-Gaby MSA, Atalla AA, Gaber AM, Abd Al-Wahab KA (2000) Studies on amino-pyrazoles: antibacterial activity of some novel pyrazolo[1,5a]pyrimidines containing sulfonamido moieties. Il Farmaco 55:596–602

El-Subbagh HI, Abadi AH, Lehmann J (1999) Synthesis and antitumor activity of ethyl 2-substitutedaminothiazole-4-carboxylate analogs. Arch Pharm Pharm Med Chem 332:137–142

Frank WB, Amanda SC, Marita HS, Richard J, Nils GJ, Christopher LJ, Michael DK, Peter L, John MM, Noreen R, Oberg B, Palkowitz JA, Parrish CA, Pranc J, Zhang H, Zhou XX (1995) Phenethylthiazolethiourea (PETT) compounds, a new class of HIV-1 reverse transcriptase inhibitors. 1. Synthesis and basic structure-activity relationship studies of PETT analogs. J Med Chem 38:4929–4936

Habeeb GA, Rao PNP, Kanus EE (2001) Design and synthesis of 4,5-diphenyl-4isoxazolines: novel inhibitors of cyclooxygenase-2 with analgesic and antiinflammatory activity. J Med Chem 44: 2921–2927

Holla BS, Malini KV, Rao BS, Sarojini BK, Kumari NS (2003) Synthesis of some new 2,4-disubstituted thiazoles as possible antibacterial and anti-inflammatory agents. Eur J Med Chem 38: 313–318

Ishida J, Ohtsu H, Tachibana Y, Nakanishi Y, Bastow KF, Nagai M, Wang H-K, Itokawa H, Lee KH (2002) Antitumor agents. Part 214: synthesis and evaluation of curcumin analogues as cytotoxic agents. Bioorg Med Chem 10:3481–3487

Jungheim L (1989) Bicyclic pyrazolidinone antibacterial agents. Synthesis of side chain analogues of carbapenems. PS-5 and thienamycin. Tetrahedron Lett 30:1889–1892

Kang YK, Shin KJ, Yoo KH, Seo KJ (2000) Synthesis and antibacterial activity of new carbapenems containing isoxazole moiety. Bioorg Med Chem Lett 10:95–99

Kaymakci oglu BK, Rollas S (2002) Synthesis, characterization and evaluation of antituberculosis activity of some hydrazones. Il Farmaco 57:595–599

Kucukguzel SG, Rollas S, Erdeniz H, Kiraz M, Ekinci AC, Vidin A (2000) Synthesis, characterization and pharmacological properties of some 4-arylhydrazono-2-pyrazolin-5-one derivatives obtained from heterocyclic amines. Eur J Med Chem 35: 761–771

Kumar S, Kumar G, Kapoor M, Surolia A, Surolia N (2006) Synthesis and evaluation of substituted pyrazoles: potential antimalarials targeting the enoyl-ACP reductase of *Plasmodium falciparum*. Syn Commun 36:215–226

Larsen JS, Zahran MA, Pedersen EB, Nielsen C (1999) Synthesis of triazinopyrazole derivatives as potential inhibitors of HIV-1. Monatsh Chem 18:203–216

Liu H, Wang HQ, Liu ZJ (2007) Synthesis and herbicidal activity of novel pyrazolo[3,4-d]pyrimidin-4-one derivatives containing aryloxyphenoxypropionate moieties. Bioorg Med Chem Lett 17: 2203–2209

Madhavi K, Bharathi K, Prasad KVSRG (2010) Synthesis and evaluation of 3-methyl-4-nitro-5-(substituted styryl) isoxazoles for antioxidant and anti-inflammatory activities. Res J Pharm Bio Chem Sci 1(4):1073–1082

Menozzi G, Mosti L, Merello L, Piana A, Armani U, Ghia M, Angiola M, Mattioli F (2000) 4-Dialkylamino-1-(5-substituted or unsubstituted 1-phenyl-1*H*-pyrazol-4-yl)-butan-1-ols: synthesis and evaluation of analgesic, anti-inflammatory and platelet anti-aggregating activities. Il Farmaco 55:219–226

Palomer A, Cabre F, Pascual J, Campos J, Trujillo MA, Entrena A, Callo MA, Garcia L, Mauleon D, Espinosa A (2002) Identification of Novel Cyclooxygenase-2 Selective inhibitors using pharmacophore models. J Med Chem 45:1402–1411

Panda SS, Chowdary PVR, Jayashree BS (2009) Synthesis, antiinflammatory and antibacterial activity of novel indolyl-isoxazoles. Ind J Pharm Sci 71(6):684–687

Park HJ, Lee K, Park SJ, Ahn B, Lee JC, Cho HY, Lee KI (2005) Identification of antitumor activity of pyrazole oxime ethers. Bioorg Med Chem Lett 15:3307–3312

Pattan SR, Reddy VV, Pawar PD, Khade AB, Desai NS, Bhat AR, Taranalli AD (2007) Synthesis and anti-inflammatory evaluation

of 4-phenylamino benzylidene(thiazolidine-2,4-dione) derivatives. Indian Drugs 44(4):253

Poreba K, Opolski A, Wietrzyk J, Kowalska M (2001) Synthesis and antiproliferative activity in vitro of new derivatives of 3-aminopyrazolo[3,4-b]pyridine. Part 1: reaction of 3-aminopyrazolo[3,4-b]pyridine with 1,3-,1,4- and α,β-unsaturated ketones. Arch Pharm Pharm Med Chem 334:219–223

Rajanarendar E, Rao EK, Shaik FP, Reddy MN, Srinivas M (2010) Design, synthesis, antibacterial and antifungal activity of novel spiroisoxazolyl-bis-[5,5′]thiazolidin-4-ones and spiro-isoxazolyl thiazolidin-4-one-[5,5′]-1,2–4 oxdiazolines. J Sulfur Chem 31 (4):263–274

Ramla MM, Omar MA, El-Khamry AMM, El-Diwan HI (2006) Synthesis and antitumor activity of 1-substituted-2-methyl-5-nitrobenzimidazoles. Bioorg Med Chem 14:7324–7332

Rathelot P, Azas N, El-Kashef H, Delmas F, Di Giorgio C, Timon-David P, Maldonado J, Vanelle P (2002) 1,3-Diphenylpyrazoles: synthesis and antiparasitic activities of azomethine derivatives. Eur J Med Chem 37:671–679

Rostom SAF (2006) Synthesis and in vitro antitumor evaluation of some indeno[1,2-c]pyrazol(in)es substituted with sulfonamide, sulfonylurea (thiourea) pharmacophores and some derived thiazole ring systems. Bioorg Med Chem 14:6475–6485

Rostom SAF, Shalaby MA, El-Demellawy MA (2003) Polysubstituted pyrazoles, part 5. Synthesis of new 1-(4-Chlorophenyl)-4-hydroxy-1H-pyrazole-3-carboxylic acid hydrazide analogs and some derived ring systems. A novel class of potential antitumor and anti-HCV agents. Eur J Med Chem 38:959–974

Rostom SAF, El-Ashmawy IM, El Razik HR, Badr MH, Ashour HMA (2009) Design and synthesis of some thiazolyl and thiadiazolyl derivatives of antipyrine as potential non-acidic anti-inflammatory, analgesic and antimicrobial agents. Bioorg Med Chem 17:882–895

Singh SP, Tarar LS, Kumar D (1993) A facile route for the synthesis of 1-[5-hydroxy-3-methyl-(2-thiazolyl)-4-pyrazolyl]-1.3-butanediones from dehydroacetic acid. Syn Commun 23(13):1855–1861

Sugiura S, Ohno S, Ohtani O, Izumi K (1977) Synthesis and antinflammatory and hypnotic activity of 5-alkoxy-3-(N-substituted carbamoyl)-1-phenylpyrazoles. J Med Chem 20:80–85

Turan-Zitouni G, Sivaci M, Kilic FS, Erol K (2001) Synthesis of some triazolyl-antipyrine derivatives and investigation of analgesic activity. Eur J Med Chem 36:685–689

Velikorodov AV, Sukhenkol LT (2003) Synthesis and antimicrobial activity of 3,5-disubstituted isoxazolines and isoxazoles with carbamate groups. Pharm Chem J 37:22–24

Venugopala KN, Jayashree BS (2003) Synthesis and characterization of carboxamides of 2′-amino-4′-(6-bromo-3-coumarinyl) thiazole for their analgesic and anti-inflammatory activity. Ind J Het Chem 12:307–310

William CP, Harriet WH, Michael DT, Michael JR, David GTJ, Cleo JCC, Annette MD, Sylvester RK, Ila S, Steinbaugh BA, Batly BL, Painchaud CA, Rapundalo ST, Michniewicz BM, Olson SCJ (1992) Structure-activity relationships of a series of 2-amino-4-thiazole-containing renin inhibitors. J Med Chem 35:2562–2572

Witherington J, Bordas V, Garland SL, Hickey DMB, Ife RJ, Liddle J, Saunders M, Smith DG, Ward RW (2003) 5-Arylpyrazolo[3,4-b]pyridines: potent inhibitors of glycogen synthase kinase-3 (GSK-3). Bioorg Med Chem Lett 13:1577–1580

Xue C, Roderick J, Mousa S, Olson RE, DeGrado WF (1998) Synthesis and antiplatelet effects of an isoxazole series of glycoprotein IIb/IIIa antagonists. Bioorg Med Chem Lett 8: 3499–3504