CHEMIJA. 2012. Vol. 23. No. 2. P. 124–130

Synthesis and antimicrobial studies of novel (2-benzylidene)-phenylureido-thiazolopyrimidine derivatives

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A new series of ethyl 2-(substitutedbenzylidene)-5-methyl-3-oxo-7-(4-(3-phenylureido) phenyl)-3,7-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxylate derivatives (7a–k) were synthesized. The newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, LCMS mass and C, H, N analyses. All newly synthesized compounds were screened for their *in vitro* antibacterial activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Streptococcus pyogenes* and for antifungal activity against *Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Penicillium marneffei* and *Mucor*. Compounds 7b, 7e, 7f, 7g, 7h and 7j showed excellent *in vitro* antibacterial activity and antifungal activity compared with the standard drugs.

Key words: 2-benzylidene-phenylureido-thiazolopyrimidine, antibacterial, antifungal activities

INTRODUCTION

Pyrimidine, being an integral part of DNA and RNA, has imparted diverse pharmacological properties as an effective bactericide and fungicide [1–3]. Certain pyrimidine derivatives were also known to exhibit analgesic [4], antihypertensive [5], anti-tumor [6], antimalarial [7], antioxidant [8], antimitotic [9], and anti-HIV activities [10]. Some of the dihydropyrimidines (DHPM) have emerged as integral backbones of several calcium channel blockers, antihypertensive agents, adrenergic and neuropeptide antagonists [11]. Several alkaloids containing dihydropyrimidine have been isolated from marine sources and, among them, the batzelladine alkaloids were found to be potent HIV-gp-120-CD4 inhibitors [12–14].

In addition to diverse biological activities of pyrimidine, other heterocycles in association with pyrimidines play an essential role in several biological processes [15, 16] and have a considerable chemical and pharmacological importance. Pyrimidines in association with thiazole have occupied a prominent place in medicinal chemistry because of their significant properties as therapeutics in clinical application. A survey of literature revealed that thiazole derivatives of pyrimidine have received much attention during recent years on account of their prominent utilization as analgesic, anti-inflammatory, ulcerogenic [17], antibacterial, antifungal [18–20], antitubercular [21], antimalarial [22], antitumor [23], cytotoxic [24], and anti cancer agents [25].

In the view of the facts mentioned above and as part of our initial efforts to discover potentially active new agents, some new 2-benzylidene-phenylureido-thiazolopyrimidine

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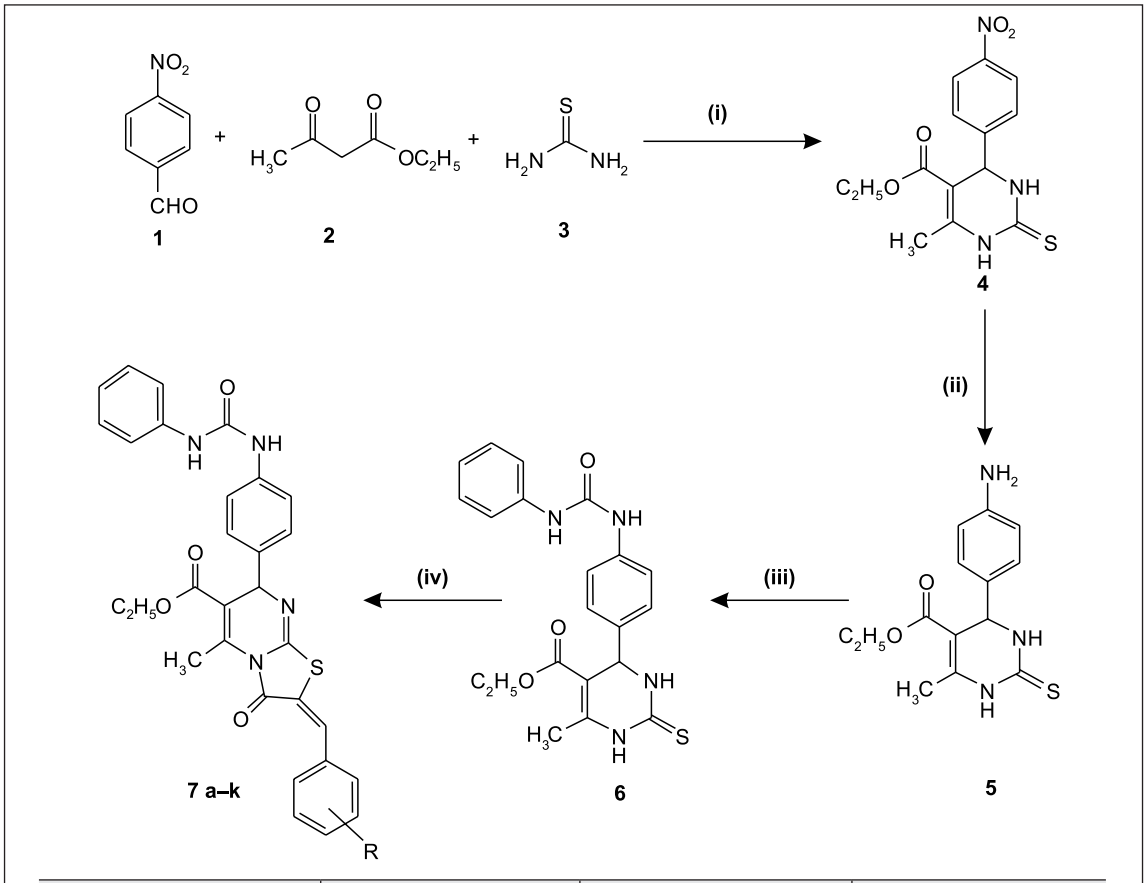
derivatives (7a–k) have been synthesized. The novel derivatives were characterized by spectral data and elemental analysis, and these compounds were tested for their antibacterial and anti-fungal screening.

RESULTS AND DISCUSSION

Chemistry

The synthesis of ethyl 2-(substitutedbenzylidene)-5-methyl-3-oxo-7-(4-(3-phenylureido) phenyl)-3,7-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxylate derivatives (7a–k) was achieved through an efficient and versatile synthetic route outlined in Scheme 1. The desired compounds were synthesized as follows: initially, nitrobenzaldehyde (1) was treated with 1,3-dicarbonyl compound (2) and thiourea (3) in the presence of iodine, which afforded the corresponding ethyl 6-methyl-4-(4-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4) [26]. The reduction of the nitro group of the compound (4) using $SnCl_2$ yielded ethyl 4-(4-aminophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (5). Then, 5 was reacted with different phenylisocynate to afford ethyl 4-(4-(3-(2-phenyl)ureido)-phenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6) [27]. Finally, the target molecules (7a–k) were synthesized by the reaction of (6) with substituted aromatic aldehydes in the presence of anhydrous sodium acetate to afford the titled compounds (Scheme).

All the synthesized compounds were obtained from moderate to high yields. Products were purified and characterized by various spectroscopic techniques. The IR spectra of compounds (7a–k) showed characteristic absorption bands at 3 332–3 304 cm^{-1}, 1 744–1 704 cm^{-1}, 1 619–1 568, and 1 546–1 513 cm^{-1} corresponding to the N-H_{str}, C=O_{str}, C=N_{str}, and C=C_{str} functions in the structures. Similarly, the ^{1}H NMR



Compound	R	Compound	R
7a	4-H	**7g**	4-NO_2
7b	2-Cl	**7h**	2,4-$(CH_3)_2$
7c	4-Cl	**7i**	4-NH_2
7d	4-CH_3	**7j**	4-C_2H_5
7e	4-CH_3O	**7k**	4-$CH(CH_3)_2$
7f	4-F	–	–

Scheme. Synthetic pathway for compound 7 a–k

Reagents and conditions: (i) – I_2, acetonitrile, reflux, 8h; (ii) – stannous chloride, ethyl acetate, rt, 3 h; (iii) – substituted phenyl isocynates, THF, rt, 6 h; (iv) – $ClCH_2COOH$, sodium acetate, Ac_2O/AcOH

spectra showed peaks due to in the range of δ 1.17–1.25 for $OCH_2\underline{CH_3}$, δ 5.04–5.16 for –CH, δ 7.96–8.15 for =CH and δ 9.29–9.71 for –NH. The mass spectrum of all the compounds showed the molecular ion peak at M^+, at M+H corresponding to its molecular formula, which confirmed its chemical structure. The IR, ^{1}H NMR, LCMS mass spectra and elemental analysis showed the structure of various novel ethyl 2-(substitutedbenzylidene)-5-methyl-3-oxo-7-(4-(3-phenylureido)phenyl)-3,7-dihydro-2*H*-thiazolo [3,2-a]pyrimidine-6-carboxylate derivatives (7a–k).

Biological assay

All the compounds were screened for their antibacterial activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Streptococcus pyogenes* and antifungal activity against *Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Penicillium marneffei* and *Mucor*. Compounds 7b–k with various stubstituents in the aromatic ring will be useful in understanding the influence of steric and electronic effects on the biological activity.

For evaluating the antibacterial activity Sparfloxacin was used as the standard drug. The observed minimum inhibitory concentrations (MIC) are given in Table 1. In general, all the synthesized compounds exert a wide range of the modest antibacterial activity *in vitro* against the tested organisms. Compound 7a without any substituent in the aryl moiety exhibits the antibacterial activity *in vitro* at 100 μg ml^{-1} against *P. aeruginosa*. It exhibits the antibacterial activity against the other tested organisms only at 200 μg ml^{-1}. However, 7b, in which hydrogen at the *para* position of the aryl moiety is replaced by chlorine, shows activity against all the tested organisms in the range of 25–50 μg ml^{-1}. Compound 7c, which has chlorine in the *ortho* position, has the same activity as 7b, which has chlorine in the *para* position. Indeed, the compounds 7b–k, bearing a substituent in the aryl group, are more active than the parent compound 7a. Compound 7a and its isopropyl analogue 7k have the same activity against *Klebsiella pneumonia*. However, 7k is more active than 7a against all the other tested organisms. Only 7f which has a nitro group at the para position, 7i having an amide group at the *para* position and 7 g with a fluorine group in the *para* position are more active than the reference drug Sparfloxacin.

For evaluating the antifungal activity, Clotrimazole is used as the standard drug. The observed minimum inhibi-

Table 1. ***In vitro*** **antibacterial activity of compounds 7a–k**

Compound	Minimum inhibitory concentration (MIC) in μg ml^{-1}				
	S. aureus	***E. coli***	***K. pneumoniae***	***P. aeruginosa***	***S. pyogenes***
7a	200	200	200	100	200
7b	25	25	50	50	25
7c	25	25	50	50	25
7d	50	50	100	100	100
7e	50	50	100	50	50
7f	12.5	25	25	25	12.5
7g	12.5	12.5	12.5	12.5	12.5
7h	100	100	100	100	50
7i	25	12.5	12.5	25	12.5
7j	50	100	50	25	100
7k	100	50	200	100	50
Sparfloxacin	**25**	**25**	**50**	25	12.5

Table 2. ***In vitro*** **antifungal activity of compounds**

Compound	Minimum inhibitory concentration (MIC) in μg ml^{-1}				
	A. flavus	***A. fumigatus***	***C. albicans***	***P. marneffei***	***Mucor***
7a	100	–	–	200	200
7b	25	50	50	25	50
7c	25	100	50	50	50
7d	100	200	50	50	100
7e	100	50	50	50	100
7f	12.5	12.5	25	25	12.5
7g	12.5	12.5	12.5	12.5	12.5
7h	100	200	100	100	200
7i	25	12.5	25	12.5	25
7j	50	100	50	50	50
7k	100	50	100	100	100
Clotrimazole	**25**	**25**	**50**	**25**	**50**

'–' No inhibition even at a higher concentration of 200 μg ml^{-1}.

tory concentrations (MIC) are given in Table 2. It is seen that 7a shows no activity against *A. fumigates* and *C. albicans* even at a concentration of 200 μg ml^{-1}. However, it shows the antifungal activity against all the other tested fungi in the range of 100–200 μg ml^{-1}. Compounds 7b–k which have substituents in the 4-aryl group are more active than the parent compound 7a against all the tested fungi. The *o*-chloro compound 7c is less active than the *p*-chloro compound 7b against *A. fumigates* and *Penicillium marneffei*. However, against all the tested fungi, both 7b and 7c have the same activity. Compounds 7f, 7g and 7i are more active than the standard drug Clotrimazole against all the tested organisms.

Influence of aromatic substituent

The results suggest that the antibacterial and antifungal activities are markedly influenced by the aromatic substituents. Thus, 7b, 7c, 7f, 7g and 7i with electron-withdrawing substituents in the aromatic ring show greater antibacterial activity than the other six compounds against all the tested organisms. Also compounds 7f, 7g and 7i show greater antifungal activity than all the other compounds against all the tested organisms. The aromatic substituents in 7b, 7c, 7f, 7g and 7i have positive values [28] for the Hammett substituent constant σ_p.

EXPERIMENTAL

Chemistry

All reagents and solvents were purchased and used without further purification. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Crude products were purified by column chromatography on silica gel of 60–120 mesh. IR spectra were obtained on a Perkin Elmer BX series FT-IR 5000 spectrometer using KBr pellet. NMR spectra were recorded on a varian 300 MHz spectrometer for ^{1}H NMR. The ^{13}C NMR spectra were recorded on JEOL. The chemical shifts were reported as ppm down field using TMS as an internal standard. LCMS mass spectra were recorded on a MASPEC low resolution mass spectrometer operating at 70 eV.

***General procedure for the preparation of ethyl 6-methyl-4-(4-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate* (4)**

4-nitrobenzaldehyde (1 mmol), ethyl acetoacetate (1 mmol), thiourea (1.1 mmol) and iodine (0.5 mmol) in CH_3CN (10 mL) were heated under reflux for 8 h in N_2 atmosphere. The reaction was monitored by TLC. After completion of the reaction the solvent was removed under reduced pressure and the residue was extracted with EtOAc. The EtOAc extracts were washed with a solution of sodium thiosulfate, subsequently with water and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure yielded the solid, which was crystallized from EtOH to afford the pure compound as a white solid. Yield: 88%; ^{1}HNMR (DMSO, 300 MHz): δ 10.44 (bs, 1H, NH), 9.68 (bs, 1H, NH), 8.20–7.12 (m, 4H, Ar-H), 5.14 (d, 1H, CH), 4.04–4.06 (q, J = 7.12, 2H), 2.27 (s, 3H, CH_3), 1.14 (t, 3H); LCMS: (*m/z*) 322 (M+H, 100%).

***General procedure for the preparation of ethyl 4-(4-aminophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate* (5)**

Ethyl 6-methyl-4-(4-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1) (1 mmol) and $SnCl_2$ (1.5 mmol) were taken in ethyl acetate. The resultant reaction mixture was stirred at room temperature and monitored by TLC. After completion, the solvent was removed under reduced pressure and the residue obtained was extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by column chromatography to afford the compound as a white solid.

^{1}HNMR (DMSO, 300 MHz): δ 10.35 (s, 1H), 9.35 (s, 1H), 6.79–7.22 (m, 4H, ArH), 5.30 (bs, 2H), 4.61 (d, 1H), 4.00 (q, 2H), 2.25 (s, 3H), 1.07 (t, 3H); LCMS: (*m/z*) 292 (M+H, 100%).

***Synthesis of ethyl 4-(4-(3-(2-phenyl)ureido)phenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate* (6)**

Ethyl 4-(4-aminophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2) (1 mmol) and substituted phenylisocynate (1.2 mmol) were taken in THF. The resultant reaction mixture was stirred at room temperature for a period of 10 h, after completion of the reaction. The reaction mixture was diluted with hexane and resultant solid was filtered to obtain the crude. This was purified by precipitation using dichloromethane and hexane to obtain a title compound as a white solid.

***General procedure for the preparation of ethyl 2-(substitutedbenzylidene)-5-methyl-3-oxo-7-(4-(3-phenylureido)phenyl)-3,7-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxylate derivatives* (7a–k)**

A mixture of compound 6 (1 mmol), chloroacetic acid (1 mmol), sodium acetate anhydrous (1.5 g) was taken in a round bottom flask, to this glacial acetic acid and acetic acid anhydride (40 mL, 3 : 1) were added and refluxed for 12 min., then to this equimolecular amount the appropriate aromatic aldehydes were added. The reaction mixture was refluxed for 2 h, allowed to cool, poured onto cold water; the formed precipitate was filtered off, dried and recrystallized from a proper solvent to give the corresponding 2-benzylidene-phenylureido-thiazolopyrimidine derivatives (7a–k), respectively.

***Ethyl 2-benzylidene-5-methyl-3-oxo-7-(4-(3-phenylureido) phenyl)-3,7-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxylate* (7a)**

Yield: 81%; mp 158–160 °C; IR (KBr) υ 3 304 (N–H), 1 704 (C=O), 1 578 (C=N), 1 527 (C=C), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (t, J = 7.12 Hz, 3H, $OCH_2\underline{CH_3}$), 2.21 (s, 3H,

Ar$\underline{CH_3}$), 4.01 (q, J = 7.12 Hz, 2H, O$\underline{CH_2}CH_3$), 5.04 (d, 1H, –CH), 7.16–7.63 (m, 14H, Ar-H), 7.96 (s, 1H, =CH), 9.58 (s, 2H, NH); ^{13}C NMR (DMSO-d_6, δ, ppm) 11.6, 13.3, 58.4, 63.0, 109.2, 117.0, 120.2, 120.6, 125.8, 127.1, 127.4, 127.8, 128.0, 128.9, 132.2, 133.3, 137.4, 139.6, 142.2, 144.5, 154.2, 161.2, 165.6, 171.1; LCMS: (*m/z*) 538 (M^+, 100%). Anal. calcd. for $C_{30}H_{26}N_4O_4S$: C, 66.89; H, 4.87; N, 10.40. Found: C, 66.93; H, 4.78; N, 10.49.

***Ethyl 2-(2-chlorobenzylidene)-5-methyl-3-oxo-7-(4-(3-phenylureido)phenyl)-3,7-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxylate* (7b)**

Yield: 76%; mp 189–190 °C; IR (KBr) υ 3 330 (N–H), 1 708 (C=O), 1 574 (C=N), 1 539 (C=C), 713 (C–Cl), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (t, J = 7.08 Hz, 3H, OCH$_2\underline{CH_3}$), 2.22 (s, 3H, Ar$\underline{CH_3}$), 4.06 (q, J = 7.14 Hz, 2H, O$\underline{CH_2}CH_3$), 5.11 (d, 1H, –CH), 7.18–7.54 (m, 13H, Ar-H), 8.15 (s, 1H, =CH), 9.71 (s, 2H, NH); LCMS: (*m/z*) 573 (M+H, 100%). Anal. calcd. for $C_{30}H_{25}ClN_4O_4S$: C, 62.88; H, 4.40; N, 9.78. Found: C, 62.95; H, 4.47; N, 9.86.

***Ethyl 2-(4-chlorobenzylidene)-5-methyl-3-oxo-7-(4-(3-phenylureido)phenyl)-3,7-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxylate* (7c)**

Yield: 73%; mp 195–197 °C; IR (KBr) υ 3 312 (N–H), 1 716 (C=O), 1 590 (C=N), 1 546 (C=C), 710 (C-Cl), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20 (t, J = 7.24 Hz, 3H, OCH$_2\underline{CH_3}$), 2.21 (s, 3H, Ar$\underline{CH_3}$), 4.04 (q, J = 7.22 Hz, 2H, O$\underline{CH_2}CH_3$), 5.08 (d, 1H, –CH), 7.23–7.60 (m, 13H, Ar-H), 8.06 (s, 1H, =CH), 9.67 (s, 2H, NH); ^{13}C NMR (DMSO-d_6, δ, ppm) 12.1, 13.8, 58.4, 63.5, 110.5, 118.2, 120.5, 120.7, 126.4, 127.4, 127.8, 128.2, 128.4, 133.3, 134.1, 137.6, 138.2, 139.6, 142.4, 144.7, 154.1, 161.3, 165.7, 171.3; LCMS: (*m/z*) 573 (M+H, 100%). Anal. calcd. for $C_{30}H_{25}ClN_4O_4S$: C, 62.88; H, 4.40; N, 9.78. Found: C, 62.93; H, 4.49; N, 9.84.

***Ethyl2-(4-methylbenzylidene)-5-methyl-3-oxo-7-(4-(3-phenylureido)phenyl)-3,7-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxylate* (7d)**

Yield: 85%; mp 221 °C; IR (KBr) υ 3 308 (N–H), 1 710 (C=O), 1 568 (C=N), 1 528 (C=C), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.17 (t, J = 6.98 Hz, 3H, OCH$_2\underline{CH_3}$), 2.18 (s, 3H, Ar$\underline{CH_3}$), 2.30 (s, 3H, Ar$\underline{CH_3}$), 4.02 (q, J = 7.12 Hz, 2H, O$\underline{CH_2}CH_3$), 5.06 (d, 1H, –CH), 7.11–7.55 (m, 13H, Ar-H), 7.98 (s, 1H, =CH), 9.29 (s, 2H, NH); ^{13}C NMR (DMSO-d_6, δ, ppm) 11.4, 13.6, 19.3, 58.1, 62.8, 109.5, 118.1, 120.3, 120.7, 127.1, 127.3, 127.8, 128.1, 128.4, 129.2, 131.4, 133.3, 136.6, 137.5, 139.7, 141.8, 144.2, 154.1, 161.2, 164.9, 171.2; LCMS: (*m/z*) 553 (M+H, 100%). Anal. calcd. for $C_{31}H_{28}N_4O_4S$: C, 67.37; H, 5.11; N, 10.14. Found: C, 67.25; H, 5.19; N, 10.21.

***Ethyl 2-(4-methoxybenzylidene)-5-methyl-3-oxo-7-(4-(3-phenylureido)phenyl)-3,7-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxylate* (7e)**

Yield: 79%; mp 189–191 °C; IR (KBr) υ 3 326 (N–H), 1 714 (C=O), 1 575 (C=N), 1 533 (C=C), 1 208 (C–O–C), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.25 (t, J = 7.14 Hz, 3H, OCH$_2\underline{CH_3}$), 2.24 (s, 3H, Ar$\underline{CH_3}$), 3.77 (s, 3H, ArO$\underline{CH_3}$), 4.08 (q, J = 7.18 Hz, 2H, O$\underline{CH_2}CH_3$), 5.13 (d, 1H, –CH), 7.01–7.58 (m, 13H, Ar-H), 8.03 (s, 1H, =CH), 9.64 (s, 2H, NH); LCMS: (*m/z*) 568 (M^+, 100%). Anal. calcd. for $C_{31}H_{28}N_4O_5S$: C, 65.48; H, 4.96; N, 9.85. Found: C, 65.57; H, 5.06; N, 9.73.

***Ethyl 2-(4-fluorobenzylidene)-5-methyl-3-oxo-7-(4-(3-phenylureido)phenyl)-3,7-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxylate* (7f)**

Yield: 90%; mp 210–212 °C; IR (KBr) υ 3 332 (N–H), 1 720 (C=O), 1 605 (C=N), 1 521 (C=C), 1 012 (C–F), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.24 (t, J = 7.14 Hz, 3H, OCH$_2\underline{CH_3}$), 2.23 (s, 3H, Ar$\underline{CH_3}$), 4.09 (q, J = 7.18 Hz, 2H, O$\underline{CH_2}CH_3$), 5.14 (d, 1H, –CH), 7.21–7.77 (m, 13H, Ar-H), 8.02 (s, 1H, =CH), 9.70 (s, 2H, NH); LCMS: (*m/z*) 557 (M+H, 100%). Anal. calcd. for $C_{30}H_{25}FN_4O_4S$: C, 64.74; H, 4.53; N, 10.07. Found: C, 64.83; H, 4.62; N, 9.95.

***Ethyl 2-(4-nitrobenzylidene)-5-methyl-3-oxo-7-(4-(3-phenylureido)phenyl)-3,7-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxylate* (7g)**

Yield: 77%; mp 231–233 °C; IR (KBr) υ 3 326 (N–H), 1 719 (C=O), 1 588 (C=N), 1 521 (C=C), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.25 (t, J = 7.14 Hz, 3H, OCH$_2\underline{CH_3}$), 2.25 (s, 3H, Ar$\underline{CH_3}$), 4.11 (q, J = 7.20 Hz, 2H, O$\underline{CH_2}CH_3$), 5.16 (d, 1H, –CH), 7.21–7.58 (m, 9H, Ar-H), 7.98–8.17 (m, 4H, Ar-H), 8.07 (s, 1H, =CH), 9.71 (s, 2H, NH); LCMS: (*m/z*) 584 (M+H, 100%). Anal. calcd. for $C_{30}H_{25}N_5O_6S$: C, 61.74; H, 4.32; N, 12.00. Found: C, 61.67; H, 4.39; N, 12.17.

***Ethyl 2-(2,4-dimethylbenzylidene)-5-methyl-3-oxo-7-(4-(3-phenylureido)phenyl)-3,7-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxylate* (7h)**

Yield: 83%; mp 202–204 °C; IR (KBr) υ 3 310 (N–H), 1 710 (C=O), 1 583 (C=N), 1 532 (C=C), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.17 (t, J = 7.18 Hz, 3H, OCH$_2\underline{CH_3}$), 2.19 (s, 3H, Ar$\underline{CH_3}$), 2.28 (s, 3H, Ar$\underline{CH_3}$), 2.41 (s, 3H, $\underline{CH_3}$), 4.02 (q, J = 7.20 Hz, 2H, O$\underline{CH_2}CH_3$), 5.02 (d, 1H, –CH), 7.03–7.55 (m, 12H, Ar-H), 8.13 (s, 1H, =CH), 9.48 (s, 2H, NH); LCMS: (*m/z*) 566 (M^+, 100%). Anal. calcd. for $C_{32}H_{30}N_4O_4S$: C, 67.82; H, 5.34; N, 9.89. Found: C, 67.74; H, 5.41; N, 10.02.

***Ethyl 2-(4-aminobenzylidene)-5-methyl-3-oxo-7-(4-(3-phenylureido)phenyl)-3,7-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxylate* (7i)**

Yield: 78%; mp 197–198 °C; IR (KBr) υ 3 328 (N–H), 1 718 (C=O), 1 619 (C=N), 1 530 (C=C), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.23 (t, J = 7.06 Hz, 3H, OCH$_2\underline{CH_3}$), 2.24 (s, 3H, Ar$\underline{CH_3}$), 4.04 (q, J = 6.98 Hz, 2H, O$\underline{CH_2}CH_3$), 5.10 (d, 1H, –CH), 6.58–7.60 (m, 13H, Ar-H), 8.01 (s, 1H, =CH), 9.69 (s, 2H, NH); LCMS: (*m/z*) 554 (M+H, 100%). Anal. calcd. for $C_{30}H_{27}N_5O_4S$: C, 65.08; H, 4.92; N, 12.65. Found: C, 65.17; H, 5.07; N, 12.73.

***Ethyl 2-(4-ethylbenzylidene)-5-methyl-3-oxo-7-(4-(3-phenylureido)phenyl)-3,7-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxylate* (7j)**

Yield: 68%; mp 216–218 °C; IR (KBr) υ 3 312 (N–H), 1 744 (C=O), 1 608 (C=N), 1 513 (C=C), cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.17–1.19 (m, 6H, $OCH_2\underline{CH_3}$ & $CH_2\underline{CH_3}$), 2.21 (s, 3H, $Ar\underline{CH_3}$), 2.48 (q, 2H, $\underline{CH_2}CH_3$), 4.03 (q, J = 7.22 Hz, 2H, $O\underline{CH_2}CH_3$), 5.04 (d, 1H, –CH), 6.96–7.60 (m, 13H, Ar-H), 7.96 (s, 1H, =CH), 9.49 (s, 2H, NH); ^{13}C NMR (DMSO-d_6, δ, ppm) 11.8, 13.1, 13.7, 27.6, 58.1, 63.2, 110.1, 118.1, 120.5, 120.6, 126.4, 127.1, 127.7, 128.3, 128.9, 133.2, 137.5, 138.8, 139.5, 142.2, 144.4, 144.9, 154.1, 161.2, 165.4, 171.3; LCMS: (*m/z*) 566 (M^+, 100%). Anal. calcd. for $C_{32}H_{30}N_4O_4S$: C, 67.82; H, 5.34; N, 9.89. Found: C, 67.76; H, 5.40; N, 10.00.

***Ethyl 2-(4-isopropylbenzylidene)-5-methyl-3-oxo-7-(4-(3-phenylureido)phenyl)-3,7-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxylate* (7k)**

Yield: 88%; mp 234–236 °C; IR (KBr) υ 3 317 (N–H), 1 704 (C=O), 1 604 (C=N), 1 524 (C=C), cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.98 (d, 6H, $CH(\underline{CH_3})_2$), 1.18 (t, J = 7.12 Hz, 3H, $OCH_2\underline{CH_3}$), 2.20 (s, 3H, $Ar\underline{CH_3}$), 2.78 (m, 1H, $\underline{CH}(CH_3)_2$), 4.04 (q, J = 7.20 Hz, 2H, $O\underline{CH_2}CH_3$), 5.06 (d, 1H, –CH), 7.19–7.58 (m, 13H, Ar-H), 7.98 (s, 1H, =CH), 9.52 (s, 2H, NH); LCMS: (*m/z*) 581 (M+H, 100%). Anal. calcd. for $C_{33}H_{32}N_4O_4S$: C, 68.25; H, 5.55; N, 9.65. Found: C, 68.33; H, 5.63; N, 9.57.

Biological screening

Antibacterial screening

The *in vitro* antibacterial activity of the compounds was tested in nutrient broth for bacteria by twofold serial dilution method [29]. The test compounds were dissolved in dimethyl sulphoxide (DMSO) to obtain 1 mg/ml stock solutions. Seeded broths (broth containing microbial spores) were prepared in NB from 24 h old bacterial cultures on nutrient agar at 37 ± 1 °C. The colony forming units (cfu) of the seeded broth were determined by the plating technique and adjusted in the range of 10^4–10^5 cfu/ml. For antibacterial assay the final inoculums size was 10^5 cfu/ml. Testing was performed at pH 7.4 ± 0.2. Exactly 0.2 ml of the solution of the test compound was added to 1.8 ml of the seeded broth to form the first dilution. One ml of this was diluted with a further 1 ml of the seeded broth to give the second dilution and so on until six such dilutions were obtained. A set of assay tubes containing only the seeded broth were kept as controls and likewise solvent controls were also run simultaneously. The tubes were incubated in biochemical oxygen demand (BOD) incubators at 37 ± 1 °C for bacteria. The minimum inhibiting concentrations (MICS) were recorded by visual observations after 24 h. Sparfloxacin was used as a standard for the antibacterial study.

Antifungal screening

The *in vitro* antifungal activity of the compounds was tested in Sabouraud's dextrose broth for fungi by twofold serial dilution method [29]. The test compounds were dissolved in dimethyl sulphoxide (DMSO) to obtain 1 mg/ml stock solutions. Seeded broth (broth containing microbial spores) was prepared by suspending 24 h to 7-days old Sabouraud's agar slant cultures in SDB. The colony forming units (cfu) of the seeded broth were determined by the plating technique and adjusted in the range of 10^4–10^5 cfu/ml. For antifungal assay the final inoculums size was $1.1–1.5 \times 10^2$ cfu/ml. Testing was carried out by the same procedure as done for antibacterial studies except the temperature which was maintained at 28 ± 1 °C for about 72–96 h of incubation. Clotrimazole was used as a standard for the antifungal study.

CONCLUSIONS

In conclusion, a simple and efficient protocol for the synthesis of novel 2-benzylidene-phenylureido-thiazolopyrimidine derivatives (7a–k) with good yields was described. All the synthesized compounds were investigated for their antibacterial and antifungal activities. With the newly synthesized compounds, it is evident that 7b, 7e, 7f, 7g, 7h and 7j have the highest antibacterial and antifungal activity. Accordingly, these novel classes of new 2-benzylidene-phenylureido-thiazolopyrimidine derivatives reported from the laboratory emerge as a valuable lead series with great potential to be used as antibacterial and antifungal agents, and as promising candidates for further efficacy evaluation.

ACKNOWLEDGEMENTS

The authors are thankful to the authorities of School of Chemistry, University of KwaZulu-Natal, Westville Campus, Durban, South Africa & Sri Venkateswara University, Tirupati, A. P. India for the facilities and encouragement.

Received 2 November 2011
Accepted 13 December 2012

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NAUJŲ (2-BENZILIDEN)-FENILUREIDO-TIAZOLOPIRIMIDINO DARINIŲ SINTEZĖ IR ANTIMIKROBINIS TYRIMAS

S a n t r a u k a

Susintetinta naujų (2-benziliden)-fenilureido-tiazolopirimidino darinių. Buvo tirtas visų naujų junginių antibakterinis aktyvumas *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* ir *Streptococcus pyogenes*, o taip pat priešgrybelinis veikimas *Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Penicillium marneffei* ir *Mucor*. Kai kurie tirti junginiai pasižymi geru antibakteriniu ir priešgrybeliniu aktyvumu, pranokstančiu standartinių preparatų aktyvumą.