Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 14 (2004) 4185–4190

Synthesis and QSAR studies of pyrimido[4,5-*d*]pyrimidine-2,5-dione derivatives as potential antimicrobial agents

Pratibha Sharma,* Nilesh Rane and V. K. Gurram

School of Chemical Sciences, Devi Ahilya University, Indore, MP 452 017, India

Received 3 May 2004; accepted 8 June 2004
Available online 2 July 2004

Abstract—A number of pyrimido[4,5-*d*]pyrimidine-2,5-dione derivatives were synthesized and screened for antibacterial and antifungal activities. All the synthesized compounds showed the potent antimicrobial activity. The quantitative structure–activity relationship investigation was applied to find a correlation between the different physicochemical parameters of the compounds studied and their biological activity.
© 2004 Elsevier Ltd. All rights reserved.

1. Introduction

Pyrimidopyrimidine, a condensed heterocycles have attracted considerable interest in the recent years. Its derivatives have been known to display a wide range of pharmacological activities, and their potent inhibitory properties as regards the tyrosine kinase domain of epidermal growth factor receptor,[1] 5-phosphoribosyl-1-pyrophosphate synthetase[2] and dihydrofolate reductase[3] have been fully demonstrated. Numerous reports delineate the antitumour,[4] antiviral[4,5] (as inhibitor of herpes simplex virus reactivation and viral protein synthesis), antioxidant[6] (as lipid peroxidation inhibitors), antifungal[7] and hepatoprotective[8] activity of these compounds.

Motivated by the aforementioned findings, and in continuation of our enduring studies[9–12] on versatile heterocycles of pharmacological importance, it was considered worthwhile to design the synthesis of some bioactive derivatives of pyrimido[4,5-*d*]pyrimidine-2,5-dione, which can be envisaged as the new lead compounds.

Further, rising prevalence of multidrug resistant pathogens has led us to screen these synthesized compounds against the panel of Gram-positive and Gram-negative bacteria and fungi in a view to search for a new class of antimicrobial agents.

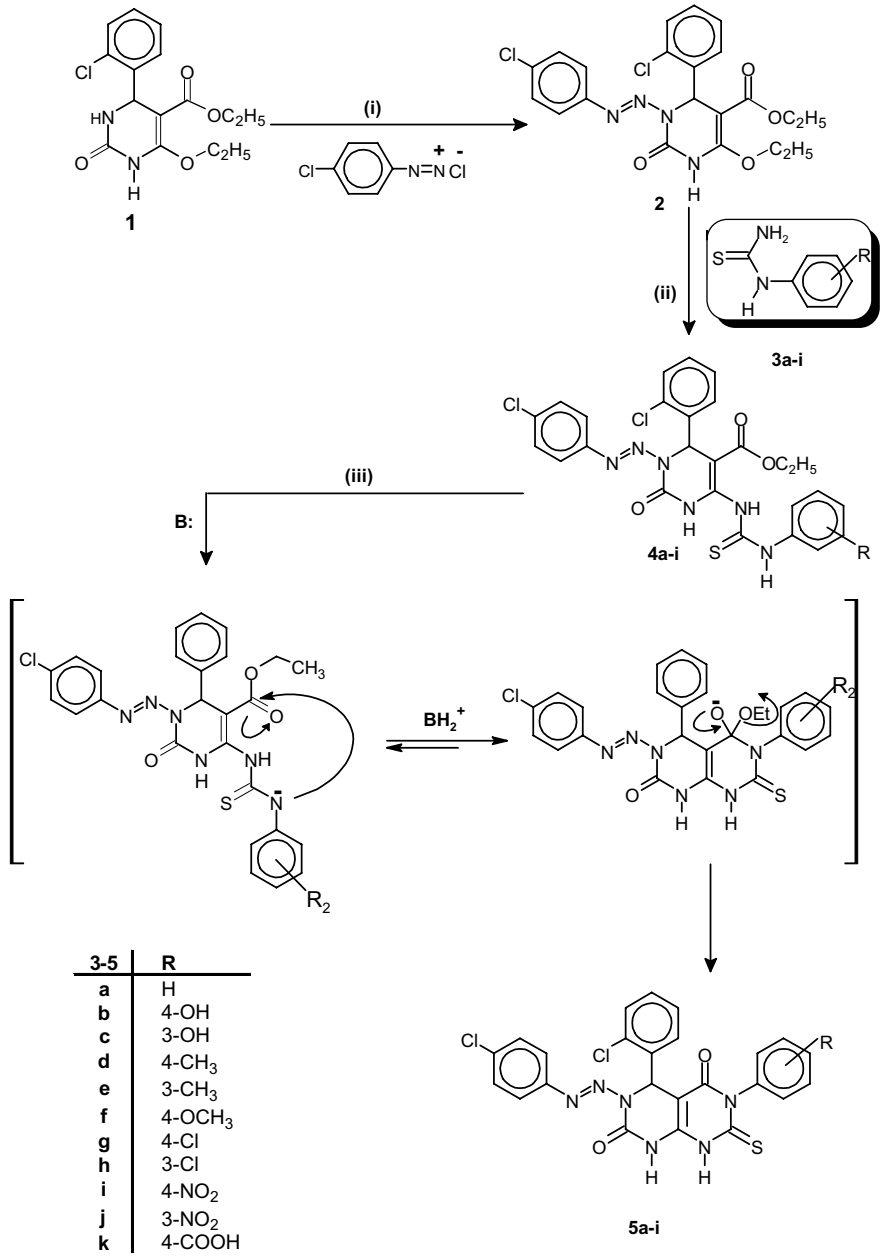
In the present study we have attempted an expeditious synthesis of a series, namely 4-(2-chlorophenyl)-3-(4-chlorophenylazo)-6-aryl-7-thioxo-4,6,7,8-tetrahydro-1*H*, 3*H*-pyrimido[4,5-*d*]pyrimidine-2,5-dione, incorporating first time biginelli compound as the precursor followed by their in vitro antibacterial and antifungal screening studies.

2. Chemistry

The most promising methods for the synthesis of pyrimido[4,5-*d*]pyrimidine are multistep synthesis starting from 1,3-disubstituted-5-cyanouracils[13] or from polymer bound 2-(alkylsulfanyl)-4-aminopyrimidine-5-carbonitrile.[14] However, our synthetic strategy commences from biginelli compound, which led to the construction of requisite pyrimido[4,5-*d*]pyrimidine nucleus. Thus the synthesis of precursor ethyl-4-(2-chlorophenyl)-6-ethoxy-2-oxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate **1** was achieved as per standard biginelli condensation reaction. This key compound was stirred (3 h) in an equimolar quantity with freshly prepared 4-chlorophenyldiazonium chloride in concentrated hydrochloric acid, under diazotization conditions to give compound **2**. Subsequently, this diazotized derivative **2** was allowed to reflux with various arylthioureas **3a**–**k** in ethanol for 3 h affording 4-(2-chlorophenyl)-2-oxo-3-(4-chlorophenylazo)-6-(*N*-arylthio ureido)-5-ethyl-carboxylato-1,2,3,4-tetrahydropyrimidine **4a**–**k** in

Keywords: Pyrimido[4,5-*d*]pyrimidine; Synthesis; Antibacterial; Antifungal; QSAR; Molar refractivity.
* Corresponding author. Tel.: +91-7312460208; fax: +91-7312470372; e-mail: drpratibhasharma@yahoo.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.06.014



Scheme 1. Reagents and conditions: (i) 0–5 °C, 3 h; (ii) EtOH, reflux, 4 h; (iii) CH_3OH/CH_3ONa, rt, 6 h.

70–80% yield. The corresponding target compounds **5a**–**k** were obtained by intramolecular cyclization of **4a**–**k** under stirring at 0 °C in a solution of sodium methoxide and methanol in an excellent yields. An overview of synthetic pathway is depicted in Scheme 1.

Conclusively, pyrimidine nucleus fused to tetrahydro pyrimidine ring was generated by cyclocondensation between C–C–C fragment derived from biginelli compound and N–C–N fragment provided by arylthiourea. Also, the key step in the cyclization involves base catalyzed intramolecular addition of the ureido nitrogen anion to the carbonyl group of ester as depicted in Scheme 1.

The IR, ^{1}H NMR and mass spectral data of all the reported compounds were found in agreement with the assigned structures.

3. Biological studies

The newly obtained derivatives were evaluated for in vitro antibacterial activity against *Escherichia coli* ATCC 13607, *Pseudomonas diminuta* MTCC 3361, *Staphylococcus aureus* ATCC 2943, *Bacillus subtilis* ATCC 6633 and antifungal activity against *Aspergillus niger* ATCC 16404 and *Candida albicans* ATCC 10231. Nutrient agar and Saboured dextrose agar were

Table 1. The in vitro antimicrobial activity of synthesized pyrimido[4,5-*d*]pyrimidine-2,5-dione derivative **5a–k**

Compd	MIC in μg/mL											
	Gram-negative bacteria				Gram-positive bacteria				Fungi			
	E. coli ATCC 13607		*P. diminuta* MTCC 3361		*S. aureus* ATCC 2943		*B. subtilis* ATCC 6633		*A. niger* ATCC 16404		*C. albicans* ATCC 10231	
	MIC	−log MIC	MIC	−log MIC	MIC	−log MIC	MIC	−log MIC	MIC	−log MIC	MIC	−log MIC
5a	40	4.116	46	4.055	32	4.213	37	4.15	57	3.962	52	4.002
5b	12	4.667	15	4.57	8	4.843	10	4.746	27	4.314	23	4.384
5c	10	4.746	13	4.632	6	4.968	8	4.843	24	4.366	20	4.445
5d	26	4.316	31	4.24	21	4.409	22	4.389	42	4.108	39	4.14
5e	22	4.389	25	4.333	18	4.476	21	4.409	39	4.14	35	4.187
5f	17	4.499	21	4.407	11	4.688	16	4.525	33	4.211	29	4.267
5g	13	4.616	14	4.583	9	4.775	12	4.65	29	4.267	24	4.349
5h	4	5.14	5	5.043	2	5.441	4	5.14	14	4.596	11	4.701
5i	7	4.909	9	4.8	4	5.152	6	4.976	19	4.475	15	4.578
5j	6	4.976	8	4.851	3	5.277	5	5.055	16	4.55	13	4.64
5k	8	4.85	11	4.712	5	5.054	7	4.908	22	4.411	17	4.523
A	49	—	15	—	50	—	10	—	NT	NT	NT	NT
C	NT	NT	NT	NT	NT	NT	NT	NT	20	—	25	—

A = ampicilin, C = clotrimazole, NT = not tested.

employed for bacterial and fungal growth, respectively. Minimal Inhibitory Concentrations (MIC) were determined by means of standard twofold serial dilution method using agar media[15] and reported in Table 1. Stock solutions of tested compounds were prepared in DMSO at a concentration of 1 mg/mL. Suspension containing approximately 10^7 CFUs/mL of bacteria and 10^6 CFUs/mL of fungi were prepared from broth cultures. Bacterial and fungal plates were made in triplicate and incubated at 37 °C within 16–24 h for bacteria and 48–72 h for fungi. Ampicillin trihydrate and clotrimazole were also screened under similar conditions as reference antibacterial and antifungal drug, respectively. MIC is defined as the lowest concentration of compound that inhibited visible growth.

All the reported compounds exhibited remarkable in vitro activity against the tested bacterial and fungal strains compared to reference drugs within a MIC range of 2–57 μg/mL. A systematic perusal of the data depicted in Table 1, reveals that in comparison to antifungal behaviour, manifested by these compounds, their antibacterial effect is observed to be highly significant and pronounced. Particularly, Gram-positive bacteria are seems to be more sensitive towards the newly synthesized pyrimido[4,5-*d*]pyrimidine analogues. However, these compounds have been found to show the least to moderate activity on the growth of *A. niger* with MIC range of 14–57 μg/mL. In general antimicrobial activity of the tested compounds follows the pattern:

$$S.\ aureus > B.\ subtilis > E.\ coli > P.\ diminuta > C.\ albicans > A.\ niger$$

Further, a close inspection of screening results reveals that the substitution in the aromatic ring attached at N-6 position of pyrimido[4,5-*d*]pyrimidine nucleus exerted significant influence on the investigated biological activity. Interestingly, an upsurge in the biological activity has been noticed owing to the methylation of 3-OH group. However, compound **5h** was found to be the most potent amongst the series members (MIC: 2–14 μg/mL). The in vitro antimicrobial profile of compounds **5a–k** is shown to be increased when groups such as –OH, –Cl, $–NO_2$ and $–CH_3$ are present at *meta* position as is also inferred by Table 1.

3.1. QSAR analysis

In an attempt to determine the role of structural features, which appears to influence the observed activity of reported compounds, QSAR studies were under taken using the linear free energy relationship (LFER) model of Hansch and Fujita.[16]

Biological activity data, reported as MIC values (Table 1) are first transformed to −log MIC on molar basis and used as dependent variable to get the linear relationship in the QSAR model. These were correlated with different molecular descriptors like energy of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), hardness, dipole, solvent-accessible surface areas (SAS), solvent excluded volume (SEV), log of octanol–water partition coefficient ($\log P$) [17] and physicochemical parameters viz. molar refractivity (M_R) and Hammett (σ) constant. The values of physicochemical parameters have been taken from the list of Skagerberg et al.[18]

To calculate electronic descriptors, geometry of all the compounds has been completely optimized by SCF calculation with the semi empirical AM1[19] method incorporated in the MOPAC 6.0[20] package. Energy minimized geometry of most active member (**5h**)[26] of the series is shown in Figure 1. SAS and SEV for the optimized geometry were subsequently computed by Chem3D[21] software. Hardness is defined as the energy

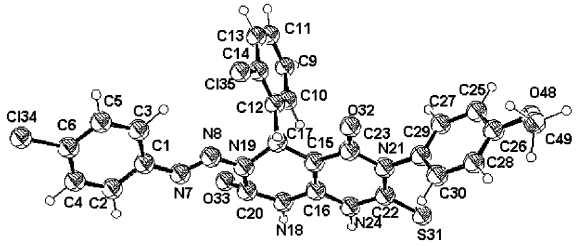


Figure 1. The ORTEP drawing of **5h**[25] (AM1 optimized geometry) with atom numbering. Thermal ellipsoids are scaled to the 50% probability. The atom numbering is arbitrary and has nothing to do with the IUPAC nomenclature.

Table 2. Values of selected descriptors with correlation ($r > 0.45$)

	MR[a]	LUMO (eV)	Hardness[b]	σ	Dipole
5a	0.103	−1.124	8.068	0	1.846
5b	0.603	−1.124	8.138	0.23	2.257
5c	0.603	−1.03	8.165	0.37	2.297
5d	0.285	−1.011	8.106	−0.37	0.934
5e	0.285	−1.018	8.127	0.12	2.153
5f	0.565	−1.017	8.008	−0.17	1.958
5g	0.565	−1.142	8.14	−0.07	1.675
5h	0.787	−1.262	7.838	−0.27	0.919
5i	0.736	−1.395	8.016	0.78	6.275
5j	0.736	−1.332	8.022	0.71	6.782
5k	0.693	−1.192	8.134	0.45	2.57

[a] MR is scaled by factor 0.1.
[b] Hardness is energy difference between HOMO and LUMO.

difference between frontier orbitals (HOMO and LUMO) (Table 2).

Firstly, correlation analysis of various descriptors used in the present study was performed and the Intercorrelated parameters were discarded depending on their individual correlation with biological activity. The resultant parameters were subjected to MLR analysis carried out by the VALSTAT[22] software using the stepwise selection and elimination procedure for variable selection.

However, no parameter showed significant correlation with the biological activity ($r < 0.73$) (Table 3), except molar refractivity for the substituents at the phenyl ring present on N-6 position have been found to exhibit best correlation ($r > 0.9$) of high statistical >99.9% significance. The resulting best-fit models, applying the Parsimony principle are reported in Eqs. 1–6 together with statistical parameters of the regression. It is noteworthy that all these equations were derived using the entire data set of compounds ($n = 11$) since no outliers were identified.

The overall quality of the models is indicated by the correlation coefficient, r, the standard deviation, s, and the Fischer statistic, F.

Qsar model for activity against *S. aureus*

$$\begin{aligned} -\log\mathrm{MIC} &= [3.95451(\pm 0.224041)] \\ &\quad + \mathrm{MR}[1.64329(\pm 0.385169)] \\ n &= 11, \quad r = 0.957, \quad s = 0.117, \\ F &= 96.793, \quad r^2_{\mathrm{cv}} = 0.911 \end{aligned} \tag{1}$$

Qsar model for activity against *B. sibtilis*

$$\begin{aligned} -\log\mathrm{MIC} &= [3.97565(\pm 0.18046)] \\ &\quad + \mathrm{MR}[1.35184(\pm 0.310245)] \\ n &= 11, \quad r = 0.958, \quad s = 0.0943, \\ F &= 100.962, \quad r^2_{\mathrm{cv}} = 0.891 \end{aligned} \tag{2}$$

Qsar model for activity against *E. coli*

$$\begin{aligned} -\log\mathrm{MIC} &= [3.93571(\pm 0.1823)] \\ &\quad + \mathrm{MR}[1.33026(\pm 0.313408)] \\ n &= 11, \quad r = 0.956, \quad s = 0.095, \\ F &= 95.802, \quad r^2_{\mathrm{cv}} = 0.895 \end{aligned} \tag{3}$$

Qsar model for activity against *P. diminuta*

$$\begin{aligned} -\log\mathrm{MIC} &= [3.89505(\pm 0.183578)] \\ &\quad + \mathrm{MR}[1.23809(\pm 0.315605)] \\ n &= 11, \quad r = 0.949, \quad s = 0.096, \\ F &= 81.834, \quad r^2_{\mathrm{cv}} = 0.898 \end{aligned} \tag{4}$$

Table 3. Correlation of biological activity[a] with molecular descriptors

	EC	PD	SA	BS	AN	CA
MR	0.956	0.949	0.957	0.958	0.952	0.956
σ	0.455	0.419	0.460	0.503	0.485	0.499
Log*P*	0.083	0.094	0.106	0.095	0.059	0.066
Dipole	0.436	0.411	0.456	0.472	0.49	0.488
SAS	0.373	0.372	0.383	0.356	0.364	0.363
SEV	0.378	0.377	0.383	0.358	0.357	0.358
E_{HOMO}	0.409	0.398	0.399	0.452	0.425	0.449
E_{LUMO}	0.704	0.703	0.713	0.699	0.716	0.728
Hardness	0.475	0.489	0.502	0.416	0.474	0.459

[a] EC = *E. coli*, PD = *P. diminuta*, SA = *S. aureus*, BS = *B. subtilis*, AN = *A. niger*, CA = *C. albicans*, SAS = solvent accessible area, SEV = solvent excluded volume.

Qsar model for activity against *A. niger*

$$-\log\mathrm{MIC} = [3.85408(\pm 0.120769)] + \mathrm{MR}[0.839611(\pm 0.207624)]$$
$$n = 11, \quad r = 0.952, \quad s = 0.063,$$
$$F = 86.960, \quad r^2_{cv} = 0.889 \qquad (5)$$

Qsar model for activity against *C. albicans*

$$-\log\mathrm{MIC} = [3.86819(\pm 0.130064)] + \mathrm{MR}[0.950431(\pm 0.223604)]$$
$$n = 11, \quad r = 0.956, \quad s = 0.068,$$
$$F = 96.072, \quad r^2_{cv} = 0.899 \qquad (6)$$

The F-value obtained in Eqs. 1–6 is found statistically significant at 99% level.

The predictive power of these equations was also checked by leave one out cross-validated r^2_{cv} value (>0.88). Figure 2 inferred that the calculated and predicted activities for the compounds were in good agreement with the observed activities.

As the molar refractivity reflects the effect of size and polarity of groups, whence it is indicated from Eqs. 1–6, that MR places positive contribution towards the expressed biological activity, possibly due to steric interaction in polar space. It has been suggested that the phenyl ring at N-6 position may be involved in binding of these molecules with the target. Conclusively, overall evaluation of antimicrobial results presented in Table 1 clearly indicate that compounds with higher molar refractivity value exhibited increased inhibitory action on the growth of test bacteria and fungi.

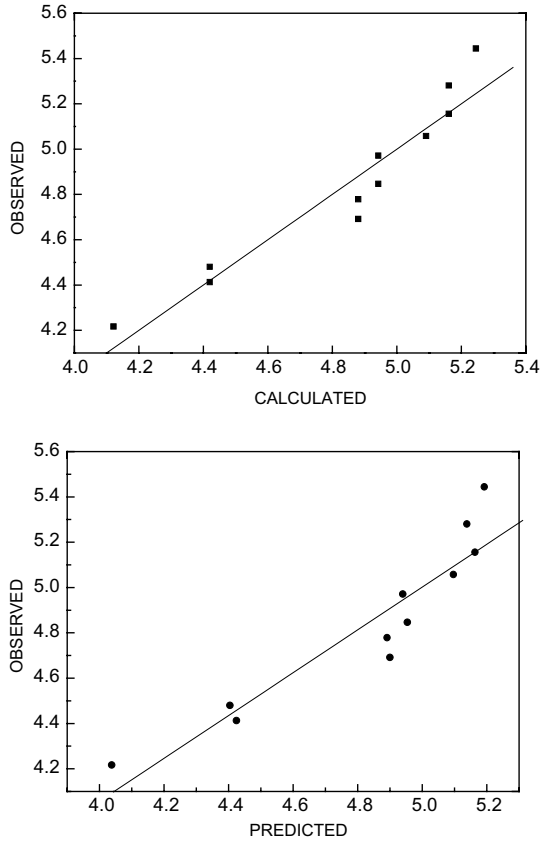


Figure 2. Plots of observed versus calculated and observed versus predicted activity of compounds **5a**–**k** screened against *S. aureus* (Eq. 1).

4. Experimental

Melting points (mp) were determined on an electrothermal apparatus by open capillary method and are uncorrected. The IR spectra were recorded with Shimadzu 460 spectrophotometer in KBr Discs, frequencies are reported in cm^{-1}. ^1H NMR spectra were run on Jeol NMR 400 MHz spectrometer in $CDCl_3$ using TMS as an internal standard. Chemical shifts are reported as δ units (ppm) values. Mass spectra were recorded on Jeol D-300 spectrometer. Purity of all the synthesized compounds were ascertained by TLC resolution on silica gel G (E Merck) using ethyl acetate–xylene (3:7, v/v) as eluent.

4.1. Synthesis of 4-(2-chlorophenyl)-3-(4-chlorophenylazo)-6-ethoxy-2-oxo-1,2,3,4-tetrahydro-5-ethylcarboxylato pyrimidine (2)

To the cold solution (0–5 °C) of **1** (6.5 g, 0.02 M) in concentrated hydrochloric acid (12 N, 5 mL), a freshly prepared 4-chlorophenyldiazonium chloride was added dropwise over a period of 10 min with constant stirring. The reaction mixture was further stirred for 3 h at the same temperature and neutralized with sodium bicarbonate solution (50%, w/v) to pH 8 under cooling to get the solid. The reaction mixture was allowed to attain the room temperature and stirred for 30 min. The solid thus obtained was filtered, washed with water, dried and recrystallized from ethanol.

4.2. Synthesis of 4-(2-chlorophenyl)-2-oxo-3-(4-chlorophenylazo)-6-(*N*-aryl-thioureido)-5-ethylcarboxylato-1,2,3,4-tetrahydro-pyrimidine (4a–k)

A mixture of **2** (9.26 g, 0.02 M) and arylthiourea **3a**–**i** (0.02 M) was refluxed in ethanol (25 mL) for a period of 8 h. The reaction mixture was allowed to cool, poured on to 25 g crushed ice. The solid separated was filtered, washed with water, dried and crystallized from ethanol.

4.3. Synthesis of 4-(2-chlorophenyl)-3-(4-chlorophenylazo)-6-aryl-7-thioxo-4,6,7,8-tetrahydro-1*H*,3*H*-pyrimido[4,5-*d*]pyrimidine-2,5-dione (5a–k)

The compound **4** in a mixture of methanol (40 mL) and sodium methoxide (28% in MeOH, 0.03 M) was stirred at room temperature for 6 h. The mixture was then acidified with 2 N HCl (20 mL) at 0 °C. The solid thus obtained was filtered, washed with water, dried and

crystallized from DMF. Structure of the synthesized compounds have been ascertain on the basis of spectroanalytical data.[23–25]

Summarizingly, a number of highly active pyrimido[4,5-*d*]pyrimidine-2,5-diones have been synthesized in efficient yields. The synthesized compound exhibited excellent in vitro antibacterial and antifungal profile. The high potencies against both Gram-positive and Gram-negative bacteria render these derivatives interesting leads for further investigations. Moreover, quantitative structure–activity relationship studies revealed that the antimicrobial activities of these synthesized derivatives against the test microorganisms are mainly governed by the molar refractivity, a polarizability parameter. Thus a proper substitution of the group with high polarizability at N-6 position probably improves the potency of these derivatives as antibacterial and antifungal agents. The effect of modification at this site will be the subject of further optimization and investigation.

References and notes

1. Rewcastle, G. W.; Bridges, A. J.; Fry, D. W.; Rubin, J. R.; Denny, W. A. *J. Med. Chem.* **1997**, *40*, 1820.
2. Fry, D. W.; Becker, M. A.; Switzer, R. L. *Mol. Pharma.* **1995**, *47*, 810.
3. Gready, J. E.; McKinlay, C.; Gebauer, M. G. *Eur. J. Med. Chem.* **2003**, *38*, 719.
4. Sanghvi, Y. S.; Larson, S. B.; Matsumoto, S. S.; Nord, L. D.; Smee, D. F.; Willis, R. C.; Avery, T. H.; Robins, R. K.; Revankar, G. R. *J. Med. Chem.* **1989**, *32*, 3629.
5. Tenser, R. B.; Gaydos, A.; Hay, K. A. *Antimicrob. Agents Chemother.* **2001**, *45*, 3657.
6. De la Cruz, J. P.; Carrasco, T.; Ortega, G.; Sanchez De la Cuesta, F. *Lipid.* **1992**, *27*, 192.
7. Nizamuddin; Mishra, M.; Srivastava, M. K.; Khan, M. H. *Indian J. Chem.* **2001**, *40*, 49.
8. Ram, V. J.; Goel, A.; Sarkhel, S.; Maulik, P. R. *Bioorg. Med. Chem.* **2002**, *10*, 1275.
9. Sharma, P.; Sharma, S.; Rane, N. *Bioorg. Med. Chem.* **2004**, *12*, 3135.
10. Sharma, P.; Kumar, A.; Mandloi, A. *Synth. Commun.* **2003**, *33*, 3.
11. Sharma, P.; Kumar, A.; Pandey, P. *Phosphorus Sulphur Silicon* **2003**, *178*, 583.
12. Sharma, P.; Kumar, A.; Sharma, R.; Mohan, P. *Indian J. Chem.* **2003**, *42B*, 416.
13. (a) Hirota, K.; Kitade, Y.; Sajiki, M.; Maki, Y. *Synthesis* **1984**, 589; (b) Hirota, K.; Kitade, Y.; Sajiki, H.; Maki, Y. *J. Chem. Soc., Perkin Trans. 1* **1990**, 123.
14. Srivastava, S. W.; Haq, W.; Chauhan, P. M. S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 965.
15. Murray, P. R.; Baron, E. J.; Faller, M. A.; Tenover, F. C.; Yolke, R. H. *Manual of Clinical Microbiology*, 6th ed.; ASM: Washington, 1995.
16. Hansch, C.; Fujita, T. *J. Am. Chem. Soc.* **1964**, *86*, 1616.
17. Hansch, C. *Acc. Chem. Res.* **1969**, *2*, 232.
18. Skagerbeg, B.; Bonelli, D.; Clementi, S.; Cruciani, G.; Ebert, C. *Quant. Struct.-Act. Relat.* **1989**, *8*, 32.
19. Dewar, M. J. S.; Zoebisch, E. G.; Healey, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
20. Stewart, J. J. P. MOPAC 6.0. QCPE 455; Indiana University: Bloomington, IN 47405, 1990.
21. CambridgeSoft corp., 100 Cambridge Park, MA 02140-2317, USA.
22. Valstat software developed at department of pharmacy, SGSITS, 23, park road, Indore, India (available on request).
23. Analytical data for compound **5a**. Mp (°C) 102–104, Yield 85%; IR (KBr) (cm^{-1}) 3250 (N–H), 1685 (C=O), 1579 (N=N), 1070 (C=S), 1564, 1514, 1452 (ring str.), ^1H NMR (δ ppm) 7.1–7.8 (m, 13H, ArH), 11.2 (s,1H, NH–C=S), 12.5 (s, 1H, NH–C=O); MS m/z (% RA) 569 (M^+, 18), 571 (M^++2, 36), 573 (M^++4, 18); Anal. Calcd for $C_{24}H_{16}Cl_2N_6O_2S$: C, 55.07; H, 3.08; N, 16.06. Found: C, 54.49; H, 3.00; N, 15.98.
24. Analytical data for compound **5e**. Mp (°C) 122–124, Yield 83%; IR (KBr) (cm^{-1}) 3420 (O–H), 3294 (N–H), 1687 (C=O), 1592 (N=N), 1090 (C=S), 1514, 1454, 1423 (ring str.), ^1H NMR (δ ppm) 6.9–7.6 (m, 12H, ArH), 10.2 (s, 1H, NH–C=S), 11.4 (s, 1H, NH–C=O), 14.2 (br s, 1H, OH); MS m/z (% RA) 585 (M^+, 12), 587 (M^++2, 24), 589 (M^++4, 12); Anal. Calcd for $C_{24}H_{16}Cl_2N_6O_3S$: C, 53.44; H, 2.99; N, 15.58. Found: C, 53.38; H, 2.92; N, 15.51.
25. Analytical data for compound **5h**. Mp (°C) 129–131, Yield 72%; IR (KBr) (cm^{-1}) 3250 (N–H), 1679 (C=O), 1597 (N=N), 1110 (C=S), 1505, 1472, 1413 (ring str.), ^1H NMR (δ ppm) 2.45 (s, 3H, CH_3), 6.8–7.4 (m, 12H, ArH), 10.1 (br s, 1H, NH–C=S), 12.3 (s, 1H, NH–C=O); MS m/z (% RA) 599 (M^+, 10), 601 (M^++2, 20), 603 (M^++4, 10); Anal. Calcd for $C_{25}H_{18}Cl_2N_6O_3S$: C, 54.26; H, 3.28; N, 15.19. Found: C, 54.18; H, 3.22; N, 15.11.
26. Important structural data for compound **5h**. Bond length (Å), C17–C12, 1.51, N21–C29, 1.43, N19–N8, 1.35, N8–N7, 1.24, N7–C1, 1.43, C6–Cl34, 1.70, C14–Cl35, 1.70; Bond angles (deg), C17–N19–N8, 114.51, N19–N8–N7, 120.64, N8–N7–C1, 119.91, C15–C17–C12, 117.72, C23–N21–C29, 118.80; Torsion angle (°), N19–N8–N7–C1, 178.25, N8–N7–C1–C3, −3.78, C22–N21–C29–C27, 91.82, C15–C17–C12–C14, −141.20.