Journal of the Association of Arab Universities for Basic and Applied Sciences (2016) **20**, 19–25

University of Bahrain

Journal of the Association of Arab Universities for Basic and Applied Sciences

www.elsevier.com/locate/jaaubas
www.sciencedirect.com

ORIGINAL ARTICLE

Computational analysis for antimicrobial active pyrano[2,3-d]pyrimidine derivatives on the basis of theoretical and experimental ground

Ajmal R. Bhat [a,*], Rajendra S. Dongre [b], Aabid H. Shalla [a], Gowhar A. Naikoo [c], Israr Ul Hassan [c]

[a] *Department of Chemistry, Islamic University of Science and Technology, Jammu and Kashmir 192122, India*
[b] *Department of Chemistry, R.T.M. Nagpur University, Nagpur 44003, India*
[c] *Maths and Sciences Unit, College of Arts and Applied Sciences, Dhofar University, Oman*

Received 22 September 2015; revised 26 November 2015; accepted 17 December 2015
Available online 25 January 2016

KEYWORDS

Pyrano[2,3-d]pyrimidines;
Pharmacophore sites;
Structural dependent biological activity;
Gram positive and Gram negative bacteria

Abstract Annulated pyrano[2,3-d]pyrimidine derivatives were synthesized with methoxy, hydroxyl, nitrile and bromine substituents in its skeleton and correlated by electronic effect of substituents on the magnitude of antimicrobial activity. The different electron donating and electron withdrawing substituents of the pyrano[2,3-d]pyrimidine derivatives exerted positive influence on its antimicrobial activity against some Gram positive and Gram negative bacteria such as, *Bacillus cereus*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aureus* and *Escherichia coli*, respectively. Antibacterial screening revealed that the presence of heteroaryl, cyano and amino groups on pyrano [2,3-d]pyrimidine skeleton increases its penetrating power on bacterial cell wall and becomes more biologically active. All the pyrano[2,3-d]pyrimidine derivatives were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectra.

1. Introduction

Certain nitrogen and oxygen annulated heterocycles have received considerable attention in medicinal chemistry such as, as inhibitors of PDE5 extracted from human platelets (Piaz et al., 2002), HIV-1 reverse transcriptase (Sweeney et al., 2008), human EPK2 (Takayoshi et al., 2006), and cyclin-dependent kinase (Brana et al., 2005). Pyrimidine rings have significant pharmacological importance as being an integral part of DNA and RNA in several biological processes (Fayed et al., 2009; Abd El-Wahab, 2002; Nargund et al., 1991) with various pharmaceutical activities such as antitumor (Grivsky et al., 1980), antihypertensive (Bennett et al., 1981), antibacterial (Ajmal et al., 2014a,b), antifungal (Bhat et al., 2014) antileishmanial activity (Agarwal et al., 2005), antiplatelet/analgesic (Bruno et al., 2004) and antiplatelet activity (Bruno et al., 2001). Therefore for the preparation of these molecules, large efforts have been taken toward the synthetic manipulation of annulated uracils that occupy a distinct and unique place in medicinal chemistry. These are synthesised

* Corresponding author.
E-mail addresses: bhatajmal@gmail.com, bhatrashid758@yahoo.com (A.R. Bhat).
Peer review under responsibility of University of Bahrain.

http://dx.doi.org/10.1016/j.jaubas.2015.12.004

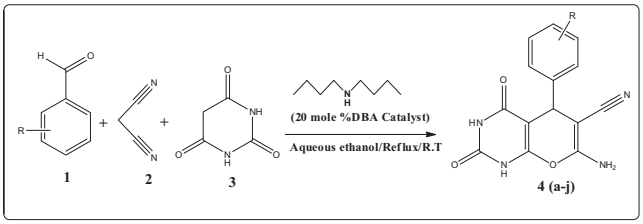
using varied multicomponent reactions based on Knoevenagel condensation, Michael addition followed by cyclodehydration strategy and finally heterocyclization (Ziarani et al., 2014).

Multicomponent reactions (MCRs) have gained significant interest in modern medicinal and combinatorial chemists (Weber et al., 1999; Armstrong et al., 1996), due to powerful bond forming efficiency, diversity-oriented synthesis (DOS), simple reaction design, atom-economy, environmental concerns, and the possibility to construct target compounds using several assorted elements in a single chemical procedure (Posner, 1986). The solvent and nature of inexpensive, mild, and reusable catalyst for MCRs play an important role in the synthesis and its selectivity of targeted products. Therefore, one of these catalysts is dibutylamine (DBA) which has received significant interest such as being highly reactive, eco-friendly, inexpensive, readily available and non-toxic for affording the corresponding products in excellent yields with high selectivity (Kalla et al., 2014).

In continuation of the current research from our laboratory to develop efficient multicomponent reactions (MCRs) for the preparation of pyrimidine annulated bioactive molecules (Ajmal et al., 2014a,b), we report here, the dibutylamine (DBA) catalyzed efficient, simple and fast synthesis of antibacterial active pyrano[2,3-d]pyrimidine derivatives via one-pot three-component domino Knoevenagel–Michael addition reaction in aqueous media (Scheme 1). A correlation structure and activity relationship of these compounds with respect to drug-likeness, Osiris and Molinspiration calculations is described and verified experimentally.

2. Materials and methods

All chemicals were obtained from Aldrich Chemical Co. and S. D. Fine Chem. Co. and used without further purification. Melting points were determined by the open capillary method and were uncorrected. IR spectra were recorded on a Perkin–Elmer 298 spectrophotometer using KBr pellet. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker instrument (^{1}H at 400 MHz and ^{13}C at 100 MHz) in DMSO-d_6 solvent and tetramethylsilane as internal standard. Chemical shifts are reported in ppm. Mass spectra were recorded on a Shimadzu GC–MS-QP-2010 model using Direct Injection Probe technique. Reactions have been monitored by thin layer chromatography on 0.2-mm precoated plates of silica gel G60 F254 (Merck). All test microorganisms were obtained from



Product	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j
R	4-NO_2	3-NO_2	2-NO_2	4-OH	3-OH	4-Br	H	4-OCH_3	2-OCH_3	3,4-OCH_3
Time (min)	103	110	101	53	67	72	58	67	59	78
Yield (%)[a]	83	86	84	94	91	89	94	91	87	93

[a]Isolated yields.

Scheme 1 General synthesis of substituted pyrano[2,3-d]pyrimidines **4(a–j)**.

Microbiology Department, Lokmanya Tilak College, Ujjain, Madhya Pradesh and were as follows: *Bacillus cereus* (ATCC-14579), *Staphylococcus aureus* (NCTC-7447), *Klebsiella pneumonia* (UC57), *Pseudomonas aureus* (ATCC 27853) and *Escherichia coli* (ATCC 14169). Streptomycin was used as antimicrobial standard drug. Antimicrobial activity was tested by the disk diffusion method (Barry, 1976-21).

2.1. General procedure for the synthesis of compounds *4(a–j)*

Substituted aromatic aldehydes **1** (1 mmol), malononitrile **2** (1 mmol), barbituric acid **3** (1 mmol) and 20 mol% dibutylamine (DBA) were taken in RB flask with 16 ml aqueous ethanol (10:6 ratio) and stirred for 43–129 min. The progress of the reaction was monitored by TLC and the solid product was filtered, washed with cold water and recrystallized from ethanol to obtain pure pyrano[2,3-d]pyrimidine derivatives with excellent yields (83–94%).

2.2. Spectral analysis

2.2.1. 7-Amino-5-(4-nitrophenyl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitril *4a*

White powder, Mp: 235–236 °C; Yield: 83%; IR (KBr (ν_{max}): 3411 (NH_2), 3209, 3163 (NH), 2989 (C—H), 2206 (C≡N), 1732 (C═O), 1461 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.01 (*s*, 1H, NH), 8.93 (*s*, 1H, NH), 7.94 (*d*, *J* = 7.1 Hz, 2H, Ar-H), 7.47 (*d*, *J* = 7.1 Hz, 2H, Ar-H), 6.79 (*s*, 2H, NH_2), 4.96 (*s*, 1H, CH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ = 176.47 (C═O), 170.60 (CNH_2), 153.93 (CONH), 151.36 (C═O), 150.32 (C-14), 147.44 (C-11), 128.99 (C-12/C-16), 123.19 (C-13/C-15), 120.41 (C≡N), 104.46 (C-5), 75.53 (C-9), 70.70 (C-10) ppm; –Ms. (m/z): 328.2 [M + H^+].

2.2.2. 7-Amino-5-(3-nitrophenyl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitril *4b*

White powder, Mp: 239-241 °C; Yield: 86%; (KBr, ν cm^{-1}): 3314 (NH_2), 3301, 3247 (NH), 2942 (C—H), 2212 (C≡N), 1605 (C═O), 1476 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.02 (*s*, 1H, NH), 10.21 (*s*, 1H, NH), 8.38 (*s*, 1H, Ar═H), 8.19 (*d*, *J* = 6.3 Hz, 2H, Ar═H), 6.82 (*s*, 2H, NH_2), 3.94 (*s*, 1H,CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.12 (C═O), 160.73 (CNH_2), 157.01 (CONH), 151.56 (C═O), 150.57 (C-14), 146.11 (C-11), 129.23 (C-12), 123.08 (C-13), 119.45 (C≡N), 104.42 (C-5), 79.32 (C-9), 71.10 (C-10); –Ms. (m/z): 328.2 [M + H^+].

2.2.3. 7-Amino-5-(2-nitrophenyl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitril *4c*

White powder, Mp: 231–234 °C; Yield: 84%; IR (KBr, ν cm^{-1}): 3302 (NH_2), 3331, 3142 (NH), 2938 (C—H), 2172 (C≡N), 1645 (C═O), 1527 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.12 (*s*, 1H, NH), 10.79 (*s*, 1H, NH), 7.69–7.63 (*m*, 3H, Ar═H), 6.82 (*s*, 2H, NH_2), 3.94 (*s*, 1H, CH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ = 179.44 (C═O), 170.14 (CNH_2), 159.59 (CONH), 156.79 (C═O), 149.87 (C-14), 134.52 (C-11), 132.12 (C-12), 130.05 (C-13), 127.31 (C≡N), 91.81 (C-5), 84.24 (C-9), 57.01 (C-10) ppm; Ms. (m/z): 350.02 [M + Na^+].

2.2.4. 7-Amino-5-(4-hydroxyphenyl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitril ***4d***

Yellow powder, Mp: 158–160 °C; Yield: 94%; IR (KBr, ν cm^{-1}): 3457 (OH), 3260 (NH_2), 3131, 3088, (NH), 2293 (C—H), 1729 (C≡N), 1678 (C═O), 1555 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.87 (*s*, 1H, NH), 10.79 (*s*, 1H, NH), 6.98 (*d*, *J* = 7.5 Hz, 2H, Ar═H), 6.82 (*s*, 2H, NH_2), 6.61 (*d*, *J* = 7.6 Hz, 2H, Ar═H), 6.05 (*s*, 1H, OH), 4.31 (*s*, 1H, CH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ = 168.93 (C═O), 157.19 (C-14), 155.61 (CNH_2), 153.38 (CONH), 152.72 (C═O), 129.32–129.20 (C-12 & C-16), 118.20 (C≡N), 115.16 (C-13), 97.22 (C-5), 58.65 (C-9), 54.87 (C-10) ppm; Ms. (m/z): 298.06.

2.2.5. 7-Amino-5-(3-hydroxyphenyl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitril ***4e***

Yellow powder, Mp: 169–171 °C; Yield 91%; IR (KBr, ν cm^{-1}): 3439 (OH), 3337 (NH_2), 3193, 3028 (NH), 2206 (C—H), 1677 (C═N), 1625 (C═O), 14741 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.04 (*s*, 1H, NH), 10.62 (*s*, 1H, NH), 6.97–6.83 (*m*, 3H, Ar═H), 6.61 (*s*, 2H, NH_2), 6.09 (*s*, 1H, OH), 3.94 (*s*, 1H, CH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ = 179.43 (C═O), 170.23 (C-14), 159.49 (CNH_2), 157.19 (CONH), 155.08 (C═O), 129.31 (C-16), 129.20 (C≡N), 115.15 (C-13), 93.41 (C-5), 84.21 (C-9), 52.03 (C-10) ppm; Ms. (m/z): 299.02 [M + H^+].

2.2.6. 7-Amino-5-(4-bromophenyl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitril ***4f***

White powder, Mp: 228–232 °C; Yield: 89%; IR (KBr, ν cm^{-1}): 3370 (NH_2), 3340, 3189 (NH), 3080 (C—H), 2220 (C═N), 1684 (C═O), 1567 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 14.10 (*s*, 1H, NH), 13.01 (*s*, 1H, NH), 7.75 (*d*, *J* = 7.1 Hz, 2H, Ar═H), 7.33 (*d*, *J* = 7.1 Hz, 2H, Ar═H), 6.82 (*s*, 2H, NH_2), 3.90 (*s*, 1H, CH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.47 (C═O), 160.34 (CNH_2), 153.89 (CONH), 145.91 (C═O), 138.53 (C-11), 131.97 (C-13 & C-15), 129.69 (C-12 & C-16), 124.03 (C-14), 121.28 (C≡N), 105.25 (C-5), 69.60 (C-9), 50.10 (C-10) ppm; Ms. (m/z): 384.12 [M + Na^+].

2.2.7. 7-Amino-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile ***4g***

Yellow powder, Mp: 236–238 °C; Yield: 94%; IR (KBr, ν cm^{-1}): 3371 (NH_2), 3301, 3212 (NH), 3114 (C—H), 2129 (C≡N), 1694 (C═O), 1572 (C═C) ppm; ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.98 (*s*, 1H, NH), 10.80 (*s*, 1H, NH), 7.31 (*t*, *J* = 7.3 Hz, 2H, Ar═H), 7.14–7.06 (*m*, 3H, Ar═H), 6.82 (*s*, 2H, NH_2), 4.29 (*s*, 1H, CH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ = 160.39 (C═O), 155.61 (CNH_2), 153.91 (CONH), 151.36 (C═O), 145.92 (C-11), 128.68 (C-12), 128.49 (C-13), 127.61 (C-14), 118.21 (C≡N), 93.41 (C-5), 58.66 (C-9), 50.89 (C-10) ppm; Ms. (m/z): 283.08 [M + H^+].

2.2.8. 7-Amino-5-(4-methoxyphenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-carbonitrile ***4h***

Dark yellow powder, Mp: 289–293 °C; Yield: 91%; IR (KBr, ν cm^{-1}): 3317 (NH_2), 3282, 3145 (NH), 3063 (C—H), 2215 (C≡N), 1743 (C═O), 1668 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.98 (*s*, 1H, NH), 10.80 (*s*, 1H, NH), 7.14 (*d*, *J* = 7.5 Hz, 2H, Ar═H), 6.86–6.82 (*m*, 4H, Ar═H & NH_2), 4.16 (*s*, 1H, CH), 3.81 (*s*, 3H, OCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ = 161.99 (C═O), 159.11 (C-14), 155.61 (CNH_2), 151.97 (CONH), 151.36 (C═O), 130.34 (C-11), 129.50 (C-12), 123.56 (C≡N), 113.14 (C-13), 93.41 (C-5), 58.66 (C-9), 57.46 (CH_3), 53.46 (C-10) ppm; Ms. (m/z): 313.01 [M + H^+].

2.2.9. 7-Amino-5-(2-methoxyphenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-carbonitrile ***4i***

Yellow powder, Mp: 301–303 °C; Yield: 87%; IR (KBr, ν cm^{-1}): 3419 (NH_2), 3202, 3137 (NH), 3016 (C—H), 2272 (C≡N), 1765 (C═O), 1609 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.93 (*s*, 1H, NH), 10.71 (*s*, 1H, NH), 7.44 (*d*, *J* = 4.6 Hz, 1H, Ar═H), 7.14–7.7.11 (*m*, 4H, Ar═H & NH_2), 4.16 (*s*, 1H, CH), 3.73 (*s*, 3H, OCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ = 169.87 (C═O), 161.02 (C-14), 158.21 (CNH_2), 152.46 (CONH), 151.82 (C═O), 131.15 (C-11), 129.09 (C-12), 124.02 (C≡N), 113.18 (C-13), 94.39 (C-5), 58.93 (C-9), 57.41 (CH3), 53.57 (C-10) ppm; Ms. (m/z): 313.05 [M + H^+].

2.2.10. 7-Amino-5-(3,4-dimethoxyphenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-carbonitrile ***4j***

Yellow powder, Mp: 312–314 °C; Yield: 93% ; IR (KBr, ν cm^{-1}): 3194 (NH_2), 3103, 2223 (NH), 1734 (C—H), 1662 (C≡N), 1576 (C═O), 1262 (C═C); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.98 (*s*, 1H, NH), 10.80 (*s*, 1H, NH), 6.83–6.80 (*m*, 5H, Ar═H & NH_2), 4.29 (*s*, 1H, CH), 3.83 (*s*, 3H, OCH_3), 3.75 (*s*, 3H, OCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ = 164.65 (C═O), 155.61 (CNH_2), 153.94 (CONH), 151.97 (C═O), 149.25 (C-14), 148.54 (C-13), 131.34 (C-11), 126.92 (C-16), 119.53 (C≡N), 116.52 (C-12), 114.27 (C-15), 93.40 (C-5), 58.66 (C-9), 57.91 (CH_3), 56.78 (CH_3), 51.23 (C-10) ppm; Ms. (m/z): 342.

2.3. Biological evaluation

All the products were dissolved in N,N-dimethylformamide (DMF) for dilution to prepare stock solutions of 20 mg/mL for antimicrobial assay. Agar plates were uniformly surface inoculated with fresh broth culture of Gram positive and negative bacteria such as *B. cereus* (ATCC-14579), *S. aureus* (NCTC-7447), *K. pneumonia* (UC57), *P. aureus* (ATCC 27853) and *E. coli* (ATCC 14169). These impregnated disks were placed on medium suitably spaced apart and plates were incubated at 30 °C for 1 h to permit good diffusion and were then transferred to an incubator at 37 ± 2 °C for 24 h. The zones of inhibition were measured on mm scale. Streptomycin was used as standard antimicrobial drug. Antimicrobial activity test results are shown in Table 3. All the synthesized pyrano [2,3-d]pyrimidine derivatives showed good antimicrobial activity, due to similarity of synthesized compounds to the substrate of targeted enzyme. The compounds with electron donating group at the different positions on phenyl ring have subsequent hydrophobic interaction with active site of enzyme increase the antibacterial activity.

Table 1 Effect of catalysts and solvents for model product **4d** using aqueous ethanol.[a]

Entry	Catalyst	Catalyst (mol%)	Time (mins)	Yield (%)[b]	Solvent	Time (mins)	Yield (%)[b]
1	N-methylamidazole	20	114	79	EtOH–H_2O	53	94
2	Triethylamine	20	83	90	DMF	59	61
3	Piperidine	20	110	71	CH_2Cl_2	103	58
4	Morpholine	20	140	68	1,4-Dioxane	72	63
5	Dibutylamine	5	105	74	Solvent-free	192	57
6	Dibutylamine	10	68	86	EtOH	45	87
7	Dibutylamine	15	53	87	H_2O	68	83
8	Dibutylamine	20	53	94			
9	Catalyst free	–	189	37			

[a] Reaction conditions: hydroxybenzaldehyde (1 mmol), malononitrile (mmol), barbituric acid (1 mmol), catalyst DBA 20 mol%, stirring/reflux, r.t.
[b] Isolated yields.

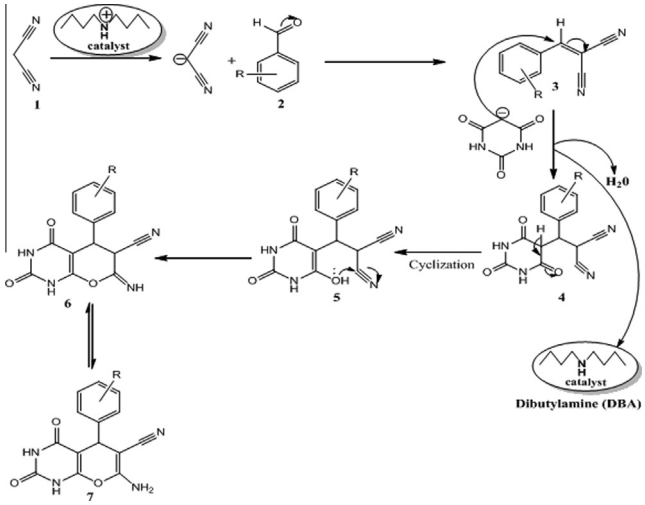
Table 2 Comparative synthesis of pyrano[2,3-d]pyrimidines using DBA catalyst versus reported literature.

Entry	Catalyst	Solvent	Condition	Time	Yield (%)	Reference
1	DABCO	EtOH: H_2O	Reflux	30–40 min	82–94	Ajmal et al. (2014a,b)
2	Catalyst-free	H_2O	MW	3–5 min	82–94	Bhat et al. (2014)
3	Catalyst-free	H_2O	60 °C	1–4 h	71–86	Bhat et al. (2014)
4	Catalyst-free	H_2O	48 °C	2–5 h	69–83	Bhat et al. (2014)
5	SBA-Pr-SO3H	Solvent-free	60 °C	5–45 min	69–86	Ziarani et al. (2014)
6	Catalyst-free	Solvent-free	Ball-milling	30–90 min	> 99	Sara and Naimi-Jamal (2009)
7	Catalyst-free	H_2O	MW	3–5 min	86–94	Yuan et al. (2004)
8	DBA	EtOH: H_2O	Reflux	53 min	94	Present Work

3. Results and discussion

Herein, we report the synthesis of annulated pyrano[2,3-d] pyrimidine derivatives via one pot three component domino Knoevenagel–Michael addition reaction. In initial studies, we found the base catalysts such as N-methylamidazole, triethylamine, piperidine, morpholine and dibutylamine (Table 1, entries **1**–**8**), were equally competent in furnishing the desired model product **4** in good yields. Among these basic catalysts, 20 mol% of dibutylamine (DBA) gave the best result in terms of time of completion and the product yields 94% (Table 1, entry **8**). In the absence of catalyst, the reaction was completed after 189 min of reflux (Table 1, entry **9**) and yield of product obtained only 37%. Therefore, dibutylamine (DBA) catalyst appears to be superior to any of the other tested catalysts. Further, we focused on systematic evaluation of different solvents like ethanol: H_2O, DMF, CH_2Cl_2 and 1,4-Dioxane (Table 1, entry **1**–**5**), for the synthesis of model product **4**. We found that the best conversion was observed when the reaction was proceeded in Ethanol: H_2O (Table 1, entry **1**). The reported literature revealed the synthesis of pyrano[2,3-d]pyrimidine derivatives under different conditions. The overall reported result was compared with DBA catalyst (Table 2, entry **1**–**8**). Plausible mechanism for the synthesis of pyrano[2,3-d] pyrimidines is shown in Scheme 2.

The different substituents of the phenyl, OH, OCH_3, NO_2 and Br group on annulated pyrimidines are very essential for activity against some Gram positive and Gram negative bacteria (Kamlesh et al., 2014). These substituents have further showed extensive effect on the membrane potential associated with bactericidal activity (Vinita et al., 2014). The relevant studies showed that steric, electronic effects and polar parameters of the phenyl in pyrano[2,3-d]pyrimidine were important for antimicrobial activity (Table 3). These findings suggest that rather than disrupting cell membranes, the compounds acted outside the cell and became attached to surface groups of the bacterial cells increases its activity. Compound **4g** exhibited broad-spectrum activity against *B. cereus*, *P. aureus* and *E. coli* bacteria, Compound **4e** showed maximum antimicrobial activity against *S. aureus*, *P. aureus* and *E. coli*, Compound **4d** showed activity against *B. cereus*, Compound **4h** and **4i** exhibited maximum activity against



Scheme 2 Plausible mechanism for the synthesis of pyrano[2,3-d]pyrimidines.

Table 3 Antibacterial activity of annulated pyrano[2,3-d] pyrimidine derivatives **4(a-j)**.

Compd.	R	Zones of inhibition*				
		Gram positive		Gram negative		
		A	B	C	D	E
4a	4-NO_2	11	4	4	3	12
4b	3-NO_2	14	7	6	11	11
4c	2-NO_2	10	5	6	9	11
4d	4-OH	14	15	10	15	15
4e	3-OH	10	15	8	15	14
4f	4-Br	4	12	3	4	6
4g	H	12	8	5	14	13
4h	4-OCH_3	11	14	14	5	8
4i	2-OCH_3	11	15	13	7	9
4j	3,4-OCH_3	13	8	14	8	12
SD	–	16	18	18	16	18

SD: *Streptomycin*, A = *Bacillus cereus* (ATCC-14579), B = *Staphylococcus aureus* (NCTC-7447), C = *Klebsiella pneumonia* (UC57), D = *Pseudomonas aureus* (ATCC 27853), E = *E. coli* (ATCC 14169).

* Inhibition zone around the disks for antibacterial activity: 11–18 mm: very strong activity; 5–10 mm: moderate activity; 1–4 mm: weak activity.

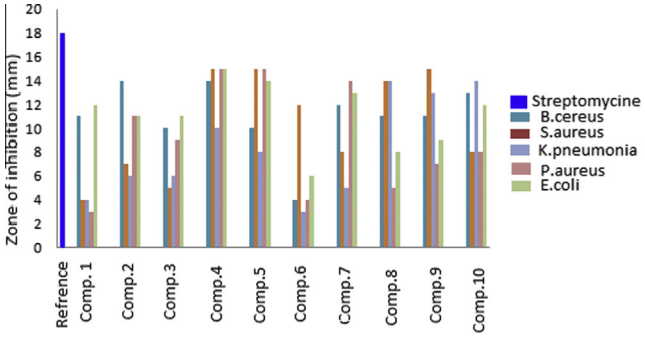


Figure 1 Antibacterial activity of annulated pyrano[2,3-d]pyrimidine derivatives.

B. cereus, *S. aureus* and *K. pneumonia* bacterial strains. Compound **4j** showed maximum activity against *B. cereus*, *K. pneumonia* and *E. coli*. Electron withdrawing groups reduced antimicrobial activity due to decreasing partial charge on nitrogen atoms of barbiturate moiety on pyrano[2,3-d] pyrimidine ring, leads to a decrease the antimicrobial activity. Compounds **4a**, **4b** and **4c** were found to have good activity against *B. cereus* and *K. pneumonia*, compound **4c** showed also activity against *P. aureus* and compound **4f** exhibited maximum activity against *S. aureus* (Table 3 and Fig. 1). The annulated pyrano[2,3-d]pyrimidine products as antimicrobial agents are excellent derivatives for drug resistance issues in clinically used therapeutics and furnish motivating model for studying interaction with antimicrobial targets, as possible charge modification of the substituents and O/N of pharmacophore groups present in the skeleton.

The results of the present study revealed the following order of bactericidal activity intensities elicited by the annulated pyrano[2,3-d]pyrimidine derivatives: 4-OH > 3-OH > 4-OCH_3 > -H > 2-OCH_3 > 3,4-OCH_3 > 4-NO_2 > 3-NO_2 > 2-NO_2 > 4-Br. It is found that the negative and positive charges of the nitrogen of the CN group and hydrogen of the NH group contribute positively in favor of an antibacterial activity, and this is in good agreement with the mode of antibacterial action of the compounds bearing ($X^{\delta-}$–$Y^{\delta+}$) pharmacophore site. It was hypothesized by Osiris calculations software that difference in charges between two terminal groups of dipolar pharmacophore site ($X^{\delta-}$–$Y^{\delta+}$) may facilitate the inhibition of bacteria, more than viruses. It is further found that the activity decreases with a decrease in negative charge of the CN group of the common pharmacophore fragment of the series **4(a–j)** (HN–CN) Table 4. Further the prediction results of compounds **4(a–j)** on the basis of molecular properties (TPSA, GPCR ligand and ICM) are valued by Molinspiration calculations software (Table 5). The computational analysis such as drug-likeness, Osiris and Molinspiration calculations were used for the targeted bio active pyrano [2,3-d]pyrimidine derivatives.

Table 4 Osiris calculations of compounds **4(a–j)** and standard reference.

Compd.	Toxicity risks[a]				Drug-score[b]			
	MUT	TUMO	IRRI	REP	CLP	S	DL	DS
4a					0.42	−6.14	−10.59	0.29
4b					0.42	−6.14	−10.59	0.29
4c					0.42	−6.14	−10.59	0.29
4d					0.25	−5.38	−0.20	0.49
4e					0.25	−5.38	−0.69	0.45
4f					1.25	−6.51	−2.22	0.30
4g					0.55	−5.68	−0.30	0.46
4h					0.45	−5.70	−0.35	0.45
4i					0.45	−5.70	−0.35	0.45
4j					0.34	−5.71	1.25	0.55
Strept					−7.83	−0.96	0.83	0.43

: not toxic; : slightly toxic; : highly toxic.

[a] MUT: mutagenic; TUMO: tumorigenic; IRRI: irritant; REP: reproductive effective.

[b] CLP: cLogP, S: Solubility, DL: Druglikeness, DS: Drug-Score.

Table 5 Molinspiration calculations of molecular properties and drug-likeness of compounds **4(a–j)** and standard reference.

Compd.	Molecular properties calculations[a]						Drug-likeness[b]					
	MW (g/mole)	TPSA	OH–NH interact.	Violation	ROTB	VOL	GPCRL	ICM	KI	NRL	PI	EN
4a	327	171	4	0	2	257	−1.36	−1.34	−1.23	−0.91	−1.38	−0.81
4b	327	171	4	0	2	257	−1.37	−1.36	−1.22	−0.91	−1.39	−0.83
4c	327	171	4	0	2	257	−1.38	−1.35	−1.32	−0.92	−1.50	−0.86
4d	298	145	5	0	1	242	−1.27	−1.35	−1.14	−0.74	−1.37	−0.69
4e	298	145	5	0	1	242	−1.28	−1.37	−1.16	−0.74	−1.38	−0.69
4f	361	125	4	0	1	252	−1.43	−1.49	−1.24	−1.02	−1.51	−0.83
4g	282	125	4	0	1	234	−1.41	−1.47	−1.27	−0.97	−1.48	−0.78
4h	312	134	4	0	2	259	−1.29	−1.42	−1.16	−0.86	−1.35	−0.76
4i	312	134	4	0	2	259	−1.31	−1.45	−1.21	−0.86	−1.44	−0.79
4j	342	143	4	0	3	285	−1.20	−1.32	−1.07	−0.82	−1.25	−0.71
Strept[c]	581	336	16	3	9	497	0.09	−0.16	−0.17	−0.18	0.65	0.38

[a] TPSA: total polar surface area, O/NH: O–HN interaction, Violation: number of violation, VOL: volume.
[b] GPCR: GPCR ligand, ICM: Ion channel modulator, KI: Kinase inhibitor, NRL: nuclear receptor ligand, PI: protease inhibitor, EN: enzyme inhibitor.
[c] Strept: *Streptomycin*.

4. Conclusion

The antimicrobial study revealed that targeted compounds were more active with OH than the OCH_3 group, irrespective of the position of substitution on the benzene ring. Since hydrophobic property is important for the drugs to diffuse through the pathogenic biological system. The different electron withdrawing subsistents of the pyrano[2,3-d]pyrimidine skeleton exerted less influence on its antimicrobial activity against some Gram positive and Gram negative bacteria like *P. aureus, E. coli, S. aureus, K. pneumonia* and *B. cereus*. Hence, this study have supportive for the medicinal chemists in understanding antimicrobial activity of annulated pyrano[2,3-d] pyrimidine products, one with different inter atomic distances (linkers) steric, electronic effects, polar parameters and with different electronic environments. The anti-Kinase pharmacophore site (O═C─NH─C═O) should be evaluated in coming step as continuation of these investigations on this series.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgements

Authors would like to thank ACTELION; the Biopharmaceutical Company of Swiss, for the online molecular properties calculations.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jaubas.2015.12.004.

References

Abd El-Wahab, A.H.F., 2002. Activated nitriles in heterocyclic synthesis: synthesis of new [1]benzopyrano[30,40:5,6]pyrano[2,3-d] pyrimidine and [1]benzopyrano[30,40:5,6]pyrano [3,2-e][1,2,4]triazolo[1,5-c]pyrimidine derivatives with promising antibacterial activity. Acta Pharm. 52, 269–280.

Agarwal, A., Ashutosh, R., Goyal, N., Chauhan, P.M.S., Gupta, S., 2005. Dihydropyrido [2,3-d]pyrimidines as a new class of antileishmanial agents. Bioorg. Med. Chem. 24, 6678–6684.

Ajmal, R.B., Rajendra, S.D., Rupali, S.S., 2014a. Potent in vitro antibacterial and antifungal activities of pyrano[2,3-d]pyrimidine derivatives with quantitative yield. Int. J. Pharma. Bio. Sci. 5, 422–430.

Ajmal, A.R., Aabid, H.S., Rajendra, D.S., 2014b. Synthesis of new annulated pyrano[2,3-d]pyrimidine derivatives using organo catalyst (DABCO) in aqueous media. J. Saudi Chem. Soc. http://dx.doi.org/10.1016/j.jscs.2014.03.008, in press.

Armstrong, R.W., Comba, A.P., Tempst, P.A., Brown, S., Keating, T. A., 1996. Multiple-component condensation strategies for combinatorial library synthesis. Acc. Chem. Res. 29, 123–131.

Barry, A.L., 1976. The Antimicrobial Susceptibility Test: Principle and Practices. Illus Lea and Febiger, Philadelphia, PA180.

Bennett, L.R., Blankely, C.J., Fleming, R.W., Smith, R.D., Tessonam, D.K., 1981. Antihypertensive activity of 6-arylpyrido[2,3-d]pyrimidin-7-amine derivatives. J. Med. Chem. 24, 382–389.

Bhat, R.A., Aabid, H.S., Rajendra, S.D., 2014. Microwave assisted one-pot catalyst free green synthesis of new methyl-7-amino-4-oxo-5-phenyl-2-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carboxylates as potent in vitro antibacterial and antifungal activity. J. Adv. Res. 6 (6), 941–948. http://dx.doi.org/10.1016/j.jare.2014.10.007.

Brana, M.F., Cacho, M., Garcia, M.L., Mayoral, E.P., Lopez, B., Pascual-Teresa, B.D., 2005. Pyrazolo[3,4-c]pyridazines as novel and selective inhibitors of cyclin-dependent kinases. J. Med. Chem. 48, 6843–6854.

Bruno, O., Brullo, C., Ranise, A., Schenone, S., Bondavalli, F., Barocelli, E., Ballabeni, V., Chiavarini, M., Tognolini, M., Impicciatore, M., 2001. Synthesis and pharmacological evaluation of 2,5-cycloamino-5H-[1]benzopyrano[4,3-d]pyrimidines endowed with in vitro antiplatelet activity. Bioorg. Med. Chem. Lett. 11, 1397–1400.

Bruno, O., Brullo, C., Schenone, S., Bondavalli, F., Ranise, A., Tognolini, M., Ballabeni, V., Barocelli, E., 2004. Synthesis and pharmacological evaluation of 5H-[1]benzopyrano[4,3-d]pyrimidines effective as antiplatelet/analgesic agents. Bioorg. Med. Chem. Lett. 12, 553–561.

Fayed, A.A., Hosni, H.M., Flefel, E.M., Amr, A.E., 2009. Synthesis and pharmacological activities of some new thieno [2,3-d] pyrimidine and pyrimidino pyrazolo thieno pyrimidine derivatives. World J. Chem. 4, 58–65.

Grivsky, E.M., Lee, S.C., Sigel, W., Duch, D.S., Nichol, C.A., 1980. Synthesis and antitumor activity of 2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-d]pyrimidine. J. Med. Chem. 23, 327–329.

Kalla, R.M.N., Jin-Seok, C., Jin-Wook, Y., Byeon, S.J., Heo, M.S., Kim, I.I., 2014. Synthesis of 2-amino-3-cyano-4*H*-chromen-4-ylphosphonates and their anticancer properties. Eur. J. Med. Chem. 76, 61–66.

Kamlesh, M.K., Ravindra, M.G., Taslimahemad, T.K., Praful, K.P., 2014. Microwave and conventional synthesis of novel pyrido[2,3-d] pyrimidine scaffold as an antimicrobial agent. Chem. Biol. Interface 4 (2), 119–130.

Nargund, L.V.G., Reddy, Y.S.R., Jose, R., 1991. Synthesis and antibacterial activity of pyrido[1, 2-a]pyrimidin-4 (1H)–ones. Indian Drugs 29, 45–46.

Piaz, V.D., Castellana, M.C., Vergelli, C., Giovannoni, M.P., Gavalda, A., Segarra, V., Beleta, J., 2002. Synthesis and evaluation of some pyrazolo[3,4-d]pyridazinones and analogues as PDE 5 inhibitors potentially useful as peripheral vasodilator agents. J. Enzyme. Inhib. Med. Chem. 17, 227–233.

Posner, G.H., 1986. Multicomponent one-pot annulations forming three to six bonds. Chem. Rev. 86, 831–844.

Sara, M., Naimi-Jamal, M.R., 2009. Mechanochemical solvent-free and catalyst-free one-pot synthesis of pyrano[2,3-d]pyrimidine-2,4 (1H,3H)-diones with quantitative yields. Molecules 14, 474–479.

Sweeney, Z.K., Harris, S.F., Arora, N.N., Javanbakht, H.H., Li, Y.Y., Fretland, J.J., 2008. Design of annulated pyrazoles as inhibitors of HIV-1 reverse transcriptase. J. Med. Chem. 51, 7449–7458.

Takayoshi, K., Masaichi, W., Makoto, O., Kentaro, S., Masahiro, N., Takashi, F., 2006. Crystal structure of human ERK2 complexed with a pyrazolo[3, 4-c]pyridazine derivative. Bioorg. Med. Chem. Lett. 16, 55–58.

Vinita, S., Nitin, C., Ajay, K.A., 2014. Significance and biological importance of pyrimidine in the microbial world. Int. J. Med. Chem. http://dx.doi.org/10.1155/2014/202784, 31p. Article ID 202784.

Weber, L., Illegel, K., Almstetter, M., 1999. Discovery of new multi component reactions with combinatorial method. Synlett, 366–374.

Yuan, G., Shujang, T., Tuanjie, L., Xiaojing, Z., Songlei, Z., Fang, F., Daqing, S., 2004. Effective synthesis of 7-Amino-6-cyano-5-aryl-5H-pyrano[2,3-d]pyrimidine-2,4(1H,3H)-diones under microwave irradiation. Synth. Commun. 7, 1295–1299.

Ziarani, Ghodsi M., Nasab, Narges H., Mahshid, R., Ali, A.S., 2014. One-pot synthesis of pyrido[2,3-d]pyrimidine derivatives using sulfonic acid functionalized SBA-15 and the study on their antimicrobial activities. J. Saud. Chem. Soc. 19 (6), 676–681. http://dx.doi.org/10.1016/j.jscs.2014.06.007.