تحليل حسابي للفعالية المضادة للمكروبات لمشتقات البايرانو [2,3-d] بايريميدين بناء على أسس نظرية وعملية

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الملخص:

تم تخليق مشتقات بايرانو[2,3-d] بايريميدين حلقية ذات مستبدلات من نوع ميثوكسي، هايدروكسي، نتزيل و برومين على هيكلها وتم قياس مدى ارتباط تأثيراتها الالكترونية على قوة نشاطيتها ضد الميكروبات. لقد برهنت الدراسة على أن مجموعات المستبدلات المختلفة سواء كانت ذات تأثير الكتروني حاث أو مثبط لها تأثير ايجابي على النشاطية ضد الميكروبات من نوع جرام موجب وجرام سالب مثل باسيللوس سيريوس، ستاقيلوكوككس اوريوس، كليبشيلا نيمونيا، سودوموناس اوريوس واسريشيا كولاى، على التوالي. وقد بين المسح المضاد للباكتيريا على أن وجود مجموعات العطري الحلقي غير المتجانس والسيانو والأمينو على هيكل البايرانو[2,3-d] بايريميدين يزيد من قوة نفاذية المركب إلى داخل جدار الخلية البكتيرية وبذلك يصبح الهيكل أكثر فعالية بيولوجية. تم تشخيص جميع مشتقات البايرانو[2,3-d] بايريميدين بواسطة الإشعة تحت الحمراء وبروتون وكربون-13-الرنين النووي المغنطيسي ومطياف الكتلة.

ORIGINAL ARTICLE

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

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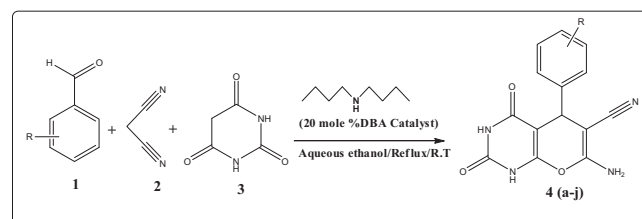
using varied multicomponent reactions based on Knoevenagel condensation, Michael addition followed by cyclodehydration strategy and finally heterocyclization (Ziarani et al., 2014).

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. ^1H and ^{13}C NMR spectra were recorded using a Bruker instrument (^1H 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

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 (v_{max}): 3411 (NH₂), 3209, 3163 (NH), 2989 (C—H), 2206 (C≡N), 1732 (C=O), 1461 (C=C); ^1H 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, NH₂), 4.96 (*s*, 1H, CH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ = 176.47 (C=O), 170.60 (CNH₂), 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, v cm⁻¹): 3314 (NH₂), 3301, 3247 (NH), 2942 (C—H), 2212 (C≡N), 1605 (C=O), 1476 (C=C); ^1H 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, NH₂), 3.94 (*s*, 1H,CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.12 (C=O), 160.73 (CNH₂), 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, v cm⁻¹): 3302 (NH₂), 3331, 3142 (NH), 2938 (C—H), 2172 (C≡N), 1645 (C=O), 1527 (C=C); ^1H 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, NH₂), 3.94 (*s*, 1H, CH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ = 179.44 (C=O), 170.14 (CNH₂), 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⁺].

Product	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j
R	4-NO₂	3-NO₂	2-NO₂	4-OH	3-OH	4-Br	H	4-OCH₃	2-OCH₃	3,4-OCH₃
Time (min)	103	110	101	53	67	72	58	67	59	78
Yield (%)ᵃ	83	86	84	94	91	89	94	91	87	93

ᵃIsolated yields.

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

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, v 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, v 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, v 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, v 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, v 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, v 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$_3$); ^{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, v 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$_2$O	53	94
2	Triethylamine	20	83	90	DMF	59	61
3	Piperidine	20	110	71	CH$_2$Cl$_2$	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$_2$O	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.

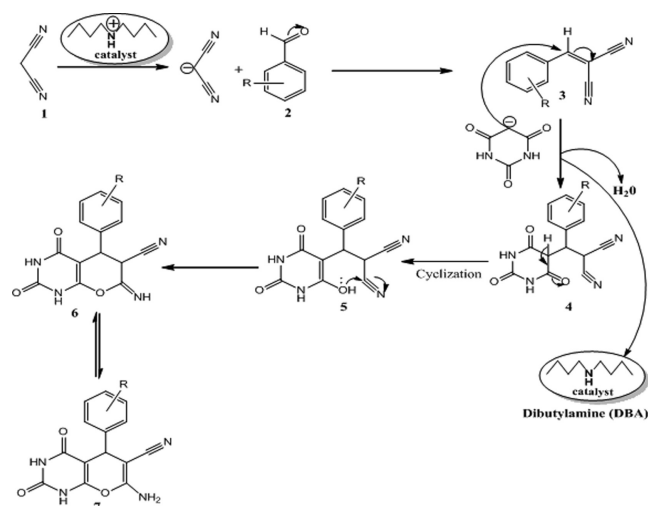**Table 2** Comparative synthesis of pyrano[2,3-d]pyrimidines using DBA catalyst versus reported literature.

Entry	Catalyst	Solvent	Condition	Time	Yield (%)	Reference
1	DABCO	EtOH: H$_2$O	Reflux	30–40 min	82–94	Ajmal et al. (2014a,b)
2	Catalyst-free	H$_2$O	MW	3–5 min	82–94	Bhat et al. (2014)
3	Catalyst-free	H$_2$O	60 °C	1–4 h	71–86	Bhat et al. (2014)
4	Catalyst-free	H$_2$O	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$_2$O	MW	3–5 min	86–94	Yuan et al. (2004)
8	DBA	EtOH: H$_2$O	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$_2$O, DMF, CH$_2$Cl$_2$ 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$_2$O (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-NO₂	11	4	4	3	12
4b	3-NO₂	14	7	6	11	11
4c	2-NO₂	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-OCH₃	11	14	14	5	8
4i	2-OCH₃	11	15	13	7	9
4j	3,4-OCH₃	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.
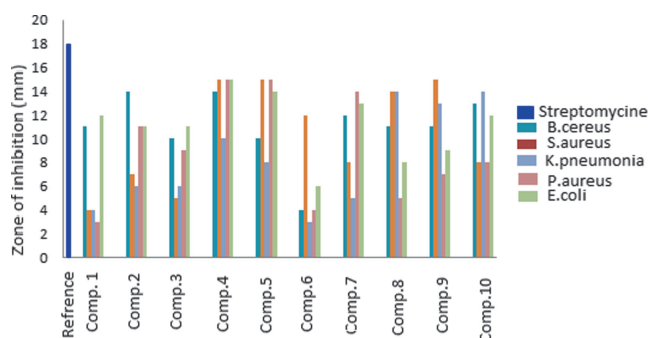
Figure 1 Antibacterial activity of annulated pyrano[2,3-d]pyrimidine derivatives.

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-OCH₃ > -H > 2-OCH₃ > 3,4-OCH₃ > 4-NO₂ > 3-NO₂ > 2-NO₂ > 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.

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.

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