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Design, synthesis and *in vitro* antimicrobial activity of fused pyridine-pyrimidine hybrids

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The antimicrobial resistance (AMR) is defined as the rising global concern of infectious illnesses that are resistant to all known antimicrobial agents. As the microbial resistance is increasing recently against the present therapeutics, there is an urgency to discover the novel antimicrobial agents. Due to this reason, we have synthesized a series of novel pyridine-pyrimidine derivatives (**4a-o**) for the development of antimicrobial agents. The structures of these bioactive molecules were determined using IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy. The titled compounds were tested for antimicrobial activity against various bacterial and fungal strains using Chloramphenicol and Griseofulvin as standard drugs. Compounds **4b, 4d** and **4m** exhibited excellent antibacterial activity with MIC = 62.5 µg/mL against *E. coli, S. aureus* and *S. pyogenes* respectively. Compounds **4g** and **4o** showed prominent activity against *C. albicans* with MIC value of 250 µg/mL.

Keywords: Pyridine, Pyrimidine, Antibacterial activity, Antifungal activity

Bacterial resistance is a known phenomenon and due to this resistance, the existing antibiotics are continuing to be a major public health problem around the globe. Antimicrobial resistance (AMR), especially multidrug resistance (MDR), is the leading cause of increased morbidity and mortality[1,2]. The overdose of antibiotics, as well as a lack of genuine efforts in the discovery of new medicines is responsible for the current situation in the field of pharmaceutical industries[3,4]. The Centers for Disease Control and Prevention (CDC) has designated a number of bacteria as an urgent and worrying hazard, with several of them are already posing a significant burden on the health-care system[5]. In this perspective, one of the most powerful techniques to overcome existing resistance mechanisms and treat life-threatening diseases produced by these pathogenic strains is responsible for the discovery and development of new chemical entities[6,7].

The pyridine moiety fused with the pyrimidine ring is a significant *N*-heterocyclic class that has been studied as bioactive agents and in the drug chemistry. Pyridine and pyrimidine ring systems are extremely important classes of compounds because of their broad range of biological activities[8]. In nature, nitrogen containing pyridine, pyrimidine and their analogs exist, and they played an important role in synthetic heterocyclic chemistry[9]. Plenty of their derivatives are active as antimicrobial[10,11], antitubercular[12,13], antiviral[14], anticancer[15], and anti-oxidant[16], anti-inflammatory[17] agents etc. A variety of novel fused pyridine-pyrimidine derivatives were synthesized and evaluated for their *in vitro* antimicrobial activity as a continuation of our research efforts on the design and synthesis of new heterocyclic compounds with biological efficacy[18,19].

The concept of drug design for the development of antimicrobial agents from commercially available drug ocinaplon is described in Figure 1. We have modified the structure of approved drug ocinaplon by making structural changes to increase the efficacy of our reported hybrids. For the rational of our work we have replaced the pyrazolo[1,5-*a*]pyrimidine moiety by 1,3-dimethylpyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione motif for enhancing antimicrobial potency. The

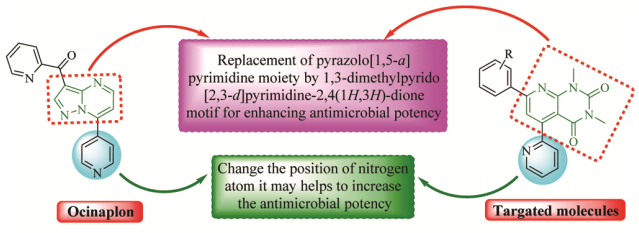


Fig. 1 — The concept of drug designed from commercially available drug verses targeted molecules **(4a-o)**

modification of nitrogen atom from 4th position to 2nd position was embarked in the synthesized hybrids for enhancing the antimicrobial activity. Over and above, we have replaced the pyridine ring by substituted phenyl ring for enhancing the antimicrobial potency.

Our present work was justified by examining bioactive molecules exhibiting antibacterial activities (Figure 1). Thakur *et al.*[20] developed and tested several pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione hybrids for antibacterial activity against *B. subtilis, S. aureus, K. pneumonia,* and *E. coli*. The most active derivative **I**, 1,3,7-trimethyl-6-nitro-5-phenylpyrido [2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione, possessed MIC in the range of 0.375-0.75 mg/mL against tested bacterial strains. The derivative **II**, 1,3,7-trimethyl-5-(naphthalen-1-yl)-6-nitropyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione possessed MIC in the range of 0.375-3.0 mg/mL against tested bacterial strains. Structural alterations like installation of substituted phenyl ring at 7th position and introducing pyridine moiety instead of phenyl ring in the previously published results in the target molecules showed enhanced antimicrobial profile. Figure 2 displays the rationale based on the above discussion.

Results and Discussion

Chemistry

In the presence of methanolic KOH, substituted chalcones (**3a-o**) were synthesized by Aldol condensation of picolinaldehyde (**1**) as the starting material with substituted acetophenones (**2a-o**)[23]. The desired products (**4a-o**) were synthesized by substituted chalcones (**3a-o**) reacting with 6-amino-1,3-dimethyluracil. The route of synthesized compounds (**4a-o**) is described in Scheme I. Stretching bands identified at 3189 cm^{-1} and 2849 cm^{-1} in the IR spectra of compound **4a** are evidence of -CH and -CH=CH groups respectively. Stretching vibration observed at 1763 cm^{-1}, prove the presence of the >C=O group. The presence of -C=N, -C=C, and C-N groups is confirmed by stretching vibrations at 1685 cm^{-1}, 1656 cm^{-1}, and 1254 cm^{-1} respectively. In ^{1}H NMR, the signal appearing at 8.88 ppm is the evidence of secondary amine proton in the pyrimidine ring. Furthermore, evidence in the range of 8.37-7.89 ppm revealed the presence of sixteen aromatic protons. Moreover, the pyridine ring was confirmed from the signals that showed in the region of 3.45-3.36 ppm. Chemical shifts of 162.6 ppm, 159.3 ppm, and 157.1 ppm in ^{13}C NMR corresponded to the carbons of pyrimidine ring. The pyridine ring's carbons were also confirmed to be the carbons of pyridine ring gave the signals at 150.0 ppm, 151.3 ppm, 116.6 ppm, and 109.6 ppm. Mass spectra at m/z = 344 (M^{+}) is in agreement with the proposed structure.

Antimicrobial assay

Antibacterial bioassay

The newly synthesized compounds were screened for their antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* (MTCC-96), *Streptococcus pyogenes* (MTCC-442)) and Gram-negative bacteria (*Escherichia coli* (MTCC-443),

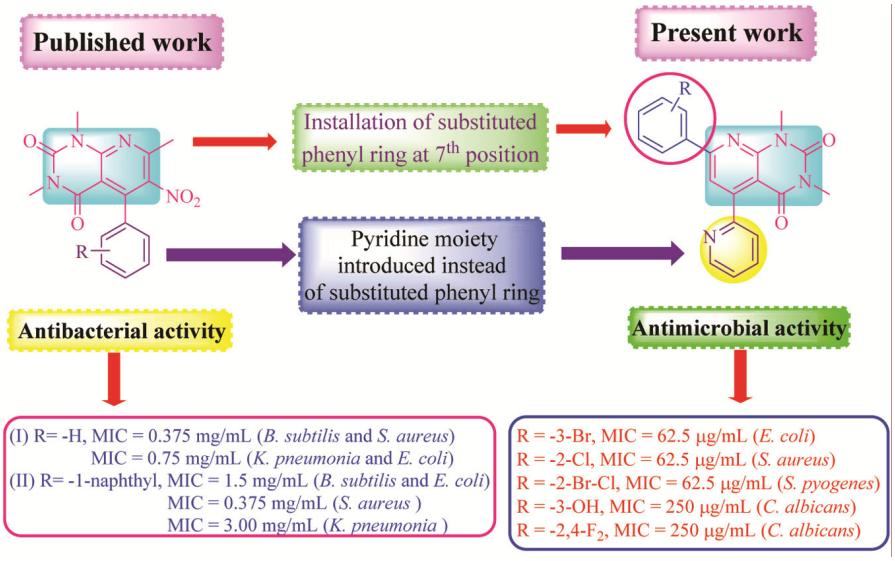
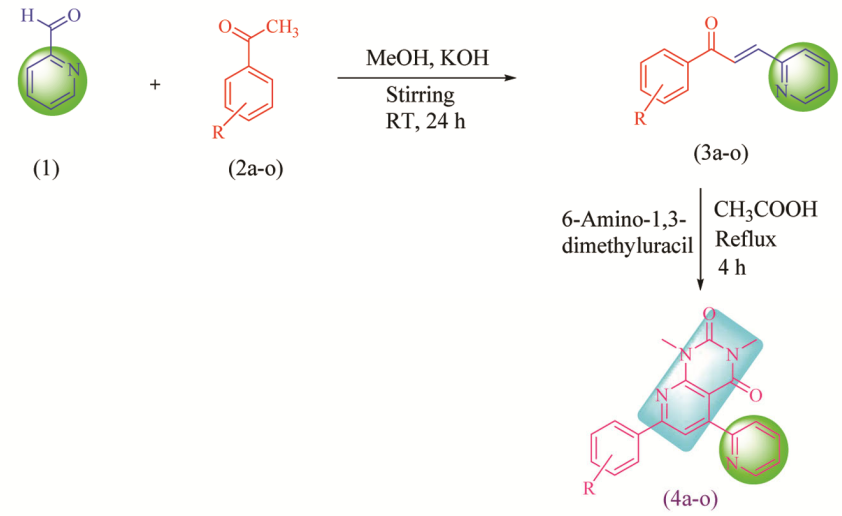


Fig. 2 — The rationale for the present study is based on a survey of previously published work



Scheme 1 — Synthesis of fused pyridine-pyrimidine hybrids (**4a-o**)

Pseudomonas aeruginosa (MTCC-1688)). The strains used for antimicrobial activity of newly synthesized compounds were procured from the IMTECH, Chandigarh. Mueller Hinton Broth dilution method (Becton Dickinson, USA) was used for the antibacterial assay of synthesized compounds. The compounds (**4a-o**) were screened for their antibacterial activity in triplicate sets against these bacteria at different concentrations of 1000, 500, 250, and 200 µg/mL. The drugs which were found to be active in primary analysis were further diluted and evaluated. 10 µg/mL suspensions were further inoculated on appropriate media and the growth was noted after one or two days. Minimum inhibitory concentration is the lowest concentration, which shows no growth of microbes after spot subculture for each drug. The test mixture should contain 10^8 cells/mL. In this study, DMSO and sterilized distilled water were used as negative controls while chloramphenicol was used as a positive control for evaluating the antibacterial activity[21].

Antifungal bioassay

The same newly synthesized compounds **(4a-o)** were screened for their antifungal activity against in six sets against *Candida albicans* (MTCC-227), *Aspergillus niger* (MTCC-282), *Aspergillus clavatus* (MTCC-1323) at various primary concentrations of 1000, 500 and 250 µg/mL. The primary screen active compounds were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25, and 12.5 µg/mL concentrations for secondary screening to test in a second set of dilution against all microorganisms. 'griseofulvin' was used as a standard drug for antifungal activity, which showed 500, 100, and 100 µg/mL MIC against *Candida albicans* (MTCC-227), *Aspergillus niger* (MTCC-282), *Aspergillus clavatus* (MTCC-1323) respectively. For growth of fungi, in the present procedure, we have used Sabourauds dextrose broth at 28 °C in aerobic condition for 48 h. DMSO and sterilized distilled water used as negative controls while 'griseofulvin' (1 U strength) was used as a positive control[21].

Discussion on antimicrobial activity

The *in vitro* antimicrobial activity results are given in Table I which revealed that the majority of the tested compounds displayed excellent to moderate antimicrobial activity. The antibacterial activity data revealed that compounds **4b, 4d** and **4m** exhibited promising antibacterial activity towards *E. coli, S. aureus*, and *S. pyogenes* strains respectively with MIC = 62.5 µg/mL. Furthermore, compounds **4g** and **4o** found to be the most prominent antifungal agents against *C. albicans* with MIC = 250 µg/mL which was more effective than reference drug griseofulvin. Moreover, when compared to the standard drug griseofulvin, the compounds **4b, 4c, 4e, 4f, 4h, 4i, 4j,** and **4n** exhibited equal activity toward *C. albicans* at MIC value of 500 µg/mL.

Table I — Antimicrobial activity of 1,3-dimethyl-7-aryl-5-(pyridin-2-yl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones (4a-o)

Sr No.	-R	Minimum inhibitory concentration (MIC) in µg/mL				Minimum inhibitory concentration (MIC) in µg/mL		
		E. c.	*P. a.*	*S. a.*	*S. p.*	*C. a.*	*A. n.*	*A. c.*
4a	-H	250	200	250	200	1000	500	500
4b	-3-Br	62.5	100	500	250	500	1000	1000
4c	-4-Br	100	100	100	200	500	1000	1000
4d	-2-Cl	250	125	62.5	100	1000	500	500
4e	-4-Cl	250	100	200	200	500	>1000	>1000
4f	-4-F	200	250	250	250	500	>1000	>1000
4g	-3-OH	200	250	200	250	250	>1000	>1000
4h	-4-OH	250	200	250	200	500	1000	1000
4i	-2-OCH_3	200	250	125	100	500	1000	1000
4j	-3-OCH_3	125	200	100	200	500	1000	1000
4k	-4-CH_3	100	250	200	250	>1000	250	>1000
4l	-3-NO_2	100	125	250	100	>1000	>1000	>1000
4m	-2-Br-4-Cl	250	200	100	62.5	>1000	1000	>1000
4n	-3,4-Cl_2	250	250	125	100	500	500	1000
4o	-2,4-F_2	250	125	500	500	250	>1000	500
Chloramphenicol		50	50	50	50		-	
Griseofulvin				-		500	100	100

Escherichia coli (*E.c.*) MTCC-442; *Pseudomonas aeruginosa* (*P.a.*) MTCC-441; *Staphylococcus aureus* (*S.a.*) MTCC-96; *Streptococcus pyogenes* (*S.p.*) MTCC-443; *Candida albicans* (*C.a.*) MTCC-227; *Aspergillus niger* (*A.n.*) MTCC-282; *Aspergillus clavatus* (*A.c.*) MTCC-1323.

Structure-activity relationship (SAR) study

On the basis of *in vitro* antimicrobial data, the structure–activity relationship (SAR) of the synthesized hybrids (**4a-o**) is given in the Figure 3. The SAR results show that the synthesized hybrids exhibited potent inhibitory growth, especially compound containing electron-withdrawing groups i.e., 3-Br (**4b**, MIC = 62.5 µg/mL), -2-Cl **(4d,** MIC = 62.5 µg/mL), and -2-Br-4-Cl **(4m,** MIC = 62.5 µg/mL) exhibited excellent antibacterial activity against *E. coli, S. aureus*, and *S. pyogenes* strains respectively. Moreover, electron-donating **-3-OH** (**4g**) and electron-withdrawing groups **-2,4-F_2** (**4o**) effects enhanced antifungal activity with MIC value of 250 µg/mL against *C. albicans* which was more potent as compared to the standard drug griseofulvin.

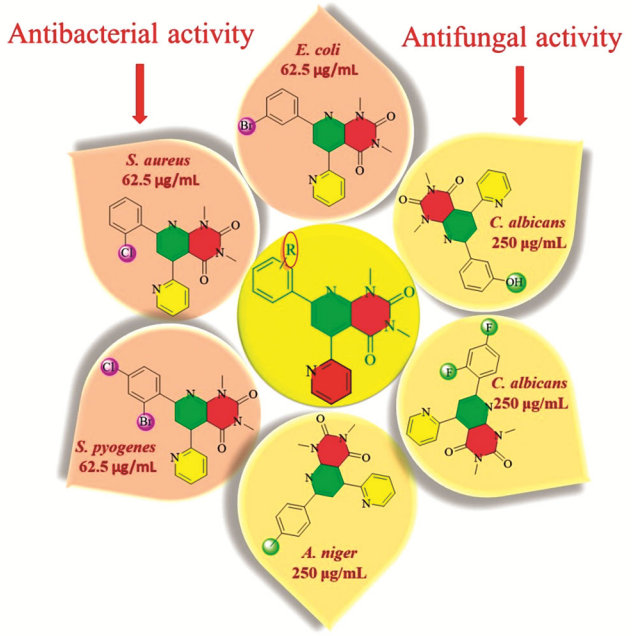


Fig. 3 — The structure-activity relationship (SAR) of the synthesized compounds (**4a-o**)

Experimental Section

The required chemicals were purchased from Aldrich and E. Merck and used without further purification. Buchi Rota Vapor was used for distillation. Melting points were determined in the Gallenkamp apparatus and were uncorrected. The completion of the reaction and the purity of all compounds was checked on aluminum-coated TLC plates 60, F_{254} (E. Merck) using n-hexane and ethyl acetate (7:3) as eluent and visualized under ultraviolet (UV) light, or iodine vapor. Elemental analysis was carried out by a Perkin-Elmer 2400 CHN analyzer. ^{1}H NMR spectra were recorded on a Bruker Avance II 400 MHz and ^{13}C NMR spectra on Varian Mercury - 400, 100 MHz in $CDCl_3$ as a solvent and tetramethylsilane (TMS) as an internal standard using 5mm tube. Chemical shifts were reported in parts per million (ppm). IR spectra were recorded on a

Shimadzu FT-IR spectrophotometer while mass spectra were scanned on a Shimadzu LC-MS 2010 spectrometer[22].

Compounds 1-aryl-3-(pyridin-2-yl)prop-2-en-1-ones (3a-o) were prepared as per the method given in literature[23].

General preparation of 1,3-dimethyl-7-aryl-5-(pyridin-2-yl)pyrido[2,3-d]pyrimidine-2,4(1H,3H)-diones (4a-o)

Chalcone derivatives **(3a-o)** were dissolved in 30 mL of ethanol (95%) and 6-amino-1, 3-dimethyluracilwas added to it. After the addition of CH_3COOH in a catalytic amount, the reaction mixture was refluxed for 4 h. The reaction mixture was allowed to cool at room temperature and poured into crushed ice. Solid formed was filtered, dried, and recrystallized from ethanol (95%).

Characterization of 1,3-dimethyl-7-phenyl-5-(pyridin-2-yl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4a)

Yield: 67%; Solid; M.P.: 111-113 °C; IR (KBr, cm^{-1}): 3189, 2849 (C-H, -CH=CH-), 1763 (>C=O), 1685, 1656, 1255 (C=N, C=C, C-N); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.22 (s, 1H, Ar-H), 7.97 – 7.92 (m, 3H, Ar-H), 7.88 – 7.81 (m, 3H, Ar-H), 7.76 – 7.68 (m, 3H, Ar-H), 3.46 (s, 3H, Ar-CH_3), 3.36 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ =162.7 (C_4 of pyrimidine), 159.3 (4C_6 of pyrimidine), 157.2 (C_2 of pyrimidine), 155.1 (C_2 of pyridine), 151.3 (C_4 of pyridine), 150.4 (C_2 & C_6 of pyridine), 144.2 (C_4 of pyridine), 138.4 (Ar-C), 137.9 (Ar-C), 129.9 (Ar-C), 129.4 (Ar-C), 127.9 (Ar-C), 124.4 (Ar-C), 122.6 (Ar-C), 116.6 (C_3 of pyridine), 109.6 (C_5 of pyridine), 29.2 (-CH_3), 28.3 (-CH_3); MS (*m/z*): 344.13 (M^+); Elements analysis calculated (%) for $C_{20}H_{16}N_4O_2$: C 69.76, H 4.68, N 16.27; Found: C 70.45, H 5.35, N 16.97.

Characterization of 7-(3-bromophenyl)-1,3-dimethyl-5-(pyridin-2-yl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4b)

Yield: 65%; Solid; M.P.:117-119 °C; IR (KBr, cm^{-1}): 3185, 2854 (C-H, -CH=CH-), 1764 (>C=O), 1688, 1654, 1258 (C=N, C=C, C-N), 685 (-Br); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.19 (s, 1H, Ar-H), 8.01 – 7.99 (m, 2H, Ar-H), 7.89 – 7.85 (m, 3H, Ar-H), 7.79 – 7.74 (m, 3H, Ar-H), 3.62 (s, 3H, Ar-CH_3), 3.16 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ =162.2 (C_4 of pyrimidine), 159.6 (C_6 of pyrimidine), 157.6 (C_2 of pyrimidine), 155.3 (C_2 of pyridine), 151.7 (C_4 of pyridine), 150.3 (C_2 & C_6 of pyridine), 144.5 (C_4 of pyridine), 138.7 (Ar-C), 137.8 (Ar-C), 129.7 (Ar-C), 129.5 (Ar-C), 127.6 (Ar-C), 124.4 (Ar-C), 122.8 (Ar-C), 122.6 (Ar-C), 116.4 (C_3 of pyridine), 109.8 (C_5 of pyridine), 29.3 (-CH_3), 28.6 (-CH_3); MS (*m/z*): 424.04 (M^+); Elements analysis calculated (%) for $C_{20}H_{15}BrN_4O_2$: C 56.75, H 3.57, N 13.24; Found: C 56.78, H 3.61, N 13.27.

Characterization of 7-(4-bromophenyl)-1,3-dimethyl-5-(pyridin-2-yl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4c)

Yield: 71%; Solid; M.P.:120-122 °C; IR (KBr, cm^{-1}): 3192, 2844 (C-H, -CH=CH-), 1768 (>C=O), 1678, 1657, 1252 (C=N, C=C, C-N), 676 (-Br); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.22 (s, 1H, Ar-H), 8.03 – 7.96 (m, 2H, Ar-H), 7.91 – 7.87 (m, 3H, Ar-H), 7.82 – 7.73 (m, 3H, Ar-H), 3.65 (s, 3H, Ar-CH_3), 3.11 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ =162.8 (C_4 of pyrimidine), 158.4 (C_6 of pyrimidine), 156.5 (C_2 of pyrimidine), 151.3 (C_2 of pyridine), 150.7 (C_4 of pyridine), 148.9 (C_2 & C_6 of pyridine), 141.5 (C_4 of pyridine), 138.8 (Ar-C), 137.5 (Ar-C), 134.9 (Ar-C), 129.9 (Ar-C), 129.5 (Ar-C), 127.4 (Ar-C), 124.8 (Ar-C), 112.4 (C_3 of pyridine), 107.4 (C_5 of pyridine), 29.8 (-CH_3), 28.4 (-CH_3); MS (*m/z*): 424.04 (M^+); Elements analysis calculated (%) for $C_{20}H_{15}BrN_4O_2$: C 56.75, H 3.57, N 13.24; Found: C 56.78, H 3.61, N 13.29.

Characterization of 7-(2-chlorophenyl)-1,3-dimethyl-5-(pyridin-2-yl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4d)

Yield: 74%; Solid; M.P.:102-104 °C; IR (KBr, cm^{-1}): 3188, 2851 (C-H, -CH=CH-), 1776 (>C=O), 1679, 1660, 1255 (C=N, C=C, C-N), 744 (-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.17 (s, 1H, Ar-H), 8.07 – 8.04 (m, 3H, Ar-H), 7.88 – 7.84 (m, 3H, Ar-H), 7.70 – 7.66 (m, 2H, Ar-H), 3.44 (s, 3H, Ar-CH_3), 3.16 (s, 3H, Ar-CH_3);^{13}C NMR (100 MHz, $CDCl_3$): δ =162.8 (C_4 of pyrimidine), 158.5 (C_6 of pyrimidine), 156.8 (C_2 of pyrimidine), 151.8 (C_2 of pyridine), 150.9 (C_4 of pyridine), 148.4 (C_2 & C_6 of pyridine), 141.8 (C_4 of pyridine), 138.6 (Ar-C), 137.4 (Ar-C), 135 (Ar-C), 129.8 (Ar-C), 129.5 (Ar-C), 127.7 (Ar-C), 124.3 (Ar-C),124.2 (Ar-C),113 (C_3 of pyridine), 107.6 (C_5 of pyridine), 29.8 (-CH_3), 28.5 (-CH_3); MS (*m/z*): 378.09 (M^+); Elements analysis calculated (%) for $C_{20}H_{15}ClN_4O_2$: C 63.41, H 3.99, N 14.79; Found: C 63.45, H 4.03, N 14.83.

Characterization of 7-(4-chlorophenyl)-1,3-dimethyl-5-(pyridin-2-yl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4e)

Yield: 75%; Solid; M.P.:112-114 °C; IR (KBr, cm^{-1}): 3187, 2848 (C-H, -CH=CH-), 1761 (>C=O), 1675, 1654, 1257 (C=N, C=C, C-N), 754 (-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.14 (s, 1H, Ar-H), 8.08 – 8.02 (m, 3H, Ar-H), 7.89 – 7.82 (m, 3H, Ar-H), 7.75 – 7.67 (m, 2H, Ar-H), 3.46 (s, 3H, Ar-CH_3), 3.15 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ =162.2 (C_4 of pyrimidine), 158.8 (C_6 of pyrimidine), 156.5 (C_2 of pyrimidine), 151.9 (C_2 of pyridine), 150.6 (C_4 of pyridine), 148.3 (C_2 & C_6 of pyridine), 141.2 (C_4 of pyridine), 138.8 (Ar-C), 137.3(Ar-C), 134.3 (Ar-C), 129.9 (Ar-C), 129.6 (Ar-C), 127.9 (Ar-C), 124.5 (Ar-C), 112.3 (C_3 of pyridine), 107.9 (C_5 of pyridine), 29.5 (-CH_3), 28.9 (-CH_3); MS (*m/z*): 378.09 (M^+); Elements analysis calculated (%) for $C_{20}H_{15}ClN_4O_2$: C 63.41, H 3.99, N 14.79; Found: C 63.46, H 4.05, N 14.82.

Characterization of 7-(4-fluorophenyl)-1,3-dimethyl-5-(pyridin-2-yl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4f)

Yield: 83%; Solid; M.P.:127-129 °C; IR (KBr, cm^{-1}): 3181, 2857 (C-H, -CH=CH-), 1762 (>C=O), 1688, 1647, 1258 (C=N, C=C, C-N), 1249 (-F); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.02 – 7.95 (m, 4H, Ar-H), 7.83 – 7.74 (m, 4H, Ar-H), 3.41 (s, 3H, Ar-CH_3), 3.27 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ =162.5 (C_4 of pyrimidine), 158.6 (C_6 of pyrimidine), 156.4 (C_2 of pyrimidine), 151.9 (C_2 of pyridine), 150.8 (C_4 of pyridine), 148.5 (C_2 & C_6 of pyridine), 141.9 (C_4 of pyridine), 138.5 (Ar-C), 137.9 (Ar-C), 134.5 (Ar-C), 129.8 (Ar-C), 129.4 (Ar-C), 127.2 (Ar-C), 124.8 (Ar-C), 112.4 (C_3 of pyridine), 108.8 (C_5 of pyridine), 29.7 (-CH_3), 28.6 (-CH_3); MS (*m/z*): 362.12 (M^+); Elements analysis calculated (%) for $C_{20}H_{15}FN_4O_2$: C 66.29, H 4.17, N 15.46; Found: C 66.34, H 4.22, N 15.50.

Characterization of 7-(3-hydroxyphenyl)-1,3-dimethyl-5-(pyridin-2-yl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4g)

Yield: 72%; Solid; M.P.:106-108 °C; IR (KBr, cm^{-1}): 3554 (-OH), 3182, 2848 (C-H, -CH=CH-), 1763 (>C=O), 1679, 1652, 1259 (C=N, C=C, C-N); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.14 (s, 1H, Ar-OH), 8.22 (s, 1H, Ar-H), 8.14 – 8.11 (m, 1H, Ar-H), 7.95 – 7.87 (m, 4H, Ar-H), 7.76 – 7.70 (m, 3H, Ar-H), 3.45 (s, 3H, Ar-CH_3), 3.33 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.6 (C_4 of pyrimidine), 158.9 (C_6 of pyrimidine), 157 (C_2 of pyrimidine, Ar-C), 151.6 (C_2 of pyridine), 150.5 (C_4 of pyridine), 148.8 (C_2 & C_6 of pyridine), 141.6 (C_4 of pyridine), 138.9 (Ar-C), 137.6 (Ar-C), 134.4 (Ar-C), 129.9 (Ar-C), 129.5 (Ar-C), 127.7 (Ar-C), 124.3 (Ar-C), 112.5 (C_3 of pyridine), 107.8 (C_5 of pyridine), 29.8 (-CH_3), 28.6 (-CH_3); MS (*m/z*): 360.12 (M^+); Elements analysis calculated (%) for $C_{20}H_{16}N_4O_3$: C 66.66, H 4.48, N 15.55; Found: C 66.70, H 4.52, N 15.59.

Characterization of 7-(4-hydroxyphenyl)-1,3-dimethyl-5-(pyridin-2-yl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4h)

Yield: 77%; Solid; M.P.:112-114 °C; IR (KBr, cm^{-1}): 3548 (-OH), 3197, 2844 (C-H, -CH=CH-), 1763 (>C=O), 1671, 1658, 1255 (C=N, C=C, C-N); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.17 (s, 1H, Ar-OH), 8.25 (s, 1H, Ar-H), 8.18 – 8.12 (m, 1H, Ar-H), 7.98 – 7.84 (m, 4H, Ar-H), 7.78 – 7.75 (m, 3H, Ar-H), 3.42 (s, 3H, Ar-CH_3), 3.39 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ =162.4 (C_4 of pyrimidine), 158.6 (C_6 of pyrimidine), 156.4 (C_2 of pyrimidine), 151.7 (C_2 of pyridine), 150.5 (C_4 of pyridine), 148.8 (C_2 & C_6 of pyridine), 141.5 (C_4 of pyridine), 138.5 (Ar-C), 137.8 (Ar-C), 134.4 (Ar-C), 129.8 (Ar-C), 129.2 (Ar-C), 127.5 (Ar-C), 124.5 (Ar-C), 112.4(C_3 of pyridine), 107.8 (C_5 of pyridine), 29.4 (-CH_3), 28.5 (-CH_3); MS (*m/z*): 360.12 (M^+); Elements analysis calculated (%) for $C_{20}H_{16}N_4O_3$: C 66.66, H 4.48, N 15.55; Found: C 66.71, H 4.52, N 15.60.

Characterization of 7-(2-methoxyphenyl)-1,3-dimethyl-5-(pyridin-2-yl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4i)

Yield: 62%; Solid; M.P.:123-125 °C; IR (KBr, cm^{-1}): 3188, 2848 (C-H, -CH=CH-), 1762 (>C=O), 1675, 1658, 1254 (C=N, C=C, C-N),1144 (-OCH_3); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.27 (s, 1H, Ar-H), 8.06 – 7.91 (m, 3H, Ar-H), 7.86 – 7.80 (m, 2H, Ar-H), 7.68 – 7.60 (m, 3H, Ar-H), 3.64 (s, 3H, Ar-OCH_3), 3.44 (s, 3H, Ar-CH_3), 3.21 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ =162.7 (C_4 of pyrimidine), 158.4 (C_6 of pyrimidine), 156.7 (C_2 of pyrimidine), 151.5 (C_2 of pyridine), 150.8 (C_4 of pyridine), 148.5 (C_2 & C_6 of pyridine), 141.8 (C_4 of pyridine), 138.4 (Ar-C), 137.5 (Ar-C), 134.4 (Ar-C), 129.8 (Ar-C), 129.5 (Ar-C), 127.9 (Ar-C), 124.5 (Ar-C), 112.6 (C_3 of pyridine), 107.5 (C_5 of pyridine), 55.7 (Ar-OCH_3),

29.4 (-CH_3), 28.6 (-CH_3); MS (*m/z*): 374.17(M^+); Elements analysis calculated (%) for $C_{21}H_{18}N_4O_3$: C 67.37, H 4.85, N 14.96; Found: C 67.40, H 4.90, N 14.99.

Characterization of 7-(3-methoxyphenyl)-1,3-dimethyl-5-(pyridin-2-yl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4j)

Yield: 66%; Solid; M.P.:121-123 °C; IR (KBr, cm^{-1}): 3196, 2840 (C-H, -CH=CH-), 1760 (>C=O), 1675, 1650, 1253 (C=N, C=C, C-N), 1154 (-OCH_3); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.23 (s, 1H, Ar-H), 8.09 – 7.94 (m, 3H, Ar-H), 7.87 – 7.85 (m, 2H, Ar-H), 7.66 – 7.62 (m, 3H, Ar-H), 3.66 (s, 3H, Ar-OCH_3), 3.46 (s, 3H, Ar-CH_3), 3.25 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ =163.2 (C_4 of pyrimidine), 158.9 (C_6 of pyrimidine), 156.8 (C_2 of pyrimidine), 151.6 (C_2 of pyridine), 150.8 (C_4 of pyridine), 148.6 (C_2 & C_6 of pyridine), 141.8 (C_4 of pyridine), 138.5 (Ar-C), 137.5 (Ar-C), 134.4 (Ar-C), 129.9 (Ar-C), 129.5 (Ar-C), 127.9 (Ar-C), 127.6 (Ar-C), 124.7 (Ar-C),124.6 (Ar-C), 112.7 (C_3 of pyridine), 107.6 (C_5 of pyridine), 55.8 (Ar-OCH_3), 29.5 (-CH_3), 28.7 (-CH_3); MS (*m/z*): 374.14 (M^+); Elements analysis calculated (%) for $C_{21}H_{18}N_4O_3$: C 67.37, H 4.85, N 14.96; Found: C 67.40, H 4.89, N 15.00.

Characterization of 1,3-dimethyl-5-(pyridin-2-yl)-7-(*p*-tolyl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4k)

Yield: 85%; Solid; M.P.:108-110 °C; IR (KBr, cm^{-1}): 3195, 2846 (C-H, -CH=CH-), 1767 (>C=O), 1679, 1653, 1253 (C=N, C=C, C-N); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.27 (s, 1H, Ar-H), 8.03 – 7.95 (m, 3H, Ar-H), 7.86 – 7.80 (m, 2H, Ar-H), 7.68 – 7.63 (m, 3H, Ar-H), 3.47 (s, 3H, Ar-CH_3), 3.21 (s, 3H, Ar-CH_3), 2.44 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ =162.8 (C_4 of pyrimidine), 158.6 (C_6 of pyrimidine), 156.7 (C_2 of pyrimidine), 151.4 (C_2 of pyridine), 150.7 (C_4 of pyridine), 148.5 (C_2 & C_6 of pyridine), 141.6 (C_4 of pyridine), 138.8 (Ar-C), 137.6 (Ar-C), 134.6 (Ar-C), 129.9 (Ar-C), 129.1 (Ar-C), 127.8 (Ar-C), 124.5 (Ar-C), 112.5 (C_3 of pyridine), 107.8 (C_5 of pyridine), 29.4 (-CH_3), 28.6 (-CH_3) 21.9 (Ar-CH_3); MS (*m/z*): 358.14 (M^+); Elements analysis calculated (%) for $C_{21}H_{18}N_4O_2$: C 70.38, H 5.06, N 15.63; Found: C 70.41, H 5.10, N 15.66.

Characterization of 1,3-dimethyl-7-(3-nitrophenyl)-5-(pyridin-2-yl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4l)

Yield: 81%; Solid; M.P.:121-123 °C; IR (KBr, cm^{-1}): 3196, 2843 (C-H, -CH=CH-), 1768 (>C=O), 1672, 1659, 1253 (C=N, C=C, C-N), 753 (-NO_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.20 (s, 1H, Ar-H), 8.11 – 8.07 (m, 2H, Ar-H), 7.94 (m, 3H, Ar-H), 7.79 – 7.72 (m, 3H, Ar-H), 3.59 (s, 3H, Ar-CH_3), 3.22 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ =162.7 (C_4 of pyrimidine), 158.5 (C_6 of pyrimidine), 156.6 (C_2 of pyrimidine), 151.4 (C_2 of pyridine), 150.5 (C_4 of pyridine), 148.9 (C_2 & C_6 of pyridine), 141.5 (C_4 of pyridine), 138.7 (Ar-C), 137.3 (Ar-C), 134.7 (Ar-C), 129.8 (Ar-C), 129.4 (Ar-C), 127.6 (Ar-C), 127.5 (Ar-C),124.8 (Ar-C),124.5 (Ar-C),112.4 (C_3 of pyridine), 107.5 (C_5 of pyridine), 29.2 (-CH_3), 28.3 (-CH_3); MS (*m/z*): 389.11 (M^+); Elements analysis calculated (%) for $C_{20}H_{15}N_4O_2$: C 61.69, H 3.88, N 17.99; Found: C 61.72, H 3.92, N 18.04.

Characterization of 7-(2-bromo-4-chlorophenyl)-1,3-dimethyl-5-(pyridin-2-yl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4m)

Yield: 61%; Solid; M.P.:129-131 °C; IR (KBr, cm^{-1}): 3190, 2841 (C-H, -CH=CH-), 1764 (>C=O), 1677, 1651, 1252 (C=N, C=C, C-N), 760 (-Cl), 673 (-Br);^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.82-8.44 (s, 5H, Ar-CH), 8.30 - 7.85 (m, 3H, Ar-H), 3.44(s, 3H, Ar-CH_3), 3.21 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ =162.9 (C_4 of pyrimidine), 158.4 (C_6 of pyrimidine), 156.7 (C_2 of pyrimidine), 151.5 (C_2 of pyridine), 150.5 (C_4 of pyridine), 148.2 (C_2 & C_6 of pyridine), 141.6 (C_4 of pyridine), 138.4 (Ar-C), 137.7 (Ar-C), 134.3 (Ar-C), 129.7 (Ar-C), 129.3 (Ar-C), 127.5 (Ar-C), 124.4 (Ar-C), 112.2 (C_3 of pyridine), 107.1 (C_5 of pyridine), 29.5 (-CH_3), 28.3 (-CH_3); MS (*m/z*): 458.0 (M^+); Elements analysis calculated (%) for $C_{20}H_{14}Cl_2N_4O_2$: C 52.48, H 3.08, N 12.24; Found: C 52.50, H 3.09, N 12.25.

Characterization of 7-(3,4-dichlorophenyl)-1,3-dimethyl-5-(pyridin-2-yl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4n)

Yield: 61%; Solid; M.P.:129-131 °C; IR (KBr, cm^{-1}): 3195, 2849 (C-H, -CH=CH-), 1769 (>C=O), 1676, 1658, 1259 (C=N, C=C, C-N), 765 (-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.84-8.46 (s, 5H, Ar-CH), 8.10 - 7.89 (m, 3H, Ar-H), 3.42(s, 3H, Ar-CH_3),

3.27 (s, 3H, Ar-CH_3);^{13}C NMR (100 MHz, $CDCl_3$): δ =162.8 (C_4 of pyrimidine), 158.4 (C_6 of pyrimidine), 156.8 (C_2 of pyrimidine), 151.4 (C_2 of pyridine), 150.8 (C_4 of pyridine), 148.4 (C_2 & C_6 of pyridine), 141.8(C_4 of pyridine), 138.8 (Ar-C), 137.4 (Ar-C), 134.7 (Ar-C), 129.4 (Ar-C), 129.2 (Ar-C), 127.8 (Ar-C), 124.4 (Ar-C), 112.8 (C_3 of pyridine), 107.4 (C_5 of pyridine), 29.8 (-CH_3), 28.4 (-CH_3); MS (*m/z*): 412.05 (M^+); Elements analysis calculated (%) for $C_{20}H_{14}Cl_2N_4O_2$: C 58.13, H 3.41, N 13.56; Found: C 58.18, H 3.44, N 13.58.

Characterization of 7-(2,4-difluorophenyl)-1,3-dimethyl-5-(pyridin-2-yl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4o)

Yield: 87%; Solid; M.P.:122-124 °C; IR (KBr, cm^{-1}): 3197, 2845 (C-H, -CH=CH-), 1764 (>C=O), 1677, 1651, 1258 (C=N, C=C, C-N), 1244 (-F); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.87-8.39 (s, 5H, Ar-CH), 8.24 - 7.81 (m, 3H, Ar-H), 3.45 (s, 3H, Ar-CH_3), 3.23 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ =162.6 (C_4 of pyrimidine), 158.3 (C_6 of pyrimidine), 156.7 (C_2 of pyrimidine), 151.5 (C_2 of pyridine), 150.3 (C_4 of pyridine), 148.4 (C_2 & C_6 of pyridine), 141.8 (C_4 of pyridine), 138.4 (Ar-C), 137.3 (Ar-C), 134.7 (Ar-C), 129.6 (Ar-C), 129.2 (Ar-C), 127.5 (Ar-C), 124.4 (Ar-C), 112.7 (C_3 of pyridine), 107.3 (C_5 of pyridine), 29.6 (-CH_3), 28.3 (-CH_3); MS (*m/z*): 380.11 (M^+); Elements analysis calculated (%) for $C_{20}H_{14}F_2N_4O_2$: C 63.16, H 3.71, N 14.73; Found: C 63.20, H 3.75, N 14.76.

Conclusions

The chalcone derivatives were synthesized using base mediated Aldol condensation protocol. The titled compounds (**4a-o**) were synthesized by reaction of chalcone derivatives with 6-amino-1,3-dimethyluracil in presence of acetic acid as a catalyst. Fused pyridine-pyrimidine hybrids were evaluated for their antibacterial and antifungal activities against various strains. Based on the antimicrobial results we concluded that compounds **4b, 4d** and **4m** containing electron-withdrawing groups were most potent against bacteria with MIC value of 62.5 µg/mL towards *E. coli, S. aureus*, and *S. pyogenes* strains respectevely. Moreover, compounds **4g** and **4o** containing electron-withdrawing as well as electron donating groups were found to exhibit most prominant antifungal activity having MIC value of 250 µg/mL against *C. albicans* strain. These finding pave the way to the discovery and development of new antimicrobial agents.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/ 123456789/ 58776

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

References

1 Marepu N, Yeturu S, & Pal M, *Bioorg Med Chem Lett*, 28 (2018) 3302.
2 Desai N C, Rupala Y M, Khasiya A G, Shah K N, Pandit U P & Khedkar V M, *J Heterocycl Chem,* 59 (2022) 75.
3 Fayed E A, Nosseir E S, Atef A & El-Kalyoubi S A, *Mol Divers,* (2021) 1.
4 David B, Wolfender J L & Dias D A, *Phytochem Rev,* 14 (2015) 299.
5 Ventola C L, *PharmTher,* 40 (2015) 277.
6 Somashekara B, Vijayakumar G R, *Indian J Chem,* 60B (2021) 1601.
7 Luepke K H, Suda K J, Boucher H, Russo R L, Bonney M W, Hunt T D & Mohr III J F, *Pharmacotherapy*, 37 (2017) 71.
8 Radwan M A A, Alshubramy M A, Abdel-Motaal M, Hemdan B A & El-Kady D S, *Bioorg chem*, 96 (2020) Article 103516.
9 Othman I M M, Gad-Elkareem M A M, Anouar E H, Snoussi M, Aouadi K & Kadri A, *J Mol Struct*, 1219 (2020) 128651.
10 Malani A, Makwana A, Monapara J, Ahmad I, Patel H & Desai N C, *J Biochem Mol Toxicol*, 35 (2021) e22903.
11 Desai N C, Vaghani H V, Jethawa A M & Khedkar V M, *Arch Pharm (Weinheim),* 354 (2021) e2100134.
12 Desai N C, Bhatt K, Jadeja D J, Mehta H K, Khedkar V M & Sarkar D, *Drug Dev Res*, 83 (2022) 416.
13 Abhale Y K, Deshmukh K K, Sasane A V, Chavan A P & Mhaske P C, *J Heterocycl Chem*, 53 (2016) 229.
14 Bai S, Liu S, Zhu Y & Wu Q, *Tetrahedron Lett*, 59 (2018) 3179.
15 Xie H, Lin X, Zhang Y, Tan F, Chi B, Peng Z, Dong W &An D, *Bioorganic Med Chem Lett*, 30 (2020) Article 127459.
16 El-Mekabaty A, Fouda A E A S & Shaaban I E. *J Heterocycl Chem*, 57 (2020) 2928.
17 Abdelgawad M A, Bakr R B & Azouz A A, *Bioorg chem*, 77 (2018) 339.
18 Showalter H D, *Molecules*, 25 (2020) Article 4137.

19 Reddyrajula R & Dalimba U K, *New J Chem*, 43 (2019) 16281.
20 Saikia L, Das B, Bharali P & Thakur A J, *Tetrahedron Lett*, 55 (2014) 1796.
21 Desai N C, Shihory N, Khasiya A, Pandit U & Khedkar V, *Phosphorus Sulfur Silicon Relat Elem*, 196 (2021) 569.
22 Desai N C, Bhatt K, Monapara J, Pandit U & Khedkar V M, *ACS Omega*, 6 (2021) 28270.
23 Desai N C, Joshi S B & Khedkar V M, *Anal Chem Lett*, 10 (2020) 307.