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POTENT IN-VITRO ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF PYRANO[2,3-D]PYRIMIDINE DERIVATIVES WITH QUANTITATIVE YIELD

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ABSTRACT

A series of Pyrano[2,3-d]Pyrimidine derivatives were synthesis using Knoevenagel and Michael addition reaction. Investigation of antimicrobial properties were done against gram positive bacteria Staphylococcus(NCTC-7447),Bacillus cereus(ATCC-14579) and gram negative bacteria Serratia maresens(IMRU-70), Proteus merabitis (NTCC-289) using Ampicillin as standard drugs. These synthesized compounds were also screened for their antifungal activity against two species of fungi Aspergillus ochraceus Wilhelm (AUCC-230 and Penicillium chrysogenum thom (AUCC-530) using Mycostatin as standard drugs. All the tested compounds shown moderate activity. The structures of the synthesized compounds were elucidated by spectral analysis (IR, 1HNMR and 13CNMR).

KEYWORDS: Pyrano[2,3-d]Pyrimidine derivatives, Ethylcyanoacetate, barbituric acid, aromatic aldehydes, N-base Triethylamine, Biological activity.

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INTRODUCTION

Over the past few decades, health-related quality of human life benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses, not only because many of them produce venomous reactions but also due to emergence of drug resistant microbes. The study of aromatic six membered annulated heterocyclic rings are always of great importance in pharmaceutical sector as owes bio-isosteric factor which is as logical as large interest both from theoretical as well as practical importance. Pyrano[2,3-d]pyrimidine moieties clubbed in to one molecule, then resultant derivative enhances its pharmaceutical activity as abundant in biologically active compounds such as antitumour[1], cardiotonic[2], and antifungal activity[3]. Some of them exhibit antihypertensive activity[4], antagonist, antimalarial or antimetabolite[5] and antiviral evaluation[6] properties. pyrano[2,3-d]pyrimidines are building blocks used to evaluate their antibacterial activities[7] and various derived natural products are also used as drug for insomnia treatment[8]. Annulated pyrano[2,3-d]pyrimidines is unsaturated N and O type heterocyclic as a fusion of pyran and pyramidine rings, consisting of one oxygen atom at 8 and two nitrogen atoms at 1 and 3 positions respectively. Annulated pyrano[2,3-d]pyrimidines are synthesized by diverse procedures based on Knoevenagel condensation, Michael addition followed by cyclodehydration strategy and finally heterocyclization[9]. The development of environmentally benign and clean synthetic procedures has become the goal of present day organic synthesis. Water plays an essential role in life processes and also as a medium for organic reactions[10,11]. The organic reactions in aqueous media have attracted much attention in organic synthesis, not only because water is one of the most abundant, cheapest and environmentally friendly solvent, but also because this green solvent is highly polar and therefore immiscible with most organic compounds. Reactions in aqueous media are environmentally safe, devoid of any carcinogenic effects, have a simple work up and especially are important in industry[12]. Moreover the water-soluble catalyst resides and operates in the aqueous media, and separation of organic compounds is thus easy. Thus, there is a need for developing multicomponent reactions (MCRs) in water and without the use of any harmful organic solvent. Multi-component reactions have emerged as an important tool in the context of modern combinatorial synthesis. Moreover, high productivity, facile execution and simple reaction profile are one of the vital strategies in multi-component reactions, which have expanded rapidly in organic chemistry [13-16]. We use Triethylamine moiety as catalyst which is N-type base has been extensively investigated in catalyzing Knoevenagel condensation and Michael addition reaction. Triethylamine is a simple aliphatic tertiary amine type with three alkyl groups attached to the basic lone pair containing nitrogen atom. This basic nature amine is successfully used for the reaction for synthesis of annulated uracil because this is simple amine where the study of result of the alkyl group attached to the nitrogen atom could be done. As a base, triethylamine makes the Knoevenagel condensation and Michael addition reaction at room temperature with good to excellent yields (Table 1).In this regard, the synthetic exploitation of nucleophilic double bond of annulated pyrano[2,3-d]pyrimidine is an important synthetic strategy. Therefore, for the preparation of these complex molecules large efforts have been directed towards the synthetic manipulation of Pyrano[2,3-d] PyrImidine derivatives that occupy a distinct and unique place in medicinal chemistry.

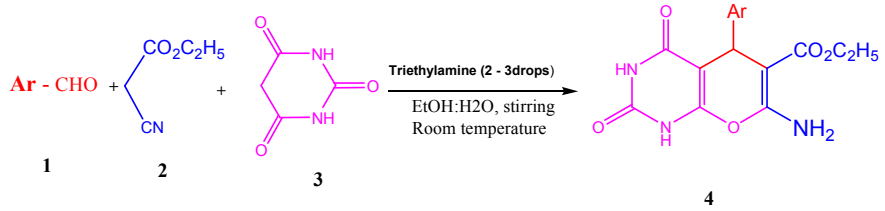
MATERIALS AND METHODS

Melting points were determined by open capillary method and were uncorrected. ^{1}H NMR spectra were obtained on a BRUKER instrument (300 MHz). IR spectra were recorded on a Perkin–Elmer 298 spectrophotometer using KBr pellet and ^{13}C-NMR (100 MHz) spectra were recorded in DMSO-*d6* as solvent with TMS as internal standard. Chemical shifts are reported in ppm.

Reactions have been monitored by thin layer chromatography on 0.2-mm precoated plates of silica gel G60 F254 (Merck). All gram positive and gram negative microbes and fungal strains were obtained from Pharmacy Department, university of Kashmir. Antimicrobial standard drug was used as Ampicillin and antifungal standard was used as Mycostatin. The potent antimicrobial and antifungal activities was tested by the disk diffusion method[17,18].

(i) General Synthetic procedure

Aromatic aldehydes (**1**) (1mmol), Ethylcyanoacetate (**2**) (1.2mmol) barbituric acid (**3**) (1mmol) and 2- 3 drops of Triethylamine base catalyst taken in R.B flask with 10-15 ml ethanol**:** water (1:1 ratio) solvent mixture and stirred for 43-110 minutes at room temperature. The reaction was monitored by TLC (thin layer chromatography). The solid compound was filtered, washed with cold water and recrystallization from 95% ethanol to obtain pure product Pyrano[2,3-d] PyrImidine derivatives. (Scheme 1)



Scheme 1

Quantitative synthesis of Pyrano [2,3-d]pyrimidiones.

(ii) Spectral data for synthesizes Pyrano[2,3-d]pyrimidine

Ethyl 7-amino-5-(4-methylphenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-6-carboxylate (4a).

IR (KBr, cm^{-1}): 3495, 3103, 2221,1912,1845, 1662,1567, 1734,; 1H NMR (300 MHz, DMSO): *δ* 2.36 (s, 3H, CH_3), 2.6(s, 3H, CH_3), 4.13 (s, 1H, H-5), 5.21(s, 2H, CH_2), 7.12 (s,2H, H-Ar), 7.20 (s,2H, H-Ar), 7.60 (br s, 2H, NH_2), 10.89 (s, 1H, NH), 11.43 (s, 1H, NH); ^{13}C NMR (100MHz, DMSO-d_6, δ, ppm) δ: 20.9, 88.7, 98.3, 115.5, 127.5, 128.1, 133.7, 137.4, 150.1, 155.5,155.9 159.1, 159.9, 160.8 ppm.

Ethyl 7-amino-2,4-dioxo-5-phenyl-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-carboxylate (4b).

IR (KBr, *v*cm^{-1}): 3381, 3168, 2289, 2202, 1664,1708, 1560; ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.6(s, 3H, CH_3),4.19 (s, 1H, H-5), 4.31(s, 2H, CH_2),6.07 (s,1H,H-Ar),7.10 (s, br, 2H, NH_2), 6.51- 8.13 (s, 5H-Ar), 11.12 (s, 1H, NH), 12.14 (br, s, 1H, NH); ^{13}CNMR (100MHZ, DMSOd_6,δppm) δ: 30.02, 129.8, 156.3, 152.8, 135.4, 128.6, 60.2, 69.2 ppm.

Ethyl 7-amino-5-(4-methoxyphenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-6-carboxylate (4c).

IR (KBr, *v* cm^{-1}): 3413,3278, 2239, 2165, 1878,1662,1543; ^{1}H NMR (300 MHz, DMSO-d_6,δ,ppm): 3.32 (s, 3H, OCH_3), 4.41 (1H, s, H-5),3.71(s, 2H,CH_2),2.49(s, 3H,CH_3) 6.93 (m, 2H, H-Ar), 7.65 (m, 2H, H-Ar), 9.07 (2H, br, s, NH_2), 11.09-10.03(s, br, 2H, NH); ^{13}CNMR(100MHz,DMSOd_6,δppm)δ:150.5,162.4, 167.3,157.2,143.1,134.3,130.1,114.2,75.6,55.8,37.2,33.03ppm.

Ethyl 7-amino-5-(3,4-dimethoxyphenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-carboxylate (4d).

IR (KBr, *v* cm^{-1}): 3495, 3303, 3123, 2987,2164,1662, 1576; ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm) δ:3.12 (s, 3H, CH_3), 3.5 (s, 2H, CH_2), 3.6 - 4.02 (s, 3H, OCH_3), 4.2 (s, 1H, H-5), 7.1 (s, 2H, NH_2), 11.1 (s, 1H, NH), 11.4 (s,1H,NH), 8.27 (m, 2H, H-Ar), 8.47 (m, 2H,H-Ar); ^{13}C NMR (100MHz, DMSO-d_6, δ, ppm) δ: 37.9, 135.8, 56.3, 163.8,57.4, 79.7, 150.1, 114.2,146.9,149.4 ppm.

*Ethyl 7-amino-5-(3-hydroxyphenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-*carboxylate (4e).

IR (KBr, *v* cm^{-1}):3439, 3337, 3193, 3028, 2206, 1677, 1625; ^1H NMR (300MHz, DMSO-d_6, δ, ppm) 3.6 (s, 3H, CH_3), 3.91 (s, 2H, CH_2), 4.10 (s, 1H, H-5), 6.56 (br s, 2H, NH_2), 6.59(m, 1H, H-Ar), 7.04-7.10 (m, 3H, H-Ar), 9.33 (br s, 1H, OH),11.09 (br s, 1H, NH), 12.07 (br s, 1H, NH); ^{13}C NMR (100MHz, DMSO-d_6, δ, ppm) δ: 35.6, 59.9, 89.5, 114.7, 114.9, 118.8, 120.1,130.1, 146.5, 150.4, 153.1, 158.1, 158.5, 163.3 ppm.

Ethyl7-amino-5-(4-hydroxyphenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-6-carboxylate (4f).

IR (KBr, *v* cm^{-1}): 3343, 3191, 3142, 2209, 1909, 1796, 1685; ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.69 (s, 1H, H-5),3.17s,2H,CH_2),2.43(s,3H,CH_3), 7.31 (br s, 2H, NH_2), 5.97(m, 2H, H-Ar), 6.74 (m, 2H, H-Ar), 6.07 (br s, 1H, OH),10.47 (br s, 1H, NH), 11.03 (br s, 1H, NH); ^{13}C NMR (100MHz, DMSO-d_6, δ, ppm)δ:150.4,160.1,163.5,155.5,142.3,134.4,115.3,79.9,75.2,61.5,37.2,29.03ppm.

Ethyl7-amino-5-(4-chlorophenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-6-carboxylate (4g).

IR (KBr, cm^{-1}):3311, 3188, 3091, 2228, 1899, 1648, 1543. ^1H NMR (100 MHz, DMSO-d_6, δ, ppm): 2.17(3H,s,CH_3),4.8(2H,s,CH_2), 5.28 (s, 1H, H-5) 4.11(2H,s,CH_2), 2.29 (3H, s, CH_3), 7.28 (m, H-Ar), 7.38 (m, 2H, H-Ar), 7.75 (br s, 2H, NH_2), 10.99 (s, 1H, NH), 11.55 (s, 1H, NH);^{13}C NMR (100MHz, DMSO-d_6, δ, ppm) δ: 88.3, 98.5, 114.8, 126.9, 128.8, 129.0, 129.9, 130.5, 135.9, 150.1, 155.4, 155.8, 159.7, 160.9 ppm.

7-amino-5-(4-bromophenyl*)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-*carboxylate (4h).

IR (KBr, *v* cm^{-1}): 3340,3370, 3189, 3080, 2220, 1684, 1567; ^1H NMR (300 MHz, DMSO-d_6, δ, ppm) 3.2(s,3H,CH_3), 3.8(s,2H,CH_2),4.26 (s, 1H, H-5), 7.17 (s, 2H, NH_2) 7.20 (m, 2H, H-Ar), 7.48 (m, H-Ar), 12.45 (m s, 1H, NH), 13.66 (m s, 1H, NH); ^{13}C NMR (100MHz, DMSO-d_6, δ, ppm) δ: 35.3, 58.5, 82.8, 119.0,120.0, 129.9, 132.2, 132.7, 143.0, 157.4, 160.3, 174.0 ppm.

*Ethyl 7-amino-5-(3-nitrophenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-*carboxylate (4i).

IR (KBr, *v* cm^{-1}): 3380, 3321, 3182, 2896, 1796, 1640,1519; ^1H NMR (300MHz, DMSO-d_6, δ, ppm): 3.6(3H,s,CH_3), 4.1(2H,s,CH_2),4.82 (s, 1H, H-5),7.26 (s, 2H, NH_2), 7.52 (m, 2H, H-Ar), 8.14 (m, 2H, H-Ar), 11.12 (s,1H, NH), 12.17 (s, 1H, NH); ^{13}C NMR (100MHz, DMSO-d_6, δ, ppm) δ: 35.7, 57.5, 87.5, 119.0, 124.3, 130.7, 146.4, 149.6, 151.9, 152.7, 157.8, 162.6 ppm.

Ethyl 7-amino-5-(4-nitrophenyl)-2,4-dioxo-1,3,4,5-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-6-carboxylate (4j).

IR (KBr, *v* cm^{-1}): 3420, 3367, 3106, 2986, 1978, 1749,1604; ^1H NMR (300MHz, DMSO-d_6, δ, ppm): 3.92 (s, 1H, H-5),7.26 (br s, 2H, NH_2), 7.32 (m, 2H, H-Ar), 8.09 (m, 2H,H-Ar), 9.67(s,1H, NH),10.15(s,1H,NH),4.12(s,2H,CH_2),3.09(3H,s,CH_3);^{13}CNMR (100MHZ,DMSOd_6,δppm)δ:150.5,160.3,162.3,167.2,163.8,130.0,148.3,145.4,121.0,37.2,61.7,14.2,79.5ppm.

Table 1
Synthesis of pyrano[2, 3-d]pyrimidine derivatives.

Sr.no.	Product	Ar	X	Time (min)	Yield (%)[a]	M.P.(° C)
1	4a	4-Me-C_6H_4	$CO_2C_2H_5$	55	72	293
2	4b	C_6H_5	$CO_2C_2H_5$	43	69	207
3	4c	4-MeO-C_6H_4	$CO_2C_2H_5$	80	83	293
4	4d	3,4-MeO-C_6H_4	$CO_2C_2H_5$	67	79	305
5	4e	3-OH-C_6H_4	$CO_2C_2H_5$	48	92	270
6	4f	4-OH-C_6H_4	$CO_2C_2H_5$	55	94	247
7	4g	4-Cl-C_6H_4	$CO_2C_2H_5$	92	86	295
8	4h	3-Br-C6H6	$CO_2C_2H_5$	110	84	235
9	4i	3-NO_2-C_6H_4	$CO_2C_2H_5$	97	82	237
10	4j	4-NO_2-C_6H_4	$CO_2C_2H_5$	105	83	289

(iii) Antibacterial activity (in vitro)

Antimicrobial activity of annulated pyrano[2,3-d]pyrimidine derivatives were tested by the disk diffusion method where sterilized Whattman No. 1 filter paper disks were autoclave for 1.5 hour at 140°C. 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 Staphylococcus (NCTC-7447), Bacillus cereus (ATCC-14579) and gram negative bacteria Serratia maresens(IMRU-70), and Proteus merabitis (NTCC-289). These impregnated disks were placed on medium suitably spaced apart and plates were incubated at 110 °C for 20 min. to permit good diffusion and were then transferred to an incubator at 37±2 °C for 24 hrs. The zones of inhibition were measured on mm scale. Ampicillin used as standard antimicrobial drug. Antimicrobial activity test results as shown in Table 2.

Table 2

In-vitro antibacterial activity (MIC) values of pyrano[2,3-d]pyrimidine derivatives 1-10.

Sr. no.	Comp.	Minimum inhibitory concentration (MIC) in µg/Ml			
		Gram positive bacteria		Gram negative bacteria	
		Staphylococcus	B. cereus	S. maresens	P. merabitis
1	4a	11	12	13	14
2	4b	12	11	14	12
3	4c	11	12	17	13
4	4d	13	12	12	14
5	4e	17	14	15	16
6	4f	19	15	17	16
7	4g	10	13	11	9
8	4h	6	8	11	8
9	4i	11	9	8	10
10	4j	9	7	11	9
SD.	Ampicillin	21	23	22	22

(iv) Antifungal (in vitro)

Newly prepared annulated pyrano[2,3-d]pyrimidine derivatives were screened separately in-vitro for their antifungal activity against cultures of two fungal species, namely, Aspergillus ochraceus Wilhelm (AUCC-230) and Penicillium chrysogenum thom (AUCC-530). The antifungal activity was detected by agar well diffusion method by the following procedure Sabouraud dextrose agar plates: A homogeneous mixture of glucoseepeptoneeagar (40:10:15) was sterilized by autoclaving at 121^{O}C for 20 min. The sterilized solution (25 mL) was poured in each sterilized petridish in laminar flow and left for 20 min to form the solidified sabouraud dextrose agar plate. These plates were inverted and kept at 30 ^{0}C in incubator to remove the moisture and to check for any contamination. Antifungal assay: Fungal strain was grown in 5 mL sabouraud dextrose broth (glucose: peptone; 40:10) for 3-4 days to achieve 105 CFU/mL cells. The fungal culture (0.1 mL) was spread out uniformly on the Sabouraud dextrose agar plates by sterilized triangular folded glass rod. Plates were left for 5-10 min so that culture is properly adsorbed on the surface of sabouraud dextrose agar plates. Now small wells of size (2 mm) were cut into the plates with the help of well cutter and bottom of the wells were sealed with 0.8% soft agar to prevent the flow of test sample at the bottom of the well. 100 ml of the tested samples (10 mg/mL) were loaded into the wells of the plates. All compounds was prepared in dimethyl sulfoxide (DMSO), DMSO was loaded as control. The plates were kept for incubation at 30 ^{0}C for 3-4 days and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured in mm three times by caliper to get an average value. Ampicillin used as references to evaluate the potency of the tested compounds under the same conditions. Zones of inhibition were determined for all compounds and the results are summarized in Table 3.

Table 3
In-vitro antifungal activity (MIC) values of pyrano[2,3-d]pyrimidine derivatives 1-10.

Sr.no.	Compound	Minimum Inhibitory Concentration (MIC) in µg/mL	
		Aspergillus ochraceus Wilhelm (AUCC-230	Penicillium chrysogenum thom (AUCC-530)
1	4a	13	16
2	4b	12	14
3	4c	17	16
4	4d	13	18
5	4e	19	17
6	4f	18	19
7	4g	11	13
8	4h	7	11
9	4i	9	7
10	4j	12	10
SD.	Mycostatin	26	24

(v) Minimum inhibitory concentration (MIC)
Minimum inhibitory concentration (MIC) of µg/mL values is the lowest product concentration preventing visible bacterial growth. MICs of selected products 1-10 were determined by taking different concentrations of the product in DMF and add to different sample test tubes containing sterilized broth test organism.DMF alone showed no inhibition zone. Then all the test tubes were incubated at 37 °C for 24 h and after incubation period, the presence of growth (turbidity) was observed.

RESULTS AND DISCUSSION

Herein, we use N-type Triethylamine as base catalyst and its efficient application to the Knoevenagel condensation and Michael addition reaction involves multicomponent by stirring in aqueous medium at room temperature. The products were obtained in high yield and very good purity. Catalyst gets easily removed by aqueous washing due to its solubility in water; hence no need of further neutralization and work-up is accomplished by simple filtration and recrystallization by ethanol. The structure function analysis contributes to efforts designed to elucidate the effects of structures and action mechanisms of antibacterial activities of annulated pyrano[2,3-d] pyrimidine derivatives. The good activity is attributed in the presence of pharmacologically active benzaldehyde, -OH, -OCH_3,-Cl,-Br and –NO_2 groups attached to phenyl ring on the pyran ring shows extensive effect on the membrane potential associated with bactericidal activity. The relevant studies showed that steric, electronic effects and polar parameters of the benzaldehydes substituent on pyrane ring were important for both antimicrobial and antifungal activities. 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. The annulated pyrano[2,3-d]pyrimidine products as an antibacterial agents are excellent derivatives for drug resistance issues in clinically used therapeutics and furnishes motivating model for studying interaction with antimicrobials and antifungal target as possible charge modification of substituent and O/N of pharmacophore groups present in skeleton. Electron donating substituent's viz; –OH, -OCH_3 on the annulated pyrano[2,3-d]pyrimidine skeleton exerted positive influence on its antimicrobial activity against Staphylococcus (NCTC-7447), Bacillus cereus(ATCC-14579), Serratia maresens(IMRU-70), and Proteus merabitis (NTCC-289) [Fig. 1]. The –OH,-OCH_3 meta and Para positions substituent's shows moderate antifungal activity against Aspergillus ochraceus Wilhelm (AUCC-230 and Penicillium chrysogenum thom (AUCC-530) strains [Fig.2] when they are attached to phenyl ring. The electron withdrawing substituents at meta and Para positions like – Br, -Cl and -NO_2 skeleton attached to phenyl ring exerted positive influence on its both antimicrobial and antifungal activities. The compounds 4a, 4b, 4c, 4d, 4e and 4f showed comparatively good antimicrobial and antifungal activities against different strains due

to presence of electron donating moieties on the phenyl ring. Compounds 4g, 4h, 4i and 4j exhibited less bacterial activities as the presence of different electron withdrawing nature groups. Newly prepared annulated pyrano[2,3-d]pyrimidine derivatives possess phenyl moiety are tested for potential antibacterial activities and antibacterial strains (both antimicrobial and antifungal) reveal that the presence of heteroaryl ring, cyano, amino and ester groups on pyran ring make these more basic on increases its penetrating power on bacterial cell wall and the compounds becomes more active. In these cases, the hydroxyl, methoxy, nitril and bromil heteroaryl part is associated with the bacterial cell wall which makes them more active. Future flexible pharmacophore sites geometric conformation enables to prepare derivatives for multi-therapeutic annulated Pyrano[2,3-d]pyrimidine products with high selectivity.

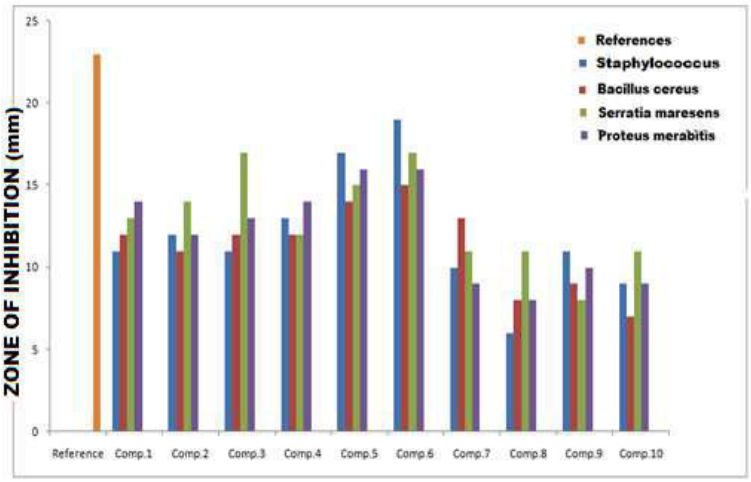


Figure 1
Antimicrobial activity of pyrano[2,3-d]pyrimidine derivatives 1- 10.

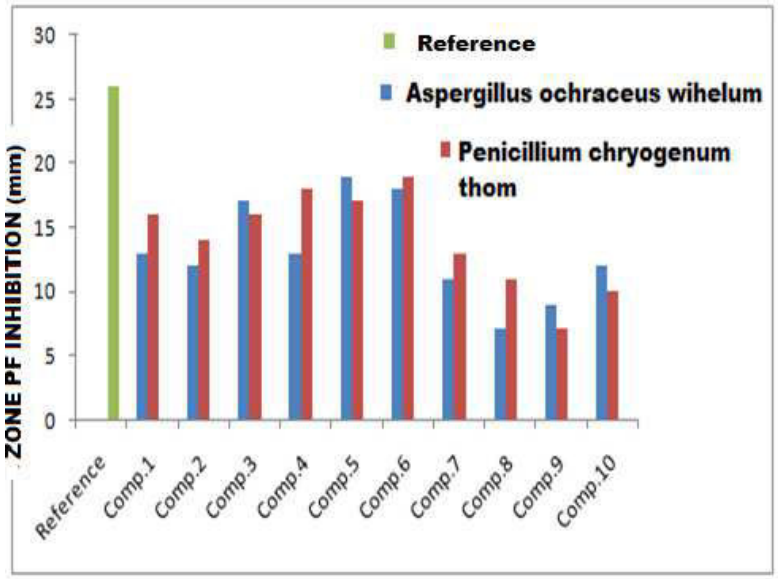


Figure 2
Antifungal activity of pyrano[2,3-d]pyrimidine derivatives 1- 10.

CONCLUSION

The research study reports the successful synthesis and antibacterial activity of new annulated pyranopyrimidine derivatives bearing phenyl ring moiety on pyran. The biological activity study revealed that all the tested compounds showed moderate to good antibacterial and antifungal activities against pathogenic strains. Structure and biological activity relationship of title compounds showed that the presence of biologically active groups like –OH,-OCH_3, -Cl,-Br,-$N0_2$, -NH_2 and –$CO_2C_2H_5$ groups attached to pyran ring and oxygen groups attached to pyrimidine moiety are responsible for the good antibacterial activity. pyrano[2,3-d]pyrimidine were more active with OH than with OCH_3, 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. Annulated pyrano[2,3-d]pyrimidine contains 4-NO_2, 4Cl and 4-Br groups exhibited less activity as electron withdrawing nature. Hence, this study may be helpful for the medicinal chemists in understanding antimicrobial and antifungal 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.

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REFERENCES

1. Grivsky, E.M., Lee, S., Sigel, C.W., Duch, D.S., Nichol, C.A., Synthesis and antitumor activity of 2,4-diamino-6-(2,5-dimethoxybenzyl)-5- methylpyrido[2,3-d]pyrimidine. *J. Med. Chem*, 23, 327–329, (1980).
2. Heber, D., Heers, C., Ravens, U.., *Positive inotropic activity of 5-amino-6-cyano-1, 3-dimethyl-1, 2, 3, 4-tetrahydropyrido [2, 3-d] pyrim idine-2, 4-dione in cardiac muscle from guinea-pig and man. Part 6: compounds with positive inotropic activity*. *Die Pharmazie,* 48,537–541, (1993).
3. Sakuma, Y., Hasegawa, M., Kataoka, K., Hoshina, K.,Kadota, N., *1, 10-Phenanthroline Derivatives. WO 91/05785 PCT Int. Appl.,* 1989 May 2. Chem Abstr, 115,71646,(1991).
4. Bennett, L. R., Blankely, C. J., Fleming, R. W., Smith, R. D., Tessonam, D. K., Antihypertensive activity of 6-arylpyrido[2,3-d]pyrimidin-7-amine derivatives. J. Med. Chem, 24, 382, (1981).
5. Davoll, J., Clarke, J., Eislager, F. E., *Antimalarial,antagonists, and antimetabolite effects of 2,4-diamino-6-[(benzyl)amino]pyrido[2,3-d]pyrimidines*. J. Med. Chem, 15,837, (1972).
6. (a) Kretzschmer, E., Pharmazie 35, 253, (1980). (b) Shigo, S., Hiroshi, I., Yakugaku Zasshi. 89, 266, (1969)
7. Ghorab, M.M.,Hassan, A.A. Synthesis and antibacterial properties of new dithienyl containing pyran, pyrano[2,3-*b*] pyridine, pyrano[2,3- *d*]pyrimidine and pyridine derivatives. Phosphorus, Sulfur, Silicon & Related Elem*,* 141, 251–261, (1998).
8. Musstazza, C., Guidice, M.R.D., Borioni, A., Gatta, F., Synthesis of pyrazolo[1,5-*a*]-, 1,2,4-triazolo[1,5-*a*]- and imidazo[1,2-*a*]pyrimidines related to zaleplon, a new drug for the treatment of insomni*.* J.Heterocycl.Chem*,* 38,1119-1130, (2001).
9. El-Assierygalal, S.A., Sayed, H., Fouda, A., Synthesis of some new annulated pyrazolo-pyrido (or pyrano) pyrimidine, pyrazolopyridine and pyranopyrazole derivatives.Acta Pharm, 54, 143–150, (2004).

10. Grieco, P.A. Organic Synthesis in Water. Thomson Science, London, 1–278, (1998).
11. Li, C.J., Organic reactions in aqueous media with a focus on carbon carbon bond formations: A decade update. Chem. Rev, 105, 3095–3165,(2005).
12. Lubineau, J., Auge, Y., Queneau, Water-promoted Organic Reactions. Synthesis,741- 1998, (1994).
13. Park, S.J., Keum, G., Kang, S.B., Koh, H.Y., Kim, Y., Lee, D.H., Tetrahedron Lett, 39, 7109-7112, (1998).
14. Zhu, J., Bienayme, H., Multicomponent Reactions, Wiley-VCH, Weinheim, Germany (2005).
15. Hasaninejad, Shekouhy, M., Golzar, N., Zare, A., Doroodmand, M.M.., Appl. Catal. A: Gen, 402, 11-22, (2011).
16. Tamaddon, F., Farahi, M., Karami, B., J. Mol. Catal. A: Chem. 356, 85-89, (2012).
17. Barry, A.L., the Antimicrobial Susceptibility Test: Principle and Practices, Illus Lea and Febiger, Philadelphia, p. 180, PA (1976). b) European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution, Clin. Microbial. Infect. Suppl. 6, 509-515, (2000).
18. National Committee for Clinical Laboratory Standards (NCCLS). Methods for Dilution, Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 5th ed.; Approved Standard M7-A5. NCCLS: Wayne, PA, USA, (2000).