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Antibacterial activity of synthetic pyrido[2,3-d]pyrimidines armed with nitrile group: POM analyses and identification of pharmacophore sites of nitriles as important *pro*-drugs

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The clean and simple one-pot multi-component methodology was developed for the preparation of 7-amino-2,4-dioxo-5-aryl-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-6-carbonitriles via reaction of 6-amino uracil, aromatic aldehydes and malononitrile using triethylamine (TEA) base as a catalyst in aqueous ethanol medium at NTP. The reaction protocol own assorted advantageous viz; mild reaction conditions, short reaction time, environmentally friendly procedure, low cost chemicals, and easy isolation of derivatives with excellent yields of bioactive products (85-95%). All the synthesised pyrido[2,3-d]pyrimidines bearing nitrile compounds (**4a-4h**) showed comparatively good *in-vitro* antibacterial activities against Staphylococcus, Bacillus cereus (Gram-positive bacteria) and P. merabitis S.maresens (Gram-negative bacteria). Nevertheless, compound **4h** exhibited highest antibacterial activity and owing excellent % inhibition as compared to standard Streptomycin. Overall, this compound **4h** enhanced antibacterial activity as compared to its positional isomers compound **4f** and compound **4g**. This increase bioactivity going from **4f** (R = 4-Nitro) to **4h** (R = 2-Nitro) is attributed to hydrolysis of C≡N to amide which restores vital intramolecular interaction between *ortho*-Nitro-phenyl and amide linkages ($ONO^{\delta-}$--$H^{\delta+}N$) and offers crucial template for antibacterial NH, O-phramacophore sites. The synthesized compound **4h** was tested to verify it fewer side effects than that of standard/Streptomycin. But, it's possible inclusion in selective fungal/viral media viz HIV, *Hepatitis* B/C phenomenon is a subject of further research. This multi-component synthetic protocol is using cheap ingredients like Et_3N catalyst and ethanol as the green solvent.

***Keywords*:** Pyrido[2,3-*d*]pyrimidines, uracil, one pot, multi-component, triethylamine, antibacterial, Petra-Osiris-Molinspiration (POM) analyses, nitrile, prodrug, pharmacophore site identification.

Introduction

Multi component reaction (MCR) is a powerful tool in organic reactions to be used for making more than one carbon–carbon or carbon–heteroatom bond in one-pot single step synthetic procedures. Worldwide organic chemists are ever enthusiastic to design new environmentally clean and economically useful synthetic methods which avoids large solid wastes after each step of multi-step reactions.[1]

Uracil and its derivatives own diverse therapeutic properties which makes them biomimetic reactive pharmacophore sites and imparts the vital role in natural and synthetic organic chemistry.[2,3] Such, heterocyclic compounds have received considerable attention in recent years due to their assorted biological activities viz; HIV-1 reverse transcriptase,[4] especially as inhibitors of PDE5 extracted from human platelets,[5] human EPK2,[6] and cyclin-dependent kinase.[7] In fact the basic skeleton of the genetic materials is made up of adenine, guanine, cytosine, thymine and uracil heterocycles illustrates their significance. Furthermore, these nitrogen containing heterocyclic rings plays pivotal role in medicinal chemistry.

Pyrido[2,3-d]pyrimidine is a bicyclic nitrogen containing heterocyclic compounds based on uracil skeleton are investigated thoroughly for their inherent antibacterial and antifungal activities,[8] antihypertensive,[9] antimicrobial,[10] cardiotonic,[11] tyrosine kinase,[12] anti-inflammatory,[13] and analgesic,[14] calcium channel antagonists,[15] tuberculostatic,[16] antilieshmanial activity,[17]. These pyrido[2,3-*d*]pyrimidine derivatives were synthesized using various catalytic systems like diammonium hydrogen phosphate (DHAP),[18] palladium-catalysed oxidative coupling,[19] *L*-proline,[20] KF-Al_2O_3,[21] nucleophilic-induced ring transformation,[22] and sometimes without catalyst,[23, 24]

On other hand, most of the frequently used drugs such as amphotericin B, bacitracin, cephaloridine, cisplatin, colistin, cyclosporine, ethacrynic acid, furosemide, paromomycin, polymyxin B, vancomycin, viomycin and streptomycin causes nerve, kidney, hearing issues, besides severe muscle and severe breathing problems. Especially, streptomycin own greatest sides effects and three violations of Lipinski five rule's; N violations = 3, as shown in below Figure 1. For these major reasons, we have prepared some pyrido[2,3-d]pyrimidine derivatives and investigated their antibacterial activities, besides compared them with virtual Petra/Osiris/Molinspiration (POM) analysis. To the best of our knowledge, this is the first research study exhibiting quantitative structure activity relationship of pharmacophore sites of pyrido[2,3-d]pyrimidine asan important pro-drugs.

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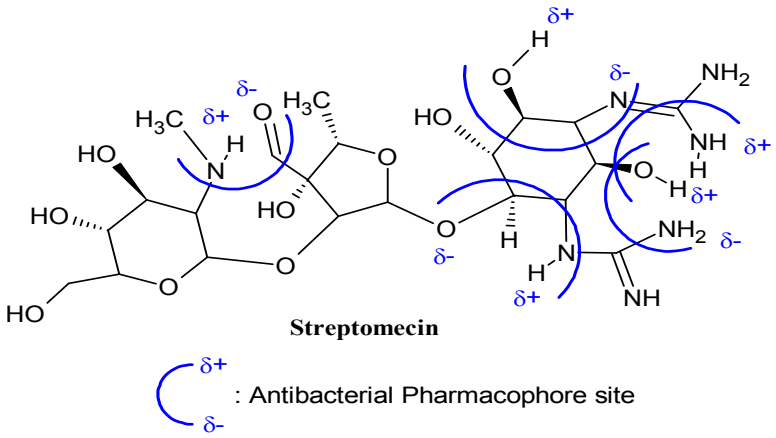

Molecular properties				Drug score			
TPSA	333	N OHNH	16	GPCR ligand	0.17	Nuclear receptor ligand	-0.32
MW	581	N violations	3	Ion channel modulator	-0.09	Protease inhibitor	0.78
nON	19	volume	497.40	Kinase inhibitor	-0.24	Enzyme inhibitor	0.46

Fig. 1 Structure of Streptomycin (**SD1**) bearing multi combined antibacterial ($X^{\delta+}$----$Y^{\delta-}$) pharmacophore sites.

Materials and methods

All chemicals and solvents were obtained from Aldrich Chemical Co. and S. D. Fine chem Co., and are used in research as such without further purification. The melting points were determined by open capillary method and were uncorrected. All the ^{1}H-NMR spectra of products were obtained on a BRUKER instrument (300 MHz). FTIR spectra were recorded on a Perkin–Elmer 298 spectrophotometer using KBr disc and all ^{13}C-NMR (100 MHz) spectra were recorded in DMSO-d_6 as solvent with TMS as internal standard. Chemical shifts are reported in ppm and the mass spectra were measured using high resolution GC–MS (DFS) thermo spectrometers with EI (70 EV). All the reactions were monitored frequently by thin layer chromatography (TLC) on 0.2-mm pre-coated plates of silica gel G60 F254 (Merck).

Spectral data for synthesised pyrido[2,3-d]pyrimidine products

7-Amino-5-(4-methyl)-1,3-dimethyl-2,4-dioxo-1,2,3,4tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile **(4a)**

Yellow powder. Yield 85 %. IR (KBr, cm^{-1}): 3395 (-NH_2), 2220 (-CN), 1799 (-C=O), 1715 (-C=O);^{1}H NMR (300 MHz, DMSO-d_6, ppm): 2.36 (s, 3H, CH_3), 3.25 (s, 3H, CH_3), 3.45 (s, 3H, CH_3), 7.12 (d, 2H, H_{Ar}), 7.20 (d, 2H, H_{Ar}), 7.68 (br s, 2H, NH_2), ppm; ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 160.8, 159.9, 155.5, 150.1, 137.4, 129.7, 128.2, 125.4, 114.1, 98.3, 88.7, 57.3, 31.2, 29.5, 20.9ppm; EI-MS (m/z) = 321 (M^+)

7-Amino-5-(phenyl)-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile **(4b)**

White powder. Yield 90%. IR (KBr, cm^{-1}): 3400 ($-NH_2$), 2222 (-CN),1710 (-C=O), 1625 (-C=O);^{1}H NMR (300 MHz, DMSO-d_6, ppm): 3.35 (s, 3H, CH_3), 3.65 (s, 3H, CH_3),7.24 (d,2H,H_{Ar}), 7.45 (t, 3H, H_{Ar}), 7.60 (br s, 2H, NH_2) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 160.9 160.1, 159.0, 158.3, 150.7, 134.2, 127.9, 127.7, 116.7, 115.1, 88.8, 40.1, 35.0, 24.1, ppm; EI-MS(m/z) = 307 (M^{+1}).

7-Amino-5-(4-methoxyphenyl)-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile **(4c)**

Yellow powder. Yield 93%. IR (KBr, cm^{-1}): 3420($-NH_2$), 2215(-CN), 1760 (-C=O), 1635 (-C=O); ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 3.15 (s, 3H, CH_3), 3.47 (s, 3H, CH_3), 4.25 (s, 3H, OCH_3), 7.05 (d, 2H, H_{Ar}), 7.25 (br s, 1H, NH_2), 7.9 (d, 2H, H_{Ar}); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 160.8, 160.0, 159.9, 158.8, 155.5, 150.1, 129.5, 128.6, 115.7, 112.9, 98.3, 88.8, 62.5, 23.4, 28.3 ppm; EI-MS (m/z) = 337 (M^+).

7-Amino-5-(4-hydroxyphenyl)-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3d]pyrimidine-6-carbonitrile **(4d)**

Yellow powder. Yield 91%. IR (KBr, cm^{-1}): 3391 ($-NH_2$), 2237 (-CN), 1786 (-C=O), 1624 (-C=O); ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 3.1 (s, 3H, CH_3), 3.3 (s, 3H, CH_3), 6.59 (s, 1H, H_{Ar}), 6.62 (d, 1H, H_{Ar}), 6.78 (d, 1H, H_{Ar}), 7.18 (t, 1H, H_{Ar}), 7.58 (br s, 2H, NH_2), 9.46 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 160.8, 159.8, 155.4, 150.2, 130.9, 121.8, 118.1, 115.4, 115.2, 98.3, 88.5, 28.3, 26.4 ppm.; EI-MS (m/z) = 323 (M^+).

7-Amino-5-(4-chlorophenyl)-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile **(4e)**

White powder. Yield 92%. IR (KBr, cm^{-1}): 3377 ($-NH_2$), 2206 (-CN), 1759 (-C=O), 1618 (-C=O); ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 3.2 (s, 3H, CH_3), 3.55 (s, 3H, CH_3), 7.34 (d, 1H, H_{Ar}), 7.50 (d, 1H, H_{Ar}), 7.72 (br s, 1H, H_{Ar}),7.81 (br s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 170, 165, 155.4, 154.6, 150.1, 135.0, 133.7, 131.7, 70, 55, 28, 25; EI-MS (m/z) = 341 (M^+).

7-Amino-5-(4-nitrophenyl)-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile **(4f)**

White powder. Yield 95%. IR (KBr, cm^{-1}): 3297 ($-NH_2$), 2222 (-CN),, 1758 (-C=O), 1624 (-C=O); ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 3.3 (s, 3H, CH_3), 3.7 (s, 3H, CH_3), 5.78 (s, 2H, NH_2), 7.45 (d, 2H, H_{Ar}), 8.38 (d, 2H, H_{Ar}) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 166.1, 164.9, 162.5, 157.8, 156.7, 148.8, 143.7, 127.3, 123.1, 113.2, 98.4, 84.2, 36.8 and 28.7; EI-MS (m/z) = 353 (M^+).

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7-Amino-5-(3-nitrophenyl)-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-6- carbonitrile **(4g)**

White powder. Yield 86%. IR (KBr, cm^{-1}): 3384 (-NH_2), 2216 (-CN), 1768 (-C=O), 1622 (-C=O); ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 3.3 (s, 3H, CH_3), 3.7 (s, 3H, CH_3), 7.75 (m, 2H, H_{Ar}), 7.77 (br s, 2H, NH_2), 8.19 (s, 1H, H_{Ar}), 8.29 (qd, 1H, H_{Ar}), ppm; ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 160.8, 160.2, 156.1, 159.0, 155.5, 150.1, 147.1, 138.4, 129.4, 134.8, 98.3, 88.3, 25.4, 29.3; EI-MS (m/z) = 353 (M^+).

7-Amino-5-(2-nitrophenyl)-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile **(4h)**

Yellow powder. Yield 89%. IR (KBr,cm^{-1}): 3403 (-NH_2), 2225 (-CN) , 1707 (-C=O), 1613 (-C=O); ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 3.3 (s, 3H, CH_3), 3.7(s, 3H, CH_3), 7.75 (s, 2H, NH_2), 7.51 (d,1H,H_{Ar}), 7.43 (dt, 1H, H_{Ar}), 7.38 (t, 1H, H_{Ar}), 7.28 (dd, 1H, H_{Ar}); ^{13}C NMR (100 MHz, DMSO-d_6, ppm), 167.50, 173.50, 158.15, 156.02, 130.63, 129.53, 128.70, 128.37, 126.65, 114.50, 75, 53.3 ,29.49, 27.56; EI-MS (m/z) = 353 (M^+).

Minimum Inhibitory concentration: (MIC)

Pyrido[2,3-d]pyrimidines were dissolved in DMF to prepare stock solutions of 20 mg/mL for antimicrobial activity. Agar plates were uniformly inoculated with fresh culture of both strains. These impregnated disks were incubated at 30 °C for 60 mints to permit good diffusion and transfer in to incubator for 24 hrs at 37±2 °C. The inhibition zone of compounds around the disk was measured in mm scale as shown in Table 3 and these observations are compared with standard Streptomycine.

Results and discussion

Substituted aromatic aldehydes **1a-1h** (1 mmol), malononitrile **2** (1 mmol), 6-amino 1, 3-dimethyl uracil **3** (1 mmol) and triethyl amine (10 mol%) as organo catalyst were taken in an R.B. flask with solvent aqueous ethanol (1:1 ratio, 10 mL) and stirred for 30–50 min at room temperature. The reaction was monitored by thin layer chromatography. The solid product was filtered, washed with cold water several times, and re-crystallized from ethanol to obtain pure pyrido[2,3-*d*]pyrimidine derivatives with excellent yield in below Scheme 1.

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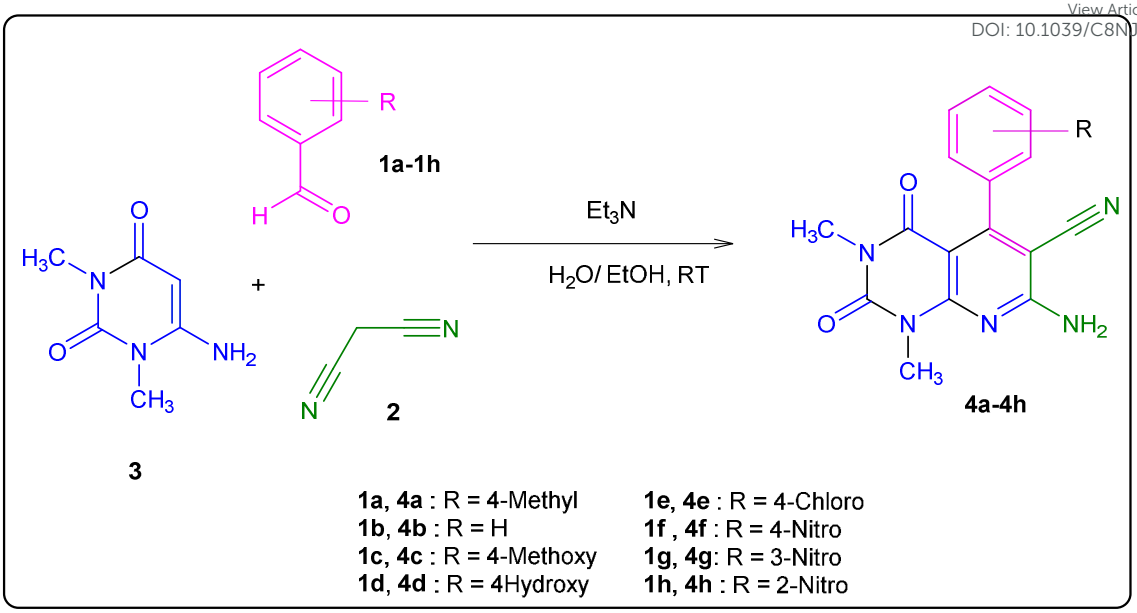


Scheme 1 General procedure for the preparation of pyrido[2,3-*d*]pyrimidines **4a-4h**.

Annulated pyrido[2,3-*d*]pyrimidines (**4a-4h**) synthesised via Knoevenagel-Michael condensation reaction pathways. (Table 1) In initial studies, 4-nitrobenzaldehyde 1 (1.0 mmol), malononitrile **2** (1.0 mmol), 6-amino-1,3-dimethyl uracil **3** (1.0 mmol) with aqueous ethanol (5:5) were taken in Round bottom (R.B) flask mounted over magnetic stirrer. The catalyst triethylamine (TEA) with 10 mol % concentrations showed the best result among these different used catalyst in terms of reaction time and product yield (95%).

Table 1 Synthesis of pyrido[2,3-d]pyrimidine derivatives **4a-4h**

Product	R	Time (mins)	Yield (%)[a]	Mp (°C)	Mp (Lit.)
4a	4-Methyl	50	85	>300	>300
4b	H	45	90	>300	>300
4c	4-Methoxy	55	93	>300	>300
4d	4-Hydroxy	58	91	>300	___
4e	4-Chloro	55	92	>300	>300
4f	4-Nitro	50	95	>300	>300
4g	3-Nitro	65	86	>300	>300
4h	2-Nitro	75	89	>300	___

[a] isolated yields

In the absence of catalyst, the reaction was completed in 185 minutes with product yield mere 35%. Therefore, 10% triethylamine (TEA) catalyst concentration found superior to other mole

% of the catalysts. The focused is given on systematic usage of different solvents combinations like ethanol:water, DMF, CH_2Cl_2 and 1,4-Dioxane for the model reaction **4f** using 4-nitrobenzaldehyde **1f** (1.0 mmol), malononitrile **2** (1.0 mmol) and 6-amino 1,3-dimethyl uracil **3** (1.0 mmol) in presence of 10 mol % triethylamine (TEA) catalyst as shown in Table 2.

Table 2 Optimization of different solvents for the model product **4f**

Entry	Solvents	Time (mins)	Yield (%)[a]
1	EtOH-H_2O	50	95
2	DMF	70	61
3	CH_2Cl_2	105	55
4	1,4-Dioxane	85	65
5	Solvent less	195	57

[a] isolated yields

The optimization results of different solvents for the model product indicated major role of the solvent on the yield of product and overall reaction span. It was found that the best conversion was observed when the reaction performed in Ethanol: water/aqueous ethanol. Aqueous ethanol solvent imparts key advantages such as, environment safety, clean/green solvent, cheap operation cost, simple work-up and harmful effects. Mild base TEA catalyst facilitates proton removal from active methylene compound/malononitrile **2** thereby enhances reaction rate and ultimately facilitating Knoevenagel condensation between aryl aldehydes and active methylene compound, proceeds via intermediate, undergoes dehydration and finally heterocyclization and thus, its successfully synthesis of annulated pyrido[2,3-*d*]pyrimidine in quantitative yields.

Biological evaluation

Antibacterial activity: All the synthesised compounds (**4a-4h**) were screened in vitro for their antibacterial activity against Staphylococcus, Bacillus cereus (Gram-positive bacteria) and P. merabitis, S. maresens (Gram-negative bacteria). The minimum inhibitory concentration (MIC) of µg/mL values is calculated out by the disc-diffusion technique in order to check the activity of all synthesised pyrido[2,3-*d*]pyrimidines. The synthesized pyrido[2,3-*d*]pyrimidines were dissolved in dimethyl sulfoxide (DMSO) for dilution purpose to prepare stock solution of 1 mg/mL and then were impregnated onto Whatman filter papers (No.1). The impregnated Whatman filter papers were placed on the surface of solidified nutrient agar dishes seeded by the test bacteria. Medium containing plates were allowed to stand at room

temperature for 10 minutes and were set to solidify in a refrigerator for 30 mints. The MIC's of studied pyrido[2,3-*d*]pyrimidines were measured in millimetres by the end of the incubation period 48 h carried at 37 °C. Streptomycin (25µg/mL) is used as standard for bacterial studies. The zones of inhibition were measured on mm scale and shown in following Table 3.

Table 3 Antibacterial activity values of pyrido[2,3-d]pyrimidines [Minimum inhibitory concentration (MIC) in µg/mL]

Compd.	R	cLog *P*	Gram positive bacteria		Gram negative bacteria	
			B. cereus	*Staphylococcus*	*P. merabitis*	*S.maresens*
4a	4-CH_3	1.54	12	11	14	13
4b	H	1.10	11	12	12	14
4c	4-OCH_3	1.15	12	11	13	17
4d	4-OH	0.62	18	15	16	17
4e	4-Cl	1.77	13	10	10	11
4f	4-NO_2	1.05	11	9	8	10
4g	3- NO_2	1.03	9	7	11	9
4h	2- NO_2	1.01	7	6	10	7
SD1[a]	—	---	21	23	22	22

[a] **SD1**: Standard drug (**Streptomycin**).

The pyrido[2,3-*d*]pyrimidines **4a-4d** showed the maximum antibacterial activity against *Staphylococcus*, *B.cereus* and *P.merabitis*, *S.maresens*. These maximum activities exerted due to extensive effect on the membrane potential associated with bactericidal activity attributed pharmacologically active electron donating functionalities like benzaldehyde, -OH, -CH_3 and -OCH_3 attached to phenyl ring on the fused pyridine skeleton of annulated pyrido[2,3-*d*]pyrimidines. There are no antibacterial pharmacophore sites in structure of compounds **4a-4h** bearing nitrile group as the simple and evident. Rather compounds **4e** and **4f** showed moderate activity and **4g** and **4h** shown relatively low bioactivity due to electron withdrawing groups. Such contrasting bioactivity pattern of pyrido[2,3-*d*]pyrimidines led us to investigate and identify real pharmacophore site by using bioinformatics Petra-Osiris-Molinspiration (POM) analysis. The data evaluated in Table 3, noted that 8/8 structures are supposed to be more efficient than clinical drugs which make this research more curious to study their side effects.

POM Analyses of Compounds 4a-4h and their metabolites 4aa-4hh

The analysis of antibacterial activity for the series **4a-4h** using the Petra-Osiris-Molinspiration (POM) programs showed that compound **4h** and certainly the rest of series **4a-4h** of pyrido[2,3-*d*]pyrimidines are more active than standard clinical drug/Streptomycin (**SD1**) as

shown in Figure 1. Pyrido[2,3-*d*]pyrimidines **4a-4h** represent no violation (NV = 0) of five Lipinski rules, so compounds **4a-4h** can be used as antibiotics with some limited restriction as depicted in Table 4.

Table 4 Molinspiration calculations of compounds bearing nitrile group (**4a-4h**)

Compd.	Molinspiration calculations [a]				Drug-likeness [b]					
	TPSA	NONH	NV	VOL	GPCRL	ICM	KI	NRL	PI	EI
4a	107	2	0	282	-0.07	-0.70	0.00	-0.65	-0.56	**0.07**
4b	107	2	0	265	-0.04	-0.65	0.05	-0.65	-0.54	**0.13**
4c	116	2	0	300	-0.07	-0.67	-0.01	-0.60	-0.53	**0.07**
4d	127	3	0	273	0.01	-0.58	0.09	-0.47	-0.49	**0.18**
4e	107	2	0	279	-0.03	-0.63	**0.03**	-0.64	-0,55	**0.09**
4f	153	2	0	289	-0.18	-0.63	-0.10	-0.67	-0.60	0.00
4g	153	2	0	289	-0.18	-0.64	-0.07	-0.67	-0.60	**0.02**
4h	153	2	0	289	-0.23	-0.64	-0,11	-0.78	-0.65	-0.04
SD1 [c]	334	16	3	497	**0.17**	-0.09	-0.24	-0.32	**0.78**	**0.46**

[a] TPSA: Total molecular polar surface area; NONH: number of OH---N or O---NH interaction, NV: number of violation of five Lipinsky rules; VOL: volume. [b] GPCRL: GPCR ligand; ICM: Ion channel modulator; KI: Kinase inhibitor; NRL: Nuclear receptor ligand; PI: Protease inhibitor; EI: Enzyme inhibitor. [c] **SD1**: Streptomycin/standard drug (Antibacterial).

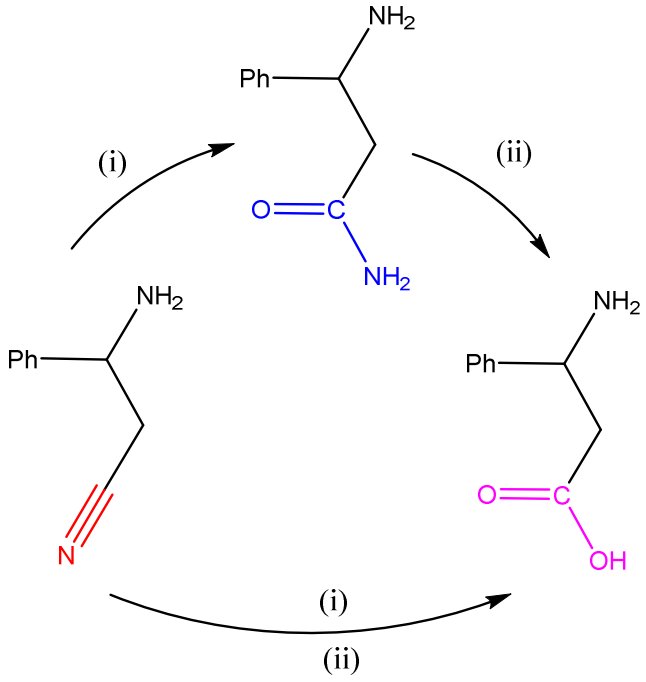
The pyrido[2,3-*d*]pyrimidines **4a-4h** series needs to be tested for their possible mutagenicity, irritating and reproductive effects as should have lower risks comparable with used standard drugs. The hydrophilicity character of each compound of**4a-4h** series has been expressed in terms of the cLog*P* value. Rather, absorption/permeation of drug gets greatly affected by the hydrophilicity i.e., value of cLog*P*. Accordingly, when cLog*P* is higher than 5, the absorption or permeation of drug found to be decreased. On this basis, compound **4a–4h series** and their corresponding metabolites **4aa-4hh** have cLog*P* values within the acceptable criteria, yet few crucial parameters should be considered. Since, the geometrical conformation of pharmacophore sites has not been concerned because it's fixed for all compounds **4a–4h series**. The distribution characteristics, absorption pattern and bioactivity proven depend on the geometrical parameter and the aqueous solubility of each compound of **4a–4h series**. Further, drug-likeness of compounds **4a–4h** series (Table 4) can't lies in the comparable zone with standard/Streptomycin drugs. The overall drug-score (DS) have been calculated for compounds **4a–4h series** and compared with that of Streptomycin (**SD1**). Furthermore, DS combines drug-likeness, cLog*P*, log*S*, molecular weight, and toxicity risks, in one handy

values, all are used to judge the synthesized compound's overall potential to qualify it for a marketed drug. The reported compounds **4a–4h series** showed low to moderate DS as compared to standard/Streptomycin drug (Table 4).

All these research findings and POM analyses suggest that compounds **4a-4h** series are worthy for its screening against other pathogenic bacteria and for future clinical trials. Any pathogenic bacterium is a common mould pathogen of human causing persistent diseases e.g., 4% patients dying in European hospitals hold persistent *aspergillosis* which is the leading transferable cause of death in leukaemia conditions. The compounds **4a-4h** series own low drug-score (DS) and weak drug-likness (DL) features as compared to the standard Streptomycin and such divergent values makes **4a-4h** quite at par with experimental antibacterial screening data. Indeed, supplementary POM calculations of metabolites of compounds **4a-4h** needs to clarify contrasting results. Thus, this research study suggested the real bioactive metabolites coexistence.

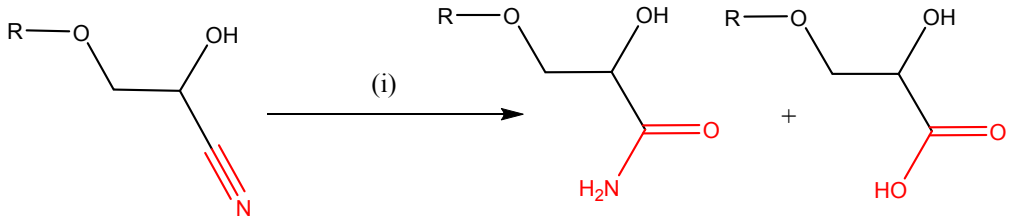
Stability of* 4a–4h series *nitrile derivatives withmicroorganisms

The bio-activity evaluation in the literature states that structures bearing nitrile on enzymatic/chemical hydrolysis undergoes transformation to yield corresponding amide and carboxylic acids as shown in Scheme 2.[25-30] Since 2001, Wegman et al.[25] identified *Rhodococcus* sp. as highly active bacterial strain, able to hydrolyse nitrile by using the nitrile hydratase enzyme.



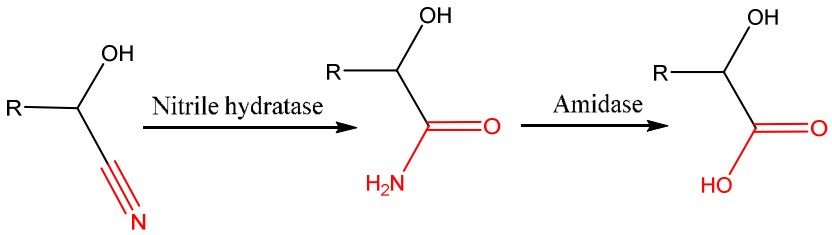
Scheme 2 Hydration and subsequent hydrolysis of D,L-phenylglycine nitrile.[25] (i) Nitrile hydralase, (ii) Amidase.

More recently, Kinfe et al.[26] confirmed the nitriles gets transformed to the corresponding carboxyamides and carboxyacids at NTP under bacterial (*Rhodococcus rhodochrous ATCC BAA-870*) hydrolysis. The bacteria expressed nitrile hydratase and amidase activities as responsible for this bio-conversion of nitriles shown in Scheme 3.



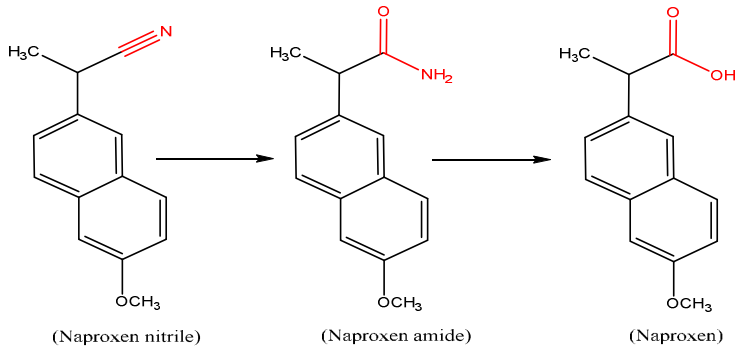
Scheme 3 (i) R. *Rhodochrous sp*., MeOH/potassium phosphate buffer (1:10), 30 °C, 3 h.[26]

Osprian et al. [27] indicated a second bacteria*; the Rhodococcus erythropolis NCIMB11540* as a source of highly active nitrile hydratase/amidase enzymatic hydrolysis of nitriles to amides and carboxylic acids as shown in Scheme 4.



Scheme 4 Cyanohydrin hydrolysis catalyzed by *R. erythropolis NCIMB 11540*.[27]

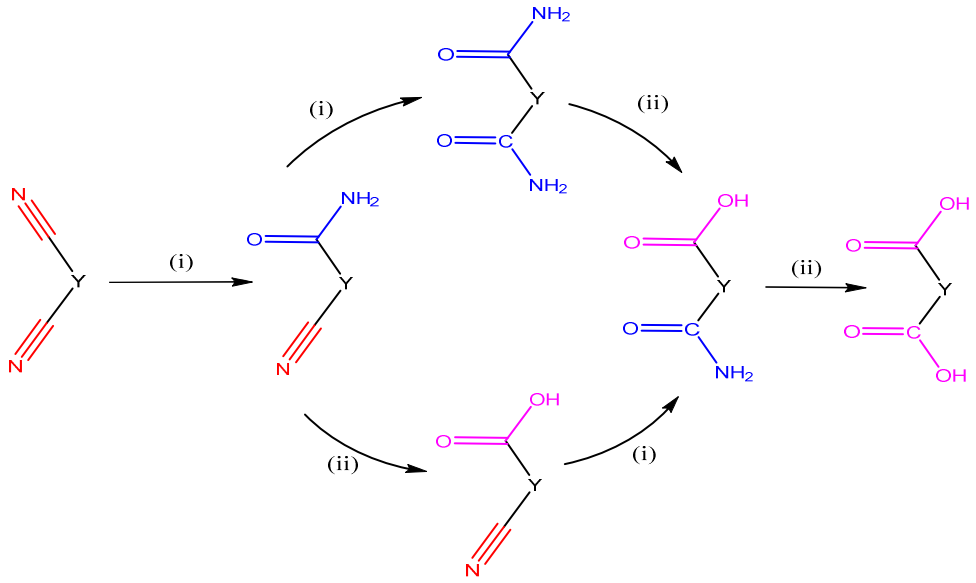
Cells of *Rhodococci* strains converted naproxen nitrile via naproxen amide to naproxen. *Rhodococcus sp. MP50* converted racemic naproxen nitrile predominantly to R-naproxen amide and racemic naproxen amide to S-naproxen as shown in Scheme 5.



Scheme 5 Naproxen nitrile hydrolysis catalyzed by *Rhodococcus sp. MP50*.[28]

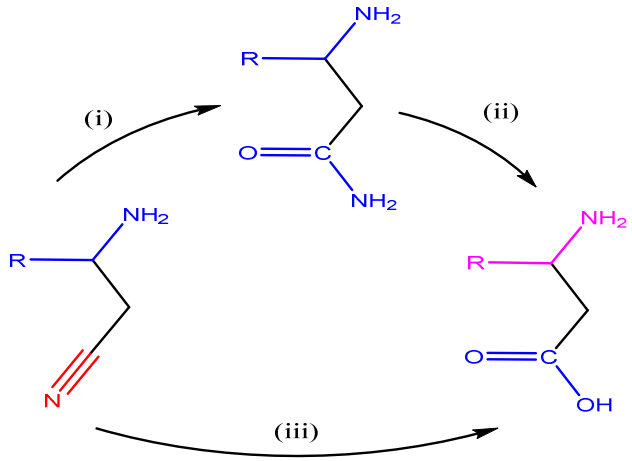
The bacterial strains viz; *Rhodococcus* sp. C3II and *Rhodococcus erythropolis* MP50 family are especially selected for enantioselective hydrolysis of pharmaceutically interesting 2-

arylpropionitriles like naproxen nitrile by Effenberger, Graef for hydrolysis of various aliphatic and aromatic nitriles and carboxylic acids and amides as shown in Scheme 6.[29]



Scheme 6 Dinitriles hydrolusis catalyzed by hydratase and amidase of *Rhodococcus sp. C3II* and *Rhodococcus erythropolis MP50* bacterial strains.[29] (i) Nitrile hydratase (ii) Amidase.

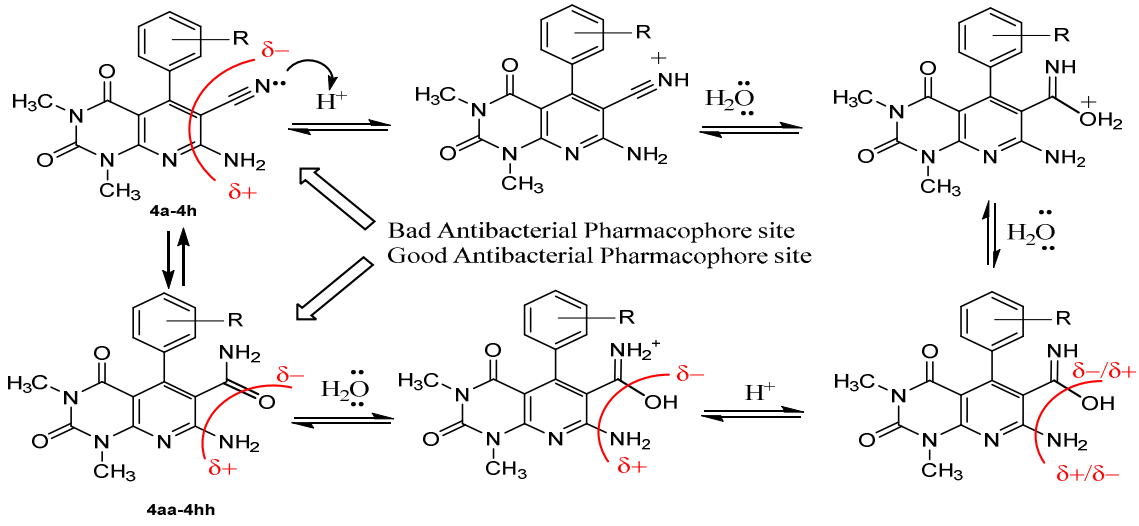
The amidase from *Rhodococcus rhodochrous J1*, which hydrolyzes amide to an acid and ammonium, was surprisingly found to catalyze the hydrolytic cleavage of the C-N triple bond in a nitrile,[30] to form an acid and ammonium stoichiometrically. An amidase plays a crucial role in the hydrolysis of nitriles as well as amides. The β-amino-nitrile compounds undergoes enzymatic hydrolysis to the corresponding amides,[31] using the nitrile biocatalytic activity of *Rhodococccus rhodochrous ATCC BAA-870*. Results showed that the nitrile hydratase enzyme was enantioselective for these compounds as shown in Scheme 7.



Scheme 7 Biocatalytic conversion of _-aminonitriles to _-amino amides and acids,[31] (i) Nitrile hydratase, (ii) Amidase, (iii) Nitrilase.

The contradiction between experimental screening data (Table 3) and POM virtual screening data of series **4a-4h** (Table 4) is of a great importance for both Organic Chemists and Pharmacologists as explained on via enzymatic and bioinformatics approaches. The analysis of theoretical drug scores for compounds **4a-4h** series using the Petra-Osiris-Molinspiration (POM) program showed that the antibacterial pharmacophore site $X^{\delta+}$----$Y^{\delta-}$ (where X, Y = O, N, S, P) is completely missing and this series represent awful antibacterial pharmacophore site. Its due to large distance between terminal nitrile and amine groups at antibacterial pharmacophore site ($d_{N---N} > 2.8$ Å) as affixed by POM theory.[32-38]

While the experimental antibacterial screening data as shown in Table 1 indicated more bio-activity of compounds **4a-4h** series than clinical drug standard/Streptomycin wherein antibacterial $X^{\delta+}$----$Y^{\delta-}$ pharmacophore sites gets combind as shown in Figure 1. The hydrophilicity of metabolites **4aa-4hh** is expressed in terms of the cLog*P* value. The absorption or permeation of **4a-4h** series are greatly affected by the hydrophilicity (value of cLog*P*). Accordingly, when cLog*P* is higher than 5, the absorption or permeation decreases. On this basis, most of tested **4a-4h** compound series have cLog*P* values within the acceptable criteria and are active at different concentration because another crucial parameter is considered. This does not concern the geometrical conformation of pharmacophore because it is resolved and fixed for compound **4aa-4hh** via $C{=}O^{\delta-}$---$^{\delta+}$HNH intramolecular interaction as shown in Scheme 8. This intra-template effect in favour of constitution of vital O^{1}---HN^{1} antibacterial pharmacophore as hypothesized by POM theory.[32-38] This calculated overall drug-score (DS) for metabolites **4aa-4hh** (Scheme 8) now compared with that of standard drug as shown in Table 5. The DS combines drug-likeness, cLog*P*, log*S*, molecular weight, and toxicity risks, in one handy value that may be used to judge the compound's overall potential to qualify for a drug. The reported compounds **4aa-4hh** showed excellent DS as compared with the standard drug used in commerce.[32-38]



Scheme 8 Mechanism of transformation of pro-drugs **4a-4h** to **4aa-4hh** via bio-catalyzed hydrolysis of nitrile group and identification of antibacterial pharmacophore site.

Table 5 Molinspiration calculations of metabolites (**4aa-4hh**)

Compd.	Molinspiration calculations [a]				Drug-likeness [b]					
	TPSA	NONH	NV	VOL	GPCRL	ICM	KI	NRL	PI	EI
4aa	126	4	0	295	-0.09	-0.65	-0.02	-0.02	-0.68	**0.10**
4bb	126	4	0	278	-0.04	-0.60	-0.03	-0.68	-0.37	**0.16**
4cc	135	4	0	304	-0.09	-0.62	-0.01	-0.64	-0.39	**0.10**
4dd	146	5	0	286	-0.00	-0.53	-0.07	-0.51	-0.34	**0.20**
4ee	126	4	0	292	-0.05	-0.53	-0.01	-0.67	-0.39	**0.12**
4ff	172	4	1	302	-0.19	-0.59	-0.11	-0.70	-0.47	**0.03**
4gg	172	4	1	302	-0.18	-0.59	-0.09	-0.70	-0.47	**0.04**
4hh	172	4	1	302	-0.23	-0.60	-0.12	-0.81	-0.51	**0.01**
SD1 [c]	334	16	3	497	**0.17**	-0.09	-0.24	-0.32	**0.78**	**0.46**

[a] TPSA: Total molecular polar surface area; NONH: number of OH---N or O---NH interaction, NV: number of violation of five Lipinsky rules; VOL: volume. [b] GPCRL: GPCR ligand; ICM: Ion channel modulator; KI: Kinase inhibitor; NRL: Nuclear receptor ligand; PI: Protease inhibitor; EI: Enzyme inhibitor. [c] **SD1**: Streptomycin (Antibacterial).

Conclusion

In this research study, a clean, simple facile and conveniently practical methodology was developed for synthesis of annulated pyrido[2,3-*d*]pyrimidines/uracils using mild base TEA as a novel organo-catalyst in green solvent aqueous ethanol. This synthesized methodology own few advantageous like, performed unedr mild conditions, excellent yields, operational simplicity, simple separation, clean, energy-efficiency, high atom-economy, no special work up required, products are filtered and purified only by simple crystallization.

The Quantitative structure–activity relationship (QSAR) of synthesized compounds **4a-4d** series own excellent antibacterial profile and pyrido[2,3-*d*]pyrimidines **4a-4d** series can serve as the prospective prodrugs. They are substances described in an inactive form that is then metabolized via enzymatic action into the active compounds **4aa-4dd**. The rationale behind administering prodrugs is to optimize absorption, distribution, metabolism, and excretion of these drugs. In addition, these prodrugs undergo enzymatic biotransformation in the presence of fungus which makes them good therapeutic agents against fungus.[39]

Pyrido[2,3-*d*]pyrimidines **4a-4d** series have exhibited varying degree of inhibitory effect on the growth of *Rhodococcus sp. C3II* and *Rhodococcus erythropolis MP50* bacterial strains and

found superior than that of clinical drug standard/Streptomycin. Calculated overall drug-score for metabolites are superior to standard/streptomycin drug. Drug score combines drug-likeness, cLog*P*, log*S*, molecular weight, and toxicity risks to judge overall potential as a prodrug. These synthesized compounds showed excellent drug score than that of standard drug streptomycin as supported by POM analyses.[32-38] This research work provided with structure–activity information, indeed proved a simple control of nature of few numbers of substituents leads to product/metabolites owing high bio-activity.

Acknowledgments

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Conflicts of interest

There are no conflicts to declare.

Notes and References

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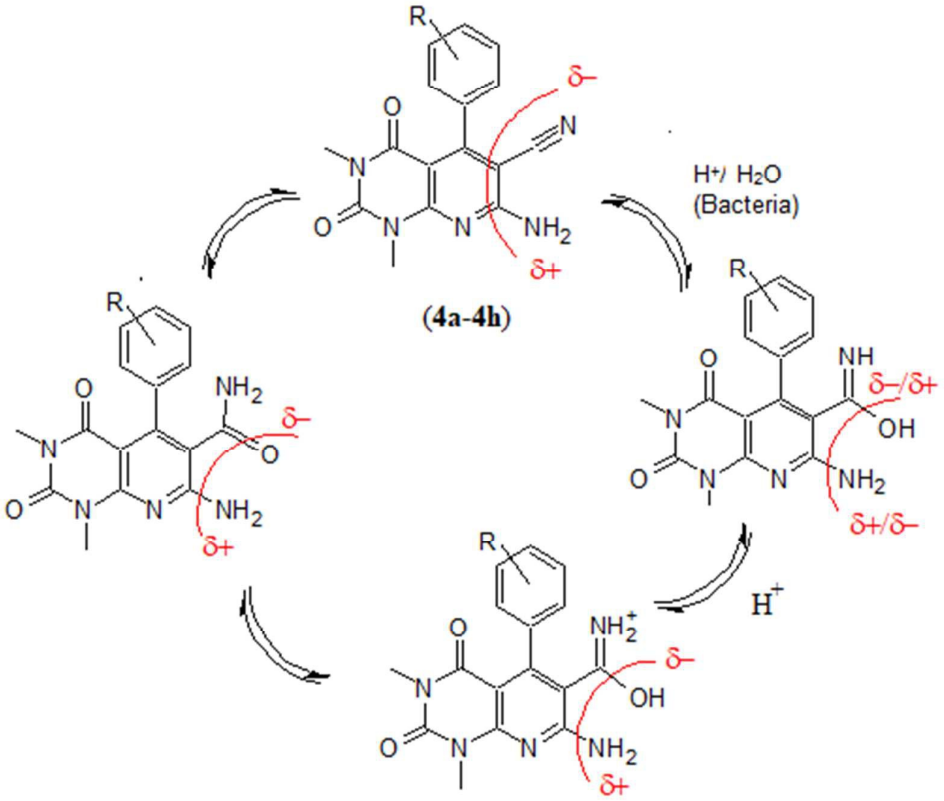
Graphical Abstract



The clean and simple one-pot multi-component methodology was developed for the preparation of 7-amino-2,4-dioxo-5-aryl-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-6-carbonitriles with excellent yields (85-95%) of compounds (**4a-4h**) is described. All the synthesised pyrido[2,3-d]pyrimidines bearing nitrile compounds (**4a-4h**) showed comparatively good *in-vitro* antibacterial activities against Staphylococcus, Bacillus cereus (Gram-positive bacteria) and P. merabitis S.maresens (Gram-negative bacteria). Pyrido[2,3-*d*]pyrimidines **4a-4d** series have exhibited varying degree of inhibitory effect on the growth of *Rhodococcus sp. C3II* and *Rhodococcus erythropolis MP50* bacterial strains and found superior than that of clinical drug standard/Streptomycin. Calculated overall drug-score for metabolites are superior to standard/streptomycin drug. Drug score combines drug-likeness, cLog*P*, log*S*, molecular weight, and toxicity risks to judge overall potential as a prodrug. These synthesized compounds showed excellent drug score than that of standard drug streptomycin as supported by bio-informatic POM analyses.

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List of Figures

Fig. 1 Structure of Streptomycin (**SD1**) bearing multi combined antibacterial ($X^{\delta+}$----$Y^{\delta-}$) pharmacophore sites.

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List of Schemes

Scheme 1 General procedure for the preparation of pyrido[2,3-*d*]pyrimidines **4a-4h**.

Scheme 2 Hydration and subsequent hydrolysis of D,L-phenylglycine nitrile.[25] (i) Nitrile hydralase, (ii) Amidase.

Scheme 3 (i) R. *Rhodochrous sp*., MeOH/potassium phosphate buffer (1:10), 30 °C, 3 h.[26]

Scheme 4 Cyanohydrin hydrolysis catalyzed by *R. erythropolis NCIMB 11540*.[27]

Scheme 5 Naproxen nitrile hydrolysis catalyzed by *Rhodococcus sp. MP50*.[28]

Scheme 6 Dinitriles hydrolusis catalyzed by hydratase and amidase of *Rhodococcus sp. C3II* and *Rhodococcus erythropolis MP50* bacterial strains.[29] (i) Nitrile hydratase (ii) Amidase.

Scheme 7 Biocatalytic conversion of _-aminonitriles to _-amino amides and acids,[31] (i) Nitrile hydratase, (ii) Amidase, (iii) Nitrilase.

Scheme 8 Mechanism of transformation of pro-drugs **4a-4h** to **4aa-4hh** via bio catalyzed hydrolysis of nitrile group and identification of antibacterial pharmacophore site.

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