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Novel isoniazid cyclocondensed 1,2,3,4-tetrahydropyrimidine derivatives for treating infectious disease: a synthesis and *in vitro* biological evaluation

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ABSTRACT

Objective: To synthesize new congeners by incorporating isoniazid with 1,2,3,4-tetrahydropyrimidinones moieties in a single molecular frame work and to evaluate their antimicrobial and antimycobacterial activity. **Methods:** A new series of some novel isoniazid cyclocondensed 1,2,3,4-tetrahydropyrimidines was prepared by N´-acetoacetylisonicotinohydrazide with urea/thiourea and appropriate aldehyde in the presence of catalytic amount of laboratory made benzenesulphonic acid. Confirmation of the chemical structure of the synthesized compounds (4a-n) was substantiated by melting point, TLC, different spectral data IR, ^{1}H-NMR, and Mass spectra were done. The synthesized compounds were evaluated for *in vitro* antimicrobial and antimycobacterial activity against *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), *Mycobacterium tuberculosis* (*M. tuberculosis*) CIP and H37Rv strain. **Results:** The titled compounds exhibited weak, moderate, or high antimicrobial and antimycobacterial activity. Compounds 4l, 4m, and 4n, exhibited potential antimicrobial and antimycobacterial action, when compare with the current therapeutic agent of Norfloxacin and Rifampicin. **Conclusions:** Compound 4l, 4m, and 4n is arguably the most potent, our present study makes it an interesting compound when compared to the current therapeutic agents and are considered the candidates to investigate further for the same.

1. Introduction

Organic compound synthesis has been promoted by microwave assisted methods by which small molecules are built up into large polymers in a fraction of time when compared to thermal methods ensuring the acceptance of Microwave assisted irradiation reactions as a valuable tool for acceleration of a wide variety of organic molecules development[1-3]. The advent of microwave assisted technology in organic chemistry dates back to the mid 1980s and since the 1990s there has been a significant increase in the number of publications on Microwave Assisted Organic Reactions (MAOS) due to increased benefits associated with the process[4-6]. The promotion of microwave assisted reactions in organic chemistry has improved the speed, reduced cost, reduced energy spent making it a sustainable process and is widely heralded as "green chemistry" measures whose applications are promoted today to minimize the use of non renewable resources as well as polluting solvent, to reduce generation of secondary products which are often toxic and to reduce the emission of harmful gases[7-9]. Microwave assisted reactions in organic chemistry achieve the same by ensuring facilitation of faster reactions under bulk conditions as well as promoting reduction of reaction time[10].

Pyrimidine derivatives comprise a diverse and interesting group of drugs are extremely important for their biological activities. Dihydropyrimidine and their derivatives have attracted increasing interest owing to their therapeutic and pharmaceutical properties, such as antiviral, antibacterial, antitubercular[11-13], antagonists of the human adenosine A2A receptor[14], cyclooxygenase-2 inhibitory activity[15-16], tyrosine kinase inhibitors, antiamoebic activity[17-18]and anticancer activities[19]. Recently, functionalized dihydropyrimidinones have been successfully used

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as antihypertensive agents, calcium channel blockers, adrenergic and neuropeptide Y (NPY) antagonists[20]. In addition, some alkaloids containing the dihydropyrimidine core unit which also exhibit interesting biological properties have been isolated from marine sources. Most notably, among these are the batzelladine alkaloids, which were found to be potent HIV gp-120-CD4 inhibitors[21-22]. The original protocol for the synthesis of dihydropyrimidinones, reported by Biginelli in 1893, involves a one-pot reaction of benzaldehyde, ethyl acetoacetate and urea in ethanol under strongly acidic conditions[23]. However, this method suffers from drawbacks such as low yields (20%-40%) of the desired products, particularly in case of substituted aldehydes, and loss of acid sensitive functional groups during the reaction. This has led to multi-step synthetic strategies that produce somewhat better yields, but which lack the simplicity of the original one-pot Biginelli protocol[24]. The discovery during the 1930s that a dihydropyridine (dihydronicotinamide derivative, NADH), ''hydrogen-transferring coenzyme'' consequently became important in biological system, has generated numerous studies on the biochemical properties of dihydropyridines and their bioisosteres dihydropyrimidines. The search for more suitable preparation of dihydropyrimidinones continues today.

The chemical structure of isoniazid provides a most valuable molecular template for the development of agents able to interact with a wide variety of biological activities. Tetrahydropyrimidines are structurally similar to dihydropyrimidines. The synthesis of isoniazid condensed with 1, 2, 3, 4-tetrahydropyrimidinones are not reported so for. Hence, it was thought worthwhile to synthesize new congeners by incorporating isoniazid with 1, 2, 3, 4-tetrahydropyrimidinones moieties in a single molecular frame work and to evaluate their antimicrobial and antimycobacterial activity.

2. Materials and methods

2.1. Experimental

All chemicals were supplied by E. Merck (Germany) and S.D fine chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in the solvent system ethanol, chloroform, ethylacetate (6:3:1); the spots were located under iodine vapors or UV light. IR spectrums were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr Pellets). ^{1}H-NMR spectra were recorded or a Bruker AC 300 MHz spectrometer using TMS as internal standard in DMSO/ $CDCl_3$. Mass spectra were obtained using Shimadzu LCMS 2010A under ESI ionization technique.

2.2. General procedure

2.2.1. Preparation of N′-acetoacetylisonicotinohydrazide (3)

Isoniazid 1 (0.01 M) and ethyl acetoacetate 2 (0.01 M) were mixed and refluxed for approximately 7 h. The colorless liquid formed was then heated on a water bath to remove the alcohol formed during the reaction. After allowing the reaction mixture to cool, crude crystals were obtained. Purification was performed by stirring crude crystals with cold diethyl ether for approximately 15 min using a mechanical stirrer. Allowing it to stand for 15 min, followed by filtration, resulted in the third compound in a pure form of N′-acetoacetylisonicotinohydrazide 3.

2.2.2. Preparation of 1,2,3,4-tetrahydropyrimidines by microwave irradiation method(4a-n)

The mixture of N′-acetoacetylisonicotinohydrazide (0.005 M), urea/thiourea (0.007 5 M), and appropriate aldehyde (0.005 M) with catalytic amount of benzenesulphonic acid in 10 ml of ethanol was subjected to microwave irradiation (300 W) for 8 min at the interval of 10 s. The reactions were monitored through TLC using 25 percent ethyl acetate in pet ether as solvent system. After the reaction was complete, the reaction mixture was cooled in a refrigerator and filtered. The precipitate obtained was washed thoroughly with water to remove unreacted urea/thiourea and dried. The crude solid product was recrystallized with ethanol to give the pure compounds (4a-n).

2.3. Analytical data

2.3.1. N′-(3-oxobutanoyl) pyridine-4-carbohydrazide (3)

Colourless crystalline solid, M.P: 174-176 ℃, Yield 68%, IR (KBr, cm^{-1}): 3 312 (N-H), 3 052 (Ht-ArC-H), 2 864 (AliC-H), 1 732 (C=O, ketone), 1 652 (C=O, amide), 1 563 (C=C), 1 348 (C-N), ^{1}H-NMR (DMSO-d6) δ: 2.28 (s, 3H, CH_3), 3.36 (s, 2H, CH_2), 7.32 (d, 2H, ArH), 9.38 (s, 1H, NH), 9.38 (s, 1H, NH), MS (m/z): M+ calculated 221.07, found 221.24.

2.3.2. 6-methyl-2-oxo-4-(pyridin-4-yl)-N′-(pyridin-4-ylcarbonyl)-1,2,3,4 tetrahydropyrimidine-5-carbohydrazide (4a)

Pale-bluish colored solid, M.P: 236-238 ℃, Yield 72%, IR (KBr, cm^{-1}): 3 296 (N-H), 3 136(ArC-H), 2 942 (AliC-H), 1 675 (C=O, amide), 1 556 (C=C), 1 210 (O-C), ^{1}H-NMR (DMSO-d6) δ : 2.05 (s, 3H, CH3), 5.42 (s, 1H, CH), 6.52-6.67(d, 2H, ArH), 7.24-7.35 (m, 5H, ArH), 7.54 (d, 2H, ArH), 8.71 (s, 1H, NH), 8.85 (s,1H, NH), 9.42 (s, 1H, NH), 9.86 (s, 1H, NH). MS (m/z): M+ calculated 352.12, found 351.92.

2.3.3. 6-methyl-4-(pyridin-4-yl)-N′-(pyridin-4-ylcarbonyl)-2-thioxo-1,2,3,4 tetrahydropyrimidine-5-carbohydrazide(4b)

Pale-yellowish solid, M.P: 248-251 ℃, Yield 70%, IR (KBr, cm^{-1}): 3 264 (N-H), 3 178(ArC-H), 2 956 (AliC-H), 1

685 (C=O, amide), 1 574 (C=C), 1 872 (C=S), 1 169 (O-C), 1H-NMR (DMSO-d6) δ : 2.07 (s, 3H, CH_3), 5.53(s, 1H, CH), 6.57 (d, 2H, ArH), 7.31-7.45 (m, 5H, ArH), 7.82 (d, 2H, ArH), 9.22 (s, 1H, NH), 9.58 (s, 1H, NH), 9.85 (s, 1H, NH), MS (m/z): M+ calculated 353, found 353.75. MS (m/z): M+ calculated 368.10, found 368.40.

2.3.4. 6-methyl-4-(3-nitrophenyl)-2-oxo-N'-(pyridin-4-ylcarbonyl)-1,2,3,4 tetrahydropyrimidine-5-carbohydrazide (4c)

Light-greenish colored solid, M.P: 256-259 ℃, Yield 78%, IR (KBr, cm^{-1}): 3 314 (N-H), 3 210 (ArC-H), 2 936 (AliC-H), 1 684 (C=O, amide), 1 568 (C=C), 1 324 (O-C), ^{1}H-NMR (DMSO-d6) δ : 2.05 (s, 3H, CH_3), 5.62 (s, 1H, CH),6.53 (d, 2H, ArH), 6.74-7.21 (m, 4H, ArH), 7.56 (d, 2H, ArH), 8.52 (s, 1H, NH), 8.76 (s, 1H, NH), 9.54 (s, 1H, NH). MS (m/z): M+ calculated 396.11, found 395.96.

2.3.5. 6-methyl-4-(3-nitrophenyl)-N'-(pyridin-4-ylcarbonyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (4d)

Dark-brownish solid, M.P: 278-280 ℃, Yield 74%, IR (KBr, cm^{-1}): 3 228 (N-H), 3 146(ArC-H), 2 930 (AliC-H), 1 672 (C=O, amide), 1 584 (C=C), 1858 (C=S), 1 210(O-C), 1H-NMR (DMSO-d6) δ : 2.04 (s, 3H, CH3), 5.38 (s, 1H, CH), 6.59 (d, 2H, ArH), 6.84 (d, 2H, ArH), 7.32 (d, 2H, ArH), 7.64 (d, 2H, ArH), 9.24 (s, 1H, NH), 9.46 (s, 1H, NH), 9.88(s, 1H, NH). MS (m/z): M+ calculated 412.09, found 413.26.

2.3.6. 4-(3-chlorophenyl)-6-methyl-2-oxo-N'-(pyridin-4-ylcarbonyl)-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (4e)

Light-ash-colored solid, M.P: 263-266 ℃, Yield 81%, IR (KBr, cm^{-1}): 3 322 (N-H), 3178 (ArC-H), 2 942 (AliC-H), 1 667 (C=O amide), 1 568 (C=C), 1 276 (C-O), ^{1}HNMR (DMSO-d6) δ :2.01 (s, 3H, CH_3), 5.61 (s, 1H, CH), 6.72 (d, 2H, ArH), 7.54 (m, 3H, ArH), 7.78 (d, 2H, ArH), 8.75 (s, 1H, NH), 9.44 (s, 1H, NH), 9.88 (s, 1H, NH). MS (m/z): MS (m/z): M+ calculated 385.09, found 386.12.

2.3.7. 4-(3-chlorophenyl)-6-methyl-N'-(pyridin-4-ylcarbonyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (4f)

Ash-colored solid, M.P: 284-287 ℃, Yield 78%, IR (KBr, cm^{-1}): 3 257 (N-H), 3 156(ArC-H), 2 966 (AliC-H), 1 648 (C=O, amide), 1 588 (C=C), 1 846 (C=S), 1 177 (O-C), ^{1}H-NMR (DMSO-d6) δ : 2.07 (s, 3H,CH3), 5.58 (s, 1H, CH), 6.72 (d, 2H, ArH), 6.89 (d, 2H, ArH),7.66-7.82 (m, 3H, ArH), 9.24 (s, 1H, NH), 9.47 (s, 1H, NH), 10.01 (s, 1H, NH), MS (m/z): M+ calculated 401.07, found 400.94.

2.3.8. 4-(furan-2-yl)-6-methyl-2-oxo-N'-(pyridin-4-ylcarbonyl)-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (4g)

Light-yellowish solid, M.P: 242-245 ℃, Yield 77%, IR (KBr, cm^{-1}): 3 252 (N-H), 3 146(ArC-H), 2 954 (AliC-H), 1 664 (C=O, amide), 1 586 (C=C), 1 174 (O-C), ^{1}H-NMR (DMSO-d6) δ : 2.01 (s, 3H, CH_3), 5.43 (s, 1H, CH), 6.54 (d, 2H, ArH), 7.39-7.63 (m, 4H, ArH), 8.92 (s, 1H, NH), 9.44 (s, 1H, NH), 9.92 (s, 1H, NH). MS (m/z): M+ calculated 341.11, found 340.86.

2.3.9. 4-(furan-2-yl)-6-methyl-N'-(pyridin-4-ylcarbonyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (4h)

Light-greenish solid, M.P: 268-271 ℃, Yield 75%, IR (KBr, cm^{-1}): 3 246 (N-H), 3 164(ArC-H), 2 968 (AliC-H), 1 638 (C=O, amide), 1 566 (C=C), 1 846 (C=S), 1 154 (O-C), ^{1}H-NMR (DMSO-d6) δ : 2.08 (s, 3H, CH3), 5.73 (s, 1H, CH), 6.51 (d, 2H, ArH), 7.38-7.56 (m, 4H, ArH), 8.94 (s, 1H, NH), 9.28 (s, 1H, NH), 9.93 (s, 1H, NH). MS (m/z): M+ calculated 357.08, found 356.93.

2.3.10. 4-(2-chlorophenyl)-6-methyl-2-oxo-N'-(pyridin-4-ylcarbonyl)-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (4i)

Light-bluish solid, M.P: 282-285 ℃, Yield 76%, IR (KBr, cm^{-1}): 3 243 (N-H), 3 153(ArC-H), 2 938 (AliC-H), 1 663 (C=O, amide), 1 568 (C=C), 1 187 (O-C), ^{1}H-NMR (DMSO-d6) δ : 2.07 (s, 3H, CH_3), 5.38 (s, 1H, CH), 6.69 (d, 2H, ArH), 7.53-7.68 (m, 4H, ArH), 8.82 (s, 1H, NH), 9.51 (s, 1H, NH), 9.87 (s, 1H, NH). MS (m/z): M+ calculated 385.09, found 384.88.

2.3.11. 4-(2-chlorophenyl)-6-methyl-N'-(pyridin-4-ylcarbonyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (4j)

Ash-colored solid, M.P: 289-291 ℃, Yield 72%, IR (KBr, cm^{-1}): 3 247 (N-H), 3 148(ArC-H), 2 928 (AliC-H), 1 658 (C=O, amide), 1 568 (C=C), 1 834 (C=S), 1 157 (O-C), ^{1}H-NMR (DMSO-d6) δ : 2.06 (s, 3H, CH_3), 5.63 (s, 1H, CH), 6.59 (d, 2H, ArH), 7.42-7.67 (m, 4H, ArH), 8.76 (s, 1H, NH), 9.39 (s, 1H, NH), 9.95 (s, 1H, NH). MS (m/z): M+ calculated 401.07, found 401.76.

2.3.12. 4-(4-chlorophenyl)-6-methyl-2-oxo-N'-(pyridin-4-ylcarbonyl)-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (4k)

Light-bluish colored solid, M.P: 294-296 ℃, Yield 79%, IR (KBr, cm^{-1}): 3 258 (N-H), 3 152(ArC-H), 2 934 (AliC-H), 1 686 (C=O, amide), 1 568 (C=C), 1 149 (O-C), ^{1}H-NMR (DMSO-d6) δ : 2.03 (s, 3H, CH_3), 5.39 (s, 1H, CH), 6.72 (d, 2H, ArH), 7.46-7.72 (m, 4H, ArH), 8.88 (s, 1H, NH), 9.38 (s, 1H, NH), 9.74 (s, 1H, NH). MS (m/z): M+ calculated 385.09, found 385.84.

2.3.13. 4-(4-chlorophenyl)-6-methyl-N'-(pyridin-4-ylcarbonyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (4l)

Light-yellowish solid, M.P: 312-315 ℃, Yield 73%, IR (KBr, cm^{-1}): 3 256 (N-H), 3 174(ArC-H), 2 966 (AliC-H), 1 672 (C=O, amide), 1 574 (C=C), 1 858 (C=S),1 134 (O-C), ^{1}H-NMR (DMSO-d6) δ : 2.06 (s, 3H, CH_3), 5.72 (s, 1H, CH), 6.49 (d, 2H,

ArH), 7.38-7.64 (m, 4H, ArH), 8.72 (s, 1H, NH), 9.68 (s, 1H, NH), 9.96 (s, 1H, NH). MS (m/z): M+ calculated 401.07, found 400.92.

2.3.14. 4-(4-fluorophenyl)-6-methyl-2-oxo-N′-(pyridin-4-ylcarbonyl)-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (4m)

Light-greenish solid, M.P: 328-331 ℃, Yield 82%, IR (KBr, cm^{-1}): 3 246 (N-H), 3 178(ArC-H), 2 936 (AliC-H), 1 646 (C=O, amide), 1 571 (C=C), 1 154 (O-C), ^{1}H-NMR (DMSO-d6) δ: 2.08 (s, 3H, CH_3), 5.58 (s, 1H, CH), 6.44 (d, 2H, ArH), 7.36-7.69 (m, 4H, ArH), 8.84 (s, 1H, NH), 9.58 (s, 1H, NH), 9.98 (s, 1H, NH). MS (m/z): M+ calculated 369.12, found 369.26

2.3.15. 4-(4-fluorophenyl)-6-methyl-N′-(pyridin-4-ylcarbonyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (4n)

Light-bluish colored solid, M.P: 344-346 ℃, Yield 78%, IR (KBr, cm^{-1}): 3 246 (N-H), 3 134(ArC-H), 2 972 (AliC-H), 1 646 (C=O, amide), 1 578 (C=C), 1 826 (C=S), 1 184 (O-C), ^{1}H-NMR (DMSO-d6) δ: 2.09 (s, 3H, CH_3), 5.74 (s, 1H, CH), 6.68 (d, 2H, ArH), 7.34-7.66 (m, 4H, ArH), 8.82 (s, 1H, NH), 9.56 (s, 1H, NH), 9.96 (s, 1H, NH). MS (m/z): M+ calculated 385.10, found 385.84.

2.4. Antimicrobial activity

The *in vitro* antibacterial activities were tested against Gram-positive bacteria *Bacillus subtilis* (*B. subtilis*) and Gram-negative bacteria *Escherichia coli* (*E. coli*) by standard serial dilution method using a stock solution of 100 μg/mL concentration[25,26]. Double strength nutrient broth was used as culture media and dimethyl sulphoxide (DMSO) was used as solvent control. The stock solutions of the test compounds were serially diluted in test tubes containing 1 mL of sterile medium to get the different concentrations and then inoculated with 100 μL of suspension of respective microorganism in sterile saline. Norfloxacin was used as standard drug. The inoculated test tubes were incubated at (37 ± 1) ℃ for 24 h.

2.5. Antimycobacterial activity

Antimycobacterial activity was performed following a protocol previously reported[27]. Compounds (4a - n) were preliminarily assayed against to freshly isolate clinical strains, *M. furtuitum* and *M. tuberculosis*, according to the dilution method in agar. Growth media were Mueller-Hilton (Difco) containing 10% of OADC (oleic acid, albumin and dextrose complex) for *M. furtuitum* and Middle brook 7H11 agar (Difco) with 10% of OADC for *M. tuberculosis*. Substances were tested at single dose of 100 μg/mL. The compounds were then assayed for inhibitory activity against a panel of mycobacterial (*M. tuberculosis* CIP, *M. tuberculosis* H37Rv) in Middle brook 7H11 agar by a standard twofold dilution method. Plates were incubated at 37 ℃ for 3 or 28 d. Rifampicin was used as reference compound. After cultivation, MICs were read as minimal concentrations of drugs completely inhibiting visible of mycobacterial growth (Table 1).

3. Results

A series of 14 novel isoniazid cyclocondensed 1,2,3,4-tetrahydropyrimidines of biological interest were synthesized and evaluated for antimicrobial and anti-mycobacterial activity, all the compounds were characterized by IR, ^{1}H NMR, MS for their structures.

3.1. Chemistry

Synthesis of 1,2,3,4-tetrahydropyrimidines by microwave irradiation method reaction was performed by following steps as outlined in Figure 1. In the first step, ethylacetoacetate 2 and isoniazid 1 reacted under neat conditions resulting in the formation of N′-acetoacetylisonicotinohydrazide 3 with the yield of 68 percent. The N′-acetoacetylisonicotinohydrazide was further taken for the condensation reaction by reacting it with urea/thiourea and appropriate aldehyde in the presence of catalytic amount of benzenesulphonic acid. The reaction times were found to be 8 minutes. Totally, fourteen compounds 4a-n, various substituted 1,2,3,4-tetrahydropyrimidines, were synthesized with the yield ranging from 70 to 82 percent. These conditions enable this method to be applicable for the synthesis of 1,2,3,4-tetrahydropyrimidines based heterocyclic compounds. The 1,2,3,4-tetrahydropyrimidines, (4a-n) were synthesized relatively easily by using laboratory made benzenesulphonic acid as an efficient catalyst compared with Lewis acid. The present protocol best describes the synthesis of 1,2,3,4-tetrahydropyrimidines. All the reported 1,2,3,4-tetrahydropyrimidines compounds were found to be novel and not reported elsewhere.

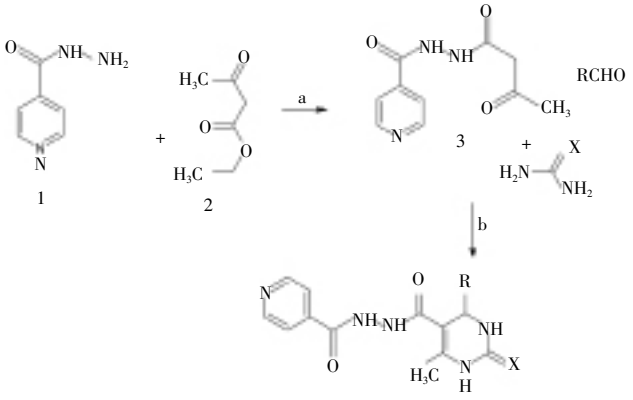

Figure 1. Synthesis of compounds (4a - 4n).
Reagents and conditions: (a) Reflux 6 h; (b) C_2H_5OH, Benzenesulphonic acid, microwave irradiation (300 W) for 8 min.

Table 1
Synthesized 1,2,3,4-tetrahydropyrimidines: Antimicrobial and antimycobacterial activity.

S.No	Compound	R	X	*B. subtilis* MIC (μ mol/mL)	*E. coli* MIC (μ mol/mL)	*M. tuberculosis* CIP MIC (μ g/mL)	*M. tuberculosis* H37Rv MIC (μ g/mL)
1	4a	4-pyridyl	O	0.012 9	0.013 0	1.41	1.47
2	4b	4-pyridyl	S	0.012 6	0.012 8	1.24	1.21
3	4c	3-nitrophenyl	O	0.014 1	0.014 3	2.73	2.97
4	4d	3-nitrophenyl	S	0.137 0	0.013 9	2.62	2.66
5	4e	3-chlorophenyl	O	0.013 9	0.014 0	2.34	2.36
6	4f	3-chlorophenyl	S	0.013 5	0.013 8	2.13	2.18
7	4g	2-furyl	O	0.015 7	0.016 3	5.28	5.35
8	4h	2-furyl	S	0.015 3	0.015 8	4.62	4.87
9	4i	2-chlorophenyl	O	0.013 7	0.014 1	2.04	2.07
10	4j	2-chlorophenyl	S	0.013 4	0.013 9	1.92	1.96
11	4k	4-chlorophenyl	O	0.011 6	0.012 3	1.07	1.09
12	4l	4-chlorophenyl	S	0.011 1	0.012 0	1.40	1.06
13	4m	4-flurophenyl	O	0.011 8	0.012 1	1.03	1.07
14	4n	4-flurophenyl	S	0.011 2	0.011 9	1.01	1.04
15	Norfloxacin	Standard	–	0.012 4	0.023 7	–	–
16	Rifampicin	Standard	–	–	–	1.06	1.08

3.2. Antimicrobial and antimycobacterial activity

The synthesized compounds were subjected to *in vitro* antimicrobial activity against Gram-positive bacteria *B. subtilis*, Gram-negative bacteria *E. coli* and antimycobacterial against *M. tuberculosis* CIP and H37RV strain. The motive is to check the antimicrobial and antimycobacterial activity for the synthesized compounds. Almost all the titled compounds exhibited weak, moderate, or high antimicrobial and antimycobacterial activity.

Analyzing the activities of the synthesized compounds, the following structure activity relationships (SARs) were obtained. The fifth position of 1,2,3,4-tetrahydropyrimidines contain N′-acetoacetylisonicotinohydrazide group contributed toward antimicrobial and antimycobacterial and forth positions of 1,2,3,4-tetrahydropyrimidines contain substituted phenyl and hetero aromatic ring responsible antimicrobial and antimycobacterial potency. Substituted atom or group of atom must be strong electron withdrawing nature for potent activity because it decreases electron density in the ring due to inductive effect. Fluoride and chloride substitution at fourth position of phenyl ring showed potent antimicrobial and antimycobacterial action because of strong electron withdrawing nature. Substitution of chloro group at third position of phenyl ring showed potent action when compare with nitro atom. Among all the substituted phenyl ring, the activity order was F>Cl>NO_2. Introduction of heterocyclic ring at forth position it showed moderate antimicrobial and antimycobacterial activity. Among the compounds reported here in, compound (4l, 4m, 4n) is arguably the most potent when compare with current therapeutic agent norfloxacin and rifampicin because fluride and chloride substituted phenyl ring present at 4th position of 1,2,3,4-tetrahydropyrimidines it enhance the antimicrobial and antimycobacterial activity (Table 1).

4. Discussion

A series of novel 1,2,3,4-tetrahydropyrimidines of biological interest were synthesized and analyzed for their structures. The libraries of compounds were prepared by using laboratory made benzenesulphonic acid as an efficient catalyst. The importance of substitutions at the fourth and fifth positions of 1,2,3,4-tetrahydropyrimidines was studied toward the antimicrobial and anti mycobacterial activity. The antimicrobial and anti mycobacterial activity data revealed that the all synthesized compounds proved to be active against the test organism *B. subtilis*, *E. coli*, *M. tuberculosis* CIP and H37RV strain. Almost all of the titled compounds exhibited weak, moderate, or high antimicrobial and antimycobacterial activity. Some of new derivatives showed an *in vitro* antimicrobial activity against *B. subtilis*, *E. coli* better than that of norfloxacin and antimycobacterial activity against *M. tuberculosis* CIP and H37RV strain better than that of antitubercular drug rifampicin. Among the compounds reported here in, compound (4l, 4m, 4n) is arguably the most potent, our present study makes it an interesting compound when compared to the current therapeutic agents and are considered the candidates to investigate further for the same.

Conflict of interest statement

The authors report no conflict of interest.

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