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Synthesis and Antitubercular Evaluation of Some Novel 1,2,3,6-tetrahydropyrimidine-5-carbonitrile

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ABSTRACT

In an attempt to find a new class of antitubercular agents, a series of 1,2,3,6-tetrahydropyrimidine-5-carbonitrile were prepared via the reaction of ethyl N-ethoxycarbonylbenzimidate **2a-b** with cyanoacetanilide derivatives **1a-c**. These compounds were screened for their antitubercular activity against *M. tuberculosis*. Several analogues, such as 2,6-dioxo-1-phenyl-4-p-tolyl-1,2,3,6-tetrahydropyrimidine-5-carbonitrile **3a**, 1-benzyl-2, 6-dioxo-4-p-tolyl-1,2,3,6-tetrahydropyrimidine-5-carbonitrile **3c** and 1-benzyl-2, 6-dioxo-4-phenyl-1,2,3,6-tetrahydropyrimidine-5-carbonitrile **3d** exhibited a potent antitubercular activity with an MIC values ranging from 10-35 µg/ml. Structures of the newly synthesized compounds were established by spectral data and HRMS.

Indexing terms/Keywords

1,2,3,6-tetrahydropyrimidine-5-carbonitrile; ethyl N-ethoxycarbonylbenzimidate; cyanoacetanilide derivatives; antitubercular activity.

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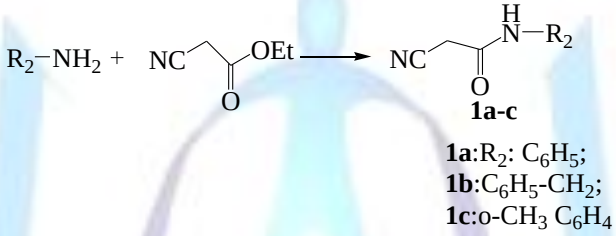
INTRODUCTION

Human tuberculosis (TB), mainly caused by Mycobacterium tuberculosis, is a major cause of illness and death worldwide[1]. In 2008, there were an estimated 8.9–9.9 million incident cases of TB, 9.6–13.3 million prevalent cases of TB, 1.1–1.7 million deaths from TB among HIV-negative people, and an additional 0.45–0.62 million TB deaths among HIV-positive people[2]. During the recent years, pyrimidine derivatives have attracted organic chemists very much due to their antitubercular activity[3-7]. A series of 1,2,3,6-tetrahydropyrimidine-5-carbonitrile have demonstrated diverse pharmacological activities. The most pronounced of which are antitubercular[8] anticancer[9.10], antimicrobial[11] and anti-inflammatory [12-15]. In connection with our previous work and continuing interest in the synthesis of valuable heterocycles [16-18], we report here the synthesis of some novel 1,2,3,6-tetrahydropyrimidine-5-carbonitrile where we have examined the reaction of alkyl N-cyanobenzimidate, phenyl N-acetybenzimidate with cyanoacetanilide derivatives.

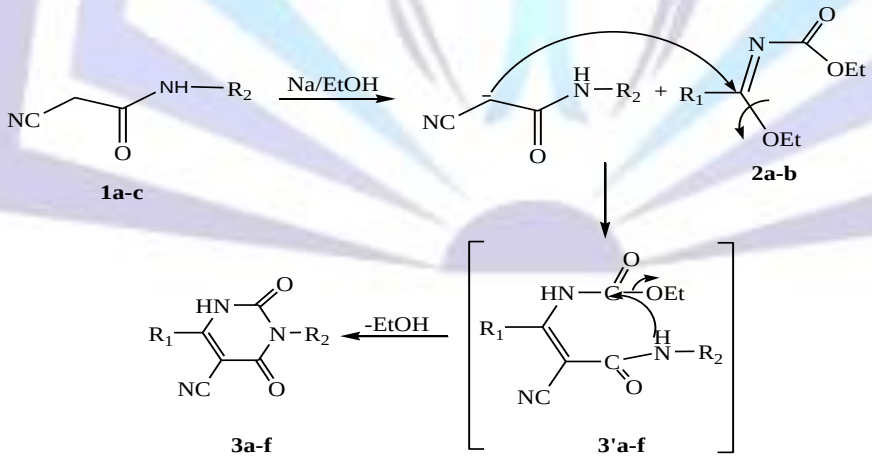
RESULTS AND DISCUSSION

The solvent-free reaction of arylamines with ethyl cyanoacetate constitutes one of the most widely used methods for the preparation of cyanoacetanilides. Thus, fusion of aromatic amines with an excessive amount of ethyl cyanoacetate at 150°C afforded cyanoacetanilide derivatives **1a-c** [19], (Scheme 1).



Scheme 1: Synthesis of cyanoacetanilide derivatives

Our approach to the target heterocyclic compounds was achieved by the synthesis of 1,2,3,6-tetrahydropyrimidine-5-carbonitrile derivatives **3a-f** which were prepared by stirring equimolar amounts of cyanoacetanilide derivatives **1a-c** with ethyl N-ethoxycarbonylbenzimidate **2a-b,** under basic medium. A reasonable mechanism for the formation of the products is outlined in scheme 2. Initially, the nonisolable intermediate **3' a-f** is formed by condensation of the cyanoacetanilide salt and ethyl N-ethoxycarbonylbenzimidate **2**, then, followed by nucleophilic attack (by the NH group) on the ester group with the loss of an ethanol molecule to give **3a-f** (Scheme 2).



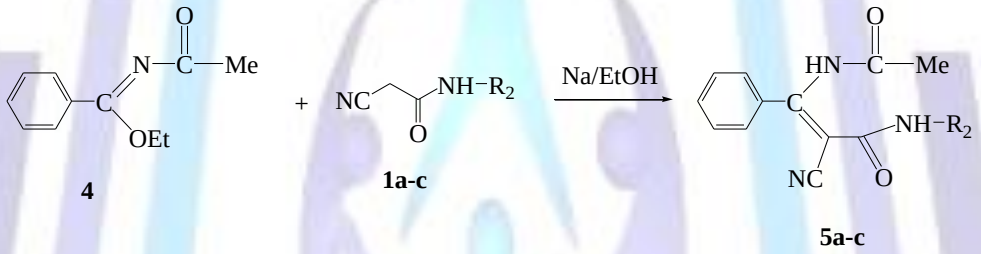
Scheme 2: Synthetic route to 1,2,3,6-tetrahydropyrimidine-5-carbonitrile **3a-f**

Table 1: Chemical structure of target compounds

Compound	R_1	R_2	Yields (%)
3a	p-CH_3 C_6H_4	C_6H_5	78
3b	C_6H_5	C_6H_5	65
3c	p-CH_3 C_6H_4	C_6H_5-CH_2	62
3d	C_6H_5	C_6H_5-CH_2	66
3e	p-CH_3 C_6H_4	o-CH_3 C_6H_4	57
3f	C_6H_5	o-CH_3 C_6H_4	50

The structures of compounds **3a-f** are in accordance with their spectroscopic data. These new products were assigned by IR, NMR and mass spectroscopy. The IR spectra showed essentially the characteristic absorption bands of cyano group at around 2225 cm^{-1}. In the ^{1}HNMR spectra we have noticed the disappearance of the triplet and the quadruplet of ethoxy groups of the starting reagent. HRMS gave the molecular ion peak for all compounds.

Under the same experimental conditions, we have studied the condensation of compound **1a-c** with ethyl N-acetylbenzimidate **4**. The formed products are acetamido-2-cyano-phenylacrylamide **5a-c**. The reaction is drawn in schema 3. The intracyclisation observed in scheme 2 is not present here.



Scheme 3: Synthetic route to acetamido-2-cyano-phenylacrylamide **5a-c**

IR spectra showed the characteristic absorption bands corresponding to NH and CN functionalities respectively at around 3312 and 2212 cm^{-1}. Besides, two bands at 1714 and 1670 cm^{-1} attributed to C=O groups (**5a-c**). Additionally ^{1}H NMR spectra indicated a singlet at around 2.4ppm due to CH_3 protons (**5a-c**) and the most significant information was the disappearance of the triplet and quadruplet of ethoxy groups present in the starting reagent **4**.

Susceptibility of *M. tuberculosis*to 1,2,3,6-tetrahydropyrimidine-5-carbonitrile analogues

We have evaluated the potential of 1,2,3,6-tetrahydropyrimidine-5-carbonitrile compounds in inhibiting growth of *M. tuberculosis.* Minimal inhibitory concentrations (MICs) were determined and are provided scheme 4. Several analogues, such as **3a**, **3c** and **3d** exhibited a potent antitubercular activity with an MIC values ranging from 10-35 µg/ml, whereas **3b**, **3f** and **5c** exhibited only a modest activity. Compound **5a** failed to show any growth inhibition activity even at 100µg/ml, the highest concentration tested.SAR studies seem to indicate that the presence of methyl group on first ring improves the activity of the molecules (compare **3a** and **3b**). Additionally, the presence of the methylene group next to the second aromatic ring appears to slightly improve the activity (compare **3c** and **3a**).

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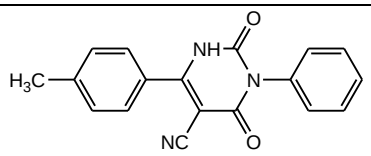
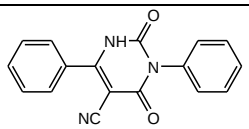
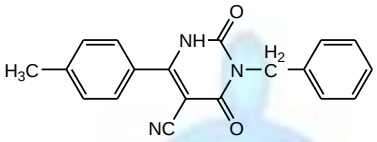
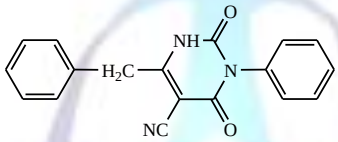
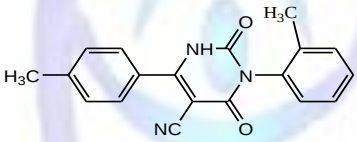
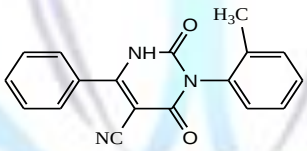
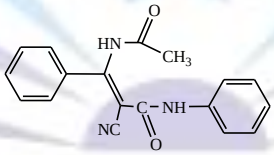
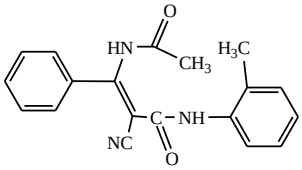
Table 2: Structures of the novel 1,2,3,6-tetrahydropyrimidine-5-carbonitrile and their corresponding minimum inhibitory concentrations in *M. tuberculosis*

Compound	*Structure*	*MIC (ug/ml)*
3a		25
3b		50-70
3c		10-25
3d		25-35
3e		35-50
3f		85-100
5a		>100
5c		85

CONCLUSION

In conclusion, we have developed a simple, quick and efficient method for the synthesis of new 1,2,3,6-tetrahydropyrimidine-5-carbonitrile and acetamido-2-cyano-N,3 diphenylacrylamide using catalytic amounts of sodium ethoxide. Our results demonstrate that ethyl N-ethoxycarbonylbenzimidate and ethyl N-acetylbenzimidate react differently with the **3**. Different skeletons of heterocyclic compounds, obtained in reasonably good yields. The potent antitubercular activity recorded for some of these compounds may provide further insight into a novel mechanism by which these heterocycling compounds are involved in diverse pharmacological processes. **3c**, the most active compound may

represent a scaffold for subsequent structure-activity relationship studies for subsequent pharmacological improvements against *M. tuberculosis*.

EXPERIMENTAL

Melting points are recorded in degrees Celsius on a Kofler apparatus. All reactions were followed by TLC (E. Merck Kieselgel 60 F-254), with UV detection at 254 nm. The IR spectra were recorded in the solid state as KBr discs on a Perkin-Elmer PARAGON 1000 FT-IR spectrometer. ^{1}H and ^{13}C NMR were determined in solution in DMSO-d_6 with an AC Bruker spectrometer at 300 MHz using TMS as an internal standard. High resolution mass were recorded on a spectrometer JEOL JMS-Gemate II.

PROCEDURE FOR THE SYNTHESIS OF COMPOUNDS 3a-f AND 5a-c

To a magnetically stirred solution of sodium (10^{-2}mol) in dry ethanol (30 mL) and cyanoacetanilide derivatives **1a-c** (10^{-2}mol), the appropriate imidate (10^{-2}mol) were added and the reaction mixture stirred for 12 h at room temperature. The progress of the reaction was monitored by TLC (mobile phase, diethyl ether: hexane; 80/20;v/v). The solvent was removed in vacuo. The contents of the flask were neutralized by a saturated solution of NH_4Cl, and then extracted by diethyl ether. The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed in vacuo. The precipitate formed was isolated by filtration and washed with diethyl ether to obtain the pure product.

Spectral Data of New Compounds

2,6-dioxo-1-phenyl-4-p-tolyl-1,2,3,6-tetrahydropyrimidine-5-carbonitrile **(3a):** Yield (78%), mp >264°C, IR (KBr) v: 3414 (NH); 2222 (CN); 1742 (C=O); 1648 (C=O) cm^{-1}, ^{1}H NMR (DMSO-d6) : δ=2.50 (s,3H,CH_3); 7.31-7.72 (m,9H,H_{arom}); 12.39(s,1H,NH), ^{13}C-NMR (DMSO-d6) δ = 21.0 (**C**H_3); 86.2 (C5); 115.0 (**C**N); 149.8 (C6); 160.6 (C2); 160.9 (C4); 126.8-142.6(C_{arom}), HRMS: (M+) calcd 303.1008 found 303.0998.

2,6-dioxo-1,4-diphenyl-1,2,3,6-tetrahydropyrimidine-5-carbonitrile **(3b):** Yield (65%), mp >264°C, IR (KBr) v: 3204 (NH); 2239 (CN); 1731 (C=O); 1692 (C=O) cm^{-1}, ^{1}H NMR (DMSO-d6) : δ=7.45-7.91 (m,10H,H_{arom}); 8.63 (s,1H,NH), ^{13}C-NMR (DMSO-d_6): δ = 86.4 (C5); 114.7 (**C**N); 149.7 (C6); 161.0 (C2); 162.2 (C4); 128.1-134.6(C_{arom}), HRMS:(M+) calcd 289.0851 found 289.0847.

1-benzyl-2, 6-dioxo-4-p-tolyl-1,2,3,6-tetrahydropyrimidine-5-carbonitrile **(3c):** Yield (62%), mp: >264°C, IR (KBr) v: 3201 (NH); 2228 (CN); 1722 (C=O); 1712 (C=O) cm^{-1}, ^{1}HNMR (DMSO-d6): δ=2.48 (s,3H,CH_3); 5.13(s,2H,CH_2); 7.23-7.77(m,9H,H_{arom}); 10.75(s,1H,NH), ^{13}C-NMR (DMSO-d6): δ = 21.7 (**C**H_3), 44.5 (**C**H_2), 87.5 (C5), 114.0 (**C**N), 151.2 (C6), 158.7 (C2), 160.1 (C4), 126.0-144.5(C_{arom}); HRMS: (M+) calcd 317.1164 found 317.1161**.**

1-benzyl-2, 6-dioxo-4-phenyl-1,2,3,6-tetrahydropyrimidine-5-carbonitrile **(3d):** Yield (66%), mp: >260°C, IR (KBr) v: 3204 (NH); 2239 (CN); 1731 (C=O); 1692 (C=O) cm^{-1}, ^{1}H NMR (DMSO-d6) : δ=5.01 (s,2H,CH_2); 7.24-7.77(m,10H,H_{arom}); 8.32(s,1H,NH), ^{13}C-NMR (DMSO-d6): δ = 43.2 (**C**H_2), 84.0 (C5); 116.3 (**C**N); 151.9 (C6); 161.6 (C2); 163.2 (C4); 126.9-136.9(C_{arom}), HRMS: (M+) calcd 303.1008 found 303.1005.

2, 6-dioxo-1-o-tolyl-4-p-tolyl-1,2,3,6-tetrahydropyrimidine-5-carbonitrile **(3e):**Yield (57%), mp: >260°C, IR (KBr) v: 3223 (NH); 2227(CN); 1721 (C=O); 1656 (C=O) cm^{-1}, ^{1}HNMR(DMSOd6):δ=1.98(s,3H, CH_3); 2.05(s,3H, CH_3); 6.81-7.61(m,8H,H_{arom}); 12.04(s,1H,NH), ^{13}C-NMR(DMSO-d6): δ = 19.4 (**C**H_3); 19.5 (**C**H_3); 85.6 (C5); 114.4 (**C**N); 149.3 (C6); 161.3 (C2); 161.7 (C4); 126.3-142.3(C_{arom}), HRMS: (M+) calcd 317.1164 found 317.1143.

2, 6-dioxo-4-phenyl-o-tolyl-1,2,3,6-tetrahydropyrimidine-5-carbonitrile **(3f):** Yield (50%), mp>260°C, IR (KBr) v: 3223 (NH); 2227 (CN); 1721 (C=O); 1656 (C=O) cm^{-1}, ^{1}HNMR(DMSOd6): δ=2.14(s,3H,CH_3); 7.27-7.81(m,9H,H_{arom}); 12.60(s,1H,NH), ^{13}C-NMR(DMSO-d_6): δ = 16.9 (**C**H_3); 86.4 (C5); 115.0 (**C**N);149.3 (C6); 160.2 (C2); 161.3 (C4); 126.7-135.7(C_{arom}), HRMS: (M+) calcd 303.1008 found 303.1004.

3-acetamido-2-cyano-N,3-diphenylacrylamide (5a): Yield (25%), mp=252°C, IR (KBr) v: 3296 (NH); 2209 (CN); 1701 (C=O); 1636 (C=O) cm^{-1}, ^{1}HNMR(DMSOd_6): δ=2.46 (s,3H, CH_3); 7.06-8.04(m,12H,H_{arom} + 2NH). ^{13}CNMR(DMSO-d_6): 24.4(**C**H_3); 95.6 (C2); 115.4 (**C**N), 160.1(C_1), 164.3(C_3), 167.0 (**C**O); 124.9-136.3(C_{arom}), HRMS: (M+) calcd 305.1164 found 305.1168.

3-acetamido-N-benzyl-2-cyano-3-phenylacrylamide **(5b):** Yield (32%), mp >260°C, IR (KBr) v: 3312 (NH); 2212 (CN); 1696 (C=O); 1661 (C=O) cm^{-1}, ^{1}HNMR(DMSOd_6): δ=2.49 (s,3H, CH_3), 5.37 (s, 2H, CH_2), 7.27-8.04 (m,12H,H_{arom} + 2NH), ^{13}CNMR(DMSO-d_6): 23.5 (**C**H_3), 47.3 (**C**H_2); 85.4 (C2); 115.5 (**C**N); 160.4 (C1); 164.5 (C3); 166.7 (**C**O); 126.7-138.7(C_{arom}), HRMS: (M+) calcd 319.1321 found 319.1319.

3-acetamido-2-cyano-3-phenyl-N-o-tolylacrylamide **(5c):** Yield (22%), mp >260°C, IR (KBr) v: 3174 (NH); 2231 (CN); 1734 (C=O); 1672 (C=O) cm^{-1}, ^{1}HNMR(DMSOd_6):δ=2.22(s,3H, CH_3); 2.39(s,3H, CH_3); 7.07-8.00 (m,11H,H_{arom}+ 2NH), ^{13}CNMR(DMSO-d_6):20.7(**C**H_3); 24.5(**C**H_3); 95.5 (C2); 115.6 (**C**N); 160.3(C1); 163.3 (C3); 167.0 (**C**O); 127. 2-139.2(C_{arom}).

Mycobacterial strain and growth conditions

M. tuberculosis mc^{2}6230 strain was grown at 37°C in Sauton's medium supplemented with 24 µg/ml of pantothenic acid.

Drug susceptibility testing or MIC determination

The susceptibility to the various compounds was assessed visually by growth inhibition on Middlebrook 7H10 plates supplemented with oleic-albumin-dextrose-catalase enrichment (OADC), pantothenic acid and increasing concentrations of each test compound including a control plate without antimicrobial agent. Chemicals stocks were prepared in DMSO for dilution. Tenfold serial dilutions of each actively growing culture were plated and incubated at 37°C for 10 to 14 days. The minimal inhibitory concentration (MIC) was determined as the minimum concentration required to inhibit 99% of the bacterial growth.

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