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RESEARCH ARTICLE

One-Pot Synthesis and Antimicrobial Activity of Novel Pyrazole-Pyridine Hybrid Analogs

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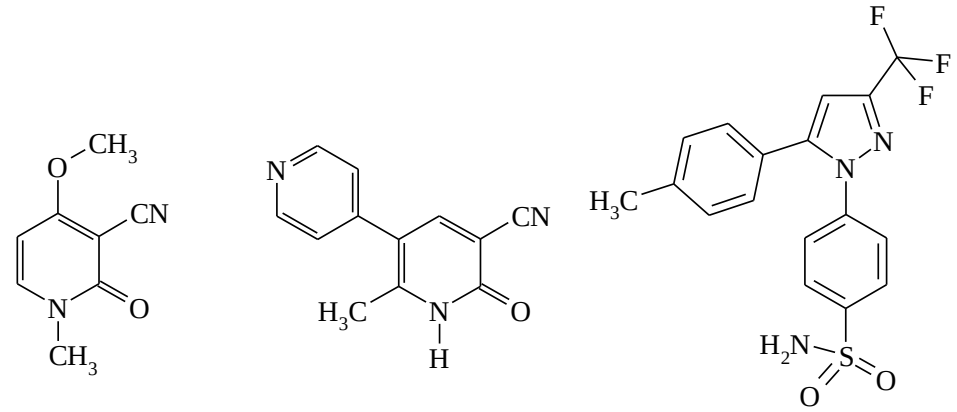
Abstract

A series of 3-cyano-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-2-pyridinones and 2-amino-3-cyano-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-pyridine derivatives has been synthesized. An efficient one-pot-4-component reaction involving pyrazole-4-carboxaldehyde along with acetyl compounds, ethyl cyanoacetate or malononitrile, and ammonium acetate has been adopted. All new compounds have been evaluated for their antimicrobial activity and most of them showed moderate to excellent inhibition against tested microorganisms.

1. Introduction

Multi-component reactions (MCRs) are elegant and economic chemical processes that involve the simultaneous molecular interaction of three or more components to afford a product containing segments of all the starting substrates [1-4]. These reactions have found widespread use in organic diversity-oriented synthesis as they provide access highly functionalized molecules in a simple and straight-forward one-step transformation and can offer expedient synthesis of libraries of drug like heterocyclic compounds [5, 6]. Many naturally occurring and synthetic pyridinone compounds display a broad spectrum of pharmacological properties. The central nervous system stimulant Ricinine (isolated from Ricinuscommunis) and the cardiotonic agents Milrinone and Loprinone (Figure 1), practically in use for treatment of patients with chronic heart failure, comprise the 3-cyano-pyridinone core ring as a pharmacophor [7- 9]. In recent years, much attention has been focused on the development of new 3-cyanopyridine and 2-amino-3-cyanopyridine analogs as they are endowed with interesting pharmaceutical activities such as antimicrobial [10-12]; analgesic anti-inflammatory [13]; and antitumor agents [14].



Ricinine Milrinone Celecoxib

Figure 1: Structures of Ricinine, Milrinone and Celecoxib

On the other hand, pyrazole derivatives are attractive targets in organic synthesis due to their significant biological activities. The well-known, pyrazole-containing, anti-inflammatory drug Celecoxib (Figure 1) has effectively been combined with other chemotherapeutic agents in clinical trials for both prevention and treatment of a diverse range of human cancers [15]. Recently, intensive studies devoted to compounds including pyrazole nucleus have disclosed their broad spectrum of pharmacological applications as antimicrobial [16-18], antiviral [19], antitubercular [20], anti-inflammatory [21] and antitumor activities [22].

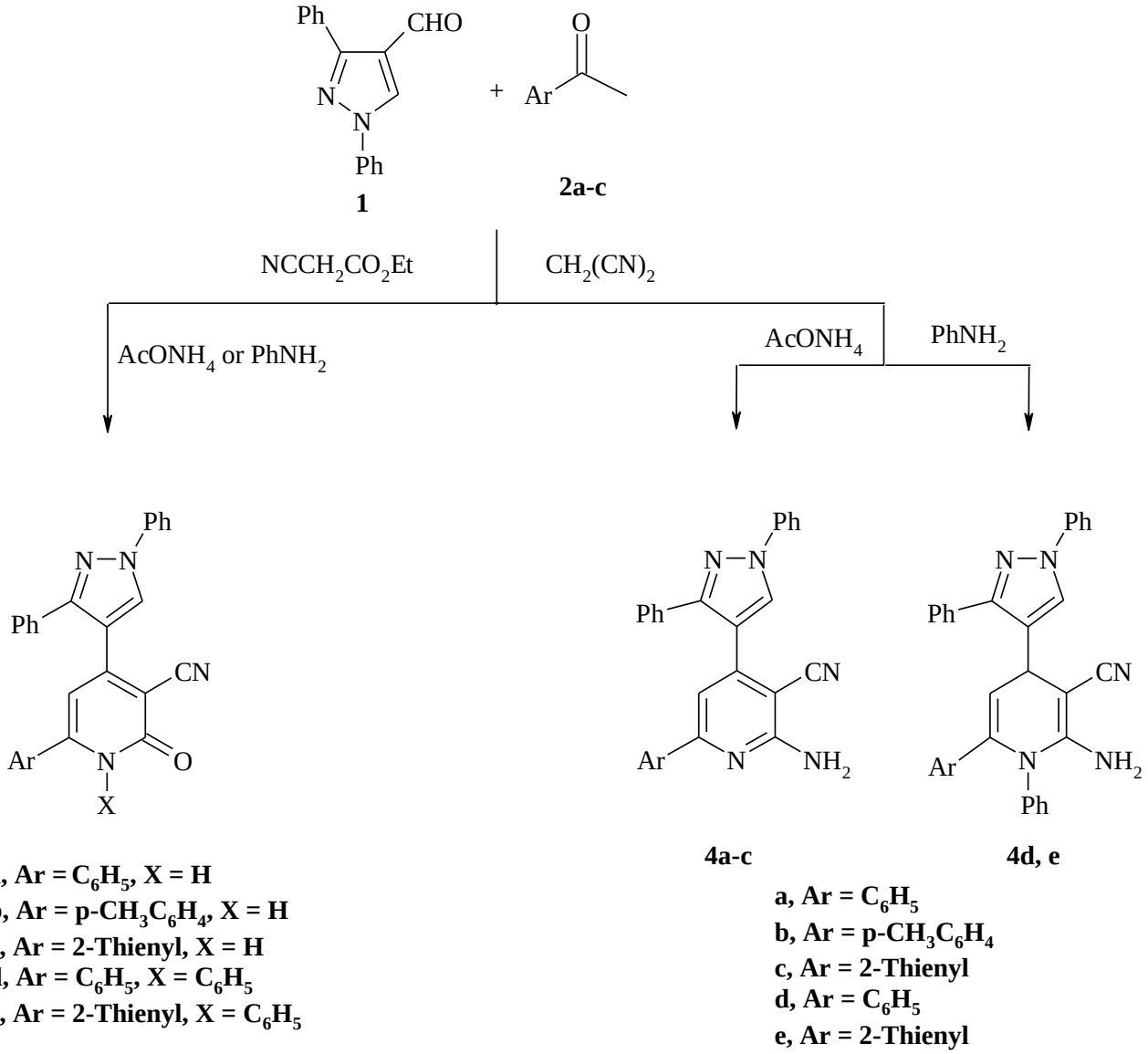
In view of the above mentioned facts, and giving attention to the predictable synergistic antimicrobial effects of both pyridine and pyrazole moieties presence in hybrid molecule, we herein, report an efficient one-pot synthesis of new 4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-6-aryl/heteroaryl-3-cyanopyridine derivatives. The target compounds were rationalized so as to incorporate entities, such as the alkoxy, chloro, morpholino and piperidino functionalities, that are prone to enhancing the biological activity of some relevant chemotherapeutic agents.

2. Results and discussion

2.1. Chemistry

The synthetic strategies adopted for the synthesis of the target compounds were depicted in schemes1-4. The 3-cyano-4-pyrazolylpyridine core structures **3** and **4** were simply constructed using the efficient, high yielding and time saving, one-pot four component coupling reaction (Scheme 1). The reaction of equimolecular amounts of 1,3-diphenyl-1*H*-pyrazole-4-carboxaldehyde **1**; appropriate acetyl derivative **2a-c**, namely acetophenone, p-methylacetophenone or 2-acetylthiophene; and ethyl cyanoacetate; in the presence of ammonium acetate (or aniline) in refluxing ethanol afforded the cyanopyridinones **3a-e**,respectively. Elemental analyses and spectral data were consistent with the proposed pyrazolylpyridine structures. IR spectrum of compound **3b**, exhibited the N-H stretching frequency at 3138 cm^{-1}, besides the characteristic CN and C=O absorption bands at 2215 cm^{-1} and 1682 cm^{-1}, respectively. The identity of compound **3b**was also supported by its ^{13}C NMR spectrum which revealed signal at 162.5 ppm belonging to C=O. Moreover, ^{1}H NMR of compound **3b** showed prominent down-field D_2O exchangeable singlet at δ 10.9 ppm for its NH proton. Furthermore, mass spectra of compounds **3a-e** showed stable molecular ion peaks.

Synthesis of the corresponding 2-amino-3-cyanopyridine analogues **4a-e** was readily achieved following the aforementioned procedure replacing ethyl cyanoacetate by malononitrile (Scheme 1). The IR of the pyridines **4a-e** showed characteristic absorption bands at 3340-3120 cm^{-1} and 2225-2194 cm^{-1} corresponding to the NH_2 and CN groups respectively. Also, their NMR and mass spectra were compatible to the assigned structures.



3a, Ar = C_6H_5, X = H
b, Ar = p-$CH_3C_6H_4$, X = H
c, Ar = 2-Thienyl, X = H
d, Ar = C_6H_5, X = C_6H_5
e, Ar = 2-Thienyl, X = C_6H_5

4a-c

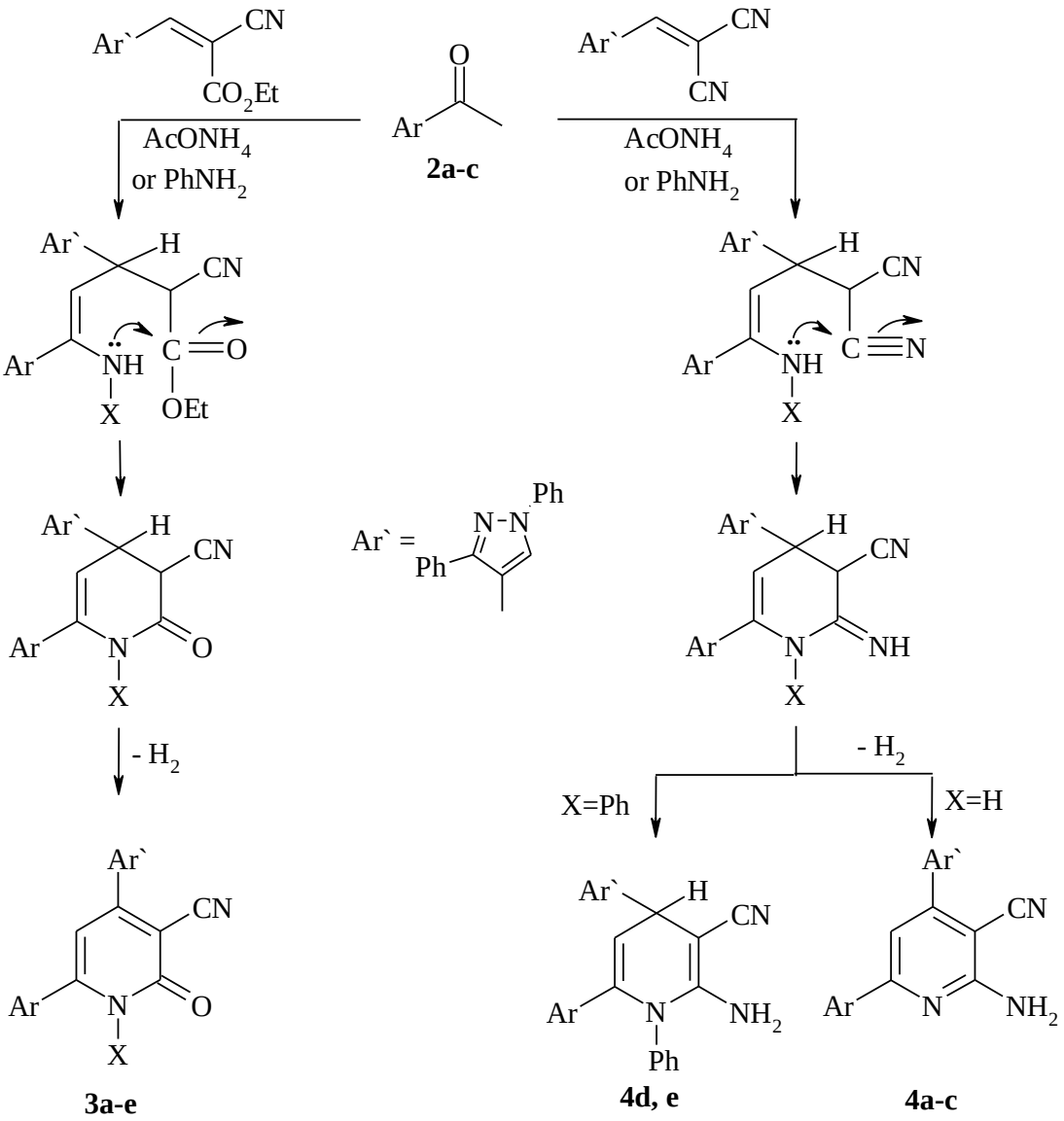
a, Ar = C_6H_5
b, Ar = p-$CH_3C_6H_4$
c, Ar = 2-Thienyl

4d, e

d, Ar = C_6H_5
e, Ar = 2-Thienyl

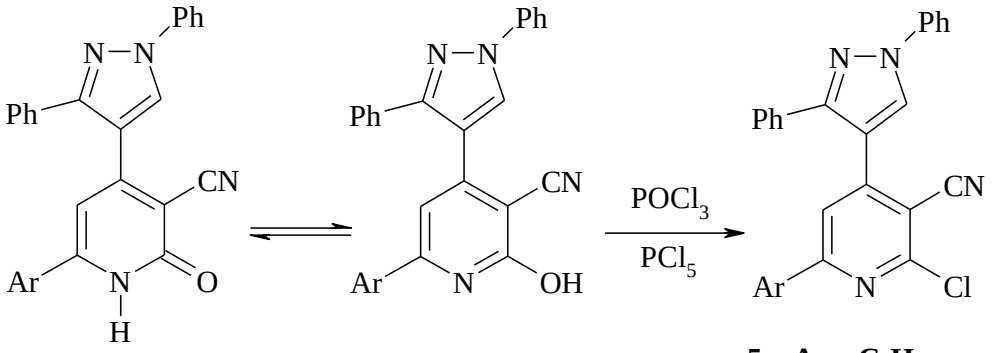
Scheme 1

The formation of the cyanopyridines **3a-e** and **4a-e** could be rationalized as depicted in Scheme 2, where the aldehyde condenses with the more reactive methylene group in ethyl cyanoacetate, or malononitrile, rather than with the less reactive methyl group in the acetyl compounds **2a-c**. The Michael addition of this latter compound on the produced unsaturated nitrile takes place, followed by enamine formation in the presence of ammonium acetate (or aniline), which undergoes cyclisation and finally dehydrogenation giving compounds **3a-e**.



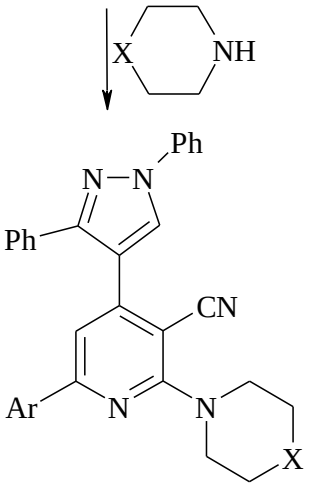
Scheme 2

Further functionalization of the desired cyanopyridine derivatives has been achieved by conversion of **3a-c** into the corresponding chloro substituted cyanopyridines **5a-c**,uponreaction with $PCl_5/POCl_3$ mixture. The absence of characteristic C=O and NH bands in the IR spectra of **5a-c**, and the appearance of a new absorption band for C-Cl confirmed their formation. These latter compounds have been utilized as starting materials for the synthesis of 2-morpholino- and 2-piperidino-cyanopyridine analogs **6a-d,** by reaction with the respective cyclic amine in refluxing ethanol (Scheme 3). Compounds **6a-d** were characterized on the bases of their analytical and spectral data.



3a, Ar = C_6H_5
b, Ar = p-$CH_3C_6H_4$
c, Ar = 2-Thienyl

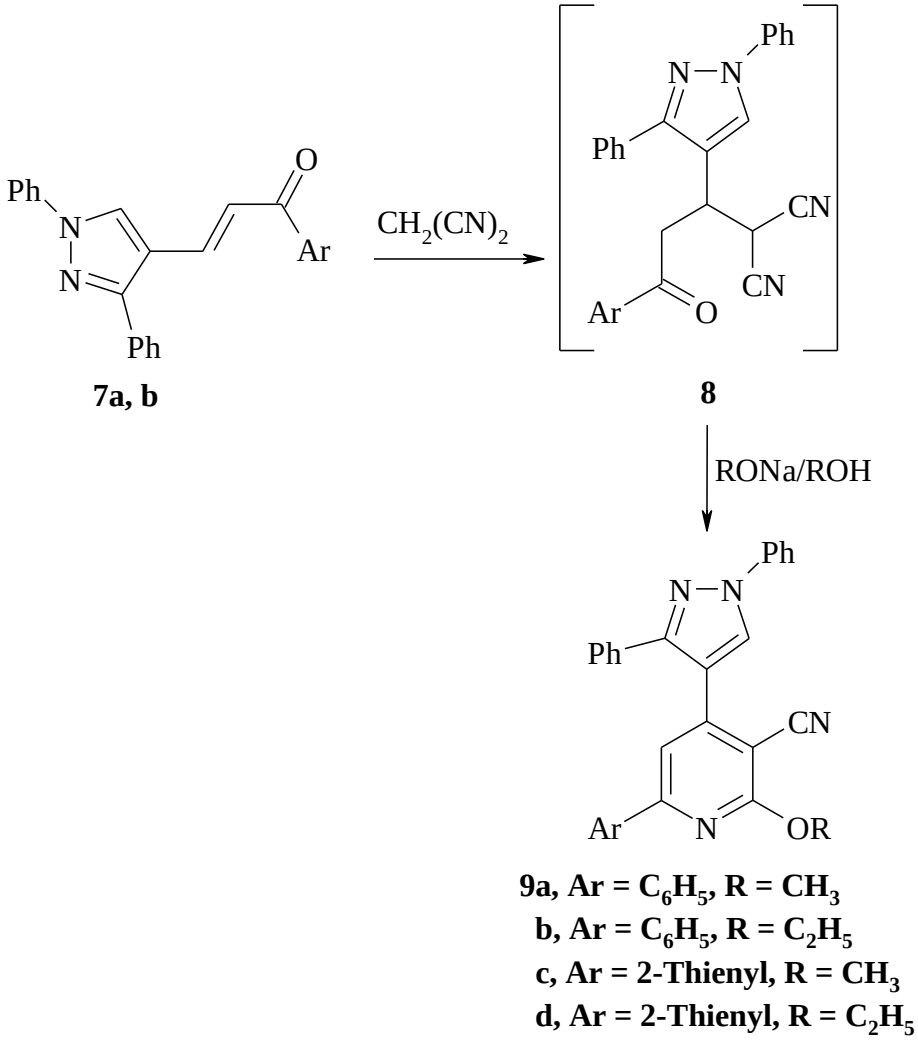
5a, Ar = C_6H_5
b, Ar = p-$CH_3C_6H_4$
c, Ar = 2-Thienyl



6a, Ar = C_6H_5, X = O
b, Ar = C_6H_5, X = CH_2
c, Ar = p-$CH_3C_6H_4$, X =CH_2
d, Ar = 2-Thienyl, X = CH_2

Scheme 3

Moreover, Michael addition of malononitrile to, the easily obtainable, α,β-unsaturated ketones **7a,b** in the presence of either sodium methoxide/methanol or sodium ethoxide/ethanol afforded 2-alkoxy cyanopyridines **9a-d** in a reasonable yield. According to Scheme 4, the intermediate adduct **8** undergoes nucleophilic attack by alkoxide anion followed by cyclization to afford 2-alkoxy cyanopyridines **9a-d**. Elemental analyses and spectral data were consistent with the assigned structures of compounds **9a-d**. ^{1}H NMR spectrum of **9b** displayed signals at 1.42 and 4.61 ppm characteristic of the ethyl group. Additionally, its mass spectrum showed molecular ion at m/z 442.05 (60%) corresponding to $C_{29}H_{22}N_4O$.



7a, b **8**

9a, Ar = C_6H_5, R = CH_3
b, Ar = C_6H_5, R = C_2H_5
c, Ar = 2-Thienyl, R = CH_3
d, Ar = 2-Thienyl, R = C_2H_5

Scheme 4

2.2. Antimicrobial activity

The results of antimicrobial testing of synthesized compounds against selected gram positive, gram negative bacteria and fungus are illustrated in Table 1.

The antimicrobial activity of the test compounds was investigated with regard to the nature of aryl group at C-6 and diversified substituents at C-2 on the cyanopyridine scaffold. Results have shown that most of the newly synthesized compounds possess moderate to high antimicrobial activity against tested microorganisms. Preliminary Structure Activity Relationship (SAR) study for the new derivatives revealed the pronounced inhibitory potential of compounds **9a-d** as compared with other series. The synergetic antimicrobial effect of thiophene moiety introduced at C-6 is clearly demonstrated from the greater potency of the 6-(2-thienyl) analog within each series. Particularly, compound **6d**, comprising thiophene moiety at C-6 and piperidine moieties and C-2 on the pyridine nucleus exhibited excellent activity against all tested microbial strains at concentrations of 2-6 µg/mL.

3. Experimental protocols

3.1. Chemistry

Melting points of the synthesized compounds were determined in open-capillaries on a Stuart electric melting point apparatus and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on a silica gel coated aluminum sheet (silica gel F254). Elemental analyses were performed by the Microanalysis center, Cairo University.

Infrared spectra were recorded on Satellite 2000 spectrometer using KBr discs. Mass spectra were determined on GC-MS (QP/000 EX) Shimadzu spectrometer at an ionizing voltage of 70 eV. ^{1}H NMR and ^{13}C NMR were recorded on Bruker 400 MHz and 100 MHz spectrometer, respectively. 1,3-Diphenyl-1*H*-pyrazole-4-carboxaldehyde **1** has been synthesized according to literature procedure [23]. Compound **1**underwent aldol condensation reaction with methyl ketones to give chalcones**7a**, **b** [24].

3.1.1. General procedure for the synthesis of pyridines (3a-e) and (4a-e)

A mixture of an appropriate acetyl compound (0.01 mol), ethyl cyanoacetate and/or malononitrile (0.01 mol), 1,3-diphenylpyrazole-4-carboxaldehyde (0.01 mol) and ammonium acetate (or aniline) (0.05 mol) in ethanol (30 mL) was refluxed for 5 h. The crystalline product that formed upon cooling was filtered, washed successively with ethanol (3 x 10 mL), dried and recrystallized from dioxane.

4-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-2-oxo-6-phenyl-1,2-dihydropyridine-3-carbonitrile (3a)

Yellow solid (88%); m.p. 150 ^{o}C. IR (KBr, ν, cm^{-1}): 3150 (NH), 2215 (CN), 1685 (CO). ^{1}H NMR ($CDCl_3$, δ ppm): 7.40-7.43 (3H, m, Ar-H), 7.48-7.57 (7H, m, Ar-H), 7.63-7.66 (5H, m, Ar-H), 7.85 (1H, d, Ar-H), 8.33 (1H, s, pyrazole H-5), 9.16 (1H, s, NH).Anal.Calcd. (%) for $C_{27}H_{18}N_4O$: C, 78.24; H, 4.38; N, 13.52. Found C, 78.22; H, 4.25; N, 13.41.MS *m/z* (%): 414 (M^+, 0.04%), 272 (100%).

4-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-2-oxo-6-(p-tolyl)-1,2-dihydropyridine-3-carbonitrile (3b)

Yellow solid (95%): m.p. 161 ^{o}C. IR (KBr, ν, cm^{-1}): 3138 (NH), 2215 (CN), 1682 (CO).
^{1}H NMR (DMSO-d_6, δ ppm): 2.44. (3H, s, CH_3), 7.11-7.57 (9H, m, Ar-H), 7.86-7.90 (6H, m, Ar-H), 8.63 (1H, s, pyrazole H-5), 10.98 (1H, s, NH).^{13}C NMR (DMSO-d_6, δ ppm):14.53, 100.53, 114.83, 116.54. 120.54, 128.7, 129.48, 129.59, 130, 130.27, 130.34, 131.07, 138.98, 146.19, 155.78, 162.51).Anal.Calcd. (%) for $C_{28}H_{20}N_4O$: C, 78.49; H, 4.70; N, 13.08. Found C, 78.44; H, 4.65; N, 13.17.MS *m/z* (%): 428 (M^+, 1%), 77 (100%).

4-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-2-oxo-6-(2-thienyl)-1,2-dihydropyridine-3-carbonitrile (3c)

Yellow solid (85%): m.p. 176 ^{o}C. IR (KBr, ν, cm^{-1}): 3154 (NH), 2215 (CN), 1682 (CO).
^{1}H NMR (DMSO-d_6, δ ppm): 7.22-7.24 (1H, dd, arH), 7.41-7.47 (5H, m, Ar-H), 7.50-7.57 (3H, m, Ar-H), 7.59-8.62 (2H, m, Ar-H), 7.69 (1H, s, pyridine H-5), 7.86-7.90 (2H, m, Ar-H) 8.55 (1H, s, pyrazole H-5), 9.99 (1H, s, NH).Anal.Calcd. (%) for $C_{25}H_{16}N_4OS$: C, 71.41; H, 3.84; N, 13.32; S, 7.63. Found C, 71.32; H, 3.93; N, 13.30; S, 7.66. MS *m/z* (%): 420 (M^+, 1%), 52 (100%).

4-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-2-oxo-1,6-diphenyl-1,2-dihydropyridine-3-carbonitrile (3d)

Yellow solid (81%); m.p. 166 ^{o}C. IR (KBr, ν, cm^{-1}): 2215 (CN), 1679(CO). ^{1}H NMR (DMSO-d_6, δ ppm): 7.52-7.99 (9H, m, Ar-H), 8.01-8.24 (6H, m, Ar-H), 8.28-8.30 (4H, d, Ar-H), 8.39-8.42 (2H, m, Ar-H), 8.47 (1H, s, pyrazole H-5). Anal.Calcd. (%) for $C_{33}H_{22}N_4O$: C, 80.80; H, 4.52; N, 11.42. Found C, 81.00; H, 4.55; N, 11.34. MS *m/z* (%): 490 (M^+, 0.52%), 351 (41%), 270 (100%), 103 (11%), 71 (93%).

4-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-2-oxo-1-phenyl-6-(2-thienyl)-1,2-dihydropyridine-3-carbonitrile (3e)

Yellow solid (74%); m.p. 172 ^{o}C. IR (KBr, ν, cm^{-1}): 2218 (CN), 1673(CO).^{1}H NMR (DMSO-d_6, δ ppm): 7.01 (1H, dd, Ar-H), 7.09-7.12 (4H, m, Ar-H), 7.25-7.29 (5H, m, Ar-H), 7.33-7.37 (3H, m, Ar-H), 7.40-7.43 (2H, m, Ar-H), 7.55 (1H, s, pyridine H-5), 7.81-7.85 (3H, t, Ar-H), 8.56 (1H, s, pyrazole H-5). Anal.Calcd. (%) for $C_{31}H_{20}N_4OS$: C, 74.98; H, 4.06; N, 11.28; S, 6.46. Found C, 75.02; H, 4.11; N, 11.30; S, 6.44. MS *m/z* (%): 496 (M^+, 3%), 344 (100%).

2-Amino-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-6-phenyl pyridine-3-carbonitrile (4a)

Yellow solid (87%); m.p. 224 ^{o}C. IR (KBr, ν, cm^{-1}): 3338, 3266 (NH_2), 2215 (CN). ^{1}H NMR ($CDCl_3$, δ ppm): 6.99 (1H, d, Ar-H), 7.35-7.38 (5H, m, Ar-H), 7.40 (1H, s, pyridine H-5), 7.44-7.54 (7H, m, Ar-H), 7.81 (2H, d, Ar-H), 8.42 (1H, s, pyrazole H-5). ^{13}C NMR ($CDCl_3$, δ ppm): 80.49, 113.31, 113.79, 118.43, 119.28, 119.66, 123.77, 127.01, 127.92, 128.67, 128.87, 128.95, 129.07, 129.11, 129.56, 129.84, 131.24, 131.69, 133.19, 139.24, 140.47, 154.24. Anal.Calcd. (%) for $C_{27}H_{19}N_5$: C, 78.43; H, 4.63; N, 16.94. Found C, 78.32; H, 4.53; N, 16.87. MS *m/z* (%): 413 (M^+, 25%), 414 (M^+ + 1, 6%), 415 (M^+ + 2, 13%), 219 (4 %), 71 (100%).

2-Amino-4-(1,3-diphenyl-1*H*-pyrazolyl)-6-(p-tolyl) pyridine-3-carbonitrile (4b)

Yield: (89%); m.p.232^{o}C IR (KBr, ν, cm^{-1}): 3340, 3281 (NH_2), 2194 (CN). ^{1}H NMR ($CDCl_3$, δ ppm): 2.42 (3H, s, CH_3), 6.61 (1H, s, Ar-H), 7.29 (2H, d, Ar-H), 7.35 (3H, br., Ar-H), 7.42 (1H, s, pyridine H-5), 7.51-7.55 (4H, m, Ar-

H), 7.60 (2H, br., Ar-H), 7.80 (2H, br., Ar-H), 8.38 (1H, s, pyrazole H-5). Anal.Calcd. (%) for $C_{28}H_{21}N_5$: C, 78.67; H, 4.95; N, 16.38. Found C, 78.72; H, 4.83; N, 16.41. MS *m/z* (%): 427 (M^+, 4%), 401 (M^+ - CN, 3%), 77 (100%).

2-Amino-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-6-(2-thienyl) pyridine-3-carbonitrile (4c)
Yellow solid (83%): m.p. 194 ^oC. IR (KBr, ν, cm^{-1}): 3322, 3227 (NH_2), 2204 (CN). ^1H NMR (DMSO-d_6, δ ppm) 7.12 (1H, dd, Ar-H), 7.22-7.39 (6H, m, Ar-H), 7.41-7.43 (4H, m, Ar-H), 7.66 (1H, s, pyridine H-5), 7.99-8.08 (2H, d, Ar-H), 8.41 (1H, s, pyrazole H-5).Anal.Calcd. (%) for $C_{25}H_{17}N_5S$: C, 71.58; H, 4.08; N, 16.69; S, 7.64. Found C, 71.56; H, 4.04; N, 16.77; S, 7.60. MS *m/z* (%): 419 (M^+, 6%), 77 (100%).

2-Amino-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1,6-diphenyl-1,4-dihydropyridine-3-carbonitrile (4d)
Yellow solid (85%): m.p. 205 ^oC. IR (KBr, ν, cm^{-1}): 3222, 3120 (NH_2), 2220 (CN). ^1H NMR ($CDCl_3$, δ ppm): 4.85 (1H, d, pyridine H-4), 5.01 (1H, d, pyridine H-5), 6.47 (2H, m, arH), 7.30 (3H, m, Ar-H), 7.40-7.58 (12H, m, Ar-H), 7.62-7.69 (3H, m, Ar-H), 7.86 (1H, s, pyrazole H-5). Anal.Calcd. (%) for $C_{33}H_{25}N_5$: C, 80.63; H, 5.13; N, 14.25. Found C, 80.52; H, 5.22; N, 14.31. MS *m/z* (%):491 (M^+, 0.49%), 462 (0.7%), 284 (46%), 105 (13%), 62 (100%).

2-Amino-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1-phenyl-6-(2-thienyl)-1,4-dihydropyridine-3-carbonitrile (4e)
Yellow solid (70%): m.p. 211 ^oC. IR (KBr, ν, cm^{-1}): 3227, 3125 (NH_2), 2221 (CN). ^1H NMR (DMSO-d_6, δ ppm): 4.53 (1H, d, pyridine H-4), 4.88 (1H, d, pyridine H-5), 6.51 (2H, m, Ar-H), 7.27 (4H, m, arH), 7.30-7.47 (10H, m, Ar-H), 7.59-7.66 (2H, m, Ar-H), 7.91 (1H, s, pyrazole H-5). Anal.Calcd. (%) for $C_{31}H_{23}N_5S$: C, 74.82; H, 4.66; N, 14.07; S, 6.44. Found C, 74.84; H, 4.74; N, 14.21; S, 6.48. MS *m/z* (%): 497 (M^+, 7%), 77 (100%).

3.1.2. General procedure for the synthesis of 2-chloropyridines (5a-c)
A mixture of compounds **3a-c** (0.01 mol), PCl_5 (0.01 mol) and $POCl_3$ (5 mL) was heated on a water bath for 3 h. The reaction mixture was poured gradually onto ice-cold water (50 mL) and neutralized with diluted ammonia solution. The separated solid was filtered off and recrystallized from dioxane.

2-Chloro-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-6-phenyl pyridine-3-carbonitrile (5a)
Yellow solid (70%): m.p. 198 ^oC. IR (KBr, ν, cm^{-1}): 2221 (CN). ^1H NMR ($CDCl_3$, δ ppm): 7.22-7.35 (7H, m, Ar-H), 7.41-7.54 (4H, m, Ar-H), 7.66 (2H, d, Ar-H), 7.79 (2H, d, Ar-H), 8.22 (1H, s, pyridine H-5), 8.40 (1H, s, pyrazole H-5). Anal.Calcd. (%) for $C_{27}H_{17}ClN_4$: C, 74.91; H, 3.96; Cl, 8.19; N, 12.94. Found C, 75.02; H, 4.11; Cl, 8.24; N, 13.12. MS *m/z* (%): 432 (M^+, 22%), 433 (M^+ +1, 7%), 434 (M^+ +2, 7%), 77 (100%).

2-Chloro-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-6-(p-tolyl) pyridine-3-carbonitrile (5b)
Yellow solid (71%): m.p. 205 ^oC. IR (KBr, ν, cm^{-1}): 2201 (CN). ^1H NMR ($CDCl_3$, δ ppm): 2.44 (3H, s, CH_3), 7.01 (2H, d, Ar-H), 7.24-7.32 (8H, m, Ar-H), 7.47 (2H, d, Ar-H), 7.71 (2H, d, Ar-H), 8.09 (1H, s, pyridine H-5), 8.42 (1H, s, pyrazole H-5).Anal.Calcd. (%) for $C_{28}H_{19}ClN_4$: C, 75.25; H, 4.28; Cl, 7.93; N, 12.54. Found C, 75.23; H, 4.30; Cl, 7.83; N, 12.40. MS *m/z* (%): 446 (M^+, 32%), 447 (M^+ + 1, 11%), 448 (M^+ + 2, 10%), 91 (100%).

2-Chloro-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-6-(2-thienyl) pyridine-3-carbonitrile (5c)
Yellow solid (67%): m.p. 215 ^oC. IR (KBr, ν, cm^{-1}): 2199 (CN). ^1H NMR (DMSO-d_6, δ ppm): 7.21 (1H, dd, Ar-H), 7.28-7.42 (9H, m, Ar-H), 7.71-7.75 (3H, m, Ar-H), 8.77 (1H, s, pyrazole H-5), 9.01 (1H, s, pyridine H-5), Anal. Calcd. (%) for $C_{25}H_{15}ClN_4S$: C, 68.41; H, 3.44; Cl, 8.08; N, 12.76; S, 7.31. Found C, 68.54; H, 3.23; Cl, 8.11; N, 12.77; S, 7.45. MS *m/z* (%): 438 (M^+, 8%), 439 (M^+ +1, 3%), 440 (M^+ +2, 3%), 219 (100%).

3.1.3. General procedure for the synthesis of (6a-d)
A solution of **5a-c** (0.01 mol), morpholine or piperidine (0.02 mol) in ethanol (25 mL), containing triethylamine (0.03 mol), was refluxed for 48 h. The precipitated Et_3NHCl was filtered off, washed with ethanol (10 mL). The filtrate and washings were concentrated to one third of its volume and left to cool. The solid separated was filtered and recrystallized from dioxane.

4-(1,3-Diph1enyl-1*H*-pyrazol-4-yl)-2-morpholino-6-phenyl pyridine-3-carbonitrile (6a)
Yellow solid (55%): m.p. 220 ^oC. IR (KBr, ν, cm^{-1}): 2217 (CN). ^1H NMR (DMSO-d_6, δ ppm): 3.77(4H, m, Morph.), 3.84 (4H, m, Morph.), 7.27-7.40 (11H, m, ArH), 7.61-7.98(4H, m, ArH), 7.98(1H, s, pyridine H-5), 8.20(1H, s, pyrazole H-5). Anal.Calcd. (%) for $C_{31}H_{25}N_5O$: C, 77.00; H, 5.21; N, 14.48. Found C, 77.12; H, 5.23; N, 14.54. MS *m/z* (%): 483 (M^+, 4%), 77(100%).

4-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-6-phenyl-2-(piperidino) pyridine-3-carbonitrile (6b)
Yellow solid (67%): m.p. 186 ^{o}C. IR (KBr, ν, cm^{-1}): 2220 (CN). ^{1}H NMR (DMSO-d$_6$, δ ppm): 1.65-1.69 (6H, m, Pip.), 3.84-3.89 (4H, m, Pip.), 7.22-7.38 (11H, m, ArH), 7.68-7.70 (4H, m, ArH), 7.92 (1H, s, pyridine H-5), 8.22 (1H, s, pyrazole H-5). Anal.Calcd. (%) for $C_{32}H_{27}N_5$: C, 79.81; H, 5.65; N, 14.54. Found C, 79.88; H, 5.55; N, 14.43. MS *m/z* (%): 481(M$^+$, 8%), 77(100%).

4-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-2-(piperidino)-6-(p-tolyl)pyridine-3-carbonitrile (6c)
Yellow solid (64%): m.p. 190 ^{o}C. IR (KBr, ν, cm^{-1}): 2221 (CN). Anal.Calcd. (%) for $C_{33}H_{29}N_5$: C, 79.97; H, 5.90; N, 14.13. Found C, 80.10; H, 5.93; N, 14.22. MS *m/z* (%): 495 (M$^+$, 3%), 105 (100%).

4-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-2-(piperidino)-6-(2-thienyl)pyridine-3-carbonitrile (6d)
Yellow solid (60%): m.p. 179 ^{o}C. IR (KBr, ν, cm^{-1}): 2191 (CN). Anal.Calcd. (%) for $C_{30}H_{25}N_5S$: C, 73.89; H, 5.17; N, 14.36; S, 6.58. Found C, 73.68; H, 5.13; N, 14.33; S, 6.62. MS *m/z* (%): 487 (M$^+$, 18%), 77 (100%).

3.1.4. General procedure for the synthesis of (9a-d)
The enones **7a,b** (0.01 mol) were added to a stirred solution of sodium alkoxides (prepared from 0.01 mole sodium in 50 mL of either absolute methanol or ethanol). Malononitrile (0.01 mol) was then added with continuous stirring at room temperature until the precipitate was separated out. The solid that separated was collected by filtration and crystallized from suitable solvent.

4-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-2-methoxy-6-phenylpyridine-3-carbonitrile (9a)
Yellow solid (dioxane), yield 75%; m.p. 240 ^{o}C. IR (KBr, ν, cm^{-1}): 2220 (CN). ^{1}H NMR ($CDCl_3$, δ ppm): 4.26 (3H, S, CH_3), 7.32 (1H, s, ArH), 7.43-7.49 (7H, m, ArH), 7.57(2H, t, ArH), 7.64-7.66 (2H, m, ArH), 7.78-7.81(2H, m, ArH), 7.90 (2H, d, ArH), 8.60 (1H, s, pyrazole H-5). ^{13}C NMR ($CDCl_3$, δ ppm): 54.63, 92.57, 114.26, 116.13, 116.90, 119.75, 127.26, 127.46, 128.66, 128.77, 128.87, 129.69, 130.44, 132.45, 137.34, 139.56, 147.90, 152.00, 157.54, 165.22. Anal.Calcd. (%) for $C_{28}H_{20}N_4O$: C, 78.49; H, 4.70; N, 13.08. Found C, 78.52; H, 4.74; N, 13.11. MS *m/z* (%): 428 (M$^+$, 2%), 429 (M$^+$ + 1, 0.5%). 430 (M$^+$ + 2, 0.25%), 402 (0.8%), 351 (0.7%), 77 (100%).

4-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-2-ethoxy-6-phenylpyridine-3-carbonitrile (9b)
Yellow solid (ethanol), yield 80%; m.p. 205 ^{o}C. IR (KBr, ν, cm^{-1}): 2224 (CN). ^{1}H NMR (DMSO-d$_6$, δ ppm):, 1.42 (3H, t, $CH_2C\mathit{H}_3$, J =7.07 Hz), 4.61 (2H, q, $C\mathit{H}_2CH_3$, J=7.07 Hz), 7.38-7.43 (4H, m, ArH), 7.50-7.52 (5H, m, ArH), 7.67(1H, s, pyridine H-5), 7.98 (2H, d, ArH), 8.05-8.07(2H, m, ArH), 9.04 (1H, s, pyrazole H-5). ^{13}C NMR (DMSO-d6, δ ppm): 14.30, 63.08, 93.15, 114.46, 114.86, 116.92, 118.64, 127.09, 127.15, 127.58, 128.43, 128.69, 128.93, 129.74, 130.14, 130.68, 132.07, 136.58, 138.96, 148.65, 150.30, 157.06, 163.79. Anal.Calcd. (%) for $C_{29}H_{22}N_4O$: C, 78.71; H, 5.01; N, 12.66. Found C, 78.64; H, 4.97; N, 12.72. MS *m/z* (%): 442 (M$^+$, 60%), 443.50 (M$^+$ + 1, 24%), 164 (100%).

4-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-2-methoxy-6-(2-thienyl)pyridine-3-carbonitrile (9c)
Yellow solid (DMF), yield 70%; m.p. 210 ^{o}C. IR (KBr, ν, cm^{-1}): 2217 (CN). ^{1}H NMR (DMSO-d$_6$, δ ppm):, 4.07 (3H, s, CH_3), 7.20-7.22 (1H, m, ArH), 7.37-7.43 (4H, m, ArH), 7.49-7.52 (2H, m, ArH), 7.56-7.60 (2H, m, ArH), 7.65(1H, s, pyridine H-5), 7.81-7.85(2H, m, ArH), 7.97(2H, d, ArH), 9.02 (1H, s, pyrazole H-5). ^{13}C NMR (DMSO-d$_6$, δ ppm): 54.48, 92.77, 113.07, 114.82, 116.69, 118.64, 127.10, 127.47, 128.34, 128.44, 128.70, 129.00, 129.75, 130.06, 131.31, 131.98, 138.95, 142.31, 148.57, 150.19, 152.67. Anal.Calcd. (%) for $C_{26}H_{18}N_4OS$: C, 71.87; H, 4.18; N, 12.89; S, 7.38. Found C, 71.76; H, 4.24; N, 12.85; S, 7.41.MS *m/z* (%): 434 (M$^+$, 3%), 215 (6%), 77 (100%).

4-(1,3-Diphenyl-1H-pyrazol-4-yl)-2-ethoxy-6-(2-thienyl) pyridine-3-carbonitrile (9d)
Yellow solid (dioxane), yield 65%; m.p. 200 ^{o}C. IR (KBr, ν, cm^{-1}): 2217 (CN). ^{1}H NMR (DMSO-d$_6$, δ ppm):, 1.41 (3H, t, $CH_2C\mathit{H}_3$, J= 7.05 Hz), 4.55 (2H, q, $C\mathit{H}_2CH_3$, J =7.05 Hz), 7.19-7.21 (1H, m, ArH), 7.37-7.42 (4H, m, ArH), 7.49-7.52(2H, m, ArH), 7.58 (2H, t, ArH), 7.63 (1H, s, pyrazole H-5), 7.80-7.82 (2H, m, ArH), 7.95 (2H, d, ArH), 9.0 (1H, s, pyrazole H-5). ^{13}C NMR (DMSO-d$_6$, δ ppm): 14.22, 63.22, 92.81, 112.86, 114.85, 116.74, 118.62, 127.08, 127.48, 128.23, 128.43, 128.69, 129.00, 129.74, 130.03, 131.26, 131.99, 138.95, 142.42, 148.59, 150.17, 152.65, 163.69. Anal.Calcd. (%) for $C_{27}H_{20}N_4OS$: C, 72.30; H, 4.49; N, 12.49; S, 7.15. Found C, 72.31; H, 4.43; N, 12.57; S, 7.19. MS *m/z* (%): 448 (M$^+$, 60%), 404 (23%), 77 (100%).

3.2. Antimicrobial screening

The newly synthesized compounds were screened for their antimicrobial activity against Bacillus subtilis, and Staphylococcus aureus (Gram positive bacteria), Klebsiella pneumonia and Escherichia coli (Gram negative bacteria) and Aspergillusniger, Candida tropicalis (fungus), by the paper disc agar diffusion method and minimal inhibitory concentration (MIC) determination [25, 26]. Ciprofloxacin and Fluconazole were used as reference for the antibacterial and antifungal activity, respectively. The test microorganisms were sub-cultured using nutrient agar medium (for bacteria) and Sabouraud-dextrose agar medium (for fungus). After incubation at 37^{o}C for 24 h (for bacteria) and 24^{o}C for 48 h (for fungus), the inhibition zone diameters were measured in millimeters. Concentrations of tested compounds were in the range: 128, 64, 32 16, 8, 4, 2 and 1 ug/ mL. All the tests were carried out in triplicate and the average value was taken as final reading. The MIC (µg/mL) was determined by Broth dilution technique. The minimum inhibitory concentration values are given in Table 1.

4. Conclusion

A series of novel pyridines were synthesized and characterized by NMR, mass spectrometry and IR studies. Our aim has been realized by the synthesis of diversely substituted cyanopyridines as novel pyrazole/pyridine hybrid analogs. All the newly synthesized compounds have moderate to excellent inhibition of bacteria and fungi growth. The thiophene nucleus, as well as, the alkoxy, piperidino and morpholino moieties contribute to the antimicrobial potential of the biheteroaryl system. As a general conclusion, compounds with the pyrazole / pyridine motif could of interest for the development of pharmacological agents.

Table 1.Antimicrobial activity results (MIC values in µg/mL) for synthesized compounds with the standard drugs

Sample	Ar	R	X	Microorganisms					
				Bs.	Sa.	Kp.	Ec.	Ct.	An.
3a	Ph	-	H	128	128	64	64	32	32
3b	p-$CH_3C_6H_4$	-	H	64	128	128	128	128	128
3c	2-Thienyl	-	H	8	16	32	8	64	64
3d	Ph	-	Ph	128	64	64	128	128	64
3e	2-Thienyl	-	Ph	4	16	16	16	64	32
4a	Ph	-	-	64	32	128	128	64	128
4b	p-$CH_3C_6H_4$	-	-	64	64	128	128	64	64
4c	2-Thienyl	-	-	8	8	64	64	16	32
4d	Ph	-	-	64	32	128	64	32	32
4e	2-Thienyl	-	-	4	8	16	16	16	32
5a	Ph	-	-	4	16	8	8	8	16
5b	p-$CH_3C_6H_4$	-	-	16	16	16	16	32	8
5c	2-Thienyl	-	-	4	8	4	8	8	8
6a	Ph	-	O	8	8	8	16	32	16
6b	Ph	-	CH_2	8	8	8	64	16	8
6c	p-$CH_3C_6H_4$	-	CH_2	8	16	16	8	32	16
6d	2-Thienyl	-	CH_2	2	4	4	8	2	2
9a	Ph	CH_3	-	4	8	8	16	8	4
9b	Ph	C_2H_5	-	4	4	16	8	8	8
9c	2-Thienyl	CH_3	-	2	4	8	8	16	8
9d	2-Thienyl	C_2H_5	-	4	4	4	8	32	4
Ciprofloxacin	-	-	-	1	4	1	1	-	-
Fluconazole	-	-	-	-	-	-	-	1	1
DMF	-	-	-	na	na	na	na	na	na

Bs.; Bacillus subtilis, Sa.; Staphylococcus aureus, Kp.; Klebsiellapneumoniae, Ec.; Escherichia coli Ct.; Candida tropicalis, An.; Aspergillusniger, na; no activity.

5. References

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