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Synthesis of pyrazole encompassing 2-pyridone derivatives as antibacterial agents

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ABSTRACT

A series of novel compounds 6-amino-1-((1,3-diphenyl-1*H*-pyrazole-4-yl)methyleneamino)-4-(aryl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitriles (**4a**–**t**) were synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. These compounds were screened for their in vitro antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes* (Gram positive), *Escherichia coli*, *Pseudomonas aeruginosa* (Gram negative) by serial broth dilution and cytotoxic activity (NIH 3T3 & HeLa) by MTT assay. The results indicated that compounds **4g**, **4i**, **4m**, **4o**, **4r** and **4t** exhibit potent antibacterial activity against bacterial strains at non-cytotoxic concentrations.

Since resistance of pathogenic bacteria towards available antibiotics is rapidly becoming a major worldwide problem, the design of new compounds to deal with resistant bacteria has become one of the most important areas of antibacterial research today.[1] Despite the development of several new antibacterial agents, their clinical value is limited to treating an increasing array of life-threatening systemic infections because of their relatively high risk of toxicity, emergence of drug resistant strains, pharmacokinetic differences, and/or insufficiencies in their activity.

Pyridone and their derivatives play an essential role in several biological processes and have considerable chemical and pharmacological importance.[2–4] 2-Pyridones represent a unique class of pharmacophores, which are observed in various therapeutic agents[5] and antibiotics.[6] In recent years, 2-pyridones have captivated much importance as these compounds have been found to exhibit several biological activities, such as HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs),[7] antifungals,[8] sedatives[9] and cardiotonic agents.[10–12] Moreover, 2-pyridones are a class of recently discovered potent antibacterial agents that are of particular interest due to their in vitro and in vivo antibacterial potencies against the bacterial type II DNA topoisomerases, which includes two highly homologous enzymes DNA gyrase and topoisomerase IV.[13] Furthermore, pyrazoles occupy a distinct place in heterocyclic chemistry and represent a key motif in medicinal chemistry due to their capability to exhibit an array of bioactivities such as antimicrobial,[14–16] anticancer,[17] anti-inflammatory,[18] antidepressant,[19] anticonvulsant,[20] antipyretic[21] and selective enzyme inhibitory activities.[22] Recently, we have reported pyrazole containing compounds as antibacterial agents (NCD_{1-17}) (Fig. 1).[23] Recognizing these facts and in continuation of our previous work[24,25] to look for new antibacterial agents with possible novel mechanisms of action, it was thought worthwhile to synthesize some new congeners of 2-pyridones by incorporating 2-pyridones and pyrazoles in a single molecular framework.

The quest for synthesis of 6-amino-1-((1,3-diphenyl-1*H*-pyrazole-4-yl)methyleneamino)-4-(aryl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitriles (**4a**–**t**) was accomplished in two steps as outlined in Scheme 1. In the first step, 1,3-diphenylpyrazole-4-carboxaldehyde (**1**), prepared according to previously published methods[26] was condensed with cyanoacetic acid hydrazide using 1,4-dioxane as a solvent to yield the intermediate 2-cyano-*N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methylene)acetohydrazide (**2**).[27] In the second step, the intermediate **2** was further reacted with (2-arylidene)malononitriles (**3a**–**t**) in the presence of catalytic amount of piperidine utilizing ethanol (95%) as a solvent to yield the desired products 6-amino-1-((1,3-diphenyl-1*H*-pyrazole-4-yl)methyleneamino)-4-(aryl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitriles (**4a**–**t**).[28] The yields of the products were obtained in the range of 54–65%. Designed series of molecules **4a**–**t** were characterized by IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry techniques before evaluating for in vitro antibacterial and cytotoxicity essays.

IR spectrum of compound **4o** showed stretching band of carbonyl group at 1684 cm^{-1} and absorption bands of NH_2 group at 3439 and 3448 cm^{-1}. Strong intense absorption bands were observed at 2210 and 2214 cm^{-1} due to stretching vibration of –C≡N groups. In ^{1}H NMR spectra of **4o**, characteristic signal of

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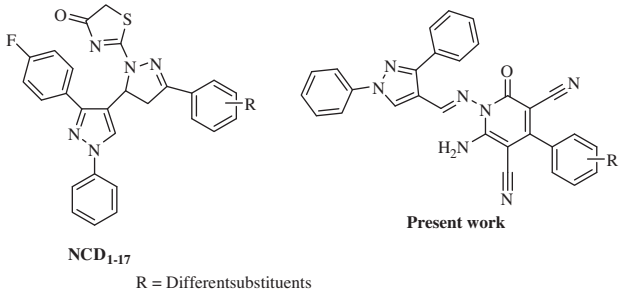


Figure 1. Structural similarity between newly synthesized compounds and previously synthesized compounds.

primary amine showed a broad singlet at δ = 5.93 ppm which is exchangable with D_2O. The aromatic and CH═N protons appeared at δ = 7.29–8.50 ppm. The ^{13}C NMR spectrum of compound **4o** displayed characteristic signal of carbonyl carbon at δ = 171.2 ppm. Carbons of –C≡N group showed a chemical shift at δ = 115.6 ppm. Carbon of nitrophenyl ring showed a chemical shift at δ = 148.2 ppm. The mass spectrum revealed a molecular ion peak at m/z = 526. In mass spectra, molecular ion peak is in agreement with proposed molecular weight and elemental analysis.

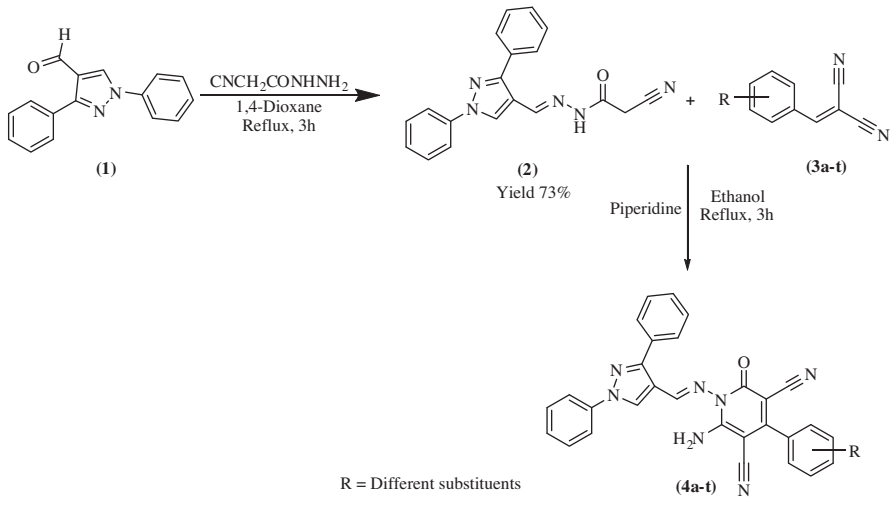
A plausible mechanism for the formation of compounds (**4a**–**t**) is suggested in Scheme 2. Firstly, the Michael addition of Schiff base cyanoacetic hydrazide **a** to Knovenagel product **b** yielded the intermediate **c**, which further underwent intramolecular nucleophilic attack on cyano carbon followed by annulation to give intermediate **e**. The compound **e** was converted to final compound by intramolecular electron transfer to nitrogen atom.

All the newly synthesized compounds (**4a**–**t**) were initially screened for their in vitro antibacterial activity against Gram positive bacteria (*Staphylococcus aureus* (MTCC-96), *Streptococcus pyogenes* (MTCC-442)) and Gram negative bacteria (*Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC-1688)) using conventional broth-dilution method.[29] The MIC (minimum inhibitory concentration) values were determined by using ciprofloxacin as reference drug. The results of antibacterial studies of newly synthesized compounds revealed that these compounds exhibited significant antibacterial activity. From antibacterial activity data (Table 1), it was observed that compounds **4f** (2-Cl), **4g** (2-F), **4i** (4-F), **4m** (2-NO_2), **4o** (4-NO_2), **4p** (2-Br), **4r** (4-Br) and **4t** (4-CF_3) were most active compounds. Compound **4m** (2-NO_2) exhibited inhibition at MIC = 25 μg/mL and compounds **4i** (4-F) and **4o** (4-NO_2) displayed strong inhibition action at MIC = 12.5 μg/mL against *E. coli*. Compounds **4i** (4-F) and **4o** (4-NO_2) showed appreciable activity at MIC = 25 μg/mL against *P. aeruginosa*. Compounds **4g** (2-F), **4m** (2-NO_2) and **4t** (4-CF_3) exhibited significant potential at MIC = 25 μg/mL while compounds **4i** (4-F), **4p** (2-Br) and **4r** (4-Br) exerted highest inhibition at MIC = 12.5 μg/mL against *S. aureus*. Compound **4g** (2-F) indicated good inhibition at MIC = 25 μg/mL and compounds **4f** (4-Cl) and **4o** (4-NO_2) showed remarkable activity at MIC = 12.5 μg/mL against *S. pyogenes*.

As shown in our results, some analogues of this series were found to have more potency than the standard drug ciprofloxacin while some of them have comparable potency. The antimicrobial activity was considerably affected by substitution pattern on the phenyl ring and the most active compounds contained an electron withdrawing substituent at *ortho* and *para* positions of the phenyl ring. The role of electron withdrawing group in improving antimicrobial activity is very well supported by previous studies.[30,31] Compounds **4g**, **4i**, **4m, 4o, 4r** and **4t** bearing F, Cl, NO_2, Br, and CF_3 groups were most potent. Thus, compounds bearing substituents at 2- or 4-position of the terminal benzene ring of 2-pyridone part were found to have higher potency than compounds bearing groups at 3-position or at both. Moreover all active compounds were effective against Gram negative bacterial strain *S. aureus*. Rest of the compounds bearing these substituents at 3-position showed moderate activity with respect to standard drug against the test strains.

All the newly synthesized compounds **4a**–**t** were tested for cytotoxic activity on Mouse embryonic fibroblasts cell line (NIH 3T3) and human cervical cancer cell line (HeLa) using MTT colorimetric assay.[32] The IC_{50} values obtained for these compounds are shown in Table 2. Pyrazole analogues **4g**, **4i**, **4m**, **4r** and **4t** showed no toxicity at concentration of 200 μg/mL (IC_{50} >200 μg/mL), while other compounds showed moderate toxicity against NIH 3T3 cells. Moreover, none of the derivatives showed cytotoxicity against HeLa cells (IC_{50} >200 μg/mL).

In conclusion, our attempts at exploring pyrazole based 2-pyridone derivatives have unexpectedly led to the identification of a novel chemotype with substantial antibacterial activity against various bacterial strains. Among the newly synthesized compounds **4a**–**t**, analogues **4g** (2-F), **4i** (4-F), **4m** (2-NO_2), **4o** (4-NO_2), **4r** (4-Br) and **4t** (4-CF_3) showed the highest inhibition against nearly all of the tested bacteria, superior to the reference drugs, and displayed antibacterial activity at non-cytotoxic concentrations. Our preliminary results suggest that the mechanism of action may be distinct from that of known antibacterial agents.



Scheme 1. Synthesis of title compounds **4a**–**t**.