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Synthesis and antibacterial survey of some nicotinonitriles clubbed with pyrazolone and/or thiazole

Ehab Abdel-Latif*, Moged A. Berghot, Ebrahim Abdel-Galil, Alshimaa I. Zaki

Department of Chemistry, Faculty of Science, Mansoura University, Mansoura 35516, Egypt

**** Correspondence to:*** *Ehab Abdel-Latif: ehabattia00@gmx.net*

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Abstract: The chlorine atom of 2-chloronicotinonitrile derivative **2** has been substituted via its heating with ethyl 4-aminobenzoate to give the conforming ethyl 4-(3-cyano-pyridin-2-ylamino)benzoate scaffold **3**, which underwent refluxing with hydrazine hydrate to produce the hydrazide derivative **4**. The reaction of hydrazide **4** with ethyl acetoacetate furnished the corresponding 2-((4-(3-methyl-5-oxo-1*H*-pyrazole-1-carbonyl)phenyl)-amino)nicotinonitrile derivative **6** which reacted with phenyl isothiocyanate and chloroacetone, phenacyl chloride or ethyl bromoacetate to give the corresponding 2-((4-(4-(3-phenylthiazolylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-phenyl)amino)-4,6-dimethyl-nicotinonitriles **10a**, **10b** and **11**. In general, all synthesized pyridine scaffolds revealed better activity against the Gram-positive bacterium (*B. subtilis*) rather than the Gram-negative bacterium (*E. coli*).

keywords: 2-Chloronicotinonitrile, Ethyl 4-aminobenzoate, Hydrazine, Phenyl isothiocyanate, Escherichia coli

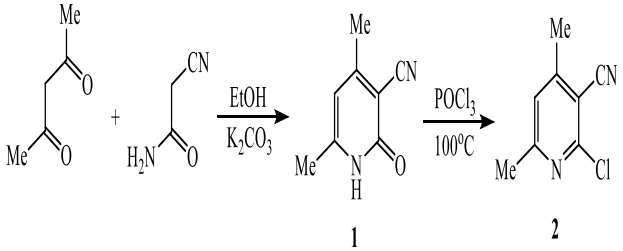
1.Introduction

Pyridine derivatives play diverse roles in organic chemistry. The moiety of pyridine is a key constituent in a range of bioactive compounds either synthetically or naturally occurring. Pyridine derivatives constitute important class of heterocyclic compounds due to their miscellaneous biological activities that have created significant interest in the pharmaceutical industry [1]. In addition, pyridine derivatives have been widely studied for over a century because of their utilization in many branches of chemistry, such as catalysis and drug design [2]. Many pyridine-containing compounds display fabulous medicinal properties, including hypnotic and sedative [3], HIV antiviral [4], bone calcium regulator [5], cholesterol and triglyceride regulator [6], antidiabetic [7], antihistaminic [8], antiulcerant [9,10], antineoplastic, and anticancer activities [11].

Pyridine is generally constructed by two approaches that based on the reaction of carbonyl compounds with amines, and the cycloaddition of azadienes and nitriles with alkenes and alkynes, respectively [12]. Therefore, it is a real challenge to combine pyridine rings and different heterocycles together in a molecular framework to see the additive effect of these rings toward the antimicrobial activities. The present work is aiming to the use of 2-hydroxynicotinonitrile in the synthesis of some new pyridine scaffolds and screening their antibacterial activity against a range of Gram-positive and Gram-negative bacteria.

Results and discussion

The present research study starts by the preparation of 4,6-dimethyl-3-cyanopyridin-5-one **(1)** from acetylacetone and cyanoacetamide in ethyl alcohol and potassium carbonate [13] (Scheme 1). Heating of this pyridone **1** with phosphorus oxychloride furnished 2-chloro-4,6-dimethylnicotinonitrile **(2)** [13].

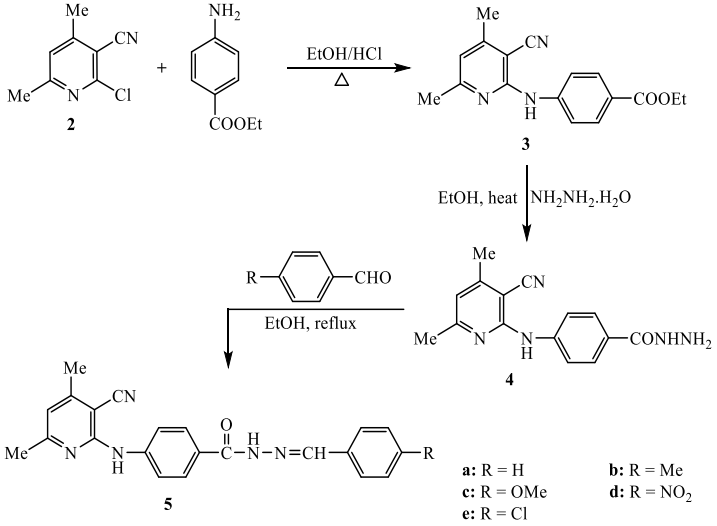


Scheme (1)

The chlorine atom of 2-chloronicotinonitrile derivative **2** proved to be reactive towards nucleophilic substitution by nitrogen nucleophile such as ethyl 4-aminobenzoate to afford ethyl 4-(3-cyano-pyridin-2-ylamino)benzoate scaffold **3** (Scheme 2). The compatible elemental and spectral analyses supported the proposed structure of benzoate compound **3**. Its infrared spectrum displayed absorptions at 3309, 2223 and 1712 cm^{-1} to indicate the presence of N-H, nitrile (C≡N) and carbonyl of ester (COOEt) groups. The ^1H NMR signals were identified as triplet at 1.31 ppm and quartet at 4.28 ppm for the protons of ethoxy group (-O-CH_2-CH_3). The singlet signals at 2.39 and 2.41 ppm indicated the protons of methyl groups. The proton of pyridine C-5 resonated as singlet at 6.87 ppm, while the aromatic protons resonated as two doublet signals at 6.74 and 7.87 ppm. The proton of N-H function resonated as singlet at 9.34 ppm.

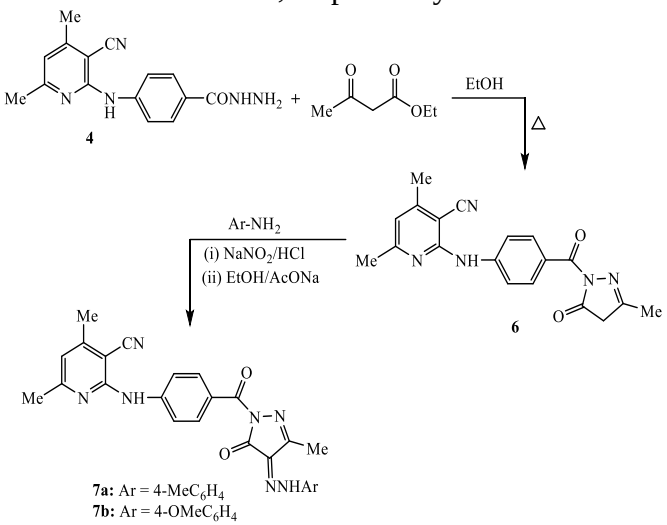
The hydrazide derivative **4** was achieved by refluxing ethyl 4-(3-cyano-pyridin-2-ylamino)benzoate compound **3** with hydrazine hydrate in ethyl alcohol for two hours. The structure of compound **4** was confirmed by spectroscopic techniques including IR and mass analyses. Its IR spectrum exhibited absorption bands at 3340 and 3287 cm^{-1} for the N-H stretching of NH and NH_2 groups. The absorptions at 2217 and 1611 cm^{-1} are referring to the nitrile (C≡N) and carbonyl (N-C=O) groups, respectively. The ^1H NMR spectrum indicated the absence of any signal related to the ethoxy group and displayed the singlet signals of the hydrazide moiety ($CONHNH_2$) at 4.44 ppm (NH_2) and 9.14 ppm (NH).

Condensation of hydrazide **4** with different aromatic benzaldehydes (namely; benzaldehyde, 4-methylbenzaldehyde, 4-methoxybenzaldehyde, 4-nitrobenzaldehyde and 4-chlorobenzaldehyde) proceeded by boiling in ethyl alcohol to afford the conforming Schiff's bases **5**. The structure of **5** was confirmed because of their spectral data and satisfactorily elemental analyses. Their IR spectra showed absorption band at wave number ranged from 1638 to 1666 cm^{-1} corresponding to the carbonyl group. The proton of azomethine group (N-N=CH-) resonated as singlet signal in the ^1H NMR spectra in the range 8.39-8.52 ppm.



Scheme (2)

The reaction of hydrazide **4** with ethyl acetoacetate was carried out in boiling ethyl alcohol to furnish the corresponding 2-((4-(3-methyl-5-oxo-1*H*-pyrazole-1-carbonyl)phenyl)amino)nicotinonitrile derivative **6** (Scheme 3). Diazocoupling of **6** with two types of diazotized para aromatic amine (namely; *p*-toluidine and *p*-anisidine) was performed in ethanol and sodium acetate to give the corresponding arylhydrazone derivatives **7a** and **7b**, respectively.

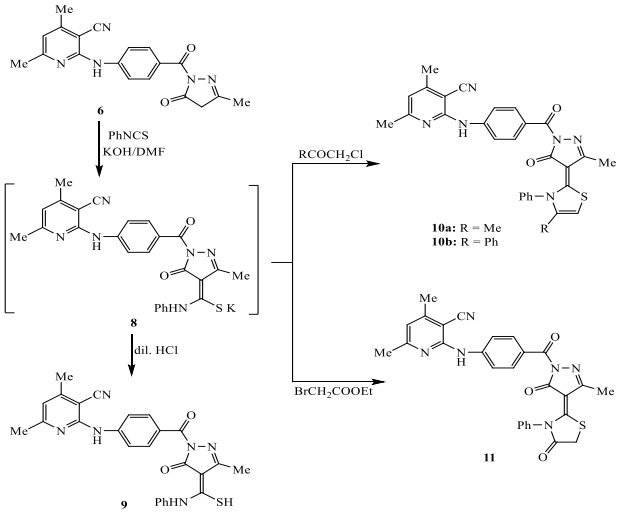


Scheme (3)

The addition of **6** through its active methylene group to phenyl isothiocyanate proceeded by stirring in dimethylformamide and potassium hydroxide to give the non-isolated sulfide salt **8**, which when neutralized with dilute HCl gave 2-((4-(4-(mercapto(phenylamino)methylene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)phenyl)amino)-4,6-

dimethylnicotinonitrile **9** (Scheme 4). The structure of **9** was secured because of its spectral data and satisfactorily elemental analysis. (*c.f.* experimental)

In situ addition of α-chloroketone reagents (namely; chloroacetone and phenacyl chloride) to the non-isolated potassium salt **8** has been achieved by stirring at room temperature, and yielded corresponding thiazoline derivatives **10a** and **10b**, respectively. The structure of **10a** and **10b** were confirmed because of their agreement spectral data and satisfactorily elemental analyses. The infrared spectrum of **10b** exhibited absorptions at υ = 3326, 2212 and 1658 cm^{-1} to indicate the presence of N-H, C≡N and C=O groups, respectively. The proton of thiazoline ring at carbon-5 resonated as singlet signal in the ^{1}H NMR spectrum at 5.66 ppm. While in situ addition of ethyl bromoacetate to the non-isolated potassium salt **8** furnished the corresponding thiazolidine-4-one scaffold **11**. The structure of **11** was confirmed because of its spectral data and satisfactorily elemental analysis. Its infrared spectrum exhibited absorptions at υ = 3327, 2218 and 1724 & 1690 cm^{-1} related to the N-H, C≡N and C=O groups, respectively. The proton of cyclic methylene group (thiazolidine-4-one ring system) resonated as singlet signal in the ^{1}H NMR spectrum at 4.15 ppm.



Scheme (4)

Antibacterial activity of newly synthesized pyridine scaffolds:

The anti-bacterial properties of the constructed pyridine scaffolds have been estimated against Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Bacillus subtilis*) according to the previously described method [14]. The common antibiotic ampicillin was utilized as standard reference for results of antibacterial activity. The % activity index for the complex was calculated by the formula:

$$\% \text{ Activity Index} = \frac{\text{Zone of inhibition by test compound (diameter)}}{\text{Zone of inhibition by standard (diameter) x 100}}$$

The results for antibacterial activities were depicted in table (1) and revealed that there are significant differences in the diameter of inhibition zones. Through survey the results that obtained about the antibacterial activity of our synthesized pyridine compounds against *E. coli* (as an example of Gram-negative bacteria), the best activity was displayed by hydrazide derivative **4** which inhibit the growth of *E. coli* with diameter zone (23 mm, activity index = 95.8%). Among the tested compounds, the hydrazide derivative **4** displayed excellent antibacterial property against *B. subtilis*, it inhibited the growth of *B. subtilis* bacterium with value inhibition zone of diameter 23 mm similar to that of the corresponding reference antibiotic, Ampicillin (23 mm). Finally, the 2-((4-(4-(mercapto(phenylamino)methylene)-3-methyl-1*H*-pyrazole-1-carbonyl)phenyl)amino)-nicotinonitrile scaffold **9** displayed moderate activity to inhibit the growth of *B. subtilis* with diameter zone (18 mm, activity index = 78.3%).

Table (1): Antimicrobial activity (inhibition zone, mm) of the synthesized pyridine scaffolds.

Cpd No.	*E. coli*		*B. subtilis*	
	Diameter (mm)inhibition zone	Activity index(%)	Diameter (mm)inhibition zone	Activity index (%)
3	NA	----	NA	----
4	23	95.8	23	100.0
5a	NA	----	NA	----
5b	NA	----	NA	----
5c	NA	----	NA	----
5d	NA	----	NA	----
5e	9	37.5	8	34.8
6	20	83.3	22	95.6
7a	4	16.7	3	13.0
7b	5	20.8	4	17.4
9	14	58.3	18	78.3
10a	9	37.5	14	60.8
10b	NA	----	NA	----
11	5	20.8	13	56.5
Ampicillin	24	100.0	23	100.0

- NA → No Activity.

Experimental

(1) Synthesis of ethyl 4-((3-cyano-4,6-dimethylpyridin-2-yl)amino)-benzoate (3):

To a suspension of 2-chloro-4,6-dimethylnicotinonitrile **(2)** (1.66 g, 0.01 mol) and ethyl *p*-aminobenzoate (1.65 g, 0.01 mol) in ethyl alcohol (30 ml), 1 ml conc. HCl was added. The reaction mixture was subjected to heating under reflux for eight hours and then cooled to 25°C. The white precipitate that formed was filtered, dried and recrystallized from ethyl alcohol.

White solid, yield 40%, m.p. = 140-141°C. IR (KBr): 3309 (N-H), 2223 (C≡N), 1712 cm^{-1} (C=O). ^1H NMR (DMSO-d_6): 1.31 (t, J = 7.0 Hz, 3H), 2.39 (s, 3H), 2.41 (s, 3H), 4.28 (q, J = 7.0 Hz, 2H), 6.87 (s, 1H, pyridine H-5), 7.74 (d, J = 8.4 Hz, 2H, Ar-H), 7.87 (d, J = 8.4 Hz, 2H, Ar-H), 9.34 ppm (s, 1H, NH). Analysis for $C_{17}H_{17}N_3O_2$ (295): Calcd: C, 69.14; H, 5.80; N, 14.23%. Found: C, 69.14; H, 5.80; N, 14.23%.

(2) Synthesis of 4-((3-cyano-4,6-dimethylpyridin-2-yl)amino)-benzohydrazide (4):

To a suspension of ethyl benzoate derivative **3** (2.95 g, 0.01 mol) in ethyl alcohol (30 ml), hydrazine hydrate (1 ml, 0.01 mol) was added. The reaction components were heated under reflux for 12 hours and then cooled to 25°C. The precipitate that formed was collected by filtration techniqueand then recrystallized from ethyl alcohol.

White crystals, yield 51%, m.p. = 240-242°C. IR (KBr): 3340, 3287 (NH and NH_2), 2217 (C≡N), 1611 cm^{-1} (C=O). ^1H NMR (DMSO-d_6): 2.35 (s, 3H), 2.37 (s, 3H), 4.44 (s, 2H, NH_2), 6.80 (s, 1H, pyridine H-5), 7.65 (d, J = 9.0 Hz, 2H), 7.74 (d, J = 8.0 Hz, 2H), 9.14 (s, 1H, NH), 9.60 ppm (s, 1H, NH). Analysis for $C_{15}H_{15}N_5O$ (281): Calcd: C, 64.04; H, 5.37; N, 24.90%. Found: C, 64.15; H, 5.34; N, 24.96%.

(3) Synthesis of *N′*-arylidene-4-((3-cyano-4,6-dimethylpyridin-2-yl)amino)-benzohydrazide 5a-e:

A mixture of benzohydrazide derivative **4** (0.56 g, 0.002 mol) and 0.002 mol of the appropriate para substituted benzaldehyde (namely; benzaldehyde, 4-methylbenzaldehyde, 4-methoxybenzaldehyde, 4-nitro-benzaldehyde and 4-chlorobenzaldehyde) in 20 ml ethyl alcohol was refluxed for two hours. The crystalline solid that formed upon cooling was filtered to afford the *N′*-arylidene derivatives **5a-e**.

N′-Benzylidene-4-((3-cyano-4,6-dimethylpyridin-2-yl)amino)-benzohydrazide (5a):

White solid, yield 60%, m.p. = 260-261°C. IR (KBr): 3412, 3257 (N-H), 2208 (C≡N), 1648 cm^{-1} (C=O). Analysis for $C_{22}H_{19}N_5O$ (369): Calcd: C, 71.53; H, 5.18; N, 18.96%. Found: C, 71.71; H, 5.15; N, 18.85%.

4-((3-Cyano-4,6-dimethylpyridin-2-yl)amino)-N'-(4-methyl-benzylidene)benzohydrazide (5b):

White solid, yield 56%, m.p. = 270-271°C. IR (KBr): 3337, 3220 (N-H), 2219 (C≡N), 1640 cm^{-1} (C=O). ^1H NMR (DMSO-d_6): 2.33 (s, 3H),2.37 (s, 3H), 2.39 (s, 3H), 6.83 (s, 1H, pyridine H-5), 7.26 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 8.0 Hz, 2H), 7.73 (d, J = 8.5 Hz, 2H), 7.85 (d, J = 8.5 Hz, 2H), 8.39 (s, 1H, CH=N), 9.27 (s, 1H, NH), 11.67 ppm (s, 1H, NH). Analysis for $C_{23}H_{21}N_5O$ (383): Calcd: C, 72.04; H, 5.52; N, 18.26%. Found: C, 72.16; H, 5.45; N, 18.18%.

4-((3-Cyano-4,6-dimethylpyridin-2-yl)amino)-*N′*-(4-methoxy-benzylidene)benzohydrazide (5c):

White solid, yield 55%, m.p. > 300°C. IR (KBr): 3331, 3252 (N-H), 2219 (C≡N), 1638 cm^{-1} (C=O). ^1H NMR (DMSO-d_6): 2.39 (s, 3H), 2.41 (s, 3H), 3.81 (s, 1H, OCH_3), 6.85 (s, 1H, pyridine H-5), 7.02 (d, J = 8.4 Hz, 2H), 7.67 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 8.4 Hz, 2H), 7.86 (d, J = 8.4 Hz, 2H), 8.39 (s, 1H, CH=N), 9.25 (s, 1H, NH), 11.60 ppm (s, 1H, NH). Analysis for $C_{23}H_{21}N_5O_2$ (399): Calcd: C, 69.16; H, 5.30; N, 17.53 %. Found: C, 69.06; H, 5.33; N, 17.59 %.

4-((3-Cyano-4,6-dimethylpyridin-2-yl)amino)-*N′*-(4-nitrobenzylidene)-benzohydrazide (5d):

Yellow solid, yield 78%, m.p. = 295-298°C. IR (KBr): 3292, 3206 (N-H), 2228 (C≡N), 1666 cm^{-1} (C=O). ^1H NMR (DMSO-d_6): 2.38 (s, 3H), 2.40 (s, 3H), 6.86 (s, 1H, pyridine H-5), 7.75 (d, J = 9.0 Hz, 2H), 7.87 (d, J = 8.0 Hz, 2H), 7.98 (d, J = 8.0 Hz, 2H), 8.29 (d, J = 9.0 Hz, 2H), 8.52 (s, 1H, CH=N), 9.30 (s, 1H, NH),

12.03 ppm (s, 1H, NH). Analysis for: $C_{22}H_{18}N_6O_3$ (414): Calcd: C, 63.76; H, 4.38; N, 20.28%. Found: C, 63.88; H, 4.34; N, 20.20%.

***N'*-(4-Chlorobenzylidene)-4-((3-cyano-4,6-dimethylpyridin-2-yl)-amino)benzohydrazide (5e):**

White solid, yield 71%, m.p. = 269-270°C. IR (KBr): 3302, 3214 (N-H), 2209 (C≡N), 1600 cm^{-1} (C=O). MS *m/z* (%): 405 (M^+ + 2, 33.26), 404 (M^+ + 1, 43.47), 403 (M^+, 100.00), 251 (16.44), 250 (32.45), 90 (17.43), 77 (10.27). Analysis for $C_{22}H_{18}ClN_5O$ (403.5): Calcd: C, 65.43; H, 4.49; N, 17.34%. Found: C, 65.55; H, 4.43; N, 17.46%.

(4) Synthesis of 4,6-dimethyl-2-((4-(3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)phenyl)amino)nicotinonitrile (6):

A solution of 4-((3-cyano-4,6-dimethylpyridin-2-yl)amino)-benzohydrazide **(4)** (1.40 g, 0.005 mol) and ethyl acetoacetate (0.65 ml, 0.005 mol) in 40 ml ethyl alcohol was heated under reflux for four hours. The reaction mixture was cooled to 25°C, and the resulting precipitate was collected by filtration technique and then recrystallized from ethyl alcohol.

White powder, yield 61%, m.p. = 170-172°C. IR (KBr): 3353 (N-H), 2219 (C≡N), 1735, 1648 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$): 2.11 (s, 3H), 2.30 (s, 3H), 2.33 (s, 3H), 3.31 (s, 2H), 6.50 (s, 1H, pyridine H-5), 7.64-7.76 (m, 4H, Ar-H), 11.73 ppm (s, 1H, NH). Analysis for $C_{19}H_{17}N_5O_2$ (347): Calcd: C, 65.69; H, 4.93; N, 20.16%. Found: C, 65.49; H, 4.86; N, 20.25%.

(5) Synthesis of 4,6-dimethyl-2-((4-(3-substituted-5-oxo-4-(2-(aryl-hydrazono)-4,5-dihydro-1*H*-pyrazole-1-carbonyl)phenyl)-amino)-nicotinonitriles 7a and 7b:

A suspension of aromatic amine (namely; *p*-toluidine and *p*-anisidine) (0.002 mol) in concentrated HCl (0.6 ml) was cooled in an ice-bath at 0-5°C, and then diazotized with a solution of sodium nitrite (0.14 g in 15 ml H_2O) by stirring for 30 min. The diazonium solution was added dropwise to a solution of 4,6-dimethyl-2-(4-(3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)phenyl)amino)nicotinonitrile **(6)** (0.7 g, 0.002 mol) in 25 ml pyridine with stirring in an ice-bath at 0-5°C for 1 hour and then kept in refrigerator for 12 hours. The obtained precipitate was filtered and recrystallized from EtOH-DMF mixture (2:1) to afford **7a** and **7b**, respectively.

4,6-Dimethyl-2-((4-(3-methyl-5-oxo-4-(2-(*p*-tolyl)hydrazono)-4,5-dihydro-1*H*-pyrazole-1-carbonyl)phenyl)amino)nicotinonitrile (7a):

Orange solid, yield 86%, m.p. = 230-232°C. IR (KBr): 3421, 3349 (N-H), 2208 (C≡N), 1711, 1657 cm^{-1} (C=O). MS *m/z* (%): 465 (M^+, 22.85), 430 (56.39), 405 (35.09), 404 (63.34), 311 (34.06), 310 (100.00), 290 (19.28), 167 (23.47), 93 (23.76), 56 (16.45). Analysis for $C_{26}H_{23}N_7O_2$ (465): Calcd: C, 67.08; H, 4.98; N, 21.06%. Found: C, 67.19; H, 4.95; N, 20.97%.

2-((4-(4-(2-(4-Methoxyphenyl)hydrazono)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)phenyl)amino)-4,6-dimethylnicotinonitrile (7b):

Orange solid, yield 83%, m.p. = 249-250°C. IR (KBr): 3349 (N-H), 2211 (C≡N), 1704, 1653 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$): 2.37 (s, 3H), 2.44 (s, 3H), 2.50 (s, 3H), 3.83 (s, 3H, OCH_3), 6.65 (s, 1H, pyridine H-5), 6.95 (d, J = 8.4 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 7.82 (d, J = 8.4 Hz, 2H), 7.93 (d, J = 8.4 Hz, 2H), 13.53 (s, 1H, NH), 13.94 ppm (s, 1H, NH). Analysis for $C_{26}H_{23}N_7O_3$ (481): Calcd: C, 64.85; H, 4.81; N, 20.36%. Found: C, 64.78; H, 4.75; N, 20.45%.

(6) Synthesis of 2-((4-(4-(mercapto(phenylamino)methylene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)phenyl)amino)-4,6-dimethylnicotinonitrile (9):

To a stirred solution of **6** (0.69 g, 0.002 mol) and potassium hydroxide (0.12 g, 0.002 mol) in 20 ml dimethylformamide, phenyl isothiocyanate (0.24 ml, 0.002 mol) was added dropwise and permitted to stir at an ambient temperature for six hours. The reaction mixture was then poured into ice water drop by drop and neutralized by dilute HCl. The precipitate that formed was filtered, dried and purified by re-precipitation from ethyl acetate to afford compound **9**.

Yellow solid, yield 33%, m.p. = 170-172°C. IR (KBr): 3349 (N-H), 2211(C≡N), 1658 cm^{-1} (C=O). ^{1}H NMR (DMSO-d_6): 2.31 (s, 3H), 2.42 (s, 3H), 2.48 (s, 3H), 6.67 (s, 1H, pyridine H-5), 7.12-7.78 (m, 9H), 10.46 (s, 1H, NH), 11.84

ppm (s, 1H, NH). Analysis for: $C_{26}H_{22}N_6O_2S$ (482): Calcd: C, 64.71; H, 4.60; N, 17.42%. Found: C, 64.48; H, 4.68; N, 17.60%.

(7) Synthesis of 2-((4-(4-(3-phenylthiazolylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)phenyl)amino)-4,6-dimethyl-nicotinonitriles 10a, 10b and 11:

To a stirred solution of **6** (0.69 g, 0.002 mol) and potassium hydroxide (0.12 g, 0.002 mol) in 20 ml dimethylformamide, phenyl isothiocyanate (0.24 ml, 0.002 mol) was added dropwise. The reaction mixture was stirred at 30°C for six hours. After which, the appropriate α-halogenated reagent (namely; chloroacetone, phenacyl chloride, ethyl bromoacetate) (0.002 mol) was added and stirring was continued for additional 4 hours. The reaction mixture was then poured drop by drop into ice water. The precipitate that formed was collected by filtration technique and recrystallized by heating in ethyl alcohol.

4,6-Dimethyl-2-((4-(3-methyl-4-(4-methyl-3-phenylthiazol-2(3*H*)-ylidene)-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)phenyl)amino)-nicotinonitrile (10a):

Yellow solid, yield 34%, m.p. = 200-202°C. IR (KBr): 3327 (N-H), 2218 (C≡N), 1692, 1661 cm^{-1} (C=O). ^1H NMR (DMSO-d_6): 2.31 (s, 6H, $2CH_3$), 2.40 (s, 6H, $2CH_3$), 5.84 (s, 1H, thiazole H-5), 6.91 (s, 1H, pyridine H-5), 7.04-7.89 (m, 9H, Ar-H), 9.49 ppm (s, 1H, NH). Analysis for $C_{29}H_{24}N_6O_2S$ (520): Calcd: C, 66.91; H, 4.65; N, 16.14%. Found: C, 66.70; H, 4.56; N, 16.22%.

2-((4-(4-(3,4-Diphenylthiazol-2(3*H*)-ylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)phenyl)amino)-4,6-dimethyl-nicotinonitrile (10b):

Yellow solid, yield 28%, m.p. = 290-291°C. IR (KBr): 3326 (N-H), 2212 (C≡N), 1703, 1658 cm^{-1} (C=O). ^1H NMR (DMSO-d_6): 2.20 (s, 3H), 2.36 (s, 3H), 2.37 (s, 3H), 5.66 (s, 1H, thiazole H-5), 6.79 (s, H, pyridine H-5), 7.65-7.91 (m, 10H, Ar- H), 9.33 ppm (s, 1H, NH). Analysis for $C_{34}H_{26}N_6O_2S$ (582): Calcd: C, 70.09; H, 4.50; N, 14.42%. Found: C, 70.31; H, 4.42; N, 14.54%.

4,6-Dimethyl-2-((4-(3-methyl-5-oxo-4-(4-oxo-3-phenylthiazolidin-2-ylidene)-4,5-dihydro-1*H*-pyrazole-1-carbonyl)phenyl)amino)-nicotinonitrile (11):

Yellow solid, yield 34%, m.p. = 160-162°C. IR (KBr): 3327 (N-H), 2218 (C≡N), 1724, 1690 cm^{-1} (C=O). ^1H NMR (DMSO-d_6): 2.32 (s, 3H), 2.41 (s, 6H, $2CH_3$), 4.15 (s, 2H, thiazolidinone-CH_2), 6.85 (s, 1H, pyridine H-5), 7.08-7.76 (m, 9H, Ar- H), 9.49 ppm (s, 1H, NH). Analysis for $C_{28}H_{22}N_6O_3S$ (522): Calcd: C, 64.35; H, 4.24; N, 16.08%. Found: C, 64.51; H, 4.30; N, 15.94%.

Conclusion

Ethyl4-(3-cyano-pyridin-2ylamino)benzoate scaffold **3** has been prepared by substitution of the chlorine atom from 2-chloronicotinonitrile derivative **2** via heating with ethyl 4-aminobenzoate and then refluxed with hydrazine hydrate to produce the hydrazide derivative **4**. The reaction of hydrazide **4** with ethyl acetoacetate furnished the corresponding 2-((4-(1*H*-pyrazole-1-carbonyl)phenyl)-amino)-nicotinonitrile derivative **6** which reacted with phenyl isothiocyanate and chloroacetone, phenacyl chloride or ethyl bromoacetate to give thecorresponding 2-((4-(4-(3-phenylthiazolylidene)-1*H*-pyrazole-1-carbonyl)phenyl)amino)-4,6-dimethyl-nicotinonitriles **10a**, **10b** and **11**. All synthesized pyridine scaffolds revealed better activity against the Gram-positive bacterium (B. subtilis) rather than the Gram-negative bacterium (E. coli).

4. References

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