HETEROCYCLES, Vol. 100, No. 11, 2020, pp. 1883 - 1901 © 2020 The Japan Institute of Heterocyclic Chemistry
Received, 5th June, 2020, Accepted, 13th August, 2020, Published online, 21st August, 2020
DOI: 10.3987/COM-20-14298

# SYNTHESIS AND ANTIBACTERIAL SURVEY OF SOME NEW PYRIDINE-BASED HETEROCYCLES

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

Department of Chemistry, Faculty of Science, Mansoura University, 35516 Mansoura, Egypt; E-mail: ehabattia00@gmx.net

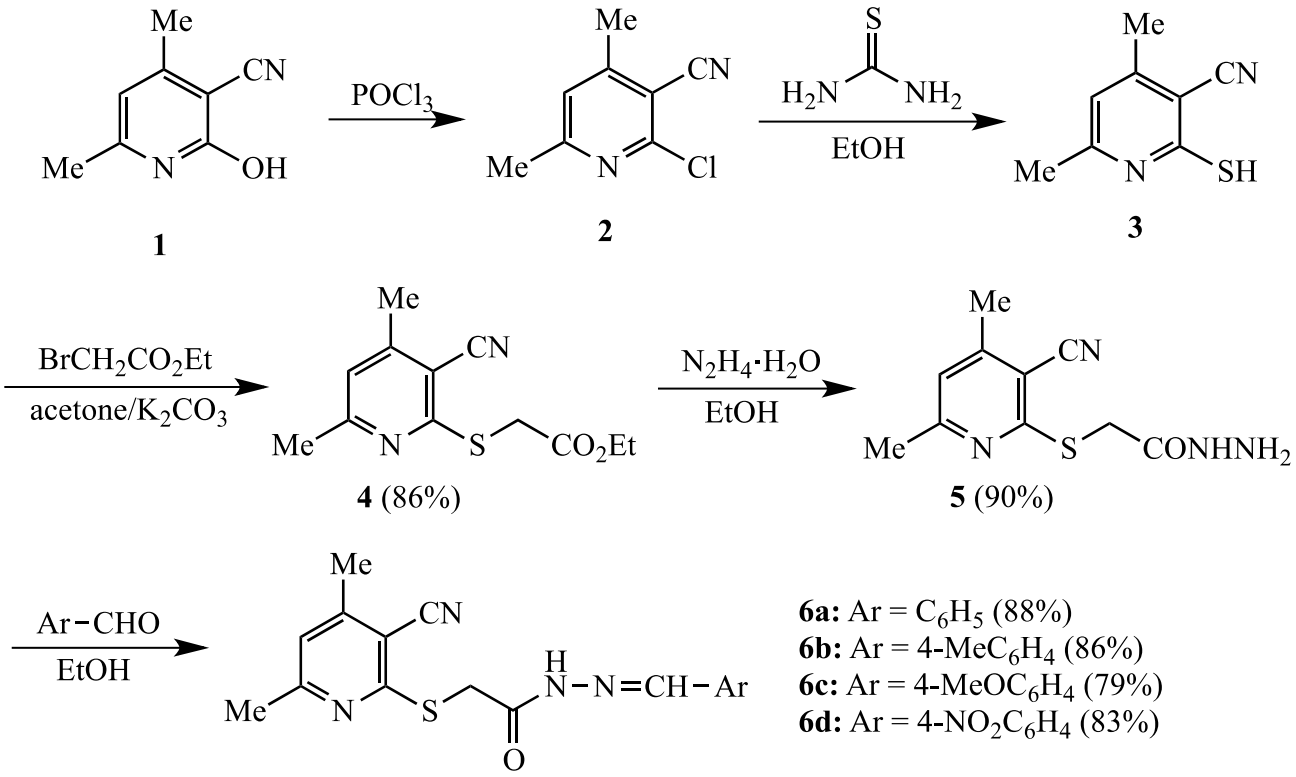
**Abstract** – Treatment of ethyl 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetate (**4**) with hydrazine hydrate furnished 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)-acetohydrazide (**5**) which underwent condensation with five benzaldehydes to afford the corresponding *N'*-arylidene-2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)-acetohydrazides **6a-e**. The reaction of hydrazide **5** with ethyl acetoacetate yielded the expected 2-((2-(3-methyl-5-oxo-pyrazolyl)-2-oxoethyl)thio)nicotinonitrile derivative **7** which diazo-coupled with different diazonium chlorides to furnish the corresponding 2-(4-(2-arylhydrazono)pyrazolyl)-2-oxoethyl)thio)nicotinonitriles **8a-c**. In addition, the nucleophilic substitution of chlorine from 2-chloroacetamide derivative **16** by various types of nucleophiles (salicylaldehyde, ethyl thioglycolate, 2-mercaptobenzoxazole, ammonium thiocyanate and/or malononitrile) was investigated. In general, all synthesized pyridine scaffolds revealed better activity against the Gram-positive bacterium (*Bacillus subtilis*) rather than the Gram-negative bacterium (*Escherichia coli*).

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.[1] In addition, pyridine derivatives have been widely studied because of their utilization in many branches of chemistry.[2] Many pyridine-containing compounds display fabulous medicinal properties, including antibacterial,[3-5] hypnotic and sedative,[6] HIV antiviral,[7] bone calcium regulator,[8] cholesterol and triglyceride regulator,[9] antidiabetic,[10] antihistaminic,[11] antiulcerant,[12,13] antineoplastic, and anticancer activities.[14-17] Moreover, several compounds containing the pyrazole,[18,19] thiazole,[20,21] benzofuran,[22] benzoxazole[23] and/or pyrrole[24] ring system were reported to possess antibacterial activity. Thus, the

present work is aiming to the following objectives: (i) The conversion of 2-hydroxynicotinonitrile derivative to new functionalized pyridine scaffolds **5** and **15**, which allow for further modification through its hydrazide and amino functions, respectively. (ii) Molecular hybridization of pyridine nucleus with other effective antibacterial moieties such as pyrazole, thiazole, furan, oxazole and/or pyrrole. (iii) Screening the antibacterial activity of the synthesized pyridine hybrids against Gram-positive and Gram-negative bacteria.

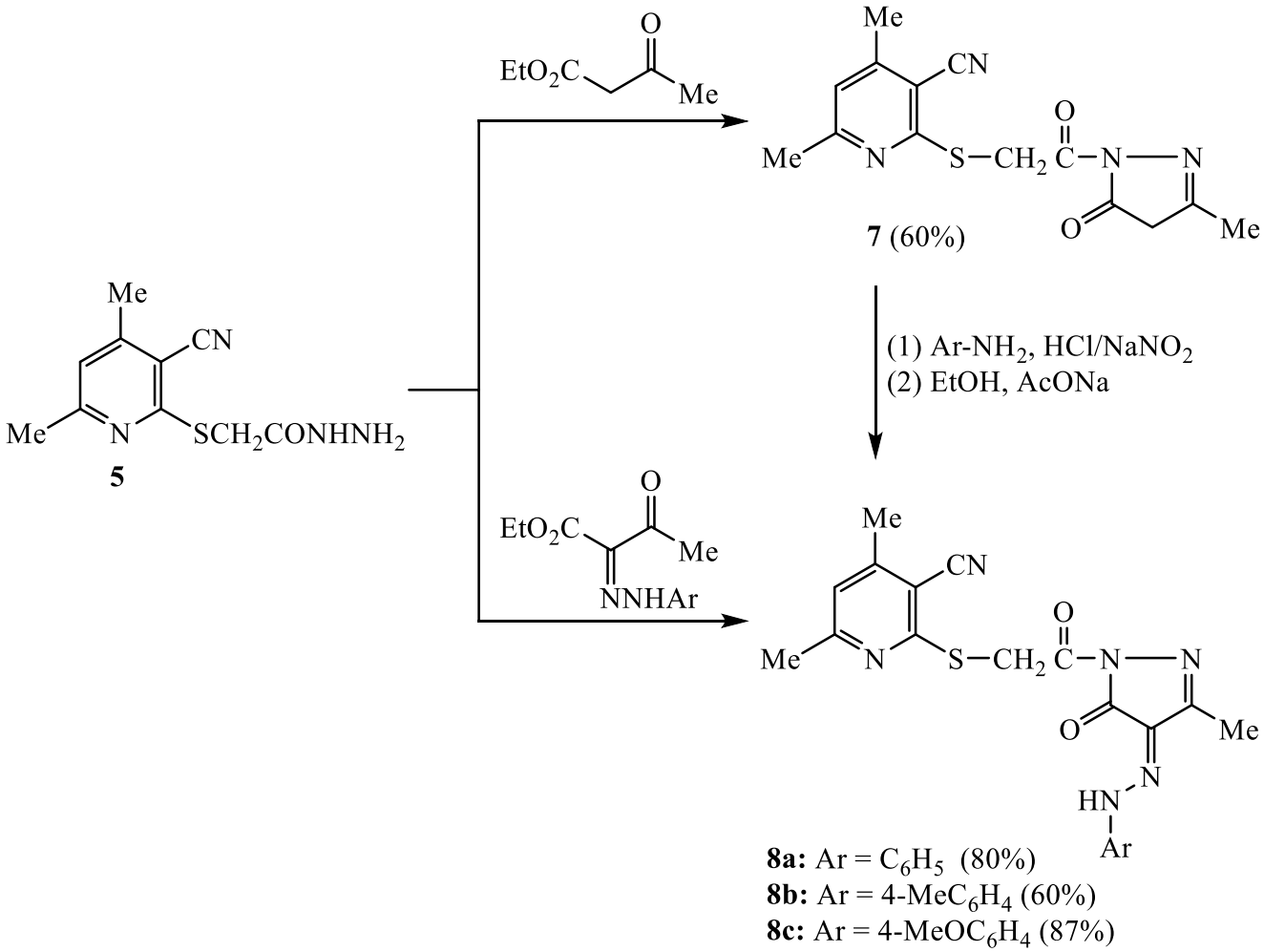
The present research study starts by heating of 2-hydroxy-4,6-dimethylnicotinonitrile (**1**) with phosphorus oxychloride to furnish 2-chloro-4,6-dimethylnicotinonitrile (**2**),[25] which has been utilized as a precursor for the synthesis of 2-mercapto-4,6-dimethylnicotinonitrile (**3**) thought its refluxing with thiourea in ethanol.[25] Nucleophilic substitution reaction between 2-mercaptonicotinonitrile compound **3** and ethyl bromoacetate proceeded in acetone in the presence of anhydrous potassium carbonate to afford the corresponding sulfide compound **4** in 86% yield.[25] Treatment of ethyl 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetate (**4**) with hydrazine hydrate by stirring at room temperature in ethanol furnished 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetohydrazide (**5**) in high yield (90%) (Scheme 1). The analyses of IR and $^1$H NMR were employed to establish the structure of acetohydrazide **5**. The IR spectrum of **5** announced stretching absorptions at 3334, 3287 cm$^{-1}$ (-NH-$NH_2$ of hydrazide moiety), 2213 cm$^{-1}$ (C≡N) and 1644 cm$^{-1}$ (C=O of hydrazide moiety). The $^1$H NMR spectrum of **5** identified singlet integrated for four protons, at δ 3.89 ppm refereed to the methylene (-$CH_2$) and amino (-$NH_2$) functions.

Many hydrazides and their derivatives as hydrazones, semicarbazides and thiosemicarbazides have been reported as biologically active compounds.[26,27] Furthermore, acyl hydrazides are privileged starting materials for the synthesis of various heterocycles such as pyrazoles, triazoles, thiazoles, thiadiazoles and oxadiazoles.[28,29] The previous observations motivated us to explore the reactivity of acetohydrazide derivative **5** towards the reaction with aldehydes, ketoesters, phenyl isothiocyanate, and benzenesulfonyl chloride. Thus, condensation of hydrazide **5** with five substituted benzaldehydes (namely; benzaldehyde, 4-methylbenzaldehyde, 4-methoxybenzaldehyde and 4-nitrobenzaldehyde) afforded the corresponding *N'*-arylidene-2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetohydrazides **6a-d** in good yields (79-88%). Assignment the structures of compounds **6** were secured by IR, $^1$H NMR, mass analysis and satisfactorily elemental analyses. The infrared absorption, which ranged from 1661 to 1685 cm$^{-1}$ through the IR spectra of compounds **6a-d**, identified the carbonyl group. The presence of singlet for one proton at 7.98 ppm in the proton-nuclear magnetic resonance spectrum of **6c** referred to the azomethine moiety (N=CH).




**Scheme 1.** Reaction of 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetohydrazide (**5**) with aromatic aldehydes

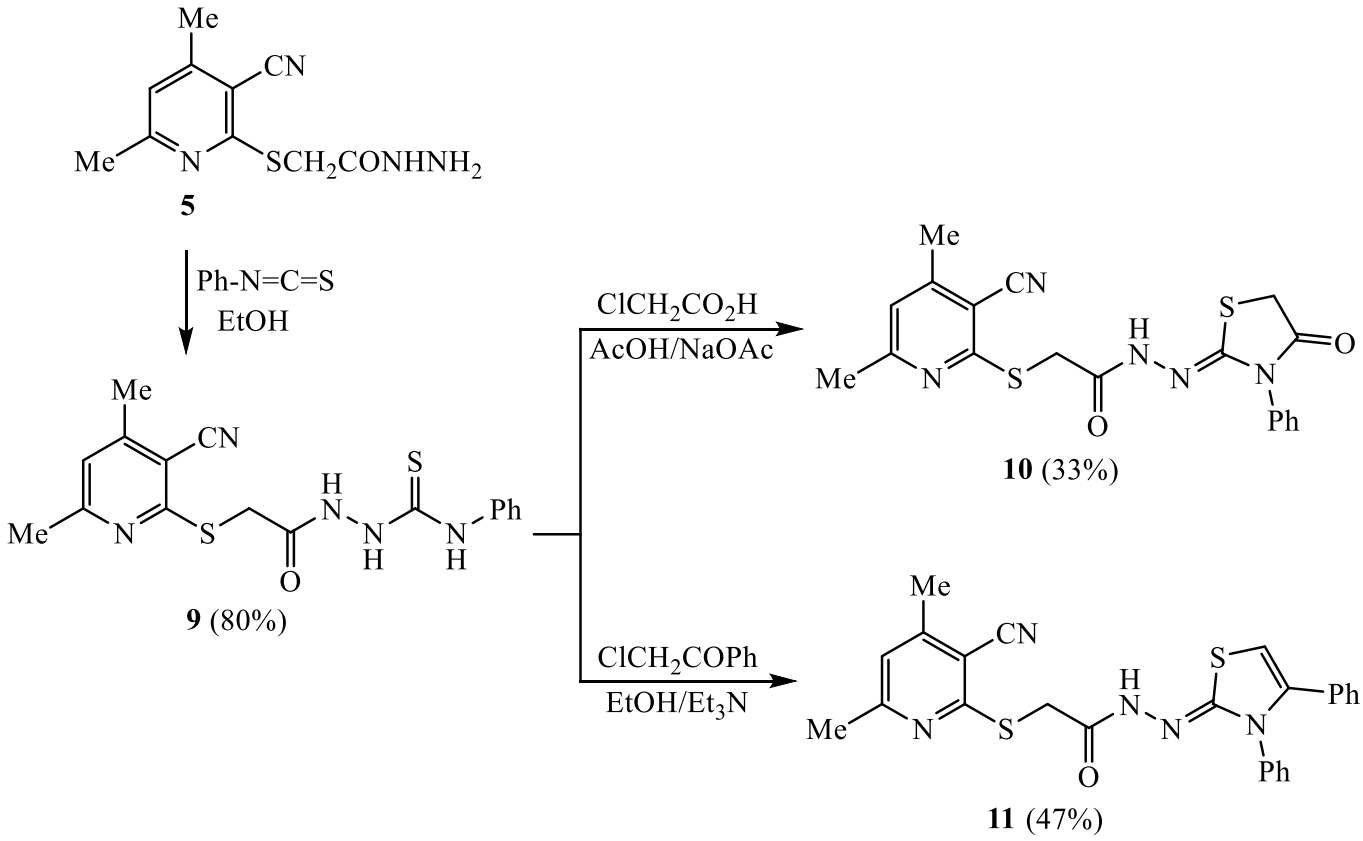
The reaction of hydrazide **5** with ethyl acetoacetate has been explored in boiling ethanol to furnish the expected 2-((2-(3-methyl-5-oxo-pyrazolyl)-2-oxoethyl)thio)nicotinonitrile derivative **7** in 60% yield. The cyclic methylene group of compound **7** (at the pyrazole moiety) proved to be reactive towards the diazo-coupling reaction with three different diazonium salts (derived from aniline, 4-toluidine and 4-anisidine). The reaction proceeded in ethanol and sodium acetate to furnish the corresponding 2-(5-oxo-4-(2-arylhydrazono)pyrazolyl)-2-oxoethyl)thio)nicotinonitriles **8a-c** with 60-87% yields (Scheme 2). The formation of nicotinonitrile scaffolds **8a-c** found support from their spectral data, satisfactorily elemental analyses and their alternative synthesis through the condensation of hydrazide **5** with ethyl (2-arylhydrazono)acetoacetates. The latter reaction has been carried out by boiling the hydrazide **5** with ethyl (2-arylhydrazono)acetoacetate derivatives in ethanol. The infrared spectrum of **8c** displayed absorptions at 3194, 2218 and (1732 & 1657) $cm^{-1}$ which refers to the -N-H, -C≡N and C=O groups, respectively. The $^1H$ NMR signals of **8c** in were four singlet signals at 2.26, 2.29, 2.39 and 3.77 ppm (four $–CH_3$ groups). The singlet at 4.69 ppm referred to the methylene group ($-CH_2-$). The aromatic protons resonated as two doublet signals (7.02 and 7.65 ppm) and integrated for four protons. The singlet signals at 7.08 and 13.20 ppm referred to the protons of pyridine C-5 and NH.




**Scheme 2.** Synthesis of 2-(5-oxo-4-(2-arylhydrazono)pyrazolyl)-2-oxoethyl)thio)nicotinonitriles **8a-c**

The reaction of hydrazide **5** with isothiocyanatobenzene was performed by reflux in ethanol to give the conforming 2-(2-((3-cyanopyridin-2-yl)thio)acetyl)-*N*-phenylhydrazine-1-carbothioamide scaffold **9** in 80% yield (Scheme 3). The IR spectrum of compound **9** exhibited absorptions at 3319, 3221, 3155 & 2217 and 1663 $cm^{-1}$ corresponding to the N-H, -C≡N and C=O groups, respectively. The $^1H$ NMR spectrum showed singlet signals at 2.40, 2.46 and 4.09 ppm (two $–CH_3$ and one $–CH_2$-groups). The singlet at 7.10 ppm referred to the proton of pyridine C-5, while the aromatic protons resonated as multiplet between 7.16 and 7.38 ppm. The three singlet signals at 7.57, 9.72 and 10.29 ppm were attributed to the NH groups. Refluxing the thiourea scaffold **9** with chloroacetic acid has been achieved in acetic acid and sodium acetate to give the corresponding 2-((3-cyanopyridin-2-yl)thio)-*N′*-(4-oxothiazolidin-2-ylidene)acetohydrazide derivative **10** in 33% yield. The infrared spectrum of **10** displayed absorptions at wavenumbers 3235, 2216 and 1718 $cm^{-1}$, which refer to the N-H, nitrile (C≡N) and carbonyl (C=O, ring) respectively. In the $^1H$ NMR spectrum, the two protons of methylene group (thiazolidinone ring) resonated as singlet at 3.95 ppm. The reaction of **9** with

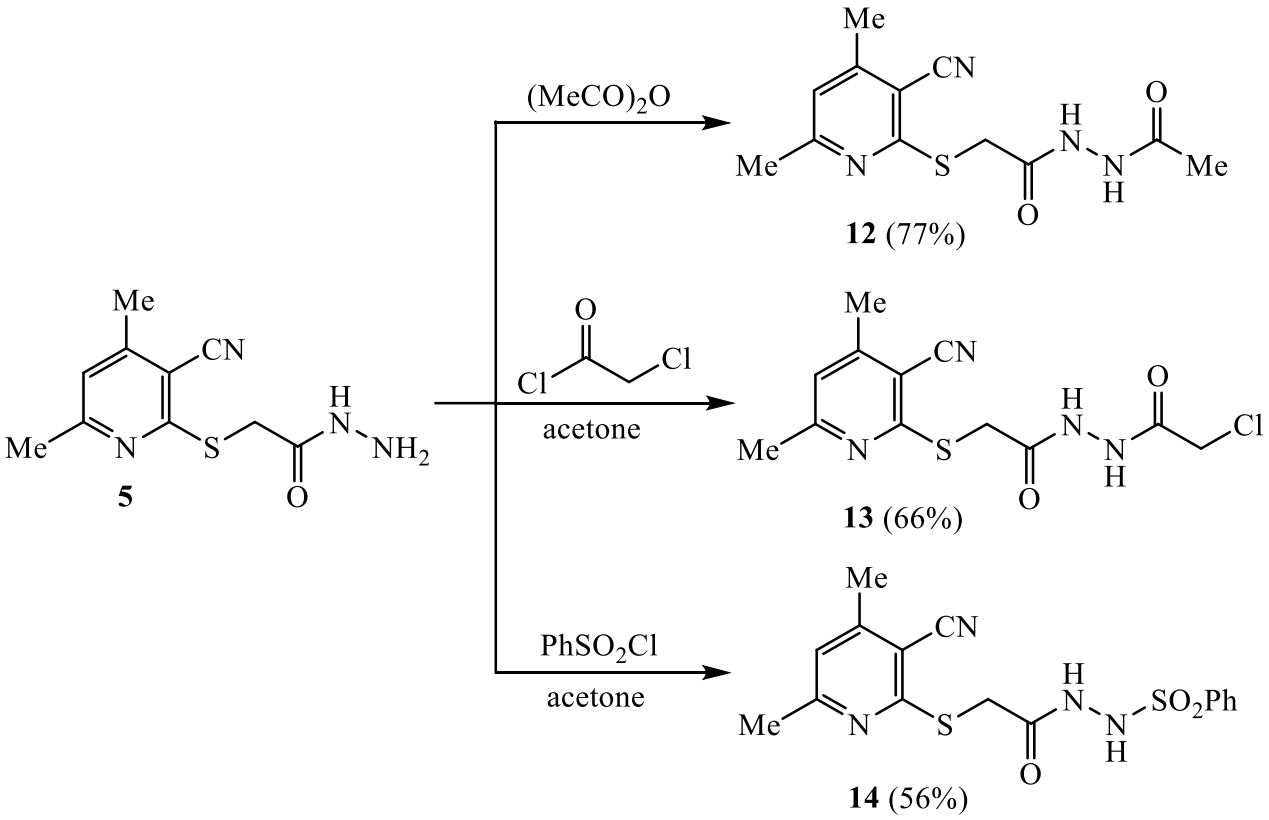
phenacyl chloride has been carried out by boiling in ethanol and trimethylamine to produce the conforming 2-((3-cyanopyridin-2-yl)thio)-*N*′-(3,4-diphenylthiazol-2(3*H*)-ylidene)acetohydrazide derivative **11** in 47% yield. Infrared and $^1$H NMR spectroscopic tools elucidated the structure of compound **11**. The $^1$H NMR spectrum showed singlet signals at 2.40 ppm (2 Me), 4.67 ppm ($CH_2$) and 4.95 ppm (CH=C). The proton of pyridine C-5 resonated as singlet at 6.76 ppm.




**Scheme 3.** Synthesis of *N*′-(4-oxothiazolidin-2-ylidene)acetohydrazide derivative **10** and *N*′-(3,4-diphenylthiazol-2(3*H*)-ylidene)acetohydrazide derivative **11**

Acetylation of hydrazide **5** to pick up its corresponding *N*-acetylation product, 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)-*N*-(2-oxopropyl)acetohydrazide **12** in 77% yield, has been achieved by heating hydrazide **5** with acetic anhydride at 90-95 °C (Scheme 4). The structure of **12** was assigned on the bases of its satisfactorily spectroscopic analyses. For example, the $^1$H NMR displayed the singlet for the three protons at acetyl group at 2.21 ppm (COMe). Chloroacetylation reaction of hydrazide **5** was carried out by its treatment with chloroacetyl chloride in acetone to afford the 2-chloro-*N*-(2-((3-cyanopyridinyl)thio)acetyl)acetohydrazide derivative **13** in 66% yield. The existence of a broad absorption centered at $\upsilon$ = 1612 cm$^{-1}$ (infrared spectrum) referred to the carbonyl group. The $^1$H NMR spectrum displayed singlet for the methylene group related to the chloroacetamide moiety at $\delta$ 4.03

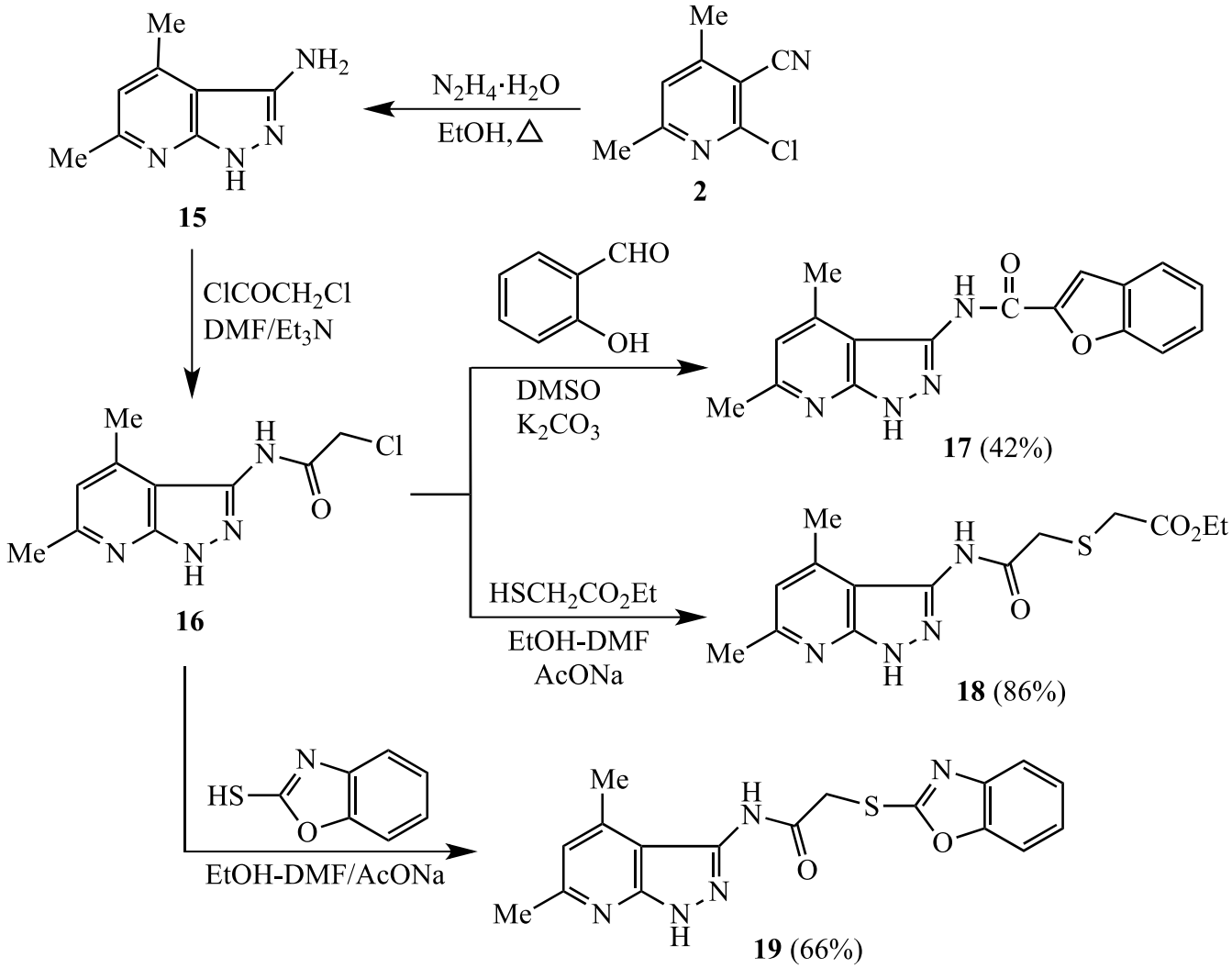
ppm ($NCOCH_2Cl$). Treatment of hydrazide **5** with benzenesulfonyl chloride proceeded in acetone to yield the corresponding *N*-(2-((3-cyanopyridinyl)-thio)acetyl)benzenesulfonohydrazide derivative **14** in 56% yield. The $^1H$ NMR spectrum exhibited singlet at δ 4.06 ppm, which refer to $–CH_2-$ group.




**Scheme 4.** Acylation of 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetohydrazide (**5**)

Pyrazolo[3,4-*b*]pyridines comprise a very interesting class of compounds because of their significant and versatile biological and pharmacological activities.[30-33] They were reacted as a forerunner to synthesize many fused, binary, and poly heterocycles compounds with potent biological activities.[34] Taking into account the previous importance of pyrazolopyridine derivatives, we synthesized and evaluated a series of new benzofuryl-, benzoxazolyl-, thiazolyl-, and pyrrolyl-pyrazolo[3,4-*b*]pyridine hybrids as antibacterial agents. The key of these targeted hybrids, 2-amino-4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine (**15**)[25] has been prepared as reported in the literature by the reaction of 2-chloro-4,6-dimethylnicotinonitrile (**2**) with hydrazine hydrate in hot ethanol. As shown in Scheme 5, the synthetic strategy was initiated by chloroacetylation of the key **15** through its treatment with chloroacetyl chloride in DMF and triethylamine to produce the corresponding chloroacetamide derivative **16**.[35] Condensation of 2-chloroacetamide derivative **16** with salicylaldehyde was carried out by stirring in DMSO and potassium carbonate to afford the corresponding *N*-(4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)benzofuran-2-carboxamide (**17**) in

42% yield. The infrared spectrum of compound **17** showed the characteristic absorptions for the required functional groups as N-H and C=O at 3380 & 3257 and 1690 $cm^{-1}$, respectively. The $^1H$ NMR spectrum exhibited singlet at 4.97 ppm attributed to the proton of furan C-3. Furthermore, the nucleophilic substitution of chlorine from 2-chloroacetamide derivative **16** by various types of sulfur nucleophiles was investigated. The reaction of **16** with ethyl thioglycolate was carried out in hot ethanol/DMF mixture (1:1) containing sodium acetate to afford the 2-((2-((4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)amino)-2-oxoethyl)thio)acetate (**18**) in 86% yield (Scheme 5).

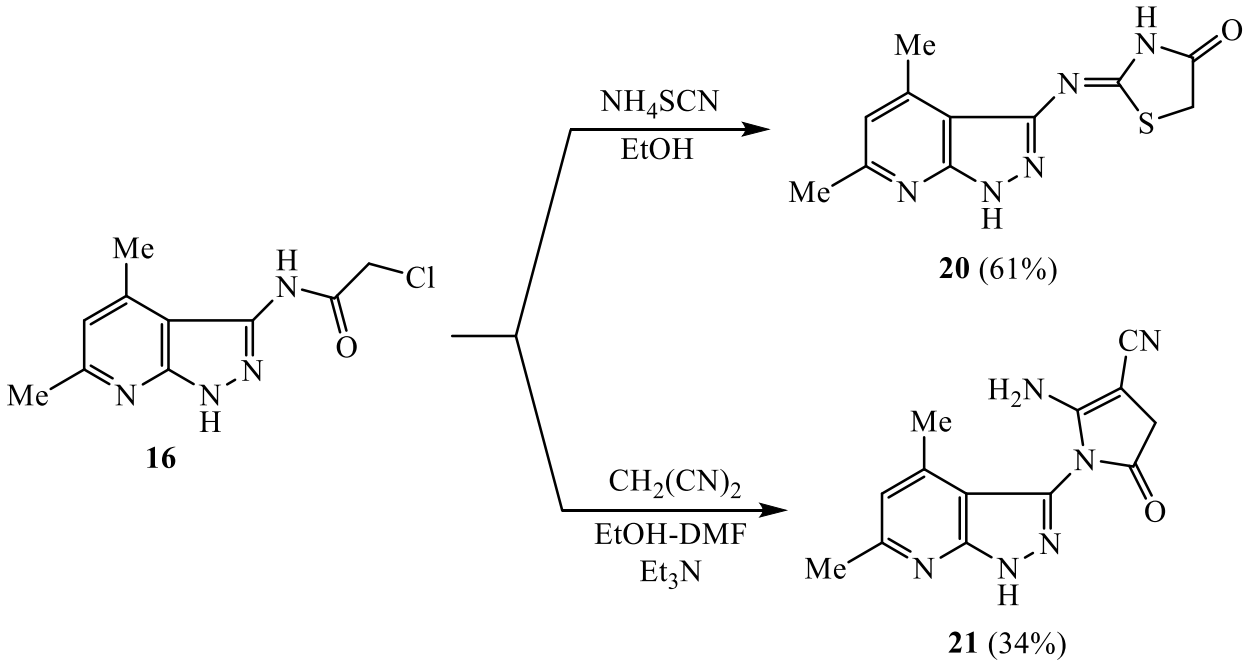



**Scheme 5.** Synthesis of 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine derivatives **17-19**

The infrared absorption at 1724 $cm^{-1}$ clearly indicated the presence of carbonyl-ester ($CO_2Et$). The $^1H$ NMR signals were triplet at 1.21 ppm (-Me), singlet at 2.46 ppm (-Me), singlet at 2.51 ppm (-Me), singlet at 3.50 ppm ($-CH_2$), singlet at 3.54 ppm ($-CH_2$), quartet at 4.13 ppm ($-OCH_2$), singlet at 6.84 ppm (pyridine H-5), singlet at 10.19 ppm (NH) and singlet at 13.13 ppm (NH). The reaction of **16** with

2-mercaptobenzoxazole proceeded successfully in hot ethanol/DMF mixture (1:1) containing sodium acetate to afford the corresponding sulphide, 2-(benzoxazol-2-ylthio)-*N*-(4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)acetamide (**19**) in 66% yield. The infrared absorptions at 3262 & 3202 and 1659 $cm^{-1}$ assigned the N-H and carbonyl groups, respectively. The methylene group of the sulphide moiety resonated as singlet ($^1$H NMR spectrum) for two protons at 3.92 ppm.

Treatment of 2-chloroacetamide derivative **16** with ammonium thiocyanate was performed by boiling in ethanol and DMF mixture (1:1) to afford 2-((4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)imino)-thiazolidin-4-one (**20**) in 61% yield. The proposed structure of **20** was supported by its compatible spectral data (Scheme 6). The carbonyl group of the thiazolidin-4-one ring was identified by the IR absorption at 1717 $cm^{-1}$. The methylene group of the thiazoliodin-4-one ring was secured by the $^1$H NMR singlet signal at 3.98 ppm. Heterocyclization reaction of **16** with malononitrile (as an example of activated nitriles) proceeded in hot ethyl ethanol/DMF mixture (1:1) and triethylamine to afford the corresponding 2-amino-1-(1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-5-oxo-1*H*-pyrrole derivative **21** in 34% yield (Scheme 6). The infrared spectrum of **21** showed the characteristic absorptions 3284 & 3212 (NH and $NH_2$), 2200 (C≡N) and 1741 $cm^{-1}$ (C=O). The methylene group of the pyrrole ring resonated as singlet ($^1$H NMR spectrum) at 3.52 ppm.




**Scheme 6.** Reactions of 2-chloroacetamide derivative **16** with ammonium thiocyanate and malononitrile

## ANTIBACTERIAL ACTIVITY

The antibacterial properties of the constructed pyridine scaffolds have been estimated against Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Bacillus subtilis*). Through survey the results that obtained (Table 1) 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 the acetohydrazide derivative **5** which inhibit the growth of *E. coli* with diameter zone (17 mm, activity index = 70.8%). Among the tested compounds, the acetohydrazide scaffold **5** displayed excellent antibacterial property against *B. subtilis* (inhibition zone = 24 mm). It was even more active than Ampicillin, which inhibits the growth of bacteria with inhibition zone 23 mm.

Structure-activity relationship (SAR) was generated by the conversion of our key building block, acetohydrazide derivative **5**, into its corresponding hydrazones **6a-d** that unfortunately did not show any antibacterial activity. The incorporation of pyridine with pyrazolone ring system in compound **7** resulted in decrease in the antibacterial activity from 104.3% into 73.9% against *B. subtilis.* While the pyridine-pyrazolone hybrids **8a-c** displayed weak activity against both types of bacteria. Although the formation of thiosemicarbazide derivative **9** and the benzenesulfonohydrazide derivative **14** exhibited good antibacterial activity; they inhibit the growth of *B. subtilis* bacterium with activity indices 91.3% and 86.9%, respectively. The chemical transformation that carried out to incorporate various heterocyclic moieties (furan as in compound **17**, oxazole as in compound **19**, thiazole as in compound **20**) to the second key building block **15**, did not bear any improvement in the antibacterial activity. Only the incorporation of pyrrole with the pyrazolopyridine ring system in compound **21** kept the inhibition activity to moderate with activity index 69.6% (16 mm zone diameter).

**Table 1.** Antibacterial activities of the constructed pyridines.

| | ***E. coli*** | | ***B. subtilis*** | |
|---|---|---|---|---|
| **Cpd. No.** | **Mean* of zone diameter (mm)** | **Activity index (%)** | **Mean* of zone diameter (mm)** | **Activity index (%)** |
| **4** | NA | ---- | NA | ---- |
| **5** | 17 | 70.8 | 24 | 104.3 |
| **6a-d** | NA | ---- | NA | ---- |
| **7** | 12.5 | 52.1 | 17 | 73.9 |
| **8a** | 3 | 12.5 | 6 | 26.1 |
| **8b,c** | NA | ---- | 3 | 13.0 |
| **9** | 15 | 62.5 | 21 | 91.3 |
| **10** | 2 | 8.3 | 3 | 13.0 |

| | | | | |
|---|---|---|---|---|
| **11** | 5 | 20.8 | 11 | 47.8 |
| **12** | 8.5 | 35.4 | 17 | 73.9 |
| **13** | NA | ---- | 2 | 8.7 |
| **14** | 13 | 54.2 | 20 | 86.9 |
| **17** | 5 | 20.8 | 5 | 21.7 |
| **18** | NA | ---- | NA | ---- |
| **19** | NA | ---- | 11.5 | 50.0 |
| **20** | 2.5 | 10.4 | NA | ---- |
| **21** | 12 | 50.0 | 16 | 69.6 |
| **Ampicillin** | 24 | 100.0 | 23 | 100.0 |

* Calculated from three values. NA → No Activity.

The absence of improving the biological efficiency from starting material may be due to different causes, which sometimes happened. i- The polarity over the compound due to the intensive addition for function groups which lead to lower lipophilic feature in cell-lipid. ii- The big size of compounds may prevent the ease of penetration from the cell membrane and lower their biological efficiency. Our comments to make active compounds in the future projects involve the following points:

[1] In general, most of synthesized compounds are practically inactive except compounds **5** and **9** and **14**. Thus, the skeleton of nicotinonitrile compounds, which is comprised of two cores, pyridine and hydrazide, thiosemicarbazide or sulfonohydrazide, was shown to be important for antimicrobial activity.

[2] Pyridine-pyrazoles **7-8** and pyridine-thiazole analogs **10-11** were less efficient as antibacterial agents. Therefore, a change in the skeleton may bring better antibacterial activity.

[3] Kind of ring system also significantly affected the antimicrobial activity. The combination of pyrrole with the pyrazolopyridine ring system improved the antibacterial activity.

In the present work, synthesis and utilization of 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)-acetohydrazide (**5**) and 3-amino-4,6-dimethylpyrazolo[3,4-*b*]pyridine (**15**) in preparation of multifunctionalized organic compounds contain pyridine moiety were investigated. All synthesized pyridine scaffolds revealed better activity against the Gram-positive bacterium (*B. subtilis*) rather than the Gram-negative bacterium (*E. coli*).

## EXPERIMENTAL

**General.** An electrothermal Gallenkamp apparatus (Weiss-Gallenkamp, Loughborough, UK) has been used to measure the melting points. The infrared spectra have been obtained by Thermo Scientific Nicolet

iS10 FTIR spectrometer (Waltham, MA). The $^1$H NMR spectra have been obtained by Bruker WP spectrometer (Rheinstetten, Germany) at 400 MHz. Quadrupole GC/MS Thermo Scientific Focus/DSQII (Waltham, MA) has been used to carry out the mass analyses. Perkin-Elmer 2400 analyzer (PerkinElmer Instruments, Shelton, CT) has been used to determine the elemental analyses. Antibacterial assessments were carried out at Microbiology Unit, Faculty of Science, Mansoura University, Egypt.

**Antibacterial method.**[36] Each of the tested compound was dissolved in DMSO and solutions of the concentration 1 mg/mL were prepared separately. Paper discs of Whatman filter paper were prepared with standard size (5 cm) were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the tested solution and placed aseptically in the petri dishes containing nutrient agar media (agar 20 g + beef extract 3 g + peptone 5 g) seeded with *Escherichia coli* and *Bacillus subtilis*. The petri dishes were incubated at 36 °C and the inhibition zones were recorded after 24 h of incubation. Each treatment was replicated three times and the results were recorded as average diameter of inhibition zones. The antibacterial activity of a common standard antibiotic ampicillin was utilized as reference for results of antibacterial activity using the same procedure as above at the same concentration and solvents. The % activity index for the test compound was calculated by the formula:

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

**Synthesis of ethyl 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetate (4).** To a suspension of 2-mercaptonicotinonitrile compound **3** (0.82 g, 0.005 mol) and potassium carbonate (0.69 g, 0.005 mol) in 20 mL acetone, 0.005 mol of ethyl bromoacetate (0.84 mL) was added and stirred overnight. The reaction suspension was added drop by drop into ice water, and the precipitate that obtained was filtered and recrystallized from EtOH. White crystals, yield 86%, mp 87-88 °C, lit. mp 90 °C.[25] IR (KBr): 2217 (C≡N), 1735 cm$^{-1}$ (C=O, ester). Anal. Calcd for $C_{12}H_{14}N_2O_2S$ (250.32): C, 57.58; H, 5.64; N, 11.19%. Found: C, 57.64; H, 5.67; N, 11.12%.

**Synthesis of 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetohydrazide (5).** A suspension of **4** (2.50 g, 0.01 mol) and hydrazine hydrate (0.75 mL, 0.015 mol) was stirred in 20 mL EtOH at 25-30 °C for 2 h. The white solid that formed was filtered and recrystallized from EtOH to give the acetohydrazide derivative **5**. White solid, yield 90%, mp 140-141 °C. IR (KBr): 3334, 3287 (NH, $NH_2$), 2213 (C≡N), 1644 cm$^{-1}$ (C=O). $^1$H NMR ($CDCl_3$) *δ/ppm*: 2.47 (s, 3H, $CH_3$), 2.55 (s, 3H, $CH_3$), 3.89 (s, 4H, $NH_2$ and $CH_2$), 6.90 (s, 1H, pyridine H-5), 8.17 (s, 1H, NH). Anal. Calcd for $C_{10}H_{12}N_4OS$ (236.29): C, 50.83; H, 5.12; N, 23.71%. Found: C, 50.70; H, 5.16; N, 23.82%.

**Synthesis of *N′*-arylidene-2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetohydrazides 6a-e.** A suspension of hydrazide **5** (0.47 g, 0.002 mol) and 0.002 mol of the appropriate *para*-substituted benzaldehydes (namely; benzaldehyde, 4-methylbenzaldehyde, 4-methoxybenzaldehyde, 4-nitrobenzaldehyde and 4-chlorobenzaldehyde) in 20 mL EtOH was refluxed for 2 h and then allowed to cool to room temperature. The crystalline solid that formed was filtered to afford the *N′*-(arylidene)acetohydrazides **6a-e**.

***N′*-Benzylidene-2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetohydrazide (6a).** White crystals, yield 88%, mp 240-242 °C. IR (KBr): 3197 (N-H), 2214 (C≡N), 1667 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$) *δ/ppm*: 2.36 (s, 3H, $CH_3$), 2.44 (s, 3H, $CH_3$), 4.41 (s, 2H, $CH_2$), 7.51-7.88 (m, 5H, Ar-H), 7.02 (s, 1H, pyridine H-5), 8.12 (s, 1H, CH=N), 11.25 (s, 1H, NH). MS *m/z* (%): 325 ($M^+$+1, 37.84), 324 ($M^+$, 100.00), 293 (13.57), 205 (59.29), 178 (25.06), 177 (90.79), 164 (25.16), 131 (40.16), 119 (29.63), 104 (49.74), 92 (50.54), 90 (70.27), 89 (82.43), 77 (73.78), 65 (58.92), 63 (29.88), 51 (31.62). Anal. Calcd for $C_{17}H_{16}N_4OS$ (324.40): C, 62.94; H, 4.97; N, 17.27%. Found: C, 62.76; H, 4.93; N, 17.39%.

**2-((3-Cyano-4,6-dimethylpyridin-2-yl)thio)-*N′*-(4-methylbenzylidene)acetohydrazide (6b).** White crystals, yield 85%, mp 224-225 °C. IR (KBr): = 3198 (N-H), 2217 (C≡N), 1663 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$) *δ/ppm*: 2.31 (s, 3H, $CH_3$), 2.44 (s, 3H, $CH_3$), 2.53 (s, 3H, $CH_3$), 4.43 (s, 2H, $CH_2$), 7.31 (d, *J* = 8.40 Hz, 2H, Ar-H), 7.06 (s, 1H, pyridine H-5), 7.78 (d, *J* = 8.40 Hz, 2H, Ar-H), 8.08 (s, 1H, CH=N), 11.35 (s, 1H, NH). MS *m/z* (%): 339 ($M^+$+1, 17.27), 338 ($M^+$, 100.00), 250 (17.23), 205 (43.14), 177 (60.75), 133 (24.81), 118 (25.47), 106 (36.70), 104 (81.04), 103 (54.37), 90.96 (31.01), 90.11 (32.45), 79 (32.87), 78 (71.23), 77 (66.37), 65 (29.03), 51 (27.15). Anal. Calcd for $C_{18}H_{18}N_4OS$ (338.43): C, 63.88; H, 5.36; N, 16.56%. Found: C, 63.96; H, 5.38; N, 16.48%.

**2-((3-Cyano-4,6-dimethylpyridin-2-yl)thio)-*N′*-(4-methoxybenzylidene)acetohydrazide (6c).** White crystals, yield 79%, mp 196-197 °C. IR (KBr): 3194 (N-H), 2214 (C≡N), 1661 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$) *δ/ppm*: 2.38 (s, 3H, $CH_3$), 2.43 (s, 3H, $CH_3$), 3.78 (s, 3H, $OCH_3$), 4.52 (s, 2H, $CH_2$), 6.97 (d, *J* = 8.00 Hz, 2H, Ar-H), 7.04 (s, 1H, pyridine H-5), 7.60 (d, *J* = 8.00 Hz, 2H, Ar-H), 7.98 (s, 1H, CH=N), 11.47 (s, 1H, NH). Anal. Calcd for $C_{18}H_{18}N_4O_2S$ (354.43): C, 61.00; H, 5.12; N, 15.81%. Found: C, 61.19; H, 5.07; N, 15.90%.

**2-((3-Cyano-4,6-dimethylpyridin-2-yl)thio)-*N′*-(4-nitrobenzylidene)acetohydrazide (6d).** Pale yellow crystals, yield 83%, mp 220-221 °C. IR (KBr): 3208 (N-H), 2216 (C≡N), 1685 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$) *δ/ppm*: 2.38 (s, 3H, $CH_3$), 2.44 (s, 3H, $CH_3$), 4.55 (s, 2H, $CH_2$), 7.82 (d, *J* = 8.50 Hz, 2H, Ar-H), 7.08 (s, 1H, pyridine H-5), 8.21 (d, *J* = 8.50 Hz, 2H, Ar-H), 8.38 (s, 1H, CH=N), 11.56 (s, 1H, NH). MS *m/z* (%): 369 (5.07), 204 (35.33), 177 (100.00), 164 (58.13), 131(27.83), 104 (9.18), 63 (10.97). Anal. Calcd for $C_{17}H_{15}N_5O_3S$ (369.40): C, 55.28; H, 4.09; N, 18.96%. Found: C, 55.13; H, 4.12; N, 18.88%.

**Synthesis of 4,6-dimethyl-2-((2-(3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethyl)thio)-nicotinonitrile (7).** A suspension of 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetohydrazide **5** (0.47 g, 0.002 mol) and ethyl acetoacetate (0.26 mL, 0.002 mol) in EtOH (15 mL) was refluxed for 4 h. The precipitate that formed upon cooling to 25 °C was filtered, dried and recrystallized from 20 mL EtOH. White solid, yield 60%, mp 128-130 °C. IR (KBr): 2216 (C≡N), 1741 and 1666 $cm^{-1}$ (C=O). $^1$H NMR ($CDCl_3$) *δ/ppm*: 1.95 (s, 3H, $CH_3$), 2.49 (s, 3H, $CH_3$), 2.57 (s, 3H, $CH_3$), 3.45 (s, 2H, $CH_2$), 4.43 (s, 2H, $CH_2$), 6.94 (s, 1H, pyridine H-5). Anal. Calcd for $C_{14}H_{14}N_4O_2S$ (302.35): C, 55.62; H, 4.67; N, 18.53%. Found: C, 55.50; H, 4.70; N, 18.61%.

**Synthesis of 4,6-dimethyl-2-((5-oxo-4-(2-arylhydrazono)-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethyl)-thio)nicotinonitriles 8a-c.**

**Method (A).** A well-stirred suspension of the appropriate aromatic amine (namely; aniline, *p*-toluidine and *p*-anisidine) (0.002 mol) in concentrated HCl (0.6 mL) was cooled in an ice-bath at 0-5 °C, then solution of $NaNO_2$ (0.14 g in 15 ml $H_2O$) was gradually added. The obtained cold solution was added dropwise to a well-stirred suspension of 4,6-dimethyl-2-((2-(3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethyl)thio)nicotinonitrile (**7**) (0.6 g, 0.002 mol) and sodium acetate (0.6 g) in 25 mL EtOH with stirring in an ice-bath at 0-5 °C for 1 h and then kept in refrigerator for 12 h. The precipitate that obtained was separated by filtration technique and then recrystallized from EtOH-DMF mixture (2:1).

**Method (B).** A solution of 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetohydrazide (**5**) (0.47 g, 0.002 mol) ) and the appropriate ethyl 3-oxo-2-(2-arylhydrazono)butanoate (0.002 mol) in 20 mL EtOH was heated under reflux for 2 h. The reaction mixture was cooled to 25 °C and the resulting precipitate was filtered and dried.

**4,6-Dimethyl-2-((2-(3-methyl-5-oxo-4-(2-phenylhydrazono)-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxo-ethyl)thio)nicotinonitrile (8a).** Orange powder, yield 80%, mp 195-200 °C. IR (KBr): 3172 (N-H), 2215 (C≡N), 1754 and 1663 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$) *δ/ppm*: 2.30 (s, 3H, $CH_3$), 2.41 (s, 3H, $CH_3$), 2.46 (s, 3H, $CH_3$), 4.62 (s, 2H, $CH_2$), 7.06 (s, 1H, pyridine H-5), 7.16-7.40 (m, 5H, Ar-H), 12.87 (s, 1H, NH). MS *m/z* (%): 406 ($M^+$, 2.91), 205 (95.51), 204 (100.00), 202 (50.48), 177 (50.61), 176 (20.10), 164 (13.30), 131 (18.75), 125 (47.47), 104 (7.94), 77 (7.95). Anal. Calcd for $C_{20}H_{18}N_6O_2S$ (406.46): C, 59.10; H, 4.46; N, 20.68%. Found: C, 59.27; H, 4.40; N, 20.79%.

**4,6-Dimethyl-2-((2-(3-methyl-5-oxo-4-(2-(*p*-tolyl)hydrazono)-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxo-ethyl)thio)nicotinonitrile (8b).** Orange powder, yield 60%, mp 189-190 °C. IR (KBr): 3189 (NH), 2218 (C≡N), 1753 and 1661 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$) *δ/ppm*: 2.26 (s, 3H, $CH_3$), 2.30 (s, 3H, $CH_3$), 2.39 (s, 3H, $CH_3$), 2.44 (s, 3H, $CH_3$), 4.68 (s, 2H, $CH_2$), 7.08 (s, 1H, pyridine H-5), 7.24 (d, *J* = 8.50 Hz,

2H, Ar-H), 7.55 (d, *J* = 8.50 Hz, 2H, Ar-H), 13.11 (s, 1H, NH). Anal. Calcd for $C_{21}H_{20}N_6O_2S$ (420.49): C, 59.98; H, 4.79; N, 19.99%. Found: C, 59.85; H, 4.76; N, 19.86%.

**2-((2-(4-(2-(4-Methoxyphenyl)hydrazono)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethyl)-thio)-4,6-dimethylnicotinonitrile (8c).** Reddish orange powder, yield 87%, mp 190-191 °C. IR (KBr): 3194 (NH), 2218 (C≡N), 1732 and 1657 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$) *δ/ppm*: 2.26 (s, 3H, $CH_3$), 2.29 (s, 3H, $CH_3$), 2.39 (s, 3H, $CH_3$), 3.77 (s, 3H, $OCH_3$), 4.69 (s, 2H, $CH_2$), 7.02 (d, *J* = 9.00 Hz, 2H, Ar-H), 7.08 (s, 1H, pyridine H-5), 7.65 (d, *J* = 9.00 Hz, 2H, Ar-H), 13.20 (s, 1H, NH). Anal. Calcd for $C_{21}H_{20}N_6O_3S$ (436.49): C, 57.79; H, 4.62; N, 19.25%. Found: C, 57.79; H, 4.62; N, 19.25%.

**Synthesis of 2-(2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetyl)-*N*-phenylhydrazine-1-carbothioamide (9).** To a suspension of 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetohydrazide (**5**) (0.47 g, 0.002 mol) in 15 mL EtOH, phenyl isothiocyanate (0.24 mL, 0.002 mol) was added, and the reaction mixture was refluxed for 2 h. After which, the reaction mixture was left to cool to 25 °C and the precipitate that formed was filtered off and dried to furnish compound **9**. White crystals, yield 80%, mp 198-200 °C. IR (KBr): 3319, 3221, 3155 (N-H), 2217 (C≡N), 1663 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$) *δ/ppm*: 2.40 (s, 3H, $CH_3$), 2.46 (s, 3H, $CH_3$), 4.09 (s, 2H, $CH_2$), 7.10 (s, 1H, pyridine H-5), 7.16-7.38 (m, 5H, Ar-H), 7.57 (s, 1H, NH), 9.72 (s, 1H, NH), 10.29 (s, 1H, NH). Anal. Calcd for $C_{17}H_{17}N_5OS_2$ (371.48): C, 54.97; H, 4.61; N, 18.85%. Found: C, 54.79; H, 4.65; N, 18.74%.

**Synthesis of 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)-*N′*-(4-oxo-3-phenylthiazolidin-2-ylidene)-acetohydrazide (10).** A suspension of 2-(2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetyl)-*N*-phenyl-hydrazine-1-carbothioamide **(9)** (1.11 g, 0.003 mol) and chloroacetic acid (0.28 g, 0.003 mol) in 15 mL acetic acid containing anhydrous sodium acetate (0.5 g) was heated under reflux for 3 h. After which, the reaction mixture was cooled to 25 °C and then diluted by ice water. The yellow solid that obtained was separated by filtration technique filtered, and then recrystallized from EtOH to furnish acetohydrazide derivative **10**. Yellow solid, yield 33%, mp 119-120 °C. IR (KBr): 3235 (N-H), 2216 (C≡N), 1718 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$) *δ/ppm*: 2.31 (s, 3H, $CH_3$), 2.35 (s, 3H, $CH_3$), 3.95 (s, 2H, thiazolidinone-$CH_2$), 4.61 (s, 2H, $CH_2$), 7.04 (s, 1H, pyridine H-5), 7.41-7.55 (m, 5H, Ar-H), 10.30 (s, 1H, NH). Anal. Calcd for $C_{19}H_{17}N_5O_2S_2$ (411.50): C, 55.46; H, 4.16; N, 17.02%. Found: C, 55.31; H, 4.11; N, 17.11%.

**Synthesis of 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)-*N′*-(3,4-diphenylthiazol-2(3*H*)-ylidene)-acetohydrazide (11).** To a suspension of 2-(2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetyl)-*N*-phenylhydrazine-1-carbothioamide **(9)** (0.37 g, 0.001 mol) and phenacyl chloride (0.15 g, 0.001 mol) in

15 mL EtOH, five drops of triethylamine were added. The reaction suspension was subjected to heating under reflux for 2 h, and then cooled to 25 °C. The precipitate that formed was filtered off, dried and recrystallized from EtOH. Yellow crystals, yield 47%, mp 160-161 °C. IR (KBr): 3439 (N-H), 2218 (C≡N), 1684 $cm^{-1}$ (C=O). $^1$H NMR ($CDCl_3$) *δ/ppm*: 2.40 (s, 6H, $2CH_3$), 4.67 (s, 2H, $CH_2$), 4.95 (s, 1H, CH=C), 6.76 (s, 1H, pyridine H-5), 7.33-7.64 (m, 9H, Ar-H and NH), 8.03 (d, 2H, Ar-H). Anal. Calcd for $C_{25}H_{21}N_5OS_2$ (471.60): C, 63.67; H, 4.49; N, 14.85%. Found: C, 63.49; H, 4.41; N, 14.96%.

**Synthesis of *N'*-acetyl-2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetohydrazide (12).** A suspension of 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetohydrazide (**5**) (0.47 g, 0.002 mol) in 10 mL acetic anhydride was heated on water bath for 3 h. After which, the reaction mixture was left to cool to 25 °C. The precipitate that formed, upon dilution with 30 mL ice water was filtered, dried and recrystallized from EtOH. White solid, yield 77%, mp 200-202 °C. IR (KBr): 3230 (N-H), 2215 (C≡N), 1727, 1679 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$) *δ/ppm*: 2.21 (s, 3H, $CH_3$), 2.40 (s, 3H, $CH_3$), 2.45 (s, 3H, $CH_3$), 4.14 (s, 2H, $CH_2$), 7.12 (s, 1H, pyridine H-5), 10.74 (s, 1H, NH). Anal. Calcd for $C_{12}H_{14}N_4O_2S$ (278.33): C, 51.78; H, 5.07; N, 20.13%. Found: C, 51.91; H, 5.10; N, 20.21%.

**Synthesis of 2-chloro-*N'*-(2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetyl)acetohydrazide (13).** To a suspension of 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetohydrazide (**5**) (0.47 g, 0.002 mol) in 15 mL acetone, chloroacetyl chloride (0.24 mL, 0.003 mol) was added, and the reaction mixture was stirred for 2 h. After which, the solid product that formed, upon dilution with 30 mL ice water, was filtered and purified by heating in EtOH. White solid, yield 66%, mp 198-200 °C. IR (KBr): 3177, (N-H), 2216 (C≡N), broad centered at 1612 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$) *δ/ppm*: 2.39 (s, 3H, $CH_3$), 2.47 (s, 3H, $CH_3$), 4.03 (s, 2H, $CH_2$), 4.11 (s, 2H, $CH_2$), 7.10 (s, 1H, pyridine H-5), 10.38 (s, 2H, 2NH). Anal. Calcd for $C_{12}H_{13}ClN_4O_2S$ (312.77): C, 46.08; H, 4.19; N, 17.91%. Found: C, 46.20; H, 4.14; N, 17.78%.

**Synthesis of *N'*-(2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetyl)benzenesulfonohydrazide (14).** A suspension of 2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetohydrazide **5** (0.24 g, 0.001 mol) and benzenesulfonyl chloride (0.14 mL, 0.001 mol) was stirred in 20 mL acetone for 2 h at room temperature . The precipitate that formed was filtered and purified by heating in 20 mL EtOH. White solid, yield 56%, mp 239-240 °C. IR (KBr): 3223 (N-H), 2214 (C≡N), 1644 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$): 2.40 (s, 3H, $CH_3$), 2.45 (s, 3H, $CH_3$), 4.06 (s, 2H, $CH_2$), 6.38 (s, 1H, NH), 7.09 (s, 1H, pyridine H-5), 7.31-7.88 (m, 5H, Ar-H), 10.31 ppm (s, 1H, NH). Anal. Calcd for $C_{16}H_{16}N_4O_3S_2$ (376.45): C, 51.05; H, 4.28; N, 14.88%. Found: C, 51.17; H, 4.22; N, 14.96%.

**Synthesis of *N*-(4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)benzofuran-2-carboxamide (17).** To a stirred solution of chloroacetamide derivative **16** (0.71 g, 0.003 mol) in 25 mL DMSO, salicylaldehyde (0.36 mL, 0.003 mol) and anhydrous potassium carbonate (0.82 g, 0.006 mol) were added. The reaction mixture was stirred overnight, and then poured into ice water drop by drop. The resulting precipitate was filtered, and then recrystallized from mixture of EtOH and DMF (2:1). Deep green powder, yield 42%, mp > 300 °C. IR (KBr): 3380, 3257 (N-H), 1690 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$) *δ/ppm*: 2.35 (s, 3H, $CH_3$), 2.48 (s, 3H, $CH_3$), 4.97 (s, 1H, furan H-3), 6.83 (s, 1H, pyridine H-5), 7.13-7.75 (m, 4H, Ar-H), 10.52 (s, 1H, NH), 13.20 (s, 1H, NH). Anal. Calcd for $C_{17}H_{14}N_4O_2$ (306.33): C, 66.66; H, 4.61; N, 18.29%. Found: C, 66.78; H, 4.54; N, 18.42%.

**Synthesis of ethyl 2-((2-((4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)amino)-2-oxoethyl)-thio)acetate (18).** A suspension of chloroacetamide derivative **16** (0.47 g, 0.002 mol), ethyl 2-mercaptoacetate (0.24 mL, 0.002 mol) and sodium acetate (0.25 g, 0.003 mol) in a mixture of 15 mL EtOH and 5 mL *N*,*N*-dimethylformamide (DMF) were refluxed for 4 h. The precipitate that formed, upon dilution with 30 mL ice water, was filtered and dried. White solid, yield 86%, mp 188-190 °C. IR (KBr): 3255 (N-H), 1724, 1665 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$) *δ/ppm*: 1.21 (t, *J* = 7.0 Hz, 3H, $CH_3$), 2.46 (s, 3H, $CH_3$), 2.51 (s, 3H, $CH_3$), 3.50 (s, 2H, $CH_2$), 3.54 (s, 2H, $CH_2$), 4.13 (q, *J* = 7.0 Hz, 2H, $CH_2$), 6.84 (s, 1H, pyridine H-5), 10.19 (s, 1H, NH), 13.13 (s, 1H, NH). Anal. Calcd for $C_{14}H_{18}N_4O_3S$ (322.38): C, 52.16; H, 5.63; N, 17.38%. Found: C, 52.02; H, 5.70; N, 17.25%.

**2-(Benzo[*d*]oxazol-2-ylthio)-*N*-(4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)acetamide (19).** A suspension of chloroacetamide derivative **16** (0.47 g, 0.002 mol), 2-mercaptobenzoxazole (0.30 g, 0.002 mol) and sodium acetate (0.25 g, 0.003 mol) in a mixture of 15 mL EtOH and 5 mL DMF was refluxed for 4 h. The precipitate that formed, upon dilution with 30 mL ice water, was filtered and dried. Pale brown solid, yield 66%, mp > 300 °C. IR (KBr): 3262, 3202 (N-H), 1659 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$) *δ/ppm*: 2.41 (s, 3H, $CH_3$), 2.48 (s, 3H, $CH_3$), 3.92 (s, 2H, $CH_2$), 6.88 (s, 1H, pyridine H-5), 7.30-7.72 (m, 4H, Ar-H), 10.28 (s, 1H, NH), 13.04 (s, 1H, NH). Anal. Calcd for $C_{17}H_{15}N_5O_2S$ (353.40): C, 57.78; H, 4.28; N, 19.82%. Found: C, 57.97; H, 4.36; N, 19.68%.

**Synthesis of 2-((4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)imino)thiazolidin-4-one (20).** To suspension of chloroacetamide derivative **19** (1.19 g, 0.005 mol) in a mixture of 15 mL EtOH and 5 mL DMF, ammonium thiocyanate (0.53 g, 0.007 mol) was added and refluxed for 4 h. The obtained precipitate that formed upon cooling was filtered, dried and recrystallized from AcOH. Brown powder, yield 61%, mp > 300 °C. IR (KBr): 3282, 3171 (N-H), 1717 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$) *δ/ppm*:

2.51 (s, 3H, $CH_3$), 2.62 (s, 3H, $CH_3$), 3.98 (s, 2H, $CH_2$), 6.82 (s, 1H, pyridine H-5), 11.85 (s, 1H, NH), 12.94 (s, 1H, NH). Anal. Calcd for $C_{11}H_{11}N_5OS$ (261.31): C, 50.56; H, 4.24; N, 26.80%. Found: C, 50.40; H, 4.31; N, 26.94%.

**Synthesis of 2-amino-1-(4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-5-oxo-4,5-dihydro-1*H*-pyrrole-3-carbonitrile (21).** To a solution of chloroacetamide derivative **19** (0.47 g, 0.002 mol) and malononitrile (0.13 g, 0.002 mol) in a mixture of 15 mL EtOH and 5 mL DMF, five drops of triethylamine were added, then the reaction mixture was refluxed for 4 h. After which, the reaction mixture was poured into ice-water drop by drop and neutralized with dilute HCl. The precipitate that formed was filtered and dried. Reddish brown solid, yield 34%, mp > 300 °C. IR (KBr): 3284, 3212 (NH and $NH_2$), 2200 (C≡N), 1741 $cm^{-1}$ (C=O). $^1$H NMR (DMSO-$d_6$) *δ/ppm*: 2.62 (s, 3H, $CH_3$), 2.71 (s, 3H, $CH_3$), 3.52 (s, 2H, $CH_2$), 6.91 (s, 2H, $NH_2$), 7.00 (s, 1H, pyridine H-5), 10.92 (s, 1H, NH). Anal. Calcd for $C_{13}H_{12}N_6O$ (268.28): C, 58.20; H, 4.51; N, 31.33%. Found: C, 58.32; H, 4.47; N, 31.24%.

## REFERENCES


1. L. D. Quin and J. A. Tyrell, *Fundamentals of Heterocyclic Chemistry: Importance in Nature and in the Synthesis of Pharmaceuticals*, John Wiley & Sons, 2010.
2. C. González-Bello and L. Castedo, *Six-Membered Heterocycles: Pyridines.* Modern Heterocyclic Chemistry, 2011, 1431.
3. M. A. Radwan, M. A. Alshubramy, M. Abdel-Motaal, B. A. Hemdan, and D. S. El-Kady, *Bioorg. Chem*., 2020, **96**, 103516.
4. S. Eryılmaz, E. T. Çelikoğlu, Ö. İdil, E. İnkaya, Z. Kozak, E. Mısır, and M. Gül, *Bioorg. Chem*., 2020, **95**, 103476.
5. N. F. Neamah, A. R. N. Khudair, and S. Al-Jadaan, *Egyptian J. Chem*., 2019, 18.
6. C. S. McCrae, A. Ross, A. Stripling, and N. D. Dautovich. *Clin. Intervent. Aging*, 2007, **2**, 313.
7. T. S. Harrison and L. J. Scott, *Drugs*, 2005, **65**, 2309.
8. R. Eastell, B. Vrijens, D. L. Cahall, J. D. Ringe, P. Garnero, and N. B. Watts, *J. Bone Miner. Res*., 2011, **26**, 1662.
9. G. F. Watts and D. C. Chan, *Arterioscler.Thromb. Vasc. Biol*., 2008, **28**, 1892.
10. P. S. Gillies and C. J. Dunn, *Drugs*, 2000, **60**, 333.
11. F. Horak, U. P. Stübner, R. Zieglmayer, and A. G. Harris, *Allergy Clin. Immunol*., 2002, **109**, 956.
12. D. C. Metz, M. Vakily, T. Dixit, and D. Mulford, *Aliment. Pharmacol. Ther*., 2009, **29**, 928.
13. G. Sachs, J. M. Shin, and R. Hunt, *Curr. Gastroenterol. Rep*., 2010, **12**, 437.
14. F. Stegmeier, M. Warmuth, W. R. Sellers, and M. Dorsch, *Clin. Pharmacol. Ther*., 2010, **87**, 543.

15. J. A. Bull, J. J. Mousseau, G. Pelletier, and A. B. Charette, *Chem. Rev*., 2012, **112**, 2642.
16. M. H. Ahmed, M. A. El‐Hashash, M. I. Marzouk, and A.M. El‐Naggar, *J. Heterocycl. Chem*., 2019, **56**, 114.
17. H. N. Hafez and A. R., El-Gazzar, *Curr. Org. Synth.*, 2020, **17**, 55.
18. T. Sharma, V. Kumar, S. Bawa, V. Kumar, J. Singh, R. Kataria, B. Singh, and V. Kumar, *Chem. Data Coll.*, 2020, **28**, 100408.
19. H. Majed, T. Johnston, C. Kelso, E. Monachino, S. Jergic, N. E. Dixon, E. Mylonakis, and M. J. Kelso, *Bioorg. Med. Chem. Lett.*, 2018, **28**, 3526.
20. A. Ahmed, K. I. Molvi, H. M. Patel, R. Ullah, and A. Bari, *J. Infect. Public Heal.*, 2020, **13**, 472.
21. E. Abdel-Latif, A. A. Almatari, and G. E. Abd-ElGhani, *J. Heterocycl. Chem*., 2019, **56**, 1978.
22. Z. Xu, S. Zhao, Z. Lv, L. Feng, Y. Wang, F. Zhang, L. Bai, and J. Deng, *Eur. J. Med. Chem.*, 2019, **162**, 266.
23. N. R. Stokes, N. Baker, J. M. Bennett, P. K. Chauhan, I. Collins, D. T. Davies, M. Gavade, D. Kumar, P. Lancett, R. Macdonald, and L. MacLeod, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 353.
24. S. D. Joshi, H. M. Vagdevi, V. P. Vaidya, and G. S. Gadaginamath, *Eur. J. Med. Chem.*, 2008, **43**, 1989.
25. F. A. Yassin, *Chem. Heterocycl. Compd*., 2009, **45**, 35.
26. H. A. Abel-Aziza, B. F. Abel-Wahab, and F. A. Badira, *Arch. Pharm.*, 2010, **343**, 152.
27. M. J. Ahsan, J. G. Samy, C. B. Jain, K. R. Dutt, H. Khalilullah, and M. S. Nomani, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 969.
28. E. M. Sarshira, N. M. Hamada, Y. M. Moghazi, and M. M. Abdelrahman, *J. Heterocycl. Chem.*, 2016, **53**, 1970.
29. Y.-W. Ho and M.-C. Suen, *J. Chin. Chem. Soc.*, 2009, **56**, 408.
30. X.-E. Jian, F. Yang, C.-S. Jiang, W.-W. You, and P.-L. Zhao, *Bioorg. Med. Chem. Lett.*, 2020, **30**, 127025.
31. C. Chen, P. Pan, Z. Deng, D. Wang, Q. Wu, L. Xu, T. Hou, and S. Cui, *Bioorg. Med. Chem. Lett.*, 2019, **29**, 912.
32. N. S. El-Gohary, M. T. Gabr, and M. I. Shaaban, *Bioorg. Chem.*, 2019, **89**, 102976.
33. L. Li, W. Zhang, F. Lin, X. Lu, W. Chen, X. Li, X. Zhou, R. Su, L. Wang, Z. Zheng, and S. Li, *Eur. J. Med. Chem.*, 2019, **173**, 107.
34. G. G. El-Bana, H. H. Zoorob, M. E. Ibrahim, and W. S. Hamama, *Synth. Commun.*, 2020, https://doi.org/10.1080/00397911.2020.1786126.
35. M. A. Metwally, H. A. Etman, H. E. Gafer, and A. M. Khali, *Chem. Heterocycl. Compd*., 2008, **44**, 715.

36. S. I. Stylianakis, A. Kolocouris, N. Kolocouris, G. Fytas, G. B. Foscolos, E. Padalko, J. Neyts, and E. De Clercq, *Bioorg. Med. Chem. Lett*., 2003, **13**, 1699.