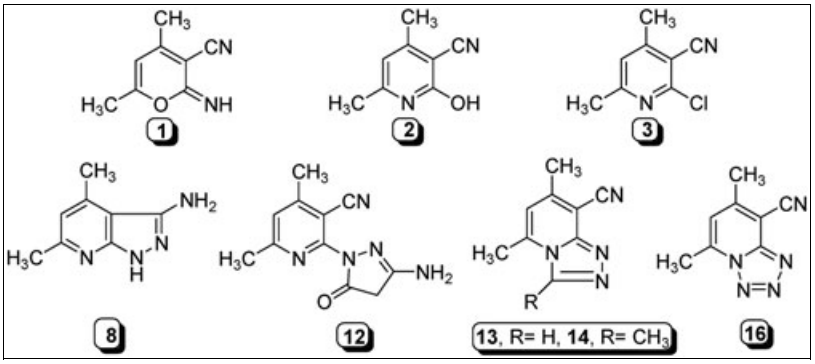
Synthesis and Antitumor Evaluation of Some New Fused and Binary Pyridines

Mohamed A. Waly,[a] Ibrahim I. EL-Hawary,[a] Wafaa S. Hamama,[b]* and Hanafi H. Zoorob[a]

[a]Department of Chemistry, Faculty of Science (Damietta), Mansoura University, Egypt
[b]Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, ET-35516, Egypt
*E-mail: wshamama@yahoo.com

Received December 21, 2010
DOI 10.1002/jhet.1020
Published online 10 April 2013 in Wiley Online Library (wileyonlinelibrary.com).



Syntheses of some new heterocyclic compounds containing pyridone, thioxopyridine, halogenated-pyridine-carbonitriles, pyrazolopyridine, and pyridine derivatives were achieved. Besides, a modified synthetic method for the synthesis of 2-chloro-4,6-dimethyl-nicotinonitrile (**3**) through the reaction of acetylacetone and malononitrile as starting materials was implemented. The reaction of 2-chloronicotinonitrile **3** with substituted amines to 2-aminonicotinonitrile were also investigated. Fused or binary pyridines were tested for cytotoxicity against well-known established model Ehrlich ascites cells *in vitro*. Compound **13** exhibited a high antitumor activity compared with 5-fluorouracil.

J. Heterocyclic Chem., **50**, E12 (2013).

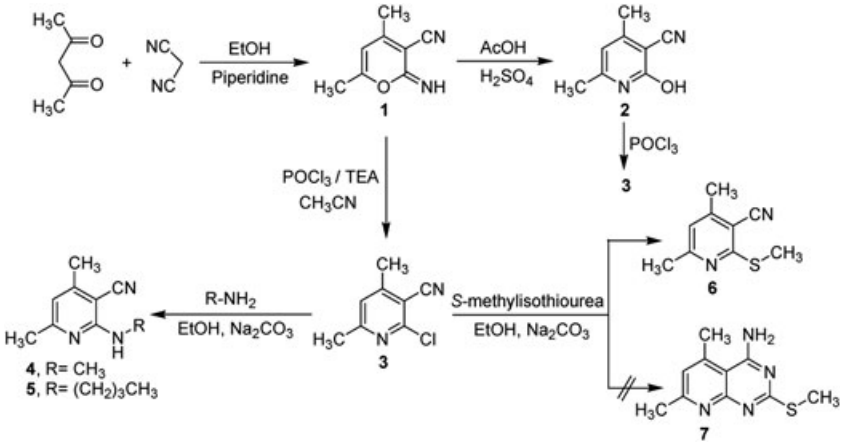
INTRODUCTION

Synthesis of the pyridine ring system and its derivatives occupy an important place in the realm of synthetic organic chemistry due to their therapeutic and pharmacological properties [1–3]. They have emerged as integral backbones of over 7000 existing drugs [4]. The pyridine ring is also an integral part of anticancer and anti-inflammatory agents [5, 6]. Moreover, tetralins "tetrahydronaphthalene" derivatives are of increasing interest as many of these compounds play a vital role in the biological activities, because of their biological potentialities; for example, as potent agonists for D2-type receptors [7], as a treatment of Alzheimer's disease [8], cardiovascular diseases [9], and as a preventer of dopamine-induced cell death [10]. On the other hand, cyanopyridone and cyanopyridine derivatives have promising antimicrobial [11] and anticancer activities [12]. The interest in 3-cyano-2(1*H*)-pyridone and their derivatives is due to their wide range of practical uses as medicinal compounds. Recently, new pyridine carbonitriles were reported as anti-inflammatory agents [13] and pyrazolopyridine derivatives have been recently reported as antitumor agents [14].

RESULTS AND DISCUSSION

In this work, we developed a convenient method for the synthesis of 2-chloro-4,6-dimethylpyridine-3-carbonitrile (**3**) [15], through a reduced reaction sequence. Its synthesis including the condensation of acetyl acetone with malononitrile in the presence of a catalytic amount of piperidine afforded 2-imino-4,6-dimethyl-2*H*-pyran-3-carbonitrile (**1**) followed by rearrangement of the iminopyran derivative **1** in a mixture of acetic acid and sulfuric acid gave to afford hydroxynicotinonitrile derivative **2**. Moreover, refluxing of compound **2** with phosphorous oxychloride in acetonitrile catalyzed by triethylamine (TEA) afforded 2-chloronecotinonitrile derivative **3** in good yield (82%). The conversion of **1** to **2** was attributed to the diministring yield of **2** (30%) which reduces the yield of its conversion to 2-chloronicotinonitrile **3**, thus, lowering the overall yield of obtaining **3** through this synthetic pathway. Consequently, in this work, we alleviated the rearrangement step of compounds **1–2** by chlorination of **1** with phosphorous oxychloride in acetonitrile catalyzed by TEA where by 2-chloronicotinonitrile derivative **3** was obtained with remarkably improved yield up to 96%

Scheme 1. Synthesis of 2-chloro-4,6-dimethylnicotinonitrile (**3**) and its reactions with amines.



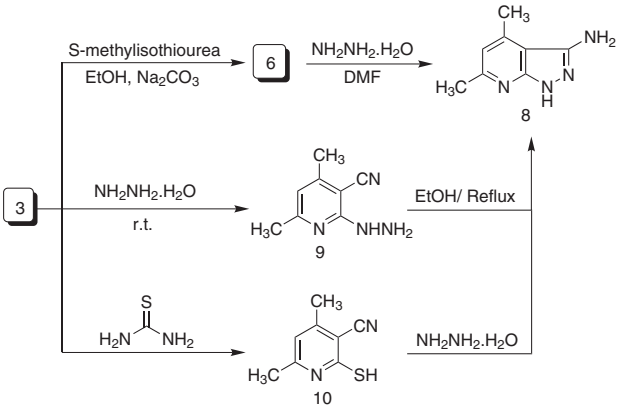
(Scheme 1). The ^{1}H-NMR spectra of iminopyran derivative **1** and hydroxynicotinonitrile **2** showed the presence of exchangeable (NH) and hydroxy protons at δ 12.3 and 12.2 ppm, respectively. Accordingly, 2-aminonicotinonitrile derivatives were synthesized through the condensation of the corresponding amines with 2-chloronicotinonitrile derivative **3**.

Aminonicotinonitriles were used as inhibitors for nitrogen activated protein kinase-2 for treating TNFα-mediated diseases in particular inflammations such as arthritis [16]. Thus, the reactions of 2-chloronicotinonitrile derivative **3** with methylamine, *n*-butylamine, ethylenediamine, and *S*-methylisothiourea were implemented. Refluxing of **3** with methylamine or butyl amine in ethanol-containing Na_2CO_3 afforded the 2-aminonicotinonitrile derivatives **4** and **5**, respectively (Scheme 1). Their ^{1}H-NMR spectra showed exchangeable (NH) protons at 4.0 and 5.0 ppm, respectively. The IR spectra of **4** and **5** showed absorption bands at 2203 and 2206 cm^{-1} attributable to cyano groups, respectively. Furthermore, condensation of **3** with *S*-methylisothiourea in ethanol and in the presence of sodium carbonate afforded the *S*-methylnicotinonitrile derivative **6** instead of the fused pyridopyrimidine derivative **7** (Scheme 1). The IR spectrum of **6** showed the presence of the cyano absorption band at 2200 cm^{-1}. Its ^{1}H-NMR spectrum showed signals at δ 2.3 (s, 3H, CH_3-C═N), 2.5 (s, 3H, CH_3-C═C), and 2.6 ppm (s, 3H, *S*-CH_3; Scheme 1).

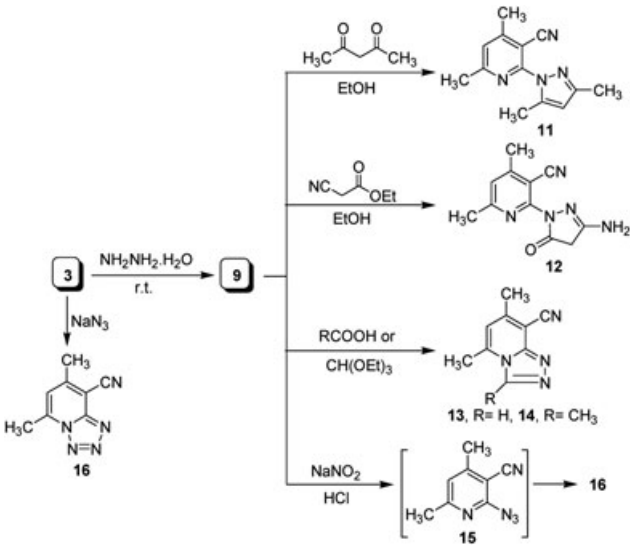
On the other hand, the preparation of 2-hyrazinoylnicotinonitrile **9** was achieved by condensation of **3** with hydrazine hydrate at room temperature. However, the formation of pyrazolopyridine derivative **8** was conducted by refluxing of **9** in ethanol (Scheme 2). Its IR spectrum showed the absorption bands at 2212 cm^{-1} attributable to cyano group besides absorption bands at 3392, 3327, and 3290 cm^{-1} corresponding to (NH) and (NH_2) groups, respectively. The ^{1}H-NMR spectrum showed two singlet signals at δ 5.0 and 11.1 ppm due to (NH_2) and (NH) protons, respectively. On the other hand, it has been reported that the reaction of **3** with thiourea afforded 2-mercapto-4,6-dimethyl-nicotinonitrile (**10**) which reacted with hydrazine hydrate to afford the corresponding pyrazolopyridine derivative **8** [15] (Scheme 2).

Once more, insecticides, such as imidacloprid and acetamprid (nicotinoids) [17], derived from pyridine act on the central nervous system of insects causing irreversible blockage of postsynaptic nicotenergic acetylcholine receptor. Fipronil (fiproles) [18] derived from pyrazole blocks the γ-aminobutyric acid (GABA) which regulated chloride channel in neurons, there by antagonizing the calming effects of GABA. The substitution pattern for the pyridine nucleus at the 2- or 3-position by different heterocyclic moieties markedly modulates its biological properties. Also, it has been found that pyridine derivatives work as insecticidal

Scheme 2. Synthetic pathways for 4,6-dimethyl-1H-pyrazolo[3,4-b] pyridin-3-amine (**8**).



Scheme 3. Synthesis of fused or binary diazole, triazole, and tetrazole derivatives.



[19] and antifungal agents [20]. Furthermore, pyrazole and pyrazoline derivatives have also been found to exhibit insecticidal [21], antifungal [22], and antibacterial activities [23]. The effects of pyrazoline and pyrazole rings at position 2 of the pyridine nucleus enhanced insecticidal, antifungal, and antibacterial profiles of the compounds as compared to their parent nucleolus [24]. Hence, it may be concluded that pyrazolines and pyrazoles increases the insecticidal, antifungal, and antibacterial activities. These findings prompted us to synthesize a new series of pyridine derivatives by incorporating pyrazole and pyrazoline moieties at position 2, with a hope to get a better potential insecticidal along with, antifungal, antibacterial, and biological activities. Subsequently, refluxing of 2-hydrazinyl-4,6-dimethylpyridine-3-carbonitrile (**9**) with acetylacetone or ethyl cyanoacetate in ethanol gave 4,6-dimethyl-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)pyridine-3-carbonitrile (**11**) and 2-(3-amino-4,5-dihydro-5-oxopyrazol-1-yl)-4,6-dimethylpyridine-3-carbonitrile (**12**), respectively (Scheme 3). The IR spectra of **11** and **12** showed cyano absorption bands at 2227 and 2260 cm^{-1}, respectively. These data reflected that the condensation of the hydrazide moiety could condense faster with the activated methylene carbonyl compounds than with the cyano group. Their ^{1}H-NMR spectra indicated the presence of the pyrazolyl C(4′)-H protons at δ 6.0 and 4.0 ppm, respectively, besides two singlet signals for compound **11** at δ 2.3 and 2.4 ppm due to C(3′)-CH_3 and C(5′)-CH_3 protons, respectively, finally, exchangeable protons for compound **12** at δ 3.8 and 4.2 ppm for (NH_2) and methylene protons, respectively.

Triazolopyridines are important class of biologically active heterocyclic compounds, which possess bactericidal [25], anxiolitic [26], and as inhibitors of mitogen-activated protein kinases [27, 28] or as growth hormone secretagogues [29]. They also can be used for the treatment of gastrointestinal disorders [30] and as antithrombotic agents [31].

Therefore, versatile and widely applicable methods for the synthesis of these heterocycles are of considerable interest. The preparations of these compounds are based on heterocyclic hydrazides as precursors. The [1,2,4]triazolo[4,3-*a*]pyridine ring system was prepared by different procedures starting from the hydrazide derivative **9**. Thus, the triazolopyridine derivative **13** was synthesized through refluxing of hydrazide **9** with triethylorthoformate in dimethylformamide. Alternatively, the hydrazide derivative **9** with excess formic or acetic acid afforded the [1,2,4]triazolo[4,3-a]pyridine derivatives **13** and **14**, respectively (Scheme 3). The IR spectra of **13** and **14** showed the cyano absorption bands at 2210 and 2213 cm^{-1}, respectively. The ^{1}H-NMR spectra of **13** showed the presence of C(1)-H proton at δ 6.4 ppm, whereas the ^{1}H-NMR spectra of **14** provided singlet signal at δ 2.9 ppm due to C(1)-CH_3 protons.

The tetrazole unit that is not commonly used in chemistry of natural products may be seen as a promising new un-natural pharmacopoeiain medicinal chemistry because of its metabolic stability and its unique structural and electronic feature [32]. The corresponding *N*-alkylated salts are used in photography, agriculture, and dyes [33]. The synthesis of tetrazolopyridines cited in literature from 2-chloro-4,6-diarylnicotininitrile and sodium azide in chlorobenzene and water catalyst by tricapryl-methylammonium chloride (Aliquat 336®) [34]. Phase transfer catalysis makes it possible to carry out azidolysis using sodium azide in a nonpolar solvent such as chlorobenzene. The products are obtained in quantitative yield in most cases; moreover, the solvent was also recoverable [34]. The same reaction was tried with different catalysts, such as tetrabutylammonium bromide, benzyltriethylammonium chloride, tributylbenzyl ammonium chloride, tetraethylammonium bromide, and cetyltrimethyl ammonium chloride, but none of these catalysts gave satisfactory results. Similar reaction conditions using toluene, 18-crown-6, and sodium azide found to increase the reaction time by 1 to 2 h [34].

Our attempts for the preparation of tetrazolopyridine derivatives by methods that do not contain catalyst were successful. Accordingly, the synthesis of tetrazolopyridine ring system **16** based on **3** was discussed. 5,7-Dimethyltetrazolo[1,5-*a*]pyridine-8-carbonitrile (**16**) was synthesized either by refluxing of **3** with sodium azide or by diazotization of the hydrazinyl derivative **9** with sodium nitrite in diluted hydrochloric acid afforded the same tetrazolopyridine derivative **16**. The last procedure passed through the formation of the intermediate **15**, which was cyclized *in situ* to the tetrazolopyridine derivative **16** (Scheme 3). The IR spectra of **16** indicated

Table 1

In vitro cytotoxicity of pyridine derivatives using EAC assay.

	% Dead		
Compounds	ED_{100} (μL)	ED_{50} (μL)	ED_{25} (μL)
5-FU	95.2	62.0	38.0
3	46.0	28.9	20.0
4	54.1	29.0	13.0
5	33.0	18.0	10.0
6	57.2	30.0	17.1
8	61.7	31.3	17.2
9	87.3	47.6	25.0
11	42.0	23.0	11.0
13	50.0	29.0	13.3
14	96.0	6.3	36.0
16	58.0	31.0	18.0

ED_{100}, ED_{50}, and ED_{25} are the effective doses at 25, 50, and 100 μL, respectively, of the compounds used. The dead % refers to the % of the dead tumor cells and **5-FU** is 5-fluorouracil as a well-known cytotoxic agent.

the presence of absorption band of cyano group at 2210 cm^{-1} and the tetrazolo ring at 3390 cm^{-1}. Its mass spectrum showed the molecular ion peak at *m/z* 173 (M^+, 86%; Scheme 3).

Biological activity. To examine whether the newly prepared compounds have a direct cytotoxic effect on Ehrlich ascites cells (EAC) viability, the percentage of viable cells was estimated by the trypan blue [35] exclusion test. Ten fused or binary pyridines were tested for cytotoxicity against well-known established model EAC *in vitro*. Results for the ED_{100}, ED_{50}, and ED_{25} values of the active compounds are summarized in Table 1. The data showed clearly that compounds **8**, **9**, and **13** showed high activities, whereas compounds **4**, **14**, and **16** showed moderate activity; on the other hand, the rest of compounds have weak activities. From the structure activity relationship (SAR), we found that incorporation of cyclic and acyclic (N-N) moiety into the position 2 of pyridine ring increases the activity of the tested compounds at high concentration. Methyl group in compound **14** decreases the activity by about its half-value than compound **13** at high concentration (Table 1).

CONCLUSION

The prepared new ring systems seem to be interesting for biological studies. Furthermore, the present investigation offers rapid and effective new procedures for the synthesis of a new class of fused or binary pyridines. The new compounds were investigated for their cytotoxicity against well-known established model EAC *in vitro*. Compound **13** exhibited a high antitumor activity compared with 5-fluorouracil.

EXPERIMENTAL

The completion of reaction was checked by tetramethylsilane (TMS) by using Silica gel (Merck F, 254) and spots were exposed to iodine vapor. Melting point (uncorrected) and were determined on a Gallenkamp melting point apparatus. The IR spectra were recorded on a Jasco 4100 FTIR spectrophotometer in KBr discs ($\upsilon_{max.}$ in cm^{-1}). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance 300 spectrometer operating at 300 and 75 MHz, respectively. Chemical shifts were expressed in δ scale downfield as part per million (ppm) and TMS is used as an internal standard. The mass spectrum was recorded on a Shimadzu GCMS-QP 1000EX mass spectrometer at 70 ev electron-impact ionization. Elemental analysis was recorded on PERKIN-ELMER 2400 (C, H, N, S, and O) elemental analyzer.

2-Imino-4,6-dimethyl-2*H*-pyran-3-carbonitrile (1). A mixture of acetylacetone (10.2 mL, 0.1 mol) and malononitrile (6.6 g, 0.1 mol) dissolved in ethanol (50 mL) and a catalytic amount of piperidine (three drops) was refluxed for 3 h. After cooling, the reaction mixture was poured into ice-cold water then the formed precipitate was filtered off, dried, and recrystallized from methanol to give compound **1**.

Yield (95%), mp 194–196°C; yellow powder; ^{1}H-NMR ($CDCl_3$): δ, 2.3 (s, 3H, CH_3-C═C), 2.5 (s, 3H, CH_3-C═N), 6.1 (s, H, pyran), 12.3 (s, H, NH).

2-Hydroxy-4,6-dimethylpyridine-3-carbonitrile (2). A mixture of **1** (3 g, 20 mmol), conc H_2SO_4 (1 mL), and acetic acid (10 mL) was refluxed for 3 h. The reaction mixture was evaporated under vacuum to remove excess acetic acid followed by addition of ethanol where by compound **2** was precipitated which was purified by crystallization from methanol.

Yield (30%); mp 285–287°C, (Lit. [15(a)] 286°C); white crystalline solid; ^{1}H-NMR ($CDCl_3$): δ, 2.3 (s, 3H, CH_3-C═C), 2.4 (s, 3H, CH_3-C═N), 6.1 (s, H, pyridyl), 12.2 (s, H, OH).

2-Chloro-4,6-dimethylpyridine-3-carbonitrile (3). ***Method (A).*** A mixture of 2-hydroxy-4,6-dimethylpyridine-3-carbonitrile (**2**) (3 g, 20 mmol) in acetonitrile (20 mL), excess $POCl_3$ (6 mL, 40 mmol), and a catalytic amount of TEA (2 mL, 20 mmol) was added, and the reaction mixture was refluxed for 3 h. After cooling, the mixture was left in air until complete evaporation of excess $POCl_3$. The reaction mixture was poured onto ice-cold water then the formed precipitate was filtered off, dried, and recrystallized from hexane to give compound **3**.

Yield (82%); mp 95–96°C, white crystals.

Method (B). A mixture of **1** (3.9 g, 20 mmol), acetonitrile (20 mL), TEA (2 mL, 20 mmol), and excess $POCl_3$ (6 mL, 40 mmol) was added, and the reaction mixture was refluxed for 2 h. After cooling, the reaction mixture was poured onto ice water; the formed precipitate was filtered off, dried, and recrystallized from hexane to give **3**.

Yield (96%); mp 95–96°C, [Lit. [15(b)] 94–95°C; Yield (77%)]; IR (KBr) ύ (cm^{-1}), 2223 (CN); ^{1}H-NMR ($CDCl_3$): δ, 2.4 (s, 3H, CH_3-C═C), 2.5 (s, 3H, CH_3-C═N), 7.0 (s, H, pyridyl).

Reaction of 2-chloronicotinonitrile derivative 3 with amines. ***General procedure.*** A mixture of **3** (20 mmol) and appropriate amine (1 mL), namely; methylamine or butylamine in ethanol (20 mL) containing Na_2CO_3 (1 g) was refluxed for 3 h. After cooling, the reaction mixture was poured into ice-cold water, and then the formed precipitate was filtered off,

dried, and recrystallized from aqueous ethanol to give the corresponding amino derivatives **4** and **5**, respectively.

4,6-Dimethyl-2-(methylamino)pyridine-3-carbonitrile (4). Yield (81%); mp 70–72°C; IR (KBr) ύ (cm^{-1}), 2202 (CN); 3390 (NH); ^{1}H-NMR ($CDCl_3$): δ, 2.3 (s, 3H, CH_3-C═C), 2.5 (s, 3H, NH-CH_3), 3 (s, 3H, CH_3-C═N), 4 (s, H, NH), 6.3 ppm (s, H, pyridyl); MS: (*m/z*, %): 161 (M^+, 100), 146 (2.5), 131 (4.6); Calcd. for $C_9H_{11}N_3$ (161.2): C 67.06, H 6.88, N 26.07%. Found: C 67.16, H 6.80, N 26.09%.

2-(Butylamino)-4,6-dimethylpyridine-3-carbonitrile (5). Yield (75%), mp 126–128°C; IR (KBr) ύ (cm^{-1}), 2206 (CN); 3367 (NH); ^{1}H-NMR ($CDCl_3$): δ, 0.9 (t, 3H, CH_2-CH_3), 1.3 (s, 2H, CH_2-CH_3), 1.6 (p, 2H, CH_2-CH_2-CH_3), 2.4 (s, 3H, CH_3-C═C), 2.5 (s, 3H, CH_3-C═N), 3.5 (t, 2H, NH-CH_2), 5.0 (s, H, NH), 6.3 (s, H, pyridyl), MS: (*m/z*, %): 203 (M^+, 100), 188 (18.5), 174 (13.8), 160 (31.1), 146 (28), 131 (2.4); Calcd. for $C_{12}H_{17}N_3$ (203.28): C 70.90, H 8.43, N 20.67%. Found: C 70.97, H 8.48, N 20.77%.

3-Cyano-2-methylmercapto-4,6-dimethylpyridine (6). A mixture of **3** (3.2 g, 20 mmol), *S*-methylisothiourea sulphate (5.56 g, 20 mmol), and Na_2CO_3 (3 g) in ethanol (20 mL) was refluxed for 4 h. The reaction mixture was left in air until complete evaporation of excess ethanol; the precipitate formed was collected and recrystallized from ethanol to give **6**.

Yield (70%); mp 81–83°C; IR (KBr) ύ (cm^{-1}), 2200 (CN); ^{1}H-NMR ($CDCl_3$): δ, 2.3 (s, 3H, CH_3-C═C), 2.5 (s, 3H, CH_3-C═N), 2.6 (s, 3H, S-CH_3), 6.7 (s, H, pyridyl), MS: (*m/z*, %): 178 (M^+, 100), 163 (25.3), 148 (33.6), 131 (2.5); Calcd. for $C_9H_{10}N_2S$ (178.25): C 60.64, H 5.65, N 15.72, S 17.99%. Found: C 60.69, H 5.75, N 15.70, S 17.91%.

4,6-Dimethyl-3-amino-3H-pyrazolo[3,4-b]pyridine (8). *Method (A).* A mixture of 2-mercapto-4,6-dimethylpyridine-3-carbonitrile (**10**) [15], (3.2 g, 20 mmol) or 2-methylmercapto-4,6-dimethylpyridine-3-carbonitrile (**6**) (3.6 g, 20 mmol), and hydrazine hydrate (50%, 50 mL) was refluxed in dimethylformamide (20 mL) for 3 h. The reaction mixture was left to cool, poured into ice water. The precipitate formed was filtrated off, washed with water, and recrystallized from ethanol to give **8**.

Yield (85%), (92%); mp 280–282°C, (Lit. [15] 280°C); IR (KBr) ύ (cm^{-1}), 1620 (C═N), 3179 (NH), 3286, 3387 (NH_2).

Method (B) A mixture of **9** (3.2 g, 20 mmol) and hydrazine hydrate (50%, 50 mL) in ethanol (20 mL) was refluxed for 3 h. The reaction mixture was left to cool and poured into ice water. The precipitate formed was filtrated off, washed with water, dried, and recrystallized from *n*-butanol to give **8**.

Yield (80%); yellow crystalline solid, identical data to that obtained in Method (A).

2-Hydrazinoyl-4,6-dimethylpyridine-3-carbonitrile (9). A mixture of **3** (3.2 g, 20 mmol) and hydrazine hydrate (5 mL, 100 mmol) in ethanol (20 mL) was stirred for 5 h, and the obtained solid was collected by filtration and dried to give **9** as yellow crystalline solid.

Yield (91%) with colored change, mp 130°C; IR (KBr) ύ (cm^{-1}), 2212 (CN), 3392 (NH), 3290, 3327 (NH_2); ^{1}H-NMR ($CDCl_3$): δ, 2.4 (s, 3H, CH_3-C═C), 2.5 (s, 3H, CH_3-C═N), 5.0 (s, 2H, NH_2), 6.5 (s, H, pyridyl), 11.7 (s, H, NH). MS: (*m/z*, %): 162 (M^+, 100), 131 (32.5); Calcd. for $C_8H_{10}N_4$ (162.19): C 59.24, H 6.21, N 34.54%. Found: C 59.33, H 6.28, N 34.64%.

Reaction of 2-hydrazinoylnicotinonitrile derivative 9 with active methylene compounds. ***General procedure.*** A mixture of **9** (3.3 g, 20 mmol) and acetylacetone or ethyl cyanoacetate (2 mL, 20 mmol) in ethanol (20 mL) was refluxed with stirring for 3 h. After cooling, the reaction mixture was poured into ice water. The formed precipitate was filtered off, dried, and recrystallized from methanol to give **11** and **12**, respectively.

4,6-Dimethyl-2-(3,5-dimethyl-1H-pyrazol-1-yl)pyridine-3-carbonitrile (11). Yield (89%); mp 83–85°C; IR (KBr) ύ (cm^{-1}), 2227 (CN); ^{1}H-NMR ($CDCl_3$): δ, 2.3 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 2.4 (s, 3H, CH_3-C═C), 2.5 (s, 3H, CH_3-C═N), 6.0 (s, 1H, ═CH-), 7.0 (s, H, phenyl); ^{13}C-NMR ($CDCl_3$): δ, 160.24 (C), 160.20 (C), 154.10 (C), 150.10 (C), 147.17 (C), 122.10 (CH), 114.41 (C), 108.46 (CH), 101.10 (C), 23.79 (CH_3), 19.99 (CH_3), 19.90 (CH_3), 12.50 (CH_3). MS: (*m/z*, %): 226 (M^+, 100), 211 (4.5), 186 (14.6), 159 (28.1), 145 (23.8), 131 (12.4); Calcd. for $C_{13}H_{14}N_4$ (226.28): C 69.00, H 6.24, N 24.76%. Found: C 69.07, H 6.34, N 24.71%.

2-(3-Amino-4,5-dihydro-5-oxopyrazol-1-yl)-4,6-dimethylpyridine-3-carbonitrile (12). Yield (80%); mp 160°C; IR (KBr) ύ (cm^{-1}), 1673 (CO), 2260 (CN), 3428, 3517 (NH_2); ^{1}H-NMR ($CDCl_3$): δ, 2.3 (s, 3H, CH_3-C═C), 2.5 (s, 3H, CH_3-C═N), 3.7 (s, 2H, NH_2), 4.0 (s, 2H, CH_2), 6.6 (s, H, pyridyl); ^{13}C-NMR ($CDCl_3$): δ, 171.8 (C), 162.83 (C), 162.80 (C), 155.10 (C), 152.70 (C), 120.79 (CH), 119.54 (C), 85.35 (C), 72.10 (CH_2), 23.21 (CH_3), 17.3 (CH_3). MS: (*m/z*, %): 229 (M^+, 100), 201 (35.3), 131 (25.4); Calcd. for $C_{11}H_1N_5O$ (229.24): C 57.63, H 4.84, N 30.55, O 6.98%. Found: C 57.65, H 4.80, N 30.65, O 6.95%.

5,7-Dimethyl-[1,2,4]triazolo[4,3-a]pyridine-8-carbonitrile (13). A mixture of **9** (3.3 g, 20 mmol) and triethylorthoformate (2 mL) or formic acid (3 mL) was refluxed in DMF (20 mL) for 3 or 2 h, respectively. The reaction mixture was left to cool, poured into ice water. The formed precipitate was filtered off, dried, and recrystallized from ethanol to give **13**.

Yield (90%) and (85%); mp 150–152°C; IR (KBr) ύ (cm^{-1}), 2210 (CN), 3270 (═CH); ^{1}H-NMR ($CDCl_3$): δ, 2.3 (s, 3H, CH_3-C═C), 2.4 (s, 3H, CH_3-C═N), 6.4 (s, H, CH), 6.4 (s, H, pyridyl); Calcd. for $C_9H_8N_4$ (172.19): C 62.78, H 4.68, N 32.54%. Found: C 62.74, H 4.61, N 32.50%.

3,5,7-Trimethyl-[1,2,4]triazolo[4,3-a]pyridine-8-carbonitrile (14). A mixture of **9** (3.3 g, 20 mmol) and acetic acid (3 mL) was refluxed for 2 h. The reaction mixture was left in air until complete evaporation of excess acetic acid. The formed precipitate was collected and recrystallized from ethanol to give **14**.

Yield (80%); mp 135–137°C; IR (KBr) ύ (cm^{-1}), 2213 (CN); ^{1}H-NMR ($CDCl_3$): δ, 2.4(s, 3H, CH_3-C═C), 2.5 (s, 3H, CH_3-C═N), 2.9 (s, 3H, CH_3), 7.0 (s, H, pyridyl); Calcd. for $C_{10}H_{10}N_4$ (186.21): C 64.50, H 5.41, N 30.09%. Found: C 64.61, H 5.55, N 30.18%.

5,7-Dimethyltetrazolo[1,5-a]pyridine-8-carbonitrile (16). *Method (A)* A mixture of **3** (3.2 g, 20 mmol), sodium azide (2 g) in ethanol (20 mL) was refluxed for 3 h. After cooling, the reaction mixture was poured into ice water. The formed precipitate was filtered off, dried, and recrystallized from ethanol to give **16**, yield (86%); mp > 300°C.

Method (B) Compound **9** (3.3 g, 20 mmol) with $NaNO_2$ (1 g, 15 mmol) and HCl (3 mL) was stirred in an ice bath. The formed precipitate was filtered off and recrystallized from ethanol to give **16**.

Yield (75%); mp > 300°C; IR (KBr) ύ (cm^{-1}), 2220 (CN); ^{1}H-NMR ($CDCl_3$): δ, 2.46 (3H, s, CH_3-C═N), 2.53 (3H, s, CH_3-C═C), 6.8 (H, s, pyridyl); MS: (*m/z*, %): 173 (M^+, 100), 145 (4.6), 131 (2.5), 119 (6.3); Calcd. for $C_8H_7N_5$ (173.17): C 55.48, H 4.07, N 40.44%. Found: C 55.41, H 4.16, N 40.51%.

Antitumor activity. Different concentrations of the tested compounds were prepared (ED_{100}, ED_{50}, and ED_{25} 1 g mL^{-1} dimethyl sulfoxide (DMSO)). The amount of DMSO was adjusted to give a final concentration of 0.1%. Ascites fluid obtained was aseptically aspirated from the peritoneal cavity of the donor animal (National Cancer Institute, Cairo, Egypt), which contains Ehrlich cell. The cells were grown partially floating and attach in AQ_4 a suspension culture (RPMI 1660 medium, Sigma Chemical, St. Louis), supplemented with 10% fetal bovine serum (GIBCO, UK). They were maintained at 37° C in humidified atmosphere with 5% CO_2 for 2 h. The viability of the cell used in control experiments (DMSO only without drug) exceeded 95% as determined by microscopic examination using a hemocytometer and trypan blue stain (stain only the dead cells).

REFERENCES AND NOTES

[1] (a) Henry, G. D. Tetrahedron 2004, 60, 6043; (b) Hamdya, N. A.; Gamal-Eldeen, A. M. Eur J Med Chem 2009, 44, 4547.

[2] Bagley, M. C.; Chapaneri, K.; Dale, D. W.; Xiong, X.; Bower, J. J Org Chem 2005, 70, 1389.

[3] Gilchrist, T. L. J Chem Soc Perkin Trans 2001, 2491.

[4] Vacher, B.; Bonnand, B.; Funes, F.; Jubault, N.; Koek, W.; Assie, M. B.; Cosi, C.; Kleven, M. J Med Chem 1999, 42, 1648.

[5] Son, J. K.; Zhao, L. X.; Basnet, A.; Thapa, P.; Karki, R. Y.; Jahng, Y.; Jeong, T. C.; Jeong, B. S.; Lee, C. S.; Lee, E. S. Eur J Med Chem 2008, 43, 675.

[6] Amr, A. G.; Abdulla, M. M. Bioorg Med Chem 2006, 14, 4341.

[7] Dutta, A. K.; Fei, X.; Reith, M. E. A. Bioorg Med Chem Lett 2002, 12, 619.

[8] Taddei, P.; Torreggiani, A.; Fini, G. Biopolymers 2002, 67, 289.

[9] Hatori, H.; Sato, B.; Sato, I.; Shibata, T.; Tsurumi, Y.; Sakamoto, K.; Takase, S.; Veda, H.; Hino, M.; Fujii, T. J Antibiot 2004, 57, 264.

[10] Song, J. H.; Slot, A. J.; Ryan, R. W. J.; Ross, G. M. Neuropharm 2004, 46, 984.

[11] Hammam, A. G.; Abdel Hafez, N. A.; Midura, W. H.; Mikolajczyk, M. Z. Z Naturforsch 2000, 55, 417.

[12] Abo-Ghalia, M.; Abdulla, M. M. Z.; Amr, A. E. Z Naturforsch 2003, 58, 903.

[13] Ismail, M. M.; Ammar, Y. A.; El-Zahaby, H. S.; Eisa, S. I.; Barakat, E. S. Arch Pharm 2007, 340, 476.

[14] Lu, Z.; Ott, G. R.; Anand, R.; Liu, R. Q.; Covington, M. B.; Vaddi, K.; Qian, M.; Newton, R. C.; Christ, D. D.; Trzaskos, J.; Duan, J. J. Bioorg Med Chem Lett 2008, 18, 1958.

[15] (a) Kalme, Z. A.; Roloff, B.; Pelchers, Yu. E.; Popelis, Yu, Yu.; Hagen, F.; Duburs, G. J. Khimiya Geterotsiklicheskikh Soedinenii 1992, 9, 1218; (b) Mariella, R. P.; Leech, J. L. J Am Chem Soc 1949, 71, 331; (c) Yassin, F. A. Chem Heterocycl Comp 2009, 45, 1.

[16] Anderson, D. R.; Stehle, N. W.; Kolodziej, S. A.; Reinhard, E. J. Pharmacia Corporation, USA; Lee, Len, F. PCT Int Appl 2004, 227.

[17] Yamada, T.; Takahashi, H.; Hatano, R. Nicotinoid Insecticides and the Nicotine Acetylcholine Receptor; Yamamoto, I.; Casida, J. E., Eds.; Springer–Verlag: Hong Kong, 1999, 149.

[18] (a) Balanca, G.; deVisscher, M. M. Crop Prot 1997, 16, 553; (b) Singh, T.; Sharma, S.; Srivastava, V. K.; Kumar, A. Arch Pharm 2006, 339, 24.

[19] Kagabu, S.; Azuma, A.; Nishimura, K. J Pesticide Sci 2002, 27, 267.

[20] Raj, M. P. P.; Rao, J. T. Asian J Chem 2003, 25, 492.

[21] Shiga, Y.; Okada, I.; Ikeda, Y.; Takizawa, E.; Fukuchi, T. J Pesticide Sci 2003, 28, 313.

[22] Mohan, J. Indian J Chem 2003, 42B, 1460.

[23] Srivastava, S. D.; Guru, N. J Indian Chem Soc 2000, 77, 400.

[24] Singh, T.; Sharma, S.; Srivastava, V. K.; Kumar, A. Arch Pharm 2006, 339, 24.

[25] Sadana, A. K.; Mirza, Y.; Aneja, K. R.; Prakash, O. Eur J Med Chem 2003, 38, 533.

[26] Moulin, A.; Martinez, J.; Fehrentz, J. -A. Tetrahedron Lett 2006, 47, 7591.

[27] Dombroski, M. A.; Letavic, M. A.; McClure, K. F. US Patent Appl Publ 2004, 077682.

[28] Buzon, R. A. S.; Castaldi, M. J.; Li, Z. B.; Ripin, D. H. B.; Tao, Y. PCT Int Appl 2004, 020438.

[29] Yu, G.; Li, J.; Ewing, W. R.; Sulsky, R. B.; Li, J. J.; Tino, J. A. PCT Int Appl 2004, 021984.

[30] Zimmermann, P. J.; Brehm, C.; Palmer, A.; Chiesa, M. V.; Simon, W. A.; Postius, S.; Kromer, W.; Buhr, W. PCT Int Appl 2005, 077947.

[31] Lawson, E. C.; Maryanoff, B. E.; Hoekstra, W. J. Tetrahedron Lett 2000, 41, 4533.

[32] Berionni, G.; Pegot, B.; Goumont, R. Magn Reson Chem 2010, 48, 101.

[33] Moderhack, D. J Prakt Chem 1998, 340, 687.

[34] Shah, H. C.; Shah, V. H.; Desai, N. D. Arkivoc 2009, ii, 76.

[35] Sheeja, K. R.; Kuttan, G.; Kuttan, R. Amala Res Bull 1997, 17, 73.