An Efficient Synthesis of Novel Pyrazole-Based Heterocycles as Potential Antitumor Agents

Magda A. Abdallah 1, Sobhi M. Gomha 1,*, Ikhlass M. Abbas 1, Mariam S. H. Kazem 2, Seham S. Alterary 3 and Yahia N. Mabkhot 3,*

1 Department of Chemistry, Faculty of Science, Cairo University, Giza 12613, Egypt; mkmalh2009@yahoo.com (M.A.A.); son2karim@gmail.com (I.M.A.)
2 Department of Chemistry, Faculty of Dentistry, October University for Modern Science & Arts, Giza 12613, Egypt; mariamkazem@hotmail.com
3 Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia; salterary@ksu.edu.sa
* Correspondence: s.m.gomha@gmail.com (S.M.G.); yahia@ksu.edu.sa (Y.N.M.);
Tel.: +20-237-400-304 (S.M.G.); +966-11-467-5898 (Y.N.M.);
Fax: +20-025-685-799 (S.M.G.); +966-11-467-5992 (Y.N.M.)

Received: 22 July 2017; Accepted: 31 July 2017; Published: 3 August 2017

Abstract: A new series of pyrazolylpyridines was prepared by reaction of ethyl-3-acetyl-1,5-diphenyl-1H-pyrazole-4-carboxylate with the appropriate aldehyde, malononitrile, or ethyl acetoacetate and an excess of ammonium acetate under reflux in acetic acid. Similarly, two novel bipyridine derivatives were prepared by the above reaction using terephthaldehyde in lieu of benzaldehyde derivatives. In addition, a series of 1,2,4-triazolo[4,3-a]pyrimidines was synthesized by a reaction of 6-(pyrazol-3-yl)pyrimidine-2-thione with a number of hydrazonoyl chlorides in dioxane and in the presence of triethylamine. The structure of the produced compounds was established by elemental analyses and spectral methods, and the mechanisms of their formation was discussed. Furthermore, the pyrazolyl-pyridine derivatives were tested as anticancer agents and the results obtained showed that some of them revealed high activity against human hepatocellular carcinoma (HEPG2) cell lines.

Keywords: pyrazoles; pyridines; multicomponent reactions; pyrazolyl-pyridines; antitumor activity

1. Introduction

A literature survey revealed that compounds, including the pyrazole nucleus, are extensively used as a precursor for the synthesis of compounds presenting many applications, such as electrolyte additives in batteries [1], catalysis [2], photographic materials [3], agrochemicals [4], and dyes [5]. The chemical versatility of the pyrazole and its analogues has attracted interest because it allows a range of applications in the pharmaceutical industry. Many pyrazole-derived compounds are known to exhibit anticancer [6–10], antimicrobial [11,12], antiviral [13], antiparasitic [14], anti-inflammatory [15,16], antipyretic [17], analgesic [18], anticoagulant [19], and anti-obesity [20] biological activities. The pyridine nucleus is a key constituent, present in a range of bioactive compounds, occurring both synthetically and naturally with wide range of biological applications [21,22]. Among the successful examples as drug candidates possessing pyridine nuclei are streptonigrin, streptonigrone, and lavendamycin, which are described in the literature as anticancer drugs. Some pyridine derivatives were studied for their topoisomerase inhibitory activity and cytotoxicity against several human cancer cell lines for the development of novel anticancer agents. As a result, it has been reported that various pyridine derivatives, as bioisosteres of α-terthiophene (potent protein kinase C inhibitor) [23], have significant topoisomerase I and/or II inhibitory activity, and cytotoxicity against several human cancer cell lines [24–28]. Early reports on the ability of α-terpyridine to form metal complexes [29] and to
bind with DNA/RNA [30] have been the base for the study on pyridine derivatives as antitumor agents. On the other hand, multicomponent reactions (MCRs) are powerful tools in modern medicinal chemistry, facilitating the lead generation by providing access to drug-like compounds, helping in drug discovery [31–33]. Additionally, the utility of MCR under microwave irradiation in the synthesis of heterocyclic compounds enhanced the reaction rates and improved the regioselectivity [34,35]. Over the last decade, several research groups adopted a hybridization approach for the design of pyrazole-pyridine hybrid analogs and illuminated their synthetic and medicinal importance [36–42].

In light of the above findings and in continuation of our efforts to synthesize new anticancer compounds [43–52], the aim of presented report is to synthesize a new series of pyrazolyl-pyridines via multicomponent reactions which are expected to be active as antitumor agents.

2. Results and Discussion

2.1. Chemistry

Ethyl 3-acetyl-1,5-diphenyl-1H-pyrazole-4-carboxylate (1) [53] was used as the starting compound for the preparation of a number of novel pyrazolyl-pyridine derivatives via one-pot multicomponent reactions. For example, a series of novel 2-amino-3-cyano-6-(pyrazol-3-yl)-pyridines 4a–f was prepared by a one-pot reaction of 3-acetylpyrazole derivative 1 with the appropriate aldehyde 2, malononitrile 3, and ammonium acetate under reflux in acetic acid (Scheme 1). Both elemental analyses and spectral data were used to elucidate the structures of the products 4a–f. The IR spectra of compounds 4a–f revealed in each case three absorption bands in the regions \( \nu \) 3431–3211, 2218–2210, 1715–1709 cm\(^{-1}\) attributed to the NH\(_2\), CN and C=O groups. The \(^1\)HNMR spectrum of compound 4a taken as a typical example of the products 4, revealed two signals at \( \delta = 6.93 \) (brs, 2H, NH\(_2\)) and 8.11 (s, 1H, pyridine-H5), in addition to the expected signals for the aryl and ester protons. Moreover, the mass spectra of product 4 showed in each case the respective molecular ion peak which is consistent with the assigned structure.

![Scheme 1. Synthesis of pyridine derivatives 4a–f.](image)

In a similar manner, another series of pyrazolylpyridines 6a–f was synthesized using ethyl acetooacetate in lieu of malononitrile. Thus, the reaction of 3-acetylpyrazole derivative 1 with the appropriate aldehyde 2, ethyl acetooacetate 5, and ammonium acetate in refluxing acetic acid afforded the corresponding products 6a–f (Scheme 2). The structure 6 assigned for the obtained products was established by elemental analyses and spectral (IR, \(^1\)HNMR, and MS) data. For example, the IR spectra of products 6a–f revealed, in each case, four absorption bands assigned for the three carbonyl groups and the -NH group of the pyridinone ring (see Section 3). The \(^1\)HNMR spectra displayed three singlet signals near \( \delta = 2.58, 9.80 \) and 7.79 ppm attributed to the acetyl, NH and pyridinyl-5H protons, in addition to the expected signals due to the ester and aryl protons (see Section 3).
To account for the formation of products 4 and 6, it was suggested that the reaction proceeds by condensation of the acetyl group of Compound 1 with the aldehyde to give the corresponding chalcone which reacts with ammonium acetate to give the imino derivative, followed by tandem Michael addition of the active methylene group of 3 (or 5) to afford the non-isolable tetrahydropyridine intermediates A (or B). The latter undergo in situ auto-oxidation (followed by tautomerization in case of A) and formation of the final products 4 (or 6) (Scheme 3).

Scheme 3. Mechanism of the synthesis of pyridine derivatives 4a–f and 6a–f.

Our study was extended to prepare another new bipyridine derivatives including the pyrazole moiety via multi-component reaction. Thus, the reaction of 3-acetylpyrazole derivative 1 with terephthaldehyde 7, malononitrile 3, and ammonium acetate in acetic acid under reflux furnished the bipyridine derivative 8 (Scheme 4).

Similarly, the reaction of compound 1 with terephthaldehyde, ethyl acetoacetate 5, and ammonium acetate in acetic acid under reflux gave the respective bipyridinone 9 (Scheme 4). The structure of products 8 and 9 were confirmed by elemental analyses and spectral data (IR, 1HNMR, and MS) (see Section 3).
On the other hand, chalcone 10, prepared by the reaction of 1 with benzaldehyde in ethanol containing catalytic amounts of NaOH [54], was used for preparation of 6-(pyrazol-3-yl) pyrimidine-2-thione derivative 11 via its reaction with thiourea in ethanol containing a catalytic amount of sodium hydroxide [54]. Reaction of the latter compound 11 with a number of hydrazonoyl chlorides 12a–h [55] in dioxane in the presence of triethylamine afforded the respective products 15a–h through the non-isolated intermediates 13 and 14 (Scheme 5). The structure assigned for the products 15 was established via microanalytical and spectral data (see Section 3). For example, the IR spectra of product 15 revealed the absence of the pyrimidinyl-NH groups, and instead showed two absorption bands near \(\nu\) 1706 and 1649 cm\(^{-1}\) assigned for the two carbonyl groups. Additionally, \(^1\)HNMR spectra of product 15 showed the absence of the signals attributed to the pyrimidinyl-NH protons and, instead, revealed the signals assigned for the acetyl protons (for 15a–d) or the ethoxycarbonyl protons (for 15e–h), in addition to the characteristic signals due to the ester and aromatic protons (see Section 3). The mass spectra of product 15 showed, in each case, the respective molecular ion peak, which is consistent with the assigned structure.
2.2. Antitumor Activity

The cytotoxicity of the synthesized pyridines 4a,b,e and 6a,b,e was evaluated against the human liver carcinoma cell line (HepG2-1) using doxorubicin as a reference drug (IC50 value of doxorubicin = 0.08 ± 0.07 nM) and MTT assay. The data generated were used to plot a dose response curve of which the concentration of the tested compounds required to kill 50% of cell population (IC50) was determined. Cytotoxic activity was expressed as the mean IC50 of three independent experiments. The results are depicted in Table 1 and Figure 1.

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>IC50 (nM)</th>
<th>General Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.08 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>H</td>
<td>CN</td>
<td>NH2</td>
<td>9.7 ± 0.85</td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>Me</td>
<td>CN</td>
<td>NH2</td>
<td>1.9 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>4e</td>
<td>Cl</td>
<td>CN</td>
<td>NH2</td>
<td>17.2 ± 0.83</td>
<td></td>
</tr>
<tr>
<td>6a</td>
<td>H</td>
<td>MeCO</td>
<td>OH</td>
<td>12.3 ± 0.37</td>
<td></td>
</tr>
<tr>
<td>6b</td>
<td>Me</td>
<td>MeCO</td>
<td>OH</td>
<td>2.4 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>6e</td>
<td>Cl</td>
<td>MeCO</td>
<td>OH</td>
<td>22.3 ± 0.36</td>
<td></td>
</tr>
</tbody>
</table>

The results revealed that the descending order of the antitumor activity of the tested compounds against HepG2-1 cell line is as follow: 4b > 6b > 4a > 6a > 4e > 6e.

The pyridine derivatives 4b and 6b (IC50 = 1.9 ± 0.16 and 2.4 ± 0.29 nM, respectively) have promising antitumor activity against HepG2-1. On the other hand, pyridine derivatives 4e and 6e have poor inhibitory activity (IC50 > 17 nM) compared with doxorubicin which used as reference drug.

**Structural Activity Relationship SAR**

Examination of the SAR led to the following conclusions:

The activity of the synthesized compounds 4 and 6 against hepatocellular carcinoma depends on the structural skeleton and electronic environment of the molecules. For example, the activity of the tested compounds 4a,b,e and 6a,b,e were found to be highly related to their structures since replacement of electron-donating groups in the two aryl groups in compounds 4b and 6b with electron-withdrawing groups in compounds 4e and 6e dramatically decreases their cytotoxicity against HepG2-1. On the other hand, the cytotoxicity of compounds 4a and 6a whose structures contain two phenyl groups (no substituent), is intermediate between the highly-potent and the weakly-potent compounds (See Table 1).
3. Experimental

3.1. Chemistry

Melting points were measured on an Electrothermal IA 9000 series (Bibby Sci. Lim. Stone, Staffordshire, UK) digital melting point apparatus. The IR spectra were recorded in potassium bromide discs on a Pye Unicam SP 3300 (Cambridge, UK) and a Shimadzu FT IR 8101 PC infrared (Shimadzu, Tokyo, Japan) spectrophotometer. $^1$H-NMR spectra were recorded in deuterated dimethyl sulfoxide (DMSO-d$_6$) using a Varian Gemini 300 NMR spectrometer (Varian, Inc., Karlsruhe, Germany). Mass spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer (Tokyo, Japan) at 70 eV. Elemental analysis was carried out at the Microanalytical Centre of Cairo University, Giza, Egypt. All reactions were followed by TLC (Silica gel, Merck, Darmstadt, Germany).

3.1.1. Synthesis of Tetra-Substituted Pyridine Derivatives (4a–f and 6a–f)

General procedure: A mixture of ethyl 3-acetyl-1,5-diphenyl-1H-pyrazole-4-carboxylate (1) (0.334 g, 1 mmol), the appropriate aldehyde 2a–f (1 mmol) and malononitrile (3), or ethyl acetoacetate (5) (1 mmol) in glacial acetic acid (20 mL) containing ammonium acetate (0.616 g, 8 mmol) was refluxed for 6–8 h (monitored by TLC). After complete reaction, the mixture was cooled and the precipitated products were filtered, washed with water, dried, and crystallized from ethanol to give the pyridine derivatives 4a–f and 6a–f, respectively. Compounds 4a–f and 6a–f together with their physical and spectral data are listed below:

**Ethyl 3-(6-amino-5-cyano-4-phenylpyridin-2-yl)-1,5-diphenyl-1H-pyrazole-4-carboxylate (4a).** Brown solid, (70% yield), mp 169–171 ºC; IR (KBr) $\nu_{max}$ 3364, 3208 (NH$_2$), 2218 (CN), 1715 (C=O) cm$^{-1}$; $^1$H NMR (DMSO-d$_6$) $\delta$ 1.02 (t, $J$ = 7.2 Hz, 3H, CH$_3$), 4.13 (q, $J$ = 7.2 Hz, 2H, CH$_2$), 6.93 (s, br, 2H, NH$_2$), 7.18–7.90 (m, 15H, Ar-H), 8.11 (s, 1H, Pyridine-H5); MS m/z (%) 485 (M$^+$, 14), 322 (47), 252 (29), 167 (38), 77 (52), 43 (100). Anal. Calcd. for C$_{30}$H$_{23}$N$_3$O$_2$ (485.55): C, 74.21; H, 4.77; N, 14.42. Found: C, 74.05; H, 4.52; N, 14.26%.

**Ethyl 3-(6-amino-5-cyano-4-(p-tolyl)pyridin-2-yl)-1,5-diphenyl-1H-pyrazole-4-carboxylate (4b).** Brown solid, (72% yield), mp 180–182 ºC; IR (KBr) $\nu_{max}$ 3379, 3211 (NH$_2$), 2210 (CN), 1712 (C=O) cm$^{-1}$; $^1$H NMR (DMSO-d$_6$) $\delta$ 1.01 (t, $J$ = 7.2 Hz, 3H, CH$_3$), 2.36 (s, 3H, CH$_3$), 4.12 (q, $J$ = 7.2 Hz, 2H, CH$_2$), 6.92 (s, br, 2H, NH$_2$), 7.14–7.94 (m, 14H, Ar-H), 8.15 (s, 1H, Pyridine-H5); MS m/z (%) 499 (M$^+$, 15), 468 (32), 364 (39), 209 (42), 104 (38), 78 (72), 43 (100). Anal. Calcd. for C$_{31}$H$_{25}$N$_3$O$_2$ (499.57): C, 74.53; H, 5.04; N, 13.85. Found: C, 74.37; H, 5.00; N, 13.85%.

**Ethyl 3-(6-amino-5-cyano-4-(4-methoxyphenyl)pyridin-2-yl)-1,5-diphenyl-1H-pyrazole-4-carboxylate (4c).** Pale green solid, (68% yield), mp 154–156 ºC; IR (KBr) $\nu_{max}$ 3367, 3219 (NH$_2$), 2210 (CN), 1714 (C=O) cm$^{-1}$; $^1$H NMR (DMSO-d$_6$) $\delta$ 1.02 (t, $J$ = 7.2 Hz, 3H, CH$_3$), 3.78 (s, 3H, OCH$_3$), 4.15 (q, $J$ = 7.2 Hz, 2H, CH$_2$), 6.93 (s, br, 2H, NH$_2$), 7.18–7.80 (m, 14H, Ar-H), 8.12 (s, 1H, Pyridine-H5); MS m/z (%) 515 (M$^+$, 9), 452 (42), 316 (100), 234 (51), 182 (37), 118 (50), 76 (66). Anal. Calcd. for C$_{31}$H$_{25}$N$_3$O$_5$ (515.57): C, 72.22; H, 4.89; N, 13.58. Found: C, 72.01; H, 4.77; N, 13.30%.

**Ethyl 3-(6-amino-5-cyano-4-(4-dimethylamino)phenyl)pyridin-2-yl)-1,5-diphenyl-1H-pyrazole-4-carboxylate (4d).** Dark yellow solid, (73% yield), mp 150–152 ºC; IR (KBr) $\nu_{max}$ 3431, 3212 (NH$_2$), 2210 (CN), 1709 (C=O) cm$^{-1}$; $^1$H NMR (DMSO-d$_6$) $\delta$ 1.01 (t, $J$ = 7.2 Hz, 3H, CH$_3$), 2.97 (s, 6H, 2CH$_3$), 4.11 (q, $J$ = 7.2 Hz, 2H, CH$_2$), 6.82 (s, br, 2H, NH$_2$), 7.14–7.82 (m, 14H, Ar-H), 8.10 (s, 1H, Pyridine-H5); MS m/z (%) 528 (M$^+$, 14), 416 (80), 212 (100), 170 (27), 105 (48), 76 (63). Anal. Calcd. for C$_{32}$H$_{26}$N$_3$O$_2$ (528.62): C, 72.71; H, 5.34; N, 15.90. Found: C, 72.59; H, 5.30; N, 15.73%.

**Ethyl 3-(6-amino-4-(4-chlorophenyl)-5-cyanoypyridin-2-yl)-1,5-diphenyl-1H-pyrazole-4-carboxylate (4e).** Dark yellow solid, (76% yield), mp 181–183 ºC; IR (KBr) $\nu_{max}$ 3362, 3218 (NH$_2$), 2213 (CN), 1712 (C=O) cm$^{-1}$; $^1$H NMR (DMSO-d$_6$) $\delta$ 1.02 (t, $J$ = 7.2 Hz, 3H, CH$_3$), 4.14 (q, $J$ = 7.2 Hz, 2H, CH$_2$), 6.98 (s, br, 2H, NH$_2$), 7.17–7.84 (m, 14H, Ar-H), 8.17 (s, 1H, Pyridine-H5); MS m/z (%) 521 (M$^+$, 23), 519 (M$^+$, 8), 397 (32), 316...
Ethyl 3-(6-amino-5-cyano-4-(2,4-dichlorophenyl)pyridin-2-yl)-1,5-diphenyl-1H-pyrazole-4-carboxylate (4f).

Yellow solid, (75% yield), mp 197–199 °C; IR (KBr) ν max 3367, 3215 (NH2), 2214 (CN), 1714 (C=O) cm⁻¹; 1H NMR (DMSO-d₆) δ 1.04 (t, J = 7.2 Hz, 3H, CH₃), 4.15 (q, J = 7.2 Hz, 2H, CH₂), 7.06 (s, br, 2H, NH₂), 7.28–7.85 (m, 13H, Ar-H), 8.14 (s, 1H, Pyridine-H₅); MS m/z (%) 554 (M⁺, 100), 316 (77), 281 (41), 193 (71), 105 (33), 58 (72). Anal. Calcd. for C₃₀H₂₁Cl₂N₅O₄ (554.43): C, 64.99; H, 3.82; N, 12.63. Found: C, 64.80; H, 3.61; N, 12.44%.

Ethyl 3-(5-acetyl-6-oxo-4-phenyl-1,6-dihydropyridin-2-yl)-1,5-diphenyl-1H-pyrazole-4-carboxylate (6a).

Brown solid, (68% yield), mp 186–188 °C; IR (KBr) ν max 3367 (NH), 1722, 1690, 1657 (3C=O) cm⁻¹; 1H NMR (DMSO-d₆) δ 1.03 (t, J = 7.2 Hz, 3H, CH₃), 2.58 (s, 3H, CH₃), 4.12 (q, J = 7.2 Hz, 2H, CH₂), 7.24–7.49 (m, 15H, Ar-H), 7.77 (s, 1H, Pyridine-H₅), 9.63 (s, br, 1H, NH); MS m/z (%) 503 (M⁺, 48), 458 (27), 334 (52), 232 (46), 99 (54), 57 (68), 43 (100). Anal. Calcd. for C₃₁H₂₃N₃O₅ (503.56): C, 73.94; H, 5.00; N, 8.34. Found: C, 73.73; H, 4.86; N, 8.17%.

Ethyl 3-(5-acetyl-6-oxo-4-(p-tolyl)-1,6-dihydropyridin-2-yl)-1,5-diphenyl-1H-pyrazole-4-carboxylate (6b).

Brown solid, (66% yield), mp 134–136 °C; IR (KBr) ν max 3409 (NH), 1718, 1681, 1662 (3C=O) cm⁻¹; 1H NMR (DMSO-d₆) δ 1.02 (t, J = 7.2 Hz, 3H, CH₃), 2.35 (s, 3H, CH₃), 2.56 (s, 3H, CH₃), 4.11 (q, J = 7.2 Hz, 2H, CH₂), 7.19–7.49 (m, 14H, Ar-H), 7.79 (s, 1H, Pyridine-H₅), 9.81 (s, br, 1H, NH); MS m/z (%) 517 (M⁺, 23), 385 (33), 294 (38), 147 (50), 120 (100), 76 (62). Anal. Calcd. for C₃₂H₂₅N₃O₅ (517.59): C, 74.26; H, 5.26; N, 8.12. Found: C, 74.20; H, 5.14; N, 8.03%.

Ethyl 3-(5-acetyl-4-(methoxyphenyl))-6-oxo-1,6-dihydropyridin-2-yl)-1,5-diphenyl-1H-pyrazole-4-carboxylate (6c).

Pale brown solid, (67% yield), mp 141–143 °C; IR (KBr) ν max 3425 (NH), 1715, 1687, 1660 (3C=O) cm⁻¹; 1H NMR (DMSO-d₆) δ 1.00 (t, J = 7.2 Hz, 3H, CH₃), 2.57 (s, 3H, CH₃), 3.77 (s, 3H, OCH₃), 4.01 (q, J = 7.2 Hz, 2H, CH₂), 6.78–7.39 (m, 14H, Ar-H), 7.72 (s, 1H, Pyridine-H₅), 9.80 (s, br, 1H, NH); MS m/z (%) 533 (M⁺, 14), 423 (37), 313 (51), 279 (100), 105 (36), 76 (43). Anal. Calcd. for C₃₂H₂₅N₃O₅ (533.58): C, 72.03; H, 5.10; N, 7.88. Found: C, 71.85; H, 5.02; N, 7.63%.

Ethyl 3-(5-acetyl-4-(4-(dimethylamino)phenyl))-6-oxo-1,6-dihydropyridin-2-yl)-1,5-diphenyl-1H-pyrazole-4-carboxylate (6d).

Brown solid, (69% yield), mp 141–143 °C; IR (KBr) ν max 3425 (NH), 1721, 1682, 1657 (3C=O) cm⁻¹; 1H NMR (DMSO-d₆) δ 1.00 (t, J = 7.2 Hz, 3H, CH₃), 2.58 (s, 3H, CH₃), 2.99 (s, 6H, 2CH₃), 4.11 (q, J = 7.2 Hz, 2H, CH₂), 6.78–7.39 (m, 14H, Ar-H), 7.72 (s, 1H, Pyridine-H₅), 9.73 (s, br, 1H, NH); MS m/z (%) 546 (M⁺, 14), 406 (36), 349 (55), 241 (49), 121 (36), 76 (30), 43 (100). Anal. Calcd. for C₃₃H₃₀N₄O₄ (546.63): C, 72.51; H, 5.53; N, 10.25. Found: C, 72.39; H, 5.38; N, 10.02%.

Ethyl 3-(5-acetyl-4-(4-chlorophenyl))-6-oxo-1,6-dihydropyridin-2-yl)-1,5-diphenyl-1H-pyrazole-4-carboxylate (6e).

Brown solid, (68% yield), mp 170–172 °C; IR (KBr) ν max 3366 (NH), 1720, 1680, 1663 (3C=O) cm⁻¹; 1H NMR (DMSO-d₆) δ 1.06 (t, J = 7.2 Hz, 3H, CH₃), 2.58 (s, 3H, CH₃), 4.14 (q, J = 7.2 Hz, 2H, CH₂), 7.24–7.59 (m, 14H, Ar-H), 7.78 (s, 1H, Pyridine-H₅), 10.06 (s, br, 1H, NH); MS m/z (%) 540 (M⁺, 2), 538 (M⁺, 3), 368 (53), 214 (100), 120 (55), 40 (79). Anal. Calcd. for C₃₁H₂₃ClN₃O₄ (538.00): C, 69.21; H, 4.50; N, 7.81. Found: C, 69.46; H, 4.35; N, 7.66%.

Ethyl 3-(5-acetyl-4-(2,4-dichlorophenyl))-6-oxo-1,6-dihydropyridin-2-yl)-1,5-diphenyl-1H-pyrazole-4-carboxylate (6f).

Brown solid, (69% yield), mp 197–199 °C; IR (KBr) ν max 3414 (NH), 1720, 1683, 1659 (3C=O) cm⁻¹; 1H NMR (DMSO-d₆) δ 1.10 (t, J = 7.2 Hz, 3H, CH₃), 2.61 (s, 3H, CH₃), 4.15 (q, J = 7.2 Hz, 2H, CH₂), 7.26–7.52 (m, 13H, Ar-H), 7.76 (s, 1H, Pyridine-H₅), 10.24 (s, br, 1H, NH); MS m/z (%) 572 (M⁺, 12), 388 (64), 256 (44), 207 (67), 125 (50), 83 (42), 55 (100). Anal. Calcd. for C₃₁H₂₃Cl₂N₃O₄ (572.44): C, 65.04; H, 4.05; N, 7.34. Found: C, 65.24; H, 4.02; N, 7.16%.
3.1.2. Synthesis of Bipyridine Derivatives 8 and 9

A mixture of 3-acetylpyrazole derivative 1 (0.668 g, 2 mmol), terephthalaldehyde 7 (0.134 g, 1 mmol), and malononitrile 3 or ethyl acetoacetate 5 (2 mmol) in acetic acid (30 mL) containing ammonium acetate (1.232 g, 16 mmol) was refluxed for 8 h. After cooling the reaction mixture it was poured into an ice-water mixture, the formed a precipitate that was collected by filtration, then crystallized from dioxane to give the bipyridine products 8 and 9, respectively.

**Diethyl 3,3’-(1,4-phenylenebis(6-amino-5-cyanopyridine-4,2-diyl))bis(1,5-diphenyl-1H-pyrazole-4-carboxylate) (8).** Brown solid, (68% yield), mp 187–189 °C; IR (KBr) ν max 3378, 3201 (NH2), 2211 (CN), 1709 (C=O) cm⁻¹; 1H NMR (DMSO-d6) δ 1.03 (t, J = 7.2 Hz, 6H, 2CH3), 4.14 (q, J = 7.2 Hz, 4H, 2CH2), 6.93 (s, br, 4H, 2NH2), 7.18–7.49 (m, 20H, Ar-H), 7.85 (s, 4H, Ar-H), 8.10 (s, 2H, 4Pyridine-H3); MS m/z (%) 892 (M⁺, 39), 724 (48), 622 (63), 368 (39), 82 (60), 76 (57), 43 (100). Anal. Calcd. for C₅₄H₄₀N₁₀O₄ (892.98): C, 72.63; H, 4.52; N, 15.69. Found: C, 72.69; H, 4.36; N, 15.47%.

**Diethyl 3,3’-(1,4-phenylenebis(5-acetyl-6-oxo-1,6-dihydropyridine-4,2-diyl))bis(1,5-diphenyl-1H-pyrazole-4-carboxylate) (9).** Brown solid, (66% yield), mp 207–209 °C; IR (KBr) ν max 3423 (NH), 1723, 1677, 1653 (3C=O) cm⁻¹; 1H NMR (DMSO-d6) δ 1.11 (t, J = 7.2 Hz, 6H, 2CH3), 2.58 (s, 6H, 2CH3), 4.14 (q, J = 7.2 Hz, 4H, 2CH2), 7.24–7.48 (m, 20H, Ar-H), 7.27 (s, 4H, Ar-H), 10.06 (s, br, 2H, 2NH); MS m/z (%) 929 (M⁺, 17), 776 (41), 509 (37), 386 (55), 267 (40), 148 (32), 77 (100), 43 (68). Anal. Calcd. for C₅₆H₄₄N₁₈O₈ (929.00): C, 72.40; H, 4.77; N, 9.05. Found: C, 72.17; H, 4.62; N, 9.01%.

3.1.3. Synthesis of 1,5-Diphenyl-1,5-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine derivatives 15a–h

General procedure: Triethylamine (0.14 mL, 1 mmol) was added to a mixture of equimolar amounts of thione 11 (0.480 g, 1 mmol) and the appropriate hydrazonoyl halides 12a–h (1 mmol) in dioxane (20 mL) at room temperature. The reaction mixture was then refluxed for 10–15 h until all hydrogen sulfide gas stopped evolving. The solid that formed after concentration of the reaction mixture was filtered and crystallized from the proper solvent to give the products 15a–h, respectively.

**Ethyl 3-(3-acetyl-1,5-diphenyl-1,5-dihydro-[1,2,4]triazolo[4,3-alpyrimidin-7-yl]-1,5-diphenyl-1H-pyrazole-4-carboxylate (15a).** Yellow solid, (74% yield), mp 233–235 °C (DMF); IR (KBr) ν max 3026, 2956 (C-H), 1706, 1649 (2C=O), 1595 (C=N) cm⁻¹; 1H NMR (DMSO-d6) δ 1.15 (t, J = 7.2 Hz, 3H, CH3), 2.43 (s, 3H, CH3), 4.19 (q, J = 7.2 Hz, 2H, CH2), 5.33 (d, J = 4 Hz, 1H, CH), 6.62 (d, J = 4Hz, 1H, CH), 7.03–7.80 (m, 20H, Ar-H); MS m/z (%) 606 (M⁺), 540 (36), 287 (29), 247 (75), 194 (37), 92 (71), 65 (60), 43 (100). Anal. Calcd. for C₅₇H₄₆N₁₂O₃ (606.69): C, 73.25; H, 4.98; N, 13.85. Found: C, 73.07; H, 4.84; N, 13.67%.

**Ethyl 3-(3-acetyl-5-phenyl-(p-tolyl)-1,5-dihydro-[1,2,4]triazolo[4,3-alpyrimidin-7-yl]-1,5-diphenyl-1H-pyrazole-4-carboxylate (15b).** Yellow solid, (72% yield), mp 211–213 °C (DMF); IR (KBr) ν max 3030, 2951 (C-H), 1697, 1642 (2C=O), 1597 (C=N) cm⁻¹; 1H NMR (DMSO-d6) δ 1.04 (t, J = 7.2 Hz, 3H, CH3), 2.24 (s, 3H, CH3), 2.44 (s, 3H, CH3), 4.16 (q, J = 7.2 Hz, 2H, CH2), 5.32 (d, J = 4 Hz, 1H, CH), 6.61 (d, J = 4Hz, 1H, CH), 7.05–7.73 (m, 19H, Ar-H); MS m/z (%) 620 (M⁺, 7), 498 (27), 390 (35), 285 (60), 105 (41), 77 (100), 43 (92). Anal. Calcd. for C₅₈H₅₂N₁₂O₃ (620.71): C, 73.53; H, 5.20; N, 13.54. Found: C, 73.39; H, 5.38; N, 13.36%.

**Ethyl 3-(3-acetyl-5-phenyl-1,5-dihydro-[1,2,4]triazolo[4,3-alpyrimidin-7-yl]-1,5-diphenyl-1H-pyrazole-4-carboxylate (15c).** Yellow solid, (74% yield), mp 242–244 °C (DMF/EtOH); IR (KBr) ν max 3028, 2963 (C-H), 1707, 1641 (2C=O), 1597 (C=N) cm⁻¹; 1H NMR (DMSO-d6) δ 1.02 (t, J = 7.2 Hz, 3H, CH3), 2.44 (s, 3H, CH3), 4.15 (q, J = 7.2 Hz, 2H, CH2), 5.36 (d, J = 4 Hz, 1H, CH), 6.69 (d, J = 4Hz, 1H, CH), 7.27–7.70 (m, 19H, Ar-H); MS m/z (%) 643 (M⁺ + 2, 4), 641 (M⁺, 13), 499 (57), 322 (39), 180 (28), 105 (35), 77 (100). Anal. Calcd. for C₃₇H₃₉C₁₁N₄O₃ (641.13): C, 69.32; H, 4.56; N, 13.11. Found: C, 69.19; H, 4.51; N, 13.00%.

**Ethyl 3-(3-acetyl-1-(4-nitrophenyl)-5-phenyl-1,5-dihydro-[1,2,4]triazolo[4,3-alpyrimidin-7-yl]-1,5-diphenyl-1H-pyrazole-4-carboxylate (15d).** Yellow solid, (75% yield), mp 204–206 °C (EtOH); IR (KBr) ν max 3031, 2950
Ethyl 7-(4-(ethoxycarbonyl)-1,5-diphenyl-1H-pyrazol-3-yl)-1-(4-nitrophenyl)-5-phenyl-1,5-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine-3-carboxylate (15e). Yellow solid, (72% yield), mp 180–182 °C (DMF/EtOH); IR (KBr) νmax 3056, 2973 (C-H), 1710, 1699 (2C=O), 1596 (C-N) cm−1; 1H NMR (DMSO-d6) δ 1.02 (t, J = 7.2 Hz, 3H, CH3), 3.96 (q, J = 7.2 Hz, 2H, CH2), 4.12 (q, J = 7.2 Hz, 2H, CH2), 4.66 (d, J = 4Hz, 1H, CH), 6.78–7.79 (m, 20H, Ar-H); MS m/z (%) 561 (M+ 4), 484 (48), 400 (71), 252 (39), 179 (42), 105 (100), 57 (83). Anal. Calcd. for C37H26N3O6 (661.68): C, 68.19; H, 4.49; N, 15.05. Found: C, 68.04; H, 4.33; N, 14.92%.

Ethyl 7-(4-(ethoxycarbonyl)-1,5-diphenyl-1H-pyrazol-3-yl)-5-phenyl-1-(p-tolyl)-1,5-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine-3-carboxylate (15f). Brown solid, (71% yield), mp 206–208 °C (EtOH); IR (KBr) νmax 3030, 2948 (C-H), 1713, 1644 (2C=O), 1593 (C-N) cm−1; 1H NMR (DMSO-d6) δ 1.07 (t, J = 7.2 Hz, 3H, CH3), 2.95 (t, J = 7.6 Hz, 3H, CH3), 4.12 (q, J = 7.2 Hz, 2H, CH2), 4.27 (q, J = 7.6 Hz, 2H, CH2), 5.46 (d, J = 4 Hz, 1H, CH), 6.94 (d, J = 4 Hz, 1H, CH), 7.25–8.42 (m, 19H, Ar-H); MS m/z (%) 673 (M+ 6), 577 (73), 390 (66), 327 (95), 115 (100), 83 (52). Anal. Calcd. for C36H31N2O6 (681.71): C, 66.95; H, 4.58; N, 14.38. Found: C, 66.77; H, 4.42; N, 14.23%.

3.2. Cytotoxic Activity

The cytotoxic evaluation of the synthesized compounds was carried out at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt according to the reported method [56].

4. Conclusions

Two series of functionalized pyrazolyl-pyridines were prepared by multi-component reaction of 3-acetylpyrazole derivative with the appropriate aldehyde, malononitrile (or ethyl acetoacetate) in acetic acid in the presence of excess ammonium acetate. The mechanism of formation of the novel products was also discussed. Additionally, two novel bipyridine derivatives were synthesized by the above described reaction and under the same reaction conditions using terephthaldehyde in lieu of benzaldehyde derivatives. Another series of 1,2,4-triazole[4,3-a]pyrimidines, including a pyrazole moiety, was prepared by the reaction of a pyrazolopyrimidine-2-thione derivative with a variety of hydrazonoyl chlorides under reflux in dioxide in the presence of triethylamine. The assigned structure for the products was elucidated based on elemental analyses and spectral data (IR, 1H NMR, MS).
Moreover, the novel pyrazolyl-pyridines were tested for their reactivity as antitumor agents and the results obtained revealed high potency of some of them against HEPG2-1 compared with doxorubicin used as the reference drug.

Acknowledgments: The authors extend their sincere appreciation to the Deanship of Scientific Research at the King Saud University for its funding this Prolific Research group (PRG-1437-29).

Author Contributions: Magda A. Abdallah, Sobhi M. Gomha, and Ikhlash M. Abbas designed research; Mariam S. H. Kazem, Seham S. Alterary and Yahia N. Mabkhot performed the research, analyzed the data, wrote the paper, and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interests.

References


35. Bortolini, O.; D’Agostino, M.; De Nino, A.; Maiuolo, L.; Nardi, M.; Sindona, G. Solvent-free, microwave assisted 1,3-cycloaddition of nitrones with vinyl nucleobases for the synthesis of N-O-nucleosides. *Tetrahedron* **2008**, *64*, 8078–8081. [CrossRef]


