Design, Synthesis, Characterization, Biological Evaluation and Docking Studies of Some New Synthesized 6–Phenyl Pyridine Derivatives

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Citation

Received: September 16, 2018; Accepted: October 31, 2018; Published: January 30, 2019

Abstract: A new series of 6–phenyl pyridine derivatives were synthesized by using (2E)–1–phenyl–3–(thiophen–2–yl) prop–2–en–1–one (1) reacted with 2–cyanoethane–thio amide (2) to afford the corresponding 6–phenyl–4–(thiophen–2–yl) 2–thioxo–1,2–dihydropyridine–3–carbonitriles (6). The synthetic potentiality of compound 6 was investigated in the present study via their reactions with active–hydrogen containing compounds ethyl chloroacetate 8 aiming to synthesize 4–(thiophen–2–yl)–6–phenylthieno [2, 3–b] pyridin–3–amines 10 via ethyl {[3–cyano–6–phenyl–4–(thiophen–2–yl) pyridin–2–yl] sulfanyl}acetate 9. Compounds 9 and10 reacted with hydrazine hydrate in EtOH to afford the corresponding 3–amino–6–phenyl–4–(thiophen–2–yl) thieno [2, 3–b] pyridine–2–carbohydrazide 11. Structures of the title compounds were characterized by UV, IR, $^1$H/$^13$C-NMR and mass spectrometric methods as well as that of elemental analyses. The compound 11 have been screened for anti–bacterial activity and molecular docking studies. UV, IR, $^1$H/$^13$C-NMR and mass spectral data, the assigned structure were established. The synthesized compound 11 showed potent antimicrobial activity against the selected strains of Gram-positive Gram-negative bacteria. Furthermore, The molecular docking technique was utilized to ascertain the mechanism and mode of action towards the molecular target cyclin–dependent kinase (CDK) inhibitor protein indicating that compound 11 has been exhibit inhibitory activity against CDK inhibitors via H–bonds and electrostatic interactions. The main purpose of this paper is synthesis of new 6–phenyl pyridine derivatives to provide an insight into its biological activities which contain the carbohydrazide moiety. The synthesized of new 6–phenyl pyridine derivatives which contain the carbohydrazide moieties have shown promising antimicrobial activities and inhibition activity against cyclin–dependent kinase (CDK) protein.

Keywords: 6–Phenyl Pyridine, Antimicrobial, Molecular Docking

1. Introduction

A large number of heterocyclic compounds, in particular, containing pyridine rings are exhibited wide spectrum of biological activities ranging from antibacterial to anticancer [1, 2].

Many of 6–phenyl pyridine compounds have been possess high range of pharmacological activities including antimicrobial, anti–inflammatory, antioxidant and anticancer activities and form an essential part of the molecular structure of important drugs [3–6]. Furthermore, many researchers have focused on design and development of synthetic new pyridine derivatives to achieve better biological activities. Although new and more expensive drugs have been developed, their cost is beyond the common man’s reach. As a consequence, these trends have emphasized the pressing need for new, more effective, cheaper and safe antimicrobial agents.

Prompted by recent literature observations, some new 6–phenyl pyridine derivatives were synthesized, leading to interesting heterocyclic scaffolds that are particularly useful for the creation of diverse chemical libraries of drug–like
molecules for biological screening. In view of the above facts, and in continuation of our search on pharmacologically active heterocyclic compounds, we report herein synthesis, anti-bacterial activity and molecular docking studies of 6-phenyl pyridine derivatives. The structures of all compounds have been evaluated by elemental analysis and spectral analysis (IR 1H NMR, Mass and elemental analyzer). The compound 11 have been screened for anti-bacterial activity and molecular docking studies.

2. Material and Methods

2.1. Materials

All chemicals which used for synthesis compounds, Biological studies were purchased from Sigma. Doubly distilled water was used as the solvent throughout the experiments. All reagents were of the best commercial grade and used without further purification.

2.2. Apparatus

All melting points were uncorrected. IR (KBr discs) spectra were recorded on a Shimadzu FTIR–8201PC Spectrophotometer. 1H NMR spectra were recorded on a Varian Mercury 300 MHz., and a Varian Gemini 200 MHz. spectrometers using TMS as an internal standard and CDCl3, DMSO-d6, and (CD3)2CO as solvents. Chemical shifts were expressed as δ (ppm) units. Mass spectra were recorded on Shimadzu GCMS–QP1000EX using an inlet type at 70 eV. The Micro analytical Center of Cairo University performed the microanalyses. Emission spectra were recorded with a Hitachi F–2500 fluorescence spectrophotometer.

2.3. Synthesis of 6 (General Method)

Method A

A solution of each of 2 (2.6mmol) and each of 1 (0.56g; 2.6mmol) in 30 ml of absolute ethanol containing a catalytic amount of piperidine (0.4 ml) heated under reflux for 5 hours. The reaction mixture then evaporated, cooled, triturated with ethanol. The products so formed collected by filtration, washed with cold ethanol, and then crystallized from the proper solvent to give the corresponding 6.

Method B

A mixture of dispersed sulfur (0.67g; 19mmol) and morpholine (1.7ml, 19mmol) in 50 ml of ethanol refluxed for 20 minutes. Add mononitrile (7) (1.3g, 19mmole) and 1 (0.4g, 19mmol) and the mixture refluxed for 2 hours. The mixture cooled to ~ 20°C, and 10% HCl added to reach pH 5–6. The precipitates so formed filtered off and washed with water and cooled ethanol then crystallized from dioxane to give the corresponding 6.


Orange crystals in colour, solid, yield–73%, melting point (218°C). FT–IR (KBr) (ν) 3160 (NH), 3130 (aromatic–CH) and 2220 (CN). Mass ES–MS m/z (%)(M+, 100%) corresponding to the molecular weight 294, (M+–H, 14.2%) 293, (M+–CN, 1.3%) 268, (M+–H, CN, 3.2%) 267, (M+–SH, 3.2%) 261, (M+–H2S, CN 4.5%) 234. Elemental analysis Calculated (found) for C16H15N2S2 (%): C, 65.28 (65.30); H, 3.42 (3.45); N, 9.52 (9.50); S, 21.78 (21.75).

2.4. Synthesis of 9: (General Procedure)

A solution of each of 6 (0.294g, 1mmole) and ethyl chloroacetate (8) (0.122g 1mmol) in sodium methoxide (prepared from 0.14g of sodium and methanol 25ml) was stirring at room temperature for 15 minutes. The formed precipitate was collected by filtration, washed with water and crystallized from the proper solvent to give 9.


Bright orange crystals in colour, solid, yield–84%, melting point (154°C). FT–IR (KBr) (ν) 3120 (C–H, aromatic), 2220 (CN), 1750 (ester CO). 1H NMR (DMSO–d6) δ 1.66 (t, 3H, CH2CH3), 2.498 (s, 2H, S–CH2), 4.300 (q, 2H, CH2CH3), 7.565–7.796 (m, 5H, phenyl), 8.070–9.402 (m, sH, thiophene H, s). Elemental analysis Calculated (found) for C20H16N2O2S2 (%): C, 63.13 (63.22); H, 4.24 (4.3); N7.36 (7.26); O, 8.41 (8.19); S, 16.85 (16.79).

2.5. The Synthesis of 10

Method A:

A solution of each of 9 (0.38g, 1mmol) in sodium ethoxide solution (prepared from 0.10g of sodium and 25ml ethanol) heated under reflux for 30 minutes. The solid that formed after cooling, collected by filtration, washed with water and ethanol then crystallized from the proper solvent to afford 10.

Method B:

A solution of each of 6 (0.32g, 1mmol) and ethyl–chloroacetate (8) (0.122g, 1 mmol) in sodium methoxide (prepared from 0.10g of sodium and 25ml ethanol) heated under reflux for 2 hours. The solid products so formed after cooling, collected by filtration, washed with water and ethanol and dried then crystallized from the proper solvent to afford 10.


Yellow crystals in colour, solid, yield–90%. melting point (254°C); FT–IR (KBr) (ν) 3478.5, 3333.3 (NH2), 1600 (C=O). FT–IR (KBr) (ν) 3478.5, 3333.3 (NH2), 1600 (C=O). 1H NMR (DMSO–d6) δ 1.052 (t, 3H, CH3–CH–), 3.067 (q, 2H, CH2–CH3), 4.300 (q, 2H, CH2–CH3), 6.469 (s, 2H, NH2), 7.483–7.897 (m, 5H, phenyl H, s) and 8.572–9.388 (m, sH, thiophene H, s). Elemental analysis Calculated (found) for C20H16N2O2S2 (%): C, 65.28 (63.22); H, 4.24 (4.3); N7.36 (7.26); O, 8.41 (8.19); S, 16.85 (16.79).

2.6. Synthesis of 11

Method A:

A solution of each of 9 (0.38g, 1mmol) in hydrazine hydrate (15ml) and ethanol (20ml) was heated under reflux for 5 hours; the excess solvents were evaporated and cooled. The solid was collected by filtration, dried, and crystallized...
from the acetic acid to give 11.

Method B

A solution of each of 10 (0.38g, 1mmol) in hydrazine hydrate (15ml) and ethanol (20ml) was heated under reflux for 4 hours, the excess solvents were evaporated and cooled. The solid was collected by filtration, dried, and crystallized from the acetic acid to give 11.


Yellow crystals in colour, solid, yield–73%, melting point (260°C). FT–IR (KBr) (v): 3463, 3320, 3301 (NH & NH\textsubscript{2}), 3044 (aromatic–CH). Mass ES–MS m/z (%)(M+, 34.3% which corresponding to the molecular weight of the molecular formula C\textsubscript{16}H\textsubscript{14}N\textsubscript{2}O\textsubscript{2} of the assigned structure) 366, (M+–NH\textsubscript{2}H\textsubscript{2}, 100%) 335, (M+–CONHNH\textsubscript{2}, 7.4%) 307.

\textsuperscript{1}H NMR (DMSO–d\textsubscript{6}), δ 4.47 (br, s, 2H, NH\textsubscript{2}), 6.824 (br, s, 2H, NH\textsubscript{3}), 7.336–8.700 (m, 8H, phenyl H, s, thiophen H, s), 9.393 (br, 1H, NH) and 9.400 (s, 1H, pyridine C\textsubscript{5}H).

Elemental Analysis Calculated (found) for C\textsubscript{16}H\textsubscript{14}N\textsubscript{2}O\textsubscript{2}:

\(\text{C: } 58.99 \text{ (59.10)}; \text{ H: } 3.85 \text{ (3.88)}; \text{ N: } 15.29 \text{ (15.2)}; \text{ O: } 4.37 \text{ (4.3)}; \text{ S: } 17.50 \text{ (17.52).}

2.7. Antimicrobial Assay

Antimicrobial screening of the compound 11 has been performed by the agar well diffusion method (Udupa et al., 1995). The discs measuring 8 mm in diameter were prepared from Whatman No. 1 filter paper sterilized by dry heat at 140°C for 1 h. The sterile discs previously soaked in two different concentrations (100 mg/ml, 200 mg/ml and 400 mg/ml), of the test compound was placed in a nutrient agar medium. The plates were inverted and kept in an incubator at 37±1°C. The inhibition zone thus formed was measured (in mm) after 24 h and Nystatin was used as the standard. The absence of inhibition against the test organism.

2.8. Molecular Docking Studies

The rigid molecular docking studies were performed using HEX 6.1 software, which is an interactive molecular graphics program to understand the drug–protein interaction [7]. The Structure of the compound was sketched by CHEMSKETCH (http://www.acdlabs.com) and converts it into pdb format from mol format by OPENBABEL (http://www.vcclab.org/ lab/babel/). The crystal structure of the CDK inhibitors (PDB ID: 2XMY) was downloaded from the protein data bank (http://www.rcsb.org/pdb). All calculations were carried out on an Intel Pentium 4, 2.4 GHz based machine running MS Windows XP SP2 as operating system. Visualization of the docked pose has been done by using PyMol (http://pymol.sourceforge.net/) molecular graphic program.

3. Results and Discussion

3.1. Chemistry

\((2E)–1–\text{phenyl–3–(thiophen–2–yl)} \text{ prop–2–en–1–one (1)} \text{ reacted with 2–cyanoethane–thioamide (2) in absolute ethanol containing a catalytic amount of piperidine under reflux to afford a reaction product. Such reaction product formed via a Michael addition of } \text{–CH=CH–} \text{ in 2 on } \text{–CH=CH–} \text{ of 1 to give the non–isolable products 3, 4, 5 followed by cyclisation via dehydration and dehydrogenation to give 6 (Figure 1). The IR (cm\textsuperscript{-1}) of this reaction product showed the bands of } \text{–CONHNH–} \text{ of } \text{CN (2220) and CO (1750) of the newly introduced COOEt group. Its } \text{IR spectrum gave m/z = 294 (100%) which corresponding to the molecular weight of the molecular formula C\textsubscript{16}H\textsubscript{10}N\textsubscript{2}S\textsubscript{2} of the assigned structure as well as m/z = 261 (12.5%) which corresponding to (M+ – SH).}

A further confirmation of 6 arose from their synthesis through other pathway via the reaction of each of 1 and malononitrile (7) in a dispersed sulfur, morpholine and ethanol under reflux 2 hours (Figure 1). It important to refer here that 6 obtained by the two pathways are identical in all physical and chemical properties.

The synthetic potentiality of each of 6 investigated through electrophilic substitution reactions using several electrophile C–species. Thus, it has been found that 6 reacted with ethyl chlorooacetate (8) in stirred methanol sodium methoxide at room temperature for 15 minutes to give a reaction product. The IR (cm\textsuperscript{-1}) of this reaction product showed the bands of CN (2220) and CO (1750) of the newly introduced COOEt group. Its \textsuperscript{1}H NMR (6 ppm) spectrum revealed the bands of \text{–CONHNH–} \text{protons and this confirm the good nucleophilicity of } \text{S} \text{ in 6 that facilitate the electrophilic attack of 8 to afford 9 in very pure state and a good yield. Furthermore, 9 structure elucidated through its cyclisation in ethanolic sodium ethoxide under reflux for 30 minutes to give a reaction product (Figure 2) whose IR spectrum showed no bands of CN group and instead the bands of the newly formed NH\textsubscript{2} group detected. Also, the \textsuperscript{1}H NMR spectrum of this reaction product revealed no signals of \text{–CONHNH–} \text{protons while that of NH\textsubscript{2} detected. Considering the data of both IR and } \text{infrared NMR we concluded that both --SCH\textsubscript{2}-- and CN functional groups in 9 involved in the cyclisation step to give the finally isolated 10. A further confirmation of 10 structure obtained through its preparation authenticly via the reaction of 6 with 8 in ethanolic sodium ethoxide under reflux for 2 hours.}
Figure 1. Synthesis of the newly 6-Phenyl Pyridine derivatives (1–6).
3.2. Biological Activity

3.2.1. Anti-Bacterial Activity

The compound 11 has been screened for in vitro antibacterial activity against Gram-negative (bacillus subtilis, staphylococcus aureus) and gram-negative (escherichia coli, pseudomonas aeruginosa), the relevant data are presented in table 1. The MIC (Minimum inhibitory concentration) was defined as the lowest concentration of the tested compound 11 at which no growth of the strain was observed in a period of time and under specified experimental conditions. The inhibition MIC values for the screening was reported in Table 1 and Figure 3. It was noticed that the synthesized compound significantly inhibited the high activity against E. coli in compare with antibiotic Nystatin. All the tested compounds recorded high to moderate activities against bacteria used S. aureus, P. aeruginosa and S. aureus in compare with Nystatin 100 µg/discs. Compound 11 showed higher activities against bacteria E. coli at concentration 200 µg/disc, the higher activity of the compound 11 is mainly due to contain 6–Phenyl Pyridine which is responsible for the enhanced activity of the compound.

Table 1. The Antimicrobial Activity Screening Compound 11.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Zoneofinhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B.subtilis</td>
</tr>
<tr>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Nystatin</td>
<td>20</td>
</tr>
</tbody>
</table>

Figure 2. Synthesis of the newly 6–Phenyl Pyridine derivatives (6–11).

Figure 3. Antibacterial activity of compound 11 against Staphylococcus aureus.
3.2.2. Molecular Docking Studies

The biological activity of compound 11 which act as antibacterial, anti–tubercular, antiviral, anti–inflammatory and anticancer has been investigated by studied the interaction between compound 11 and cyclin–dependent kinase (CDK) inhibitor protein [8].

The high resolution crystal structure of CDK inhibitors (PDB ID: 2XMY) was downloaded from the protein data bank (http://www.rcsb.org). The protein was prepared for docking by removing the co–crystallized ligands waters and co–factors [9, 10]. The resulting docked pattern (Figure 4) suggesting the existence there are a number of specific electrostatic interactions and hydrogen bonds, because several ionic and polar residues in the proximity of the ligand play an important role in stabilizing the molecule via H–bonds and electrostatic interactions. As shown in Figure 5, there are hydrogen bond interactions between the main amino acid residues of Thr–165, Gln–131, Val–163 and Val–164 forms H–bond with OH and NH moieties of compound 11, the binding energy of docked compound 11 with CDK was found to be $-226.78 \text{ kJ mol}^{-1}$ which indicating that compound 11 has been exhibit inhibitory activity against CDK inhibitors (Figure 6).

![Figure 4. Molecular docked model of compound 11 (stick representation) located within the hydrophobic pocket in cyclin–dependent kinase (CDK) inhibitor of protein.](image1)

![Figure 5. Docking model of compound–enzyme complex. Representation of compound 11 interacting with amino acid residues in the binding site of protein (PDB: 2XMY). The compound is shown in green stick model. Pictures are created with Discover Studio 4.0. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).](image2)
4. Conclusion

In the present work, we have synthesized a new series of 6-phenyl pyridine derivatives and characterized by using UV, IR, $^1$H/$^1$C-NMR and mass spectra as well as elemental analyzer techniques. In the biological applications, Compound 11 which have carbohydrazide moiety has exhibited excellent antibacterial activity against two gram-positive bacteria (e.g. B. subtilis, S. aureus) and two gram-negative bacteria (escherichia coli, pseudomonas aeruginosa), compound 11 showed higher activities against bacteria E. coli at concentration 200 µg/disc. Furthermore, molecular docking study were performed, the binding energy of docked compound 11 with cyclin–dependent kinase (CDK) protein was found to be $\sim$226.78 kJ mol$^{-1}$ which indicating that compound 11 has been exhibit inhibitory activity against CDK inhibitors to provide valuable information about pharmaceutical potential of the investigated molecule. Finally, these encouraging results are helpful in different biological applications in future endeavors.

References