DESIGN, SYNTHESIS, AND ANTIMICROBIAL EVALUATION OF NOVEL THIENOPYRIMIDINES AND TRIAZOLOTHIENOPYRIMIDINES

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GRAPHICAL ABSTRACT

Abstract 2-Cyanoacetohydrazide and 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene derivatives were exploited as starting materials for the syntheses of novel thienopyrimidines and triazolothienopyrimidines. The proclivity of these compounds toward one-carbon donor reagents such as carbon disulfide, phenyl isothiocyanate, and aromatic aldehydes was investigated. The structures of all synthesized compounds were ascertained by spectral and analytical data. The antimicrobial activity of the target synthesized compounds was tested against various microorganisms.

Keywords Antimicrobial activity; 2-cyanoacetohydrazide; thienopyrimidines; triazolothienopyrimidines

Received October 16, 2014.
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INTRODUCTION

Heterocyclic compounds with thienopyrimidine molecules have received considerable interest because of their interesting pharmacological and biological activities. These derivatives have also displayed analgesic, anti-inflammatory, antimicrobial, and platelet aggregation inhibition activities and demonstrated antagonism of α-adrenoceptors and prevention of cartilage destruction in articular diseases. In view of these fascinating and encouraging results and in continuation of our work on biologically active nitrogen and sulfur heterocycles, we have synthesized some thienopyrimidines and triazolothienopyrimidines by adopting a different methodology and evaluated them for their antimicrobial properties. The well-known Gewald reaction was adopted for the synthesis of the precursor 2-amino-4,5,6,7-tetrahydrobenzo [b]thiophene derivatives.

RESULTS AND DISCUSSION

In this investigation, condensation of thieno[2,3-d]1,3-oxazine with 2-cyanoacetohydrazide in dioxane resulted in the formation of thieno[2,3-d]pyrimidine derivative (Scheme 1).

The structure was deduced from the study of 1H NMR spectrum. Thus, the presence of δ-1H-CH2 cited for the acetamido methylene protons as singlet at 4.15 ppm, together with the methyl and cyclohexyl protons, confirm the structure. Moreover, the mass spectrum of compound 3 showed the respective molecular ion peak at m/z = 302 (29.3%), which upon loss of water molecule afforded the base peak at m/z = 284. The formation of 3 could be explained on the basis of nucleophilic attack by nitrogen nucleophile of 2 on the positively polarized carbonyl carbon of the oxazinone ring followed by 1,6-exo-trig cyclization and dehydration (Scheme 2).

The functionalities in 2-methyl-3-cyanoacetamido-5,6,7,8-tetrahydro-4H-benzo-thieno[2,3-d]pyrimidin-4-one 3 made it valuable key precursors for the formation of fused heterocyclic compounds. The reactivity of compound 3 toward various chemical reagents was investigated with the aim to producing thienopyrimidine derivatives with potential biological activities. Thus, the reaction of 3 with salicylaldehyde gave the coumarin derivative 4. On the other hand, the reaction of 3 with either 1,3-diphenyl pyrazole-4-carboxaldehyde or p-anisaldehyde yielded the arylidene derivatives 5 and 6, respectively (Scheme 3).

The appearances of a high-frequency C=O stretching at 1723 cm⁻¹ cited for the coumarin oxo function besides the absence of the cyano acetamido CN observed in the starting material 3 and coumarin C₄-H proton at 8.3 ppm in the 1H NMR spectrum of 4 confirm the expected structure. Furthermore, compound 4 revealed a molecular ion peak [M⁺] at m/z = 407, which represents the base peak corresponding to
to the molecular formula C₂₁H₁₇N₃O₄S. A clear-cut clue for the condensation products 5 and 6 is forthcoming from the study of their spectral data. The observed δ-¹H olefinic C=CH at 7.9 ppm and 7.72 ppm in ¹H NMR spectra, the appearance of conjugated nitriles at lower frequencies of 2212 and 2218 cm⁻¹ than those observed with the starting compound 3, and the presence of the correct molecular ion peaks in the mass spectra at m/z = 531 and 420 were consistent with the assigned structures 5 and 6, respectively.

In this context, it was claimed that the reaction of arylidene cyano acetamido system like 5 with hydrazine hydrate yielded the respective pyrazole derivative. Herein treatment of 5 with hydrazine hydrate in boiling dioxane afforded an unexpected product with molecular formula C₃₂H₂₄N₆ [mol. wt. = 492], which was identified as 1E,2E-1,2-bis(1,3-diphenyl-1H-pyrazol-4-yl)methylene]hydrazine 7. The azine structure was confirmed by comparison (thin-layer chromatography, TLC; infrared, IR; melting point, mp) with an authentic sample prepared from the condensation of

Scheme 2. Mechanistic pathway for formation of compound 3.

Scheme 3. Reactions of compound 3 with aromatic and heterocyclic aldehydes.
1,3-diphenyl pyrazol-4-carboxaldehyde with hydrazine hydrate in refluxing ethanol (Scheme 3). A plausible mechanism for the formation of compound 7 is depicted in Scheme 4.

The reactivity of 3 toward active methylene reagents such as malononitrile and ethyl acetoacetate was investigated. Thus, the reaction of compound 3 with malononitrile and/or ethyl acetoacetate in the presence of a catalytic amount of piperidine in boiling dioxane afforded the 2-pyridone derivatives 8 and 10, respectively (Scheme 5).

All data for the product obtained were consistent with assigned structure 8 and ruled out structure 9. Thus, $^1$H NMR spectrum revealed the absence of $\delta$-$^1$H of the pyridine C$_5$-H at 6.9 ppm and the presence of $\delta$-$^1$H CH$_2$ cited for the acetamido methylene protons observed with 3 at 4.11 ppm. Moreover, the highest recorded peak in the mass spectrum at $m/z = 368$ (9.1%) represents the molecular ion peak, which upon loss of H$_2$N-C≡C-CN fragment and water molecule afforded the radical cation at $m/z = 284$ attributable for the base peak. The reaction pathway for the conversion of 3 to the 2-pyridone derivative 8 could be explained on the basis of nucleophilic attack by the carbanion derived from malononitrile on the electropositive carbon of the nitrile group in compound 3, whereas the formation of 2-pyridone derivative 10 seemed to proceed through the anion of compound 3, which effected nucleophilic attack on the carbonyl keto group of ethyl acetoacetate followed by base-catalyzed dehydration to give the corresponding condensation product, which when subjected to 1,6-exo-trig cyclization afforded the product 10. The structure 10 was confirmed using analytical and spectroscopic data (IR, $^1$H NMR, and MS).

It was claimed that treatment of cyanoacetamido compounds with benzylidene carbonitrile reagents gave the 2-pyridone derivative.[21] Herein, treatment of compound 3 with 4-methoxybenzylidene malononitrile in refluxing dioxane in the presence of a catalytic amount of piperidine afforded the 2-pyridone derivative 11 with elimination of the heteryl group (Scheme 5). The IR spectrum revealed the absence of $\nu$C-H (cyclohexane group) and the presence of two CN stretching bands at 2215 and 2188 cm$^{-1}$. The $^1$H NMR spectrum lacked the upfield signals characteristic for

![Scheme 4](image-url)  
**Scheme 4.** A plausible mechanism for formation of the azine 7.
the cyclohexyl and methyl protons. Moreover, the analytical and elemental tests showed the absence of elemental sulfur. All these facts were in agreement with the assigned structure 11. The formation of compound 11 could be explained as shown in Scheme 6.

Our study moved to the reactivity of active methylene of the key precursor 3 toward phenyl isothiocyanate in basic dimethylformamide. Thus, subjecting 3 to react with phenyl isothiocyanate followed by acidification of the potassium sulfide salt yielded 2-methyl-3-(2-cyano-3-mercapto-3-phenylamino)acrylamido)-5,6,7,8-tetrahydro-4H-benzothieno[2,3-d]pyrimidin-4-one 12. Moreover, the reaction of 3 with phenyl isothiocyanate in ethanolic potassium hydroxide, dimethylformamide, and chloroacetyl chloride yielded the thiazolidinone derivative 13. The structures of compounds 12 and 13 were deduced from the analytical and spectral data (Scheme 7).

α-Aminocarbonitriles[22,23] were used as general precursors for the synthesis of a broad range of biologically active thienopyrimidines and triazolothienopyrimidines. 2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile[19] 14 has attracted a great deal of interest over the years. The reaction of 14 with triethylorthoformate in the presence of freshly distilled acetic anhydride (drops) yielded ethyl N-3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-ylformimidate 15, which when subjected to react with 2-cyanoacetohydrazide in refluxing dioxane afforded 2-cyanomethyl-8,9,10,11-tetrahydro benzothieno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine 16 (Scheme 8).

The structure 16 was substantiated from the study of analytical and spectroscopic data. Thus, the IR spectrum displayed $\nu_{C-H(\text{arom.})}$ at 3059 cm$^{-1}$, $\nu_{C-H}$ (aliph) at 2970, 2942, 2920 cm$^{-1}$, $\nu_{C=N}$ at 2256 cm$^{-1}$ (saturated nitrile), and $\nu_{C=N}$ at 1610 cm$^{-1}$. $^1$H NMR spectrum [CDCl$_3$] revealed the presence of three types of protons attributable for the cyclohexyl, methylene, and C$_2$-H pyrimidine protons.
Scheme 6. Mechanistic pathway for formation of 2-pyridone derivative 11.

Scheme 7. Reaction of compound 3 with phenyl isothiocyanate.
Moreover, the mass spectrum of 16 shows the respective molecular ion peak at $m/z = 269$ (100%), which also represents the base peak.

The reaction mechanism for the combination of 15 with 2-cyanoacetohydrazide is depicted in Scheme 9.

The structure of compound 16 got further chemical support through its reaction with aromatic and heterocyclic aldehydes. Thus, the reaction of 16 with 1,3-diphenyl pyrazol-4-carboxaldehyde and/or 4-chlorobenzaldehyde afforded the corresponding condensation products 17a,b. On the other hand, the reaction of 16 with salicylaldehyde gave the coumarin derivative 18 (Scheme 8). The structures of compounds 17a,b and 18 were deduced from their spectroscopic and analytical data.

Compelling evidence for the structure 16 is forthcoming from its reaction with one-carbon reagents such as carbon disulfide. Thus, stirring compound 16 with carbon disulfide in ethanolic potassium hydroxide in dimethylformamide yielded

Scheme 8. Synthesis of triazolothienopyrimidines 16–18.

Scheme 9. A plausible mechanism for formation of compound 16.
the dipotassium sulfide salt 19, which in situ reacted with dimethyl sulfate and/or 1,3-dibromopropane to give 20 and 21, respectively (Scheme 10).

Compounds 20 and 21 show similar IR spectra, displayed ν\(_{C≡N}\) at 2202 and ν\(_{C=N}\) at 1647 cm\(^{-1}\). The mass spectra of both revealed the molecular ion peaks at \(m/z = 373\) (9.2\%) and 385 (63.9\%), respectively. The reaction seemed to proceed via nucleophilic addition of the carbanion derived from 16 on electrophilic carbon of the carbon disulfide to give the dipotassium salt 19, which undergoes double S\(_2\) mechanism, affording the S-alkylated products 20 and 21.

**ANTIMICROBIAL EVALUATION**

The most important part of the results that were obtained from antimicrobial activities of synthesized compounds screened against two fungal species, namely *Aspergillus flavus* and *Candida albicans*, as well as two bacteria species, namely *Escherichia coli* and *Staphylococcus aureus*. The antimicrobial activity was biologically assayed using the diffusion plate technique. Preliminary screening of the synthetic derivatives and standard drugs were performed at fixed concentration of 20 mg/ml, and inhibition was recorded by measuring the diameter of the inhibition zone at the end of 18 h for bacteria. *Amphotericin B* as an antifungal agent and *Ampicillin* as an antibacterial agent were used as references to evaluate the potency of the tested compounds under the same conditions. The investigation of antibacterial and antifungal screening data revealed that the tested compounds 3–6, 8, 10, and 12 showed comparatively good activity against all the bacterial strains. Only compounds 5, 8, and 17b exhibited moderate activity against *Candida albicans*. Compounds 16, 17a, and 18 exhibited no antimicrobial activity. The results are depicted in Table 1.
CONCLUSION

The research study reported the successful synthesis and antimicrobial activity of new thienopyrimidines and triazolothienopyrimidines. The antimicrobial activity study revealed that the tested compounds showed moderate (compounds 3, 4, 6, 8, 10, 12) to good (compound 5) antibacterial activity, whereas some of them (compounds 5, 8, 17b) had antifungal activities against pathogenic strains, and all tested compounds have no activity against Aspergillus flavus.

EXPERIMENTAL

All melting points were taken on a Griffin and Geory melting-point apparatus and are uncorrected. IR spectra were recorded on Pye Unicam SP1200 spectrophotometer using the KBr wafer technique. $^1$H NMR spectra were determined on a Varian Gemini 300 MHz using tetramethylsilane (TMS) as internal standard (chemical shifts in $\delta$ scale). EI-MS were measured on a Schimadzu-GC-MS operating at 70 eV. Elemental analyses were carried out at the Microanalytical Unit, Faculty of Science, Ain Shams University, using a Perkin-Elmer 2400 CHN elemental analyzer, and satisfactory analytical data (+0.4) were obtained for all compounds. Biological activities were carried out at the Microanalytical Center, Faculty of Science, Cairo University. The homogeneity of the synthesized compounds was controlled by thin-layer chromatography (TLC) using aluminum sheet silica gel F$_{254}$ (Merck).

**Synthesis of 2-Methyl-3-cyanoacetamido-5,6,7,8-tetrahydro-4H-benzo[2,3-d]pyrimidin-4-one 3**

A mixture of thieno[2,3-d]1,3-oxazine 1 (2.21 g, 10 mmol) and 2-cyanoacetohydrazide 2 (1 g, 10 mmol) in dioxane (10 ml) was heated under reflux for 1 h. The deposited solid was filtered off, dried, and recrystallized from dioxane to give 3 as

| Sample  | Inhibition zone diameter (mm/mg sample) | Escherichia coli (G–) | Staphylococcus aureus (G+) | Aspergillus flavus (fungus) | Candida albicans (fungus) |
|---------|----------------------------------------|-----------------------|--------------------------|---------------------------|--------------------------|
| Control: DMSO | | 0.0 | 0.0 | 0.0 | 0.0 |
| 3       | | 11  | 12  | 0.0 | 0.0 |
| 4       | | 9   | 9   | 0.0 | 0.0 |
| 5       | | 15  | 17  | 0.0 | 11 |
| 6       | | 12  | 12  | 0.0 | 0.0 |
| 8       | | 10  | 11  | 0.0 | 9  |
| 10      | | 10  | 9   | 0.0 | 0.0 |
| 12      | | 10  | 11  | 0.0 | 0.0 |
| 16      | | 0.0 | 0.0 | 0.0 | 0.0 |
| 17a     | | 0.0 | 0.0 | 0.0 | 0.0 |
| 17b     | | 0.0 | 0.0 | 0.0 | 12 |
| 18      | | 0.0 | 0.0 | 0.0 | 0.0 |
| Ampicillin | | 12  | 18  | 0.0 | 0.0 |
| Amphotericin B | | 0.0 | 0.0 | 16  | 19 |
white crystals; mp: 278–280 °C, yield: 66%. Anal. calcd. for C_{14}H_{14}N_{4}O_{2}S (302.35): C, 55.61; H, 4.67; N, 18.53; S, 10.61. Found: C, 55.54; H, 4.51; N, 18.44; S, 10.52. IR (μ/cm⁻¹): 3264, 3182 (NH), 2948, 2885, 2862 (CH aliph), 2262 (CH/CN), 1677 (C=O).

Supporting Information

Full experimental details and spectroscopic data for compounds 4–21 can be accessed on the publisher’s website.

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