Facile synthesis of quinazoline, oxazine and triazole derivatives as potential antimicrobial agents based on isothiocyanate moiety
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Abstract
A novel series of heterocyclic compounds like quinazoline, triazole, thiazolidine and oxazine derivatives was prepared via treatment of isothiocyanate with nitrogen, sulfur and carbon nucleophiles. The chemical structures of all products were confirmed on the basis of their elemental analyses and spectroscopic data (IR, MS, \( ^1 \)H NMR, \( ^{13} \)C NMR).

Keywords: Quinazoline, oxazine, triazole, thiazolidine, isothiocyanate.

1. Introduction

Heteroallenesisothiocyanates are an interesting group of organic compounds that are used as reactive key precursors in the synthesis of heterocyclic molecules due to their diverse reactions and also to the easiness of availability. [1-4]

Isothiocyanates are often prepared from the reaction ofaminoderivatives with carbon disulfide [5-8] or thiophosgene, [9-11] as well as treatment of the carboxylic acid derivatives with ammonium thiocyanate [12-17].

In addition, isothiocyanates are a class of organic compounds with well-known potent pharmaceutical applications [18,19] and are very popular in drug discovery [20-22].

On the basis of these experiences and in continuation of our ongoing interest in the design of bioactive heterocyclic molecules [23-27], the present work involves a facile synthesis of a series of five and six-membered heterocyclic compounds as triazole, quinazoline, thiazolidine and oxazine derivatives using 2-(4-phenoxybenzoyl)benzoyl isothiocyanate (2).

2. Results and Discussion:

Heating of acid 1 in thionyl chloride under reflux furnished the corresponding acid chloride which in turn reacted with ammonium thiocyanate in dry acetone to give 2-(4-phenoxybenzoyl)benzoylisothiocyanate (2).

The prepared 2-(4-phenoxybenzoyl)benzoyl isothiocyanate (2) was used in situ as a reactive key precursor for synthesis of variety of heterocyclic molecules via its reaction with various nucleophiles like nitrogen nucleophiles, carbon nucleophiles and sulfur nucleophiles.

Thus, heating of isothiocyanate (2) with antranilic acid as a nitrogen nucleophile under reflux at 40 C furnished thiourea derivative 3. Heating of the latter with acetic anhydride resulted in the formation of 3-(2-(4-phenoxybenzoyl)benzoyl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (4).

The reactivity of isothiocyanatederivate (2) towards nitrogen nucleophilic reagents was investigated also through its reaction with benzoylhydrazine. Heating of the reaction mixture under reflux at 40 C gave thiosemicarbazide derivative 5 while its formulation of triazole (6) is temperature dependent and proceed via heating at 80 C furnished triazole (6).
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In addition, benzoxazole derivatives are an interesting group of heterocyclic molecules due to their wide range of pharmaceutical applications. This encouraged us to synthesize N-(benzo[d]oxazol-2-yl)-2-(4-phenoxybenzoyl)benzamide (8) in similar way to the previous behavior of isothiocyanate 2 toward nitrogen nucleophiles via treatment of isothiocyanate with o-aminophenol as amphoteric nucleophile in dry acetone under reflux.

Similarly, 2-(4-phenoxybenzoyl)benzoylisothiocyanate (2) reacted with thioglycolic acid as sulfur nucleophile in dry acetone under reflux to give 2-(((2-(4-phenoxybenzoyl) benzoyl) carbamothioyl) thio) acetic acid (10). In addition, thiazolidine 11 can be formed in a good yield upon heating of acid 10 in acetic anhydride under reflux.

On the other hand, treatment of isothiocyanate 2 with benzylidenemalononitrile (12) as a carbon nucleophile in dry toluene gave 2-(2-(4-phenoxybenzoyl)phenyl)-6-phenyl-4-thioxo-4H-1,3-oxazine-5,5(6H)-dicarbonitrile (13) via [4+2]cycloaddition mechanism.

Antimicrobial activity

The synthesized molecules were screened and tested in vitro for their antimicrobial activity against Bacillus subtilis and Staphylococcus coeci as Gram-positive bacteria in addition to Klebsiella bacilli and Escherichia coli as Gram-negative bacteria. The products were also evaluated for their antifungal activity against Candida albican and Aspergillus niger. Both of Ampicillin trihydrate and Terbinafine were used as standard drugs to evaluate the potency of the tested products under the same conditions.

Determination of the preliminary antibacterial and antifungal activity was carried out by agar diffusion method and the results were presented for the tested products as the average diameter of inhibition zones (r) of bacterial or fungal growth around the disks in millimeters at 100 μg concentrations in DMSO.

The observed data on the antimicrobial activity of the products and standards are given in Table 1. It was observed from the results given in Table 1 that most of the synthesized products exhibited varying degrees of inhibition against the tested microorganisms.

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<tr>
<th>compound</th>
<th>B. subtilis</th>
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Note: Numbers in the table represent the inhibition zone diameter (r, mm) of either bacterial or fungal growth for each compound; r > 25 mm, highly active; r > 14 mm, moderately active; r > 10 mm, slightly active; (-), no inhibition was observed; A = Ampicillin trihydrate as the standard antibacterial agent and T = Terbinafine as the standard antifungal agent.

It has been observed from data shown in Table 1 most of the synthesized compounds exhibited varying degrees of inhibition against the tested microorganisms in comparison with the standard antibacterial and antifungal. Quinazoline 4 and thiazolidine 11 showed potent activity against Gram-negative bacteria Escherichia coli while compound 13 showed the lowest activity. Also, triazole 6 and benzoxazole 8 exhibited the highest activity against Gram-positive bacteria Bacillus subtilis but other compounds showed moderate activity. On the other hand, triazoles 6 and 9 exhibited high activity against fungal strains Candida Albican and Aspergillus Niger. Additionally, all the tested compounds showed lower to moderate activity against Candida Albican except triazole 6.

From the previous results, it was observed that, the presence of triazole moiety in compounds 6 and 9 enhanced their antifungal activity and the presence of quinazoline, benzoxazole and thiazolidine enhanced their antibacterial activity.

Experimental

Melting points were determined by the capillary tube method and were uncorrected. IR spectra in KBr were recorded using (Perkin – Elmer 298) spectrophotometer. 1H NMR spectra were recorded on a varian-Gemini 400 MHZ instrument. CDCl3 and DMSO-d6 were used as solvents, chemical shifts (δ) were reported in ppm relative to internal TMS. Mass spectra were obtained by Schimadzu single focusing mass spectrum at abeam energy 70ev. Microanalytical data were obtained from the microanalytical center at Cairo University.
4.2. Synthesis of 2-(4-phenoxybenzoyl)benzoylisothiocyanate (2)

Acid $1^{[27]}$ (0.01 mol) in little amount of thionyl chloride was heated under reflux at lower than 70 °C for 2 hrs. Excess of thionyl chloride was removed by evaporation under vacuum to leave solid acid chloride. Solid ammonium thiocyanate (0.01 mol) was added to stirred solution of acid chloride in dry acetone (15 ml). The reaction mixture was stirred for 1.5 hrs. at room temperature. The precipitated ammonium chloride was filtered off, then the solvent was removed under vacuum and the crude product was used in the next reaction without purification.

**Synthesis of 2-(3-(2-(4-phenoxybenzoyl)benzoyl)thioureo) benzoic acid (3)**

A mixture of isothiocyanate 2 (0.01 mol) and antranilic acid (0.01 mol) in dry acetone was heated under reflux for 4 hrs. After the completion of reaction (the reaction progress was monitored by TLC), the precipitated solid was filtered, dried and recrystallized from toluene.

Yield, 79%; mp 212°C-214 °C; IR (Cm-1): $\nu_{\max}$: 3425-3100(0H, NH), 1687-1660(2CO), 1631 (C=O), 1307 (C=S); Ms: m/z 496 (M+) $^1$H NMR(DMSO-d6) δ: 6.89-8.50 (m, 17H, Ar-H), 9.35, 10.10 (2s, 2H, 2NH, exchangeable), 12.12 (s, 1H, OH, exchangeable); $^{13}$C NMR: 117.5, 118.3, 118.8, 119.8, 120.9, 121.6, 122.6, 126.5, 127.3, 128.3, 129.5, 130.2, 130.7, 131.3, 135.5, 148.2, 152.5, 161.3, 165.5, 171.4, 178.5, 190.4. Anal. Calcd. for C$_{33}$H$_{24}$O$_{4}$S: C, 70.37; H, 3.92; N, 8.73%.

4.3. Synthesis of N-(2-benzoylhydrazine-1-carboxothioyl)-2-(4-phenoxybenzoyl) benzamide (5)

A mixture of isothiocyanate 2 (0.01 mol) and benzoylhydrazine (0.01 mol) was heated in dry acetone for 4 hrs. After cooling the precipitated product was filtered, dried and recrystallized from ethanol.

Yield, 81%; mp 193°C-195 °C; IR (Cm-1): $\nu_{\max}$: 3415 (NH), 3030(CH aromatic), 1685-1664(C=O), 1262 (C=S); Ms: m/z 495 (M+); $^1$H NMR (DMSO-d6) δ: 7.18-8.42 (m, 18H, Ar-H), 9.38, 10.49, 10.82 (3s, 3H, 3NH, exchangeable); $^{13}$C NMR: 117.8, 118.3, 118.7, 121.3, 122.5, 124.2, 124.8, 126.5, 126.8, 127.2, 127.7, 128.6, 129.8, 130.6, 131.5, 135.7, 148.5, 150.5, 168.7, 170.2 181.5, 192.5; Anal. Calcd. for C$_{32}$H$_{29}$N$_5$O$_7$: C, 67.87; H, 4.27; N, 8.48; Found: C, 67.80; H, 4.18; N, 8.42%.

4.4. Synthesis of (2-(4-phenoxybenzoyl)phenyl)(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)methane (6)

The same procedures described for the synthesis of compound 4. The product was crystallized from methanol.

Yield, 74%; mp 157°C-159 °C; IR (Cm-1): $\nu_{\max}$: 3380 (NH), 1630(CH aromatic), 1692-1670(C=O), 1315(C=S); Ms: m/z 477 (M+); $^1$H NMR (DMSO-d6) δ: 7.13-8.36 (m, 18H, Ar-H), 12.82 (s, 1H, NH, exchangeable); $^{13}$C NMR: 118.2, 118.5, 119.3, 121.5, 121.8, 124.6, 125.5, 126.2, 126.9, 127.5, 128.3, 128.8, 129.2, 131.1, 137.1, 135.8, 145.2, 155.4, 170.3, 182.2, 189.5; Anal. Calcd. for C$_{33}$H$_{23}$N$_5$O$_7$: C, 70.43; H, 4.01; N, 8.80 Found: C, 70.29; H, 3.92; N, 8.73%.

4.5. Synthesis of N-((2-hydroxyphenyl)carbamothioyl)-2-(4-phenoxybenzoyl) benzamide (7)

Heat a mixture of isothiocyanate 2 (0.01 mol) and o-aminophenol (0.01 mol) in dry acetone for 3 hrs. After cooling the precipitated product was filtered, dried and recrystallized from benzene.

Yield, 77%; mp 181°C-183 °C; IR (Cm-1): $\nu_{\max}$: 3430 – 3180 (OH, NH), 3035 (CH aromatic), 1683-1660 (C=O), 1306 (C=S); Ms: m/z 468 (M+); $^1$H NMR (DMSO-d6) δ: 6.98-8.13 (m, 17H, Ar-H), 10.34 (s, 1H, OH, exchangeable), 11.51, 12.82 (2s, 2H, 2NH, exchangeable); $^{13}$C NMR: 118.4, 118.6, 118.9, 121.3, 122.5, 123.3, 126.5, 127.7, 128.2, 128.7, 129.4, 130.5, 131.5, 133.4, 134.2, 150.3, 154.7, 168.5, 173.1, 184.5; Anal. Calcd. for C$_{32}$H$_{23}$N$_5$O$_7$: C, 69.22; H, 4.30; N, 5.98; Found: C, 69.28; H, 4.42; N, 5.93%.


Heat compound 7 above its melting point for 2 hrs., the product was recrystallized from cyclohexane.

Yield, 68%; mp 172°C-174 °C; IR (Cm-1): $\nu_{\max}$: 3400 (NH), 3013 (CH aromatic), 1690-1673 (C=O), 1240 (C=S); Ms: m/z 434 (M+); $^1$H NMR (CDCl$_3$) δ: 5.64 (s, 1H, NH, exchangeable), 6.89-7.95 (m, 18H, Ar-H); Anal. Calcd. for C$_{32}$H$_{29}$N$_5$O$_7$: C, 74.65; H, 4.18; N, 6.45; Found: C, 74.58; H, 4.10; N, 6.37.
4.7. Synthesis of (4-phenoxypyphenyl)(2-(2-phenyl-5-thiooxo-2,5-dihydro-1H,1,2,4-triazol-3-y1)phenyl)methanone (9)

A mixture of isothiocyanate 2 (0.01 mol) and phenylhydrazine (0.01 mol) in dry aceton was heated under reflux for 4 hrs. After cooling the precipitated product was filtered, dried and recrystallized from ethanol.

Yield, 80%; mp 179°C; IR (cm⁻¹): vmax: 3348 (NH), 3050 (CH-aromatic), 1686 (C=O), 1295 (C=S); Ms: m/z 449 (M+); ¹H NMR (DMSO-d₆) δ: 6.67-7.89 (m, 17H, Ar-H), 7.54 (s, 1H, NH, exchangeable); ¹³C NMR: 118.3, 118.7, 119.8, 121.7, 124.3, 124.6, 126.6, 129.1, 129.2, 129.3, 136.7, 138.4, 140.8, 152.6, 156.6 (Ar-C), 172.4 (C=O, CS); Anal. Calc. for C₂₀H₁₄N₂O₂S (Mol. wt. 449): C, 72.14; H, 4.26; N, 9.35; Found: 72.03; H, 4.17; N, 9.28%.

4.8. Synthesis of 2-(((2-(4-phenoxypybenzoyl) benzoyl)carbamothioyl)thio)acetic acid (10)

A mixture of isothiocyanate 2 (0.01 mol) and thioglycolic acid (0.01 mol) in dry aceton was heated under reflux for 4 hrs. After cooling, the precipitated product was filtered, dried and recrystallized from ethanol.

Yield, 76%; mp 203°C- 205°C; IR (cm⁻¹): vmax: 3450-3215 (OH, NH), 3038 (CH-aromatic), 2938, 2850 (CH-aliphatic), 1710-1670 (CO), 1290 (C=S); Ms: m/z 451 (M+); ¹H NMR (DMSO-d₆) δ: 2.32 (s, 2H, SCH₂), 6.93-7.86 (m, 13H, Ar-H), 9.83 (s, 1H, NH, exchangeable), 10.40 (s, 1H, OH, exchangeable); ¹³C NMR: 41.3 (SCH₂), 118.1, 118.5, 119.3, 121.3, 126.2, 128.7, 129.1, 129.6, 130.4, 135.2, 140.8, 148.5, 155.3, 155.5 (Ar-C), 168.5, 177.6, 185.5, 190.7 (CO, CS); Anal. Calc. for C₂₅H₁₅NO₅S₂ (Mol. wt. 451): C, 61.18; H, 3.80; N, 3.10 Found: C, 61.11; H, 3.73; N, 2.98%.

4.9. Synthesis of 3-(2-(4-phenoxypybenzoyl)benzoyl)-2-thioxothiazolidin-4-one (11)

Acid 3 (0.01mol) was heated in aceticanhydride (5ml) under reflux for 4 hrs. The separated solid was filtered, dried and recrystallized from ethanol.

Yield, 74%; mp 184°C-186°C; IR (cm⁻¹): vmax: 3050 (CH-aromatic), 2915, 2875 (CH-aliphatic), 1740-1685 (CO), 1310 (C=S); Ms: m/z 433 (M+); ¹H NMR (DMSO-d₆) δ: 4.19 (s, 2H, SCH₂), 7.11-7.81 (m, 13H, Ar-H); ¹³C NMR: 38.8 (SCH₂), 117.5, 118.2, 118.4, 122.3, 127.6, 128.2, 128.6, 129.8, 131.8, 133.5, 142.4, 148.1, 154.6, 156.2 (Ar-C), 170.3, 173.5, 187.2, 192.6 (CO, CS); Anal. Calc. for C₂₃H₁₆N₂O₂S₂ (Mol. wt. 433): C, 63.73; H, 3.49; N, 3.23 Found: C, 63.65; H, 3.39; N, 3.16%.

4.10. Synthesis of 2-(2-(4-phenoxypybenzoyl)phenyl)-6-phenyl-4-thioxo-4H-1,3-oxazine-5,5-(6H)-dicarbonitrile (13)

A mixture of isothiocyanate 2 (0.01 mol) and benzylidenemalononitrile (12) (0.01 mol) in dry toluene was heated under reflux for 4 hrs. After cooling, the precipitated product was filtered, dried and recrystallized from benzene.

Yield, 78%; mp 213°C- 215°C; IR (cm⁻¹): vmax: 3007 (CH-aromatic), 2248 (C=S), 1603 (C=S), 1267 (C=S); Ms: m/z 513 (M+); ¹H NMR (DMSO-d₆) δ: 4.87 (s, 1H, CH), 7.32-8.18 (m, 18H, Ar-H); Anal. Calc. for C₂₅H₁₅N₂O₂S (Mol. wt. 513.57): C, 72.50; H, 3.73; N, 8.18; Found: C, 72.42; H, 3.61; N, 8.07%.

References
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