RESEARCH ARTICLE

An Efficient Synthesis of Biologically Active Tetrachloroquinazolin-2,4-dione
An Efficient Synthesis of Biologically Active Tetrachloroquinazolin-2,4-dione

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Abstract
Pyrimidine is a prominent member of the diazine family of heterocyclics. Pyrimidine compounds have been explored for use as histamine and adenosine receptor antagonists as well as among several other biological receptors and modulators. The aim of the current research work was to synthesize a new set of tetrachloroquinazolin-2,4-dione derivatives by treatment of N-phenylsulphonyloxytetrachlorophthalimide with some primary aliphatic and aromatic amines via Lossen Rearrangement. The structures of the synthesized compounds were characterized using physical and spectral data such as IR, 1H NMR and mass spectral studies. The newly synthesized compounds were evaluated for their preliminary in vitro antibacterial activity towards Salmonella typhi, Staphylococcus aureus and Bacillus cereus. This study leads us to conclude that quinazolinediones have interesting biological and pharmacological properties towards bacteria.

Keywords: 3-Alkyl(1H,3H)tetrachloroquinazolin-2,4-diones; 3-benzyl(1H,3H)tetrachloroquinazolin-2,4-dione; p-anisidine; pyrimidine ring; antibacterial.

1. Introduction
The quinazolinedione template occurs in a large number of bioactive molecules including serotonergic, dopaminergic and adrenergic receptor ligands and inhibitors of aldose reductase, lipoxygenase, cyclooxygenase, collagenase and carbonic anhydrase [1]. Furthermore, quinazolinediones have demonstrated utility in a diverse range of medical and biological applications. Pyrimidine and condensed pyrimidines have received much attention over the years because of their interesting biological and pharmacological properties as sedatives [2], antibacterials [3–9], antimalarial [2], analgesic [2, 4, 8], anti-inflammatory [2, 3, 8], anticonvulsant [9], antipyretic [5], antiparastic [5, 7], antifungal [6, 10, 11], antitoxic [12], antiviral [9, 12, 13], anticancers [14–17] ad DNA-binding activities [18]. In addition, pyrimidine and quinazolinediones have been found as a key component of many biologically active and pharmaceutical compounds. For this, our strategy was to prepare the targeted 3-substituted tetrachloroquinazoline from N-phenylsulphonyloxytetrachlorophthalimide.

2. Methods
2.1. Instrumentation
The main experimental challenge appears to be in fine tuning of experimental parameters (solvent, ratio of reagents, catalysis, etc.) so that pure crystalline material can be isolated. 1H NMR (200 MHz) spectra were recorded on a Varian EM 390 spectrometer. Chemical shift values were recorded in δ units (ppm) relative to tetramethylsilane (TMS) as internal standard. Melting points were determined using an electric melting point apparatus (Kofler). Infrared spectra (IR) were recorded using KBr pellets on a Shimadzu 408 spectrometer. Electron impact mass spectra were obtained at 70 eV using a GCMS sp.1000 Shimadzu. Thin layer chromatography (TLC) was performed on silica gel 60 PF254 plates or aluminum oxide plates obtained from Merck. Elemental analyses were carried out at a Microanalysis Unit at Cairo University, Egypt.
2.2. Chemical procedure

2.2.1. General procedure for the preparation of substituted (alkyl, cyclohexyl and benzyl) tetrachloroquinazolin-2,4-dione (2a–f)
A mixture of N-phenylsulphonyloxytetrachlorophthalimide (1) (0.44 gm, 1 mmol) and aliphatic amines, namely, methylamine, n-propylamine, isobutylamine and n-butylamine, cyclohexylamine and benzylamine (2 mmol) in the presence of anhydrous sodium acetate as a basic catalyst (0.12 gm, 1.5 mmol) in glacial acetic acid (20 ml) was refluxed for 6–12 h. After cooling, the reaction mixture was poured on ice water, where a white solid product was formed which filtered off and crystallized from appropriate solvent to give 3-alkyl(1H,3H)tetrachloroquinazolin-2,4-diones (2a–f) as white crystals (Scheme 2).

2.2.2. General procedure for the preparation of substituted (aryl)tetrachloroquinazolin-2,4-dione (3a–h)
To a solution of N-phenylsulphonyloxytetrachlorophthalimide (1) (0.44 gm, 1 mmol), aromatic amines (1.2 mmol), namely, aniline, p-toulidine, p-aminophenol, p-anisidine, p-chloroaniline, p-aminoacetophenone, p-aminobenzoic acid and p-nitroaniline were added; the reaction mixture was refluxed for 8 h. The reaction mixture was poured into water, and then the mixture was allowed to stand at room temperature overnight. The collected solid was filtered off and crystallized from the appropriate solvent to give 3-aryl(1H,3H)tetrachloroquinazolin-2,4-diones (3a–h).

2.3. Characterization data

2.3.1. 3-Methyl(1H,3H)tetrachloroquinazolin-2,4-dione (2a)
White crystal (0.28 gm, 72%), m.p. 280–282°C. FT-IR (KBr, cm⁻¹): 3200 (ν NH), 1740, 1660 (ν C=O’s). ¹H NMR spectrum: (300 MHz, DMSO-d₆): 0.9 (t, 3H, CH₃); 1.6 (m, 2H, CH₂); 3.8 (t, 2H, CH₂); 11.1 (s, 1H, NH). Anal. calcd. for C₁₂H₁₀Cl₄N₂O₂: C, 44.04%; H, 2.72%; N, 7.89%. Found: C, 44.20%; H, 2.72%; N, 7.89%.

2.3.2. 3-Propyl(1H,3H)tetrachloroquinazolin-2,4-dione (2b)
White crystal (0.35 gm, 67%), m.p. 250–252°C. FT-IR (KBr, cm⁻¹): 3200 (ν NH), 1740, 1660 (ν C=O’s). ¹H NMR spectrum: (300 MHz, DMSO-d₆): 0.9 (t, 3H, CH₃); 1.6 (m, 2H, CH₂); 3.8 (t, 2H, CH₂); 11.1 (s, 1H, NH). MS (m/z, %): 340 (1.26%) (M⁺) in addition to the characteristic peaks for compounds containing four chlorine atoms [19] at m/z = 318 (49.18%) (M+2); 316 (23.29%) (M+4) and 314 (14.48%) (M+6). The main fragment ions for compound 2a gives an excellent confirmation for the proposed structures, as shown in Scheme 1. Anal. calcd. for C₁₂H₂₀Cl₄N₂: C, 38.64%; H, 2.36%; N, 8.19%. Found: C, 38.92%; H, 2.36%; N, 8.20%.

2.3.3. 3-Isobutyl(1H,3H)tetrachloroquinazolin-2,4-dione (2c)
White crystal (0.41 gm, 83%), m.p. 280–282°C. FT-IR (KBr, cm⁻¹): 3206 (ν NH), 1726, 1669 (ν C=O’s). ¹H NMR spectrum: (300 MHz, DMSO-d₆): 0.9 (d, 6H, 2CH₃); 2.0 (m, 1H, CH); 3.7 (d, 2H, CH₂); 11.1 (s, 1H, NH). Anal. calcd. for C₁₂H₂₀Cl₄N₂O₂: C, 40.49%; H, 2.83%; N, 7.87%. Found: C, 40.55%; H, 2.72%; N, 7.89%.

2.3.4. 3-Butyl(1H,3H)tetrachloroquinazolin-2,4-dione (2d)
White crystal (0.34 gm, 78%), m.p. 280–282°C. FT-IR (KBr, cm⁻¹): 3201 (ν NH), 2960 (ν CH aliph.), 1726, 1660 (ν C=O’s). ¹H NMR spectrum: (300 MHz, DMSO-d₆): 0.9 (t, 3H, CH₃); 1.2 (m, 2H, CH₂); 1.5 (m, 2H, CH₂); 3.8 (t, 2H, CH₂); 11.1 (s, 1H, NH). MS (m/z, %): 354 (M⁺) in addition to the characteristic peaks for compounds containing four chlorine atoms at (M+2), (M+4) and (M+6). Anal. calcd. for C₁₂H₂₀Cl₄N₂O₂: C, 40.49%; H, 2.83%; N, 7.87%. Found: C, 40.22%; H, 2.91%; N, 7.69%.

2.3.5. 3-Cyclohexyl(1H,3H)tetrachloroquinazolin-2,4-dione (2e)
White crystal (0.16 gm, 53%), m.p. 306–308°C. FT-IR (KBr, cm⁻¹): 3201 (NH), 2924, 2852 (CH aliph.), 1740, 1659 (C=O’s). ¹H NMR spectrum: (200 MHz, DMSO-d₆): 1.0–2.5 (m, 10H, 5CH₂); 4.61 (m, 1H, N–CH); 11.1 (s, 1H, NH). MS (m/z, %): 380 (1.26%) (M⁺) in addition to the characteristic peaks for compounds containing four chlorine atoms at (M+2), (M+4) and (M+6). Anal. calcd. for C₁₄H₁₂Cl₄N₂O₂: C, 44.04%; H, 3.16%; N, 7.33%. Found: C, 44.20%; H, 3.17%; N, 7.41%.

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2.3.6. 3-Benzyl(1H,3H)tetrachloroquinazolin-2,4-dione (2f)
White crystal (0.24 gm, 76%), m.p. 262–263°C. FT-IR (KBr, cm\(^{-1}\)): 3211 (NH), 3032 (CH arom.), 1721, 1658 (C=O's).

\[\text{\textsuperscript{1}H NMR spectrum: (200 MHz, DMSO-d\textsubscript{6}): 7.2–7.35 (m, 5H, arom.); 5.1 (s, 2H, CH\textsubscript{2}); 11.1 (s, 1H, NH).}\]

MS (m/z, %): 388 (9.43%) (M\textsuperscript{+}) in addition to the characteristic peaks for compounds containing four chlorine atoms at (M\textsuperscript{+}2), (M\textsuperscript{+}4) and (M\textsuperscript{+}6). Anal. calcd. for C\textsubscript{15}H\textsubscript{8}Cl\textsubscript{4}N\textsubscript{2}O\textsubscript{2}: C, 46.20%; H, 2.06%; N, 7.18%. Found: C, 46.32%; H, 2.07%; N, 7.26%.

2.3.7. 3-Phenyl(1H,3H)tetrachloroquinazolin-2,4-dione (3a)
White crystal (0.22 gm, 68%), m.p. 328–329°C. \textsuperscript{1}H NMR spectrum: (300 MHz, DMSO-d\textsubscript{6}): 7.3–7.5 (m, 5H, arom.); 11.3 (s, 1H, NH). MS (m/z, %): 374 (M\textsuperscript{+}) in addition to the characteristic peaks for compounds containing four chlorine atoms at (M\textsuperscript{+}2), (M\textsuperscript{+}4) and (M\textsuperscript{+}6). Anal. calcd. for C\textsubscript{14}H\textsubscript{6}Cl\textsubscript{4}N\textsubscript{2}O\textsubscript{2}: C, 44.73%; H, 1.60%; N, 7.45%. Found: C, 44.94%; H, 1.61%; N, 7.56%.

2.3.8. 3-Toly(1H,3H)tetrachloroquinazolin-2,4-dione (3b)
White crystal (0.33 gm, 69%), m.p. 325–327°C. FT-IR (KBr, cm\(^{-1}\)): 3200 (\nu NH), 1740, 1660 (\nu C=O's). \textsuperscript{1}H NMR spectrum: (300 MHz, DMSO-d\textsubscript{6}): 2.2 (s, 3H, CH\textsubscript{3}); 7.1–7.3 (2d, 4H, A\textsubscript{2}B\textsubscript{2} arom.); coupling constant for aromatic protons = 0.0005 Hz, 11.2 (s, 1H, NH). MS (m/z, %): 388 (M\textsuperscript{+}) in addition to the characteristic peaks for compounds containing four chlorine atoms at (M\textsuperscript{+}2), (M\textsuperscript{+}4) and (M\textsuperscript{+}6). Anal. calcd. for C\textsubscript{15}H\textsubscript{8}Cl\textsubscript{4}N\textsubscript{2}O\textsubscript{2}: C, 46.20%; H, 2.06%; N, 7.18%. Found: C, 46.41%; H, 2.07%; N, 7.21%.

Scheme 1: The main fragmentations for compound 2a.
2.3.9. 3-Hydroxyphenyl(1H,3H)tetrachloroquinazolin-2,4-dione (3c)
Grey crystal (0.45 gm, 81%), m.p. 308–309°C. FT-IR (KBr, cm⁻¹): 3472 (ν OH), 3200 (ν NH), 1726, 1664 (ν C=O's).
1H NMR spectrum: (300 MHz, DMSO-d6): 6.8–7.1 (2d, 4H, Arom.); coupling constant for aromatic protons = 0.00077Hz, 11.2 (s, 1H, NH). MS (m/z, %): 408 (M⁺) in addition to the characteristic peaks for compounds containing four chlorine atoms at (M+2), (M+4) and (M+6). Anal. calcd. for C₁₅H₁₀Cl₁₄N₂O₂: C, 44.37%; H, 1.99%; N, 6.90%. Found: C, 44.62%; H, 2.01%; N, 7.00%.

2.3.10. 3-Anisidyl(1H,3H)tetrachloroquinazolin-2,4-dione (3d)
Buff crystal (0.49 gm, 83%), m.p. 312–314°C. FT-IR (KBr, cm⁻¹): 3196 (ν NH), 3001 (ν CH arom.), 2919 (ν CH aliph.), 1746, 1669 (ν C=O's). 1H NMR spectrum: (300 MHz, DMSO-d6): 3.8 (s, 3H, OCH₃); 7.4–8.1 (2d, 4H, Arom.); coupling constant for aromatic protons = 0.0008Hz, 11.2 (s, 1H, NH). MS (m/z, %): 416 (M⁺) in addition to the characteristic peaks for compounds containing four chlorine atoms at (M+2), (M+4) and (M+6). Anal. calcd. for C₁₅H₁₀Cl₁₄N₂O₂: C, 45.98%; H, 1.92%; N, 6.70%. Found: C, 46.35%; H, 1.94%; N, 6.92%.

3. Results and Discussion

3.1. Synthesis
The synthesis of the compounds 2a–f and 3a–h resulted from three steps [22], sequence starting from tetrachlorophthalic anhydride followed by N-hydroxytetrachlorophthalimide, then N-phenylsulphonyloxytetrachlorophthalimide (I).

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Table 1: Preparation of substituted (alkyl, cyclohexyl and aralkyl)tetrachloroquinazolin-2,4-diones (2a–f).

<table>
<thead>
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<th>Entry</th>
<th>Alkyl group</th>
<th>Product</th>
<th>Time (h)</th>
<th>Yield (%)</th>
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Scheme 2 outlines the synthetic pathway used to obtain compounds 2a–f. The starting material N-phenylsulphonyloxytetrachlorophthalimide was prepared by allowing N-hydroxytetrachlorophthalimide to react with benzenesulfonyl chloride. Upon mixing of compound 1 with primary aliphatic amines, cyclohexylamine and benzylamine (Table 1) in acetic acid, 3-alkyl(1H,3H)tetrachloroquinazolin-2,4-diones 2a–f were obtained in relatively good yields (Scheme 2).

In conjunction with our current research with the action of amines on compound 1, we study the action of primary aromatic amines, which have been found to be less basic than alkyl- and aralkylamines.

Scheme 3 outlines the synthetic pathway used to obtain compounds 3a–h, which is prepared by the treatment of compound 1 with different aromatic amines, namely, aniline, p-toulidine, p-aminophenol, p-anisidine, NOSO₂Ph

![Scheme 3](image)

Scheme 4: The main fragmentations for compound 2b.
Table 2: Preparation of substituted (aryl)tetrachloroquinazolin-2,4-dione (3a–h).

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p-chloroaniline, p-aminoaceto-phenone, p-aminobenzoic acid and p-nitroaniline (Table 2) in the presence of anhydrous sodium acetate as a base catalyst (0.12 gm, 1.5 mmol) in glacial acetic acid (20 ml) and was refluxed for 6–10 h to (Scheme 3).
4. Biological Activity
Bacterial infection causes high rate of mortality in human population and aquaculture organisms [23]. For example, B. cereus is responsible for causing foodborne diseases [24]. S. aureus causes diseases such as mastitis, abortion and upper respiratory complications, while Salmonella sp. causes diarrhea and typhoid fever [25]. The revolutionized therapy of infectious diseases by the use of antimicrobial drugs has certain limitations because of changing patterns of resistance in pathogens and side effects they produced. These limitations demand for improved pharmacokinetic properties, which necessitate continued research for new antimicrobial compounds for the development of drugs [26]. So accordingly, pharmaceutical industries are giving importance to the compounds derived from quinazolinedione sources.

4.1. Bacterial source and culture conditions
The bacteria used in this study were S. aureus, S. typhi and B. cereus (obtained from the Pathology Department, Faculty of Veterinary Medicine, South Valley University). These bacterial strains were maintained on suitable medium at 4°C and subcultured on Mueller Hinton Broth at 37°C for 18 h before testing.

4.2. Antibacterial assays
Most of the tested compounds exhibited antibacterial activity against all the tested bacterial species. The gram-negative S. typhi was the most sensitive to most of the compounds tested (2a–d and 3f). The higher antibacterial activity (indicated as zone of inhibition) was recorded for compound 3f followed by 2d and 2f (11, 10 and 10 mm), respectively (Figure 1). Hence, the susceptibility of the gram-positive B. cereus to compounds 2d, 2f, 3a, 3e–g was more pronounced when compared to the other tested compounds. The most observed antibacterial activity was recorded for compound 2d for both gram-positive and negative bacteria; this may be due to the four chlorine atoms [27] and pyrimidine ring [28–30]. On the other hand, S. aureus and B. cereus were resistant to compounds 2a–c, while they were sensitive to the other compounds. It is worthy to mention that the clear zone caused by compounds 2a, b and d with S. typhi was nearly closely to the inhibition zone caused by tetracycline disk; the sensitivity of these bacteria toward our compounds may be due to the presence of four chlorine atoms [27] and pyrimidine ring [28–30] (Figure 1). The efficiency of compounds 2a–d, and 3f as antibacterial products recorded the highest inhibition with the gram-negative bacteria; while 2d, 2f, 3a, 3e–g were more efficient for gram-positive bacteria. Thus,
the susceptibility of gram-positive bacteria to quinazolinediones was more than those of gram-negative bacteria. Many authors recorded similar observations [31]. The greater susceptibility of gram-positive bacteria to quinazolinedione compounds was because of the differences in their cell wall structure and their composition [32]. In gram-negative bacteria, the outer membrane acts as a barrier to many environmental substances, including antibiotics [33]. The presence of thick murine layer in the cell wall also prevents the entry of the inhibitors [34]. The above results confirm the broad antibacterial effect of quinazolinedione compounds.

Competing Interests
None declared.

Authors’ Contributions
All authors contributed equally to this work.

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References


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