Utilisation of bis-chloroacetamide derivative in the synthesis of new biologically active sulfide compounds

Norhan A Khalaf, Ehab Abdel-Latif*, Mohamed A Ismail and Heba M Metwally

Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, Egypt

ABSTRACT

4-Aminobenzohydrazide (I) undergoes chloroacetylation twice, at the primary amine and hydrazide-\(\text{NH}_2\) functional groups. The conforming bis-chloroacetamide derivative 3 was reacted with different sulfur reagents (namely, 2-mercaptobenzothiazole, 6-amino-2-mercaptopyrimidin-4-ol, and 2-mercapto-4,6-dimethyl-nicotinonitrile) to give new bis-sulfide compounds 5, 7 and 9, respectively. The newly synthesised bis-chloroacetamide and corresponding sulfides were screened for anti-microbial and antioxidant potential. The sulfide derivative 7 exhibited the most potent activity against \textit{Staphylococcus aureus} and \textit{Pseudomonas aeruginosa}. It shows inhibition activities of 83.4% and 78.8%, respectively. Moreover, the sulfide derivative 7 showed the highest antioxidant activity with an inhibition ratio of 85.9%, which is close to L-ascorbic acid.

KEYWORDS

4-aminobenzohydrazide, antibacterial, antioxidant chloroacetamide, sulfide

INTRODUCTION

In recent years, a notable increase in bacterial infections has occurred.\(^1\) Resistance against bacterial and fungal infections – resulting from the abuse of antibacterial and antifungal drugs – poses a serious health hazard. The resistance of known antibacterial agents was the driving force for creating and modifying new antifungal and antibacterial medications.\(^2\) Nowadays, various pharmacological actions of benzohydrazide derivatives have given them great importance. For example, they have shown analgesic\(^4\) and anti-inflammatory properties.\(^5\) Along with their promising anti-microbial activities,\(^6\) benzohydrazide derivatives act as anti-cancer drugs.\(^7\) They also possess significant anti-tubercular,\(^8\) anti-HIV,\(^9\) and antioxidant\(^10\) activities. Benzohydrazide possesses some important drug features; for example, Isoniazid (I) and its derivative Iproniazid (II) (Figure 1) have an antidepressant action and are used in pharmacologic treatments for tuberculosis and psychosis.\(^11\) Several benzohydrazide compounds have been identified to possess a wide range of biological functions. 2-Aminobenzohydrazide derivative III (Figure 1) showed a greater than 90% scavenging percentage in their antioxidant activity experiment.\(^12\) In addition, a good inhibitory activity was shown by 4-amino escitalopram benzohydrazide derivative IV (Figure 1) against cholinesterase AChE and BChE.\(^13\) Furthermore, the most potent analgesic, ulcerogenic and anti-inflammatory activity was presented by quinazoline benzohydrazide derivative V (Figure 1).\(^14\)

A well-known synthetic intermediate is chloroacetamide. It has received remarkable attention as this intermediate features various biological activities (for example, analgesic, antitumor, anti-microbial, antioxidant, hypoglycemic, and antipyretic) and applications in agriculture.\(^15\)–\(^18\) Moreover, different 2-chloroacetamide compounds have been utilised in solid-state chemistry\(^19\) and were found to produce pharmaceutically favourable compounds.\(^20\)–\(^22\) Also, various transformations of chloroacetamide compounds could lead to the preparation of sulfur-containing compounds. Growth inhibition of \textit{Escherichia coli} and \textit{Staphylococcus aureus} was achieved by the most potent antibacterial compounds, sulfide derivatives.\(^23\)

Based on the previous importance, various methods are described for the preparation of sulfides. The most common protocols include the addition of aryllithium or organocuprate to thiocarbonyl compounds, reduction of sulfoxides and sulfones;\(^24\) nucleophilic displacement of aryl and alkyl halides by thiols;\(^25\)–\(^27\) the treatment of alkyl halides with thiourea,\(^24\) or thiocarbonate;\(^28\) the reaction of halides with thiolamines;\(^29\) Michael addition of thiols\(^30\) to \(\alpha,\beta\)-unsaturated carbonyl compounds; intermolecular \(\text{S-alkylation of thiol with alcohols;}\(^29\) and the addition of thiols, or their anions to alkenes.\(^29\),\(^30\) Because of various disadvantages encountered in the reported methodologies, such as use of hazardous and toxic solvents, difficulty in recovery of high boiling solvents, use of costly catalysts, etc., eco-friendly and more convenient methods have been developed.\(^31\) These comprise the use of catalytic phenylselenyl bromide,\(^32\) nickel,\(^33\) or native silica nanoparticle\(^34\) under solvent-free conditions, \(\beta\)-cycloextrin in the presence of water and acetone or under catalyst-free conditions.\(^35\) In light of the previous statement, this study aims to prepare bis-sulfide derivatives from N-aryl bis-chloroacetamide and study the biological effects of these compounds as antioxidant and antibacterial agents.

RESULTS and DISCUSSION

Chemistry

The starting compound 4-aminobenzohydrazide (I) has been obtained by the reported experimental conditions through refluxing ethyl 4-aminobenzoate in hydradine hydrate.\(^36\) The reactivity of 4-aminobenzohydrazide (I) towards chloroacetylation was tested by treatment with chloroacetyl chloride in DMF containing anhydrous potassium carbonate (Scheme 1). It was expected that a mono-substituted chloroacetamide product 2 would be obtained through chloroacetylation at the hydrazide function (\(-\text{NH}_2\)). Unexpectedly, spectroscopic analyses indicated the formation of the bis-substituted chloroacetamide compound 3 through double chloroacetylation at both amino groups (primary amotic amine and the amino group of hydrazide function). The structure of the previously obtained compound was confirmed by spectroscopic analyses, where the IR spectrum displayed absorptions at 3323, 3257, and 1685 cm\(^{-1}\), corresponding to the (N-H) and carbonyl (C=O) groups. The \(^1\)H NMR spectrum indicated the appearance of singlet signals at \(\delta\) 4.20 and 4.28 ppm for the protons of two methylene groups, two doublet signals at \(\delta\) 7.68 and 7.85 ppm for four aromatic protons, and three singlet signals at \(\delta\) 10.33, 10.43, and 10.57 ppm for the protons of three N-H groups.
The 13C NMR spectrum confirmed the appearance of characteristic signals at δ 164.76, 165.12 and 165.44 ppm indicating the carbon atoms of three amidic carbonyl groups. It also showed the appearance of new signals at δ 41.00 and 43.64 corresponding to the two carbon atoms of the methylene groups.

The chemical behaviour of the bis-chloroacetamide derivative 3 was tested against different types of nucleophilic sulfur reagents. Thus, stirring of bis-chloroacetamide compound 3 with 2-mercaptobenzothiazole (4) yielded the corresponding benzothiazole-2-yl sulfide derivative 5 in 60% yield (Scheme 2). The IR spectrum of sulfide 5 exhibited absorptions at 3326 and 3288 (N-H), 1677 and 1656 cm\(^{-1}\) (C=O). The 1H NMR spectrum indicated singlet signals at δ 4.28 and 4.42 ppm for the protons of methylene groups. The aromatic protons resonate as a multiplet and doublet in the range from δ 7.34 to 8.02 ppm. The protons of N-H groups resonate as three singlet signals at δ 10.38, 10.42 and 10.71 ppm. The 13C NMR spectrum exhibited twenty-three carbon signals for 25 carbon atoms and indicated the characteristic signals of methylene carbon atoms at δ 34.90 and 37.76 ppm.

The reaction of one molar amount of bis-chloroacetamide derivative 3 with two molar amounts of 6-amino-2-mercaptopyrimidin-4-ol (6) and/or 2-mercapto-4,6dimethyl nicotinonitrile (8) in acetone and anhydrous K\(_2\)CO\(_3\) yielded the corresponding bis-sulfide derivatives 7 and 9 in 79% and 71% yields, respectively (Scheme 3). The reaction proceeded via attack of the sulfur nucleophile on bis-chloroacetamide derivative 3 and substitution of the chlorine atom. The IR spectrum of bis-sulfide compound 7 (as an example) exhibited absorptions at 3475, 3336 and 3228 cm\(^{-1}\) for the O-H and NH\(_2\) functions. The 1H NMR spectrum displayed singlet signals at δ 4.98 and 5.02 ppm for the protons of pyrimidine-H and a broad singlet at δ 6.57 ppm for the protons of amino groups.

### Biological activity of the prepared bis-sulfide compounds

#### Antibacterial activity

The bis-chloroacetamide compounds and their corresponding bis-sulfide compounds were examined as antibacterial agents. Two types of Gram(+)ve bacteria (Staphylococcus aureus and Bacillus subtilis) and two types of Gram(−)ve bacteria (Escherichia coli and Pseudomonas aeruginosa) were selected for this study. The methodology of the applied antibacterial assay was carried out as previously described in the literature. A primary investigation of the antibacterial activity of the prepared sulfide derivatives (Table 1) indicated that the bis-sulfide derivative 7, linked to the pyrimidine ring system, is the most active compound against S. aureus and P. aeruginosa bacteria with relatively inhibitory activity indices of 83.4% and 78.8%, respectively. It also showed relatively moderate activity against B. subtilis and E. coli with activity indices of 65.2% and 52.0% (inhibition zones of 15 and 13 mm), respectively. The bis-sulfide derivative 5, with the benzothiazole moiety, showed moderate antibacterial activity against S. aureus with...
an activity index of 62.2%. It also showed significant activity against *Pseudomonas aeruginosa* with an activity index of 51.5% (inhibition zone of 11.9 mm). On the other hand, compounds 3 and 9 are the least active compounds against all tested bacteria.

**Antioxidant assay**

The pro-oxidant activities of the produced compounds were tested using the ABTS assay. The antioxidant potential of the prepared sulfide derivatives 5, 7, and 9 was investigated (Table 2). The results indicated that the sulfide derivative 7, bearing pyrimidine rings, showed antioxidant activity with the highest percentage inhibition (85.9%), which is close to the reference, ascorbic acid (88.0%). The sulfide derivatives 5 and 9, and bis-chloroacetamide compound 3 exhibited weak antioxidant activity.

**CONCLUSION**

Chloroacetylation of 4-aminobenzohydrazide (1) was carried out at both amino groups to give bis-chloroacetamide reagent 3. Nucleophilic substitution of the mobile chlorine atom of compound 3 with thiol reagents led to the synthesis of bis-sulphide derivatives 5, 7, and 9. Antibacterial activities of the new bis-sulfide derivatives were screened against *S. aureus*, *B. subtilis*, and *P. aeruginosa*. The bis-sulphide derivative 7 containing the pyrimidine ring showed significant antibacterial and antioxidant potentialities.

**EXPERIMENTAL**

Melting points were determined on the Gallenkamp apparatus. The IR spectroscopic analysis was recorded on a Thermo Scientific Nicolet iS10 FTIR spectrometer. 1H NMR (500 MHz) and 13C NMR (125 MHz) spectra were obtained in DMSO-d$_6$ using JEOL’s spectrometer. The Perkin-Elmer 2400 analyser was used to determine the elemental analyses.

### Preparation of 2-chloro-N-(4-(2-(2 chloroacetyl) hydrazinecarbonyl)-phenyl)acetamide (3)

A mixture of 4-aminobenzohydrazide (0.30 g, 2 mmol), 2-chloroacetyl chloride (0.16 mL, 2 mmol) and potassium carbonate (0.28 g, 2 mmol) was stirred in 20 mL DMF for 4 h. The mixture was poured into ice water and subjected to neutralisation by diluted HCl. The solid was filtered and subjected to recrystallisation from ethanol to furnish the bis-chloroacetamide compound 3.

Yield 57%, m.p. 240–241°C. IR (v/cm$^{-1}$): 3232, 3257 (N-H), 1685, 1655 (C=O); 1H NMR (δ/ppm): 4.20 (s, 2H, CH$_2$), 4.28 (s, 2H), 7.68 (d, $J$ = 8.50 Hz, 2H), 7.85 (d, $J$ = 8.50 Hz, 2H), 10.33 (s, 1H), 10.43 (s, 1H), 10.57 (s, 1H). 13C NMR (δ/ppm): 41.00, 43.64, 118.65 (2C), 127.19, 128.72 (2C), 128.72, 141.70, 164.76, 165.12, 165.44. Analysis Calculated for C$_{11}$H$_{11}$Cl$_2$N$_3$O$_3$ (303.02): C, 43.44; H, 3.65; N, 12.38%. Found: C, 53.08; H, 3.39; N, 12.38%. Condition: C, 52.87; H, 3.34; N, 12.48%.

### Preparation of 2-(Benzothiazol-2-ylthio)-N-(4-(2-(benzothiazol-2-ylthio)acetyl)hydrazinecarbonyl)phenyl)acetamide (5)

Yield 63%, m.p. 195–196°C. IR (v/cm$^{-1}$): 3228, 3210 (N-H), 1677, 1656, 1625 (C=O); 1H NMR (δ/ppm): 4.28 (s, 2H), 4.42 (s, 2H), 7.36 (q, $J$ = 7.00 Hz, 2H), 7.40-7.50 (m, 2H), 7.68 (d, $J$ = 8.50 Hz, 2H), 7.80 (d, $J$ = 8.00 Hz, 1H), 7.80-7.89 (m, 3H), 8.01-8.02 (dd, $J_1 = 8.00, J_2 = 4.00$ Hz, 2H), 10.38 (s, 1H), 10.42 (s, 1H), 10.71 (s, 1H). 13C NMR (δ/ppm): 34.90, 37.76, 118.41 (2C), 121.08, 121.18, 121.84, 121.91, 124.55 (2C), 126.39, 126.43, 127.26, 127.29, 128.50, 134.77, 134.80, 141.78, 152.50, 152.52, 164.55, 165.71, 165.76, 165.91, 165.97. Analysis calculated for C$_{27}$H$_{25}$N$_7$O$_3$S$_2$ (559.15): C, 57.94; H, 4.50; N, 17.52%. Found: C, 57.73; H, 4.38; N, 17.80%.

### Experimental:

- **Preparation of 2-chloro-N-(4-(2-(2 chloroacetyl) hydrazinecarbonyl)-phenyl)acetamide (3)**
  - A mixture of 4-aminobenzohydrazide (0.30 g, 2 mmol), 2-chloroacetyl chloride (0.16 mL, 2 mmol) and potassium carbonate (0.28 g, 2 mmol) was stirred in 20 mL DMF for 4 h. The mixture was poured into ice water and subjected to neutralisation by diluted HCl. The solid was filtered and subjected to recrystallisation from ethanol to furnish the bis-chloroacetamide compound 3.
- **Preparation of 2-(Benzothiazol-2-ylthio)-N-(4-(2-(benzothiazol-2-ylthio)acetyl)hydrazinecarbonyl)phenyl)acetamide (5)**
  - A mixture of bis-chloroacetamide compound 3 (0.15 g, 0.5 mmol), 2-mercaptobenzoxazole (0.17 g, 1 mmol) or 6-amino-2-mercaptopyrimidin-4-ol (0.14 g, 1 mmol) and potassium carbonate (0.14 g, 1 mmol) was stirred in 20 mL acetone for 4 h. The mixture was poured into ice water, and the solid was collected and subjected to recrystallisation from ethanol to produce the targeted sulfides 5 and 7.

**General methodology for the production of bis-sulfide compounds 5 and 7**

A mixture of bis-chloroacetamide compound 3 (0.15 g, 0.5 mmol), 2-mercaptobenzoxazole (0.17 g, 1 mmol) or 6-amino-2-mercaptopyrimidin-4-ol (0.14 g, 1 mmol) and potassium carbonate (0.14 g, 1 mmol) was stirred in 20 mL acetone for 4 h. The mixture was poured into ice water, and the solid was collected and subjected to recrystallisation from ethanol to produce the targeted sulfides 5 and 7.

**SUPPLEMENTARY MATERIAL**

Supplementary information for this article is provided in the online supplement. This contains spectral characterization of title compounds, copies of IR, 1H NMR and 13C NMR spectra (Figures S1-S11).

**ORCID IDs**

Ehab Abdel-Latif: https://orcid.org/0000-0003-4437-6635

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**Table 2: ABTS radical scavenging activity of the prepared bis-sulfide derivatives**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Absorbance</th>
<th>Inhibition [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control of ABTS</td>
<td>0.510</td>
<td>0.0</td>
</tr>
<tr>
<td>Ascorbic-acid</td>
<td>0.061 ± 0.001</td>
<td>88.0</td>
</tr>
<tr>
<td>3</td>
<td>0.407 ± 0.085</td>
<td>20.2</td>
</tr>
<tr>
<td>5</td>
<td>0.294 ± 0.062</td>
<td>42.3</td>
</tr>
<tr>
<td>7</td>
<td>0.072 ± 0.011</td>
<td>85.9</td>
</tr>
<tr>
<td>9</td>
<td>0.430 ± 0.065</td>
<td>15.7</td>
</tr>
</tbody>
</table>

Each value = mean (± SEM) of three triplicates
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