Synthesis and Antibacterial Activity of Novel Hydroxy Semicarbazone Derivatives

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Abstract

A series of hydroxyl semicarbazone derivatives of substituted diaryl ketones and acetophenones were synthesized and their structures were confirmed by analytical and spectroscopic methods including elemental analysis, infrared and nuclear magnetic resonance spectroscopy. The derivatives were prepared by a condensation reaction between N-hydroxy semicarbazide and substituted diaryl ketones or acetophenones leading to the desired hydroxysemicarbazones with excellent purity. The synthesized hydrazones were then evaluated for their inhibitory activity against bacterial strains including \textit{S. aureus}, \textit{E. Coli}, \textit{P. aeruginosa}, \textit{K. pneumonia} and \textit{M. luteus}. Among the tested derivatives, compounds 2, 6 and 7 exhibited the highest bioactivity. Analysis of the activity data suggests that hydrophilicity is an important factor for the bioactivity of compounds 2 and 6 and also their selectivity over the gram-negative bacteria.

Keywords: \textit{N}-hydroxy semicarbazone; Antibacterial; Broth microdilution assay; Ketones; Hydrophilicity.

Introduction

In the drug discovery efforts to combat against microbial infections, (thio) semicarbazones have attracted considerable attentions and many derivatives of this class have been reported to possess promising bioactivity. In addition some (thio) semicarbazones have been marketed in some periods as antimicrobial drugs and some more are under investigations as potential ones. Because of their interference with vital biochemical processes in the living cells such as deoxyribonucleotide synthesis (1), cell wall biosynthesis (2) and maintaining thiol contents (3), (thio)semicarbazones have been reported to exert antibacterial (4), antmycobacterial (5), anticancer (1), antifungal (6) and antimalarial (7) activities. The general structure of the active (thio) semicarbazones is disclosed in Figure 1, which consists of an aromatic system linked to the (thio) semicarbazone moiety. Working on this structural backbone, many research projects have been conducted to discover novel (thio) semicarbazone derivatives with optimized potency and safety profiles.

In a research work carried out by Sriram and his colleagues, some \textit{N}-hydroxythiosemicarbazones of different aromatic systems were synthesized and tested for their antmycobacterial
activity (8). Among the employed aromatic carbonyl compounds to condense with N-hydroxythiosemicarbazide, the order of activity of the tested compounds was found to be Schiff bases of diaryl ketones > acetophenones > aromatic aldehydes (Figure 2).

Considering the above findings and in continuation of our interest to study the bioactivity of hydrazone derivatives (9-12), in the present work we describe the synthesis and antibacterial activity of some N-hydroxysemicarbazones of diaryl ketones and substituted acetophenones. These derivatives could be considered as bioisoesters of active N-hydroxysemicarbazones in which the thiocarbonyl is substituted by carbonyl functional group. The new derivatives could also be viewed as active antimicrobial semicarbazones bearing a hydroxyl substituent at their thioamide nitrogen (Figure 3).

Experimental

General

Melting points were measured using an Electrothermal 9100 apparatus and are uncorrected. The Infrared spectra were obtained with a Perkin-Elmer 843 spectrometer. Proton nuclear magnetic resonance (1H NMR) and carbon nuclear magnetic resonance (13C NMR) spectra were determined by a Bruker Avance DRX 500 MHz spectrometer and the samples were dissolved in DMSO-d6 and tetramethylsilane (0.05% v/v) as internal standard. All the compounds were analyzed for C, H and N on a Costech model 4010 and agreed with the proposed structures within ±0.4% of the theoretical values. Hydroxysemicarbazide (13) and 4,4′-dihydroxybenzophenone 2,4-dinitrophenylhydrazone (compound 7) (13) were prepared according to previously reported methodologies.

Benzophenone hydroxysemicarbazone (1)

Method A. (conventional synthesis)

Benzophenone (3.64 g, 20 mmol) was added to 50 mL of absolute ethanol and the mixture was heated at 70 °C. Then, a solution of hydroxysemicarbazide (1.82 g, 20 mmol) in 20 mL of water and 7 drops of glacial acetic acid was added dropwise to the solution via a dropping funnel. The mixture was heated under reflux for 48 h and then concentrated by vacuum distillation. The resulting precipitate was filtered and recrystallized from methanol to afford the title compound as white powder (1.27 g, 24%).

Method B. (microwave-assisted synthesis)

In a 100 mL beaker, benzophenone (3.64 g, 20 mmol) was dissolved in 50 mL of absolute ethanol by a gentle heating. A solution of hydroxysemicarbazide (1.82 g, 20 mmol) in 20 mL of water and 7 drops of glacial acetic acid was then added in three portions to the
Benzophenone solution. After addition of each portion, the beaker was placed in a microwave reactor set at 600 W for five 30-second periods. After the completion of the procedure, the beaker was cooled at room temperature and the resulting precipitate was filtered. The crude was recrystallized from 2-propanol to afford the title compound.

White powder (3.31 g, 65%): mp 162.5-165 °C; IR (KBr): 3200 (OH), 3350 (NH), 1668 (C=O); 1H NMR (CDCl3/500 MHz): δ 8.30 (br s, 1H), 7.85 (br s, 1H), 7.65-7.36 (m, 5H, Ar H), 7.21-7.17 (m, 3H, Ar H), 7.12 (d, J=8.15, Ar H); Anal. Calcd for C14H13N3O2 (255.27): C, 65.87; H, 5.13; N, 16.46. Found: C, 65.71; H, 5.14; N, 16.50.

Benzoylbenzoic acid hydroxyl semicarbazone (2)

After addition of the regents as described for compound 1 (method A), the mixture was heated under reflux for 1 h and then stirred at room temperature for 24 h. The mixture was concentrated by vacuum distillation and refrigerated for 1 h. The precipitate was filtered and recrystallized from methanol.

White powder (4.24 g, 72%): mp 230-231 °C; IR (KBr): 3000 (OH), 3160 (NH), 1670 (C=O); 1H NMR (DMSO-d6/500 MHz): δ 7.56-7.65 (m, 6H, Ar H), 7.68 (m, 1H, Ar H), 7.92 (m, 2H, Ar H), 8.36 (1H, s, Ar H), 12.88 (s, 1H); 13C NMR (CDCl3/125 MHz): δ 160.8, 148.3, 135.4, 133.6, 131.8, 130.0, 129.5, 128.7, 127.1, 77.8, 77.5, 77.3, 40.4, 40.4, 40.2; Anal. Calcd for C15H13N3O4 (299.28): C, 65.71; H, 4.38; N, 14.04. Found: C, 60.29; H, 4.38; N, 14.00.

2,4-Dihydroxybenzophenone hydroxyl semicarbazone (3)

After addition of the regents as described for compound 1 (method A), the mixture was heated under reflux for 24 h and then stirred at room temperature for 48 h. The mixture was concentrated by vacuum distillation and refrigerated for 1 h. The precipitate was filtered and the title compound with acceptable purity was obtained.

Yellow powder (4.24 g, 72%): mp 184-186 °C; IR (KBr): 3100 (OH), 3200 (NH), 1634 (C=O); 1H NMR (CDCl3/500 MHz): δ 12.40 (s, 1H), 11.95 (s, 1H), 9.9 (s, 1H), 9.27 (s), 7.39 (m, 3H, Ar H), 7.13 (d, J=7.26, 2H, Ar H), 6.51 (d, J=8.78, 1H, Ar H), 6.13 (s, 1H, Ar H), 6.03 (dd, J=8.79, J=2.33, Ar H); Anal. Calcd for C14H13N3O2 (255.27): C, 58.53; H, 4.56; N, 14.63. Found: C, 58.40; H, 4.57; N, 14.65.

4,4’-Dihydroxybenzophenone hydroxyl semicarbazone (4)

The reaction was carried out as described for compound 1 (method A), except that an appropriate amount of molecular sieve was added to the reaction mixture. After heating the mixture under reflux for 24 h, stirring for further 48 h and typical workup as described earlier, the title compound was obtained.

Yellow powder (3.50 g, 60%): mp 141-144 °C; IR (KBr): 3200 (OH), 1630 (C=O); 1H NMR (CDCl3/500 MHz): δ 12.20 (s, 1H), 10.72 (s, 1H), 7.61 (m, 3H, Ar H), 7.54 (m, 2H, Ar H), 7.37 (d, J=8.66, 1H, Ar H), 6.38 (m, 2H, Ar H); Anal. Calcd for C14H13N3O4 (299.27): C, 65.71; H, 4.38; N, 14.63. Found: C, 65.80; H, 4.56; N, 14.69.

Acetophenone hydroxysemicarbazone (5)

After addition of the regents as described for compound 1 (method A), the mixture was heated under reflux for 1 h and then stirred at room temperature for 1 h. The precipitate thus formed was filtered and recrystallized form 2-butanol.

Yellow powder (2.90 g, 75%): mp 144-146 °C; IR (KBr): 3300 (NH), 3200 (OH), 1680 (C=O); 1H NMR (CDCl3/500 MHz): δ 8.61 (br s, 1H), 8.35 (br s, 1H), 7.63 (d, J=6.96, 2H, Ar H), 7.35 (m, 1H, Ar H), 7.29 (m, 2H, Ar H), 2.15 (s, 3H, CH3), 2.1 (s); Anal. Calcd for C9H11N2O2 (193.20): C, 55.95; H, 5.74; N, 21.75. Found: C, 55.87; H, 5.75; N, 21.71.

Methoxyacetophenone hydroxysemicarbazone (6)

After addition of the regents as described for compound 1 (method A), the mixture was heated under reflux for 1 h and then stirred at room temperature for 2 h. The precipitate thus formed was filtered and the title compound with acceptable purity was obtained.

Light brown powder (3.25 g, 73%): mp 181-
In each well was calculated by the following formula:

\[
\text{IC} = \frac{\text{OD}_{\text{c}} - (\text{OD}_{\text{a}} - \text{OD}_{\text{b}})}{\text{OD}_{\text{c}}}
\]

Where ODa, ODb and ODc are the optical density of the solutions containing microorganisms and test compounds, only test compounds and only microorganisms respectively. IC50 was defined as the lowest concentration of the test compound in which the bacterial growth was completely inhibited. Amikacin and vancomycin were used as standard antibiotics. It is notable that each assay was performed as duplicates.

**Results and Discussion**

**Chemistry**

As disclosed in scheme 1, the final derivatives were prepared by a Schiff base formation reaction. The condensation between (thio) semicarbazide and aromatic ketones and aldehydes in the presence of an acidic catalyst often leads to stable crystalline products in good to excellent yields. However, in cases that (thio) semicarbazones of diaryl ketones are desired, a more challenging reaction could be anticipated and may need some modifications to force the reaction to proceed. This happens because the two \(\pi\)-donating aromatic systems make their adjacent carbonyl group less reactive towards different nucleophiles. In this project, the acetophenone derivatives 5 and 6 were prepared in good yields and the reaction time was as short as 2-3 h. However, for preparing compounds 1-4, for the...
As it appears in Table 1, compound 7 showed the highest activity against both gram-positive and gram-negative strains. The MIC values for compound 7 against E. coli, K. pneumonia, and P. aeruginosa were recorded as 12.5, 25, and 50 µg/mL, respectively. In contrast, unsubstituted benzophenone derivative 1 showed no activity even at 1000 µg/mL, indicating that the presence of hydrophilic substituents is essential for antibacterial activity. Among the substituted benzophenone derivatives, compound 2 with 2-carboxy substituent was the most active derivative, especially against E. coli, with an MIC value of 12.5 µg/mL.

Biological activity

The synthesized derivatives were evaluated for their antibacterial activity against 6 different strains and the MIC values are listed in Table 1. In addition to hydroxysemicarbazone derivatives 1-6, we were interested in bioactivity evaluation of compound 7. This compound has been reported to exhibit antitumor activity against some cancer cell lines (15). Since many antitumor derivatives have also been reported to possess antimicrobial activity (16-19), antimicrobial activity assessment of compound 7 as a previously established anticancer agent in order to evaluate its potential to be considered as a lead in antimicrobial drug discovery seems logical. Furthermore, since the hydroxysemicarbazone moiety is replaced by a phenylhydrazine derivative in compound 7, this derivative can be used to assess the role of hydroxysemicarbazone moiety in the tested derivatives.

As it appears in Table 1, compound 7 showed the highest activity against P. aeruginosa and...
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Dover LG, Alahari A, Gratraud P, Gomes JM and Bhowruth VE. EthA, a common activator of

E. Coli with IC\textsubscript{50} values of 62.5 and 31.25 µg/mL. Among the substituted benzophenone derivatives, compound 2 with 2-carboxy substituent was the most active derivative especially against gram-negative strains such as E. Coli, P. aeruginosa and K. pneumonia. While the 4-methoxyacetophenone derivative 6 showed moderate activity against E. Coli, P. aeruginosa, the acetophenone derivative 5 did not show any activity at 1000 µg/mL concentration.

Based on the analysis of the activity data, it is suggested that the presence of hydrophilic substituents on the aromatic ring is important for antibacterial activity. As evident from the activity data, 2-carboxy substituted benzophenone derivative 2 showed higher activity than the unsubstituted benzophenone derivative 1. Similarly, 4-methoxysubstituted acetophenone derivative 6 exhibited higher potency than its unsubstituted analog (compound 5). It is notable that compounds 2 and 5 demonstrated some selectivity through inhibition of gram-negative bacteria. This might be due to higher penetration of these hydrophilic derivatives inside the gram-negative bacteria through their hydrophilic porin channels which leads to availability of higher toxic concentrations of the tested derivatives inside the microorganism. Besides the hydroxysemicarbazones 1-6, phenylhydrazone derivatives 7 exhibited promising antibacterial activity which gives us a new opportunity to conduct more focused studies on the antibacterial activity of other phenylhydrazone derivatives of diaryl systems.

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