SYNTHESIS OF NOVEL 2-AMINO-N-HYDROXYBENZAMIDE ANTIMICROBIALS

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GRAPHICAL ABSTRACT

Abstract New 2-amino-N-hydroxybenzamide derivatives (3a-j) have been synthesized in good to excellent yields from the reaction of isatoic anhydride with different hydroxamic acids. All the compounds of the series were screened against both Gram-positive and Gram-negative bacteria and fungi, and some of them have antibacterial and antifungal activities compared to the standard drugs.

Keywords Antimicrobial agents; benzamide; hydroxamic acid; isatoic anhydride

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INTRODUCTION

In the recent years, isatoic anhydride synthesized from anthranil and ethyl chloroformate[1] has been extensively used for the synthesis of various families of organic compounds such as quinolines,[2] quinazolinones,[3–7] bezimidazoquinazolins,[8] 2-aminobenzothiates,[9] quinazolinediones,[10] and 2-aminobenzamides[7,11–13] because of its unique physicochemical properties and reactivity. This versatility in its chemistry is possible because of the susceptibility for the α-hetero cleavage of anthranilic C-4 carbonyl group rather than isatoic C-2 carbonyl group by the action of various nucleophiles such as amines,[7,11–13] disulfides,[9] semicarbazide,[13] hydrazine,[13] boronic acid,[14] and alkenes.[15]

Benzamide molecules have a wide range of therapeutic applications because of their anti-inflammatory,[16] antibacterial,[17] antifungal,[18] antiallergic,[19] antiarrhythmic,[20] and antileukotriene[21] activities. Apart from their direct use in medicine, their metal complexes have also found application as anthelmintic agents.[22–24]

The structurally modified benzamide forms different interesting libraries of compounds.[25,26] Among them, the N-hydroxy amides received serious attention from researchers working in coordination and medicinal chemistry because of their multidentate capacity.[27] This property made them good siderophores capable of chelating with the Fe$^{+2}$ ion and drug conjugates in vivo, which facilitates transfer of the drugs into the cells of microbes and inhibits their growth. Some important siderophores are cited in Fig. 1.

In view of their potential medical applications, we have synthesized some 2-amino-N-hydroxybenzamide analogs that are capable of acting as multidentate siderophores and thus antimicrobial agents. A solvent- and catalyst-free methodology with mild operating conditions involving simple separation and purification of the products was designed and accomplished.[28–30]

RESULTS AND DISCUSSION

Chemistry

The chemical advantages shown by isatoic anhydride as precursor for a variety of medicinally important derivatives lie mainly in its capacity to react at two

![Figure 1. Important siderophores that contain the N-hydroxy amide moiety.](image-url)
fundamental sites: (i) the C-8 position of the aromatic ring through electrophilic aromatic substitution and (ii) the more electrophilic C-4 carbonyl group though nucleophilic substitution reaction by losing a CO₂ group. In addition, it may also react through the N atom due to its nonbonded electrons to give some useful products.

A series of new 2-amino-N-hydroxybenzamide derivatives (3a–j) has been synthesized in a one-pot synthetic procedure from the reaction of isatoic anhydride (1a), 6-chloro isatoic anhydride (1b), and 5-chloro isatoic anhydride (1c) with various alkyl and aryl hydroxamic acids (2a–f) in acetonitrile at room temperature (Scheme 1).

Initially we studied the variations in product yields of 3a and their reaction times at various temperatures with different organic solvents such as EtOH, tetrahydrofuran (THF), CHCl₃, toluene, and water and then with CH₃CN by reacting simple isatoic anhydride (1a) and acetohydroxamic acid (2a) (Table 1). Finally the reaction proceeded well in CH₃CN at room temperature and hence these conditions are selected as the optimized experimental conditions. Different hydroxamic acids (2a–f) were synthesized from the corresponding carboxylic acids in a two-step reaction procedure[31] and used as substrates to accomplish the title compounds (3a–j) (Table 2). Both aliphatic and aromatic hydroxamic acids gave good yields with isatoic anhydride and chloro-substituted isatoic anhydrides in CH₃CN even at room temperature.

![Scheme 1. Synthesis of 2-amino-N-hydroxy benzamide derivatives (3a–j).](image)

**Table 1. Screening of the different solvents at varying temperatures on the reaction of isatoic anhydride and acetohydroxamic acid**

| Entry | Solvent | Temperature (°C) | Time (h) | Yield (%) |
|-------|---------|-----------------|----------|-----------|
| 1     | H₂O     | RT              | 5        | Trace     |
| 2     | H₂O     | 70              | 3        | 72        |
| 3     | EtOH    | 60              | 4        | 80        |
| 4     | THF     | 75              | 5        | 45        |
| 5     | CHCl₃   | 50              | 3        | 75        |
| 6     | Toluene | 80              | 8        | 58        |
| 7     | CH₃CN   | 75              | 3        | 85        |
| 8     | CH₃CN   | 60              | 4        | 88        |
| 9     | CH₃CN   | 40              | 5        | 90        |
| 10    | CH₃CN   | RT              | 3        | 95        |
| 11    | CH₃CN   | RT              | 3        | 98        |

aExperimental conditions: isatoic anhydride (1 mmol) and acetohydroxamic acid (1 mmol) in 10 mL of acetonitrile for 3 h at rt under reflux conditions.

bIsolated yields.

c5-Chloro isatoic anhydride is used instead of isatoic anhydride.
| Entry | Isatoicanhydride (1a–c) | Hydoxamic acid (2a–f) | Product (3a–j) | Time (h) | Yield (%) | Melting point (°C) |
|-------|------------------------|----------------------|----------------|----------|-----------|-------------------|
| a     | \[
\begin{array}{c}
\text{H} \\
\text{O}
\end{array}
\]  
(1a) | \[
\begin{array}{c}
\text{NH}_{2} \\
\text{OH}
\end{array}
\]  
(2a) | \[
\begin{array}{c}
\text{NH}_{2} \\
\text{O}
\end{array}
\]  
(3a) | 3 | 95 | 85–87 |
| b     | \[
\begin{array}{c}
\text{H} \\
\text{O}
\end{array}
\]  
(1a) | \[
\begin{array}{c}
\text{NH}_{2} \\
\text{OH}
\end{array}
\]  
(2b) | \[
\begin{array}{c}
\text{NH}
\end{array}
\]  
(3b) | 3 | 95 | 90–92 |
| c     | \[
\begin{array}{c}
\text{H} \\
\text{O}
\end{array}
\]  
(1a) | \[
\begin{array}{c}
\text{NH}_{2} \\
\text{OH}
\end{array}
\]  
(2c) | \[
\begin{array}{c}
\text{NH}
\end{array}
\]  
(3c) | 3 | 96 | 136–138 |
| d     | \[
\begin{array}{c}
\text{H} \\
\text{O}
\end{array}
\]  
(1a) | \[
\begin{array}{c}
\text{NH}_{2} \\
\text{OH}
\end{array}
\]  
(2d) | \[
\begin{array}{c}
\text{NH}
\end{array}
\]  
(3d) | 3 | 98 | 109–111 |
| e     | \[
\begin{array}{c}
\text{H} \\
\text{O}
\end{array}
\]  
(1a) | \[
\begin{array}{c}
\text{NH}_{2} \\
\text{OH}
\end{array}
\]  
(2e) | \[
\begin{array}{c}
\text{NH}
\end{array}
\]  
(3e) | 3 | 98 | 149–151 |
| f     | \[
\begin{array}{c}
\text{Cl} \\
\text{O}
\end{array}
\]  
(1a) | \[
\begin{array}{c}
\text{NH}_{2} \\
\text{OH}
\end{array}
\]  
(2f) | \[
\begin{array}{c}
\text{NH}
\end{array}
\]  
(3f) | 3 | 97 | 155–157 |
| g     | \[
\begin{array}{c}
\text{Cl} \\
\text{O}
\end{array}
\]  
(1b) | \[
\begin{array}{c}
\text{NH}_{2} \\
\text{OH}
\end{array}
\]  
(2a) | \[
\begin{array}{c}
\text{NH}
\end{array}
\]  
(3a) | 3 | 98 | 144–146 |
| h     | \[
\begin{array}{c}
\text{Cl} \\
\text{O}
\end{array}
\]  
(1b) | \[
\begin{array}{c}
\text{NH}_{2} \\
\text{OH}
\end{array}
\]  
(2f) | \[
\begin{array}{c}
\text{NH}
\end{array}
\]  
(3f) | 3 | 96 | 163–165 |
| i     | \[
\begin{array}{c}
\text{Cl} \\
\text{O}
\end{array}
\]  
(1c) | \[
\begin{array}{c}
\text{NH}_{2} \\
\text{OH}
\end{array}
\]  
(2a) | \[
\begin{array}{c}
\text{NH}
\end{array}
\]  
(3a) | 3 | 98 | 131–133 |
| j     | \[
\begin{array}{c}
\text{Cl} \\
\text{O}
\end{array}
\]  
(1c) | \[
\begin{array}{c}
\text{NH}_{2} \\
\text{OH}
\end{array}
\]  
(2f) | \[
\begin{array}{c}
\text{NH}
\end{array}
\]  
(3f) | 3 | 97 | 160–162 |
The possible mechanism for the reaction is ring opening of isatoic anhydride through decarboxylation. Hydroxamic acid itself acts as a proton donor and the nitrogen atom present in the hydroxamic acid act as a good nucleophile. Because of the more electrophilic nature of anthranilic C-4 carbonyl carbon, the nucleophilic nitrogen of hydroxamic acid attacks at C-4 instead of C-2 (Scheme 2). Moreover, strong nucleophilicity of hydroxamic acid favors subsequent decarboxylation in a smoother way even at mild conditions and forms the products in good yield. This is a better procedure because the previous reports used either a catalyst or harsh reaction condition.

All the synthesized compounds were fully characterized on the basis of their physical and spectral (infrared [IR], NMR, and mass spectrographic [MS]) data. They showed strong IR absorption bands at 3490–3300, 3230–3170, 1732–1680, 1612–1590, and 2945–2820 cm\(^{-1}\) for the amino, hydroxy, carbonyl, aromatic -C=C-, -CH\(_2\)-, and -CH\(_3\) groups respectively. Their \(^1\)H NMR spectrum gave three multiplets at \(\delta\) 6.78–9.40, which are assigned to aromatic protons, and a singlet at \(\delta\) 2.0–2.6 corresponding to hydroxyl proton. Another singlet at \(\delta\) 6.20–6.70 for two amino protons and the singlet at \(\delta\) 1.2–2.3 correspond to methyl protons. The \(^{13}\)C NMR data confirm the presence of three important carbonyl groups in the title compounds from the predicted chemical shift values in the range of 176.8–170.3 for acetyl carbon, 166.0–161.4 for benzamide carbon, and 163.6–161.1 for benzoyl carbon respectively.

Pharmacology

All the synthesized compounds were screened for antimicrobial activity by the disc diffusion technique. The antibacterial activity of the compounds was evaluated against two Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*; two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*; and two fungi, *Candida albicans* and *Candida non-albicans*. The test solutions were prepared with a concentration of 10 mg/mL of the compound in dimethylsulfoxide (DMSO) in order to obtain a final concentration of 1 mg/0.1 mL.

Test results are shown in Table 3 and it reveals that among ten synthetic compounds, 3e exhibited the greatest antibacterial activity against three test bacterial species, *Bacillus subtilis* (14.4 ± 0.12), *Escherichia coli* (16.2 ± 0.06), and *Pseudomonas aeruginosa* (15.2 ± 0.23) and both the fungi, *Candida albicans* (14.2 ± 0.22) and *Candida non-albicans* (15.8 ± 0.24). Compound 3h inhibited *Staphylococcus aureus* at maximum of 14.2 ± 0.17 and 3e inhibited it at 14.2 ± 0.14. The least growth inhibition was observed in the cases of bacteria by 3d and fungi by 3a.

Scheme 2. Plausible mechanism for formation of N-hydroxy benzamide.
The structure–activity relationship correlated from the antimicrobial activity results obtained for the title compounds and their corresponding chemical structures reveals that the compounds having N-benzoyl substitution exhibited greater antimicrobial activity than the compounds having N-acetyl substitution. It is due to the fact that the π electron delocalization enhances proton-donating capacity of the N-hydroxy group, and the multidentate nature of the compound easily facilitates their interaction with the microbes and hence inhibits their growth to act as good antimicrobial agents.

**EXPERIMENTAL**

**Chemistry**

Chemicals were procured from Sigma Aldrich and used as such without further purification. All solvents used for the spectroscopic and other physical studies were reagent grade and were further purified. Melting points were determined using a calibrated thermometer by Guna digital melting-point apparatus. They are expressed in degrees centigrade (°C) and are uncorrected. IR spectra were recorded as neat samples on Bruker Alpha-Eco ATR-FTIR (attenuated total reflection–Fourier transform infrared) interferometer with single-reflection sampling module equipped with ZnSe crystal and reported in reciprocal centimetres (cm⁻¹). ¹H and ¹³C NMR spectra were recorded as solutions in DMSO-ｄ₆ on a Bruker AMX 500-MHz spectrometer operating at 400 MHz for ¹H and 100 MHz for ¹³C NMR. The ¹H and ¹³C chemical shifts were expressed in δ with reference to tetramethylsilane (TMS) as an internal standard. Electron impact (EI) and high-resolution mass spectrographic (HRMS) data were recorded on a VG 7070 H ev instrument at 70 ev.

**General Method for Synthesis of 2-Amino-N-hydroxy-N-acetylbenzamide (3a)**

Isatoic anhydride (1 mmol) and acetohydroxamic acid (1 mmol) were taken in a 50-ml round-bottomed flask, and 10 mL of acetonitrile was added. The contents

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| Table 3. Antimicrobial activity zone of growth inhibition calculated by applying standard deviation |
|---------------------------------------------------------------|
| **Entry** | **B. subtilis** | **S. aureus** | **E. coli** | **P. aeruginosa** | **C. albicans** | **C. non-albicans** |
|-----------|----------------|----------------|-------------|------------------|------------------|------------------|
| 3a        | 8.6 ± 0.58     | 9.3 ± 0.49     | 9.8 ± 0.35  | 8.4 ± 0.14       | 9.4 ± 0.26       | 8.6 ± 0.28       |
| 3b        | 10.7 ± 0.14    | 14.5 ± 0.35    | 12.6 ± 0.08 | 10.6 ± 0.26      | 11.5 ± 0.16      | 12.5 ± 0.14      |
| 3c        | 10.2 ± 0.28    | 12.4 ± 0.15    | 10.2 ± 0.088| 8.6 ± 0.16       | 10.4 ± 0.08      | 12.4 ± 0.18      |
| 3d        | 7.9 ± 0.18     | 8.5 ± 0.20     | 7.5 ± 0.21  | 8.5 ± 0.34       | 9.2 ± 0.12       | 9.6 ± 0.34       |
| 3e        | 14.4 ± 0.12    | 14.2 ± 0.14    | 16.2 ± 0.06 | 15.2 ± 0.23      | 14.2 ± 0.22      | 15.8 ± 0.24      |
| 3f        | 10.3 ± 0.08    | 11.3 ± 0.26    | 12.4 ± 0.22 | 10.6 ± 0.16      | 10.0 ± 0.18      | 11.4 ± 0.16      |
| 3g        | 12.5 ± 0.21    | 13.4 ± 0.08    | 12.2 ± 0.18 | 12.3 ± 0.14      | 10.3 ± 0.32      | 13.2 ± 0.12      |
| 3h        | 12.8 ± 0.08    | 14.2 ± 0.17    | 10.5 ± 0.133| 11.6 ± 0.12      | 13.6 ± 0.14      | 14.8 ± 0.33      |
| 3i        | 10.4 ± 0.16    | 11.2 ± 0.06    | 9.6 ± 0.08  | 10.4 ± 0.30      | 11.8 ± 0.16      | 10.6 ± 0.08      |
| 3j        | 12.2 ± 0.14    | 10.5 ± 0.53    | 10.0 ± 0.14 | 9.2 ± 0.16       | 10.4 ± 0.24      | 10.5 ± 0.18      |
| Tetracycline| 18.2 ± 0.15    | 18.4 ± 0.32    | 20.4 ± 0.32 | 18.2 ± 0.12      | —                | —                |
| Ampoterccine B | —     | —              | —            | —                | 16.5 ± 0.33      | 17.4 ± 0.12      |
were stirred magnetically for about 3 h at room temperature. After completion of the reaction as indicated by thin-layer chromatography (TLC) using SiO$_2$ as adsorbent and 4:6 ethyl acetate/n-hexane as eluent, the crude product was separated by filtration and purified by recrystallization from EtOH. The other compounds (3b–j) were prepared by the same procedure.

**2-Amino-N-hydroxy-N-acetylbenzamide (3a)**

Yield (95%), mp 85–87°C; $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm): 2.1 (s, 3H), 5.3 (brs, 2H), 6.4 (m, $J = 8.1$ Hz, 2H), 7.2 (m, $J = 8.1$ Hz, 1H), 7.9 (m, $J = 8.1$ Hz, 1H), 9.2 (brs, 1H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm): 175.6 (acetyl -C=O), 165.2 (benzamide -C=O), 150.6 (Ar C-2), 135.7 (Ar C-4), 130.3 (Ar C-6), 115.6 (Ar C-5), 114.9 (Ar C-1), 106.9 (Ar C-3), and 23.8 (-CH$_3$); IR (neat, $\nu$, cm$^{-1}$): 3480 (-NH$_2$), 3164 (-OH), 1684 (-C=O), 1608 (Ar), 2823 (-CH$_3$); HRMS $m/z$ (EI) calcd. for C$_9$H$_{10}$N$_2$O$_3$: 194.07333; found: 194.07310.

**Pharmacology**

**In vitro antimicrobial activity.** The identified test bacterial strains were retrieved from −80°C freezers, thawed, streaked onto nutrient agar (NA), and checked for purity after incubation for 24 h at 30°C. A loop full of each test strain was inoculated in 10 mL of N-broth separately and was incubated for 24 h in an incubator at 37°C to activate the bacterial strain.[32] Following the pour plate technique, 0.2 mL ($10^6$ cells per 1 mL) of the activated strain was inoculated into the nutrient agar medium on reaching 40–45°C and allowed to solidify. This complete procedure was done in a laminar airflow to maintain strict sterile and aseptic conditions. After solidification of the media, a well was made in the media with the help of a cup-borer (0.85 cm) and then 0.1 mL of the synthetic compound (dissolved in DMSO) was inoculated into the well. For each test compound against each test strain, three replicas were maintained. Also, controls were maintained for each test bacterial strain where 0.1 mL of only the pure solvent was inoculated into the well. The plates were incubated for 24 h at 37°C. The inhibition zone formed by test compounds against the particular test bacterial strain was observed, and the mean value of the three individual replicates was calculated to determine the zone of growth inhibition of each test compound.[33]

The antifungal activity of the synthetic compounds at a concentration of 10 mg/mL in DMSO was tested in vitro against two fungi *Candida albicans* and *Candida non-albicans* using the same diffusion technique applied in case of determining antibacterial activity with slight modifications. By following the pour plate technique, 0.1 mL ($10^5$ cells per 1 mL) of the activated strain was inoculated into the potato dextrose agar medium on reaching 40–45°C and allowed to solidify. This complete procedure was done in a laminar airflow to maintain strict sterile and aseptic condition. After solidification of the media, a well was made in the media with the help of a cup-borer (0.85 cm diameter) and then 0.1 mL of the synthetic compound (dissolved in DMSO) was inoculated into the well. For each test compound against each test strain, three replicas were maintained. Also, controls were maintained for each test bacterial strain where 0.1 mL of only the pure solvent was inoculated into the well. The plates were incubated for 72 h at 30°C. The inhibition zone formed by test compounds against the particular test bacterial strain was observed, and the mean value of the three individual replicates was calculated to determine the zone of growth inhibition of each test compound.[34]
was calculated to determine the zone of growth inhibition of each test compound. Ampotercine B was used in standard antifungal agent.\\[33\\]

**CONCLUSION**

A series of new 2-amino-N-hydroxybenzamide derivatives (3a–j) has been synthesized in good to excellent yields, and their antimicrobial potentiality has identified 3e and 3h as potential antibacterial compounds and 3a and 3d as potential antifungal compounds.

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**SUPPORTING INFORMATION**

Supplemental data for this article can be accessed on the publisher’s website.

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