# Synthesis of 2-Oxo and 6-Hydroxylated Benzimidazole Analogs of DOPA as Potential Norepinephrine Antagonists



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The synthesis of benzimidazolone analogs of DOPA and α-methyl-DOPA and of the benzimidazole and benzimidazolone analogs of 6-hydroxy-DOPA is reported. These compounds showed a moderate amount of dopamine β-hydroxylase and tyrosine hydroxylase inhibitory activity but did not significantly alter tissue catacholamine levels.

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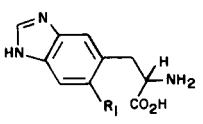
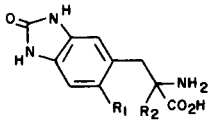
Our laboratory has long been interested in the synthesis of benzimidazole analogs of tyrosine as potential antihypertensive agents (1,2). Following the promise shown by 2-amino-3-(benzimidazol-5-yl)propionic acid (benzimidazolalanine **1**) as a tyrosine hydroxylase inhibitor (1) and as a tissue catecholamine lowering compound (3), the derived ethylamines (4) and β-hydroxyethyl amines (5) were prepared and tested. This communication describes the extension of this series to include 2-oxo and 6-hydroxy derivatives of **1**.

2-Oxobenzimidazoles exist in solution in equilibrium with the 2-hydroxybenzimidazole tautomers (6). The aromatic hydroxy moiety of 2-hydroxybenzimidazole can be thought as phenolic although benzimidazolones ($pk_a$ 11-12) (7) are less acidic than phenol or catechol. Therefore, 2-oxobenzimidazoles were chosen for synthesis as heterocyclic counterparts of the catechol antihypertensive agent methyldopa. The preparation of 2-amino-3-(2-oxo-2*H*-benzimidazol-5-yl)propionic acid (**3**) and its α-methyl analog **2** is described in this report.

6-Hydroxylated derivatives of **1** (compound **7**) and of **3** (compound **5**) which are possible metabolites (8) of their parent compounds (**1** and **3**) were prepared as potential precursor analogs of the chemical sympatholytic agent, 6-hydroxydopamine. These compounds, as well as the 6-methoxy precursors of **5** and **7** were tested for their ability to affect tissue catecholamine levels and to inhibit tyrosine hydroxylase and dopamine β-hydroxylase activity. Compounds **4-7** were also tested for their ability to affect the adrenergic nervous system of the neonatal rat. A partial description of the work has been published in the patent literature (9,10,11).





| | | |
|---|---|---|
| **1** $R_1$ = H | **2** $R_1$ = H | $R_2$ = $CH_3$ |
| **6** $R_1$ = $OCH_3$ | **3** $R_1$ = H | $R_2$ = H |
| **7** R = OH | **4** $R_1$ = $OCH_3$ | $R_2$ = H |
| | **5** $R_1$ = OH | $R_2$ = H |

## Synthesis.

Compounds **2** and **3** were synthesized from α-methylphenylalanine and phenylalanine, respectively. After protecting the amino and the carboxyl groups of the side chain, 4-acetamido and 3-nitro substituents were introduced into the phenyl ring and the urea analogs then prepared by hydrolysis, reduction, and cyclization in the presence of phosgene.

The starting point for compounds **4-7** was 2-methoxy-5-nitrobenzyl bromide which was condensed with sodioethyl-acetamidomalonate to yield diethyl 2-(2-methoxy-5-nitro-

SCHEME I

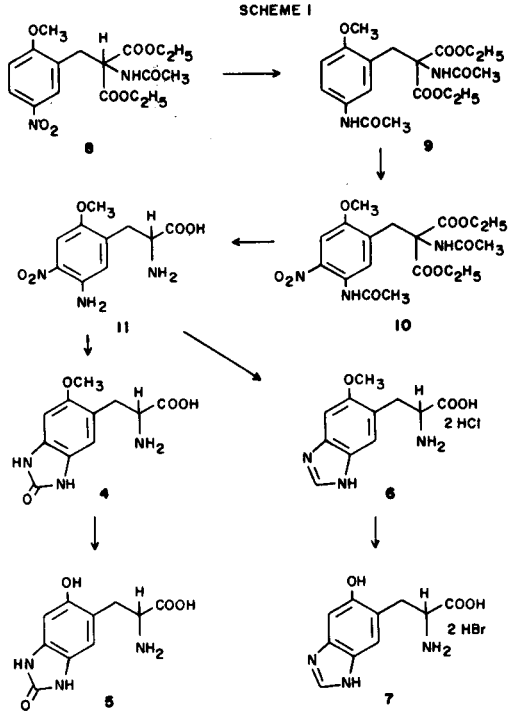


Table I

Inhibition of Tyrosine Hydroxylase and of Dopamine β-Hydroxylase Activity by Compounds **2-7** and by some Reference Compounds

| Compound Tested (a) | Percent Inhibition (b) | |
|---|---|---|
| | Tyrosine Hydroxylase (c) | Dopamine β-Hydroxylase (d) |
| **2** | 26 | 17 |
| **3** | 0 | 18 |
| **4** | 4 | 33 |
| **5** | 0 | 24 |
| **6** | 6 | 25 |
| **7** | 7 | 0 |
| 1 | 40 | 25 |
| *o*-Tyrosine | 9 | 12 |
| 6-OH-DOPA | 2 | 7 |

(a) The numbering of the compounds is the same as that in the body of the text. (b) The inhibition values listed on the table are based on the average of two or more experiments performed in triplicate (tyrosine hydroxylase) or quadruplicate (dopamine β-hydroxylase). The concentration of inhibitor during the incubation was equal to the concentration of substrate: $0.2 \times 10^{-4} M$ in tyrosine hydroxylase, $0.4 \times 10^{-4} M$ in dopamine β-hydroxylase measurements. (c) Reference 18. (d) Reference 19.

benzyl)-2-acetamidomalonate (12) (Scheme 1, Compound **8**), which was reduced and acetylated to yield diethyl 2-(2-methoxy-5-acetylaminobenzyl)-2-acetamidomalonate (**9**). Compound **9** was nitrated to yield 2-(2-methoxy-4-nitro-5-acetylaminobenzyl)-2-acetamidomalonate (**10**), which, in turn, was hydrolyzed to yield 2-amino-3-(5-amino-2-methoxy-4-nitrophenyl)propionic acid (**11**). Compound **11** was reduced and cyclized in the presence of phosgene to yield 2-amino-3-(6-methoxy-2-oxo-2*H*-benzimidazol-5-yl)propionic acid (**4**), or in the presence of formic acid to yield 2-amino-3-(6-methoxybenzimidazol-5-yl)propionic acid (**6**). Refluxing **4** and **6** with hydrobromic acid yielded 2-amino-3-(6-hydroxy-2-oxo-2*H*-benzimidazol-5-yl)propionic acid (**5**) and 2-amino-3-(6-hydroxybenzimidazol-5-yl)propionic acid (**7**), respectively.

Preliminary Biochemical Evaluation.

Compounds **2-7** were tested as inhibitors of tyrosine hydroxylase (tyrosine 3-monooxygenase) activity as inhibition of this enzyme was apparently a component of the *in vivo* activity of (**1**). The results presented (Table I) showed only α-methylbenzimidazolone (**2**) to have significant inhibitory activity; there is no indication from compounds **5** or **7**, or from 6-hydroxy-DOPA or *o*-tyrosine that a hydroxy group in position 6 enhances inhibitory activity. Compounds **2-7** were also tested for inhibition of dopamine β-hydroxylase activity, an activity that may correlate with antihypertensive activity (13). The results presented in Table I suggest that all compounds except **7** exhibit dopamine β-hydroxylase inhibitory activity. Since our previous results (3) suggested that the main component of the *in vivo* activity of **1** was the ability of a decarboxylated metabolite of that amino acid to release catecholamines from synaptosomal granules, we tested in the same system compound **3**, which differs from **1** only by the presence of a 2-oxobenzimidazole moiety, and compound **4**, which was the best dopamine β-hydroxylase inhibitor of the series (Table I). The results revealed that neither the compounds nor their possible decarboxylated metabolites had a significant catecholamine releasing effect. No other compound of this series was able to lower heart norepinephrine or brain catecholamines when administered at a dose of 0.5 mmole/kg 3 hours before sacrifice.

Injection of componds **4-7** to seven day old rats according to a dose and schedule described in the experimental section produced no change in the number or shape of the sympathetic neurons of superior cervical ganglia. With 6-hydroxydopamine, such treatment leads to extensive disappearance of the neurons (14).

The present results indicate that 2-oxo and some of the 6-substituted derivatives of benzimidazolealanine interfere with norepinephrine synthesis and warrant further evaluation as potential antihypertensive agents.

## EXPERIMENTAL

Melting points were determined on a Thomas-Hoover or Meltemp melting point apparatus and are uncorrected. The $^1H$ nmr were recorded on a Jeolco C-60 HL NMR spectrometer and the chemical shifts are expressed in parts per million relative to TMS or sodium 2,2-dimethyl-2-silapentane-5-sulfonate. A Perkin-Elmer 257 Infrared spectrometer was used to obtain ir spectra. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee.

2-Amino-2-methyl-3-(2-oxo-2*H*-benzimidazol-5-yl)propionic Acid Hydrochloride (**2**).

A mixture of 2-methyl-2-acetamido-3-(3-nitro-4-acetamidophenyl)-propionate (15) (0.8 g, 2.5 mmoles) and 4 *N* hydrochloric acid (50 ml) was held at reflux for 2 hours. The resulting red-orange solution was cooled and then reduced with hydrogen (2.5 atmospheres) over 10% palladium on carbon (0.3 g) at room temperature overnight. The mixture was then filtered under nitrogen through a bed of celite by suction and the filtrate treated with phosgene gas (60 ml/minute) for 1 hour. The white precipitate which developed was collected to yield **2** (0.4 g, 59%). Two recrystallizations from water provided an analytical sample, mp 333° dec.

*Anal.* Calcd. for $C_{11}H_{13}N_3O_3 \cdot HCl$: C, 48.63; H, 5.19; N, 15.47. Found: C, 48.39; H, 5.19; N, 15.25.

2-Amino-3-(2-oxo-2*H*-benzimidazol-5-yl)propionic Acid Hydrochloride (**3**).

Following the procedure for the production of **2**, diethyl 2-(3-nitro-4-acetamidobenzyl)-2-acetamidomalonate (16) was hydrolyzed and cyclized with phosgene to yield pure product, mp 259° dec.

*Anal.* Calcd. for $C_{10}H_{11}N_3O_3 \cdot HCl$: C, 46.61; H, 4.69; N, 16.31. Found: C, 46.84; H, 4.73; N, 16.23.

Diethyl 2-(2-Methoxy-5-acetamidobenzyl)-2-acetamidomalonate (**9**).

A solution of diethyl 2-(2-methoxy-5-nitrobenzyl)-2-acetamidomalonate (12) (**8**, 15.0 g, 39.3 mmoles) in absolute ethanol (200 ml) was hydrogenated over 10% palladium on carbon (0.8 g) at 2.7 atmospheres. After removal of the catalyst by filtration through a bed of celite, the filtrate was evaporated. The residual amine was immediately refluxed with acetic acid (5 ml) and acetic anhydride (25 ml) for 1 hour, cooled, and then

poured on ice (100 g). The precipitate which formed provided 14 g (88%) of the title compound and was used in the next step without further purification. An analytical sample was crystallized from ethyl acetate, mp 150-151°.

*Anal.* Calcd. for $C_{19}H_{26}N_2O_7$: C, 57.86; H, 6.60; N, 7.10. Found: C, 57.49; H, 6.92; N, 7.13.

Diethyl 2-(2-Methoxy-4-nitro-5-acetamidobenzyl)-2-acetamidomalonate (**10**).

To a stirred suspension of **9** (19.0 g, 48.2 mmoles) in acetic acid/acetic anhydride (1:1) maintained at 0-5° was added nitric acid (30 ml, specific gravity 1.48) during 1 hour. After stirring for an additional hour at 10-15°, the mixture was poured slowly into a rapidly stirred, ice-cold aqueous sodium bicarbonate solution (120 g, in 40 ml). The resulting dark yellow solid was collected, washed with water and crystallized from chloroform/hexanes (1:1) to give **10** (14.7 g, 88%), mp 159-161°; ir (potassium bromide): 1570 $cm^{-1}$ ($NO_2$); $^1H$ nmr (deuteriochloroform): 7.72 (s, aromatic 1H).

*Anal.* Calcd. for $C_{19}H_{25}N_3O_9$: C, 51.39; H, 5.73; N, 9.56. Found: C, 51.89; H, 5.80; N, 9.57.

2-Amino-3-(5-amino-2-methoxy-4-nitrophenyl)propionic Acid (**11**).

The diester **10** (10.0 g, 22.8 mmoles) was held at reflux with 5 *N* hydrochloric acid (90 ml) for 6 hours. After cooling, the *p*H of the solution was adjusted to 6 with 30% ammonium hydroxide. Amino acid **11** precipitated from the solution after refrigeration overnight. Crystallization from methanol/water (1:1) gave bright red crystals of **11** monohydrate (5.5 g, 94%), mp 239° dec.

*Anal.* Calcd. for $C_{10}H_{13}N_3O_5 \cdot H_2O$: C, 43.95; H, 5.53; N, 15.38. Found: C, 44.29; H, 5.59; N, 15.11.

2-Amino-3-(6-methoxy-2-oxo-2*H*-benzimidazol-5-yl)propionic Acid Hydrochloride (**4**).

Following the procedure for the preparation of **2**, compound **11** was reduced and cyclized to form **4**, mp 288° dec.

*Anal.* Calcd. for $C_{11}H_{13}O_4N_3 \cdot HCl$: C, 45.91; H, 4.87; N, 14.61; Cl, 12.35. Found: C, 46.15; H, 5.11; N, 14.42; Cl, 12.10.

2-Amino-3-(6-hydroxy-2-oxo-2*H*-benzimidazol-5-yl)propionic Acid Hydrobromide (**5**).

Treatment of **4** (0.25 g, 0.99 mmole) with refluxing hydrobromic acid (48%, 10 ml) for 6 hours under nitrogen followed by decolorization with norit yielded, upon evaporation of the volatile solvents, a residue which when crystallized from methanol/acetonitrile (1:2) gave **5** (0.11 g, 39%), mp 265° dec.

*Anal.* Calcd. for $C_{10}H_{11}O_4N_3 \cdot HBr$: C, 37.74; H, 3.77; N, 13.21; Br, 25.13. Found: C, 37.57; H, 3.90; N, 13.14; Br, 24.94.

2-Amino-3-(6-methoxybenzimidazol-5-yl)propionic Acid Dihydrochloride (**6**).

A solution of **11** (1.0 g, 3.9 mmoles) in 5 *N* hydrochloric acid (40 ml) was hydrogenated over 5% palladium on carbon (0.1 g) at 2.7 atmospheres. The mixture was filtered through celite under nitrogen into formic acid (97%, 7 ml) and then heated to reflux for 3.5 hours under nitrogen. The resulting solution was decolorized (norit) and the solvent removed. The residue was twice taken up in isopropanol (5 ml) and the solvent evaporated to yield crude **6** (970 mg, 81%). Recrystallization from ethanol/water gave pure **6** (620 mg, 51%), mp 275° dec; ir (potassium bromide): 1755 $cm^{-1}$ (COOH) and 1630 $cm^{-1}$ (C=N); $^1H$ nmr (deuterium oxide): 9.27 (s, C-2 hyrogen indicating ring closure).

*Anal.* Calcd. for $C_{11}H_{13}N_3O_3 \cdot 2HCl$: C, 42.86; H, 4.87; N, 13.64; Cl, 23.05. Found: C, 43.34; H, 4.63; N, 13.59; Cl, 22.86.

2-Amino-3-(6-hydroxybenzimidazol-5-yl)propionic Acid Dihydrobromide (**7**).

Compound **6** (1.4 g, 4.6 mmoles) was heated under reflux with hydrobromic acid (48%, 25 ml) for 6 hours under nitrogen. The precipitate which formed on cooling overnight was collected and washed successively with acetone and ether. The resulting solid was decolorized (norit) in ethanol. Pure **7** (0.9 g, 53%) was obtained by recrystallization from ethanol/acetonitrile (1:2), mp 273° dec.

*Anal.* Calcd. for $C_{10}H_{11}N_3O_3 \cdot 2HBr$: C, 31.35; H, 3.40; N, 10.97; Br, 41.75. Found: C, 31.68; H, 3.50; N, 11.00; Br, 41.58.

Biological Screening.

A tyrosine hydroxylase preparation was made as previously reported (17). The enzyme was assayed by the method of Quick and Sourkes (18). Dopamine β-hydroxylase activity was assayed by the method of Wise (19) using the 20,000 g pellet of a buffered adrenal bovine sucrose homogenate taken up in phosphate buffer containing 0.1% Triton. Sprague-Dawley rats used in the catecholamine experiments were sacrificed by decapitation, the heart tissue first chopped in a McElwain type tissue chopper (Brinkmann-Mickle), brain and heart tissue then homogenized (polytron) and processed for catecholamines by liquid chromatography and electrochemical detection according to the method of Refshauge, *et al.* (20).

To test for destruction of adrenergic tissue, compounds **4-7** were administered as aqueous solutions (5.0 mg/ml) at a rate of 50 mg/kg subcutaneously every other day for a total of 6 injections starting at 7 days of age (14). To assess possible damage, superior cervical ganglia were fixed in 2.5% buffered glutaraldehyde, dehydrated, embedded in paraffin, cut in 7μ sections and stained with toluidine blue.

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