Hydroxyindole-*O*-methyltransferase VI: Inhibitory Activities of Substituted Benzoyltryptamines and Benzenesulfonyltryptamines

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Abstract □ A number of benzoyltryptamines and benzenesulfonyltryptamines substituted on the phenyl ring with OH, NH_2, or $NHCOCH_3$ were synthesized, and their inhibitory activities on hydroxyindole-*O*-methyltransferase were evaluated. The results showed that the enzyme has tolerance for the $NHCOCH_3$ group on either the *meta*- or *ortho*-position.

Keyphrases □ Hydroxyindole-*O*-methyltransferase—tryptamines, benzoyl, benzenesulfonyl substituted, synthesis, inhibitory activities □ Tryptamines, benzoyl, benzenesulfonyl substituted, synthesis, inhibitory activities—hydroxyindole-*O*-methyltransferase □ Inhibitory activities—substituted tryptamines

A number of substituted *N*-benzoyltryptamines and benzenesulfonyltryptamines have been found to be good inhibitors of hydroxyindole-*O*-methyltransferase (1, 2). Chlorine substitution on the benzene ring of *N*-benzoyltryptamine, in general, increases inhibition of the enzyme (2). In the present work, some *N*-acetamidobenzoyltryptamines were synthesized to test tolerance of the enzyme for the $NHCOCH_3$ group at the *ortho*-, *meta*-, or *para*-position.

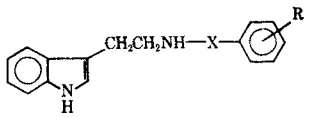
At the pH (7.8) at which the *in vitro* enzyme assay was performed, anilino groups of II, III, and IV exist mostly in the protonated form (Table I). From the observation that II and I were inhibitors of equal activity and III was only 1.2-fold less active than I (Table I), it seems that the *para*- and *meta*-NH_3^+ groups did not participate in enzyme-inhibitor attachment, nor did they exert any effect on binding of the phenyl ring to the enzyme. The phenolic OH group of VIII, however, was probably bound to the enzyme *via* hydrogen bonding and/or charge-transfer complex of the type HO:→*A*–*E*. This argument is based on the finding that the NH_3^+ of IV, which cannot be a donor, caused a 2.5-fold decrease in activity relative to I.

Acetylation of anilino groups of III and IV improved inhibitory activities 4.6 and 3.5 times, respectively, (see VI and VII), whereas the same substitution on II resulted in a 1.4-fold decrease in activity which, if significant, may suggest a limited bulk tolerance of the enzyme for the $NHCOCH_3$ group at the *para*-position. The increased activity from the acetyl group of VI and VII could be attributed to the presence of a carbonyl group whose oxygen atom is capable of serving as the donor in a hydrogen-bonding and/or charge-transfer interaction between the inhibitor and the enzyme. Attempts to prepare *N*-(*O*-acetylsalicyloyl)tryptamine were unsuccessful due to the instability of the acetoxy group; hydrolysis always occurred during workup as followed by GLC.

It was difficult to rationalize the twofold loss of inhibition when a *p*-amino group was substituted on the benzene ring of IX to give X, particularly since no difference in activity was observed between I and IX and between I and II. The *p*-$NHCOCH_3$ group gave further reduction of inhibition (see XI).

Results from the present study indicate that the enzyme has tolerance for the $NHCOCH_3$ group on either the *meta*- or *ortho*-position of the benzene ring of *N*-benzoyltryptamine. Thus, inhibitors bearing a group at one of these two positions, which is capable of forming a covalent bond with a nucleophilic group on the enzyme (3), might possibly be potential candidates for active-site-directed irreversible inhibition of hydroxyindole-*O*-methyltransferase. An irreversible inhibitor is needed to prolong the inhibition of hydroxyindole-*O*-methyltransferase *in vivo*, and an active-site-directed inhibitor would be advantageous for specificity. Syntheses of these compounds are currently being pursued in these laboratories.

Table I—Inhibition of Hydroxyindole-*O*-methyltransferase by



Compound	X	R	I_{50}[a], m*M*
I	CO	H	0.37[b]
II	CO	*p*-NH_2	0.32
III	CO	*m*-NH_2	0.46
IV	CO	*o*-NH_2	0.92
V	CO	*p*-$NHCOCH_3$	0.45
VI	CO	*m*-$NHCOCH_3$	0.10
VII	CO	*o*-$NHCOCH_3$	0.26
VIII	CO	*o*-OH	0.23
IX	SO_2	H	0.37[b]
X	SO_2	*p*-NH_2	0.75
XI	SO_2	*p*-$NHCOCH_3$	—[c]

[a] Concentration of an inhibitor giving 50% inhibition of the enzyme. [b] Data from *Reference 1*. [c] Due to limited solubility of the compound in the medium, the highest concentration tested was 1×10^{-3} *M*. A 15% inhibition was observed at this concentration.

EXPERIMENTAL[1]

***N*-*p*-Nitrobenzoyltryptamine**—To a solution of 4.7 g. (29 mmoles) of tryptamine and 3.5 g. (35 mmoles) of triethylamine in 50 ml. of chloroform was added dropwise a solution of 5.4 g. (30 mmoles) of *p*-nitrobenzoyl chloride. Precipitation took place almost immediately. After stirring the mixture at ambient temperature for 2 hr., 50 ml. of water was added. The solid was filtered and washed successively with 10% HCl (2 × 20 ml.), 2 *N* NaOH (2 × 20 ml.), and water (2 × 20 ml.); yield 8.5 g. (94.5%); m.p. 197–198°. Recrystallization from methoxyethanol–water gave 6.7 g. (74%), m.p. 200–201°.

Anal.—Calcd. for $C_{17}H_{15}N_3O_3$: C, 66.01; H, 4.89; N, 13.59. Found: C, 65.90; H, 4.75; N, 13.56.

[1] Melting points are corrected and were taken on a Mel-Temp apparatus.

N-m-**Nitrobenzoyltryptamine**—In a similar manner as described in the preparation of *N-p*-nitrobenzoyltryptamine, tryptamine was reacted with *m*-nitrobenzoyl chloride. The mixture was stirred overnight at ambient temperature, during which period precipitation occurred. The product weighed 8.4 g. (93.5%), m.p. 141.5–142°. After one recrystallization from methoxyethanol–water, the melting point was not changed.

Anal.—Calcd. for $C_{17}H_{15}N_3O_5$: C, 66.01; H, 4.89; N, 13.59. Found: C, 65.92; H, 4.69; N, 13.70.

N-o-**Nitrobenzoyltryptamine**—To a cooled solution of 16.7 g. (0.1 mole) of *o*-nitrobenzoic acid in 150 ml. of chloroform was added 10 ml. (0.13 mole) of thionyl chloride, and the mixture was stirred at 60° for 2 hr. After evaporation of the solvent and excess thionyl chloride, the oily residue was dissolved in 50 ml. of chloroform. It was added dropwise to a mixture prepared by neutralizing 20 g. (0.1 mole) of tryptamine hydrochloride in 100 ml. of water with 100 ml. 2 *N* NaOH and adding 75 ml. of chloroform to dissolve the liberated amine. Stirring the reaction mixture at ambient temperature for 1 hr. brought about the formation of an oily solid, which was filtered and washed with chloroform. The wet solid was dissolved in chloroform, and the solution was dried (Na_2SO_4), treated with charcoal, and filtered through diatomaceous earth[2]; upon chilling, it gave 20.8 g. (67%) of a bright-yellow solid, m.p. 110–112°. From the chloroform layer of the original reaction mixture and the mother liquor, an additional 20% of product was obtained (total yield 87%). Subsequent recrystallizations from chloroform gave an analytical sample as lustrous yellow platelets, m.p. 111–113°.

Anal.—Calcd. for $C_{17}H_{15}N_3O_5$: C, 66.01; H, 4.89; N, 13.59. Found: C, 65.89; H, 4.89; N, 13.62.

N-p-**Nitrobenzenesulfonyltryptamine**—A solution of 5 g. (25 mmoles) of tryptamine hydrochloride in 25 ml. of water was treated with charcoal and then filtered. The solution was shaken with 50 ml. of chloroform and 25 ml. of 2 *N* NaOH. *p*-Nitrobenzenesulfonyl chloride was added, and the shaking was continued for 5 min. A thick yellow oil, which separated from the mixture, solidified upon chilling. Recrystallization from methanol gave 7.2 g. (83.5%), m.p. 135–139°. Subsequent recrystallizations from chloroform gave an analytical sample as flocculent white needles, m.p. 138–139.5°.

Anal.—Calcd. for $C_{16}H_{15}N_3O_4S$: C, 55.64; H, 4.38; N, 12.17. Found: C, 55.49; H, 4.23; N, 12.07.

N-p-**Aminobenzoyltryptamine (II)**—A solution of 6 g. (19.4 mmoles) of *N-p*-nitrobenzoyltryptamine in 200 ml. of ethanol was shaken with hydrogen in the presence of 0.5 g. of 5% palladium on carbon catalyst until the consumption of hydrogen ceased (about 30 min.). The filtered solution was evaporated *in vacuo*, leaving 5.2 g. of product, m.p. 162–165°. Two recrystallizations from aqueous ethanol gave 3.4 g. (63%) of analytical sample, m.p. 167–169°.

Anal.—Calcd. for $C_{17}H_{17}N_3O$: C, 73.09; H, 6.13; N, 15.04. Found: C, 72.85; H, 6.14; N, 14.95.

N-m-**Aminobenzoyltryptamine (III)**—Hydrogenation of *N-m*-nitrobenzoyltryptamine was carried out in a similar manner as in the preparation of *N-p*-aminobenzoyltryptamine. The crude product (m.p. 153–155°) in 10% HCl was extracted with chloroform; the chloroform extracts were discarded, and the aqueous layer was made basic (pH 10) with 10% NaOH to precipitate the free amine. After recrystallization from aqueous ethanol, the yield of product (m.p. 156–157°) was 50%.

Anal.—Calcd. for $C_{17}H_{17}N_3O$: C, 73.09; H, 6.13; N, 15.04. Found: C, 72.95; H, 6.13; N, 14.89.

N-o-**Aminobenzoyltryptamine (IV)**—The amine, m.p. 160–161.5° (methanol), was obtained in 93% yield by catalytic reduction of *N-o*-nitrobenzoyltryptamine.

Anal.—Calcd. for $C_{17}H_{17}N_3O$: C, 73.09; H, 6.13; N, 15.04. Found: C, 73.18; H, 6.07; N, 15.08.

N-p-**Aminobenzenesulfonyltryptamine (X)**—Catalytic reduction of *N-o*-nitrobenzenesulfonyltryptamine gave 90% of the amine, m.p. 144–146° (aqueous acetone).

Anal.—Calcd. for $C_{16}H_{17}N_3O_2S$: C, 60.93; H, 5.43; N, 13.22. Found: C, 61.13; H, 5.44; N, 13.25.

N-p-**Acetamidobenzoyltryptamine (V)**—A solution of 1.4 g. (5 mmoles) of *N-p*-aminobenzoyltryptamine and 15 g. of acetic anhydride was stirred at ambient temperature for 2 hr., during which a pink precipitate deposited. The product was filtered and washed with water; yield 1.4 g. (87%); m.p. 204–205.5°. For recrystallization, the solid was dissolved in toluene with a small amount of ethanol and heptane added to turbidity. The melting point of the product remained unchanged after the recrystallization.

Anal.—Calcd. for $C_{19}H_{19}N_3O_2$: C, 71.01; H, 5.96; N, 13.08. Found: C, 71.24; H, 6.04; N, 12.91.

N-m-**Acetamidobenzoyltryptamine (VI)**—Acetylation of *N-m*-aminobenzoyltryptamine gave 88% of crude product, m.p. 158–160°. Recrystallization from the same solvent system as described for V yielded 67% of analytically pure sample, m.p. 160.5–161.5°.

Anal.—Calcd. for $C_{19}H_{19}N_3O_2$: C, 71.01; H, 5.96; N, 13.08. Found: C, 70.99; H, 5.70; N, 12.86.

N-o-**Acetamidobenzoyltryptamine (VII)**—To a chilled solution of 2.35 g. (8.5 mmoles) of *N-o*-aminobenzoyltryptamine in 50 ml. of chloroform, containing 2 ml. of pyridine, was added 4 ml. (42.4 mmoles) of acetic anhydride. The mixture was stirred overnight at ambient temperature. Chloroform was evaporated *in vacuo*, and the residual oil solidified upon trituration with ice water. Recrystallization of the crude product from ethyl acetate–ether gave 2.58 g. (96%) of white solid, m.p. 148–150°. Subsequent recrystallizations from ethyl acetate gave an analytical sample, m.p. 152–154°, as white needles.

Anal.—Calcd. for $C_{19}H_{19}N_3O_2$: C, 71.01; H, 5.96; N, 13.08. Found: C, 71.04; H, 6.09; N, 13.15.

N-p-**Acetamidobenzenesulfonyltryptamine (XI)**—A solution of 0.83 g. (2.65 mmoles) of *N-p*-aminobenzenesulfonyltryptamine in 10 ml. of acetic anhydride containing 2 ml. pyridine was stirred overnight at ambient temperature and then poured with vigorous stirring into ice water. The yellow solid was collected on a filter; yield 0.86 g. (92%); m.p. 124–129°. Recrystallizations from aqueous methanol and then chloroform gave 0.70 g. (75%) of analytically pure sample, m.p. 136–136.5°.

Anal.—Calcd. for $C_{18}H_{19}N_3O_3S$: C, 60.49; H, 5.36; N, 11.76. Found: C, 60.62; H, 5.41; N, 11.65.

N-**Salicyloyltryptamine (VIII)**—To a stirred solution of 2 g. (10 mmoles) of tryptamine in 15 ml. of benzene was added 0.8 g. (5 mmoles) of salicyloyl chloride, prepared according to the literature procedure (4). The mixture was stirred overnight at ambient temperature, and the precipitate was collected on a filter and washed successively with hot 10% HCl (two 100-ml portions) and hot water (three 100-ml. portions). Recrystallization of the crude product from benzene–petroleum ether gave 0.8 g. (57%), m.p. 143.5–144.5°.

Anal.—Calcd. for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99. Found: C, 72.61; H, 5.81; N, 9.71.

Assay—Hydroxyindole-*O*-methyltransferase was isolated from beef pineal gland and purified according to the method of Axelrod and Weissbach (5). Incubation was carried out with *N*-acetylserotonin and *S*-adenosyl-L-methionine-methyl-^{14}C, as previously described (6). The stock solutions of all the inhibitors were prepared in dimethyl sulfoxide. Previous findings showed that the same magnitude of inhibitory activity was obtained regardless of the use of the organic solvent (6).

[2] Celite, Johns-Manville.

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