Imidazole Derivatives as Inhibitors of Cyclic Nucleotide Phosphodiesterases†

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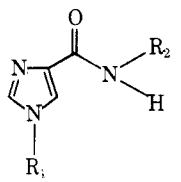
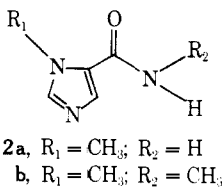
A variety of imidazole derivatives was designed with theophylline as the model and synthesized in an attempt to determine the minimum structural requirements for phosphodiesterase inhibition. Four series of imidazole derivatives were studied as inhibitors of the hydrolyses of cyclic 3′,5′-adenosine monophosphate and cyclic 3′,5′-guanosine monosphosphate by the soluble phosphodiesterases of the intima-media of pig coronary arteries. Although some of the compounds had significant inhibitory activity, none were as active as the xanthines studied.

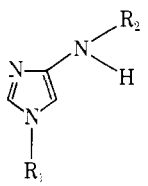
As a result of the extensive involvement of cyclic AMP in hormonal responses,[1-3] the pharmacological control of tissue levels of this cyclic nucleotide is potentially important. Phosphodiesterase[4] (PD) is the only known enzyme for degradation of cyclic AMP. Inhibition of PD was first demonstrated with methylxanthines,[4] and theophylline is now used routinely as a PD inhibitor. Inhibitory activity has also been demonstrated with a large number of other xanthine derivatives.[5-8] The aim of this study was to investigate the minimum structural requirements for inhibition of PD using theophylline as a model. Four series of imidazole derivatives were synthesized and assayed for inhibitory activity.



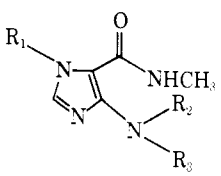
1a, $R_1 = H$; $R_2 = H$
b, $R_1 = H$; $R_2 = CH_3$
c, $R_1 = H$; $R_2 = CH(CH_3)_2$
d, $R_1 = H$; $R_2 = CH_2C_6H_5$
e, $R_1 = C(C_6H_5)_3$; $R_2 = H$
f, $R_1 = C(C_6H_5)_3$; $R_2 = CH_3$
g, $R_1 = COCH_3$; $R_2 = CH_3$



2a, $R_1 = CH_3$; $R_2 = H$
b, $R_1 = CH_3$; $R_2 = CH_3$



3a, $R_1 = CH_2C_6H_5$; $R_2 = H$
b, $R_1 = CH_2C_6H_5$; $R_2 = CHO$
c, $R_1 = CH_2C_6H_5$; $R_2 = COCH_3$
d, $R_1 = H$; $R_2 = CO_2CH_3$

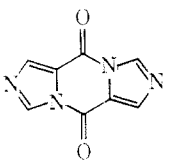


4a, $R_1 = H$; $R_2 = H$; $R_3 = H$
b, $R_1 = H$; $R_2 = CH_3$; $R_3 = H$
c, $R_1 = H$; $R_2 = CH_3$; $R_3 = COCH_3$
d, $R_1 = H$; $R_2 = CH_2C_6H_5$; $R_3 = CH_3$
e, $R_1 = CH_3$; $R_2 = CH_3$; $R_3 = H$
f, $R_1 = CH_3$; $R_2 = CH_3$; $R_3 = CHO$
g, $R_1 = CH_3$; $R_2 = CH_3$; $R_3 = COCH_3$
h, $R_1 = CH_2C_6H_5$; $R_2 = CH_3$; $R_3 = H$
i, $R_1 = CH_2C_6H_5$; $R_2 = CH_3$; $R_3 = CHO$
j, $R_1 = CH_2C_6H_5$; $R_2 = CH_3$; $R_3 = COCH_3$

It was proposed to synthesize 1a–**d** by reaction of 4(5)-carbethoxyimidazole (**5**)[9] with the appropriate amine. This procedure was successful for the preparation of 1**a**,[10] 1**b**,[11] and 1**d** (Scheme I). The synthesis of 1**c** was accomplished by hydrolysis of **5** to 4(5)-imidazolecarboxylic acid (**6**)[9] followed by successive treatment with phosphorus pentachloride and isopropylamine. Compounds 1**e** and 1**f** were obtained by reaction of 1**a** and 1**b**, respectively, with triphenylmethyl chloride in the presence of triethylamine (Scheme I). Also prepared was 1-triphenylmethyl-4-carbethoxyimidazole (**7**).

Compound 1**g** was prepared by reaction of 1**b** with acetic anhydride. It is believed that the 1,4-disubstituted imidazole was obtained. Previous nmr studies[12] on the ring acetylation of imidazoles showed that acetylation occurred farthest from any substituents. In addition, an average downfield shift of 19 Hz was observed[12] for imidazole ring protons adjacent to the acetyl group. The nmr absorptions of the ring protons of **1b** were observed at 460 and 466 Hz while those of 1**g** were observed at 481 and 504 Hz. Also prepared was 1-acetyl-4-carbethoxyimidazole (**8**).

The low yields obtained (29%) in the synthesis of 1**c** prompted a search for an improved method of preparation of the acid chloride of 4(5)-imidazolecarboxylic acid. Earlier investigators[13] have reported no reaction between thionyl chloride and **6**. However, when **6** was refluxed with thionyl chloride, a crude solid was obtained in high yields. Spectral and analytical data indicated the material was not the desired acid chloride but was, instead, 5*H*,10*H*-diimidazolo[1,5-*a*:1′,5′-*d*]pyrazine-5,10-dione (**9**)



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which could arise from dimerization of the intermediate acid chloride and subsequent intramolecular ring closure. A similar dimerization has been observed with 3(5)-pyrazolecarboxylic acid.[14] Treatment of **9** with excess methylamine gave a product which was identical in all respects with 1**b**.

The preparation of **2a** and **2b** was accomplished by reaction of 1-methyl-4-carbomethoxyimidazole (**10**)[9] with ammonia and methylamine, respectively (Scheme II).

The proposed synthetic route to **3a–c** involved benzylation of 4(5)-nitroimidazole (**11**) to give 1-benzyl-4-nitroimidazole (**12**) (Scheme III). Alkylation of **11** in the presence of base has been reported[15] to yield predominantly the 1,4-disubstituted isomer. Reduction of **12** with hydrazine and palladium on charcoal[16] gave the amine which was isolated as its hydrochloride salt (**3a**). Compounds **3b** and **3c** were prepared by acylation of the amine *in situ* with acetic formic anhydride[17] and acetic anhydride, respectively. Compound **3d** was prepared according to known procedures.[18]

The nmr spectrum of **3a** contained certain interesting characteristics. In deuterated dimethyl sulfoxide two doublets were observed at 6.67 and 8.84 ppm. The former absorption was assigned to the ring proton adjacent to the

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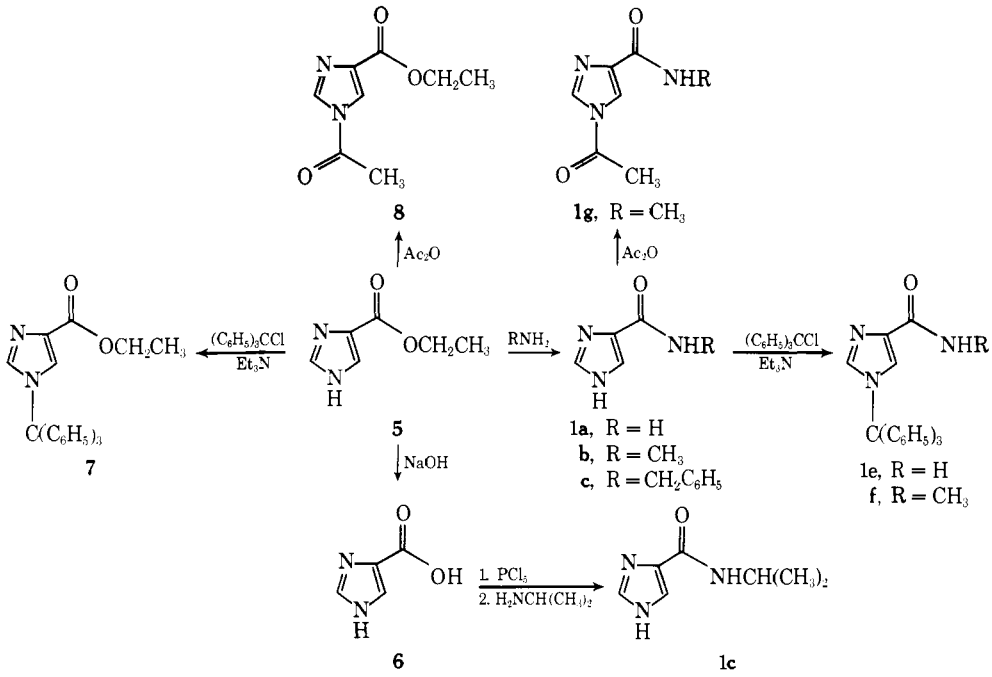
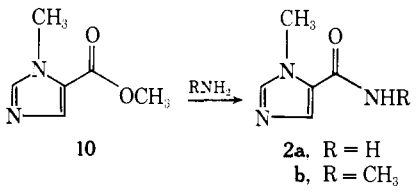
Scheme I



amino substituent and the latter one assigned to the ring proton between the two nitrogen atoms. Double resonance experiments demonstrated coupling between these two doublets. Addition of D_2O caused the downfield doublet to collapse to a singlet and the upfield doublet to disappear. These data indicated that the ring proton adjacent to the amino substituent was undergoing hydrogen–deuterium exchange.

The proposed synthesis of **4a**[19] and **4d** involved cyclization of α-amino-α-cyano-*N*-methylacetamide (**15**)[19] with formamidine (Scheme IV). The required amide **15** was prepared by the aluminum amalgam[20] reduction[21] of ethyl α-cyano-α-oximinoacetate (**13**)[22] followed by treatment of the resulting amine **14** with methylamine. Treatment of **4a** with benzaldehyde gave the Schiff base **16**, which was hydrogenated with palladium on charcoal to the benzylamine **4d**.

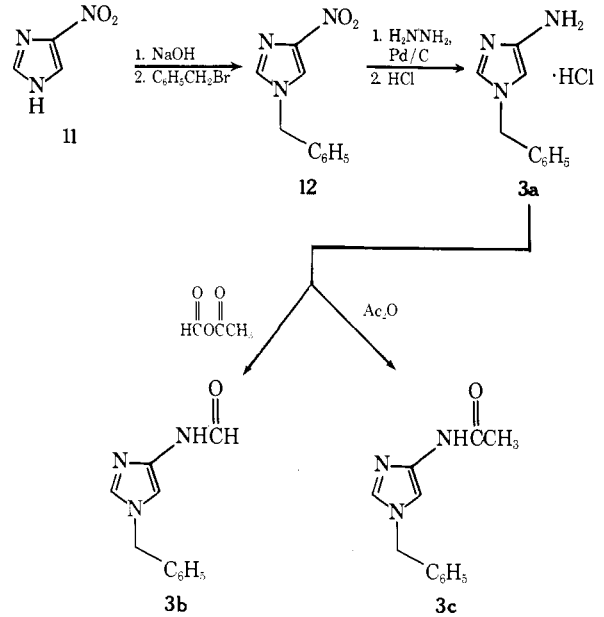
Scheme II



The synthesis of **4b**,[23] **4c**, and **4h–j** involved initial hydrolysis of 7-benzyltheophylline (**17**)[24] to give the imidazole **4h** (Scheme V). Acylation of **4h** with acetic formic anhydride[17] or acetic anhydride gave **4i** and **4j**, respectively. Compound **4b** was prepared by catalytic hydrogenolysis of **4h**. Acetylation of **4b** with acetic anhydride afforded a hygroscopic diacyl intermediate which was readily converted to **4c**.

Compounds **4e–g**[25] were prepared according to known procedures. The nmr spectrum of **4i** contained features which indicated restricted internal rotation. The tertiary amide *N*-methyl (3.19 ppm), the benzyl methylene (5.36 ppm), and the tertiary amide *N*-formyl absorptions (8.24 ppm) all appeared as doublets of unequal intensities in deuterated chloroform or deuterated dimethyl sulfoxide. Similar observations have been made for carbon–carbonyl bonds[26] and for carbonyl–nitrogen bonds[27] in di-ortho-substituted benzamides.

Scheme III



The compounds prepared in this study were assayed for inhibitory activity against a soluble (40,000g) phosphodiesterase preparation from the intima-media of pig coronary artery.[28] The assay was a modification of a procedure previously reported.[29] Assays were performed at 30° for 30 min at enzyme dilutions which would give a 20% maximum hydrolysis of substrate in the absence of inhibitor. Substrate concentrations of cyclic 3′,5′-adenosine monophosphate (cyclic AMP) and cyclic 3′,5′-guanosine monophosphate (cyclic GMP) were 1 μM. Per cent inhibition values were calculated by comparison of the hydrolysis of substrate in the presence and absence of inhibitor. Control activities were measured in the presence of the respective solvent and product accumulation was linear for more than 30 min under the conditions of the assay. Inhibitor concentrations were 10^{-4} M in all cases.

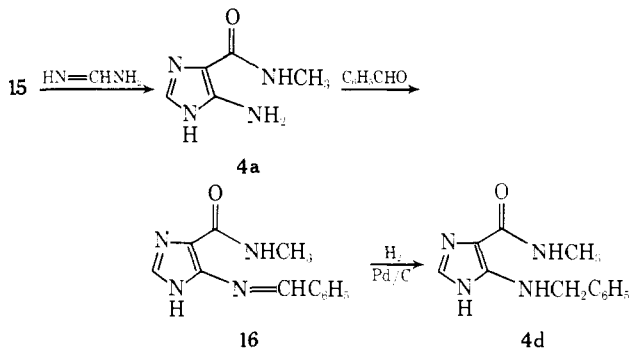
Table I contains data for those compounds which inhibited phosphodiesterase 5% or more in the presence of cyclic

Table I. Activity of Imidazole Derivatives as Inhibitors of Pig Coronary Artery Cyclic Nucleotide Phosphodiesterases

Compd (1×10^{-4} *M*)	Solvent[a]	% inhibition[b] Cyclic AMP[d]	% inhibition[b] Cyclic GMP[d]
Theophylline	H_2O	47.9 ± 3.5	42.6 ± 0.46
SC-2964[c]	1% aq DMSO	87.7 ± 0.92	94.9 ± 1.2
1c	H_2O	9.6 ± 0.47	<5
1d	1% aq DMSO	<5	7.6 ± 0.75
2a	H_2O	6.0 (0.48)	<5
2b	H_2O	6.8 (0.54)	<5
3a	H_2O	9.6 ± 2.5	18.5 ± 1.0
3b	1% aq DMSO	<5	9.4 ± 0.26
3c	1% aq DMSO	13.6 (0.31)	9.2 ± (0.25)
4b	H_2O	<5	13.9 ± 0.11
4d	1% aq DMSO	37.7 (1.77)	33.9 (0.26)
4f	H_2O	7.3 (0.29)	<5
12	1% aq DMSO	15.8 (0.01)	13.5 (0.37)
16	1% aq DMSO	33.3 (1.0)	34.3 (0.01)
17	1% aq DMSO	53.9 ± 3.0	73.9 ± 4.4

[a] In all cases per cent inhibition was calculated from control enzyme activity without inhibitor but in the presence of the solvent indicated. [b] ± standard deviation of mean of four determinations or, in parentheses, difference between duplicates. [c] 1-Methyl-3-isobutylxanthine was a gift from Searle Laboratories, Chicago, Ill. [d] Substrate concentration = 1 μM.

Scheme IV



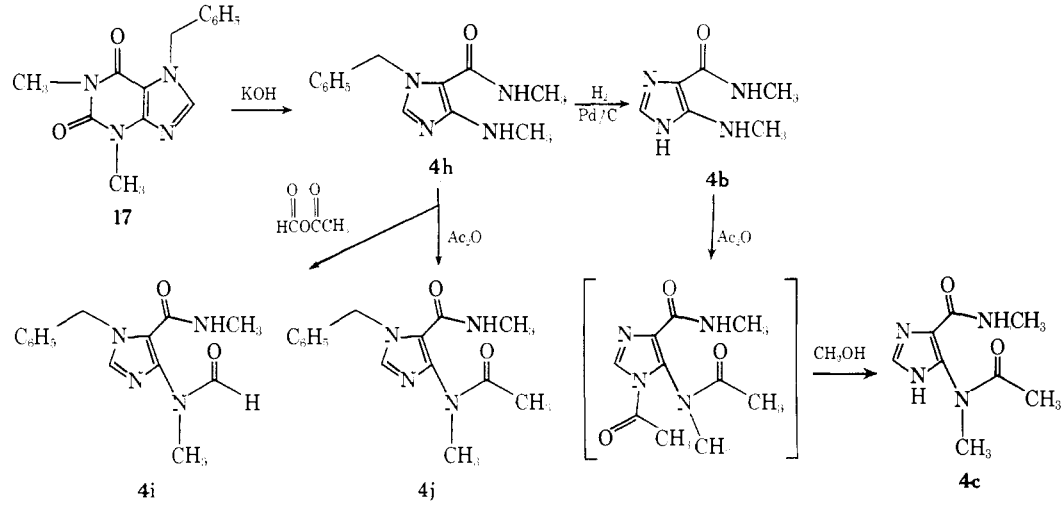
AMP or cyclic GMP as substrate. The most striking feature of the test data was the significant loss of activity which accompanied the opening of the pyrimidine ring of the xanthine nucleus. Compounds **4f** and **4c** seem to most closely approximate the structure of theophylline but the lack of activity serves to point out the effect of opening the pyrimidine ring. Series **4** was the most active series if substituted by a benzyl or benzal group on the amino function; otherwise, activity was not increased over that of the other three series.

The substitution of a benzyl group on the 7 position of theophylline increased the inhibitory activity significantly when cyclic GMP was used as substrate. This relative specificity for cyclic GMP phosphodiesterase activity is not observed with caffeine[28] and is, therefore, dependent upon the type of substituent and not just upon 7-substitution in general. We are actively pursuing the possibility of selective cyclic AMP or cyclic GMP phosphodiesterase inhibition.

Experimental Section

Melting points were determined in open capillary tubes using a Laboratory Devices Mel-Temp or a Büchi capillary melting point apparatus and are reported uncorrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knowville, Tenn., and by Midwest Microlabs, Inc., Indianapolis, Ind. Where analyses are indicated by only symbols of the elements, the analytical results for those elements were within ±0.4% of the theoretical

Scheme V



value. Ultraviolet spectra were determined on a Bausch and Lomb Model 505 or a Perkin-Elmer Coleman 124 recording spectrophotometer in 95% ethanol. Infrared spectra were obtained using a Perkin-Elmer Model 237B or Model 21 and a Beckman Model 33 infrared spectrophotometer. Nuclear magnetic reasonance spectra were recorded on a Varian Associates A-60A or HA 100 and a Japan Electron Optics Laboratory MH-60II spectrometer using tetramethylsilane as an internal standard. Mass spectra were obtained on a Hitachi Perkin-Elmer RMU-60 or a Consolidated Electronics Corp. Model 21-110B mass spectrometer. High-resolution mass spectra were obtained on the CEC 21-110B.

4(5)-(*N*-Isopropylcarbamoyl)imidazole (1c). A finely ground mixture of 4(5)-imidazolecarboxylic acid[9] (0.5 g, 4.46 mmol) and PCl_5 (0.9 g, 4.32 mmol) was heated on an oil bath in a nitrogen atmosphere at 110° (0.5 hr) and then at 130° (1 hr). A solution of isopropylamine (1.0 g, 16.9 mmol) in H_2O (1.5 ml) was added dropwise with stirring and cooling (0°). The resulting solution was stirred at room temperature overnight and then filtered. Evaporation *in vacuo* gave a yellow semisolid which was recrystallized (acetone) twice: 0.19 g (29%); mp (after an additional recrystallization from acetone) 167.5–170.5°. A small amount of colorless crystals (5 mg) separated from the mother liquors on standing for a few days. This material was collected by filtration and air-dried: mp 195.2–196.5°. The ir spectrum of this solid was identical with the one described above. This material was analyzed without further purification. *Anal.* ($C_7H_{11}N_3O$) C, H.

4(5)-(*N*-Benzylcarbamoyl)imidazole (1d). A solution of 4(5)-carbethoxyimidazole[9] (2.0 g, 14.3 mmol) in benzylamine (25 ml) was stirred under reflux (24 hr) in a nitrogen atmosphere. The solution was then cooled and diluted with hexane. The white solid which precipitated was collected by filtration and air-dried: 2.25 g (78%); mp (after recrystallization from $CHCl_3$–hexane) 143.5–144.5°. An analytical sample was prepared by recrystallization from H_2O: mp 145.5–147.0°. *Anal.* ($C_{11}H_{11}N_3O$) C, H.

4-Carbamoyl-1-triphenylmethylimidazole (1e). To a stirred solution of 4(5)-carbamoylimidazole monohydrate[10] (0.56 g, 4.34 mmol), Et_3N (0.51 g, 5.05 mmol), and DMSO (15 ml) was added a warm solution of triphenylmethyl chloride (1.40 g, 5.02 mmol) in DMSO (15 ml) dropwise with stirring. The resulting solution was stirred at room temperature (5 hr), then poured into H_2O (60 ml), and extracted three times with $CHCl_3$ (50 ml). The combined $CHCl_3$ extracts were dried (Drierite) and evaporated *in vacuo* to a yellow crystalline solid. Recrystallization (benzene–hexane) gave 0.32 g (21%), mp 228–229°. A second recrystallization (benzene) gave mp 233.0–233.5°. An analytical sample was prepared by recrystallization from benzene: mp 233.0–233.5°. *Anal.* ($C_{23}H_{19}N_3O$) C, H.

4-(*N*-Methylcarbamoyl)-1-triphenylmethylimidazole (1f). A solution of 4(5)-(*N*-methylcarbamoyl)imidazole[11] (0.85 g, 6.8 mmol), triphenylmethyl chloride (1.9 g, 6.8 mmol), Et_3N (0.69 g, 6.83 mmol), and tetrahydrofuran (150 ml) was stirred at room temperature (24 hr), then filtered, and evaporated *in vacuo* to a pink foamy solid. The solid was dissolved in $CHCl_3$ (50 ml), and the resulting solution was washed two times with H_2O (25 ml), dried (Drierite), and evaporated *in vacuo* to a pink liquid. Hexane was added and the mixture boiled on a steam bath until crystallization was complete. After cooling (0°), the solid was collected by filtration and air-dried: 1.52 g (61%); mp 172.0–172.5°. An analytical sample was prepared by recrystallization from CH_2Cl_2–hexane: mp 174.5–175.0°. *Anal.* ($C_{24}H_{21}N_3O$) C, H.

1-Acetyl-4-(*N*-methylcarbamoyl)imidazole (1g). A solution of 4(5)-(*N*-methylcarbamoyl)imidazole[11] (1.0 g, 8.0 mmol) in Ac_2O (25 ml) was stirred under reflux (0.5 hr). The solution was cooled and evaporated *in vacuo* to a tan crystalline solid. Anhydrous Et_2O was added and the solid collected by filtration, washed three times with anhydrous Et_2O, and air-dried: 0.97 g (73%); mp 183–184°. An analytical sample was prepared by recrystallization from anhydrous EtOAc followed by sublimation at 130° (0.2 mm): mp 188.8–190.0°. *Anal.* ($C_7H_9N_3O_2$) C, H.

4-Carbethoxy-1-triphenylmethylimidazole (7). To a stirred solution of 4(5)-carbethoxyimidazole[9] (14.0 g, 0.1 mol), Et_3N (11.1 g, 0.11 mol), and $CHCl_3$ (250 ml) was added triphenylmethyl chloride (27.9 g, 0.1 mol). The resulting solution was stirred at room temperature (15 hr), then washed two times with H_2O (100 ml), dried (Drierite), and evaporated *in vacuo* to a yellow liquid. Anhydrous Et_2O was added and the mixture was heated on a steam bath until crystallization was complete. After cooling (0°), the almost white crystalline solid was collected by filtration and air-dried: 28.4 g; mp 170.5–173.0°. Recrystallization (95% EtOH) gave mp 173.0–174.0°. A second crop was obtained from the mother liquors: 2.23 g. The total product weighed 30.6 g (80%). An analytical sample was prepared by recrystallization from 95% EtOH: mp 175.3–175.5°. *Anal.* ($C_{25}H_{22}N_2O_2$) C, H.

1-Acetyl-4-carbethoxyimidazole (8). A solution of 4(5)-carbethoxyimidazole[9] (2.0 g, 14.3 mmol) in Ac_2O (40 ml) was stirred under reflux (0.5 hr). The solution was cooled and evaporated *in vacuo* to a nearly colorless liquid. Anhydrous Et_2O was added until a slight turbidity was observed and the mixture stored at −15° for a few hours. The white crystalline solid which separated was collected by filtration, washed several times with anhydrous Et_2O, and air-dried: 2.01 g (77%); mp 75.5–76.0°. An analytical sample was prepared by recrystallization from anhydrous Et_2O: mp 75.0–75.5°. *Anal.* ($C_8H_{10}N_2O_3$) C, H.

5*H*,10*H*-Diimidazolo[1,5-*a*:1′,5′-*d*]pyrazine-5,10-dione (9). A slurry of 4(5)-imidazolecarboxylic acid[9] (1.0 g, 8.93 mmol) and thionyl chloride (15 ml) was stirred under reflux (21 hr). The yellow solid was collected by filtration and washed a few times with benzene: 1.01 g. Recrystallization (acetonitrile) gave the free base: sublimes by 300° without melting. An analytical sample was prepared by recrystallization from acetonitrile: sublimes by 300° without melting. *Anal.* ($C_8H_4N_4O_2$) C, H.

4(5)-(*N*-Methylcarbamoyl)imidazole (1b). A solution of the crude solid (0.25 g), from reaction of 4(5)-imidazolecarboxylic acid and thionyl chloride, in 40% aqueous methylamine (20 ml) was stirred at room temperature (3 hr) and then evaporated *in vacuo* to a light yellow solid. The solid was extracted several times with hot benzene, and the combined extracts were reduced in volume to approximately 40 ml and cooled. The white crystalline solid which separated was collected by filtration and air-dried: 70 mg; mp 146.0–156.5° (lit.[11] 145°); mixture melting point with authentic 4(5)-(*N*-methylcarbamoyl)imidazole[11] was not depressed. Spectral data were identical with that of authentic 4(5)-(*N*-methylcarbamoyl)imidazole.[11]

5-Carbamoyl-1-methylimidazole (2a). 5-Carbomethoxyl-1-methylimidazole (0.5 g, 3.57 mmol)[9] was combined with 15 ml of a solution of absolute CH_3OH (50 ml) saturated with NH_3 (9.02 g, 0.29 mol). The resulting solution was heated in a sealed vessel (100–105°, 24 hr). The solution was cooled, filtered, and evaporated *in vacuo* to a brown semisolid which was recrystallized (anhydrous EtOAc): 0.31 g (70%); mp (after two additional recrystallizations from anhydrous EtOAc) 187.5–188.0°. An analytical sample was prepared by sublimation at 105° (0.1 mm): mp 188.5–189.0°. *Anal.* ($C_5H_7N_3O$) C, H.

1-Methyl-5-(*N*-methylcarbamoyl)imidazole (2b). A solution of 5-carbomethoxy-1-methylimidazole[9] (3.0 g, 21.4 mmol) in 40% aqueous methylamine (30 ml) was heated in a sealed vessel (90–100°, 45.5 hr). The solution was cooled, filtered, and evaporated *in vacuo* to a yellow oil. The oil crystallized on standing overnight, and the solid was recrystallized (anhydrous EtOAc): 1.81 g (61%); mp 123–124°. An analytical sample was prepared by recrystallization from anhydrous EtOAc: mp 126.0–126.5°. *Anal.* ($C_6H_9N_3O$) C, H.

4-Amino-1-benzylimidazole Hydrochloride (3a). In a nitrogen atmosphere a solution of 1-benzyl-4-nitroimidazole[30] (6.1 g, 30.0 mmol) in absolute CH_3OH (50 ml) was added dropwise to 5% Pd/C (1.0 g) with stirring. Dropwise addition of a solution of hydrazine hydrate (20 ml) in absolute CH_3OH (60 ml) was begun. Gentle warming initiated gas evolution. The methanolic hydrazine solution was added until no further gas evolution was observed. The catalyst was collected by filtration and washed a few times with absolute CH_3OH and the combined filtrate and washings were poured into H_2O (250 ml). The CH_3OH was removed by evaporation *in vacuo* at room temperature. The resulting aqueous solution was extracted three times with $CHCl_3$ (100 ml) and the combined $CHCl_3$ extracts were dried (Drierite).

In previous experiments the $CHCl_3$ solution had been evaporated *in vacuo* to a brown oil. The crude 4-amino-1-benzylimidazole intermediate decomposed on standing at room temperature.

The $CHCl_3$ solution of the 4-amino-1-benzylimidazole was evaporated *in vacuo* to a final volume of approximately 75 ml and cooled (0°) and ethereal HCl was added dropwise until precipitation of the yellow solid was complete. The solid was collected by filtration, washed a few times with anhydrous Et_2O, and dried under nitrogen: 6.0 g (96%); mp [after recrystallization from acetonitrile and sublimation at 130° (0.1 mm)] 173–175° dec. An analytical sample was prepared by recrystallization from acetonitrile: mp 179–182° dec. *Anal.* ($C_{10}H_{12}ClN_3$) C, H.

1-Benzyl-4-formamidoimidazole (3b). The $CHCl_3$ solution of crude 4-amino-1-benzylimidazole, prepared from 1-benzyl-4-nitroimidazole (1.0 g, 4.92 mmol), was evaporated *in vacuo* to a

final volume of approximately 75 ml. Acetic formic anhydride[17] (5 ml) was added in one portion; the solution was stirred at room temperature (1 hr), washed three times with saturated aqueous $NaHCO_3$ (50 ml), dried (Drierite), and evaporated *in vacuo* to a yellow crystalline solid. Anhydrous Et_2O was added, and the solid was collected by filtration, washed with anhydrous Et_2O, and air-dried: 0.46 g (47%); mp 169–170°. An analytical sample was prepared by recrystallization from anhydrous EtOAc followed by sublimation at 125° (0.1 mm): mp 168.0–168.8°. *Anal.* ($C_{11}H_{11}N_3O$) C, H.

1-Benzyl-4-acetamidoimidazole (3c). The $CHCl_3$ solution of crude 4-amino-1-benzylimidazole, prepared from 1-benzyl-4-nitroimidazole (6.1 g, 30.0 mmol), was evaporated *in vacuo* to a final volume of approximately 200 ml. Ac_2O (25 ml) was added dropwise with stirring. The solution was stirred at room temperature (1 hr), washed twice with saturated aqueous $NaHCO_3$ (100 ml), dried (Drierite), and evaporated *in vacuo* to a yellow crystalline solid. Anhydrous Et_2O was added, and the solid was collected by filtration, washed with anhydrous Et_2O, and air-dried: 3.83 g (59%); mp 181.0–183.0°. An analytical sample was prepared by recrystallization from anhydrous EtOAc: mp 182.5–183.5° (lit.[31] 180–181°). *Anal.* ($C_{12}H_{13}N_3O$) C, H.

α-Amino-α-cyano-*N*-methylacetamide (15). To a stirred solution of ethyl α-cyano-α-oximinoacetate (10.0 g, 70.4 mmol)[22] in anhydrous Et_2O (150 ml) was added aluminum amalgam (prepared from 5 g of aluminum shot)[20] in one portion. H_2O (5 ml) was added dropwise at such a rate as to maintain gentle reflux (0.5 hr). After additional stirring (1 hr) the mixture was filtered, and the collected solid was washed three times with anhydrous Et_2O (50 ml). The combined filtrate and washings were dried (Drierite) and evaporated *in vacuo* at room temperature to 4.8 g of a yellow liquid. To a stirred and cooled (0°) solution of the yellow liquid in anhydrous Et_2O (50 ml) was added dropwise a cooled (0°) solution of methylamine (5.9 g, 0.19 mol) in anhydrous Et_2O (50 ml). The resulting solution was stirred at 0° (15 min) and then at room temperature (0.5 hr). The tan crystalline solid which separated was collected by filtration and air-dried: 2.2 g (28%); mp 116–117°. An analytical sample was prepared by recrystallization from absolute EtOH: mp 118.0–118.5° (lit.[19] 115–116°). *Anal.* ($C_4H_7N_3O$) C, H.

4(5)-Amino-5(4)-(*N*-methylcarbamoyl)imidazole (4a). A mixture of α-amino-α-cyano-*N*-methylacetamide (1.0 g, 8.85 mmol), formamidine acetate (1.04 g, 10.0 mmol), and absolute CH_3OH (20 ml) was stirred under reflux (1 hr), cooled, diluted with anhydrous Et_2O (130 ml), and stored at −15° for a few hours. The supernatant fluid was decanted from the small amount of black oil and solid which had separated and evaporated *in vacuo* to an orange oil. The oil was cooled (0°) and triturated with anhydrous Et_2O and a small amount of absolute EtOH. The tan solid which separated was collected by filtration and air-dried: 0.14 g (11%); mp 167–172°. An analytical sample was prepared by sublimation at 140° (0.1 mm): mp 200–203° dec. *Anal.* ($C_5H_8N_4O$) C, H.

4(5)-Benzylideneamino-5(4)-(*N*-methylcarbamoyl)imidazole (16). A mixture of α-amino-α-cyano-*N*-methylacetamide (3.0 g, 26.6 mmol), formamidine acetate (3.12 g, 30.0 mmol), and absolute CH_3OH (50 ml) was stirred under reflux (1 hr), cooled, diluted with anhydrous Et_2O until a faint turbidity was observed, and stored at −15° overnight. The supernatant fluid was decanted from the black oil which separated and evaporated *in vacuo* to a red oil. The oil and benzaldehyde (5 ml) were combined and heated gently on a steam bath until solidification was complete. Absolute EtOH (5 ml) was added and heating was continued (10 min). The mixture was cooled and the yellow crystalline solid was collected by filtration, washed twice with absolute EtOH and twice with anhydrous Et_2O, and air-dried: 2.64 g (44%); mp 230–232°. An analytical sample was prepared by recrystallization from 95% EtOH: mp 240.5–241.0° dec. *Anal.* ($C_{12}H_{12}N_4O$) C, H.

4(5)-Benzylamino-5(4)-(*N*-methylcarbamoyl)imidazole (4d). A solution of 4(5)-benzylideneamino-5(4)-(*N*-methylcarbamoyl)-imidazole (0.57 g, 2.5 mmol) in 95% EtOH (100 ml) was shaken (10 min) with 5% Pd/C (0.15 g) in a H_2 atmosphere (15 psi). The catalyst was collected by filtration and washed a few times with 95% EtOH. The combined filtrate and washings were evaporated *in vacuo* at room temperature to a green solid. Recrystallization (EtOAc) gave a green crystalline solid: 0.46 g (80%). An analytical sample was prepared by recrystallization from anhydrous EtOAc: mp 159–161° dec. *Anal.* ($C_{12}H_{14}N_4O$) C, H.

1-Benzyl-4-methylamino-5-(*N*-methylcarbamoyl)imidazole (4h). A mixture of 7-benzyltheophylline[24] (189.3 g, 0.702 mol) and 3 *N* KOH (1500 ml) was stirred under reflux (3 hr) and then cooled (0°). Concentrated HNO_3 (350 ml) was added dropwise. A vigorous evolution of gas was observed. The mixture was adjusted to pH 10 by dropwise addition at room temperature of 5 *N* NaOH (300 ml). The mixture was extracted once with 1000 ml of $CHCl_3$ and then four times with 500 ml of $CHCl_3$. The combined extracts were dried (Drierite) and evaporated *in vacuo* to a purple solid. Recrystallization (EtOAc) gave a tan crystalline solid: 83.0 g (49%); mp 113.0–114.5°. An analytical sample was prepared by recrystallization from anhydrous Et_2O: mp 115.5–116.0°. *Anal.* ($C_{13}H_{16}N_4O$) C, H.

4(5)-Methylamino-5(4)-(*N*-methylcarbamoyl)imidazole (4b). A solution of 1-benzyl-4-methylamino-5-(*N*-methylcarbamoyl)imidazole (3.0 g, 12.3 mmol) in 95% EtOH (50 ml) was shaken (48 hr) with 5% Pd/C (0.50 g) in an H_2 atmosphere (50 psi). The catalyst was collected by filtration and washed a few times with 95% EtOH. The combined filtrate and washings were evaporated *in vacuo* at room temperature. The green crystalline residue was triturated with anhydrous Et_2O, collected by filtration, and air-dried: 1.76 g (93%); mp 174–176° dec. An analytical sample was prepared by recrystallization from anhydrous EtOAc: mp 179–181° dec. *Anal.* ($C_6H_{10}N_4O$) C, H.

4(5)-(*N*-Methylacetamido)-5(4)-(*N*-methylcarbamoyl)imidazole (4c). A solution of 4(5)-methylamino-5(4)-(*N*-methylcarbamoyl)imidazole (1.0 g, 6.49 mmol) in Ac_2O (25 ml) was stirred under reflux (0.5 hr). The solution gradually changed from a green to a brown color. The solution was evaporated *in vacuo* to a brown oil. In previous experiments the diacetyl intermediate had been obtained as a hygroscopic, tan crystalline solid. A solution of the brown oil in absolute CH_3OH (20 ml) was boiled on a steam bath (0.5 hr) and evaporated *in vacuo* to a brown oil. Trituration with anhydrous Et_2O and a small amount of acetone gave a tan solid which was collected by filtration and air-dried: 0.51 g (40%); mp (after two recrystallizations from anhydrous EtOAc) 185.3–186.3°. An analytical sample was prepared by sublimation at 135° (0.1 mm): mp 185.5–186.0°. *Anal.* ($C_8H_{12}N_4O_2$) C, H.

1-Benzyl-5-(*N*-methylcarbamoyl)-4-(*N*-methylformamido)imidazole (4i). 1-Benzyl-4-methylamino-5-(*N*-methylcarbamoyl)imidazole (24.4 g, 0.1 mol) was added to cooled (0°) acetic formic anhydride[17] (200 ml) with stirring. The cooling bath was removed, and the solution was stirred at room temperature (16 hr), filtered, and evaporated *in vacuo* to a brown oil. Anydrous Et_2O was added, and the mixture was boiled on a steam bath until crystallization was complete and stored at −15° overnight. The tan crystalline solid was collected by filtration and air-dried: 22.2 g; mp (after recrystallization from benzene–anhydrous Et_2O) 102–105°. A second crop was obtained from the mother liquors: 1.45 g. The total product weighed 23.7 g (87%). An analytical sample was prepared by recrystallization from anhydrous EtOAc: mp 106.5–107.0°. *Anal.* ($C_{14}H_{16}N_4O_2$) C, H.

1-Benzyl-4-(*N*-methylacetamido)-5-(*N*-methylcarbamoyl)imidazole (4j). A solution of 1-benzyl-4-methylamino-5-(*N*-methylcarbamoyl)imidazole (8.3 g, 34.0 mmol) in Ac_2O (50 ml) was stirred under reflux (0.5 hr), cooled, and evaporated *in vacuo* to a brown liquid. The liquid was cooled (0°) and anhydrous Et_2O added until crystallization was complete. The mixture was stored in the cold (−15°) for 2 days, and the tan crystalline solid was collected by filtration, washed a few times with anhydrous Et_2O, and air-dried: 9.4 g (97%); mp 160.0–161.0°. An analytical sample was prepared by recrystallization from anhydrous EtOAc followed by sublimation at 135° (0.1 mm): mp 159.0–160.0°. *Anal.* ($C_{15}H_{18}N_4O_2$) C, H.

References

(1) J. G. Hardman, G. A. Robison, and E. W. Sutherland, *Annu. Rev. Physiol.*, **33**, 311 (1971).
(2) J. P. Jost and H. V. Rickenberg, *Annu. Rev. Biochem.*, 40, 741 (1971).
(3) G. A. Robison, R. W. Butcher, and E. W. Sutherland, "Cyclic AMP," Academic Press, New York, N. Y., 1971.
(4) R. W. Butcher and E. W. Sutherland, *J. Biol. Chem.*, **237**, 1244 (1962).
(5) E. N. Goren and O. M. Rosen, *Arch. Biochem. Biophys.*, **153**, 384 (1972).
(6) E. B. Goodsell, H. H. Stein, and K. J. Wenzke, *J. Med. Chem.*, 14, 1202 (1971).
(7) J. A. Beavo, N. L. Rogers, O. B. Crofford, J. G. Hardman, E. W. Sutherland, and E. V. Newman, *Mol. Pharmacol.*, 6, 597 (1970).

(8) A. Lespagnol, M. Debaert, J. Mizon, and C. Mizon-Capron, *Therapie*, **25**, 707 (1970); *Chem. Abstr.*, **74**, 19728t (1971).
(9) R. G. Jones, *J. Amer. Chem. Soc.*, **71**, 644 (1949).
(10) I. E. Balaban, *J. Chem. Soc.*, 2423 (1932).
(11) R. Weidenhagen and H. Wegner, *Ber.*, **70**, 2309 (1937).
(12) G. S. Reddy, L. Mandell, and J. H. Goldstein, *J. Chem. Soc.*, 1414 (1963).
(13) F. L. Pyman, *J. Chem. Soc.*, **109**, 186 (1916).
(14) C. Musante and P. Pino, *Gazz. Chim. Ital.*, **77**, 199 (1947).
(15) W. G. Forsyth and F. L. Pymann, *J. Chem. Soc.*, **127**, 573 (1925).
(16) L. P. Kuhn, *J. Amer. Chem. Soc.*, **73**, 1510 (1951).
(17) L. I. Krimen, *Org. Syn.*, **50**, 1 (1970).
(18) I. E. Balaban, *J. Chem. Soc.*, 268 (1930).
(19) G. Shaw, R. N. Warrener, D. N. Butler, and R. K. Ralph, *J. Chem. Soc.*, 1648 (1959).
(20) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Vol. 1, Wiley, New York, N. Y., 1967, p 20.
(21) G. Shaw and D. V. Wilson, *J. Chem. Soc.*, 2937 (1962).
(22) M. Conrad and A. Schulz, *Ber.*, **42**, 735 (1909).
(23) H. Auterhoff and M. F. Hebler, *Arzneim.-Forsch.*, **9**, 621 (1959).
(24) G. P. Hager, J. C. Krantz, Jr., Dranta, Jr., and J. B. Harman, *J. Amer. Pharm. Ass.*, **42**, 36 (1953).
(25) R. M. Hoskinson, *Aust. J. Chem.*, **21**, 1913 (1968).
(26) T. H. Sidall and R. H. Garner, *Tetrahedron Lett.*, 3512 (1966).
(27) H. A. Staab and D. Lauer, *Tetrahedron Lett.*, 4593 (1966).
(28) J. N. Wells, C. E. Baird, and J. G. Hardman, Abstracts of Federal Proceedings, Atlantic City, N. J., April 1974.
(29) J. A. Beavo, J. G. Hardman, and E. W. Sutherland, *J. Biol. Chem.*, **245**, 5649 (1970).
(30) C. Cosar, C. Crisan, R. Horclois, R. M. Jacqb, J. Robert, G. Tchelitcheff, and R. Vaupre, *Arzneim.-Forsch.*, **16**, 23 (1966).
(31) F. Johnson and W. A. Nasutavicus, *J. Org. Chem.*, **29**, 153 (1964).

Inhibition of Tyrosinase-Catalyzed Melanin Formation by Catechol Phenyl Sulfones

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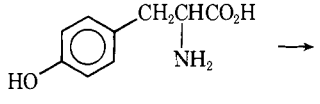
Catechol phenyl sulfones were synthesized and evaluated as potential inhibitors of tyrosinase-mediated Dopa melanin formation. The results suggest that the dopamine analogs, 2,3-dihydroxy-5-(β-aminoethyl)diphenyl sulfone hydrochloride (**9b**) and 2,3-dihydroxy-5-(β-aminoethyl)-4′-aminodiphenyl sulfone dihydrochloride (**9d**) as well as the Dopa analogs 2,3-dihydroxy-5-(β-alanyl)diphenyl sulfone hydrochloride (**10b**) and 2,3-dihydroxy-5-(β-alanyl)-4′-aminodiphenyl sulfone dihydrochloride (**10d**), are noncompetitive inhibitors of enzyme-catalyzed melanin formation. The methylcatechol analogs, 2,3-dihydroxy-5-methyldiphenyl sulfone (**8a**) and 2,3-dihydroxy-5-methyl-4′-aminodiphenyl sulfone (**8c**), had no effect on the formation of melanin, indicating that the β-aminoethyl side chain was required for inhibitory activity in this series of compounds. Although **10d** was the most effective inhibitor of melanin formation, it did not inhibit the tyrosinase-catalyzed conversion of tyrosine to Dopa.

Tyrosinase (E.C. 1.10.3.1) is an enzyme capable of performing a dual function; it catalyzes the hydroxylation of tyrosine (1) to 3,4-dihydroxyphenylalanine (**4b**, Dopa) and subsequently oxidizes the catechol to a labile *o*-quinone **7b**.[1,2] The enzyme also acts on a variety of other catechols converting them to quinones. The reactive quinones may



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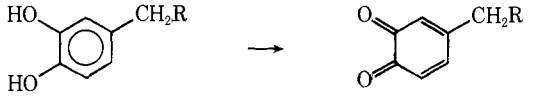
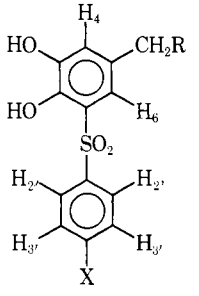


2, R = H
3a, R = CH_2NHCbz
b, R = CH_2NH_2
4a, R = $CH(NHCbz)CO_2CH_3$
b, R = $CH(NH_2)CO_2H$

5, R = H
6a, R = CH_2NHCbz
b, R = CH_2NH_2
7a, R = $CH(NHCbz)CO_2CH_3$
b, R = $CH(NH_2)CO_2H$

undergo a number of reactions including ring cyclization, addition with nucleophiles, and ring cleavage. These varied reactions usually result in the production of melanin pigments which are heterogeneous polymers. Primarily through the efforts of Raper[3,4] and Mason[5] and their coworkers, a scheme of melanogenesis has been proposed

†Dedicated to my friend and former research professor, Dr. Alfred Burger.



8a, R = H; X = H
b, R = H; X = NHAc
c, R = H; X = NH_2
9a, R = CH_2NHCbz; X = H
b, R = $CH_2\overset{+}{N}H_3Cl^-$; X = H
c, R = CH_2NHCbz; X = NHAc
d, R = $CH_2\overset{+}{N}H_3Cl^-$; X = $\overset{+}{N}H_3Cl^-$
10a, R = $CH(NHCbz)CO_2CH_3$
b, R = $CH(\overset{+}{N}H_3Cl^-)CO_2H$; X = H
c, R = $CH(NHCbz)CO_2CH_3$; X = NHAc
d, R = $CH(\overset{+}{N}H_3Cl^-)CO_2H$; X = $\overset{+}{N}H_3Cl^-$

whereby melanin is formed from Dopa proceeding through quinoidal and indolic intermediates. In the cancer, melanoblastoma (malignant melanoma), an abnormal amount of melanin is usually produced mediated by tyrosinase in an active state.[6] Unfortunately, chemotherapeutic agents