Inhibitors of Dopamine β-Hydroxylase. 3. Some 1-(Pyridylmethyl)imidazole-2-thiones

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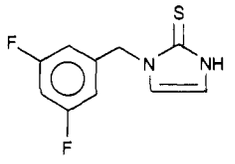
The 1-benzylimidazole-2-thione moiety has been previously shown by Kruse et al. to be broadly associated with dopamine β-hydroxylase (DBH) inhibitory activity both in vitro and in vivo in spontaneously hypertensive rats (SHR). An extension of structure–activity studies to 1-(pyridylmethyl)- and 1-(oxypyridylmethyl)imidazole-2-thiones is reported here in an attempt to exploit the pH differential that exists across the chromaffin vesicle membrane. We hypothesized that the weakly basic pyridyl compounds would diffuse into the acidic vesicles in their neutral forms where protonation and concentration would occur to enhance their in vivo effectiveness as inhibitors. To test this hypothesis, isomeric 2-, 3- and 4-(1-pyridylmethyl)imidazole-2-thiones were synthesized from the appropriate pyridinecarboxaldehydes by reductive alkylation of aminoacetaldehyde dialkyl acetal followed by imidazole-2-thione formation using acidic potassium thiocyanate. Related oxypyridyl compounds were synthesized by first preparing the appropriate aldehyde intermediate followed by conversion to the imidazole-2-thione by the same procedure. The unsubstituted pyridylmethyl compounds showed modest DBH inhibition in vitro but, consistent with a transport-mediated increase in observed potency, showed significant effects in vivo to increase the vascular ratio of dopamine to norepinephrine and to lower blood pressure.

(Arylalkyl)imidazole-2-thiones have recently been described as potent inhibitors of dopamine β-hydroxylase (DBH, E.C. 1.14.17.1) that act in a multisubstrate fashion to bind to the enzyme active site.[1,2] In particular, compound 1 was found to be a potent (K_{is} = 5 × 10^{-8} M) inhibitor of DBH that caused a fivefold increase in the dopamine/norepinephrine (DA/NE) ratio in a mesenteric artery assay and substantial blood pressure (BP) decreases upon oral administration to spontaneously hypertensive rats (SHR).[1a,2]



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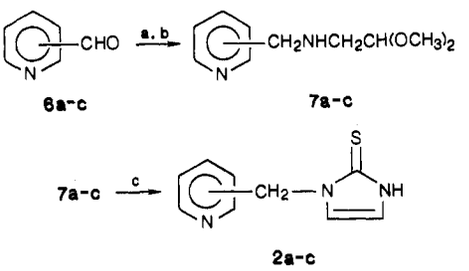
DBH inhibitors such as 1 thus appear to offer significant promise in the search for new therapeutic modalities effective in the treatment of cardiovascular diseases, in particular hypertension.

DBH is localized in chromaffin vesicles, subcellular organelles that are found within the chromaffin cells of the adrenal medulla and that also occur in presynaptic axons of noradrenergic neurons.[3] The vesicle is characterized by an acidic interior pH relative to cytosol, i.e., the cytosol of the adrenal chromaffin cell is pH 7.2 while the pH within the vesicle is 5.5.[4,5] It was anticipated that the pH differential across the vesicle membrane could be exploited by appropriate modification of the DBH inhibitor (see Figure 1). Specifically, a weakly basic inhibitor was expected to diffuse into the chromaffin cell cytosol in its un-ionized form, then be protonated during migration through the hydrophobic vesicle membrane, and finally be retained within the vesicle in the form of the conjugate acid. Support for this hypothesis may be found from the fact that isolated chromaffin vesicles have been shown to concentrate ammonia and other amines within the vesicle.[4,5] Thus, therapeutic efficacy would be enhanced by this concentration of drug in the target vesicles.

We report here an investigation of this approach with substituted pyridyl moieties as isosteres of the aryl group present in substrates such as phenethylamines and *p*-tyramine and in the 1-(arylmethyl)imidazole-2-thiones (compounds **2a–c** and **3–5**).

Scheme I. Synthesis of **2a–c**[a,b]



[a] Reagents and conditions: (a) $NH_2CH_2CH(OCH_3)_2$; (b) $NaBH_4$; (c) HCl, KSCN, H_2O, heat, then neutralization. [b] **a–c** refer to 2-, 3-, and 4-pyridyl isomers, respectively.

Chemistry. Synthesis of the compounds in Table I is shown in Schemes I–IV. All compounds were prepared by a fundamentally similar approach: pyridinecarboxaldehydes were condensed with aminoacetaldehyde dimethyl or diethyl acetal and the imines thus formed were reduced with sodium borohydride or alternately by hydrogenation using Pd/C catalyst. The N-substituted amino acetals were then condensed with acidified potassium thiocyanate in refluxing aqueous ethanol to give the 1-substituted imidazole-2-thiones. Free bases were obtained by neutralizing the reaction mixture. Pyridyl compounds **2a–c** were prepared and isolated in this manner; the hydrochloride of **2c** was also formed readily and was easily purified.

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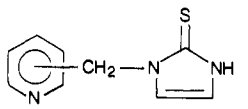
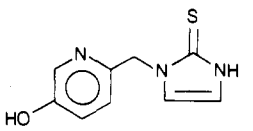


2a-c

a-c: 2-,3- and 4-pyridyl, respectively



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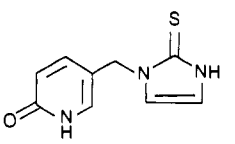

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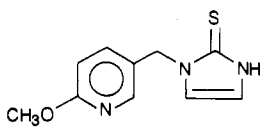

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Preparation of **3** (Scheme II) required a multistep sequence to provide the desired protected aldehyde. Following O-tosylation of 5-hydroxy-2-methylpyridine (**8**), the *N*-oxide was formed and the 2-methyl group functionalized by a Polonovski-type rearrangement using acetic anhydride.[6] The acetate ester was hydrolyzed and the resulting alcohol oxidized to the carboxaldehyde **10** by activated MnO_2. Following reductive alkylation and imidazole-2-thione formation, the protective group was removed by base and **3** was isolated upon neutralization.

To prepare the 2-pyridone **4**, the procedure described by Kompis et al.[7] was utilized to provide 2-methoxypyridine-5-carboxyaldehyde. Purified 48% HBr readily demethylated the 2-methoxy compound and provided 2-pyridone-5-carboxaldehyde (**13**), which was then used to reductively alkylate aminoacetaldehyde acetal followed by conversion to the imidazole-2-thione **4** (Scheme III). This highly polar compound was found to be difficult to purify, resulting in a low overall yield.

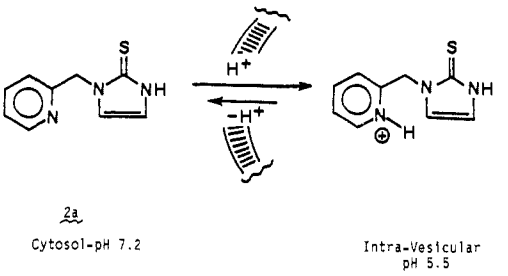
The corresponding 2-methoxy analogue **5** was prepared from 2-methoxypyridine-5-carboxaldehyde (**14**)[7] (Scheme IV).

Enzymology and Pharmacology. In vitro DBH inhibition was determined as previously reported.[1,8]

Determinations of vascular DA and NE levels and antihypertensive effects were measured in adult male Okamoto-Aoki SHR as previously reported.[1,2,9,10]

Results and Discussion

In vitro and in vivo test data are shown in Table I together with reference data for three 1-(arylmethyl)-imidazole-2-thiones[1] and a standard DBH inhibitor, fusaric acid.[11] This shows that the 2-, 3-, and 4-pyridylmethyl compounds **2a-c** are moderately potent as DBH inhibitors in vitro ($IC_{50} \sim 10^{-4}$ M) but show considerable in vivo effectivness. In particular, the 2-pyridyl compound **2a** causes in vivo effects (DA/NE ratio increase and BP decrease) comparable to those of fusaric acid. Figure 2 shows the SHR blood pressure response to **2a** and is typical for



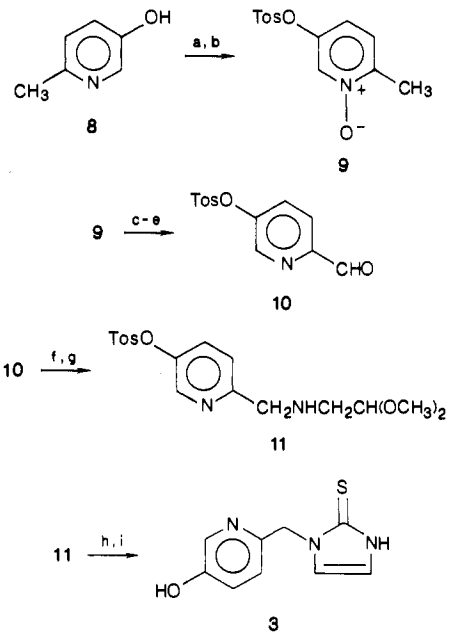
2a

Cytosol-pH 7.2

Intra-Vesicular pH 5.5

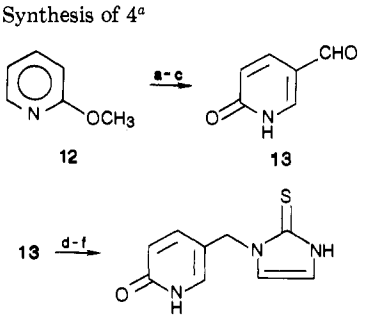
Figure 1. Hypothesized pH-gradient transport of (pyridylmethyl)imidazole-2-thiones.

Scheme II. Synthesis of **3**[a]



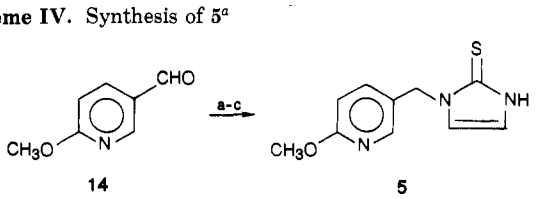
[a] Reagents and conditions: (a) *p*-toluenesulfonyl chloride; (b) *m*-chloroperbenzoic acid; (c) Ac_2O, heat; (d) CH_3OH, Et_3N; (e) MnO_2; (f) $NH_2CH_2CH(OCH_3)_2$; (g) $NaBH_4$; (h) HCl, KSCN, H_2O, heat; (i) NaOH, H_2O, then neutralization.

Scheme III. Synthesis of **4**[a]



[a] Reagents and conditions: (a) Br_2; (b) *n*-BuLi, then DMF followed by H_2O; (c) 48% HBr, heat; (d) $H_2NCH_2CH(OCH_3)_2$; (e) $NaBH_4$; (f) HCl, KSCN, H_2O, heat, then neutralization.

Scheme IV. Synthesis of **5**[a]



[a] Reagents and conditions: (a) $NH_2CH_2CH(OCH_3)_2$; (b) H_2, Pd/C; (c) HCl, KSCN, H_2O, heat, then neutralization.

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Table I. DBH Inhibition, DA/NE Ratios, and Antihypertensive Activities of 1-(Pyridylmethyl)imidazole-2-thiones

no.	R	IC_{50},[a] μM	% increase in DA/NE ratio[b]	mmHg decrease in BP[c]
2a	2-pyridyl	131 (76–220)	141 ± 6	35 ± 14
2b	3-pyridyl	104 (89–121)	52 ± 11	34 ± 11
2c	4-pyridyl	17%[d]	49 ± 8	38 ± 15
3	5-hydroxy-2-pyridyl	27 (21–35)		22 ± 17
4	2-pyridon-5-yl	7%[d]		
5	2-methoxy-5-pyridyl	17%[d]		
	C_6H_5	11 (8.9–13)		
	4-HOC_6H_4	2.6 (1.3–4.6)	95 ± 15	22 ± 4 (N = 3)
1	3,5-$F_2C_6H_3$	1.2 (1.1–1.4)	407 ± 30	50 ± 4 (N = 3)
fusaric acid		0.7 (0.4–1.1)	80 ± 35	54 ± 7 (N = 4)

[a] Values are given as IC_{50} in μM with upper and lower confidence limits, mean ± SEM, shown in parentheses. [b] These ratios were determined from assay of DA and NE in the mesenteric artery of SHR after two doses of the test comound at 50 mg/kg po, 18 h apart. Percent change (±SEM) reflects responses relative to vehicle-dosed control animals. [c] Blood pressure response was determined in SHR after dosing with test compound at 50 mg/kg ip. The value is recorded two h after dosing. Reduction (±SEM) reflects responses relative to vehicle-dosed control animals. [d] This number represents the percent inhibition observed at a concentration of 10^{-4} M.

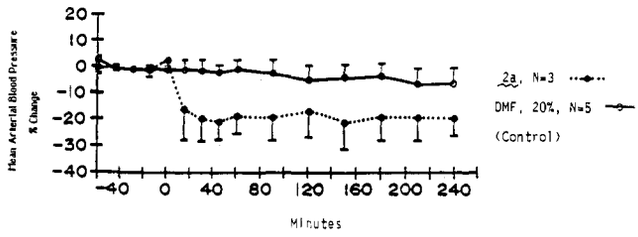

Figure 2. Effect of **2a** (50 mg/kg ip) on mean arterial blood pressure of conscious SHR.

compounds **2a–c**. The antihypertensive effect occurs shortly after dosage and remains relatively constant during the 4-h course of the experiment. The oxy-substituted compounds were included because of the increase in in vitro potency that occurs when *p*-hydroxy substitution is introduced to the aromatic moiety of 1-(arylmethyl)-imidazole-2-thiones.[1a] Of the oxy-substituted compounds **3–5**, only **3**, the 5-hydroxy-2-pyridylmethyl compound, is an effective DBH inhibitor ($IC_{50} \sim 10^{-5}$ M) in vitro, but **3** has only a marginal antihypertensive effect in vivo. Compounds **4** and **5** were not tested in either in vivo test.

These results show that, at least among the simple pyridylmethyl compounds **2a–c**, potencies in both the DA/NE ratio and hypotensive tests are enhanced over those that would be expected on the basis of in vitro potencies and compared with other known DBH inhibitors (see Table I). This potency enhancement is hypothesized to occur at least in part because of concentration of these compounds within the DBH-containing chromaffin vesicles by effects of the electrochemical pH gradient across the vesicle membrane. This is a qualitative relationship; no quantitative one may be implied due to the variety of additional factors that influence in vivo potencies. For example, the significantly improved potency of **2a** over **2b** and **2c** in increasing the DA/NE ratio cannot be explained by the pH gradient hypothesis. We conclude from this present investigation that pH gradient effects within chromaffin vesicles may be used to enhance in vivo DBH inhibitory potency.

Experimental Section

Melting points below 200 °C were taken on a capillary oil bath (Hoover-Thomas) apparatus and those over 200 °C were taken on a capillary-heated block (Mel-Temp) apparatus; data from both are uncorrected. ^{1}H NMR spectra were taken on a Varian EM 390 90-MHz spectrometer or a Hitachi Perkin-Elmer R-24 60-MHz spectrometer. IR spectra were taken on a Perkin-Elmer Model 727 or a Perkin-Elmer Model 783 instrument. Thin-layer chromatography (TLC) was performed on Analtech silica gel GF plates, 25 × 100 mm. Concentrations were performed with Büchi rotary evaporators. Flash column chromatography was performed with E. M. Science silica gel 60, 230–400 mesh. Elemental analyses (C, H, N) were performed by the Analytical, Physical and Structural Chemistry Department of Smith Kline & French Laboratories. Results were within ±0.3% of theoretical values unless otherwise indicated. Starting materials and reagents were obtained from established commercial suppliers and were used as received unless otherwise indicated.

1-(2-Pyridylmethyl)-1,3-dihydro-2*H*-imidazole-2-thione (2a). A 10.7-g (0.10 mol) quantity of pyridine-2-carboxaldehyde (**6a**) (freshly distilled) and 13.3 g (0.10 mol) of aminoacetaldehyde diethyl acetal were mixed and heated on a steam bath for a few minutes. The resulting imine was taken up in 150 mL of EtOH and the solution was allowed to cool. A 3.8-g (0.10 mol) quantity of $NaBH_4$ was added in small portions with stirring and the resulting solution was allowed to stand for several hours. It was then diluted with H_2O and extracted three times with EtOAc, and the combined EtOAc extracts were washed with water, dried over Na_2SO_4, and concentrated to give 18.9 g (84%) of crude **7a** as a yellow oil: ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.25 (6 H, t, J = 7 Hz, CH_3), 2.3 (1.5 H, s, NH), 2.85 (2 H, d, J = 5 Hz, $NHCH_2CH$), 3.4–3.9 (4 H, m, OCH_2), 4.65 (1 H, t, J = 6 Hz, CH), 7.0–7.9 (3 H, m, pyridyl), 8.55 (1 H, dd, J = 6 Hz, 4 Hz, pyridyl).

The crude **7a** was stirred with 100 mL of H_2O, 20 mL of concentrated HCl, and 12.0 g (0.124 mol) of KSCN. The mixture was heated at reflux for 1 h. The solution was cooled, neutralized to pH 8.0 with 50% NaOH, and cooled to 0 °C, which caused crystallization to occur. The solid (ca. 15 g) that precipitated was recrystallized twice from EtOH to give 11.5 g (51%) of **2a**: mp 183–186 °C; ^{1}H NMR [60 MHz, $CDCl_3$–$(CD_3)_2SO$] δ 3.5 (ca. 1 H, br s, NH), 5.3 (2 H, s, CH_2), 6.75–7.95 (5 H, m, pyridyl and imidazole), 8.55 (1 H, dd, J = 6 Hz, 3 Hz, pyridyl). Anal. ($C_9H_9N_3S$) C, H, N.

1-(3-Pyridylmethyl)-1,3-dihydro-2*H*-imidazole-2-thione (2b). A 0.050-mol quantity of pyridine-3-carboxaldehyde (**6b**) was converted to 12.8 g (67%) of the imidazole-2-thione (**2b**) by procedures analogous to those used to prepare **2a**, above: mp 148–150 °C; ^{1}H NMR [60 MHz, $CDCl_3$–$(CD_3)_2SO$] δ 5.3 (2 H, s, CH_2), 6.85 (2 H, dd, J = 14 Hz, 10 Hz, imidazole), 7.1–8.1 (2 H, m, pyridyl). Anal. ($C_9H_9N_3S$) C, H, N.

1-(4-Pyridylmethyl)-1,3-dihydro-2*H*-imidazole-2-thione (2c). A 21.4-g (0.20 mol) quantity of pyridine-4-carboxaldehyde (**6c**) and 21.03 g (0.20 mol) of aminoacetaldehyde dimethyl acetal were added to 200 mL of methanol, and the resulting solution was stirred for 1 h at ambient temperature. The reaction mixture was then cooled to −10 °C and 7.57 g (0.20 mol) of $NaBH_4$ was added in small portions as a solid with stirring. After the addition was complete, the mixture was allowed to warm to ambient temperature and stirring was continued overnight. The reaction mixture was diluted with H_2O and extracted three times with EtOAc, and the combined extracts were concentrated to give 42 g of crude **7c** as an oil. This was taken up in ether and the hydrochloride salt was formed by dropwise addition of ethereal HCl to give 40.25 g (86%) of **7c·HCl**: mp 132.5–136 °C dec.

A 37.3-g (0.16 mol) quantitiy of the salt was treated with 15.6 g (0.16 mol) of KSCN in 560 mL of H_2O and 75 mL of concen-

trated HCl as described for **2a**, above. The product precipitated in two crops (25.5 g, 83%) from the neutralized reaction mixture: mp 232–234 °C. Recrystallization from EtOH raised the mp to 233–235 °C. ^{1}H NMR [60 MHz, $(CD_3)_2CO$–$(CD_3)_2SO$] δ 5.2 (2 H, s, CH_2), 6.85 (2 H, dd, J = 12 Hz, 8 Hz, imidazole), 7.6 (4 H, dd, J = 83 Hz, 72 Hz, pyridyl). Anal. ($C_9H_9N_3S$) C, H, N.

The hydrochloride salt was formed by dissolution of the base in EtOAc and addition of etheral HCl: mp 238–242 °C dec. Anal. ($C_9H_9N_3S$·HCl) H, N; C: calcd, 47.47; found, 47.10.

1-[(5-Hydroxy-2-pyridyl)methyl]-1,3-dihydro-2*H*-imidazole-2-thione (3). A 57.55-g (0.527 mol) quantity of 5-hydroxy-2-methylpyridine (**8**) was mixed with 110.2 g (0.58 mol) of *p*-toluenesulfonyl chloride, 58.6 g (0.58 mol) of triethylamine, and 300 mL of DMF. The reaction mixture was stirred and heated to 95 °C where nearly complete solution occurred. Heating was continued for 2.5 h and the reaction mixture was cooled and diluted with H_2O and the precipitate that formed was filtered to give 112 g (81%) of a tan crystalline solid: mp 101–102 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.45 (3 h, s, CH_3), 2.55 (3 H, s, CH_3). A 111.1-g (0.42 mol) quantity of this tosylate was dissolved in 1 L of $CHCl_3$ and 94.3 g (0.464 mol) of 85% 3-chloroperbenzoic acid was added in portions over 15–20 min. An exotherm raised the temperature to 38 °C. The solution was the refluxed for 30 min and cooled and 300 mL of 20% Na_2CO_3 was added. The mixture was filtered and the filtrate was extracted twice with saturated brine and was then concentrated to give a moist solid. This was dissolved in CH_2Cl_2 and the solution was filtered through Celite and the filtrate was concentrated to a yellow oil, which crystallized rapidly on seeding. The solid was triturated with hexane to give 99.9 g (85%) of the *N*-oxide (**9**): ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.45 (6 H, s, CH_3).

A 79.6-g (0.29 mol) quantity of the *N*-oxide (**9**) was added in portions to 400 mL of Ac_2O which had been heated to 85 °C. An exotherm increased the temperature to 100–110 °C where it was maintained during the course of the 30–40-min addition period. The solution was then heated at reflux for 1 h and then was cooled and concentrated to give a dark oil. This was taken up in a mixture of CH_2Cl_2–Et_2O and the solution was decanted from a small amount of insoluble oil and concentrated. The residual oil was redissolved in CH_2Cl_2 and chromatographed on a column of 440 g of silica gel with CH_2Cl_2 as eluant to give 62.5 g (68%) of product ester: IR (film) 1740 cm^{-1} (ester carbonyl); ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.15 (3 H, s, CH_3CO), 2.4 (3 H, s, CH_3), 5.2 (2 H, s, CH_2).

The ester (0.198 mol) was dissolved in 700 mL of MeOH and 25 mL of Et_3N was added. The solution was heated at reflux for 40 h and allowed to stand at ambient temperature for 72 h. An IR spectrum of a film of an aliquot showed no ester carbonyl absorption. The reaction mixture was concentrated to a dark oil, which was triturated with boiling Et_2O and then with boiling EtOAc. The combined triturates were concentrated to a yellow oil and this was purified by flash chromatography on a column of 500 g of silica gel with 5% MeOH in $CHCl_3$ eluant to yield 50.5 g (91%) of product alcohol: TLC (10% CH_3OH in $CHCl_3$) indicated the major component at R_f 0.6; ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.45 (3 H, s, CH_3), 4.7 (2 H, s, CH_2).

A 49.5-g (0.177 mol) quantity of the above alcohol was dissolved in 1 L of $CHCl_3$ and 150 g of activated MnO_2 was added. The reaction mixture was stirred for 65 **h** at ambient temperature. The reaction mixture was filtered, the filtrate was concentrated, and the residual oil was taken up in Et_2O. The solution was filtered and then concentrated to give 28.0 g (57%) of a brown oil. This oil was redissolved in Et_2O and the solution was chilled to depost 10.3 g (21%) of **10** as a crystalline solid: mp 97–98.5 °C. Recrystallization from Et_2O–hexane gave mp 98–100 °C; IR 1720 cm^{-1} (aldehyde); ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.4 (3 H, s, CH_3), 7.2–8.5 (7 H, m, heterocyclic and aromatic protons), 10.0 (1 H, s, CHO). Anal.

An 8.90-g (0.032 mol) quantity of the above aldehyde (**10**) was treated with equimolar quantities of aminoacetaldehyde dimethyl acetal and $NaBH_4$ in 100 mL of MeOH, by the same procedure used for **2c**. The product was extracted with Et_2O. Concentration of the combined Et_2O extracts gave 9.3 g (79%) of a yellow oil: ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.45 (3 H, s, CH_3), 2.6 (2 H, d, J = 6 Hz, CH_2CH), 2.8 (1 H, s, NH), 3.4 (6 H, s, OCH_3), 3.9 (2 H, s, pyridyl CH_2), 4.5 (1 H, t, J = 6 Hz, CH), 7.2–8.3 (7 H, m, pyridyl, aromatic). This was treated with 25 mL of H_2O, 12 mL of EtOH, 3.06 g (0.032 mol, 20% excess) of KSCN, and 6 mL of 12 N HCl as described for **2a**. Upon cooling, but before neutralization, the greenish-white crystalline solid that formed was filtered and washed with ether to give 4.68 g (37%) of 1-[[5-[(*p*-tolylsulfonyl)oxy]-2-pyridyl]methyl]imidazole-2-thione hydrochloride: ^{1}H NMR [90 MHz, $(CD_3)_2SO$] δ 2.45 (3 H, s, CH_3), 3.6 (2 H, br s, NH), 5.3 (2 H, s, CH_2), 6.9–8.4 (9 H, m, imidazole, pyridyl, aromatic).

A 4.53-g (0.011 mol) quantity of the [[(tosyloxy)pyridyl]-methyl]imidazole-2-thione hydrochloride was stirred with 25 mL of 2.5 N NaOH and the mixture was heated at reflux for 1 h and then cooled and neutralized to pH 7.0 with 12 N HCl. The precipitated yellow solid was filtered and recrystallized from MeOH–MeCN and the triturated with a small amount of H_2O at ambient temperature to give 1.72 g (73%) of **3**: mp 260–263 °C dec; TLC (4% NH_4OH in 1:1 MeOH–EtOAc) R_f 0.7; IR (KBr disk) 1475 (s), 1285 (s), 1250 cm^{-1}; ^{1}H NMR [90 MHz, $(CD_3)_2SO$] δ 3.4 (2 H, br s, NH, OH), 5.15 (2 H, s, CH_2), 6.8–8.3 (5 H, m, pyridyl, imidazole). Anal. ($C_9H_9N_3SO$) C, H, N.

1-[(1,2-Dihydro-2-oxo-5-pyridyl)methyl]imidazole-2-thione (4). The method of Kompis et al.,[7] bromination, lithiation, and formylation was used to convert 2-methoxypyridine (**12**) to the 5-formyl derivative **14** in 31% overall yield: bp 115–135 °C (25 torr). The distilled liquid crystallized in the receiver and was recrystallized from Et_2O–hexane: mp 51–52 °C.

A 12.7-g (0.0927 mol) quantity of 2-methoxypyridine-5-carboxaldehyde (**14**) was stirred with 127 mL of 48% aqueous HBr (water-white, redistilled from $SnCl_2$) under argon and the mixture was heated to reflux over 45 min. Gas (MeBr) evolution began at ca. 90 °C and increased with rising temperature but diminished rapidly as reflux began. The solution was refluxed 2–3 min and was then cooled and concentrated to a tan solid. This was dissolved in MeOH and the solution was diluted with Me_2CO. Some dark, amorphous solid was filtered, and the filtrate was diluted with Et_2O, which formed a second liquid phase. The lower phase was separated and extracted with several additions portions of Et_2O, which caused precipitation of a tan solid. This was stirred with H_2O and 5% $NaHCO_3$ was added to bring the pH to 7.0, and the mixture was filtered to yield 3.95 g (35%) of **13**: mp 211–215 °C. Purification by Soxhlet extraction (Et_2O) gave mp 220.5–222 °C; IR (KBr disk) 1722 cm^{-1}; ^{1}H NMR [90 MHz, $(CD_3)_2SO$, $CDCl_3$] δ 3.3 (1 H, br s, NH), 6.1 (1 H, d, pyridyl), 7.45 (1 H, pyridyl), 7.85 (1 H, d, pyridyl) 9.25 (1 H, s, CHO). Anal. ($C_6H_5NO_2$) C, H, N.

A 3.0-g (0.024 mol) quantity of **13** was stirred with 2.56 g (0.024 mol) of aminoacetaldehyde dimethyl acetal in 30 mL of MeOH. The solution was concentrated and the solid was redissolved in MeOH and reconcentrated twice to yield a yellow semisolid. The solid was redissolved in MeOH and 0.92 g (0.024 mol) $NaBH_4$ was added in small portions at 0 °C. After the addition was complete, the solution was stirred at ambient temperature for 1 h. The MeOH solution was diluted with 30 mL of H_2O and 2.85 g (0.029 mol, 20% excess) of KSCN was added, followed by 10 mL of 12 N HCl. The solution was refluxed for 30 min, cooled, and concentrated to give a brown semisolid. This was taken up in EtOH, diluted with MeCN, filtered, and concentrated to a glass (4.5 g). This was triturated successively with MeCN and 2-PrOH, and the insoluble solid was then dissolved in 50 mL of MeOH. This solution was treated with 2 mL of concentrated NH_4OH and the resulting solution was flash chromatographed on a column of 150 g of silica gel with 4% NH_4OH in MeOH as eluant to give 1.2 g of crude **4**. This was triturated with EtOH and the filtrate yielded 164 mg (3%) of **4** as a yellow solid: mp 234–236 °C dec; TLC (4% NH_4OH in MeOH) R_f 0.8; IR (KBr disk) 1660 (vs), 1620 (vs), 1470 (s), 1270 (s) cm^{-1}; ^{1}H NMR [90 MHz, $(CD_3)_2SO$] δ 5.3 (2 H, s, CH_2), 6.75 (1 H, d, J = 9 Hz, pyridyl), 7.4 (2 H, dd, J = 24, 20 Hz, imidazole), 7.85 (1 H, s, pyridyl), 7.95 (1 H, dd, J = 14, 9 Hz, pyridyl), 12.6 (1 H, s, NH); chemical ionization (CH_4) mass spectrum, m/e 208 (M + H$^+$). Anal. ($C_9H_9N_3OS$) C, H, N.

1-[(2-Methoxy-5-pyridyl)methyl]imidazole-2-thione (5). A 1.37-g (0.01 mol) sample of 2-methoxypyridine-5-carboxaldehyde[7] (**14**) was treated with 1.33 g (0.01 mol) of aminoacetaldehyde diethyl acetal as described for **2a** and the resulting solution of imine in EtOH was reduced in a Parr low-pressure hydrogenation apparatus using 100 mg of 5% Pd/C catalyst. The catalyst was

filtered and the filtrate was concentrated and taken up in 5 mL of EtOH. This solution was diluted with 10 mL of H_2O and 1.1 g (0.011 mol, 10% excess) of KSCN and 2 mL of 12 N HCl was added. The imidazole-2-thione was formed by the same procedure used for **2a**. The crude reaction product was flash chromatographed on silica gel with EtOAc, and the chromatographed material was recrystallized from EtOAc–hexane to give 0.42 g (19%) of **5**: mp 143–145 °C; ^{1}H NMR [60 MHz, $CDCl_3$, $(CD_3)_2SO$] δ 3.9 (3 H, s, CH_3), 5.2 (2 H, s, CH_2), 6.45–6.8 (3 H, m, pyridyl, imidazole), 7.65 (1 H, dd, J = 12 Hz, 6 Hz, pyridyl), 8.2 (1 H, d, J = 3 Hz, pyridyl). Anal. ($C_{10}H_{11}N_3OS$) C, H, N.

Enzymology. In vitro IC_{50} determinations were made as previously reported.[1] The IC_{50} is defined as the concentration of compound that produces a 50% inhibition of product formation when compared to treated control.

Pharmacology. DA/NE Ratios. Mesenteric artery catecholamine levels were determined in SHR by a reported procedure.[9,10] Compounds were administered ip in two doses as suspensions in a 5% PEG 400, 1% methocel vehicle, 18 h apart.

Blood Pressure Measurements. These were determined as described previously.[1b] Compounds were administered ip as solutions in 20% DMF. Blood pressures and heart rates were monitored for 5–6 h and recorded at 0.5- intervals.

Registry No. 1, 95333-81-6; **2a**, 106984-85-4; **2b**, 106984-86-5; **2c**, 106984-87-6; **2c**·HCl, 108269-98-3; **3**, 106984-89-8; **3** (tosylate)·HCl, 108270-00-4; **4**, 106984-88-7; **5**, 108270-02-6; **6a**, 1121-60-4; **6b**, 500-22-1; **6c**, 872-85-5; **7a** (diethyl acetal), 6957-15-9; **7a** (imine, diethyl acetal), 6190-94-9; **7c**, 108269-96-1; **7c**·HCl, 108269-97-2; **8**, 1121-78-4; **8** (tosylate), 74838-56-5; **9**, 106984-92-3; **10**, 106984-95-6; **10** (2-alcohol, acetate), 106984-93-4; **10** (2-alcohol), 106984-94-5; **11**, 108269-99-4; **12**, 1628-89-3; **13**, 106984-91-2; **14**, 65873-72-5; DBH, 9013-38-1; $H_2NCH_2CH(OC_2H_5)_2$, 645-36-3; $H_2NCH_2CH(OCH_3)_2$, 22483-09-6; KSCN, 333-20-0; TosCl, 98-59-9; 5-[[(5,5-dimethoxyethyl)imino]methy]-2(1*H*)-pyridone, 108270-01-5; 1-benzyl-1,3-dihydro-2*H*-imidazole-2-thione, 23269-10-5; 1-(*p*-hydroxybenzyl)-1,3-dihydro-2*H*-imidazole-2-thione, 95333-64-5; fusaric acid, 536-69-6.

New Antitumor Agents Containing the Anthracene Nucleus

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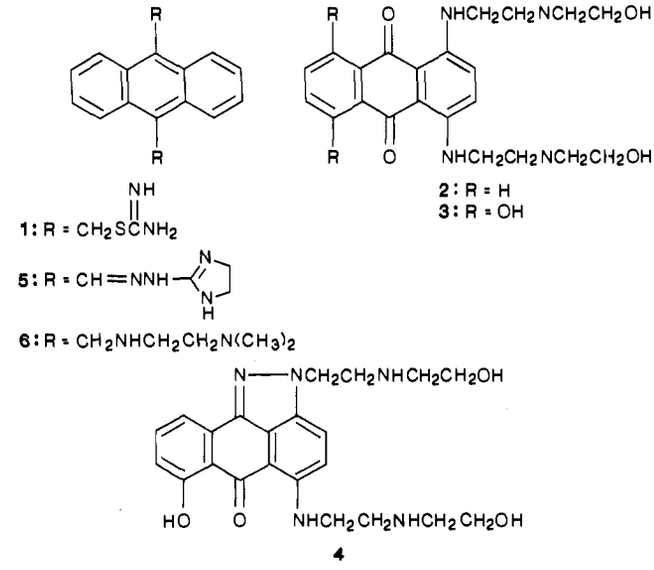
A series of 21 new compounds related to bisantrene was synthesized and tested in vitro by using clonogenic assays against a variety of human tumor cell lines, fresh human tumors, and P-388 leukemia. Those most closely related to bisantrene were less active than it was, but a subset of compounds with saturated side chains containing two basic nitrogens showed good activity. Two compounds of this subset, *N,N'*-bis[2-(dimethylamino)ethyl]-9,10-anthracenebis(methylamine) (**6**), and *N,N'*-bis(1-ethyl-3-piperidinyl)-9,10-anthracenebis(methylamine) (**19**), were very active in vitro against human tumor cell lines, but not active against fresh human tumors or P-388 leukemia cells. They had only marginal activity in mouse tumor models. Thus, the fresh human tumors and P-388 leukemia cells in vitro were better predictors than the established cell lines for the activity of these anthracene compounds in vivo against mouse tumors. These compounds appear to be distinct from bisantrene in aspects of mode of action. For example, **6** did not cause inhibition of macromolecular synthesis and promotion of DNA single strand breakage at cytotoxic drug concentrations. Toxicological studies showed that its rapid administration caused acute neurotoxicity resulting in apnea. It also produced skin ulcers on id administration, but they were less severe than those caused by bisantrene.

In recent years a variety of anthracene derivatives have received attention as potential antitumor drugs. The first such derivative was 2,2'-(9,10-anthrylenedimethylene)-bis[2-thiopseudourea] (**1**),[1,2] known as "pseudourea", which entered clinical trials, but was withdrawn because of phototoxicity.[3,4] Subsequently, a variety of substituted anthraquinones, including ametantrone (**2**) and mitoxantrone (Novantrone, **3**), have been tested in clinical trials.[5–8] The latter compound appears to be active principally in breast cancer, acute leukemias,[9] and non-Hodgkin's lymphomas.[10] It is less cardiotoxic than doxorubicin. A new system based on anthraquinone, the 5-substituted anthra[1,9-*cd*]pyrazol-6(2*H*)-one (e.g., **4**) also shows promise in cancer chemotherapy.[11] Diguanylhydrazones of anthracene-9,10-dicarboxaldehydes comprise a rather large group of compounds with antitumor activity in experimental systems.[12] The most active compound of this group, bisantrene (**5**), has proven active in patients with breast cancer and acute leukemia.[13–16]

Our initial interest in the anthracenes was to develop a more complete set of structure–activity relationships for compounds related to bisantrene and pseudourea. Although many guanylhydrazones had been prepared and tested, most of the structural variation was in the guanidinyl moiety or in substituents on the anthracene nucleus.[12]



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