gradually increasing the polarity of the eluant from chloroform to 10% methanol/chloroform to obtain 5.4 g of pure **9** and 4 g of slightly contaminated product. The impure product was subjected to a second flash column chromatography to obtain an additional 1.5 g of pure **9**; the combined yield of **9** was 6.9 g (73% yield) as a red solid: mp 240–243 °C; ^{1}H NMR δ 1.38 (t, 3 H, J = 7.5), 2.02 (s, 3 H), 3.30–3.70 (br m, 6 H), 4.36 (q, 2 H, J = 7.5), 4.55 (m, 1 H), 6.27 (br d, 1 H, J = 6), 7.05 (m, 2 H), 7.40 (m, 1 H), 7.90 (d, 1 H, J = 13.5), 8.35 (s, 1 H); mass spectrum, m/z 475 (parent + H). Anal.[15] ($C_{23}H_{21}F_3N_4O_4 \cdot {}^1/_3H_2O$) C, H, N. In a 1-L round-bottom flask were placed 5.35 g (11.3 mmol) of **9**, 100 mL of THF, and 350 mL of 0.1 M aqueous sodium hydroxide. The reaction mixture was stirred at 65 °C for 1.5 h and concentrated with a rotary evaporator. To the residue was added 400 mL of 6 M aqueous hydrochloric acid, and the reaction mixture was heated at 110 °C for 24 h under nitrogen. The reaction mixture was concentrated with a rotary evaporator, and ~70 mL of water was added to the system. This mixture was heated to boiling for approximately 5 min and chilled to 0 °C, and the precipitate was collected by suction filtration and rinsed with water, ethanol, and ether. The precipitate was recrystallized from hot ethanol, rinsed with cold ethanol and ether, and dried overnight in vacuo at 60 °C to afford 3.24 g (66% yield) of pure **1a** as a pale beige solid: mp 229–234 °C dec; $[\alpha]^{20}_D$ +14° (c 0.92, DMSO); ^{1}H NMR (DMSO-d_6) δ 2.10 (m, 2 H), 2.50 (m, 3 H), 3.30–3.40 (complex, 2 H), 3.85 (br, 2 H), 7.35 (m, 1 H), 7.60 (m, 1 H), 7.80 (m, 1 H), 8.12 (d, 1 H, J = 12), 8.83 (s, 1 H); mass spectrum, m/z 405 (parent + H). Anal. ($C_{19}H_{15}F_3N_4O_3 \cdot {}^3/_2HCl$) C, H, N.

Analogous procedures were used to convert N-benzyl-3(S)-pyrrolidinol to 3(R)-3-acetamidopyrrolidine (**2b**) and its corresponding naphthyridine analogue **1b**, $[\alpha]^{20}_D$ −15° (c 0.91, DMSO).

(3*S*)-1-Benzyl-3-[(*R*)-α-methoxy-α-(trifluoromethyl)-α-phenylacetamido]pyrrolidine (12). In a 25-mL round-bottom flask under nitrogen were placed 57 mg (0.33 mmol) of 3(S)-amino-1-benzylpyrrolidine (**10**), 0.60 mL of pyridine, and 60 μL (45 mg, 0.45 mmol) of triethylamine. To the system was added 80 μL (108 mg, 0.45 mmol) of (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride[9] (derived from (R)-(+)-MTPA). The reaction mixture was stirred at room temperature under an atmosphere of nitrogen for 5 h, diluted with ether and washed with aqueous sodium bicarbonate and brine. The organic phase was dried (Na_2SO_4) and concentrated with a rotary evaporator to afford 98 mg (76% yield) of crude **12** as a yellow oil: ^{19}F NMR (trifluoroethanol, internal standard) δ 8.29 ppm; mass spectrum, m/z 393 (parent + H).

(3*R*)-1-Benzyl-3-[(*R*)-α-methoxy-α-(trifluoromethyl)-α-phenylacetamido]pyrrolidine (13): ^{19}F NMR (trifluoroethanol, internal standard) δ 8.35 ppm.

Biological Studies. The minimum inhibitory concentrations (MIC) and ED_{50} values were obtained using standard techniques as described in ref 5. The aerobic MIC values were determined on brain–heart infusion agar, and the anaerobic MIC values were determined on Wilkins–Chalgren agar. The IC_{50} values were determined by using the technique described previously.[13]

Registry No. (±)-**1**, 114636-36-1; **1a**, 114715-36-5; **1b**, 114715-37-6; **2a**, 114636-30-5; **2b**, 114636-33-8; **3**, 100491-29-0; **5**, 101930-07-8; **6**, 114715-35-4; **7**, 114636-29-2; **8**, 114636-31-6; **9**, 114636-32-7; **10**, 114715-38-7; **11**, 114715-39-8; **12**, 114636-34-9; **13**, 114636-35-0; **16**, 109431-87-0; **17**, 114636-37-2; CIP, 85721-33-1; (±)-MTPA chloride, 20445-33-4; N-benzyl-3(S)-pyrrolidinol, 101385-90-4; norfloxacin, 70458-96-7.

Imidazo[1,2-*a*]pyrimidines and Imidazo[1,2-*a*]pyrazines: The Role of Nitrogen Position in Inotropic Activity

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Congestive heart failure is a major medical problem for which existing medicaments have provided limited benefit. Recent new experimental drugs, including imidazo[4,5-*b*]- and imidazo[4,5-*c*]pyridines, have both inotropic and vasodilatory properties. Subtle changes in nitrogen position of these compounds have been shown to dramatically affect potency.[5] We have synthesized a series of imidazo[4,5-*b*]- and -[4,5-*c*]pyridine analogues having an imidazo nitrogen relocated at the bridgehead position. The superior inotropic activity of the [4,5-*c*]pyridines as compared to [4,5-*b*]pyridines[5] is reaffirmed by the activity of our analogues. The biological equivalence of imidazo[4,5-*b*]pyridines with imidazo[1,2-*a*]pyrimidines and imidazo[4,5-*c*]pyridines with imidazo[1,2-*a*]pyrazines is demonstrated. Further, 2-[2-methoxy-4-(methylsulfenyl)phenyl]imidazo[1,2-*a*]pyrazine and 2-[2-methoxy-4-(methylsulfonyl)phenyl]-imidazo[1,2-*a*]pyrazine are potent inotropic agents both in vitro and in vivo.

Congestive heart failure is a major cause of morbidity and mortality throughout the United States and other industrialized countries.[1] While drugs such as cardiac glycosides and β-agonists are widely used to improve the performance of the failing heart, they suffer from substantial disadvantages and limited efficacy.[2] With the advancing age of the general population, the need for more effective treatment for congestive heart failure will become even more acute, and it is appropriate, therefore, that significant effort has been directed toward this medical need as evidenced by the recent appearance of several new drugs.[3]

Some of these new drugs are characterized by the advantage of having at the same time both inotropic and vasodilatory properties.[4] Two of these compounds (AR-L115BS [sulmazole], **1c**, and LY175326 [isomazole], **2c** see Figure 2) are structurally closely related being imidazo-[4,5-*b*]- and -[4,5-*c*]pyridines, respectively. Robertson et al.[5] have recently reported a comparative study of these series of compounds. They found that for a wide range of substituents, compounds from the [4,5-*c*] series routinely show superior inotropic potency. Their study clearly illustrates that subtle changes in the position of nitrogen can play an important role in affecting biological activity.

(1) (a) Braunwald, E. *Am. J. Cardiol.* **1985**, *56*, 1B. (b) *Congestive Heart Failure. Current Research and Clinical Applications*; Braunwald, E., Mock, M. B., Watson, J. T., Eds.; Grune and Stratton: New York, 1982.
(2) *Pharmacology and Therapeutics*; Bowman, W. C., Breckenridge, A. M., Sartorelli, A. S., Eds.; Pergamon: Elmsford, NY, 1982; Vol. 18.
(3) Erhardt, P. W. *J. Med. Chem.* **1987**, *30*, 231.
(4) (a) Dredesen, W.; Kadatz, R. *Arzneim-Forsch.* **1981**, *31*, 141. (b) Hayes, J. S.; Pollock, D. G.; Wilson, H.; Bowling, N.; Robertson, D. W. *J. Pharmacol. Exp. Ther.* **1985**, *233*, 318.
(5) Robertson, D. W.; Beedle, E. E.; Krushinski, J. H.; Pollock, D. G.; Wilson, H.; Wyss, V. L.; Hayes, J. S. *J. Med. Chem.* **1985**, *28*, 717.

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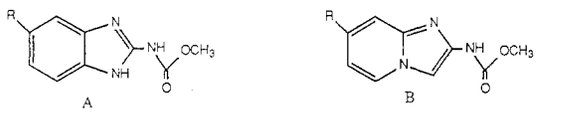
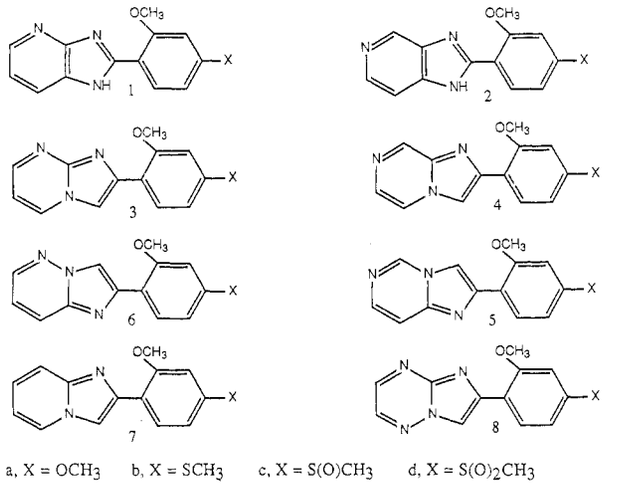


Figure 1.



a, X = OCH_3 b, X = SCH_3 c, X = $S(O)CH_3$ d, X = $S(O)_2CH_3$

Figure 2.

Several years ago Fisher and Lusi[6] demonstrated a similarity of structure–activity relationships between benzimidazole anthelmintics and their corresponding [1,2-*a*]-pyridine counterparts. This correlation has been extended by Bochis et al.[7] Thus, benzimidazoles A and corresponding [1,2-*a*]pyridines B (see Figure 1) have similar anthelmintic activity.

During the course of work in our laboratory, we have also noted that imidazo[1,2-*a*]pyridines may sometimes be substituted for benzimidazoles without dramatically altering biological activity. Bearing in mind the important role nitrogen position can play, we consequently examined a similar substitution in the inotropic compounds **1** and **2** (Figure 2) to test the effect of relocation of an imidazole nitrogen to the bridgehead position. Moving a nitrogen to the bridgehead position removes the redundency associated with structures **1** and **2**, and consequently four isomers (**3–6**, see Figure 2) are now possible.

Chemistry

The synthesis of compound **3** was carried out by reacting the appropriately substituted α-bromoacetophenone with 2-aminopyrimidine. However this procedure was less applicable to the other members of this series. In 2-aminopyrazine, 4-aminopyrimidine, and 3-aminopyridazine, the ring nitrogen that is not adjacent to the amino function is the most nucleophilic. Consequently alkylation by the α-bromoacetophenone takes place preferentially at this site, thus thwarting an effective synthesis of the desired bicyclic product. This can be overcome by placing a chlorine in the ring, adjacent to the offending nitrogen (see Figure 3 for example), thus greatly reducing the nucleophilicity of this nitrogen and returning the preferential site of alkylation to the ring nitrogen adjacent to the amino function. The desired ring systems can thus be obtained by reacting the appropriately substituted α-bromoacetophenones with 2-amino-3-chloropyrazine, 4-amino-6-chloropyrimidine, and 3-amino-6-chloropyridazine. The chlorine can then readily be removed by catalytic hydrogenation to give **4–6**, respectively.

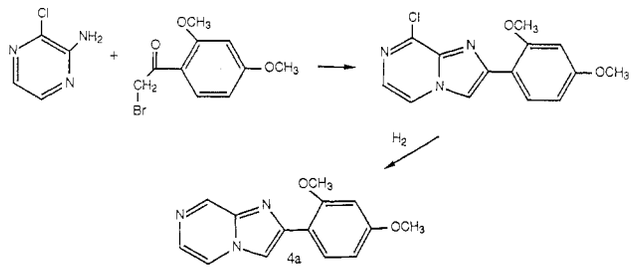


Figure 3.

Table I. Inotropic Data in Cat Papillary Muscle at 10^{-5} M

compound	% of control (*n*)	compound	% of control (*n*)
1a	146 ± 11 (2)	**4b**	140 ± 13 (6)
1c (sulmazole)	112 ± 3 (8)[5]	**4c**	161 ± 16 (11)
2a	158 ± 9 (3)[5]	**4d**	180 ± 21 (6)
2b	143 ± 15 (6)[5]	**5a**	114 ± 5 (6)
2c (isomazole)	172 ± 24 (3)[5]	**6a**	115 ± 17 (4)
2d	176 ± 18 (3)[5]	**7a**	98 ± 11 (6)
3a	136 ± 7 (5)	**8a**	130 ± 7 (4)
4a	145 ± 8 (5)		

A similar alternative procedure can be applied to the pyrazine ring system in which a carboxymethyl derivative can be employed in place of chlorine. In this case the appropriate α-bromoacetophenone is reacted with 3-aminopyrazine-2-carboxylic acid methyl ester. After formation of the desired bicyclic derivative the ester is removed by hydrolysis, and the resulting acid function decarboxylates under the hydrolysis conditions to yield the desired imidazo[1,2-*a*]pyrazine.

To complete the study on the effects of nitrogen position on inotropic activity, compounds **7** and **8** (see Figure 2) were prepared by using the same synthetic procedure as employed in the preparation of compound **3**.

Results and Discussion

The in vitro results shown here correlate with those reported by Robertson et al.[5] The inactivity of compound **7a** (see Table I) shows the importance of having a nitrogen substituent in the six-membered ring. The relevance of nitrogen position, as established by Robertson et al.,[5] is again illustrated here by the somewhat greater activity of **4a** versus **3a**. In addition, the relatively weak activity of **5a** and **6a** demonstrates a further refinement of the structural requirements for positive inotropy. Thus when N_1 of the imidazole ring is moved to the adjacent bridgehead position (for example, compound **1** going to **3** or compound **2** going to **4**) little change in inotropic activity is observed, whereas relocating N_3 (compound **1** going to **6** or compound **2** going to **5**) results in a loss of activity (see Table I), compounds **5** and **6** being relatively weakly inotropic. The relationship of the two nitrogens on the top of structures **1** and **2** (also **3** and **4**) seem to be more important to activity, whereas the location of the nitrogen on the bottom half—either imidazole ring or bridgehead—is of less importance. This concept is further supported by the fact that when an additional ring nitrogen is added to the "correct" position of weakly active compound **6a**, to form **8a** (compare **8a** with **1a** and **3a**), the inotropic activity is at least partially restored.

The relative in vitro activities in the cat papillary muscle for compounds **3a**, **4a**, **5a**, and **6a** is reinforced by their relative in vivo activities in the anesthetized dog (Figure 4). Here again pyrazine **4a** is somewhat more active than the corresponding pyrimidine **3a** whereas **5a** and **6a** are less active.

(6) Fisher, M; Lusi, A. *J. Med. Chem.* **1972**, *15*, 982.
(7) Bochis, R. J.; Oben, L. E.; Wakamunski, F. S.; Mrozek, H.; Eskola, P.; Kulsa, P; Wilks, G.; Taylor, J. E.; Egerton, J. R.; Ostlind, D. A.; Olson, G. *J. Med. Chem.* **1981**, *24*, 1518.

Table II. ED_{50}'s in Anesthetized Dog (mg/kg iv)[a]

[4,5-c]pyridines			[1,2-a]pyrazines		
compd	4'-substit	ED_{50} (n)	compd	4'-substit	ED_{50} (n)
2a	OCH_3	0.3 (4)	**4a**	OCH_3	0.7 (2)
2b	SCH_3	0.2 (2)	**4b**	SCH_3	0.5 (2)
2c	$S(O)CH_3$	0.03 (7)	**4c**	$S(O)CH_3$	0.1 (2)
2d	$S(O)_2CH_3$	0.02 (4)	**4d**	$S(O)_2CH_3$	0.02 (4)

[a] For comparison, sulmazole (**1c**), with the 4'-substituent $S(O)CH_3$ has an ED_{50} of 0.3.[5]

Note also the parallelism of series **2a–d** with **4a–d**. In these two series, the various 4-substituted derivatives have comparable inotropic activities in both the cat papillary muscle (Table I) and the anesthetized dog (Table II). Thus the relocation of the N_1 imidazole nitrogen to the bridgehead position has little adverse effect on biological activity. In addition, by comparison with **1c** (sulmazole), which has undergone extensive human clinical studies, and with **2c** (isomazole), for which human clinical studies have recently been initiated, both compounds **4c** and **4d** are potent cardiotonic agents.

In conclusion this study further clarifies the structural requirements for inotropic activity of imidazo[4,5-*b*]- and -[4,5-*c*]pyridines. As with the compounds described by Robertson et al.,[5] the position of the ring nitrogen in the imidazo[4,5-*c*] series provides a more active inotrope than the position of the ring nitrogen in the corresponding imidazo[4,5-*b*] isomer. Further, the apparent biological equivalence of imidazo[4,5-*b*]pyridines (**1**) with imidazo-[1,2-*a*]pyrimidines (**3**), and imidazo[4,5-*c*]pyridines (**2**) with imidazo[1,2-*a*]pyrazines (**4**) has been demonstrated by comparable inotropic properties both in vitro and in vivo.

Experimental Section

All reactions were followed by TLC with Merck F254 silica gel plates. ^1H NMR spectra were recorded on a Varian T-60 spectrometer, a Bruker WM270 spectrometer, a QE-300 spectrometer, or a Bruker WH360 spectrometer. Mass spectra were recorded on a CC21-110A mass spectrometer. ^1H NMR spectra, mass spectra, and microanalytical data were provided by the Physical Chemistry Department of the Lilly Research Laboratories. Microanalytical data was correct where noted. In the workup procedure where the organic phase was dried, anhydrous magnesium sulfate was employed as the drying agent.

Isolated Cat Papillary Muscles.[4b] Cats of either sex were anesthetized with methoxyflurane, their hearts were removed immediately, and the papillary muscles were isolated and suspended in individual muscle baths. A 27-gauge hook secured the muscle to an electrode mounted in the bottom of the bath, and a silk thread attached the tendon to a Statham isometric transducer. Baths contained Krebs-Henseleit solution (37.5 °C, bubbled with 95% O_2–5% CO_2) of the following millimolar composition: NaCl, 118; KCl, 4.5; $CaCl_2$, 2.5; KH_2PO_4, 1.1; $MgSO_4$, 1.2; $NaHCO_3$, 25; and glucose, 11. A resting tension of 1.0 g was applied to each tissue. Muscles were stimulated to contract by administering square-wave pulses (2.0 ms in duration, 12 times/min, 20% above threshold voltage) delivered through the hook electrode and a second electrode positioned near the top of the muscle; contractions were recorded on a Grass polygraph. To establish cumulative dose–response relationships, compounds (dissolved in DMSO) were added to baths and allowed to produce maximal responses before the addition of the next higher concentration.

Experiments in Anesthetized Dogs. Mongrel dogs of either sex (7–14 kg) were anesthetized with sodium pentobarbital (35 mg/kg iv). A positive-pressure pump was used to ventilate the dogs through an endotracheal tube (18 strokes/min, 20 mL/kg per stroke), and a heating pad maintained body temperature at 37–38 °C. Femoral arterial blood pressure was measured through a polyethylene catheter filled with heparin solution (16 units/mL) and connected to a Statham pressure transducer. The femoral vein was cannulated for iv drug administration. Heart rate was derived by means of a cardiotachometer that was triggered by the arterial pressure pulse. A Walton–Brodie strain-gauge arch sutured to the right ventricle of the heart measured cardiac contractility. Tension on the gauge was adjusted to 50 g, which corresponded to 10 mm of recorder pen deflection. Rapid iv injection of 50 mL of 5% dextran and mechanical compression of the aorta showed that contractility measurements were independent of changes in preload and afterload. Subcutaneous pin electrodes provided a lead II ECG. Increasing doses of test compounds (0.01, 0.02, 0.05, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg) were administered iv in volumes of 0.25–4.0 mL at 5-min intervals; no responses occurred with appropriate vehicle control injections.

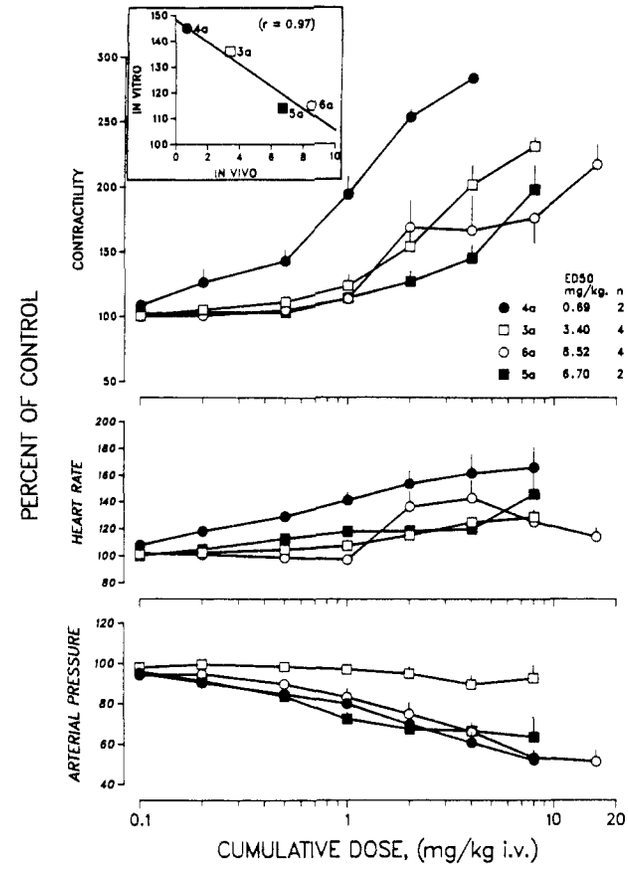


Figure 4. Dose-dependent effects of myocardial contractility, heart rate, and mean arterial blood pressure in pentobarbital anesthetized dogs. Each point is the mean ± SEM (n = 4) or mean ± range (n = 2) for peak responses during 5-min infusion. Control values were as follows: contractility, 50-g tension; heart rate, 127 ± 3 bpm; and arterial pressure, 98 ± 3 mmHg. The ED_{50} was calculated for each dog by regression analyses, and the average value for each compound is the dose in milligrams/kilogram required to increase contractility by 50%. Contractility is compared in the insert plot with a correlation value (r = 0.97) between in vitro (cat papillary muscles) and in vivo (anesthetized dog) results.

2-(2,4-Dimethoxyphenyl)imidazo[1,2-*a*]pyrimidine (3a). A solution of 1.9 g (20 mmol) of 2-aminopyrimidine and 5.2 g of α-bromo-2,4-dimethoxyacetophenone (20 mmol) in 40 mL of dimethylformamide was stirred at room temperature for approximately 60 h. The reaction mixture was poured into approximately 300 mL of ethyl acetate, and the organic solution was then washed with a saturated sodium bicarbonate solution followed by two washes with 300 mL each of saturated sodium chloride solution. The organic phase was dried, and the solvent was removed in vacuo. The resulting residue was crystallized from hot ethanol to yield 2 g (7.8 mmol or 39%) of the title product: ^1H NMR (60 MHz) ($CDCl_3/Me_4Si$) δ 3.86 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 6.51–6.88 (m, 3 H, phenyl H), 8.07 (s, 1 H, ring H), 8.33–8.63 (m, 3 H, ring H); MS, m/e (relative intensity), 255 (M^+, 100), 226 (31), 182 (29), 79 (30). Anal. ($C_{14}H_{13}N_3O_2$) C, H, N.

2-(2,4-Dimethoxyphenyl)imidazo[1,2-*a*]pyrazine (4a).

2-Aminopyrazine (1.9 g, 20 mmol) and α-bromo-2,4-dimethoxyacetophenone (5.2 g, 20 mmol) were dissolved in 40 mL of dimethylformamide and stirred at room temperature for 48 h. The reaction mixture was then added to 300 mL of ethyl acetate and washed one time with 300 mL of saturated sodium bicarbonate solution followed by two washes with 300 mL of saturated sodium chloride. The organic phase was dried, and the solvent was removed by rotary evaporation. The residue was then purified by silica gel column chromatography by elution with 10% methanol/ethyl acetate to yield 330 mg (1.3 mmol or 6%) of product: ^{1}H NMR (270 MHz) (DMSO-d_6/Me_4Si) δ 3.82 (s, 3 H, OCH_3) 3.98 (s, 3 H, OCH_3), 6.66–6.78 (m, 2 H, phenyl H), 7.85 (d, J = 5 Hz, 1 H, ring H), 8.24 (d, J = 9 Hz, 1 H, phenyl H), 8.46 (s, 1 H ring H), 8.60 (d, J = 5 Hz, 1 H, ring H), 9.03 (s, 1 H, ring H); MS, m/e (relative intensity) 255 (M^+, 100), 226 (29), 210 (30), 182 (33), 79 (41). Anal. ($C_{14}H_{13}N_3O_2$) C, H, N.

2-(2,4-Dimethoxyphenyl)imidazo[1,2-*c*]pyrimidine (5a). A. Preparation of 2-(2,4-Dimethoxyphenyl)-7-chloroimidazo[1,2-*c*]pyrimidine Hydrobromide. 4-Amino-6-chloropyrimidine (2.6 g, 20 mmol) and α-bromo-2,4-dimethoxyacetophenone (5.2 g, 20 mmol) were stirred in 10 mL of dimethylformamide for 3 days at room temperature. At this time the resulting precipatate was filtered and washed with ethyl acetate to give 7.37 g (19.8 mmol or 99%) of the desired product: ^{1}H NMR (270 MHz) (DMSO-d_6/Me_4Si) δ 3.86 (s, 3 H, OCH_3), 4.01 (s, 3 H, OCH_3) 6.68–6.79 (m, 2 H, phenyl H), 7.98 (d, J = 8 Hz, 1 H, phenyl H), 8.03 (s, 1 H, ring H), 7.98 (d, J = 8 Hz, 1 H, phenyl H), 8.03 (s, 1 H, ring H), 8.56 (s, 1 H, ring H), 9.54 (s, 1 H, ring H); MS, m/e (relative intensity) 291 (M^+, 34), 289 (M^+, 100), 260 (21), 161 (29), 82 (31), 80 (31). Anal. ($C_{14}H_{13}N_3O_2Cl$·HBr) C, H, N, Cl, Br.

B. Preparation of 2-(2,4-Dimethoxyphenyl)imidazo[1,2-*c*]pyrimidine (5a). Approximately 7.3 g (19.6 mmol) of 2-(2,4-dimethoxyphenyl)-7-chloroimidazo[1,2-*c*]pyrimidine hydrobromide was converted to the free base by suspending in ethyl acetate, adding 20 mL of propylene oxide, and stirring until dissolution had occurred. The ethyl acetate solution was washed with water and dried, and the solid was removed in vacuo to yield the free amine.

A solution of 3 g of 2-(2,4-dimethoxyphenyl)-7-chloroimidazo[1,2-*c*]pyrimidine (10.3 mmol) in ethyl acetate was treated with 1 g of 5% palladium on carbon and subjected to hydrogenation (initial pressure 60 psi) until hydrogen uptake ceased. Two grams of triethylamine was added, and hydrogenation was continued. When hydrogen uptake ceased, the catalyst was removed by filtration, and the ethyl acetate solution was washed with 300 mL of a saturated sodium chloride solution. The organic solution was evaporated in vacuo, and the resulting residue was crystallized from ethyl acetate/ether to yield 1.2 g of the title product (4.7 mmol or 46%): ^{1}H NMR (60 Hz) (DMSO-d_6/Me_4Si) δ 3.84 (s, 3 H, OCH_3), 4.02 (s, 3 H, OCH_3) 6.63–6.87 (m, 2 H, phenyl H), 7.50–7.71 (m, 1 H, ring H), 7.96 (d, J = 6 Hz, 1 H, phenyl H), 8.21–8.52 (m, 2 H, ring H), 9.49–9.59 (m, 1 H, ring H); MS, m/e (relative intensity) 255 (M^+, 100), 226 (22), 210 (23), 86 (33). Anal. ($C_{14}H_{13}N_3O_2$) C, H, N.

2-(2,4-Dimethoxyphenyl)imidazo[1,2-*b*]pyridazine (6a). A. Preparation of 6-Chloro-2-(2,4-dimethoxyphenyl)-imidazo[1,2-*b*]pyridazine Hydrobromide. Approximately 0.65 g (5 mmol) of 3-amino-6-chloropyridazine[8] and 1.3 g (5 mmol) of α-bromo-2,4-dimethoxyacetophenone were dissolved in 7 mL of dimethylformamide and stirred at 60 °C for approximately 4 h. At this time the precipatated material was collected by filtration and washed with ethyl acetate to yield 730 mg (1.96 mmol or 39%) of the desired product as the HBr salt: ^{1}H NMR (300 MHz) (DMSO-d_6/Me_4Si) δ 3.86 (s, 3 H, OCH_3), 4.00 (s, 3 H, OCH_3), 6.69–6.75 (m, 2 H, phenyl H), 7.54 (d, J = 11 Hz, 1 H, ring H), 8.13 (d, J = 9 Hz, 1 H, phenyl H) 8.29 (d, J = 11 Hz, 1 H, ring H) 8.69 (s, 1 H, ring H); MS, m/e (relative intensity), 291 (M^+, 17), 289 (M^+, 44), 277 (34), 275 (100), 254 (19), 232 (17), 162 (40). Anal. ($C_{14}H_{12}N_3O_2Cl$·HBr) C, H, N, Cl, Br.

B. Preparation of 2-(2,4-Dimethoxyphenyl)imidazo[1,2-*b*]pyridazine. 6-Chloro-2-(2,4-dimthoxyphenyl)imidazo[1,2-*b*]pyridazine hydrobromide (0.5 g, 1.56 mmol) was added to 40 mL of tetrahydrofuran along with 0.5 mL of triethylamine and 50 mg of 5% palladium on carbon. The reaction mixture was then subjected to hydrogenation at an initial pressure of 60 psi. After 30 min at room temperature, the reaction was halted, the catalyst was removed by filtration, and the reaction mixture was added to 200 mL of saturated sodium chloride solution; the organic phase was separated and dried, and the solvent was removed by rotary evaporation to give 238 mg of product (0.93 mmol or 60%): ^{1}H NMR (270 MHz) (DMSO-d_6/Me_4Si) δ 3.84 (s, 3 H, OCH_3), 3.99 (s, 3 H, OCH_3), 6.67–6.76 (M, 2 H, phenyl H), 8.23 (d, J = 9 Hz, 1 H, ring H), 8.10 (d, J = 11 Hz, 1 H, ring H), 8.23 (d, J = 9 Hz, 1 H, phenyl H), 8.47 (d, J = 5 Hz, 1 H, ring H), 8.51 (s, 1 H, ring H); MS, m/e (relative intensity) 255 (M^+, 100), 254 (100), 226 (50), 210 (57), 165 (100). Anal. ($C_{14}H_{13}N_3O_2$) C, H, N.

2-(2,4-Dimethoxyphenyl)imidazo[1,2-*a*]pyridine (7a). 2-Aminopyridine (1.84 g, 20 mmol) and α-bromo-2,4-dimethoxyacetophenone (5.2 g, 20 mmol) were dissolved in 20 mL of dimethylformamide, and the reaction mixture was stirred at room temperature for 2 h, poured into 200 mL of ethyl acetate, and washed three times with equal volumes of water (the likely initial product is the HBr salt and the yield would probably be improved by beginning with a wash with saturated sodium bicarbonate solution). The organic phase was then dried, and the solvent was removed by rotary evaporation. Further purification was carried out by silica gel column chromatography, eluting with 100% ethyl acetate to yield 664 mg (2.6 mmol or 13%) of product as a light brown crystalline material: ^{1}H NMR (270 MHz) (DMSO-d_6/Me_4Si) δ 3.82 (s, 3 H, OCH_3), 3.97 (s, 3 H, OCH_3), 6.63–6.72 (m, 2 H, phenyl H), 6.84 (t, J = 9 Hz, 1 H, ring H), 7.21 (t, J = 9 Hz, 1 H, ring H), 7.53 (d, J = 11 Hz, 1 H, phenyl H), 8.22 (d, J = 9 Hz, 1 H, ring H); MS, m/e (relative intensity) 254 (M^+, 100), 225 (30), 209 (26), 181 (21). Anal. ($C_{15}H_{14}N_2O_2$) C, H, N.

6-(2,4-Dimethoxyphenyl)imidazo[1,2-*b*][1,2,4]triazine (8a). Approximately 1.9 g (20 mmol) of 3-amino-1,2,4-triazine and 2.7 g (12.3 mmol) of α-bromo-2,4-dimethoxyacetophenone were heated for 2 h at 60 °C in 30 mL of dimethylformamide. The reaction mixture was added to 300 mL of ethyl acetate and washed with 300 mL of saturated sodium bicarbonate solution followed by two washings each with 300 mL of saturated sodium chloride. The organic layer was evaporated to dryness, and the residue was purified by chromatography over silica gel, eluting with ethyl acetate to yield 200 mg (0.78 mmol or 6%) of the title product: ^{1}H NMR (360 HZ) (DMSO-d_6/Me_4Si) δ 3.87 (s, 3 H, OCH_3), 4.02 (s, 3 H, OCH_3), 6.69–6.76 (m, 2 H, phenyl H), 8.25 (d, J = 9 Hz, 1 H, ring H), 8.53–8.63 (m, 2 H, ring and phenyl H); MS, m/e (relative intensity) 256 (M^+, 100), 202 (41), 175 (24). Anal. ($C_{13}H_{12}N_4O_2$) C, H, N.

2-Methoxy-4-(methylthio)acetophenone. A. Preparation of 3-Fluorophenyl Acetate. To a solution of 20 mL (240 mmol) of 3-fluorophenol in 200 mL of dry methylene chloride were added 19.5 mL (240 mmol) of pyridine. The solution was cooled to 0 °C, and 17.5 mL (240 mmol) of acetyl chloride was added dropwise with stirring. After the addition was complete, the reaction mixture was stirred for 1 h at 0 °C. An additional 200 mL of methylene chloride was added, and the organic solution was extracted once with 300 mL of 1 N hydrochloric acid. The organic solution was dried, and solvent was removed in vacuo to yield 34.3 g of 3-fluorophenyl acetate as an oil, which was used in the subsequent step without further purification.

B. Preparation of 2-Hydroxy-4-fluoroacetophenone. To a flask containing 34.2 g of 3-fluorophenyl acetate, which was cooled to 0 °C, was added 40 g of aluminum chloride in portions. The flask and its contents were allowed to warm to room temperature, and the reaction mixture was then placed in an oil bath and heated to 160–180 °C. The reaction mixture was then cooled to 0 °C, and ice was carefully added followed by the addition of 150 mL of concentrated hydrochloric acid and 250 mL of ethyl acetate. The mixture was stirred until complete dissolution occurred. Layers were separated, and ethyl acetate was removed in vacuo; the residue was subjected to steam distillation. The distillate was acidified with 1 N hydrochloric acid and extracted with ethyl acetate. The ethyl acetate layer was dried, and the solvent was removed in vacuo to yield 28 g (181 mmol or 75%) of 2-hydroxy-4-fluoroacetophenone as an oil, which crystallized upon cooling.

(8) Steck, A. E.; Brundage, P. R.; Fletcher, L. T. *J. Am. Chem. Soc.* **1954**, *76*, 3225.

C. Preparation of 2-Methoxy-4-fluoroacetophenone. To a solution of 13.9 g (90 mmol) of 2-hydroxy-4-fluoroacetophenone and 75 mL of dried dimethylformamide were added 30 mL of methyl iodide. The solution was cooled to 0 °C, and 4.1 g (85 mmol) of a 50% oil dispersion of sodium hydride was carefully added. After being stirred for 1 h at 0 °C, the reaction mixture was extracted with ethyl acetate. The organic extract was washed three times with 200 mL each of 1 N hydrochloric acid. The ethyl acetate solution was then dried and removed in vacuo. This reaction and a subsequent identical reaction provided a total of 28 g (166 mmol or 92%) of 2-methoxy-4-fluoroacetophenone as an oil, which could be used in the subsequent step without further purification.

D. Preparation of 2-Methoxy-4-(methylthio)acetophenone. A suspension of 17 g (213 mmol) of potassium hydroxide in 100 mL of dry dimethylformamide under a nitrogen atmosphere was cooled to −10 °C with an external ice/acetone bath. To this suspension was added 26 mL (235 mmol) of methanethiol. The reaction mixture was stirred until all the potassium hydroxide was dissolved. At this time, 33.1 g (197 mmol) of 2-methoxy-4-fluoroacetophenone was added, and the reaction mixture was stirred for 2 h at 0 °C. The reaction mixture was poured into 400 mL of ethyl acetate, and the resulting solution was washed three times with 300-mL aliquots of 1 N hydrochloric acid. The organic phase was dried and evaporated in vacuo to yield an oil. Crystallization from 50% ether/hexane afforded 21.3 g (109 mmol or 55%) of the desired 2-methoxy-4-(methylthio)acetophenone: ^{1}H NMR (60 MHz) (DMSO-d_6/Me_4Si) δ 2.52 (s, 3 H, SCH_3), 2.57 (s, 3 H, CH_3), 3.96 (s, 3 H, OCH_3) 6.97 (d, J = 8 Hz, 1 H, phenyl H), 7.03 (s, 1 H, phenyl H), 7.69 (d, J = 8 Hz, 1 H, phenyl H); MS, m/e (relative intensity) 196 (M^+, 34), 181 (100), 138 (10). Anal. ($C_{10}H_{12}O_2S$) C, H, S.

α-Bromo-2-methoxy-4-(methylthio)acetophenone. Approximately 15.6 mL of diisopropylamine was added to 500 mL of dry tetrahydrofuran under nitrogen, the temperature was reduced to −20 °C, and 112 mL of 1.6 M n-butyllithium (179 mmol) was added; the mixture was stirred for 20 min at −20 °C. The temperature was then reduced to −78 °C, and 32 g (16.3 mmol) of 2-methoxy-4-(methylthio)acetophenone in 50 mL of dry tetrahydrofuran was added. After the mixture was stirred for 30 min at −78 °C, 32 mL (252 mmol) of chlorotrimethylsilane was added. Cooling was then stopped, and the reaction mixture was allowed to stir at room temperature for 1.5 h. The solvent was removed by rotary evaporation to yield 44 g of silylated product as a clear sticky oil. This silylated material (42 g) was dissolved in 200 mL of dry tetrahydrofuran under nitrogen and cooled to −78 °C. To this was added 28 g (161 mmol) of N-bromosuccinamide in 100 mL of tetrahydrofuran. The reaction mixture was allowed to stir at reduced temperature for 30 min, and 400 mL of methylene chloride was added. The mixture was then washed with 500 mL of cold saturated sodium bicarbonate solution, the organic layer was then dried, and the solvent was removed by rotary evaporation. The product was stirred in 200 mL of glacial acetic for 16 h at room temperature. Methylene chloride (300 mL) was added, and the acetic acid was neutralized with sodium bicarbonate; 200 mL of H_2O was added, the organic layer was separated and dried, and the solvent was removed by rotary evaporation to provide the product as a crystalline solid in 70% yield: ^{1}H NMR (270 MHz) ($CDCl_3$/Me_4Si) δ 2.75 (s, 3 H, SCH_3), 3.93 (s, 3 H, OCH_3) 4.56 (s, 2 H, CH_2), 6.80 (s, 1 H, phenyl H), 6.85 (d, J = 9 Hz, 1 H, phenyl H), 7.80 (d, J = 9 Hz, 1 H, phenyl H); MS, m/e (relative intensity) 276 (M^+, 10), 274 (M^+, 10), 181 (100), 137 (10). Anal. ($C_{10}H_{11}O_2SBr$) C, H, S, Br.

α-Bromo-2-methoxy-4-(methylsulfonyl)acetophenone. A. Preparation of 2-Methoxy-4-(methylsulfonyl)acetophenone. To a solution of 3.92 g (20 mmol) of 2-methoxy-4-(methylthio)-acetophenone in 200 mL of methylene chloride was added 4 g (20 mmol of 85% purity) of m-chloroperbenzoic acid. Thin-layer chromatography indicated the formation of the intermediate sulfoxide derivative. This was followed 10 min later by a second addition of 4 g of m-chloroperbenzoic acid. After being stirred for 1 h, the reaction mixture was washed with 500 mL of saturated sodium bicarbonate. The organic phase was separated and dried over magnesium sulfate. The organic solution was then evaporated in vacuo to yield 3.61 g (15.8 mmol or 79%) of 2-methoxy-4-(methylsulfonyl)acetophenone.

B. Preparation of α-Bromo-2-methoxy-4-(methylsulfonyl)acetophenone. To a suspension of 3.61 g (15.8 mmol) of 2-methoxy-4-(methylsulfonyl)acetophenone and 100 mL of acetic acid was added enough methylene chloride to cause dissolution. While the mixture was stirred at room temperature, 0.91 mL (17.7 mmol) of bromine was added. The reaction was stirred until the bromine color was discharged. The reaction was poured into 300 mL of saturated sodium chloride, and the organic solution was further washed with 300 mL of saturated sodium bicarbonate; the organic phase was dried, and the solvent was removed in vacuo. The residual oil crystallized upon the addition of diethyl ether, yielding 4.05 g (13.2 mmol or 84%) of the desired α-bromo-2-methoxy-4-(methylsulfonyl)acetophenone: ^{1}H NMR (60 MHz) (DMSO-d_6/Me_4Si) δ 3.33 (s, 3 H, $S(O)_2CH_3$), 4.07 (s, 3 H, OCH_3), 4.83 (s, 2 H, CH_2) 7.54–8.10 (m, 3 H, phenyl H); MS, m/e (relative intensity) 213 (100), 151 (22), 134 (18). Anal. ($C_{10}H_{11}O_4SBr$) C, H, S, Br.

2-[2-Methoxy-4-(methylthio)phenyl]imidazo[1,2-*a*]pyrazine (4b). α-Bromo-2-methoxy-4-(methylthio)acetophenone (5.5 g, 20 mmol) and 3-aminopyrazine-2-carboxylic acid methyl ester (3.1 g, 20 mmol) were added to 12 mL of dimethylformamide and heated at 50 °C for 48 h. The reaction mixture was then poured into 200 mL of ethyl acetate, washed with 200 mL of saturated sodium bicarbonate solution, and dried, and the solvent was removed by rotary evaporation. The bicyclic product was partially purified by silica gel column chromatography, eluting with 100% ethyl acetate to yield 8-carbomethoxy-2-[2-methoxy-4-(methylthio)phenyl]imidazo[1,2-*a*]pyrazine that was used in the next step without further purification.

The carbomethoxy derivative from above was refluxed in 100 mL of 1 N HCl with 10 mL of methylene chloride added to dissolve the starting material. After 3 h the reaction mixture was added to 250 mL of ethyl acetate, and this was washed with 250 mL of saturated sodium bicarbonate solution. The organic phase was separated and dried, and solvent was removed by rotary evaporation. The residue was then purified by silica gel column chromatography, eluting with 100% ethyl acetate to yield 500 mg (1.85 mmol or 9%) of product: ^{1}H NMR (300 MHz) (DMSO-d_6/Me_4Si) δ 2.46 (s, 3 H, SCH_3), 4.00 (s, 3 H, OCH_3), 6.99 (d, J = 9 Hz, 1 H, phenyl H), 7.03 (s, 1 H, phenyl H), 7.88 (d, J = 5 Hz, H, ring H), 8.25 (d, J = 9 Hz, 1 H, phenyl H), 8.52 (s, 1 H, ring H), 8.61 (d, J = 5 Hz, 1 H, ring H), 9.05 (s, 1 H, ring H); MS, m/e (relative intensity) 271 (M^+, 100), 242 (33), 226 (52), 177 (37). Anal. ($C_{14}H_{13}N_3OS$) C, H, N.

2-[2-Methoxy-4-(methylsulfenyl)phenyl]imidazo[1,2-*a*]pyrazine (4c). A half gram (1.85 mmol) of 2-[2-methoxy-4-(methylthio)phenyl]imidazo[1,2-*a*]pyrazine (**4b**) was dissolved in 100 mL of chloroform under argon, and the temperature was reduced to −40 °C. A solution of 360 mg (1.8 mmol of 85% purity) of m-chloroperbenzoic acid in 100 mL of chloroform was then slowly added over a 45-min period, after which the reaction mixture was stirred at −40 °C for an additional 3 h. At this time the reaction mixture was washed two times with 200-mL portions of saturated sodium bicarbonate solution, the organic phase was dried, and the solvent was removed by rotary evaporation.

Two such runs were combined, and the material was purified by silica gel column chromotography, eluting with 15% methanol/methylene chloride. The material collected was then crystallized from ethyl acetate to yield approximately 800 mg (2.8 mmol or 32%) of product: ^{1}H NMR (270 MHz) (DMSO-d_6/Me_4Si) 2.83 (s, 3 H, $S(O)CH_3$), 4.09 (s, 3 H, OCH_3), 7.39 (d, J = 9 Hz, 1 H, phenyl H), 7.46 (s, 1 H, phenyl H), 7.89 (d, J = 5 Hz, 1 H, ring H), 8.48 (d, J = 9 Hz, 1 H, phenyl H), 8.58–8.68 (m, 2 H, ring H); MS, m/e (relative intensity) 287 (M^+, 95), 272 (100), 242 (17), 194 (13), 172 (18). Anal. ($C_{14}H_{13}N_3O_2S$) C, H, N.

2-[2-Methoxy-4-(methylsulfonyl)phenyl]imidazo[1,2-*a*]pyrazine (4d). A. Preparation of 8-Chloro-2-[2-methoxy-4-(methylsulfonyl)phenyl]imidazo[1,2-*a*]pyrazine Hydrobromide. 2-Amino-3-chloropyrazine[9] (3.88 g, 30 mmol) and α-bromo-2-methoxy-4-(methylsulfonyl)acetophenone (9.21 g, 30 mmol) were suspended in 30 mL of dimethylformamide and stirred at 40 °C for approximately 60 h. At this time the precipitate was collected by filtration and washed with ethyl acetate

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to yield 4.8 g (12 mmol or 30%) of the bicyclic product: ^{1}H NMR (270 MHz) (DMSO-d_6/Me_4Si) δ 3.32 (s, 3 H, $S(O)_2CH_3$) 4.03 (s, 3 H, OCH_3) 7.64 (s, 1 H, phenyl H) 7.68 (d, J = 8 Hz, 1 H, phenyl H), 7.76 (d, J = 5 Hz, 1 H, ring H), 8.55 (d, J = 8 Hz, 1 H, phenyl H), 8.67 (d, J = 5 Hz, 1 H, ring H), 8.84 (s, 1 H, ring H); MS, m/e (relative intensity) 337 (M^+, 100), 308 (28), 228 (84), 209 (56), 129 (83). Anal. (free amine) ($C_{14}H_{12}N_3O_3SCl$) C, H, N, S, Cl.

B. Preparation of 2-[2-Methoxy-4-(methylsulfonyl)-phenyl]imidazo[1,2-*a*]pyrazine. The chloro derivative (3.6 g, 9.1 mmol) was added to 195 mL of dimethylformamide followed by 2.7 g of triethylamine. Hydrogenation was carried out at an initial pressure of 60 psi with 1 g of 5% Pd/$BaSO_4$ as catalyst. After the mixture was shaken at room temperature for 1.5 h, 100% of theoretical hydrogen uptake had occurred. The catalyst was removed by filtration, and the reaction mixture was added to 700 mL of ethyl acetate. The ethyl acetate solution was washed four times with 250 mL of saturated NaCl solution, the ethyl acetate solution was dried, and the solvent was removed by rotary evaporation. The product was crystallized from a small volume of ethyl acetate to yield 540 mg (1.78 mmol or 20%) in the first crop: ^{1}H NMR (270 MHz) (DMSO-D_6/Me_4Si) δ 3.32 (s, 3 H, $S(O)_2CH_3$), 4.14 (s, 3 H, OCH_3), 7.64–7.70 (m, 2 H, phenyl H), 7.93 (d, J = 5 Hz, 1 H, ring H), 8.57 d, J = 9 Hz, 1 H, phenyl H), 8.64 (m, 1 H, ring H) 8.73 (s, 1 H, ring H); MS, m/e (relative intensity) 303 (M^+, 100), 274 (34), 209 (39) 194 (75). Anal. ($C_{14}H_{13}N_3O_3S$) C, H, N, S.

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Registry No. **1a**, 77303-19-6; **1c**, 73384-60-8; **2a**, 87359-11-3; **2b**, 87359-45-3; **2c**, 86315-52-8; **2d**, 87359-43-1; **3a**, 93276-53-0; **4a**, 93276-54-1; **4b**, 114552-59-9; **4c**, 102362-14-1; **4d**, 93276-60-9; **5a**, 93276-56-3; **6a**, 114552-60-2; **7a**, 114552-61-3; **8a**, 93276-55-2; 2-aminopyrimidine, 109-12-6; α-bromo-2,4-dimethoxyacetophenone, 60965-26-6; 2-aminopyrazine, 5049-61-6; 2-(2,4-dimethoxyphenyl)-7-chloroimidazo[1,2-*c*]pyrimidine hydrobromide, 93276-70-1; 4-amino-6-chloropyrimidine, 5305-59-5; 2-(2,4-dimethoxyphenyl)-7-chloroimidazo[1,2-*c*]pyrimidine, 93276-71-2; 6-chloro-2-(2,4-dimethoxyphenyl)imidazo[1,2-*b*]pyridazine hydrobromide, 114552-62-4; 3-amino-6-chloropyridazine, 5469-69-2; 2-aminopyridine, 504-29-0; 3-amino-1,2,4-triazine, 1120-99-6; 3-fluorophenyl acetate, 701-83-7; 3-fluorophenol, 372-20-3; 2-hydroxy-4-fluoroacetophenone, 1481-27-2; 2-methoxy-4-fluoroacetophenone, 51788-80-8; 2-methoxy-4-(methylthio)acetophenone, 93276-65-4; methanethiol, 74-93-1; α-bromo-2-methoxy-4-(methylthio)acetophenone, 93276-66-5; 2-methoxy-4-(methylthio)-α-(trimethylsilyl)acetophenone, 114552-63-5; 2-methoxy-4-(methylsulfonyl)acetophenone, 93276-68-7; α-bromo-2-methoxy-4-(methylsulfonyl)acetophenone, 93276-69-8; 3-aminopyrazine-2-carboxylic acid methyl ester, 16298-03-6; 8-carbomethoxy-2-[2-methoxy-4-(methylthio)phenyl]imidazo[1,2-*a*]pyrazine, 114552-64-6; 2-amino-3-chloropyrazine, 6863-73-6; 8-chloro-2-[2-methoxy-4-(methylsulfonyl)phenyl]imidazo[1,2-*a*]pyrazine hydrobromide, 93276-72-3.

Deletion Sequences of Salmon Calcitonin That Retain the Essential Biological and Conformational Features of the Intact Molecule†

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Salmon calcitonin has an amino acid sequences that would allow it to form an amphipathic helix from approximately residue 9 to residue 22. We have synthesized a number of analogues of this peptide hormone with deletions in the carboxyl terminus of this putative amphipathic helix. These analogues include deletions of single amino acid residues at positions 19, 20, 21, or 22 as well as deletions of progressively larger segments starting with residue 19 and including deletions of residues 19 and 20; 19, 20, and 21; or 19, 20, 21, and 22. There is a small decrease in the helical content of these analogues compared with the native hormone, both in the presence and absence of amphiphiles. However, the extent of formation of secondary structure, as measured by circular dichroism, is similar for these deletion sequences as it is for the native hormone. In all cases, there is a large increase in the helical content of the peptide in the presence of dimyristoylphosphatidylglycerol, lysolecithin, or sodium dodecyl sulfate. All of the analogues have hypocalcemic activity in vivo in rats, comparable to the native hormone, except for des-Leu^{19}-salmon calcitonin, which is about twice as active as the unmodified hormone. With use of an in vitro assay of adenylate cyclase activation in purified rat kidney membranes, des-Tyr^{22}-salmon calcitonin, des-Leu^{19},Gln^{20},Thr^{21}-salmon calcitonin, and des-Leu^{19}Gln^{20},Thr^{21},Thr^{22}-salmon calcitonin exhibited about one-tenth the stimulatory activity of the native hormone. Des-Tyr^{22}-sCT and des-Leu^{19},Gln^{20},Thr^{21},Tyr^{22}-sCT were also tested for their activity in inhibiting prolactin release from isolated rat pituitary cells. Both of these analogues exhibited inhibitory activity. Thus, the region of residues 19–22 does not greatly affect either the conformational or the biological properties of salmon calcitonin.

There is much current interest in "protein engineering", i.e. the design and synthesis of modified peptides or proteins with altered biological activity. Most of these studies involve substituting or deleting single amino acid residues of a native peptide chain. Deletion of amino acid residues that have important functional roles leads to a marked reduction in activity. In the case of salmon calcitonin (sCT), most of the 32 amino acid peptide is required for activity since removal of several residues from the carboxyl terminus leads to sCT(1–23)-peptide amide that has only 0.25% the potency of the native hormone while the carboxyl terminal segment sCT(12–32)-peptide amide is devoid of activity.[1] A more complete discussion of structure–activity relationships in sCT can be found in a recent review.[2] The results suggest that several regions of the peptide contribute to the activity and/or potency of the

† Abbreviations: sCT, salmon calcitonin; DMPG, dimyristoylphosphatidylglycerol; LPC, palmitoyllysophosphatidylcholine; SDS, sodium dodecyl sulfate; CD, circular dichroism; Pipes, 1,4-piperazinediethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; TRH, thyrotropin releasing hormone.

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