Arylformamidines with Antinociceptive Properties

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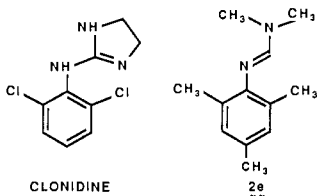
A series of formamidines structurally related to clonidine were synthesized and investigated as potential nonopiate analgesics. Several of these compounds showed potent analgesic activity (ED_{50} on HCl writhing <1.0 mg/kg) with low potential for hypotensive effects. A qualitative description of the structure–activity relationship of this series reveals that the 2,4- and 2,6-dimethylphenyl compounds are more potent analgesics than are the corresponding dichlorophenyl compounds.

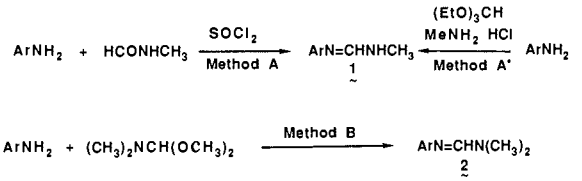
Clonidine is an antihypertensive agent that acts centrally at the α_2-adrenoreceptor.[1] It is also a potent analgesic whose action apparently is dependent on an interaction with central noradrenergic receptors[2,3] and seems not to involve an opioid mechanism.[4,5] Clonidine is not a clinically useful analgesic because it causes hypotension, sedation, and bradycardia. However, because its analgesia might not be mediated by an opioid mechanism, a clonidine-like compound without significant cardiovascular effects might be a clinically useful analgesic, since it presumably would not produce opioid physical dependence. We have investigated a series of formamidines, some of which are structurally related to clonidine but which were originally synthesized as insecticides.[6] A qualitative description of the structure–activity relationship (SAR) of the simpler members of this series reveals that the 2,4- and 2,6-dimethylphenyl compounds are more potent analgesics than are the corresponding dichlorophenyl compounds. One of these formamidines, *N,N*-dimethyl-*N'*-(2,4,6-trimethylphenyl)formamidine hydrochloride (**2e**) demonstrates good analgesic activity with virtually no hypotensive effect. Like clonidine, the antinociceptive effect of this formamidine may be mediated by α_2-receptors and not by opioid receptors. We also describe the effect of introducing larger functional groups at the para position of **2e**. One compound, **3c**, having the propionic ester structural features of known analgesic/antiinflammatory agents, has analgesic activity on the tail-flick and pinch assays.



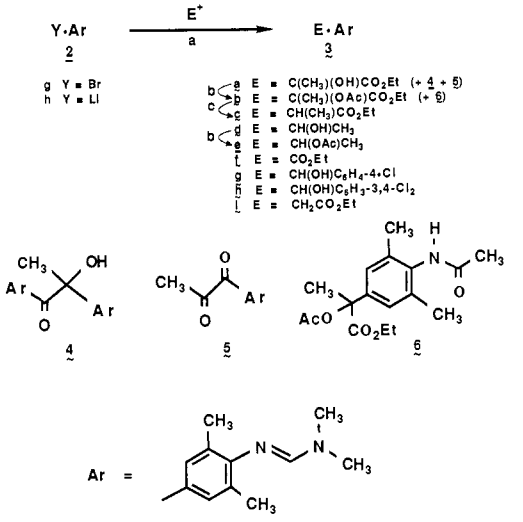
Chemistry

The syntheses of the *N*-methyl- and *N,N*-dimethyl-*N'*-arylformamidines are described in Schemes I and II. Scheme I shows that the *N*-monomethyl series was obtained by treating the anilines with (method A) thionyl chloride and *N*-methylformamide or (method A') methylamine hydrochloride and triethyl orthoformate in ethanol. *N,N*-Dimethylformamidine dimethyl acetal reacts with various anilines to give the desired dimethylformamidine products (method B).

Scheme I



Scheme II[a]



[a] (a) Method C: *n*-BuLi, E^+. (b) Method D: Ac_2O, DMAP, TEA. (c) Method E: Li/liquid NH_3.

A three-step procedure was used to prepare **3c**. First, *N'*-(4-bromo-2,6-dimethylphenyl)-*N,N*-dimethylformamidine **2g** was metalated with *n*-BuLi in THF at −78 °C and the lithiated derivative (**2h**) was treated with ethyl pyruvate to afford **3a**, **4**, and **5** (Scheme II, method C). The yield of **3a** was enhanced relative to byproducts **4** and **5** when the reaction was run by adding the aryllithium to ethyl pyruvate which contained 1–2 equiv of dry lithium bromide generated from *n*-BuLi and *tert*-butyl bromide. If the reaction was carried out by rapidly adding ethyl pyruvate to the lithium anion, so that the reaction temperature rose above −70 °C, then bis adduct **4** became a substantial byproduct, which was difficult to remove chromatographically from the desired alcohol **3a**. The presence of dry LiBr improved the yield of atrolactic ester **3a** relative to byproduct **5**. Second, the acetate **3b** was prepared by treating the corresponding alcohol with an excess of acetic anhydride, a catalytic amount of 4-(dimethylamino)pyridine (DMAP), and 1.5 equiv of triethylamine in methylene chloride at 0 °C (method D). If the reaction mixture was permitted to rise to room temperature prior to workup, hydrolysis byproduct **6** formed (see the Experimental Section). Finally, **3c** was prepared

(1) Scriabine, A., Ed. *Pharmacology of Antihypertensive Drugs*; Raven: New York, 1980; p 55–70.
(2) Schmitt, H.; LeDouarec, J. C.; Petillot, N. *Neuropharmacology* **1974**, *13*, 119.
(3) Paalzow, L. *J. Pharm. Pharmacol.* **1974**, *26*, 361.
(4) Paalzow, G.; Paalzow, L. *Naunyn-Schmiedebergs Arch. Pharmacol.* **1976**, *292*, 119.
(5) Spaulding, T. C.; Fielding, S.; Venafro, J.; Lal, H. *Eur. J. Pharmacol.* **1979**, *58*, 19.
(6) Steinhausen, A. German Patent 1172081, 1984, and U.S. Patent 3378437, 1984.

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Table I. Biological and Chemical Data: *N*-Arylformamidines (See Schemes I and II)

compd	substituents	analgesic ED_{50}[a]	hypotensive activity[b]	yield, % (method)	molecular vformula[c]	mp, °C (solvent)
clonidine	see text	0.02	−29/−19[d]			
			MeNRCH=NAr			
1a	R = H, Ar = 2,4-$Me_2C_6H_3$	0.02	−10/−19[e]	86.8 (A)	$C_{10}H_{14}N_2$·HCl	161–163 (SSB[f]–acetone)
1b	R = H, Ar = 2,5-$Cl_2C_6H_3$	>50	+3/+5	34 (A)	$C_8H_8Cl_2N_2$·HCl	172–174 (EtOH)
1c	R = H, Ar = 3,4-$Cl_2C_6H_3$	>50	0/−3	54 (A)	$C_8H_8Cl_2N_2$·HCl	139–141 (EtOH)
1d	R = H, Ar = 2,6-$Cl_2C_6H_3$	4.4	−21/−13[e]	85 (A)	$C_8H_8Cl_2N_2$·HCl	184–186.5 (EtOH)
1e	R = H, Ar = 2-ClC_6H_4	35	NT[g]	48.8 (A′)	$C_8H_9ClN_2$	64–66.5
1f	R = H, Ar = 2,3-$Cl_2C_6H_3$	0.8	NT[g]	36.9 (A′)	$C_8H_8Cl_2N_2$	132–134
1g	R = H, Ar = 2,4-$Cl_2C_6H_3$	>50	NT[g]	90 (A′)	$C_8H_8Cl_2N_2$	95–97 (C_6H_{12})
1g′	R = H, Ar = 2,4-$Cl_2C_6H_3$	NT	NT[g]	90 (A′)	$C_8H_8Cl_2N_2$·HCl[h]	215–218 (*i*-PrOH/Hex)
1h	R = H, Ar = 2,4,6-$Me_3C_6H_2$	0.11	NT[g]	49.2 (A′)[i]	$C_{11}H_{16}N_2$	117.5–119.5 (SSB[f])
2a	R = CH_3, Ar = 2,6-$Me_2C_6H_3$	0.1	−15/−21[e]	17 (B)	$C_{11}H_{16}N_2$·HCl	197.5–198.5 (EtOH/Et_2O)
2b	R = CH_3, Ar = 2,5-$Cl_2C_6H_3$	>50	−5/−4	22 (B)	$C_9H_{10}Cl_2N_2$·HCl	246–248 (EtOH/Et_2O)
2c	R = CH_3, Ar = 3,4-$Cl_2C_6H_3$	>50	0/+2	48 (B)	$C_9H_{10}Cl_2N_2$·HCl	258.5–259.5 (EtOH/Et_2O)
2d	R = CH_3, Ar = 2,6-$Cl_2C_6H_3$	3	−24/−4[e]	62 (B)	$C_9H_{10}Cl_2N_2$·HCl	241–242 (EtOH/Et_2O)
2e	R = CH_3, Ar = 2,4,6-$Me_3C_6H_2$	0.4[j]	−10/+2	41 (B)	$C_{12}H_{18}N_2$·HCl[k]	219.5–220.5 (EtOH/Et_2O)
2f	R = CH_3, Ar = 2-(3-MeC_5H_3N)	>50	+8/+9	76 (B)	$C_9H_{13}N_3$·2HCl[l]	140–145 (Et_2O)
2h	R = CH_3, Ar = 2,4-$Me_2C_6H_3$	0.10	NT[g]		$C_{11}H_{16}N_2$·HCl	205.2 (CH_3CN)
			$Me_2NCH=N(2,6\text{-}Me_2\text{-}4\text{-}E\text{-}C_6H_2)$			
3a	E = $C(OH)(CH_3)CO_2Et$	>50	NT[g]	51.8 (C)	$C_{16}H_{24}N_2O_3$·$C_7H_8SO_3$	168–171 (EtOH/EtOAc)
3b	E = $C(OAc)(CH_3)CO_2Et$	>50	NT[g]	(D)	$C_{18}H_{26}N_2O_4$[m]	oil
3c	E = $CH(CH_3)CO_2Et$	>50[n]	NT[g]	19.1 (E)	$C_{16}H_{24}N_2O_2$[o]	oil
3d	E = $CH(OH)CH_3$	40	NT[g]	74.4[p]	$C_{13}H_{20}N_2O$	134–136.5 (EtOAc/Hex)
3e	E = $CH(OAc)CH_3$	>30	NT[g]	16.2[p]	$C_{15}H_{22}N_2O_2$·$C_7H_8SO_3$·$^1/_2H_2O$	100–108 (EtOAc/Hex)
3f	E = CO_2Et	>50	NT[g]	41.1[q]	$C_{14}H_{20}N_2O_2$·HCl	90.5–92 (EtOH/Hex)
3g	E = CH(OH)-(4-Cl-C_6H_4)	>50	NT[g]	48.3[r]	$C_{18}H_{21}ClN_2O$	144–145.5 (EtOAc)
3h	E = CH(OH)-(3,4-$Cl_2C_6H_3$)	>50	NT[g]	47.3[s]	$C_{18}H_{20}Cl_2N_2O$	143–144 (EtOAc/Hex)

[a] HCl writhing ED_{50} in mg/kg. [b] Change in blood pressure (mmHg) at 4 and 24 h after 50 mg/kg po unless otherwise noted. A change of ±5 mmHg is not significant. [c] Analyses are ±0.4% unless otherwise noted. [d] 1 mg/kg po. [e] Bradycardia. [f] SSB = Skellysolve B. [g] Not tested. [h] C: calcd/found = 40.61/39.85. [i] *p*-TsCl was used in place of $SOCl_2$. [j] Subsequent retesting of **2e** gave ED_{50} = 0.37 mg/kg. [k] C: calcd/found = 60.44/61.01. [l] C: calcd/found = 45.77/46.62; the free base was taken up in Et_2O and treated with Et_2O/HCl. [m] High-resolution mass spectrometry verified the molecular formula; strong molecular ion peak at *m/e* 334.1897 (calcd for $C_{18}H_{26}N_2O_4$ 334.1892). [n] This compound was evaluated in the tail-flick, pinch, and mouse HCl writhing assays and found to have ED_{50} values of 18, 22, and 8 mg, respectively (unpublished data, P. F. VonVoigtlander). [o] High-resolution mass spectrometry verfied the molecular formula; strong molecular ion peak at *m/e* 276.1833 (calcd for $C_{16}H_{24}N_2O_2$ 276.1838). [p] See the Experimental Section. [q] Method C with diethyl carbonate. [r] Method C with 4-chlorobenzaldehyde. [s] Method C with 3,4-dichlorobenzaldehyde.

as an oil in low yield via lithium/liquid ammonia reduction of acetate **3b** (method E).

Treating anion **2h** with acetaldehyde afforded **3d** in excellent yield. Via procedure D, **3d** was converted to **3e** in good yield when the reaction temperature was maintained at 0 °C. Finally, treating **2h** with diethyl carbonate, 4-chlorobenzaldehyde, or 3,4-dichlorobenzaldehyde afforded the derivatives **3f–h** (Scheme II).

Several attempts to prepare the acetic ester derivative **3i** by coupling **2h** with methyl bromoacetate afforded only **2a** (40%) and **2g** (60%). A control aliquot indicated that **2g** had been metalated to over 86% extent by *n*-BuLi; the reappearance of **2g** on addition of the bromo ester suggested that the aryllithium reagent underwent preferential metal halogen exchange with the bromo ester. Attempts to prepare **3c** via nickel-catalyzed coupling of Reformatsky reagents with **2g** also failed (data not presented).

Physical data for compounds of structure **1–3** are summarized in Table I.

Pharmacology

The analgesic and hypotensive activities of compounds **1–3** are summarized in Table I. Analgesic activity was inferred from the mouse HCl writhing test.[7] This test involved the intraperitoneal administration of 0.2 mL of 0.08 N HCl 30 min after the subcutaneous injection of the test compound. The animals were then observed for the absence of abdominal stretching (writhing) reflex for a period of 15 min. Thus, only compounds capable of suppressing this nociceptive response for the period of 30–45 min after administration were identified as analgesics. These minimal criteria for detection exclude compounds of very short duration of action and those that might require more than 30 min for absorption of bioactivation of effective drug levels. Hypotensive activity was evaluated in the rat as the change in blood pressure (mmHg) at 4 and 24 h after a 50 mg/kg oral dose of the test compound.[8]

Results

A few qualitative statements can be made about the structure–activity relationship (see data in Table I). First, for matched pairs, the *N*-methylformamidines and the *N,N*-dimethylformamidines had comparable analgesic activity. Thus, compounds **1d** and **2d** had similar potencies in the HCl writhing assay (ED_{50} **1d** = 4.4, ED_{50} **2d** = 3.0 mg/kg) as did compounds **1h** and **2e** (ED_{50} = 0.11 and 0.4 mg/kg, respectively), **1a** and **2h** (ED_{50} = 0.02 and 0.4 mg/kg, respectively), and **1c** and **2c** (both inactive at 50 mg/kg). Second, for the chloro-substituted phenyls, 2,6-disubstitution was associated with analgesia. Thus, the 2,6-dichloro analogues (**1d** and **2d**) had good activity, but the 2,5-dichloro (**1b** and **2b**), the 3,4-dichloro (**1c** and **2c**), and the 2,4-dichloro (**1g**) analogues had ED_{50} values > 50 mg/kg. Third, the ED_{50} (0.8 mg/kg) of the 2,3-dichloro compound **1f** indicates that the 2,6-dichloro pattern is not obligatory for analgesic activity, and in the aromatic methyl series, surprisingly good activity was found in the 2,4-dimethyl analogues, **1a** (*N*-monomethyl) and **2h** (*N*,-

(7) Lednicer, D.; VonVoigtlander, P. F.; Emmert, D. E. *J. Med. Chem.* **1980**, *23*, 424.

(8) Weeks, J. R.; Jones, J. A. *Proc. Soc. Exp. Biol. Med.* **1960**, *104*, 646.

N-dimethyl) (ED_{50} = 0.02 and 0.10 mg/kg, respectively), as well as in the 2,6-dimethyl analogues **1d** and **2a**. Fourth, methyl groups on the phenyl ring apparently enhanced analgesic potency compared with the analogous chloro-substituted compounds. Thus, the *N*,*N*-dimethyl-2,6-dimethylphenyl analogue **2a** (ED_{50} = 0.10 mg/kg) was 30 times more potent as an analgesic than was its *N*,*N*-dimethyl-2,6-dichloro counterpart **2d** (ED_{50} = 3.0 mg/kg). Likewise, the *N*-monomethyl 2,4-dimethyl analogue **1a** (ED_{50} = 0.02 mg/kg) was superior to the *N*-methyl 2,4-dichlorophenyl compound **1g** (ED_{50} > 50 mg/kg) as an analgesic (vide supra). Fifth, the 2,4-dimethyl analogues seemed more potent than the 2,4,6 trimethyl analogues (cf. **1a** and **2h** vs **1h** and **2e**, respectively). Finally, larger functional groups at position 4 tended to decrease or eliminate analgesic activity (cf. compounds **3a–h**).

Because *N*,*N*-dimethyl-*N'*-(2,4,6-trimethylphenyl)formamidine (**2e**) had the best split between analgesic and hypotensive activity (a dose of 50 mg/kg po of **2e** caused only a slight lowering of blood pressure at 4 h and no change at 24 h), it was selected for further study. Compound **2e** was evaluated in several analgesic procedures, including mouse tail flick and tail pinch, and rat air writhing, warm plate, and tail flick. In these assays, the analgesic potency of compound **2e** was 7–300 times less than that of clonidine, depending on the test used.[9] Its effect on blood pressure in sodium pentobarbital anesthetized rats after sc administration was minimal. At no time did the mean arterial pressure of rats administered 10 or 30 mg/kg of compound **2e** sc differ from control values. The analgesia could be antagonized by the α-blocker yohimbine but not by the opiate antagonist naloxone.[9] These observations were all consistent with the view that lead compound **2e** exerted its analgesic effect by an α_2 mechanism, similar to the mechanism by which clonidine exerted its analgesic activity. Presumably, the same mechanism of analgesic action operated with the other formamidines.[9,10]

The SAR of formamidines **1** and **2**, and the activity of compound **2e** in particular, suggested that the presence of a 4-Me substituent on the aromatic ring reduced, but did not eliminate, analgesic activity. Compounds **3a–h** were therefore prepared as analogues of **2e** to determine the effect of functional groups larger than Me at this position. The data of Table I show that none of the compounds of structure **3** had an ED_{50} value that was less than 40 mg/kg on the *standard* HCl writhing assay. As a result, these compounds were not evaluated further in the hypotensive assay. Compound **3c** was prepared in the hope that the arylpropionic ester moiety might impart antiinflammatory and analgesic activity after in vivo hydrolysis to the acid. In fact, this compound was active on the tail-flick and pinch assays at 18 and 22 mg/kg sc, respectively. When tested in an HCl writhing assay involving a larger number of animals (female vs male) and a change in the time interval between dosing and assay, **3c** was active at 8 mg/kg sc.[11] This was still considerably less potent than the parent compound **2e**. Further work on these analogues was discontinued after the compound was found to be inactive when administered orally in the same assay.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are uncorrected. Elemental analyses and melting points are noted in Table I. NMR spectra of $CDCl_3$ solutions were recorded on a Varian A-60D or HFT-80 spectrometer and are consistent with structure. Medium-pressure liquid chromatography was carried out with EM silica gel 60 and EM RP2 silica gel. Example procedures for methods A–E are given below. The experimental procedures for other analogues did not differ appreciably from these example procedures. Analgesic activity on the HCl writhing assay was determined as described in ref 7.

Method A. *N'*-(2,6-Dichlorophenyl)-*N*-methylformamidine Hydrochloride (1d). Thionyl chloride (10.0 g, 0.0617 mol) was added dropwise to a stirred solution of 10.0 g (0.062 mol) of 2,6-dichloroaniline and 4.19 g (0.071 mol) of *N*-methylformamide in 60 mL of toluene. The resultant suspension was stirred at room temperature for 1 h and at 65 °C for 16 h. Nitrogen was bubbled through the hot solution for 2.5 h. The mixture was cooled to 5 °C and filtered to yield 12.85 (85%) of solid product, which was crystallized from ethanol, mp 184–186.5 °C. Anal. ($C_8H_8Cl_2N_2$·HCl) C, H, N, Cl.

Method A'. *N'*-(2-Chlorophenyl)-*N*-methylformamidine (1e). Methylamine hydrochloride (20.25 g, 0.30 mol) and $(EtO)_3CH$ (44.4 g, 0.30 mol) were refluxed in a few milliliters of absolute EtOH for 15 min, at which point 2-chloroaniline (48.6 g, 0.30 mol) in 150 mL of absolute EtOH was added dropwise. The addition was complete in 1.5 h. Following an additional 20-min reflux period, the reaction solution was cooled and concentrated in vacuo. The crude product was taken up in water and extracted with CCL_4. The aqueous layer was treated with $CHCl_3$ and stirred vigorously while Na_2CO_3 was cautiously added (37.2 g, 0.30 mol). The $CHCl_3$ layer was separated and dried (Na_2SO_4) and concentrated to a crude crystalline material, which was crystallized from cyclohexane containing a trace of ether, affording 24.5 g (48.8%) of product, mp 64–66.5 °C. Anal. ($C_8H_9ClN_2$) C, H, N.

Method B. *N'*-(2,4,6-Trimethylphenyl)-*N*,*N*-dimethylformamidine Hydrochloride (2e). A mixture of 5.00 g (36.9 mmol) of 2,4,6-trimethylaniline and 7.05 g (59.2 mmol) of *N*,*N*-dimethylformamide dimethyl acetal was stirred at room temperature under slight vacuum for 2 days. The mixture was chromatographed on Florisil with 10% methanol/90% methylene chloride to yield 2.85 g (41%) of product. This was converted to the hydrochloride salt with methanol and ethereal hydrogen chloride, mp 219.5–220 °C. Anal. ($C_{12}H_{18}N_2$·HCl), C, H, N, Cl.

***N'*-(2,6-Dimethyl-4-bromophenyl)-*N*,*N*-dimethylformamidine (2g).** A mixture of 40.0 g (200 mmol) of 2,6-dimethyl-4-bromoaniline and 47.86 g (400 mmol) of dimethylformamide dimethyl acetal was heated to 120 °C under nitrogen for 18 h. GLC analysis (2 ft SE-30, 100 °C, 1 min, 20 °C/min to 250 °C) indicated a 52/48 ratio of starting material to product. Methanol was removed in vacuo and the crude reaction mixture was treated with an additional 23 mL of dimethylformamide dimethyl acetal and refluxed for 2 days. This procedure was repeated three more times over 8 days until only 1% starting material was present by GLC analysis. Distillation (bp 112–113 °C/1.5 mmHg) afforded 49.1 g (96.2% yield) of product as an oil: NMR ($CDCl_3$) δ 7.11 (3 H, br, 2 aromatic and N=CH), 2.98 (6 H, S, $N(CH_3)_2$), 2.09 (6 H, s, aryl CH_3). This material was an intermediate for the preparation of compounds described in Scheme II.

Method C. 4-[[(Dimethylamino)methylene]amino]-α-hydroxy-α,3,5-trimethylbenzeneacetic Acid Ethyl Ester 4-Methylbenzenesulfonate (3a). Bromoformamidine **2g** (10.20 g, 40.0 mmol), dissolved in 80 mL of THF in a 250-mL flame-dried

(9) The specific work on compound **2e** was reported separately by J. S. Mohrland and P. F. VonVoigtlander: *Neuropharmacology* **1985**, *24*, 1207.

(10) Cheney and Kalantar recently reported on computer-analyzed structural similarities between clonidine and cyclazocine which, coupled with the fact that naloxone has been shown to reverse clonidine's hypotensive effect, suggest that "the pharmacological responses to clonidine are not mediated solely by the α_2-adrenergic receptor". Clonidine's biological activity may be potentiated or mediated by direct interaction with opioid receptors. Cheney, B. V.; Kalantar, J. *J. Mol. Graphics* **1986**, *4*, 21.

(11) The assay was conducted 45 min after sc injection. Private communication from P. F. VonVoigtlander of these laboratories.

round-bottom flask, under a nitrogen atmosphere, was treated with 30.0 mL of 1.6 M *n*-BuLi in hexane (48.0 mmol) to generate the aryl anion. In a separate 500-mL flame-dried round-bottom flask, fresh LiBr was generated at −78 °C by adding 26.0 mL of 1.6 M *n*-BuLi in hexane (41.6 mmol) to 5.48 g of *t*-BuBr (40.0 mmol) dissolved in 30 mL of THF. The solution was slowly warmed to 0 °C and then recooled to −78 °C. Ethyl pyruvate (18.58 g, 160.0 mmol) dissolved in 30 mL of THF was slowly added while the temperature was maintained at −78 °C. To this solution was added, via cannula, portions of the lithium anion generated as described above. The addition took 1 h. Stirring was maintained at −78 °C for 15 min. The reaction solution was then permitted to slowly warm to −20 °C at which point it was quenched in cold aqueous sodium bicarbonate solution, and the products were extracted into EtOAc. The extracts were dried overnight and concentrated in vacuo to 14.18 g of orange oil. VPC analysis of the reaction mixture (SE-30, 100 °C, 1 min, then 20 °C/min to 250 °C and 250 °C for 5 min) showed 7.6% of **2a** (2.63 min), 6.9% **2g** (4.36 min), 7.1% of unidentified material (4.63 min), 13.5% of **5** (5.64 min), 64.7% of **3a** (5.94 min), and no peak at ~11.7 min (corresponding to 4, see below). The crude reaction mixture was chromatographed over silica gel (60 (E Merck 0.04–0.063 mm) (flow ~1 mL/min). The products were eluted most efficiently with 1% of MeOH/99% $CHCl_3$ containing 1 mL of aqueous NH_4OH/L of solution. Following a 600-mL forerun, 20-mL fractions were collected. The product **3a**, 3.50 g of oil, was collected in fractions 81–140 and in the next 1–2 L of eluent. The total amount of product collected from both portions of the reaction was 6.06 g (51.8% yield): a small portion crystallized poorly from acetate/hexane mixture to afford a powder of mp 68–70 °C. The product was best purified as a *p*-TsOH salt, which crystallized from ethanol/ethyl acetate mixtures as prisms: mp 168–171 °C; IR (Nujol) 3275, 3135, 3058 (OH, $^+$NH) 1719 (C=O), 1699 (C=N), and 1600 cm^{-1} (C=C); UV (95% EtOH) λ max 211 nm (ε 32850); NMR (free base) ($CDCl_3$) δ 7.12, 7.15 (3 H, 2 singlets, aromatic CH and CH=N), 4.20 (2 H, q, J = 7.1 Hz, OCH_2), 1.98 (6 H, s, $N(CH_3)_2$), 2.12 (6 H, s, aromatic CH_3), 1.73 (3 H, s, CH_3), 1.23 (3 H, t, J = 7.1 Hz, CH_3); high-resolution MS, M+ = 292.1798, calcd for free base $C_{16}H_{24}N_2O_3$ 292.1787. Large fragment ions were found at m/e 219 (M^+ − 73, i.e. $M^+ - CO_2Et$) 177, and 172 (*p*-TsOH). Anal. ($C_{16}H_{24}N_2O_2 \cdot C_7H_8SO_3 \cdot 0.5H_2O$) C, H, N, S. The early fractions from the chromatography were pooled and rechromatographed over silica gel 60 (E. Merck 0.04–0.063-mm mesh) by eluting with 0.5% acetone/99.5% $CHCl_3$) containing 1 mL of aqueous NH4OH/L of solution. Fractions 21–25 contained a small amount of pure **5** (t_R by VPC was 5.70 min): IR (Nujol) 1713 (C=O), 1648 (conjugated C=O), 1584 (C=C); UV (95% EtOH) λ max 205 nm (ε 22143) and 322 (11857); NMR ($CDCl_3$) δ 7.65 (2H, s, aromatic CH), 7.15 (1 H, s, CH=N), 3.02 (6 H, s, $N(CH_3)_2$), 2.45 (3 H, s, CH_3C=O), 2.16 (6 H, s, aromatic CH_3); MS, molecular ion peak at m/e 246 with fragment ions at m/e 204 and 203 (M^+ − 43). Fractions 26–39 contained 0.95 g of oil, 55.5% **5**, 36.7% **2g**, and 7.8% of uncharacterized peak at 4.67 min. Fraction 40–70 contained 0.213 g of oil comprised primarily (83.7%) of **2g**. Fractions 181–200 contained 0.37 g of oil comprised primarily (81.2%) of **2a**: NMR ($CDCl_3$) δ 6.60–7.30 (4 H, m, aromatic CH and CH=N), 2.99 (6 H, s, $N(CH_3)_2$), 2.13 (6 H, s, aromatic CH). This was nearly identical with the oil obtained by quenching the organolithium anion, **2h**, with CH_3OH. If the reaction was run by rapidly adding ethyl pyruvate to the anion, so that the temperature rose above −70 °C, then a substantial amount of bis adduct **4** was formed. **4** was difficult to separate from **3a**, the TLC mobility being highly dependent upon the amount of dissolved aqueous NH4OH present in solution. A small amount of **4** crystallized from mixtures of **3a** and **4** in Et_2O/hexane to afford prisms: mp 152–154 °C: IR (Nujol) 2925, 1653, and 1591 cm^{-1}; UV (95% EtOH) λ max 205 nm (ε 47822), 286 (sh) (16201), 301 (17266); NMR ($CDCl_3$) δ 7.44 (2 H, s, aromatic CH, 5.61 (1 H, s, exchangeable, OH), 2.98 (12 H, s, $N(CH_3)_2$), 2.11 (CH, s, aromatic CH_3), 2.01 (6 H, s, aromatic CH_3), 1.86 (3 H, s, CCH3); MS, weak molecular ion peak at m/e 422 with fragments ions at 404 ($M^+ - H_2O$), 378 ($M^+ - N(CH_3)_2$), and 219 (base peak). Anal. ($C_{25}H_{34}N_4O_2$) C, H, N.

4-[[(Dimethylamino)methylene]amino]-α,3,5-trimethylbenzenemethanol (3d). The aryl anion generated as above from 20.0 mmol of **2g** and 22.4 mmol of *n*-BuLi in hexane/THF at −78 °C was transferred in portions over 5 min to a separate flask, containing 3.52 g (80.0 mmol) of acetaldehyde dissolved in 35 mL of THF, and the contents were cooled to −78 °C. Stirring was maintained at −78 °C for $^1/_4$ h at which time the bath was removed, and the contents of the reaction were permitted to warm to ambient temperature. The reaction mixture was quenched in cold aqueous NaCl, and the products were extracted with ethyl acetate. The organic extracts were dried over Na_2SO_4 and concentrated in vacuo to a solid. The product was crystallized from ethyl acetate/hexane mixtures to afford 3.28 g (74.4%) of prisms: mp 134–136.5 °C in two crops; IR (Nujol) 3174, 3036, 2932, 1642 cm^{-1} (C=N); UV (95% EtOH) λ max 237 nm (ε 16,600); NMR ($CDCl_3$) δ 7.09 (1 H, s, CH=N), 6.85 (2 H, s, aromatic CH), 4.71 (1 H, q, J = 6.4 Hz, CHO), 2.97 (6 H, s, $N(CH_3)_2$), 2.06 (6 H, s, aromatic CH_3); MS, strong molecular ion peak at m/e 220 with fragment ions at m/e 205 ($M^+ - CH_3$) and 132 (M^+ − (OH plus N=CHNMe$_2$)). Anal. ($C_{13}H_{20}N_2O$) C, H, N.

3f was prepared by adding the anion of starting material to a THF solution of diethyl carbonate. Alcohols **3g** and **3h** were prepared by adding the anion to respectively 4-chloro- and 3,4-dichlorobenzaldehyde.

Method D. α-Acetoxy-4-[[(dimethylamino)methylene]amino]-α,3,5-trimethylbenzeneacetic Acid Ethyl Ester (3b). Acetic anhydride (2.72 mL, 3.07 g, 30.0 mmol) was added over 1 min to a cold solution (0 °C) of 2.72 g (9.30 mmol) of starting alcohol dissolved in 20 mL of CH_2Cl_2 containing 0.244 g (2.0 mmol) of 4-(dimethylamino)pyridine and 1.41 g (13.95 mmol) of Et_3N. The reagents were stirred at 0 °C for a total of 7 h until the reaction was judged to be complete (TLC, GLC evidence). The reaction was quenched in cold aqueous $NaHCO_3$; the products were extracted with EtOAc and concentrated in vacuo to a dark red oil. The oil was chromatographed (MPLC, Michel Miller Size B column, eluting with 1% MeOH/99% $CHCl_3$ containing 1 mL of aqueous NH_4OH/L) over silica gel G (mesh 0.04–0.063 mm). Following a 400-mL forerun, 20-mL fractions were collected. Fractions 17–40 contained 2.13 g of pure product **3b** by TLC and NMR. Fractions 41–70 contained 0.36 g of product (oil), which was pure by TLC: IR (Nujol) 2940, 1742 (C=O, average), 1646 cm^{-1}; UV (95% EtOH) λ max 244 nm (ε 13100); NMR ($CDCl_3$) δ 7.15 (1 H, s, CH=N), 7.10 (2 H, s, aromatic CH), 4.15 (2 H, q, J = 7.2 Hz, OCH_2), 2.98 (6 H, s, $N(CH_3)_2$), 2.17 (3 H, s, CH_3C=O), 2.13 (6 H, s, aromatic CH), 1.91 (3 H, s, aliphatic CH), 1.18 (3 H, t, J = 7.1 Hz, CCH_3); high-resolution MS, strong molecular ion peak at 334.1897, calcd for $C_{18}H_{26}N_2O_4$ 334.1982, with fragment ions at m/e 275 and 219 (base peak). If the reaction mixture was permitted to stir at ambient temperature overnight prior to workup, a byproduct (**6**) formed which resulted from formamidine hydrolysis and aniline acylation. This material was slightly more polar than the desired product (silica gel, eluted with 1% MeOH/99%/$CHCl_3$ containing 1 mL of aqueous NH_4OH/L). It crystallized from EtOAc/Et_2O mixtures to afford a colorless powder: mp 156.5–159 °C; IR (Nujol) 2954, 2925, 1743 (C=O), 1649 cm^{-1}; UV (95% EtOH) λ max 227 nm (sh) (ε 8220), 266 (469); NMR ($CDCl_3$) δ 7.21 (2 H, s, aromatic CH), 6.5–6.75 (1 H, br exchangeable, HN(C=O), 4.15, 4.14 (2 H, 2 of q, J = 7.2 Hz, OCH_2), 2.24 (6 H, s, aromatic CH_3), 2.20 (3 H, s, CH_3C), 2.18 (3 H, s, CH_3C), 1.90 (3 H, s, aliphatic CCH_3), 1.20 (3 H, t, J = 7.2 Hz, CCH_3); MS, molecular ion peak at m/e 321 with fragment ions at m/e 261 (M^+ − CH3CO2H) and 206 (M^+ − (CH_2=C=O and CO_2Et). Anal. ($C_{17}H_{23}NO_5 \cdot 0.5H_2O$) C, H, N.

Acetate **3e** was prepared from 3.00 mmol of alcohol **3d**, 4.23 mmol of Et_3N, 0.6 mmol of 4-(dimethylamino)pyridine, and 1.0 mL of Ac_2O in CH_2Cl_2, analogous to the procedure to prepare **3b**. Anal. ($C_{15}H_{22}N_2O_2 \cdot C_7H_8O_3S \cdot 0.5H_2O$) C, H, N.

Method E. 4-[[(Dimethylamino)methylene]amino]-α,3,5-trimethylbenzeneacetic Acid Ethyl Ester (3c). A solution of 2.13 g (6.37 mmol) of starting material dissolved in 40 mL of Et_2O was added to a cold (−33 °C) solution of Li in 120 mL of liquid NH_3. The blue color of Li persisted. The color was quenched by adding a solution of 1.17 g of ethyl bromopropionate in 5.0 mL of Et_2O. The resulting yellow solution was treated with solid NH_4Cl and stirred 5 min. The dry ice was removed from the trap and N_2 was blown in to remove NH_3. The evaporation took 1 h. The resulting solution was quenched in aqueous $NaHCO_3$ and extracted with EtOAc. The combined organic extracts were concentrated in vacuo to 2.3 g of crude oil, which

by GLC analysis was 65% product (t_R 5.58 min). Impurity peaks were present at 4.34 min (13.4%), 7.13 min (11.0%), and 7.47 min (9.68%). The oil was chromatographed over silica gel (0.04–0.063 mm) in a medium-sized Michel Miller column (MPLC) by eluting with 1% MeOH/99% $CHCl_3$ containing 1 mL of saturated NH_4OH/L of solution. Following a 500-mL forerun, 20-mL fractions were collected. Fractions 51–60 contained 0.185 g (10.5%) of product (**3c**). An attempt to crystallize this material as a *p*-TsOH salt failed. Fractions 61–90 contained 0.1463 g (8.31%) of product oil, which was submitted for analysis and testing. The oil had a retention time of 5.58 min on a 2-ft SE 30 column, 100 °C/1 min, programmed 20 °C/min to 250 °C. Byproducts had t_R of 4.34, 5.02, 7.13, and 7.47 min. By TLC (silica gel, 10% MeOH/90% $CHCl_3$ containing 1 drop of NH_4OH) the R_f value was 0.46 separable from a side product with R_f 0.55. **3c**: IR (Nujol) 2933, 1730 (C=O) 1647 cm^{-1} (C=N); NMR ($CDCl_3$ δ 7.16 (1 H, s, N=CH), 6.90 (2 H, s, aromatic CH), 4.10 (2 H, q, J = 7.1 Hz, OCH_2), 3.57 (1 H, q, J = 7.1 Hz, $CHCO_2Et$), 2.97 (6 H, s, $N(CH_3)_2$), 2.10 (6 H, s, aromatic CH_3), 1.45 (3 H, d, J = 7.1 Hz, CH_3), 1.19 (3 H, t, J = 7.1 Hz, ethyl CH_3); high-resolution MS, molecular ion peak at m/e 276.1833, calcd for $C_{16}H_{14}N_2O_2$ 276.1838, with fragment ions at m/e 261 ($M^+ - CH_3$), 203 (base peak $M^+ - CO_2Et$), and 158 ($M^+ - (CO_2Et$ and $HNMe_2)$). The yield of product was higher in several smaller scale reactions.

Attempted Preparation of 4-[[(Dimethylamino)-methylene]amino]-3,5-dimethylbenzeneacetic Acid Ethyl Ester (3i). Starting material (0.255 g, 1.00 mmol) **2g** was dissolved in 3.0 mL of THF in a flame-dried 25-mL round-bottom flask with a side arm fitted with a rubber stopper. The solution was stirred with a magnetic stirrer bar and kept in a dry ice/acetone bath at −78 °C. A freshly titrated solution of 0.75 mL of *n*-BuLi (1.6 M, 1.20 mmol) was added. The reactants were stirred for 5 min, at which time an aliquot (A) was removed and quenched in aqueous NaOH, extracted with $CHCl_3$ dried over Na_2SO_4, and concentrated in vacuo. GLC analysis (see method C) showed 86% **2a**, 4.8% **2g**, and 5.7% of uncharacterized material at a retention time of 4.71 min. To the remaining solution of anion was added 0.183 g (1.20 mmol) of methyl bromoacetate dissolved in 2.0 mL of THF. The reagents were stirred for 1 min at −78 °C at which time the dry ice bath was removed. The solution was stirred until the temperature of the flask approached room temperature. The reaction mixture was then worked up as before. GLC analyses showed 39.8% (**2a**) and 60.2% (**2g**), indicating metal–halogen exchange occurred. None of the desired product **3i** was detected.

Hypotensive Activity in Conscious Rat. The blood pressure of restrained female Sprague-Dawley rats was measured directly from chronic indwelling aortic cannulas exteriorized from the nape of the neck. In order to obtain a high level of sympathetic tone, rats were restrained in a towel during the period of blood pressure measurement with a Statham transducer (P23G) and a Grass Model of 5 polygraph. Measurements were made at 4 and 24 h after the oral administration of each compound. Test compounds were suspended in a (carboxymethyl)cellulose vehicle at 10 mL/kg. Blood pressure values of two animals were averaged at each of the three measurement times.[1] An average change of at least 5 mmHg was required posttreatment for statistical significance ($p < 0.05$).

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Registry No. **1a**, 51550-40-4; **1a** (free base), 33089-74-6; **1b**, 114886-08-7; **1b** (free base), 75211-09-5; **1c**, 114886-09-8; **1c** (free base), 86538-00-3; **1d**, 114886-10-1; **1d** (free base), 67791-83-7; **1e**, 75211-12-0; **1f**, 76662-31-2; **1g**, 67346-15-0; **1g'**, 114886-11-2; **1h**, 51062-42-1; **2a**, 114886-12-3; **2a** (free base), 24053-84-7; **2b**, 2350-56-3; **2b** (free base), 15851-79-3; **2c**, 1934-03-8; **2c** (free base), 2103-44-8; **2d**, 114886-13-4; **2d** (free base), 15851-80-6; **2e**, 36192-46-8; **2e** (free base), 36192-18-4; **2f**, 114886-14-5; **2f** (free base), 36172-55-1; **2g**, 114886-15-6; **2h**, 69618-84-4; **2h** (free base), 50884-21-4; **3a**, 114886-17-8; **3a** (free base), 114886-16-7; **3b**, 114886-18-9; **3c**, 114886-19-0; **3d**, 114886-20-3; **3e**, 114886-22-5; **3f**, 114886-23-6; **3g**, 114886-24-7; **3h**, 114886-25-8; **4**, 114886-26-9; **5**, 114886-27-0; **6**, 114886-28-1; $(EtO)_3CH$, 122-51-0; 2,4-Me_2-1-$NH_2C_6H_3$, 95-68-1; 2,5-Cl_2-1-$NH_2C_6H_3$, 95-82-9; 3,4-Cl_2-1-$NH_2C_6H_3$, 95-76-1; 2,3-Cl_2-1-$NH_2C_6H_3$, 608-27-5; 2,4-Cl_2-1-$NH_2C_6H_3$, 554-00-7; 2,6-Me_2-1-$NH_2C_6H_3$, 87-62-7; clonidine, 4205-90-7; 2,6-dichloroaniline, 608-31-1; *N*-methylformamide, 123-39-7; methylamine hydrochloride, 593-51-1; 2-chloroaniline, 95-51-2; 2,4,6-trimethylaniline, 88-05-1; *N,N*-dimethylformamide dimethyl acetal, 4637-24-5; 2,6-dimethyl-4-bromoaniline, 24596-19-8; 2-amino-5-methylpyridine, 1603-41-4.

Effect of the CH_2NH and CH_2NAc Peptide Bond Isosteres on the Antagonistic and Histamine Releasing Activities of a Luteinizing Hormone-Releasing Hormone Analogue[1]

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The effect of the CH_2NH and CH_2NAc peptide bond isosteres on the antagonistic and histamine releasing activities of the LH-RH antagonist [*N*-Ac-D-Nal[1],D-Phe[2,3],D-Arg[6],Phe[7],D-Ala[10]]LH-RH was investigated. The moieties were introduced by facile, racemization-free solid-phase synthesis in an attempt to reduce the histamine releasing activity inherent to the most potent analogues while retaining high antiovulatory activity. The $\psi[CH_2NH]$ isostere was incorporated at each CONH site with the exception of 8–9, which involves Pro, by reductive alkylation with a protected amino acid aldehyde in the presence of $NaBH_3CN$ during conventional solid-phase peptide synthesis. The $\psi[CH_2NH]$ group was extremely resistant to derivatization and could only be partially acetylated to give $\psi[CH_2NAc]$. The analogues were cleaved from the resin with simultaneous deprotection by anhydrous hydrogen fluoride and purified to homogeneity in two stages: gel permeation followed by preparative reversed-phase liquid chromatography. The analogues were assayed in standard rat antiovulatory and in vitro histamine release assays. The isosteres caused a loss of the antiovulatory activity of the antagonist at the 50-μg dose when incorporated at the positions 1–2, 2–3, 3–4, and 7–8. Incorporation at the other positions resulted in a less marked reduction in activity relative to the unmodified parent analogue. No significant effect was noted on the potent histamine releasing activity of the analogues.

The current interest in antagonists of the luteinizing hormone-releasing hormone, Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (LH-RH),[2] for the potential control of fertility and hormone-dependent tumors has been tem-

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