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Application of Bioisosterism in Development of Novel Cardiotonics Based on (2′-Aminoethyl)carbostyril and (2′-Aminoethyl)-1-hydroxy-2-pyridone Systems

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Abstract

Two different types of chemical manipulations of dobutamine were investigated in order to develop novel, improved cardiotonic drugs. Three new analogues of carbostyril, in which the *m*-hydroxy group of dobutamine was isosterically modified with an amide type carbostyril system, were synthesized from *p*-methoxyphenethylamine via multi-steps. Two analogues of (2′-aminoethyl)-1-hydroxy-2-pyridone system which has isosteric structural similarity with dopamine without having the COMT vulnerable *m*-hydroxy group were synthesized via 12 synthetic steps. Their biological stabilities in various media and inotropic activities were evaluated.

1. INTRODUCTION

Although an extensive studies were focused on development of new cardiotonics in past decades,[1] it is still necessary to develop the new class of cardiotonics which would have longer duration of action and oral bioavailability. Among the currently available cardiotonics in the market, dobutamine (**1**) is the of most extensively used drugs to treat severe cardiac failure.[2] In spite of inconvenience of administration method of dobutamine, various chemical manipulations[3] of dobutamine were tried to achieve longer duration of action and/or oral bioavailability due to potentially beneficial pharmacological profile of dobutamine. As of results, the analogue of dobutamine (**2**), KM-13, which replaces the para hydroxyl group in dobutamine with carboxyamide at the end of molecule not only increased inotropic potency three fold without changing the inotropic selective profile of dobutamine but also gave a little oral effectiveness.[4] As part of developing novel, improved cardiotonic drugs, we investigated two different types of chemical manipulation of dobutamine including isosteric/isoelectronic replacement of catechol ring moiety in dobutamine by carbostyril and 1-hydroxy-2-pyridone systems. Here, we report the design, synthesis and preliminary pharmacological studies of 5-(2′-aminoethyl)carbostyril and (2′-aminoethyl)-1-hydroxy-2-pyridone systems.

2. DESIGN

2-1. Design of 5-(2′-Aminoethyl)carbostyril Compounds

Since the major reason of short duration of action of dobutamine is its fast elimination from the body by transformation in the liver to inactive glucuronide conjugates of the meta-O-methyldobutamine by catechol-O-transferase (COMT) enzyme,[5] we postulated that one of the most effective hydroxyl replacement group in catechol is the NH of a carbostyril derivative to produce longer duration of action and/or oral effectiveness because the carbostyril moiety has the isoste-

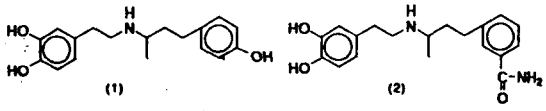


Figure 1

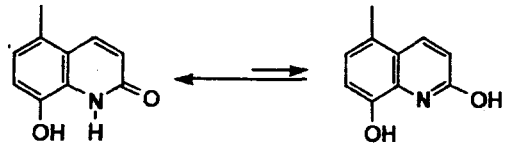


Figure 1-1—Tautomers of 8-hydroxycarbostyril

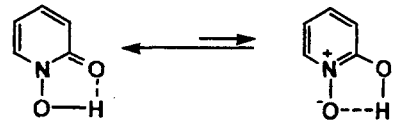


Figure 1-2—Tautomers of 1-hydroxy-2-pyridone

ric/isoelectronic structural similarity compared to catechol moiety and exists as resonance hybrids (Fig. 1-1) which possess two weekly acidic hydrogen stoms in about the same general vicinity as those in catechol without having the hydroxyl group in meta position. This postulate was reinforced by the recent results of Kaiser *et al.*[6] Based on above postulate, we have synthesized three modified 5-(2′-aminoethyl)carbostyril derivatives (**3, 4, 5**) and examined their pharmacological effects.

2-2. Design of (2′-Aminoethyl)-1-hydroxy-2-pyridone Compounds

Similarly, 1-hydroxy-2-pyridone moiety was considered to replace the catechol moiety as new class of dobutamine analogues because the 1-hydroxy-2-pyridone moiety can tautomerize to the 2-hydroxypyridine-1-oxide form[7] (Fig. 1-2), and this moiety could be isosteric/isoelectric structural similarity with catechol moiety without having the vulnerable *m*-OH group which is a substrate to COMT. Therefore, the prevention of this facile metabolism could lead the development of long-acting and/or orally effective new class of cardiotonics.

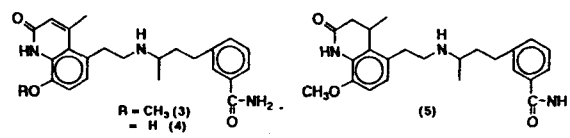


Figure 2

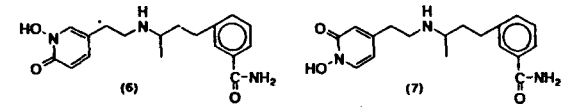


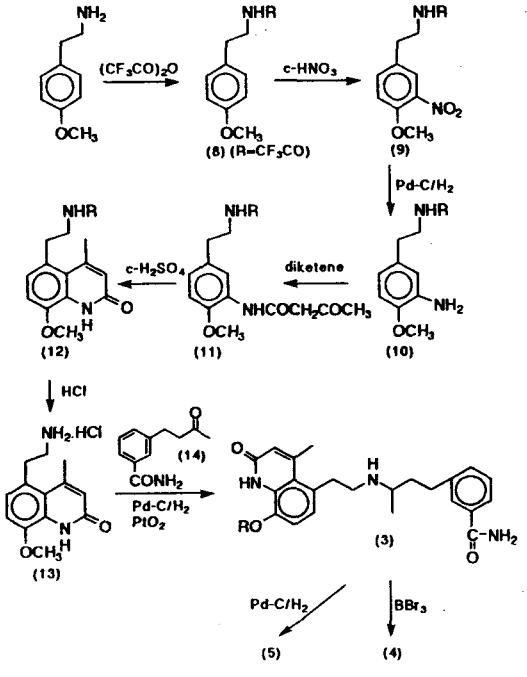
Figure 3

Accordingly, 5-(2′-aminoethyl)-1-hydroxy-2-pyridone (**6**) and 4-(2′-aminoethyl)-1-hydroxy-2-pyridone (7) were synthesized and their pharmacological effects were evaluated.

3. RESULTS AND DISCUSSION

3-1. Chemistry

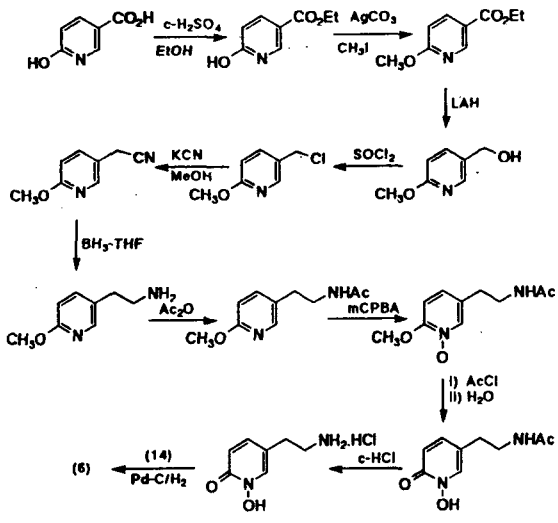
The carbostyril compounds (**3, 4, 5**) were prepared according to Scheme I. The amino group of *p*-methoxyphenethylamine was first protected with trifluoroacylation ($(CF_3CO)_2O$/CH_2Cl_2), and then nitrated. The resulting nitro compound (**9**) was reduced under hydrogenation to afford amino compound (**10**) in 67% of the total yield from starting material. After synthesis of anilide (**11**) from the reaction of **9** with diketene in good yield, ring closure of anilide was tried in various acidic conditions (c-H_2SO_4, c-HCl, PPA) and best result was obtained in concentrated sulfuric acid in 45% yield. Deprotection of the trifluoro group of **12** was under methanolic HCl condition to give the desired carbostyril amine compound (**13**) as hydrochloride salt form. Finally, the coupling reaction **13** with the corresponding keto compound (**14**), which was synthesized from *m*-cyanobenzyl bromide via three steps, was achieved by either reductive amination ($NaBH_3CN$/MeOH) method or hydrogenation (PtO_2/Pd-C/MeOH) in fairly good yield. After the synthesis of **3**, it was converted into 8-hydroxy derivative



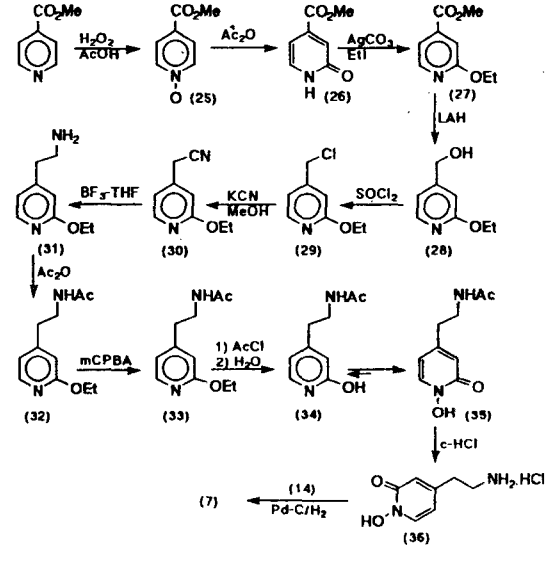
Scheme I

(4), by demethylation reaction with 1.0 M BBr_3 solution and was converted into 3,4-dihydro derivative (5) by hydrogenation method (50 psi).

Compound **6** was prepared from 6-hydroxynicotinic acid as shown in Scheme II. The key intermediate, **16**, was obtained via esterification of 6-hydroxynicotinic acid followed by O-alkylation, using a modification of the previously reported method.[8] After reduction of ester group of **16** with $LiAlH_4$, the resulting hydroxy group was converted to a chloro group with thionyl chloride, as in the chloro compound **18**. The cyano compound (**19**) was prepared from the corresponding chloromethyl derivative using either NaCN/EtOH method or NaCN/NaI/acetone as reaction condition. The NaCN/EtOH method resulted in the formation of ethoxymethylpyridine as a side product. The free amine, obtained from reduction of **19** with 1 M solution of diborane/THF, was protected by acylation. N-Oxidation of the resulting **21**, followed by rearrangement of the resulting 2-ethoxypyri-



Scheme II



Scheme III

dine-1-oxide using the reported method,[9] afforded **23**. Finally, the protecting group of compound **23** was removed by standard method to give the compound **24**, as hydrochloride salt. Finally, coupling reaction of **24** with **14** gave the desired product **6**.

Compound **7** was prepared from methyl isonicotinate as shown in Scheme III. Key intemediate, **27**, was obtained via O-alkylation of **26** which was prepared by using the

Table I—*In Vitro* Stability Studies of Compound (**3, 4, 5**) in pH 7.40 Phosphate Buffer, in 100% Human Blood, in 80% Human Plasma and in 20% Rat Liver Homogenate.

Compd.	pH 7.40	100% Human blood[a]	80% Human plasma[b]	20% Liver homogenate[c]
-OMe (**3**)	d	189[e] (0.999)	94.4 (0.995)	f
-OH (**4**)	d	g	345[e] (0.957)	f
Dihydro (**5**)	d	g	544[e] (0.999)	f

a: Whole human blood. b: Plasma from one individual. c: Liver from one rat. d: No decomposition observed over 21 day period. e: Experiment followed for < one half-life. f: No decomposition observed over 80 minutes. g: No decomposition observed over 6.6 hrs

previously reported method.[10] Compound **27** was converted into the target compound **7** by following the methods which were used for the conversion of compound **16** into compound **6**.

3-2. Stability of Carbostyril Compounds (3, 4, 5) in pH 7.4, in Whole Human Blood, 80% Human Plasma and 20% Rat Liver Homogenate

Rates of disappearance for each carbostyril analogue were measured at 37℃ in 0.05 M phosphate buffer at pH 7.40 by using HPLC method. As seen in Table I, all carbostyril compounds were extremely stable at pH 7.40, and no observed decomposition occurred over 21 day period. This result was not unexpected since the carbostyril compounds do not possess obvious chemically labile bonds in the structure such as the ester linkages or catechol groups.

In order to evaluate potential success as a useful therapeutic agent since human blood has numerous enzyme activities, all carbostyril compounds were tested and their rates of disappearance were measured in both whole human blood and human plasma. In whole blood (See Table I), all carbostyril compounds were very stable and no significant decomposition was observed over 6.6 hour period. Only, the compound (**3**) showed the decomposition with an 189 hour half-life. In plasma, a metabolite peak of the compound (**3**) were clearly observed after 23 hours and this peak increased as a function of time. Similar patterns were observed with the compound (**4, 5**). The compound (**3**) was the least stable compound tested yielding a 94.4 hour half-life. The other compounds (**4, 5**) displayed 345 and 545 hour half-lives. This trend was consistent with the rate of decomposition in human blood as the compound (**3**) had the least stability in human blood as well as plasma. These results indicated that the carbostyril compounds had few enzymatically labile bond or group and the enzymes involved in the formation of metabolite were present in plasma.

Stability test of all carbostyril compounds in 20% rat liver homogenate was performed to determine if carbostyril compounds are good substrates of hepatic enzymes, especially COMT. Again, all carbostyril compounds were extremely stable and no decomposition was observed over 80 min., at which time the homogenate denatured. This result indicated that the carbostyril ring system is metabolically insensitive to hepatic enzymes while the catechol ring system is a good substrate of hepatic enzymes, especially COMT.

From the overall results of the *in vitro* stability studies of carbostyril compounds (See Table I), it may be predicted that all carbostyril compounds may have oral bioavailability.

3-3. *In vitro* Evaluation of (2′-Aminoethyl)-1-hydroxy-2-pyridone Compounds (24, 25) as

Table II—Inhibitorary Effects of Drugs on Prolactin Secretion.[a]

Compd	Conc., μM	Prolactin[b] (ng/mg) protein (+SEM)		% Change
		Control	Compound	
24	0.001	127.73 (+20.75)	135.19 (+19.64)	+6
	0.01	88.75 (+13.72)	104.94 (+13.74)	+18
	0.1	76.23 (+ 9.80)	92.51 (+12.05)	+21
	1.0	74.00 (+16.70)	39.13 (+ 5.03)*	−47
36	0.001	91.80 (+17.94)	82.84 (+11.91)	−9
	0.01	89.59 (+12.70)	63.33 (+ 7.06)*	−29
	0.1	80.17 (+ 9.15)	68.75 (+ 5.22)	−13
	1.0	37.49 (+ 4.59)	26.90 (+ 2.23)*	−28
DA[c]	0.2	282 (+34)	121 (+38)*	−57

a: On freshly obtained anterior pituitary (AP) at 37℃. All values are average of seven separated AP-S. b: Prolactin release of the incubated AP-S. c: Cited from reference 11. *: $p<0.05$ versus control hemipituary using a paired Students' t-test.

Table III—Physiochemical Data of Compounds (**24, 36**)

Compound	H (Kcal/mol)	I_p (eV)	log P	V (A^3)	S (A^2)	D
24a	−9.71	7.15	0.651	144.2	189.5	1.425
24b	−12.10	8.90	1.299	140.6	182.4	1.392
36a	−18.39	9.00	0.393	141.1	184.6	1.408
36b	−12.52	8.86	1.309	140.9	184.6	1.410
DA	−71.49	8.70	0.763	144.8	187.8	1.408

Dopaminergic Agonist

In order to assess the dopaminergic activity of **24, 36** in comparison to dopamine (DA), *in vitro* inhibitory effects of prolactin secretion from anterior pituitary of rats were first performed by using the reported method.[11] Fresh anterior pituitaries obtained from female rats were incubated with various concentrations of **24** and **36**, respectively, and their effects on the rate of release of prolactin were measured and compared with the control anterior pituitary. As seen in Table II, **36** caused a continuous reduction of prolactin secretion at $10^{-9}-10^{-6}$ M concentration ranges while **24** showed prolactin inhibiting activity only at 10^{-6} M concentration. In comparison with dopamine, dopamine caused a 57% reduction of the prolactin secretion at a 2×10^{-7} M concentration.[11] These results indicate that both compounds, **24** and **36**, have dopaminergic activities, although less than that of dopamine. However, It is interesting to point out that **36** showed some dopaminergic activity at even lower concentration than the concentration at which dopamine revealed its inhibitory effect on prolactin secretion. From these results, it was predicted that this system may be used as new starting material for new dopaminergic agents and/or new cardiotonic agents which presumably will not be substrates for COMT.

3-4. AM-1 Calculation Studies of (2′-Aminoethyl)-1-hydroxy-2-pyridone Compounds (24, 36)

The isosteric/isoelctronic properties of **24a, b** and **36a, b** as compared to DA were eva-

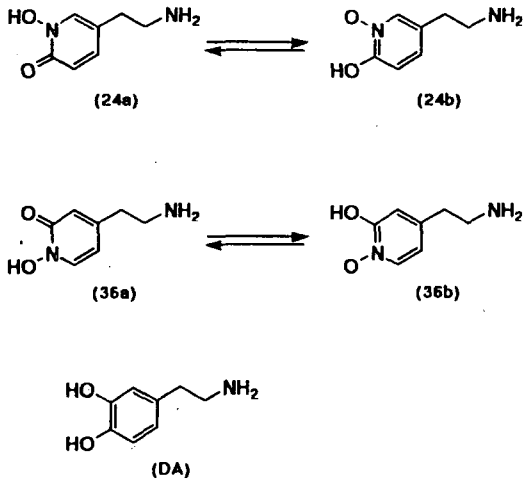


Figure 4

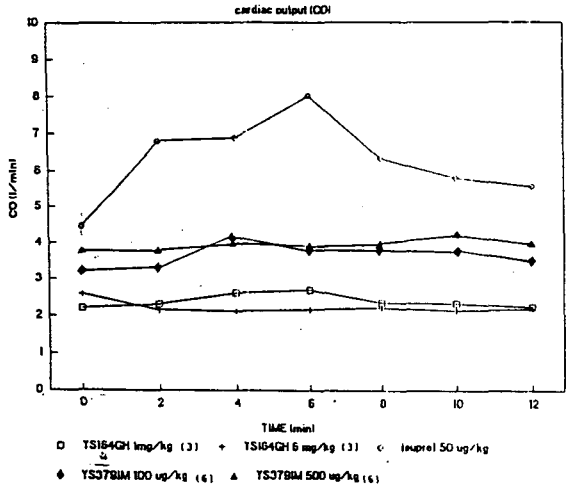


Figure 5—Cardiac output of compounds 3 and 6.

luated first optimizing the structures within the AM1 framework as shown in Table IV. First, it was concluded that the N-OH-keto tautomer **36a** is more stable than the N-oxide form **36b**, but the opposite is true for the **24a**=**24b** tautomers. The calculated partition coefficients (log P) indicate that **24a** is the closest to DA, but essentially all are within one log P unit. The corresponding molecular volumes, surface and the ovality indicate that these are indeed close isosteric/isoelectronic structures.

3-5. Evaluation of Inotropic Activities of Compounds (3, 6) in Dogs

Preliminary inotropic activities were measured by checking cardiac output in dogs after compounds were administered as an intravenous infusion for five minutes. Data comparing the cardiac output of tested compounds (**3, 6**) with dobutamine are given in Fig. 5. No significant inotropic activities were obtained with tested compounds (**3, 6**) even at higher doses.

4. CONCLUSION

As a new cardiotonic which has long duration of action as well as oral bioavailability, two different systems by chemical manipulation of dobutamine were studied. Three analogues of carbostyril were synthesized and their analytical systems were developed. The stability studies in various biological media such as pH 7.40 phosphate buffer, whole human blood, 80% human plasma and 20% rat liver homogenate showed that all carbostyril compounds (**3, 4, 5**) were extremely stable. These results leads the possibility of oral bioavailability of all carbostyril compounds, but preliminary studies in dog does not show any significant inotropic activity. Two analogues (**24, 36**) of (2′-aminoethyl)-1-hydroxy-2-pyridone were synthesized and their *in vitro* dopaminergic activities were evaluated by measuring the inhibitory effects of prolactin secretion from anterior pituitary in rats. Compound (**24**) showed its activity at 10^{-6} M concentration while compound (**36**) showed a continuous reduction at $10^{-9}-10^{-6}$ M concentration ranges. These results indicated that this system can be used as a new dopaminergic agent as starting material for new cardiotonic. The inotropic activities of compounds (**3, 6**) were evaluated by measuring cardiac output in dog and no significant activities were found. The AM-1 calculation of the pyridone compounds (**24, 36**) showed that compound (**24**) is less chemically stable than

compound (**36**), but is more lipophilic than compound (**36**).

5. EXPERIMENTAL SECTION

5-1. Materials and Methods

Melting points were determined on a Fischer-Johns melting point apparatus and were uncorrected. Ultraviolet spectroscopy was performed on a Varian Cary 210 spectrophotometer. Proton nuclear magnetic resonance spectra were recorded on a Varian EM 390 spectrophotometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The solvents used are given in parentheses for each spectrum reported. Multiplicities of proton are designated as singlet (s), doublet(d), triplet(t), quartet(q) or multiplet(m). Infrared(IR) spectra were recorded on Perkin-Elmer 281 spectrophotometer. Solid samples were run as either a KBr pellet or a nujol mull; liquid samples were analyzed neat as a thin film between NaCl plates. Mass spectra were performed by Department of Medicinal Chemistry, University of Florida, Gainesville, Florida. Elemental analyses were performed by Atlantic Microlab. Inc., Atlanta, Georgia in U.S.A. and were within +0.4% of the theoretical values unless otherwise indicated. Silica gel 60 (Merck) for column chromatography was used.

N-(2-(4′-Methoxyphenyl)ethyl)-2,2,2-trifluoroacetamide (8)

To a cold solution of 36.0 g (0.238 mol) of *p*-methoxyphenethylamine in 360 m*l* of methylene chloride under nitrogen atmosphere was added dropwise with stirring a solution of 100 g (0.476 mol) of trifluoroacetic anhydride in 50 m*l* of methylene chloride. After the mixture was stirred for 1.5 hr at room temperature, the volatile material was removed in vacuo, toluene was added and removed, and residue was crystallized from 600 m*l* of mixture of ethyl ether-petroleum ether (1:1) to give a 48.70 g of white crystal (82.8% yield).

m.p.: 84-85℃ (ref.[12]: 84℃).

^{1}H NMR($CDCl_3$): 2.80(2H, m, -$\underline{CH_2}$-), 3.60(2 H, m, -$\underline{CH_2}$-NH-), 3.80(3H, s, -O$\underline{CH_3}$), 6.95(2H, d, J_{AB}=6 Hz), 7.20(2H, d, J_{AB}=6 Hz).

N-(2-(4′-Methoxy-3′-nitrophenyl)ethyl)-2,2,2-trifluoroacetamide (9)

To a cold solution of 15.0 g (0.0605 mol) of **8** in 127 m*l* of trifluoroacetic acid under nitrogen atmosphere was added dropwise while stirring 3.9 m*l* (1.2 eq.) of concentrated nitric acid. After the mixture was stirred for 3 hrs at room temperature, the solvents were removed and the residue was dissolved in 150 m*l* of ethyl acetate, which was successively washed with 5% HCl solution, dilute sodium bicarbonate solution and brine and dried over anhydrous magnesium sulfate-activated carbon. The mixture was filtered and the filtrate was concentrated. The resulting crude reddish solid was crystallized from ethyl acetate-hexene(1:1) to give 15.0 g of product (85.2% yield).

m.p.: 92-93℃ (ref.[12]: 92.5-93℃).

^{1}H NMR($CDCl_3$): 2.93(2H, t, -$\underline{CH_2}$-), 3.62(2 H, m, -$\underline{CH_2}$-NH-), 7.15(1H, d, J_{AB}=5 Hz), 7.47 (1H, dd, J_{AB}=5 Hz, J_{AC}=1.5 Hz), 7.77(1H, d, J_{AC}=1.5 Hz).

N-(2-(3′-Amino-4′-methoxyphenyl)ethyl)-2,2,2-trifluoroacetamide (10)

A solution of 12.10 g (0.0414 mol) of compound (**9**), 1.20 g of 10% Pd-C in 160 m*l* of ethanol-ethyl acetate (1:1) was hydrogenated at room temperature and an initial pressure of 50 psi for 1 hr. After reaction completion, the reaction mixture was filtered and concentrated to give a crude product, which was crystallized from ethyl ether-hexane mixture to give 10.27 g of product (94.5% yield).

m.p.: 87-88℃ (ref.[12]: 87-88℃).

^{1}H NMR($CDCl_3$): 2.70(2H, t, -$\underline{CH_2}$-), 3.47(2 H, m, -$\underline{CH_2}$-NH-), 3.83(5H, s, -O$\underline{CH_3}$ & -$\underline{NH_2}$), 6.50-6.85(3H, m, aromatic H), 7.10(1H, br., -$\underline{NH}$-).

N-(2-(3′-(N-Acetoacetyl)amino-4′-methoxyphenyl)ethyl)-2,2,2-trifluoroacetamide (11)

The following reaction was modified from the reported method.[12] To a solution of 5.82 g (0.0222 mol) of **10** in 20 m*l* of anhydrous THF were added 2.05 m*l* (1.2 eq.) of diketene dropwise via a syringe under nitrogen atmosphere. The reaction mixture was refluxed for 3.5 hrs and solvent was removed in vacuo. The resulting reddish thick oil was subjected to column chromatography on silica-gel with ethyl acetate-hexane (1:1) to give 6.12 g of product (79.6% yield).

m.p.: 104-105℃.

^{1}H NMR($CDCl_3$): 2.32(3H, s, -CO$\underline{CH_3}$), 2.75 (2H, t, -$\underline{CH_2}$-), 3.60(2H, -CO$\underline{CH_2}$CO-), 3.50-3.70 (2H, m, -$\underline{CH_2}$-NH-), 3.90(3H, s, -O$\underline{CH_3}$), 6.75(1H, br., -$\underline{NH}$-CO-), 6.90(2H, s, aromatic $\underline{H}$), 8.23 (1H, s, aromatic $\underline{H}$), 9.27(1H, br., -$\underline{NH}$-CO-).

IR (nujol mull): 3290(amide), 1720(C=O), 1675(C=O), 1600, 1465, 1380, 1185, 1145, 1035 cm^{-1}.

Elemental analysis for $C_{15}H_{17}N_2O_4F_3$:

Cald.: C: 52.20, H: 4.95, N: 8.09

Found: C: 51.98, H: 4.96, N: 8.05.

5-(2′-Trifluoroacetamido)ethyl-8-methoxy-4-methylcarbostyril (12)

A solution of 720 mg (2.08 mmol) of **11** in 25 m*l* of concentrated sulfuric acid was heated between 80 to 90℃ overnight. After the reaction mixture was cooled to room temperature, it was carefully poured into crushed ice. The resulting precipitate was filtered, washed with cold water and dried to give 292 mg of a grey colored compound which was insoluble in most solvents except organic acids such as trifluoroacetic acid (45 % yield).

m.p.: 224-227℃.

^{1}H NMR(TFA): 2.90(3H, s, -$\underline{CH_3}$), 3.25-3.80 (4H, m, -$\underline{CH_2}$-$\underline{CH_2}$-), 3.95(3H, s, -O$\underline{CH_3}$), 7.17(1 H, s, =$\underline{CH}$-), 7.15-7.45(2H, m, aromatic $\underline{H}$), 7.70(1H, br., -$\underline{NH}$-CO-).

IR(nujol mull): 3245(br., amide), 1715(C=O), 1645(C=O), 1605, 1545, 1210, 865 cm^{-1}.

Mass spec. (70 eV): 328(M^+).

5-(2′-Aminoethyl)-8-methoxy-4-methylcarbostyril hydrogen chloride (13)

A solution of 10.50 g (0.0320 mol) of **12** in 31.6 m*l* of ethanol and 72 m*l* of water containing 36 m*l* of concentrated HCl was refluxed for 10 hrs under nitrogen atmosphere. After the solution was cooled, the resultant white precipitate was collected and the filtrate was concentrated to give another brownish solid. This solid was dissolved in a small amount of methanol and diluted with ether to give a white precipitate. The combined white material was reprecipitated from MeOH-ether solution to give 8.20 g of HCl salt (95.4% yield).

The free base of **13** was obtained as follows: 3.00 g (0.0112 mol) of **13** was dissolved in a small amount of water. The resultant solution was made basic with dilute NH_4OH, and then extracted with 200 m*l* of methylene chloride. The methylene chloride layer was dried over anhydrous magnesium sulfate, filtered and concentrated to give 2.34 g of a white product (90.2% yield).

m.p.(HCl salt): 235-245℃(dec.).

m.p.(Free base): 159-160℃.

^{1}H NMR(DMSO-d_6; HCl salt): 2.70(3H, s, -$\underline{CH_3}$-), 2.90-3.15(2H, br., -$\underline{CH_2}$-), 3.20-3.55(2H, br., -$\underline{CH_2}$-), 3.92(3H, s, -O$\underline{CH_3}$), 6.53(1H, s), 7.03-7.27(2H, m, aromatic $\underline{H}$), 8.40(3H, br., -$\underline{NH_3}^+$).

Elemental analysis for $C_{13}H_{16}N_2O_2$ (free base):

Cald.: C: 67.22, H: 6.94, N: 12.06

Found: C: 67.07, H: 7.00, N: 12.00.

Mass spec. (70 eV, free base): 232(M^+).

3-(3′-Oxobutyl)benzamide (14)

38.2 g of ethyl 2-(3′-cyanophenylmethyl)-3-oxobutyrate, which was prepared by heating *m*-cyanobenzyl bromide with acetoacetate for 2 hrs under reflux in 500 m*l* of concentrated hydrochloric acid. To the reaction mixture were added 500 m*l* of water, followed by extraction with ethyl acetate (3×500 m*l*). The organic phase was washed with water and dried over anhydrous sodium sulfate after which the solvent was removed. The resultant 3-(3′-oxobutyl)benzoic acid was mixed

with 500 m*l* of benzene and 17 m*l* of thionyl chloride and heated for 2 hrs under reflux. The reaction solution was poured into an ice-cooled concentrated aqueous ammonia solution and the resulting amide extracted three times with 500 m*l* portions of ethyl acetate. The organic layer was washed with brine and dried over anhydrous sodium sulfate. The solvent was removed and the residue was recrystallized from ethyl acetate-hexane mixture to give 23.1 g of the product (59.8% yield).

m.p.: 122-125℃.

^{1}H NMR($CDCl_3$): 2.10(3H, s, -CO$\underline{CH_3}$), 2.80 (4H, s, -$\underline{CH_2}$-$\underline{CH_2}$-), 7.32(2H, d, aromatic $\underline{H}$), 7.75(2H, m, aromatic $\underline{H}$), 7.90(2H, br., -CO$\underline{NH_2}$-)

5-(2′-(N-(1-Methyl-3-(3′-carbamylphenyl)-n-propyl))aminoethyl)-8-methoxy-4-methylcarbostyril (3)

Reductive Amination Method: To a suspension of 1.34 g (0.0050 mol) of **13** and 0.95 g (0.0050 mol) of **14** in 35 m*l* of methanol and 25 m*l* of ethanol were added 250 mg of sodium cyanoborohydride, portionwise at room temperature. The resulting solution was stirred for 24 hrs at which time another 150 mg of sodium cyanoborohydride was added and stirred for additional 24 hrs. After the reaction was completed, the solution was concentrated *in vacuo* to give a white solid which was subjected to chromatography on silica-gel with methanol-chloroform (3:1) as an eluent to give 450 mg of product and 430 mg of starting material (22.0% yield). The acetate salt as a white powder was obtained from an acetic acid-methanol solution, followed by dilution with ether.

Hydrogenation Method: A mixture of 800 mg (0.00343 mol) of the free base of **13**, 0.72 g (1.1 eq.) of **14**, 300 mg of 10% Pd-C and 20 mg of PtO_2 in 30 m*l* of MeOH and 10 m*l* of acetic acid was hydrogenated for 24 hrs at an initial pressure of 32 psi. After the reaction was completed as indicated by TLC, the mixture was filtered and concentrated to give a residue which was subjected to column chromatography on silica-gel with chloroform-methanol to yield a white solid following evaporation of the solvents. The acetate salt of the product was obtained from an acetic acid-methanol solution, followed by dilution with ether.

m.p.(AcOH salt): 141-145℃.

^{1}H NMR(AcOH salt in DMSO-d_6): 1.35(3H, d, -$\underline{CH_3}$), 1.60-2.20(3H, br., -$\underline{CH}$-$\underline{CH_2}$-CH_2-), 2.00 (3H, s, -$\underline{CH_3}$COOH), 2.80(5H, s+br., -$\underline{CH_3}$ & -$\underline{CH_2}$-CH_2-), 3.10-3.35(2H, br.), 3.50-3.75(2H, br.), 3.98(3H, s, -O$\underline{CH_3}$), 6.63(1H, s), 7.20(2H, m), 7.57(2H, m), 7.95(2H, m).

U.V.(MeOH): 258 nm (max.).

Elemental analysis for $C_{26}H_{33}N_3O_5$(AcOH salt):

Cald: C: 66.79, H: 7.11, N: 8.98

Found: C: 66.53, H: 7.18, N: 8.90.

5-(2′-(N-(1-Methyl-3-(3′-carbamylphenyl)-n-propyl))aminoethyl)-8-hydroxy-4-methylcarbostyril (4)

1) Generation of the free amine of **3**: 1.00 g of acetate salt of **3** was dissolved in minimum amount of water and made basic with dilute ammonium hydroxide. The resulting basic aqueous solution was extracted with chloroform (2×50 m*l*). The chloroform layers were washed with 10 m*l* of water, dried over anhydrous magnesium sulfate, filtered and concentrated to give 570 mg of a white solid.

2) Reaction with BBr_3: A solution of 570 mg (0.00140 mol) of free amine of **3** in 50 m*l* of dry dichloromethane was added to 30 m*l* of 1.0 M BBr_3 solution dropwise via a syringe in an ice-water bath under nitrogen atmosphere. After stirring the resulting suspension overnight at room temperature, it was carefully quenched with methanol and concentrated *in vacuo* to give a yellowish foam, which was directly subjected into column chromatography on silica-gel with chloroform-methanol (1:3) as an eluent to yield a pale yellowish product. This product was purified by reprecipitation from a methanol-ether mixture to obtain 263 mg of pure product (48.1% yield).

^{1}H NMR(CD_3OD): 1.50(3H, d, -CH-$\underline{CH_3}$), 1.80-2.20(3H, br. -$\underline{CH}$-$\underline{CH_2}$-), 2.80(3H, s, -$\underline{CH_3}$), 2.75-2.85(2H, br., -$\underline{CH_2}$-NH-), 3.10-3.70(4H, br., 2 of -$\underline{CH_2}$-ring), 6.67(1H, s), 7.20(2H, m), 7.55(2H, m), 7.97(2H, m).

Elemental analysis for $C_{23}H_{27}N_3O_3 \cdot 1.6$ $HBr \cdot 1.3H_2O$:

Cald.: C: 50.48, H: 5.72, N: 7.69, Br: 23.40

Found: C: 50.43, H: 5.55, N: 7.52, Br: 23.25.

5-(2′-(N-(1-Methyl-3-(3′-carbamylphenyl)-n-propyl))aminoethyl)-8-methoxy-4-methyl-3,4-dihydrocarbostyril (5)

A solution of 400 mg of the free amine of **3** in 100 m*l* of methanol and 400 mg of 10% Pd-C was hydrogenated at an initial pressure of 58 psi for 3 days. After the solution was filtered, the filtrate was concentrated to give a white solid which was dissolved in chloroform and filtered. The filtrate was diluted with ether to give a pale yellow precipitate.

IR(nujol mull): 3170-3450(br., -$CONH_2$), 1660(C=O), 1460, 1375 cm^{-1}.

^{1}H NMR(CD_3OD): 1.15(3H, d, -$\underline{CH_3}$), 1.45(3 H, d, -$\underline{CH_3}$), 1.75-2.40(4H, br.), 2.60-3.45(8H, br.), 3.85(3H, s, -O$\underline{CH_3}$), 6.90(2H, m), 7.43(2H, m), 7.72(2H, m).

Elemental analysis for $C_{24}H_{31}N_3O_3 \cdot 0.45$ $CHCl_3$:

Cald.: C: 63.39, H: 6.84, N: 9.07, Cl: 10.32

Foudn: C: 63.39, H: 7.17, N: 9.12, Cl: 10.14.

2-Methoxy-5-carbethoxy pyridine (16)

Silver carbonate (5.95 g, 0.02 mol) and 2-hydroxy-5-carbethoxypyridine[13] (3.35 g, 0.02 mol) were reacted with methyl iodide (20.5 g, 0.14 mol) for 24 hrs in 30 m*l* of benzene at room temperature in the dark. The reaction mixture was filtered and the filtrate was concentrated *in vacuo* to give a sticky oil which was purified by silica gel column chromatography with ethyl acetate-hexane (1:4) to yield 2.72 g of product (75.1% yield).

IR(neat): 3050, 1710(C=O), 1600, 1490, 1370, 1260, 1110, 1020, 840, 780 cm^{-1}.

^{1}H NMR($CDCl_3$): 1.45(3H, t, -CH_2-$\underline{CH_3}$), 4.00 (3H, s, -O$\underline{CH_3}$), 4.45(2H, m, -O$\underline{CH_2}$-), 6.80(1H, d, J_{AB}=6 Hz, 3-py-$\underline{H}$), 8.25(1H, dd, J_{AB}=6 Hz, J_{AC}=1.5 Hz, 4-py-$\underline{H}$), 8.87(1H, d, J_{AC}=1.5 Hz, 6-py-$\underline{H}$).

2-Methoxy-5-chloromethylpyridine (18)

To a suspension of 0.85 g of $LiAlH_4$ in 3 m*l* of anhydrous THF was added 1.38 g (0.00762 mol) of **16** in 7 m*l* of anhydrous THF dropwise in an ice-water bath under nitrogen atmosphere. The reaction mixture was stirred for 1 hr at room temperature and quenched with 0.85 m*l* of water, 0.85 m*l* of 15% NaOH solution, 3×0.85 m*l* of water. After the resulting solid was filtered and washed with ether (2×15 m*l*), the combined etherates were concentrated and redissolved in ethyl acetate, washed with brine and dried over anhydrous magnesium sulfate. After evaporation of solvent, the crude product was purified by silica gel column chromatography with ethyl acetate-hexane (1:2) to yield 870 mg of 2-methoxy-5-hydroxymethylpyridine (82.1% yield).

IR(neat): 3360(-OH), 2960, 1610, 1570, 14 90, 1390, 1285, 1210, 1150, 1150, 1015 cm^{-1}.

^{1}H NMR($CDCl_3$): 3.90(3H, s, -O$\underline{CH_3}$), 4.35(1 H, br., -$\underline{OH}$), 4.55(2H, s, -$\underline{CH_2}$-), 6.72(1H, d, 3-py-$\underline{H}$), 7.67(1H, dd, 4-py-$\underline{H}$), 8.07(1H, d, 6-py-$\underline{H}$).

The solution of 780 mg (0.00561 mol) of 2-methoxy-5-hydroxymethylpyridine (**17**) and 0.80 mg (2.0 eq.) of thionyl chloride in 5 m*l* of chloroform was stirred for 4 days at room temperature under nitrogen condition. After concentration, the resulting sticky yellowish oil was dissolved in 20 m*l* of water, made basic with dilute NH_4OH solution and then extracted with chloroform (2×30 m*l*). The combined chloroform layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated to give yellow liquid which was used in the next reaction without further purification.

IR(neat): 2960, 1605, 1570, 1490, 1385, 1285, 1255, 1120, 1020, 825, 750 cm^{-1}.

^{1}H NMR($CDCl_3$): 3.93(3H, s, -O$\underline{CH_3}$), 4.55(2 H, s, -$\underline{CH_2}$-Cl), 6.80(1H, d, 3-py-$\underline{H}$), 7.68(1H,

dd, 4-py-H), 8.20(1H, d, 6-py-H).

2-Methoxy-5-cyanomethylpyridine (19)

Method 1: The mixture of 760 mg (0.00501 mol) of **18**, a catalytic amount of sodium iodide and 0.50 g of sodium cyanide in 8 m*l* of methanol and 1.5 m*l* of water were refluxed for 1.5 hr. The resulting brownish solution was cooled and concentrated. The residue was dissolved in 30 m*l* of water and extracted with chloroform (2×20 m*l*). The combined chloroform layers were washed with water and brine, dried over anhydrous $MgSO_4$, filtered and concentrated to give yellowish oil, which was purified by silica gel column chromatography with ethyl acetate-hexane (1:3) to yield white solid. An analytically pure product was obtained from recrystallization from ether-petroleum ether mixture (400 mg, 50.5% yield).

Method 2: A mixture of 440 mg of **18**, an excess of sodium cyanide and a catalytic amount of sodium iodide in 15 m*l* of dry acetone were stirred for 2 days under nitrogen atmosphere. After the reaction was completed as indicated by TLC, the reaction mixture was concentrated and redissolved in chloroform. The chloroform layer was washed with water and brine, dried and filtered. The filtrate was concentrated to give a reddish residue, which was purified by silica gel column chromatography with ethyl acetate-hexane (2:3) to yield 220 mg of product (78.5% yield).

m.p.: 53-54℃.

IR(nujol mull): 2250(-CN) cm^{-1}

^1H NMR($CDCl_3$): 3.67(2H, s, -CH_2-CN), 3.95 (3H, s, -OCH_3), 6.80(1H, d, 3-py-H), 7.60(1H, dd, 4-py-H), 8.17(1H, d, 6-py-H).

Elemental analysis for $C_8H_8N_2O$:

Cald.: C: 64.85, H: 5.44, N: 18.90

Foudn: C: 64.92, H: 5.48, N: 18.85.

2-Methoxy-5-(2′-acetaminoethyl)pyridine (21)

The solution of 220 mg (1.39 mmol) of **19** in 8 m*l* of anhydrous THF in an ice bath was added with 7.0 m*l* (5.0 eq.) of 1.0 M BH_3-THF complex dropwise by syringe under nitrogen atmosphere. After reaction mixture stirred for 24 hrs, it was quenched with methanol and concentrated *in vacuo* to give a residue, which was subsquently dissolved in 30 m*l* of 5% HCl solution. The acidic aqueous layer was washed with ethyl acetate, and then made basic with 10% NaOH solution and finally extracted with chloroform (2×30 m*l*). The chloroform layer was washed with brine, dried, filtered and concentrated to give the yellow oil, which was used directly in the next reaction.

^1H NMR($CDCl_3$): 1.23(2H, s, -NH_2), 2.60-3.00(4H, m., -CH_2-CH_2-), 6.70(1H, d, 3-py-H), 7.40(1H, dd, 4-py-H), 8.03(1H, d, 6-py-H).

A mixture of crude free amine (**20**), 0.5 m*l* of acetic anhydride and a catalytic amount of pyridine in 5 m*l* of dichloromethane was stirred for 40 min. at room temperature under nitrogen atmosphere. The reaction mixture was concentrated *in vacuo* and stripped with toluene once. The resulting oil was dissolved in 10 m*l* of water and made basic with 10% NaOH solution, and then extracted with chloroform (2×30 m*l*). The combined chloroform layers were washed with brine, dried, filtered and concentrated to give a crude product, which was purified by column chromatography on silica gel with ethyl acetate to yield a white solid (151 mg, 52.7% yield).

m.p.: 54.5-55.5℃.

^1H NMR($CDCl_3$): 1.93(3H, s, -$COCH_3$), 2.70 (2H, t, -CH_2-), 3.45(2H, m, -CH_2-NH-), 3.90(3 H, s, -OCH_3), 6.20(1H, br., -NH-), 6.78(1H, d, 3-py-H), 7.45(1H, dd, 4-py-H), 7.97(1H, d, 6-py-H).

Elemental analysis for $C_{10}H_{14}N_2O_2$:

Cald. C: 61.83, H: 7.26, N: 14.42

Found: C: 61.87, H: 7.31, N: 14.37.

5-(2′-Acetaminoethyl)-1-hydroxy-2-pyridone (23)

A solution of 300 mg (0.00154 mol) of **21** and 0.47 g (1.5 eq.) of 85% mCPBA in 5 m*l* of dichloromethane was stirred for 24 hrs at room temperature under nitrogen atmos-

phere. After evaporation of solvent, the residue was directly subjected to column chromatogrphy on silica gel with ethyl acetate-methanol (2:1) to afford 180 mg of corresponding N-oxide (55.6% yield).

m.p.: 128-129℃.

^{1}H NMR($CDCl_3$): 1.93(3H, s, -CO$\underline{CH_3}$), 2.78 (2H, t, -$\underline{CH_2}$-), 3.45(2H, m, -$\underline{CH_2}$-NH-), 4.05(3 H, s, -O$\underline{CH_3}$), 6.95(1H, d, 3-py-$\underline{H}$), 7.30(1H, dd, 4-py-$\underline{H}$), 7.62(1H, br., -$\underline{NH}$-), 8.15(1H, d, 6-py-$\underline{H}$).

Mass spec.: 211(M^+).

To 410 mg (0.00195 mol) of a white solid N-oxide (**22**) was added 6.0 m*l* of acetyl chloride dropwise under nitrogen atmosphere and the resulting mixture was refluxed for 1 hr. After evaporation of the excess acetyl chloride, the resulting sticky yellowish residue was dissolved in 10 m*l* of water and was stirred overnight and concentrated *in vacuo* to give a sticky residue. This was stripped with methanol-toluene twice and extracted with hot chloroform. The combined chloroform layers were concentrated to give a white solid, which was crystallized from chloroform-ether mixture to afford the compound **8a** (195 mg, 51.2% yield).

m.p.: 124.5-125.5℃.

NMR(CD_3OD): 1.98(3H, s, -CO$\underline{CH_3}$), 2.70(2 H, t, -$\underline{CH_2}$-), 3.37(2H, m, -$\underline{CH_2}$-NH-), 6.82(1H, d, 3-py-$\underline{H}$), 7.15(1H, dd, 4-py-$\underline{H}$), 7.95(1H, d, 6-py-$\underline{H}$).

IR(nujol mull): 3320(N-OH), 3100(NH-C=O), 1695(NH-C=O), 1580, 1365, 910 cm^{-1}.

Elemental analysis for $C_9H_{12}N_2O_3 \cdot 0.5H_2O$:

Calcd.: C: 52.67, H: 6.36, N: 13.65

Found: C: 52.93, H: 6.06, N: 13.62.

5-(2′-Aminoethyl)-1-hydroxy-2-pyridone hydrochloride (24)

A solution of 1.10 g of **23** in 8 m*l* of MeOH-H_2O-c-HCl (1:1:2) mixture was refluxed for 10 hrs under nitrogen atmosphere. After the reaction was completed, the solution was concentrated to give crude solid product. The crude compound was recrytallized from ethanol-water mixture to yield the compound **1a** (740 mg, 76.0% yield).

m.p.: 242-244℃.

IR(nujol mull): 3150-2700(br., -OH & NH_3^+), 1650(C=O) cm^{-1}.

^{1}H NMR(DMSO-d_6): 2.65-2.72(2H, m, -$\underline{CH_2}$-), 2.85(2H, br., -$\underline{CH_2}$-NH_3^+), 6.50(1H, d, 3-py-$\underline{H}$), 7.37(1H, dd, 4-py-$\underline{H}$), 7.80(1H, d, -6-py-$\underline{H}$), 8. 25(3H, br., -$\underline{NH_3}^+$).

Mass spec.: 155(M^+).

Elemental analysis for $C_7H_{11}N_2O_2Cl$:

Cald.: C: 44.10, H: 5.81, N: 14.69, Cl: 18. 59

Found: C: 44.19, H: 5.82, N: 14.64, Cl: 18.52

5-(2′-(N-(1-Methyl-3-(3′-carbamylphenyl)-n-propyl))aminoethyl)-1-hydroxy-2-pyridone (6)

The mixture of 35 mg of **24** and 33 mg (1.1 eq.) of the corresponding ketone (**14**) in 3.0 m*l* of methanol was added with 20 mg of sodium cyanoborohydride at room temperature under nitrogen atomsphere. The resulting solution was stirred for 3 days. After solvent was evaporated, the resulting white residue was chromatographied on silica gel with ethyl acetate-methanol (1:1) to give the white product, which was purified by reprecipitation method from methanol-ether mixture.

m.p.: 110-113℃.

IR(Nujol mull): 3500-3000, 1630 cm^{-1}

^{1}H NMR(CD_3OD): 1.30(3H, d, CH-$\underline{CH_2}$-), 2.70 (4H, m, py-$\underline{CH_2}$ & -$\underline{CH_2}$-O), 3.00(3H, m -$\underline{CH_2}$-NH-$\underline{CH}$-), 6.40(1H, d, 4-py-$\underline{H}$), 7.10(1H, dd, 3-py-$\underline{H}$), 7.20(2H, m, ortho $\underline{H}$s to carbamate group), 7.65(3H, m, 2-py-$\underline{H}$, & meta, para $\underline{H}$s to carbamate group)

Elemental analysis for $C_{18}H_{23}N_3O_3 \cdot 0.6H_2O$:

Cald.: C: 63.54, H: 7.17, N: 12.35,

Found: C: 63.34, H: 6.97, N: 12.31

4-(2′-(N-(1-Methyl-3-(3′-carbamylphenyl)-*n*-propyl))aminoethyl)-1-hydroxy-2-pyridone (7)

was prepared from **27** by analogy with the methods of **6**.

4-Carbomethoxy-2-ethoxypyridine (27)

was produced in 83% yield from 4-carbomethoxy-2-pyridone (**26**) as a yellowish oil

after silica gel column chromatography with ethyl acetate-hexane (1:3) by analogy with the method of **21**.

IR(neat): 3000, 1735(C=O), 1600, 1560, 1100, 765 cm^{-1}.

^1H NMR($CDCl_3$): 1.33(3H, t, $-CH_2-\underline{CH_3}$), 3.90 (3H, s, $-O\underline{CH_3}$), 4.35(2H, m, $-O\underline{CH_2}-$), 7.27(1H, s, 3-py-H), 7.35(1H, dd, 5-py-H), 8.22(1H, d, 6-py-H).

4-Chloromethyl-2-ethoxypyridine (29)

In a similar manner, **29** was prepared from **28** in 93% yield.

IR(neat): 3020, 1615, 1570, 1170, 1050, 725 cm^{-1}.

^1H NMR($CDCl_3$): 1.42(3H, t, $-CH_2-\underline{CH_3}$), 4.35 (2H, m, $-O\underline{CH_2}-$), 4.48(2H, s, $-\underline{CH_2}-Cl$), 6.72(1 H, s, 3-py-H), 6.85(1H, dd, 5-py-H), 8.14(1H, d, 6-py-H).

4-Cyanomethyl-2-ethoxypyridine (30)

In a similar manner, **30** was prepared from **29** in 64% yield.

m.p.: 55.5-56℃.

IR(nujol mull): 2270(CN) cm^{-1}.

^1H NMR($CDCl_3$); 1.40(3H, t, $-CH_2-\underline{CH_3}$), 3.72 (2H, s, $-\underline{CH_2}-CN$), 4.35(2H, m, $-O\underline{CH_2}-$), 6.70(1 H, s, 3-py-H), 6.87(1H, s, 5-py-H), 8.17(1H, d, 6-py-H).

Mass spec.: 163(M^+).

Elemental analysis for $C_9H_{10}N_2O$:

Cald.: C: 66.64, H: 6.21, N: 17.27

Found: C: 66.53, H: 6.22, N: 17.24.

4-(2′-Acetaminoethyl)-2-ethoxypyridine (32)

In a similar manner, **32** was prepared from **30** in 57% yield.

m.p.: 84.5-85.5℃.

^1H NMR($CDCl_3$): 1.37(3H, t, $-OCH_2-\underline{CH_3}$), 1.93(3H, s, $-CO\underline{CH_3}$), 2.70(2H, t, $-\underline{CH_2}-CH_2-$ NH-), 3.40(2H, m, $-\underline{CH_2}-NH-$), 4.30(3H, qt, $-O\underline{CH_2}-CH_3$), 6.45(1H, br., -NH-), 6.52(1H, s, 3-py-H), 6.70(1H, dd, 5-py-H), 8.00(1H, d, 6-py-H).

Mass spec.(70 eV): 208(M^+)

Elemental analysis for $C_{11}H_{16}N_2O_2$:

Cald.: C: 63.44, H: 7.74, N: 13.45

Found: C: 63.51, H: 7.75, N: 13.44.

4-(2′-Acetaminoethyl)-1-hydroxy-2-pyridone (35)

In a similar manner, **35** was prepared from **32** in 44% yield.

^1H NMR(CD_3OD): 1.93(3H, s, $-CO\underline{CH_3}$), 2.72 (2H, m, $-\underline{CH_2}-CH_2-NH-$), 3.40(2H, m, $-\underline{CH_2}-$ NH-), 6.60(1H, dd, 5-py-H), 6.70(1H, s, 3-py-H), 7.97(1H, d, 6-py-H).

Mass spec.: 225(M+1).

4-(2′-Aminoethyl)-1-hydroxy-2-pyridone hydrogen chloride (36)

In a similar manner, **36** was prepared from **35** in 78% yield.

m.p.: 224-226℃.

IR(nujol mull): 2600-3300(br., -OH & $-NH_3^+$), 1650(C=O) cm^{-1}.

^1H NMR(DMSO-d_6): 2.62-3.25(4H, m, $-\underline{CH_2}-\underline{CH_2}-$), 6.17(1H, dd, 5-py-H), 6.40(1H, s, 3-py-H), 7.83(1H, d, 6-py-H), 8.25(3H, br., $-\underline{NH_3}^+$).

U.V.(MeOH): 228, 303 nm.

Mass spec.: 155(M^+).

Elemental analysis for $C_7H_{11}N_2O_2Cl$:

Cald.: C: 44.10, H: 5.81, N: 14.69, Cl: 18.59

Found: C: 44.18, H: 5.86, N: 14.62, Cl: 18.51

5-2. Stability of the Carbostyril Compounds (3, 4, 5) in pH 7.40 Phosphate Buffer

Solutions of monobasic potassium phosphate (0.2 N) and dibasic potassium phosphate (0.2 N) were made and used to prepare a pH 7.40 phosphate buffer by mixing together.

Buffer solutions (4.9 m*l*) were equilibrated at 37℃. At time zero, 50 μ*l* of a 5.3×10^{-2} M stock solution of test compound in methanol were added to a buffer solution. At designated time points, 100 μ*l* samples were removed and added to 900 μ*l* of ice-cold 40% acetonitril-water. The final concentration of test compound would be 5.3×10^{-5} M at time zero. Samples were stored at 0℃ until analyzed by HPLC.

5-3. Stability of the Carbostyril Compounds (3, 4, 5) in 100% Whole Human Blood

Freshly collected heparinized blood was obtained. The blood was stored in a refrigerator and used the next day. Thirty microliters of a freshly prepared 0.061 M solution

of test compound in methanol was added to 3.0 m*l* of blood, previously equilibrated to 37℃ in a water bath and mixed thoroughly to result in an initial concentration of 6.1×10^{-4} M. At designated time intervals, 100 μ*l* aliquots were withdrawn from the test medium, added immediately to 900 μ*l* of ice-cold acetonitrile, shaken vigorously and placed in a freezer. The final test compound concentration was 6.1×10^{-5} M at time zero. When all samples had been collected, they were centrifuged at 13000 rpm for 5 min. The samples were kept at 0℃ until analyzed by HPLC.

5-4. Stability of the Carbostyril Compounds (3, 4, 5) in 80% Human Plasma

Freshly collected plasma contained about 80% plasma diluted with anticoagulant citrate phosphate-dextrose solution U.S.P. The plasma was stored in a refrigerator and used the next day. Thirty microliters of a freshly prepared 0.061 M solution of test compound in methanol was added to 3.0 m*l* of plasma, previously equilibrated to 37℃ in a water bath and mixed thoroughly to result in an initial concentration of 6.1×10^{-4} M. At designated time intervals, 100 μ*l* aliquots were withdrawn from test medium, added immediately to 900 μ*l* of ice-cold acetonitrile, shaken vigorously and placed in a freezer. The final test compound concentration was 6.1×10^{-5} M at time zero. When all samples had been collected, they were centrifuged at 13,000 rpm for 5 min. The samples were kept at 0℃ until they were analyzed by HPLC.

5-5. Stability of the Carbostyril Compounds (3, 4, 5) in 20% Rat Liver Homogenate

The liver homogenate was prepared by the following method. One Sprague-Dawley rat was killed by decapitation, and liver was removed, weighed and homogenated in a tissue homogenizer in 0.05 M aqueous phosphate buffer (pH 7.4) to make 20% liver homogenate. Thirty microliters of 5.3×10^{-3} M solution of test compound in methanol were added to 3.0 m*l* of the homogenate, previously equilibrated to 37℃ in a water bath, to result in an initial concentration of 5.3×10^{-5} M. At various time points, 100 μ*l* of samples were withdrawn from the test medium, added immediately to 400 μ*l* of ice-cold acetonitrile, shaken vigrously and placed in a freezer. The final test sample concentration was 1.0×10^{-5} M. When all samplex had been collected, they were centrifuged at 13,000 rpm for 5 min. and were stored at 0℃ until analyzed by HPLC.

5-6. *In Vitro* Evaluation of the Prolactin Inhibitory Effects of the Pyridones (24, 36)

Adult female rats (Charles Rivers Labs), weighing 220-250 g, were maintained on food and water *ad libitum*. Animals were sacrificed by decapitation; their pituitary glands were quickly removed from the cranium. The anterior pituitary (AP) of each animal was dissected into two equal halves and placed into incubation media (media 199 supplied by Grand Island Biological Co.) The incubation was conducted at 37℃, under continuous aeration (95% O_2/5% CO_2); the pH was 7.61. After one hour preincubation, the media were discarded and replaced with fresh ones containing either compound (**24**, 1×10^{-9} M) or compound (**36**, 1×10^{-9} M). In all cases, one-half of AP received the test drug; the other, the media 199 control. After 30 minutes, samples were taken from the media, and the remaining media were discarded. Fresh media containing compound (**24**, 1×10^{-8} M), compound (**36**, 1×10^{-8} M), respectively, were then added. Thirty minutes later, the second samples were taken. Same procedure was continued through the 1×10^{-6} M doses of compounds (**24, 36**). At end of experiment, each half of the AP was weighed. The samples were diluted 1:50 with phosphate-buffered saline and then assayed in triplicate by the radioimmunoassay method described. The data are given as nanograms of prolactin released per milligram of wet weight per milligram of protein. Paired Student's t-test was used to evaluate the significance of the inhi-

bitory effects of the test drugs on prolactin secretion. The control AP half and the drug-treated half were employed in each paired comparision.

5-7. AM-1 Calculation of (2'-Aminoethyl)-1-hydroxy-2-pyridone Systems (24, 36)

The molecular properties were evaluated using the AM-1 semiempirical method,[13] while the solubility characteristics and the volume, surface and "ovality" (isosteric properties) by the recent version[14] of the theoretical method developed by Bodor *et al.*[15]

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