SYNTHESIS OF NOVEL TRYPTOPHAN DERIVATIVES OF POTENTIAL BIOLOGICAL ACTIVITY

RAFAT M. MOHAREB[A,B], NADIA A. LOUCA[C], GAMAL A. ELMEGEED[C] AND HANAA Y. HANA[C]*

[a] *Departament of Organic Chemistry, Faculty of Pharmacy, October University for Modem Sciences and Arts (MSA), El-Wahaat Road, October City, Egypt*
[b] *Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt*
[c] *Hormone Department, National Research Centre, Dokki, Giza, Egypt*
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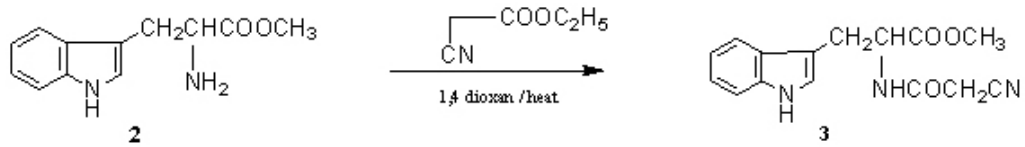
ABSTRACT

Tryptophan methyl ester **2** reacts with ethyl cyanoacetate to form acetonitrilocarbonyltryptophan methylester **3**. The latter reacts with cyanomethylene reagents, hydrazines, cyanomethylenes and sulfur to form the corresponding α-pyrido-3-indolopropanoate derivatives **6a,b**, pyrazolyltryptophan methyl ester derivatives **8a,b** and thiophenotryptophan methyl ester derivatives **10a,b**, respectively. Also compound **3** reacts with benzaldehyde to give the condensated product **12**. The reactivity of the latter product towards chemical reagents was studied to form pyridine, pyrazole and isoxazole derivatives.

Keywords: Teyptophane, pyrazd, pyridine, 1, 3 - oxazine.



2 **3**

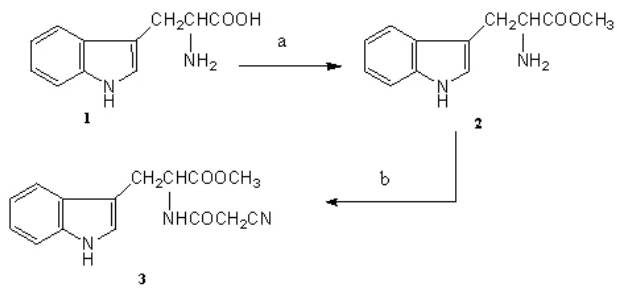
INTRODUCTION

The development of potential therapeutic agents based on the structure of peptides has stimulated an interest in the design and synthesis of unnatural amino acids [1-3]. Thus, numerous chemical transformations of amino acids have been reported [4]. Indole and it's derivatives have been a topic of research interest for over a century [5,6]. This, is in part, due to the fact that indole moieties are found in a variety of naturally occurring compounds that exhibit various physiological properties [7,8]. Tryptophan is one of the most important amino acids which containing indole moiety. However, to our knowledge, few investigations have been reported concerning its uses in the formation of cyclic tryptophan derivatives [9-12]. The reaction of tryptophan with cyanoacetates is known to produce cyanoamides in the aim of forming photo-active cross-linking bioprobes [13,14]. In view of these observations and in continuation with studies involving the synthesis of unusual hormones and amino acids derivatives [15-17], we have used in this study, *L*-tryptophan is used to form new heterocyclic compounds of potential biological activities.

RESULTS AND DISCUSSION

The reaction of tryptophan **(1)** with methanol in acetyl chloride solution afforded the corresponding tryptophan methyl ester **2** (Scheme 1). Compound **2** reacted with an equimolar amount of ethyl cyanoacetate in 1,4-dioxan under reflux to give the methyl *α*-imino(acetonitrilocarbamido)-3-indolopropanoate **(3)** via elimination of ethanol. The mass spectrum of compound **3** showed molecular ion peak at m/z 286 (25%). The IR spectrum of compound **3** revealed the presence of NH stretching at *v* 3400-3360 cm^{-1}, one CN group stretching at *v* 2220 cm^{-1} and two C=O groups stretching at *v* 1730 and 1680 cm^{-1}. Also the ^1H NMR spectrum of compound **3** showed two D_2O-exchangeable singlets at δ 8.65 and 8.76 for the two NH groups (*cf*. experimental section). Moreover, the ^{13}C NMR spectrum showed δ 22.6 (CH_3), 28.9, 44.2 (2 CH_2), 50.2 (CH), 115.1, 116.7, 118.4, 120.4, 121.4, 122.6, 142.6 (pyrole, benzene C), 172.6, 174.8 (2 C=O).

Synthesis of compound **3**



Reagents and conditions: (a) CH_3COCl, CH_3OH; (b) $NCCH_2COOC_2H_5$, heat.

Scheme (1)

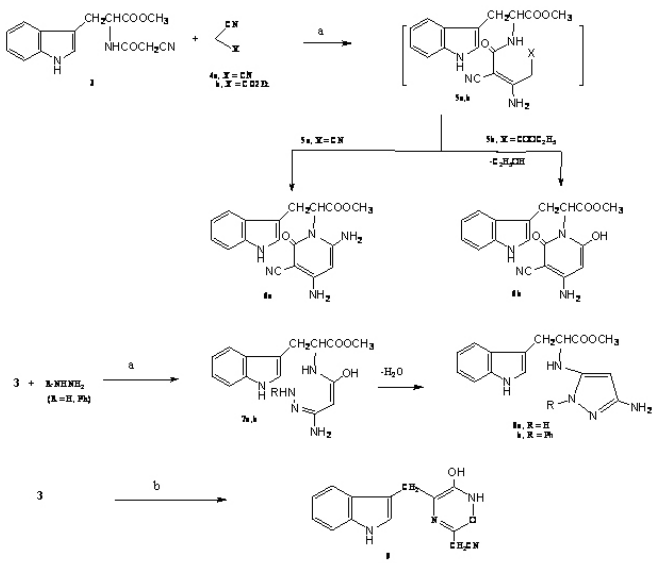
To improve the pharmaceutical properties of the tryptophan molecule, we have outlined the synthesis of several indole derivatives containing heterocyclic moiety. Therapeutic agents containing pyridine, thiophene, and pyrazole moieties have attracted the attention of researchers in pharmaceutical chemistry; these heterocycles have been found to show various biological activities [18,19]. Compound **3** seemed to be an interest candidate for further chemical transformations to form indole derivatives with potential biological activity. The reactivity of compound **3** towards the reaction with active methylene reagents was investigated. Thus, compound **3** reacted with equimolar amount of either malononitrile **(4a)** or ethyl cyanoacetate **(4b)** in ethanolic triethylamine solution gave the α-pyrido-3-indolopropanoate derivatives **6a,b**, respectively. Formation of the latter products took place via the intermediate formation of **5a,b** which underwent ready intramolecular cyclization to give compounds **6a,b** (Scheme 2). The IR spectrum of each of **6a** and **6b** showed two CN groups stretching at *v* 2200 and 2225 cm^{-1}, respectively. The ^1H NMR of compound **6a** showed a singlet at δ 4.62 (4H, D_2O-exchangeable) for the two NH_2 groups while that of compound **6b** showed a singlet at δ 4.41 (2H, D_2O-exchangeable) for one NH_2 group and a singlet at δ 10.05 for the OH group. Moreover, each of compounds **6a,b** showed a singlet at δ 6.23 and 6.34, respectively for the pyridine ring proton at C-3 (*cf*. experimental section).

The study also extended to the reactivity of compound **3** towards nitrogen nucleophilic reagents. Thus, compound **3** reacted with equimolar amounts of either hydrazine hydrate or phenylhydrazine in refluxing ethanol containing a catalytic amount of triethylamine to give the corresponding pyrazolyltryptophan methyl ester derivatives **8a,b**. The reaction took place via a simple ad-

e.mail: raafat_mohareb@yahoo.com

dition of hydrazine hydrate or phenylhydrazine to the CN group in compound **3** to give the intermediates **7a,b** which readily afforded compounds **8a,b** via water elimination (Scheme 2). Moreover, compound **3** reacted with hydroxylamine hydrochloride in cold ethanolic sodium acetate solution to give the corresponding oxadiazinomethylenoindole derivative **9** (Scheme 2). Structures of compounds **8a,b** and **9** were supported by their compatible analytical and spectral data (*cf.* experimental section).

Synthesis of compounds **6a,b** to **9**:

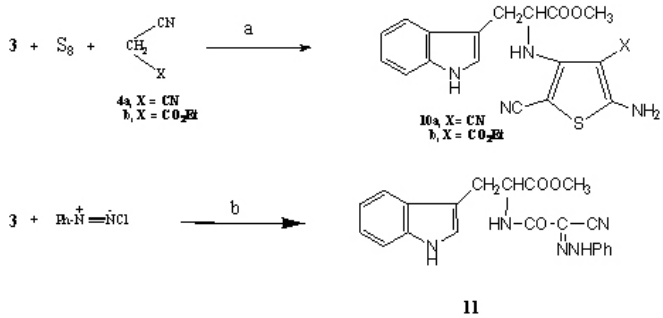


Reagents and conditions: (a) EtOH/Et3N, heat, (b) NH_2OH, EtOH/AcONa, heat

Scheme (2)

Compound **3** reacted with equimolar amounts of either malononitrile **4a** or ethyl cyanoacetate **4b** and elemental sulfur in ethanolic triethylamine solution under reflux to afford the corresponding thiophenotryptophan methyl ester derivatives **10a,b** (Scheme 3). The IR spectrum of compound **10a** revealed the presence of two CN groups stretching at ν 2210 and 2225 cm^{-1} and the ^1H NMR spectrum revealed the presence of D_2O-exchangeable singlet (2H) at δ 4.58 for the NH_2 group and two D_2O-exchangeable singlets at δ 10.78 (1H) and δ 11.94 (1H) for the two NH groups. On the other hand, the IR spectrum of **10b** showed the presence of one CN group stretching at ν 2200 cm^{-1} and its ^1H NMR spectrum showed a triplet at δ 1.23 beside a quartet at δ 4.24 due to the ethyl ester group, in addition to the NH_2 and the two NH singlet signals at δ 6.50, 10.71 and 10.88, respectively. The cyanomethylene moiety of compound **3** coupled easily with benzenediazonium chloride in cold ethanolic sodium acetate solution to give the phenylhydrazo derivative **11** based on ^1H NMR spectrum which showed, beside the expected chemical shifts, three NH singlets at δ 9.25 and 9.97-9.99 (D_2O-exchangeable) (Scheme 3).

Synthesis of compounds **10a,b** and **11**

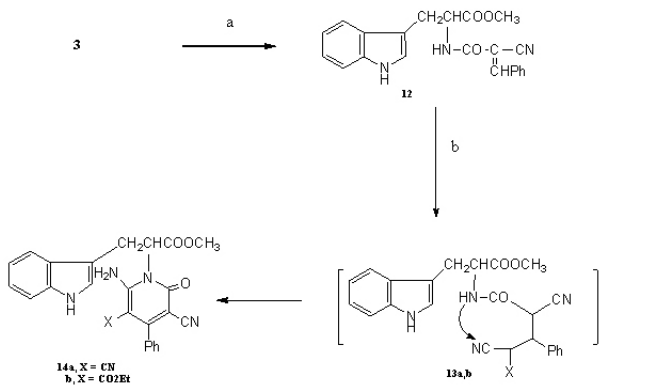


Reagents and conditions: (a) EtOH/Et_3N, heat; (b) EtOH, AcONa.

Scheme (3)

The reaction of compound **3** with equimolar amount of benzaldehyde in ethanolic triethylamine solution under reflux afforded the condensated product benzalacetonitrilocarbonyltryptophan methyl ester derivative **12** (Scheme 4). The reactivity of the *α,β*-unsaturated nitrile moiety of compound **12** towards the reaction with various chemical reagents was investigated. Thus, **12** reacted with either malononitrile **4a** or ethyl cyanoacetate **4b** in refluxing ethanolic triethylamine solution to give the methyl α-pyrido-1-yl-3-indolopropanoate derivatives **14a,b**, respectively via the intermediacy of **13a,b** (Scheme 4). The IR spectrum of compound **14a** revealed the presence of two CN groups at ν 2220 and 2215 cm^{-1} and the ^1H NMR spectrum of compound **14a** showed two D_2O-exchangeable singlets at δ 4.46 (2H) and δ 11.02 (1H) corresponding to the NH_2 and NH groups, respectively. The mass spectrum of compound **14b** showed a molecular ion peak at m/z 486 (35%) and the ^1H NMR spectrum of **14b** revealed a triplet at δ 1.33 and a quartet at δ 4.32 which is characteristic to ethyl ester group (*cf.* experimental section).

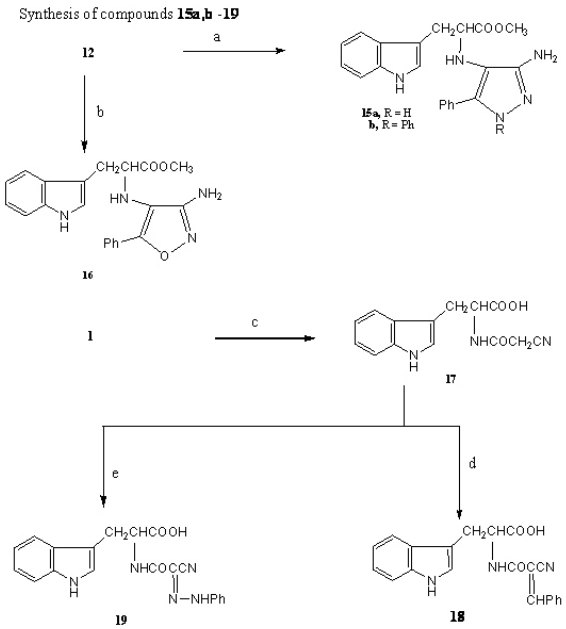
Synthesis of compounds **12**-**14a,b**



Reagents and conditions: (a) PhCHO, EtOH/Et3N, heat; (b) $NCCH_2COOC_2H_5$ or $NCCH_2CN$, EtOH, Et_3N

Scheme (4)

The reactivity of compound **12** towards the reaction with nucleophilic reagents was investigated. Thus, the reaction of **12** with equimolar amounts of either hydrazine hydrate or phenylhydrazine in ethanolic triethylamine solution under reflux afforded the corresponding carbonylpyrazolotryptophan methyl ester derivatives **15a,b**, respectively (Scheme 5). Similarly compound **12** reacted with hydroxylamine hydrochloride in ethanol containing sodium acetate solution to afford the isooxazolotryptophan methyl ester derivative **16** (Scheme 5). The chemical structures of compounds **15a,b** and **16** were confirmed via the analytical and spectral data (*cf.* experimental section).



Reagents and conditions: (a) NH_2NH_2 or $PhNHNH_2$, EtOH/Et_3N; (b) NH_2OHHCl, EtOH/AcONa; (c) $NCCH_2COOC_2H_5$, DMF, heat, (d) PhCHO, EtOH/Et_3N, heat, (e) $PhN_2^+Cl^-$, EtOH, AcONa

Scheme (5)

Tryptophan **(1)** reacted with an equimolar amount of ethyl cyanoacetate in refluxing dimethylformamide solution to give the acetonitrilocarbonyl tryptophan **17** (Scheme 5). The IR spectrum of compound **17** revealed the presence of two NH groups stretching at ν 3430-3385 cm^{-1}, and one CN group stretching at ν 2220 cm^{-1} in addition to two C=O groups stretching at ν 1705 and 1680 cm^{-1}. Also the ^1H NMR spectrum of compound **17** showed two singlets at δ 8.75 and 8.82 (D_2O-exchangeable) for the two NH groups and a singlet at δ 11.15 for the OH group (*cf.* experimental section). Compound **17** reacted with an equimolar amount of benzaldehyde in ethanol/piperidine solution under reflux to afford the condensed product benzaloacetonitrilocarbonyl-tryptophan **18** (Scheme 5). Moreover, compound **17** easily coupled with benzenediazonium chloride in cold ethanolic sodium acetate solution to afford the phenylhydrazo derivative **19** (Scheme 5), based on ^1H NMR spectrum which showed, beside the expected chemical shifts, three NH singlets at δ 8.51 and 9.32-9.36 (D_2O-exchangeable). The structures of the latter products **18** and **19** were confirmed based on their compatible elemental and spectral analyses (*cf.* experimental section).

EXPERIMENTAL SECTION

The starting pure powder of *L*-tryptophan was purchased from Sigma Company, USA. The appropriate precautions in handling moisture sensitive compounds were undertaken. All melting points of the newly synthesized compounds were measured using an electrothermal capillary melting point apparatus and are uncorrected. The IR spectra are expressed in cm^{-1} and recorded in KBr pellets on a Pa-9721 IR spectrometer (Shimadzu, Japan). ^1H- and ^{13}C NMR spectra were obtained on a Varian EM-390 90 MHz spectrometer in DMSO-d_6 as solvent, using TMS as internal reference and chemical shifts (δ) are expressed in ppm. Mass spectra were recorded on a GCMS-QP 1000 Ex spectra mass spectrometer operating at 70 eV Equipment (Germany). Elemental analyses were preformed with all final compounds by Microanalytical Data Unit at The National Research Centre, Giza, Egypt. The reactions were followed using TLC analyses which were performed using Merck 60 F254 aluminum sheets and visualized by UV light (254 nm). Although tryptophan methyl ester is commercially available in the hydrochloride form we preferred to syntheses the native methyl ester via the procedure indicated below.

Methyl *α*-amino-3-indolopropanoate (2). To a solution of tryptophan **1** (10.2 g, 0.05 mol) in methanol (5 mL) equivalent amount of acetylchloride (3.77 mL, 0.05 mol) in methanol (30 mL) was added drop wise with stirring at 0°C. The reaction mixture was stirred for 24 h, and then evaporated under vacuum. The formed solid product neutralized by sodium carbonate solution (1 N), extracted by chloroform and dried by anhydrous sodium sulfate. The solid product formed after evaporation of the solvent, filtered off, dried and crystallized from methanol. White crystals, yield 55% (6.0 g), mp 152-153°C. IR (ν/cm^{-1}): 3450-3235 (NH_2, NH), 3050 (CH-aromatic) 2985, 2865 (CH_3, CH_2) 1730 (C=O), 1620 (C=C). ^1H NMR (δ ppm): 3.35 (s, 3H, CH_3), 3.43 (m, 2H, CH_2), 3.75 (m, 1H, CH), 6.57 (s, 2H, NH_2, D_2O-exchangeable), 6.64 (s, 1H, CH), 7.51 (m, 4H, C_6H_4), 8.92 (s, 1H, NH, D_2O-exchangeable). MS (m/z, %): 218 ($M^{+\cdot}$, 45%). *Anal.* Calcd. for $C_{12}H_{14}N_2O_2$ (218.26): C, 66.03; H, 6.46; N, 12.83. Found: C, 65.79; H, 6.25; N, 12.52.

Methyl *α*-imino(acetonitrilocarbamido)-3-indolopropanoate (3). To a solution of **2** (1.09 g, 0.005 mol) in 1,4-dioxan (30 mL), equivalent amount of ethyl cyanoacetate **4b** (0.56 g, 0.005 mol) was added. The reaction mixture was heated under reflux for 3h. The solid product formed by evaporation under vacuum was filtered off, dried and crystallized. White crystals from dioxane, yield 75% (1.07 g), mp 128-130°C. IR (ν/cm^{-1}): 3400-3360 (2NH), 3050 (CH-aromatic), 2985, 2659 (CH_3, CH_2), 2220 (CN), 1730, 1680 (2C=O), 1620 (C=C). ^1H NMR (δ ppm): 3.38 (s, 3H, CH_3), 3.45 (m, 2H, CH_2), 3.48 (s, 2H, CH_2CN), 3.78 (m, 1H, CH), 6.95 (s, 1H, CH), 7.01-7.49 (m, 4H, C_6H_4), 8.65, 8.76 (2s, 2H, 2NH, D_2O-exchangeable). ^{13}C NMR (δ): 22.6 (CH_3), 28.9, 44.2 (2 CH_2), 50.2 (CH), 115.1, 116.7, 118.4, 120.4, 121.4, 122.6, 142.6 (pyrole, benzene C), 172.6, 174.8 (2 C=O). MS (m/z, %): 286 ($M^{+\cdot}$+1), 25%), 260 ($M^{+\cdot}$-CN, 25%), 203 ($M^{+\cdot}$-$NHCOCH_2CN$, 23%), 130 (indolomethylene fragment, C_9H_8N, 100%). *Anal.* Calcd. for $C_{15}H_{15}N_3O_3$ (285.30): C, 63.15; H, 5.30; N, 14.73. Found: C, 63.42; H, 5.37; N, 14.39.

Methyl*α*-(2,4-diamino-5-cyano-6-oxopyrido-1-yl)-3-indolopropanoate (6a), Methyl *α*-(4-Amino-5-cyano-2-hydroxy-6-oxopyido-1-yl) -3-indolopropanoate (6b)

General Procedure

To a solution of Compound **3** (1.42 g, 0.005 mol) in ethanol (30 mL), containing a catalytic amount of triethylamine (1 mL, 0.01 mol), equivalent amount of either malononitrile **4a** (0.33 g, 0.005 mol) or ethyl cyanoacetate **4b** (0.56 g, 0.005 mol) was added. The reaction mixture in each case was heated under reflux for 3h and then was cooled at room temperature, poured over ice/water mixture and neutralized with dilute hydrochloric acid. The formed solid product, in each case filtered off, dried and crystallized from the appropriate solvent.

Compound 6a: Yellow crystals, from MeOH, yield 77% (1.35g), mp 150-152°C. IR (ν/cm^{-1}): 3450-3250 ($2NH_2$, NH), 2980, 2865 (CH_3, CH_2), 2200 (CN), 1730, 1690 (2C=O). ^1H NMR (δ ppm): 3.33 (s, 3H, CH_3), 3.43 (m, 2H, CH_2), 3.70 (m, 1H, CH), 4.62 (s, 4H, $2NH_2$, D_2O-exchangeable), 6.23 (s, 1H, C_3-pyridine ring proton), 6.62 (s, 1H, CH), 7.00-7.32 (m, 4H, C_6H_4), 10.87 (s, 1H, NH, D_2O-exchangeable). MS (m/z, %): 351 ($M^{+\cdot}$, 55%), 325 ($M^{+\cdot}$-CN, 35%), 130 (indolomethylene fragment, C_9H_8N, 100%). *Anal.* Calcd. for $C_{18}H_{17}N_5O_3$ (351.36): C, 61.53; H, 4.87; N, 19.93. Found: C, 61.21; H, 4.73; N, 19.67.

Compound 6b: Pale yellow crystals, from MeOH, yield 82% (1.44 g), mp 198-199°C. IR (ν/cm^{-1}): 3450-3250 (NH_2, NH), 3050 (CH-aromatic), 2225 (CN), 1730, 1700 (2C=O), 1630 (C=C). ^1H NMR (δ ppm): 3.38 (s, 3H, CH_3), 3.43 (m, 2H, CH_2), 3.75 (m, 1H, CH), 4.41 (s, 2H, NH_2, D_2O-exchangeable), 6.34 (s, 1H, C_3-pyridine ring proton), 6.82 (s, 1H, CH), 7.11-7.54 (m, 4H, C_6H_4), 10.05 (s, 1H, OH). *Anal.* Calcd. for $C_{18}H_{16}N_4O_4$ (352.35): C, 61.35; H, 4.57; N, 15.90. Found: C, 61.19; H, 4.28; N, 15.73.

Methyl α-imino(5-aminopyrazolo-3-yl)-3-indolopropanoate (8a)
Methylα-imino(5-amino-2-phenylpyrazolo-3-yl)-3-indolopropanoate (8b)

General Procedure. To a solution, of compound **3** (1.42 g, 0.005 mol) in ethanol (30 mL), containing a catalytic amount of triethylamine (2 mL, 0.02 mol), equivalent amount of either hydrazine hydrate (0.25 g, 0.005 mol) or phenylhydrazine (0.54 g, 0.005 mol) was added. The reaction mixture in each case was heated under reflux for 5 h and then was cooled at room temperature, poured over ice/water mixture and neutralized with dilute hydrochloric acid.

The formed solid product, in each case, was filtered off, washed with water and crystallized from the appropriate solvent.

Compound 8a: White crystals, from EtOH, yield 72% (1.08 g), mp 154-155°C. IR (ν/cm^{-1}): 3450-3250 (NH_2, 3NH), 3050 (CH-aromatic), 2985, 2865 (CH_3, CH_2), 1720 (C=O), 1660 (C=N), 1620 (C=C). ^{1}H NMR (δ ppm): 3.35 (s, 3H, CH_3), 3.46 (m, 2H, CH_2), 3.73 (m, 1H, CH), 4.50 (s, 2H, NH_2, D_2O-exchangeable), 5.83 (s, 1H, CH pyrazole), 6.51 (s, 1H, CH), 6.98-7.40 (m, 4H, C_6H_4), 9.83, 10.80, 10.92 (3s, 3H, 3NH, D_2O-exchangeable). *Anal.* Calcd. for $C_{15}H_{17}N_5O_2$ (299.33): C, 60.18, H, 5.72, N, 23.39. Found: C, 59.90; H, 5.43; N, 23.64.

Compound 8b: Brown crystals, from EtOH, yield 75% (1.40 g), mp. 90-91°C. IR (ν/cm^{-1}): 3400-3250 (NH_2, 2NH), 3050 (CH-aromatic), 2985, 2865 (CH_3, CH_2), 1730 (C=O), 1660 (C=N), 1620 (C=C). ^{1}H NMR (δ ppm): 3.40 (s, 3H, CH_3), 3.51 (m, 2H, CH_2), 3.74 (m, 1H, CH), 4.23 (s, 2H, NH_2, D_2O-exchangeable), 5.83 (s, 1H, CH pyrazole), 6.33 (s, 1H, CH), 7.32-7.55 (m, 9H, C_6H_4, C_6H_5), 10.80, 10.92 (2s, 2H, 2NH, D_2O-exchangeable). MS (m/z, %): 374 ($M^{+\cdot}$-1; 34%), 298 ($M^{+\cdot}$- C_6H_5, 57%), 77 (C_6H_5; 70%). *Anal.* Calcd. for $C_{21}H_{21}N_5O_2$ (375.43): C, 67.18; H, 5.62; N, 18.65. Found: C, 67.17; H, 5.49; N, 18.52.

3-Acetonitrilo-6-hydroxy-3-methylenoindolo-2,1,4 oxadiazine (9). To a solution of compound **3** (1.42 g, 0.005 mol) in ethanol (30 mL), containing a catalytic amount of sodium acetate (1.0 g) equivalent amount of hydroxylamine hydrochloric acid (0.35 g, 0.005 mol) in H_2O (5 mL) was added with stirring. The reaction mixture was stirred with warming for 30min, left to cool to 20 °C. The formed solid product was triturated ice/water mixture, collected by filtration and crystallized from MeOH. White crystals, yield 73% (0.97 g), mp 94-95°C. IR (ν/cm^{-1}): 3300-3200 (2NH, OH), 3050 (CH-aromatic), 2985, 2865 (CH_3, CH_2), 2200 (CN), 1660 (C = N), 1620 (C = C). ^{1}H NMR (δ ppm): 1.25 (s, 2H, CH_2), 3.91 (s, 2H, CH_2), 6.64 (s, 1H, CH), 7.00-7.71 (m, 4H, C_6H_4), 9.22, 9.52 (2s, 2H, 2NH, D_2O-exchangeable), 10.89 (s, 1H, OH). ^{13}C NMR (d): 20.3, 21.6 (2CH2), 88.4 (oxadiazine C-4), 118.7, 119.4, 120.6, 120.9, 121.5, 130.4, 136.9 (benzene, pyrrole C), 118.7 (CN). 179.8 (oxadiazine C-3). *Anal.* Calcd. for $C_{14}H_{12}N_4O_2$ (268.28). C, 62.68; H, 4.50; N, 20.83. Found: C, 62.59; H, 4.53; N, 20.77.

Methyl *α*-*N*-(5-amino-2,4-dicyanothiopheno-3-yl)-3-indolopropanoate (10a)

Methyl*α*-*N*-(ethyl 5-amino-2-cyanothiopheno-3-yl-4-carboxylate)-3-indolo-propanoate (10b)

General Procedure

To a mixture of compound **3** (1.42 g, 0.005 mol), elemental sulfur (0.16 g, 0.005mol) and either malononitrile **4a** (0.33 g, 0.005 mol) or ethyl cyanoacetate **4b** (0.56 g, 0.005 mol) in ethanol (30 mL), triethylamine (0.5 mL, 0.005 mol) was added. The reaction mixture in each case was heated under reflux for 4 h and then was cooled at room temperature, poured over ice/water mixture and neutralized with dilute hydrochloric acid. The formed solid product was filtered off, dried and crystallized from the appropriate solvent.

Compound 10a: Brown crystals, from MeOH, yield 73% (1.33 g), mp 106-107°C. IR (ν/cm^{-1}): 3400-3250 (NH_2, 2NH), 3050 (CH-aromatic), 2965, 2865 (CH_3, CH_2), 2210, 2225 (2CN), 1730 (C = O), 1620 (C = C). ^{1}H NMR (δ ppm): 3.39 (s, 3H, CH_3), 3.53 (m, 2H, CH_2), 3.75 (m, 1H, CH), 4.58 (s, 2H, NH_2, D_2O-exchangeable), 6.53 (s, 1H, CH), 7.01-7.60 (m, 4H, C_6H_4) 10.78, 11.94 (2s, 2H, 2NH, D_2O-exchangeable). *Anal.* Calcd. for $C_{18}H_{15}N_3SO_2$ (365.42): C, 59.16; H, 4.13; N, 19.16; S, 8.77. Found: C, 58.91; H, 4.05; N, 19.02; S, 8.51.

Compound 10b: Pale brown crystals, from EtOH, yield 75% (1.54g), mp 91-92°C. IR (ν/cm^{-1}): 3400-3250 (NH_2, 2NH) 3050 (CH-aromatic), 2965, 2865 (CH_3, CH_2), 2200 (CN), 1730, 1700 (2C=O), 1620 (C=C). ^{1}H NMR (δ ppm): 1.23 (t, 3H, J = 6.8 Hz, ester CH_3), 3.41 (s, 3H, CH_3), 3.53 (m, 2H, CH_2), 3.75 (m, 1H, CH), 4.24 (q, 2H, J = 6.8 Hz, ester CH_2), 6.50 (s, 2H, NH_2, D_2O-exchangeable), 6.99 (s, 1H, CH), 7.04-7.49 (m, 4H, C_6H_4), 10.71, 10.88 (2s, 2H, 2NH, D_2O-exchangeable). MS (m/z, %): 412 ($M^{+\cdot}$, 62%). *Anal.* Calcd. for $C_{20}H_{20}N_4O_4S$ (412.47): C, 58.23; H, 4.88; N, 13.58; S, 7.77. Found: C, 58.11; H, 4.72; N, 13.43; S, 7.62.

Methyl *α*-*N*-(phenylhydrazoacetonitrilocarbamido)-3 indolopropanoate (11). A solution of compound **3** (1.42 g, 0.05 mol), in ethanol (30 mL) containing a catalytic amount of sodium acetate (1.0 g) was cooled to 0-5°C and then treated gradually with a cold solution of benzenediazonium chloride (prepared from the appropriate quantities of aniline, HCl and $NaNO_2$). After addition of the diazonium salt was completed, the reaction mixture was stirred at room temperature for 30 min. The solid product, separated upon dilution with cold water, was filtered off, washed with water several times, dried and crystallized from EtOH to yield 72% (1.40 g) pale brown crystals, mp 94-95°C. IR (ν/cm^{-1}): 3400, 3300 (3NH), 3050 (CH-aromatic), 2985, 2865 (CH_3, CH_2), 2200 (CN), 1720, 1690 (2C=O), 1660 (C=N), 1630 (C=C). ^{1}H NMR (δ ppm): 1.27 (s, 1H, CH), 3.37 (s, 3H, CH_3), 3.47 (m, 2H, CH_2), 3.70 (m, 1H, CH), 5.64 (s, 1H, CH), 7.67-7.74 (m, 9H, C_6H_4, C_6H_5), 9.25, 9.97-9.99 (3s, 3H, 3NH, D_2O-exchangeable). *Anal.* Calcd. for $C_{21}H_{19}N_5O_3$ (389.41): C, 64.77; H, 4.91; N, 17.98. Found: C, 64.67; H, 4.89; N, 17.88.

Methyl *α*-*N*-(benzaloacetonitrilocarbamido)-3-indolopropanoate (12). To a solution of compound **3** (1.42 g, 0.005 mol) in ethanol (30 mL), containing triethylamine (1 mL, 0.01 mol), equivalent amount of benzaldehyde (0.53 g, 0.005 mol) was added. The reaction mixture was heated under reflux for 3h. Then poured over ice/water mixture and neutralized with dilute hydrochloric acid. The formed solid product filtered off, dried and crystallized from EtOH. Pale brown crystals, yield 77% (1.43 g), mp 81-82°C. IR (ν/cm^{-1}): 3400-3200 (2NH), 3050 (CH-aromatic), 2985, 2865 (CH_3, CH_2), 2200 (CN), 1720, 1690 (2C=O). ^{1}H NMR (δ ppm): 3.40 (s, 3H, CH_3), 3.59 (m, 2H, CH_2), 3.72 (m, 1H, CH), 4.10 (s, 1H, CH), 7.08 (s, 1H, CH), 7.31-7.72 (m, 9 H, C_6H_4, C_6H_5), 8.42, 10.93 (2s, 2H, 2NH, D_2O-exchangeable). MS (m/z, %): 374 ($M^{+\cdot}$, 22%), 315 ($M^{+\cdot}$-$COOCH_3$, 21%), 284 ($M^{+\cdot}$- CHPh, 20%), 130, (indolomethylene fragment, C_9H_8N, 100%). *Anal.* Calcd. for $C_{22}H_{19}N_3O_3$ (373.41): C, 70.76; H, 5.12; N, 11.25. Found: C, 70.55; H, 5.11; N, 11.13.

Methyl *α*-(6-amino-3,5-dicyano-2-oxo-4-phenylpyrido-1-yl) -3-indolopropanoate (14a)

Methyl *α*-(ethyl 6-amino-3-cyano-2-oxo-4-phenylpyrido-1-yl-5-carboxylate)-3-indolopropanoate (14b)

General Procedure. To a solution of compound **12** (1.86 g, 0.005 mol) in ethanol (30 mL), containing (1 mL, 0.01 mol), equivalent amount of either malononitrile **4a** (0.33 g, 0.005 mol), or ethyl canoacetate **4b** (0.56 g, 0.005 mol) was added. The reaction mixture, in each case, was heated under reflux for 3h then poured over ice/water mixture and neutralized with hydrochloric acid. The formed solid product, in each case filtered off, dried, and crystallized from the appropriate solvent.

Compound 14a: yellow crystals, from EtOH, yield 72% (1.58g), mp 118-120°C. IR (ν/cm^{-1}): 3450-3350 (NH_2), 3300, 3200 (2NH), 3050 (CH-aromatic), 2985, 2865 (CH_3, CH_2), 2220, 2215 (2 CN), 1730, 1690 (2C = O). ^{1}H NMR (δ ppm): 3.39 (s, 3H, CH_3), 3.53 (m, 2H, CH_2), 3.75 (m, 1H, CH), 4.46 (s, 2H, NH_2, D_2O-exchangeable), 6.51, 6.57 (2d, 2H, J = 9.7 Hz, 2CH), 6.60 (s, 1H, CH), 7.02-7.60 (m, 9 H, C_6H_5, C_6H_4), 11.02 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (δ): 18.9 (CH2), 48.7 (CH3), 55.8 (CH), 80.9, 11.6, 113.4, 115.9, 118.4, 120.1, 122.7, 130.5, 133.8 (benzene, pyrrole C), 118.6, 119.2 (2 CN), 160.3, 170.4 (2CO). *Anal.* Calcd. for $C_{25}H_{21}N_5O_3$ (439.37): C, 68.32; H, 4.81; N, 15.93. Found: C, 68.25; H, 4.73; N, 15.85.

Compound 14b: White crystals, from dioxane, yield 77% (1.87 g), mp 77-78°C. IR (ν/cm^{-1}): 3450-3250 (NH_2, NH), 3050 (CH-aromatic), 2985, 2865 (CH_3, CH_2), 2200, (CN), 1735, 1730, 1700 (3C=O), 1630 (C=C). ^{1}H NMR (δ ppm): 1.33 (t, J = 6.8 Hz, 3H, ester CH_3), 3.45 (s, 3H, CH_3), 3.52 (m, 2H, CH_2), 3.68 (m, 1H, CH), 4.32 (q, 2H, J = 6.8 Hz, ester CH_2), 5.18 (s, 2H, NH_2, D_2O-exchangeable), 6.46, 6.52 (2d, 2H, J = 9.7 Hz, 2CH), 6.73 (s, 1H, CH), 7.23-7.92 (m, 9H, C_6H_5, C_6H_4), 10.93 (s, 1H, NH, D_2O-exchangeable). MS (m/z, %): 486 ($M^{+\cdot}$, 35%), 202 [$M^{+\cdot}$-$C_{15}H_{14}N_3O_3$ (substituted pyridine fragment), 34%], 130 (indolomethylene fragment, C_9H_8N, 100%). *Anal.* Calcd. for $C_{27}H_{26}N_4O_5$ (486.53): C, 66.65; H, 5.38; N, 11.51. Found: C, 66.75; H, 5.28; N, 11.31.

Methyl-*α*-iminocarbonyl(3-amino-5-phenylpyrazolo-4-yl) -3-indolopropanoate (15a)

Methyl *α*-iminocarbonyl(3-amino-1,5-diphenylpyrazolo-4-yl) -3-indolopropanoate (15b)

General Procedure

To a solution of compound **12** (1.86 g, 0.005 mol) in ethanol (30 mL), containing triethylamine (1 mL, 0.01 mol), either hydrazine hydrate (0.25 g, 0.005 mol) or phenylhydrazine (0.54 g, 0.005 mol) was added. The reaction mixture, in each case, was heated under reflux for 4h then poured over ice/water mixture and neutralized with hydrochloric acid. The formed solid prod-

uct, in each case, was filtered off, washed with water and crystallized from the appropriate solvent.

Compound 15a: Yellow crystals, from EtOH, yield 77% (1.55 g), mp 100-101°C. IR (ν/cm^{-1}): 3450-3150 (NH_2, 3NH), 3050 (CH-aromatic), 2985, 2865 (CH_3, CH_2), 1730, 1700 (2C=O), 1660 (C=N), 1630 (C=C). ^{1}H NMR (δ ppm): 3.43 (s, 3H, CH_3), 3.56 (m, 2H, CH_2), 3.75 (m, 1H, CH), 4.83 (s, 2H, NH_2, D_2O-exchangeable), 6.93 (s, 1H, CH), 7.05-7.72 (m, 9 H, C_6H_5, C_6H_4), 9.48, 10.91, 10.92 (3s, 3H, 3NH, D_2O-exchangeable). MS (m/z, %): 404 (M^{+}+1; 70%), 336 (M^{+}- C_6H_5, 50%), 77 (C_6H_5; 36%). *Anal.* Calcd. for $C_{22}H_{21}N_5O_3$ (403.44): C, 65.49; H, 5.24; N, 17.35. Found: C, 65.32; H, 5.11; N, 17.15.

Compound 15b: White crystals from MeOH, yield 72% (1.72 g), mp 136-137°C. IR (ν/cm^{-1}): 3450-3250 (NH_2, 2NH), 3050 (CH-aromatic), 2985, 2685 (CH_3, CH_2), 1730, 1690 (2C=O), 1660 (C=N), 1630 (C=C). ^{1}H NMR (δ ppm): 3.43 (s, 3H, CH_3), 3.53 (m, 2H, CH_2), 3.75 (m, 1H, CH), 4.85 (s, 2H, NH_2, D_2O-exchangeable), 6.73 (s, 1H, CH), 6.76-7.80 (m, 14H, $2C_6H_5$, C_6H_4), 10.37, 10.93, (2s, 2H, 2NH, D_2O-exchangeable). *Anal.* Calcd. for $C_{28}H_{25}N_5O_3$ (479.54): C, 70.13; H, 5.25; N, 14.60. Found: C, 69.99; H, 5.11; N, 14.42.

Methyl α-iminocarbonyl(3-amino-5-phenylisooxazolo-4-yl) -3-indolopropanoate (16). To a solution of compound **12** (1.86 g, 0.005 mol) in ethanol (30 mL), containing a catalytic amount of sodium acetate (1.0 g), equivalent amount of hydroxylamine hydrochloride acid (0.35 g, 0.005 mol) in ethanol (10 mL) was added with stirring and warming. The solid product formed at room temperature triturated with ice/water mixture and collected by filtration. Yellowish crystals, from MeOH, yield 75% (1.51 g), mp 136-137°C. IR (ν/cm^{-1}): 3450-3250 (NH_2, 2NH), 3050 (CH-aromatic), 2985, 2865 (CH_3, CH_2), 1730, 1690 (2C=O), 1660 (C=N), 1630 (C=C). ^{1}H NMR (δ ppm): 3.38 (s, 3H, CH_3), 3.55 (m, 2H, CH_2), 3.72 (m, 1H, CH), 4.83 (s, 2H, NH_2, D_2O-exchangeable), 6.31 (s, 1H, CH), 7.04-7.71 (m, 9 H, C_6H_4, C_6H_5), 10.91, 10.97 (2s, 2H, 2NH, D_2O-exchangeable). *Anal.* Calcd. for $C_{22}H_{20}N_4O_4$ (404.43): C, 65.33; H, 4.98; N, 13.85. Found: C, 65.11; H, 4.72; N, 13.79.

***α*-Imino(acetonitrilocarbamido)-3-indolopropionic acid (17).** To a solution of tryptophan **1** (1.02 g, 0.005 mol) in dimethyl formamide (30 mL), equimolar amount of ethyl cyanoacetate **4b** (0.56 g, 0.005 mol) was added. The reaction mixture was heated under reflux for 4h and then was evaporated in vacuum. The remaining product was triturated with diethyl ether, and the solid product was collected by filtration. Yellow crystals, from EtOH, yield 79% (1.07 g), mp 146-147°C. IR (ν/cm^{-1}): 3430-3385 (2NH), 3050 (CH-aromatic), 2680 (CH_2), 2220 (CN), 1705, 1680 (2C=O), 1620 (C=C). ^{1}H NMR (δ ppm): 3.43 (m, 2H, CH_2), 3.49 (s, 2H, CH_2CN), 3.75 (m, 1H, CH), 6.95 (s, 1H, CH), 7.01-7.49 (m, 4H, C_6H_4), 8.75, 8.82 (2s, 2H, 2NH, D_2O-exchangeable), 11.15 (s, 1H, OH). *Anal.* Calcd. for $C_{14}H_{13}N_3O_3$ (271.28): C, 61.98; H, 4.83; N, 15.48. Found: C, 61.77; H, 4.65; N, 15.21.

***α*-Imino(benzaloacetonitriocarbamido)-3-indolopropionic acid (18).** To a solution of compound **17** (1.35 g, 0.005 mol), in ethanol (30 mL), containing piperidine (0.85 mL, 0.01 mol), equivalent amount of benzaldehyde (0.53 g, 0.005 mol) was added. The reaction mixture was heated under reflux for 3h then poured over ice/water mixture and neutralized with dilute hydrochloric acid. The formed solid product filtered off, dried and crystallized from dilute ethanol. Pale brown crystals, yield 75% (1.21 g), mp 177-178°C. IR (γ/cm^{-1}): 3400-3200 (2NH), 3050 (CH-aromatic), 2865 (CH_2), 2200 (CN), 1720, 1690 (2C=O). ^{1}H NMR (δ ppm): 3.53 (m, 2H, CH_2), 3.72 (m, 1H, CH), 4.10 (s, 1H, CH), 6.48 (s, 1H, CH), 7.31-7.72 (m, 9H, C_6H_4, C_6H_5), 8.42, 9.11 (2s, 2H, 2NH, D_2O-exchangeable), 11.15 (s, 1H, OH). MS (m/z, %): 359 (M^+, 35%). *Anal.* Calcd. for $C_{21}H_{17}N_3O_3$ (359.38): C, 70.18; H, 4.76; N, 11.69. Found: C, 70.31; H, 4.53; N, 11.45.

***α*-Imino(phenylhydrazoacetonitrilocarbamido)-3-indolopropionic acid (19).** A solution of compound **17** (1.35 g, 0.005 mol), in ethanol (30 mL), containing a catalytic amount of sodium acetate (1.0 g) was cooled to 0-5°C and then gradually with a cold solution of benzenediazonium chloride [prepared by adding sodium nitrite solution (0.7 g, 0.01 mol) to a cold solution of aniline 0.45 g, 0.005 mol) with continuous stirring]. After addition of diazonium salt was completed, the reaction mixture was stirred at room temperature for 30 min. The solid product, separated upon dilution with cold water, was filtered off, washed with water several times, dried and crystallized. Brown crystals from EtOH, yield 75% (1.40 g), mp 79-80°C. IR (γ/cm^{-1}): 3430-3335 (3NH), 3050 (CH-aromatic), 2865 (CH_2), 2200 (CN), 1720, 1690 (2C=O), 1660 (C=N), 1630 (C=C). ^{1}H NMR (δ ppm): 1.27 (s, 1H, CH), 3.28 (m, 2H, CH_2), 3.75 (m, 1H, CH), 5.64 (s, 1H, CH), 7.67-7.74 (m, 9H, C_6H_4, C_6H_5), 8.51, 9.32-9.36 (3s, 3H, 3NH, D_2O-exchangeable), 11.40 (s, 1H, OH). ^{13}C NMR (δ): 25.0 (CH_2), 55.8 (CH), 80.7, 111.6, 113.6, 114.8, 118.6, 120.4, 122.2, 130.4, 133.9 (benzene, pyrrole C), 117.6 (CN), 150.3, 166.4 (2 CO), 177.9 (C=N). *Anal.* Calcd. for $C_{20}H_{17}N_5O_3$ (375.39): C, 63.99; H, 4.56; N, 18.65. Found: C, 63.78; H, 4.43; N, 18.59.

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REFERENCES

[1] T. R. Webb, C. Eigenbrot, J. Org. Chem. **56**, 3009, (1991).
[2] C. H. Stammer, Tetrahedron **46**, 2231, (1990).
[3] P. W. Schiller, G. Weltrowska, T. M. D. Nguyen, C. Lemieux, N. N. Chung, B. J. Marsden, B. C. Wilkes, J. Med. Chem., **34**, 3125 (1991).
[4] G.M. Coppola, H.F. Schuster, Asymmetric Synthesis and Construction of Chiral Molecules Using Amino Acid, John Wiley& Sons: New York, 1987.
[5] A. Baeyer, A. Emmerting, Ber., **2**, 679 (1869).
[6] E. Fischer, F. Jourdan, Ber., **16**, 2241 (1883).
[7] J. Bergman, E. Koch, B. Pelcman, Tetrahedron, **51**, 5631 (1995).
[8] Ninomiya, Ichiya J. Nat. prod., **55**, 541 (1992).
[9] B.A. Morgan, J. Singh, E. Baizman, H. Bently, D. Keifer, S. Ward, In Proceedings of the 10th: American Peptide Symposium; Marshall G. R., Ed., Escom, Leiden, 1988, pp 508-509.
[10] J. Y. L. Chung, J. T. Wasicak, A. M. Nadzen, Synthetic Commun, **22**, 1039 (1992).
[11] D. C. Horwell, P. D. Nichols, E. Roberts, Tetrahedron Lett., **35** (6), 939 (1994).
[12] A. Janczuk, W. Zhang, W. Xie, S. Lou, J. Cheng, P.G. Wang, Tetrahedron Lett., **43**, 4271 (2002).
[13] V. S. Goodfellow, M. Settineri, R.G. Lawton, Bioochemistry, **28**, 6346 (1989).
[14] T. Fujiwara, K. Omata, K. Kabuto, C. Kabuto, T. Takahashi, M. Segawa, Y. Takeuchi, Chem. Commun., 2694 (2001).
[15]S. H. Doss, R. M. Mohareb, G. A. Elmegeed, N. A. Louca, Pharmazie, **58**, 607 (2003).
[16] S.H. Doss, N. A. Louca, G.A. Elmegeed, R.M. Mohareb, Arch. Pharm. Res., **22**(5), 496 (1999).
[17] G.A. Elmegeed, H.H. Ahmed, J. S. Hussein, Eur. J. Med. Chem., **40**, 1283 (2005).
[18] M.J. Queiroz, I.C. Ferreira, Y.D. Gaetano, G. Kirsch, R.C. Calhelha, L.M. Estevinho, Bioorg. Med. Chem., **14**(20), 6827 (2006).
[19] R.G. Christiansen, M.R. Bell, T.E. D'Ambra, J.P. Mallamo, J.L. Herrmann, J.H. Ackerman, C. J. Opalka, R.K. Kullnig, R.C. Winneker, B.W. Snyder, F.H. Batzold, H.P. Schane, J. Med. Chem., **33**, 2094 (1990).