# Synthesis of Hydroxy-substituted Aza-analogues of Antitumor Anthrapyrazoles



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Synthetic routes have been developed which lead to ring-hydroxylated aza-analogues of antitumor anthrapyrazoles, namely, 2,5-bis[(aminoalkyl)amino] substituted 10-hydroxyindazolo[3,4-*fg*]isoquinolin-6(2*H*)-ones **1** and 7-hydroxyindazolo[4,3-*gh*]isoquinolin-6(2*H*)-ones **2**. The regiospecific synthesis of 6,9-dihalo-4-hydroxybenz[*g*]isoquinolines **3** and **4** has been accomplished. Intermediate **3** was constructed in a multistep process involving Diels-Alder chemistry of benzoylacrylates whereas **4** was assembled using Ni(II) mediated coupling of methyl 3-chloro-5-methoxyisonicotinate (**15b**) with the organic zinc reagent **18** derived from 2-fluoro-5-chlorobenzyl bromide (**17**). After protection of the hydroxy group with a *p*-methoxybenzyl moiety, the different nucleofugacities of the leaving groups present in **10** and **20** allowed sequential displacements by substituted hydrazines and amines, respectively, to lead to the desired *p*-methoxybenzyl protected analogues **12** and **22**. Deprotection led to the side arm modified compounds **1** and **2**. The displacements of **21a** and **21b** with *N,N*-dimethylethylenediamine also led to the tri[(aminoalkyl)amino]substituted analogues **23a** and **23b**, respectively, which arose from further $S_NAr$ substitutions of the *p*-methoxybenzyloxy group.

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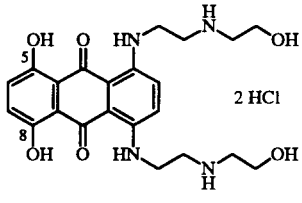
## Introduction.

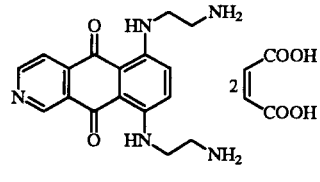
Mitoxantrone is an antitumor anthracene-9,10-dione which finds clinical use in the management of breast cancer although it is not devoid of cardiotoxicity [1]. With the goal of developing drug candidates which would represent safer substitutes for mitoxantrone, we have exploited the systematic incorporation of nitrogen atoms at different positions of the anthracene-9,10-dione chromophore. This study has led to the identification of the antitumor properties of BBR 2778 [2-5], a non-cardiotoxic benz[*g*]isoquinoline-5,10-dione which is currently in phase I clinical trials.

The introduction of a 4-hydroxy substituent [6] into the benz[*g*]isoquinoline-5,10-dione chromophore leads to compounds (Figure 1) of higher potency [3].

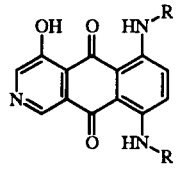
Our heteroatom manipulation approach has been expanded to anthrapyrazoles, which represent a class of chromophore-modified anthracene-9,10-diones with good clinical efficacy in the treatment of breast cancer [7,8]. We have reported the preparation and the antitumor properties of the regioisomers bearing nitrogen atoms at positions 7, 8, 9 or 10 of the anthrapyrazole chromophore [9]. The 9-aza-regioisomers represent the most active chemotypes [10]. The enhanced potency exhibited by the 4-hydroxy-6,9-bis[(aminoalkyl)amino]benz[*g*]-isoquinoline-5,10-diones, in comparison to the respective non-hydroxylated analogues, suggested that it would be of considerable interest to study the antitumor properties of compounds bearing hydroxy substitution on the aza-anthrapyrazole chromophore.



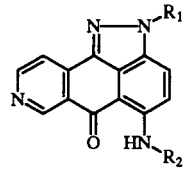

Mitoxantrone



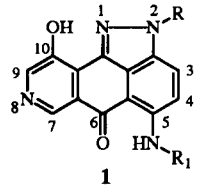

BBR 2778



4-Hydroxy-5,8-Bis[(aminoalkyl)-aminobenz[*g*]isoquinoline-5,10-diones



2,5-Bis[(aminoalkyl)amino]anthra-[1,9-*cd*]pyrazol-6(2*H*)-ones (anthrapyrazoles)



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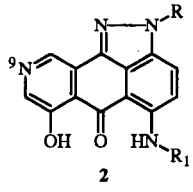

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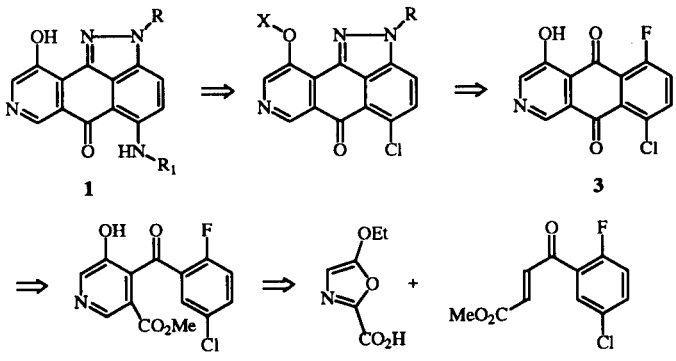
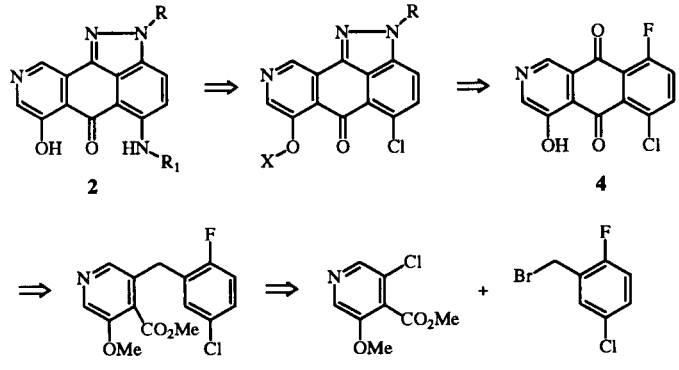
Figure 1. Structures where R, $R_1$ and $R_2$ are alkylamino groups.

Scheme 1

10-Hydroxy-8-aza-anthrapyrazoles



7-Hydroxy-9-aza-anthrapyrazoles



In this manuscript we report the synthesis of ring-hydroxylated chemotypes 2,5-bis[(aminoalkyl)amino]substituted 10-hydroxyindazolo[3,4-*fg*]isoquinolin-6(2*H*)ones **1** (10-hydroxy-8-aza-anthrapyrazoles) and 7-hydroxyindazolo[4,3-*gh*]isoquinolin-6(2*H*)ones **2** (7-hydroxy-9-azaanthrapyrazoles) [11].

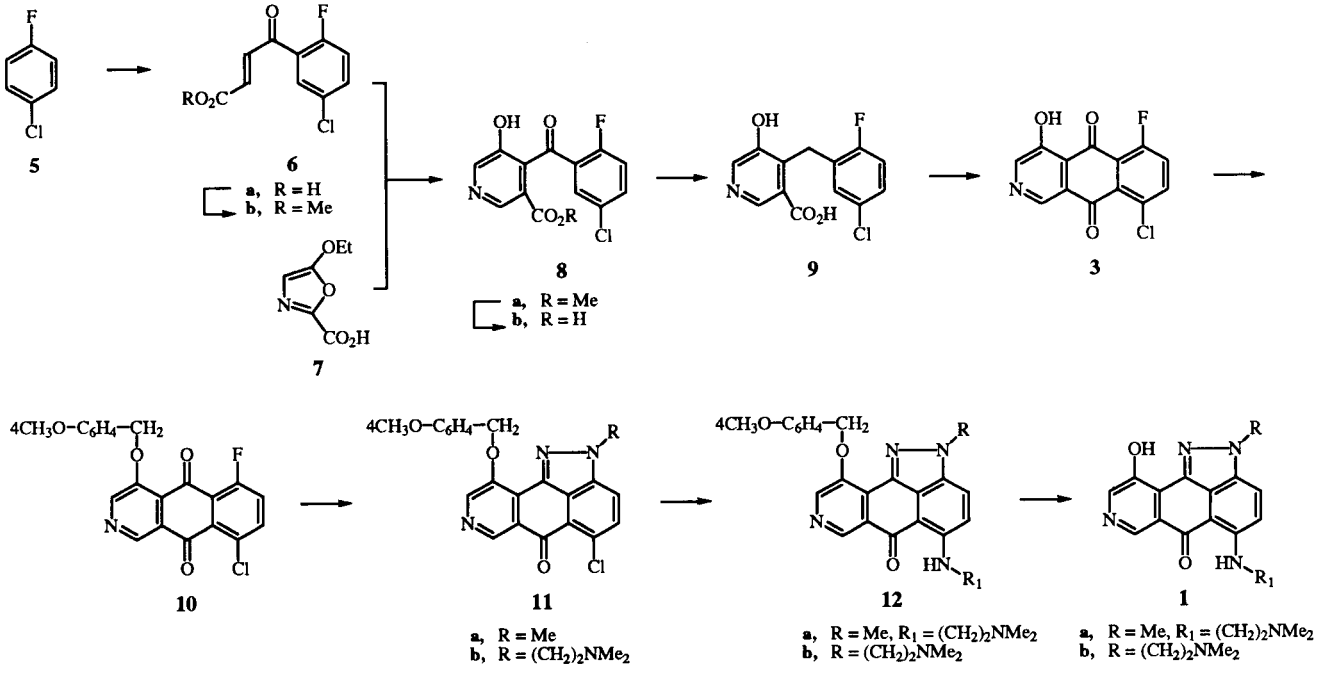
Synthesis.

Retrosynthetic analysis of 10-hydroxy-8-aza-anthrapyrazoles **1** and 7-hydroxy-9-aza-anthrapyrazoles **2** led to the recognition of 6-fluoro-9-chloro-4-hydroxybenz[*g*]isoquinoline-5,10-dione (**3**) and 6-chloro-9-fluoro-4-hydroxybenz[*g*]isoquinoline-5,10-dione (**4**) as critical intermediates (Scheme 1).

A fluorine and a chlorine atom were chosen as the substituents at positions 6 and 9 in **3** or **4**, respectively, because of their differences in nucleofugacity in $S_NAr$ displacements. Access to the hydroxybenz[*g*]isoquinoline-5,10-diones **3** or **4** could be achieved by exploiting Friedel-Crafts and Diels-Alder chemistry or Ni(II) mediated couplings, respectively. After protection of the hydroxy groups of **3** or **4** (where X = *p*-methoxybenzyl), regioselective construction of the 2-alkylaminopyrazole ring (R = alkylamino) would be accomplished by the displacement of fluoride by a hydrazine and the subsequent introduction of the aminoalkylamino side arms ($R_1$ = alkylamino) would be performed by displacement of the chloride at position 5. Removal of the protective group would lead to the desired analogues **1** and **2**.

Scheme 2

10-Hydroxy-8-aza-anthrapyrazoles



10-Hydroxy-8-aza-anthrapyrazoles.

The synthetic pathway to these analogues is outlined in Scheme 2.

Friedel-Crafts acylation of 1-chloro-4-fluorobenzene (**5**) with maleic anhydride and aluminum chloride led predominantly to the *E*-regioisomer **6a** which could be separated from a small amount of the regioisomer resulting from acylation *ortho* to the chlorine atom by recrystallizations from benzene or ethyl acetate. The assignment of *E*-stereochemistry is based on the magnitudes of the $^{1}H$ and $^{19}F$ coupling constants exhibited in the $^{1}H$ nmr spectrum. The regioisomer **6a** was found to be light sensitive with the apparent formation of [2 + 2] cycloaddition products. The conversion of acid **6a** to ester **6b** was accomplished by treatment of the acid with methanolic sulfuric acid, or, more efficiently, by treatment with methyl chloroformate, methanol and triethylamine. The ester **6b** on being heated with 5-ethoxyoxazole-2-carboxylic acid (**7**) afforded the cycloadduct **8a**. Acid **7** was readily prepared following a literature procedure [6]. The structure of **8a** was firmly established by X-ray crystallographic analysis [12]. The ORTEP diagram is shown in Figure 2 and pertinent crystallographic data are tabulated in Tables 1, 2, 3, 4 and 5.

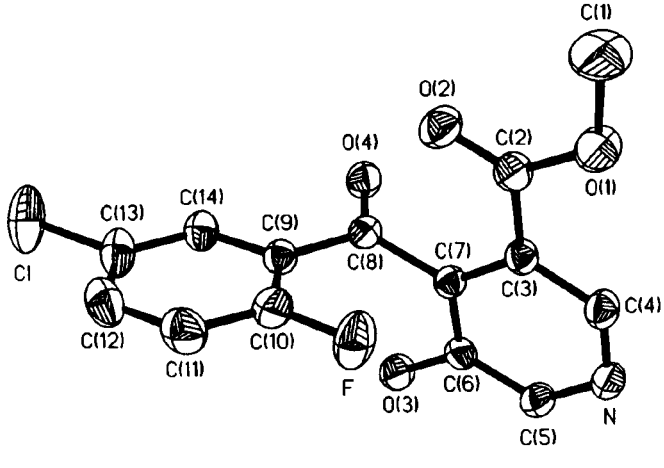



Figure 2. X-ray Crstallographic Structure of **8a**.

Ester **8a** was hydrolyzed with aqueous methanolic sodium hydroxide and after acidification afforded acid **8b**. Upon heating **8b** in fuming sulfuric acid, regioisomers **3** and **4** were formed in an 85:15 ratio, respectively. This ratio was obtained from the integration areas of the hydrogen bonded upfield -OH protons in the $^{1}H$ nmr spectra at δ 11.48 (**3**) and 11.55 (**4**). The lack of regiospecificity in the cyclization is attributable to a Hayashi-type rearrangement [13-15].

To circumvent this rearrangement, the carbonyl group present in **8b** was reduced by treatment with zinc in formic acid to yield **9**. Regioisomerically pure dione **3** was prepared by heating **9** in fuming sulfuric acid. Under these conditions, cyclization followed by oxidation of the azaanthrone (or azaanthranol) intermediate occurred.

The protection of the hydroxy group of **3** was necessary in order to avoid nucleophilic addition to the 3 position of the pyridine ring in the subsequent displacement steps [6].

Table 1

Structure Determination Summary

Crystal Data

| | |
|---|---|
| Empirical Formula | $C_{14}H_8ClFN_5$ |
| Color; Habit | colorless, irregular |
| Crystal size (mm) | .35x.25x.20 |
| Crystal System | Monoclinic |
| Space Group | $P2_1/n$ |
| Unit Cell Dimensions | a = 9.815(3) Å |
| | b = 11.665(3) Å |
| | c = 13.487(4) Å |
| | β = 109.950(0)° |
| Volume | 1451.5(7) Å$^3$ |
| Z | 4 |
| Formula weight | 324.7 |
| Density(calcd.) | 1.486 Mg/m$^3$ |
| Absorption Coefficient | 0.297 mm$^{-1}$ |
| F(000) | 660 |

Table 2

Bond lengths (Å)

| | | | | | |
|---|---|---|---|---|---|
| C1-C(13) | 1.728 | (3) | F-C(10) | 1.366 | (4) |
| O(1)-C(1) | 1.445 | (4) | O(1)-C(2) | 1.327 | (4) |
| O(2)-C(2) | 1.200 | (3) | O(3)-C(6) | 1.341 | (3) |
| O(4)-C(8) | 1.211 | (4) | N-C(4) | 1.332 | (3) |
| N-C(5) | 1.327 | (4) | C(2)-C(3) | 1.484 | (3) |
| C(3)-C(4) | 1.380 | (4) | C(3)-C(7) | 1.386 | (4) |
| C(5)-C(6) | 1.384 | (4) | C(6)-C(7) | 1.395 | (3) |
| C(7)-C(8) | 1.502 | (4) | C(8)-C(9) | 1.487 | (4) |
| C(9)-C(10) | 1.390 | (5) | C(9)-C(14) | 1.392 | (4) |
| C(10)-C(11) | 1.369 | (5) | C(11)-C(12) | 1.365 | (5) |
| C(12)-C(13) | 1.379 | (6) | C(13)-C(14) | 1.376 | (5) |

Table 3

Bond angles (°)

| | | | |
|---|---|---|---|
| C(1)-O(1)-C(2) | 116.3(3) | C(4)-N-C(5) | 118.3(2) |
| O(1)-C(2)-O(2) | 124.6(2) | O(1)-C(2)-C(3) | 111.9(2) |
| O(2)-C(2)-C(3) | 123.5(3) | C(2)-C(3)-C(4) | 121.7(2) |
| C(2)-C(3)-C(7) | 119.4(2) | C(4)-C(3)-C(7) | 118.9(2) |
| N-C(4)-C(3) | 122.8(2) | N-C(5)-C(6) | 123.2(2) |
| O(3)-C(6)-C(5) | 123.7(2) | O(3)-C(6)-C(7) | 118.1(2) |
| C(5)-C(6)-c(7) | 118.2(2) | C(3)-C(7)-C(6) | 118.5(2) |
| C(3)-C(7)-C(8) | 124.6(2) | C(6)-C(7)-C(8) | 116.9(2) |
| O(4)-C(8)-C(7) | 119.2(3) | O(4)-C(8)-C(9) | 120.6(2) |
| C(7)-C(8)-C(9) | 119.9(3) | C(8)-C(9)-C(10) | 124.9(2) |
| C(8)-C(9)-C(14) | 117.6(3) | C(10)-C(9)-C(14) | 117.5(3) |
| F-C(10)-C(9) | 120.6(3) | F-C(10)-C(11) | 116.8(3) |
| C(9)-C(10)-C(11) | 122.6(3) | C(10)-C(11)-C(12) | 119.4(4) |
| C(11)-C(12)-C(13) | 119.2(3) | C1-C(13)-C(12) | 119.6(3) |
| C1-C(13)-C(14) | 118.6(3) | C(12)-C(13)-C(14) | 121.8(3) |
| C(9)-C(14)-C(13) | 119.4(3) | | |

Table 4
Anisotropic Displacement Coefficients ($Å^2 \times 10^3$)

| | $U_{11}$ | $U_{22}$ | $U_{33}$ | $U_{12}$ | $U_{13}$ | $U_{23}$ |
|---|---|---|---|---|---|---|
| Cl | 104(1) | 33(1) | 147(1) | -6(1) | 65(1) | -1(1) |
| F | 85(1) | 40(1) | 87(1) | -7(1) | 54(1) | -17(1) |
| O(1) | 53(1) | 44(1) | 72(1) | 15(1) | -13(1) | -5(1) |
| O(2) | 42(1) | 47(1) | 57(1) | -5(1) | 3(1) | -5(1) |
| O(3) | 51(1) | 32(1) | 48(1) | 10(1) | 6(1) | 0(1) |
| O(4) | 57(1) | 38(1) | 42(1) | -4(1) | 24(1) | -5(1) |
| O(5) | 51(1) | 48(1) | 47(1) | -17(1) | 7(1) | -5(1) |
| N | 45(1) | 32(1) | 43(1) | -1(1) | 9(1) | -4(1) |
| C(1) | 60(2) | 74(3) | 85(3) | 30(2) | -20(2) | -6(2) |
| C(2) | 39(2) | 38(2) | 43(2) | 4(1) | 12(1) | 0(1) |
| C(3) | 35(1) | 30(1) | 38(1) | 2(1) | 11(1) | 1(1) |
| C(4) | 42(2) | 30(1) | 45(2) | 3(1) | 12(1) | -2(1) |
| C(5) | 39(1) | 37(2) | 38(1) | -2(1) | 5(1) | -2(1) |
| C(6) | 38(1) | 31(1) | 34(1) | 4(1) | 11(1) | 3(1) |
| C(7) | 34(1) | 31(1) | 32(1) | -3(1) | 13(1) | -1(1) |
| C(8) | 30(1) | 31(1) | 36(1) | 3(1) | 9(1) | -1(1) |
| C(9) | 33(1) | 30(1) | 38(1) | 0(1) | 10(1) | 1(1) |
| C(10) | 45(2) | 41(2) | 46(2) | -1(1) | 16(1) | -3(1) |
| C(11) | 54(2) | 64(2) | 54(2) | -5(2) | 28(2) | 5(2) |
| C(12) | 49(2) | 54(2) | 66(2) | -4(2) | 23(2) | 17(2) |
| C(13) | 47(2) | 34(2) | 75(2) | -2(1) | 19(2) | 7(1) |
| C(14) | 43(2) | 33(1) | 52(2) | 1(1) | 17(1) | 1(1) |

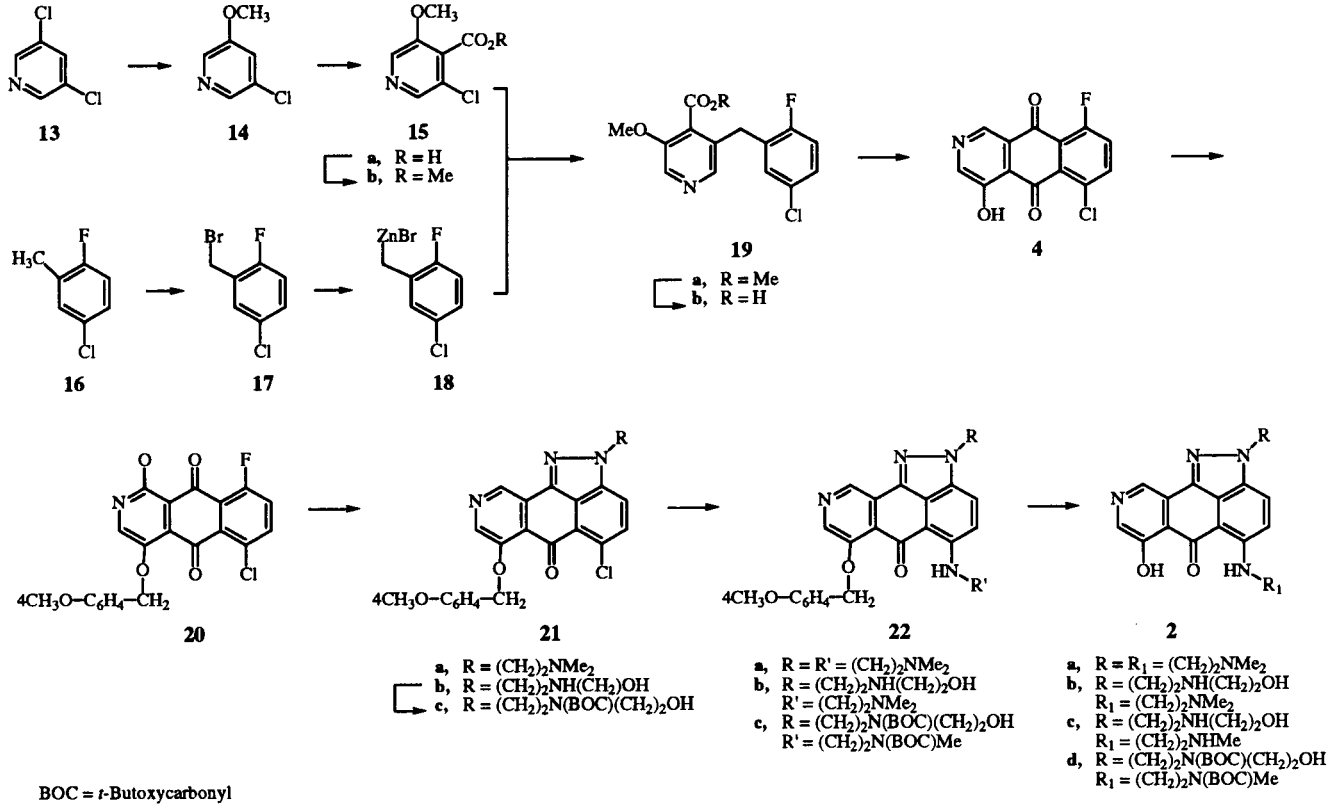
The anisotropic displacement exponent takes the form:
$-2p^2 (h^2a^{*2}U_{11} + ... + 2hka^*b^*U_{12})$

Table 5
Atomic Coordinates ($\times 10^4$) and equivalent isotropic displacement coefficients ($A^2 \times 10^3$)

| | x | y | z | u(eq) |
|---|---|---|---|---|
| Cl | 1860(1) | 2503(1) | 10223(1) | 89(1) |
| F | 2014(2) | 7284(2) | 11446(2) | 64(1) |
| O(1) | 1320(3) | 9867(2) | 9155(2) | 65(1) |
| O(2) | 1213(2) | 7961(2) | 8957(2) | 53(1) |
| O(3) | 6103(2) | 6777(2) | 11527(2) | 46(1) |
| O(4) | 3810(2) | 6344(2) | 9213(2) | 44(1) |
| O(5) | 6191(2) | 1947(2) | 2292(2) | 51(1) |
| N | 5217(3) | 9795(2) | 11484(2) | 41(1) |
| C(1) | -79(4) | 9974(4) | 8335(3) | 84(2) |
| C(2) | 1837(3) | 8811(3) | 9374(2) | 41(1) |
| C(3) | 3291(3) | 8798(2) | 10207(2) | 35(1) |
| C(4) | 3924(3) | 9786(2) | 10723(2) | 40(1) |
| C(5) | 5927(3) | 8811(3) | 11748(2) | 41(1) |
| C(6) | 5392(3) | 7776(2) | 11270(2) | 35(1) |
| C(7) | 4027(3) | 7768(2) | 10486(2) | 32(1) |
| C(8) | 3490(3) | 6637(2) | 9967(2) | 33(1) |
| C(9) | 2701(3) | 5843(2) | 10443(2) | 34(1) |
| C(10) | 1998(3) | 6168(3) | 11136(2) | 44(1) |
| C(11) | 1261(3) | 5404(3) | 11537(3) | 55(1) |
| C(12) | 1232(3) | 4274(3) | 11271(3) | 55(1) |
| C(13) | 1926(3) | 3927(3) | 10589(3) | 53(1) |
| C(14) | 2648(3) | 4690(3) | 10167(2) | 42(1) |

* Equivalent isotropic U defined as one third of the trace of the orthogonalized $U_{ij}$ tensor

Scheme 3
7-Hydroxy-9-aza-anthrapyrazoles




BOC = *t*-Butoxycarbonyl

This was accomplished by the use of *O*-(*p*-methoxybenzyl)-*N,N'*-diisopropylisourea [6] to yield the protected intermediate **10**, although in a low yield. The incorporation of the pyrazole ring was accomplished by treatment of **10** with the appropriate hydrazine to give satisfactory yields of **11a,b** after recrystallization.

Compounds **12a,b** were obtained by displacement of the 5-chloro substituent from **11a,b** with an excess of *N,N*-dimethylethylenediamine. These displacements could be performed either in pyridine or by use of the amine as the solvent, the latter conditions leading to products of higher purity and better overall yields.

The removal of the *p*-methoxybenzyl protective group was effected by use of trifluoroacetic acid or methanesulfonic acid. Treatment of **12a** with methanesulfonic acid followed by an aqueous workup led to the isolation of the free base **1a** which was then converted into the dihydrochloride salt by treatment with hydrogen chloride gas in ethanol. The trifluoroacetate salt of **1b** was isolated in high yields by treatment of **12b** with trifluoroacetic acid followed by dilution with tetrahydrofuran.

7-Hydroxy-9-aza-anthrapyrazoles.

Since the Friedel-Crafts acylation of **5** led predominantly to **6a**, insufficient amounts of the other regioisomemcould be isolated in order to prepare **4** by a reaction sequence similar to the one which was used in the preparation of **3**. Therefore, an alternative pathway which led to **4** was developed.

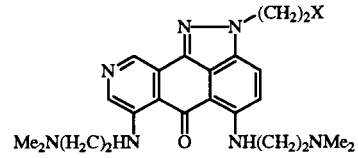
The preparative pathway to **4** is outlined in Scheme 3.

Commercially available 3,5-dichloropyridine (**13**) on treatment with sodium methoxide in dimethylformamide led to **14** [16], which on lithiation with lithium diisopropylamide at -78° followed by quenching of the resultant 3-chloro-4-lithio-5-methoxpyridine by carbon dioxide gas [17], afforded acid **15a** in good yield. The corresponding methyl ester **15b** was prepared by treatment of **15a** with thionyl chloride and quenching of the resultant acyl chloride with methanol. The conversion of acid **15a** to ester **15b** could also be readily effected by ethereal-ethanolic diazomethane.

The crucial step was the Ni mediated coupling [18] of ester **15b** with the organo zinc bromide reagent **18**. This organo zinc reagent was easily prepared by treatment of **17** with zinc dust in tetrahydrofuran. Benzylic bromide **17** [18] was prepared by treatment of **16** with *N*-bromosuccinimide in carbon tetrachloride. The addition of the tetrahydrofuran solution of **18** to ester **15b** in the presence of bis[triphenylphospine]nickel(II) chloride led to coupled product **19a**. Basic hydrolysis of **19a** led to acid **19b**. Cyclization and oxidation of **19b** with fuming sulfuric acid, with concomitant demethylation, led to **4**.

The protection of the 4-hydroxy functionality of **4** to form **20** was performed according to a procedure similar to that used for the formation of **10**. The construction of the pyrazole ring along similar lines to that developed for the regioisomeric compounds **11a,b** afforded the expected products **21a,b,** although in lower yields. The BOC-intermediate **21c** was obtained from **21b** by a standard technique in nearly a quantitative yield.

Displacements of the 5-chloro substituent of **21a,b,c** by the appropriate amines gave products **22a,b,c** in low yields after tedious purification procedures. In the displacements of **21a,b** by *N,N*-dimethylethylene diamine, the interesting tri[(aminoalkyl)amino] analogues **23a,b** (Figure 3), respectively, were also isolated and characterized. These latter compounds are formed by $S_NAr$ displacement of the *p*-methoxybenzyloxy group. Other unidentified by-products were also formed in these reactions.




**23**

**a**, X = $NMe_2$
**b**, X = $NH(CH_2)_2OH$

Figure 3. Trisubstituted analogues.

Analogue **22a** on treatment with methanesulfonic acid led to **2a**. Deprotection of **22b** with hydrochloric acid afforded **2b** as the trihydrochloride salt. Treatment of **22c** with hydrochloric acid led to the simultaneous removal of all the protecting groups to yield **2c**. Although this material was shown to be at least 95% pure by $^1H$ nmr, it failed to give satisfactory elemental analysis. Therefore it was further purified by conversion to the bis-BOC (*t*-butoxycarbonyl) analogue **2d** followed by removal of the BOC protecting groups to afford **2c** as the trihydrochloride salt.

The biological data for the hydroxy substituted aza-anthrapyrazoles which have been prepared will be reported elsewhere in a subsequent publication.

## EXPERIMENTAL

Melting points were determined on a Thomas-Hoover or Fisher-Johns apparatus and are uncorrected. Proton nmr were run on a Bruker WP-270SY, AC-200, WM-250 or ARX-500 pulsed Fourier transform spectrophotomer. The nmr spectra of the BOC-protected compounds were run at higher temperatures in order to improve the resolution and avoid (or minimize) the splitting of some signals, This latter is the effect of the hindered rotation brought about by the BOC groups. Column and flash chromatography were run using silica gel of mesh 70-230 and 230-400, respectively. Microanalyses were performed by Robertson Microlit Laboratories, Inc. Madison, NJ or by Redox s.n.c., Cologno Monzese, Milan Italy.

4-(2-Fluoro-5-chlorophenyl)-4-oxo-2*E*-butenoic Acid (**6a**).

Finely pulverized maleic anhydride (3.0 g, 0.02 mole) was added portionwise to a mixture of 1-chloro-4-fluorobenzene (**5**, 24.6 g, 20.0 ml, 0.19 mole) and anhydrous aluminum chloride (11.56 g, 0.09 mole). A gas bubbler was attached to the top of the condenser and the mixture was heated in an oil bath at 80-85° for 2 hours at which time hydrogen chloride gas evolution had ceased. The mixture was cooled and the excess 1-chloro-4-fluorobenzene was recovered by distillation at water aspirator pressure. The resultant mixture was quenched with ice and then steam distilled to remove the last traces of the 1-chloro-4-fluorobenzene. Concentrated hydrochloric acid (10 ml) was added to the cooled mixture and the resultant solid was collected by filtration (9.2 g). This material was taken up in boiling benzene (50 ml), treated with decolorizing charcoal and filtered through a celite bed. On standing overnight, the yellow solid was collected by filtration to yield 2.45 g. A second recrystallization from benzene (40 ml) led to pure regioisomer **6a** (2.1 g, 32%), mp 146-147°; $^1$H nmr (deuteriochloroform): δ 7.81 (dd, $J_{HH}$ = 15.4 Hz, $J_{HF}$ = 3.3 Hz, 1H), 7.79 (dd, $J_{HF}$ = 6.1 Hz, $J_{HH}$ = 2.7 Hz, 1H), 7.54 (m, $J_{HH}$ = 10Hz, $J_{HF}$ = 4.7 Hz, $J_{HH}$ = 2.7 Hz, 1H), 7.15 (dd, $J_{HH}$ = 10 Hz, $J_{HF}$ = 9 Hz, 1H), 6.85 ( $J_{HH}$ = 15.4 Hz, $J_{HF}$ = 1.2 Hz, 1H); in octadeuteriotetrahydrofuran the COOH proton was found at δ 12.5.

*Anal.* Calcd. for $C_{10}H_6ClFO_3$: C, 52.54: H, 2.65. Found: C, 52.26; H, 2.67.

Methyl 4-(2-Fluoro-5-chlorophenyl)-4-oxo-2*E*-butenoate (**6b**).

Method A.

A methanol (90 ml) solution of **6a** (4.6 g, 0.02 mole) containing concentrated sulfuric acid (20 drops) was heated under reflux for 2 hours. The mixture was cooled to room temperature and the removal of the methanol by rotary evaporation yielded a pale yellow solid. Ice water was added to the residue and the solid was collected by filtration and washed several times with ice water. The solid was taken up in pentane (100 ml) and the solution dried over sodium sulfate. The drying agent was removed and the pentane solution placed in the freezer for 3 days. The crystals were collected by filtration to afford pure **6b** (3.2 g, 76%), mp 42-43°; $^1$H nmr (deuteriochloroform): δ 7.78 ( dd, $J_{HF}$ 6.1 Hz, $J_{HH}$ = 2.7 Hz, 1H), 7.70 ( dd, $J_{HH}$ = 15.6 Hz, $J_{HF}$ = 3.3 Hz,1H), 7.51 (m, $J_{HH}$ = 9.8 Hz, $J_{HF}$ = 4.5 Hz, $J_{HH}$ = 2.7 Hz, 1H), 7.13 (dd, $J_{HH}$ = 9.8 Hz, $J_{HF}$ = 8.9 Hz, 1H), 6.84 (dd, $J_{HH}$ = 15.6 Hz, $J_{HF}$ = 1 3 Hz, 1H), 3.84 (s, 3H).

*Anal.* Calcd. For $C_{11}H_8ClFO_3$: C, 54.45: H, 3.32. Found: C, 54.53: H, 3.34.

Method B.

Methyl chloroformate (8.5 ml, 0.11 mole) was added dropwise in a period of 20 minutes to a mixture of **6a** ( 7.7 g, 0.08 mmole) and triethylamine (16.1 ml, 0.12 mole) in anhydrous tetrahydrofuran being stirred at -15° under a nitrogen atmosphere. The mixture was stirred for 20 minutes at -10° and then allowed to warm to 0° and held at this temperature for 1 hour. Methanol (177 ml) was added dropwise and the mixture was allowed to warm to room temperature during which time the evolution of carbon dioxide occurred. After 1 hour the mixture was concentrated to dryness and the residue was added to water and the product extracted into ethyl acetate. The extracts were dried over sodium sulfate and concentrated to dryness. The residue was recrystallized from methanol in two crops to yield **6b** (14.7 g, 78%).

Methyl 4-(2-Fluoro-5-chlorobenzoyl)-5-hydroxynicotinate (**8a**).

A mixture of **6b** (2.0 g, 8.26 mmoles) and 5-ethoxyoxazole-2-carboxylic acid (**7**,1.5 g, 9.50 mmoles) was heated at 90° for 1.75 hours at which time carbon dioxide evolution had ceased. The cooled reaction mixture (orange-red solid) was triturated with ethyl acetate (5 ml). The pale yellow solid was collected by filtration (1.43 g). Additional material could be obtained by partial concentration of the filtrate (0.4 g, total recovery 1.83 g, 70%). The analytical and X-ray samples were recrystallized from ethyl acetate to give a colorless product, mp 163-164°; $^1$H nmr (dimethyl-$d_6$ sulfoxide): δ 10.9 (br s, 1H), 8.62 (s, 1H), 8.50 (s, 1H), 7.78 (m, 2H), 7.40 (m, 1H), 3.92 (s, 3H).

*Anal.* Calcd. for $C_{14}H_9ClFNO_4 \cdot H_2O$: C, 51.31; H, 3.38; N, 4.27. Found: C, 51.61; H, 3.39; N, 4.27.

4-(2-Fluoro-5-chlorobenzoyl)-5-hydroxynicotinic Acid (**8b**).

Ester **8a** (1.2 g, 4.00 mmoles) was added to a solution of sodium hydroxide (0.67 g, 17 mmoles) in water (4 ml) and methanol (12 ml). A yellow solution was obtained after 5 minutes The mixture was stirred at room temperature for 1.25 hours. The resulting solution was acidified with 1*M* hydrochloric acid (14 ml) to *p*H 2 and the mixture was allowed to stand overnight. The white solid was collected by filtration, washed with water (3 x 5 ml) and dried to give **8b** (1.04 g, 92%), mp 262-264°; $^1$H nmr (dimethyl-$d_6$ sulfoxide): δ 13.60 (s, 1H), 10.70 (s, 1H), 8.60 (s, 1H), 8.45 (s, 1H), 7.75 (m, 2H), 7.36 (t, 1 H, J = 9.4 Hz).

*Anal.* Calcd. for $C_{13}H_7ClFNO_4$: C, 52.81; H, 2.39; N, 4.74. Found: C, 52.60; H, 2.02; N, 4.73.

4-(2-Fluoro-5-chlorobenzyl)-5-hydroxynicotinic Acid (**9**).

The crude acid **8b** (0.16 g, 0.56 mmole) was added to formic acid (3 ml, 74%) and zinc dust (0.1 g, -325 mesh, Aldrich) was added to the mixture which was magnetically stirred. The mixture was placed in a pre-heated oil bath (80°) for 0.5 hour and an additional amount of zinc dust (0.1 g) was added. The mixture was heated for an additional 3.5 hours, then cooled to room temperature and partitioned between aqueous hydrochloric acid (10 ml, 0.5 *M*) and ethyl acetate (10 ml). The aqueous layer was extracted with ethyl acetate (2 x 10 ml) and the combined organic extracts were washed with brine (10 ml) and dried over magnesium sulfate. The drying agent was removed by filtration and the filtrate was concentrated. Methanol (0.5 ml) was added to the residue and the mixture was allowed to stand overnight. The white crystalline product was collected by filtration and dried to yield **8b** (0.07 g, 43%) which was used without further purification, mp >280°; $^1$H nmr (dimethyl-$d_6$ sulfoxide): δ 13.25 (s, 1H), 10.34 (s, 1H), 8.47 (s, 1H), 8.30 (s, 1H), 7.25 (m, 2H), 6.80 (dd, 1H), 4.27 (s, 2H).

4-Hydroxy-6-fluoro-9-chlorobenz[*g*]isoquinoline-5,10-dione (**3**).

Acid **9** (150 mg, 0.55 mmole) was placed in fuming sulfuric acid (2 ml, 18-24% sulfur trioxide) and heated for 45 minutes in an oil bath held at 125°. The dark brown solution was cautiously quenched over ice (20 g) and the resultant mixture neutralized with solid sodium bicarbonate to *p*H 7. The dione was extracted with dichloromethane (2 x 20 ml) and the extracts dried over magnesium sulfate. The solvent was removed by rotary evaporation to yield **3** (120 mg, 81%) as a yellow solid which darkened on standing in air, mp 227-228°; $^1$H nmr (deuteriochloroform): δ 11.48 (s, 1H), 9.02 (s, 1H), 8.86 (s, 1H), 7.88 (dd, $J_{HH}$ = 9.1 Hz, $J_{HF}$ = 4.5 Hz, 1H), 7.47 (dd, $J_{HF}$ = 10.2, $J_{HH}$ = 9.1 Hz,1H).

*Anal.* Calcd. for $C_{13}H_5ClFNO_3$: C, 56.24; H, 1.81: N, 5.04. Found: C, 56.43; H, 2.02; N, 5.25.

4-[[(4-Methoxyphenyl)methyl]oxy]-6-fluoro-9-chlorobenz-[*g*]isoquinoline-5,10-dione (**10**).

Dione **3** (50 mg, 0.18 mmole) and *O*-(*p*-methoxybenzyl)-*N,N'*-diisopropylisourea [6] (95 mg, 0.35 mmole) in dichloromethane (1 ml) was stirred at room temperature for 3.5 hours under a nitrogen blanket. The dark reddish brown mixture was purified by flash chromatography over silica gel (2 cm by 12 cm) using gradient elution commencing with 100:2 followed by 100:4 mixtures of chloroform: *t*-butyl methyl ether. Removal of the eluent yielded **10** (32 mg, 45%) as yellow-brown solid. Attempts at further purification led to decomposition and the sample was used as is, mp 155-157°; $^1$H nmr (deuteriochloroform): δ 9.06 (s,1 H), 8.78 (s, 1 H), 7 75 (dd, $J_{HH}$ = 9 0 Hz, $J_{HF}$ = 4.6 Hz, 1H), 7.42 (d, $J_{HH}$ = 8.3 Hz, 2H), 7.38 (t, $J_{HF}$ = 9.8 Hz, $J_{HH}$ = 9.0 Hz, 1H), 6.93 (d, $J_{HH}$ = 8.3 Hz, 2H), 5.36 (s, 2H), 3.82 (s, 3H).

2-Methyl-5-chloro-10-[[(4-methoxyphenyl)methyl]oxy]-lindazolo[3,4-*fg*]isoquinolin-6(2*H*)-one (**11a**).

A solution of methylhydrazine (15 mg, 0.36 mmole) in pyridine (0.6 ml) was added over a 2 minute period to a solution of **10** (55 mg, 0.13 mmole) in pyridine (0.5 ml) at 0° and the mixture was stirred for 1 hour 20 minutes. The pyridine was removed by a slow nitrogen stream and the resultant residue was placed under vacuum for 2 hours. The residue was purified *via* a chromatotron (2 mm silica gel plate) using gradient elution commencing with chloroform (100 ml), then 1% methanol: 99% chloroform (75 ml) followed by 2% methanol: 98% chloroform (175 ml). Upon removal of the final eluent, **11a** (25 mg, 46%) was obtained as a bright yellow solid, mp 181-183°; $^1$H nmr (deuteriochloroform): δ 9.28 (s, 1H), 8.62 (s, 1H), 7.66 (d, $J_{HH}$ = 8.3 Hz,1H), 7.62 (d, $J_{HH}$ = 8.4 Hz, 2H), 7.57 (d, $J_{HH}$ = 8 3 Hz, 1H), 6 94 (d, $J_{HH}$ = 8.4 Hz, 2H), 5.44 (s, 2H), 4.31 (s, 3H), 3.83 (s, 3H).

*Anal.* Calcd. for $C_{22}H_{16}ClN_3O_3$: C, 65.11; H, 3.97; N, 10.35. Found: C, 65.25; H, 3.82; N, 10.21.

2-Methyl-5-[[2-(dimethylamino)ethyl]amino]-10-[[(4-methoxyphenyl)methyl]oxy]indazolo[3,4-*fg*]isoquinolin-6(2*H*)-one (**12a**).

A solution of **11a** (53 mg, 0.13 mmole) and *N,N*-dimethylethylenediamine (115 mg, 1.31 mmoles) in pyridine (0.5 ml) was heated at 120° for 3.5 hours under a nitrogen atmosphere. The excess diamine and pyridine were removed by a slow stream of nitrogen and the resultant dark orange residue was placed under vacuum for 3 hours. The crude material was purified *via* a chromatotron (silica gel, 2 mm thickness) using gradient elution commencing with 2% methanol:98% chloroform (100 ml) to mixtures containing 4% methanol (75 ml) to 6% methanol (60 ml). Upon removal of the 4% methanol:96% chloroform eluent, starting material was recovered (10 mg). Removal of the 6% methanol:94% chloroform eluent led to the orange product **12a** (22 mg, 45%), mp 231-232°; $^1$H nmr (deuteriochloroform): δ 9.42 (s, 1H), 9.35 (t, 1H), 8.55 (s, 1H), 7.66 (d, $J_{HH}$ = 9.1 Hz, 1H), 7.61 (d, $J_{HH}$ = 8.5 Hz, 2H), 7.0 (d, $J_{HH}$ = 9.1 Hz, 1H), 6.93 (d, $J_{HH}$ = 8.5 Hz, 2H), 5.45 (s, 2H), 4.31 (s, 3H), 3.82 (s, 3H), 3.56 (q, $J_{HH}$ = 6.5 Hz, $J_{HH}$ = 6.2 Hz, 2H), 2.71 (t, $J_{HH}$ = 6.5 Hz, 2H), 2.37 (s, 6H).

*Anal.* Calcd. for $C_{26}H_{27}N_5O_3 \cdot 0.5H_2O$: C, 66.98; H, 5.84; N, 15.02. Found: C, 66.52, H, 5.72, N, 14.79.

2-Methyl-5-[[2-(dimethylamino)ethyl]amino]-10-hydroxyindazolo-[3,4-*fg*]isoquinolin-6(2*H*)-one (**1a**).

Method A (Trifluoroacetic Acid).

To a mixture of **12a** (10 mg, 0.02 mmole) in dichloromethane (1 ml), trifluoroacetic acid (1 ml) was added upon which all solid was in solution. After 1 hour, the mixture was diluted with dichloromethane (3 ml) and a saturated solution of sodium bicarbonate (5 ml) was added. The dichloromethane layer was separated and the aqueous phase extracted with dichloromethane (3 x 8 ml). The extracts were dried over magnesium sulfate and the solvent removed under a slow nitrogen stream. The material was placed under vacuum for 2 hours. The $^1$H nmr indicated the presence of the desired product along with a small amount of impurity. All attempts to purify the product by preparative chromatography led to decomposition and use of smaller amounts of trifluoroacetic acid for longer times led to the desired deprotected product along with other side products.

Method B. (Methanesulfonic Acid).

Methanesulfonic acid (20 drops) was added to the orange solution of **12a** (20 mg, 0.04 mmole) in dichloromethane (0.75 ml) upon which a fluorescent hue appeared. The mixture was stirred at room temperature for 1 hour and then quenched by the addition of a saturated sodium bicarbonate solution (2 ml). The dichloromethane layer was separated and the aqueous phase extracted with dichloromethane (3 x 7 ml). The extracts were washed with water 2 x 1 ml), dried over magnesium sulfate and concentrated to dryness to yield **1a** (2 mg, 85%) as an orange solid: mp 211-212°; $^1$H nmr (deuteriochloroform): δ 9.26 (s, 1H), 9.19 (m, 1H), 8.59 (s, 1H), 7.60 (d, J = 9.1 Hz, 1H), 7.01 (d, J = 9.1 Hz, 1H), 4.24 (s, 3H), 3.55 (q, J = 6.2 Hz, 2H), 2.71 (t, J = 6.2 Hz, 2H), 2.38 (s, 6H).

*Anal.* Calcd. for $C_{18}H_{19}N_5O_2$: C, 64.08; H, 5.68; N, 20.76. Found: C, 63.80; H, 5.55; N, 20.37.

The dihydrochloride salt of **1a** was prepared by dissolving the free base **1a** (12 mg, 0.03 mmole) in dichloromethane: methanol 96:4) and bubbling hydrogen chloride gas into the solution for 5 minutes. The resultant orange-red solid was collected by filtration to afford the salt **1a** (11 mg, 76%); $^1$H nmr (deuterium oxide): δ 8.51 (s, 1 H), 8.19 (s, 1 H), 7.63 (m, 1H), 6.82 (m, 1H), 4.16 (s, 3H), 3.96 (t, J = 6.0 Hz, 2H), 3.64 (t, J = 6.0 Hz, 2H), 3.12 (s, 6H).

2-[(2-Dimethylamino)ethyl]-5-chloro-10-[[(4-methoxyphenyl)-methyl]oxy]indazolo[3,4-*fg*]isoquinolin-6(2*H*)-one (**11b**).

A solution of *N*-[(2-dimethylamino)ethyl]hydrazine (69 mg, 0.67 mmole) in pyridine (0.7 ml) was added to **10** (107 mg, 0.29 mmole) over a period of 2 minutes at 0° under a nitrogen blanket and the mixture was stirred for 2 hours. The excess pyridine and some excess hydrazine were removed under a nitrogen stream. The residue was placed under vacuum for 0.5 hour. The product was purified *via* thick layer chromatography (silica gel, 10 cm by 20 mm) using 6% methanol:94% dichloromethane (about 10 color zones were evident on the plate). The major yellow band was scraped from the plate and the yellow compound extracted into 25% methanol:75% dichloromethane. Removal of the solvents led to **11b** (47 mg, 38%), mp 192-193°; $^1$H nmr (deuteriochloroform): δ 9.29 (s, 1 H), 8.64 (s, 1 H), 7.71 (d, J = 8.6 Hz, 1H), 7.61 (m, 4H), 6.95 (d, J = 8.6 Hz, 1H), 5.42 (s, 2H), 4.65 (t, J = 6.6 Hz, 2H), 3.84 (s, 3H), 2.98 (t, J = 6.6 Hz, 2H), 2.33 (s, 6H).

*Anal.* Calcd. for $C_{25}H_{23}N_4O_3Cl$: C, 64.86; H, 5.01; N, 12.10. Found: C, 65.02; H, 4.76; N, 11.89.

2-[(2-Dimethylamino)ethyl]-5-[[2-(dimethylamino)ethyl]amino]-10-[[(4-methoxyphenyl)methyl]oxy]indazolo[3,4-*fg*]isoquinolin-6(2*H*)-one (**12b**).

A solution of **11b** (47 mg, 0.10 mmole) in pyridine (1 ml) and *N,N*-dimethylethylenediamine (179 mg, 2.03 mmoles) was heated at 110° for 3 hours under a nitrogen blanket. The excess diamine and pyridine were removed under a slow stream of nitrogen and the resultant dark red residue was placed under vacuum for 1 hour. The material was purified by column chromatography (1.75 cm x 14 cm of silica gel) using gradient elution commencing with 2% methanol to 4%, 8%, 12%, 18% and 25% methanol in dichloromethane. Removal of the eluent from the major yellow band yielded **12b** (21 mg, 40%), mp 204-205°; $^1$H nmr (deuteriochloroform): δ 9.43 (s, 1H), 9.36 (m, 1H), 8.58 (s, 1H), 7.71 (d, J = 9.1 Hz, 1H), 7.66 (d, J = 8.6 Hz, 2H), 7.00 (d, J = 9.1 Hz, 1H), 6.94 (d, J = 8.6 Hz, 2H), 5.43 (s, 2H), 4.66 (t, J = 6.8 Hz, 2H), 3.83 (s, 3H), 3.57 (q, J = 6.5 Hz, 2H), 3.00 (t, J = 6.8 Hz, 2H), 2.71 (t, J = 6.5 Hz, 2H), 2.37 (s, 6H), 2.35 (s, 6H).

2-[(2-Dimethylamino)ethyl]-5-[[2-(dimethylamino)ethyl]-amino]-10-hydroxyindazolo[3,4-*fg*]isoquinolin-6(2*H*)-one (**1b**).

Methanesulfonic acid (15 drops) was added to the orange solution of anthrapyrazole **12b** (28 mg, 0.05 mmole) in dichloromethane (1 ml) upon which an immediate red coloration developed. The mixture was stirred at room temperature for 1 hour and quenched by the addition of a saturated sodium bicarbonate solution (1 ml). The dichloromethane layer was separated and the aqueous phase extracted with dichloromethane (3 x 5 ml). The combined organic phases were washed with water (2 x 1 ml), dried over magnesium sulfate and concentrated to yield **1b** (20 mg, 93%) as a yellow-brown solid, mp 176-177°; $^1$H nmr (deuteriochloroform): δ 9.28 (s, 1H), 9.21 (m, 1H), 8.60 (s, 1H), 7.69 (d, J = 9.1 Hz, 1H), 7.04 (d, J = 9.1 Hz, 1H), 4.61 (t, J = 6.6 Hz, 2H), 3.55 (q, J = 6.45 Hz, 2H), 2.92 (t, J = 6.6 Hz, 2H), 2.70 (t, J = 6.45 Hz, 2H), 2.37 (s, 6H), 2.32 (s, 6H).

*Anal.* Calcd. for $C_{21}H_{26}N_6O_2 \cdot H_2O$: C, 61.15; H, 6.84; N, 20.37. Found: C, 61.02; H, 6.54; N, 20.01.

The free base **1b** (15 mg, 0.04 mmole) was dissolved in a methanol: dichloromethane mixture (5:95, 1 ml) and hydrogen chloride gas was bubbled in for 5 minutes. The mixture was allowed to stand at room temperature for 3 hours and the supernatant liquid was removed by pipet. The residue was washed with dichloromethane (3 ml) and the resultant trihydrochloride salt was placed under vacuum for 3 hours to yield the salt (17 mg, 81%) as a dark orange solid; $^1$H nmr (deuterium oxide): δ 8.99 (s, 1 H), 8.40 (s, 1 H), 8.07 (d, J = 9.45 Hz, 1H), 7.28 (d, J = 9.45, 1H), 5.11 (t, J = 5.8 Hz, 2H), 4.09 (t, J = 6.3 Hz, 2H), 3.95 (t, J = 5.8 Hz, 2H), 3.61 (t, J = 6.3 Hz, 2H), 3.03 (s, 6H), 3.02 (s, 6H).

2-[2-Dimethylamino)ethyl]-5-[[2-(dimethylamino)ethyl]amino]-10-hydroxyindazolo[3,4-*fg*]isoquinolin-6(2*H*)-one Tristrifluoroacetate.

The protected anthrapyrazole **12b** (1.0 g, 1.95 mmoles) was suspended in trifluoroacetic acid (8.0 ml) and stirred at 5° under a nitrogen atmosphere for 30 minutes. Anhydrous tetrahydrofuran was added to the resultant solution and the reaction mixture was stirred at 10° for 2 hours. The orange solid was collected by filtration, washed with tetrahydrofuran and dried under vacuum to constant weight to afford the salt (1.20 g, 83%), mp 223° dec; $^1$H nmr (dimethyl-$d_6$ sulfoxide): δ 11.20 (br s, 1H), 9.90 (br s, 2H), 9.22 (br t, 1H), 9.05 (s, 1H), 8.54 (s, 1H), 8.21 (d, 1H), 7.38 (d, 1H), 5.07 (t, 2H), 3.95 (q, 2H), 3.72 (t, 2H), 3.38 (t, 2H), 2.90 (s, 6H), 2.80 (s, 6H).

*Anal.* Calcd. For $C_{21}H_{26}N_6O_2 \cdot 3C_2HF_3O_2$: C, 44.03; H, 3.97; N, 11.41; F, 23.22. Found: C, 43.75; H, 3.90; N, 11.25; F, 23.38.

3-Chloro-5-methoxypyridine (**14**).

Sodium metal (6 g, 0.26 mole) was added to dry methanol (120 ml) under a nitrogen blanket. Upon complete reaction of the metal, the methanol was removed under reduced pressure. Dimethyl formamide (120 ml) and 3,5-dichloropyridine (**13**, 19.0 g, 0.13 mole) were added to the residual dry sodium methoxide and the resultant suspension was placed in an oil bath at 70°. The oil bath was maintained at 70-75° for 1.5 hours and then the flask was removed from the bath and cooled to room temperature. The mixture was quenched over ice (200 ml) and cooled in an ice bath. The precipitate was collected and dried (7.2 g, tlc pure product, silica gel, hexane:ethyl acetate 9:1). The filtrate was extracted with ether (5 x 70 ml) and the ethereal extracts washed with cold brine (2 x 50 ml). Removal of the ether led to a white solid (10 g) which on nmr analysis showed only trace amounts of 3,5-dimethoxypyridine. The crude solid was taken up in cold hexane (50 ml) and the solution dried over sodium sulfate. Cooling in the freezer followed by collection of the crystals by filtration led to **14** (7.8 g), total weight of **14** (15.0 g, 81%), mp 39-40°; lit mp 40-41° [16]; $^1$H nmr (deuteriochloroform): δ 8.21 (d, 1H), 8.19 (d, 1H), 7.20 (dd, 1H), 3.86 (s, 3H).

3-Chloro-5-methoxyisonicotinic Acid (**15a**).

Diisopropylamine (2.82 g, 0.03 mole) and tetrahydrofuran (25 ml) were placed in a 250 ml 3-necked flask which was cooled in a dry ice-acetone bath to -78° under an argon atmosphere. A solution of *n*-butyllithium (8.7 ml, 1.6*M*, in hexane) was added *via* a syringe over a 10 minute period and the mixture was stirred at -78° for 20 minutes. A solution of **14** (4.0 g, 0.03 mole) in tetrahydrofuran (12 ml) was added *via* a cannula under a slight argon pressure over a 20 minute interval and the resultant pale yellow solution was stirred for 20 minutes. Carbon dioxide gas, scrubbed by passage through a cold trap (-55°), was bubbled into the mixture for 1 hour at -78° and the bubbling continued for an additional 1.5 hours as the mixture was removed from the cold bath and allowed to warm to room temperature. The pale yellow solution was concentrated to dryness and water (10 ml) was added. The mixture was acidified to *p*H 2 with 10% hydrochloric acid. The white precipitate was collected and dried. Additional product was collected from the filtrate on standing to yield **15a** (total of 3.7 g, 70%), mp 205-206°. Recrystallization could be effected from 95% ethanol or aqueous methanol; $^1$H nmr (dimethyl-$d_6$ sulfoxide): δ 13.97 (br s, 1H), 8.46 (s, 1H), 8.34 (s, 1H), 3.94 (s, 3H).

Methyl 3-Chloro-5-methoxyisonicotinate (**15b**).

Method A. Diazomethane Route.

An ethereal-ethanol solution of diazomethane was added to a suspension of **15a** (0.5 g, 2.67 mmoles) in ether (2 ml) until the evolution of nitrogen ceased. The light yellow solution was decanted from a small amount of insoluble residue and concentrated by rotary evaporation to yield crude **15b** (0.24 g, 62%) as a pale yellow solid. Recrystallization could be effected by dissolution in ether and cooling in the freezer (-15°) to yield colorless needles, mp 65-66°; $^1$H nmr (deuteriochloroform): δ 8.30 (s, 1H), 8.26 (s, 1H), 3.96 (s, 3H), 3.95 (s, 3H).

*Anal.* Calcd. for $C_8H_8ClNO_3$: C, 47.66; H, 4.00; N, 6.95. Found: C, 47.73; H, 3.94; N, 6.76.

Method B. Acid chloride: Methanol Route.

A mixture of **15a** (14.61 g, 0.07 mole) and thionyl chloride (85 ml, 1.16 moles) was heated at reflux for 2.5 hours. The excess

thionyl chloride was removed by distillation under reduced pressure. The residue was treated with dry methanol (246 ml) during which a slightly exothermic reaction occurred. The reaction flask was stoppered with a calcium chloride drying tube and the mixture was stirred at room temperature for 20 minutes, then cooled to 0° and triethylamine (1.9 ml, 0.09 mole) was added. After 1 hour the mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed by rotary evaporation and the residue partitioned between diethyl ether (500 ml) and water (200 ml). The organic layer was washed with a saturated solution of sodium bicarbonate (100 ml), a solution of disodium hydrogen phosphate (1*M*, 100 ml) and brine (100 ml). The aqueous phases were further extracted with diethyl ether (100 ml). The combined ethereal extracts were dried over sodium sulfate and concentrated. The resultant oil crystallized on cooling to yield **15b** (15.36 g, 98%).

Methyl 3-(2-Fluoro-5-chlorobenzyl)-5-methoxyisonicotinate (**19a**).

A solution of **17** [18] (1.64 g, 7.30 mmoles) in tetrahydrofuran (5 ml) was added dropwise to activated zinc (0.58 g, 8.80 mmoles) which was cooled in an ice bath at 0° and the mixture was stirred at this temperature for 3 hours under a nitrogen atmosphere. The organo zinc reagent **18** which was formed was added *via* a cannula under nitrogen pressure to methyl 3-chloro-5-methoxyisonicotinate (**15b**, 1 g, 4.90 mmoles) and bis[triphenylphosphine]nickel(II)-chloride (0.64 g, 0.97 mmole) in tetrahydrofuran (50 ml). The mixture was stirred at room temperature for 3 hours and the resultant brown mixture was quenched with aqueous ammonium chloride (10%, 25 ml) and ethyl acetate (40 ml) was added. The organic layer was separated and washed with brine (2 x 25 ml) and dried over sodium sulfate. The solvents were removed by rotary evaporation to yield a yellow oil which was purified by column chromatography (2.75 id by 12 cm) using gradient elution commencing with hexane: ethyl acetate mixtures 4:1, 200 ml, 3:1, 100 ml and 1:1 100 ml, which eluted the major yellow band. On concentration a pale yellow oil was obtained which was crystallized at low temperature from ethyl acetate to yield **19a** (1.1 g, 73%), mp 98-99°; $^1$H nmr (deuteriochloro-form): δ 8.29 (s, 1H), 8.19 (s, 1H), 7.16 (ddd, $J_{HH}$ = 9.0 Hz, $J_{HF}$ = 4.4 Hz, $J_{HH}$ = 2.6 Hz, 1H), 7.05 (dd, $J_{HF}$ = 6.5 Hz, $J_{HF}$ = 2.6 Hz, 1H), 6.96 (dd, $J_{HH}$ = 9.0 Hz, $J_{HF}$ = 4.0 Hz, 1H), 3.96 (s, 2H), 3.93 (s, 3H), 3.83 (s, 3H).

*Anal.* Calcd. for $C_{15}H_{13}ClFNO_3$: C, 58.17; H, 4.23; N, 4.52. Found: C, 58.32; H, 4.27; N, 4.39.

3-(2-Fluoro-5-chlorobenzyl)-5-methoxyisonicotinic Acid (**19b**).

Ester **19a** (500 mg, 1.62 mmoles) was added to a solution of sodium hydroxide (291 mg, 7.21 mmoles) in water (1.6 ml) and methanol (4 ml) and the mixture was refluxed for 2 hours. The cooled solution was acidified with concentrated hydrochloric acid and concentrated to one-half volume by rotary evaporation. The solid was collected by filtration to afford **19b** (430 mg, 90%), mp 199-200° dec; $^1$H nmr (dimethyl-$d_6$ sulfoxide): δ 12.80 (br s, 1H), 8.38 (s, 1H), 8.14 (s, 1H), 7.33 (m, $J_{HH}$ = 9.2 Hz, $J_{HF}$ = 4.5 Hz, $J_{HH}$ = 2.7 Hz, 1H), 7.22 (dd, $J_{HH}$ = 9.2 Hz, $J_{HF}$ = 4.5 Hz, 1H), 7.22 (dd, $J_{HF}$ = 6.5 Hz, $J_{HH}$ = 2.7 Hz, 1H), 3.97 (s, 2H), 3.90 (s, 3H).

4-Hydroxy-6-chloro-9-fluorobenz[*g*]isoquinoline-5,10-dione (**4**).

Isonicotinic acid **19b** (200 mg, 0.67 mmole) was added to fuming sulfuric acid (30% sulfur trioxide, 2 ml) and the mixture was heated for 0.75 hours in an oil bath at 125-130°. The brownish red solution was cautiously quenched over ice (10 g) and extracted with dichloromethane (40 ml). The aqueous layer was further extracted with dichloromethane (3 x 30 ml), the extracts dried over magnesium sulfate and the solvent removed by rotary evaporation to yield **4** (130 mg, 70%) as a yellow solid. Purification could be accomplished by chromatography over silica gel with ethyl acetate as the eluent, mp 220-222°; $^1$H nmr (deuteriochloroform): δ 11.55 (s, 1H), 8.99 (s, 1H), 8.84 (s, 1H), 7.84 (dd, $J_{HH}$ = 9.0 Hz, $J_{HF}$ = 4.5 Hz, 1H), 7.51 (dd, $J_{HF}$ = 10.0 Hz, $J_{HH}$ = 4.0 Hz, 1H)

*Anal.* Calcd. for $C_{13}H_5ClFNO_3$: C, 56.24; H, 1.81; N, 5.04. Found: C, 55.90; H, 1.91; N, 4.64.

4-[[(4-methoxyphenyl)methyl]oxy]-6-chloro-9-fluorobenz[*g*]-isoquinoline-5,10-dione (**20**).

Dione **4** (200 mg, 0.73 mmole) and *O*-(*p*-methoxybenzyl)-*N,N'*-diisopropylisourea (381 mg, 1.44 mmoles) in dichloromethane (2 ml) were stirred at room temperature under a nitrogen blanket. The dark reddish mixture was concentrated to 1 ml and purified by flash chromatography (14 cm x 2 cm id) eluting with dichloromethane:*t*-butyl methyl ether 100:2 (500 ml). Removal of the eluents yielded **20** (120 mg, 42%) as a yellowish-brown solid which was somewhat unstable in air, mp 132-134°; $^1$H nmr (deuteriochloroform): δ 9.03 (s, 1H), 8.77 (s, 1H), 7.77 (dd, $J_{HH}$ = 8 9 Hz, $J_{HF}$ = 4 4 Hz, 1H), 7.45 (d, $J_{HH}$ = 8.7 Hz, 2H), 7.39 (dd, $J_{HH}$ = 8 9 Hz, $J_{HF}$ = 4.5 Hz, 1H), 6.94 (d, J = 8.7 Hz, 2H), 5.38 (s, 2H), 3.82 (s, 3H).

2-[2-(Dimethylamino)ethyl]-5-chloro-7-[[(4-methoxyphenyl)-methyl]oxy]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (**21a**).

A solution of *N*-[(2-dimethylamino)ethyl]hydrazine (79 mg, 0.76 mmole) in pyridine (1 ml) was added to **20** (20 mg, 0.30 mmole) in pyridine (0.6 ml) at 0° over a period of 3 minutes and the mixture was stirred for 2 hours at this temperature. The pyridine was removed by a slow nitrogen stream and the residue was placed under vacuum for 0.5 hour. The product was obtained *via* thick layer chromatography (silica gel, 2 mm thickness, 10 x 20 cm plate) using 5% methanol:95% dichloromethane as eluent. The major yellow band was scraped from the plate and the product extracted into 10% methanol:90% dichloromethane (30 ml). Removal of the solvents led to **21a** (44 mg, 32%), mp 121-123°; $^1$H nmr (deuteriochloroform): δ 9.15 (s, 1H), 8.51 (s, 1H), 7.64 (d, J = 8.7 Hz, 1H), 7.55 (d, J = 8.7 Hz, 1H), 7.52 (d, J = 8.7 Hz, 2H), 6.94 (d, J = 8.7 Hz, 2H), 5.36 (s, 2H), 4.59 (t, J = 6.6 Hz, 2H), 3.82 (s, 3H), 2.92 (t, J = 6.6 Hz, 2H), 2.31 (s, 6H).

2-[2-(Dimethylamino)ethyl]-5-[[2-(dimethylamino)ethyl]amino]-7-[[(4-methoxyphenyl)methyl]oxy]indazolo[4,3-*gh*]isoquinolin-6-(2*H*)-one (**22a**).

Run 1.

A solution of **21a** (37 mg, 0.08 mmole) and *N,N*-dimethylethylenediamine (141 mg, 1.60 mmoles) in pyridine (1 ml) was heated at 1 05-110° for 3 hours under a nitrogen blanket. The pyridine and excess amine were removed under a stream of nitrogen and the dark residue placed under vacuum overnight. The crude product was purified by flash chromatography (12 cm x 1.75 cm id) using gradient elution commencing with 2%, 4%, 8%, 12%, 16%, 22% and 25% methanol in dichloromethane. Upon concentration of the major reddish-yellow fraction an orange solid was obtained (24 mg). Analysis by tlc still showed impurities and further purification was accomplished *via* a chromatotron (2 mm plates) using gradient elution mixtures of 4%, 6%. 8%, 10% and 12% methanol in dichloromethane. Concentration led to **22a** (13 mg, 32%) as an orange solid, mp 185-186°; $^1$H nmr (deuteriochloroform): δ 9.51 (m, 1H), 9.30 (s, 1H), 8.43

(s, 1H), 7.66 (d, J = 9.1Hz, 1H), 7.56 (d, J = 8.6 Hz, 2H), 7.01 (d, J = 9.1 Hz, 1H), 6.94 (d, J = 8.6 Hz, 2H), 5.36 (s, 2H), 4.62 (t, J = 6.7 Hz, 2H), 3.82 (s, 3H), 3.58 (m, 2H), 2.94 (t, J = 6.7 Hz, 2H), 2.71 (t, J = 6.8 Hz, 2H), 2.38 (s, 6H), 2.33 (s, 6H).

*Anal.* Calcd. for $C_{29}H_{34}N_6O_3 \cdot 3H_2O$: C, 61.25; H, 7.09; N, 14.77. Found: C, 60.89; H, 6.67; N, 14.45.

Run 2.

Compound **22a** and 2-[2-(Dimethylamino)ethyl]-5,7-bis[[(2-(dimethylamino)ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (**23a**).

Chloro analogue **21a** (300 mg, 0.64 mmole) was suspended in *N,N*-dimethylethylenediamine (3 ml, 27 mmoles) and the resultant mixture was heated to 90° for 3 hours (all the starting material dissolved at 70°). The cooled mixture was poured into aqueous sodium bicarbonate and extracted with dichloromethane (3 x 20 ml). The combined organic extracts were dried over sodium sulfate and concentrated under reduced pressure. The oily residue was purified by column chromatography using dichloromethane: methanol 7:3 as the eluent. Recrystallization from tetrahydrofuran led to **22a** (72 mg, 22%). A red spot with a lower $R_f$ relative to **22a** was also isolated (100 mg, 36%). This material could be crystallized from dichloromethane:*t*-butyl methyl ether 1:1 (10 ml) to yield an orange solid characterized as **23a** on the basis of $^1$H nmr, mp 153-155°; $^1$H nmr (dimethyl-$d_6$ sulfoxide): δ 9.70 (br t, 1H), 9.15 (br t, 1H), 8.6 (s, 1H), 8.2 (s, 1H), 8.05 (d, 1H), 7.15 (d, 1H), 4.65 (br t, 2H), 3.6 (br q, 2H), 3.4 (m, 2H), 2.8 (br t, 2H), 2.6 (br t, 4H), 2.1-2.3 (2s, 18H).

2-[2-(Dimethylamino)ethyl]-5-[[2-(dimethylamino)ethyl]amino]-7-hydroxyindazolo[4,3-*gh*]isoquinolin-4-(2*H*)-one (**2a**).

Methanesulfonic acid (1.97 g, 20 mmole) was added to a solution of **22a** (70 mg, 0.14 mmole) in dichloromethane (3 ml) and the mixture was stirred at room temperature for 1 hour and then poured into ice-cold water (10 ml) containing sodium hydrogen carbonate (2.1 g, 24.50 mmole). The aqueous layer was further extracted with dichloromethane. The combined organic extracts were dried over sodium sulfate and concentrated. The residue was purified by column chromatography on Florisil eluting with chloroform:methanol 90:10. The product **2a** (20 mg, 37%) was obtained as an orange solid, mp 192-194°; $^1$H nmr (deuteriochloroform): δ 13.12 (br s, 1H), 8.80 (br t, 1H), 8.75 (s, 1H), 8.03 (s, 1H), 7.48 (d, 1H), 6.67 (d, 1H), 4.32 (t, 2H), 3.30 (q, 2H), 2.65 (t, 2H), 2.47 (t, 2H), 2.10 (s, 6H), 2.00 (s, 6H); ms: (electron impact) m/z 464 ($M^+$, 15%), 406 (60%), 72 (36%), 59 (100%).

2-[2-[(2-Hydroxyethyl)amino]ethyl]-5-chloro-7-[[(4-methoxyphenyl)methyl]oxy]indazolo[4,3-*gh*]isoquinolin-9(2*H*)-one (**21b**).

A solution of 2-[(2-hydroxyethyl)amino]ethylhydrazine (49 mg, 0.41 mmole, 2.5 molar excess) in pyridine (0.8 ml) was added to **20** (65 mg, 0.16 mmole) in pyridine (0.6 ml) at 0° over a 2 minute period and the mixture was stirred for 2 hours. The pyridine was removed under a slow nitrogen stream and the residue placed under vacuum for 1 hour. The residue was purified by thick layer chromatography (silica gel, 2 mm thickness, 10 x 20 cm plate) using 12% methanol: 88% dichloromethane as eluent. The second yellow band was scraped from the plate and the product extracted with 20% methanol: 80% dichloromethane (30 ml). Removal of the solvents led **21b** (15 mg, 20%), mp 96-97°; $^1$H nmr (deuteriochloroform): δ 9.14 (s, 1H), 8.52 (s, 1H), 7.64 (d, $J_{HH}$ = 8.7 Hz, 1H), 7.57 (d, $J_{HH}$ = 8.7 Hz, 1H), 7.52 (d, $J_{HH}$ = 8.7 Hz, 2H), 6.94 (d, $J_{HH}$ = 8.7 Hz, 2H), 5.37 (s, 2H), 4.61 (t, $J_{HH}$ = 5.7 Hz, 2H), 3.82 (s, 3H), 3.62 (t, $J_{HH}$ = 5.1 Hz, 2H), 3.29 (t, $J_{HH}$ = 5.7 Hz, 2H), 2.83 (t, $J_{HH}$ = 5.1 Hz, 2H).

*Anal.* Calcd. for $C_{25}H_{23}N_4O_4Cl$: C, 62.70; H, 4.84; N, 11.70. Found: 62.33; H, 4.92; N, 11.50.

2-[2-[(2-Hydroxyethyl)amino]ethyl]-5-[[2-(dimethylamino)ethyl]-amino]-7-[[(4-methoxyphenyl)methyl]oxy]indazolo[4,3-*gh*]-isoquinolin-6(2*H*)-one (**22b**).

Run 1.

A solution of **21b** (18 mg, 0.04 mmole) and *N,N*-dimethylethylenediamine (66 mg, 0.75 mmole) was heated at 105-110° for 3 hours under a nitrogen blanket. The pyridine and excess amine were removed under a slow stream of nitrogen and the dark residue was placed under vacuum overnight. The crude product was purified *via* thick layer chromatography (silica gel, 2 mm thickness, 10 x 20 cm plates) using 20% methanol:80% dichloromethane as eluent. The reddish-orange band was scraped from the plate and the product extracted into methanol:dichloromethane 1:3 (30 ml). Removal of the solvents led to **22b** (6 mg, 31%), mp 175-176°; $^1$H nmr (deuteriochloroform): δ 9.52 (m, 1H), 9.29 (s, 1H), 8.44 (s, 1H), 7.65 (d, J = 9.1 Hz, 1H), 7.60 (d, J = 8.7 Hz, 2H), 7.01 (d, J = 9.1 Hz, 1H), 6.93 (d, J = 8.7 Hz, 2H), 5.37 (s, 2H), 4.62 (t, J = 5.8 Hz, 2H), 3.82 (s, 3H), 3.61 (t, J = 5.1 Hz, 2H), 3.55 (m, 2H), 3.28 (t, J = 5.8 Hz, 2H), 2.83 (t, J = 5.1 Hz, 2H), 2.70 (t, 6.8 Hz, 2H), 2.37 (s, 6H); $^{13}$C nmr (deuteriochloroform): δ 182.1, 159.4, 154.7, 149.6, 138.8, 134.5, 130.8, 128.8 (2 closely spaced), 126.4, 122.9, 119.0, 114.0 (2 closely spaced), 106.0, 72.1. 60.9, 58.3, 55.3, 50.8, 50.4, 49.1, 45.6, 41.4 (5 overlapping resonances).

*Anal.* Calcd. for $C_{29}H_{34}N_6O_4$: C, 65.65; H, 6.46; N, 15.84. Found: C, 65.26; H, 6.47; N, 15.52.

Run 2.

Compound **22b** and 2-[(2-Hydroxyethyl)amino]ethyl]-5,7-bis-[[2-(dimethylamino)ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (**23b**).

A mixture of **21b** (500 mg, 1.04 mmoles) and *N,N*-dimethylethylenediamine (4.6 ml, 41.80 mmoles) in dimethyl sulfoxide (1.5 ml) was heated for 15 hours at 50° under a nitrogen atmosphere. The excess amine was removed by rotary evaporation and the residue was partitioned between chloroform (100 ml) and water (50 ml). The phases were separated and the aqueous layer was further extracted with chloroform (50 ml). The organic extracts were washed with water (2 x 50 ml) and brine (50 ml), then combined, dried over sodium sulfate and concentrated to dryness. The residue was purified by flash chromatography eluting with chloroform:methanol; concentrated ammonium hydroxide mixtures from 95:5:0.5 to 90:10:1. The fractions containing the first orange band were pooled and concentrated to dryness. The residue was recrystallized twice from ethanol to give **22b** (188 mg, 34%). The chromatographic fractions containing the slower moving red band were pooled and concentrated to dryness. The residue was recrystallized from ethyl acetate (2 ml) to give **23b** (58 mg, 8%) as a red solid; tlc (silica gel, chloroform:methanol: ammonium hydroxide 80:20:1) $R_f$ 0.25, mp 135-138°; $^1$H nmr (deuteriochloroform): δ 9.65 (br t, J = 5.0 Hz, 1H), 9.13 (br t, J = 4.9 Hz, 1H), 8.77 (s, 1H), 8.17 (s, 1H), 7.57 (d, J = 9.0 Hz, 1H), 6.91 (d, J = 9.0 Hz, 1H), 4.58 (t, J = 5.6 Hz, 2H), 3.63 (t, J = 5.2 Hz, 2H), 3.58-3.40 (m, 4H), 3.26 (t, J = 5.6 Hz,

2H), 2.83 (t, J = 5.0 Hz, 2H), 2.76-2.62 (m, 4H), 3.38 (s, 6H), 2.37 (s, 6H); $^{13}$C nmr (deuteriochloroform): δ 186.3, 148.9, 145.9, 135.4, 132.9, 130.8, 130.6, 125.0, 122.8, 118.8, 118.5, 113.8, 105.6, 60.9, 58.2, 50.8, 50.2, 49.1, 45.6, 45.7, 41.2, 40.8 (3 apparently overlapping signals).

*Anal.* Calcd. for $C_{25}H_{36}N_8O_2 \cdot 0.2H_2O$: C, 62.01; H, 7.58; N, 23.14. Found: C, 62.42; H, 7.61; N, 22.79.

2-[2-[(2-Hydroxyethyl)amino]ethyl]-5-[[2-(dimethylamino)ethyl]-amino]-7-hydroxyindazolo[4,3-*gh*]isoquinolin-6(2*H*)-one Trihydrochloride (**2b**).

Concentrated hydrochloric acid (1.0 ml) was added to a stirred suspension of **22b** (246 mg, 0.46 mmole) in ethanol: water 85:15 (12 ml). The mixture was stirred for 21 hours at room temperature and at 50° for an additional 4 hours. The resultant reddish solid was collected by filtration, washed with ethanol:water 9:1 and absolute ethanol and dried under vacuum at 50° to constant weight to yield **2b** (208 mg, 86%), mp (dsc) 190° dec; $^1$H nmr (deuterium oxide): δ 8.35 (s, 1H), 7.91 (s, 1H), 7.85 (d, J = 9.3 Hz, 1H), 7.06 (d, J = 9.3 Hz, 1H), 4.91 (t, J = 5.8 Hz, 2H), 4.03 (t, J = 6.5 Hz, 2H), 3.96-3.85 (m, 2H), 3.79 (t, J = 5.8 Hz, 2H), 3.58 (t, J = 6.5 Hz, 2H), 3.29 (m 2H), 3.05 (s, 6H); $^{13}$C nmr (deuterium oxide): δ 186.1, 159.9, 153.3, 136.6, 135.9, 134.2, 133.9, 127.6, 125.7, 125.0, 124.3, 118.2, 106.7, 59.4, 58.8, 52.4, 49.5, 48.7, 46.3, 41.0 (one overlapping resonance).

*Anal.* Calcd. for $C_{21}H_{26}N_6O_3 \cdot 3HCl \cdot 0.5H_2O$: C, 47.17; H, 5.59, N, 16.05. Found: C, 48.20; H, 5.85; N, 15.33.

2-[2-[*N*-[(1,1-Dimethylethoxy)carbonyl]-*N*-(2-hydroxyethyl)-amino]ethyl]-5-chloro-7-[[(4-methoxyphenyl)methyl]oxy]-indazolo[4,3-*gh*]isoquinolin-6(2*H*)one (**21c**).

A solution of di-*tert*-butyl dicarbonate (48 mg, 0.68 mmole) in tetrahydrofuran (1 ml) was added to a stirred solution of **21b** (250 mg, 0.52 mmole) in tetrahydrofuran (4 ml) and the mixture was stirred at room temperature for 30 hours. The reaction mixture was concentrated to dryness and the residue was purified by flash chromatography by elution with ethyl acetate: methanol 96:4. The chromatographic fractions containing the product were pooled and concentrated to dryness to yield **21c** (302 mg, quantitative) as a brownish-yellow foam; $^1$H nmr (deuteriochloroform, 55°): δ 9.10 (s, 1H), 8.52 (s, 1H), 7.65-7.47 (m, 4H), 6.99-6.88 (m, 2H), 5.35 (s, 2H), 4.72 (t, J = 5.9 Hz, 2H), 3.82 (s, 3H), 3.80 (t, J = 5.9 Hz, 2H), 3.68 (t, J = 4.9 Hz, 2H), 3.21 (t, J = 4.9 Hz, 2H), 1.28 (s, 9H).

2-[2-[*N*-[(1,1-Dimethylethoxy)carbonyl]-*N*-(2-hydroxyethyl)-amino]ethyl]-5-[[2-[*N*-[(1,1-dimethylethoxy)carbonyl]-*N*-methyl-amino]ethyl]amino]-7-[[(4-methoxyphenyl)methyl]oxy]indazolo-[4,3-*gh*]isoquinolin-6(2*H*)-one (**22c**).

The *N*-*tert*-butoxycarbonyl-*N*-methylethylenediamine [19] (1.92 g, 11.00 mmoles) was added to a solution of **21c** (300 mg, 0.52 mmole) in dichloromethane (5 ml). The solvent was removed by rotary evaporation and the resulting residue was kept at 50° for 18 hours and at 70° for 24 hours. The reaction mixture was partitioned between ethyl acetate (50 ml) and a phosphate buffer made from water (45 ml), a saturated solution of dihydrogen sodium phosphate (5 ml) and 6 *N* hydrochloric acid (1 ml). The organic layer was separated and washed with an additional amount of the phosphate buffer (50 ml) and with brine (50 ml). The aqueous layer were further extracted with ethyl acetate (25 ml). The combined extracts were dried over sodium sulfate and concentrated to dryness. The residue was purified by flash chromatography eluting with ethyl acetate:methanol 96:4. The chromatographic fractions containing the product were pooled and concentrated to dryness to yield **22c** (108 mg, 29%) as an orange foam. Chromatographic purification of the mixed fractions from the first chromatography yielded a second crop of product (104 mg, 28%) as an orange foam.

Although the product was chromatographically homogeneous (tlc), nmr analysis showed small amounts of one or more impurities which could not be identified or quantified. The product was used in the subsequent step without further purification; $^1$H nmr (deuteriochloroform, 55°): δ 9.47 (t, 1H), 9.26 (s, 1H), 8.45 (s, 1H), 7.62 (d, 1H), 7.53 (d, 2H), 7.11 (d, 1H), 6.94 (d, 2H), 5.35 (s, 2H), 4.71 (t, 2H), 3.82 (s, 3H), 3.80 (t, 2H), 3.75-3.45 (m, 6H), 3.23 (br t, 2H), 2.95 (s, 3H), 1.45 (br s, 9H), 1.27 (br s, 9H).

2-[2-[(2-Hydroxyethyl)amino]ethyl]-5-[[2-(methylamino)ethyl]-amino]-7-hydroxyindazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (**2c**).

A solution of hydrogen chloride in ethanol (4.1 *M*, 2 ml) was added to a solution of **22c** (230 mg, 0.32 mmole) in absolute ethanol (2 ml). The mixture was stirred at room temperatrue for 18 hours upon which hydrochloric acid (6 *N*, 0.5 ml) was added and the mixture was heated at 50° for 6 hours. The resultant precipitate was collected by filtration, washed with ethanol and dried under vacuum at 40°; analysis by nmr indicated this solid was a 3:1 mixture of the desired product and an unknown impurity. The product was purified by flash chromatography by elution with chloroform:methanol:concentrated ammonium hydroxide mixtures from 85:15:1 to 60:40:3. The chromatographic fractions containing the product were pooled and concentrated to dryness. The solid which was obtained was insoluble in the common organic solvents. Reverse phase chromatography (LiChro-prep RP-18 40-63 micrometers, 12 g; eluents water, water:methanol 1:1 and methanol) followed by recrystallization from ethanol:water afforded **2c** (75 mg, 58%). This product failed to give a satisfactory elemental analysis even though nmr analysis was consistent with a single product with a purity of >95%; $^1$H nmr (deuterium oxide): δ 7.84 (s, 1H), 7.63 (d, 1H), 7.45 (s, 1H), 6.85 (d, 1H), 4.73 (t, 2H), 4.95-5.85 (m 2H), 3.83 (t, 2H), 3.70 (t, 2H), 3.50-3.30 (m, 4H), 2.84 (s, 3H); $^1$H nmr (deuterium oxide, 55°): 8.47 (s, 1H), 8.09 (d, 1H), 8.02 (s, 1H), 7.30 (d, 1H), 5.15 (t, 2H), 4.32-4.18 (m, 4H), 4.11 (t, 2H), 3.80 (t, 2H), 3.72 (t, 2H), 3.21 (s, 3H); $^{13}$C nmr (deuterium oxide, 55°): δ 182.0, 158.6, 153.1, 139.8, 136.6, 134.0, 125.4, 125.3, 124.2, 123.4, 118.4, 106.5, 59.8, 52.8, 51.0, 49.9, 48.7, 42.3, 36.6 (one peak missing or overlapping).

2-[2-[*N*-[(1,1-Dimethylethoxy)carbonyl]-*N*-(2-hydroxyethyl)-amino]ethyl]-5-[[2-[*N*-[(1,1-dimethylethoxy)carbonyl]-*N*-methyl-amino]ethyl]amino]-7-hydroxyindazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (**2d**).

To a solution of **2c** (70 mg, 0.18 mmole) in water (1 ml) sodium hydroxide (1*N*, 0.5 ml) was added followed by a solution of di-*tert*-butyl dicarbonate (95 mg, 0.893 mmole) in tetrahydrofuran (0.5 ml). The mixture was stirred at room temperature for 3 hours and then partitioned between water (10 ml) and ethyl acetate (15 ml). The organic layer was washed with water (10 ml) and brine (10 ml). The aqueous layers were further extracted with ethyl acetate (10 ml). The combined organic extracts were dried over sodium sulfate and concentrated to dryness. The residue was purified by flash chromatography eluting with ethyl acetate:methanol from 90:20 to 85:15. The chromatographic fractions containing the product were pooled and concentrated to dryness to afford **2d**

(42 mg, 40%) as an orange foam, which was used without further purification in the next step; $^1$H nmr (deuteriochloroform, 55°): δ 13.05 (s, 1H), 8.96 (s, 1H), 8.87 (br t, J = 5.3 Hz, 1H), 8.36 (s, 1H), 7.59(d, J = 9.1Hz, 1H), 6.94(d, J = 9.1Hz, 1H), 4.69 (t, J = 5.9 Hz, 2H), 3.79 (t, J = 5.9 Hz, 2H), 3.73-3.53 (m, 6H), 3.20 (t, J = 5.9 Hz, 2H), 2.96 (s, 3H), 1.49 (s, 9H), 1.28 (s, 9H).

2-[2-[(2-Hydroxyethyl)amino]ethyl]-5-[[2-(methylamino)-ethyl]amino]-7-hydroxyindazolo[4,3-*gh*]isoquinolin-6(2*H*)-one Trihydrochloride (**2c** Trihydrochloride).

A hydrogen chloride solution in ethanol (4.1 *M*, 0.5 ml) was added to a solution of **2d** (42 mg, 0.07 mmole) in ethanol (1.5 ml) and the mixture was stirred at room temperature for 57 hours. The precipitated solid was collected by filtration, washed with ethanol and dried under vacuum at 40° to yield the trihydrochloride salt (25 mg, 70%) as a dark red solid; $^1$H nmr (deuterium oxide): δ 8.54 (s, 1H), 8.07 (s, 1H), 7.92 (d, 1H), 7.11 (d, 1H), 4.95 (t, 2H), 3.99 (t, 2H), 3.89 (t, 2H), 3.81 (t, 2H), 3.46 (t, 2H), 3.34 (t, 2H), 2.82 (s, 3H).

*Anal.* Calcd. for $C_{20}H_{24}N_6O_3 \cdot 3HCl \cdot 0.57H_2O$: C, 46.54; H, 5.50; N, 16.28; Cl, 20.61. Found: C, 46.39; H, 5.48; N, 15.61; Cl, 20.13.

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