Synthesis and Antitumor Evaluation of 2,5-Disubstituted-Indazolo[4,3-*gh*]isoquinolin-6(2*H*)-ones (9-Aza-anthrapyrazoles)

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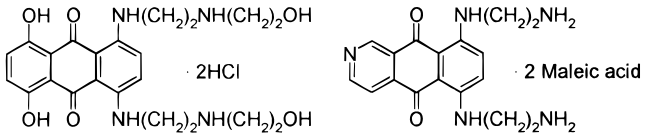
The synthesis and antitumor evaluation of 2,5-disubstituted-indazolo[4,3-*gh*]isoquinolin-6(2*H*)-ones (9-aza-APs) are described. The key intermediates in the synthesis are benz[*g*]isoquinoline-5,10-diones which are substituted at positions 6 and 9 with groups of different nucleofugacity for S_NAr displacements. The initial displacement of fluoride by a substituted hydrazine leads to the pyrazole analogues. Substitution of the remaining leaving group by an amine or BOC-protected amines leads to the 9-aza-APs **12**. These analogues were converted into their maleate or hydrochloride salts **13**. In two cases, namely, **13x** and **13z**, sidearm buildup was also employed in the synthetic pathway. In vitro evaluation of 9-aza-APs against the human colon tumor cell line LoVo uncovered for most of the compounds a cytotoxic potency lower than that of DuP-941 or mitoxantrone and comparable to that of doxorubicin. Only analogues **13c**, **13n**, and **13ff** were as cytotoxic as DuP-941. Interestingly, while DuP-941 was highly cross-resistant in the LoVo cell line resistant to doxorubicin (LoVo/Dx), the 9-aza-APs carrying a distal lipophilic tertiary amine moiety in both chains were capable of overcoming the MDR resistance induced in this cell line. The 9-aza-APs show outstanding in vivo antitumor activity against both systemic P388 murine leukemia and MX-1 human mammary carcinoma transplanted in nude mice. At their optimal dosages, congeners **13a**−**c**, **13f**, **13n**, **13q**, **13x**, and **13dd** were highly effective against P388 leukemia with T/C% of 200−381, while the T/C% value of DuP-941 was 147. In the MX-1 tumor model, 24 compounds elicited percentages of tumor weight inhibitions (TWI) ranging from 50% to 99%. Congeners **13d**, **13k**, **13l**, **13x**, **13z**, and **13ee** emerged as the most effective ones, with TWI% 96, simliar to that of DuP-941 (TWI% = 95). On the basis of their efficacy profile in additional experimental tumors and lack of cardiotoxicity in preclinical models, two congeners have surfaced as potential clinical candidates.

Introduction

Although potential drug targets only present in cancerous cells have surfaced, the design of a drug which is selectively toxic to a tumor and not to the host organism is still very difficult. Many useful therapeutic drugs have their discoveries rooted in serendipity. In particular, one might note the development of the important anticancer drugs doxorubicin and mitoxantrone (Chart 1).[1−4] Mitoxantrone, an anthracene-9,10-dione, has gained an important position in the clinical management of leukemia and lymphomas as well as in combination therapy of advanced breast and ovarian cancers.[5−7] Although mitoxantrone is endowed with an improved tolerance profile when compared with doxorubicin and other anthracyclines,[8] it is not devoid of significant toxic side effects, especially those associated with myelosuppression and cardiotoxicity.[9,10] Mitoxantrone also shows a cross-resistance to cell histotypes developing resistance against doxorubicin mediated by overexpression of glycoprotein P.[11−12] The cell-killing effects elicited by mitoxantrone are probably multimodal in nature.[13] Several studies suggest that intercalation into DNA is a major cellular event and this intercalative interaction may serve as an anchor for the drug at specific base pair sites,[14,15] which is then followed by the critical cell-killing events.[16−19]

Chart 1. Structures of Mitoxantrone and BBR 2778



Mitoxantrone Dihydrochloride BBR 2778 Dimaleate

The biochemical basis for the cardiotoxicity exhibited by mitoxantrone is not fully understood. It is generally believed that the in vivo reduction of the quinone moiety is probably more related to the cardiotoxic side effects of mitoxantrone than to its mechanism of cytotoxicity. The generation of free radicals occurs by addition of an electron to the quinone to form semi-quinone free radicals which then transfer an electron to molecular oxygen to afford superoxide radical anions. These radi-

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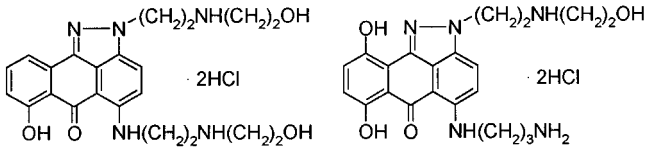
Chart 2. Structures of DuP-941 and DuP-942



DuP-941 DuP-942

cal anions ultimately lead to hydroxyl radicals which can damage cardiac tissue. The reaction of the quinone with singlet oxygen followed by electron transfer to form the destructive radical species has been advanced as an alternative hypothesis.[20] It has also been suggested that the affinity of molecules such as mitoxantrone for electron-transferring flavo-proteins, instead of their redox properties, may play the most important role in free radical formation and their cardiotoxic side effects.[21−23] In addition, the presence of the hydroxy groups in mitoxantrone can facilitate metal ion binding and enhance redox cycling by metal-catalyzed reactions.[24]

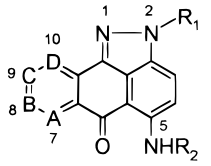
Despite the attempts to rationalize the cardiotoxicity of anthracene-9,10-dione antitumor agents, few compounds have been shown to possess both good antitumor activity and little or no cardiotoxicity. Consequently there appears to be no way to predict which compounds will be cardiotoxic and which compounds will not. One is thus confronted with the major problem of designing molecules with high efficacy and no toxicity.

Over the past few years, the introduction of heteroatoms into different positions of the anthracene-9,10-dione chromophore has been investigated by our group as a means of identification of effective second-generation anthracene-9,10-dione chemotypes lacking the 5,8-dihydroxy substitution pattern.[25−29] These bioisosteres would clearly differ from their carbocyclic counterparts in their interactions with DNA and enzymes, as well as in their reduction potentials. A number of aza-bioisosteric chemotypes related to mitoxantrone have been synthesized, and the position of the nitrogen atom was found to exert a profound effect on the antitumor activity. This has led to the discovery of 6,9-bis[(2-aminoethyl)amino]benz[*g*]isoquinoline-5,10-dione dimaleate salt (BBR 2778; Chart 1), an aza-anthracene-9,10-dione which is currently in phase I clinical trials. This chemotype, whose profile of preclinical antitumor activity is comparable to that of mitoxantrone, is devoid of any significant toxic effect on cardiac tissue, after both single- and multiple-dose treatment, respectively, in the rat and mouse.[30]

Anthrapyrazoles were initially developed as chromophore-modified anthracene-9,10-diones with the goals of increasing the spectrum of antitumor activity and reducing the cardiotoxic potential exhibited by quinone analogues.[31−38] Compounds of this class, such as DuP-941 and DuP-942 (Chart 2), were expected to be less prone to undergo bioreduction to anion radicals and thus could inhibit radical-cycling processes which might be responsible for cardiotoxicity.

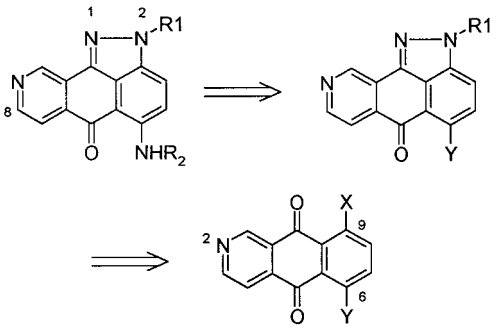
It has also been proposed that the reduced peroxidizing activity may be a result of the decreased affinity of these molecules for NADH dehydrogenase responsible for the one-electron transfer to the chromophore.[23] In addition, it has been noted that while molecules such as DuP-941 are quite resistant to metabolic reduction they are susceptible to facile oxidation.[32] However, whereas DuP-941 has demonstrated good clinical efficacy in treatment of breast cancer,[36] cardiac toxicitiy has been observed during clinical trials with both DuP-941 and DuP-942.[39−44]

Chart 3. Regioisomeric 7-, 8-, 9-, and 10-Aza-anthrapyrazoles



7-Aza A = N, B = C = D = CH
8-Aza B = N, A = C = D = CH
9-Aza C = N, A = B = D = CH
10-Aza D = N, A = B = C = CH

Scheme 1



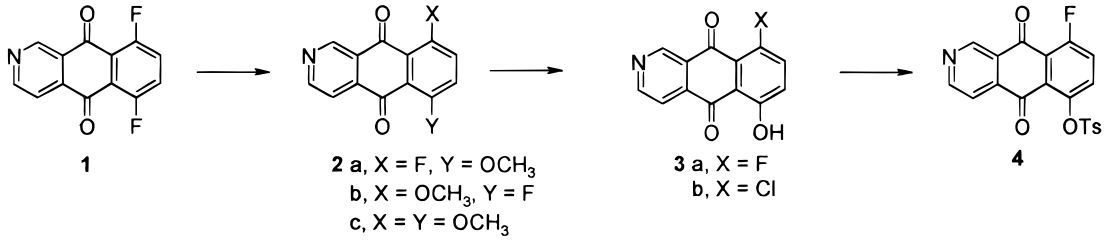
Of particular interest to us was the preparation of aza analogues related to the anthrapyrazoles (Chart 3). We have substituted the CH moieties at positions 7, 8, 9, and 10 by a nitrogen atom to afford the 7-aza-, 8-aza-, 9-aza-, and 10-aza-anthrapyrazoles while simultaneously removing the OH moieties at positions 7 and 10.[45−52]

Comparative antitumor evaluations of these four regioisomers in several cell lines in vitro and in vivo clearly showed that the cytotoxic effects were critically dependent on the position of the nitrogen atom.[45] The 9-aza regioisomers (2,5-disubstituted indazolo[4,3-*gh*]isoquinolin-6(2*H*)-ones), hereinafter referred to as 9-aza-APs, surfaced as the most potent group of compounds, and this manuscript will focus on the synthesis and antitumor evaluations of these chemotypes. The synthesis of the regioisomeric aza-anthrapyrazoles and several hydroxy-substituted analogues[52] and their biological evaluations will be reported in subsequent publications.

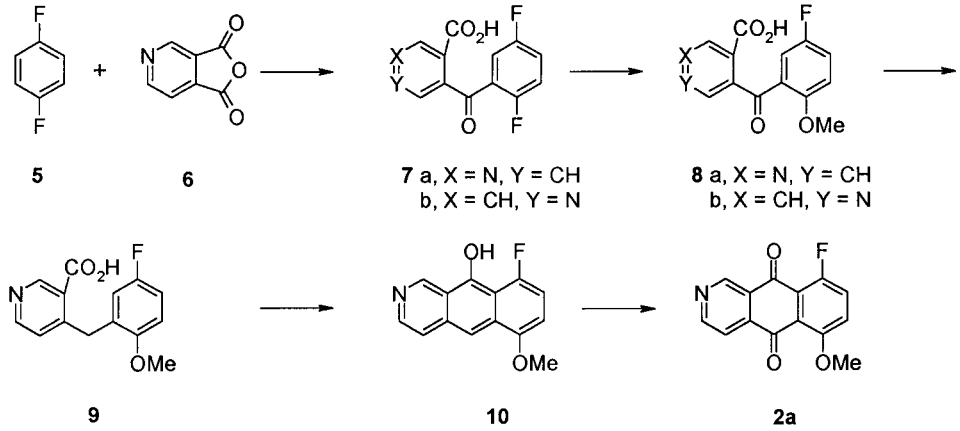
Chemistry

The synthesis of indazolo[4,3-*gh*]isoquinolin-6(2*H*)-ones (9-aza-anthrapyrazoles, 9-aza-APs) required as key intermediates benz[*g*]isoquinoline-5,10-diones substituted at the 6 and 9 positions with groups which differ in their S_NAr displacement rates. The retrosynthetic strategy is illustrated in Scheme 1. On treatment of these intermediates with a substituted hydrazine, the more readily displaced group X would be regioselectively

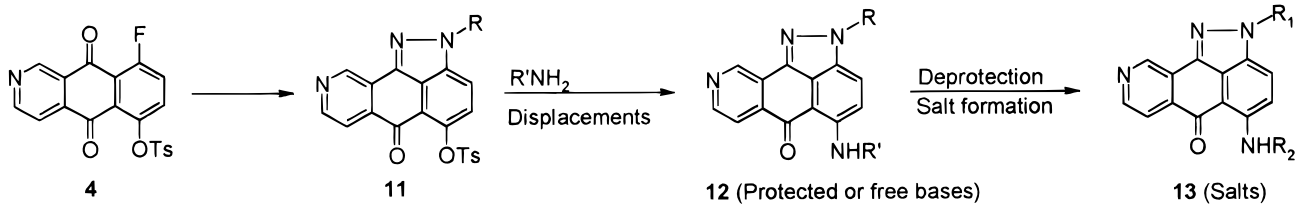
Scheme 2



Scheme 3



Scheme 4



substituted by the nitrogen atom of the hydrazine bearing the substituent to yield the pyrazole ring. Treatment of these pyrazole derivatives with an appropriately substituted amine would lead to the substitution of the remaining leaving group Y at position 5 to lead to the desired 9-aza-APs.

Pathway 1. The critical starting materials for the synthesis of the 9-aza-APs were prepared following the route shown in Scheme 2. Although treatment of **1**[25] with sodium methoxide in tetrahydrofuran did not show any regioselectivity in S_NAr displacements of the fluorides, the regioisomer **2a** preferentially separated from the reaction mixture and could be obtained in satisfactory purity by recrystallizations. Chromatography of the soluble material led to the isolation of the regioisomer **2b** along with the bis-substitution product **2c**.

Initial attempts at demethylations of **2a** with aluminum trichloride in dichloromethane at reflux led to mixtures of **3a** and **3b** (from S_NAr displacement of fluoride by chloride) which could be separated by chromatography. The product ratios **3a**:**3b** were dependent upon the amount of aluminum trichloride used and the refluxing time. The demethylation of **2a** by methanesulfonic acid at 110 °C led to pure **3a** (89%) which was used in the tosylation step to afford **4**.

The structural assignment for regioisomer **2a** was confirmed by an unambiguous synthesis following the pathway shown in Scheme 3. Friedel−Crafts acylation of 1,4-difluorobenzene (**5**) with anhydride **6** led to a regioisomeric mixture of **7a**:**7b** (ratio 4:1).[25] This mixture on treatment with sodium methoxide in methanol led to **8a** and **8b** by regioselective displacements of the fluoride adjacent to the carbonyl group. The reduction of the carbonyl groups in the **8a**:**8b** mixture was accomplished by zinc powder in formic acid, and chromatographic purification gave pure **9**. Cyclization of **9** by polyphosphoric acid led to the aza-anthranol **10** which upon treatment with ceric ammonium nitrate led to regioisomer **2a**. This product was identical in its spectroscopic properties to that obtained from the methoxide displacement from **1**.

The synthesis of the 9-aza-APs followed the route illustrated in Scheme 4. Reactions of **4** with the appropriately substituted hydrazine led to the regioselective displacement of the fluoride to yield the 5-tosyloxy-9-aza-APs **11** which are tabulated in Table 1. Tosylate **11d** was obtained from **11c** by standard BOC protection of the sidearm amino functionality. Reactions of the tosylates **11** with the appropriately substituted amine or BOC-protected amine led to ipso substitution of the tosylate group to afford the free bases or BOC-protected bases **12** (Table 2) as red-orange powders or glassy solids in reasonable yields. The final compounds **13** (Table 3) were obtained either as maleate salts by treatment of the free bases **12** with maleic acid or as

Scheme 5

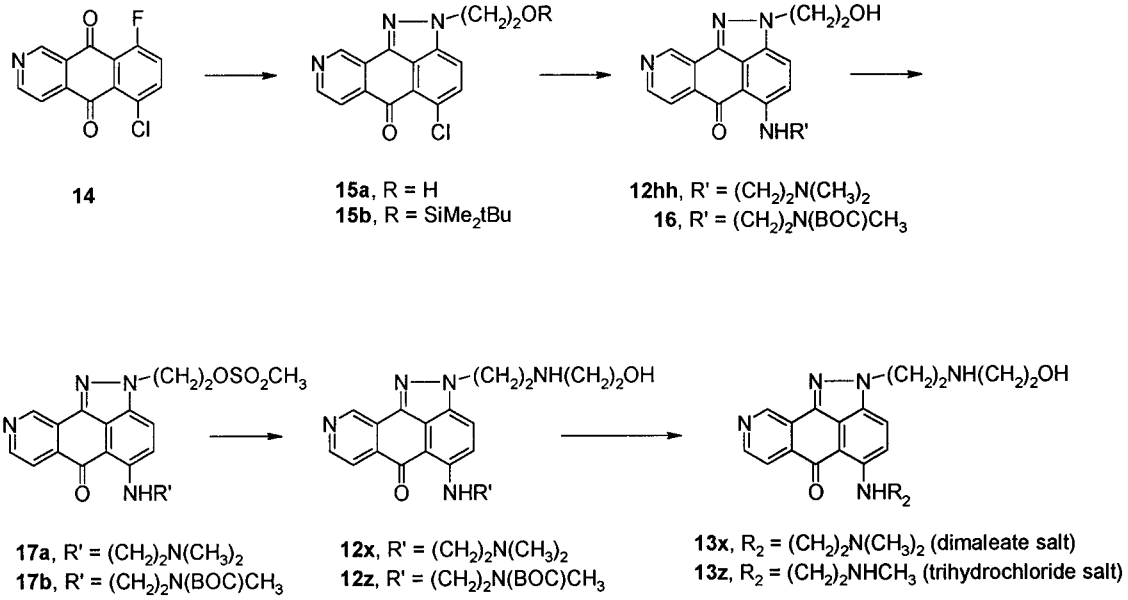
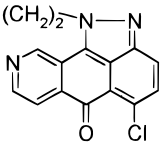


Chart 4. Alternative Structural Formulation



18

hydrochloride salts by treatment of the free bases or the BOC-protected bases **12** with hydrochloric acid.

Pathway 2. An alternative route to the 9-aza-APs was also developed which involved the sequential displacements of the halo substituents from dione **14.**[53] This intermediate was converted to **13x** and **13z** by the sequence of reactions illustrated in Scheme 5. Treatment of **14** with commercially available (2-hydroxyethyl)hydrazine led to **15a** which on reaction with *N*,*N*-dimethylethylenediamine or *N*-BOC-*N*-methylethylenediamine led to **12hh** and **16**, respectively. Conversion of **12hh** and **16** to the mesylates **17a** and **17b** followed by displacements of the mesyl group by 2-aminoethanol afforded **12x** and **12z**, respectively. Treatment of **12x** with maleic acid led to dimaleate **13x**, while treatment of **12z** with hydrochloric acid led to trihydrochloride **13z**.

To firmly establish the structure for the pyrazole moiety, formed from the initial hydrazine displacement, and to rule out structure **18** (Chart 4), **15a** was converted to the silylated derivative **15b** by treatment with *tert*-butyldimethylsilyl chloride. Crystallization from a dichloromethane/hexane mixture by the "double-layer" technique yielded a large single crystal which on X-ray crystallographic analysis confirmed the structure as **15b**.

Biological Results and Discussion

The in vitro antiproliferative activity values for analogues **13** against human colon adenocarcinoma (LoVo) and its subline resistant to doxorubicin (LoVo/Dx) with comparative data for DuP-941, mitoxantrone, and doxorubicin are listed in Table 4. On the LoVo cell line, the 9-aza-APs show cytotoxicity lower than that of DuP-941 or mitoxantrone and similar to that of doxorubicin. The most potent compounds are **13c**, **13n**, and **13ff**, which retain cytotoxic levels similar to that of DuP-941. With a few exceptions (such as **13ff**), congeners with a tertiary amino substituent in R_1 are generally more potent than their primary or secondary amine analogues. Sidearm homologation in R_2 (**13a** and **13g**, **13b** and **13h**) or R_1 (**13a** and **13dd**, **13d** and **13ee**) has a variable effect on the potency against LoVo cells which depends on the nature of the R_2 substituent. With the exception of **13k**, a nonbasic sidearm in R_1 (**13hh**, **13ii**) or R_2 (**13 cc**, **13o**) significantly reduces or abolishes the cytotoxicity on LoVo cells.

Table 1. Tosylate Intermediates **11**

11	R
a	$CH_2CH_2NMe_2$
b	CH_2CH_2NHBOC
c	$CH_2CH_2NHCH_2CH_2OH$
d[a]	$CH_2CH_2N(BOC)CH_2CH_2OH$
e	$CH_2CH_2CH_2NMe_2$
f	CH_2CH_2OH
g	$CH_2CH_2N(BOC)CH_3$

[a] Prepared by treatment of **11c** with di-*tert*-butyl dicarbonate.

The introduction of a second basic site in R_2 appears to diminish the activity significantly (**13d** and **13p**, **13f** and **13j**). Compounds holding a distal, lipophilic tertiary amino moiety in both R_1 and R_2 (**13a**, **13e**, **13f**, **13g**, and **13i**) are capable of overcoming the MDR resistance induced in the LoVo/Dx cell line.

The antitumor activity of the 9-aza-APs against systemic murine leukemia P388 is shown in Table 5. At their optimal dosages (OD) several 9-aza-APs show significant antileukemia efficacy (T/C% > 200): **13a**−**c**, **13f**, **13n**, **13q**, **13x**, and **13dd**, with the most active compound being the bis[2-(dimethylamino)ethyl] analogue **13a**. High levels of activity (T/C% = 194) are also shown by compounds **13d**, **13r**, **13t**, and **13w**. Many analogues are much more effective than DuP-941 and as active as or more active than mitoxantrone or doxorubicin. Seven of the eight chemotypes with T/C%

Table 2. Intermediates **12** Prepared from Tosylates **11**

12	method	reaction conditions (% yield)	R	R′
a	A	**11a**, $NH_2CH_2CH_2NMe_2$, pyd, 80 °C (61%)	$CH_2CH_2NMe_2$	$CH_2CH_2NMe_2$
b	A	**11a**, $NH_2CH_2CH_2NH_2$, pyd, 80 °C (70%)	$CH_2CH_2NMe_2$	$CH_2CH_2NH_2$
c	A	**11a**, $NH_2CH_2CH_2N(BOC)Me$, pyd, 75% (nd)	$CH_2CH_2NMe_2$	$CH_2CH_2N(BOC)Me$
d	A	**11a**, $NH_2CH_2CH_2NHCH_2CH_2OH$, pyd, 75 °C (nd)	$CH_2CH_2NMe_2$	$CH_2CH_2NHCH_2CH_2OH$
e	A	**11a**, $NH_2CH_2CH_2NEt_2$, pyd, 70 °C (64%)	$CH_2CH_2NMe_2$	$CH_2CH_2NEt_2$
f	A	**11a**, $NH_2CH_2CH_2N(CH_2CH_2)_2O$, pyd, 70 °C (60%)	$CH_2CH_2NMe_2$	$CH_2CH_2N(CH_2CH_2)_2O$
g	A	**11a**, $NH_2CH_2CH_2CH_2NMe_2$, pyd, 70 °C (46%)	$CH_2CH_2NMe_2$	$CH_2CH_2CH_2NMe_2$
h	A	**11a**, $NH_2CH_2CH_2CH_2NH_2$, pyd, 70 °C (67%)	$CH_2CH_2NMe_2$	$CH_2CH_2CH_2NH_2$
i	A	**11a**, 1-(2-aminoethyl)piperidine, pyd, 75 °C (nd)	$CH_2CH_2NMe_2$	$CH_2CH_2N(CH_2CH_2)_2CH_2$
j	A	**11a**, 1-(2-aminoethyl)-4-BOC-piperazine, pyd, 60 °C (56%)	$CH_2CH_2NMe_2$	$CH_2CH_2N(CH_2CH_2)_2NBOC$
k	A	**11a**, $NH_2CH_2CH_2OH$, pyd, 75 °C (nd)	$CH_2CH_2NMe_2$	CH_2CH_2OH
l	A	**11a**, $NH_2CH_2CH_2N(CH_2CH_2OH)_2$, pyd, 75 °C (nd)	$CH_2CH_2NMe_2$	$CH_2CH_2N(CH_2CH_2OH)_2$
m	B	**11a**, 2-(imidazol-1-yl)ethylamine, DMSO, 50 °C (nd)	$CH_2CH_2NMe_2$	2-(imidazol-1-yl)ethyl
n	A	**11a**, $NH_2CH_2CH_2N(Me)CH_2OH_2OH$, pyd, rt (61%)	$CH_2CH_2NMe_2$	$CH_2CH_2N(Me)CH_2CH_2OH$
o	C	(i) **11a**, $NH_2CH_2CH_2NH_2$, pyd, 60 °C; (ii) $MeSO_2Cl$, TEA, CH_2Cl_2, 40 °C (64%)	$CH_2CH_2NMe_2$	$CH_2CH_2NHSO_2Me$
p	A	**11a**, $NH_2CH_2CH_2NHCH_2CH_2NMe_2$, pyd, 60 °C (66%)	$CH_2CH_2NMe_2$	$CH_2CH_2NHCH_2CH_2NMe_2$
q	A	**11b**, $NH_2CH_2CH_2NMe_2$, pyd, 80 °C (70%)	CH_2CH_2NHBOC	$CH_2CH_2NMe_2$
r	A	**11b**, $NH_2CH_2CH_2NHBOC$, pyd, 80 °C (61%)	CH_2CH_2NHBOC	CH_2CH_2NHBOC
s	B	**11b**, $NH_2CH_2CH_2N(BOC)Me$, DMSO, 60 °C (60%)	CH_2CH_2NHBOC	$CH_2CH_2N(BOC)Me$
t	A	**11b**, $NH_2CH_2CH_2NHCH_2CH_2OH$, pyd, 50 °C (55%)	CH_2CH_2NHBOC	$CH_2CH_2NHCH_2CH_2OH$
u	A	**11b**, $NH_2CH_2CH_2NEt_2$, pyd, 70 °C (53%)	CH_2CH_2NHBOC	$CH_2CH_2NEt_2$
v	A	**11b**, $NH_2CH_2CH_2N(CH_2CH_2)_2O$, pyd, 70 °C (61%)	CH_2CH_2NHBOC	$CH_2CH_2N(CH_2CH_2)_2O$
w	A	**11b**, $NH_2CH_2CH_2N(CH_2CH_2OH)_2$, pyd, 70 °C (53%)	CH_2CH_2NHBOC	$CH_2CH_2N(CH_2CH_2OH)_2$
x	A	**11c**, $NH_2CH_2CH_2NMe_2$, pyd, 50 °C (59%)	$CH_2CH_2NHCH_2CH_2OH$	$CH_2CH_2NMe_2$
y	D	(i) **11c**, $NH_2CH_2CH_2NH_2$, pyd, 50 °C; (ii) $(BOC)_2O$, THF, 1 N NaOH (38%)	$CH_2CH_2N(BOC)CH_2CH_2OH$	CH_2CH_2NHBOC
z	A	**11c**, $NH_2CH_2N(BOC)Me$, pyd, 50 °C (44%)	$CH_2CH_2NHCH_2CH_2OH$	$CH_2CH_2N(BOC)Me$
aa	A	**11d**, $NH_2CH_2CH_2NHCH_2CH_2OH$, pyd, rt (60%)	$CH_2CH_2N(BOC)CH_2CH_2OH$	$CH_2CH_2NHCH_2CH_2OH$
bb	A	**11d**, $NH_2CH_2CH_2N(CH_2CH_2OH)_2$, pyd, rt (47%)	$CH_2CH_2N(BOC)CH_2CH_2OH$	$CH_2CH_2N(CH_2CH_2OH)_2$
cc	A	**11d**, $NH_2CH_2CH_2OH$, pyd, 70 °C (72%)	$CH_2CH_2N(BOC)CH_2CH_2OH$	CH_2CH_2OH
dd	A	**11e**, $NH_2CH_2CH_2NMe_2$, pyd, 40 °C (65%)	$CH_2CH_2CH_2NMe_2$	$CH_2CH_2NMe_2$
ee	A	**11e**, $NH_2CH_2CH_2NHCH_2CH_2OH$, pyd, 50% (52%)	$CH_2CH_2CH_2NMe_2$	$CH_2CH_2NHCH_2CH_2OH$
ff	E	(i) **11f**, $NH_2CH_2CH_2OH$, pyd, 70 °C (61%); (ii) $MeSO_2Cl$, TEA (64%); (iii) $MeNH_2$, then $(BOC)_2O$/THF (71%)	$CH_2CH_2N(BOC)Me$	$CH_2CH_2N(BOC)Me$
gg	A	**11g**, $NH_2CH_2CH_2NHCH_2CH_2OH$, pyd, 50 °C (59%)	$CH_2CH_2N(BOC)Me$	$CH_2CH_2NHCH_2CH_2OH$
hh	A	**11f**, $NH_2CH_2CH_2NMe_2$, pyd, 80 °C (nd)	CH_2CH_2OH	$CH_2CH_2Me_2$
ii	B	**11f**, $NH_2CH_2CH_2NHBOC$, DMSO, 70 °C (50%)	CH_2CH_2OH	CH_2CH_2NHBOC

greater than 200 hold at least one distal dimethylamino moiety in the R_1 or R_2 sidearm. With the exception of **13k,** which has borderline activity**,** the presence of a nonbasic sidearm in R_1 or R_2 (compounds **13o**, **13cc**, **13hh**, and **13ii**) abolished antileukemic activity. Introduction in R_2 of a second basic site also greatly diminishes activity (compare **13d** with **13p** or **13f** with **13j**).

With the possible exception of the *N*-bis(2-hydroxyethyl) analogue **13w**, steric effects on the distal nitrogen atom of the R_2 residues result in a significantly reduced activity and/or potency (compare **13a** with **13e** or **13l**, **13q** with **13u** or **13w**, **13x** with **13bb**).

Side-chain homologation in R_2 from a C2 to a C3 methylene spacer results in a significant decrease of activity (compare **13a** and **13g**), whereas the same homologation in R_1 seems to retain activity (comparison of **13a** with **13dd** or **13d** with **13ee**). The exchange of the basic R_1 and R_2 substituents in general does not affect activity significantly (comparison of **13b** with **13q**, **13d** with **13x**, **13t** with **13y**, **13z** with **13gg**).

The in vivo antitumor activity of the 9-aza-APs against MX-1 human mammary carcinoma subcutaneously transplanted in nude mice is also tabulated in Table 5. Twenty-four compounds were found to be active in this model, with tumor weight inhibition (TWI%) ranging from 50% to 99%. Several analogues (**13d**, **13k**, **13l**, **13x, 13z**, and **13ee**) show outstanding activity, with TWI% values greater than 96 and comparable to those of DuP-941 (95%). As in the P388 model, side-chain homologation in R_1 (compare **13d** with **13ee** or **13a** with **13dd**) retains activity, whereas it has a variable effect in R_2 (compare **13a** with **13g** or **13b** with **13h**). It might be of interest to note that the presence of a nonbasic side chain such as R_2 (**13k** and **13o**), but not R_1 (**13hh**), is compatible with high levels of activity.

Conclusions

The 9-aza-anthrapyrazoles (9-aza-APs) emerged as the most interesting chemotypes from a series of isosteres obtained by systematic introduction of nitrogen atoms in the anthrapyrazole chromophore with concomitant deletion of ring hydroxyl substituents. From a synthetic point of view, accessibility of this class of compounds has required the establishment of nonobvious synthetic methodologies, which feature the critical intermediacy of benz[*g*]isoquinoline-5,10-diones such as **4** and **14** suitably substituted at positions 6 and 9 with leaving groups of differential reactivity in S_NAr reactions.

In vitro screening of 9-aza-APs against the human colon tumor cell line LoVo uncovered for most of the compounds a decreased cytotoxic potency in comparison with DuP-941. This reduced potency is likely due to the lack of the hydroxyl substituent(s) typically present in the anthrapyrazoles selected for clinical trials since the introduction of a hydroxyl group in the 7 position of the 9-aza-anthrapyrazole chromophore results in a remarkable enhancement of potency.[52]

Table 3. 9-Aza-anthrapyrazole Salts **13** Prepared from **12**

13	method	mp, °C	reaction conditions from **12** (yield)	salt	R_1	R_2
a	F	189−191	maleic acid, EtOH, rt (91%)	dimaleate	$CH_2CH_2NMe_2$	$CH_2CH_2NMe_2$
b	G	172−173	maleic acid, EtOH/$CHCl_3$, 50 °C (57%)	dimaleate	$CH_2CH_2NMe_2$	$CH_2CH_2NH_2$
c	H	166−167	(i) TFA, CH_2Cl_2 (57%); (ii) maleic acid, EtOH, 50 °C (68%)	dimaleate	$CH_2CH_2NMe_2$	CH_2CH_2NHMe
d	F	145[a]	maleic acid, EtOH, 50 °C (85%)	dimaleate	$CH_2CH_2NMe_2$	$CH_2CH_2NHCH_2CH_2OH$
e	F	144−145	maleic acid, EtOH, 50 °C (58%)	dimaleate	$CH_2CH_2NMe_2$	$CH_2CH_2NEt_2$
f	F	180−182	maleic acid, EtOH, 50 °C (75%)	dimaleate	$CH_2CH_2NMe_2$	$CH_2CH_2N(CH_2CH_2)_2O$
g	F	139−141	maleic acid, EtOH, 50 °C (90%)	dimaleate	$CH_2CH_2NMe_2$	$CH_2CH_2CH_2NMe_2$
h	F	181[a,b]	maleic acid, EtOH, 50 °C (34%)	dimaleate	$CH_2CH_2NMe_2$	$CH_2CH_2CH_2NH_2$
i	F	165−167	maleic acid, EtOH, 50 °C (47%)	dimaleate	$CH_2CH_2NMe_2$	$CH_2CH_2N(CH_2CH_2)_2CH_2$
j	I	150[b]	(i) HCl/EtOH−H_2O; (ii) $NaHCO_3$; (iii) maleic acid, EtOH, 70 °C	dimaleate	$CH_2CH_2NMe_2$	$CH_2CH_2N(CH_2CH_2)_2NH$
k	F	213−215	maleic acid, EtOH, 50 °C (50%)	dimaleate	$CH_2CH_2NMe_2$	CH_2CH_2OH
l	F	150−151	maleic acid, EtOH, 50 °C (75%)	dimaleate	$CH_2CH_2NMe_2$	$CH_2CH_2N(CH_2CH_2OH)_2$
m	F	118−122[b]	maleic acid, EtOH, 50 °C (42%)	dimaleate	$CH_2CH_2NMe_2$	2-(imidazol-1-yl)ethyl
n	F	118−121[b]	maleic acid, EtOH, 78 °C (88%)	dimaleate	$CH_2CH_2NMe_2$	$CH_2CH_2NMeCH_2CH_2OH$
o	F	178−180	maleic acid, EtOH, 78 °C (89%)	maleate	$CH_2CH_2NMe_2$	$CH_2CH_2NHSO_2Me$
p	F	186−188	maleic acid, EtOH, 78 °C (74%)	dimaleate	$CH_2CH_2NMe_2$	$CH_2CH_2NHCH_2CH_2NMe_2$
q	J	224[b]	HCl/EtOH−$CHCl_3$, rt (75%)	3HCl	$CH_2CH_2NH_2$	$CH_2CH_2NMe_2$
r	K	258−260	HCl/EtOH, rt (90%)	3HCl	$CH_2CH_2NH_2$	$CH_2CH_2NH_2$
s	K	>250	HCl/EtOH, rt (93%)	3HCl	$CH_2CH_2NH_2$	CH_2CH_2NHMe
t	L	>250	HCl/EtOH−H_2O, 50 °C (85%)	3HCl	$CH_2CH_2NH_2$	$CH_2CH_2NHCH_2CH_2OH$
u	K	222−224	HCl/EtOH, 40 °C (84%)	3HCl	$CH_2CH_2NH_2$	$CH_2CH_2NEt_2$
v	K	238−240	HCl/EtOH, 40 °C (82%)	3HCl	$CH_2CH_2NH_2$	$CH_2CH_2N(CH_2CH_2)_2O$
w	M	116−118[b]	(i) HCl/EtOH; (ii) maleic acid, EtOH (55%)	dimaleate	$CH_2CH_2NH_2$	$CH_2CH_2N(CH_2CH_2OH)_2$
x	F	170.6[a,b]	maleic acid, EtOH, 50 °C (85%)	dimaleate	$CH_2CH_2NHCH_2CH_2OH$	$CH_2CH_2NMe_2$
y	K	>200	HCl/EtOH (80%)	3HCl	$CH_2CH_2NHCH_2CH_2OH$	$CH_2CH_2NH_2$
z	L	270−273	HCl/EtOH−H_2O, 50 °C (83%)	3HCl	$CH_2CH_2NHCH_2CH_2OH$	CH_2CH_2NHMe
aa	L	>200[b]	HCl/EtOH−H_2O, rt (83%)	3HCl	$CH_2CH_2NHCH_2CH_2OH$	$CH_2CH_2NHCH_2CH_2OH$
bb	L	207−210[b]	HCl/EtOH−H_2O, 60 °C (96%)	3HCl	$CH_2CH_2NHCH_2CH_2OH$	$CH_2CH_2N(CH_2CH_2OH)_2$
cc	L	>230	HCl/EtOH−H_2O, 50 °C (96%)	2HCl	$CH_2CH_2NHCH_2CH_2OH$	CH_2CH_2OH
dd	F	164−166	maleic acid, EtOH, rt (quant)	dimaleate	$CH_2CH_2CH_2NMe_2$	$CH_2CH_2NMe_2$
ee	K	260−262	HCl/EtOH, rt (72%)	3HCl	$CH_2CH_2CH_2NMe_2$	$CH_2CH_2NHCH_2CH_2OH$
ff	K	>250	HCl/EtOH, rt, 30 h (95%)	3HCl	CH_2CH_2NHMe	CH_2CH_2NHMe
gg	L	227[b]	HCl/EtOH−H_2O, 50 °C (91%)	3HCl	CH_2CH_2NHMe	$CH_2CH_2NHCH_2CH_2OH$
hh	F	105−107	maleic acid, EtOH, 50 °C (58%)	maleate	CH_2CH_2OH	$CH_2CH_2NMe_2$
ii	K	>200	HCl/EtOH, 36 h, rt (88%)	2HCl	CH_2CH_2OH	$CH_2CH_2NH_2$

[a] Differential scanning calorimetry. [b] With decomposition.

Perhaps the most significant result of this in vitro evaluation is the recognition that 9-aza-APs carrying a distal, lipophilic tertiary amine moiety in both side chains are capable of overcoming the MDR resistance induced in the LoVo/Dx cell line. Despite the generally reduced cytotoxic potency in vitro, the 9-aza-APs are a family of compounds characterized by remarkable in vivo antitumor activities in a series of preclinical models. The results presented here show that these chemotypes are highly effective against the systemic P388 murine leukemia, where many analogues are more active than DuP-941. More importantly, in the in vivo screening against the MX-1 human mammary carcinoma, several 9-aza-APs were uncovered which clearly were as effective as DuP-941 in terms of their tumor weight inhibitions (TWI% > 95). On the basis of their efficacy in the MX-1 model, **13b**, **13d**, **13k**, **13l**, **13x**, and **13z** entered a secondary in vivo screening against a panel of human solid tumors and were simultaneously assessed for their cardiotoxicity profile in the mouse.[45,51] This evaluation confirmed the potential of these aza bioisosteres as effective antitumor agents characterized by minimal or negligible delayed cardiotoxicity and ultimately led to the identification of **13x** and **13z** as potential clinical candidates. The corresponding dihydrochloride salts BBR 3576 and BBR 3438 are undergoing further investigations toward possible clinical development. The above results represent an additional example of the value of the aza-bioisosteric modification as a useful tool for the identification of effective chemotherapeutic agents.

Experimental Section

General. Melting points were determined with a Thomas-Hoover or a Fisher-Johns melting point apparatus and are uncorrected. ^1H and ^{13}C NMR were run on a Bruker AC-200 or ARX-500 pulsed Fourier transform spectrometer. The ^1H NMR spectra of several BOC-substituted analogues exhibited two singlets due to restricted bond rotation. Column chromatography and TLC monitoring were performed with silica gel 60. All hydrazines were prepared via literature procedures or were commercially available except for [2-[[(1,1-dimethylethoxy)carbonyl]amino]ethyl]hydrazine and [2-[*N*-[(1,1-dimethylethoxy)carbonyl]-*N*-methylamino]ethyl]hydrazine. Mitoxantrone (Novantrone, Lederle, Gosport, U.K.) and doxorubicin (Adriablastina, Farmitalia-C. Erba, Milan, Italy) were purchased. The DuP-941 used as the reference compound was prepared by the published procedure[54] with a purity of >98% (HPLC). Microanalysis were performed by Robertson Microlit Laboratories, Inc., Madison, NJ, or Redox s.n.c., Cologno Monzese, Milan, Italy, and are indicated only by elemental symbols when the results were within ±0.4%.

(A) Biological Methods. (a) In Vitro Studies. Tumor cell lines used were human colon adenocarcinoma (LoVo) and human colon adenocarcinoma resistant to doxorubicin (LoVo/Dx). The cells (2.5×10^4 cells/mL) were placed in 96-well plates and preincubated for 24 h in complete medium. The drug concentration inhibiting 50% of cellular growth (IC_{50}, μg/mL) was determined by MTT assay[25] following 1 h of drug exposure, and the resistance index RI (IC_{50}(LoVo/Dx)/IC_{50}(LoVo)) was calculated. The results are the mean of at least three independent experiments unless otherwise indicated. Standard deviations (SD) are shown in parentheses.

(b) In Vivo Studies. (1) P388 Murine Leukemia. Cells (10^6 cells/mouse) were injected iv in CD2F1 male mice. Drug treatment was iv on days 1, 4, and 7 after tumor transplanta-

Table 4. Cytotoxic Activity of **13** on LoVo and LoVo/Dx Tumor Cell Lines

			IC_{50}, μg/mL (SD)[a]		
13	R_1	R_2	LoVo	LoVo/Dx	RI[b]
a	$CH_2CH_2NMe_2$	$CH_2CH_2NMe_2$	0.35 (0.17)	1.0 (0.53)	2.8
b	$CH_2CH_2NMe_2$	$CH_2CH_2NH_2$	0.16 (0.09)	4.3 (1.4)	27
c	$CH_2CH_2NMe_2$	CH_2CH_2NHMe	0.03 (0.01)	3.78 (1.78)	126
d	$CH_2CH_2NMe_2$	$CH_2CH_2NHCH_2CH_2OH$	0.2 (0.1)	41.2 (34.6)	206
e	$CH_2CH_2NMe_2$	$CH_2CH_2NEt_2$	0.37 (0.06)	0.83 (0.3)	2.2
f	$CH_2CH_2NMe_2$	2-(4-morpholinyl)ethyl	1.35[c]	3.63 (0.8)	2.7
g	$CH_2CH_2NMe_2$	$CH_2CH_2CH_2NMe_2$	0.33 (0.06)	2.13 (0.5)	6.4
h	$CH_2CH_2NMe_2$	$CH_2CH_2CH_2NH_2$	1.65 (0.68)	12.5 (1.6)	7.6
i	$CH_2CH_2NMe_2$	2-(1-piperidinyl)ethyl	0.35 (0.23)	0.39 (0.21)	1.1
j	$CH_2CH_2NMe_2$	2-(1-piperazinyl)ethyl	4.83 (1.0)	>100	21
k	$CH_2CH_2NMe_2$	CH_2CH_2OH	0.32 (0.14)	13.6 (3.7)	42.5
l	$CH_2CH_2NMe_2$	$CH_2CH_2N(CH_2CH_2OH)_2$	2.13 (1.19)	>100	>47
m	$CH_2CH_2NMe_2$	2-(imidazol-1-yl)ethyl	1.1 (0.3)	>100	>91
n	$CH_2CH_2NMe_2$	$CH_2CH_2N(Me)CH_2CH_2OH$	0.049 (0.003)	1.55 (0.7)	31.6
o	$CH_2CH_2NMe_2$	$CH_2CH_2NHSO_2Me$	4.1 (1.1)	>100	>24
p	$CH_2CH_2NMe_2$	$CH_2CH_2NHCH_2CH_2NMe_2$	3.2 (0.25)	>100	>31
q	$CH_2CH_2NH_2$	$CH_2CH_2NMe_2$	0.3 (0.17)	15.9 (8)	53
r	$CH_2CH_2NH_2$	$CH_2CH_2NH_2$	0.86 (0.06)	15.4 (8.9)	18
s	$CH_2CH_2NH_2$	CH_2CH_2NHMe	0.21 (0.07)	10.2 (2.7)	49
t	$CH_2CH_2NH_2$	$CH_2CH_2NHCH_2CH_2OH$	1.63 (0.32)	>100	>61
u	$CH_2CH_2NH_2$	$CH_2CH_2NEt_2$	0.52 (0.59)	15.7 (10.5)	30
v	$CH_2CH_2NH_2$	2-(4-morpholinyl)ethyl	1.76 (1.4)	>100	>57
w	$CH_2CH_2NH_2$	$CH_2CH_2N(CH_2CH_2OH)_2$	46.4[d]	>100	2.1
x	$CH_2CH_2NHCH_2CH_2OH$	$CH_2CH_2NMe_2$	0.3 (0.14)	57.0 (37.1)	190
y	$CH_2CH_2NHCH_2CH_2OH$	$CH_2CH_2NH_2$	2.17 (0.64)	>100	>46
z	$CH_2CH_2NHCH_2CH_2OH$	CH_2CH_2NHMe	0.54 (0.17)	>100	>185
aa	$CH_2CH_2NHCH_2CH_2OH$	$CH_2CH_2NHCH_2CH_2OH$	4.5 (2.26)	>100	>22
bb	$CH_2CH_2NHCH_2CH_2OH$	$CH_2CH_2N(CH_2CH_2OH)_2$	>100	>100	
cc	$CH_2CH_2NHCH_2CH_2OH$	CH_2CH_2OH	53.1 (8.9)	>100	>1.9
dd	$CH_2CH_2CH_2NMe_2$	$CH_2CH_2NMe_2$	0.24 (0.21)	2.0 (0.79)	8.3
ee	$CH_2CH_2CH_2NMe_2$	$CH_2CH_2NHCH_2CH_2OH$	0.3 (0.17)	90 (11.7)	300
ff	CH_2CH_2NHMe	CH_2CH_2NHMe	0.04 (0.007)	3.14 (1.22)	78
gg	CH_2CH_2NHMe	$CH_2CH_2NHCH_2CH_2OH$	0.29 (0.01)	31.6 (1.16)	109
hh	CH_2CH_2OH	$CH_2CH_2NMe_2$	18.5 (15.7)	>100	>5.4
ii	CH_2CH_2OH	$CH_2CH_2NH_2$	28.6 (14.6)	>100	>3.5
DuP-941			0.053 (0.03)	44.5 (39.1)	840
mitoxantrone			0.024 (0.016)	0.67 (0.28)	28
doxorubicin			0.58 (0.42)	53.0 (29.3)	91.3

[a] IC_{50}, drug concentration inhibiting 50% of cellular growth following 1 h of drug exposure; SD, standard deviation. [b] RI, resistance index = IC_{50}(LoVo/Dx)/IC_{50}(LoVo). [c] Mean of two experiments. [d] Single experiment.

tion (day 0). The effectiveness of the drug on mice survival (T/C%) was evaluated from the following formula:

$$T/C\% = (\text{median survival time in treated mice})/(\text{median survival time in controls}) \times 100$$

(2) MX-1 Human Mammary Carcinoma. Tumor fragments were implanted sc in CD1 *nu/nu* female nude mice and allowed to grow to a palpable size (100−200 mg). Drug treatment was iv once a week for 3 weeks. The effectiveness of each compound on tumor weight inhibition (TWI) was evaluated 7 days after the last treatment and once a week thereinafter, by means of the following formula:

$$TWI\% = (\text{mean } R_v \text{ of treated mice})/(\text{mean } R_v \text{ of control mice}) \times 100$$

where $R_v = V_t$ (tumor weight on day t)/V_o (initial tumor weight at the onset of the treatment). The TWI% value at the optimal dose (OD, expressed as mg/kg/day) is shown.

(B) Synthesis. 9-Fluoro-6-methoxybenz[*g*]isoquinoline-5,10-dione (2a). Procedure 1: From 1. Under a nitrogen atmosphere, a solution of sodium methoxide prepared by dissolution of sodium metal (10.7 g, 0.465 mol) in MeOH (520 mL) was dropwise added to a solution of **1** (103.8 g, 0.42 mol) in THF (4.7 L) during a period of about 4 h, while cooling at 15 °C. The reaction mixture was stirred overnight at room temperature. The solid was recovered by filtration and suspended in water (550 mL), and the mixture was stirred for 1 h. Crude **2a** was recovered by filtration and suspended in boiling dichloromethane (370 mL). The mixture was stirred for 30 min and then allowed to cool at room temperature. The solid was collected by filtration, washed with dichloromethane (2 × 10 mL), and dried under vacuum at 40 °C. This material was suspended in boiling THF (300 mL); the mixture was stirred for 2 h and then allowed to cool to room temperature. The solid was collected by filtration and dried under vacuum at 50 °C to constant weight to afford **2a** (28.2 g, 26%) in satisfactory purity: mp 250−252 °C; HPLC (column Supelco RP8 15 cm × 4.6 mm, 3 μm, eluant H_2O/CH_3CN (80:20), KH_2PO_4 (30 mM), pH 3.1 with H_3PO_4, flow rate 1 mL/min, detection wavelength 245 nm) **2a** t_R = 15.1 min, 89% area, **2b** t_R = 16.0 min, **2c** t_R = 5.1 min, **1** t_R = 21.8 min; TLC (ethyl acetate/dichloromethane, 30:70) **2a** R_f = 0.40, **2b** R_f = 0.35, **2c** R_f = 0.85, **1** R_f = 0.90; ^{1}H NMR ($CDCl_3$) δ 9.49 (d, J = 0.8 Hz, 1H), 9.07 (d, J = 5.1 Hz, 1H), 8.00 (dd, J = 5.1, 0.8 Hz, 1H), 7.55 (dd, J = 10.4, 9.4 Hz, 1H), 7.39 (dd, J = 3.4, 9.4 Hz, 1H), 4.05 (s, 3H). Anal. ($C_{14}H_8FNO_3$) C, H, F, N.

6-Fluoro-9-methoxybenz[*g*]isoquinoline-5,10-dione (2b): isolated via column chromatography (ethyl acetate/dichloromethane, 30:70); mp 168−170 °C; ^{1}H NMR ($CDCl_3$) δ 9.51 (d, J = 0.8 Hz, 1H), 9.05 (d, J = 5.1 Hz, 1H), 7.98 (dd, J = 5.1, 0.8 Hz, 1H), 7.50 (dd, J = 10.4, 9.4 Hz, 1H), 7.41 (dd, J = 3.4, 9.4 Hz, 1H), 4.05 (s, 3H). Anal. ($C_{14}H_8FNO_3$) C, H, F, N.

6,9-Dimethoxybenz[*g*]isoquinoline-5,10-dione (2c): isolated via column chromatography (ethyl acetate/dichloromethane, 30:70); mp 188−190 °C; ^{1}H NMR ($CDCl_3$) δ 9.45 (s, 1H), 9.01 (d, J = 5.1 Hz, 1H), 7.95 (d, J = 5.1 Hz, 1H), 7.43 (part A of AB system, J = 9.4 Hz, 1H), 7.41 (part B of AB system, J = 9.4 Hz, 1H), 4.06 (s, 3H).

Table 5. Antitumor Activity of **13** on Systemic P388 Leukemia and MX-1 Human Mammary Carcinoma

13	P388 T/C% (OD)[a]	MX-1 TWI% (OD)[b]
a	381 (4)	56 (2)
b	213 (2)	87 (4.5)
c	262 (13)	68 (1.3)
d	194 (6)	99 (9)
e	175 (4)	35 (9)
f	256 (24)	48 (27)
g	138 (40)[c]	141 (90)[c]
h	125 (18)[d]	73 (8)
i	113 (3)[c]	6 (24)
j	113 (18)[c]	14 (18)
k	167 (12)[c]	97 (40)
l	175 (40)[c]	97 (90)
m	150 (40)[c]	70 (120)
n	280 (12)	75 (12)
o	111 (20)[c]	90 (60)
p	133 (40)[c]	25 (60)[c]
q	231 (9)	80 (11)
r	194 (7.5)	70 (13)
s	153 (3)	70 (6)
t	194 (24)	62 (27)
u	147 (12)[c]	51 (40)
v	129 (40)[c]	32 (40)
w	194 (7.5)	50 (150)
x	200 (12)	99 (27)
y	175 (12)	74 (20)
z	167 (7.5)	96 (17.5)
aa	165 (40)[c]	89 (40)
bb	111 (120)	45 (130)
cc	113 (90)[c]	49 (120)
dd	270 (12)	55 (24)
ee	166 (7.5)	98 (10)
ff	167 (1.5)[d]	nt[e]
gg	181 (6)	nt[e]
hh	112 (40)[c]	16 (90)[c]
ii	106 (60)[c]	nt[e]
DuP-941	147 (13.5)	95 (13.5)
Mitox[f]	197 (3)	57 (3)
Dx[g]	200 (7.5)	64 (7.5)

[a] T/C%, (median survival time of treated mice)/(median survival time of controls) × 100; OD, optimal dose (mg/kg/day). [b] TWI%, tumor weight inhibition at the optimal dose (OD). [c] Maximum tested dose. [d] Toxic deaths observed at the indicated treated doses. [e] Not tested. [f] Mitoxantrone. [g] Doxorubicin.

Procedure 2: From CAN Oxidation of 10. A solution of ceric ammonium nitrate (1.37 g, 2.50 mmol) in water (5 mL) was added over a 2-min period to a stirring suspension of **10** (0.122 g, 0.47 mmol) in acetonitrile (15 mL). The suspension was then heated at 60° C for 2 h to give a clear, dark solution which was cooled to room temperature and diluted with water (10 mL). After removing the acetonitrile by distillation at reduced pressure, the aqueous phase was saturated with sodium chloride and extracted with dichloromethane (3 × 15 mL). The organic extracts were dried over sodium sulfate and the solvent removed under reduced pressure. The residue was chromatographed over silica gel (eluant dichloromethane/ethyl acetate from 85:15 to 75:25) to yield **2a** (0.05 g, 44%) as a brown-yellowish solid.

9-Fluoro-6-hydroxybenz[*g*]isoquinoline-5,10-dione (3a). A solution of **2a** (29 g, 0.112 mol) in methanesulfonic acid (245 mL) was stirred at 110 °C for 2 h. The reaction mixture was allowed to cool to room temperature and poured into cold water (3000 mL), and the resultant suspension was stirred for 2 h. The solid was collected by filtration and washed with water (300 mL). The material was suspended in water (200 mL) and stirred at room temperature for 1 h and the solid collected by filtration. The wet solid was suspended in 2-propanol (400 mL) and stirred for 1 h. The product was collected by filtration and dried under vacuum at 40 °C to constant weight to yield **3a** (24 g, 89%): R_f 0.66 (silica gel, ethyl acetate/dichloromethane, 30:70); mp 189−191 °C; ^{1}H NMR ($CDCl_3$) δ 12.56 (s, 1H), 9.57 (d, 1H), 9.15 (d, 1H), 8.09 (dd, 1H), 7.53 (dd, 1H), 7.38 (dd, 1H).

9-Fluoro-6-[[(4-methylphenyl)sulfonyl]oxy]benz[*g*]isoquinoline-5,10-dione (4). To a solution of **3a** (18.5 g, 0.076 mol) in dichloromethane (50 mL) were added *p*-toluenesulfonyl chloride (29.3 g, 0.152 mol) and triethylamine (31.7 mL, 0.228 mol). The mixture was stirred for 1 h at room temperature and then poured into water (1300 mL). The layers were separated, and the aqueous phase was extracted with dichloromethane (2 × 300 mL). The combined extracts were dried over sodium sulfate and concentrated to about 150 mL. The product, which crystallized on addition of hexane (500 mL), was collected by filtration, washed with cold dichloromethane, and dried under vacuum at 50 °C for 4 h. This material (33 g) was recrystallized from dichloromethane (65 mL) at 0 °C and dried under vacuum to constant weight to afford **4** (23.5 g, 78%): TLC (silica gel, hexane/dichloromethane, 50:50) R_f = 0.50; mp 167−169 °C (a sample purified by flash chromatography gave mp 173−174 °C); ^{1}H NMR ($CDCl_3$) δ 9.48 (s, 1H), 9.08 (d, 1H), 7.94−7.82 (m, 3H), 7.57−7.50 (m, 2H), 7.35 (d, 2H), 2.45 (s, 3H). Anal. ($C_{20}H_{12}FNO_5S$) C, H, N, S.

4-(2-Methoxy-5-fluorobenzoyl)nicotinic Acid (8a) and 3-(2-Methoxy-5-fluorobenzoyl)isonicotinic Acid (8b). The mixture of **7a** and **7b** (14.1 g, 0.053 mol) was added to a solution of sodium methoxide, prepared by portionwise addition of sodium metal (6.8 g, 0.290 mol) to dry methanol (140 mL). The mixture was held at reflux for 8.5 h and then kept at room temperature overnight. The mixture was concentrated to about one-third volume, water (100 mL) was added, and the methanol was removed by distillation. Concentrated hydrochloric acid (25 mL) was slowly added to the residue which was being cooled at 10 °C, and the precipitate was collected by filtration and washed with hydrochloric acid to afford an approximately 4:1 mixture of **8a** and **8b** (11.31 g, 89%): mp > 230 °C; ^{1}H NMR (DMSO-d_6) δ 13.55 (br s), 8.83 (d), 8.60 (s), 7.26 (d), 7.63−7.42 (m), 7.37 (d), 7.15 (m), 3.45 (s), 3.40 (s).

4-(2-Methoxy-5-fluorobenzyl)nicotinic Acid (9). A zinc−copper couple[55] (1.5 g) was added to a stirred suspension of the mixture of **8a** and **8b** (1.22 g, 4.43 mmol) in formic acid (15 mL) and water (5 mL). After 15 min at room temperature, the mixture was heated for 2 h in an oil bath held at 80 °C. After cooling to room temperature the mixture was filtered through a sintered glass funnel, and the residue was thoroughly washed with aqueous formic acid (75%, 10 mL) and ethyl acetate (10 mL). The combined filtrates were concentrated to about 5 mL, treated with hydrochloric acid (0.5 N, 15 mL), and extracted with ethyl acetate (3 × 20 mL). After saturation of the aqueous phase with sodium chloride, it was further extracted with ethyl acetate/1,2-dimethoxyethane (3:1) (2 × 20 mL). The combined organic phases were dried over sodium sulfate, and the solvent was removed under reduced pressure to yield a yellow residue. Silica gel chromatography (eluant ethyl acetate/methanol/acetic acid from 96:4:0 to 90:10:1) led to **9** (0.80 g): mp > 220 °C; R_f 0.5 (silica gel, ethyl acetate/methanol/acetic acid, 85:15:1); ^{1}H NMR (DMSO-d_6) δ 8.97 (s, 1H), 8.61 (d, 1H), 7.21 (d, 1H), 7.12−6.91 (m, 3H), 4.33 (s, 2H), 3.70 (s, 3H).

9-Fluoro-10-hydroxy-6-methoxybenz[*g*]isoquinoline (10). A mixture of **9** (0.63 g, 2.41 mmol) and polyphosphoric acid (15 g) was heated at 110−120 °C for 2 h with stirring. Water (50 mL) was added to the mixture while it was still warm (60 °C), and the mixture was cooled to 0 °C. Upon neutralization with sodium hydroxide (20%), the mixture was stirred at room temperature for 1.5 h and then extracted with methanol (4%) in chloroform (4 × 75 mL). The combined organic phases were washed with brine and dried over sodium sulfate and the solvents removed under reduced pressure to give **10** (0.40 g, 68%) as a reddish-purple solid: mp > 210 °C (from ethanol); ^{1}H NMR (DMSO-d_6) δ 12.30 (br s, 1H), 8.75 (s, 1H), 7.50 (d, 1H), 7.30 (d, 1H), 7.00−6.92 (m, 1H), 6.83−6.70 (m, 2H), 3.87 (s, 3H).

2-[2-(Dimethylamino)ethyl]-5-[[(4-methylphenyl)sulfonyl]oxy]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (11a). A solution of [2-(dimethylamino)ethyl]hydrazine [56] (1.86 g, 18 mmol) in anhydrous THF (6.0 mL) was added during 30 min

to a solution of **4** (2.4 g, 6.0 mmol) and diisopropylethylamine (1.1 mL, 6.3 mmol) in anhydrous THF (24 mL). The mixture was stirred at room temperature for 1 h and poured into water (240 mL), and the resulting suspension was stirred at room temperature for 30 min. The solid was collected by filtration, washed with water, and dried under vacuum at 40 °C to constant weight to give **11a** (1.44 g, 52%): mp 139−141 °C; ^{1}H NMR ($CDCl_3$) δ 9.55 (s, 1H), 8.80 (d, 1H), 8.10 (d, 1H), 7.95 (d, 2H), 7.80 (d, 1H), 7.52 (d, 1H), 7.35 (d, 2H), 4.65 (t, 2H), 2.95 (t, 2H), 2.45 (s, 3H), 2.35 (s, 6H).

2-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]ethyl]-5-[[(4-methylphenyl)sulfonyl]oxy]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (11b). A solution of [2-[[(1,1-dimethoxyethoxy)carbonyl]amino]ethyl]hydrazine (4.73 g, 27 mmol) in anhydrous THF (9.5 mL) was added to a solution of **4** (7.18 g, 18 mmol) and diisopropylethylamine (3.30 mL, 18.9 mmol) in anhydrous THF (54 mL). The mixture was stirred at room temperature for 3 h; then the solid was collected by filtration, washed with THF and THF/hexane, and dried under vacuum at 40 °C to constant weight to afford **11b** (4.75 g, 49%): mp 205−207 °C; ^{1}H NMR ($CDCl_3$) δ 9.50 (s, 1H), 8.78 (d, 1H), 8.09 (d, 1H), 7.95 (d, 2H), 7.75 (d, 1H), 7.50 (d, 1H), 7.35 (d, 2H), 4.92 (br, 1H), 4.70 (t, 2H), 3.75 (q, 2H), 2.45 (s, 3H), 1.4 (s, 9H). Anal. ($C_{27}H_{26}N_4O_6S$) H, S; C: calcd, 60.66; found, 60.06. N: calcd, 10.48; found, 9.98.

[2-[[(1,1-Dimethylethoxy)carbonyl]amino]ethyl]hydrazine. To a stirred solution of di-*tert*-butyl dicarbonate (107 g, 0.475 mol) in THF (213 mL) were simultaneously added a solution of 2-chloroethylamine hydrochloride (58.6 g, 0.50 mol) in water (150 mL) and a solution of triethylamine (69.6 mL, 0.50 mol) in THF (150 mL), during 1 h, while keeping the bulk temperature between 20 and 30 °C. The mixture was stirred at room temperature for 1 additional hour, and the layers were separated. The aqueous layer was saturated with sodium chloride and extracted with THF (2 × 100 mL). The combined organic phases were dried over sodium sulfate and concentrated giving 90 g of crude 2-[[(1,1-dimethylethoxy)carbonyl]amino]ethyl chloride. This material was dissolved in 95% ethanol (900 mL), and 40% hydrazine hydrate (291 mL, 2.37 mol) was added, followed by a solution of potassium carbonate (131 g, 0.95 mol) in water (263 mL); the mixture was stirred overnight at room temperature and for 5 h at reflux temperature. Ethanol was removed by rotary evaporation, then 40% sodium hydroxide solution (740 mL) and solid sodium hydroxide (100 g, 2.5 mol) were added, and the aqueous layer was extracted with diethyl ether (3 × 300 mL). The combined organic extracts were dried over sodium sulfate and concentrated. The resulting oily residue (81.5 g) was purified by column chromatography (silica gel, 1.63 kg) eluting with dichloromethane/methanol mixtures from 98:2 to 95:5. The chromatographic fractions containing the product were concentrated giving 36.5 g (44%) of product as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 5.26 (br t, 1H, exchangeable with D_2O), 3.55 (br s, 3H, exchangeable with D_2O), 3.28 (q, 2H), 2.86 (t, 2H), 1.41 (s, 9H).

2-[2-[(2-Hydroxyethyl)amino]ethyl]-5-[[(4-methylphenyl)sulfonyl]oxy]indazolo[4,3-*g*]isoquinolin-6(2*H*)-one (11c). A solution of [2-[(2-hydroxyethyl)amino]ethyl]hydrazine[54] (2.65 g, 22.2 mmol) in ethanol (20 mL) was added dropwise to a solution of **4** (5.9 g, 14.8 mmol) and triethylamine (2.2 g, 22.2 mmol) in THF (60 mL) while keeping the internal temperature below 5 °C. The mixture was allowed to warm to room temperature. Ethanol (40 mL) was added to the resulting thick suspension, and stirring was continued for a further 3 h. The reaction mixture was concentrated under vacuum to about one-half volume and poured in water (1200 mL). The resultant suspension was stirred overnight at room temperature. The dark-brownish solid was collected by filtration, washed with cold water, and dried under vacuum at 40 °C to constant weight to yield **11c** (2.95 g, 42%): mp 129−131 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 9.40 (s, 1H), 8.80 (d, 1H), 8.15 (d, 1H), 7.95 (d, 1H), 7.75 (d, 2H), 7.40 (d, 2H), 7.25 (d, 1H), 4.65 (t, 2H), 3.40 (t, 2H), 3.13 (t, 2H), 2.62 (t, 2H), 2.45 (s, 3H). Anal. ($C_{24}H_{22}N_4O_5S$) H, N; C: calcd, 60.24; found, 58.81.

2-[2-[*N*-[(1,1-Dimethylethoxy)carbonyl]-*N*-(2-hydroxyethyl)amino]ethyl]-5-[[(4-methylphenyl)sulfonyl]oxy]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (11d). To a stirred solution of di-*tert*-butyl dicarbonate (1.12 g, 5.12 mmol) in a mixture of THF (20 mL) and water (4 mL) was added solid **11c** (2.132 g, 4.46 mmol) portionwise during 2 min. The transfer was completed by rinsing the container with a further amount of THF (2 mL). The reaction mixture was stirred at room temperature for 1 h and then concentrated to a small volume. The residue was repeatedly taken up with ethanol and concentrated almost to dryness. The residue was triturated with boiling methyl *tert*-butyl ether; then dichloromethane was added to the mixture until complete dissolution of the solid. Dichloromethane was then removed by distillation under a gentle nitrogen flow. The mixture was allowed to cool to room temperature. The solid was collected by filtration, washed with plenty of methyl *tert*-butyl ether, and dried under vacuum at 40 °C to constant weight. The product **11d** (1.91 g, 74%) was obtained as a green solid: mp 175−178 °C; ^{1}H NMR (DMSO-d_6, 323 K) δ 9.43 (s, 1H), 8.85 (d, 1H), 8.17 (d, 1H), 7.98 (d, 1H), 7.78 (d, 2H), 7.50−7.30 (m, 3H), 4.8 (t, 2H), 4.67 (br s, 1H), 3.75 (br s, 2H), 3.57−3.35 (br m, 2H), 3.30−2.95 (br m, 2H, partially hidden by the water signal), 2.38 (s, 3H), 1.17 (br s, 4H, part of the *tert*-butyl signal), 0.90 (br s, 5H, part of the *tert*-butyl signal).

2-[3-(Dimethylamino)propyl]-5-[[(4-methylphenyl)sulfonyl]oxy]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (11e). A solution of [3-(dimethylamino)propyl]hydrazine[56] (1.77 g, 15.1 mmol) in anhydrous THF (5.4 mL) was added to a mixture of **4** (2.00 g, 5.03 mmol) and diisopropylethylamine (0.923 mL, 5.28 mmol) in anhydrous THF (40 mL). The mixture was stirred at room temperature for 1 h and poured in water (450 mL), and the resulting suspension was stirred at room temperature for 30 min. The solid was collected by filtration, washed with water, and dried under vacuum at 40 °C. The crude product was purified by recrystallization from 2-propanol (19.5 mL). The solid was collected by filtration, washed with 2-propanol, and dried under vacuum at 40 °C to constant weight to afford **11e** (1.12 g, 47%): mp 149−151 °C; ^{1}H NMR ($CDCl_3$) δ 9.55 (s, 1H), 8.82 (d, 1H), 8.10 (d, 1H), 7.97 (d, 2H), 7.83 (d, 1H), 7.52 (d, 1H), 7.35 (d, 2H), 4.64 (t, 2H), 2.45 (s, 3H), 2.31−2.11 (m, 10H).

2-(2-Hydroxyethyl)-5-[[(4-methylphenyl)sulfonyl]oxy]-indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (11f). A solution of (2-hydroxyethyl)hydrazine (0.966 mL, 12.8 mmol) in THF/MeOH (4:1) (5 mL) was added to a solution of **4** (3.0 g, 7.55 mmol) and triethylamine (1.78 mL, 12.8 mmol) in THF (63 mL). The mixture was stirred at room temperature for 2 h and then concentrated. The residue was partitioned between dichloromethane (200 mL) and 1 N NaOH (50 mL). The aqueous phase was further extracted with dichloromethane (6 × 200 mL). The organic phases were washed with a phosphate buffer solution made from water (50 mL), saturated solution of sodium dihydrogen phosphate (7 mL), and 6 N hydrochloric acid (4 mL). The combined organic phases were dried over sodium sulfate and concentrated. The resulting residue was triturated with methyl *tert*-butyl ether at reflux temperature; the solid was collected by filtration, washed with methyl *tert*-butyl ether, and dried under vacuum at 40 °C. This material was triturated with hot ethanol, and the mixture was allowed to cool to room temperature. The greenish solid was collected by filtration, washed with ethanol, and dried under vacuum at 50 °C to constant weight to afford **11f** (1.92 g, 58%): mp 211−213 °C; ^{1}H NMR (DMSO-d_6) δ 9.43 (s, 1H), 8.84 (d, J = 5.2 Hz, 1H), 8.22 (d, J = 9.2 Hz, 1H), 7.97 (d, J = 5.2 Hz, 1H), 7.81 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 9.2 Hz, 1H), 5.00 (br t, J = 5.4 Hz, 1H), 4.68 (t, J = 5.0 Hz, 2H), 3.91 (q, J = 5.0 Hz, 2H), 2.38 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 178.78, 149.77, 145.93, 144.73, 142.26, 138.18, 137.16, 135.13, 131.71, 130.00, 128.43, 124.40, 123.92, 122.78, 120.67, 119.74, 116.43, 60.30, 52.86, 21.12 (2 overlapping resonances).

2-[2-[*N*-[(1,1-Dimethylethoxy)carbonyl]-*N*-methylamino]ethyl]-5-[[(4-methylphenyl)sulfonyl]oxy]indazolo[4,3-

***gh*]isoquinolin-6(2*H*)-one (11g).** A solution of crude [2-[*N*-[(1,1-dimethylethoxy)carbonyl]-*N*-methylamino]ethyl]hydrazine (2.39 g, 3.78 mmol) in anhydrous THF (5.0 mL) was added to a mixture of **4** (0.75 g, 1.89 mmol) and diisopropylethylamine (0.34 mL, 1.98 mmol) in anhydrous THF (20 mL). The mixture was stirred overnight at room temperature and partitioned between water (100 mL) and ethyl acetate (50 mL). The aqueous phase was further extracted with ethyl acetate (2 × 50 mL), and the combined organic phases were dried over sodium sulfate and concentrated. The residue was purified by column chromatography (silica gel) eluting with ethyl acetate/hexane mixture (2:1). Upon concentration of the chromatographic fractions a solid crystallized, which was collected by filtration, washed with hexane, and dried under vacuum at 40 °C to constant weight to yield **11g** (0.35 g, 34%): mp 186−188 °C; ^{1}H NMR ($CDCl_3$) δ 9.55 (s, 1H), 8.82 (d, 1H), 8.09 (d, 1H), 7.95 (d, 2H), 7.82−7.60 (m, 1H), 7.51 (d, 1H), 7.34 (d, 2H), 4.80−4.60 (m, 2H), 3.78 (t, 2H), 2.78 (br s, ∼1H, part of the split methyl signal), 2.55 (br s, ∼2H, part of the split methyl signal), 2.44 (s, 3H), 1.42 (br s, ∼6H, part of the *tert*-butyl signal), 1.24 (br s, ∼3H, part of the *tert*-butyl signal).

(2-Chloroethyl)methylamine Hydrochloride. Thionyl chloride (58.8 mL, 0.80 mol) was dropwise added to a stirred solution of 2-(methylamino)ethanol (32.4 g, 0.4 mol) in anhydrous toluene (324 mL) while cooling at 0 °C. The mixture was then heated at 70 °C until complete dissolution of the white waxy solid (about 1 h). The solvent was removed by rotary evaporation. The residue was triturated in diethyl ether (300 mL), the mixture was stirred for 3 h at room temperature, and the solid was collected by filtration. The solid was suspended in diethyl ether, and stirring was continued for 1 h. The solid was collected by filtration and dried under vacuum to constant weight to yield the product (41.9 g, 81%): mp 111−113 °C; ^{1}H NMR (DMSO-d_6) δ 9.25 (br s, 2H), 3.93 (t, 2H), 3.27 (t, 2H), 2.56 (s, 3H).

1,1-Dimethylethyl *N*-(2-Chloroethyl)-*N*-methylcarbamate. To a stirred solution of di-*tert*-butyl dicarbonate (70.9 g, 0.315 mol) in THF (141 mL) were simultaneously added a solution of (2-chloroethyl)methylamine hydrochloride (41.0 g, 0.315 mol) in water (103 mL) and a solution of triethylamine (43.8 mL, 0.315 mol) in THF (103 mL), during 1 h, while keeping the bulk temperature between 5 and 10 °C. The mixture was stirred at room temperature for 2 further hours; then the layers were separated. The aqueous layer was saturated with sodium chloride and extracted with THF (150 mL). The combined organic phases were dried over sodium sulfate and concentrated giving an oily residue which was taken up with diethyl ether. The mixture was stirred for 30 min; then the solid was removed by filtration. The filtrate was concentrated to yield the product (60 g) as a yellow oil: ^{1}H NMR (DMSO-d_6) δ 3.68 (t, 2H), 3.47 (t, 2H), 2.82 (br s, 3H), 1.40 (s, 9H).

[2-[*N*-[(1,1-Dimethylethoxy)carbonyl]-*N*-methylamino]-ethyl]hydrazine. A mixture of 1,1-dimethylethyl *N*-(2-chloroethyl)-*N*-methylcarbamate (4.95 g, 25.5 mmol), 80% hydrazine (19.8 mL, 0.51 mol), and potassium carbonate (7.2 g, 52 mmol) in absolute ethanol (40 mL) was refluxed for 2 h. The solid was removed by filtration, and the filtrate was diluted with diethyl ether (150 mL) and washed with a saturated solution of sodium chloride brought to pH 14 with solid sodium hydroxide (2 × 50 mL). The organic phase was dried over sodium sulfate and concentrated giving the crude product as an oil (2.39 g). ^{1}H NMR analysis of this material showed a content of the expected product of about 30%; the remainder was starting material. This material was used in the subsequent step without further purification: ^{1}H NMR ($CDCl_3$) δ 3.35 (t, 2H), 2.87−2.79 (m, 5H), 1.45 (s, 9H).

Typical Procedures for the Preparation of Analogues 12. 2-[2-(Dimethylamino)ethyl]-5-[[2-(dimethylamino)-ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (12a). Method A. A solution of **11a** (0.463 g, 1.00 mmol) and 2-(dimethylamino)ethylamine (0.80 mL, 7.2 mmol)) in dry pyridine (4.63 mL) was heated at 80 °C for 1.5 h under a nitrogen blanket. The solution was concentrated to dryness, and the dark residue was partitioned between brine (30 mL) and ethyl acetate (4 × 25 mL). The combined organic extracts were dried over sodium sulfate and concentrated to about 3 mL. After addition of hexane (12 mL) and stirring for 1 h, the precipitate was collected by filtration and dried at 40 °C under vacuum to give **12a** (0.23 g, 61%): mp 150−152 °C; ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 6H), 2.40 (s, 6H), 2.70 (t, 2H), 2.95 (t, 2H), 3.60 (q, 2H), 4.65 (t, 2H), 6.98 (d, 1H), 7.70 (d, 1H), 8.30 (d, 1H), 8.80 (d, 1H), 9.30 (br, 1H), 9.65 (s, 1H).

5-[[2-[[(1,1-Dimethylethoxy)carbonyl]amino]ethyl]amino]-2-(2-hydroxyethyl)indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (12ii). Method B. A solution of **11f** (505 mg, 1.16 mmol) and *N*-Boc-ethylenediamine (1.30 g, 8.11 mmol) in dimethyl sulfoxide (12 mL) was stirred at 70 °C for 3 h. The mixture was partitioned between water and ethyl acetate, and the aqueous phase was further extracted with ethyl acetate. The combined organic extracts were dried over sodium sulfate and concentrated. The resulting residue was purified by flash chromatography (silica gel, 230−400 mesh, 50 g) eluting with dichloromethane/methanol (90:10). The chromatographic fractions containing the product were pooled and concentrated to dryness. The residue was triturated with diethyl ether, and the resulting solid was collected by filtration and dried under vacuum to yield **12ii** (246 mg, 50%) as an orange solid: mp 178−180 °C; TLC (silica gel, dichloromethane/methanol, 90:10) R_f = 0.3; ^{1}H NMR (DMSO-d_6) δ 9.55 (br s, 1H), 9.26 (br t, J = 5.5 Hz, 1H), 8.80 (br s, 1H), 8.24 (d, J = 4.9 Hz, 1H), 8.08 (d, J = 9.2 Hz, 1H), 7.26 (d, J = 9.2 Hz, 1H), 7.09 (br t, 1H), 4.65 (t, J = 5.1 Hz, 2H), 3.89 (t, J = 5.1 Hz, 2H), 3.61 (q, J = 5.9 Hz, 2H), 3.23 (q, J = 5.9 Hz, 2H), 1.35 (s, 9H).

2-[2-(Dimethylamino)ethyl]-5-[[2-[(methylsulfonyl)amino]ethyl]amino]indazolo-[4,3-*gh*]isoquinolin-6(2*H*)-one (12o). Method C. A mixture of **11a** (0.50 g, 1.08 mmol) and ethylenediamine (0.58 mL, 8.65 mmol) in anhydrous pyridine (5.0 mL) was stirred at 60 °C for 4.5 h under a nitrogen atmosphere. The solvent and the excess of diamine were removed by rotary evaporation. The residue was taken up with anhydrous dichloromethane (5.0 mL), and to the resulting suspension was added triethylamine (0.452 mL, 3.25 mmol). Addition of methanesulfonyl chloride (0.252 mL, 3.25 mmol) brought about an exothermic reaction and the increase of the reaction temperature to boiling point. After 5 min the exothermic reaction subsided, and a very thick suspension formed. After 5 more min, additional anhydrous dichloromethane (5.0 mL) was added to the reaction mixture. The mixture was stirred for 35 min and then quenched with a saturated solution of sodium bicarbonate (6 mL). The layers were separated, and the aqueous phase was extracted with dichloromethane (5 × 10 mL). Brine (6 mL) was added to the aqueous phase which was further extracted with dichloromethane (2 × 10 mL). The combined organic layers were dried over sodium sulfate and concentrated to dryness. The residue was purified by column chromatography (silica gel, 230−400 mesh, 36 g) eluting with chloroform/methanol/concentrated ammonium hydroxide mixtures from 95:5:0.25 to 80:20:1. The chromatographic fractions containing the product were pooled and concentrated to dryness. The residue, which contained 0.5 mol equiv of methanesulfonic acid (^{1}H NMR analysis), was partitioned between dichloromethane (20 mL) and a sodium bicarbonate/carbonate-buffered solution at pH 9−9.5 (15 mL). The layers were separated, and the aqueous phase was thoroughly extracted with dichloromethane. The organic extracts were washed with a saturated solution of sodium chloride (10 mL) made slightly basic (pH 9−9.5) with bicarbonate/carbonate buffer, then combined, dried over sodium sulfate, and concentrated to dryness. The product **12o** (0.298 g, 64%) was obtained as an orange solid: ^{1}H NMR (DMSO-d_6) δ 9.51 (d, 1H), 9.29 (t, 1H), 8.79 (d, 1H), 8.21 (dd, 1H), 8.15 (d, 1H), 7.36 (t, 1H), 7.26 (d, 1H), 4.70 (t, 2H), 3.68 (q, 2H), 3.27 (q, 2H, partially hidden by the water signal), 2.93 (s, 3H), 2.82 (t, 2H), 2.20 (s, 6H).

5-[[2-[[(1,1-Dimethylethoxy)carbonyl]amino]ethyl]amino]-2-[2-[*N*-(2-hydroxyethyl)-*N*-[(1,1-dimethylethoxy)-carbonyl]amino]ethyl]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-

one (12y). Method D. A mixture of **11c** (600 mg. 1.25 mmol) and ethylenediamine (0.50 mL, 7.52 mmol) in anhydrous pyridine (5 mL) was stirred at 50 °C for 5 h. The solvent and the excess of ethylenediamine were removed by rotary evaporation, and the resulting residue was partially purified by flash chromatography (silica gel, 230−400 mesh, 55 g) eluting with chloroform/methanol/concentrated ammonium hydroxide mixtures from 90:10:0.5 to 75:25:3. The chromatographic fractions containing the product were pooled and concentrated to dryness. The resulting brown solid was suspended in tetrahydrofuran (15 mL); then 1 N sodium hydroxide (4.2 mL) and a solution of di-*tert*-butyl dicarbonate (1.16 g, 5.32 mmol) in tetrahydrofuran (5 mL) were added. The reaction mixture was stirred at room temperature for 1.5 h and then partitioned between water (100 mL) and dichloromethane (100 mL). The aqueous phase was neutralized with sodium dihydrogen phosphate and further extracted with dichloromethane (2 × 50 mL). The organic extracts were washed with water (100 mL) and brine (100 mL), then combined, dried over sodium sulfate, and concentrated to dryness. The residue was purified by flash chromatography (silica gel, 230−400 mesh, 23 g) eluting with dichloromethane/methanol mixtures from 100:3 to 90:10. The chromatographic fractions containing the product were pooled and concentrated to dryness. The product **12y** (269 mg, 38%) was obtained as a red solid: ^{1}H NMR ($CDCl_3$) δ 9.43 (s, 1H), 9.23 (t, 1H), 8.77 (d, 1H), 8.29 (d, 1H), 7.63−7.44 (m, 1H), 7.03 (d, 1H), 5.50 (br t, 1H), 4.70 (br t, 2H), 3.86−3.60 (m, 6H), 3.58−3.42 (m, 2H), 3.34−3.11 (m, 2H), 1.49 (s, 9H), 1.12 (br s, 4H, Boc signal), 0.96 (br s, 5H, Boc signal).

5-[[2-[[(1,1-Dimethylethoxy)carbonyl]methylamino]-ethyl]amino]-2-[2-[[(1,1-dimethylethoxy)carbonyl]methylamino]ethyl]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (12ff). Method E. Step i. 2-(2-Hydroxyethyl)-5-[(2-hydroxyethyl)amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (12jj). A mixture of **11f** (900 mg. 2.07 mmol) and ethanolamine (0.998 mL, 16.5 mmol) in anhydrous pyridine (8 mL) was stirred at 70 °C for 6 h. The reaction mixture was concentrated to small volume and partitioned between water (15 mL) and ethyl acetate (6 mL). The resulting solid was collected by filtration, washed with water and ethyl acetate, and dried under vacuum. The product **12jj** (406 mg, 61%) was obtained as an orange solid: TLC (chloroform/methanol/ammonium hydroxide, 90:10:0.5) R_f = 0.22; ^{1}H NMR (DMSO-d_6) δ 9.47 (d, J = 0.8 Hz, 1H), 9.31 (t, J = 5.6 Hz, 1H), 8.77 (d, J = 5.3 Hz, 1H), 8.17 (dd, J = 0.8, 5.3 Hz, 1H), 8.02 (d, J = 9.2 Hz, 1H), 7.19 (d, J = 9.2 Hz, 1H), 5.00 (br s, 2H), 4.62 (t, J = 5.2 Hz, 2H), 3.89 (br s, 2H), 3.72 (br s, 2H), 3.60 (t, J = 5.2 Hz, 2H).

Step ii. To a stirred suspension of **12jj** (530 mg, 1.63 mmol) in anhydrous pyridine (5 mL), under a nitrogen atmosphere at 0 °C, was added methanesulfonyl chloride (0.285 mL, 3.68 mmol). The mixture was stirred for 1 h at room temperature. The resulting thick suspension was diluted with anhydrous dichloromethane (5 mL) and stirred at room temperature for 4 h. Water (10 mL) was added to the reaction mixture, and stirring was continued for an additional 10 min. The solid was collected by filtration, washed with water and ethyl acetate, and dried under vacuum. The product, 2-[2-[(methylsulfonyl)oxy]ethyl]-5-[[2-[(methylsulfonyl)oxy]ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one, was obtained as a brownish-red solid (595 mg, 76%) and used for the subsequent reaction without further purification: TLC (silica gel, chloroform/methanol/ammonium hydroxide, 90:10:0.5) R_f = 0.56; ^{1}H NMR (DMSO-d_6) δ 9.51 (s, 1H), 9.26 (br t, 1H), 8.82 (d, 1H), 8.21 (d, 1H), 8.13 (d, 1H), 7.35 (d, 1H), 4.99 (t, 2H), 4.72 (t, 2H), 4.50 (t, 2H), 3.94 (q, 2H), 3.22 (s, 3H), 3.08 (s, 3H).

Step iii. To a solution of 2-[2-[(methylsulfonyl)oxy]ethyl]-5-[[2-[(methylsulfonyl)oxy]ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (640 mg, 1.33 mmol) in anhydrous dimethyl sulfoxide (4.5 mL) was added a 33% ethanol solution of methylamine (4.5 mL, 36.1 mmol), and the mixture was stirred at room temperature for 24 h. Ethanol and the excess of methylamine were removed by rotary evaporation. The resulting residue was diluted with tetrahydrofuran (4 mL), and a solution of di-*tert*-butyl dicarbonate (580 mg, 2.66 mmol) in tetrahydrofuran (1.5 mL) was added, followed by 1 N sodium hydroxide (2.0 mL). The reaction mixture was stirred at room temperature for 6 h, then concentrated, and partitioned between water (50 mL) and dichloromethane (50 mL). The aqueous layer was further extracted with dichloromethane (3 × 50 mL). The organic extracts were washed with water (50 mL), dried over sodium sulfate, and concentrated to dryness. The residue was purified by flash chromatography (silica gel, 230−400 mesh, 50 g) eluting with dichloromethane/ethyl acetate mixtures from 70:30 to 25:75 followed by ethyl acetate/methanol mixtures from 100:0 to 100:1. The chromatographic fractions containing the product were pooled and concentrated to dryness. The product **12ff** (530 mg, 71%) was obtained as an orange foam: TLC (silica gel, ethyl acetate/methanol, 99:1) R_f = 0.30; ^{1}H NMR ($CDCl_3$, 313 K) δ 9.62 (s, 1H), 9.22 (br t, 1H), 8.78 (d, 1H), 8.25 (d, 1H), 7.60 (br s, 1H), 7.05 (br d, 1H), 4.65 (br t, 2H), 3.74 (t, 2H), 3.64 (t, 2H), 3.57−3.47 (m, 2H), 2.92 (s, 3H), 2.55 (br s, 3H), 1.47 (s, 9H), 1.36 (br s, 9H).

Typical Procedures for the Preparation of Salts 13 (melting points in Table 3). 2-[2-(Dimethylamino)-ethyl]-5-[[2-[(2-hydroxyethyl)methylamino]ethyl]amino]-indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one Dimaleate (13n). Method F. A solution of maleic acid (0.156 g, 1.35 mmol) in ethanol (1.5 mL) was rapidly added to a stirred solution of **12n** (0.250 g, 0.612 mmol) in ethanol (5 mL). The reaction mixture was stirred for 17 h at room temperature under a nitrogen atmosphere. The resulting suspension was heated at the boiling point until the solid dissolved, and the reaction mixture was allowed to cool to room temperature while stirring. The orange solid was collected from the resulting thick suspension by filtration, washed with ethanol and methyl *tert*-butyl ether, and dried under vacuum at 40 °C to yield dimaleate **13n** (0.345 g, 88%): ^{1}H NMR (D_2O) δ 9.01 (s, 1H), 8.57 (d, 1H), 7.83 (d, 1H), 7.73 (d, 1H), 6.99 (d, 1H), 6.00 (s, 4H), 4.89 (t, 2H), 4.10−3.90 (m, 4H), 3.82 (t, 2H), 3.63 (br t, 2H), 3.47 (br t, 2H), 3.07 (s, 3H), 3.03 (s, 6H); ^{1}H NMR (DMSO-d_6) δ 9.58 (s, 1H), 9.24 (t, 1H), 8.30−8.20 (m, 2H), 7.41 (d, 1H), 6.04 (s, 4H), 5.35 (br s, 1H), 5.03 (t, 2H), 3.98 (br q, 2H), 3.80−3.65 (br m, 4H), 3.60−3.15 (br m, 4H, partially overlapped with the broad water signal), 2.89 (s, 9H). Anal. ($C_{22}H_{28}N_6O_2 \cdot 2.2C_4H_4O_4 \cdot 2H_2O$) C, H, N.

2-[2-(Dimethylamino)ethyl]-5-[[2-(dimethylamino)ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one dimaleate (13a): Method F; ^{1}H NMR (DMSO-d_6) δ 9.55 (s, 1H), 9.20 (br t, 1H), 8.85 (d, 1H), 8.20 (dd, 1H), 7.38 (d, 1H), 6.00 (s, 4H), 5.01 (t, 2H), 3.95 (q, 2H), 3.70 (t, 2H), 3.37 (t, 2H), 2.88 (s, 12H). Anal. ($C_{21}H_{26}N_6O \cdot 2C_4H_4O_4 \cdot 1.5H_2O$) C, H, N.

2-[2-(Dimethylamino)ethyl]-5-[[2-[(2-hydroxyethyl)amino]ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one dimaleate (13d): Method F; ^{1}H NMR (D_2O) δ 9.05 (s, 1H), 8.57 (d, 1H), 7.83 (dd, 1H), 7.70 (d, 1H), 7.00 (d, 1H), 6.00 (s, 4H), 4.96 (t, 2H), 4.03 (t, 2H), 3.88 (m, 4H), 3.54 (t, 2H), 3.30 (m, 2H), 3.05 (s, 6H). Anal. ($C_{21}H_{26}N_6O_2 \cdot 2.1C_4H_4O_4 \cdot H_2O$) C, H, N.

2-[2-(Dimethylamino)ethyl]-5-[[2-(diethylamino)ethyl]-amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one dimaleate (13e): Method F; ^{1}H NMR (D_2O) δ 9.11 (s, 1H), 8.63 (d, 1H), 7.95 (d, 1H), 7.78 (d, 1H), 7.03 (d, 1H), 6.07 (s, 4H), 4.93 (t, 2H), 4.01 (t, 2H), 3.84 (t, 2H), 3.55 (t, 2H), 3.35 (q, 4H), 3.00 (s, 6H), 1.31 (t, 6H). Anal. ($C_{23}H_{30}N_6O \cdot 2C_4H_4O_4$) H, N; C: calcd, 58.30; found, 60.22.

2-[2-(Dimethylamino)ethyl]-5-[2-(4-morpholinyl)ethyl]-indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one dimaleate (13f): Method F; ^{1}H NMR (D_2O) δ 9.05 (s, 1H), 8.60 (d, 1H), 7.88 (d, 1H), 7.74 (d, 1H), 7.00 (d, 1H), 6.01 (s, 4H), 4.89 (t, 2H), 4.01 (m, 6H), 3.81 (t, 2H), 3.57 (t, 2H), 3.49 (m, 4H), 3.01 (s, 6H). Anal. ($C_{23}H_{28}N_6O_2 \cdot 2C_4H_4O_4 \cdot 0.5H_2O$) C, H, N.

2-[2-(Dimethylamino)ethyl]-5-[3-(dimethylamino)propyl]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one dimaleate (13g): Method F; ^{1}H NMR (D_2O) δ 9.10 (s, 1H), 8.63 (d, 1H), 7.91 (d, 1H), 7.73 (d, 1H), 6.99 (d, 1H), 6.14 (s, 4H), 4.87, t, 2H), 3.83 (t, 2H), 3.67 (t, 2H), 3.35 (t, 2H), 3.00 (s, 6H), 2.94 (s, 6H), 2.24 (m, 2H). Anal. ($C_{22}H_{28}N_6O \cdot 2C_4H_4O_4 \cdot 2H_2O$) C, H, N.

2-[2-(Dimethylamino)ethyl]-5-[(3-aminopropyl)amino]-indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one dimaleate (13h):

Method F; ^{1}H NMR (D_2O) δ 9.02 (s, 1H), 8.59 (d, 1H), 7.82 (d, 1H), 7.68 (d, 1H), 6.94 (d, 1H), 6.09 (s, 4H), 4.85 (t, 2H, D_2O overlapping), 3.81 (t, 2H), 3.62 (t, 2H), 3.20 (t, 2H), 3.00 (s, 6H), 2.15 (m, 2H). Anal.($C_{20}H_{24}N_6O\cdot 2C_4H_4O_4\cdot 0.7H_2O$) C, H, N.

2-[2-(Dimethylamino)ethyl]-5-[2-(1-piperidinyl)ethyl]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one dimaleate (13i): Method F; ^{1}H NMR (D_2O) δ 9.34 (s, 1H), 8.73 (d, 1H), 8.23 (d, 1H), 7.90 (d, 1H), 7.14 (d, 1H), 6.17 (s, 4H), 4.99 (t, 2H), 4.06 (t, 2H), 3.87 (t, 2H), 3.75−3.60 (m, 2H), 3.54 (t, 2H) 3.18−2.98 (m, 2H), 3.04 (s, 6H), 2.07−1.40 (m, 6H). Anal. ($C_{24}H_{30}N_6O\cdot 3C_4H_4O_4$) C, H, N.

2-[2-(Dimethylamino)ethyl]-5-[(2-hydroxyethyl)amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one maleate (13k): Method F; ^{1}H NMR (D_2O) δ 8.57 (s, 1H), 8.42 (d, 1H), 7.42 (d, 1H), 7.40 (d, 1H), 6.62 (d, 1H), 6.21 (s, 4H), 4.70 (t, 2H), 3.92 (t, 2H), 3.78 (t, 2H), 3.47 (t, 2H), 3.07 (s, 6H). Anal. ($C_{19}H_{21}N_5O_2\cdot C_4H_4O_4$) C, H, N.

2-[2-(Dimethylamino)ethyl]-5-[[2-[bis(2-hydroxyethyl)amino]ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one dimaleate (13l): Method F; ^{1}H NMR (D_2O) δ 9.32 (s, 1H), 8.73 (d, 1H), 8.24 (d, 1H), 7.89 (d, 1H), 7.16 (d, 1H), 6.14 (s, 4H), 4.98 (t, 2H), 4.12 (t, 2H), 3.99 (t, 4H), 3.88 (t, 2H), 3.76 (t, 2H), 3.58 (t, 4H), 3.05 (s, 6H). Anal. ($C_{23}H_{30}N_6O_3\cdot 3C_4H_4O_4$) C, H, N.

2-[2-(Dimethylamino)ethyl]-5-[[2-(imidazol-1-yl)ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one dimaleate (13m): Method F; ^{1}H NMR (D_2O/DMSO-d_6) δ 8.98 (s, 1H), 8.73 (s, 1H), 8.55 (d, 1H), 7.78 (d, 1H), 7.63 (d, 1H), 7.58 (s, 1H), 7.43 (s, 1H), 6.84 (d, 1H), 6.02 (s, 4H), 4.83 (t, 2H), 4.60 (t, 2H), 4.05 (t, 2H), 3.70 (t, 2H), 3.01 (s, 6H). Anal. ($C_{22}H_{23}N_7O\cdot 2C_4H_4O_4\cdot 1.8H_2O$) C, H, N.

2-[2-(Dimethylamino)ethyl]-5-[[2-[(methylsulfonyl)amino]ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one maleate (13o): Method F; ^{1}H NMR (DMSO-d_6) δ 9.54 (d, 1H), 9.30 (t, 1H), 8.83 (d, 1H), 8.25−8.15 (m, 2H),7.40−7.30 (m, 2H), 6.03 (s, 2H), 5.00 (t, 2H), 3.75−3.60 (m, 4H), 3.28 (q, 2H, partially hidden by the water signal), 2.93 (s, 3H), 2.87 (s, 6H). Anal. ($C_{20}H_{24}N_6O_3S\cdot C_4H_4O_4\cdot H_2O$) C, H, N, S.

2-[2-(Dimethylamino)ethyl]-5-[[2-[[2-(dimethylamino)ethyl]amino]ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one dimaleate (13p): Method F; ^{1}H NMR (D_2O) δ 9.05 (s, 1H), 8.63 (d, 1H), 7.86 (d, 1H), 7.70 (d, 1H), 6.96 (d, 1H), 5.95 (s, 4H), 4.87 (t, 2H), 3.97 (br t, 2H), 3.81 (t, 2H), 3.60−3.42 (m, 4H), 3.04 (s, 6H), 2.91 (s, 6H). Anal. ($C_{23}H_{31}N_7O\cdot 2.8C_4H_4O_4\cdot H_2O$) C, H, N.

2-[2-[(2-Hydroxyethyl)amino]ethyl]-5-[[2-(dimethylamino)ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one dimaleate (13x): Method F; ^{1}H NMR (D_2O) δ 8.83 (s, 1H), 8.48 (d, J = 5.5 Hz, 1H), 7.65 (d, J = 5.5 Hz, 1H), 7.61 (d, J = 9.4 Hz, 1H), 6.85 (d, J = 9.4 Hz, 1H), 5.92 (s, 4H), 4.80 (t, J = 5.6 Hz, 2H, partially hidden by HOD signal), 3.96 (t, J = 6.1 Hz, 2H), 3.89 (dd, J = 4.8, 6.7 Hz, 2H), 3.78 (t, J = 5.6 Hz, 2H), 3.53 (t, J = 6.1 Hz, 2H), 3.36 (dd, J = 4.8, 6.7 Hz, 2H), 3.02 (s, 6H). Anal. ($C_{21}H_{26}N_6O_2\cdot 2C_4H_4O_4$) C, H, N.

2-[3-(Dimethylamino)propyl]-5-[[2-(dimethylamino)ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one dimaleate (13dd): Method F; ^{1}H NMR (D_2O) δ 9.11 (s, 1H), 8.68 (d, 1H), 8.08 (d, 1H), 7.29 (d, 1H), 7.05 (d, 1H), 6.10 (s, 4H), 4.61 (t, 2H), 4.05 (t, 2H), 3.60 (t, 2H), 3.30−3.16 (m, 2H), 3.06 (s, 6H), 2.90 (s, 6H), 2.53−2.33 (m, 2H). Anal. ($C_{22}H_{28}N_6O\cdot 2.6C_4H_4O_4\cdot 0.5H_2O$) C, H, N.

2-(2-Hydroxyethyl)-5-[[2-(dimethylamino)ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one maleate (13hh): Method F; ^{1}H NMR (D_2O) δ 8.42 (s, 1H), 8.20 (d, 1H), 7.44 (d, 1H), 7.28 (d, 1H), 6.62 (d, 1H), 6.09 (s, 2H), 4.32 (t, 2H), 4.0 (t, 2H), 3.82 (t, 2H), 3.48 (t, 2H), 3.00 (s, 6H). Anal. ($C_{19}H_{21}N_5O_2\cdot C_4H_4O_4\cdot 0.9H_2O$) C, H, N.

2-[2-(Dimethylamino)ethyl]-5-[(2-aminoethyl)amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one Dimaleate (13b). Method G. To a stirred suspension of **12b** (295 mg, 0.84 mmol) in ethanol (25 mL) and chloroform (5 mL) at 50 °C was added a solution of maleic acid (224 mg, 1.93 mmol) in ethanol (5 mL). The resulting dark-red solution was stirred for a further 5 min at 50 °C and allowed to cool to room temperature. The solid was collected by filtration, washed with absolute ethanol and methyl *tert*-butyl ether, and dried under vacuum at 40 °C to constant weight to yield **13b** (280 mg, 57%) as a brick-red solid: ^{1}H NMR (D_2O) δ 9.00 (s, 1H), 8.55 (d, 1H), 7.80 (d, 1H), 7.70 (d, 1H), 7.00 (d, 1H), 6.00 (s, 4H), 4.90 (t, 2H), 3.93 (t, 2H), 3.80 (t, 2H), 3.40 (t, 2H), 3.00 (s, 6H). Anal. ($C_{19}H_{22}N_6O\cdot 2C_4H_4O_4\cdot H_2O$) C, H, N.

2-[2-(Dimethylamino)ethyl]-5-[[2-(methylamino)ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one Dimaleate (13c). Method H. To a stirred solution of **12c** (518 mg, 1.12 mmol) in dichloromethane (50 mL) at 0 °C was added trifluoroacetic acid (20 mL) dropwise during a 20-min period. After 1 h the solvents were removed by rotary evaporation at room temperature. The residue was taken up with ethanol, and the solution was concentrated to dryness by rotary evaporation at room temperature. The resulting trifluoroacetate salt was partitioned between dichloromethane and a saturated solution of sodium hydrogen carbonate. The aqueous phase was thoroughly extracted with dichloromethane; the combined organic extracts were dried over sodium sulfate and concentrated to dryness. The crude free base was obtained as a red viscous oil (230 mg, 0.631 mmol, 57%). This material was dissolved in absolute ethanol (7 mL), and the solution was heated to 50 °C. A solution of maleic acid (168 mg, 1.45 mmol) in absolute ethanol (2 mL) was added, and the mixture was stirred for about 5 min at 50 °C and then allowed to cool to room temperature. After 2 h the solid was collected by filtration, washed with absolute ethanol and diethyl ether, and dried under vacuum at 40 °C. The product **13c** (256 mg, salt formation, 68%; 38% overall) was obtained as a dark-red solid: ^{1}H NMR (D_2O) δ 9.04 (s, 1H), 8.59 (d, 1H), 7.89 (d, 1H), 7.74 (d, 1H), 6.98 (d, 1H), 5.99 (s, 4H), 4.90 (t, 2H), 3.95 (t, 2H), 3.82 (t, 2H), 3.44 (t, 2H), 3.04 (s, 6H), 2.80 (s, 3H). Anal. ($C_{20}H_{24}N_6O\cdot 2.4C_4H_4O_4\cdot H_2O$) C, H, N.

2-[2-(Dimethylamino)ethyl]-5-[[2-(piperazin-1-yl)ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one Dimaleate (13j). Method I. To a stirred solution of **12j** (340 mg, 0.654 mmol) in 96% ethanol (20 mL) was slowly added concentrated hydrochloric acid (2.0 mL). To the resulting thick suspension was added water (2.0 mL). The reaction mixture was stirred for 18 h at room temperature and for 5 h at 50 °C. The solid was collected by filtration, washed with absolute ethanol and diethyl ether, and dried under vacuum at room temperature. The crude 2-[2-(dimethylamino)ethyl]-5-[[2-(piperazin-1-yl)ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one trihydrochloride salt (342 mg) was obtained as a red solid, which failed to give satisfactory elemental analysis. This material was partitioned between dichloromethane (50 mL) and a buffer solution made from sodium hydrogen carbonate-saturated solution (20 mL), brine (20 mL), and 20% sodium hydroxide (4 mL). The phases were separated, and the aqueous layer was further extracted with dichloromethane/methanol mixtures (100:1) (3 × 50 mL). The organic extracts were washed with brine (20 mL) and concentrated to dryness. The free base of compound **13j** (257 mg) was obtained as an orange solid. This material was dissolved in absolute ethanol (2.5 mL) at 75 °C, and a solution of maleic acid (161 mg, 1.39 mmol) in absolute ethanol (1.5 mL) was added. The resulting thick suspension was diluted with additional absolute ethanol (2 mL), stirred for a further 5 min at 75 °C, and allowed to cool to room temperature. The solid was collected by filtration, washed with absolute ethanol and diethyl ether, and dried under vacuum at 50 °C to constant weight. The product **13j** (391 mg, 91%) was obtained as an orange solid: ^{1}H NMR (DMSO-d_6) δ 9.57 (s, 1H), 9.36 (br t, 1H), 8.84 (d, 1H), 8.24 (d, 1H), 8.19 (d, 1H), 7.33 (d, 1H), 6.05 (s, 4H), 5.02 (t, 2H), 3.80−3.58 (m, 4H), 3.21−3.05 (m, 4H), 2.88 (s, 6H), 2.82−2.67 (m, 6H). Anal. ($C_{23}H_{29}N_7O\cdot 2.3C_4H_4O_4\cdot H_2O$) C, H, N.

2-(2-Aminoethyl)-5-[[2-(dimethylamino)ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one Trihydrochloride (13q). Method J. To a stirred suspension of **12q** (201 mg, 0.46 mmol) in anhydrous chloroform (20 mL) was added dropwise a mixture of 6.5 N hydrochloric acid in ethanol (5.1 mL) and

chloroform (2.5 mL) during a 10-min period. The reaction mixture was stirred at room temperature for 30 min and diluted with diethyl ether, and the solid was collected by filtration, washed with diethyl ether, and dried under vacuum at room temperature for 30 min. The solid was suspended in absolute ethanol (3 mL), and the mixture was stirred at room temperature for 10 min. The solid was collected by filtration, washed with diethyl ether, and dried under vacuum at room temperature for 24 h. The pure product **13q** (159 mg, 75%) was obtained as a dark-red solid: ^{1}H NMR (D_2O) δ 9.43 (s, 1H), 8.77 (d, 1H), 8.43 (dd, 1H), 7.95 (d, 1H), 7.20 (d, 1H), 4.93 (t, 2H), 4.07 (t, 2H), 3.70 (t, 2H), 3.60 (t, 2H), 3.00 (s, 6H). Anal. ($C_{19}H_{22}N_6O\cdot3HCl\cdot2.1H_2O$) C, H, Cl, N.

2-(2-Aminoethyl)-5-[(2-aminoethyl)amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one Trihydrochloride (13r). Method K. Under a nitrogen atmosphere a 5 M ethanolic solution of anhydrous HCl (0.40 mL) was added dropwise to a stirred suspension of **12r** (0.104 g, 1.99 mmol) in absolute ethanol (2.1 mL). Initial dissolution of the starting material followed by precipitation of a dark-red solid was observed during the addition of ethanolic HCl. This solid was redissolved in absolute ethanol, and the solution was heated to 40 °C for 2.5 h. The gradual formation of a red-amaranth precipitate was observed during the heating. After the mixture cooled to room temperature, diethyl ether (10 mL) was added, and the precipitate was collected by suction filtration under a nitrogen blanket to give **13r** (0.070 g, 81%): ^{1}H NMR (D_2O) δ 9.10 (s, 2H), 8.62 (d, 1H), 8.04 (dd, 1H), 7.78 (d, 1H), 7.05 (d, 1H), 4.87 (t, 2H), 3.95 (t, 2H), 3.70 (t, 2H), 3.40 (t, 2H). Anal. ($C_{17}H_{18}N_6O\cdot2.5HCl\cdot2.4H_2O$) C, N, Cl; H: calcd, 5.58; found, 5.12.

2-(2-Aminoethyl)-5-[[2-(methylamino)ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one trihydrochloride (13s): Method K; ^{1}H NMR (D_2O) δ 9.35 (s, 1H), 8.76 (d, 1H), 8.38 (d, 1H), 7.89 (d. 1H), 7.11 (d, 1H), 4.89 (t, 2H), 4.0 (t, 2H), 3.70 (t, 2H), 3.45 (t, 2H), 2.80 (s, 3H). Anal. ($C_{18}H_{20}N_6O\cdot3HCl\cdot0.6H_2O$) C, H, Cl, N.

2-(2-Aminoethyl)-5-[[2-(diethylamino)ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one trihydrochloride (13u): Method K; ^{1}H NMR (D_2O) δ 9.50 (s, 1H), 8.83 (d, 1H), 8.52 (d, 1H), 8.02 (d, 1H), 7.23 (d, 1H), 4.97 (t, 2H), 4.11 (t, 2H), 3.75 (t, 4H), 3.61 (t, 2H), 3.49 (q, 4H), 1.34 (t, 6H). Anal. ($C_{21}H_{26}N_6O\cdot3HCl\cdot2.5H_2O$) C, H, Cl, N.

2-(2-Aminoethyl)-5-[[2-(4-morpholinyl)ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one trihydrochloride (13v): Method K; ^{1}H NMR (D_2O) δ 9.47 (s, 1H), 8.81 (d, 1H), 8.49 (d, 1H), 8.00 (d, 1H), 7.21 (d, 1H), 4.95 (t, 2H), 4.20−3.82 (m, 6H), 3.80−3.20 (m, 8H). Anal. ($C_{21}H_{24}N_6O_2\cdot2.9HCl\cdot3.1H_2O$) C, N; Cl: calcd, 18.56; found, 18.03. H: calcd, 6.02; found, 5.32.

2-[2-[(2-Hydroxyethyl)amino]ethyl]-5-[(2-aminoethyl)amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one trihydrochloride (13y): Method K; ^{1}H NMR (D_2O) δ 9.28 (s, 1H), 8.69 (d, 1H), 8.25 (dd, 1H), 7.88 (d, 1H), 4.93 (t, 2H), 7.10 (d, 1H), 3.95 (t, 2H), 3.88 (t, 2H), 3.80 (t, 2H), 3.34 (t, 2H), 3.31 (t, 2H). Anal. ($C_{19}H_{22}N_6O_2\cdot2.8HCl\cdot2.4H_2O$) C, H, Cl, N.

2-[3-(Dimethylamino)propyl]-5-[[2-[(2-hydroxyethyl)amino]ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one trihydrochloride (13ee): Method K; ^{1}H NMR (D_2O) δ 9.19 (s, 1H), 8.72 (d, 1H), 8.28 (d, 1H), 7.83 (d, 1H), 7.07 (d, 1H), 4.61 (t, 2H), 4.00 (t, 2H), 3.88 (t, 2H), 3.50 (t, 2H), 3.30 (t, 2H), 3.24−3.11 (m, 2H), 2.85 (s, 6H), 2.50−2.31 (m, 2H). Anal. ($C_{22}H_{28}N_6O_2\cdot2.9HCl\cdot2H_2O$) C, H, Cl, N.

2-[2-(Methylamino)ethyl]-5-[[2-(methylamino)ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one trihydrochloride (13ff): Method K; ^{1}H NMR (D_2O) δ 9.42 (s, 1H), 8.83 (d, 1H), 8.44 (d, 1H), 7.93 (d, 1H), 7.14 (d, 1H), 4.97 (t, 2H), 4.03 (t, 2H), 3.77 (t, 2H), 3.48 (t, 2H), 2.82 (s, 3H), 2.80 (s, 3H); ^{13}C NMR (D_2O) δ 179.96, 153.90, 144.62, 142.89, 142.31, 135.36, 134.21, 129.22, 126.73, 125.54, 125.37, 118.18, 107.91, 51.23, 50.77, 49.09, 42.17, 36.29. Anal. ($C_{19}H_{22}N_6O\cdot2.9HCl\cdot0.7H_2O$) C, H, Cl, N.

2-[2-[(2-Hydroxyethyl)amino]ethyl]-5-[[2-[bis(2-hydroxyethyl)amino]ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one Trihydrochloride (13bb). Method L. Concentrated hydrochloric acid (2.5 mL) was added to a stirred suspension of **12bb** (0.870 g, 1.57 mmol) in 96% ethanol (25 mL) heated at 40 °C. The resulting purple-red solution was stirred for 6 h at 60 °C. The red solid was collected by filtration, washed with plenty of absolute ethanol, and dried under vacuum at 50 °C to yield **13bb** (0.851 g, 96%): ^{1}H NMR (D_2O) δ 9.35 (s, 1H), 8.77 (d, 1H), 7.93 (d, 1H), 7.38 (d, 1H), 7.15 (d, 1H), 4.97 (t, 2H), 4.13 (t, 2H), 4.00−3.70 (m, 10H), 3.62−3.52 (m, 4H), 3.40−3.30 (m, 2H). Anal. ($C_{23}H_{30}N_6O_4\cdot3HCl\cdot2H_2O$) C, H, Cl, N.

2-[2-[(2-Hydroxyethyl)amino]ethyl]-5-[[2-[(2-hydroxyethyl)amino]ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one trihydrochloride (13aa): Method L; ^{1}H NMR (D_2O) δ 9.33 (s, 1H), 8.74 (d, 1H), 8.35 (d, 1H), 7.91 (d, 1H), 7.12 (d, 1H), 4.96 (t, 2H), 4.02 (t, 2H), 3.87 (m, 6H), 3.51 (t, 2H), 3.32 (m, 4H). Anal. ($C_{21}H_{26}N_6O_3\cdot3HCl\cdot H_2O$) C, H, Cl, N.

2-(2-Aminoethyl)-5-[[2-[(2-hydroxyethyl)amino]ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one trihydrochloride (13t): Method L; ^{1}H NMR (D_2O) δ 9.77 (s, 1H), 8.72 (d, 1H), 8.35 (dd, 1H), 7.70 (d, 1H), 7.03 (d, 1H), 4.90 (t, 2H), 3.85 (m, 4H), 3.31 (m, 4H), 3.28 (t, 2H). Anal. ($C_{19}H_{22}N_6O_2\cdot3HCl\cdot0.6H_2O$) C, H, Cl, N.

2-[2-[(2-Hydroxyethyl)amino]ethyl]-5-[[2-(methylamino)ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one trihydrochloride (13z): Method L; ^{1}H NMR (D_2O) δ 9.34 (s, 1H), 8.77 (d, J = 6.0 Hz, 1H), 8.39 (d, J = 6.0 Hz, 1H), 7.91 (d, J = 9.3 Hz, 1H), 7.11 (d, J = 9.3 Hz, 1H), 4.97 (t, J = 5.7 Hz, 2H), 4.00 (t, J = 6.0 Hz, 2H), 3.94−3.86 (m, 2H), 3.83 (t, J = 5.7 Hz, 2H), 3.45 (t, J = 6.0 Hz, 2H), 3.40−3.31 (m, 2H), 2.80 (s, 3H); ^{13}C NMR (D_2O) δ 180.20, 153.86, 144.41, 143.25, 142.54, 135.40, 134.25, 129.08, 126.52, 125.54, 118.15, 59.45, 52.41, 50.74, 49.61, 48.85, 42.12, 36.23. Anal. ($C_{20}H_{24}N_6O_2\cdot3HCl\cdot0.7H_2O$) C, H, Cl, N.

2-[2-[(2-Hydroxyethyl)amino]ethyl]-5-[(2-hydroxyethyl)amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one dihydrochloride (13 cc): Method L; ^{1}H NMR (D_2O) δ 8.77 (s, 1H), 8.58 (d, 1H), 7.90 (d, 1H), 7.53 (d, 1H), 6.70 (d, 1H), 4.77 (t, 2H), 4.00−3.85 (m, 4H), 3.79 (t, 2H), 3.50 (t, 2H), 3.41 (t, 2H). Anal. ($C_{19}H_{21}N_5O_3\cdot2HCl\cdot0.25H_2O$) C, H, N, Cl.

2-[2-(Methylamino)ethyl]-5-[[2-[(2-hydroxyethyl)amino]ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one trihydrochloride (13gg): Method L; ^{1}H NMR (D_2O) δ 9.37 (s, 1H), 8.76 (d, J = 6.0 Hz, 1H), 8.37 (d, J = 6.0 Hz, 1H), 7.90 (d, J = 9.3 Hz, 1H), 7.13 (d, J = 9.3 Hz, 1H), 4.93 (t, J = 5.7 Hz, 2H), 4.02 (t, J = 5.7 Hz, 2H), 3.89−3.83 (m, 2H), 3.72 (t, J = 5.7 Hz, 2H), 3.50 (t, J = 5.7 Hz, 2H), 3.32−3.24 (m, 2H); ^{13}C NMR (D_2O) δ 171.82, 162.37, 161.09, 160.49, 152.12, 147.05, 144.43, 143.42, 136.08, 77.41, 70.41, 69.10, 66.97, 66.91, 59.99, 54.09 (4 overlapping signals). Anal. ($C_{20}H_{24}N_6O_2\cdot3HCl\cdot2.4H_2O$) C, H, N, Cl.

2-(2-Hydroxyethyl)-5-[(2-aminoethyl)amino]indazolo-[4,3-*gh*]isoquinolin-6(2*H*)-one dihydrochloride (13ii): Method L; ^{1}H NMR (D_2O) δ 8.99 (s, 1H), 8.58 (d, 1H), 8.02 (d, 1H), 7.71 (d, 1H), 6.94 (d, 1H), 4.55 (t, 2H), 4.06 (t, 2H), 3.88 (t, 2H), 3.39 (t, 2H). Anal. ($C_{17}H_{17}N_5O_2\cdot2HCl\cdot0.75H_2O$) C, H, Cl, N.

2-(2-Aminoethyl)-5-[[2-[bis(2-hydroxyethyl)amino]ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one Dimaleate (13w). Method M. To a stirred suspension of **12w** (360 mg, 0.705 mmol) in absolute ethanol (15 mL) was added 6.5 N hydrochloric acid in ethanol (5 mL). The reaction mixture was stirred at room temperature for 6 h and then concentrated to dryness. The residue was triturated with diethyl ether, and the solid was collected by filtration and dried under vacuum at 40 °C. This material was purified by column chromatography (silica gel, 230−400 mesh, 20 g) eluting with dichloromethane/methanol/concentrated ammonium hydroxide (90:10:2). The chromatographic fractions containing the product were pooled and concentrated to dryness. The free base of **13w** (218 mg, 75%) was obtained as an orange solid. This material was suspended in absolute ethanol (17 mL) at 60 °C and maleic acid (135 mg, 1.16 mmol) was added. The resulting solution was stirred for further 5 min at 60 °C, and allowed to cool to room temperature. The solid was collected by filtration,

washed with absolute ethanol, and dried under vacuum at 40 °C overnight. The dimaleate **13w** (250 mg, 55%) was obtained as a brick-red solid: ^{1}H NMR (D_2O) δ 9.05 (s, 1H), 8.60 (d, 1H), 7.90 (d, 1H), 7.72 (d, 1H), 6.98 (d, 1H), 6.06 (s, 4H), 4.78 (t, 2H, partially hidden by the HOD signal), 4.13−3.93 (m, 6H), 3.80−3.52 (m, 8H). Anal. ($C_{21}H_{26}N_6O_3 \cdot 2.4C_4H_4O_4 \cdot 2H_2O$) C, N; H: calcd, 5.50; found, 4.99; inorganic residue 1.7%.

2-(2-Hydroxyethyl)-5-chloroindazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (15a). A mixture of **14** (915 mg, 3.5 mmol) and diisopropylethylamine (0.76 mL, 4.38 mmol) in THF (27 mL) was heated at 30 °C to ensure complete dissolution of the solid. Commercially available (2-hydroxyethyl)hydrazine (0.416 mL, 5.95 mmol) was added to the resulting solution. The reaction mixture was stirred for 1 h at 30 °C. The green solid was collected by filtration, washed with a small amount of THF, and suspended in water (5 mL). The suspension was stirred at room temperature for 1 h. The solid was collected by filtration and washed with water (5 mL). This crude material was dissolved in methanol (5 mL) and crystallized by addition of hexane (15 mL) to afford **15a** (570 mg, 54%): TLC (methanol/dichloromethane/ammonium hydroxide, 10:90:2.5) $R_f = 0.55$; mp 231−233 °C; ^{1}H NMR (DMSO-d_6) δ 9.4 (s, 1H), 8.8 (d, 1H), 8.15 (d, 1H), 8.05 (d, 1H), 7.7 (d, 1H), 5.0 (t, 1H), 4.65 (t, 2H), 3.9 (q, 2H).

5-Chloro-2-[2-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-ethyl]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (15b). A mixture of **15a** (176 mg, 0.857 mmol), imidazole (60 mg, 0.880 mmol), and *tert*-butyldimethylchlorosilane (111 mg, 0.734 mmol) in anhydrous dichloromethane (4 mL) was stirred for 3 h at room temperature. The reaction mixture was partitioned between water (10 mL) and a 2:1 mixture of hexane and ethyl acetate. The organic phase was washed with phosphate buffer (20 mL, pH 4−5), water (25 mL), and brine (25 mL), then dried over sodium sulfate, and concentrated. The residue was purified by flash chromatography eluting with a 90:10 dichloromethane/methyl *tert*-butyl ether mixture. The product **15b** (201 mg, 83%) was obtained as a yellow crystalline solid: mp 162.4−163.1 °C; TLC (dichloromethane/methyl *tert*-butyl ether, 90:10) $R_f = 0.37$; Crystals for X-ray analysis were obtained by crystallization from ethyl acetate/hexane by the double-layer technique; ^{1}H NMR ($CDCl_3$) δ 9.53 (d, J = 0.8 Hz, 1H), 8.85 (d, J = 5.1 Hz, 1H), 8.20 (dd, J = 5.1, 0.8 Hz, 1H), 7.74 (d, J = 8.6 Hz, 1H), 7.57 (d, J = 8.6 Hz, 1H), 4.63 (t, J = 5.0 Hz, 2H), 4.11 (t, J = 5.0 Hz, 2H), 0.71 (s, 9H), −0.20 (s, 6H).

5-[[(2-Dimethylamino)ethyl]amino]-2-(2-hydroxyethyl)indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (12hh from 15a). A mixture of **15a** (300 mg, 1.0 mmol) and 2-(dimethylamino)-ethylamine (0.78 mL, 7.0 mmol) in anhydrous pyridine (4.5 mL) was stirred for 15 h at 90 °C. The reaction mixture was poured in brine (22 mL), ethyl acetate (5 mL) was added, and the mixture was stirred at room temperature for 1 h. The orange solid was collected by filtration and then suspended in water (20 mL), and the mixture was stirred at room temperature for 30 min. The solid was collected by filtration and dried under vacuum at 60 °C to yield **12hh** (220 mg, 63%): mp 211−213 °C; spectroscopic and chromatographic properties of this material were found to be identical to those of the same compound obtained through pathway 1 (method A); TLC (dichloromethane/methanol/ammonium hydroxide, 90:10:2.5); $R_f = 0.35$; ^{1}H NMR (DMSO-d_6) δ 9.51 (d, J = 0.98 Hz, 1H), 9.3 (br t, J = 5.18 Hz, 1H), 8.79 (d, J = 5.28 Hz, 1H), 8.22 (d, J = 5.28, 0.98 Hz, 1H), 8.08 (d, J = 9.19 Hz, 1H), 7.21 (d, J = 9.19 Hz, 1H), 4.98 (t, J = 5.47 Hz, 1H), 4.65 (t, J = 5.38 Hz, 2H), 3.90 (q, J = 5.28 Hz, 2H), 3.58 (q, J = 5.74 Hz, 2H), 2.60 (t, J = 5.97 Hz, 2H), 2.26 (s, 6H).

5-[[2-[*N*-[(1,1-Dimethylethoxy)carbonyl]-*N*-methylamino]ethyl]amino]-2-(2-hydroxyethyl)indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (16). A mixture of **15a** (5 g, 16.6 mmol) and 2-[*N*-methyl-*N*-[(1,1-dimethylethoxy)carbonyl]amino]ethylamine (24 g, 0.166 mol) in pyridine (50 mL) was stirred at 100 °C (internal bulk temperature) for 5 h and at 90 °C overnight, under a nitrogen atmosphere. The reaction mixture was concentrated to one-half volume and poured into brine (250 mL). The aqueous phase was extracted with chloroform/methanol (90:10) (5 × 70 mL), and the combined organic phases were dried over sodium sulfate and concentrated. The residue was taken up in ethyl acetate (60 mL), hexane (60 mL) was added, and the mixture was stirred at room temperature for 2 h. The solid was collected by filtration, washed with water, and dried under vacuum at 40 °C to constant weight. The latter material (6.65 g) was recrystallized from 2-propanol (195 mL) to yield **16** (4.8 g, 66%): mp 199−201 °C; TLC (methanol/dichloromethane/ammonium hydroxide, 10:200:2.5) $R_f = 0.30$; ^{1}H NMR ($CDCl_3$) δ 9.25 (s, 1H), 9.1 (m, 1H), 8.25 (d, 1H), 7.8 (d, 1H), 7.6 (m, 1H), 6.85 (m, 1H), 4.6 (m, 2H), 4.35 (m, 2H), 3.5−3.75 (m, 4H), 1.5 (s, 9H).

5-[[2-(Dimethylamino)ethyl]amino]-2-[2-[(methylsulfonyl)oxy]ethyl]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (17a). Methanesulfonyl chloride (0.864 mL, 11 mmol) was added to a stirred suspension of **12hh** (3.58 g, 10 mmol) and triethylamine (1.67 mL, 12 mmol) in anhydrous dichloromethane (105 mL) while cooling to 0 °C. The cooling bath was removed, and the mixture was stirred at room temperature overnight. The reaction mixture was partitioned between dichloromethane (200 mL) and 1 N sodium hydroxide (100 mL). The aqueous phase was extracted with dichloromethane (100 mL); the organic phases were combined, dried over sodium sulfate, and concentrated. The residue was suspended in ethyl acetate (90 mL) and stirred at room temperature for 0.5 h. Hexane (30 mL) was added, the mixture was stirred at 0 °C for 1 h, and the solid was collected by filtration and dried under vacuum at 50 °C to constant weight to yield **17a** (3.45 g, 80.3%): TLC (dichloromethane/methanol/ammonium hydroxide, 90:10:2.5) $R_f = 0.55$; this material was used in the subsequent step without further purification; mp 251−253 °C; ^{1}H NMR (DMSO-d_6) δ 9.49 (s, 1H), 9.27 (t, J = 5.09 Hz, 1H), 8.80 (d, J = 5.28 Hz, 1H), 8.20 (d, J = 5.28 Hz, 1H), 8.08 (d, J = 9.19 Hz, 1H), 7.23 (d, J = 9.19 Hz, 1H), 4.97 (t, J = 4.89 Hz, 2H), 4.72 (t, J = 4.70 Hz, 2H), 3.58 (q, J = 5.67 Hz, 2H), 3.06 (s, 3H), 2.60 (t, J = 5.87 Hz, 2H), 2.26 (s, 6H). Anal. ($C_{20}H_{23}N_5O_4S$) H; S: calcd, C 55.93; found, 55.11. N: calcd, 16.37; found, 15.54.

5-[[2-[*N*-[(1,1-Dimethylethoxy)carbonyl]-*N*-methylamino]ethyl]amino]-2-[2-[(methylsulfonyl)oxy]ethyl]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (17b). Methanesulfonyl chloride (0.97 mL, 12.3 mmol) was added to a stirred suspension of **16** (4.16 g, 9.5 mmol) and triethylamine (1.98 mL, 114.2 mmol) in dichloromethane (85 mL) while cooling to 0 °C. The cooling bath was removed, and the mixture was stirred at room temperature overnight. Additional triethylamine (1.00 mL, 7.12 mmol) and methanesulfonyl chloride (0.49 mL, 6.17 mmol) were added, and the mixture was stirred at reflux temperature for 1 h. The mixture was partitioned between dichloromethane (200 mL) and 1 N sodium hydroxide (100 mL). The aqueous phase was diluted with additional 1 N sodium hydroxide (100 mL) and extracted with dichloromethane (2 × 100 mL). The organic phases were combined, dried over sodium sulfate, and concentrated. The residue was suspended in ethyl acetate (90 mL) and stirred at room temperature for 0.5 h. Hexane (30 mL) was added, the mixture was stirred at 0 °C for 1 h, and then the solid was collected by filtration and dried under vacuum at 50 °C to constant weight to afford **17b** (4.00 g, 82%) of satisfactory purity. This material was used in the subsequent step without further purification: TLC (methanol/dichloromethane/ammonium hydroxide, 10:200:2.5) $R_f = 0.65$; mp 117−119 °C; ^{1}H NMR (DMSO-d_6) δ 9.5 (s, 1H), 9.2 (m, 1H), 8.8 (d, 1H), 8.2 (d, 1H), 8.1 (d, 1H), 5.0 (m, 2H), 4.7 (m, 2H), 3.7 (m, 2H), 3.5 (m, 2H), 3.3 (s, 3H), 3.1 (s, 3H), 1.4−1.2 (br s, 9H). Anal. ($C_{24}H_{29}N_5O_6S$) H, N, S; C: calcd, 55.91; found, 55.11.

5-[[2-(Dimethylamino)ethyl]amino]-2-[2-[(2-hydroxyethyl)amino]ethyl]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (12x). A suspension of **17a** (3.35 g, 7.8 mmol) in ethanolamine (100 mL) was heated at 50 °C for 3.5 h. The mixture was allowed to cool to room temperature, and the solid was collected by filtration and washed with brine. The filtrate was poured into brine (600 mL), and the aqueous phase was extracted with dichloromethane (5 × 200 mL). The previously

collected solid was dissolved in dichloromethane (200 mL), and the resulting solution was combined with the extracts, dried over sodium sulfate, and concentrated. The residue was recrystallized from 2-propanol (21 mL). The mixture was stirred at 0 °C for 1 h and the solid collected by filtration, washed with cold 2-propanol, and dried under vacuum at 40 °C to constant weight to yield **12x** (2.50 g, 81%) as a red solid (identical to the product obtained from pathway 1, method A): TLC (dichloromethane/methanol/ammounium hydroxide, 90:10:2.5) $R_f = 0.25$; mp 184−186 °C; ^{1}H NMR (DMSO-d_6) δ 9.50 (d, $J = 0.78$ Hz, 1H), 9.30 (t, $J = 5.18$ Hz, 1H), 8.78 (d, $J = 5.28$, 1H), 8.20 (dd, $J = 5.28$, 0.98 Hz, 1H), 8.12 (d, $J = 9.19$, 1H), 7.20 (d, $J = 9.19$, 1H), 4.65 (t, $J = 6.06$ Hz, 2H), 4.43 (t, $J = 5.37$ Hz, 1H), 3.58 (q, $J = 5.80$ Hz, 2H), 3.38 (q, $J = 5.41$ Hz, 2H), 3.08 (t, $J = 5.97$ Hz, 2H), 2.60 (t, $J = 6.06$ Hz, 2H), 2.59 (t, $J = 5.57$ Hz, 2H), 2.26 (s, 6H). Anal. ($C_{21}H_{26}N_6O_2$) C, H, N.

2-[2-[(2-Hydroxyethyl)amino]ethyl]-5-[[2-[*N*-[(1,1-dimethylethoxy)carbonyl]-*N*-methylamino]ethyl]amino]indazolo[4,3-*gh*]isoquinolin-6(2*H*)-one (12z). A mixture of **17b** (3.9 g, 7.5 mmol) and ethanolamine (78 mL) was stirred at 50 °C for 2 h. The reaction mixture was allowed to cool to room temperature and then poured into brine (600 mL). The aqueous phase was extracted with dichloromethane (4 × 200 mL). The organic extracts were combined, dried over sodium sulfate, and concentrated. The dark oily residue was recrystallized from 2-propanol (20 mL). The mixture was stirred at 0 °C for 2 h, and the solid was collected by filtration, washed with cold 2-propanol, and dried under vacuum at 40 °C to constant weight to afford **12z** (2.35 g, 65%) as an orange solid (identical to the product obtaind from pathway 1, method A): TLC (methanol/dichloromethane/ammonium hydroxide, 10:90:2.5) $R_f = 0.50$; mp 133−135 °C; ^{1}H NMR ($CDCl_3$) δ 9.6 (s, 1H), 9.3 (m, 1H), 8.8 (d, 1H), 8.25 (d, 1H), 7.7 (d, 1H), 7.1 (m, 1H), 4.6 (t, 2H), 3.6−3.7 (m, 6H), 3.3 (t, 2H), 2.95 (s, 3H), 2.8 (t, 2H), 1.5 (s, 9H). Anal. ($C_{25}H_{32}N_6O$) C, H, N.

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Supporting Information Available: ^{1}H NMR spectra of compounds **12** not included in the Experimental Section along with some melting points, UV data and HPLC data for final compounds **13**, and X-ray crystallographic stucture of **15b** and structural parameters (19 pages). Ordering information is given on any current masthead page.

References

(1) Krapcho, A. P.; Maresch, M. J.; Hacker, M. P.; Hazelhurst, L.; Menta, E.; Oliva, A.; Spinelli, S.; Giuliani, F. C.; Beggiolin, G.; Pezzoni, G.; Tognella, S. Anthracene-9,10-diones and Aza Bioisosteres as Antitumor Agents. *Curr. Med. Chem.* **1995**, *2*, 803−824.

(2) Lown, J. W., Ed. *Bioactive molecules. Anthracycline and Anthracenedione-based Anticancer Agents*; Elsevier: New York, 1988; p 6.

(3) Zee-Cheng, R. K. Y.; Cheng, C. C. Anthraquinone Anticancer Agents. *Drugs Future* **1983**, *8*, 229−248.

(4) Sengupta, S. K. Topoisomerase II Inhibitors. In *Cancer Chemotherapeutic Agents*; Foye, W. O., Ed.; American Chemical Society: Washington, DC, 1995; pp 205−260.

(5) Faulds, D.; Balfour, J. A.; Chrisp, P.; Langtry, H. D. Mitoxantrone, a Review of its Pharmacodynamic and Pharmacokinetic Properties, and Therapeutic Potential in the Treatment of Cancer. *Drugs* **1991**, *41*, 400−449.

(6) Dunn, C. J.; Goa, K. L. Mitoxantrone: a Review of its Pharmacological Properties and use in Acute Nonlymphoblastic Leukaemia. *Drugs Aging* **1996**, *9*, 122−147.

(7) Wiseman, L. R.; Spencer, C. M. Mitoxantrone. A Review of its Pharmacology and Clinical Efficacy in the Management of Hormone-resistant Advanced Prostate Cancer. *Drugs Aging* **1997**, *10*, 473−485.

(8) Fukushima, T.; Kawai, Y.; Nakayama, T.; Yamauchi, T.; Yoshida, A.; Urasaki, Y.; Imamura, S.; Kamiya, K.; Tsutani, H.; Ueda, T.; Nakamura, T. Superior Cytotoxic Potency of Mitoxantrone in Interaction with DNA: Comparison with that of Daunorubicin. *Oncol. Res.* **1996**, *8*, 95−100.

(9) Umemoto, M.; Kawasaki, H.; Azuma, E.; Komada, Y.; Ito, M.; Sakurai, M. Bradycardia due to Mitoxantrone Exacerbated by Previous Anthracycline Therapy. *Am. J. Hematol.* **1996**, *52*, 327−328.

(10) Benekli, M.; Kars, A.; Guter, N. Mitoxantrone-induced Bradycardia. *Ann. Intern. Med.* **1997**, *126*, 409.

(11) Pincus, R.; Goldman, I. D. Evidence for Impaired Mitoxantrone and Vinblastine Binding in P388 Murine Leukemia Cells with Multiple Drug Resistance. *Biochem. Pharmacol.* **1990**, *40*, 2625−2635.

(12) Bailly, J. D.; Skladanowski, A.; Bettaieb, A.; Mansat, V.; Larsen, A. K.; Laurent, G. Natural Resistance of Acute Myeloid Leukemia Cell Lines to Mitoxantrone is Associated with Lack of Apoptosis. *Leukemia* **1997**, *11*, 1523−1532.

(13) Krapcho, A. P.; Getahun, Z.; Avery, K. L., Jr.; Vargas, K. J.; Hacker, M. P.; Spinelli, S.; Pezzoni, G.; Manzotti, C. Synthesis and Antitumor evaluations of Symmetrically and Unsymmetrically substituted 1,4-Bis[(aminoalkyl)amino]anthracene-9,10-diones and 1,4-Bis[(aminoalkyl)amino]-5,8-dihydroxyanthracene-9,10-diones. *J. Med. Chem.* **1991**, *34*, 2373−2380.

(14) Bailly, C.; Routier, S.; Bernier, J.-L.; Waring, M. J. DNA Recognition by Two Mitoxantrone Analogues: Influence of the Hydroxy Groups. *FEB Lett.* **1996**, *379*, 269−272.

(15) De Isabella, P.; Capranico, G.; Palumbo, M.; Sissi, C.; Krapcho, A. P.; Zunino, F. Sequence Specificity of Topoisomerase II DNA Cleavage Stimulated by Mitoxantrone Derivatives: Relationship to Drug DNA Binding and Cellular Effects. *Mol. Pharmacol.* **1993**, *43*, 715−721.

(16) Ho, C. K.; Law, S. L.; Chiang, H.; Hsu, M. L.; Wang, C. C.; Wang, S. Y. Inhibition of Microtubule Assembly is a Possible Mechanism of Action of Mitoxantrone. *Biochem. Biophys. Res. Commun.* **1991**, *180*, 118−123.

(17) Takeuchi, N.; Nakamura, T.; Takeuchi, F.; Hashimoto, E.; Yamamura, H. Inhibitory effect of Mitoxantrone on Activity of Protein Kinase C and Growth of HL60 cells. *J. Biochem.* **1992**, *112*, 762−767.

(18) Mewes, K.; Blanz, J.; Ehninger, G.; Gebhardt, R.; Zeller, K. P. Cytochrome P-450- Induced Cytotoxicity of Mitoxantrone by Formation of Electrophilic Intermediates. *Cancer Res.* **1993**, *53*, 5135−5142.

(19) Kapuscinski, J.; Darzynkiewicz, Z. Relationship between the Pharmacological Activity of Antitumor Drugs Ametantrone and Mitoxantrone (Novantrone) and their Ability to Condense Nucleic Acids. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6302−6306.

(20) Liwo, A.; Jeziorek, D.; Ossowski, T.; Dyl, D.; Tempczyk, A.; Tarasiuk, J.; Nowacka, M.; Borowski, E.; Woznicki, W. Molecular Modeling of Singlet-oxygen Binding to Anthraquinones in Relation to the Peroxidating Activity of Antitumor Anthraquinone Drugs. *Acta Biochem. Polon.* **1995**, *42*, 445−456.

(21) Tarasiuk, J.; Garnier-Suillerot, A.; Stefanska, B.; Borowski, E. The Essential Role of Anthraquinones as Substrates for NADH Dehydrogenase in their Redox Cycling Ability. *Anti-Cancer Drug Des.* **1992**, *7*, 329−340.

(22) Tarasiuk, J.; Stefanska, B.; Borowski, E. The Direct Reduction of Cytochrome *c* by some Anthraquinone Antitumor Compounds. *Anticancer Drug Des.* **1996**, *11*, 183−192.

(23) Stefanska, B.; Dzieduszycka, M.; Martelli, S.; Tarasiuk, J.; Bontemps-Gracz, M.; Borowski, E. 6-[(Aminoalkyl)amino]-substituted 7H-benzo[*e*]perimidin-7-ones as Novel Antineoplastic Agents. Synthesis and Biological Evaluations. *J. Med. Chem.* **1993**, *36*, 38−41.

(24) Shipp, N. C.; Dorr, R. T.; Alberts, D. S.; Dawson, B. V.; Hendrix, M. Characterization of Experimental Mitoxantrone Cardiotoxicity and its Partial Inhibition of ICRF-187 in Cultural Neonatal Rat Cells. *Cancer Res.* **1993**, *53*, 550−556.

(25) Krapcho, A. P.; Petry, M. E.; Getahun, Z.; Landi, J. J., Jr.; Stallman, J.; Polsenberg, J. F.; Gallagher, C. E.; Maresch, M. J.; Hacker, M. P.; Giuliani, F. C.; Beggiolin, G.; Pezzoni, G.; Menta, E.; Manzotti, C.; Oliva, A.; Spinelli, S.; Tognella, S. 6,9-Bis[(aminoalkyl)amino]benz[*g*]isoquinoline-5,10-diones. A Novel Class of Chromophore- Modified Antitumor Anthracene-9,10-diones: Synthesis and Antitumor Evaluations. *J. Med. Chem.* **1994**, *37*, 828−837.

(26) Krapcho, A. P.; Maresch, M. J.; Hacker, M. P.; Menta, E.; Oliva, A.; Giuliani, F. C.; Spinelli, S. Aza and Diazabioisosteric Anthracene-9,10-diones as Antitumor Agents. *Acta Biochem. Polon.* **1995**, *42*, 427−432.
(27) Hazlehurst, L. A.; Krapcho, A. P.; Hacker, M. P. Correlation of DNA Reactivity and Cytotoxicity of a New Class of Anticancer Agents: Aza-anthracenediones. *Cancer Lett.* **1995**, *91*, 115−124.
(28) De Isabella, P.; Palumbo, M.; Sissi, C.; Capranico, G.; Carenini, N.; Menta, E.; Oliva, A.; Spinelli, S.; Krapcho, A. P.; Zunino, F. Topoisomerase II Cleavage Stimulation, DNA Binding Activity, Cytotoxicity, and Physicochemical Properties of 2-Aza- and 2-Aza- oxide-anthracene-9,10-dione Derivatives. *Mol. Pharmacol.* **1995**, *48*, 30−38.
(29) Pezzoni, G.; Beggiolin, G.; Manzotti, C.; Spinelli, S.; Tognella, S.; Giuliani, F. C. BBR 2778, a Novel Aza-analogue of Anthracenediones Endowed with Preclinical Anticancer Activity. *Proc. Am. Assoc. Cancer Res.* **1993**, *34*, Abstr 2226.
(30) Cavalletti, E.; Crippa, L.; Melloni, E.; Bellini, O.; Tognella, S.; Giuliani, F. C. BBR 2778, a Novel Aza-anthracenedione: Preclinical Toxicological Evaluation. *Proc. Am. Assoc. Cancer Res.* **1993**, *34*, Abstr 2227.
(31) Showalter, H. D. H.; Johnson, J. L.; Hoftiezer, J. M.; Turner, W. R.; Werbel, L. M.; Leopold, W. R.; Shillis, J. L.; Jackson, R. C.; Elslager, E. F. Anthrapyrazole Anticancer Agents. Synthesis and Structure−activity Relationship against Murine Leukemias. *J. Med. Chem.* **1987**, *30*, 121−131.
(32) Patterson, L. H.; Newell, D. R. Cellular and Molecular Pharmacology of the Anthrapyrazole Antitumor Agents. In *Molecular Aspects of Anticancer Drug−DNA Interactions*; Neidle, S., Waring, M., Eds.; Macmillan: New York, 1994; Vol. 2, pp 96−129.
(33) Judson, I. R. Anthrapyrazoles: True Successors to the Anthracyclines? *Anti-Cancer Drugs* **1991**, *2*, 223−231.
(34) Gogas, H.; Mansi, J. L. The Anthrapyrazoles. *Cancer Treat. Rev.* **1995**, *21*, 541−552.
(35) Berg, S. Status of new anthrapyrazoles and pyrazoloacridine derivatives. *Crit. Rev. Oncol./Hematol.* **1996**, *22*, 79−87.
(36) Talbot, D. C.; Smith, I. E.; Mansi, J. L.; Hudson, I.; Calvert, A. H.; Ashley, S. E. Anthrapyrazole CI 941: a Highly Active New Agent in the Treatment of Advanced Breast Cancer. *J. Clin. Oncol.* **1991**, *9*, 2141−2147.
(37) Hortobagyi, G. N. Chemotherapy of Breast Cancer: a Historical Perspective. *Semin. Oncol.* **1997**, *24* (Suppl. 17), S17-1−S17-4.
(38) Barbu, M.; Azarnia, N.; Calvert, H. The Safety Profile of Losoxantrone (DuP-941), a New Anthrapyrazole Administered on a q.3.w. Schedule in Patients with Advanced Breast Cancer. *Ann. Oncol.* **1994**, *5* (Suppl. 5), Abstr 288.
(39) Vandenberg, T.; ten Bokkel Huinink, W.; Hedley, D.; Panasci, L.; Verma, S.; Calvert, H.; Francher, D.; Azarnia, N.; Sisk, R.; Symes, A. A Phase II Study of DuP-941 in Advanced Breast Cancer Patients with no Prior Chemotherapy. *Chemotherapy Foundation Symposium XI*; Innovative Cancer Chemotherapy For Tomorrow: New York, 1993; Abstr 10.
(40) Walsh, S. M.; Walley, V. M.; Chandra, L.; Huan, S. D.; Veinot, J. P.; Higginson, L. A. J. Potential Cardiotoxicity with the use of DuP-941: a Case Report. *Can. J. Cardiol.* **1995**, *11*, 419−422.
(41) Zalupski, M. M.; Benedetti, J.; Balcerzak, S. P.; Hutchins, L. F.; Belt, R. J.; Hantel, A.; Goodwin, J. W. Phase II Trial of Piroxantrone for Advanced or Metastatic Soft Tissue Sarcomas. A Southwest Oncology Group Study. *Invest. New Drugs* **1993**, *11*, 337−341.
(42) Hantel, A.; Donehower, R. C.; Rowinsky, E. K.; Vance, E.; Clarke, B. V.; McGuire, W. P.; Ettinger, D. S.; Noe, D. A.; Grochow, L. B. Phase I Study and Pharmacodynamincs of Piroxantrone (NSC 349174), a New Anthrapyrazole. *Cancer Res.* **1990**, *50*, 3284−3288.
(43) Ingle, J. N.; Kuross, S. A.; Mailliard, J. A.; Loprinzi, C. L.; Jung, S. H.; Nelimark, R. A.; Krook, J. E.; Long, H. J. Evaluation of Piroxantrone in Women with Metastatic Breast Cancer and Failure on Nonanthracycline Chemotherapy. *Cancer* **1994**, *74*, 1733−1738.
(44) Sosman, J. A.; Flaherty, L. E.; Liu, P. Y.; Fletcher, W.; Thompson, J. A.; Hantel, A.; Sondak, V. A Phase II Study of Piroxantrone in Disseminated Malignant Melanoma. A Southwest Oncology Group Study. *Invest. New Drugs* **1995**, *13*, 83−87.
(45) Krapcho, A. P.; Menta, E. Antitumor Aza-anthrapyrazoles. *Drugs Future* **1997**, *22*, 641−646.
(46) Krapcho, A. P.; Maresch, M. J.; Gallagher, C. E.; Menta, E.; Di Domenico, R.; Oliva, A.; Spinelli, S.; Beggiolin, G.; Giuliani, F. C.; Hacker, M. P. Synthesis and Antitumor Studies of Azabioisosteric Anthrapyrazoles. 211th ACS National Meeting, March 24−29, 1996, New Orleans, LA; Abstr MEDI 003.
(47) Menta, E.; Krapcho, A. P.; Maresch, M. J.; Gallagher, C. E.; Di Domenico, R.; Oliva, A.; Spinelli, S.; Beggiolin, G.; Giuliani, F. C.; Hacker, M. P. Synthesis and Antitumor Evaluations of 2,5-Disubstituted-indazolo[4,3-gh]isoquinoline-6(2H)-ones (9-Aza-anthrapyrazoles). 211th ACS National Meeting, March 24−29, 1996, New Orleans, LA; Abstr MEDI 076.
(48) Krapcho, A. P.; Maresch, M. J.; Gallagher, C. E.; Di Domenico, R.; Menta, E.; Oliva, A.; Da Re, G.; Fiocchi, L.; Spinelli, S. 6,9-Disubstituted Benz[*g*]isoquinoline-5,10-diones, Key Intermediates for the Synthesis of Antitumor 2,5-Disubstituted-indazolo[4,3-gh]isoquinoline-6(2H)-ones (9-Aza-anthrapyrazoles). 25th National Medicinal Chemistry Symposium, June 18, 1996, Ann Arbor, MI; Abstr 15.
(49) Menta, E.; Di Domenico, R.; Oliva, A.; Spinelli, S.; Beggiolin, G.; Pezzoni, G.; Giuliani, F. C.; Krapcho, A. P. Design, Synthesis and Antitumor Evaluations of 9-Aza- anthrapyrazoles. *Proc. Am. Assoc. Cancer Res.* **1996**, *37*, Abstr 2015.
(50) Beggiolin, G.; Pezzoni, G.; Torriani, D.; Ruggeri, F.; Randisi, E.; De Giorgi, M.; Krapcho, A. P.; Giuliani, F. C. 9-Aza-anthrapyrazoles: New Agents Endowed with "in vivo" Antitumor Activity. *Proc. Am. Assoc. Cancer Res.* **1996**, *37*, Abstr 2663.
(51) Cavalletti, E.; Crippa, L.; Bellini, O.; Menta, E.; Cavagnoli, R.; Delbo, D. 9-Aza-anthrapyrazoles Devoid of Cardiotoxic Effects. *Proc. Am. Assoc. Cancer Res.* **1996**, *37*, Abstr 2662.
(52) Menta, E.; Krapcho, A. P.; Maresch, M. J.; Gallagher, C. E.; Hammach, A.; Di Domenico, R.; Oliva, A.; Da Re, G.; Spinelli, S.; Beggiolin, G.; Giuliani, F. C.; Hacker, M. P. Hydroxy-Substituted Aza-analogues of Antitumor Anthrapyrazoles. 211th ACS National Meeting, March 24−29, 1996, New Orleans, LA; Abstr MEDI 077.
(53) Krapcho, A. P.; Gallagher, C. E.; Hammach, A.; Ellis, M.; Menta, E.; Oliva, A. Synthesis of Regioisomeric 6,9-(chlorofluoro)-substituted Benzo[*g*]quinoline-5,10-diones, Benzo[*g*]isoquinoline-5,10-diones and 6-Chloro-9-fluorbenzo[*g*]quinoxaline. *J. Heterocycl. Chem.* **1997**, *27*, 27−32.
(54) Beylin, V. G.; Colbry, N. L.; Goel, O. P.; Haky, J. E.; Johnson, D. R.; Johnson, J. L.; Kanter, G. D.; Leeds, R. L.; Leja, B.; Lewis, E. P.; Rithner, C. D.; Showalter, H. D. H.; Sercel, A. D.; Turner, W. R.; Uhlendorf, S. E. Anticancer Anthrapyrazoles. Improved Synthesis of Clinical Agents CI-937, CI-941, and Piroxantrone Hydrochloride. *J. Heterocycl. Chem.* **1989**, *26*, 85−96.
(55) Shank, R. S.; Shechter, H. Simplified Zinc−Copper Couple for use in Preparing Cyclopropanes from Methylene Iodide and Olefins. *J. Org. Chem.* **1959**, *24*, 1825−1826.
(56) Elslager, E. F.; Weinstein, E. A.; Worth, D. F. Synthesis and Biological Properties of Aminoalkylhydrazines. A Unique Nitrogen−nitrogen Scission of 1-(2-Diethylaminoethyl)-2-(1-phenyl-2-propyl)hydrazine. *J. Med. Chem.* **1964**, *7*, 493−500.

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