Benzothiopyranoindazoles, a New Class of Chromophore Modified Anthracenedione Anticancer Agents. Synthesis and Activity against Murine Leukemias

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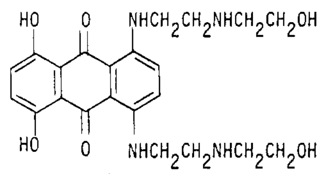
The synthesis of the benzothiopyranoindazoles (**3**), a new class of chromophore modified anthracenediones related to mitoxantrone (**1**), is described. In this structural class the quinone moiety, which is believed to be responsible for the cardiotoxicity of the anthracyclines, has been designed out. The synthesis of the benzothiopyranoindazoles was carried out by a multistep sequence from requisite 1-chloro-4-nitro-9*H*-thioxanthen-9-one precursors (**5**). Reaction with a monoalkylhydrazine gave a 5-nitrobenzothiopyranoindazole adduct (**6**), which was catalytically reduced to a corresponding C-5 anilino intermediate (**7**). Alkylation of **7** with a requisite $X(CH_2)_nNR_1R_2$ (X = Cl, Br; R_1, R_2 = H, alkyl, acyl; n = 2,3) provided target "two-armed" benzothiopyranoindazoles (**3**) or A-ring methoxy and/or side chain acyl intermediates, which could be converted to **3** by appropriate deprotection methodologies. Alternatively, certain target compounds **3** were synthesized by reaction of **7** with appropriately functionalized glycine precursors under Schotten–Bauman or BOP chloride condensation conditions to provide C-5 acylamino intermediates (**11**), followed by Red-Al reduction and deprotection steps. Described also is the synthesis of selected benzothiopyranoindazole congeners with proximal acylamino side chains at C-5 (**12**) and B-ring sulfone functionality at S-6 (**4**). Potent activity was demonstrated against murine L1210 leukemia in vitro ($IC_{50} = 10^{-7}$–10^{-9} M) as well as against P388 leukemia in vivo over a wide range of structural variants. In general, activity against the P388 line was maximized by (a) a basic side chain at N-2 and a dibasic side chain at C-5 with primary or secondary distal amine substitution, (b) certain patterns of A-ring hydroxylation with 8-OH and 9-OH most favorable, and (c) sulfide oxidation state at S-6. Besides having curative activity against the P388 line, the more active compounds were curative against murine B-16 melanoma in vivo. On the basis of their exceptional broad-spectrum in vivo anticancer activity, selected compounds in this series have been chosen for development toward clinical trials.

DNA-complexing agents have been established as one of the most effective classes of anticancer agents in clinical use today with broad application against a number of malignant diseases. The anthracyclines, primarily doxorubicin, are respesentative of this class and are widely utilized clinically.[1,2] Cumulative cardiotoxicity, however, has limited their prolonged use.

We have recently reported on the design rationale,[3] synthesis,[4] tumor biology,[5,6] and biochemical pharmacology[7] of the anthrapyrazoles (**2**), a novel class of chromophore-modified anthracenediones related to mitoxantrone (**1**).[8] Relative to doxorubicin, the modification of the central quinone to a quasi-iminoquinone results in a considerably reduced superoxide dismutase sensitive oxygen consumption in a rat liver microsomal system and a much greater resistance to electrochemical reduction.[3] This apparent suppression of redox cycling and radical generation may be indicative of a reduced liability for cardiotoxicity. Because of their unique biochemistry and exceptional in vivo anticancer activity, three members of the anthrapyrazoles have been entered into clinical trials.[6,9]

In this paper, we report the synthesis and biological evaluation against murine L1210 leukemia in vitro and P388 leukemia and B-16 melanoma in vivo for a large series of 2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazoles (**3**, hereafter referred to as benzothiopyranoindazoles) possessing deshydroxyl or variable hydroxylation patterns in the A ring and varied basic substituents on the N-2 and C-5 positions[10] and a small series of corresponding sulfones (**4**). Relative to the anthrapyrazoles, the substitution of sulfur for carbonyl at C-6 in this new structural class virtually eliminates the possibility of redox cycling and subsequent radical formation in vivo.[11] Our interest in this class was spurred by the earlier studies of Elslager et al. of the synthesis of benzothiopyranoindazole congeners[12] of the antischistosomal agent hycanthone[13] and by the more re-



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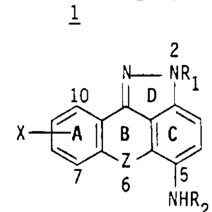

2: Z=C=O; anthrapyrazoles
3: Z=S; benzothiopyranoindazoles
4: Z=SO_2; benzothiopyranoindazole dioxides

X=H, $(OH)_n$ where n=1,2

R_1,R_2=H, alkyl, substituted aminoalkyl

cent reports of Canadian workers of the synthesis of anthracyclinone congeners in which one of the quinone

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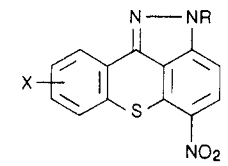
Table I. 2-Substituted 5-Nitro-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazoles



compd	X	R	mp, °C	method	yield,[a] %	recrystn solvent	molecular formula[b]
32	H	$CH_2CH_2NH_2$	>300[c]	A	46	MeOH–ether	$C_{15}H_{12}N_4O_2S \cdot HCl \cdot 0.4H_2O$
33	H	CH_2CH_2NHAc[d]	259–263	*e*	45	DMF	$C_{17}H_{14}N_4O_3S$
34	H	$CH_2CH_2NHCH_2CH_2OH$	293–295[f,g]	C	91	DMF	$C_{17}H_{16}N_4O_3S \cdot HCl$
				A	88		
35	H	$CH_2CH_2N(Ac)CH_2CH_2OAc$[d]	165–168	*e*	93		$C_{21}H_{20}N_4O_5S \cdot 0.2HOAc \cdot 0.3H_2O$[h]
36	H	$CH_2CH_2NMe_2$	266–270[f,i]	B	78	MeOH	$C_{17}H_{16}N_4O_2S \cdot MeSO_3H$
				A	100		
37	H	$(CH_2)_3NMe_2$	309[f,j]	B	73	EtOH[k]	$C_{18}H_{18}N_4O_2S \cdot HCl \cdot 0.3H_2O$
38	H	$CH_2CH_2NEt_2$	154–157	B	84	$CHCl_3$	$C_{19}H_{20}N_4O_2S$
39	7-OMe	$CH_2CH_2NEt_2$	275–280[f,l]	A	87	2-PrOH[k]	$C_{20}H_{22}N_4O_3S \cdot HCl$
40	8-OMe	$CH_2CH_2NEt_2$	269–270[f]	A	91	2-PrOH[k]	$C_{20}H_{22}N_4O_3S \cdot HCl$
41	9-OH	$CH_2CH_2NHCH_2CH_2OH$	265–267	A	59	MeOH[k]	$C_{17}H_{16}N_4O_4S \cdot 0.9MeSO_3H \cdot 0.4H_2O$
42	9-OMe	$CH_2CH_2NHCH_2CH_2OH$	195–197	A[m]	75	DMF	$C_{18}H_{18}N_4O_4S \cdot 0.1H_2O$
43	9-OH	$CH_2CH_2NEt_2$	152–155[n]	A	65	2-PrOH[k]	$C_{19}H_{20}N_4O_3S \cdot 1.3MeSO_3H \cdot 0.2$ 2-PrOH[o]
44	9-OMe	$CH_2CH_2NEt_2$	281–283[p,q]	A	93		$C_{20}H_{22}N_4O_3S \cdot HCl$
45	10-OH	$CH_2CH_2NEt_2$	138–140	B	79	CH_2Cl_2[k]	$C_{19}H_{20}N_4O_3S$
				A	47		
46	10-OMe	$CH_2CH_2NEt_2$	140–147	A	56	DMF	$C_{20}H_{22}N_4O_3S \cdot 0.2H_2O$
47	10-OH	$CH_2CH_2NHCH_2CH_2OH$	207–210	A	75	2-PrOH[k]	$C_{17}H_{16}N_4O_4S \cdot 0.3H_2O$
48	7,10-$(OMe)_2$	$CH_2CH_2NEt_2$	188–190	C	81	DMF	$C_{21}H_{24}N_4O_4S \cdot 0.1DMF$[r]
				A	75		

[a] Yields were not optimized. [b] Unless otherwise stated, the analyses are within ±0.4% of the theoretical values. [c] Free base, mp 199–203 °C. [d] Ac = acetyl. [e] Synthesized via acetylation (NaOAc, Ac_2O) of precursor amine or aminoalcohol. [f] With decomposition. [g] Free base, mp 183–186 °C. [h] ^1H NMR indicates the presence of acetic acid. [i] Free base, mp 184–189 °C. [j] Free base, mp 132–135 °C. [k] With trituration. [l] Free base, mp 144–145 °C. [m] Heating at 80 °C required to complete reaction. [n] Hydrochloride, mp 284 °C. [o] ^1H NMR indicates the presence of 2-propanol. [p] Methanesulfonate salt, mp 240–244 °C. [q] Free base, mp 154–157 °C. [r] ^1H NMR indicates the presence of DMF.

carbonyl moieties has been replaced by thioether[14] or sulfone functionality,[15] respectively.

Chemistry. The benzothiopyranoindazoles bearing basic side chains at the N-2 and C-5 positions were synthesized via two reaction manifolds shown in Scheme I. Both emanate from the C-5 anilino intermediate **7** to which the lower side chain was attached by C-5 aminoalkylation (Scheme Ia) or by C-5 aminoacylation/reduction (Scheme Ib) methodologies. The anilino intermediate **7** was derived from a two-stage sequence in which a requisite 1-chloro-4-nitro-9*H*-thioxanthen-9-one (**5**) was first condensed with a monoalkylhydrazine to give the 2-substituted 5-nitrobenzothiopyranoindazole intermediate **6** (methods A–C) followed by catalytic reduction to give **7** (method D). Methods A–C differ primarily by the solvent and reaction temperatures utilized, and for cases where different methods were applied to the same compound, the yields were generally comparable. Intermediate 2-substituted 5-nitro-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazoles (**6**) obtained by these methodologies are listed in Table I as compounds **32**–**48**. The anilino intermediates **7** were derived from **6** in good to excellent yields and are listed in Table II as compounds **49**–**65**. Because of their oxidative instability, they were generally stored as hydrochloride salts. Alkylation of **7** was carried out by condensation with a requisite $Br(CH_2)_nNR_1R_2$ or $Br(CH_2)_nNH_2$, $n = 2,3$, in a suitable solvent (methods F, G) to lead directly to target benzothiopyranoindazoles **3**. For cases in which reaction with $Br(CH_2)_nNH_2$ resulted in difficulties in product purification due to incomplete reaction of the anilino substrate, we synthesized **3** via neat reaction of $X(CH_2)_nNR$(acyl), X = Cl, Br, with **7** or **9** to give acylated intermediates **8** and **10**, respectively (method E), that were then hydrolyzed to **3** (methods I, J). For anilino intermediates **7** with A-ring monomethoxylation, alkylation was carried out either prior to or after demethylation (method H) with comparable two-step yields via either manifold.

All target compounds **3** obtained via alkylation utilized commercially available reagents to install the C-5 side

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chain. The $NH(CH_2)_2NH(CH_2)_2OH$ side chain was introduced by chemistry previously described for 3-(2-chloroethyl)-2-oxazolidinone (13),[16] and $NH(CH_2)_nNH_2$ (n = 2, 3) from **14a–d**.

The introduction of the lower side chain via acylation methodology (Scheme Ib) was performed on anilino intermediate **7** via Schotten–Bauman reaction with acid chloride **15a**,[17] or by BOP chloride condensation[18] with commercially available *t*-BOC-glycine (**15b**) (method K). Reduction of the derived **11** to give intermediate **8** was carried out initially with AlH_3. However, yields were variable because of incomplete reduction. After evaluating numerous hydride reducing agents, we determined that Red-Al, (Aldrich) was optimal for product yield, ease of scale-up, and reaction reproducibility (method M). For intermediate **8** (X = OMe and NPQ = NH-*t*-BOC), simultaneous cleavage of the protecting groups was carried out with BBr_3 (method H) to give target benzothiopyranoindazoles **3** (X = OH). For the synthesis of **3** (X = OMe), selective *t*-BOC hydrolysis was performed with concentrated HCl in ethanol (method L). Alternatively, target benzothiopyranoindazoles (**12**) with a C-5 proximal acylamino side chain could be obtained from **11** via three methods of hydrolysis (methods H, J, L) mentioned above, the choice of which depended on the targeted A-ring functionality and the nature of the distal amine protecting group.

All A-ring deshydroxy, methoxyl, and hydroxylated benzothiopyranoindazoles, with either C-5 proximal aminoalkyl or aminoacyl appendages, are listed in Table II as compounds **66–97**.

The synthesis of target benzothiopyranoindazole sulfones is outlined in Scheme II. This sequence parallels chemistry that we developed previously for the anthrapyrazoles.[4] Briefly, reaction of 1,4-dichloro-9*H*-thioxanthen-9-one 10,10-dioxide (**16a**)[19] with a monoalkylhydrazine (method A) gave the 2-substituted 5-chlorobenzothiopyranoindazole sulfone **17a**, which was then condensed with a primary substituted alkylamine (method N) to give the "two-armed" target **4**. We attempted to utilize the same chemistry for the synthesis of a selected number of target sulfoxides, but there was insufficient activation of the C-5 position in **17b** for chloride displacement, even at elevated temperatures.[20] We also investigated briefly the reduction of target sulfones to their corresponding sulfides. Such a conversion would offer a more direct route to the benzothiopyranoindazoles and circumvent side chain protection/deprotection steps associated with Scheme I. Of the hydride reagents evaluated, diisobutylaluminum hydride in refluxing toluene effected reduction but with only ~50% conversion. All benzothiopyranoindazole sulfoxides and sulfones synthesized in this study are listed in Table II as compounds **98–103**.

The synthesis of all 9*H*-thioxanthen-9-one precursors utilized in this study is delineated in Scheme IIIa–c. 1-Chloro-4-nitro-9*H*-thioxanthen-9-ones (**5**) were derived from two major pathways. The first (Scheme IIIa) utilized the base-catalyzed condensation of thiosalicyclic acids **19a–c** with commercially available 2,4-dichloronitrobenzoic acid (**20**) to give the substituted 2-(phenylthio)benzoic acids **21a–c** (method O) followed by Friedel–Crafts ring closure of the preformed acid chloride with $AlCl_3$ (TFA/TFAA for **21c**) to give tricyclic 9*H*-thioxanthen-9-ones **5**. Thiosalicylic acid (**19a**) is commercially available while **19b,c** were synthesized from substituted anthranilic acid precursors **18b**[21] and **18c**,[22] respectively. An alternative route to 2-(phenylthio)benzoic acids **21**, as was applied to the synthesis of **21c**, was to employ conditions reported in the early chemical literature.[23] In situ conversion of **18c** to diazonium salt **22** followed by condensation with 5-chloro-2-nitrothiophenol (**23**)[24] gave **21c** (method P) in comparable overall yield to **21c** obtained by method O.

The second major route to **5** (Scheme IIIb) utilized commercially available methoxybenzenethiols (**24a–c**) for condensation (Method Q) with 2,6-dichloro-3-nitrobenzoic acid (**25**), which is easily derived from the nitration of commercially available 2,6–dichlorobenzoic acid.[25] Friedel–Crafts ring closure of the resultant substituted 2-(phenylthio)benzoic acids **26a–c** with TFA/TFAA gave **5**. In comparing these two major routes for the synthesis of methoxy-substituted 1-chloro-4-nitro-9*H*-thioxanthen-9-ones **5**, we prefer the latter because of its convergency, higher overall yields, and ease of scale-up.

We examined also the demethylation of methoxy-9*H*-thioxanthen-9-ones **5d,e** to their corresponding phenols **5g,h**. While reaction of **5e** with BBr_3 (method H) proceeded uneventfully to give **5h**, similar reaction with **5d** failed to afford **5g**. After evaluating unsuccessfully a number of classically utilized demethylation reagents for this transformation, we discovered that reaction of **5d** with $AlCl_3$ in refluxing 1,2-dichloroethane gave an almost quantitative yield of **5g**.

The synthesis of 1,4-dichloro-9*H*-thioxanthen-9-one 10-oxides **16a,b**, precursors to benzothiopyranoindazole sulfones (**4**) and sulfoxides, respectively, is delineated in Scheme IIIc. We initially carried out the synthesis of substituted (phenylthio)benzoic acid **29** by a literature procedure via the copper-catalyzed coupling of 2-iodobenzoic acid (**27**) and 2,5-dichlorobenzenethiol (**28**).[26] A more economical route to **29** was the coupling of thiosalicyclic acid (**19a**) with 1,4-dichloro-2-iodobenzene (**30**) to give the product in good yield and purity (method S). Friedel–Crafts ring closure of **29** afforded 1,4-dichloro-9*H*-thioxanthen-9-one (**31**) of which oxidation by the literature procedure[19] gave the sulfone **16a**. Selective oxidation of **31** to give sulfoxide **16b** was performed with 20% aqueous $TiCl_3$/30% H_2O_2 (method S).

All substituted 2-(phenylthio)benzoic acids and 9*H*-thioxanthen-9-ones synthesized by the routes shown in Scheme IIIa–c and utilized in this study are listed in Tables III and IV, respectively.

The color of the target benzothiopyranoindazoles as salts

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(20) Reaction of compound **98** with neat *N,N*-diethylethylenediamine, either at 160 °C and catalyzed by KF or at 100 °C and catalyzed by Cu/CuBr resulted in sulfoxide reduction.
(21) (a) Bennett, O. F.; Johnson, J.; Tramondozzi, J. *Org. Prep. Proced. Int.* 1974, *6*, 287–293. (b) 5-Methoxyanthranilic acid is synthesized also via Cu/Cu(OAc)$_2$-catalyzed amination of commercially available 2-bromo-5-methoxybenzoic acid. Unpublished work of D. E. Butler.
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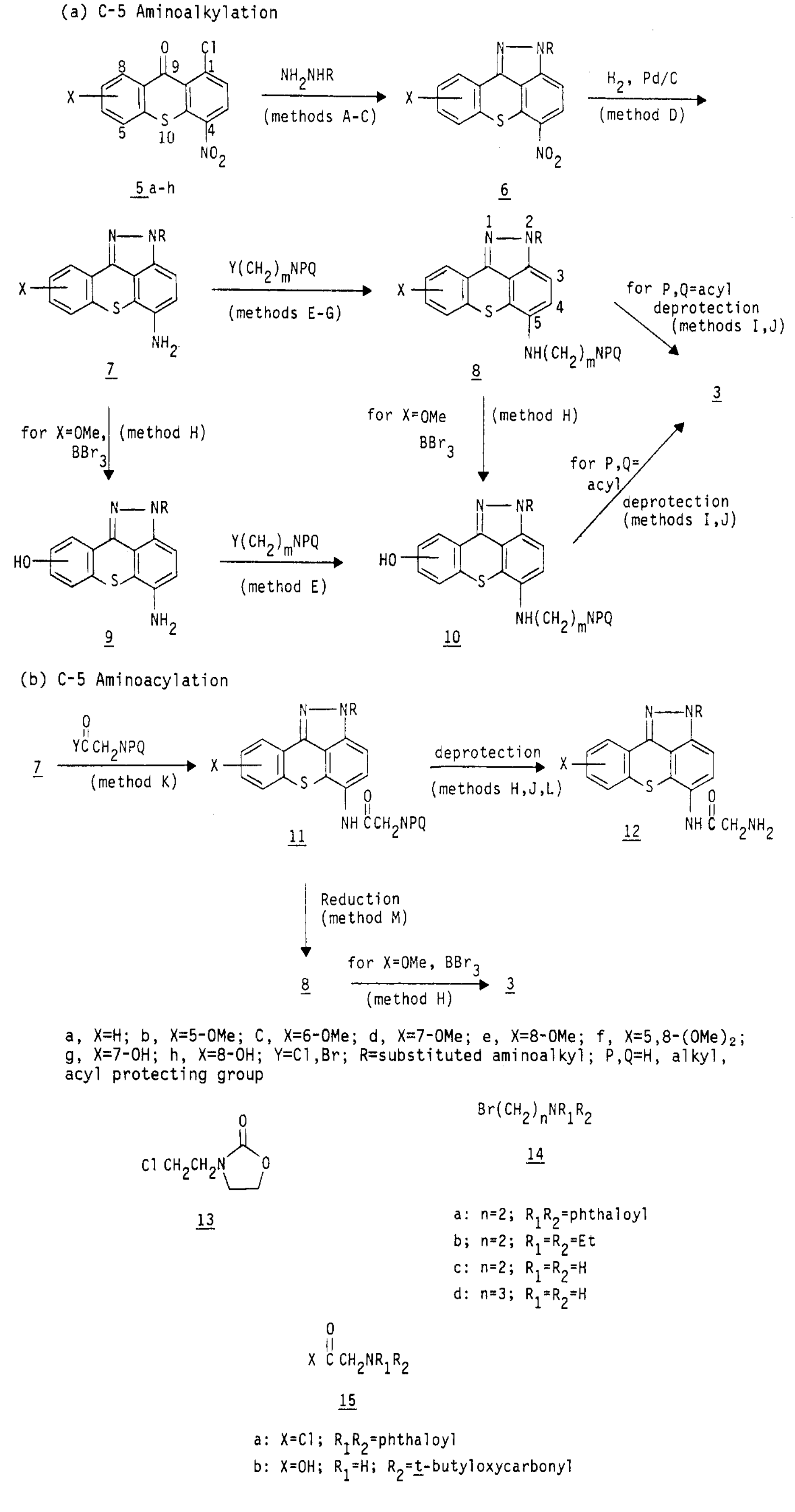
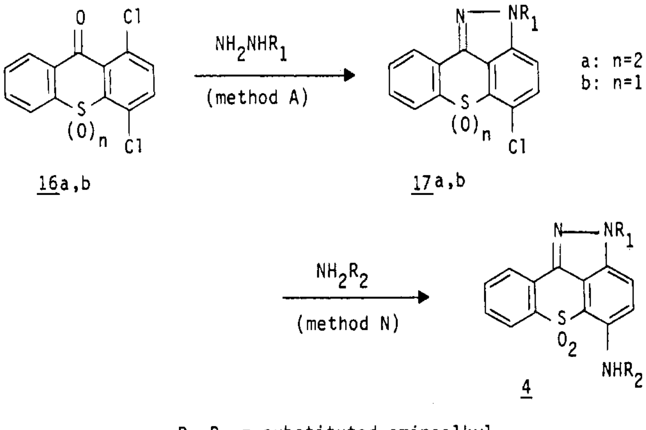
Scheme I. Synthetic Routes to Benzothiopyranoindazoles



in aqueous solution is yellow. Aqueous solutions are unstable to heat, light, alkaline pH, and oxidizing agents.

Biological Activity. Because of the extensive structure–activity relationship (SAR) developed previously for the anthrapyrazoles,[4a] we concentrated primarily on the synthesis of benzothiopyranoindazoles with N-2 and C-5 chains that possessed distal basic amine moieties (Table II).[27] While many of the C-5 anilino precursors showed

Scheme II. Synthetic Route to Benzothiopyranoindazole Sulfones



good activity, most of the corresponding intermediate 2-substituted 5-nitrobenzothiopyranoindazoles listed in Table I revealed minimal in vitro or in vivo activity.

Most of the compounds listed in Table II were tested in vitro against murine L1210 leukemia as described by Baguley.[28] Among compounds with the NH_2 or dibasic $NH(CH_2)_nNR_1R_2$ substituents at C-5, potent activity (IC_{50} = 10^{-7}–10^{-9} M) was associated with specific hydroxylation patterns in the A ring, especially at C-9 (e.g., **59**, **61**, **87**, **89**). The effects of corresponding methoxylation seemed to be deleterious (compare **57** vs **58**, **61** vs **62**, **81** vs **83**, **87** vs **88**). In contrast, 7-OH (**79**) and 7,10-$(OH)_2$ (**96**) patterns abolished activity. Compounds with C-5 proximal or distal amide functionality (compare **81** vs **85**, **89** vs **90**, respectively) showed little or no reduction in activity. Oxidation of the B-ring sulfide to the sulfone greatly diminished activity (compare **68** vs **101**).

Among the 40 analogues listed in Table II prepared and tested in vivo against P388 leukemia in mice (IP/IP; D1-5),[29] 32 of the compounds demonstrated a $T/C > 125$ and seven compounds a $T/C \geq 200$ with one or more cures at optimal doses that ranged from 1.5–12.5 mg/kg per injection.

In evaluating the structure–activity relationships of the benzothiopyranoindazoles against P388 leukemia, several trends are evident. First, benzothiopyranoindazoles at a higher oxidation state generally demonstrated considerably lowered potency and efficacy at the maximum tolerated dose relative of their less oxidized congeners. This was evident for most of the 5-nitrobenzothiopyranoindazoles listed in Table I (in vivo data not given) compared to the C-5 NH_2 analogues in Table II. An exception to this was found in comparing nitro compound **43** [$T/C = 234$ (2/5 cures) at 25 mg/kg per injection] with anilino **61**, which displayed lowered efficacy but greater potency, and nitro **34** vs anilino **51**, which demonstrated equivalent efficacy and potency. The general trend of reduced potency and efficacy is clearly evident in comparing analogues in which the B-ring sulfide has been modified to sulfone (**68** vs **101**, **73** vs **102**). This diminution of activity can be ascribed to both electronic and steric effects, which for both the nitro and sulfone compounds results in a lowered electron density in the chromophore and the presence of functionality that would distort the intercalating chromophore out of plane.

Certain patterns of A-ring hydroxylation were associated with increased potency but not increased efficacy, a trend observed for the anthracenediones.[8] In all instances where there were identical substituents at the N-2 and C-5 positions, the 8-OH (**73** vs **81**) and 9-OH (**73** vs **87**, **76** vs **89**) derivatives were more potent. The effects of 10-OH substitution (**73** vs **93**) were minimal while corresponding 7-OH (**73** vs **79**) or 7,10-$(OH)_2$ (**76** vs **96**) substitutions were clearly deleterious. For all cases evaluated, methoxylated compounds possessed lowered potency and efficacy relative to their hydroxylated analogues (**81** vs **83**, **87** vs **88**, **89** vs **91**).

The nature of the substitution pattern at C-5 has a pronounced effect on activity. Chain extension of dibasic NH_2 precursors to tribasic two-armed compounds generally resulted in an increase in both potency and efficacy for deshydroxy (**51** vs **68**, **55** vs **73**, and others) and to a lesser degree for hydroxylated compounds (**61** vs **87**, **89**). Variation in the nature of the C-5 side chain was not extensively explored, but for compounds with the same N-2 upper side chain, there was a marked reduction in activity with a progression from compounds with a primary or secondary to a tertiary distal amine substituent (compare **68** vs **70**, **73** vs **78**, **87** vs **89**), an effect observed earlier for the anthrapyrazoles.[4a] A reduction in activity was also observed for compounds in which the C-5 proximal (**73** vs **74**, **81** vs **85**) or distal (**89** vs **90**) amine had been modified to acyl functionality. A single example of chain extension from two to three methylene spacers (**73** vs **75**) suggested that both efficacy and potency would decrease with an increase in the length of the side chain.

The effects of variation on the N-2 upper side chain are not nearly as pronounced, especially the degree of substitution of the distal amine functionality (**66** vs **68** vs **76**, **71** vs **73**, **70** vs **78**), and side chain length (**71** vs **72**).

In summary, on the basis of these studies, antitumor activity in vivo against P388 leukemia is generally maximized by (a) a basic side chain at N-2 and a dibasic side chain at C-5 with primary or secondary distal amine substitution, (b) certain patterns of A-ring hydroxylation (for constant side chains at N-2 and C-5, activity decreases in the order 8-OH $\cong$ 9-OH > 10-OH $\cong$ H $\gg$ 7-OH $\cong$ 7,10-$(OH)_2$), and (c) sulfide oxidation state at S-6.

Besides having curative activity against P388 leukemia, several of the compounds in Tables I and II were curative against murine B-16 melanoma in vivo. The data for these compounds are given in Table V. All but two compounds showed significant activity [$T/C > 135\%$ (IP/IP; D1-9)]. The most active compounds demonstrated a $T/C > 260\%$ and one or more cures at 1.5–12 mg/kg per injection. Additionally, many of the compounds in Table II showed curative activity against one or more tumors of the National Cancer Institute tumor panel, including L1210 leukemia, amelanotic melanoma, colon 38, M5076 sarcoma, and the MX-1 mammary xenograft in nude mice, and outstanding broad-spectrum activity in the Parke-Davis tumor panel.[5a,30]

On the basis of their exceptional in vivo anticancer activity, possible lack of cross-resistance with the anthracyclines,[31] and potential for lowered cardiotoxicity relative

(27) We synthesized a number of compounds with no basic side chains or one basic side chain at N-2 or C-5. For the compounds with no basic side chains, none were active in vivo against P388 leukemia. For those with one basic side chain, none demonstrated a $T/C \geq 200$.

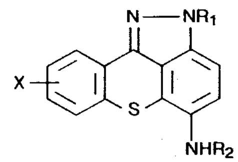
(28) Baguley, B. C.; Nash, R. *Eur. J. Cancer* 1981, *17*, 671–679.

(29) (a) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* 1972, *3*, 1–85. (b) NIH Publication No. 84-2635, 1984.

(30) Leopold, W. R., manuscript in preparation.

(31) Berman, E.; Klohs, W.; Leopold, W. R.; Plowman, J.; Sercel, A. D.; Shillis, J. L.; Showalter, H. D. H.; Werbel, L. M. *Proc. Am. Assoc. Cancer Res.* 1987, *28*, 302.

Table II. Activity of 2-Substituted 5-Amino-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazoles against Murine L1210 Leukemia in Vitro and P388 Leukemia in Vivo



compd	X	R_1	R_2	mp, °C	method	yield,[a] %	recrystn solvent	molecular formula[b]	L1210 leukemia[c] in vitro: IC_{50}, M	P388 leukemia in vivo[d]: opt dose (mg/kg/ per inj)	P388 leukemia in vivo[d]: net $\log_{10}$ tumor cell kill	P388 leukemia in vivo[d]: % T/C (day 30 surv)
doxorubicin									6.9×10^{-8}	2.0		232
1 (mitoxantrone)									1.6×10^{-9}	1.25	>6.8	291 (4/5)
49	H	$CH_2CH_2NH_2$	H	>300	D	76	EtOH[e]	$C_{15}H_{14}N_4S\cdot 2HCl$	1.0×10^{-7}	12.5	3.1	181
50	H	CH_2CH_2NHAc[f]	H	387[g]	D	76	EtOH[e]	$C_{17}H_{16}N_4OS\cdot HCl$	inactive	100	−1.7	100
51	H	$CH_2CH_2CHCH_2CH_2OH$	H	285[g]	D	74	MeOH[e]	$C_{17}H_{18}N_4OS\cdot 1.6HCl\cdot 0.2H_2O$	1.6×10^{-1}	25	4.3	188 (1/5)
52	H	$CH_2CH_2N(Ac)CH_2CH_2OAc$[f]	H	247–249[g]	D	79	EtOH[e]	$C_{21}H_{22}N_4O_3S\cdot HCl$	inactive	400	−1.3	117
53	H	$CH_2CH_2NMe_2$	H	273–282[g]	D	80	MeCN[e]	$C_{17}H_{18}N_4S\cdot 2HCl\cdot 1.5H_2O$	6.3×10^{-8}	12.5	1.8	165
54	H	$(CH_2)_3NMe_2$	H	264–274	D	56	EtOH	$C_{18}H_{20}N_4S\cdot 2HCl\cdot 1.1H_2O$	2.0×10^{-6}	100	4.0	197
55	H	$CH_2CH_2NEt_2$	H	100–104	D	75	MeCN	$C_{19}H_{22}N_4S$	1.0×10^{-7}	25	2.2	162
56	7-OMe	$CH_2CH_2NEt_2$	H	250[g]	D	87	2-PrOH[e]	$C_{20}H_{24}N_4OS\cdot 2.2HCl$	inactive	25	−1.5	109
57	8-OH	$CH_2CH_2NEt_2$	H	>300	H[h]	96	MeOH[e]	$C_{19}H_{22}N_4OS\cdot 2HBr\cdot 0.3H_2O$	1.9×10^{-7}	nt[ae]		
58	8-OMe	$CH_2CH_2NEt_2$	H	260–265[g]	D	76	2-PrOH[e]	$C_{20}H_{24}N_4OS\cdot 2.3HCl\cdot 0.9H_2O$	inactive	nt[ae]		
59	9-OH	$CH_2CH_2NHCH_2CH_2OH$	H	>310	H[h]	72	CH_2Cl_2/ MeOH[e]	$C_{17}H_{18}N_4O_2S\cdot 2.3HBr\cdot 0.4H_2O$	2.3×10^{-8}	3.1	4.2	192
60	9-OMe	$CH_2CH_2NHCH_2CH_2OH$	H	266–274[g]	D	71	2-PrOH[e]	$C_{18}H_{20}N_4O_2S\cdot 2HCl\cdot 0.7H_2O$	nt[ae]	nt[ae]		
61	9-OH	$CH_2CH_2NEt_2$	H	280[g,i]	H	78	EtOH	$C_{19}H_{22}N_4OS\cdot 2HCl\cdot 1.4H_2O$	1.1×10^{-8}	6.3	>6.7	238
62	9-OMe	$CH_2CH_2NEt_2$	H	275[g,j]	D	92	EtOH[e]	$C_{20}H_{24}N_4OS\cdot 2HCl$	1.5×10^{-6}	50	1.4	154
63	10-OH	$CH_2CH_2NEt_2$	H	240[g]	D	77	MeOH[e]	$C_{19}H_{22}N_4OS\cdot 1.9HCl\cdot H_2O$	6.1×10^{-8}	nt[ae]		
64	10-OMe	$CH_2CH_2NEt_2$	H	233–241[g]	D	70	2-PrOH[e]	$C_{20}H_{24}N_4OS\cdot 2.6HCl\cdot 0.9H_2O\cdot 0.1$ 2-PrOH[k]	2.0×10^{-6}	nt[ae]		
65	7,10-$(OMe)_2$	$CH_2CH_2NEt_2$	H	134–139	D	85	toluene/ cyclohexane	$C_{21}H_{26}N_4O_2S\cdot 0.2H_2O$	nt[ae]	nt[ae]		
66	H	$CH_2CH_2NH_2$	$CH_2CH_2NHCH_2CH_2OH$	264[g]	I[l]	78	MeOH/ 2 N HCl (1:1)	$C_{19}H_{23}N_5OS\cdot 3HCl$	2.2×10^{-8}	12.5	>6.7	233
67	H	CH_2CH_2NHAc[f]	CH_2CH_2Ox[m]	143–147	E	30	acetone/ Et_2O (1:6)	$C_{22}H_{23}N_5O_3S$	inactive	12.5	−1.6	100
68	H	$CH_2CH_2NHCH_2CH_2OH$	$CH_2CH_2NHCH_2CH_2OH$	222–225	E,[n] I	10	MeOH	$C_{21}H_{27}N_5O_2S\cdot 2.9HCl\cdot 0.5H_2O$	3.2×10^{-7}	12.0	>6.6	270 (4/6)

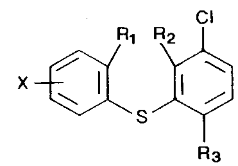
69	H	$CH_2CH_2NHCH_2CH_2OH$	CH_2CH_2Ox[m]	180–182	*o*	1	2-PrOH[e]	$C_{22}H_{25}N_5O_3S$·HCl·0.5H_2O	5.4 × 10^{-7}	nt[ae]		
70	H	$CH_2CH_2NHCH_2CH_2OH$	$CH_2CH_2NEt_2$	208–212	E[p]	17		$C_{23}H_{31}N_5OS$·2.9HCl·1.9H_2O	2.4 × 10^{-7}	25.0	0.5	147
71	H	$CH_2CH_2NMe_2$	$CH_2CH_2NH_2$	246[g]	G	34	2-PrOH[e]	$C_{19}H_{23}N_5S$·2.9HCl·0.9H_2O	1.5 × 10^{-8}	12.5	>6.7	270 (3/6)
72	H	$(CH_2)_3NMe_2$	$CH_2CH_2NH_2$	276[g]	G	17	MeOH	$C_{20}H_{25}N_5S$·3HCl·0.1H_2O	2.7 × 10^{-8}	12.5	>6.7	298 (3/6)
73	H	$CH_2CH_2NEt_2$	$CH_2CH_2NH_2$	140[g,q]	G	43	EtOH[e]	$C_{21}H_{27}N_5S$·2HBr·0.2EtOH·0.3H_2O[k]	3.0 × 10^{-8}	12.5	>6.6	297 (4/6)
74	H	$CH_2CH_2NEt_2$	$COCH_2NH_2$	195[g]	J[r]	58	2-PrOH[e]	$C_{21}H_{25}N_5OS$·2HCl·H_2O	1.5 × 10^{-8}	20	2.4	184
75	H	$CH_2CH_2NEt_2$	$(CH_2)_3NH_2$	222[g]	G	26	EtOH	$C_{22}H_{29}N_5S$·3HCl·2.3H_2O	2.6 × 10^{-7}	50	>6.6	262
76	H	$CH_2CH_2NEt_2$	$CH_2CH_2NHCH_2$-CH_2OH	223–229[g]	I	50	EtOH[e]	$C_{23}H_{31}N_5OS$·3HCl·0.3H_2O	2.8 × 10^{-8}	12.5	>6.8	280 (1/5)
77	H	$CH_2CH_2NEt_2$	CH_2CH_2Ox[m]	90–94	E	63	acetone	$C_{24}H_{29}N_5O_2S$·0.2H_2O	8.1 × 10^{-7}	100	6.0	218
78	H	$CH_2CH_2NEt_2$	$CH_2CH_2NEt_2$	234–236	F	52	MeCN/EtOH	$C_{25}H_{35}N_5S$·2HCl	5.0 × 10^{-8}	25	1.6	155
79	7-OH	$CH_2CH_2NEt_2$	$CH_2CH_2NH_2$	263–264[g]	H,[s] J	50	MeOH	$C_{21}H_{27}N_5OS$·3.1HCl·0.7H_2O·0.5MeOH	inactive	6.3	−0.9	122
80	7-OMe	$CH_2CH_2NEt_2$	CH_2CH_2NPht[t]	176–178	E	70	2-PrOH[e]	$C_{30}H_{31}N_5O_3S$·0.6H_2O	inactive	12.5	−1.5	107
81	8-OH	$CH_2CH_2NEt_2$	$CH_2CH_2NH_2$	263–265[g]	J	73	EtOH/H_2O (4:1)	$C_{21}H_{27}N_5OS$·2.8HCl·0.5H_2O	7.1 × 10^{-8}	6.3		280 (3/6)
82	8-OH	$CH_2CH_2NEt_2$	CH_2CH_2NPht[t]	232–234	H[h]	71	MeOH	$C_{29}H_{29}N_5O_3S$·2HBr	nt[ae]	nt[ae]		
83	8-OMe	$CH_2CH_2NEt_2$	$CH_2CH_2NH_2$	235[g]	L	56	EtOH[e]	$C_{22}N_{29}N_5OS$·3HCl·H_2O	inactive	25	−0.4	133
84	8-OMe	$CH_2CH_2NEt_2$	CH_2CH_2NHBOC[u]	133–134	M	66	MeCN	$C_{27}H_{37}N_5O_3S$·1.7H_2O	inactive	6.3	−1.7	93
85	8-OH	$CH_2CH_2NEt_2$	$COCH_2NH_2$	210[g]	H[h]	66	MeOH[e]	$C_{21}H_{25}N_5O_2S$·2.5HCl·1.6H_2O	1.4 × 10^{-7}	100[v]		195
86	8-OMe	$CH_2CH_2NEt_2$	$COCH_2NHBOC$[u]	131–133	K	63	MeCN	$C_{27}H_{35}N_5O_4S$	inactive	nt[ae]		
87	9-OH	$CH_2CH_2NEt_2$	$CH_2CH_2NH_2$	271–274[g]	E,[s,w] J	22	MeOH	$C_{21}H_{27}N_5OS$·2.8HCl·0.4H_2O	2.6 × 10^{-9}	0.8		186
88	9-OMe	$CH_2CH_2NEt_2$	$CH_2CH_2NH_2$	246[g]	G	22	EtOH[e]	$C_{22}H_{29}N_5OS$·3HCl·0.3H_2O	2.1 × 10^{-7}	12.5	3.9	177
89	9-OH	$CH_2CH_2NEt_2$	$CH_2CH_2NHCH_2$-CH_2OH	250–252[g]	I	73	MeOH/H_2O (4:1)	$C_{23}H_{31}N_5O_2S$·3HCl·H_2O	4.8 × 10^{-9}	1.5	4.5	200 (1/6)
90	9-OH	$CH_2CH_2NEt_2$	CH_2CH_2Ox[m]	223–226[x]	H	76	EtOH[e]	$C_{24}H_{29}N_5O_3S$·2HCl·0.2H_2O	4.3 × 10^{-9}	12.5[v]		175
91	9-OMe	$CH_2CH_2NEt_2$	$CH_2CH_2NHCH_2$-CH_2OH	235[g]	E; I	60; 80	MeOH	$C_{24}H_{33}N_5O_2S$·3HCl	2.0 × 10^{-7}	25	3.2	177
92	9-OMe	$CH_2CH_2NEt_2$	CH_2CH_2Ox[m]	212[g,y]	E	73	EtOH	$C_{25}H_{31}N_5O_3S$·2HCl·0.3H_2O	inactive	100	6.0	220
93	10-OH	$CH_2CH_2NEt_2$	$CH_2CH_2NH_2$	274–280[g]	H[h]	45	aq EtOH	$C_{21}H_{27}N_5OS$·2.9HBr·0.5H_2O	4.8 × 10^{-8}	12.5	2.8	177

Table II (Continued)

compd	X	R_1	R_2	mp, °C	method	yield,[a] %	recrystn solvent	molecular formula[b]	L1210 leukemia[c] in vitro: IC_{50}, M	P388 leukemia in vivo[d]: opt dose (mg/kg/per inj)	P388 leukemia in vivo[d]: net $\log_{10}$ tumor cell kill	P388 leukemia in vivo[d]: % T/C (day 30 surv)
94	10-OMe	$CH_2CH_2NEt_2$	CH_2CH_2NHBOC[u]	169–172	M	72	EtOAc/$CHCl_3$ (4:1)	$C_{27}H_{37}N_5O_3S{\cdot}0.3H_2O$[z]	inactive	nt[ae]		
95	10-OMe	$CH_2CH_2NEt_2$	$COCH_2NHBOC$[u]	157–158	K	79	MeCN	$C_{27}H_{35}N_5O_4S$	nt[ae]	nt[ae]		
96	7,10-$(OH)_2$	$CH_2CH_2NEt_2$	$CH_2CH_2NHCH_2$-CH_2OH	262[g]	H	80	MeOH	$C_{23}H_{31}N_5O_3S{\cdot}3HBr{\cdot}0.8H_2O$	inactive	50	0.1	138
97	7,10-$(OMe)_2$	$CH_2CH_2NEt_2$	$CH_2CH_2NHCH_2$-CH_2OH	123–125	E,[s] I	17	MeCN	$C_{25}H_{35}N_5O_3S{\cdot}0.3H_2O$	nt[ae]	nt[ae]		
98[af]	H	$CH_2CH_2NEt_2$	NHR_2 = Cl	110–113	A	16	2-PrOH	$C_{19}H_{20}N_3ClOS$	inactive	nt[ae]		
99[ag]	H	$CH_2CH_2NHCH_2C$-H_2OH	NHR_2 = Cl	285–290[g]	A	30	DMF	$C_{17}H_{16}N_3ClO_3S$	9.2×10^{-7}	nt[ae]		
100[ag]	H	$CH_2CH_2NEt_2$	NHR_2 = Cl	107–109	A[aa]	74	MeCN	$C_{19}H_{20}N_3ClO_3S$[ab]	2.2×10^{-6}	nt[ae]		
101[ag]	H	$CH_2CH_2NHCH_2C$-H_2OH	$CH_2CH_2NHCH_2$-CH_2OH	225–227	N	48	MeOH	$C_{21}H_{27}N_5O_4S{\cdot}2.1HCl{\cdot}1.1H_2O$	inactive	200	2.5	168
102[ag]	H	$CH_2CH_2NEt_2$	$CH_2CH_2NH_2$	241–246[g]	N[ac,ad]	19	2-PrOH[e]	$C_{21}H_{27}N_5O_2S{\cdot}2HCl{\cdot}0.6H_2O$	1.2×10^{-6}	100	1.8	159
103[ag]	H	$CH_2CH_2NEt_2$	$CH_2CH_2NEt_2$	142–144	N[ac]	57	MeCN	$C_{25}H_{35}N_5O_2S$	inactive	12.5	−1.6	101

[a] See footnote *a*, Table I. [b] See footnote *b*, Table I. [c] See ref 28. [d] Optimum response; carried out by the National Cancer Institute testing protocol (Q01D×5, ip schedule).[29] Net kill calculated by method of Schabel et al. detailed in ref 32, except that "cured" mice are included in calculations of %T/C and net kill. %T/C values ≥ 125 are considered indicative of significant activity. [e] With trituration. [f] Ac acetyl. [g] With decomposition. [h] Reaction carried out in refluxing CH_2Cl_2. [i] Free base, mp 229–230 °C. [j] Free base, mp 152–157 °C. [k] ^1H NMR indicates the presence of crystallization solvent. [l] From hydrolysis of peracylated precursor. [m] Ox = 2-oxo-3-oxazolidinyl. [n] Intermediate formed from alkylation of **52** with 3-(2-chloroethyl)-2-oxazolidinone (**13**) not characterized. [o] Derived from incomplete hydrolysis of peracylated precursor to **68**. [p] Intermediate formed from alkylation of **52** with (diethylamino)ethyl bromide hydrobromide (**14b**) not characterized but subjected to hydrolysis with refluxing 2 N HCl. [q] Trihydrobromide, mp 295 °C.[g] [r] Reaction carried out with 54% aqueous hydrazine; precursor (mp 176–178 °C) synthesized in 51% yield from reaction of **55** with *N*-phthaloylglycine acid chloride (**15a**)[17] in pyridine. [s] Intermediate not characterized. [t] Pht = phthaloyl. [u] BOC = *tert*-butyloxycarbonyl. [v] D 3–7 dosing schedule. [w] From alkylation of **61** with **14a**. [x] Free base, mp 192–198 °C. [y] Free base, mp 135–138 °C. [z] N: calcd, 13.54; found, 12.92. [aa] Use of 2 equiv of substrate hydrazine; reaction heated at 80 °C. [ab] C: calcd, 58.53; found, 58.99. [ac] Heated at reflux. [ad] Catalyzed with 6 equiv of ahydrous KF. [ae] Not tested. [af] Example **98**, S = SO. [ag] Examples **99–103**, S = SO_2.

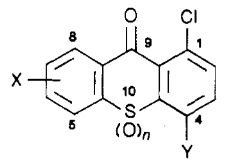
Table III. Substituted 2-(Phenylthio)benzoic Acids



compd	X	R_1	R_2	R_3	mp, °C	method	yield,[a] %	molecular formula[b]
21a	H	CO_2H	H	NO_2	188–191[c]	O	83	$C_{13}H_8ClNO_4S$
21b	5-OMe	CO_2H	H	NO_2	182–186	O	36	$C_{14}H_{10}ClNO_5S$
21c	3,6-$(OMe)_2$	CO_2H	H	NO_2	218–220	O	44[d]	$C_{15}H_{12}ClNO_6S{\cdot}0.8H_2O$[e]
						P	45	
26a	H	OMe	CO_2H	NO_2		Q	95[f,g]	$C_{14}H_{10}ClNO_5S$
26b	3-OMe	H	CO_2H	NO_2		Q	97[g,h]	$C_{14}H_{10}ClNO_5S$
26c	4-OMe	H	CO_2H	NO_2	154–157	Q	68	$C_{14}H_{10}ClNO_5S$
29	H	CO_2H	H	Cl	223–225[i]	S	79	$C_{13}H_8Cl_2O_2S$

[a] See footnote *a*, Table I. [b] See footnote *b*, Table I. [c] Lit.[33] mp 187–190 °C. [d] Crystallized from CH_3CN. [e] S: calcd, 8.35; found, 8.93. [f] After crystallization from toluene/CH_3CN. [g] Mixture of product and bis[(methoxyphenyl)thio] addition product (~5:1). This mixture was used directly for cyclization to corresponding thioxanthenone 5. [h] Mixture is an oil. [i] Lit.[26] mp 225–227 °C.

Table IV. 1-Chloro-4-nitro-9*H*-thioxanthen-9-ones (**5a–h**), 1,4-Dichloro-9*H*-thioxanthen-9-one (**31**), and 1,4-Dichloro-9*H*-thioxanthen-9-one 10-Oxides (**16a,b**)



compd	*n*	X	Y	mp, °C	crystn solvent	yield,[a] %	method	molecular formula[b]
5a	0	H	NO_2	205–207[c]	MeOH[d]	77	O[e]	$C_{13}H_6ClNO_3S$
5b	0	5-OMe	NO_2	248–250	MeCN[d]	88	Q	$C_{14}H_8ClNO_4S\cdot 0.2CH_3CN$[f,g]
5c	0	6-OMe	NO_2	253–256	$CHCl_3$[d]	64	Q[h]	$C_{14}H_8ClNO_4S$
5d	0	7-OMe	NO_2	243–247		95	Q	$C_{14}H_8ClNO_4S$
				235–240	MeOH[d]	65	Q	
5e	0	8-OMe	NO_2	216–221	DMF[i]		Q	$C_{14}H_8ClNO_4S$
5f	0	5,8-$(OMe)_2$	NO_2	234–236	MeCN[d]	61	Q[j]	$C_{15}H_{10}ClNO_5S$
5g	0	7-OH	NO_2	290[k]	2-PrOH[d]	97	R	$C_{13}H_6ClNO_4S\cdot 0.2$ 2-PrOH[f]
5h	0	8-OH	NO_2	212–216	MeOH[d]	62	H[l]	$C_{13}H_6ClNO_4S\cdot 0.4MeOH$[f]
31	0	H	Cl	177–179[m]	DMF	83	S	$C_{13}H_6Cl_2OS$
16a	2	H	Cl	190–192[n]	HOAc	83	S	$C_{13}H_6Cl_2O_3S$
16b	1	H	Cl	171–173	EtOH	61	S[o]	$C_{13}H_6Cl_2O_2S$

[a] See footnote *a*, Table I. [b] See footnote *b*, Table I. [c] Lit.[33] mp 201–203 °C. [d] With trituration. [e] Chlorobenzene used as reaction solvent. [f] ^1H NMR indicates the presence of trituration solvent. [g] Cl: calcd, 10.74; found, 9.90. [h] SiO_2 TLC (CH_2Cl_2) of reaction mixture indicates **5c**/**5e** (7–8:1). [i] Crystallization of ca. 1:1 mixture of **5c**/**5e** removes >90% **5c**. [j] TFA/TFAA (1:1) cyclization of **21c** at 50 °C. [k] With decomposition. [l] Reaction carried out in refluxing CH_2Cl_2. [m] Lit.[26] mp 181–183 °C. [n] Lit.[19] mp 184–185 °C. [o] Oxidation with *m*-chloroperbenzoic acid in CH_2Cl_2 at 25 °C gives a 3:7 mixture of **16a**/**16b**.

Table V. Activity of Substituted 2*H*-[1]Benzothiopyrano[4,3,2-*cd*]indazoles against Murine B-16 Melanoma in Vivo[a]

no.	opt dose (mg/kg per inj)	% T/C (day 60 surv)	no.	opt dose (mg/kg per inj)	% T/C (day 60 surv)
doxorubicin	1.0	434 (5/10)	**68**	12	261 (1/10)
1 (mitoxantrone)	0.6	265 (4/6)	**73**	6.3	159
44	25	126	**76**	12	273 (1/10)
51	25	259	**81**	7.5	281 (3/10)
55	12	112	**89**	1.5	183 (2/10)
61	1.8	264 (1/10)			

[a] Optimum response; carried out according to standard NCI protocol.[29] Cured animals are included in calculations of % T/C. % T/C values > 135 indicate significant activity.

to doxorubicin,[11] selected compounds in this series have been chosen for development toward clinical trials. The results of more advanced preclinical activities with the benzothiopyranoindazoles, including extensive toxicology and molecular pharmacology studies, as well as the synthesis and preclinical evaluation of congeneric oxygen and selenium benzochalcogenoindazoles will be the subject of future publications.

Experimental section

General Procedures. Melting points were taken on a Thomas-Hoover Unimelt capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were determined on a Digilab FTS-14 or Nicolet MX-1 FT-IR spectrometer system. Ultraviolet (UV) spectra were taken on a Cary Model 118C recording spectrophotometer. ^1H nuclear magnetic resonance (^1H NMR) spectra were recorded at 90 MHz on a Varian EM-390 or a Bruker WH-90 instrument, at 100 MHz on a Bruker WP100SY instrument, or at 200 MHz on a Varian XL-200 instrument. Chemical shifts are reported as δ values (parts per million) downfield from internal tetramethylsilane on samples of ~1%, w/v. Combustion analyses were performed on a Perkin-Elmer 240 elemental analyzer and are reported within ±0.4% of the theoretical values. Water of crystallization was determined by Karl Fischer titration. pK_a values were determined on a Copenhagen Radiometer TTT60 titrator.

Chromatography was carried out with E. Merck products with use of silica gel 60 catalog no. 5760 for TLC, catalog no. 7734 for open column chromatography, and catalog no. 9385 for flash chromatography. Charcoal refers to activated "Darco" G-60. All solvents and reagents were reagent grade unless otherwise noted.

Method A. *N*,*N*-Diethyl-9-methoxy-5-nitro-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazole-2-ethanamine Monohydrochloride (44). A slurry of 65 g (202 mmol) of thioxanthenone **5d** suspended in 500 mL of DMF was treated with the dropwise addition of 27 g (220 mmol) of *N*,*N*-diethyl-2-hydrazinoethanamine[34] such that the temperature remained below 35 °C. The viscous slurry was stirred at ambient temperature for 18 h. The bright orange solids were collected by filtration, washed successively with DMF and ether, and dried to give 82 g of **44**: ^1H NMR (TFA) δ 1.63 (t, 6, J = 7 Hz), 3.72 (m, 4), 4.10 (s, 3), 5.20 (t, 2, J = 5 Hz), 7.22–7.44 (m, 3), 7.87 (d, 1, J = 1.5 Hz), 8.26 (d, 1, J = 9 Hz); IR (KBr) 1658, 1586, 1484, 1316, 1286, 1233 cm^{-1}. Anal. ($C_{20}H_{22}N_4O_3S\cdot HCl$) C, H, N, Cl^-, S.

Method B. *N*,*N*-Diethyl-5-nitro-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazole-2-ethanamine (38). A mixture of 409 g (1.4 mol) of thioxanthenone **5a**, 230 g (1.77 mol) of *N*,*N*-diethyl-2-hydrazinoethanamine,[34] 230 g (1.7 mol) of anhydrous K_2CO_3, and 7 L of xylene was heated at reflux for 4 h. The mixture was cooled to 100 °C and then filtered. Upon the mixture being cooled to 25 °C, crystallization occurred. The solids were collected by filtration, washed with MeOH, and dried to give 404 g of **38**. The filter cake from the hot filtration was stirred with ca. 1 L of boiling $CHCl_3$. The solids were filtered off, and the filtrate was concentrated to ca. 0.5 L and let stand to crystallize. Further processing as above afforded 32 g of additional **38**: pK_a (67% aqueous DMF) 7.1; ^1H NMR ($CDCl_3$) δ 1.93 (t, 6, J = 7 Hz), 2.52 (q, 4, J = 7 Hz), 2.97 (t, 2, J = 7 Hz), 4.37 (t, 2, J = 6 Hz), 6.91 (d, 1, J = 9 Hz), 7.18–7.60 (m, 3), 8.02–8.26 (m, 2); IR (KBr) 1610, 1588, 1503, 1303, 1290, 1143 cm^{-1}; UV λ_{max} (1 N aqueous HCl) 220 nm (ϵ 25 240), 242 (13 120), 276 (16 065), 303 (10 720), 349 (6375), 435 (7810). Anal. ($C_{19}H_{20}N_4O_2S$) C, H, N.

Method C. 2-[[2-(5-Nitro-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-2-yl)ethyl]amino]ethanol Monohydrochloride (34). A mixture of 300 g (1.02 mol) of thioxanthenone **5a**, 126 g (1.06 mol) of 2-[(2-hydrazinoethyl)amino]ethanol,[4b] and 7 L of THF/MeOH (4:3) was stirred at ambient temperature under N_2

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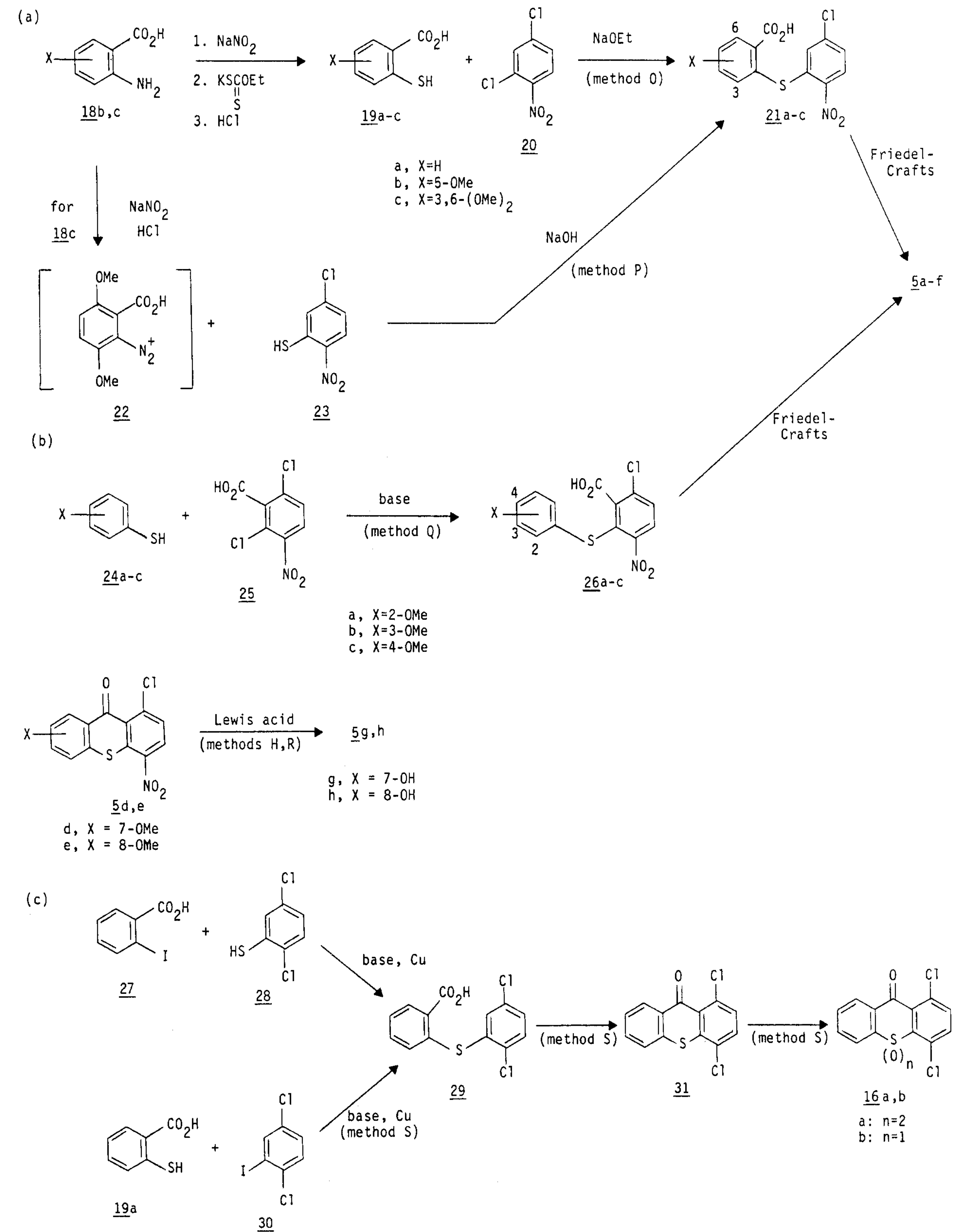
(33) Archer, S.; Suter, C. M. *J. Am. Chem. Soc.* **1952**, *74*, 4296–4309.
(34) Elslager, E. F.; Weistein, E. A.; Worth, D. F. *J. Med. Chem.* **1964**, *7*, 493–500.

Scheme III. Synthesis of 9*H*-Thioxanthen-9-one Intermediates



for 3 days. The suspension was diluted with 4 L of ether, and the solids were collected by filtration, washed with ether, and dried to give 367 g of **34**. Recrystallization of a small portion from DMF gave pure **34**: pK_a (67% aqueous DMF) 7.6; ^{1}H NMR [$(CD_3)_2SO$] δ 3.07 (t, 2, J = 7 Hz), 3.41–3.82 (m, 4), 4.85 (t, 2, J = 6 Hz), 5.28 (t, 1, J = 5 Hz, exchanges D_2O), 7.40–7.65 (3, m), 7.65–7.98 (m, 1), 8.00–8.33 (2, m), 9.05 (br s, 1, exchanges D_2O); IR (KBr) 1605, 1588, 1503, 1300, 755 cm^{-1}; UV λ_{max} (MeOH) 219 nm (ϵ 30 605), 241 (15 635), 273 (22 470), 348 (9230), 415 (9980). Anal. ($C_{17}H_{16}N_4O_3S{\cdot}HCl$) C, H, N, Cl$^-$, S.

Method D. 5-Amino-*N*,*N*-diethyl-9-methoxy-2*H*-[1]-benzothiopyrano[4,3,2-*cd*]indazole-2-ethanamine Dihydrochloride (62). A slurry of 12 g (27.6 mmol) of *N*,*N*-diethyl-9-methoxy-5-nitro-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazole-2-ethanamine hydrochloride (**44**), 500 mg of 20% Pd/C, and 120 mL of glacial HOAc was hydrogenated at 50 psi for 18 h. The mixture was concentrated to remove most of the HOAc, diluted with 1 L of 5% aqueous $NH_4OH/CHCl_3$ (1:1), and filtered over Celite. The phases were separated, and the $CHCl_3$ layer was washed successively with H_2O and then brine, dried, and concentrated. The off-white solids were triturated in EtOH/ether to afford 9.4 g of **62** as the base. Dissolution in hot EtOH followed by treatment with excess 2-propanolic HCl gave **62**: ^{1}H NMR (free base; $CDCl_3$) δ 1.00 (t, 6, J = 7 Hz), 2.55 (q, 4, J = 7 Hz), 2.88 (t, 2, J = 7 Hz), 3.81 (s, 3), 4.28 (t, 2, J = 7 Hz), 6.60–6.88 (m, 3), 7.12 (d, 1, J = 9 Hz), 7.53 (d, 1, J = 3 Hz); IR (KBr) 1608, 1502, 1472, 1290, 1230 cm^{-1}. Anal. ($C_{20}H_{24}N_4OS{\cdot}2HCl$) C, H, N.

Method E. 3-[2-[[2-[2-(Diethylamino)ethyl]-9-methoxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]amino]ethyl]-2-oxazolidinone Dihydrochloride (92). A intimate mixture of 16 g (43.4 mmol) of 5-amino-*N*,*N*-diethyl-9-methoxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazole-2-ethanamine (**62**) and 32 g of 3-(2-chloroethyl)-2-oxazolidinone (**13**) was heated under nitrogen at 100 °C for 18 h. The cooled mixture was diluted with 1 L of CH_2Cl_2/saturated aqueous $NaHCO_3$ (1:1). The organic layer was washed with H_2O (three times), dried ($MgSO_4$), and concentrated to an oil, which solidified. The solids were triturated in 2-PrOH and collected by filtration. Crystallization from hot 2-PrOH gave 15.2 g of **92** as the free base. A small sample was dissolved in EtOH and treated with an excess of 2-propanolic HCl to afford the dihydrochloride: ^{1}H NMR [$(CD_3)_2SO$; free base] δ 0.85 (t, 6, J = 7 Hz), 2.40–2.60 (m, 6), 2.79 (t, 2, J = 6 Hz), 3.31 (t, 2, J = 7 Hz), 3.59 (t, 2, J = 7.5 Hz), 3.80 (s, 3), 4.17–4.32 (m, 4), 4.43 (t, 1, J = 6 Hz, exchanges D_2O), 6.86 (dd, 1, J = 8.9, 2.9 Hz), 6.93 (d, 1, J = 8.8 Hz), 7.09 (d, 1, J = 8.9 Hz), 7.28 (d, 1, J = 8.8 Hz), 7.38 (d, 1, J = 2.9 Hz); IR (KBr) 1735, 1510, 1475, 1275, 1230 cm^{-1}. Anal. ($C_{25}H_{31}N_5O_3S{\cdot}2HCl{\cdot}0.3H_2O$) C, H, N, Cl$^-$, H_2O.

Method F. *N'*-[2-[2-(Diethylamino)ethyl]-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]-*N*,*N*-diethyl-1,2-ethanediamine Dihydrochloride (78). A mixture of 3.0 g (8.9 mmol) of 5-amino-*N*,*N*-diethyl-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazole-2-ethanamine (**55**), 3.5 g (13 mmol) of 2-(diethylamino)ethyl bromide hydrobromide (**14b**), 4.6 g (34 mmol) of K_2CO_3, and 120 mL of toluene was heated at reflux for 8 h, cooled to room temperature, and filtered. The solids were triturated in boiling CH_3CN and filtered, and the filtrate was concentrated to dryness. The residue was dissolved in acetone and treated with excess 2-propanolic HCl. The precipitated solid was collected by filtration and recrystallized from CH_3CN/EtOH to give 2.4 g of **78**: ^{1}H NMR [$(CD_3)_2SO$] δ 1.17–1.29 (m, 12), 3.10–3.31 (m, 12), 3.50–3.68 (m, 2), 4.79 (t, 2, J = 6 Hz), 7.14 (d, 1, J = 9 Hz), 7.28–7.50 (m, 4), 7.90–8.00 (m, 1); IR (KBr) 1521, 1460 cm^{-1}. Anal. ($C_{25}H_{35}N_5S{\cdot}2HCl$) C, H, N.

Method G. *N*-[2-[2-(Diethylamino)ethyl]-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]-1,2-ethanediamine Dihydrobromide (73). A mixture of 188 g (0.56 mol) of 5-amino-*N*,*N*-diethyl-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazole-2-ethanamine (**55**), 340 g (1.66 mol) of 2-bromoethylamine hydrobromide (**14c**), and 3.5 L of absolute EtOH was heated at reflux under N_2 for 7 days, during which time a precipitate gradually formed. The reaction mixture was filtered hot, and the collected solids were washed thoroughly with EtOH and dried to leave 153 g of **73** as the trihydrobromide. The product was dissolved in 2.9 L of H_2O, and the solution was treated with 58 mL of NH_4OH. The precipitated solids were extracted into $CHCl_3$, and the organic layer was washed with brine, dried, and concentrated to a brown oil. The oil was dissolved into 2.3 L of absolute EtOH, and the vigorously stirring solution was treated dropwise with 405 mmol of 23% ethanolic HBr. The precipitated yellow solids were collected by filtration and dried to afford 67 g of **73** as the dihydrobromide: ^{1}H NMR [$(CD_3)_2SO + D_2O$] δ 1.21 (t, 6, J = 7 Hz), 3.02 (t, 2, J = 6 Hz), 3.25 (q, 4, J = 7 Hz), 3.38 (t, 2, J = 6 Hz), 3.63 (t, 2, J = 6 Hz), 4.71 (t, 2, J = 6 Hz), 7.06 (d, 1, J = 9 Hz), 7.27–7.52 (m, 4), 7.91–8.02 (m, 1); IR (KBr) 1520, 1460, 1243, 1165, 780, 750 cm^{-1}. Anal. ($C_{21}H_{27}N_5S{\cdot}2HBr{\cdot}0.2EtOH{\cdot}0.3H_2O$) C, H, N, Br$^-$.

Method H. 5-Amino-2-[2-(diethylamino)ethyl]-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-9-ol Dihydrochloride (61). A solution of 12 g (34.4 mmol) of 5-amino-*N*,*N*-diethyl-9-methoxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazole-2-ethanamine (**62**) in 1 L of 1,2-dichloroethane under N_2 was treated dropwise with 50 mL (50 mmol) of BBr_3 (1 M solution in CH_2Cl_2). The resultant slurry was heated at reflux for 1.5 h, and then ~8 mL of MeOH was added dropwise to the hot solution. The cooled suspension was concentrated to a greenish-yellow residue that was dissolved in H_2O. The aqueous solution was filtered over Celite and then treated with saturated aqueous $NaHCO_3$. The resultant oil crystallized to a solid that was collected by filtration, washed with water, and dried to give 9.5 g of **61** as the free base. The solids were dissolved in hot EtOH, and the solution was treated with an excess of 2-propanolic HCl and then stirred at 25 °C for 2 h. The precipitated solids were collected by filtration, washed successively with EtOH and ether, and dried to give 7.9 g of **61**: ^{1}H NMR [$(CD_3)_2SO + D_2O$] δ 1.22 (t, 6, J = 7 Hz), 3.24 (q, 4, J = 7 Hz), 3.63 (t, 2, J = 6 Hz), 4.79 (t, 2, J = 6 Hz), 6.87 (dd, 1, J = 3, 9 Hz), 7.26–7.44 (m, 3), 7.46 (d, 1, J = 3 Hz); IR (KBr) 1605, 1504, 1436, 1234, 1116 cm^{-1}. Anal. ($C_{19}H_{22}N_4OS{\cdot}2HCl{\cdot}1.4H_2O$) C, H, N, S, Cl$^-$.

Method I. 2-[2-(Diethylamino)ethyl]-5-[[2-[(2-hydroxyethyl)amino]ethyl]amino]-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-9-ol Trihydrochloride (89). A solution of 6.0 g (12.5 mmol) of 3-[2-[[2-[2-(diethylamino)ethyl]-9-hydroxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]amino]ethyl]-2-oxazolidinone (**90**) in 250 mL of 2 M methanolic KOH was heated at reflux under N_2 for 18 h, cooled, and then treated with 200 mL of saturated aqueous NH_4Cl. The product was extracted into ethyl acetate (5 × 200 mL), and then the combined extracts were dried, clarified with charcoal, and filtered. The filtrate was treated dropwise with an excess of 2-propanolic HCl. The precipitated solids were collected by filtration, washed with ether, and then recrystallized from warm 20% aqueous MeOH to afford 5.2 g of **89** after drying: ^{1}H NMR [$(CD_3)_2SO + D_2O$] δ 1.17 (t, 6, J = 7 Hz), 3.01–3.23 (m, 8), 3.41–3.70 (m, 6), 4.71 (t, 2, J = 6 Hz), 6.79 (dd, 1, J = 2.5, 8.7 Hz), 7.07 (d, 1, J = 8.8 Hz), 7.23 (d, 1, J = 8.5 Hz), 7.27 (d, 1, J = 8.3 Hz), 7.38 (d, 1, J = 2.6 Hz); IR (KBr) 1512, 1477, 1438, 1222 cm^{-1}. Anal. ($C_{23}H_{31}N_5O_2S{\cdot}3HCl{\cdot}H_2O$) C, H, N, S, Cl$^-$, H_2O.

Method J. 5-[(2-Aminoethyl)amino]-2-[2-(diethylamino)ethyl]-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-9-ol Trihydrochloride (87). A mixture of 2.4 g (4.4 mmol) of 2-[2-[[2-[2-(diethylamino)ethyl]-9-hydroxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]amino]ethyl]-1*H*-isoindole-1,3-(2*H*)-dione,[35] 5.25 mL of anhydrous methylhydrazine, and 100 mL of MeOH was stirred overnight at 25 °C under N_2. The mixture was filtered through Celite, and the filtrate was concentrated. The oily residue was diluted with 25 mL of MeOH, and the solution was treated with an excess of 2-propanolic HCl and then stored in the cold. The precipitated solids were collected by filtration and then recrystallized from hot MeOH to afford 2.2 g of **87** in two crops: ^{1}H NMR [$(CD_3)_2SO + D_2O$] δ 1.20 (t, 6, J = 7 Hz), 3.03 (t, 2, J = 6 Hz), 3.20 (q, 4, J = 7 Hz), 3.43 (t, 2, J = 6 Hz), 3.57 (t, 2, J = 6 Hz), 4.77 (t, 2, J = 6 Hz), 6.83 (dd, 1, J = 2.7, 8.7 Hz), 7.14 (d, 1, J = 9.3 Hz), 7.27 (d, 1, J = 8.9 Hz), 7.33 (d, 1, J = 9 Hz), 7.42 (d, 1, J = 2.7 Hz); IR (KBr) 1598, 1511, 1476, 1291, 1224 cm^{-1}. Anal. ($C_{21}H_{27}N_5OS{\cdot}2.8HCl{\cdot}0.4H_2O$) C, H, N, S, Cl$^-$.

Method K. [2-[[2-[2-(Diethylamino)ethyl]-10-methoxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]amino]-2-oxoethyl]carbamic Acid 1,1-Dimethylethyl Ester (95). A suspension of 6.82 g (14 mmol) of 5-amino-*N*,*N*-diethyl-10-methoxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazole-2-ethanamine dihydrochloride (**64**), 4.23 g (24 mmol) of *N*-BOC-glycine (**15b**), 6.13 g (24 mmol) of bis(2-oxo-3-oxazolidinyl)phosphinic chloride, 10.8 mL (62 mmol) of diisopropylethylamine, and 55 mL of CH_2Cl_2 was stirred under N_2 at 25 °C. An additional 1.2 mL of base was added after 2.5 h, and the mixture was stirred for an additional 4.5 h. The solution was poured into 1 M aqueous K_2CO_3, the mixture was vigorously stirred for 15 min, and the layers were separated. The organic phase was dried and concentrated to leave a solid residue. Crystallization from CH_3CN afforded 5.8 g of **95**: ^{1}H NMR ($CDCl_3 + D_2O$) δ 1.03 (t, 6, J = 7 Hz), 1.52 (s, 9), 2.61 (q, 4, J = 7 Hz), 2.95 (t, 2, J = 7 Hz), 4.00 (s, 2), 4.03 (s, 3), 4.41 (t, 2, J = 7 Hz), 6.80–6.94 (m, 3), 7.15 (t, 1, J = 8.1 Hz), 7.35 (d, 1, J = 8.8 Hz); IR (KBr) 1716, 1697, 1661, 1498, 1268, 1173 cm^{-1}. Anal. ($C_{27}H_{35}N_5O_4S$) C, H, N, S.

Method L. *N*-[2-[2-(Diethylamino)ethyl]-8-methoxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]-1,2-ethanediamine Trihydrochloride (83). A mixture of 4 g (7.8 mmol) of [2-[[2-[2-(diethylamino)ethyl]-8-methoxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]amino]ethyl]carbamic acid 1,1-di-

(35) See Footnotes *s* and *w* of Table II.

methylethyl ester (**84**), 10 mL of concentrated HCl, and 60 mL of a EtOH was maintained at 40 °C overnight. The cooled suspension was filtered, and the solids were washed with EtOH and dried to give 2.35 g of **83**: ^{1}H NMR [$(CD_3)_2SO$] δ 1.18 (t, 6, *J* = 7 Hz), 2.90–3.22 (m, 6), 3.40–3.55 (m, 4), 3.79 (s, 3), 4.78 (t, 2, *J* = 6.5 Hz), 4.95 (br s, exchanges D_2O), 6.89–7.00 (m, 2), 7.20 (d, 1, *J* = 8.8 Hz), 7.36 (d, 1, *J* = 8.9 Hz), 7.86 (d, 1, *J* = 8.6 Hz), 8.33 (br s, 3, exchanges D_2O), 11.10 (br s, 1, exchanges D_2O); IR (KBr) 1608, 1561, 1475, 1295, 1244, 1037 cm^{-1}. Anal. ($C_{22}H_{29}N_5OS·3HCl·H_2O$) C, H, N, S, Cl$^-$.

Method M. [2-[[2-[2-(Diethylamino)ethyl]-10-methoxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]amino]ethyl]carbamic Acid 1,1-Dimethylethyl Ester (94). To a stirred suspension of 5.73 g (11 mmol) of crude **95** in 20 mL of toluene at 60 °C was added dropwise during 15 min 16 mL (54 mmol) of sodium bis(2-methoxyethoxy)aluminum hydride (3.4 M in toluene). The resultant solution was heated for an additional 1.75 h, cooled, and treated cautiously with saturated aqueous NH_4Cl. The mixture was diluted with CH_2Cl_2 and then filtered through Celite. The organic phase was dried and concentrated to a solid, which was purified by flash SiO_2 chromatography, eluting sequentially with 0, 1.5, 2, 3, 4, 6, 8, and 20% MeOH in CH_2Cl_2. The product fractions were pooled and concentrated to a solid. Crystallization from ethyl acetate/$CHCl_3$ (4:1) afforded 4.0 g of **94** in two crops: ^{1}H NMR ($CDCl_3$) δ 1.05 (t, 6, *J* = 7 Hz), 1.47 (s, 9), 2.63 (q, 4, *J* = 7 Hz), 2.98 (t, 2, *J* = 7 Hz), 3.35 (br s, 4), 4.02 (s, 3), 4.40 (t, 2, *J* = 7 Hz), 6.78–6.95 (m, 4), 7.14 (t, 1, *J* = 8.1 Hz); IR (KBr) 1678, 1565, 1531, 1259, 1173, 1041 cm^{-1}. Anal. ($C_{27}H_{37}N_5O_3S·0.3H_2O$) C, H, S.

Method N. *N'*-[2-[2-(Diethylamino)ethyl]-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]-*N*,*N*-diethyl-1,2-ethanediamine *S*,*S*-Dioxide (103). An intimate mixture of 25 g (58.6 mmol) of 5-chloro-*N*,*N*-diethyl-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazole-2-ethanamine 6,6-dioxide hydrochloride (**100**) and 62 mL (645 mmol) of *N*,*N*-diethylethylenediamine was heated at reflux under N_2 for 32 h. The mixture was concentrated at 0.5 mm to leave a semisolid residue that was triturated in 2-propanol. The solids were collected by filtration, washed with 2-propanol, and then recrystallized from CH_3CN to give 15.6 g of **103** after drying: ^{1}H NMR [$(CD_3)_2SO$] δ 0.80 (t, 6, *J* = 7 Hz), 1.02 (t, 6, *J* = 7 Hz), 2.43–2.58 (m, 8), 2.68 (t, 2, *J* = 6 Hz), 2.87 (t, 2, *J* = 6 Hz), 3.34 (t, 2, *J* = 6 Hz), 4.53 (t, 2, *J* = 6 Hz), 6.28 (t, 1, *J* = 5 Hz, exchanges D_2O), 7.18 (d, 1, *J* = 9 Hz), 7.58–7.80 (m, 2), 7.94 (d, 1, *J* = 9 Hz), 8.08–8.14 (m, 2); IR (KBr) 1636, 1542, 1520, 1466, 1276, 1119 cm^{-1}. Anal. ($C_{25}H_{35}N_5O_2S$) C, H, N, S.

Method O. 2-[(5-Chloro-2-nitrophenyl)thio]-5-methoxybenzoic Acid (21b). A solution of 27.6 g (0.16 mol) of 2-amino-5-methoxybenzoic acid[21] (**18b**), 16.0 mL (0.38 mol) of 50% aqueous NaOH, 220 mL of H_2O, and 11.4 g (0.16 mol) of $NaNO_2$ was added slowly to a –5 °C mixture of 50 mL of concentrated HCl and 65 g of ice chips. Good stirring was maintained throughout the addition, and the temperature was kept below 5 °C. Following addition, the mixture was stirred at 0 °C for 1 h, neutralized (pH 5.1) with potassium acetate, and added while cold in a thin stream to an 80 °C solution of 76.9 g (0.48 mol) of *O*-ethylxanthic acid potassium salt in 275 mL of H_2O under N_2. Copious N_2 evolution (foaming) occurred during the addition, and heat was applied as needed to maintain the temperature at 75–80 °C. The reaction mixture was cooled to 20 °C and acidified (pH 3) with concentrated HCl. The mixture was treated with 200 mL of CH_2Cl_2, stirred, and filtered to remove an insoluble solid shown by NMR to be the disulfide of 2-mercapto-5-methoxybenzoic acid.[36] The layers were separated, and the aqueous phase was extracted with a second 200-mL portion of CH_2Cl_2, keeping contact with air to a minimum. The extracts were combined, dried under N_2, and concentrated to dryness.

The crude 2-mercapto-5-methoxybenzoic acid (**19b**) was immediately dissolved in 140 mL of hot anhydrous EtOH and added to a premixed, 25 °C mixture of 31.7 g (0.16 mol) of 2,4-dichloronitrobenzene (**20**) in NaOEt, which was made by dissolving 7.6 g (0.33 g-atom) of Na spheres in 330 mL of anhydrous EtOH. The resulting suspension was heated at reflux for 1 h, concentrated to dryness, and distributed between ether and H_2O. The aqueous layer was extracted twice with ether and then made acidic (pH 1) with concentrated HCl. The precipitate was collected by filtration, dried, and recrystallized from EtOH to give, in two crops, 19.8 g of **21b**. Silica gel TLC (EtOAc/MeOH/Et_3N, 75:25:1) showed one spot, $R_f \cong 0.2$ with a trace origin impurity. The product was sufficiently pure for direct use in the next reaction: ^{1}H NMR [$(CD_3)_2SO$] δ 3.85 (s, 3), 6.65 (d, 1, *J* = 2 Hz), 7.05–7.60 (m, 4), 8.12 (d, 1, *J* = 9 Hz); IR (KBr) 1695, 1590, 1560, 1510, 1330, 1235, 860 cm^{-1}.

1-Chloro-7-methoxy-4-nitro-9*H*-thioxanthen-9-one (5d). A mixture of 118.5 g (0.35 mol) of **21b**, 600 mL of toluene, and 131 mL (0.43 mol) of thionyl chloride was heated at reflux for 1.5 h, concentrated to dryness, and dissolved in 950 mL of nitrobenzene. The solution was cooled to 0 °C and then treated portionwise with 44.2 g (0.39 mol) of anhydrous $AlCl_3$, keeping the temperature below 35 °C during the addition. The mixture was stirred at room temperature for 20 h and poured into 5 L of ice-cold H_2O. The mixture was stirred for 1 h, and the H_2O was decanted from the tarry residue. The residue was washed and decanted with 1- and then 2-L portions of MeOH. The oil was layered with 4 L of MeOH and stored at 25 °C until crystallization set in. The solids were collected by filtration, washed with MeOH, and dried to give 73 g of **5d** as a yellow solid; silica gel TLC (CH_2Cl_2) showed one spot.

Method P. 2-[(5-Chloro-2-nitrophenyl)thio]-3,6-dimethoxybenzoic Acid (21c). A solution of 1 g (5 mmol) of 2-amino-3,6-dimethoxybenzoic acid (**18c**),[22] 400 mg (5.8 mmol) of $NaNO_2$, and 6.5 mL of 2.93 M aqueous NaOH was added dropwise to a solution of 4.7 mL of 4.34 M aqueous HCl, while the temperature was maintained at 0 to –5 °C. The mixture was stirred for 10 min and then added dropwise to a stirred mixture of 1.2 g (6 mmol) of 5-chloro-2-nitrothiophenol[24] (**23**) and 1.3 g of NaOH in 10 mL of H_2O maintained at 52–54 °C (brisk N_2 evolution). The mixture was stirred for 15 min, cooled to 25 °C, and acidified to pH 2. The solids were collected by filtration, triturated in CH_2Cl_2/CH_3CN, and dried to leave 830 mg of product, identical by ^{1}H NMR and TLC analyses with **21c** synthesized by method O.

Method Q. 6-Chloro-2-[(4-methoxyphenyl)thio]-3-nitrobenzoic Acid (26c). A 0–5 °C suspension of 3.0 g (125 mmol) of NaH in 100 mL of THF was treated portionwise during 10 min with 12.2 g (52 mmol) of 2,6-dichloro-3-nitrobenzoic acid[25] (**25**). After being stirred for 10 min, the suspension was treated dropwise with 7.0 g (50 mmol) of 4-methoxybenzenethiol (**24c**) in 50 mL of THF. After being stirred for 30 min at 0 °C, the mixture was maintained at 25 °C for 12 h. The mixture was acidified with 10% aqueous HCl and then extracted with ethyl acetate. The combined organic phases were dried and concentrated to a yellow solid that was purified by SiO_2 flash chromatography, with CH_2Cl_2/MeOH (8:1) as eluting solvent, to give 11.9 g of **26c**, following crystallization from toluene.[37] Silica gel TLC (CH_2Cl_2/MeOH/HOAc, 90:10:1) showed one spot: ^{1}H NMR [$CDCl_3$ + $(CD_3)_2SO$] δ 3.71 (s, 3), 6.72 (d, 2, *J* = 8 Hz), 7.1–7.7 (m, 4), 11.25 (br s, exchanges D_2O, 1); IR (KBr) 1710, 1570, 1360, 1270, 1250, 1030, 830 cm^{-1}. Anal. ($C_{14}H_{10}ClNO_5S$) C, H, Cl, N, S.

Alternatively, acid **26c** of sufficient purity for direct conversion to thioxanthenone **5d** was prepared as follows:

A mixture of 76 g (542 mmol) of 4-methoxybenzenethiol (**24c**), 127.5 g (538 mmol) of 2,6-dichloro-3-nitrobenzoic acid (**25**), 150 g of anhydrous K_2CO_3, and 1.5 L of DMF was stirred at 25 °C under N_2 for 18 h. The suspension was filtered over Celite, and the filtrate was concentrated in vacuo to a residue that was distributed between 1 N aqueous HCl and $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and then treated with 100 mL of 37% NH_4OH. The precipitated ammonium salt of **26c** was collected by filtration and then slurried in 1 N aqueous HCl. The solids were collected and washed with H_2O to provide crude **26c**. Processing of the ammonium salt filtrate provided additional

(36) Archer, S.; Miller, K. J.; Rej, R.; Periana, C.; Fricker, L. *J. Med. Chem.* 1982, *25*, 220–227.

(37) The higher R_f impurity is the bis[(4-methoxyphenyl)thio] addition product derived from displacement of both chlorines of **25**: ^{1}H NMR ($CDCl_3$) δ 3.53 (s, 3), 3.71 (s, 3), 6.3–6.9 (m, 5), 6.95–7.4 (m, 5).

material. Crystallization of the combined crops from 2 L of hot toluene provided 107.5 g of **26c**. TLC indicated only a trace impurity.

1-Chloro-7-methoxy-4-nitro-9*H*-thioxanthen-9-one (5d). A slurry of 107.5 g (317 mmol) of **26c** in 400 mL of trifluoroacetic acid was treated with 200 mL of trifluoroacetic anhydride to give a red solution. After the mixture had stirred at room temperature for 18 h, the resulting bright yellow solids were collected by filtration, washed successively with EtOH and ether, and dried to provide 97 g of **5d**: ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 3), 7.33 (d, 1, J = 2 Hz), 7.5–7.7 (m, 2), 7.81 (d, 1, J = 2 Hz), 8.50 (d, 1, J = 8 Hz); IR (KBr) 1646, 1581, 1511, 1338, 1029 cm^{-1}. Anal. ($C_{14}H_8ClNO_4S$) C, H, Cl, N, S.

Method R. 1-Chloro-7-hydroxy-4-nitro-9*H*-thioxanthen-9-one (5g). A 25 °C solution of 16.3 g (50.7 mmol) of thioxanthenone **5d** in 200 mL of dichloroethane was treated portionwise with 20.9 g (157 mmol) of anhydrous $AlCl_3$. The red-purple solution was heated at reflux for 1.5 h, cooled, and concentrated. The solid residue was treated with 500 mL of 6 N aqueous HCl, and the mixture was heated at reflux for 4 h. After the mixture was cooled to 25 °C, the solids were collected by filtration, washed with water and then 2-PrOH, and dried to give 15.6 g of **5g**: ^{1}H NMR [$(CD_3)_2SO$] δ 7.23 (dd, 1, J = 9 Hz, 3 Hz), 7.51 (d, 1, J = 3 Hz), 7.69 (d, 1, J = 9 Hz), 7.78 (d, 1, J = 9 Hz), 8.55 (d, 1, J = 9 Hz), 10.33 (br s, 1, exchanges D_2O); IR (KBr) 1653, 1603, 1578, 1438, 1341, 917 cm^{-1}. Anal. ($C_{13}H_6ClNO_4S\cdot 0.22$-PrOH) C, H, N, S, Cl.

Method S. 2-[(2,5-Dichlorophenyl)thio]benzoic Acid (29). A solution of 22.4 g (145 mmol) of thiosalicyclic acid (**19a**), 20 g (357 mmol) of KOH, and 232 mL of H_2O was heated at 60 °C under N_2 for 15 min and then treated with 0.8 g of Cu powder followed by 40 g (147 mmol) of 1,4-dichloro-2-iodobenzene (**30**). The mixture was heated at reflux for 1 day and then filtered while hot. The filtrate was acidified with 27 mL of concentrated HCl, and the precipitated solids were collected by filtration, washed with H_2O, and dried. Crystallization from 800 mL of ethyl acetate gave 34.1 g of **29**. Anal. ($C_{13}H_8Cl_2O_2S$) C, H, Cl, S.

1,4-Dichloro-9*H*-thioxanthen-9-one (31). A mixture of 25.0 g (84 mmol) of 2-[(2,5-dichlorophenyl)thio]benzoic acid (**29**) and 150 mL of thionyl chloride was heated at reflux for 2 h. The solution was concentrated to a solid that was dissolved in 300 mL of 1,2-dichloroethane. The well-stirred solution was treated portionwise at 25 °C with 33.4 g (250 mmol) of anhydrous $AlCl_3$. The mixture was stirred at 25 °C for 2 h, poured into 1 L of 10% aqueous HCl, and extracted with CH_2Cl_2. The combined organic extracts were washed successively with H_2O and 5% aqueous $NaHCO_3$, dried, and concentrated to a solid that was recrystallized from DMF to give 19.6 g of **31**: ^{1}H NMR ($CDCl_3$) δ 7.4–7.9 (m, 5), 8.43 (d, 1, J = 7 Hz); IR (KBr) 1651, 1565, 1414, 1299, 821, 743 cm^{-1}. Anal. ($C_{13}H_6Cl_2OS$) C, H, Cl, S.

1,4-Dichloro-9*H*-thioxanthen-9-one 10,10-Dioxide (16a). Oxidation of **31** by the literature procedure[19] gave a 96% yield of **16a**, mp 178–181 °C, shown by TLC to contain a trace impurity. Purification by flash silica gel chromatography with CH_2Cl_2 elution gave a 87% recovery of pure **16a** after crystallization from acetic acid: IR (KBr) 1679, 1425, 1324, 1298, 1163, 950 cm^{-1}. Anal. ($C_{13}H_6Cl_2O_3S$) C, H, Cl, S.

1,4-Dichloro-9*H*-thioxanthen-9-one 10-Oxide (16b). A suspension of 10 g (35.6 mmol) of thioxanthenone **31**, 40 mL of 20% aqueous $TiCl_3$, and 280 mL of CH_3CN/MeOH (5:2) was brought to reflux and treated dropwise during 15 min with 15 mL of 30% H_2O_2. The suspension was maintained at reflux until silica gel TLC (CH_2Cl_2) showed complete consumption of **31**. The mixture was diluted with H_2O and then extracted with CH_2Cl_2. The combined extracts were dried and concentrated to give 8.9 g of crude **16b**. Crystallization from EtOH gave 6.4 g of pure **16b**: ^{1}H NMR ($CDCl_3$) δ 7.62 (s, 2), 7.67–8.05 (m, 3), 8.05–8.30 (m, 1); IR (KBr) 1685, 1591, 1431, 1300, 1096, 1042, 759 cm^{-1}. Anal. ($C_{13}H_6Cl_2O_2S$) C, H, Cl, S.

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Registry No. **5a**, 41215-88-7; **5b**, 94635-55-9; **5c**, 94635-45-7; **5d**, 94636-19-8; **5e**, 94635-57-1; **5f**, 94636-42-7; **5g**, 100332-33-0; **5h**, 114615-18-8; **13**, 2508-01-2; **14a**, 574-98-1; **14b**, 1069-72-3; **14c**, 2576-47-8; **14d**, 18370-81-5; **15a**, 6780-38-7; **15b**, 4530-20-5; **16a**, 29941-56-8; **16b**, 114615-62-2; **18b**, 6705-03-9; **18c**, 50472-10-1; **19a**, 147-93-3; **19b**, 16807-37-7; **19c**, 94636-43-8; **20**, 611-06-3; **21a**, 54920-86-4; **21b**, 94636-18-7; **21c**, 94635-51-5; **23**, 14371-79-0; **24a**, 7217-59-6; **24b**, 15570-12-4; **24c**, 696-63-9; **25**, 55775-97-8; **26a**, 114615-19-9; **26b**, 114615-65-5; **26c**, 94636-20-1; **27**, 88-67-5; **28**, 5858-18-4; **29**, 50900-44-2; **30**, 29682-41-5; **31**, 39657-89-1; **32**, 94635-96-8; **32** (free base), 94636-62-1; **33**, 94636-31-4; **34**, 94636-11-0; **34** (free base), 94636-63-2; **35**, 114615-20-2; **36**, 94635-86-6; **36** (free base), 94635-85-5; **37**, 94635-87-7; **37** (free base), 94636-64-3; **38**, 94636-61-0; **39**, 114615-21-3; **39** (free base), 94635-53-7; **40**, 114615-22-4; **41**, 114615-23-5; **42**, 114615-24-6; **43**, 114615-27-9; **43** (hydrochloride), 114615-78-0; **44**, 114615-28-0; **44** (free base), 94635-71-9; **44** (methanesulfonate), 94635-78-6; **45**, 114615-29-1; **46**, 94636-04-1; **47**, 114615-30-4; **48**, 94636-41-6; **49**, 94636-48-3; **49** (free base), 94635-90-2; **50**, 94636-49-4; **50** (free base), 94635-46-8; **51**, 94636-45-0; **51** (free base), 94635-81-1; **52**, 94654-42-9; **52** (free base), 114615-66-6; **53**, 94636-46-1; **53** (free base), 94635-88-8; **54**, 94636-47-2; **54** (free base), 94635-89-9; **55**, 94636-44-9; **56**, 114615-31-5; **56** (free base, 94635-54-8; **57**, 114615-32-6; **57** (free base), 94635-63-9; **58**, 114615-33-7; **58** (free base), 94654-43-0; **59**, 114615-34-8; **59** (free base), 114615-67-7; **60**, 114615-35-9; **61**, 94636-27-8; **61** (free base), 94636-26-7; **62**, 94635-79-7; **62** (free base), 94635-72-0; **63**, 114615-36-0; **63** (free base), 94635-64-0; **64**, 114615-37-1; **64** (free base), 94635-56-0; **65**, 94636-12-1; **66**, 94636-38-1; **66** (free base), 94635-91-3; **67**, 94635-43-5; **68**, 94636-36-9; **68** (free base), 94635-83-3; **69**, 94636-37-0; **69** (free base), 94635-84-4; **70**, 94636-33-6; **70** (free base), 94636-32-5; **71**, 114615-38-2; **71** (free base), 114615-68-8; **72**, 94635-68-4; **72** (free base), 94635-69-5; **73**, 114615-39-3; **73**·3HBr, 94636-51-8; **73** (free base), 94635-44-6; **74**, 114615-40-6; **74** (free base), 114615-69-9; **75**, 94635-95-7; **75** (free base), 94635-92-4; **76**, 114615-41-7; **76** (free base), 94635-82-2; **77**, 94636-56-3; **78**, 94636-50-7; **78** (free base), 94938-76-8; **79**, 114615-42-8; **79** (free base), 114615-70-2; **80**, 114615-43-9; **81**, 114615-44-0; **81** (free base), 113457-05-9; **82**, 114615-45-1; **83**, 114615-46-2; **83** (free base), 114615-71-3; **84**, 114615-47-3; **85**, 114615-48-4; **85** (free base), 114615-72-4; **86**, 114615-49-5; **87**, 114615-50-8; **87** (free base), 113457-06-0; **88**, 94635-76-4; **88** (free base), 94635-74-2; **89**, 114615-51-9; **89** (free base), 94636-28-9; **90**, 94635-97-9; **90** (free base), 94636-29-0; **91**, 94635-75-3; **91** (free base), 94635-73-1; **92**, 94636-24-5; **92** (free base), 94636-23-4; **93**, 114615-52-0; **93** (free base), 114615-73-5; **94**, 114615-53-1; **95**, 114615-54-2; **96**, 114615-55-3; **96** (free base), 94635-94-6; **97**, 94636-15-4; **98**, 114615-56-4; **99**, 114615-57-5; **100**, 114615-58-6; **100** (free base), 114615-76-8; **101**, 114615-59-7; **101** (free base), 114615-74-6; **102**, 114615-60-0; **102** (free base), 114615-75-7; **103**, 114615-61-1; $NH_2NHCH_2CH_2NEt_2$, 924-29-8; $NH_2NHCH_2CH_2NHCH_2CH_2OH$, 88303-65-5; $NH_2NHCH_2CH_2NH_2$, 14478-61-6; $NH_2NHCH_2CH_2NMe_2$, 1754-57-0; $NH_2NH(CH_2)_3NMe_2$, 3762-38-7; $H_2N(CH_2)_2NH(CH_2)_2OH$, 111-41-1; $H_2N(CH_2)_2NH_2$, 107-15-3; *o*-$MeOC_6H_4SSC_6H_4OMe$-*o*, 13920-94-0; *m*-$MeOC_6H_4SSC_6H_4OMe$-*m*, 59014-89-0; 2-[2-[[2-[2-(diethylamino)ethyl]-9-hydroxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]amino]ethyl]-1*H*-isoindole-1,3(2*H*)-dione, 114615-63-3; bis(2-oxo-3-oxazolidinyl)phosphinic chloride, 68641-49-6; *N*,*N*-diethylethylenediamine, 100-36-7; bis(1-carboxy-5-methoxyphenyl-2-yl) disulfide, 19532-69-5; 2-[2-[[2-[2-(diethylamino)ethyl]-8-methoxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]amino]ethyl]-1*H*-isoindole-1,3(2*H*)-dione, 114651-79-5; 2-[2-[[2-[2-(diethylamino)ethyl]-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]amino]-2-oxo-ethyl]-1*H*-isoindole-1,3(2*H*)-dione, 114615-64-4; 2,6-bis[(4-methoxyphenyl)thio]-3-nitrobenzenecarboxylic acid, 114615-77-9.