# Synthesis and Antitumor Activity of 4-Aminomethylthioxanthenone and 5-Aminomethylbenzothiopyranoindazole Derivatives

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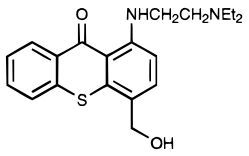
Two new series of antitumor agents, 4-aminomethylthioxanthenones (**6**−**50**) and 5-aminomethylbenzothiopyranoindazoles (**51**−**61**), are described and compared. Nearly all members of both series display excellent in vivo activity versus murine pancreatic adenocarcinoma 03 (Panc03) although there is little to distinguish the two series from each other. In both series there is no discernible relationship between structure and in vivo efficacy. Selected analogues were evaluated in vitro; all were observed to have moderate to strong DNA binding via intercalation. However, varying degrees of in vitro P388 cytotoxicity and topoisomerase II inhibition were seen. In general, those molecules which exhibited strong topoisomerase II inhibition were significantly more cytotoxic than those which did not. In both series, those derivatives (**48**−**50**, **60**, and **61**) having a phenolic hydroxy substitution exhibited the most potent P388 cytotoxicity and topoisomerase II inhibition.

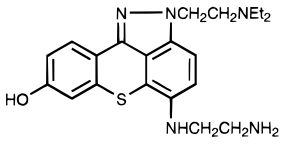
## Introduction

Numerous reasons have been offered for the lack of significant advances in the treatment of most solid neoplasms over the last 30 years.[1] Part of the difficulty may lie in the historical preference for carrying out cytotoxic screening regimens in vivo or in vitro with leukemia cell lines. A screening program designed to identify compounds which are selectively toxic to solid tumor cell lines, as opposed to leukemias or normal cell lines, has the effect of searching a completely different region of structure space than conventional (i.e., leukemia L1210) screening would have provided. Assuming some correlation between in vitro and in vivo activity, the desired result is that positive responses would be obtained for compounds which are effective versus solid tumors in vivo and which could have been missed in earlier screens.[2]

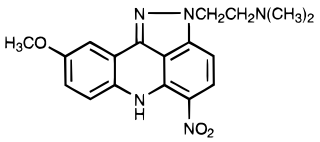
Using this strategy to screen in-house compound libraries, we recently reported[3] the curative antisolid tumor activity of a series of 4-aminomethylthioxanthenone derivatives, related to the known antitumor agent hycanthone (**1**).[4] One member of this series, SR 233377 (WIN 33377, **12**), a compound that displayed broad spectrum murine solid tumor activity, has completed phase I clinical trials. In this paper we describe, in detail, the synthesis and antitumor properties of a novel series of compounds structurally similar to SR 233377 (**12**)[3] and also discuss a new structurally related series incorporating a pyrazolo ring fusion (**51**−**61**).[5] This structural modification was commonly used to study structure−activity relationships of similar antitumor series such as the benzothiopyranoindazole CI 958 (**2**)[6] and the pyrazoloacridine PD 115934 (**3**).[7] In addition to showing good activity in murine tumor models, the pyrazoloacridines (e.g., **3**) also display selectivity against solid tumor cell lines in vitro similar to SR 233377.[8] We now present comparative in vitro and in vivo antitumor data for the novel 5-aminomethylbenzothiopyranoindazoles **51**−**61** and the structurally related 4-aminomethylthioxanthenones **6**−**50**.




**1**: Hycanthone




**2**: CI-958




**3**: PD 115934

## Chemistry

The syntheses of tricyclic derivatives **6**−**50** were achieved via elaboration of the 4-position of the thioxanthenone nucleus as shown in Schemes 1 and 2. The known aldehydes **4**[9] and **63**[4b] were converted, via Leuckart reaction, to *N*-methylformamide derivatives **5** and **64**, respectively, using *N*-methylformamide/formic

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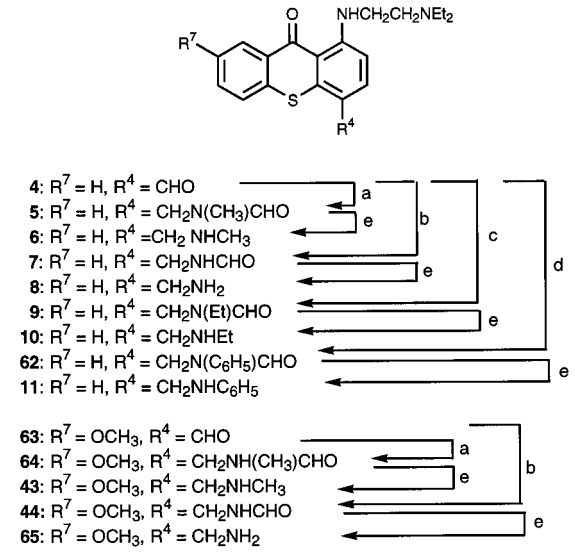
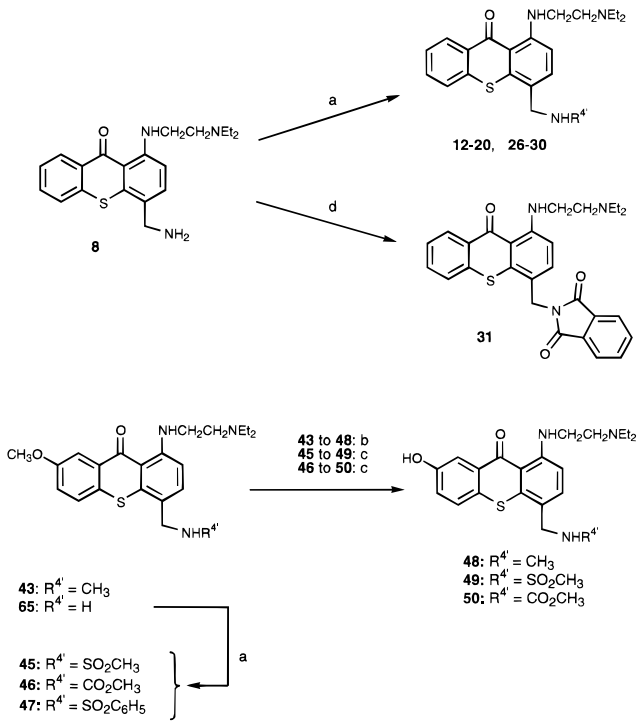
**Scheme 1**[a]




[a] (a) $HCONCH_3$, HCOOH; (b) $HCONH_2$, HCOOH; (c) HCONHEt, HCOOH; (d) $HCONHC_6H_5$, HCOOH; (e) 2 N HCl.

**Scheme 2**[a]

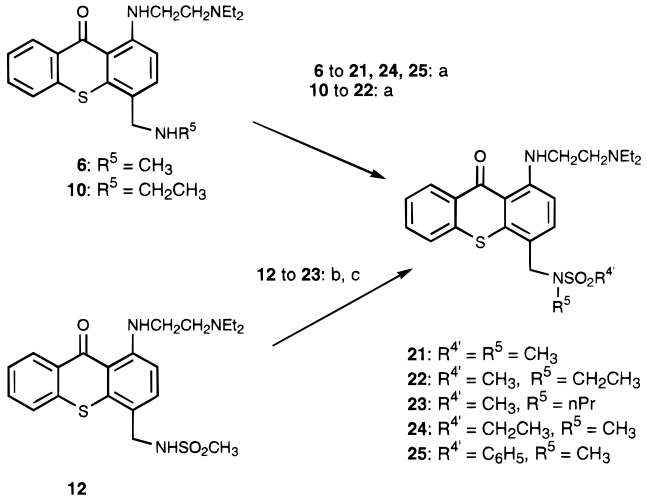



[a] (a) R4′Cl, $Et_3N$, $CH_2Cl_2$; (b) 48% HBr; (c) $BBr_3$, $CH_2Cl_2$; (d) phthalic anhydride.

acid at 160 °C (Scheme 1). Substituting formamide, *N*-ethylformamide, and *N*-phenylformamide for *N*-methylformamide under identical conditions afforded **7**, **9**, and **62**, respectively (from **4**), and **44** (from **63**). Subsequent hydrolysis of **5**, **7**, **9**, **62**, **64**, and **44** with hot 2 N aqueous HCl afforded high yields of **6**, **8**,[3] **10**, **11**, **43**, and **65**, respectively.

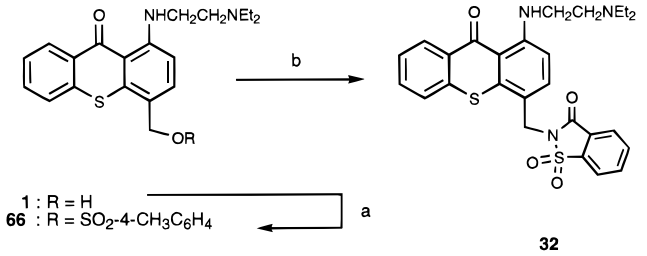
Treatment of **8** with a variety of sulfonyl chlorides afforded **12**−**20** (Scheme 2) in generally good yields. Analogously, the 7-methoxy derivative **65** was reacted with methanesulfonyl and benzenesulfonyl chlorides to give **45** and **47**, respectively. Amide derivatives **26**−**28** were prepared from **8** and the corresponding acid

**Scheme 3**[a]




[a] (a) R4′$SO_2Cl$, $Et_3N$, $CH_2Cl_2$; (b) NaH, THF; (c) *n*-PrI.

**Scheme 4**[a]




[a] (a) *p*-Toluenesulfonyl chloride, pyridine; (b) sodium saccharin, tetra-*n*-butylammonium bromide.

chlorides under the same conditions as for sulfonamides **12**−**20**. Likewise, phosphoramide **30** was produced from the reaction of **8** and diethylphosphoryl chloride. Two methyl carbamate derivatives, **29** and **46**, were synthesized from **8** and **65**, respectively, by treatment with methyl chloroformate. Phthalimide **31** was also obtained cleanly from **8** and phthalic anhydride.

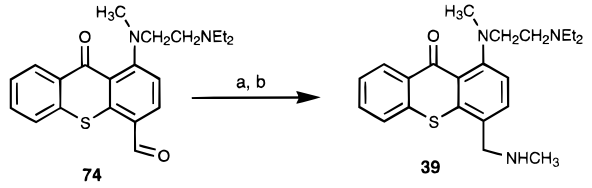
*N*-Methylsulfonamide analogues **21**, **24**, and **25** were prepared in a fashion similar to sulfonamides (**12**−**20**) from **6**, while **22** was synthesized from **10** (Scheme 3). Alternatively, deprotonation of **12** with NaH followed by quenching with *n*-PrI gave **23**.

Demethylation of the 7-methoxy group of **43**, **45**, and **46** to give the corresponding phenols **48**−**50** was effected via standard conditions (Scheme 2). Treatment of **45** and **46** with $BBr_3$ in $CH_2Cl_2$ at −78 °C followed by warming to room temperature resulted in the formation of **49** and **50**, respectively. When **43** was allowed to react under these same conditions, no identifiable products were obtained. Phenol **48** was obtained by heating **43** at 110 °C in 48% HBr for 5 h.

In an effort to incorporate both a sulfonamide and an amide at the 4′-position of the thioxanthenone, the saccharin derivative **32** was assembled by $S_N2$ displacement of tosylate from **66** with sodium saccharin and catalytic tetra-*N*-butylammonium bromide. The unstable tosylate **66** was readily prepared from tosyl chloride and hycanthone (**1**) in pyridine (Scheme 4).

Syntheses of analogues where the length of the 1-position side chain was varied was accomplished using the same standard procedures as were used for making

**Scheme 5**[a]




[a] (a) $HCOO^-NH_4^+$, $HCONHCH_3$; (b) NaOH, aq MeOH.

the (diethylamino)ethylthioxanthenones.[3] For example, the reaction of **67** (Scheme 6) with (diethylamino)-propylamine or (dimethylamino)propylamine followed by elaboration of the 4-position according to the published route[3] afforded **34** and **38**, respectively. This standard methodology failed, however, when an attempt was made to convert **74**[10] to **39** (Scheme 5). In this instance, Leuckart conditions resulted in the loss of side chain leaving only $NHCH_3$ at the 1-position. To circumvent this presumed acid-catalyzed result, the Leuckart reaction was run under neutral conditions with tetra-*N*-butylammonium formate and methylformamide. The desired formamide was obtained along with a small amount of the dealkylated product. Subsequent hydrolysis of the intermediate formamide afforded **39** (Scheme 5).

Three derivatives (**40**−**42**) with a 7-bromo substitution were prepared from the 7-bromo analogue of **4** using methodology[4] previously described (Scheme 1) for making the corresponding 7-H compounds **6**, **8**, and **12**, respectively.

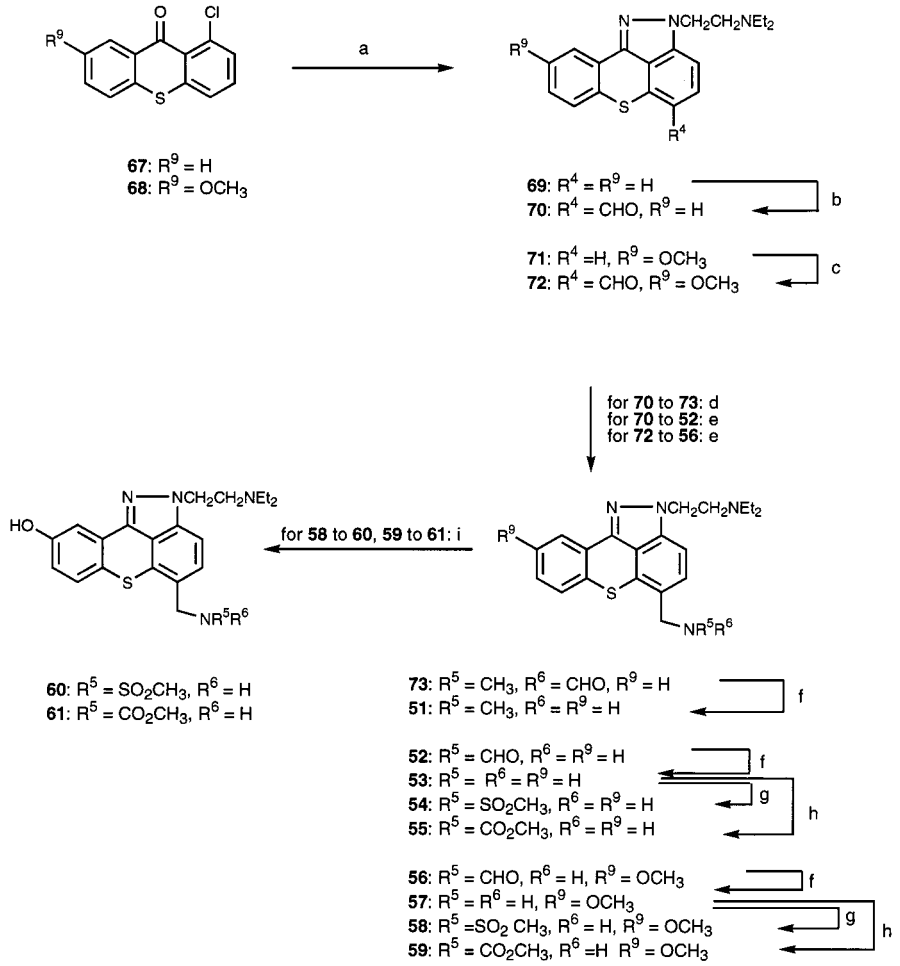
The benzothiopyranoindazole ring system, which can be considered a rigid variant of the parent 1-aminothioxanthenone derivatives, was assembled as shown in Scheme 6. Condensation of the 2-chlorothioxanthenones **67**[10] and **68**[4b] with *N*,*N*-diethylaminoethylhydrazine[11] in pyridine at 100 °C afforded **69** and **71**, respectively. Formation of the desired regioisomer is indicated by analogous chemical shifts of the ring proton at C-2 of **4** and the C-3 proton of **70**. Elaboration of the 5-position of **69** and **71** was initiated by introduction of a formyl group by two different methods. Formylation of **69** to **70** was carried out using $CHCl_2OCH_3$ and $AlCl_3$ followed by aqueous workup,[12] while reaction of **71** via a standard Vilsmeier reaction ($POCl_3$/DMF) gave **72**. From this point on, the syntheses paralleled the preparation of the thioxanthenone targets previously described in Scheme 1. A Leuckart reaction effected the transformation of aldehydes **70** to **52** and **72** to **56** using formamide and formic acid (vide supra). Using identical methodology as for the preparation of **5, 70** was converted to **73** with *N*-methylformamide and formic acid. The resulting formamide derivatives **52**, **56**, and **73** were hydrolyzed in high yield to amines **53**, **57**, and **51**, respectively, under basic conditions (NaOH, $CH_3OH$/$H_2O$). Treatment of **53** with methanesulfonyl chloride ($Et_3N$, $CH_2Cl_2$) gave sulfonamide **54**. Similarly, **58** was obtained from **57** in good yield. Treatment of **53** and **57** with methyl chloroformate ($Et_3N$, $CH_2Cl_2$) gave carbamates **55** and **59,** respectively, in good yield. Analogous to the preparation of **49** and **50**, demethylation of the 7-methoxy substituents of both **58** and **59** was effected in moderate yield with $BBr_3$ in $CH_2Cl_2$ to give **60** and **61**, respectively.

## In Vivo Properties

All compounds underwent in vivo antitumor evaluation versus subcutaneously implanted murine pancreatic adenocarcinoma 03 (Panc03)[2] regardless of their in vitro profile versus topoisomerase II, P388, or as intercalators. Tumor growth inhibition as expressed as a %T/C value was measured at the maximum tolerated dose (MTD) for each compound dosed intravenously; these data and a more detailed description of these and other terms are found in Table 1. Two other measures of antitumor efficacy, long-term cures (LTC) and the clonogenic log cell kill (LCK), are also presented in Table 1. Long-term cures require a minimum of 86 tumor free days and an LCK > 4.5. Within both series of compounds (thioxanthenones **6**−**50** and benzothiopyranoindazoles **51**−**61**) in vivo activity versus Panc03 was generally high with 32 of 57 compounds showing complete tumor growth inhibition (%T/C = 0). Long-term cures were obtained in 27 of those 32 cases. In general, this activity is similar to that of adriamycin (%T/C = 0, with 1 LTC out of 5 test mice) and superior to that of hycanthone (%T/C = 4, no cures). The MTDs ranged from 18 mg/kg for **61** to 2594 mg/kg for **19**. The MTD, defined as the $LD_{10}$ or less, did not correlate with LTC or LCK in vivo. With the exception of phenolic derivatives **48**−**50**, **60**, and **61**, all other compounds tested required a higher total dose (i.e., less potent) than adriamycin and mAMSA, as measured by the MTD. Except for **53,** the most potent compounds (**48**−**50**, **52**−**55**, **60**, and **61**) (MTDs < 100) were not curative although significant antitumor activity was observed. The log cell kill ranged from <0.5 to 8.8 (cures were excluded from this calculation). In general, cures represent >4.5 log kill in this tumor system. Compounds which were curative represent an improvement over hycanthone (**1**) and mAMSA which were not curative in this model. Within the entire series (**6**−**61**) the range of %T/C values, MTDs, the number of LTCs, or LCKs did not correlate with structural modifications.

Substitution of the 4′-position nitrogen of the thioxanthenone ring system (**6**−**50**, Table 1) with a variety of functional groups resulted in a large percentage of compounds (26 of 47) displaying high activity (%T/C < 10) with 22 compounds providing curative activity. Particularly effective (%T/C = 0) were sulfonamides **12**−**14** and **21**−**25**; however, not all sulfonamides (e.g., **15**, **16**, and **18**) were active. While there is no discernible trend in the activity of the sulfonamide derivatives, phenylsulfonamides having a para substituent other than H (e.g., **15**−**18** and **20**) were generally the least active. Formamides **7**, **9**, and **44** were also highly active. Basic amines **6**, **8**, and **10** were curative while **11** was inactive. The inactivity of the NHPh derivative **11** is probably not related to steric bulk of the phenyl ring since benzenesulfonamide derivative **14** was highly active. The known tertiary amine **33**[13] was inactive. While the acetamide **26** was curative, the trifluoroacetamido derivative **27** and benzamide **28** displayed only moderate activity (%T/C = 21 and 26, respectively). A methyl carbamate analogue, **29**, was also highly active and provided an exceptionally high LCK of 8.8. Since both methylsulfonamide **12** and acetamide **26** displayed excellent activity, a saccharin derivative (**32**) which incorporates both an amide and a sulfonamide moiety

**Scheme 6**[a]




[a] (a) $H_2NNHCH_2CH_2NEt_2$, pyridine; (b) $CHCl_2OCH_3$, $AlCl_3$, $CH_2Cl_2$; (c) $POCl_3$, DMF; (d) $HCONHCH_3$, HCOOH; (e) $HCONH_2$, HCOOH; (f) NaOH, $CH_3OH$; (g) $CH_3SO_2Cl$, pyridine; (h) $CH_3OCOCl$, $Et_3N$, $CH_2Cl_2$; (i) $BBr_3$, $CH_2Cl_2$.

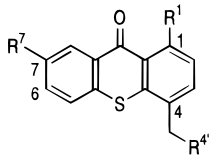
was prepared. Unfortunately **32** displayed no antitumor activity at its MTD of 740 mg/kg. A phthalimide derivative, **31**, showed marginal activity (%T/C = 38).

The benefits of 7-methoxy and 7-hydroxy substitutions were suggested by the reported activity of similarly substituted hycanthone[4b] and ellipticine derivatives.[14,15] While maintaining groups at the 4′-position that had previously shown good activity (i.e., methylamino, formamido, methylsulfonamido, and carbamoyl), incorporation of the 7-methoxy substituent provided compounds **43**−**47** which displayed uniformly high activity and were very well-tolerated with rapid host recovery times. Demethylation of **43**, **45**, and **46** gave phenols **48**−**50**, respectively, which retained significant activity; however, maximum tolerated doses dropped 4−7-fold, and the agents were poorly tolerated with slow host recovery. None of these phenols were curative. Replacement of methoxy at the 7-position with bromine abolished activity in **40**−**42**.

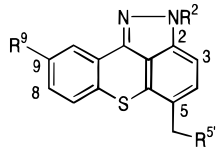
Varying the (*N*,*N*-dialkylamino)alkylamino side chain at the 1-position, while maintaining known active substituents at the 4′-position, provided compounds **34**−**39** that were generally less active in vivo than the 1-(2-diethylamino)ethylamino derivatives (**6**−**33**). Only sulfonamide **37** was curative although **38** (propyl versus ethyl side chain) also gave %T/C = 0. N-Methylation of the highly active derivative **6** provided **39** which was inactive. This observation could be rationalized as follows: The putative active conformation of these agents is stabilized by an intramolecular H-bond between the N−H (donor) at position 1 and the carbonyl group (acceptor) of the thioxanthenone ring. This planar conformation locks the amine into conjugation with the aromatic ring which in turn may affect the reactivity of the 4-methylene group (e.g., enhance the leaving group ability of the 4′-substituent in an $S_N2$ alkylation of a biological nucleophile). The additional methyl group of **39** obviously cannot form such an H-bond; thus the planar conformation is relatively destabilized resulting in loss of activity.

Incorporation of a pyrazolo ring into the thioxanthenone ring provided **51**−**61** (Table 1) which did not significantly alter the in vivo properties of the series. In this 5-aminomethylbenzothiopyranoindazole series, all compounds displayed high levels of activity. In fact, with the exception of two analogues, **53** and **61**, %T/C values were zero indicating complete growth inhibition. For identical $R^7$ and $R^4$ substituents, changing from the thioxanthenone ring system to the benzothiopyranoindazole system did not appreciably change in vivo efficacy in most cases. Pyrazolo analogues **51**−**55** of thioxanthenones **6**−**8**, **12**, and **29**, respectively, shared the same excellent in vivo efficacy; however, activity was not curative. The benzothiopyranoindazole derivatives were

**Table 1.** Physical and Antitumor Properties of 4-Aminomethylthioxanthenones and 5-Aminomethylbenzothiopyranoindazoles



**6-50**

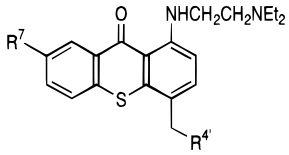


**51-61**

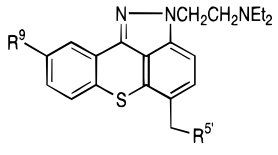
| | | | | | | murine antitumor activity vs Panc03[a] | | | |
|---|---|---|---|---|---|---|---|---|---|
| compd | $R^7$ | $R^{4'}$ | $R^1$ | mp, °C | formula[b] | %T/C[c] | MTD[d] | LTC[e] | LCK[f] |
| **1** | H | OH | $NHCH_2CH_2NEt_2$ | | | 4 | 290 | 0/3 | 3.4 |
| adriamycin | | | | | | 0 | 18 | 1/5 | 2.9 |
| mAMSA | | | | | | 0 | 48 | 0/5 | 1.8 |
| **6** | H | $NHCH_3$ | $NHCH_2CH_2NEt_2$ | 237−239 | $C_{21}H_{27}N_3OS\cdot 2HCl\cdot {}^1/_2H_2O$ | 0 | 800 | 1/3 | 3.1 |
| **7** | H | NHCHO | $NHCH_2CH_2NEt_2$ | 154−155 | $C_{21}H_{25}N_3O_2S$ | 0 | 576 | 1/5 | 3.0 |
| **8** | H | $NH_2$ | $NHCH_2CH_2NEt_2$ | 270−272 | $C_{20}H_{25}N_3OS\cdot 2HCl$ | 0 | 270 | 4/5 | >4 |
| **9** | H | N(Et)CHO | $NHCH_2CH_2NEt_2$ | 75−77 | $C_{23}H_{29}N_3O_2S\cdot CH_3SO_3H\cdot {}^1/_2H_2O$ | 0 | 431 | 2/5 | 1.1 |
| **10** | H | NHEt | $NHCH_2CH_2NEt_2$ | >160 dec | $C_{22}H_{29}N_3OS\cdot 2HCl$ | 0 | 448 | 0/5 | 1.8 |
| **11** | H | $NHC_6H_5$ | $NHCH_2CH_2NEt_2$ | 133−135 | $C_{26}H_{29}N_3OS$ | *g* | 674 | | |
| **12** | H | $NHSO_2CH_3$ | $NHCH_2CH_2NEt_2$ | 169−170 | $C_{21}H_{27}N_3O_3S_2$ | 0 | 124 | 3/5 | 2.0 |
| **13** | H | $NHSO_2Et$ | $NHCH_2CH_2NEt_2$ | >135 dec | $C_{22}H_{29}N_3O_3S_2\cdot CH_3SO_3H$ | 0 | 203 | 0/6 | 3.7 |
| **14** | H | $NHSO_2C_6H_5$ | $NHCH_2CH_2NEt_2$ | 164−167 | $C_{26}H_{29}N_3O_3S_2\cdot CH_3SO_3H$ | 0 | 840 | 1/5 | 2.9 |
| **15** | H | $NHSO_2$−4-$CH_3$−$C_6H_4$ | $NHCH_2CH_2NEt_2$ | >58 dec | $C_{27}H_{31}N_3O_3S_2\cdot CH_3SO_3H\cdot {}^3/_2H_2O$ | *g* | >1532 | | |
| **16** | H | $NHSO_2$−4-Cl−$C_6H_4$ | $NHCH_2CH_2NEt_2$ | >57 dec | $C_{26}H_{28}ClN_3O_3S_2\cdot CH_3SO_3H\cdot {}^3/_2H_2O$ | *g* | >1500 | | |
| **17** | H | $NHSO_2$−3,4-Cl−$C_6H_3$ | $NHCH_2CH_2NEt_2$ | >109 dec | $C_{26}H_{27}Cl_2N_3O_3S_2\cdot CH_3SO_3H\cdot {}^1/_2H_2O$ | 13 | 654 | 1/5 | *h* |
| **18** | H | $NHSO_2$−4-F−$C_6H_4$ | $NHCH_2CH_2NEt_2$ | 118−128 | $C_{26}H_{28}FN_3O_3S_2$ | *g* | >1597 | | |
| **19** | H | $NHSO_2$−2-F−$C_6H_4$ | $NHCH_2CH_2NEt_2$ | 125−127 | $C_{26}H_{28}FN_3O_3S_2$ | 5 | 2594 | 1/5 | 2.6 |
| **20** | H | $NHSO_2$−4-$CH_3O$−$C_6H_4$ | $NHCH_2CH_2NEt_2$ | 133−137 | $C_{27}H_{31}N_3O_4S_2\cdot CH_3SO_3H$ | 20 | 1212 | 0/5 | h |
| **21** | H | $N(CH_3)SO_2CH_3$ | $NHCH_2CH_2NEt_2$ | 175−177 | $C_{22}H_{29}N_3O_3S_2$ | 0 | 304 | 1/5 | 2.5 |
| **22** | H | $N(Et)SO_2CH_3$ | $NHCH_2CH_2NEt_2$ | 172−176 | $C_{23}H_{31}N_3O_3S_2$ | 0 | 254 | 3/5 | 3.1 |
| **23** | H | $N(nPr)SO_2CH_3$ | $NHCH_2CH_2NEt_2$ | 42−43 | $C_{24}H_{33}N_3O_3S_2$ | 0 | 552 | 2/5 | 3.2 |
| **24** | H | $N(CH_3)SO_2Et$ | $NHCH_2CH_2NEt_2$ | 159−161 | $C_{23}H_{31}N_3O_3S_2\cdot CH_3SO_3H$ | 0 | 465 | 4/5 | 5.5 |
| **25** | H | $N(CH_3)SO_2C_6H_5$ | $NHCH_2CH_2NEt_2$ | 171−174 | $C_{27}H_{31}N_3O_3S_2\cdot CH_3SO_3H$ | 6 | 2403 | 2/5 | 1.0 |
| **26** | H | $NHCOCH_3$ | $NHCH_2CH_2NEt_2$ | 182−183 | $C_{22}H_{27}N_3O_2S$ | 0 | 540 | 3/5 | 2.7 |
| **27** | H | $NHCOCF_3$ | $NHCH_2CH_2NEt_2$ | 152−154 | $C_{22}H_{24}N_3F_3O_3S_2$ | 21 | 1171 | 0/6 | 1.5 |
| **28** | H | $NHCOC_6H_5$ | $NHCH_2CH_2NEt_2$ | 161−163 | $C_{27}H_{29}N_3O_2S$ | 26 | 252 | 1/5 | 0.5 |
| **29** | H | $NHCO_2CH_3$ | $NHCH_2CH_2NEt_2$ | 129−131 | $C_{22}H_{27}N_3O_3S$ | 0 | 246 | 2/5 | 8.8 |
| **30** | H | $NHPO(OEt)_2$ | $NHCH_2CH_2NEt_2$ | 108−110 | $C_{24}H_{34}N_3O_4PS$ | 36 | 1298 | 0/5 | <0.5 |
| **31** | H | 2-phthalimidoyl | $NHCH_2CH_2NEt_2$ | 212−214 | $C_{28}H_{27}N_3O_3S\cdot CH_3SO_3H\cdot {}^1/_2H_2O$ | 38 | 2201 | 0/5 | *h* |
| **32** | H | 2-saccharinyl | $NHCH_2CH_2NEt_2$ | >103 dec | $C_{27}H_{27}N_3O_4S_2\cdot CH_3SO_3H$ | *g* | 740 | | |
| **33** | H | $NEt_2$ | $NHCH_2CH_2NEt_2$ | 205−207[13] | $C_{24}H_{33}N_3OS\cdot 2HCl$ | *g* | 1405 | | |
| **34** | H | $NHCH_3$ | $NHCH_2CH_2CH_2NEt_2$ | 222−224 | $C_{22}H_{29}N_3OS\cdot 2HCl\cdot {}^3/_2H_2O$ | 17 | 1060 | 0/5 | 1.0 |
| **35** | H | $NHCH_3$ | $NHCH_2CH_2NMe_2$ | >177 dec | $C_{19}H_{23}N_3OS\cdot 2HCl\cdot {}^5/_4H_2O$ | 17 | 608 | 0/5 | 1.6 |
| **36** | H | $NHCH_3$ | $NHCH_2CH_2CH_2NMe_2$ | 228−229 | $C_{20}H_{25}N_3OS\cdot 2HCl\cdot H_2O$ | 16 | 952 | 0/5 | 1.8 |
| **37** | H | $NHSO_2CH_3$ | $NHCH_2CH_2NMe_2$ | >168 dec | $C_{19}H_{23}N_3O_3S_2\cdot CH_3SO_3H$ | 0 | 160 | 1/5 | 3.0 |
| **38** | H | $NHSO_2CH_3$ | $NHCH_2CH_2CH_2NMe_2$ | >103 dec | $C_{20}H_{25}N_3O_3S_2\cdot CH_3SO_3H\cdot {}^1/_2H_2O$ | 0 | 256 | 0/5 | 5.1 |
| **39** | H | $NHCH_3$ | $N(CH_3)CH_2CH_2NEt_2$ | oil | $C_{22}H_{29}N_3OS\cdot {}^1/_4H_2O$ | *g* | 1506 | | |
| **40** | Br | $NHCH_3$ | $NHCH_2CH_2NEt_2$ | oil | $C_{21}H_{26}BrN_3OS$ | *g* | 345 | | |
| **41** | Br | $NH_2$ | $NHCH_2CH_2NEt_2$ | 79−82 | $C_{20}H_{24}BrN_3OS$ | *g* | 967 | | |
| **42** | Br | $NHSO_2CH_3$ | $NHCH_2CH_2NEt_2$ | 134−139 | $C_{21}H_{26}BrN_3O_3S_2$ | *g* | 1281 | | |
| **43** | $OCH_3$ | $NHCH_3$ | $NHCH_2CH_2NEt_2$ | 55−56 | $C_{22}H_{29}N_3O_2S$ | 0 | 880 | 1/5 | 5.1 |
| **44** | $OCH_3$ | NHCHO | $NHCH_2CH_2NEt_2$ | 95−99 | $C_{22}H_{27}N_3O_3S$ | 0 | 763 | 5/5 | >4.5 |
| **45** | $OCH_3$ | $NHSO_2CH_3$ | $NHCH_2CH_2NEt_2$ | >144 dec | $C_{22}H_{29}N_3O_4S_2$ | 0 | 248 | 1/5 | 6.5 |
| **46** | $OCH_3$ | $NHCO_2CH_3$ | $NHCH_2CH_2NEt_2$ | 139−140 | $C_{23}H_{29}N_3O_4S$ | 0 | 240 | 5/5 | >4.5 |
| **47** | $OCH_3$ | $NHSO_2C_6H_5$ | $NHCH_2CH_2NEt_2$ | 134−137 | $C_{27}H_{31}N_3O_4S_2$ | 6 | 382 | 0/3 | *h* |
| **48** | OH | $NHCH_3$ | $NHCH_2CH_2NEt_2$ | 167−169 | $C_{21}H_{27}N_3O_2S$ | 0 | 60 | 0/5 | 4.6 |
| **49** | OH | $NHSO_2CH_3$ | $NHCH_2CH_2NEt_2$ | >78 dec | $C_{21}H_{27}N_3O_4S_2\cdot {}^3/_4H_2O$ | 11 | 47 | 0/5 | 3.7 |
| **50** | OH | $NHCO_2CH_3$ | $NHCH_2CH_2NEt_2$ | 149−157 | $C_{22}H_{27}N_3O_4S$ | 12 | 34 | 0/5 | 3.0 |
| | $R^9$ | $R^{5'}$ | $R^2$ | | | | | | |
| **51** | H | $NHCH_3$ | $CH_2CH_2NEt_2$ | 189−193 | $C_{21}H_{26}N_4S\cdot 2HCl\cdot {}^3/_4H_2O$ | 0 | 326 | 0/5 | 3.0 |
| **52** | H | NHCHO | $CH_2CH_2NEt_2$ | 160−161 | $C_{21}H_{24}N_4OS$ | 0 | 81 | 0/5 | 2.4 |
| **53** | H | $NH_2$ | $CH_2CH_2NEt_2$ | 197−199.5 | $C_{20}H_{24}N_4S\cdot HCl\cdot {}^1/_2H_2O$ | 25[i] | 72 | 0/5 | *h* |
| **54** | H | $NHSO_2CH_3$ | $CH_2CH_2NEt_2$ | 119−123 | $C_{21}H_{26}N_4O_2S_2\cdot {}^1/_2H_2O$ | 0 | 72 | 0/5 | 2.3 |
| **55** | H | $NHCO_2CH_3$ | $CH_2CH_2NEt_2$ | 130−132 | $C_{22}H_{26}N_4O_2S$ | 0 | 78 | 0/5 | 2.5 |
| **56** | $OCH_3$ | NHCHO | $CH_2CH_2NEt_2$ | 125−131 | $C_{22}H_{26}N_4O_2S\cdot {}^1/_4H_2O$ | 0 | 231 | 3/5 | 3.6 |
| **57** | $OCH_3$ | $NH_2$ | $CH_2CH_2NEt_2$ | 74−76 | $C_{21}H_{26}N_4OS$ | 0[i] | 240 | 3/4 | >4.5 |
| **58** | $OCH_3$ | $NHSO_2CH_3$ | $CH_2CH_2NEt_2$ | 125−126 | $C_{22}H_{28}N_4O_3S_2$ | 0 | 208 | 3/5 | 2.2 |
| **59** | $OCH_3$ | $NHCO_2CH_3$ | $CH_2CH_2NEt_2$ | 125−127 | $C_{23}H_{28}N_4O_3S$ | 0[i] | 170 | 4/4 | >4.5 |
| **60** | OH | $NHSO_2CH_3$ | $CH_2CH_2NEt_2$ | 215−218 | $C_{21}H_{26}N_4O_3S_2\cdot {}^1/_4H_2O$ | 0[i] | 31 | 0/5 | 2.0 |
| **61** | OH | $NHCO_2CH_3$ | $CH_2CH_2NEt_2$ | 239−240 | $C_{22}H_{26}N_4O_3S$ | 13[i] | 18 | 0/5 | *h* |

[a] See ref 2 for the methods of tumor implantation, end point determination, and quantification. Mice were implanted bilaterally sc on day 0 with 30-60-mg tumor fragments, and chemotherapy (iv) was started 3 days later. Animal use was approved by the Wayne State University IACUC. [b] Proton NMR, IR, and mass spectra were consistent with the assigned structures of all new compounds. Carbon, hydrogen, and nitrogen elemental analyses were obtained for all new targets and most intermediates and were within ±0.4% of the theoretical values. [c] T/C value, tumor growth inhibition, where T is the median tumor burden in the treatment group × 100 at evaluation and C is the median tumor burden in the control group at evaluation. [d] MTD, maximum tolerated dose administered intravenously in mg/kg. [e] LTC, long-term cures, the number of mice in a treatment group with no palpable tumor evident after a minimum of 86 days/total number in treatment group. [f] LCK, log cell kill of tumor-bearing mice (cures excluded), a calculation based on tumor growth delay; cures require >4.5 log kill. [g] Not active, %T/C > 42. [h] Not calculated. [i] First dose administered 4 days after implantation.

**Table 2.** In Vitro Properties of 4-Aminomethylthioxanthones and Benzothiopyranoindazoles




**6-50**




**51-61**

| compd | $R^7$ | $R^{4'}$ | cytotoxicity[a] $IC_{50}$, $\mu$M | topo II[b] $EC_{50}$, $\mu$M | intercalation[c] $EC_{50}$, $\mu$M |
|---|---|---|---|---|---|
| **1** | H | OH | 28 | *d* | 0.80 |
| adriamycin | | | 0.83 | *d* | *d* |
| mAMSA | | | 0.15 | 0.72 | 11 |
| **6** | H | $NHCH_3$ | 9.5 | >270 | 0.42 |
| **7** | H | NHCHO | 12 | 510[e] | 17 |
| **8** | H | $NH_2$ | 11 | 90[e] | 0.48 |
| **12** | H | $NHSO_2CH_3$ | 0.30 | 3.0[e] | 18 |
| **29** | H | $NHCO_2CH_3$ | 0.86 | 20 | 2.5 |
| **43** | $OCH_3$ | $NHCH_3$ | 21 | >500 | 0.65 |
| **44** | $OCH_3$ | NHCHO | 106 | >480 | 3.0 |
| **45** | $OCH_3$ | $NHSO_2CH_3$ | 82 | >210 | 4.4 |
| **46** | $OCH_3$ | $NHCO_2CH_3$ | 49 | >220 | 37 |
| **48** | OH | $NHCH_3$ | 0.024 | 10[e] | 3.1 |
| **49** | OH | $NHSO_2CH_3$ | 0.025 | 0.50 | 2.2 |
| **50** | OH | $NHCO_2CH_3$ | 0.022 | 1.6 | *d* |
| | $R^9$ | $R^{5'}$ | | | |
| **51** | H | $NHCH_3$ | 16 | >220 | 0.18 |
| **52** | H | NHCHO | 0.33 | >260 | 1.5 |
| **53** | H | $NH_2$ | 5.8 | >250 | 0.12 |
| **54** | H | $NHSO_2CH_3$ | 0.15 | >230 | 1.8 |
| **55** | H | $NHCO_2CH_3$ | 0.5 | >240 | 2.6 |
| **56** | $OCH_3$ | NHCHO | 55 | >240 | 1.9 |
| **57** | $OCH_3$ | $NH_2$ | 13 | >260 | 0.13 |
| **58** | $OCH_3$ | $NHSO_2CH_3$ | 30 | >210 | 1.6 |
| **59** | $OCH_3$ | $NHCO_2CH_3$ | 45 | >220 | 0.79 |
| **60** | OH | $NHSO_2CH_3$ | 0.0044 | 0.55[e] | 2.4 |
| **61** | OH | $NHCO_2CH_3$ | 0.011 | 0.33[e] | 1.5 |

[a] In vitro cytotoxicity was measured by quantifying clonogenic survival in soft agar following a 1-h transient exposure of P388 mouse leukemia cells to drug. The $IC_{50}$ is the concentration of drug which reduced clonogenic survival by 50%. $IC_{50}$ values were an average of a minimum of two separate tests which were generally within 2−3-fold agreement with one another. [b] Topoisomerase II inhibition: the quantification of topoisomerase II covalent complex formation between $^{32}$P-end-labeled pB*R*322 DNA and extensively purified HeLa cell topo II was determined by the method of Trask and Miller (ref 19) as modified and fully described in ref 16. $EC_{50}$ values were calculated to be the concentration of test compound at which the amount of DNA precipitated was equivalent to 50% of the maximum precipitated by mAMSA in a concomitant control experiment. The $EC_{50}$ for each test compound is based on the average of at least two tests which were generally within 2-fold of one another. However, reproducibility was not as great for strong intercalators. [c] DNA intercalation: a known ethidium bromide displacement assay was used to determine intercalation potency (see ref 16). The $EC_{50}$ value is the concentration of test agent that causes a 50% reduction in the fluorescence of the calf thymus DNA/ethidium bromide complex. [d] Not determined. [e] Bell-shaped dose−response curve (see ref 20).

generally less potent than their corresponding thioxanthenone analogues.

## In Vitro Properties

Selected compounds were evaluated in vitro for cytotoxicity against a P388 leukemia cell line,[16] as intercalators in calf thymus DNA,[17] and as inhibitors of human topoisomerase II[16] (Table 2). In vitro cytotoxic potency spanned 4 orders of magnitude from 0.0044 $\mu$M for **60** to 106 $\mu$M for **44**. Activity of the methoxy derivatives **43**−**46** and **56**−**59** was similar to that of **1**, but they were consistently less active than the corresponding unsubstituted derivatives **6**, **7**, **12**, **29**, and **51**−**55**. Hydroxy derivatives **48**−**50**, **60**, and **61** had similar potency to each other and were more cytotoxic than mAMSA and adriamycin. There is little or no correlation between the in vitro P388 data and in vivo activity, although there is some correlation between the P388 cytotoxicity and the in vivo MTD. The most potent compounds versus P388 were the hydroxy derivatives **48**−**50**, **60**, and **61** which also displayed the lowest MTDs of all the compounds studied. The P388 cytotoxicity data for the benzothiopyranoindazole series (**51**−**56** and **59**−**61**) did not correlate with the cytotoxicity data for similarly substituted thioxanthenone analogues (**6**−**8**, **12**, **29**, **44**−**46**, **49**, and **50**, respectively).

Recent molecular dynamics calculations suggest that increased intercalative binding energy enhances antitumor activity.[18] As might be expected compounds with basic amine substituents attached at the pendant 4′-methylene carbon, **6**, **8**, **43**, **51**, and **53**, are more potent intercalators than their nonbasic counterparts probably due to the additional site of electrostatic attraction between the phosphate backbone of DNA and the protonated nitrogen atom. For unknown reasons, the 7-OH substituent of **48** diminishes the intercalative binding relative to the corresponding 7-$OCH_3$ (**43**) and 7-H (**6**) derivatives. In general, benzothiopyranoindazole analogues were more potent DNA intercalating agents than their thioxanthenone counterparts. The potency difference was as great as 47-fold as seen with the pair **59** and **46**.

As shown in Table 2, most analogues did not act as topo II poisons in the assay system used. The only nonphenolic compound that approached the potency of mAMSA ($IC_{50}$ = 0.72 $\mu$M) was **12** having an $IC_{50}$ value of 3.0 $\mu$M. However, in both the benzothiopyranoindazole and thioxanthenone series, the phenolic substitution had a profound effect on topo II inhibition. Potency for phenolic compounds having the methanesulfonamido (**49** and **60**) and methylcarbamido (**50** and **61**) groups was very close to that of the standard, mAMSA. It is unclear why the addition of the OH group is so beneficial.

## Conclusions

Within these two series of new antitumor agents, the only group that has a substantial impact on structure−cytotoxicity function is the phenolic OH group (7-position for the thioxanthenones or 9-position for the benzothiopyranoindazoles); these compounds (**48**−**50**, **60**, and **61**) displayed greatly enhanced P388 cytotoxicity, topo II inhibition potency, and DNA binding relative to all other analogues. Otherwise, no relationship between structure and topoisomerase II inhibition, P388 cytotoxicity, or DNA binding was discernible. Ring substituents (e.g., $OCH_3$) and the 4-aminomethyl appendage that contributed to the high in vivo activity of the thioxanthenones (i.e., **6**) also contributed to the activity of the benzothiopyranoindazoles (e.g., **51**). Within both series, in vivo activity was observed in analogues having varied physicochemical properties; thus no clear relationship evolved between structure and activity.

In vivo efficacy did not correlate with in vitro P388 cytotoxicity. Of note is the fact that the 7-$OCH_3$

**Table 3.** In Vivo Activity (%T/C) of SR 233377 (**12**) versus Murine Solid Tumors[a]

| | Panc02 | Mam 16/C | Colon 38 | Colon 26/A | Colon 51 |
|---|---|---|---|---|---|
| SR 233377 (**12**) | 23 | 6 | 0[b] | 29 | 14 |
| adriamycin | 35 | 0[b] | 0 | 7 | 21 |
| cytoxan | 36 | 0 | *c* | 8 | *c* |

[a] See refs 2 and 21 for the methods of tumor implantation, end point determination, and quantification. %T/C value, tumor growth inhibition, where T is the median tumor burden in the treatment group × 100 at evaluation and C is the median tumor burden in the control group at evaluation. Mice were implanted bilaterally sc on day 0 with 30-60-mg tumor fragments, and chemotherapy (iv) was started 3 days later. [b]Curative activity. [c] Not tested.

derivatives **43**−**46** in the thioxanthenone series and the three 9-$OCH_3$ analogues in the benzothiopyranoindazole series (**56**−**58**) showed exceptional in vivo activity (%T/C = 0 with long-term cures) despite being weakly potent in the P388 cytotoxicity assay. It is likely that for the phenolic derivatives **48**−**50**, **60**, and **61**, their inherent potent topoisomerase II inhibition properties and associated in vitro P388 cytotoxicity account for the excellent in vivo activity (%T/C ≤ 13) observed for these analogues. However, these phenolic derivatives were clearly less active and less well-tolerated than the corresponding methoxy derivatives. For compounds with moderate P388 cytotoxicity (e.g., 7-H derivatives **12** and **29** and 9-H analogues **52, 54,** and **55),** it is unclear whether their inherent cytotoxicity or metabolism of parent drug to a more cytotoxic species is responsible for the observed highly efficacious in vivo activity.

The methoxy derivatives **43**−**46** and **56**−**59** displayed a very low level of P388 in vitro cytotoxicity and topoisomerase II inhibition. Nonetheless, they exhibited a high level of antitumor activity in in vivo murine tumor models. It is likely that this activity is a consequence of metabolism to an active topo II inhibitor, presumed to be the corresponding 7(or 9)-OH derivatives (e.g., **45** → **49** and **59** → **61**). The consistently high levels of in vivo activity associated with most of these compounds render it difficult to elucidate a structure−activity relationship particularly in the absence of pharmacokinetic and tumor cell/normal cell metabolism data.

In conclusion, two related series of hycanthone derivatives have been prepared and evaluated in vivo and in vitro as antitumor agents. Both series were shown to be efficacious and broadly active in vivo but displayed varying in vitro properties. This work has led to the clinical development of SR 233377 (**12**) based on its breadth of in vivo activity and favorable toxicological profile. The selection of SR 233377 was based not only on its in vivo Panc03 and P388 in vitro cytotoxicity data but also on a favorable comparison with adriamycin and cytoxan across a range of in vivo solid tumor models. As summarized in Table 3, SR 233377 was active against the five murine solid tumors Panc 02, Mam 16/C, Colon 38, Colon 26/A, and Colon 51. As previously reported,[21] SR 233377 showed no significant in vivo activity against adriamycin-resistant or adriamycin-sensitive P388.

## Experimental Section

**General.** Melting points were determined on a Mel-Temp melting point apparatus in open capillaries and are uncorrected. Proton NMR spectra, obtained on a GE QE 300 NMR spectrometer, and chemical ionization mass spectra, obtained on a Nermag R 10-10 C spectrometer, were consistent with the assigned structures. Proton NMR multiplicity data are denoted by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Coupling constants are reported in hertz. Combustion analyses (C, H, N) were performed by Quantitative Technologies, Inc. (Whitehouse, NJ) and were within 0.4% of theoretical values. Reactions were performed under an $N_2$ atmosphere.

***N*-[[1-[[2-(Diethylamino)ethyl]amino]-9-oxo-9*H*-thioxanthen-4-yl]methyl]-*N*-methylformamide (5).** A solution of **4** (35 g, 0.10 mol) in *N*-methylformamide (394 mL) and formic acid (50 mL) was heated at 160 °C for 1 h, allowed to cool to ambient temperature, and poured into water (2 L). The aqueous solution was made basic with 35% aqueous NaOH; the precipitate was collected and dried in vacuo. Recrystallization from acetone afforded pure **5** (24.6 g, 62%): mp 127−130 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.40 (m, 1H), 8.50 and 8.18 (s and s, 1H, amide rotamers), 7.62−7.36 (m, 4H), 7.31−7.20 (m, 1H), 6.60 (dd, 1H), 4.65 and 4.48 (s and s, 2H, amide rotamers), 3.35 (q, 2H), 3.80 (m, 5H), 2.64 (q, 4H), 1.09 (t, 6H). Anal. ($C_{22}H_{27}N_3O_2S$) C, H, N.

***N*-[[1-[[2-(Diethylamino)ethyl]amino]-7-methoxy-9-oxo-9*H*-thioxanthen-4-yl]methyl]formamide (44)** was prepared in 69% yield from **63** via the same procedure as for **5** except formamide was used instead of *N*-methylformamide. The crude material was purified by flash chromatography (1% isopropylamine in 1:1 $CHCl_3$/hexane): mp 95−99 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.37 (br t, 1H), 8.28 (d, 1H), 7.97 (d, 1H), 7.37 (dd, 2H), 7.15 (dd, 1H), 6.54 (d, 1H), 4.56 and 4.45 (d and d, 2H, amide rotamers), 3.91 (s, 6H), 3.32 (q, 2H), 2.82 (t, 2H), 2.64 (q, 4H), 1.09 (t, 6H); MS *m*/*z* 413 ($M^+$). Anal. ($C_{22}H_{27}N_3O_3S$) C, H, N.

***N*-[[1-[[2-(Diethylamino)ethyl]amino]-9-oxo-9*H*-thioxanthen-4-yl]methyl]methanamine (6).** A solution of **5** (9.34 g, 23 mmol) in 2 N aqueous HCl was refluxed for 3 h, allowed to cool to ambient temperature, neutralized with 35% aqueous NaOH, and chilled at 0 °C for 1 h. The resulting precipitate was collected and dried in vacuo at 80 °C. The crude product was passed through a pad of silica gel (isopropylamine/MeOH/$CHCl_3$-1:5:94). After concentration in vacuo the orange solid was dissolved in MeOH, treated with 4 N HCl/$Et_2O$, and chilled at 0 °C. Ice cold *i*-PrOH was added and the precipitate collected and dried in vacuo to yield **6** (12.81 g 58%) as the dihydrochloride salt: mp 237−239 °C; $^1$H NMR ($CD_3OD$) $\delta$ 8.31 (d, 1H), 7.55−7.30 (m, 4H), 6.76 (d, 1H), 4.14 (s, 2H), 4.73 (t, 2H), 3.27−3.05 (m, 6H), 2.58 (s, 3H), 1.26 (t, 6H). Anal. ($C_{21}H_{27}N_3OS\cdot 2HCl\cdot {}^1/_2H_2O$) C, H, N.

**[1-[[2-(Diethylamino)ethyl]amino]-7-methoxy-9-oxo-9*H*-thioxanthen-4-yl]methylamine (65).** A solution of **44** (1.02 g, 2.5 mmol) in 35 mL of 2 N HCl was heated on a steam bath for 1 h. The reaction mixture was cooled to room temperature and made basic with 10% aqueous sodium hydroxide, and the product was extracted into $CHCl_3$. The organic portion was washed with water and brine, dried over $Na_2SO_4$, and concentrated in vacuo to give crude **65**. Flash chromatography (1:1 $CHCl_3$/hexanes then 1% isopropylamine in 1:1 $CHCl_3$/hexane) afforded pure **65** (0.58 g, 60%): mp 75−78 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.28 (br t, 1H), 7.99 (d, 1H), 7.41 (t, 2H), 7.18 (dd, 1H), 6.59 (d, 1H), 3.95 (s, 2H), 3.92 (s, 3H), 3.36 (m, 2H), 2.82 (m, 2H), 2.64 (q, 4H), 1.09 (t, 6H); MS *m*/*z* 386 ($MH^+$). Anal. ($C_{21}H_{27}N_3O_2S$) C, H, N.

***N*-[[1-[[2-(Diethylamino)ethyl]amino]-7-methoxy-9-oxo-9*H*-thioxanthen-4-yl]methyl]methanesulfonamide (45).** A solution of **65** (5.60 g, 14.5 mmol) in dry pyridine (75 mL) was cooled in an ice bath, and methanesulfonyl chloride (1.25 mL, 16 mmol) was added dropwise. The reaction mixture was allowed to warm to ambient temperature over 2 h, poured into ice water (800 mL), and extracted with $CHCl_3$ (4 × 200 mL). The combined extracts were washed with water (200 mL) and brine (200 mL), dried over $Na_2SO_4$, and concentrated in vacuo to afford crude **45.** Purification by flash chromatography (1:1 $CHCl_3$/hexane followed by 1% isopropylamine in 1:1 $CHCl_3$/

hexane then 1% isopropylamine in $CHCl_3$) gave a solid which was suspended in 1:1 EtOAc−hexane. The solid was collected and dried in vacuo to afford (4.86 g, 78%): mp 144 °C dec; $^1$H NMR ($CDCl_3$) $\delta$ 10.42 (br t, 1H), 7.97 (d, 1H), 7.40 (dd, 2H), 7.18 (dd, 1H), 6.59 (d, 1H), 4.43 (d, 2H), 3.94 (s, 3H), 3.41 (br q, 2H), 2.88 (m, 2H), 2.85 (s, 3H), 2.72 (q, 4H), 1.12 (t, 6H); MS *m*/*z* 463 ($M^+$). Anal. ($C_{22}H_{29}N_3O_4S_2$) C, H, N.

**General Procedure for Compounds 12−30, 46, and 47: Carbamic Acid, [[1-[[2-(Diethylamino)ethyl]amino]-9-oxo-9*H*-thioxanthe*N*-4-yl]methyl]-, Methyl Ester (29).** A solution of **8** (prepared according to the same procedure as for **65**) (2.94 g, 8.3 mmol) in $CH_2Cl_2$ (50 mL) containing triethylamine (5 mL) was cooled in an ice bath, treated with methyl chloroformate (0.7 mL, 9.1 mmol, or the corresponding chloroformate or acid chloride), and stirred for 2.5 h. The solvent was removed in vacuo, and the residue was flash-chromatographed ($CHCl_3$ then 1% isopropylamine/$CHCl_3$) to afford **29** (2.36 g, 69%): mp 129−131 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.32 (br t, 1H), 8.48 (dd, 1H), 7.50−7.28 (m, 4H), 6.56 (d, 1H), 4.43 (d, 2H), 3.69 (s, 3H), 3.33 (q, 2H), 2.79 (t, 2H), 2.63 (q, 4H); MS *m*/*z* 414 ($MH^+$). Anal. ($C_{22}H_{27}N_3O_3S$) C, H, N.

***N*-[[1-[[2-(Diethylamino)ethyl]amino]-7-hydroxy-9-oxo-9*H*-thioxanthen-4-yl]methyl]methanamine (48).** A solution of **43** (1.6 g, 4 mmol) in 48% HBr (10 mL) was heated to 110 °C for 5 h. After cooling, the reaction mixture was neutralized with saturated $NaHCO_3$ and extracted into $CHCl_3$ (3 × 100 mL). The residual dark gum from the reaction vessel was dissolved in MeOH and combined with the $CHCl_3$ solution. The solvents were removed in vacuo to afford 1.67 g of a dark-orange solid. The crude product was purified by flash chromatography (isopropylamine/MeOH/$CHCl_3$-1:1:98) followed by a second silica gel column eluting with isopropylamine/MeOH/$CHCl_3$ (2:2:96) to afford **48** (0.56 g, 36%): mp 167−169 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.23 (br t, 1H), 7.64 (d, 1H), 7.30 (d, 1H), 7.19 (d, 1H), 6.93 (dd, 1H), 6.43 (d, 1H), 3.88 (s, 2H), 3.30 (q, 2H), 2.84 (t, 2H), 2.69 (q, 4H), 2.57 (s, 3H), 1.12 (t, 6H). Anal. ($C_{21}H_{27}N_3O_2S\cdot{}^1/_4H_2O$) C, H, N.

***N*-[[1-[[2-(Diethylamino)ethyl]amino]-7-hydroxy-9-oxo-9*H*-thioxanthen-4-yl]methyl]methanesulfonamide (49).** To a solution of **45** (0.5 g, 1.1 mmol) in $CH_2Cl_2$ (45 mL) at −78 °C was added, dropwise, $BBr_3$ (1.75 mL). The mixture was warmed to room temperature, stirred overnight, and then poured into ice−water (250 mL) containing 35% NaOH (8 mL). The mixture was acidified with dilute HCl, neutralized with solid $Na_2CO_3$, and extracted with EtOAc. The organic layer was separated, washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was purified by column chromatography (5% MeOH/EtOAc) to afford **49** (0.28 g, 58%): mp 78 °C dec; $^1$H NMR ($CDCl_3$) $\delta$ 10.35 (br t, 1H), 10.06 (br s, 1H), 7.86 (d, 1H), 7.55 (m, 2H), 7.23 (dd, 1H), 6.74 (d, 1H), 4.27 (d, 2H), 3.39 (s, 3H), 3.35 (m, 2H), 2.77 (t, 2H), 2.58 (m, 4H), 1.11 (t, 6H); MS *m*/*z* 449 ($M^+$). Anal. ($C_{21}H_{27}N_3O_4S_2\cdot{}^3/_4H_2O$) C, H, N.

**Carbamic Acid, [[1-[[2-(Diethylamino)ethyl]amino]-7-hydroxy-9-oxo-9*H*-thioxanthen-4-yl]methyl]-, Methyl Ester (50)** was prepared from **46** in 52% yield via the same procedure as for **49**. The crude product was purified by flash chromatography (isopropylamine/MeOH/$CHCl_3$-1:1:98): mp 149−157 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.33 (br t, 1H), 7.70 (d, 1H), 7.34 (d, 1H), 6.78 (dd, 1H), 6.46 (d, 1H) 4.37 (d, 2H), 3.73 (s, 3H), 3.39 (q, 2H), 2.90 (t, 2H), 2.75 (q, 4H), 1.16 (t, 6H). Anal. ($C_{22}H_{27}N_3O_4S$) C, H, N.

***N*-[[1-[[2-(Diethylamino)ethyl]amino]-9-oxo-9*H*-thioxanthen-4-yl]methyl]phthalimide (31).** A mixture of **8** (1.67 g, 4.7 mmol) and phthalic anhydride (0.80 g, 5.4 mmol) in toluene (200 mL) was refluxed with a Dean−Stark trap for 6 h. The resulting solution was filtered through a pad of silica gel eluting with $CHCl_3$ to remove an impurity followed by 1% isopropylamine/$CHCl_3$ to afford 1.92 g (84%) of **31** as an orange solid. Recrystallization from *i*-PrOH/*i*-PrOAc/MeOH containing methanesulfonic acid afforded **31** as its methanesulfonate salt: mp 212−214 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.46 (d, 1H), 7.90 (m, 2H), 7.75 (m, 2H), 7.60−7.38 (m, 4H), 6.74 (d, 1H), 4.96 (s, 2H), 3.89 (t, 2H), 3.40−3.17 (m, 6H), 2.85 (s, 3H), 1.40 (t, 6H). Anal. ($C_{28}H_{27}N_3O_3S\cdot CH_3SO_3H\cdot{}^1/_2H_2O$) C, H, N.

***N*-[[1-[[2-(Diethylamino)ethyl]amino]-9-oxo-9*H*-thioxanthen-4-yl]methyl]-*N*-(1-propyl)sulfonamide (23).** To a mixture of NaH (0.20 g of 60% oil suspension washed with pentane) in dry DMF (40 mL) was added **12** (2.00 g, 4.61 mmol), and the resulting mixture was heated to 50 °C for 2 h. The reaction mixture was chilled in an ice bath for 15 min, treated with *N*-propyl iodide (0.87 g, 5.12 mmol), and stirred at room temperature overnight. Water was added (35 mL) with rapid stirring. The resulting precipitate was washed with additional water and dried in vacuo (50 °C) to give **23** (2.17 g, 99%): mp 142−143 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.51 (d, 1H), 7.62−7.38 (m, 4H), 6.65 (d, 1H), 4.46 (s, 2H), 3.38 (m, 2H), 3.15 (m, 2H), 2.89 (s, 3H), 2.80 (t, 2H), 2.63 (q, 4H), 1.70−1.45 (m, 2H), 1.05 (t, 6H), 0.83 (t, 3H). Anal. ($C_{24}H_{33}N_3O_3S_2$) C, H, N.

***N*-[[1-[[2-(Diethylamino)ethyl]amino]-9-oxo-9*H*-thioxanthen-4-yl]methyl]saccharin (32).** A solution of hycanthone (**1**) (2.00 g, 5.6 mmol) in dry pyridine (50 mL) at 0 °C was treated with *p*-toluenesulfonyl chloride (1.15 g, 6.0 mmol) and stirred at ambient temperature for 90 min. The resulting tosylate (**66**) was collected and dried under high vacuum. A solution of **66** (2.04 g, 4.0 mmol) with sodium saccharin (1.94 g, 9.5 mmol) and tetra-*n*-butylammonium bromide (0.20 g) in DMF (50 mL) was heated at 100 °C for 2 h. After cooling to ambient temperature the solution was diluted with $CHCl_3$ (500 mL) and filtered through a pad of silica gel. The product was eluted with 2% isopropylamine/$CHCl_3$ to give **32** (1.66 g, 80%) as a yellow solid. Recrystallization from methanol containing methanesulfonic acid afforded **32** as its methanesulfonate salt (1.35 g); mp 103 °C dec; $^1$H NMR ($CDCl_3$) $\delta$ 8.49 (d, 1H), 8.15 (m, 1H), 7.96−7.83 (m, 3H), 7.65−7.53 (m, 3H), 7.45 (m, 1H), 6.77 (d, 2H) 5.09 (s, 2H), 3.90 (t, 2H), 3.40−3.18 (m, 6H), 2.85 (s, 3H), 1.40 (t, 6H). Anal. ($C_{27}H_{27}N_3O_4S_2\cdot CH_3SO_3H$) C,H,N.

***N*-[[1-[[2-(Diethylamino)ethyl]-1-methylamino]-9-oxo-9*H*-thioxanthen-4-yl]methyl]methanamine (39).** A mixture of **74**[10] (3.05 g, 8.3 mmol) and ammonium formate (3.00 g) in *N*-methylformamide (50 mL) was heated to 160 °C for 2 h, diluted with water (400 mL), and extracted with $CHCl_3$ (5 × 100 mL). The combined extracts were flash chromatographed on silica gel ($CHCl_3$ then 2% isopropylamine/$CHCl_3$). The resulting orange gum was dissolved in MeOH (30 mL), treated with 2 N NaOH (25 mL), and refluxed for 3 h. The crude product was extracted with $CHCl_3$ and flash chromatographed (0.5% isopropylamine/1% MeOH/$CHCl_3$). Final purification by MPLC (2% $Et_3N$/$CHCl_3$) furnished **39** (0.62 g, 19%) as an orange gum: $^1$H NMR ($CDCl_3$) $\delta$ 8.21 (d, 1H), 7.51−7.42 (m, 2H), 7.38−7.27 (m, 2H), 6.88 (dd, 1H), 3.85 (s, 2H), 3.35 (t, 2H), 2.85 (s, 3H), 2.67 (t, 2H), 2.46 (q, 4H), 2.44 (s, 3H) 0.92 (t, 6H). Anal. ($C_{22}H_{29}N_3OS\cdot{}^1/_4H_2O$) C, H, N.

***N*,*N*-Diethyl-2*H*-(1)benzothiopyrano[4,3,2-*cd*]indazole-2-ethanamine (69).** A solution of **67**[10] (as a mixture with the corresponding 3-Cl isomer; 6.20 g, 25.2 mmol), *N*,*N*-diethyl-2-hydrazinoethanamine[11] (4.20 g, 32.0 mmol), and pyridine (50 mL) was heated at reflux for 24 h, and the solvent was removed in vacuo. The residue was dissolved in $CHCl_3$ and washed with water (3×). The organic layer was dried over $Na_2SO_4$ and concentrated in vacuo, and the residue was flushed through a pad of silica gel eluting with EtOAc/hexane (10−40%) to give **69** (2.42 g, 30%). Recrystallization from hexanes gave the analytical sample: mp 49−50 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.02 (dd, 1H), 7.22−7.10 (m, 4H), 6.84 (d, 1H), 6.64 (d, 1H), 4.30 (t, 2H), 2.90 (t, 2H), 2.56 (q, 4H), 1.00 (t, 6H); MS *m*/*z* 324 ($MH^+$). Anal. ($C_{19}H_{21}N_3S$) C, H, N.

**2-[2-(Diethylamino)ethyl]-2*H*-(1)benzothiopyrano[4,3,2-*cd*]indazole-5-carboxaldehyde (70).** A suspension of aluminum chloride (1.76 g, 12.8 mmol) in $CH_2Cl_2$ (25 mL) was stirred at room temperature for 15 min, and a solution of **69** (2.07 g, 6.38 mmol) in $CH_2Cl_2$ (15 mL) was added at 5 °C. The resulting mixture was stirred for 10 min and cooled to 0 °C. A solution of dichloromethyl methyl ether (1.52 g, 17.4 mmol) in $CH_2Cl_2$ (10 mL) was added dropwise over a period of 15 min. The mixture was allowed to warm to ambient temper-

ature, stirred overnight, and diluted with of 2 N HCl (10 mL) and cool water (100 mL). The mixture was poured into $CHCl_3$ (100 mL) and basified with 2 N NaOH solution to pH 8−9, and the layers were separated. The aqueous layer was extracted with $CHCl_3$ (40 mL). The combined organic layers were washed with water, dried over $Na_2SO_4$, and concentrated in vacuo to give crude **70** (2.16 g, 96%) which could be used without additional purification. The analytical sample was obtained by silica gel chromatography (EtOAc) following by recrystallization (1:2 EtOAc/hexane): mp 81−82 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.02 (s, 1H), 8.20−8.17 (m, 1H), 7.64 (d, 1H), 7.53 (m, 1H), 7.31 (m, 2H), 7.02 (d, 1H), 4.40 (t, 2H), 2.95 (t, 2H), 2.54 (q, 4H), 0.95 (t, 6H); MS *m*/*z* 352 ($MH^+$). Anal. ($C_{20}H_{21}N_3OS$) C, H, N.

***N*,*N*-Diethyl-9-methoxy-2*H*-(1)benzothiopyrano[4,3,2-*cd*]indazole-2-ethanamine (71)** was prepared from **68**[4b] (as a mixture with the corresponding 3-Cl isomer) and purified via the same procedure as for **69** in 39% yield as a yellow oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.58 (d, 1H), 7.08 (2d, 2H), 6.80 (d, 1d), 6.76 (dd, 1H), 6.62 (d, 1H), 4.30 (t, 2H), 3.82 (s, 3H), 2.90 (t, 2H), 2.56 (q, 4H), 0.98 (t, 6H); MS *m*/*z* 354 ($MH^+$). Anal. ($C_{20}H_{23}N_3OS$) C, H, N.

**2-[2-(Diethylamino)ethyl]-9-methoxy-2*H*-(1)benzothiopyrano[4,3,2-*cd*]indazole-5-carboxaldehyde (72).** To a stirred solution of **71** (1.60 g, 4.19 mmol) in DMF (45 mL) at room temperature was added dropwise a solution of phosphorus oxychloride (1.6 mL) in DMF (5 mL). The reaction mixture was heated at 95−100 °C for 18 h. Heat was removed, and ice (50 g) was added. The mixture was stirred for 10 min and basified with 2 N NaOH solution to pH 8−9. The basic solution was extracted with $CHCl_3$, and the extracts were washed with water (3×), dried over $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (hexane, hexane/EtOAc-60:40, then EtOAc) to give **72** as a yellow oil (1.06 g, 61%): $^1$H NMR ($CDCl_3$) $\delta$ 10.04 (s, 1H), 7.72−7.62 (dd, 2H), 7.48 (d, 1H), 7.06−6.96 (m, 2H), 4.42 (t, 2H), 3.94 (s, 3H), 2.96 (t, 2H), 2.56 (q, 4H), 0.98 (t, 6H); MS *m*/*z* 382 ($MH^+$). Anal. ($C_{21}H_{23}N_3O_2S\cdot{}^1/_2H_2O$) C, H, N.

***N*-[[2-[2-(Diethylamino)ethyl]-2*H*-(1)benzothiopyrano-[4,3,2-*cd*]indazol-5-yl]methyl]formamide (52).** A solution of **70** (1.64 g, 4.67 mmol), formic acid (1.8 g, 39 mmol), and formamide (30 mL) was heated at 140−150 °C for 5 h. The reaction mixture was poured into 40 mL of ice−water and basified with 2 N NaOH solution to pH 8−9. The basic aqueous solution was extracted with $CHCl_3$. The organic layer was washed with water, dried, and concentrated to give the crude product. The pure formamide was obtained by silica gel chromatography, eluting with 0.5% isopropylamine in EtOAc to afford pure **52** (1.20 g, 67%): mp 160−161 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.26 (s, 1H), 8.18 (m, 1H), 7.24 (m, 3H), 7.15 (d, 1H), 6.83 (d, 1H), 4.42 (d, 2H), 4.36 (t, 2H), 2.92 (t, 2H), 2.56 (q, 4H), 0.95 (t, 6H); MS *m*/*z* 381 ($MH^+$). Anal. ($C_{21}H_{24}N_4OS$) C, H, N.

***N*-[[2-[2-(Diethylamino)ethyl]-9-methoxy-2*H*-(1)benzothiopyrano[4,3,2-*cd*]indazol-5-yl]methyl]formamide (56)** was prepared in quantitative yield via the same procedure used to synthesize **52** and could be used without additional purification: mp 125−131 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.08 (s, 1H), 7.42 (d, 1H), 7.10 (m, 2H), 6.75 (m, 2H), 4.28 (d, 2H), 4.22 (t, 2H), 3.80 (s, 3H), 2.82 (t, 2H), 2.48 (q, 4H), 0.92 (t, 6H); MS *m*/*z* 412 ($MH^+$). Anal. ($C_{22}H_{26}N_4O_2S\cdot{}^1/_4H_2O$) C, H, N.

**2-[2-(Diethylamino)ethyl]-2*H*-(1)benzothiopyrano[4,3,2-*cd*]indazole-5-methanamine Hydrochloride Hemihydrate (53).** To a solution of **52** (2.76 g, 7.25 mmol) in MeOH (100 mL) was added 10% NaOH solution (50 mL), and the mixture was heated at reflux for 5 h. The reaction mixture was cooled to room temperature and extracted with $CHCl_3$. The organic layers were washed with water, dried over $Na_2SO_4$, and concentrated in vacuo to give the crude product. Purification by chromatography (2% $Et_3N/CHCl_3$) afforded pure **53** as a viscous oil (1.78 g 62%). Recrystallization from methanolic HCl/EtOAc (2:10) gave the analytical sample: mp 197−199.5 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.01 (m, 1H), 7.10−7.30 (m, 4H), 6.87 (d, 1H), 4.34 (t, 2H), 3.79 (s, 2H), 2.93 (t, 2H), 2.54 (q, 4H), 0.99 (t, 6H); MS *m*/*z* 353 ($MH^+$). Anal. ($C_{20}H_{24}N_4S\cdot HCl\cdot{}^1/_2H_2O$) C, H, N.

**2-[2-(Diethylamino)ethyl]-9-methoxy-2*H*-(1)benzothiopyrano[4,3,2-*cd*]indazole-5-methanamine (57)** was prepared from **56** via the same procedure as for **53**. Pure material was obtained by flash chromatography (hexane/EtOAc-1:1, followed by 100% EtOAc, followed by 100% $CH_2Cl_2$, and then 0.5−1% isopropylamine in $CH_2Cl_2$) in 90% yield: mp 74−76 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.62 (d, 1H), 7.10 (d, 1H), 6.75 (m, 2H), 4.28 (t, 2H), 3.82 (s, 3H), 3.72 (s, 2H), 2.88 (t, 2H), 2,52 (q, 4H), 0.96 (t, 6H); MS *m*/*z* 383 ($MH^+$). Anal. ($C_{21}H_{26}N_4OS$) C, H, N.

***N*-[[2-[2-(Diethylamino)ethyl]-2*H*-(1)benzothiopyrano-[4,3,2-*cd*]indazol-5-yl]-methyl]methanesulfonamide (54).** To a solution of methanesulfonyl chloride (172 mg, 1.50 mmol) in $CH_2Cl_2$ (30 mL) were added the free base of **53** (0.51 g, 1.28 mmol) and pyridine (0.4 mL) with stirring at 0 °C. After the mixture was allowed to warm to ambient temperature over 5 h, $CHCl_3$ (30 mL) and 2 N NaOH (5 mL) were added to the mixture. The organic layers were separated, washed with water, dried over $Na_2SO_4$, and concentrated in vacuo to give the crude product. Purification by chromatography (0.5% $Et_3N/CH_2Cl_2$) gave 650 mg of gum. Crystallization from ether/MeOH (90:10) gave **54** (460 mg, 84%): mp 119−123 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.06 (m, 1H), 7.19 (m, 4H), 6.90 (d, 1H), 4.48 (d, 1H), 4.38 (t, 2H), 4.26 (d, 2H), 2.92 (t, 2H), 2.84 (s, 3H), 2,58 (q, 4H), 0.98 (t, 6H); MS *m*/*z* 431 ($MH^+$). Anal. ($C_{21}H_{26}N_4O_2S_2\cdot{}^1/_2H_2O$) C, H, N.

***N*-[[2-[2-(Diethylamino)ethyl]-9-methoxy-2*H*-(1)benzothiopyrano[4,3,2-*cd*]indazol-5-yl]methyl]methanesulfonamide (58)** was prepared from **57** via the same procedure as for **54**. Flash chromatography (hexane followed by 2:3 hexane/EtOAc and then EtOAc) gave **58** as a gum (785 mg, 66%). Crystallization from ether and a few drops of $CH_2Cl_2$ provided the analytical sample: mp 125−126 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.60 (d, 1H), 7.22 (d, 1H), 7.20 (d, 1H), 6.86 (m, 2H), 4.72 (t, 1H), 4.26 (d, 2H), 3.88 (s, 3H), 2.90 (t, 2H), 2.84 (s, 3H), 2.58 (q, 4H), 0.98 (t, 6H); MS *m*/*z* 461 ($MH^+$). Anal. ($C_{22}H_{28}N_4O_3S_2$) C, H, N.

**Carbamic Acid, [[2-[2-(Diethylamino)ethyl]-2*H*-(1)benzothiopyrano[4,3,2-*cd*]indazol-5-yl]methyl]-, Methyl Ester (55).** To a solution of the free base of **53** (974 mg, 2.45 mmol) in $CH_2Cl_2$ were added methyl chloroformate (0.23 mL) and $Et_3N$ (0.8 mL) with stirring at 0 °C. The mixture was stirred for 5 h and allowed to warm to room temperature. The reaction mixture was partitioned between $CHCl_3$/water and basified to pH 10 by adding a few drops of 10% aqueous NaOH solution. The organic layer was separated, washed with water, dried over $Na_2SO_4$, and concentrated in vacuo to give crude **55**. Purification by flash chromatography (0.5% isopropylamine/EtOAc) gave pure **55** (0.62 g, 55%): mp 130−132 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.04 (m, 1H), 7.19 (m, 4H), 6.86 (d, 1H), 4.98 (d, 1H), 4.30 (m, 4H), 3.66 (s, 2H), 2.90 (t, 2H), 2.56 (q, 4H), 0.98 (t, 6H); MS *m*/*z* 411 ($MH^+$). Anal. ($C_{22}H_{26}N_4O_2S$) C, H, N.

**Carbamic Acid, [[2-[2-(Diethylamino)ethyl]-2*H*-(1)-9-methoxybenzothiopyran[4,3,2-*cd*]indazol-5-yl]methyl]-, Methyl Ester (59)** was prepared from **57** via the same procedure as for **55**. The crude product was flash chromatographed (5% MeOH/EtOAc) to afford pure **59** (1.10 g, 63%): mp 125−127 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.60 (d, 1H), 7.22 (d, 2H), 7.85 (m, 2H), 4.89 (s, 3H), 3.70 (s, 3H), 4.35 (m, 4H), 2.95 (t, 2H), 2.59 (q, 4H), 0.98 (t, 6H). Anal. ($C_{23}H_{28}N_4O_3S$) C, H, N.

***N*-[[2-[2-(Diethylamino)ethyl]-9-hydroxy-2*H*-(1)benzothiopyrano[4,3,2-*cd*]indazol-5-yl]methyl]methanesulfonamide (60).** A solution of **58** (1.02 g, 2.22 mmol) in $CH_2Cl_2$ (50 mL) was cooled to −50 °C and treated dropwise with $BBr_3$ (15 mL of 1 M solution in $CH_2Cl_2$, 15 mmol) forming a thick orange suspension. The mixture was allowed to warm to −10 °C and stirred for an additional 2 h. The reaction mixture was diluted with $CH_2Cl_2$ (100 mL) and the reaction quenched with MeOH (10 mL). The resulting mixture was poured into ice water, neutralized with aqueous NaOH, and

extracted with 10% MeOH/$CHCl_3$ (5×). The organic phase was separated, washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo to afford a yellow solid (0.87 g). Dry flash chromatography (EtOAc followed by 1% MeOH/EtOAc and 1% MeOH/0.2% IPA/EtOAc) provided **60** as a white solid (0.44 g, 45%): mp 215−218 °C dec; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.78 (s, 1H), 7.44 (t, $J$ = 6.0 Hz, 1H), 7.37 (d, $J$ = 2.6 Hz, 1H), 7.26 (d, $J$ = 8.3 Hz, 2H), 7.16 (d, $J$ = 8.6 Hz, 1H), 6.74 (dd, $J$ = 2.7, 8.7 Hz, 1H), 4.33 (t, $J$ = 6.1 Hz, 2H), 4.02 (d, $J$ = 6.0 Hz, 1H), 2.86 (s, 3H), 2.82 (t, $J$ = 6.3 Hz, 2H), 2.44 (q, $J$ = 7.1 Hz, 4H), 0.80 (t, $J$ = 7.1 Hz, 6H); MS $m/z$ 447 ($MH^+$). Anal. ($C_{21}H_{26}N_4O_3S_2 \cdot {}^1/_4H_2O$) C, H, N.

**Carbamic Acid, [[2-[2-(Diethylamino)ethyl]-9-hydroxy-2*H*-(1)benzothiopyrano[4,3,2-*cd*]indazol-5-yl]methyl]-, Methyl Ester (61)** was prepared from **59** via the same procedure as for **49**. The crude product was purified by dry flash chromatography (EtOAc followed by 2% MeOH/EtOAc and 1.8% MeOH/0.2% IPA/EtOAc) to afford **61** as an off-white solid (0.42 g, 61%): mp 239−240 °C dec; $^1$H NMR ($CDCl_3$) $\delta$ 7.43 (d, $J$ = 2.9 Hz, 1H), 7.23 (m, 1H), 7.14 (d, $J$ = 8.6 Hz, 1H), 6.85 (d, $J$ = 8.6 Hz, 1H), 6.76 (dd, $J$ = 7.2, 8.5 Hz, 1H), 5.15 (br s, 1H), 4.25 (m, 2H), 3.65 (s, 3H), 2.86 (t, $J$ = 7.2 Hz, 2H), 2.54 (q, $J$ = 7.1 Hz, 4H), 0.98 (t, $J$ = 7.2 Hz, 6H); MS $m/z$ 427 ($MH^+$). Anal. ($C_{22}H_{26}N_4O_3S$) C, H, N.

**In Vivo Testing**. BDF1 mice were implanted bilateraly sc with 30−60-mg fragments of Panc03. Beginning 3 days following tumor implantation, chemotherapy was administered intravenously at the maximum tolerated dose. Detailed descriptions of tumor implantation, end point determination, and quantification of tumor cell kill have been previously reported.[2]

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