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ANTI-SOLID TUMOR EFFICACY AND PREPARATION OF *N*-[[1-[[2-(DIETHYLAMINO)ETHYL]AMINO]-9-OXO-9*H*-THIOXANTHEN-4-YL]METHYL]METHANESULFONAMIDE (WIN 33377) AND RELATED DERIVATIVES.[1]
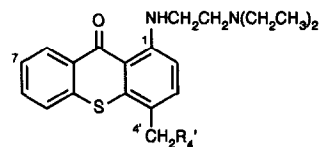
Mark P. Wentland,[*,†] Robert B. Perni,[†] Ronald G. Powles,[†] Allan G. Hlavac,[‡] Kenneth C. Mattes,[£] Thomas H. Corbett,[¥] Susan A. Coughlin,[¶] and James B. Rake[¶]

Departments of Medicinal Chemistry,[†] Molecular Characterization,[‡] and Oncopharmacology,[¶] Sterling Winthrop Pharmaceuticals Research Division, Sterling Winthrop Inc., Collegeville, PA 19426, Eastman Kodak Co.,[£] Rochester, NY 14650, and Department of Internal Medicine, Division of Hematology and Oncology, Wayne State University,[¥] Detroit, MI 48202

INTRODUCTION - Highly efficacious and curative murine anti-solid tumor activity has been identified in *N*-[[1-[[2-(diethylamino)ethyl]amino]-9-oxo-9*H*-thioxanthen-4-yl]methyl]methanesulfonamide (WIN 33377, **1**) and related congeners. These novel 4-aminomethylthioxanthones are structurally related to hycanthone (**2**) and its corresponding carbamate derivative **3** that previously have been shown to have antitumor properties in mice.[2,3] Hycanthone, widely known for its antiparasitic properties, was evaluated in humans as a potential antitumor agent, however, dose-limiting hepatotoxicity precluded its development.[4]

1; R₄' = NHSO₂CH₃
2; R₄' = OH
3; R₄' = OCONHCH₃
14b; R₄' = NHCH₃

The 4-aminomethylthioxanthones were discovered as antitumor agents during random screening of the Eastman Kodak and Sterling Winthrop compound collections. The first screen hit was **14b**. When tested in an agar disk diffusion assay,[5] **14b** was found to be selectively cytotoxic for the pancreatic ductal adenocarcinoma 03 (Panc 03) murine tumor over normal and leukemia cell lines. Subsequent *in vivo* testing of **14b** in a model[5-7] where Panc 03 was implanted subcutaneously in BDF₁ mice and drug was administered iv at the maximum tolerated dose, showed complete tumor regressions, with animals tumor free at day 171 (representative of cures). Owing to the high level of activity seen with the prototype, **14b**, further *in vitro* screening was not performed, rather all derivatives, obtained from analogue searching of compound libraries or from synthesis, were tested directly *in vivo*. A number of other analogues, including **1**, were also found have curative activity in the Panc 03 model. With the lead structures **1** and **14b** in hand, we prepared and evaluated analogues modified at the 4'- and 7-positions as probes to define the structural requirements necessary for murine anti-solid tumor activity.
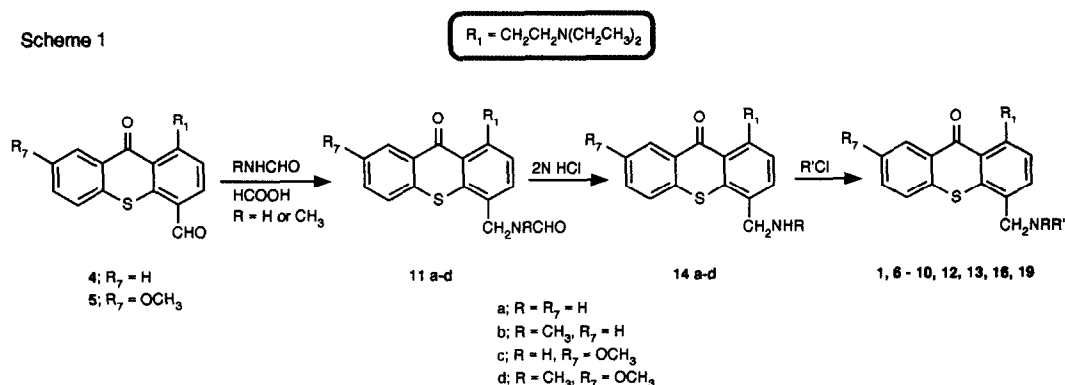
CHEMISTRY - The target 4-aminomethylthioxanthones were made using the standard methodology shown in Scheme 1. The structures, formulas, and melting points are summarized in Table 1. Reductive amination of the known carboxaldehydes **4**[8] and **5**[3] with formic acid and formamide or N-methylformamide (Leuckart
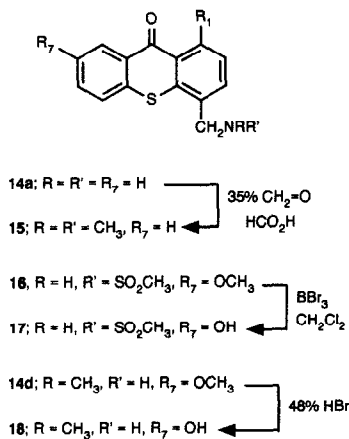
conditions) provided **11a-d**. Hydrolysis of **11a-d** in 2N HCl gave amine derivatives **14a-d** which were subsequently treated with the appropriate sulfonyl chloride, acid chloride, or phosphoryl chloride to give targets **1, 6-10, 12, 13, 16,** and **19**. Details for the synthesis of **1** using this sequence are described in the experimental section. The dimethylamino analogue **15** was made by treating **14a** with formic acid/aqueous formaldehyde at 100 °C. Phenolic derivative **17** was made by exposing **16** to BBr_3/CH_2Cl_2 (-78 °C → 25 °C) and **18** was made from the reaction of **14d** with 48% HBr at 100 °C.

Scheme 1

$$R_1 = CH_2CH_2N(CH_2CH_3)_2$$

4; R_7 = H
5; R_7 = OCH₃

11 a-d

14 a-d

1, 6 - 10, 12, 13, 16, 19

a; R = R_7 = H
b; R = CH₃, R_7 = H
c; R = H, R_7 = OCH₃
d; R = CH₃, R_7 = OCH₃

For 14a → 1; CH₃SO₂Cl, Et₃N, CH₂Cl₂; 14a → 6; C₆H₅SO₂Cl, pyr: 14b → 7; CH₃SO₂Cl, pyr: 14a → 8; CH₃COCl, pyr: 14a → 9; C₆H₅COCl, pyr:
14a → 10; CF₃COCl, tol, CH₂Cl₂: 14a → 12; CH₃OCOCl, Et₃N, CH₂Cl₂: 14a → 13; (EtO)₂POCl, Et₃N, CH₂Cl₂: 14c → 16; CH₃SO₂Cl, pyr:
14c → 19; CH₃OCOCl, Et₃N, CH₂Cl₂

14a; R = R' = R_7 = H ——┐
                                    │ 35% CH₂=O
15; R = R' = CH₃, R_7 = H ◄——┘ HCO₂H

16, R = H, R' = SO₂CH₃, R_7 = OCH₃ ——┐
                                                      │ BBr₃
17; R = H, R' = SO₂CH₃, R_7 = OH ◄——┘ CH₂Cl₂

14d; R = CH₃, R' = H, R_7 = OCH₃ ——┐
                                                    │ 48% HBr
18; R = CH₃, R' = H, R_7 = OH ◄——┘

Results and Discussion - The effect of varying the 4'-nitrogen of **1** and **14b** on antitumor efficacy in the murine Panc 03 model is shown Table 1. In addition, the 7-H in selected analogues was replaced with CH_3O or OH. Evaluations of *in vivo* antitumor activity were conducted at Wayne State University in mice implanted bilaterally s.c. with 30-60 mg tumor fragments of Panc 03.[6] Chemotherapy was administered intravenously at the maximum tolerated dose starting 3 days after tumor implantation. The methods of tumor implantation, end point determination and quantification of tumor cell kill have previously been described.[5-7]

In this model, compound **1** had a %T/C (definition of terms can be found in the footnotes of Table 1) of zero with 3 of 5 mice tumor free on day 245. This high level of activity is comparable to **14b** and adriamycin and somewhat greater than hycanthone. Activity was sustained when the methanesulfonamido group of **1** was replaced with a benzenesulfonamido group (**6**) or N-methylated to give **7**. High activity with cures was also seen in amide derivatives **8** and **11a** and urethane **12**. The benzamide **9**, trifluoroacetamide **10** and phosphoramide **13** analogues were less efficacious.

Excellent efficacy, comparable to **14b**, was observed for the primary amino analogue, **14a**. The dimethylamino derivative **15**, however, had diminished activity. The observations that an appropriately placed methoxy or hydroxy group was beneficial for antitumor activity for certain hycanthone[3] and ellipticine[9-11] derivatives prompted the synthesis of **14d** and **16-19**. Activity was generally sustained relative to their 7-H counterparts.

It is difficult, at best, to relate structure to efficacy within this small series since the primary assay used to evaluate the targets was done *in vivo* and the pharmacokinetic properties (elimination half-life, volume of distribution, rate or extent of metabolism, tumor distribution) were not measured. In addition, where data are available, the relationship of structure to *in vitro* cytotoxicity potency or interaction with suspected molecular targets [e.g., topoisomerase II or DNA (via intercalative binding or alkylation)] is also not well understood.

In conclusion, we have identified a new series of cytotoxic agents having excellent solid tumor selectivity and activity. Within this small series, efficacy in mice versus Panc 03 did not correlate well to the nature of the R_4' and R_7 groups in that all compounds evaluated had activity (%T/C < 42) in the model. Compound **1** (WIN 33377) is in the preclinical phase of development as a human therapeutic based on high activity observed in the Panc 03 and other anti-solid tumor murine models (Mam 16/C, Colon 38 (curative), Colon 51, Colon 26, and Panc 02). Safety studies of **1** showed that dose-limiting toxicity in mice was myelosuppression and GI intolerance while no evidence of hepatotoxicity was observed.[12]

Table 1. Physical and antitumor properties of 4-aminomethylthioxanthones.

cmpd	R	R'	R_7	mp, °C	formula[b]	murine antitumor activity versus Panc 03[a] %T/C[c]	MTD[d]	LTC[e]	LCK[f]
1	H	SO_2CH_3	H	169-170	$C_{21}H_{27}N_3O_3S_2$	0	124	3/5	2.0
6	H	$SO_2C_6H_5$	H	164-167	$C_{26}H_{29}N_3O_3S_2 \cdot CH_3SO_3H$	0	840	1/5	2.9
7	CH_3	SO_2CH_3	H	175-177	$C_{22}H_{29}N_3O_3S_2$	0	304	1/5	2.5
8	H	$COCH_3$	H	182-183	$C_{22}H_{27}N_3O_2S$	0	540	3/5	2.7
9	H	COC_6H_5	H	161-163	$C_{27}H_{29}N_3O_2S$	26	252	1/5	0.5
10	H	$COCF_3$	H	152-154	$C_{22}H_{24}F_3N_3O_2S_2 \cdot CH_3SO_3H$	21	1171	0/6	1.5
11a	H	CHO	H	154-155	$C_{21}H_{25}N_3O_2S$	0	576	1/5	3.0
12	H	CO_2CH_3	H	129-131	$C_{22}H_{27}N_3O_3S$	0	248	2/5	8.8
13	H	$PO(OEt)_2$	H	108-110	$C_{24}H_{34}N_3O_4PS$	36	1298	0/5	<0.5
14a	H	H	H	270-272	$C_{20}H_{25}N_3OS \cdot 2HCl$	0	270	4/5	>4
14b	CH_3	H	H	237-239	$C_{21}H_{27}N_3OS \cdot 2HCl$	0	800	1/3	3.1
15	CH_3	CH_3	H	256-257	$C_{22}H_{29}N_3OS \cdot 2HCl$	23	544	0/6	0.9
16	H	SO_2CH_3	OCH_3	144 (dec)	$C_{22}H_{29}N_3O_4S_2$	0	248	1/5	6.5
17	H	SO_2CH_3	OH	78 (dec)	$C_{21}H_{27}N_3O_4S_2 \cdot 0.75H_2O$	11	47	0/5	3.7
14d	CH_3	H	OCH_3	55-56	$C_{22}H_{29}N_3O_2S$	0	880	1/5	5.1
18	CH_3	H	OH	167-169	$C_{21}H_{27}N_3O_2S$	0	60	0/5	4.6
19	H	CO_2CH_3	OCH_3	139-140	$C_{23}H_{29}N_3O_4S$	0	240	5/5	>4.5
hycanthone (2)						4	290	0/3	3.4
adriamycin						0	18	1/5	2.9

[a]See Refs. 5-7. [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 X 100 at evaluation and C is the median tumor burden in the control group at evaluation. A T/C value <42% is considered significant antitumor activity. [d]MTD = maximum tolerated total dose administered intraveneously in mg/kg. [e]LTC = long term cures, the number of mice in the treatment group with no palpable tumor evident after a minimum of 100 days/total number in treatment group. [f]LCK = log_{10} cell kill of tumor bearing mice (cures excluded), a calculation based on tumor growth delay; cures for this tumor require >4.5 log kill: See refs. 5 and 7.

Experimental Section

N-[[1-[[2-(Diethylamino)ethyl]amino]-9-oxo-9*H*-thioxanthen-4-yl]methyl]formamide (11a). A mixture of 4 (35.4 g, 0.10 mol), formamide (420 mL), and formic acid (50 mL) was heated at 160 °C for 1 h. After cooling, the reaction mixture was poured into 2 L water and made basic with 35% NaOH. The solid that separated was collected, washed with H_2O and dried to give 37.4 g of crude product. Recrystallization of this material from EtOAc (1500 mL) provided 29.0 g (76%) of 11a: mp 154-155 °C; ^1H NMR ($CDCl_3$) δ 10.33 (br t, 1H), 8.45 (d, 1H), 8.27 (s, 1H), 7.53-7.29 (m, 4H), 6.52 (d, 1H), 5.98 (br s, 1H), 4.54 (d, 2H), 3.31 (m, 2H), 2.80 (apparent t, 2H), 2.63 (q, J = 6.9 Hz, 4H), 1.09 (t, J = 6.9 Hz, 6H). Anal. ($C_{21}H_{25}N_3O_2S$) C, H, N.

4-(Aminomethyl)-1-[[2-(diethylamino)ethyl]amino]-9-oxo-9*H*-thioxanthen-4-one Dihydrochloride (14a). A mixture of 11a (24.4 g, 0.064 mol) and 240 mL 2N HCl was heated at 100 °C for 1 h. After cooling, the mixture was made basic with 35% NaOH and a crude yellow solid was collected. This solid was washed with dilute NH_4OH, dried, and dissolved in benzene. The resulting solution was treated with charcoal and $MgSO_4$, filtered and concentrated. The residue was dissolved in 1500 mL of hexane, filtered and concentrated to a volume of 900 mL. The solid that separated upon standing was collected and dried to give 17.6 g (77%) of the neutral form of 14a. A portion of this material was converted to the dihydrochloride salt 14a: mp 270-272 °C (methanol); ^1H NMR (CF_3CO_2D) δ 8.67 (d, 1H), 8.21-8.18 (m, 1H), 7.98-7.76 (m, 4H), 4.92 (s, 2H), 4.24 (br t, J = 5.8 Hz, 2H), 4.04 (br t, J = 5.8 Hz, 2H), 3.55 (m, 4H), 1.53 (t, J = 5.2 Hz, 6H) Anal. ($C_{20}H_{25}N_3OS \cdot 2HCl$) C, H, N.

N-[[1-[[2-(Diethylamino)ethyl]amino]-9-oxo-9*H*-thioxanthen-4-yl]methyl]methanesulfonamide (1). Methanesulfonyl chloride (4.01 g, 0.035 mol) was added to a cold (0 °C) solution of neutral form 14a (10.7 g, 0.03 mol) in 100 mL pyridine. The resulting mixture was stirred 2 h while warming to 25 °C and poured into 700 mL water containing 2.0 g (0.05 mol) of NaOH. The yellow solid that separated was collected, washed with H_2O, dried, and recrystallized from benzene to give 6.4 g (51%) of 1: mp 169-170 °C; ^1H NMR ($CDCl_3$) δ 10.37 (br t, 1H), 8.49 (d, 1H), 7.57-7.39 (m, 4H), 6.56 (d, 1H), 4.84 (br s, 1H), 4.41 (s, 2H), 3.32 (m, 2H), 2.84 (s, 3H), 2.82 (apparent t, 2H), 2.64 (q, J = 6.8 Hz, 4H), 1.09 (t, J = 6.8 Hz, 6H). Anal. ($C_{21}H_{27}N_3O_3S_2$) C, H, N.

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