Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 12, pp. 1345-1350, 1996

0960-894X/95 $15.00 + 0.00

PII: S0960-894X(96)00230-2

CYCLIC VARIATIONS OF SR 233377 (WIN 33377) AND EFFECTS ON ANTITUMOR ACTIVITY.[1]

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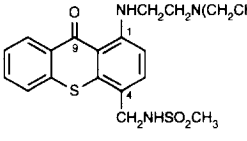
Abstract. Novel derivatives of SR 233377 (**1**, WIN 33377) where a pyrazolo ring fusion has been incorporated at the 1- and 9-positions of the thioxanthone ring displayed outstanding in vivo efficacy against the murine solid tumor Panc 03 (T/C values of 0% with log cell kill ≥ 2.0). No relationship between structure and Panc 03 activity was observed because all analogues studied were highly active.

Introduction. We recently described the outstanding in vivo antitumor activity of novel 4-aminomethylthioxanthones versus the murine solid tumor Panc 03.[2] As a result of the high and, in several instances, curative activity against this and other murine solid tumors, the methanesulfonamido analogue SR 233377 (**1**, WIN 33377), is undergoing clinical evaluation in humans. Relative to many known tricyclic DNA-interactive antitumor agents (e.g., mitoxantrone), **1** has a unique substitution at the 4-position, namely a 4-aminomethyl appendage (C-C attachment) rather than a (di)alkylaminoalkylamino DNA groove-stabilizing group (via an N-C bond). A common and productive variation of known tricyclic antitumor agents has been incorporation of a pyrazolo ring fusion at the 1- and 9-positions; examples of these highly active tetracyclic antitumor agents are the anthrapyrazoles (CI-941 and CI-942),[3] pyrazoloacridines (PD 115,934)[4] and benzothiopyranoindazoles (CI-958).[5] The objective of the study we are now reporting was to incorporate a similar pyrazolo ring fusion into the thioxanthone ring of **1** and related analogues and study the topoisomerase II inhibitory, DNA binding, P388 cytotoxicity, and Panc 03 in vivo antitumor properties of these new derivatives.



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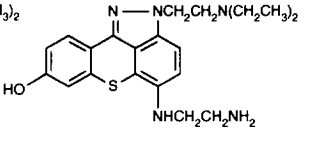

CI-958

Chemistry - The target 5-aminomethylbenzothiopyranoindazoles were made using the methodology shown in Scheme I. Treatment of thioxanthones **2a**[6] and **2b**[7] (each as a ca. 1:1 mixture with their corresponding

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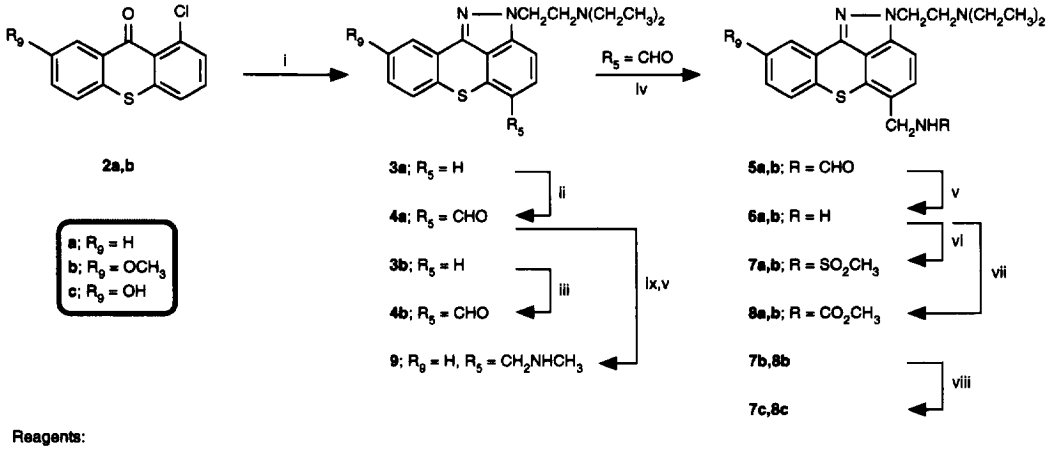
3-chloro regioisomer) with diethylaminoethylhydrazine[5] in refluxing pyridine provided the core heterocyclic systems **3a** and **3b**, respectively in yields ranging from 30 to 40%. Compound **3a** was treated with $CHCl_2OCH_3/AlCl_3$[8] to give the 5-formyl derivative **4a** in 96% yield while **3b** was subjected to Vilsmeier conditions to give **4b** (61%). Treatment of **4a** and **4b** with formamide/formic acid (Leuckart conditions) provided **5a** and **5b**, respectively; these formamides were hydrolyzed to the corresponding amines **6a** and **6b** using aqueous methanolic NaOH. The yields in each of these two steps were approximately 65%. Using standard methodology, **6a** and **6b** were converted to the corresponding methanesulfonamido and urethane derivatives **7a**, **7b**, **8a**, and **8b** in yields ranging from 55-73%. The phenolic analogues **7c** (45%) and **8c** (61%) were made by treating **7b** and **8b**, respectively, with BBr_3/CH_2Cl_2 at -78 °C. Target compound **9** was made by treating **4a** with $HCONHCH_3/HCO_2H$ followed by base hydrolysis in 67% overall yield. The structures, melting points and formulas of these target compounds are shown in Table 1.

Scheme I

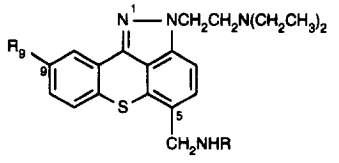


Reagents:

(i) $H_2NNHCH_2CH_2N(CH_2CH_3)_2$, pyr, 115 °C; (ii) $CHCl_2OCH_3$, $AlCl_3$, CH_2Cl_2, 25 °C; (iii) $POCl_3$, DMF, 100 °C; (iv) $HCONH_2$, HCO_2H, 140 °C; (v) NaOH, MeOH, H_2O, 70 °C; (vi) CH_3SO_2Cl, pyr, CH_2Cl_2, 25 °C; (vii) CH_3OCOCl, Et_3N, CH_2Cl_2, 25 °C; (viii) BBr_3, CH_2Cl_2, -78 °C; (ix) $HCONHCH_3$, HCOOH, 140 °C

Results. The effect of incorporating the pyrazolo ring fusion into the thioxanthone ring of **1** on inhibition of mammalian topoisomerase II, P388 in vitro cytotoxicity, intercalative DNA binding and antitumor efficacy in mice is shown in Tables 1 and 2. In addition, pyrazolo analogues of other highly active thioxanthones[2] were made and evaluated; these include replacing the methanesulfonamido group with $NHCO_2CH_3$, NH_2, $NHCH_3$ or NHCHO and replacing the 9-H in certain analogues with OCH_3 or OH. Data for three reference compounds (**1**, adriamycin and mAMSA) are also given. Biochemical and cellular data are found in Table 1 along with an explanation (footnotes) of these assays.

Table 1. Physical, biochemical and in vitro antitumor properties of 5-aminomethylbenzothiopyranoindazoles.



cmpd	R	R_9	mp, °C	formula[d]	in vitro cytox[a] IC_{50}-μM	topo II inhibition.[b] EC_{50}-μM	inter-calation[c] EC_{50}-μM
5a	CHO	H	160-161	$C_{21}H_{24}N_4OS$	0.33	>260	1.5
5b	CHO	OCH_3	125-131	$C_{22}H_{26}N_4O_2S \cdot 0.25H_2O$	55	>240	1.9
6a	H	H	173 (dec)	$C_{20}H_{24}N_4S \cdot 2HCl$	5.8	>250	0.12
6b	H	OCH_3	74-76	$C_{21}H_{26}N_4OS$	13	>260	0.13
7a	SO_2CH_3	H	119-123	$C_{21}H_{26}N_4O_2S_2$	0.15	>230	1.8
7b	SO_2CH_3	OCH_3	125-126	$C_{22}H_{28}N_4O_3S_2 \cdot 0.25H_2O$	30	>210	1.6
7c	SO_2CH_3	OH	215-218	$C_{21}H_{26}N_4O_3S_2 \cdot 0.25H_2O$	0.0044	0.55[e]	2.4
8a	CO_2CH_3	H	130-132	$C_{22}H_{26}N_4O_2S$	0.50	>240	2.6
8b	CO_2CH_3	OCH_3	125-127	$C_{23}H_{28}N_4O_3S$	45	>220	0.79
8c	CO_2CH_3	OH	239-240	$C_{22}H_{26}N_4O_3S$	0.011	0.33[e]	1.5
9	CH_3	H	189-193	$C_{21}H_{26}N_4S \cdot 2HCl \cdot 0.75H_2O$	16	>220	0.18
1					0.30	3.0[e]	18
adriamycin					0.83	[f]	[f]
mAMSA					0.15	0.72	11

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[a]In Vitro Cytotoxicity was measured by quantifying clonogenic survival in soft agar following a 1 hour transient exposure of P388 mouse leukemia cells to drug. The IC_{50} value is the concentration of drug which reduced clonogenic survival by 50%.[9] [b]Topoisomerase II Inhibition[9] - Promotion by test agent of covalent complex formation between [^{32}P]-end labeled pBR322 DNA and extensively purified HeLa cell topo II was determined by the SDS/K^+ precipitation method. 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. [c]DNA Intercalation - A known ethidium bromide displacement assay was used to determine intercalation potency.[10] 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]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. [e]Bell-shaped dose response curve was noted when determining the EC_{50} (see Ref. 11). [f]Not determined.

Evaluations of in vivo antitumor activity (Table 2) were conducted at Wayne State University in mice implanted bilaterally s.c. with 30-60 mg tumor fragments of Panc 03.[12] Chemotherapy was administered intravenously at the maximum tolerated dose starting 3 or 4 days after tumor implantation. The rationale for the use of the murine solid tumor Panc 03 for drug discovery as well as the methods of tumor implantation, end point determination, and quantification of tumor cell kill have previously been described.[12-14] In this model, compound **1** had a %T/C (definition of terms can be found in the footnotes of Table 2) of zero with 3 of 5 mice tumor free on day 245. A summary of these data is as follows:

In vitro cytotoxicity - Both 9-OH derivatives, **7c** and **8c** showed outstanding potency that was much greater than the three reference compounds. Compounds **5a**, **7a**, and **8a** displayed good activity that was similar to the three reference compounds. All four 9-methoxy derivatives (**5b**, **6b**, **7b**, and **8b**) as well as **6a** and **9** displayed considerably reduced potency.

Topoisomerase II inhibition - The only targets to show activity were the two 9-OH analogues **7c** and **8c**; potency was substantially greater than **1** and was similar to mAMSA. It has been reported that certain topoisomerase II interactive antitumor agents that bind to DNA *via* intercalation inhibit topoisomerase II-DNA complex formation at high concentrations.[11] Consistent with that observation, **7c** and **8c** exhibit a bell-shaped dose response curve.

DNA binding via intercalation - All targets displayed DNA binding activity; potency in this assay was considerably greater than **1** and mAMSA.

Murine antitumor activity versus Panc 03 - Significant activity (%T/C < 42) was observed for all analogues in the series with the majority of target compounds having %T/C values of zero; long term curative activity was noted for **5b**, **6b**, **7b**, **8a**, **8b**, and **9**.

Conclusions. Structure-Activity Studies - Within this small series, the only group that has a substantial impact on structure-activity function is the 9-OH appendage; compounds **7c** and **8c** displayed greatly enhanced in vitro cytotoxicity and topo II inhibition potencies. Otherwise, no relationship between structure and topoisomerase II inhibition, P388 cytotoxicity, or DNA binding was discernable. The same functional groups (e.g., OCH_3 and OH ring substituents and the aminomethyl appendage) that contributed to the high in vivo activity of the thioxanthones (i.e., **1**) also contributed to the activity of the benzothiopyranoindazoles . Since all compounds in the series had significant activity, no relationship between structure and Panc 03 efficacy emerged.

In vivo Panc 03 Efficacy - Efficacy did not correlate with in vitro P388 cytotoxicity. Of note is the fact that all four 9-OCH_3 derivatives (**5b**, **6b**, **7b**, and **8b**) 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 the inherent potent topoisomerase II inhibition and associated in vitro P388 cytotoxicity of **7c** and **8c** accounts for the excellent *in vivo* activity (%T/C < 42) observed for these two 9-OH analogues. For compounds with moderate P388 cytotoxicity (e.g., 9-H analogues **5a**, **7a**, and **8a**), 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.

Topoisomerase II inhibition does not play a significant role in the mechanism of action of the parent drugs. Since the 9-OCH_3 derivatives **5b**, **6b**, **7b**, and **8b** displayed a very low level of P388 in vitro cytotoxicity, it is likely that the very high level of antitumor activity observed in mice is a result of metabolism to a cytotoxic species, presumed to be the corresponding 9-OH derivatives (e.g., **7b** → **7c** and **8b** → **8c**). If this is, in fact, the case, the greater in vivo efficacy observed for **8b** (relative to **8c**) may be due to a fortuitous controlled release of **8c** such that its circulating plasma levels are relatively low at a given time point resulting in greater tolerance while its distribution into tumor is relatively high. These hypotheses will be tested in the appropriate pharmacokinetic studies should the antitumor activities observed in subsequent murine models warrant development of these agents.

Table 2. Murine antitumor activity of 5-aminomethylbenzothiopyranoindazoles versus Panc 03.[a]

cmpd	%T/C[b]	MTD[c]	LTC[d]	LCK[e]
5a	0	81	0/5	2.4
5b	0	231	3/5	3.6
6a	25[f]	72	0/5	[g]
6b	0[f]	240	3/4	>4.5
7a	0	72	0/5	2.3
7b	0	208	3/5	2.2
7c	0[f]	31	0/5	2.0
8a	0	78	3/5	2.5
8b	0[f]	170	4/4	>4.5
8c	13[f]	18	0/5	[g]
9	0	326	1/5	3.6
1	0	124	3/5	2.0
adriamycin	0	18	1/5	2.9
mAMSA	0	48	0/5	1.8

[a]See Refs. 12-14 for the methods of tumor implantation, end point determination and quantification. Animal use was approved by the Wayne State University IACUC. [b]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. [c]MTD = maximum tolerated total dose administered intravenously in mg/kg. [d]LTC = long term cures, the number of mice in the treatment group with no palpable tumor evident after a minimum of 150 days/total number in treatment group. [e]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. [f]First dose of drug administered 4 days after tumor implantation. [g]Not calculated.

Acknowledgements. The contributions of Chiab Panchapor, Susan Pugh, Lisa Polin, Kathryn White, Juiwanna Knight, Lisa Demchik, and Lynne Jones for generating in vivo tumor biology data at Wayne State University are gratefully acknowledged. Support: In vivo studies at WSU were supported by CA 46560.

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(*Received in USA* 15 *March* 1996; *accepted* 14 *May* 1996)