J. Med. Chem. 1990, 33, 2852-2856

3.84 (dd, 1, $C_{4'}$-H), 3.97 (dd, 1, $C_{3'}$-H), 4.28 (dd, 1, $C_{2'}$-H), 4.67 (d, 1, $J_{1,2}$ = 6.4 Hz, $C_{1'}$-H), 4.80 (t, 1, $C_{5'}$-CH_2), 5.12 and 5.35 (2 d, 2, $C_{2',3'}$-OH), 7.5 and 7.68 (2 s, 2, $CONH_2$), 8.58 (s, 1, C_5-H). Anal. ($C_9H_{12}N_2O_6$) C, H, N.

5-Amino-2-[5′-(hydroxymethyl)furan-2′-yl]oxazole-4-carboxamide (12). A solution of **9** (100 mg, 0.28 mmol) in $MeOH/NH_3$ (10 mL, saturated at 0 °C) was stirred at room temperature for 53 h in a pressure bottle. The solvent was evaporated to dryness and the residue was purified on a silica gel column using $CHCl_3$–MeOH–NH_4OH (80:19:1 v/v) as eluent. The homogeneous fractions were pooled and evaporated to dryness, and the residue was crystallized from methanol to give 25 mg (40%) of **12**; mp 132–135 °C; IR ν 1605 and 1688 (C=O), 3318 (NH_2), cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.43 (d, CH_2OH), 5.37 (t, 1, OH), 6.41 (d, 1, $J_{4',3'}$ = 3.0 Hz, H-4 furan ring), 7.02 (d, 1, $J_{3',4'}$ = 3.0 Hz, H-3 furan ring), 7.30 and 7.70 (2 br s, 4, NH_2, $CONH_2$). Anal. ($C_9H_9N_3O_4$) C, H, N.

Ethyl 2-[5′-[(Benzoyloxy)methyl]furan-2′-yl]oxazole-4-carboxylate (13). Compound **9** (0.5 g, 1.4 mmol) in THF (18 mL) was added to a precooled (−20 °C) stirred solution of hypophosphorous acid (50%, 14 mL) containing a few drops of hydrochloric acid. To the above yellow solution, a solution of $NaNO_2$ (250 mg, 3.62 mmol) in water (3 mL) was added slowly (8 min). The stirring was continued for 3.5 h at −20 °C. The reaction mixture was adjusted to pH 6 by careful addition of a saturated solution of sodium bicarbonate and extracted with ethyl acetate (4 × 30 mL). The organic layer in turn was washed thoroughly with water, and the ethyl acetate portion was dried (Na_2SO_4); after evaporation to dryness, the crude residue was chromatographed on a silica gel column eluting with C_6H_6–EtOAc (80:20, v/v). The homogeneous solid that was obtained after evaporation of the solvent was crystallized from ethyl acetate–petroleum ether to give 180 mg (37%) of **13** as a white solid: mp 107–109 °C; ^1H NMR (Me_2SO-d_6) δ 1.30 (t, 3, CH_3), 4.32 (q, 2, CH_2), 5.46 (s, 2, CH_2), 6.90 (d, 1, $J_{4',3'}$ = 3.1 Hz, H-4 furan ring), 7.31 (d, 1, $J_{3',4'}$ = 3.1 Hz, H-3 furan ring), 7.50–8.0 (m, 5, C_6H_5), 8.93 (s, 1 C_5-H). Anal. ($C_{18}H_{15}NO_6$) C, H, N.

2-[5′-(Hydroxymethyl)furan-2′-yl]oxazole-4-carboxamide (14). Treatments of compound **13** (150 mg, 0.43 mmol) with MeOH–NH_3 (40 mL, saturated at 0 °C) for 5 days at room temperature and evaporation to dryness yielded a product which was purified on a silica gel column using $CHCl_3$–MeOH (90:10, v/v) as eluent. The homogeneous solid was crystallized from EtOH to yield 40 mg (36%) of **14**: mp 193–195 °C; IR ν 1610 and 1660 (C=O), 3290 and 3340 (NH_2) cm^{-1}; UV (EtOH) λ_{max} 206 (ϵ 11 700), 282 nm (ϵ 19 200); ^1H NMR (Me_2SO-d_6) δ 4.48 (d, 2, CH_2), 5.47 (t, 1, OH), 6.56 (d, 1, $J_{4',3'}$ = 3.4 Hz, H-4 furan ring), 7.14 (d, 1, $J_{3',4'}$ = 3.4 Hz, H-3 furan ring), 7.57 and 7.70 (br 2 s, 2, NH_2), 8.63 (s, 1, H-5 oxazole ring). Anal. ($C_9H_8N_2O_4$) C, H, N.

Antitumor Evaluation. The following cell lines were used: P388 murine lymphocytic leukemia, L1210 murine lymphocytic leukemia, B16 murine melanoma, and HL-60 human promyelocytic leukemia. Cell lines, maintained in vitro in exponential growth, were cultured in RPMI-1640 supplemented with antibiotics (penicillin 100 units/mL, streptomycin 100 μg/mL, gentamicin 50 μg/mL), g/mL), 3 mM glutamine, 10 mM HEPES buffer, and 15% (for P388 and L1210 cell lines) heat-inactivated new born calf serum or 10% (for B16 cell line) or 15% (for HL-60 cell line) heat-inactivated fetal calf serum. In order to determine cell growth inhibition, an antimetabolic assay was performed. Tiazofurin (**1**)[9] and compound **3** were solubilized in water and then in culture medium. Compounds **12** and **14** were solubilized in DMSO, and then water and culture medium were added; the final concentration of DMSO (not more than 0.5%) had no cytotoxic effect in our testing system. Various concentrations of each compound were placed, in quadruplicate, in flat-bottomed microculture wells with tumor cell suspensions for 48 h at 37 °C. Cells were placed in aliquots of 0.2 mL at the following concentrations: P388, 10^5 cell/well; L1210, 5 × 10^4 cell/well; B16, 3 × 10^3 cell/well; HL 60, 10^5 cell/well. Antiproliferative activity was determined by adding to the cultured cells [^{125}I]-5-iodo-2′-deoxyuridine together with 5-fluoro-2′-deoxyuridine, for an additional 18 h. Harvesting was performed by a multiple suction filtration apparatus on a fiber-glass filter. Immediately before harvesting, B16 cells were treated with trypsin 0.05% plus EDTA 0.02%. The filter paper was washed six times with 0.85% NaCl solution and the paper disks containing the aspirates cells were read in a γ-scintillation counter. At each dose level of compounds tested, cell-growth inhibition was expressed as a percentage of inhibition of radioisotope incorporation in the treated cultures with respect to control cultures. A dose resulting in 50% inhibition of radioisotope incorporation (ID_{50}) was determined; ID_{50} mean of at least three experiments was reported.

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8-Substituted 5-[(Aminoalkyl)amino]-6*H*-*v*-triazolo[4,5,1-*de*]acridin-6-ones as Potential Antineoplastic Agents. Synthesis and Biological Activity

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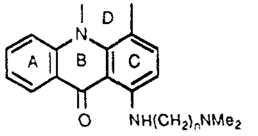
A series of 8-substituted 5-[(aminoalkyl)amino]-6*H*-*v*-triazolo[4,5,1-*de*]acridin-6-ones (**2**), structurally related to the imidazoacridinones (**1**), was synthesized and tested for cytotoxic and antineoplastic activity. Preliminary biological results indicated that the 8-OH derivatives possess the highest antitumor activity. No relationship has been found between the nature of the C-8 substituent and antitumor activity.

Among the antineoplastic compounds, a growing interest has been observed in recent years in the development of synthetic DNA-interacting agents. They have as a common general structural feature a tri- or tetracyclic chromophore bearing one or two side chains containing an (aminoalkyl)amino residue. Anthracenediones (ametantrone, mitoxantrone),[1] anthrapyrazoles,[2] pyrazoloacridines,[3] and acridine-4-carboxamides[4] belong, among others, to this broad class of compounds. We recently described a further example of active compounds in this class, i.e. the imidazoacridinones (**1**).[5]



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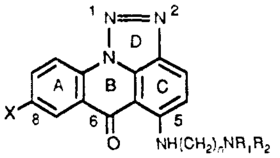

2

The results obtained so far indicate that the presence of an (aminoalkyl)amino side chain is crucial for the bio-

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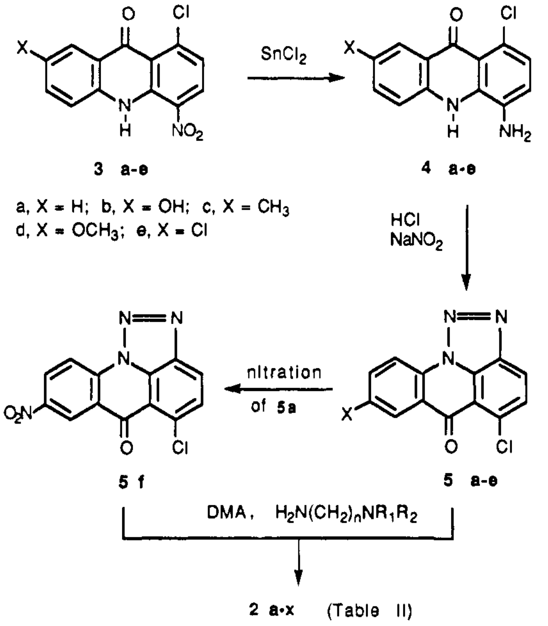
(1) (a) Zee-Cheng, R. K.-Y.; Podrebarac, E. G.; Menon, C. S.; Cheng, C. C. *J. Med. Chem.* **1979**, *22*, 501. (b) Murdock, K. C.; Child, R. G.; Fabio, P. F.; Angier, R. B.; Wallace, R. E.; Durr, F. E.; Citarella, R. V. *J. Med. Chem.* **1979**, *22*, 1024.

Scheme I



logical activity of these compounds, and the distance between the amino groups plays an important role.[1b,6,7] Depending on the kind of the chromophore, the optimal distance is equal to two or three methylene units. It is additionally known that the substituents at the distal amino group are also important and that the side chain must be attached to the chromophore at a strictly defined position.[4a,8]

It has been suggested that the distal amino group binds electrostatically to the phosphate moiety of DNA[9,10,11] or that the side chain has the function of additionally stabilizing a drug–DNA complex.[8,12]

No general structure–activity dependences have been found so far for modified polycyclic chromophores in this broad class of compounds although within some classes certain relationships have been shown.[4b,13]

Table I. Physical Properties of 7-Substituted 4-Amino-1-chloro-9(10*H*)-acridinones (4) and 8-Substituted 5-Chloro-6*H*-*υ*-[4,5,1-*de*]acridin-6-ones (5)

no.	% yield	mp,[a] °C	formula[b]
4a	91	232–234 dec[c]	$C_{13}H_9ClN_2O$
4b	93	235–238 dec	$C_{13}H_9ClN_2O_2$
4c	88	242–244 dec	$C_{14}H_{11}ClN_2O$
4d	88	215–217 dec	$C_{14}H_{11}ClN_2O_2$
4e	90	290–293 dec	$C_{13}H_8Cl_2N_2O$
5a	89	222–223[d]	$C_{13}H_6ClN_3O$
5b	90	>350 dec	$C_{13}H_6ClN_3O_2$
5c	88	253–255	$C_{14}H_8ClN_3O$
5d	76	263–266	$C_{14}H_8ClN_3O_2$
5e	90	299–302	$C_{13}H_5Cl_2N_3O$
5f	68	>350 dec	$C_{13}H_5ClN_4O_3$

[a] Recrystallization solvents in all cases were DMF + H_2O. [b] All compounds were analyzed for C, H, N and the results were within ± 0.4% of the theoretical values. [c] Literature[14] mp 224–227 °C. [d] Literature[14] mp 218 °C.

Following our previous work on the imidazoacridinones,[5] we now report on the synthesis and in vitro and in vivo antineoplastic activity for a new group of acridine derivatives (**2**) in which the acridine chromophore is modified by addition of an extra five-membered triazolo ring and variable substitution at C-8. The (aminoalkyl)amino side chains, which are crucial for biological activity, were retained.

Chemistry

The general synthetic route leading to the 8-substituted 5-[(aminoalkyl)amino]-6*H*-*υ*-triazolo[4,5,1-*de*]acridin-6-ones (designated hereafter as triazoloacridinones) is presented in Scheme I.

The 1-chloro-4-nitro-9(10*H*)-acridinones **3a–d** were prepared according to the methods described earlier.[14,15] The **3e** derivative was prepared by cyclization of 6-chloro-2-[(4-chlorophenyl)amino]-3-nitrobenzoic acid (**6**) with $POCl_3$. Compound **6** was obtained by condensation of 2,6-dichloro-3-nitrobenzoic acid[14] with 4-chloroaniline. The 4-nitroacridinones were reduced to 4-aminoacridinones **4a–e** in very good yields by means of $SnCl_2$ under conditions similar to those given by Lehmstedt and Schrader[14] for preparation of **4e**; only the method of isolation and purification was slightly modified. The obtained derivatives, diazotized under conditions similar to those described already for **5a**,[14] yielded, after crystalization from DMF–H_2O, pure compounds **5a–e**. Their structure was confirmed by NMR spectroscopy. Compound **5a** was transformed into **5f** by nitration with sodium nitrate in sulfuric acid. Intermediate 7-substituted 4-amino-1-chloro-9(10*H*)-acridinones (**4a–e**) as well as 8-substituted 5-chloro-6*H*-*υ*-triazolo[4,5,1-*de*]acridin-6-ones (**5a–f**) are listed in Table I.

The condensation of **5a–f** with suitable amines (3–4-fold excess), carried out at 60 °C using *N,N*-dimethylacetamide

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(2) (a) Showalter, H. D. H.; Fry, D. W.; Leopold, W. R.; Lown, J. W.; Plambeck, J. A.; Reszka, K. *Anti-Cancer Drug Des.* **1986**, *1*, 73. (b) Showalter, H. D. H.; Johnson, J. L.; Hoftiezer, J. M.; Turner, W. R.; Werbel, L. M.; Leopold, W. R.; Shillis, J. L.; Jackson, R. C.; Elslager, E. F. *J. Med. Chem.* **1987**, *30*, 121.

(3) (a) Capps, D.; Kesten, S. R.; Shillis, J.; Plowman, J. *Proc. Am. Assoc. Cancer Res.* **1986**, *27*, 277. (b) Sebolt, J. S.; Scavone, S. V.; Pinter, C. D.; Hamelehle, K. L.; Von Hoff, D. D.; Jackson, R. C. *Cancer Res.* **1987**, *47*, 4299.

(4) (a) Atwell, G. J.; Rewcastle, G. W.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1987**, *30*, 664. (b) Palmer, B. D.; Rewcastle, G. W.; Atwell, G. J.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1988**, *31*, 707.

(5) Cholody, W. M.; Martelli, S.; Paradziej-Lukowicz, J.; Konopa, J. *J. Med. Chem.* **1990**, *33*, 49.

(6) Johnson, R. K.; Zee-Cheng, R. K.-Y.; Lee, W. W.; Acton, E. M.; Henry, D. W.; Cheng, C. C. *Cancer Treat. Rep.* **1979**, *63*, 425.

(7) White, R. J.; Durr, F. E. *Invest. New Drugs* **1985**, *3*, 8.

(8) Wakelin, L. P. G.; Atwell, G. J.; Rewcastle, G. W.; Denny, W. A. *J. Med. Chem.* **1987**, *30*, 855.

(9) Zee-Cheng, R. K.-Y.; Cheng, C. C. *J. Med. Chem.* **1978**, *21*, 291.

(10) Kapuscinski, J.; Darzynkiewicz, Z.; Traganos, F.; Melamed, R. R. *Biochem. Pharm.* **1981**, *30*, 231.

(11) Palumbo, M.; Antonello, C.; Viano, I.; Santiano, M.; Gia, O.; Gastaldi, S.; Marciani Magno, S. *Chem. Biol. Interact.* **1983**, *44*, 207.

(12) Feigon, J.; Denny, W. A.; Leupin, W.; Kearns, D. R. *J. Med. Chem.* **1984**, *27*, 450.

(13) Showalter, H. D. H.; Angelo, M. M.; Berman, E. M.; Kanter, G. D.; Otwine, D. F.; Ross-Kesten, S. G.; Sercel, A. D.; Turner, W. R.; Werbel, L. M.; Worth, D. F.; Elslager, E. F.; Leopold, W. R.; Shillis, J. L. *J. Med. Chem.* **1988**, *31*, 1527.

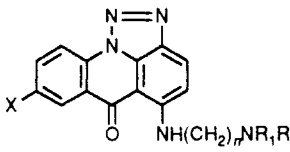
(14) Lehmstedt, K.; Schrader, K. *Chem. Ber.* **1937**, *70*, 1526.

(15) (a) Capps, D. B. European Patent Appl. E. P. 138302, 1985; *Chem. Abstr.* **1985**, *103*, 196074. (b) Capps, D. B. European Patent Appl. E. P. 145226, 1985; *Chem. Abstr.* **1985**, *103*, 215182s.

(16) Pawlak, K.; Matuszkiewicz, A.; Pawlak, J. W.; Konopa, J. *Chem. Biol. Interact.* **1983**, *43*, 131.

(17) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 1.

Table II. Physical Properties and Cytotoxic and Antineoplastic Activity of 8-Substituted 5-[(Aminoalkyl)amino]-6*H*-*v*-triazolo[4,5,1-*de*]acridin-6-ones



no.	X	*n*	R_1	R_2	reactn solvent mixture[a]	% yield	mp, °C	formula[b]	in vitro[c] HeLa S_3 IC_{50}, μg/mL	in vivo opt. dose, mp/kg per injection	P388[d] % T/C (day 30 surv)
ametantrone									0.17 ± 0.09	12.5	300 (3/6)
2a	NO_2	2	CH_3	CH_3	C-H	86	195-197 dec	$C_{17}H_{16}N_6O_3$	0.11 ± 0.02	25	130
2b	NO_2	2	C_2H_5	C_2H_5	B-H	92	218-220 dec	$C_{19}H_{20}N_6O_3$	0.12 ± 0.03	12.5	122
2c	NO_2	2	H	CH_2CH_2OH	D-W	78	168-169 dec	$C_{17}H_{16}N_6O_4$	0.12 ± 0.01	25	133
2d	NO_2	3	CH_3	CH_3	C-H	84	200-202 dec	$C_{18}H_{18}N_6O_3$	0.12 ± 0.03	25	110
2e	Cl	2	CH_3	CH_3	E-W	82	196-198	$C_{17}H_{16}N_5OCl$	0.10 ± 0.03	25	110
2f	Cl	2	C_2H_5	C_2H_5	E-W	79	159-161	$C_{19}H_{20}N_5OCl$	0.13 ± 0.06	12.5	111
2g	Cl	2	H	CH_2CH_2OH	T-H	80	145-146	$C_{17}H_{16}N_5O_2Cl$	0.16 ± 0.05	25	133
2h	Cl	3	CH_3	CH_3	E-W	77	125-127	$C_{18}H_{18}N_5OCl$	0.13 ± 0.01	50	111
2i	H	2	CH_3	CH_3	B-H	81	128-129	$C_{17}H_{17}N_5O$	0.21 ± 0.01	100	180
2j	H	2	C_2H_5	C_2H_5	B-H	83	158-159	$C_{19}H_{21}N_5O$	0.42 ± 0.17	100	160
2k	H	2	H	CH_2CH_2OH	D-A	90	164-165	$C_{17}H_{17}N_5O_2$	0.29 ± 0.12	50	133
2l	H	3	CH_3	CH_3	B-H	79	111-112	$C_{18}H_{19}N_5O$	0.28 ± 0.20	100	133
2m	CH_3	2	CH_3	CH_3	A-W	80	180-182	$C_{18}H_{19}N_5O$	0.17 ± 0.06	100	130
2n	CH_3	2	C_2H_5	C_2H_5	A-W	92	132-133	$C_{20}H_{23}N_5O$	0.21 ± 0.09	40	133
2o	CH_3	2	H	CH_2CH_2OH	D-H	77	164-166	$C_{18}H_{19}N_5O_2$	0.24 ± 0.15	not tested	
2p	CH_3	3	CH_3	CH_3	A-W	74	87-90	$C_{19}H_{21}N_5O$	0.15 ± 0.01	not tested	
2q	OCH_3	2	CH_3	CH_3	C-H	84	173-174	$C_{18}H_{19}N_5O_2$	0.10 ± 0.02	25	110
2r	OCH_3	2	C_2H_5	C_2H_5	A-W	82	138-139	$C_{20}H_{23}N_5O_2$	0.16 ± 0.04	12.5	120
2s	OCH_3	2	H	CH_2CH_2OH	D-A	81	176-177	$C_{18}H_{19}N_5O_3$	0.09 ± 0.01	12.5	120
2t	OCH_3	3	CH_3	CH_3	A-W	79	119-121	$C_{19}H_{21}N_5O_2$	0.17 ± 0.13	not tested	
2u	OH	2	CH_3	CH_3	A-C	80	218-220	$C_{17}H_{17}N_5O_2$	0.10 ± 0.03	25	260 (2/6)
2v	OH	2	C_2H_5	C_2H_5	A-C	81	211-213	$C_{19}H_{21}N_5O_2$	0.05 ± 0.02	8	190
2w	OH	2	H	CH_2CH_2OH	D-W	80	190-192	$C_{17}H_{17}N_5O_3$	0.12 ± 0.03	12.5	210
2x	OH	3	CH_3	CH_3	A-C	83	228-230	$C_{18}H_{19}N_5O_2$	0.08 ± 0.01	25	320 (3/6)

[a] A, acetone; B, benzene; C, chloroform; D, dioxane; E, ethanol; H, hexane; T, toluene; W, water. [b] All compounds were analyzed for C, H, N, and the results were within ±0.4% of the theoretical values. [c] Cytotoxic assay is based on a 72-h exposure time. For a detailed description of the assay see ref 16. A criterion for significant cytotoxic activity is $IC_{50} < 1$ μg/mL. Results represent the mean value from three independent experiments. The solutions for this test were prepared by dissolving the compounds in a volume of 0.2% aqueous L-lactic acid containing 2 equiv of acid, and subsequent addition of EtOH to final concentration of 40% (v/v). [d] BDF1 mice were implanted ip with 10^6 P388 cells. Compounds were administrated ip as aqueous solutions containing 3 equiv of L-lactic acid on days 1-5. Values of T/C ≥125% indicate statistically significant antileukemic activity. For the general procedure, see ref 17.

(DMA) as solvent, readily gave the triazoloacridinones **2a-x** in good yields. Significant differences were found in reactivity of compounds **5a-f**, depending on the substituent at position 8. The 8-nitro and 8-chloro derivatives required 15-30 min of heating, the unsubstituted and 8-methyl derivatives needed about 60 min, and the 8-methoxy and 8-hydroxy derivatives required 2-4 h.

Two general methods were applied for isolation of pure final products. The first method consisted of crystallization of the product from the reaction mixture after addition of ethanol and subsequent recrystallization from a suitable solvent mixture. This method was not applied in the case of derivatives which were quite soluble in alcohol. Instead, such compounds were isolated by standard extractive methods as outlined in the Experimental Section.

The physicochemical properties and yields of the triazoloacridinones are listed in Table II. All compounds are yellow in color. The **2e-t** derivatives show blue fluorescence in the longwave UV. The 8-nitro derivatives darken in the light.

Results and Discussion

All the compounds listed in Table II were tested in vitro against HeLa S_3 cells and showed significant cytotoxic activity, comparable to that of ametantrone used as positive control. No marked differences in cytotoxic activity were noted for variable substituents at C-8 or for different side chains. In vivo antineoplastic activity was evaluated against murine P 388 leukemia (ip/ip; D 1-5). Contrary to the in vitro results, significant differences in activity were observed in this test, although no clear structure-activity relationship can be shown. All four 8-OH derivatives (**2u-x**) revealed excellent activity with cures in the case of **2u** and **2x**, the potency and efficacy depending on the nature of the side chain. Marked activity has also been found for two unsubstituted compounds (**2i** and **2j**), while the remaining derivatives showed borderline activity or were inactive.

The derived results clearly indicate that high antineoplastic activity is related to the A ring hydroxylation. This finding is consistent with the large, positive influence of A ring hydroxylation on the activity of anthracenediones,[7] pyrazoloacridines,[3b,18] benzothiopyranoindazoles,[13] and synthetic analogues of ellipticine.[19] The role played by the hydroxy group on antileukemic activity of these compounds is not clear. The **2x** derivative containing three methylene units between nitrogen atoms in the side chain showed the highest activity. This remains in contrast to 1,8-naphthalimides[20] and imidazoacridinones,[5] where

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(18) (a) Jackson, R. C.; Leopold, W. R.; Sebolt, J. S. *Proc. Am. Assoc. Cancer Res.* **1988**, *29*, 536. (b) Sebolt, J.; Havlick, M.; Hamelehle, K.; Nelson, J.; Leopold, W.; Jackson, R. *Cancer Chemother. Pharmacol.* **1989**, *24*, 219.

(19) Bisagni, E.; Nguyen, C. H.; Pierre, A.; Pepin, O.; de Cointet, P.; Gros, P. *J. Med. Chem.* **1988**, *31*, 398.

(20) Zee-Cheng, R. K.-Y.; Cheng, C. C. *J. Med. Chem.* **1985**, *28*, 1216.

compounds with more than two carbon atoms separating the nitrogens were either inactive or greatly diminished in activity.

Conclusions

The results of our studies demonstrated that a chromophore modification of the previously described imidazoacridinones, in which the D ring is replaced with a five-membered triazolo ring, gives compounds with preserved antineoplastic activity. This activity is significantly enhanced by a presence of OH group at position C-8 and decreased by OCH_3, CH_3, Cl, and NO_2 substituents at this position.

No significant differences in in vivo antileukemic activity has been found in the group of active 8-OH derivatives containing an ethylenediamine or propylenediamine side chain.

As in the case of imidazoacridinones, there is no correlation between the in vitro and in vivo antineoplastic activity of the triazoloacridinones.

Experimental Section

Melting points were determined with a Boetius PHMK 05 apparatus and are uncorrected. NMR spectra were obtained with a Varian VXR-300 spectrometer using TMS as internal standard and are reported as δ (ppm) values. NMR abbreviations used are as follows: s (singlet), d (doublet), t (triplet), qu (quartet), qt (quintet), m (multiplet), ex (exchangeable with deuterium oxide). Coupling constants are given in hertz. Quartets that are transformed into triplets by addition of deuterium oxide are labeled with a *. Elemental analyses were performed by the Laboratory of Elemental Analysis, Institute of Chemistry, University of Gdansk and were within ±0.4% of the calculated values.

6-Chloro-2-[(4-chlorophenyl)amino]-3-nitrobenzoic Acid (6). 4-Chloroaniline (19.1 g, 0.15 mol) was heated at 110 °C under stirring until completely melted, and 2,6-dichloro-3-nitrobenzoic acid[14] (7.08 g, 0.03 mol) was subsequently added in small doses during 20 min. Stirring was continued for 2 h, and the mixture was then heated for an additional 5 h. The solidified mass was cooled and 2 N aqueous NaOH (150 mL) was added. The mixture was thoroughly crushed and vigorously stirred for 30 min. The unreacted aniline was filtered off and washed with water. The filtrate was acidified with diluted hydrochloric acid to pH = 2. The precipitate was filtered off, washed with water, and dried to give chromatographically pure, yellow-orange **6** (7.11 g, 72%): mp 204–207 °C, a purified sample from toluene recrystallization melts at 206–208 °C; ^{1}H NMR (Me_2SO-d_6), 8.54 (1 H, s, ex, N*H*), 8.05 (1 H, d, J = 8.9), 7.48 (2 H, d, J = 8.9), 7.18 (2 H, d, J = 8.9), 6.77 (2 H, d, J = 8.9). Anal. ($C_{13}H_8Cl_2N_2O_4$) C, H, N.

1,7-Dichloro-4-nitro-9(10*H*)-acridinone (3e). A mixture of 6-chloro-2-[(4-chlorophenyl)amino]-3-nitrobenzoic acid (6.54 g, 0.02 mol) and $POCl_3$ (20 mL) was refluxed for 1 h. Excess of $POCl_3$ was removed under reduced pressure and 1,4-dioxane (30 mL) was added to the residue. The product was filtered and washed thoroughly with water and ethanol to give **3e** as red crystals (5.06 g, 82%): mp 305–307 °C; ^{1}H NMR (Me_2SO-d_6), 11.58 (1 H, s, ex, N*H*), 8.55 (1 H, d, J = 8.8, 3-H), 8.11 (1 H, d, J = 1.5, 8-H), 8.09 (1 H, d, J = 9.0, 5-H), 7.85 (1 H, dd, J_0 = 8.9, J_m = 1.5, 6-H), 7.44 (1 H, d, J = 8.8, 2-H). Anal. ($C_{13}H_6Cl_2N_2O_3$) C, H, N.

General Procedure for the Preparation of 4. Example: 4-Amino-1,7-dichloro-9(10*H*)-acridinone (4e). A solution of $SnCl_2 \cdot 2H_2O$ (20.31 g, 0.09 mol) in concentrated hydrochloric acid (25 mL) was added at room temperature with stirring to a suspension of finely powdered **3e** (6.28 g, 0.02 mol) in ethanol–concentrated hydrochloric acid (20 mL:20 mL). The vigorously stirred mixture was heated under reflux for 6 h. After cooling, the precipitate was filtered, poured into water (200 mL), made basic with NaOH, and stirred vigorously for 30 min. The product was collected, washed with water, and crystallized from DMF–H_2O to give yellow crystals of **4e** (5.02 g, 90%): mp 290–293 °C; ^{1}H NMR (Me_2SO-d_6), 10.64 (1 H, s, ex, NH), 8.07 (1 H, d, J = 1.6, 8-H), 7.71 (2 H, m, 5-H and 6-H), 7.03 (1 H, d, J = 8.3, 1-H), 6.96 (1 H, d, J = 8.3, 3-H), 5.62 (2 H, s, ex, NH_2).

In the case of **4b**, the product obtained after reduction was stirred with pure water without alkalization.

General Procedure for the Preparation of 5a–e. Example: 5-Chloro-8-hydroxy-6*H*-*v*-triazolo[4,5,1-*de*]acridin-6-one (5b). A suspension of finely powdered **4b** (7.8 g, 0.03 mol) in concentrated hydrochloric acid (100 mL) was stirred vigorously at room temperature for 30 min. A solution of $NaNO_2$ (2.76 g, 0.04 mol) in water (60 mL) was added to the suspension in small doses and the mixture was stirred for 4 h. The precipitate was collected, washed with water, and crystallized from DMF–H_2O to give **5b** as yellow needles (7.34 g, 90%): mp >350 °C dec; ^{1}H NMR (Me_2SO-d_6), 10.38 (1 H, s, ex, O*H*), 8.55 (1 H, d, J = 8.6, 3-H), 8.27 (1 H, d, J = 8.8, 10-H), 7.77 (1 H, d, J = 8.6, 4-H), 7.60 (1 H, d, J = 1.7, 7-H), 7.38 (1 H, dd, J_0 = 8.8, J_m = 1.7, 9-H). Anal. ($C_{13}H_6ClN_3O_2$) C, H, N.

5-Chloro-8-nitro-6*H*-*v*-triazolo[4,5,1-*de*]acridin-6-one (5f). To a solution of **5a** (2.04 g, 0.008 mol) in concentrated H_2SO_4 (25 mL) was added finely powdered $NaNO_3$ (740 mg, 0.009 mol) and the mixture was vigorously stirred at room temperature for 1.5 h. The mixture was poured into cold water (100 mL). The precipitate was collected, washed with water, and crystallized from DMF–EtOH to give **5f** as almost colorless crystals (1.88 g, 78%): mp >350 °C dec; ^{1}H NMR (Me_2SO-d_6), 9.02 (1 H, d, J = 2.5, 7-H), 8.81 (1 H, dd, J_0 = 9.2, J_m = 1.7, 9-H), 8.74 (1 H, d, J = 9.2, 10-H), 8.73 (1 H, d, J = 8.5, 3-H), 7.93 (1 H, d, J = 8.5, 4-H). Anal. ($C_{13}H_5ClN_4O_3$) C, H, N.

General Procedure for the Preparation of 2a,c,g,k,o,s,u–x. Example: 5-[[3-(Dimethylamino)propyl]amino]-8-hydroxy-6*H*-*v*-triazolo[4,5,1-*de*]acridin-6-one (2x). 3-(Dimethylamino)propan-1-amine (5.8 mL, 0.045 mol) was added to a suspension of **5b** (4.08 g, 0.015 mol) in freshly distilled DMA (25 mL) and the mixture was heated at 60 °C for 4 h. The reaction mixture, after addition of ethanol (75 mL), was left overnight in a refrigerator. The crystallized product was filtered off and washed with water (2 × 100 mL) and methanol (50 mL). Then, it was recrystallized from aqueous DMA to give **2x** as yellow-orange needles (4.2 g, 83%): mp 228–230 °C; ^{1}H NMR (Me_2SO-d_6), 10.26 (1 H, s, ex, O*H*), 9.43 (1 H, t, ex, N*H*), 8.33 (1 H, d, J = 8.8, 10-H), 8.27 (1 H, d, J = 9.2, 3-H), 7.73 (1 H, d, J = 2.8, 7-H), 7.41 (1 H, dd, J_0 = 8.8, J_m = 2.8, 9-H), 7.14 (1 H, d, J = 9.3, 4-H), 3.58 (2 H, qu*, $NHCH_2CH_2$), 2.36 (2 H, t, $CH_2CH_2NMe_2$), 2.18 (6 H, s, $N(CH_3)_2$), 1.82 (2 H, qt, $CH_2CH_2CH_2$). Anal. ($C_{18}H_{19}N_5O_2$) C, H, N.

General Procedure for the Preparation of 2b,d–f,h–j,l–n,p,r,t. Example: 5-[[3-(Dimethylamino)propyl]amino]-8-methoxy-6*H*-*v*-triazolo[4,5,1-*de*]acridin-6-one (2t). 3-(Dimethylamino)propan-1-amine (0.6 mL, 4.7 mmol) was added to a suspension of **5d** (432 mg, 1.5 mmol) in freshly distilled DMA (3 mL) and the mixture was heated at 60 °C for 3 h. Chloroform (100 mL) and water (50 mL) were added, and the reaction mixture was vigorously shaken. The chloroform layer was separated; water (100 mL) was added and acidified with L-lactic acid. After shaking, the water layer was separated, made basic with aqueous NaOH, and extracted with chloroform. The chloroform extract was evaporated and the crude product was crystallized from a acetone–water mixture to give yellow needles (420 mg, 79%): mp 119–121 °C; ^{1}H NMR (Me_2SO-d_6), 9.36 (1 H, t, ex, N*H*), 8.37 (1 H, d, J = 8.9, 10-H), 8.27 (1 H, d, J = 9.2, 3-H), 7.74 (1 H, d, J = 2.8, 7-H), 7.56 (1 H, dd, J_0 = 8.9, J_m = 2.9, 9-H), 7.12 (1 H, d, J = 9.3, 4-H), 3.94 (3 H, s, OCH_3), 3.57 (2 H, qu*, $NHCH_2CH_2$), 2.36 (2 H, t, $CH_2CH_2NMe_2$), 2.19 (6 H, s, $N(CH_3)_2$), 1.83 (2 H, qt, $CH_2CH_2CH_2$). Anal. ($C_{19}H_{21}N_5O_2$) C, H, N.

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Registry No. **2a**, 128112-99-2; **2b**, 128113-01-9; **2c**, 128113-01-9; **2d**, 128113-02-0; **2e**, 128113-03-1; **2f**, 128113-04-2; **2g**, 128113-05-3; **2h**, 128113-06-4; **2i**, 128113-07-5; **2j**, 128113-08-6; **2k**, 128113-09-7; **2l**, 128113-10-0; **2m**, 128113-11-1; **2n**, 128113-12-2; **20**, 128113-13-3;

2p, 128113-14-4; **2q**, 128113-15-5; **2r**, 128113-16-6; **2s**, 128113-17-7; **2t**, 128113-18-8; **2u**, 128113-19-9; **2v**, 128113-20-2; **2w**, 128113-21-3; **2x**, 128113-22-4; **3a**, 20621-51-6; **3b**, 99009-49-1; **3c**, 21814-53-9; **3d**, 21814-48-2; 3e, 21814-67-5; **4a**, 128113-23-5; **4b**, 128113-24-6; **4c**, 128113-25-7; **4d**, 128113-26-8; **4e**, 128113-27-9; **5a**, 76181-06-1; **5b**, 128113-28-0; **5c**, 128113-29-1; **5d**, 128113-30-4; **5e**, 128113-31-5; **5f**, 128113-32-6; **6**, 55776-14-2; $H_2N(CH_2)_2NMe_2$, 108-00-9; $H_2N(CH_2)_2NEt_2$, 100-36-7; $H_2N(CH_2)_2NH(CH_2)_2OH$, 111-41-1; $H_2N(CH_2)_3NMe_2$, 109-55-7; 4-chloroaniline, 106-47-8; 2,6-dichloro-3-nitrobenzoic acid, 55775-97-8.

Propenyl Carboxamide Derivatives as Antagonists of Platelet Activating Factor

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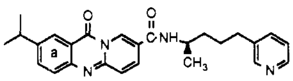
A series of *N*-[4-(3-pyridinyl)butyl] 3-substituted propenyl carboxamide derivatives bearing an unsaturated bicyclic moiety in the 3-position was prepared and evaluated for PAF (platelet activating factor) antagonist activity. These compounds represent conformationally constrained direct analogues of the corresponding potent 5-arylpentadienecarboxamides (**5**). Most of the new compounds were active in a PAF-binding assay employing whole, washed dog platelets as the receptor source and inhibited PAF-induced bronchoconstriction in guinea pigs after intravenous administration. However, oral activity in the PAF-induced bronchoconstriction model was highly sensitive to the nature and substitution of the bicyclic ring system. The most interesting compounds included [*R*-(*E*)]-(1-butyl-6-methoxy-2-naphthyl)-*N*-[1-methyl-4-(3-pyridinyl)butyl]-2-propenamide (**4b**), [*R*-(*E*)]-(3-butyl-6-methoxy-2-benzo[*b*]thiophene-yl)-*N*-[1-methyl-4-(3-pyridinyl)butyl]-2-propenamide (**4k**), and [*R*-(*E*)]-(3-butyl-6-methoxy-1-methyl-2-indolyl)-*N*-[1-ethyl-4-(3-pyridinyl)butyl]-2-propenamide (**4l**) which inhibited PAF-induced bronchoconstriction in guinea pigs with IC_{50}s of 3.0–5.4 mg/kg, when the animals were challenged 2 h after drug treatment. They were also highly effective 6 h after a 50 mg/kg oral dose. This study supports the notion that the key remote aromatic ring present in the 5-arylpentadienecarboxamides (**5**) is preferentially coplanar with the diene system for good PAF antagonist activity.

In the relatively short period since the discovery of platelet activating factor (PAF), considerable effort has been invested in determining the pathophysiological role of this ether phospholipid, particularly as a mediator of allergic[1-4] and inflammatory disease states.[5,6] The search for PAF antagonists has led to the identification of a wide assortment of structural types that exhibit potent inhibitory activity in both in vitro and in vivo screening models. Several of these PAF antagonists are currently being evaluated in man.[7]

In preceding papers from these laboratories,[8-10] we have described the synthesis and pharmacological evaluation of several related series of PAF antagonists exemplified by pyridoquinazolinecarboxamide 1, biphenyl carboxamide 2, and (*E*,*E*)-5-phenyl-2,4-pentadienamide 3, a compound that was ultimately selected for clinical development. In these reports, we discussed in detail the key structural features common to 1–3 that are apparently required for PAF inhibition. These include an aromatic ring "a" attached through an extended π-system to a carboxamide group, connected in turn with an appropriate spacer to a 3-pyridyl moiety.



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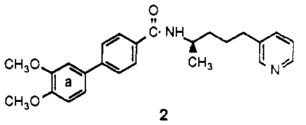

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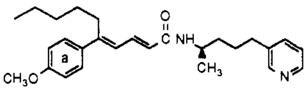

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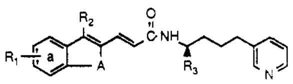

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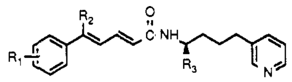
The pyridoquinazolines in which the key aromatic ring "a" is part of a planar heteroaromatic ring are generally less potent PAF antagonists than the biphenylcarboxamides or the pentadienamides in which rotation of the corresponding aromatic ring out of conjugation with the remainder of the π-system is possible. We were thus interested to determine the effect of constraining analogues of 3 such that the aromatic ring would be held in conjugation with the olefin and amide portions of the molecule. In the present study, we have prepared a number of propenamide derivatives of general formula 4 in which an ortho position of the aromatic ring has been fused to C_4 of the pentadienamide moiety through a one or two atom linking unit "A".

Much of the information elicited from structure–activity studies on the 5-phenyl-2,4-pentadienamide series was available when the present program was initiated. With reference to 5, structural elements shown to be required



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(1) Morley, J.; Smith, D. *Agents Actions* **1989**, *26*, 31.
(2) Page, C. P. *J. Allergy Clin. Immunol.* **1988**, *81*, 144.
(3) Barnes, P. J. *J. Allergy Clin. Immunol.* **1988**, *81*, 152.
(4) Townley, R. G.; Hopp, R. J.; Agrawal, D. K.; Bewtra, A. K. *J. Allergy Clin. Immunol.* **1989**, *83*, 997.
(5) Braquet, P.; Touqui, L.; Shen, T. Y.; Vargaftig, B. B. *Pharmacol. Rev.* **1987**, *39*, 97.
(6) Braquet, P.; Paubert-Braquet, M.; Koltai, M.; Rourgain, R.; Bussolino, F.; Hosford, D. *Trends Pharmacol. Sci.* **1989**, *10*, 23.
(7) Cooper, K.; Parry, M. J. *Annu. Rep. Med. Chem.* **1989**, *24*, 81.
(8) Tilley, J. W.; Burghardt, B.; Burghardt, C.; Mowles, T. F.; Lienweber, F.-J.; Klevans, L.; Young, R.; Hirkaler, G.; Fahrenholtz, K.; Zawoiski, S.; Todaro, L. J. *J. Med. Chem.* **1988**, *31*, 466.
(9) Tilley, J. W.; Clader, J. W.; Zawoiski, S.; Wirkus, M.; LeMahieu, R. A.; O'Donnell, M.; Crowley, H.; Welton, A. F. *J. Med. Chem.* **1989**, *32*, 1814.
(10) Guthrie, R. W.; Kaplan, G.; Mennona, F.; Tilley, J. W.; Kierstead, R. W.; Mullin, J.; Zawoiski, S.; LeMahieu, R. A.; O'Donnell, M.; Crowley, H.; Yaremko, B.; Welton, A. F. *J. Med. Chem.* **1989**, *32*, 1820.

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