as part of a synthetic program or by fermentation, in order to increase β-lactamase stability. For the case of penicillin, however, no general solution to combining both high intrinsic activity and β-lactamase stability has yet emerged. These data should also be useful as a catalogue of substructures to be correlated with other aspects of the interactions among β-lactams, β-lactamases, and β-lactamase-producing bacteria. These include the ability to induce the formation of β-lactamase by Gram-negative bacteria, to bind tightly to the inducible β-lactamases, and to be excluded, by permeability changes, from entry into Gram-negative organisms. Each of these phenomena has been implicated as a mechanism of resistance of Gram-negative bacteria to β-lactamase-stable β-lactam antibiotics.[31]

Acknowledgment. Drs. K. Bush and W. H. Koster critically read and commented on this manuscript while in preparation. Drs. K. Bush and D. P. Bonner contributed most of the unpublished observations made at the Squibb Institute. The author's appreciation for and perspective on the importance of β-lactamases has been influenced by his collaboration with Dr. R. B. Sykes.

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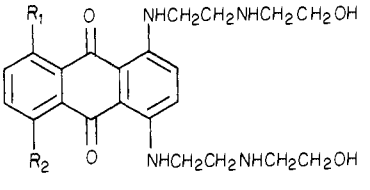
Communications to the Editor

5-[(Aminoalkyl)amino]-Substituted Anthra[1,9-*cd*]pyrazol-6(2*H*)-ones as Novel Anticancer Agents. Synthesis and Biological Evaluation

Sir:

The anthracycline antitumor antibiotics daunorubicin and doxorubicin have a well-established place in the clinical treatment of various malignant diseases.[1] Unfortunately, these drugs possess some serious toxicities, especially cardiotoxicity, ranging from delayed and insidious cardiomyopathy to irreversible congestive heart failure. Recent research efforts have directed attention toward developing new DNA-complexing agents that minimize this side effect, notably second-generation anthracyclines, such as aclacinomycin A,[1b] and several anthracenediones.[2,3] Of the latter, ametantrone (1) and



1, $R_1 = R_2 = H$ (ametantrone)
2, $R_1 = R_2 = OH$ (mitoxantrone)

mitoxantrone (2) are currently undergoing phase II clinical trials. In-depth studies on mitoxantrone (2)[4] have suggested activity principally in the treatment of breast cancer and the acute leukemias, with less encouraging results against other tumors when compared to doxorubicin. However, cardiotoxicity studies show that while occasional episodes have been reported in patients on prolonged treatment with 2, the overall incidence of cardiac failure has been relatively low.

We now report the synthesis and initial biological evaluation of two representatives, compounds 8 and 10, of a novel class of highly active anticancer DNA-complexing agents, namely, 5-[(aminoalkyl)amino]anthra[1,9-*cd*]pyrazol-6(2*H*)-ones (hereafter referred to as anthrapyrazoles). It was anticipated that such chromophore modification of the anthracenedione nucleus might provide a unique class of DNA-complexing agents with diminished or absent cardiotoxicity vis-a-vis a reduced tendency to semiquinone free radical formation. Such rationale has been applied to the synthesis of chromophore-modified anthracyclines.[5-7]

Chemistry. Scheme I delineates the synthetic pathway to the target anthrapyrazoles, 8 and 10.[8]

Alkylation of 1,4-dichloro-5-hydroxy-9,10-anthracenedione (3)[9] with powdered K_2CO_3 and benzyl bromide in refluxing acetone gave a 93% yield of the benzyl ether 4, mp 124–127 °C. Condensation of dichloroether 4 with 3 equiv of 2-[(2-hydrazinoethyl)amino]ethanol[10] in anhydrous Me_2SO afforded a 50% crude yield of regioisomers 5 and 6 in ca. 4:1 ratio, respectively, by HPLC. Partial separation of isomers was achieved by flash silica gel chromatography. The faster eluting component was the minor isomer, 6, mp 172–175 °C, and the slower eluting component was the major isomer, 5, mp 142–143 °C. Reaction of 5 with an excess of 2-[(2-aminoethyl)amino]ethanol in refluxing pyridine gave the "two-armed" compound 7, mp 157–159 °C. Hydrogenolysis of the benzyl protecting group with Pearlman's catalyst[11] in glacial AcOH, followed by dihydrochloride salt formation, gave the target 7-hydroxy compound 8 in 88% yield, mp 265–270 °C dec. Application of the same transformations to 6 afforded the 10-hydroxy isomer 10, mp 260–267 °C dec, via benzylated intermediate 9, mp 178–180 °C.

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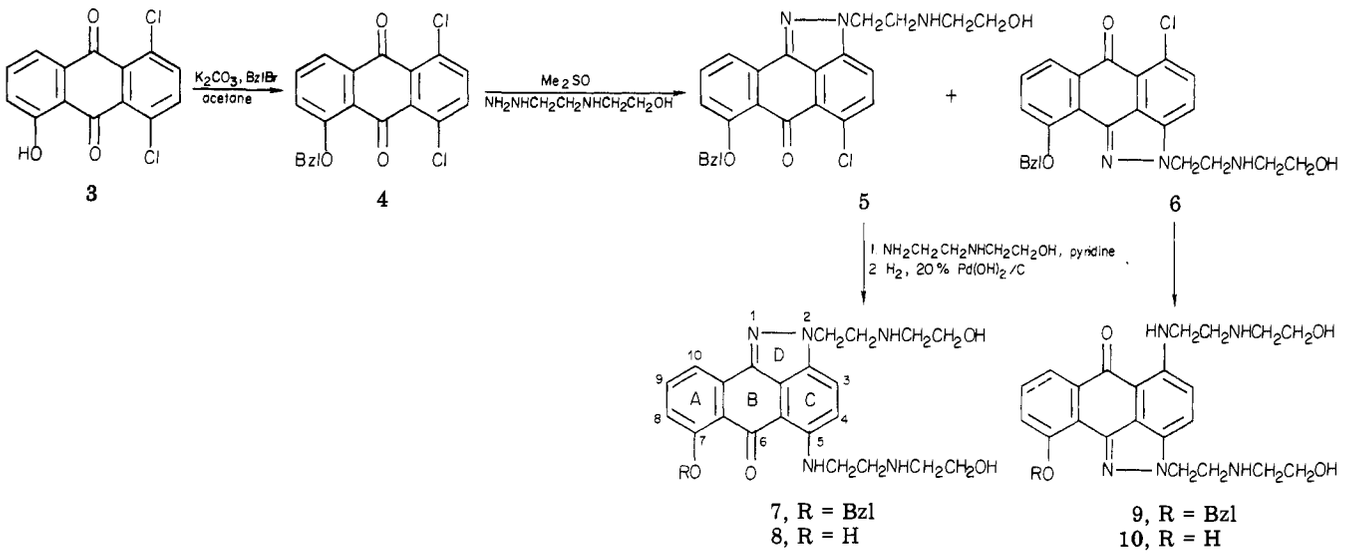
Scheme I[a]



[a] Bzl = benzyl.

Table I. In Vitro and in Vivo Anticancer Activity of Anthrapyrazoles 8 and 10

		murine in vivo act.				
		dose,[b] mg/kg		P388[c,e] leukemia % T/C	mammary adenocarcinoma 16C[d,e]	
compd[a]	L1210 leukemia in vitro: ID_{50}, M	single injn	total		T − C days	gross log kill
8	5.13×10^{-9}	25	50	248 (5/6)		
		12.5	25	219 (2/6)		
		12.5	37.5		11.4	3.1
		6.25	18.8		7.0	1.9
10	1.80×10^{-7}	100	200	221	not tested	
		50	100	172		
mitoxantrone (2)	1.55×10^{-9}	1.5	3.0	278 (3/6)		
		0.75	1.5	231 (2/6)		
		2.5	7.5		4.1	1.1
		1.25	3.75		3.0	0.8

[a] Tested as the dihydrochloride salt. [b] The highest doses listed are the maximum tolerated dose ($\leq LD_{10}$) for mammary 16C and the dose to give the optimum response for P388. [c] Carried out by a slight modification (q04dX 02, ip schedule) of the National Cancer Institute testing protocol described in ref 13. Mice surviving 30 days are considered cured. [d] Testing and evaluation carried out as described in ref 14 and 15. The biology and therapeutic response characteristics of mammary adenocarcinoma 16C have been previously described in ref 16. Mice were inoculated sc on day 0 and treated iv on days 1, 5, and 9. Tumors were measured in two dimensions, three times weekly to assess tumor growth delay (T − C). [e] Numbers in parentheses are number of mice cured/number of mice in test. Cures are excluded from calculations of % T/C, T − C, and log kill. % T/C values >125 and gross log kill values >0.7 indicate significant activity.

Biology. Table I lists in vitro and optimal in vivo antitumor activity for target anthrapyrazoles **8** and **10** and for mitoxantrone (**2**), which was utilized as a positive control. Compound **8** was essentially equipotent with mitoxantrone in the in vitro L1210 lymphocytic leukemia assay,[12] whereas the activity of **10** was 100-fold lower. Both agents had excellent activity against P388 leukemia, with compound **8** giving cures at 12.5–25 mg/kg. Mitoxantrone (**2**) had comparable efficacy but was considerably more potent than either anthrapyrazole. Compound **8** also displayed strong activity against mammary adenocarcinoma 16C, surpassing that of mitoxantrone by 2–3 logs of tumor cell kill. Compound **10** was not tested against this tumor. The position of the ring hydroxy substitution did not have a strong effect on in vivo activity in the P388 system but did have a significant influence on potency, the anthrapyrazole **8** being approximately 4-fold more potent than **10**.

Future reports will detail the synthesis and broad-spectrum anticancer activity of a large series of anthrapyrazoles characterized by aminoalkyl and hydroxyalkyl substitutents at N-2, (aminoalkyl)amino substituents at C-5, and optimal A-ring substitution ranging from no substitution to C-7, C-10, C-7,10, C-7,8,10, and C-7,9,10 hydroxylation patterns. In addition, detailed biological and biochemical studies will be presented supporting the selection of specific compounds from this series for preclinical toxicology studies as a prelude to clinical trials.

Registry No. **3**, 6770-15-6; **4**, 88303-56-4; **5**, 88303-57-5; **6**, 88303-58-6; **7**, 88303-59-7; **8**, 88303-60-0; **8**·2HCl, 88303-61-1; **9**, 88303-62-2; **10**, 88303-63-3; **10**·2HCl, 88303-64-4; NH_2NHCH_2C-

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$H_2NHCH_2CH_2OH$, 88303-65-5; $NH_2CH_2CH_2NHCH_2CH_2OH$, 111-41-1.

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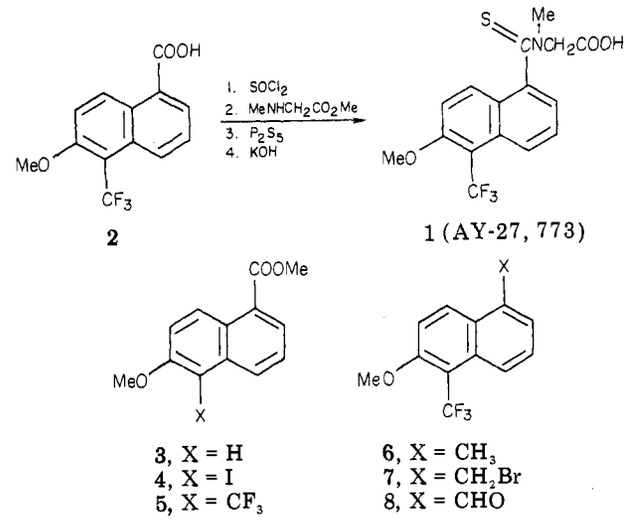
N-[[5-(Trifluoromethyl)-6-methoxy-1-naphthalenyl]thioxomethyl]-*N*-methylglycine (Tolrestat),[1] a Potent, Orally Active Aldose Reductase Inhibitor[2]

Sir:

Insulin therapy is effective for the primary control of glucose levels and has considerably reduced mortality from acute effects of diabetes. However, the complications of chronic diabetes, e.g., neuropathy, nephropathy, retinopathy, and cataracts, are, in practice, not controlled by insulin. The tissues involved (nerve, kidney, retina, and lens) do not require insulin for glucose uptake and, hence, are exposed to a high concentration of glucose, which enters the sorbitol pathway and is reduced by aldose reductase to sorbitol. The intracellular accumulation of sorbitol and its metabolite fructose can eventually result in a loss of osmotic integrity and cellular damage. These events have been linked to the development of some complications of chronic diabetes,[3] and, consequently, inhibition of the enzyme aldose reductase should provide a pharmacological approach to the treatment of these complications. Earlier work has appeared describing the biochemical, pharmacological, and clinical properties of alrestatin,[4-13] an aldose reductase inhibitor of relatively low potency developed in these laboratories. Efforts to develop a potent, orally active aldose reductase inhibitor have continued, and this report describes the chemistry, biochemistry, and pharmacology of *N*-[[5-(trifluoromethyl)-6-methoxy-1-naphthalenyl]thioxomethyl]-*N*-methylglycine (tolrestat,[1] 1).

Scheme I



2 1 (AY-27,773)

3, X = H
4, X = I
5, X = CF_3

6, X = CH_3
7, X = CH_2Br
8, X = CHO

Chemistry.[14] Tolrestat (1) was synthesized from 6-methoxy-5-(trifluoromethyl)naphthalene-1-carboxylic acid (2), mp 218–219 °C, by treatment of its acid chloride with methyl sarcosinate (Scheme I). The resulting carboxamide, mp 70–71 °C, was heated with phosphorus pentasulfide to afford 1 methyl ester, mp 109–110 °C, which on hydrolysis with KOH gave 1:[15] mp 164–165 °C; homogeneous by HPLC.[16]

The key intermediate 2, as its methyl ester, was obtained in 87% yield via the iodination of methyl 6-methoxy-1-naphthalenecarboxylate (3)[17] with I_2/HIO_3 in an acetic acid–sulfuric acid mixture. The position of iodination was confirmed by the synthesis of 2 from authentic 2-methoxy-5-methyl-1-(trifluoromethyl)naphthalene (6)[18] (see below). The iodo derivative 4, mp 98–99 °C, was reacted with trifluoromethyl iodide and copper powder in pyridine in an autoclave at 120 °C for 20 h to give 5, mp 75–78 °C, in 93% yield. Hydrolysis of 5 afforded the key intermediate 2, mp 221–222 °C. Alternatively, 2 was obtained directly from 6[18] in 55% yield by potassium permanganate oxidation. Compound 6 was also brominated with NBS to afford 7, mp 97–99 °C, which was converted to aldehyde 8, mp 98–100 °C, in high yield under the conditions of the Sommelet reaction. Oxidation of 8 with potassium permanganate gave 2.

Biochemistry and Pharmacology. Biochemical and pharmacological properties of tolrestat (1) were investigated and compared with data obtained with alrestatin (vide supra) or sorbinil,[19] the two aldose reductase inhib-

(1) Nonproprietary name adopted by the USAN Council; also known by the Ayerst code number AY-27,773.
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