Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 21, pp. 2473-2476, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00426-2

METHANODIBENZOSUBERYLPIPERAZINES AS POTENT MULTIDRUG RESISTANCE REVERSAL AGENTS[1]

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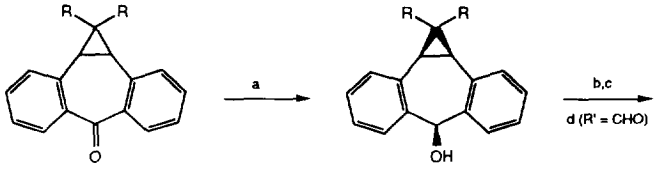
Abstract. Annellation of a cyclopropyl group to the dibenzosuberane moiety of the prototypical agent MS-073 afforded potent multiple drug resistance modulators. The chiral differentiation observed for the enantiomers of some of these compounds points to a specific interaction with the drug efflux pump, glycoprotein p170, in contrast to the less specific membrane interaction seen with less potent agents.

Introduction. The multiple drug resistance (MDR) phenotype involves intrinsic and acquired cross-resistance to structurally and mechanistically diverse agents such as anthracyclines, vinca alkaloids, epipodophyllotoxins, colchicine and taxol. While drug uptake appears normal, accumulation is reduced as a result of increased efflux due to overexpression of glycoprotein p170 (P-gp), an energy-dependent pump.[4] Although MDR can be reversed by calcium entry blockers, phenothiazines, progesterone, quinidine, cyclosporin A and a host of other, structurally unrelated pharmacological agents,[5] their clinical use could be severely limited because of undesirable side effects. Recent efforts have therefore focused on minimizing unwanted biological activities in existing series, while attempting to boost MDR reversal potency.[6] Due to the lack of understanding of the interaction of MDR reversal agents with glycoprotein p170, rational drug design stratagems have so far not led to more potent compounds.[7] While embarked on a screening program, our attention was drawn to a report on MS-073, an agent with noteworthy MDR reversal properties containing a dibenzosuberylpiperazine group attached to the 5-position of quinoline *via* a 2-hydroxypropoxy spacer.[8] The relatively low oral bioavailability reported for this compound is presumably a consequence of its acid lability ($t_{1/2}$ at pH 2.0 and 37 °C ca. 15 min.); the dibenzosuberyl group has in fact been used as an amine protecting group which can be removed under mildly acidic conditions.[9]

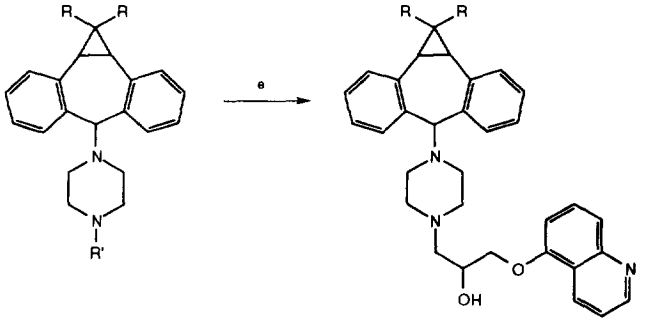
Extensive structural modifications of MS-073 invariably resulted in a loss of potency, usually at least by a factor of ten. However, annellation of a cyclopropyl group to the dibenzosuberane improved or maintained activity. Additionally, difluoro or dichloro substitution of the cyclopropane conferred excellent acid stability to these compounds ($t_{1/2}$ at pH 2.0 and 37 °C >> 72 h), whereas hydrogen substitution provided only intermediate stability ($t_{1/2}$ ca. 3 h).

Chemistry. Reduction of the methanodibenzosuberones **1-3**[10] with $NaBH_4$ gave exclusively the *syn*-alcohols **4-6** (Scheme I), which were converted to the corresponding chloro compounds (*syn/anti* mixtures) with $SOCl_2$. Treatment with 1-formylpiperazine afforded predominantly the *anti*-products **7a**, **8**, and **9**. In the difluoro series, the *syn*-analog **7b** was also isolated; stereochemical assignments could readily be made based on NOE experiments. Base-induced deformylation provided the piperazines **10-12**, which were reacted with racemic 5-(2,3-epoxypropoxy)quinoline or its individual enantiomers[11] to give the desired products **13-15**.

Scheme 1



1	R = F	
2	R = Cl	
3	R = H	
4	R = F	
5	R = Cl	
6	R = H	



7a	R = F	R' = CHO	*anti*
7b	R = F	R' = CHO	*syn*
8	R = Cl	R' = CHO	*anti*
9	R = H	R' = CHO	*anti*
10a	R = F	R' = H	*anti*
10b	R = F	R' = H	*syn*
11	R = Cl	R' = H	*anti*
12	R = H	R' = H	*anti*

13a	R = F	*anti*-(*R,S*)	**13f**	R = F	*syn*-(*S*)
13b	R = F	*anti*-(*R*)	**14a**	R = Cl	*anti*-(*R,S*)
13c	R = F	*anti*-(*S*)	**14b**	R = Cl	*anti*-(*R*)
13d	R = F	*syn*-(*R,S*)	**14c**	R = Cl	*anti*-(*S*)
13e	R = F	*syn*-(*R*)	**15**	R = H	*anti*-(*R,S*)

Reagents and Conditions:

(a) $NaBH_4$, THF/MeOH, 20 °C, 2 h (b) $SOCl_2$, dioxane, 50 °C, 4 h (c) 1-formylpiperazine, MeCN, 80 °C, 20 h (d) KOH, aq. EtOH, 80 °C, 1 h (e) 5-(2,3-epoxypropoxy)quinoline, 2-PrOH, 80 °C, 20 h

Results and Conclusions. The effect of the test compounds on the proliferation and viability of multidrug resistant CH^RC5 Chinese hamster cells in the presence and absence of doxorubicin was assessed in a modified MTT assay[12] *in vitro*. At 1 μM, **13a**, **13b**, and **13c** alone did not show any cytotoxicity.

Table I. MDR reversal EC_{50} values *in vitro*[13]

Ex.	R	Stereochem.	EC_{50} (nM)
13a	F	anti-(*R,S*)	50
13b	F	anti-(*R*)	20.7±3.8
13c	F	anti-(*S*)	60
13d	F	syn-(*R,S*)	200
13e	F	syn-(*R*)	180
13f	F	syn-(*S*)	190
14a	Cl	anti-(*R,S*)	70
14b	Cl	anti-(*R*)	55
14c	Cl	anti-(*S*)	100
15	H	anti-(*R,S*)	40
MS-073			65
S 9788[a]			290
Ro 11-2933[b]			540
Verapamil			1200

[a]Ref. 6h [b]Ref. 6f

A notable facet of these data is the superior potency of the *anti*-compounds **13a-c** (piperazine axial[14]) over their *syn*-counterparts **13d-f** (piperazine equatorial), which may be a result of more efficient π-stacking of the rigid *anti*-methanodibenzosuberyl group with overlapping phenylalanine side chains extending outwards from the α-helical backbone of P-gp. Such phenylalanine repeat motifs are in fact present in several transmembrane regions of human and murine P-gp.[15] It is also noteworthy that in the *anti*-series, compounds with the (*R*)-configuration in the hydroxypropoxy spacer unit are more potent than the (*S*)-enantiomers (**13b** > **13c**; **14b** > **14c**), whereas there is virtually no difference between the *syn*-enantiomers **13e** and **13f**. It has previously been reported that stereoisomers of calcium antagonists such as verapamil analogs and 1,4-dihydropyridines, which differed markedly in their potencies as calcium entry blockers, were equally effective in modulating drug transport by P-gp.[16] These observations, combined with the finding that compounds with a permanent positive charge such as the N-methyl quaternary ammonium derivatives of verapamil and chlorpromazine are ineffective in blocking MDR,[7a] implicate a "membrane bilayer pathway"[17] for the binding of MDR reversal agents to P-gp. According to this model, the drug molecule would partition to a well-defined, energetically favorable location, orientation, and conformation in the membrane bilayer before laterally diffusing to an intrabilayer P-gp binding site. Thus, it is plausible that the above-mentioned calcium entry blockers simply partition to a certain depth of the bilayer, resulting in modulation of P-gp function *via* a relatively non-specific membrane effect. Based on the high binding

affinity of **13b** to P-gp (IC_{50} = 40 nM vs. 22.6 μM for verapamil)[18], the methanodibenzosuberylpiperazines described here very likely interact with P-gp in a more specific manner.

Due to its high potency, benign receptor binding profile (no $pK_i > 6.3$), and effectiveness as an MDR modulator i.p. and p.o. in several *in vivo* models,[19] compound **13b** (RS 33295-198) represents a new benchmark clinical candidate.

References and Notes.

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13. The concentration of test compound required to reduce cell proliferation by 50% when used in combination with doxorubicin (1 μg/ml) was measured on day 3 in an MTT assay. Eight replicates for each of at least three drug concentrations were plated, and the EC_{50} interpolated from a graph of percent of control absorbance at 570 nm vs. drug concentration.
14. MM2 calculations indicate that in the *anti*-isomer **13a** the axial conformer is lower in energy than the equatorial conformer, whereas in the *syn*-isomer **13d** the conformational preference is reversed such that the equatorial conformer is lower in energy than the axial conformer.
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(*Received in USA* 31 *March* 1995; *accepted* 20 *September* 1995)