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SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF THE STEREOISOMERS OF GP-88, A PROPAFENONE-TYPE MODULATOR OF MULTIDRUG RESISTANCE

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Abstract: All four stereoisomers of the propafenone-type MDR-modulator GP-88 (1) were synthesized using a combined approach with chiral pool building blocks and an acetalic protective group, which allows not only diastereoseparation but also assignment of absolute configuration via NMR spectroscopy. Those isomers with different configuration on the center of chirality in the propanolamine side chain showed statistically different PGP-inhibitory activity. Generally, the (R)-configured isomers were by a factor of nearby two higher active than the (S)-isomers. No differences in activity were observed for isomers with different configuration on the benzylic center of chirality.

Active efflux of chemotherapeutic drugs gains increasing attention as basic mechanism responsible for development of multidrug resistance (MDR) both in tumor therapy and in therapy of bacterial and viral infections. In human tumor cells predominantly P-glycoprotein (PGP) and multidrug resistance related proteins (MRPs) account for decreased accumulation of natural product toxins.[1]

We recently showed, that both within a series of analogous propafenone derivatives and a series of conformationally constrained benzofurane analogs PGP inhibitory activity excellently correlates with overall lipophilicity of the molecules, whereby benzofuranes generally showed lower activity/lipophilicity ratios than propafenones. NMR and differential scanning calorimetry (DSC) experiments showed that this difference is not due to different interaction with phosphatidylcholine vesicles, which indicates a direct protein interaction as basic mechanism of action for propafenone-type modulators of MDR.[2] Synthesis of a series of deshydroxy derivatives demonstrated the importance of the propanolamine hydroxy group for protein interaction. Reduction of the carbonyl group in propafenones showed, that this substructure also seems to be important for high activity of the compounds.[3] Thus, we synthesized all four stereoisomeres of GP-88 (1) and tested their ability to inhibit PGP-mediated daunomycin transport in CEM vcr1000 cells.

For synthesis of the desired compounds, we applied an approach using a chiral auxiliary group, which allows also assignment of absolute configuration via NMR-spectrometry. This approach was recently used for synthesis of the enantiomers both of propafenone and corresponding benzofuranes.[4] Thus, the racemic phenol **2**

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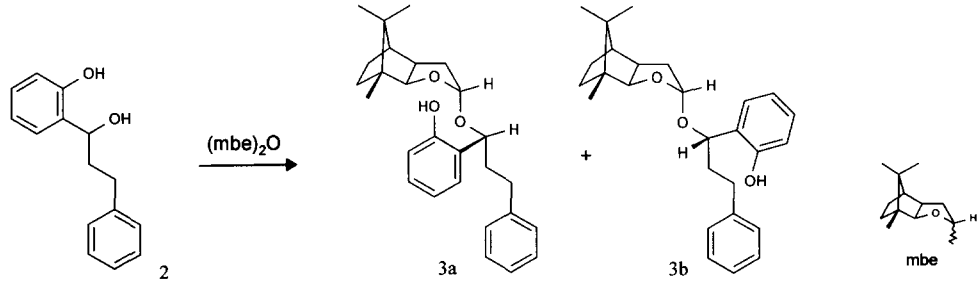
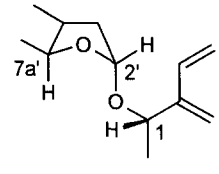
was reacted with [2S-[2α(2R*,3a'S*,4'S*,7'S*,7a'S*),3aα,4β,7β,7aα]]-2,2'-oxy-bis(octahydro-7,8,8-trimethyl-4,7-methanobenzofuran)[5] (mbe-dilactol) to yield a mixture of the desired diastereomers **3a** and **3b**. Separation of **3a** and **3b** was achieved via column chromatography on silica gel using petroleum ether/diethyl ether as eluent. To control diastereomeric purity, each fraction obtained was analyzed by achiral HPLC on reversed phase material. Only fractions with a *de* greater than 98% were collected as "pure" **3a** or **3b**. Mixtures were evaporated and kept for further separation runs.



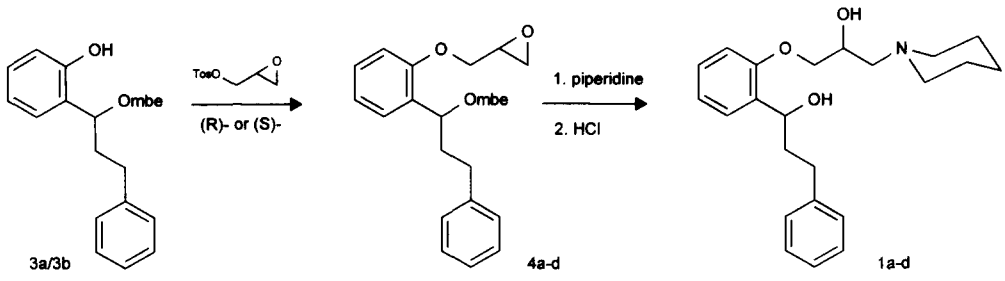
Assignment of the absolute configuration of the resolved diastereomers was undertaken via comparison of the ^{1}H and ^{13}C NMR spectra of **3a** and **3b** according to Noe's bulky-planar-H model.[6] Thus, the signal of the benzylic proton in **3b** was downfield shifted in comparison to **3a** due to the influence of the tetrahydrofurane oxygen. Furthermore, the signal of H-2' of the lactol moiety exhibits in **3b** a shift to higher field due to the ring current caused by the aromatic ring. Identical effects are responsible for a shift to higher field of H-7a' and the lactol methyl signals of **3a** in comparison to **3b**.

Table 1: Chemical shift (δ_{ppm}) of protons used for assignment of absolute configuration of **3a** and **3b**

	H-1	H-2'	H-7a'
3a	4.66	5.32	3.63
3b	4.79	5.13	4.00



Reaction of **3a** and **3b** with either (R)- or (S)-glycidyl tosylate lead to enantiomerically pure epoxides **4a-d**, which were reacted with piperidine to yield the amines **5a-d**. Cleavage of the protecting group was performed in analogy to Noe et al. under extremely mild conditions to avoid racemisation of the chiral center in the benzylic position.[7]



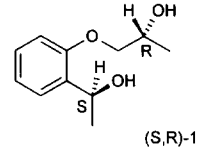
During the whole synthetic route control of diastereomeric purity is easily achieved via NMR spectroscopy. Nevertheless, reconfirmation of enantiomeric purity after cleavage of the mbe-protective group using chiral HPLC failed. Neither modified cyclodextrine columns like CYCLOBOND® RSP or CYCLOBOND® SN2000 nor a vancomycine column showed any separation. Even the diastereomers could not be separated. Thus, we used ^{13}C-NMR spectroscopy to prove diastereomeric purity of the products. The center of chirality in the propanolamine moiety remains stable during the cleavage of the mbe-protective group. Signals of Ph-O-$\underline{C}H_2$-, and Ph-$\underline{C}$H(OH)-$\underline{C}H_2$- were taken as probes for diastereomeric impurities. All four stereoisomers showed a diastereomeric purity greater than 90%.

MDR-modulating activity was estimated using the daunomycin efflux assay. This is a direct and accurate functional method to measure inhibition of PGP-mediated transmembrane transport. The resistant human T-lymphoblast cell line CEM vcr1000 was used in our studies. The time dependent decrease in mean cellular fluorescence was determined in the presence of various concentrations of modifier and the first order rate constants (V_{max}/K_m) were calculated by nonlinear regression analysis. Correction for simple diffusion was achieved by subtracting the efflux rates observed in the parental line. EC_{50} values of modifiers were calculated from dose response curves of V_{max}/K_m vs. modifier concentration, whereby the previously described procedure of simultaneous analysis of sigmoidal dose response curves was applied.[8]

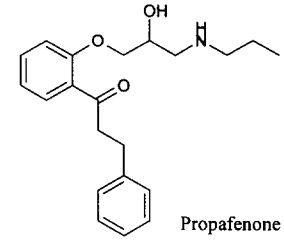
It is apparent from the data in Table 2 that (R,R)-1 and (S,R)-1 are by a factor of two higher active than the corresponding (R,S)- and (S,S)-stereoisomers. This indicates that the hydroxy group of the propanolamine side chain is involved in interaction with P-glycoprotein. To further support this finding, we also tested the enantiomers of propafenone (for an improved synthesis see ref. [9]). In analogy to **1a-d**, (R)-propafenone showed slightly, but nevertheless statistically significant higher activity than (S)-propafenone. This might have some implications for future clinical testing of propafenone-type MDR modulators, because the unwanted β-receptor blocking activity is mainly present in the corresponding (S)-isomers.

Table 2: MDR-modulating activity of stereoisomers **1a-d** and (R)- and (S)-propafenone

	EC_{50} (μM)[b]
(R,R)-1[a]	0.78 ± 0.07
(R,S)-1	1.40 ± 0.13
(S,R)-1	0.58 ± 0.05
(S,S)-1	1.56 ± 0.14
(R)-propafenone	0.26 ± 0.02
(S)-propafenone	0.37 ± 0.03



(S,R)-1



Propafenone

[a] the first assignment corresponds to the benzylic center of chirality;
[b] mean ± 95% confidence interval of at least 5 experiments is given

Interestingly, no differences in activity were observed with regard to the center of chirality on the benzylic carbon atom in **1a-d**. This might indicate that this position of the molecule is not important for protein interaction. Nevertheless, modifications thereof influenced activity remarkably with carbonyl being superior to alkoxy and hydroxy. The lack of stereoselectivity in this position of the molecule might be due to the extremely high flexibility of the compounds. This is also reflected by the fact that resolution of the diastereomers could not be achieved, although different chiral columns were used.

Acknowledgment

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