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Chirality of Azelastine and Flezelastine: Investigation of Their Enantiomers

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ABSTRACT The racemic phthalazinone derivatives azelastine and flezelastine were resolved via formation of diastereomeric salts and fractional crystallization thereof. The optical purity of the enantiomers was checked by HPLC. Pharmacological investigations in vitro and in vivo related to antiallergic/antiasthmatic activity revealed some stereospecific differences. However, chiral discrimination could not be observed with regard to overall activity of azelastine and flezelastine.

KEY WORDS: azelastine, flezelastine, fractional crystallization, antiallergic/antiasthmatic, chiral discrimination

The phthalazinone derivative azelastine has successfully been introduced into therapy for treatment of allergic rhinitis and asthma. The antiasthmatic/antiallergic activity is not solely explained by its antihistaminic properties, but also involves other qualities such as leukotriene inhibition, bronchodilatation, and calcium antagonism.[1]

Flezelastine (code: D-18024) has been selected as back-up candidate because of its improved physicochemical and pharmacological properties. It is currently under clinical investigation.

Due to the 7-membered ring moiety both compounds have a chiral center (Fig. 1). They are synthesized as racemates.[2] In order to evaluate the pharmacological profile of the enantiomers in comparison to that of the corresponding racemate, optical resolution was necessary.

MATERIAL AND METHODS

Chemistry

Optical resolution of azelastine[2] was achieved by fractional crystallization. The racemic compound was treated with equimolar amounts of (+)-(1R,3S)-camphoric acid in methanol. The camphorate of (+)-azelastine crystallized first and was recrystallized from methanol. The diastereomeric salt, (−)-azelastine-(+)-camphorate, was isolated from the mother liquor of the first crystallization and crystallized from 2-propanol.

Each salt was treated with aq. NH_3, then with isopropanolic HCl to give the pure enantiomers of azelastine as hydrochlorides (+)-**1a** and (−)-**1a**. The overall yield was 36 and 32%, respectively. Melting points and optical rotation are given in Table 1. Optical purity (Table 1) was checked by HPLC using a chiral AGP phase. The retention times for (+)-azelastine and (−)-azelastine were 10.1 and 14.7 min, respectively. For structure determination of (−)-azelastine [(−)-**1a**] by X-ray crystallography the substance was crystallized from dichloromethane/*n*-hexane. The absolute configuration was determined to be R and was checked by means of Hamilton's R test ($P > 99.5\%$) (Fig. 3).[3]

Resolution of flezelastine (D-18024) was achieved by using its *N*-methyl precursor (±)-**1b** and transferring the method already applied for the resolution of azelastine. After separation of the diastereomeric salts of (±)-**1b** formed with (+)-(1R,3S)-camphoric acid and isolation of both enantiomers of **1b**, the final products (+)-**2b** and (−)-**2b** were obtained by a demethylation–alkylation procedure in two steps (Fig. 2). The antipodes of flezelastine were isolated as hydrochlorides [overall yield starting from (±)-**1b**: 25% for (+)-**2b** and 15% for (−)-**2b**] exhibiting excellent optical purities (Table 1). HPLC (AGP phase) retention times were 8.5 min for (+)-flezelastine [(+)-**2b**] and 12.5 min for (−)-flezelastine [(−)-**2b**].

Pharmacology

The influence of the test substances on the mediators of arachidonic acid metabolism (5-HETE, LTB_4, LTC_4) was investigated using peritoneal cells of rats. After addition of [^{3}H]arachidonic acid and the test compound the cells were stimulated by Ca-ionophore. The arachidonic acid metabolites in the extract were measured using a scintillation counter and characterized by HPLC. Half-maximal inhibitory concentrations (IC_{50} values) were calculated by log-probit analysis using an IBM-AT.

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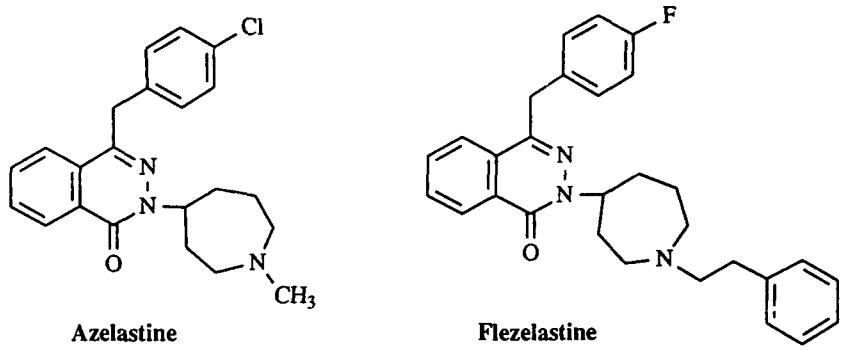


Fig. 1. Structural formulas of azelastine and flezelastine.

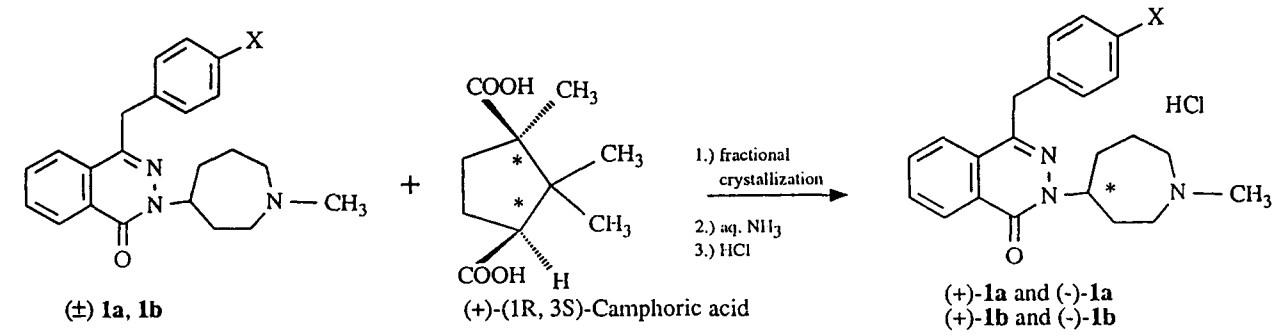
TABLE 1. Optical rotation, optical purity, and absolute configuration of the enantiomers

Enantiomer	$[\alpha]_D^{20}$ (in MeOH)	ee (by HPLC)	Absolute configuration
(+)-Azelastine [(+)-**1a**]	+31.0°	>99%	S
(−)-Azelastine [(−)-**1a**]	−26.0°	94%	R
(+)-Flezelastine [(+)-**2b**]	+21.8°	>99%	
(−)-Flezelastine [(−)-**2b**]	−20.9°	>99%	

The in vitro model of inhibition of allergic induced histamine release is based on the method described by Jasani and Stanworth.[4a] Histamine release from rat peritoneal cells induced by whole egg white is measured by fluorometric assay.[4b]

For measuring H_1-receptor binding the method described by Calcutt et al.[5] was modified. [^{3}H]Pyrilamine and the test substance were added to the receptor suspension isolated from rat brain. Displacement of the radioactive ligand was measured, and IC_{50} values were calculated by log-probit analysis.

Inhibition of "early phase reaction" was measured on guinea pigs which were actively sensitized by one i.m. injection of 70 mg ovalbumin. Three weeks later they got either vehicle (1% methocel) or the test compound p.o. 2 h before allergen challenge. Allergen challenge was performed in boxes by nebulizing 5% ovalbumin solution for a maximum of 4 min or until dyspnoea occurred. Animals not showing any respiratory symptoms during 4 min of inhalative challenge and 10 min



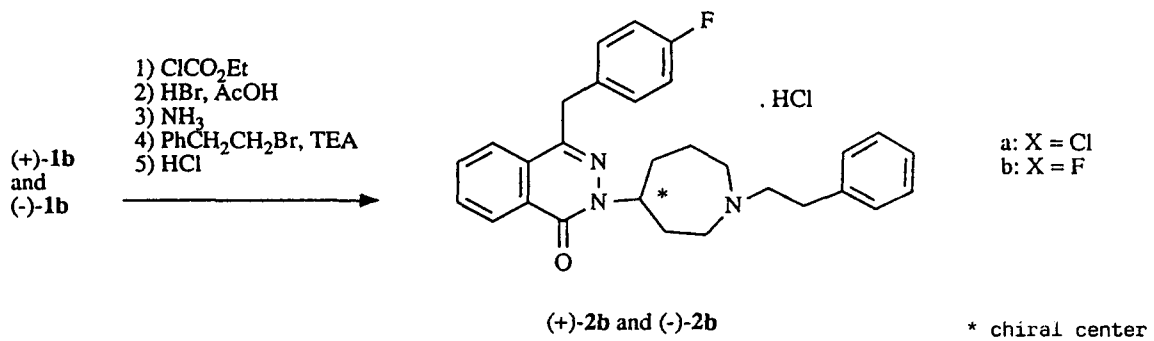


Fig. 2. Optical resolution of azelastine (**1a**) and flezelastine (**2b**).

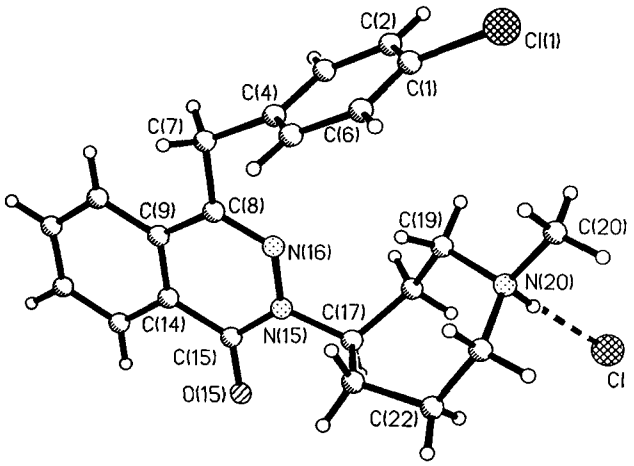


Fig. 3. Structure of (−)-azelastine [(−)-**1a**] as indicated by X-ray analysis: R-configuration at C-17.

thereafter were considered to be protected. Percentage protection in groups of 6–12 animals was determined, and ED_{50} values and confidence limits ($P \leq 0.05$) were calculated according to Litchfield and Wilcoxon.

For evaluating the influence on spontaneous mobility of rats the behavior of the animals was registered over 60 min after oral administration of the drug (40 mg/kg) using infrared light.[6] The calculation was done by a computer system (ANAMOS, Gerb Electronic, Berlin, Germany).

RESULTS AND DISCUSSION

Comparative pharmacological studies were performed for both enantiomers of azelastine and flezelastine, respectively. The results of selected in vitro and in vivo models are shown in Tables 2 and 3.

As azelastine is known to interfere with the metabolites of arachidonic acid, the enantiomers were tested with regard to inhibition of mediator formation. Whereas generation of 5-HETE was influenced by both enantiomers and the racemate to the same extent, slight differences were observed with LTB_4 and LTC_4 inhibition. The (+)-enantiomer of **1a** was found to have approximately the same IC_{50} as the racemate regarding LTB_4 inhibition. However, this antipode showed higher activity in inhibiting LTC_4 formation when comparing with the (−)-enantiomer (−)-**1a** and the racemate which were almost equipotent. No enantiospecifity was observed concerning inhibition of histamine release from mast cells. However, binding to H_1 receptors was stronger for the (+)-enantiomer (+)-**1a**. It also proved to be somewhat more effective in protecting sensitized guinea pigs against ovalbumin challenge ("early phase reaction").

Side effects as sedation are reflected by the influence on spontaneous motility. Although the reduction of motility is still in the same range, its sequence [(±) = (+) > (−)] correlates with H_1 receptor binding affinity.

For flezelastine [(±)-**2b**] the results of the H_1 receptor assay and the in vivo model of "early phase reaction" showed an analogous tendency as for azelastine: the (−)-enantiomer (−)-**2b** exhibited less affinity and less prophylactic activity than the (+)-enantiomer (+)-**2b** which caused almost identical effects as the racemate. However, other in vitro models revealed no significant enantiospecifity. In addition, no difference was seen when measuring the reduction of spontaneous motility.

CONCLUSION

The investigation of azelastine, flezelastine, and the corresponding enantiomers in a series of in vitro and in vivo models related to allergy/asthma revealed no correlation between chirality and activity in most tests. However, it is remarkable that in those tests where slight differences between the enantiomers were observed, one enantiomer—either the (+)- or the (−)-antipode—showed the same activity as the racemate, whereas the other one had a stronger or weaker effect.

Thus, taking into account biological variations, there is no indication that either enantiomer is superior in preclinical overall activity. As the pathophysiology of asthma and allergic disorders cannot be attributed to a single mode of action, but involves various factors, our investigations suggest that both

TABLE 2. Comparison of azelastine (1a) and its enantiomers

Test model	(±)-**1a**	(+)-**1a**	(−)-**1a**
In vitro			
Inhibition of formation of			
5-HETE (IC_{50}, μmol/liter)	55.2	51.8	62.4
LTB_4 (IC_{50}, μmol/liter)	42.8	44.2	75.9
LTC_4 (IC_{50}, μmol/liter)	21.2	9.6	22.7
Inhibition of histamine release at 10 μmol/liter ± SEM	47 ± 1%	55 ± 5%	57 ± 8%
H_1-receptor binding (IC_{50}, nmol/liter ± SEM)	13.0 ± 1.3	12.5 ± 0.8	81.3 ± 3.1
In vivo			
Inhibition of ovalbumin spasm (early phase, ED_{50}, mg/kg p.o.)	0.93 (0.69–1.25)	0.29 (0.05–1.81)	1.24 (0.49–3.23)
Influence on spont. motility at 40 mg/kg p.o. ± SEM	−58 ± 15%	−46 ± 11%	−27 ± 5%

TABLE 3. Comparison of flezelastine (2b) and its enantiomers

Test model	(±)-**2b**	(+)-**2b**	(−)-**2b**
In vitro			
Inhibition of formation of			
5-HETE (IC_{50}, μmol/liter)	17.0	19.2	31.2
LTB_4 (IC_{50}, μmol/liter)	16.0	16.8	21.5
LTC_4 (IC_{50}, μmol/liter)	19.6	20.3	28.8
Inhibition of histamine release			
(IC_{50}, μmol/liter ± SEM)	6.4 ± 0.4	6.6 ± 0.1	6.3 ± 0.4
H_1-receptor binding			
(IC_{50}, nmol/liter ± SEM)	189 ± 22	176 ± 30	501 ± 41
In vivo			
Inhibition of ovalbumin spasm			
(early phase, ED_{50}, mg/kg p.o.)	0.2 (0.7–0.48)	0.2 (0.12–0.36)	2.0 (0.32–12.3)
Influence on spont. motility at 40 mg/kg p.o. ± SEM	−18 ± 9%	−31 ± 8%	−30 ± 12%

enantiomers contribute to the prophylactic and therapeutic efficacy of azelastine and flezelastine.

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