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Direct high-performance liquid chromatographic resolution of a novel benzothiazine Ca^{2+} antagonist and related compounds

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ABSTRACT

Enantiomers of 3,4-dihydro-2-{5-methoxy-2-[3-(N-methyl-N-{2-[(3,4-methylenedioxy)phenoxy]ethyl}amino)propoxy]phenyl}-4-methyl-3-oxo-2H-1,4-benzothiazine hydrogen fumarate (I), a novel and potent Ca^{2+} antagonist, and its synthetic precursors, phenol (II) and bromide (III), were directly resolved by high-performance liquid chromatography on a chiral column, with a stationary phase of cellulose carbamate-coated silica gel. Further, the resolution of some 2-(substituted-phenyl)benzothiazines (IVa–IVl) was investigated to study the effects of 2-phenyl ring substituents on chiral recognition. As an index of the characteristics of substituents, substituent constants for quantitative structure–activity relationship studies were used. The correlations between the resolution efficiency (R_s) and the substituent constants for these benzothiazines were investigated by regression analysis. As a result, for 2-(4-substituted-phenyl)-benzothiazines, R_s showed good correlation with E_s, Taft's steric parameter [correlation coefficient (r) = 0.99]. It was also shown that R_s correlated with R, an electronic constant for the resonance effect, for 2-(2-hydroxy-5-substituted-phenyl)benzothiazines (r = 0.92). These findings suggest that the 2-phenyl ring plays an important role in chiral recognition in the resolution of these benzothiazines.

INTRODUCTION

In the course of our recent studies on sulphur-containing heterocyclic compounds [1–4], we found a novel 2-aryl-3-oxo-2H-1,4-benzothiazine derivative I (Fig. 1) with a potent Ca^{2+} antagonistic activity [5]. Ca^{2+} antagonists are useful in the treatment of hypertension, angina pectoris and certain cardiac arrhythmias [6–8]. Compound I has an asymmetric carbon on the C-2 position of the benzothiazine ring. In an *in vitro* study, the Ca^{2+} antagonistic activity of the (*R*)-enantiomer was about seven times more than that of the (*S*)-enantiomer. The (*R*)-enantiomer (SD-3211) is now undergoing clinical trials.

During the progress of this research, an analytical method for the determination of optical purity was required for I and its synthetic precursors II and III (Fig. 1). In the present study, the direct resolution of enantiomers of these compounds with high-performance liquid chromatography (HPLC) on a commercially available chiral column (Chiralcel OG) was investigated. The stationary phase of this chiral column is cellulose carbamate-coated silica gel [9]. Further, we became interested in the chiral recognition mechanism for these benzothiazine

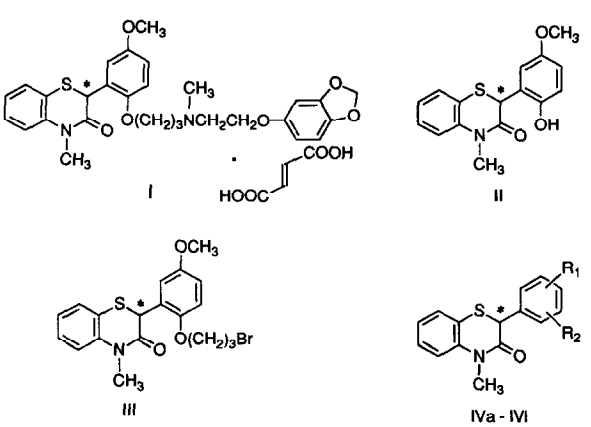

Fig. 1. Structures of benzothiazine derivatives I, II, III and IV.

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derivatives. Since we had already developed a convenient method of synthesizing 2-(substituted-phenyl)benzothiazines [3], it was intended to study the effects of 2-phenyl ring substituents on chiral recognition by using these benzothiazines. In the present study, the correlations between the resolution efficiency and physicochemical properties of the substituents on the 2-phenyl ring for benzothiazines IVa–IVl (Fig. 1) were studied by applying the quantitative structure–activity relationship (QSAR) procedure.

EXPERIMENTAL

Materials

The benzothiazine derivatives I–III [5] and IVa–IVl [3], except IVj, were prepared as reported previously. Compound IVj was synthesized from a mandelic acid derivative, which was prepared from *m*-anisaldehyde and 2-methylaminobenzenethiol in accordance with the preparation of IVl.

Liquid chromatography

Chiralcel OG (250 mm × 4.6 mm I.D.) was purchased from Daicel Chemical Industries. The chromatographic system was from Shimadzu (Kyoto, Japan) and consisted of a solvent-delivery pump (Model LC-6A) equipped with a UV detector (Model SPD-6A). The column was maintained at 30°C by a column oven (Model CTO-2A). The flow-rate was adjusted to 1.5 ml/min, and the detector was set to 238 nm. Chromatograms were recorded on a recorder (Model C-R2AX Chromatopac). Ethanol (1 ml) solutions of the samples (10 μmol) were diluted with mobile phase to give a 1 mM solution. Aliquots of 20 μl of the solutions were injected. The dead time (t_0) of the column was estimated to be 2.2 min with 1,3,5-tri-*tert.*-butylbenzene as a non-retained compound [10]. Capacity factors (k'_1, k'_2) were estimated as $(t_1 - t_0)/t_0$ and $(t_2 - t_0)/t_0$, respectively. The separation factor (α) was calculated as k'_2/k'_1. Resolution (R_s) was conveniently calculated as $2(t_2 - t_1)/(W_1 + W_2)$, where t_1 and t_2 are elution

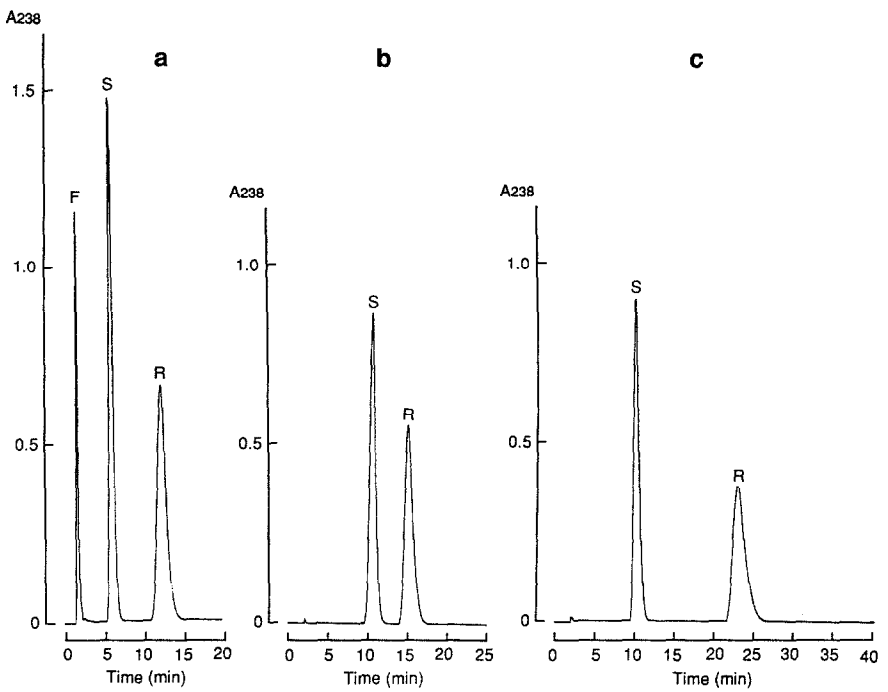

Fig. 2. Chromatograms of benzothiazine derivatives I (a), II (b) and III (c). In each chromatogram, R and S are (*R*)- and (*S*)-enantiomers, respectively. In the chromatogram of I (a), F is fumaric acid.

times and W_1 and W_2 are band widths. Subscripts 1 and 2 represent the first- and the second-eluting enantiomers, respectively.

RESULTS AND DISCUSSION

Resolution of enantiomers of I and its synthetic precursors, II and III

In the investigation of resolution, *n*-hexane and alcohol (methanol and/or ethanol and/or 2-propanol) mixtures were examined as eluents. Of these, a *n*-hexane–ethanol (85:15) mixture gave the best results for II and III. Under this condition, enantiomers of II and III were completely resolved. k'_1 and k'_2 were estimated as 3.63 and 5.69, respectively for II, and as 3.63 and 9.29, respectively for III; α was found to be 1.57 and 2.56, respectively. R_s values were 2.63 and 6.28, respectively. In the case of I, addition of a small amount of diethylamine to the eluent was required. Without diethylamine, I could not be eluted. Enantiomers of I were completely resolved by using a *n*-hexane–ethanol–diethylamine (50:450:1) mixture as the eluent, with k'_1 = 1.64, k'_2 = 4.39, α = 2.67 and R_s = 4.31. Chromatograms of I–III are shown in Fig. 2. In all cases, the (*S*)-enantiomer was the first-eluting isomer.

Resolution of enantiomers of 2-(substituted-phenyl)-benzothiazines IVa–IVl

The resolution of the enantiomers of 2-(substituted-phenyl)benzothiazines IVa–IVl was investigated under the same condition as that for II and III. The results are summarized in Table I. For most of the compounds, the enantiomers were partially or completely resolved.

In order to study the effects of 2-phenyl ring substituents on chiral recognition, the QSAR procedure was applied. Of the QSAR procedures, the Hansch–Fujita approach has been most widely used [11]. It assumes that the potency of a certain biological activity is expressible in terms of a function of the substituent constants [12], which represent the various physicochemical characteristics. Thus, correlations between the resolution efficiency, which was used instead of biological activity, and several substituent constants were investigated for these 2-(substituted-phenyl)benzothiazines by regression analysis. As a result, the following correlations were found. First, as shown in Fig. 3a, R_s showed good correlation with E_s, Taft's steric parameter, for 2-(4-substituted-phenyl)benzothiazines IVa–IVe [correlation coefficient (r) = 0.99]. The compounds whose *para*-substituent on the 2-phenyl ring had an E_s value close to 0 gave better resolution. It seems

TABLE I

RESOLUTION OF ENANTIOMERS (COMPOUNDS II AND IVa–IVl)

Compound	R_1	R_2	k'_1	k'_2	α	R_s
IVa	H	H	1.51	1.94	1.28	1.33
IVb	4-OH	H	3.66	4.34	1.18	0.99
IVc	4-OCH_3	H	2.33	2.69	1.16	0.86
IVd	4-Cl	H	1.26	1.45	1.15	0.60
IVe	4-CH_3	H	1.31	1.43	1.10	0.30
IVf	2-OH	H	1.90	2.25	1.18	0.83
II	2-OH	5-OCH_3	3.63	5.69	1.57	2.63
IVg	2-OH	5-Cl	1.48	1.69	1.14	0.50
IVh	2-OH	5-CH_3	1.56	1.81	1.16	0.62
IVi	2-OH	5-NO_2	2.85	2.95	1.04	~0
IVj	H	3-OCH_3 (5-OCH_3)	2.24	3.03	1.35	1.86
IVk	2-OCH_3	5-OCH_3	3.20	5.93	1.85	3.76
IVl	2-OBz[a]	5-OCH_3	3.39	5.46	1.61	2.76

[a] Bz = benzyl group.

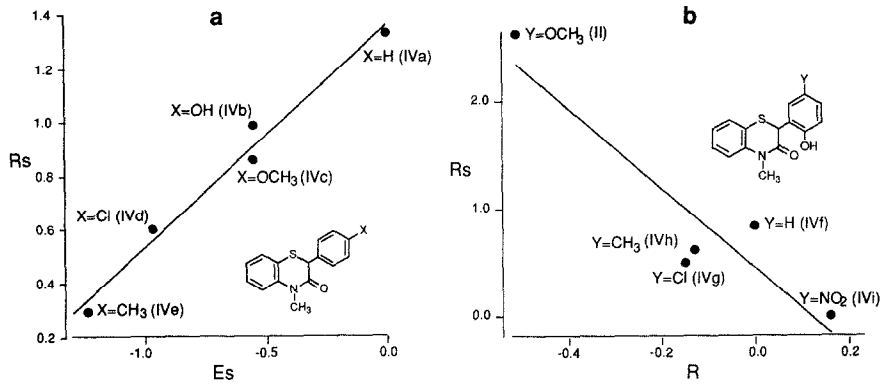

Fig. 3. Plots of resolution factor (R_s) against substituent constant. (a) Correlation between R_s and Taft's steric parameter (E_s) for 4-(substituted-phenyl)benzothiazines (IVa–IVe). (b) Correlation between R_s and Swain–Lupton's constant for resonance effect (R) for 2-(2-hydroxy-5-substituted-phenyl)benzothiazines (II and IVf–IVi).

that a smaller *para*-substituent is better for resolution. Second, as shown in Fig. 3b, R_s showed a correlation with R, an electronic constant for the resonance effect, for 2-(2-hydroxy-5-substituted-phenyl)benzothiazines II and IVf–IVi (r = 0.92). The compounds in which the substituent on the 5-position of 2-phenyl ring had a smaller R were resolved more efficiently. This suggested that substituents donating their electrons to the 2-phenyl ring by the resonance effect are favourable for good resolution. The fact that IVj gave better resolution than IVa might be also explained by this correlation. These findings suggest that the electron-rich 2-phenyl ring is desirable for resolution. This could be supported by the fact that 2-(2-substituted-5-methoxyphenyl)benzothiazines with a hydroxy or alkoxy group as a substituent, *i.e.* II, IVk and IVl, showed significant resolution.

Okamoto *et al.* [9] showed the importance of hydrogen bonding between the carbamate group of the stationary phase and the solute for chiral recognition. Since the benzothiazine derivatives examined in this study have an amido carbonyl group, such hydrogen bonding is likely to occur. This interaction might play an important role in chiral recognition. In addition, Okamoto *et al.* [9] also pointed out the participation of π–π interaction of phenyl groups on the stationary phase with aromatic groups of the solute. The data shown in Fig. 3b, in which the electronic character is correlated with resolution efficiency, show the possibility that the 2-phenyl ring of these benzothiazines could be involved in π–π interaction.

The present data suggest that the 2-phenyl ring of these benzothiazines is an important recognition site for resolution.

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REFERENCES

1 M. Oya, T. Baba, E. Kato, Y. Kawashima and T. Watanabe, *Chem. Pharm. Bull.*, 30 (1982) 440.
2 K. Yamamoto, M. Fujita, K. Tabashi, Y. Kawashima, E. Kato, M. Oya, T. Iso and J. Iwao, *J. Med. Chem.*, 31 (1988) 919.
3 M. Fujita, A. Ota, S. Ito, K. Yamamoto and Y. Kawashima, *Synthesis*, (1988) 599.
4 M. Fujita, A. Ota, S. Ito, K. Yamamoto, Y. Kawashima, T. Iso and J. Iwao, *Chem. Pharm. Bull.*, 38 (1990) 936.
5 M. Fujita, S. Ito, A. Ota, N. Kato, K. Yamamoto, Y. Kawashima, H. Yamauchi and J. Iwao, *J. Med. Chem.*, 33 (1990) 1898.
6 A. Fleckenstein, *Circ. Res.*, 52 (Suppl. I) (1983) 3.
7 R. A. Janis and D. J. Triggle, *J. Med. Chem.*, 26 (1983) 775.
8 H. Purcell, D. G. Waller and K. Fox, *Br. J. Clin. Pract.*, 43 (1989) 369.
9 Y. Okamoto, M. Kawashima and K. Hatada, *J. Chromatogr.*, 363 (1986) 173.
10 H. Koller, K.-H. Rimbock and A. Mannschreck, *J. Chromatogr.*, 282 (1983) 89.
11 C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, 86 (1964) 1616.
12 C. Hansch and A. J. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979.