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Resolution of Some 4-Benzofurazanyl and 4-Benzofuroxanyl 1,4-Dihydropyridine Derivatives by Chiral HPLC on Whelk-O1 and Some Polysaccharide Chiral Stationary Phases

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ABSTRACT The chromatographic chiral resolution of racemic methyl 1,4-dihydro-2,6-dimethyl-5-nitro-4-benzofurazanyl-3-carboxylates **1** and **2** and their benzofuroxanyl analogues **3** and **4** were studied on Whelk-O1, Chiralcel OD-H, Chiralcel OJ, and Chiralpak AD and AS. These CSPs were selected on the basis of the results of structural searches in Chirbase. Examination of the data and cluster analysis pointed out the influence of benzofurazane–benzofuroxane change versus α – β connection change on retention and enantioselectivity, respectively. The major contribution to the retention change arose from the type of heterocycle, whereas the major contribution to the enantioselectivity change came from the mode of connection (α or β) almost irrespective of the nature of the heterocycle. It resulted in a similarity of behaviour between **1** and **2** on one hand and **3** and **4** on the other as far as capacity factors were concerned, and in a similarity of behaviour between **1** and **3** on the one hand and **2** and **4** on the other as far as enantioselectivities were concerned. Chiralpak AS was selected for semipreparative resolution of the enantiomers. The study of several CSPs allowed us to obtain correlations of structure with retention and enantioselectivity as well as the choice of a semipreparative support to provide the quantities for biological tests. *Chirality* 11:602–608, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS: analytical and semipreparative chiral HPLC; 1,4-dihydropyridines; benzofurazane; benzofuroxane; cluster analysis

INTRODUCTION

1,4-Dihydropyridines (1,4-DHPs) are an important class of drugs which are potent blockers of Calcium (Ca^{2+}) currents through voltage-dependent L class calcium channels. Several are well established in the treatment of cardiac arrhythmias, peripheral vascular disorders, and hypertension.¹ Enantiomers of chiral DHPs have opposite pharmacological profiles. One of the antipodes is a calcium entry blocker, while the other is a calcium entry activator. As progression of our work on 1,4-DHPs bearing at the 4-position benzofurazanyl and benzofuroxanyl substructures,² we have synthesised the racemic mixtures of derivatives **1–4** (Chart 1).

The study of the pharmaceutical properties of single DHP enantiomers implies the resolution of racemic mixture. Several methods have been used to reach this goal,³ among them chromatographic chiral separation which has been developing rapidly in recent years.⁴ In this paper, we report analytical and semipreparative chiral HPLC separation of DHPs **1–4**.

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EXPERIMENTAL

Chemicals

Racemic mixtures of derivatives **1**, **2** and **4** were prepared by a modified Hantzsch method. Racemic mixture of derivative **3** was obtained by irradiation of methyl 1,4-dihydro-2,6-dimethyl-5-nitro-4-(2-azido-3-nitrophenyl)pyridine-3-carboxylate dissolved in THF, with a medium pressure Hg lamp. Details of the syntheses will appear elsewhere.⁵ Benzofuroxan derivatives **3** and **4** exhibit tautomerism. ¹H- and ¹³C-NMR spectra show broad peaks, indicating extensive benzofuroxan tautomerism. At low temperature (0°C for **3** and –30°C for **4**), the ratio of isomers can be determined as well as the barriers to tautomerisation (ΔG^* in the range of 14.3–15.8 kcal/mol. A

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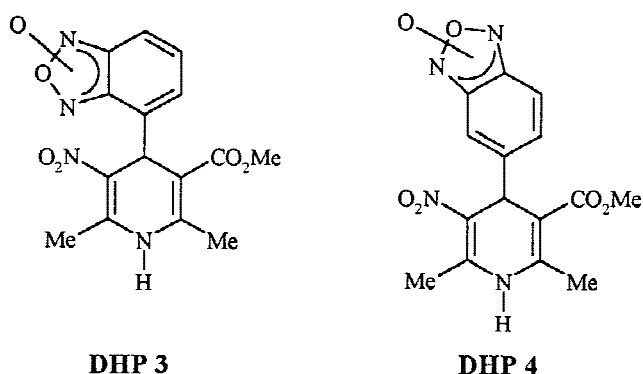
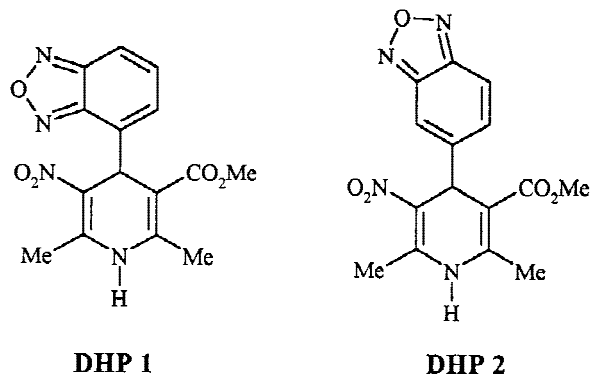


Chart 1

detailed structural study of the benzofuroxan derivatives described in this paper and in Ref. 2 will be published later.

Liquid Chromatography

Obtaining enriched samples of the (+) and (–) enantiomers by preparative resolution on microcrystalline cellulose triacetate is a very attractive methodology since both CSP and eluent (EtOH/H₂O 96:4 v/v) are very cheap and easily handled. The liquid chromatographic system for semipreparative resolution using microcrystalline cellulose triacetate (Merck 15–25 μ m) has been already described, chromatographic separation being monitored by UV and polarimetric detection (Perkin-Elmer 241 MC).^{6a} For semipreparative injection 10–50 mg of the racemates were injected in a 5 mL loop (eluent 96:4 EtOH/H₂O) in a (200 \times 25 mm) column and the fractions manually or automatically collected according to the sign of the rotatory power at 346 nm (Hg lamp). Unfortunately, none of DHP 1–4 was baseline resolved, but nevertheless the enriched fractions were used for the determination of the order of elution on other analytical columns.

HPLC studies on Chiralcel OJ^{6b} (cellulose tris(4-methylbenzoate) coated on silica, Daicel Co. Tokyo (250 \times 4.6 mm, 10 μ m)), Chiralpak AS (amylose tris[(S)- α -phenylethylcarbamate] coated on silica, Daicel Co. Tokyo, (250 \times 4.6 mm, 10 μ m)), Chiralcel OD-H (cellulose tris(3,5-dimethylphenylcarbamate) coated on silica, Daicel Co. Tokyo, (250 \times 4.6 mm, 5 μ m)), Chiralpak AD (amylose

tris(3,5-dimethylphenylcarbamate) coated on silica, Daicel Co. Tokyo (250 \times 4.6 mm, 10 μ m)), (so-called (R,R)Whelk-01 ((3S,4R)-4-(3,5-dinitrobenzamido)-3-[3-(dimethylsilyloxy)propyl]-1,2,3,4-tetrahydrophenanthrene reacted with silica, E. Merck Darmstadt (250 \times 4.6 mm, 5 μ m)) were performed with a Merck-Hitachi LiChrograph Model L-6000 HPLC pump at a controlled temperature, a Merck-Hitachi LiChrograph L-4000 UV detector (λ = 254 nm) and a Merck D-2500 recorder. Dead volume was determined by coinjection of 1,3,5-tri-*tert*-butylbenzene. Successful semipreparative separations were performed on Chiralpak AS column (Daicel Co. Tokyo, 250 \times 10 mm, 10 μ m). The eluents were HPLC grade from Merck (Darmstadt, Germany) and SDS (Peypin France).

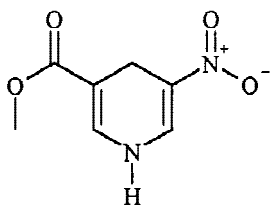
RESULTS AND DISCUSSION

Direct chromatographic chiral resolution of dihydropyridines has attracted a lot of interest since Goldmann and Stoltefuss reviewed the effects of chirality and conformation on dihydropyridine activities in 1991.³ A short paragraph in this review was devoted to the already published reports on the technique for the preparation of small quantities of enantiomers for initial pharmacological studies. Since that time, several dihydropyridines have been separated on the semipreparative scale for the study of their inversion by oxidation and electroreduction;⁷ fluorenone DHP derivatives were separated on Chiralpak AD⁸ giving sizeable amounts of the individual enantiomers; two couples of optically active labelling precursors of SN11568 have been preparatively resolved on home-made Chiralcel OF whereas the analytical check of the enantiomeric purity was performed on a Chiralpak AD column.⁹ Semipreparative chromatographic purification of the enantiomers of amlodipine was obtained on a semipreparative Chiral-AGP column.¹⁰ These selected examples deal with actual semipreparative resolutions, however one may take advantage of the numerous examples of analytical separations of DHP in literature, provided they are available in a molecularly oriented factual database format. These analytical separations may be scaled-up to semipreparative levels. The other alternative is the automatic screening of various CSPs under different eluting conditions. The automatic screening proved to be very efficient and should be an invaluable source of (positive and negative) data to feed a molecularly oriented internal database to manage these high throughput chromatographic results.^{11a}

Inspection of Chirbase,^{11b} the molecular database for chiral separations by HPLC, afforded examples of successful separations of DHP on most of the commercially available CSPs as long as the substructure structural query is performed on the dihydropyridine minimal framework (unsubstituted 4-aryldihydropyridine). Chirbase provides 289 successful or unsuccessful combinations of DHP and CSP corresponding to 499 combinations of DHP, CSP, and conditions. In order to reduce the number of hits, one has to keep close to the actual structures under study.

Inspection of the structural features of compounds 1–4 reveals that the chirality arises from the spatial arrangement of a nitro and an ester group bonded to the dihydropyridine in positions 3 and 5, the common structure of all

Substructure A



Substructure B

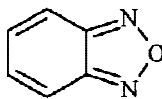


Chart 2

four derivatives, whereas a benzofurazanyl or benzofuroxanyl differing on the position of attachment differentiate the four derivatives. Furthermore, the chirality introduced by the benzofurazanyl or benzofuroxanyl is not unique since these planar substituents may adopt two conformations (syn- or antiperiplanar) in which they are perpendicular or nearly perpendicular to the plane formed by the sp^2 carbon atoms 2, 3, 5, and 6 of the dihydropyridine moiety. Considering what is well documented¹² on the sp^2 – sp^3 pivot bond conformational analysis, both rotamers will be strongly populated in the case of DHPs **2** and **4**, which lack ortho-substitution on the benzofurazanyl or benzofuroxanyl groups, whereas a strong conformational preference for one rotamer is expected in the cases of DHPs **1** and **3**. In addition, for DHP **3** and **4**, the diversity of 3-D structures is doubled due to tautomerism.

A substructure search in Chirbase was performed on two structures **A** and **B** (Chart 2).

The substructure search on **A** afforded only four compounds: Bay K 8644, two analogues differing by the ester residue, and SDZ 202-701 and seven CSPs (Chiral-AGP, Cyclobond I, Cyclobond I SP, Cyclobond I RSP, Chiralcel OJ, Chiralpak OT(+), and Ultron ES-OVM) leading to 12 and 16 DHP, CSP, and conditions combinations.

The substructure search on **B** afforded 31 compound/CSP combinations which correspond to 19 compounds separated on various CSPs: four DHP derivatives, two 3,4-epoxy-2,2-dimethylchromane annelated to benzofurazane or benzofuroxane, respectively, and a series of amino acid and pyrrolidine derivatised with a substituted benzofurazane.

Examination of the separation of these compounds on their respective CSP afforded some interesting leads for the separation of DHP **1–4**:

(1) Derivatisation of amino acid or pyrrolidine with benzofurazane¹³ introduces in the sample π -accepting or donating sites as well as hydrogen-bond accepting sites suitable for successful separation on Sumichiral OA 2500. Sumichiral OA 2500 is composed of *N*-3,5-dinitrobenzoyl-1-naphthylglycine covalently bonded to silica. The same *N*-3,5-dinitrobenzoyl and (1)-naphthyl connectivity relationship is also found for the Whelk-O1 column in a more rigid spatial arrangement (Chart 3).

From these data as well as the perpendicular arrangement of benzofurazanyl or benzofuroxanyl and dihydropyridine framework, Whelk-O1 appeared to be a candidate

CSP for a tentative separation of compounds **1–4**: Whelk-O1 has been reported in a single example in 1997¹⁴ for isradipine in reverse phase conditions.

(2) The excellent separations of benzofurazane epoxychromene and tautomerisable benzofuroxane epoxychromene derivatives on Chiralcel OJ ($\alpha = 1.6$ and 2.68, respectively)¹⁵ showed that the benzofurazane and tautomerisable benzofuroxane moieties are compatible with Chiralcel OJ (Chart 4).

(3) Inspection of the separations of the four DHP derivatives was particularly instructive. We learnt from isradipine separation attempts on polysaccharide carbamates that Chiralcel OD gave no separation¹⁶ whereas some non-commercially available cellulose carbamates gave positive results^{16,17} while Chiralpak AD gave partial separation.¹⁶ Isradipine was particularly well separated on Chiralcel OJ¹⁸, Chiral-AGP,^{19,20} Ultron ES-OVM,¹⁹ Chiralpak OT(+) moderately resolves the four DHP derivatives at low temperature (5°C).²¹ Chiral-AGP, Ultron ES-OVM, and Chiralpak OT(+) are not in our opinion suitable for preparative purposes.

The DHP derivative SDZ 202-791 belongs to both substructure sets **A** and **B** and differs from compound **1** by the presence of an isopropyl instead of a methyl group in the ester residue. SDZ 202-791 was partially separated on Chiralcel OJ.¹⁸

It emerged from that structural analysis that Chiralcel OJ could be suitable for tentative separation of compounds **1–4** whereas Chiralcel OD would not be suitable.

Chirbase provided 289 successful or unsuccessful DHP/CSP combinations corresponding to 499 combinations of DHP, CSP, and conditions. To our surprise only 3 combinations of DHP/Chiralpak AS/conditions were reported in two articles dealing with enzymatic resolution.^{22,23} The absence of any reported unsuccessful DHP/Chiralpak AS/conditions combination was noteworthy. It can be concluded that Chiralpak AS has been overlooked until now in the field of DHP chromatographic chiral separations.

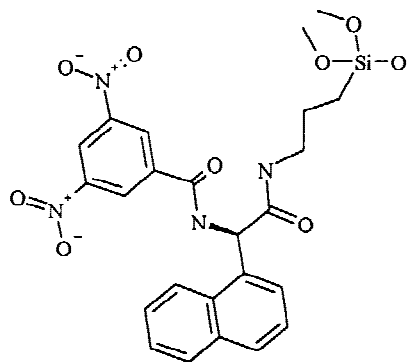
Chiralpak AD has been used for the separation attempts of 13 DHP derivatives bearing two different ester groups in positions 3 and 5 with contrasting results.^{13,16,24,25}

We thus decided to explore the separating abilities of two CSPs: Whelk-O1 and Chiralpak AS for DHPs **1–4**, the first with the one on a rational structural base (*vide supra*), the second to gain experience in almost undocumented DHP/Chiralpak AS combination. Separation attempts on Chiralcel OD, Chiralpak AD and Chiralcel OJ were performed to confirm what was inferred from CHIRBASE.

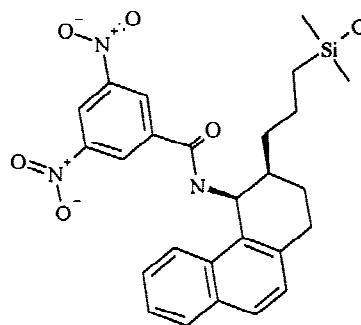
All the data for separation attempts are reported in Table 1.

A common feature to all the reported data is the higher retention for tautomerisable benzofuroxanes **3** and **4** compared to benzofurazanes **1** and **2** whatever the nature of the chiral selector involved on the CSP: ester for Chiralcel OJ, carbamate for Chiralcel OD, Chiralpak AD and AS, amide, and π donor and π acceptor for Whelk-O1.

As expected Chiralcel OD-H failed to separate the enantiomers of DHPs **1–4**. Interestingly DHPs **1** and **2** and DHP **3** and **4** exhibit very similar retention (**1**, **2**, 1.56–1.6; **3**, **4**, 2.57–2.29) pointing out that retention is dependent on



Sumichiral OA 2500



Whelk-O1

Chart 3

the constitutive parts **A** (common dihydropyridine part) and **B** (benzofurazanyl versus benzofuroxanyl) but is insensitive to the α or β connection between them.

As expected, Chiralcel OJ succeeded for three out of the four DHPs. However the retention times were far too long, even at 40°C, for convenient semipreparative separations. Attempts to reduce the retention by an increase of 2-PrOH content reduced resolution as well. When pure EtOH was used separation was lost. On Chiralcel OJ, the (+) enantiomers are eluted first for all DHPs **1–4**. On this CSP, retentions are dependent on both the nature of the substructure **B** and on the connection: the β connection in DHPs **2** and **4** gives shorter retention than the α linkage found in DHPs **1** and **3**. Enantioselectivity is strictly related to the connection and independent upon the nature of the substructure **B**: baseline separation for DHPs **1** and **3** and partial separation for DHPs **2** and **4**. These results differ from those reported for benzofurazane epoxy-chromene and tautomerisable benzofuroxane epoxy chromene derivatives on Chiralcel OJ, which were strongly dependent on the nature of the heterocycle ($\alpha = 1.6$ and 2.68 respectively).¹⁵ Chiralcel OJ is suitable for chiral analysis in DHPs **1** and **3**, but analysis of DHPs **2** and **4** would require further eluent optimization.

As predicted, Whelk-O1 separated all four DHP derivatives. Similar enantioselectivities and resolutions are observed for the four DHPs. However, the order of elution on Whelk-O1 is related to the connection (α or β): first eluted (–) enantiomer in DHP **1** and **3** and first eluted (+) enantiomer in DHP **2** and **4** independent of the nature of the heterocycle (benzofurazane or benzofuroxane), which has a strong effect on retention. This is in sharp contrast with the unique order of elution found on each polysaccharide CSP.

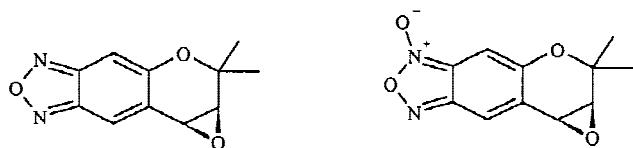


Chart 4

Chiralpak AD separated all the four DHPs with almost identical enantioselectivities ($\alpha = 1.23 \pm 0.02$) and the same order of elution: (+) before (–) as on Chiralcel OJ. Enantioselectivities are independent of the substructure **B** (benzofurazane or benzofuroxane) and its linkage (α or β) to the dihydropyridine. On this CSP, retentions depend on both the nature of the substructure **B** and the linkage: β connection in DHPs **2** and **4** gives higher retention than α connection found in DHPs **1** and **3** (in opposition to Chiralcel OJ).

Chiralpak AS separated all the four DHPs using 2-PrOH or EtOH in hexane. Better enantioselectivities were obtained using EtOH, so these conditions were selected for semipreparative separation of the enantiomers. The same order of elution is observed for all four DHPs: (–) enantiomers eluted first (opposite to Chiralcel OJ and Chiralpak AD).

Enantioselectivity is strictly related to the connection and independent upon the nature of the heterocycle: β connection results in better enantioselectivities than α connection whereas the opposite was found for Chiralcel OJ.

There are two ways to associate in pairs the DHPs **1–4**. One is to associate DHP **3** with DHP **4** and DHP **1** with DHP **2**, which emphasises the role of the heterocycle (benzofurazane versus benzofuroxane). The other is to associate DHP **1** with DHP **3** and DHP **2** with DHP **4**, this pairing emphasising the role of the connection between the dihydropyridine and heterocycle (α or β connection). For discrimination on the chiral stationary phases we have examined, the main effect comes from the α or β connection almost independently of the nature of the heterocycle. As pointed out earlier, the conformational repartition about the pivot bond is strongly dependent on the α or β connection, and might be at the origin of the observed pairing.

In order to support our analysis of Table 1, we have performed cluster analysis with standard software using the agglomeration method standardised by mean/SD. The first clustering analysis was performed using capacity factors (k values) taken from Table 1 whatever the CSP, the

TABLE 1. Chiral chromatographic data for DHP 1–4.

CSP	Eluent	Flow rate	<i>T</i> (°C)		DHP-1	DHP-2	DHP-3	DHP-4
Chiralcel OD-H	Hex/2-PrOH 85:15	1 mL/min	40	Rt1	7.3	7.41	10.18	9.38
				Rt2	7.3	7.41	10.18	9.38
				k1	1.56	1.6	2.57	2.29
				k2	1.56	1.6	2.57	2.29
				α	1	1	1	1
				Rs	0	0	0	0
Chiralcel OJ	Hex/2-PrOH 85:15	1 mL/min	40	Rt1	29.54	26.16	56.32	46.46
				Rt2	35.23	28.7	71.47	48.11
				k1	9.78 (+)	8.51 (+)	19.41 (+)	15.83 (+)
				k2	11.86	9.44	24.89	16.43
				α	1.21	1.11	1.28	1.04
				Rs	1.67	0.98	2.53	0.3
<i>(R,R)</i> -Whelk-O1	Hex/2-PrOH 80:20	2 mL/min	25	Rt1	11.9	13.4	25.1	22.5
				Rt2	15.38	16.19	29.99	26.43
				k1	11.14 (–)	12.7 (+)	24.6 (–)	21.96 (+)
				k2	14.7	15.5	29.6	25.97
				α	1.32	1.22	1.2	1.18
				Rs	1.3	0.8	1.2	1.25
Chiralpak AD	Hex/2-PrOH 85:15	1 mL/min	40	Rt1	6.67	7.58	8.58	9.94
				Rt2	7.47	8.8	9.82	11.7
				k1	1.37 (+)	1.69 (+)	2.06 (+)	2.54 (+)
				k2	1.66	2.12	2.51	3.16
				α	1.21	1.26	1.21	1.25
				Rs	1	1.53	1.55	1.6
Chiralpak AS	Hex/2-PrOH 85:15	1 mL/min	25	Rt1	17.07	20.18	28.79	29.15
				Rt2	18.44	24.91	32.94	37.1
				k1	4.52 (–)	5.47 (–)	8.32 (–)	8.43 (–)
				k2	4.97	7.06	9.66	11.01
				α	1.1	1.28	1.16	1.31
				Rs	0.6	0.85	0.8	1.1
Chiralpak AS	Hex/2-PrOH 85:15	1 mL/min	40	Rt1	12.96	14.15	19.08	19.44
				Rt2	14.68	17.82	22.72	25.11
				k1	3.33 (–)	3.72 (–)	5.38 (–)	5.48 (–)
				k2	3.91	4.92	6.6	7.37
				α	1.17	1.33	1.23	1.34
				Rs	1.43	2.29	2.02	2.58
Chiralpak AS	Hex/EtOH 85:15	1 mL/min	25	Rt1	8.26	8.51	10.87	11.31
				Rt2	9.19	10.98	12.63	15.14
				k1	1.67 (–)	1.75 (–)	2.52 (–)	2.66 (–)
				k2	1.97	2.55	3.09	3.9
				α	1.18	1.46	1.23	1.47
				Rs	0.7	1.23	1.2	1.5
Chiralpak AS (semi-prep)	Hex/EtOH 85:15	3 mL/min	25	Rt1	12.59	13	19.2	20.92
				Rt2	14.31	17.02	22.48	29.07
				k1	1.68 (–)	1.77 (–)	3.09 (–)	3.45 (–)
				k2	2.04	2.62	3.78	5.19
				α	1.22	1.48	1.23	1.5
				Rs	0.66	1.03	0.82	1.1

conditions, and the actual enantiomer. The results of this cluster analysis are reported in Fig. 1. This points out clearly the similarity between DHP 1 and DHP 2 on one hand and DHP 3 and DHP 4 on the other hand as far as retention is concerned, pointing out the pairing of the DHP based on the nature of the heterocycle. The second clustering analysis was performed taking into account the enantioselectivity alone, and is reported in Fig. 2. This shows clearly the similarity between DHP 1 and DHP 3 in one hand and DHP 2 and DHP 4 on the other hand as far as

enantioselectivity is concerned, pointing out the pairing of the DHP based on the α or β connection.

Chiralpak AS was chosen for the semipreparative separation of the DHP 1–4. Ca 50 mg of each enantiomers were obtained by repetitive injections using hexane/EtOH 85:15 as eluent. EtOH was chosen for improved solubility of the compounds. All the enantiomers were obtained with an optical purity higher than 99%: (+)1 ($c = 0.5$, EtOH): $[\alpha]^{25/589} = +37$; (–)1 ($c = 0.55$, EtOH): $[\alpha]^{25/589} = -36$; (+)2 ($c = 0.4$, EtOH): $[\alpha]^{25/589} = +11$; (–)2 ($c = 0.4$, EtOH):

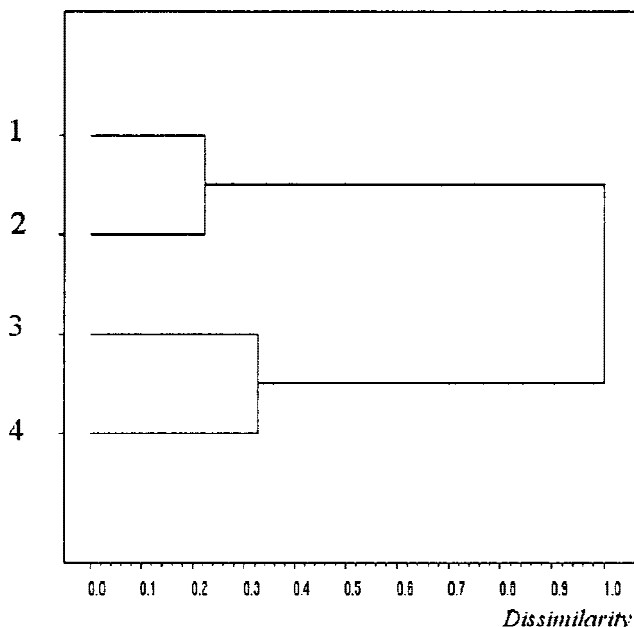


Fig. 1. Cluster analysis based on k reported in Table 1, showing the similarity between DHP 1 and DHP 2 on the one hand and DHP 3 and DHP 4 on the other, pointing out the importance of the nature of the heterocycle on retention.

$[\alpha]^{25/589} = -11$; (+)3 ($c = 0.5$, EtOH): $[\alpha]^{25/589} = +36.2$; (–)3 ($c = 0.47$, EtOH): $[\alpha]^{25/589} = -38.2$; (+)4 ($c = 0.55$, EtOH): $[\alpha]^{25/589} = +8.18$; (–)4 ($c = 0.525$, EtOH): $[\alpha]^{25/589} = -8.19$. Whole-cell voltage-clamp studies on L-type Ca^{2+} channels expressed by rat insulinoma cell line (RINm5F) showed that all the dextrorotatory antipodes were effective ago-

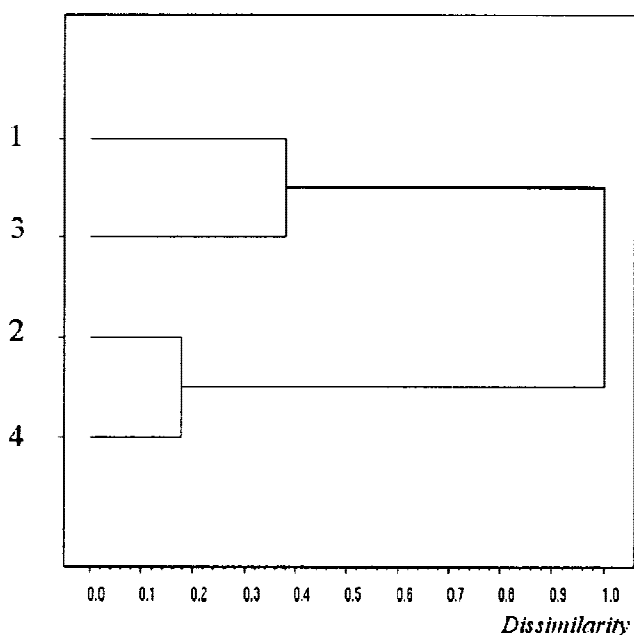


Fig. 2. Cluster analysis based on enantioselectivities reported in Table 1, showing the similarity between DHP 1 and DHP 3 on the one hand and DHP 2 and DHP 4 on the other and pointing out the determining role of the α or β connection on enantioselectivities.

nists of L-type Ca^{2+} currents, while the levorotatory ones were weak Ca^{2+} entry blockers.⁵

In summary, this study on several CSPs type gave structural correlations with retention and enantioselectivity as well as information about the choice of a semipreparative support to provide the pure enantiomers for biological tests.

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