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Reversed-phase high-performance liquid chromatographic separation of diastereomers of (*R*,*S*)-mexiletine prepared by microwave irradiation with four new chiral derivatizing reagents based on trichloro-*s*-triazine having amino acids as chiral auxiliaries and 10 others having amino acid amides

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ABSTRACT

A new series of chiral derivatizing reagents (CDRs) consisting of four dichloro-*s*-triazine reagents was synthesized by nucleophilic substitution of one chlorine atom in trichloro-*s*-triazine with amino acids, namely L-Leu, D-Phg, L-Val and L-Ala as chiral auxiliaries. Two other sets of CDRs consisting of four dichloro-*s*-triazine (DCT) and six monochloro-*s*-triazine (MCT) reagents were also prepared by nucleophilic substitution of chlorine atom(s) with different amino acid amides as chiral auxiliaries in trichloro-*s*-triazine and its 6-methoxy derivative, respectively. These 14 CDRs were used for the synthesis of diastereomers of (*R*,*S*)-mexiletine under microwave irradiation (*i.e.* 60 s and 90 s at 85% power (of 800 W) using DCT and MCT reagents, respectively), which were resolved by reversed-phase high-performance liquid chromatography using C18 column and gradient eluting mixtures of methanol with aqueous trifluoroacetic acid (TFA) with UV detection at 230 nm. The resolution (R_s), difference between retention times of resolved diastereomers (Δt) and retention factors (k) obtained for the three sets of diastereomers were compared among themselves and among the three groups. Explanations have been offered for longer retention times and better resolution of diastereomers prepared with DCT reagents in comparison of their MCT counterparts and, for the influence of hydrophobicity of the side chain R of the amino acid in the CDRs on retention times and resolution. The newly synthesized CDRs were observed to be superior as compared to their amide counterparts in terms of providing better resolution and cost effectiveness. The method was validated for limit of detection, linearity, accuracy and precision.

1. Introduction

The physiological environment within a living organism is chiral, and therefore the pharmacodynamic, pharmacokinetic and toxicological activities of enantiomers of a drug can differ dramatically. However, many drugs are administered as a racemic mixture which may cause undesired biological processes resulting in catastrophic side effects. Therefore, enantiomeric separation and analysis of chiral drugs are significant areas of research in pharmaceutical chemistry. The resolution of a pair of enantiomers by reacting them with an optically pure chiral derivatizing reagent (CDR), *i.e.* the formation of diastereomers followed by their chromatographic separation in an achiral environment, is considered as an indirect approach. Its advantages include availability of a variety of CDRs either commercially or via simple synthetic sequence, presence of easily derivatizable and compatible functional groups leading to diastereomeric derivatives with excellent separation and detection possibilities and, a relatively wide choice of chromatographic conditions in comparison to direct methods for chiral resolution [1–3].

Mexiletine [1-(2,6-dimethylphenoxy)-2-amino propane, MEX] (Fig. 1) is classified as a class Ib antiarrhythmic drug and clinically administered as antiarrhythmic, antimyotonic, and analgesic agent in its racemic form [4]. Several lines of evidences have shown that mexiletine enantiomers differ in their pharmacodynamic, pharmacokinetic and receptor binding properties [5,6]. The aliphatic and aromatic hydroxylations of mexiletine in human microsomes are reported to be stereoselective; aliphatic hydroxylation is predominant for the (*R*)-enantiomer while the aromatic hydroxylation is favored for the (*S*)-enantiomer [7]. The (*R*)-isomer of mexiletine is also reported to be more potent than the (*S*)-isomer in the treatment of experimental arrhythmias [8].

Indirect enantioseparation of (*R*,*S*)-MEX using high-performance liquid chromatography (HPLC) has been reported with CDRs like (*S*)-1-(l-naphthyl)ethyl isocyanate (NEIC) [9], (1*S*,

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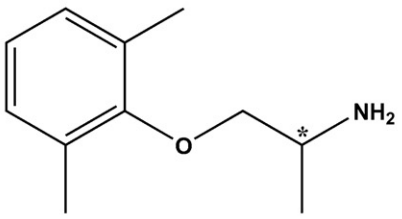
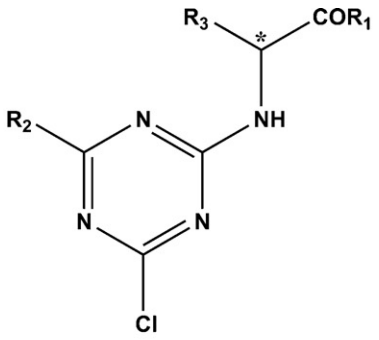


Mexilitine



CDR (1-14)

CDR	R_1	R_2	R_3
1, *N*-(4,6-Dichloro-[1,3,5]triazine-2-yl)- L-Leu	-OH	-Cl	$-CH_2CH(CH_3)_2$
2, *N*-(4,6-Dichloro-[1,3,5]triazine-2-yl)- D-Phg	-OH	-Cl	$-C_6H_5$
3, *N*-(4,6-Dichloro-[1,3,5]triazine-2-yl)- L-Val	-OH	-Cl	$-CH(CH_3)_2$
4, *N*-(4,6-Dichloro-[1,3,5]triazine-2-yl)- L-Ala	-OH	-Cl	$-CH_3$
5, *N*-(4,6-Dichloro-[1,3,5]triazine-2-yl)- L-Leu-NH_2	$-NH_2$	-Cl	$-CH_2CH(CH_3)_2$
6, *N*-(4,6-Dichloro-[1,3,5]triazine-2-yl)- D-Phg-NH_2	$-NH_2$	-Cl	$-C_6H_5$
7, *N*-(4,6-Dichloro-[1,3,5]triazine-2-yl)- L-Val-NH_2	$-NH_2$	-Cl	$-CH(CH_3)_2$
8, *N*-(4,6-Dichloro-[1,3,5]triazine-2-yl)- L-Ala-NH_2	$-NH_2$	-Cl	$-CH_3$
9, *N*-(4-Chloro-6-methoxy-[1,3,5]-triazine-2-yl)-L-Leu-NH_2	$-NH_2$	$-OCH_3$	$-CH_2CH(CH_3)_2$
10, *N*-(4-Chloro-6-methoxy-[1,3,5]-triazine-2-yl)-D-Phg-NH_2	$-NH_2$	$-OCH_3$	$-C_6H_5$
11, *N*-(4-Chloro-6-methoxy-[1,3,5]-triazine-2-yl)-L-Val-NH_2	$-NH_2$	$-OCH_3$	$-CH(CH_3)_2$
12, *N*-(4-Chloro-6-methoxy-[1,3,5]-triazine-2-yl)-L-Met-NH_2	$-NH_2$	$-OCH_3$	$-CH_2CH_2SCH_3$
13, *N*-(4-Chloro-6-methoxy-[1,3,5]-triazine-2-yl)-L-Ala-NH_2	$-NH_2$	$-OCH_3$	$-CH_3$
14, *N*-(4-((S)-1-Carbamoyl-2-methyl-propylamino)-6-chloro-[1,3,5]triazine-2-yl]-L-Phe	$-NH_2$	$-NHCH(CH_2C_6H_5)CONH_2$	$-CH(CH_3)_2$

Fig. 1. Structures of MEX and chiral derivatizing reagents (CDR 1–14).

2*S*)-*N*-[(2-isothiocyanato)-cyclohexyl]-pivalinoyl amide (PDITC) and 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate (GITC) [10], (−)-4-(6-methoxy-2-naphthyl)-2-butyl chloroformate (NAB-C) [11] and *o*-phthaldialdehyde combined with *N*-acetyl-L-cysteine [12]. Direct HPLC resolution of enantiomers of (*R*,*S*)-MEX and its derivatives has been achieved on various chiral stationary phases (CSPs); these include CSP based on 1,1′-binaphthyl crown ether [13] and (18-crown-6)-2,3,11,12-tetracarboxylic acid [14,15] for (*R*,*S*)-MEX, Pirkle ionic column based on (*R*)-(−)-3,5-dinitrobenzoylphenylglycine for naphthoyl [16] and *N*-anthroyl derivatives [17] and, CSP based on amylose *tris*(3,5-dimethylphenylcarbamate) for resolution of (*R*,*S*)-MEX and its main metabolites in plasma and urine [18]. Chiral separation of mexiletine has also been reported employing cyclodextrins [19] and (18-crown-6)-tetracarboxylic acid [20] as chiral additives using capillary electrophoresis (CE).

Cyanuric chloride (2,4,6-trichloro-1,3,5-triazine; trichloro-*s*-triazine; *s*-triazine chloride; CC) has the prospect of easy and controlled sequential substitution of its chlorine atoms by nucleophiles attributed to its trifunctional high reactivity [21]. It is commercially available and is an inexpensive reagent that makes its applications even more attractive. Enantioresolution of amino acids and amino alcohols has been reported on a series of CSPs prepared by immobilization of chloro-*s*-triazines bearing amines or amino acids as chiral selectors on solid supports such as aminopropylsilica [22–24]. Brückner and co-workers [25,26] synthesized chiral monochloro-*s*-triazine reagents from cyanuric chloride and used them for enantioseparation of only a few selected amino acids by HPLC.

Indirect enantioresolution of *α*-amino acids has been reported from this laboratory using four dichloro-*s*-triazine (DCT) and six monochloro-*s*-triazine (MCT) reagents as CDRs prepared by the nucleophilic substitution of chlorine atom(s) with different amino acid amides moieties as chiral auxiliaries in trichloro-*s*-triazine and its 6-methoxy derivative, respectively [27]. Enantioseparation of 18 proteinogenic and 8 non-proteinogenic amino acids has also been reported with two cyanuric chloride based CDRs having piperidinyl as achiral auxiliary and, L-leucine amide and L-leucine as chiral auxiliaries, respectively [28].

DFDNB (1,5-difluoro-2,4-dinitrobenzene) has a unique feature of allowing the substitution of its fluorine atoms with nucleophiles. For this feature, Marfey prepared the reagent 1-fluoro-2,4-dinitophenyl-5-L-alanine amide (FDNP-L-Ala-NH_2, FDAA, MR) by substituting one of its fluorine atoms with L-Ala-NH_2 [29]. Using DFDNB, several CDRs considered as structural variants of MR (having amino acid amides, amino acids and amines as chiral auxiliaries) have been synthesized and used in this laboratory for indirect enantioseparation of a variety of compounds such as proteinogenic and nonproteinogenic amino acids [30], amino alcohols [31] and (*R*,*S*)-MEX [32,33]. These studies have proved superiority of structural variants of MR having amino acid moieties as chiral auxiliaries over to those having amino acid amide moieties, in terms of providing better resolution of analytes and cost effectiveness. The structures of the diastereomers formed by compounds possessing amino groups with *s*-triazine reagents show similarities with those prepared with Marfey's reagent [1].

Keeping in view the literature mentioned above and the references cited therein, three sets of CDRs were synthesized from trichloro-*s*-triazine, (A) four new DCT reagents having amino acid moieties (viz., L-Leu, D-Phg, L-Val and L-Ala), (B) four DCT reagents having amino acid amide moieties and, (C) six MCT reagents having amino acid amide moieties, as chiral auxiliaries. The CDRs were