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Supercritical fluid chromatographic chiral separation of potential P2RX7 antagonists containing one, two and three chiral centers

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ABSTRACT

Eleven polysaccharide-based chiral stationary phases were screened to separate stereoisomers of six lactam derivatives by supercritical fluid chromatography (SFC). Those molecules are antagonists of the P2×7 receptor with potential anti-inflammatory activity. They present a mixture of either two enantiomers, or four or eight stereoisomers depending on the number of chiral centers in their structure. The effect of chiral stationary phase, either amylose- or cellulose-based, coated or immobilized, chlorinated or non-chlorinated, on retention and selectivity of those compounds was studied. The effect of the co-solvent nature (methanol, ethanol, propan-2-ol and acetonitrile) and percentage (15 to 40 %), temperature (20 to 45 °C) and backpressure (8 to 20 MPa) on the enantioseparation and retention of 1-(2-chlorobenzyl)-5-((4-methoxyphenyl)amino)pyrrolidin-2-one (compound 4) was thoroughly studied. Thus, optimal conditions were defined for each derivative in order to implement the preparative separation of the whole series, except of 1-((3,4-dimethoxyphenyl)(phenyl)methyl)-5-oxo-N-(1-phenylethyl)pyrrolidine-2-carboxamide (compound 6) for which none of the eleven columns could resolve the eight stereoisomers. The small-scale preparative separation of the enantiomers of the most promising derivative, *i.e.* 3-acetyl-1-(4-chlorophenyl)-N-(2,4-dichlorobenzyl)-5-oxopyrrolidine-2-carboxamide (compound 2), is presented.

1. Introduction

Given the significance of chirality in influencing the pharmacological properties of molecules, the separation of enantiomers (and stereoisomers in the broader sense) has been the subject of extensive research. High-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) are preferred methods/techniques for enantiomeric analysis due to their preparative scalability and other advantages [1]. Polysaccharide-based chiral stationary phases (CSPs) are widely used in both, SFC and HPLC due to their versatility, diverse chemical moieties, and high loading capacity, which are particularly beneficial for preparative applications [2,3]. The group of Okamoto introduced the "golden four" stationary phases, including *tris*(3,5-dimethylphenyl)carbamate of cellulose and amylose, cellulose *tris*(4-methylbenzoate), and amylose *tris*((S)- α -methylbenzyl)carbamate, as

well as chlorinated polysaccharide derivatives. The hybrid chloromethylphenylcarbamates of amylose and cellulose were proposed as useful chiral selectors by Chankvetadze *et al.* since 1993 [4–7]. These materials were then marketed by several companies. For more information about polysaccharide-based CSPs for liquid-phase separations one can refer to a recent review [8]. Various chromatographic mobile phases, such as polar aqueous-organic solvent (in reversed-phase chromatography) [9,10], polar organic solvents [11,12], and alcohol-hydrocarbon solvent (in normal-phase chromatography) [13], can be used with these CSPs in liquid chromatography. These boundaries disappear when implementing SFC since a single mobile phase consisting of carbon dioxide is used. The inherent properties of supercritical CO₂, including low viscosity and high diffusivity of the mobile phase, as well as reduced pressure drop, make it a particularly convenient tool for preparative scale, as stated above.

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Following the results previously obtained on experimental P2RX7 ligands with promising anti-inflammatory, anticancer and lung fibrosis activities [14–16], the pharmacomodulations were continued on different positions of the lactam ring of the best antagonists previously discovered (GSK 1370319A and HEI 2401, Fig. 1) in order to improve desired pharmacological/therapeutic effect. It should be noted that other ligands obtained via different pharmacomodulations have been studied previously by our group [17,18]. In this work, the central lactam heterocycle of these P2RX7 antagonists offers numerous modulation possibilities and different substituents have been added on positions 1, 4 and 5 of the reference molecules (Fig. 1). In the position 1, bulkier substituents such as 4-chlorophenyl, benzyl, 2-chlorobenzyl or benzhydryl substituents have been added (e.g. compounds 2–4, 5 and 6). Then, modulations were carried out on the position 4 of the lactam ring by adding an acetyl group in compound 2. Finally, several modulations were realized on the position 5 where the amide linker of the reference compounds has been replaced by an amino, an ester and a different amide group (compounds 1 and 3–6). All these compounds contain one, two or three asymmetric carbons in their structure and were obtained as a mixture of stereoisomers. Significant analytical effort was then

deployed to separate every mixture of stereoisomers to obtain pure enantiomers and/or stereoisomers for their biological evaluation. This operation is not easy even when two enantiomers are present, and it becomes more complicated when four or eight stereoisomers must be isolated. Chiral columns have been shown in early studies to be suitable for HPLC and SFC separation of stereoisomers of chiral compounds having multiple centers of chirality. In 2021, in our group, SFC and HPLC were used for separation of stereoisomers of chiral analytes having multiple centers of chirality [19].

Thus, the goals of this work were i) to screen chiral columns and co-solvents to separate the potential P2RX7 antagonists, ii) to illustrate the influence of the main parameters such as the type and percentage of the co-solvent, the flow-rate, the temperature and the backpressure on retention and selectivity for compound 4 chosen as a probe, and iii) to scale-up the separation to recover each enantiomer for the most promising antagonist, the compound 2, showing better P2RX7 affinity, predicted in molecular docking studies, than the reference compound GSK 1370319A, studied for treating inflammatory pain such as arthritis (Fig. 1). The additional carbonyl unit of compound 2 is providing an additional hydrogen bond acceptor (HBA) in the binding site and

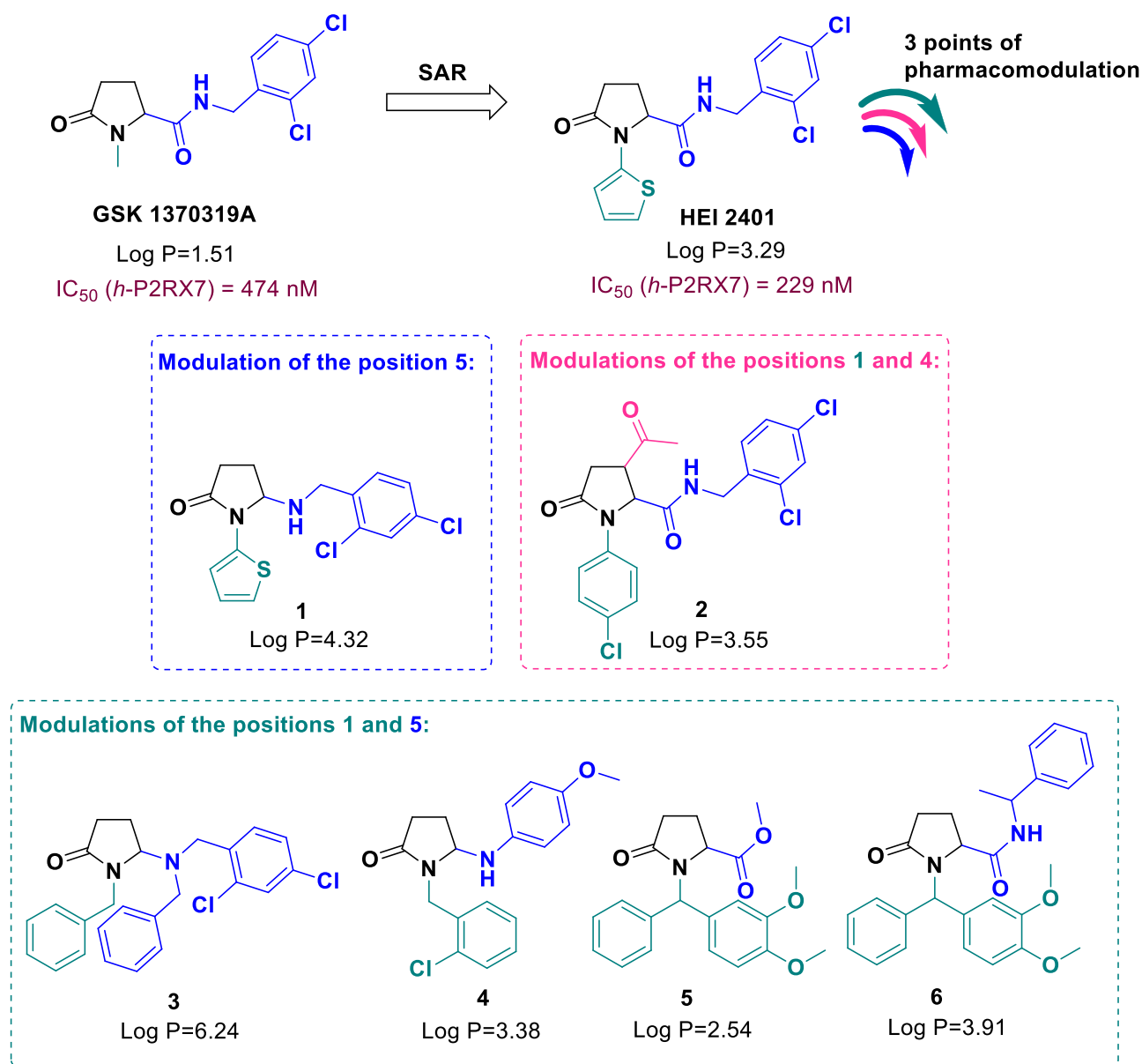


Fig. 1. Chemical structure of the reference (GSK 1370319A and HEI 2401)¹⁴ and newly synthesized P2RX7 antagonists investigated in this study (compounds 1–6).

consequently, additional interaction with the biological target.

2. Material and methods

2.1. Chemicals

The lactam derivatives (compound **1**: 5-((2,4-dichlorobenzyl)amino)-1-(thiophen-2-yl)pyrrolidin-2-one, compound **2**: 3-acetyl-1-(4-chlorophenyl)-*N*-(2,4-dichlorobenzyl)-5-oxopyrrolidine-2-carboxamide, racemic compound **3**: 1-benzyl-5-(benzyl(2,4-dichlorobenzyl)amino)pyrrolidin-2-one, racemic compounds **4**: 1-(2-chlorobenzyl)-5-((4-methoxyphenyl)amino)pyrrolidin-2-one, compound **5**: methyl 1-((3,4-dimethoxyphenyl)(phenyl)methyl)-5-oxopyrrolidine-2-carboxylate, compound **6**: 1-((3,4-dimethoxyphenyl)(phenyl)methyl)-5-oxo-*N*-(1-phenylethyl)pyrrolidine-2-carboxamide) described were synthesized in the frame of a structure-activity relationship study, by a chemical pathway reported elsewhere [14]. The methanol (MeOH), propan-2-ol (2-PrOH), ethanol (EtOH), acetonitrile (ACN) and heptane (Hept) were of HPLC grade and were purchased from Carlo-Erba Reagents (Val-de-Reuil, France). Carbon dioxide (CO₂, 99.995 % purity) was purchased from Air Liquide (Loos, France).

2.2. Solutions

For analytical screening, solutions of the samples were prepared in ethanol at 2 mg/mL. The solutions were degassed by an ultrasonic bath and filtered through a 0.45 μm PTFE syringe-filter (15 mm diameter) prior to use.

2.3. Supercritical fluid chromatography apparatus

2.3.1. Chiral stationary phases

Eleven chiral analytical columns were used for this study. Chiralpak AS-H based on amylose *tris*(*S*)- α -methylbenzylcarbamate, Chiralcel OJ-H based on cellulose *tris*(4-methylbenzoate), Chiralcel OD-H based on cellulose *tris*(3,5-dimethylphenylcarbamate), Chiralpak AD-H based on amylose *tris*(3,5-dimethylphenylcarbamate), Chiralpak IA based on amylose *tris*(3,5-dimethylphenylcarbamate) and Chiralpak IB based on cellulose *tris*(3,5-dimethylphenylcarbamate) were purchased from Chiral Technologies Europe (Illkirch, France). Lux Cellulose-2 based on cellulose *tris*(3-chloro-4-methylphenylcarbamate), Lux Amylose-2 based on amylose *tris*(5-chloro-2-methylphenylcarbamate), Lux i-Amylose-3 based on amylose *tris*(3-chloro-5-methylphenylcarbamate) and Lux i-Cellulose-5 based on cellulose *tris*(3,5-dichlorophenylcarbamate) were from Phenomenex® (Le Pecq, France). All columns had dimensions of 250 mm x 4.6 mm i.d. with 5 μm particle size and were coated silica-gel phases, except for the Lux i-Cellulose-5, Lux i-Amylose-3, Chiralpak IA and Chiralpak IB which were immobilized silica-gel phases. The coated amylose *tris*(3-chloro-5-methylphenylcarbamate), stationary phase was synthesized as described in reference [7] with slight modifications. Chiralpak AD-H (from Chiral Technologies) and Lux i-Cellulose-5 (from Phenomenex Inc.) semi-preparative columns had dimensions of 250 mm x 10 mm i.d. with 5 μm particle size.

2.3.2. Chromatographic systems and conditions

The chromatographic system used was an SFC-PICLAB hybrid 10–20 apparatus (PIC Solution, Avignon, France) equipped with an autosampler comprising a 48-vial plate (model Alias, Emmen, Netherlands), three model 40 P pumps (two for CO₂ and a third for the modifier (Knauer, Berlin, Germany)), a column oven with a Valco ten-position column selection valve, and a Valco six-position solvent switching valve. The system was also composed of a Smartline 2600 diode array detector (DAD) (Knauer, Berlin, Germany). Detection wavelength was set at 210 nm. After the detector, the outlet pressure was controlled by a back-pressure regulator (BPR). The pressure regulator pipe is heated to 55 °C to avoid ice formation during CO₂ depressurization. The system

was controlled and the data was acquired using the SFC PicLab Analytic Online v.3.1.2 software (PIC Solution, Avignon, France). The volume injected was 20 μL. During the separation optimization, the mobile phase was always a CO₂-co-solvent mixture with 40 % of MeOH, 2-PrOH, EtOH or ACN as co-solvent, at 4 mL/min flow rate. All analyses were performed in isocratic mode. The hold up time (t_0) was considered equal to the peak of the solvent front and was calculated from each run. It was equal to the value obtained by injection of 1,3,5-tri-*tert*-butylbenzene used as a non-retained sample. Retention times were the average values of two replicate determinations. The column oven temperature was 40 °C and the outlet pressure was maintained at 15 MPa (150 bar) for all experiments.

3. Results and discussion

With the aim of separating potential P2RX7 antagonists with either one, two or three chiral centers, eleven chiral columns differing in their polysaccharide backbone (cellulose or amylose), type of derivatives (carbamate or ester) and nature of the substituent, were used for analytical screening in SFC. The screening was carried out at 4 mL/min flow-rate, with four mobile phases consisting of CO₂/2-PrOH, CO₂/EtOH, CO₂/MeOH, CO₂/ACN 60:40 (v/v), at 40 °C and a back-pressure of 15 MPa.

3.1. SFC chiral separation method development

3.1.1. Effect of the nature of the stationary phase

Among the various commercially available CSPs, those based on polysaccharides, especially those coated with amylose or cellulose phenylcarbamate derivatives, are widely used due to their broad applicability to a wide range of chiral compounds and their high capacity for preparative-scale separations [2]. West's group has extensively characterized these CSPs [20–25] and they have been studied by many researchers. Introduced in the 1990s by Chankvetadze's group [4-7,26], CSPs coated with polysaccharide phenylcarbamates containing both chloro- and methyl-substituents are also popular [27]. The literature suggests that these CSPs, featuring both electron-donating and electron-withdrawing substituents, demonstrate complementary chiral selectivity compared to non-chlorinated phenylcarbamate-coated phases. The results obtained for each compound are presented in Table S1 provided in the Supplementary Information. It should be noted that compound **2**, presenting potential interesting biological activity in its racemic mixture, was only screened on Chiralpak AD-H and Lux i-Cellulose-5 columns which were available in our labs in semi-preparative dimensions. In this particular case, very good baseline resolutions were obtained on the Chiralpak AD-H (except with acetonitrile). Considering compounds **1**, **3** and **4** for which two enantiomers are expected, we compared the amylose-backbone stationary phases with each other. Among the coated ones, the Chiralpak AD-H and Lux Amylose 2 had a surprisingly low success rate, while the amylose *tris*(3-chloro-5-methylphenylcarbamate) and Chiralpak AS-H stationary phases were able to separate all compounds. This illustrates that the substitution on the phenylcarbamate moiety (*i.e.* 5-chloro-2-methylphenyl versus 3-chloro-5-methylphenyl) is important in this case. Among the immobilized phases, the Lux i-Amylose-3 separated all compounds, while compound **1** was not resolved on Chiralpak IA. Now comparing cellulose-backbone polysaccharide stationary phases, among the coated ones, the chlorinated columns (Lux Cellulose-2 and Lux Cellulose-5) led to enantioresolution for all compounds for the first and compounds **1** and **3** for the second. This can be explained by the electron withdrawing substituent on the phenylcarbamate group which increases the acidic strength of the NH group, thereby improving the resolution capacity of the four carboxamido analytes interacting with this latter group [24]. Non-chlorinated stationary phases, Chiralcel OD-H and Chiralcel OJ-H separated compounds **1** and **3** and compound **4**, respectively. Comparing the effect of the substituents, on the cellulose backbone

(3-chloro-4-methylphenyl) versus (3,5-dimethylphenyl) *i.e.* Lux Cellulose-2 against Chiralcel OD-H column, the higher abilities of the former to resolve all the amide derivatives can be explained by the presence of more acidic NH groups in larger proportions as noted above. In addition, the phenyl groups in the analytes form π - π interactions with the phenyl moiety of all the CSPs. Finally, the cellulose-based polysaccharide derivative immobilized on silica (Chiralpak IB) had the lower ability to resolve the stereoisomers. It is known that coated and immobilized CSPs can exhibit different enantioselectivities as the conformation of the polymer is influenced by the linkage between the stationary phase and the silica. Concerning compound 5, for which four stereoisomers were expected, baseline resolutions were obtained with all the CSPs tested, except for Chiralcel OJ-H and Chiralpak IB. An example is given in Fig. 2. Lastly, compound 6 was the most difficult case since eight stereoisomers were expected. From Table 1, it can be seen that at the best, whatever the co-solvent, five isomers are separated on the Lux i-Amylose-3 column. For this compound, coupling two or more stationary phases should be considered, as this is a very powerful tool [28], when CSP has failed to resolve the stereoisomers.

3.1.2. Effect of the nature and percentage of the co-solvent

As discussed in the previous section, CSP facilitates chiral resolution by engaging in hydrogen bonding, dipole-dipole, and π - π interactions

with amide derivatives. Likewise, pivotal in the process, the mobile phase, like the stationary phase, plays an important role. The polarity and density of the mobile phase are governed by the nature and proportion of the modifier. Additionally, the adsorption of mobile phase components also influences the character of the stationary phase (thus affecting the interaction environment), changes the phase ratio (thus impacting polymer chain solvation), and modifies the three-dimensional structure (thereby affecting the accessibility and size of the chiral cavities) [25]. The compound 4 was, due to its baseline separation, chosen for further SFC parameter optimization.

The nature of the organic co-solvent was studied in comparison with the identical proportions of different co-solvents: acetonitrile, methanol, ethanol and 2-PrOH, without any additives. This effect on retention and selectivity is illustrated in Fig. 3.

It is found that 2-PrOH, ethanol and methanol lead to similar retention times. The use of 2-PrOH, methanol and ethanol results in shorter retention time and lower enantioselectivity, while the use of ACN leads to highest retention times and resolution. This could be explained by the insufficient ability of ACN to form hydrogen bonds, compared to protic organic modifiers.

The percentage of co-solvent was tested varying from 20 % to 60 % of 2-PrOH (to maintain a short analysis time and sufficient resolution of the compound) on the Lux i-Amylose 3 CSP. The corresponding

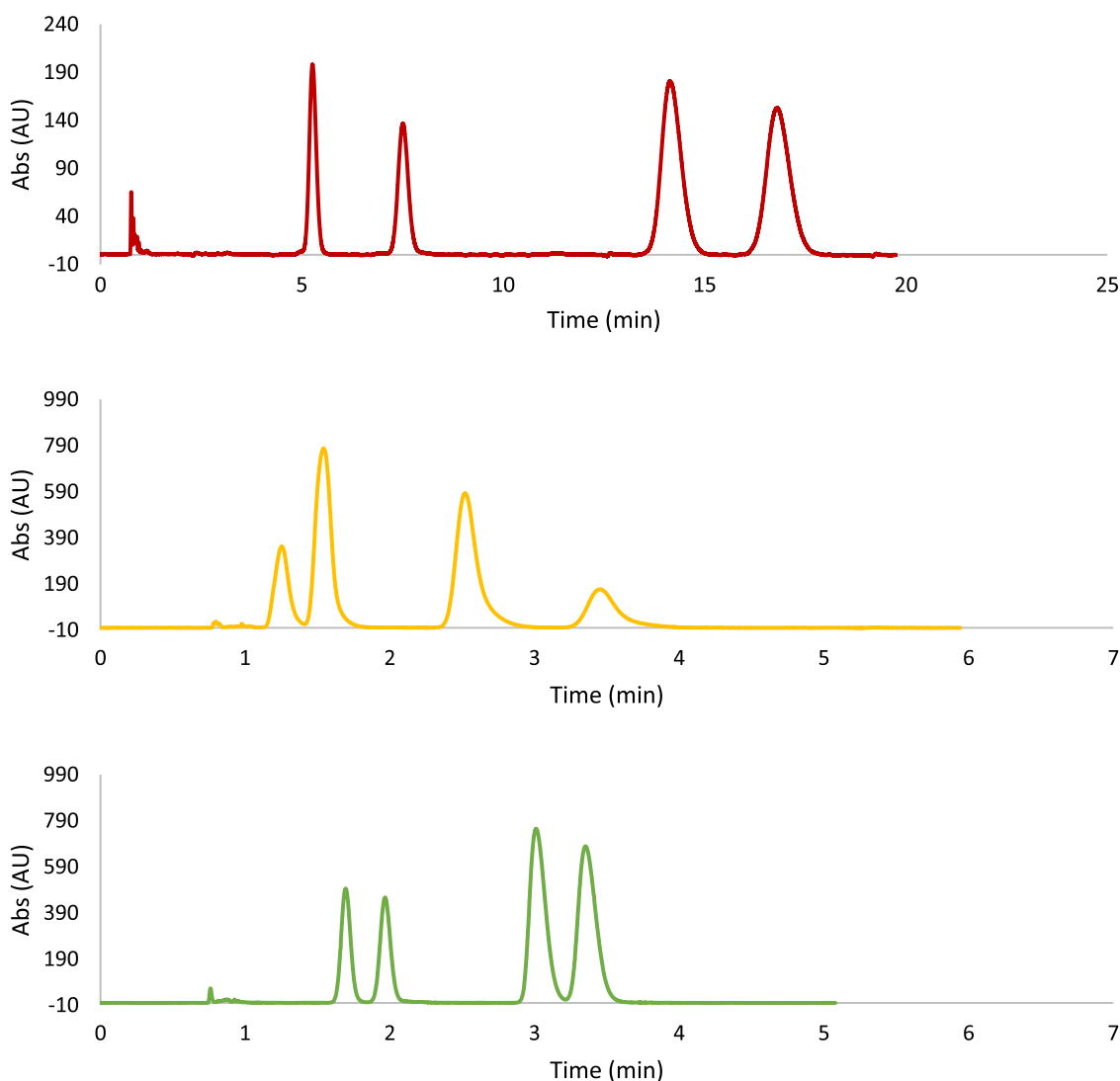


Fig. 2. Influence of the type of stationary phase Lux i-Cellulose 5 (red line), Chiralpak AS-H (yellow line) and Lux Amylose-2 (green line) on the enantioseparation of compound 5, 40 % 2-PrOH in CO₂, 4 mL/min, 40 °C, 15 MPa.

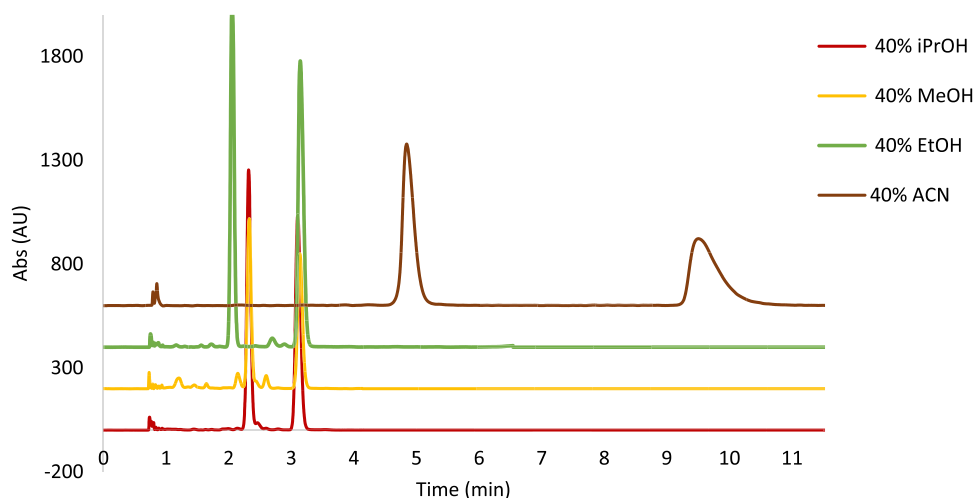


Fig. 3. Influence of the type of co-solvent in CO₂ on the enantioseparation of compound 4 on Lux i-Amylose-3 CSP, 4 mL/min, 40 °C, 15 MPa.

chromatograms are presented in Fig. 4. At constant conditions (pressure and temperature), increasing the proportion of co-solvent in the mobile phase increases the dielectric constant as well as the density of the mobile phase. Due to the fluid density increase, the elution force of the mobile phase increases, thereby promoting a decrease of the retention time of the analyte and the enantioselectivity as well. These experimental data show that modifier percentage is indeed the most powerful tool for modifying retention.

3.1.3. Effect of the flow-rate

Six flow-rates were tested, 2, 3, 4, 5, and 6 mL/min. Those high flow-rates can be implemented due to the low viscosity of the fluids. These variations are shown in Figure S1 (in Supplementary Information) for compound 4. It is observed that as the flow-rate increases, the retention factors decrease. As the fluid is compressible, changing its linear velocity in the column affects its density and, therefore, its eluting strength. Thus, the resolution decreases with increasing flow-rates. Therefore, flow-rate can be used to reduce analysis time but attention should be paid to the loss of resolution.

3.1.4. Effect of the temperature

The effect of the temperature was studied on the chosen analyte with 40 % 2-PrOH from 25 to 45 °C in increments of 5 °C at 4 mL/min on the previously indicated column. A lower temperature was not possible to achieve with the available oven and a higher temperature was not

recommended for the polysaccharide stationary phases. Figure S2 (in Supplementary Information) shows that temperature has little effect on retention and selectivity. This can be easily understood as temperature acts in two opposite directions on the retention. With a constant pressure drop, increasing temperature reduces analyte retention. At the same time, the density of carbon dioxide (and therefore the elution force) decreases, resulting in higher retention.

3.1.5. Effect of the back-pressure

Four different backpressures were tested: 8, 12, 15 and 20 MPa on the compound 4 with 40 % 2-PrOH at 4 mL/min on the column indicated previously. Stacked chromatograms are shown in Figure S3 (in Supplementary Information). Retention times decrease, while the resolution decreases slightly. Indeed, as pressure increases, the fluid density and hence the elution force also increase, leading to a decrease in retention times, while the separation factors may decrease to a lesser extent or remain constant. The magnitude of the retention variation depends on the composition of the mobile phase. Specifically, the impact of pressure on resolution is substantial when the fluid is more a gas-like, such as when pure carbon dioxide or low percentages of modifier (less than 5 %) are used as the mobile phase. Conversely, for a liquid type of fluid (above 10 % of co-solvent), the effect of pressure is less pronounced. Additionally, as diffusivity decreases under high-pressure conditions, column efficiency may be affected. However, the influence on selectivity and column efficiency is generally less significant than on retention,

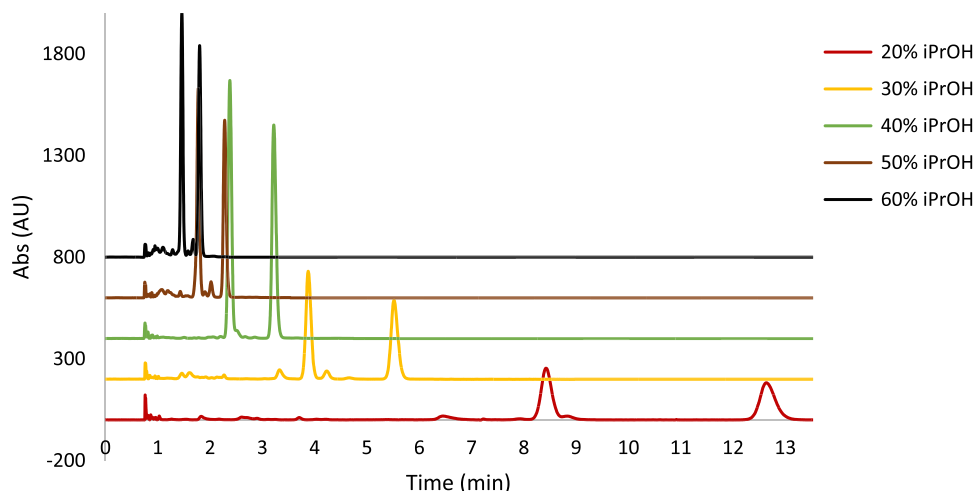


Fig. 4. Influence of the percentage of 2-PrOH in CO₂ on the enantioseparation of compound 4 on Lux i-Amylose-3 CSP, 4 mL/min, 40 °C, 15 MPa.

resulting in less impact on resolution due to pressure changes. This observation also applies to Figure 4S (in Supplementary Information) at less than 20 % of 2-PrOH in carbon dioxide.

In SFC, as in HPLC, chiral separation is mainly based on the optimization of the stationary phase, as well as on the determination of the type and percentage of co-solvent in the CO₂-based mobile phase. Flow rate and temperature also require optimization. However, a key distinction from HPLC is the application of backpressure after the column in SFC to maintain a consistent pressure drop throughout the column, making this parameter also crucial for optimization.

3.2. Scale-up

In liquid chromatography, the scale-up from the analytical to the preparative scale is tedious, as it relies on scaling the volumetric flow rate and injection volume relative to the ratio of the column radius. In SFC, scale-up is simple when column, particle size and mobile-phase velocity in the column remain identical during the two steps, but can be complicated when more variations are implemented, which is sometimes the case. Analytical optimization and scale-up to preparative scale were performed in our case on the same SFC device. It must also be kept in mind that the viability and success of a chromatographic process to reach the industrial scale essentially depends on the right choice of the operating conditions, such as the choice of CSP, nature and percentage of co-solvent, flow-rate, backpressure regulation, temperature. However, the scale-up in SFC can appear more complex compared to HPLC due to the compressibility of the mobile phase which changes of density along the column. It is very important to maintain equivalent mobile phase density in the analytical and preparative systems, otherwise retention time variation may occur.

3.2.1. Method optimization for scale-up

To find optimal separation conditions, the Chiralpak AD-H and Lux i-Cellulose-5 analytical columns were tested. Good resolution was obtained on Chiralpak AD-H with 40 % MeOH, EtOH and 2-PrOH, but slightly longer retention times were obtained, therefore Lux i-Cellulose 5 with 2-PrOH as organic co-solvent was chosen to further optimize the method to obtain better separation. The screening results for compound 2 are presented in Figure 5S (in Supplementary Information).

A decrease of the percentage of organic modifier was performed. The best resolution with acceptable retention times was obtained with 15 % 2-PrOH on Lux i-Cellulose 5 as shown in Fig. 5.

3.2.2. Semi-preparative separation

After setting the method, we performed the injection of compound 2 at the highest concentration around 11 mg/mL in EtOH, with three

increased injection volumes: 20 μ L, 100 μ L, and 250 μ L on the analytical column Lux i-Cellulose-5. It is worth mentioning that polysaccharide CSPs have high loading capacity with a saturation capacity ranging from 7.5 to more than 100 mg/g CSP [29]. When resolution was lost on the analytical dimensions' column, injection of the previous volume was performed on the semi-preparative column. No baseline separation was observed when injecting 250 μ L of sample onto the analytical column. Therefore, upscaling to preparative column was performed by injecting 250 μ L and 485 μ L sample solution. To maintain approximately the same retention times, it is necessary to calculate the transposition factor. With a value of 4.72, we therefore expect that working under 18.9 mL/min would lead to a similar chromatogram. However, the maximal flow-rate achievable by the device is equal to 16 mL/min (so four times the analytical flow-rate). The corresponding chromatograms obtained at 4 and 16 mL/min are shown in Fig. 6.

Before performing stacked injection on the SFC system, some modifications had to be made to the PrepSFC system. In "Injection Type", the total number of stacked injections and other related parameters in the software system had to be indicated. Three stacked injections of compound 2 on Lux i-Cellulose-5 semi-preparative column were performed. A customized injector sequence is implemented to run the injector in stacking mode. In Fig. 7, a thrice-stacked injection of 485 μ L is shown, and five additional stacked injections of 485 μ L were performed. Each enantiomer collected in a small fraction of 2-PrOH after depressurization of the CO₂, is evaporated to dryness and approximately 20 mg were recovered for the two enantiomers.

4. Conclusion

SFC is a convenient technology as high flow-rate can be used to reduce the analysis time, when many experiments need to be implemented. In this work, all the newly synthesized P2 \times 7 receptor antagonists were successfully separated, except for compound 6, bearing three chiral centers for which only 5 stereoisomers were separated. In our future study HPLC and column coupling in SFC will be tested on this compound.

Here, it is shown that only one type of CSP is not capable to resolve the whole series of the lactam derivatives, but that each of them can act in a complementary way to achieve this goal. It should be noted that, in this work, the different chlorinated polysaccharide-based stationary phases (either based on cellulose or on amylose) strongly contributed to the separation of the molecules studied. Additionally, an amount of 20 mg of each enantiomer of compound 2 was recovered for subsequent pharmacological studies. Other compounds are currently being separated on the preparative scale.

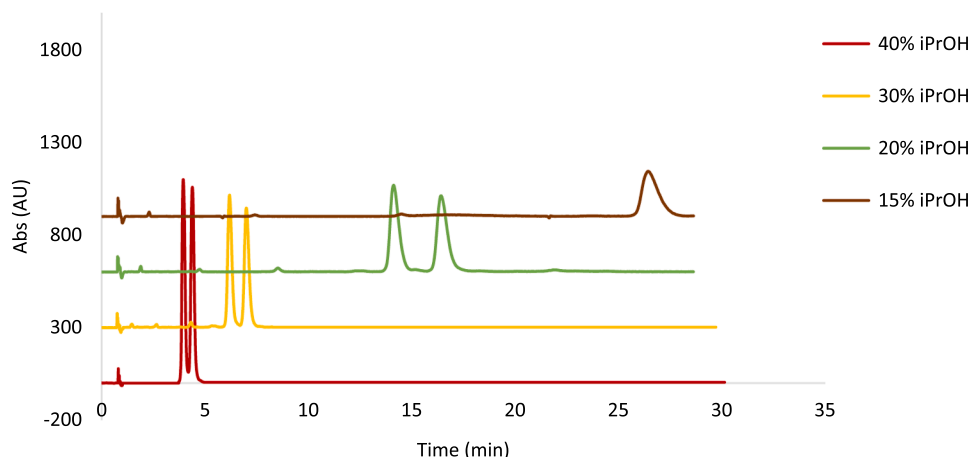


Fig. 5. Influence of the percentage of 2-PrOH in CO₂ on the enantioseparation of compound 4 on Lux i-Amylose-3 CSP, 4 mL/min, 40 °C, 15 MPa.

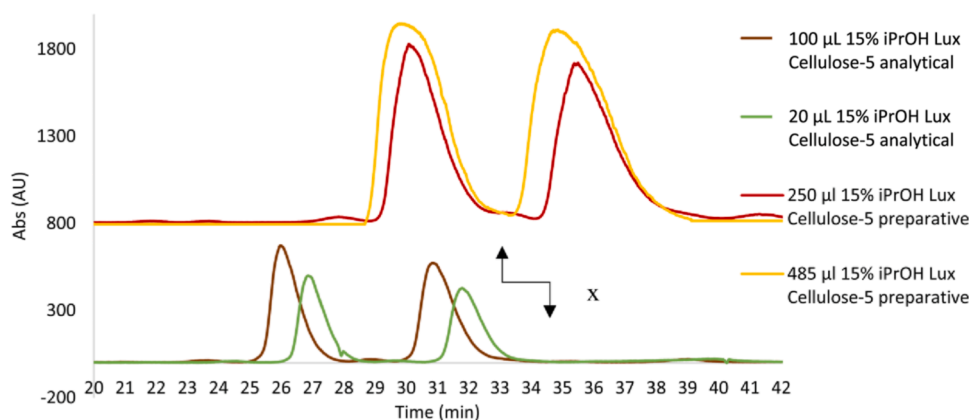


Fig. 6. 20 μL and 100 μL volume injection of compound 2 (concentration 11 mg/mL) on analytical column Lux i-Cellulose-5, 15 % 2-PrOH, 4 mL/min, 40 $^{\circ}\text{C}$, 15 MPa and 250 μL and 485 μL volume injection of compound 2 (concentration 11 mg/mL) on preparative column Lux i-Cellulose-5, 15 % 2-PrOH, 16 mL/min, 40 $^{\circ}\text{C}$, 15 MPa.

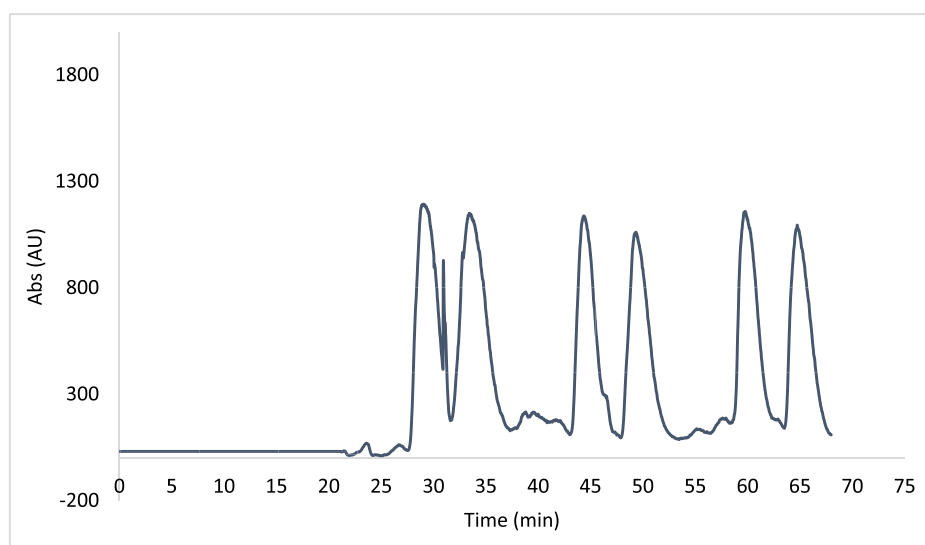


Fig. 7. Thrice-stacked injections of compound 2 at 11 mg/mL on preparative column Lux i-Cellulose 5, 15 % 2-PrOH, 16 mL/min, 40 $^{\circ}\text{C}$, 15 MPa.

CRedit authorship contribution statement

Naomi KNAVS: Investigation, Formal analysis. **Andreea ZUBAŞ:** Resources. **Georgiana APOSTOL:** Resources. **Christophe FURMAN:** Investigation. **Adrian Sorin NICA:** Investigation. **Bezhn CHANKVETADZE:** Writing – review & editing, Resources. **Alina GHINET:** Writing – review & editing, Resources. **Emmanuelle LIPKA:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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