Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 602–606

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Chiral separation of potent corticotropin-releasing factor-1 receptor antagonists by supercritical fluid chromatography

Jingfang Qian-Cutrone[a,*], Richard Hartz[a], Vijay T. Ahuja[a], Vivekananda M. Vrudhula[a], Dauh-Rurng Wu[b], Richard A. Dalterio[a], David Wang-Iverson[b], Joanne J. Bronson[a]

[a] Bristol-Myers Squibb Co., Research and Development, 5 Research Parkway, Wallingford, CT 06492, USA
[b] Bristol-Myers Squibb Co., Research and Development, P.O. Box 4000, Princeton, NJ 08543, USA

ARTICLE INFO

Article history:
Received 7 July 2010
Received in revised form
18 September 2010
Accepted 21 September 2010
Available online 30 October 2010

Keywords:
Enantiomeric separation
Supercritical fluid chromatography
Pyrazinone
CRF1 antagonist
Drug discovery

ABSTRACT

Pyrazinones bearing an *N*-1-alkyl chain with a chiral center have been reported as potent antagonists of the corticotropin-releasing factor-1 receptor (CRF1R). Separation of individual enantiomers for preclinical testing was an important aspect of lead optimization. To evaluate the applicability and efficiency of supercritical fluid chromatography (SFC) for enantiomeric resolution of this class of compounds, enantiomeric pairs of eight pyrazinones with different structural characteristics were tested under an array of SFC conditions. The results showed that pyrazinones with a 1-cyclopropyl-2-methoxyethyl substituent were readily separated with a Chiralpak AD-H or Chiralcel OD-H column with ethanol as the modifier. On the other hand, analogs with a less polar alkyl substituent were not amenable to the general method and required further optimization of the chromatographic conditions. In addition, structural variations on the pyrazinone core and aromatic moiety had an impact on the chiral resolution of this class of compounds. This investigation led to the development of efficient chiral SFC methods for separating all eight pyrazinone enantiomeric pairs encompassing an array of structural variations.

1. Introduction

Corticotropin releasing factor (CRF), a 41 amino acid peptide hormone, plays a major role in the regulation of the endocrine, behavioral and autonomic responses to stress, and is believed to be involved in the etiology of anxiety and depression [1,2]. There are two types of CRF receptors in mammals, CRF1R and CRF2R, which belong to the class B group of G-protein coupled receptors. CRF1R-selective antagonists are of considerable interest for use as a potential novel treatment of stress-related disorders such as anxiety and depression [3,4]. In our search for such agents, a class of compounds containing the pyrazinone core was found to have potent antagonistic activity toward CRF1R [5]. As part of the process to optimize the potency, pharmacokinetic and metabolic properties of this series of compounds, a significant number of analogs were synthesized for evaluation in bioassays [6–8]. Many of these analogs contained a chiral center on the side chain at *N*-1, as shown in Fig. 1. In the absence of an asymmetric synthesis, fast and efficient chiral separation methods for resolution of racemic analogs were essential for providing optically pure materials for bioassays and *in vivo* studies. A challenge facing separation scientists in the drug discovery environment is to quickly find the most efficient way to resolve a large number of structurally diverse compounds. One way to tackle this challenge is to shorten the cycle time for method development by implementing more efficient separation technologies such as supercritical fluid chromatography (SFC), which has rapidly become a preferred technology for chiral separation in drug discovery and development [9–14]. Another way to address the challenge is to establish a generic method for a class of compounds by attempting to correlate structural features with separation efficiency. To investigate the applicability of SFC to separation of pyrazinone enantiomers, an SFC study on eight enantiomeric pairs of pyrazinones with varying structural substitutions was conducted using various chiral stationary phases (CSPs) in combination with three alcoholic modifiers (methanol, ethanol and 2-propanol). Pyrazinones **1**–**8** (Fig. 1), which demonstrated potent CRF1R antagonist activity in *in-vitro* biological assays [6–8], differ structurally in the chiral alkyl substituent at *N*-1 as well as in the 5-substituent on the pyrazinone core and the aromatic amine at the 3-position. In this article, we report the separation results of this SFC study on these CRF1 receptor antagonists, and discuss the effects of structural differences, solvents and columns on the chiral resolution.

* Corresponding author. Tel.: +1 203 677 7828; fax: +1 203 677 7702.
E-mail addresses: jingfang.cutrone@bms.com, jfqiancutrone@yahoo.com (J. Qian-Cutrone).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.09.031

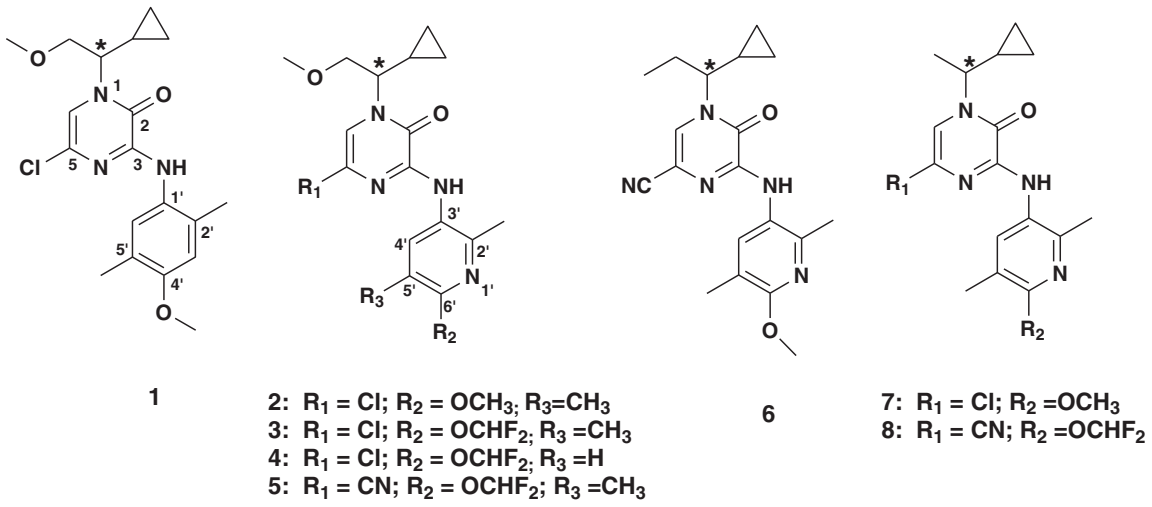

Fig. 1. Chemical structures of compounds **1**–**8**.

2. Experimental

2.1. Reagents and compounds

Methanol and 2-propanol were HPLC grade and purchased from J.T. Baker (Phillipsburg, NJ). Ethanol, 200 proof (99.98%), was purchased from Pharmco-AAPER (Brookfield, CT). The compounds (Fig. 1) studied are listed as follows: 5-chloro-1-(1-cyclopropyl-2-methoxyethyl)-3-(4-methoxy-2,5-dimethylphenylamino)pyrazin-2(1*H*)-one (**1**), 5-chloro-1-(1-cyclopropyl-2-methoxyethyl)-3-(6-methoxy-2,5-dimethylpyridin-3-ylamino)pyrazin-2(1*H*)-one (**2**), 5-chloro-1-(1-cyclopropyl-2-methoxyethyl)-3-(6-(difluoromethoxy)-2,5-dimethylpyridin-3-ylamino)pyrazin-2(1*H*)-one (**3**), 5-chloro-1-(1-cyclopropyl-2-methoxyethyl)-3-(6-(difluoromethoxy)-2-methylpyridin-3-ylamino)pyrazin-2(1*H*)-one (**4**), 4-(1-cyclopropyl-2-methoxyethyl)-6-(6-(difluoromethoxy)-2,5-dimethylpyridin-3-ylamino)-5-oxo-4,5-dihydropyrazine-2-carbonitrile (**5**), 4-(1-cyclopropylpropyl)-6-(6-methoxy-2,5-dimethylpyridin-3-ylamino)-5-oxo-4,5-dihydropyrazine-2-carbonitrile (**6**), 5-chloro-1-(1-cyclopropylethyl)-3-(6-methoxy-2-methylpyridin-3-ylamino)pyrazin-2(1*H*)-one (**7**) and 4-(1-cyclopropylethyl)-6-(6-(difluoromethoxy)-2,5-dimethylpyridin-3-ylamino)-5-oxo-4,5-dihydropyrazine-2-carbonitrile (**8**). These substances were synthesized and purified as previously described [6–8]. All were ≥95% pure as determined by HPLC with UV detection.

2.2. Instrumentation and method for chiral SFC screening

All analytical SFC experiments were performed on a Berger analytical SFC system (Mettler-Toledo Autochem, Newark, DE, USA) equipped with a FCM1200 dual pump fluid control module with a 6-position modifier switching valve, a thermal column module TCM-2000 loop, as well as an Agilent diode-array detector G1315A with a high-pressure flow cell (Agilent Technologies, Palo Alto, CA, USA.). Chromatographic data were acquired and processed with Berger SFC ProNTo software (Version 92.1). Liquid CO_2 was directly delivered from a dip-tube cylinder (SFC-grade CO_2, Airgas, CT, USA). All analyses were operated under isocratic conditions at a backpressure of 150 bar, a temperature of 35 °C, a flow rate of 2 ml/min, and UV detection at 230 and 350 nm. Four coated polysaccharide-based chiral SFC columns (Chiralpak AD-H and AS-H, Chiralcel OD-H and OJ-H, 4.6 mm × 250 mm, 5 μ) and three immobilized polysaccharide SFC columns (Chiralpak IA, IB and IC 4.6 mm × 250 mm, 5 μ) from Chiral Technologies (West Chester, PA, USA) were tested. Three organic modifiers (10% methanol, 10% ethanol and 10% 2-propanol) were employed in our SFC study. The equilibration time for each chromatographic condition was 10 min. The compounds were dissolved in ethanol at 1 mg/ml, and sample injection volume was 5 μl. All racemic samples were initially tested on AD-H, OD-H, OJ-H and AS-H, using 10% methanol, 10% ethanol and 10% 2-propanol as the modifiers. Compounds without baseline separation using the first set of conditions were further chromatographed on IA, IB and IC, using the same three modifiers. To determine the

Table 1
Retention times of the *S*- and *R*-enantiomers ($t_{R,S}$, $t_{R,R}$), separation factor (α) and resolution (R_s) of **1**–**8** on Chiralpak AD-H and AS-H, Chiralcel OD-H and OJ-H, for **8** also on Chiralpak IA, IB and IC columns, using 10% methanol in carbon dioxide at 2 ml/min, 35 °C, 230 nm detection, 150 bar back pressure.

Compound	Column	$t_{R,S}$ (min)	$t_{R,R}$ (min)	α	R_s
1	AD-H	8.35	10.43	1.33	2.97
	OD-H	7.48	7.87	1.07	0.98
	OJ-H	6.31	6.55	1.06	0.60
	AS-H	4.82	–	1.00	0.00
2	AD-H	7.39	9.59	1.41	4.40
	OD-H	6.75	7.82	1.23	2.68
	OJ-H	5.25	–	1.00	0.00
	AS-H	4.52	–	1.00	0.00
3	AD-H	4.59	5.01	1.18	1.05
	OD-H	5.52	6.73	1.36	3.46
	OJ-H	4.47	4.61	1.06	0.35
	AS-H	4.05	–	1.00	0.00
4	AD-H	5.36	5.66	1.09	0.67
	OD-H	5.67	7.13	1.42	3.74
	OJ-H	4.96	5.09	1.05	0.26
	AS-H	4.16	–	1.00	0.00
5	AD-H	5.06	–	1.00	0.00
	OD-H	6.07	7.43	1.35	3.02
	OJ-H	5.15	–	1.00	0.00
	AS-H	4.13	–	1.00	0.00
6	AD-H	6.98	6.65	1.07	0.33
	OD-H	5.58	5.83	1.07	0.63
	OJ-H	4.30	3.70	1.40	2.40
	AS-H	2.91	–	1.00	0.00
7	AD-H	6.31	6.85	1.13	0.68
	OD-H	4.95	–	1.00	0.00
	OJ-H	3.25	–	1.00	0.00
	AS-H	2.77	–	1.00	0.00
8	AD-H	3.90	–	1.00	0.00
	OD-H	5.10	–	1.00	0.00
	OJ-H	3.67	–	1.00	0.00
	AS-H	2.55	2.63	1.23	0.26
	IA	9.73	–	1.00	0.00
	IB	4.20	–	1.00	0.00
	IC	5.03	5.27	1.08	0.80

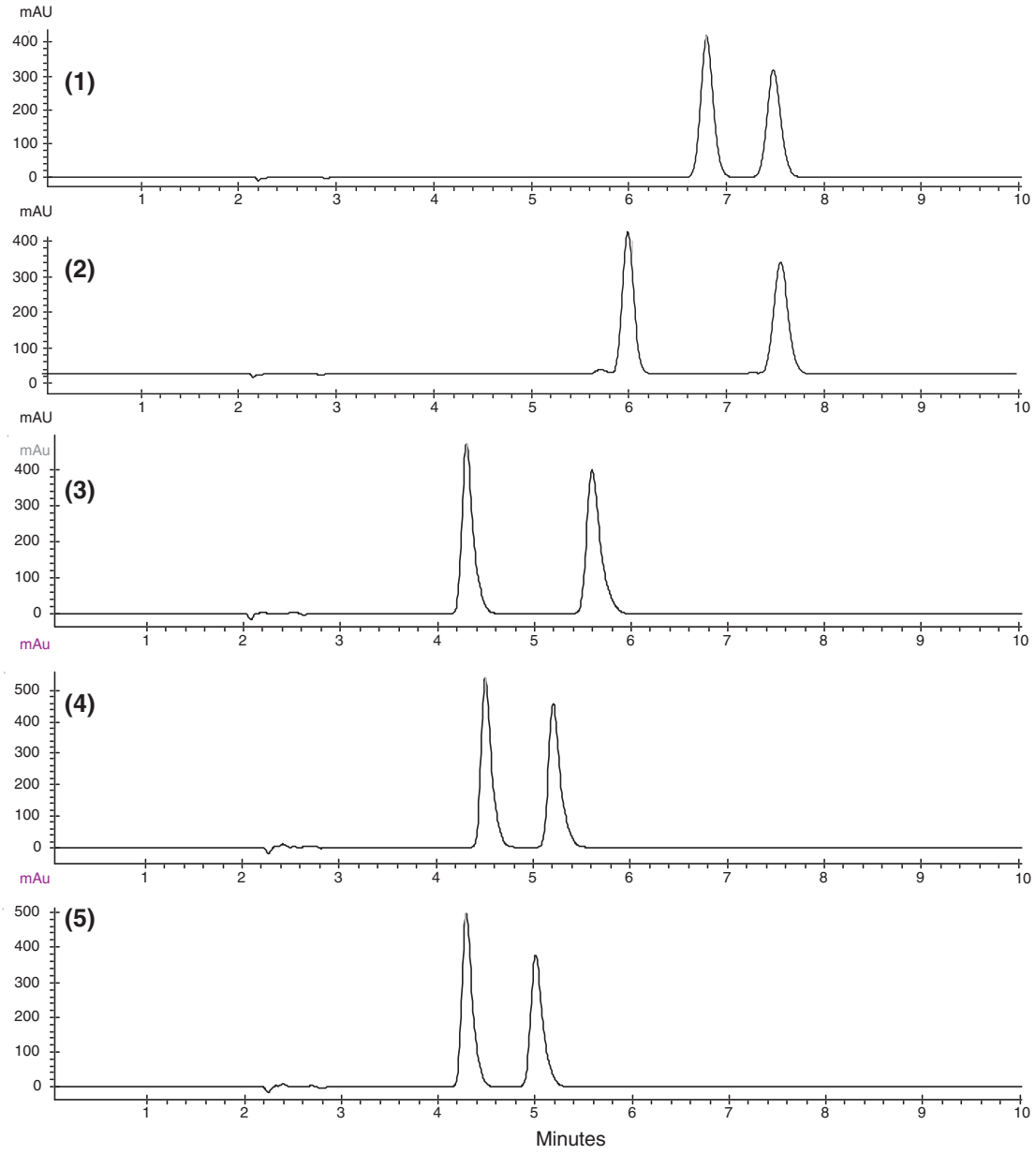

Fig. 2. Chromatograms of **1** and **2** on Chiralcel OD-H column, and **3**–**5** on AD-H column (4.6 mm × 250 mm, 5 μm), with 10% ethanol in carbon dioxide at 2 ml/min, 35 °C, 350 nm detection, 150 bar back pressure.

elution order, individual single enantiomers were also analyzed under the same conditions. The retention times of the *S*- and *R*-enantiomers were represented as $t_{R,S}$ and $t_{R,R}$. The chromatographic results including retention times ($t_{R,S}$ and $t_{R,R}$), separation factor (α) and resolution (R_s) for all compounds were recorded in Tables 1–3, while the most efficient chiral separations for **1**–**8** were shown in Figs. 2 and 3.

3. Results and discussion

3.1. SFC separation of pyrazinones with 1-cyclopropyl-2-methoxyethyl group at N-1

Separation of pyrazinones **1**–**5**, with an identical 1-cyclopropyl-2-methoxyethyl substituent at the *N*-1 position, but with varying substituents on the pyrazinone core and the aromatic amine moiety, were well-resolved under multiple SFC conditions. The polar nature of the chiral side chain proved to be an important factor in the facile separation of these compounds. Among the four coated column, OD-H column was found to be the choice of the column, since base-line separations were observed for all five enantiomeric pairs, regardless of which modifier was used. On the AD-H column, when ethanol was used as the modifier, all five pyrazinones were also well resolved. When the modifier was switched to methanol or 2-propanol, base-line separations were not achieved for **4** and **5**. Furthermore, the structural difference of the analytes showed an effect on the chiral resolution on the AD-H column, since the decreasing R_s values from **1** to **5** were observed using all three modifiers. Replacement of the phenylamino with a pyridylamino group at *C*-3, the chloro group with a cyano group at *C*-5, as well as a methoxy group with a difluoromethoxy group at *C*-6′, led to a decrease of the chiral resolution of the corresponding analogs. On the OJ-H column, baseline separation was obtained only for the phenylaminopyrazinone (**1**), while there was no chiral reso-

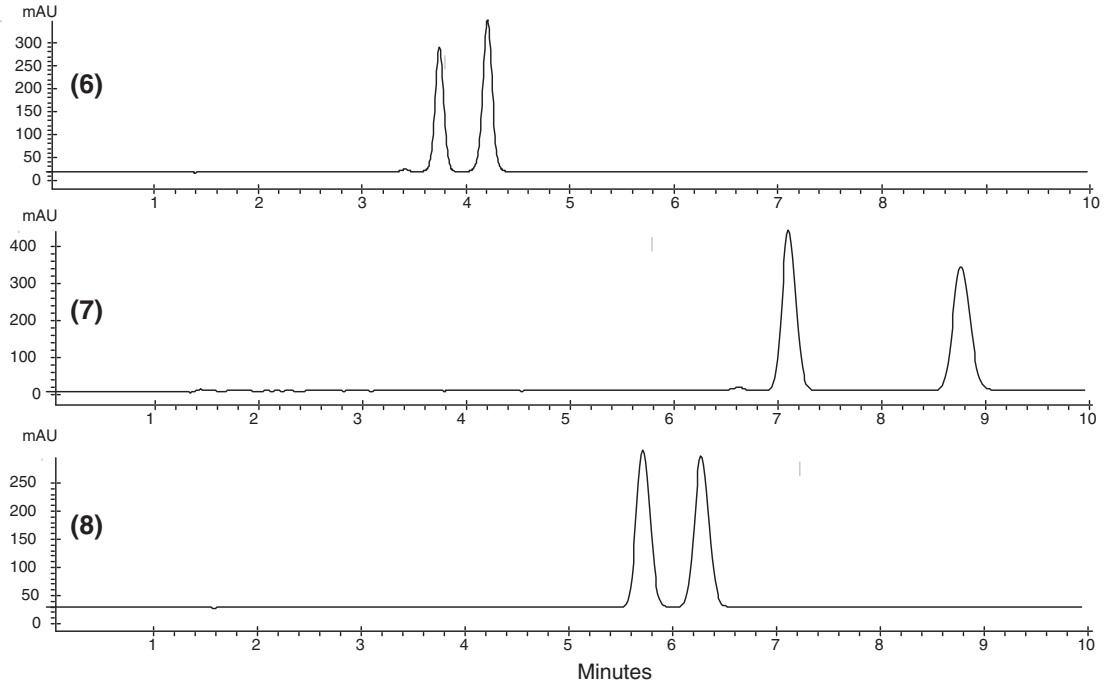

Fig. 3. Chromatograms of **6** (Chiracel OJ-H with 10% methanol), **7** (Chiralpak AD-H with 10% 2-propanol) and **8** (Chiralpak IC with 10% 2-propanol), 2 ml/min, 35 °C, 350 nm detection, 150 bar back pressure.

Table 2
Retention times of the *S*- and *R*-enantiomers ($t_{R,S}$, $t_{R,R}$), separation factor (α) and resolution (R_s) of **1**–**8** on Chiralpak AD-H and AS-H, Chiralcel OD-H and OJ-H, for **8** also on Chiralpak IA, IB and IC columns, using 10% ethanol in carbon dioxide at 2 ml/min, 35 °C, 230 nm detection, 150 bar back pressure.

Compound	Column	$t_{R,S}$ (min)	$t_{R,R}$ (min)	α	R_s
1	AD-H	7.99	12.35	1.75	5.95
	OD-H	6.80	7.48	1.58	2.27
	OJ-H	5.71	6.22	1.15	1.28
	AS-H	4.25	–	1.00	0.00
2	AD-H	6.88	10.65	1.81	5.38
	OD-H	6.04	7.61	1.41	3.93
	OJ-H	4.49	4.62	1.03	0.33
	AS-H	3.93	–	1.00	0.00
3	AD-H	4.35	5.65	1.60	3.33
	OD-H	4.90	6.61	1.63	5.70
	OJ-H	4.29	4.43	1.07	0.35
	AS-H	3.43	–	1.00	0.00
4	AD-H	4.55	5.25	1.30	1.84
	OD-H	5.03	7.21	1.77	5.45
	OJ-H	4.59	–	1.00	0.00
	AS-H	3.57	–	1.00	0.00
5	AD-H	4.33	5.05	1.34	1.84
	OD-H	5.53	7.51	1.59	5.21
	OJ-H	5.75	–	1.00	0.00
	AS-H	3.68	–	1.00	0.00
6	AD-H	5.76	6.56	1.22	1.33
	OD-H	5.61	5.93	1.09	0.80
	OJ-H	4.27	3.86	1.25	1.64
	AS-H	3.05	–	1.00	0.00
7	AD-H	6.57	7.22	1.15	2.03
	OD-H	4.84	–	1.00	0.00
	OJ-H	3.27	–	1.00	0.00
	AS-H	2.77	–	1.00	0.00
8	AD-H	3.41	3.53	1.10	0.34
	OD-H	5.03	5.21	1.06	0.47
	OJ-H	3.80	–	1.00	0.00
	AS-H	3.62	–	1.00	0.00
	IA	5.21	–	1.00	0.00
	IB	4.67	–	1.00	0.00
	IC	4.43	4.64	1.09	0.70

Table 3
Retention times of the *S*- and *R*-enantiomers ($t_{R,S}$, $t_{R,R}$), separation factor (α) and resolution (R_s) of **1**–**8** on Chiralpak AD-H and AS-H, Chiralcel OD-H and OJ-H, for **8** also on Chiralpak IA, IB and IC columns, using 10% 2-propanol in carbon dioxide at 2 ml/min, 35 °C, 230 nm detection, 150 bar back pressure.

Compound	Column	$t_{R,S}$ (min)	$t_{R,R}$ (min)	α	R_s
1	AD-H	9.12	10.42	1.30	2.17
	OD-H	7.63	9.27	1.30	4.10
	OJ-H	4.48	5.23	1.33	2.50
	AS-H	3.52	–	1.00	0.00
2	AD-H	7.88	9.10	1.21	2.03
	OD-H	6.87	9.73	1.61	5.20
	OJ-H	3.21	3.39	1.18	0.60
	AS-H	3.08	–	1.00	0.00
3	AD-H	5.16	5.60	1.15	0.88
	OD-H	4.85	8.68	2.45	8.51
	OJ-H	2.61	–	1.00	0.00
	AS-H	2.42	–	1.00	0.00
4	AD-H	5.56	–	1.00	0.00
	OD-H	5.09	10.28	2.80	8.95
	OJ-H	2.95	3.07	1.16	0.34
	AS-H	2.57	–	1.00	0.00
5	AD-H	4.44	4.57	1.06	0.29
	OD-H	6.32	11.16	2.17	6.91
	OJ-H	3.51	3.68	1.13	0.57
	AS-H	2.93	–	1.00	0.00
6	AD-H	5.51	5.87	1.11	0.72
	OD-H	6.66	7.03	1.08	0.80
	OJ-H	4.49	4.13	1.17	1.28
	AS-H	3.47	–	1.00	0.00
7	AD-H	7.28	8.93	1.32	5.50
	OD-H	5.54	–	1.00	0.00
	OJ-H	3.40	–	1.00	0.00
	AS-H	3.06	–	1.00	0.00
8	AD-H	3.72	4.01	1.19	0.76
	OD-H	6.17	–	1.00	0.00
	OJ-H	4.18	–	1.00	0.00
	AS-H	2.94	3.09	1.20	0.50
	IA	4.72	–	1.00	0.00
	IB	5.13	–	1.00	0.00
	IC	6.33	5.79	1.15	1.93

lution on the AS-H column for all tested pyrazinones. Among all conditions, the most efficient chiral resolution for this subgroup of pyrazinones was found on the either OD-H column or AD-H column with ethanol as the modifier.

3.2. SFC separation of pyrazinones with a methyl/ethyl group next to the cyclopropyl group at the chiral center of the N-1 substituent

Pyrazinones **6**, **7** and **8** are structurally similar to **1**–**5** on the pyrazinone core and aromatic moiety; however, replacement of the methoxymethyl group with a less polar alkyl group at the chiral center in these pyrazinones significantly affected their chiral resolution. Removal of the ether-containing side chain and replacement with an alkyl group resulted in the loss of potential hydrogen-bonding interactions between the *N*-1 side chain and the CSPs, and consequently decreased their chiral resolution on these CSPs. Pyrazinone **6**, with an ethyl group at the chiral center on the *N*-1 side-chain, was resolved by four SFC methods. The OJ-H column appeared to be the column of the choice, yielding good separation with all three modifiers. In addition, baseline resolution of the enantiomers of **6** was also achieved on the AD-H column when ethanol was used as the modifier. Pyrazinone **7**, with a methyl group next to the cyclopropyl group at the chiral side-chain, was well resolved only on the AD-H column using either ethanol or 2-propanol as the modifier. For pyrazinone **8**, all 12 SFC conditions used during our primary evaluation failed to achieve satisfactory separation, probably because of the combined effects of a less polar *N*-1 side-chain, a smaller alkyl group (methyl) at the chiral center, a *C*-5 cyano group on the pyrazinone core, and a *C*-6′ difluoromethoxy group. Consequently, **8** was further tested on the three immobilized CSPs (IA, IB and IC) using the three alcoholic modifiers. While the CSPs IA and IB did not afford any separation with all three modifiers, the IC column, a unique chiral selector based on the 3,5-dichlorophenylcarbamate of cellulose, provided baseline separation of **8** when 2-propanol was used as the modifier.

4. Conclusion

Our study showed that the enantiomers of eight pyrazinones (**1**–**8**), which belong to a promising class of potent CRFR1 antagonists, can be efficiently resolved using supercritical fluid chromatography. Within this class of compounds, the polarity of the substituents at the chiral center of the *N*-1 side-chain appeared to have the most significant effect on the chiral separation. Pyrazinones **1**–**5**, with a methoxyethyl group at the chiral center, were readily separated by SFC. In addition, the vast difference in chiral resolution efficiency among four coated CSPs suggested that the meta-substitution of methyl groups on the phenyl rings of the CSPs as in OD-H and AD-H was vital for the interaction with the individual enantiomers of this sub-group of pyrazinones. On the other hand, compounds with less polar alkyl substituents at the chiral center as in **6**–**8** were generally more challenging to separate and required individual method development. Furthermore, structural variations on the pyrazinone core and aromatic moiety also showed an effect on the chiral resolution of this class of compounds. Since replacement of the phenylamino with a pyridylamino group at *C*-3, the chloro group with a cyano group at *C*-5, as well as a methoxy group with a difluoromethoxy group at *C*-6′, had a detrimental impact on chiral resolution, the enantiomers of pyrazinone **8** were the most challenging to resolve among all the pyrazinones tested in this study. The IC column, with its unique ability to form additional dipole interactions with the individual enantiomers due to its dichlorophenylcarbamate moiety, was the only CSP that achieved a baseline separation of **8**. Nevertheless, our study demonstrated that SFC can serve as a powerful and efficient separation tool for this important class of CRFR1 antagonists, and it can potentially be used to resolve increasingly more challenging compounds thanks to the availability of diverse SFC stationary phases.

References

[1] E.B. De Souza, D.E. Grigoriadis, Corticotropin-releasing factor: physiology, pharmacology, and role in central nervous system and immune disorders, in: F.E. Bloom, D.J. Kupfer (Eds.), Psychopharmacology: The Fourth Generation of Progress, Raven, New York, 1995, pp. 505–517.
[2] M.J. Owens, C.B. Nemeroff, Physiology and pharmacology of corticotrophin-releasing factor, Pharmacol. Rev. 43 (1991) 425–473.
[3] F. Holsboer, The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety, J. Psychiatr. Res. 33 (1999) 181–214.
[4] C.D. Dzierba, R.A. Hartz, J.J. Bronson, Recent advances in corticotropin-releasing factor receptor antagonists, in: J.E. Macor (Ed.), Anuual Reports in Medcinal Chemistry, vol. 43, Academic, San Diego, 2008, pp. 1–23.
[5] A.G. Arvanitis, R.E. Olson, C.R. Arnold III, W.E. Frietze, Pyrazinones and triazinones and their derivatives thereof. World Patent Appl. WO 98/11075 A1 (1998).
[6] R.A. Hartz, V.T. Ahuja, M. Rafalski, E.W. Yue, D.J. Denhart, W.D. Schmitz, J.L. Ditta, J.A. Deskus, A.B. Brenner, A.G. Arvanitis, A.P. Combs, F.W. Hobbs, J. Payne, S. Lelas, Y. Li, T.F. Molski, G.K. Mattson, Y. Peng, H. Wong, J.E. Grace, K.A. Lentz, J. Qian-Cutrone, N.J. Lodge, R. Zaczek, J.J. Bronson, R.E. Olson, R.J. Mattson, J.E. Macor, Synthesis, structure-activity relationships, and in vivo evaluation of N^3-phenylpyrazinones as novel corticotropin-releasing factor-1 (CRF1) receptor antagonists, J. Med. Chem. 52 (2009) 4173–4191.
[7] R.A. Hartz, V.T. Ahuja, R.J. Mattson, D.J. Denhart, J.A. Deskus, J.L. Ditta, V. Vrudhula, S. Pan, X. Zhuo, Y. Shu, J.E. Grace, K.A. Lentz, S. Lelas, Y. Li, T.F. Molski, S. Krishnananthan, H. Wong, J. Qian-Cutrone, R. Schartman, R. Denton, N.J. Lodge, R. Zaczek, J.E. Macor, J.J. Bronson, A strategy to minimize reactive metabolite formation: discovery of (*S*)-4-(1-cyclopropyl-2-methoxyethyl)-6-[6-(difluoromethoxy)-2,5-dimethylpyridin-3-ylamino]-5-oxo-4,5-dihydropyrazine-2-carbonitrile, a potent, orally bioavailable corticotropin-releasing factor-1 (CRF) receptor antagonist, J. Med. Chem. 52 (2009) 7653–7668.
[8] R.A. Hartz, V.T. Ahuja, M. Rafalski, W.D. Schmitz, A.B. Brenner, D.J. Denhart, J.L. Ditta, J.A. Deskus, E.W. Yue, A.G. Arvanitis, S. Lelas, Y. Li, T.F. Molski, H. Wong, J.E. Grace, K.A. Lentz, J. Li, N.J. Lodge, R. Zaczek, A.P. Combs, R.E. Olson, R.J. Mattson, J.J. Bronson, J.E. Macor, In vitro intrinsic clearance-based optimization of arylpyrazinones as corticotropin-releasing $factor_1$ receptor antagonist, J. Med. Chem. 52 (2009) 4161–4172.
[9] T.A. Berger, Packed Column SFC, The Royal Society of Chemistry, Cambridge, UK, 1995.
[10] K. Anton, C. Berger (Eds.), Supercritical Fluid Chromatography with Packed Columns: Techniques and Application, Marcel Dekker, Inc., New York, 1998.
[11] L.T. Taylor, Supercritical fluid chromatograph, Anal. Chem. 80 (2008) 4285–4294.
[12] L. Miller, M. Potter, Preparative chromatographic resolution of racemates using HPLC and SFC in a pharmaceutical environment, J. Chromatogr. B 875 (2008) 230–236.
[13] D. Wu, L. Leith, B. Balasubramanian, T. Palcic, D. Wang-Iverson, The impact of chiral supercritical fluid chromatography in drug discovery: from analytical to multigram scale, Am. Lab. 38 (2006) 24–26.
[14] J. Qian-Cutrone, B. Dasgupta, E. Kozlowski, R. Dalterio, D. Wang-Iverson, V. Vrudhula, Separation of maxi-K channel opening 3-substitued-4-arylquinolinone atropisomers by enantioselective supercritical fluid chromatography, J. Pharm. Biomed. Anal. 48 (2008) 1120–1126.