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Screening of thiourea derivatives and carbonyl-2-aminothiazole derivatives for potential CCR4 antagonists using capillary zone electrophoresis

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ABSTRACT

CC chemokine receptor 4 (CCR4) is a kind of G-protein-coupled receptors with a characteristic seven-transmembrane structure and selectively expressed on Th2-type CD4+ T-cells. CCR4 has been identified as a potentially important drug target for the treatment of T cell-mediated allergic inflammatory diseases. In this study, a novel series of CCR4 antagonists were screened by investigating the interactions between the compounds and the human CCR4 N-terminal peptide ML40 using capillary zone electrophoresis (CZE) for the first time. Both qualitative and quantitative characterizations of the compound-peptide binding were determined. The results showed that, compared with positive control, ten of the compounds were interacted with ML40, which were A3C223, A3C231, A4C238, A3C241, A4C241, A4C239, ZXF0337, ZXF0432, ZXF0519 and ZXF0637A, and their binding constants were calculated from the Scatchard plot by regression. The binding constants of the compounds to ML40 were calculated and the binding constant of ZXF0432 was the largest among them [$(7.6334 \pm 0.1907) \times 10^4\ M^{-1}$]. Here, a sensitive and selective high-performance analytical method based on CZE was developed for screening of thiourea derivatives and C-arbonyl-2-aminothiazole derivatives for potential CCR4 antagonists for the first time. The methodology presented should be generally applicable to study compounds-ML40 interactions as a powerful, sensitive and fast screening method for CCR4 antagonist discovery.

1. Introduction

Chemokines and their receptors act as a communication and signaling network between cells, organs and regions of the body to facilitate immunological responses [1–4]. The CKLF1 is a novel human cytokine isolated from PHA-stimulated U937 cells. The CKLF1 protein exhibits chemotactic activities on leukocytes [5]. The previous study showed that CKLF1 is a novel functional ligand of CCR4 [6]. CC chemokine receptor 4 (CCR4) is a kind of G-protein-coupled receptors with a characteristic seven-transmembrane structure and selectively expressed on Th2-type CD4+ T-cells, which plays a pivotal role in allergic inflammation such as asthma and atopic dermatitis [7–9]. Evidence exists that CCR4 and its ligands play a role in Th2-type CD4+ T-lymphocyte-mediated inflammation. Current treatments for allergic inflammation include antihistamines and bronchodilators, which control symptoms but not disease progression, in addition to corticosteroids, which specifically target the disease. However, because of the subsequent development of steroid resistance and the side effects associated with long-term steroid use, other novel therapies that provide safe and effective treatments for these diseases have been explored [10–12]. Several approaches are being developed to block the effects of chemokines, including small-molecule antagonists of chemokine receptors, modified chemokines, and antibodies directed against chemokine receptors. Lactam analog, i.e., 2-(2-(2,4-dichloro-phenyl)-4-{[(2-methyl-3-chloro-phenyl)-1-ylmethyl]-carbamoyl}-methyl)-5-oxo-pyrrole-1-yl)-N-(3-piperidinyl-propyl)-acetamide (S009, structure as shown in Fig. 1), as a novel antagonist of CCR4, displays high-binding affinities, excellent chemotaxis inhibitory activity and selectivity toward CCR4 [13]. Previous studies demonstrated that the amino termini of the chemokine receptors are important in ligand binding using the cell-based binding assays or multi-dimensional heteronuclear NMR spectroscopy, such as the interactions of CCR4 agonists, stromal cell-derived factor-1, eotaxin, interleukin-8, fractalkine, and monocyte chemotactic protein-1 with the amino terminus of their cognate receptors [14], with high-binding affinities, respectively [15–19].

In order to seek novel CCR4 antagonists, Prof. Song Li's group imitated the interaction of CCR4 and CCL22, CCL17 or CKLF1-C19 using computer aided drug design (CADD) strategy and found

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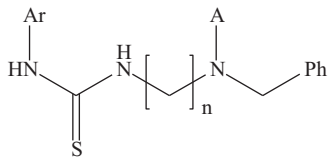
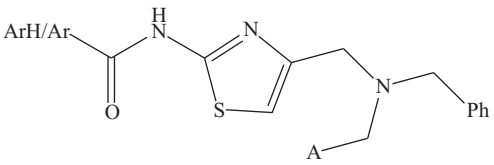
Table 1
Structures of the synthetic compounds.



nuclear structure I (compound 1-28)



nuclear structure II (compound 29-44)

Ar=ArCO,ArCH$_2$,Ar ; HAr=heteroAr ; A=Aliphatic radical ; n=2~4

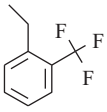
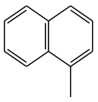
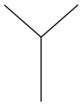
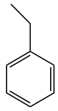
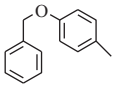
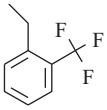
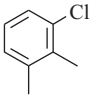
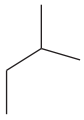
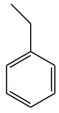
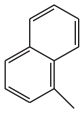
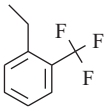
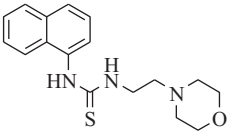
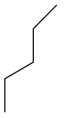
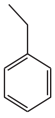
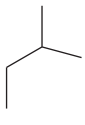
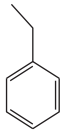
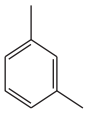
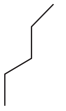
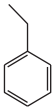
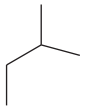
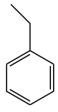
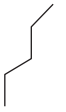
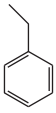
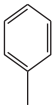
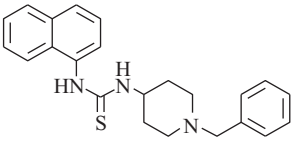
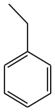
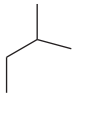
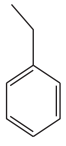
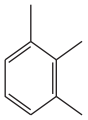
No.	Compound	A	Ph	Ar	*n*	No.	Compound	A	Ph	Ar	*n*
1	A3C11				2	15	A4C239				2.5
2	A3C112				2	16	ZXF0339				1.5
3	A3C113				2	17	ZXF0437				
4	A3C221				2	18	ZXF0336				1.5
5	A3C223				2	19	ZXF0337				1.5
6	A3C222				2	20	ZXF0432				
7	A3C231				2	21	ZXF0405				1.5
8	A3C232				2	22	ZXF0435				
9	A3C233				2	23	ZXF0416				2.5

Table 1 (*Continued*)

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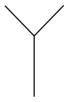
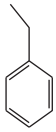
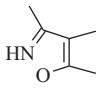
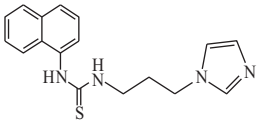
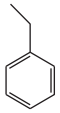
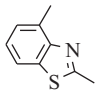
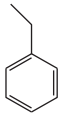
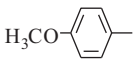
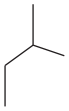
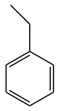
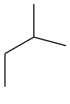
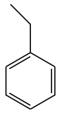
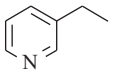
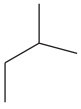
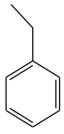
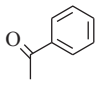
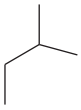
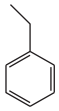
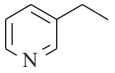
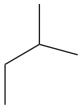
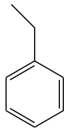
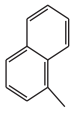
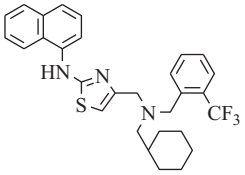
10	A4C236				2.5	24	ZXF0436				
11	A4C238				2.5	25	ZXF0311				2.5
12	A3C241				2	26	ZXF0319				2.5
13	A3C242				2	27	ZXF0320				2
14	A4C241				2.5	28	ZXF0740				

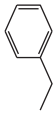
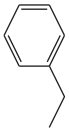
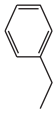
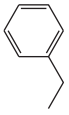
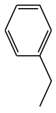
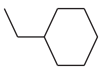
No.	Compound	Ph	A	R_3	No.	Compound	Ph	A	R_3
29	ZXF0636A				37	ZXF0640B			
30	ZXF0630A				38	ZXF0636D			
31	ZXF0636C				39	ZXF0710			
32	ZXF0636B				40	ZXF0639A			
33	ZXF0519				41	ZXF0639B			
34	ZXF0640A				42	ZXF0719A			

Table 1 (*Continued*)

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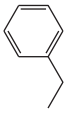
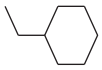
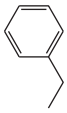
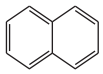
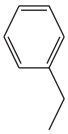
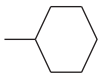
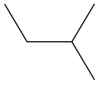
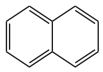
35	ZXF0637B				43	ZXF0719B			
36	ZXF0637A				44	ZXF0719C			

the key residues for the interaction. By getting the coordinate of these residues, two novel skeletal of CCR4 antagonists were built [20]. The thiourea derivatives and carbonyl-2-aminothiazole derivatives were designed according to these two respective skeletal structures. There were 44 synthetic compounds in total which named A3C11, A3C112, A3C113, A3C221, A3C223, A3C222, A3C231, A3C232, A3C233, A4C236, A4C238, A3C241, A3C242, A4C241, A4C239, ZXF0339, ZXF0437, ZXF0336, ZXF0337, ZXF0432, ZXF0405, ZXF0435, ZXF0416, ZXF0436, ZXF0311, ZXF0319, ZXF0320, ZXF0740, ZXF0636A, ZXF0630A, ZXF0636C, ZXF0636B, ZXF0519, ZXF0640A, ZXF0637B, ZXF0637A, ZXF0640B, ZXF0636D, ZXF0710, ZXF0639A, ZXF0639B, ZXF0719A, ZXF0719B and ZXF0719C. The chemical structures of these compounds are shown in Table 1 .

Many methods have been performed to study the interactions between protein receptor and different compounds, such as nuclear magnetic resonance (NMR), Fourier transform infrared spectrometry (FTIR), affinity chromatography (AC), mass spectrometry (MS), X-ray, fluorescence, surface plasmon resonance spectrometry (SPR), polyacrylamide gel electrophoresis (PAGE), equilibrium dialysis, and capillary electrophoresis (CE) [21]. NMR, X-ray, MS, fluorescence, and FTIR require an experiment and elaborated procedures for sample purification before analysis. SPR, AC, PAGE, and equilibrium dialysis are time-consuming procedures and demand a large sample size for analysis. In this study, CE was chosen to investigate the binding between synthetic compounds and ML40 due to its high speed, excellent resolution, low sample size, high reproducibility and flexibility. CE is able to study the interaction of individual components in a mixture as well as to determine binding parameters in a single step, a unique feature as compared with other techniques for the study of noncovalent interactions [22]. CE has been used in a wide range of binding studies between biologically important molecules, such as protein–protein, protein–DNA, protein–drug, protein–sugar, DNA–peptide, peptide–drug, peptide–peptide, antibody–antigen, and peptide–carbohydrate [23–29]. Among the various CE modes [30,31], CZE is the most appropriate one for the study of molecular interactions if only small amounts of samples are available.

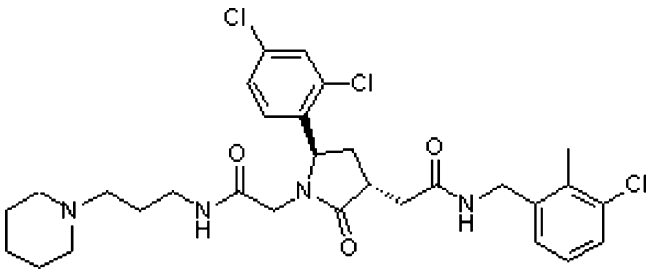


Fig. 1. Structures of 2-(2-(2,4-dichloro-phenyl)-4-{[(2-methyl-3-chloro-phenyl)-1-yl-methyl]-carbamoyl}-methyl)-5-oxo-pyrrole-1-yl)-N-(3-piperidinyl-propyl)-acetamid (Compound S009, trans diastereomer)-S009.

2. Methods and materials

2.1. Apparatus and CE conditions

All experiments were performed on a Beckman P/ACE^{TM} MDQ system (Beckman Coulter, Inc., Fullerton, CA, USA) equipped with a photodiode array detector as well as the 32 Karat^{TM} software version 5.0 (Beckman). A capillary tube (Yongnian Optical Fibre Corp., Hebei, China) with an internal diameter of 75 μm was used. The total and effective lengths of the capillary were 30.2 cm and 20 cm, respectively. Before using, the new capillaries were rinsed with 0.1 mol L^{-1} NaOH solution for 20 min, and subsequently with deionized water for 5 min.

2.2. Chemicals and materials

All chemicals were of analytical grade unless otherwise indicated. Tris base (ultrapure) and acetic acid used in this study were from Beijing Chemical Reagent Factory (Beijing, China). Tris–HAc that served as running buffer was prepared by dissolving 0.9105 g of Tris in 250 mL of deionized water and adjusting to pH 7.309 at 25 °C using diluted acetic acid.

Compounds of thiourea derivatives and C-arbonyl-2-aminothiazole derivatives were synthesized in Professor Song Li' laboratory from Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences (Beijing, China). Their structures were confirmed by MS and ^{1}H NMR. Dissolve compounds in methanol to the concentration of 5 mg/mL as stock solution which would be evaporated under nitrogen stream, and then the residual was reconstituted by adding appropriate amounts of Tris–HAc running buffer (30 mmol L^{-1} Tris, the pH was adjusted to 7.309 by acetic acid) diluting to different concentrations.

The equivalent peptide derived from ML40 was synthesized from Chinese Peptide Company in Hangzhou, China by RP HPLC and MS. The synthesized amino acid sequence of the human CCR4 amino terminus was MNPTDIADTTLDESIYSNYYLYESIPKPC-TKEGIKAFGEL.

Dissolve ML40 in Tris–HAc buffer (30 mmol L^{-1} Tris, the pH was adjusted to 7.309 by acetic acid) to the concentration of 4 mg/mL as stock solution which would be dilute to concentrations needed by adding appropriate amounts of Tris–HAc running buffer.

Deionized water was used to prepare buffer solutions and obtained using a Millipore Milli Q-Plus system (Millipore, Bedford, MA). The injected solutions were diluted with running buffer to different concentrations. All buffers and solutions used in the study were filtered through 0.45 μm membranes (Agilent, Germany) before using.

2.3. Sample preparation

To investigate compound-ML40 interaction, different concentrations of ML40 were tested for the formation of complex, which

Table 2
Compounds and the binding constants.

Compounds	Regression equation	r	Binding constants ($\times 10^4$ M^{-1})	RSD
A3C223	$y = -0.8183x + 0.8781$	0.8647	0.8183	0.1187
A3C231	$y = -2.8109x + 1.5427$	0.8923	2.8109	0.6522
A4C238	$y = -2.7145x + 2.7921$	0.9642	2.7145	0.3400
A3C241	$y = -1.4691x + 1.2219$	0.9405	1.4691	0.1262
A4C241	$y = -1.4784x + 0.5019$	0.8604	1.4784	0.1109
A4C239	$y = -2.4649x + 3.2773$	0.8174	2.4649	0.2851
ZXF0337	$y = -2.6238x + 2.4168$	0.7655	2.6238	0.3090
ZXF0432	$y = -7.6334x + 8.4604$	0.8838	7.6334	0.1907
ZXF0519	$y = -5.2072x + 3.3208$	0.8512	5.2072	0.2226
ZXF0637A	$y = -3.8148x + 7.9841$	0.8512	3.8148	0.1684

was formed by mixing compounds with ML40 with the running buffer. It was incubated for 20 min at 25 °C before CE analysis [32]. All solutions were prepared with doinized water.

2.4. CZE conditions

To study the interaction of compounds and ML40, the temperatures of the cartridge and sample room were kept at 25 °C and 4 °C, respectively. Before each measurement, the capillary was rinsed with running buffer (30 mmol L^{-1} Tris–HAc, pH 7.309). Samples containing the mixtures of compounds and ML40 were injected using the pressure injection mode at 0.5 p.s.i. for 5 s (1 p.s.i. = 6894.76 Pa). The applied voltage was 15 kV. The capillary was washed between runs with the running buffer for 5 min at 20 p.s.i. Each concentration was run in duplicate.

2.5. Quantitative model for the binding study

In the binding studies, the binding constant and the stoichiometry are important parameters to be determined. Scatchard analysis is a common way to linearize the binding data, as expressed in the following equation:

$$\frac{r}{C_f} = -Kr + nk \quad (1)$$

Assuming that there are n binding sites for the binding and each binding site does not influence the bindings on the other sites (noncooperative binding), K is defined as the intrinsic association constant of certain type of binding, C_f as the concentration of unbound ligand, and r as the average number of bound ligand per receptor molecule. The plot of r/C_f versus r gives a linear curve [33]. In this study, r is the concentration ratio of the bound synthetic compounds to the total synthetic compounds and C_f is the unbound ML40 concentration.

In this method, the peak height linearly depends on the concentration of the analyte. Therefore, a standard curve for synthetic compound concentration is created in order to measure the concentration of unbound synthetic compound by the peak height exhibited in the electropherograms. Usually, most of publications in binding studies with CE assume that the stoichiometry of the binding between receptor and ligand is 1:1 to establish a simple model ($n = 1$) [34–36]. In the following session, therefore, it is also assume the equations for the simple model to estimate binding constants so that the concentration of bound synthetic compound can be obtained and is equal to the concentration of bound ML40 according to the assumption ($n = 1$). Consequently, C_f as the concentration of unbound ML40 can be calculated, r is the concentration ratio of the bound synthetic compound to the total synthetic compound and the binding constant is calculated from the slope ($-K$) of the linear regression curve fitting from acquired data with Eq. (1).

3. Results and discussion

3.1. Optimization of the CZE conditions

A PDA was selected in this binding assay so that the structure information and absorption spectrum can be obtained simultaneously. The other electrophoretic conditions including buffer type, concentration of the buffer used, buffer pH value, applied voltage and the length of the capillary remained the same as the previous study [37]. Under the CE conditions employed in this paper, the baseline and peak shape were good enough for the quantification. No significant change was observed in the area of peak due to complex formation in 20 min, 30 min, 40 min, 50 min and 60 min, indicating that equilibrium had been reached in solution within 30 min. All samples were, therefore, incubated for at least 30 min before CE analysis.

3.2. Interactions between compounds and ML40 by CZE

Lactam analog, 2-(2-(2,4-dichloro-phenyl)-4-{[(2-methyl-3-chloro-phenyl)-1-ylmethyl]-carbamoyl}-methyl)-5-oxo-pyrrole-1-yl)-N-(3-piperidinyl-propyl)-acetamide (S009, structure as shown in Fig. 1), as a known antagonist of CCR4, displays high-binding affinities, excellent chemotaxis inhibitory activity and selectivity toward CCR4 in vitro [13]. In this study we used S009 as the positive control. The interactions between different compounds and ML40 at pH 7.3 were studied. Fig. 2 shows the electropherograms of A3C223, A3C231, A4C238, A3C241, A4C241, A4C239, ZXF0337, ZXF0432, ZXF0519, ZXF0637A and their mixtures. Curve A is for each compound alone and curves B–D for the mixtures containing a fixed concentration of corresponding compound with increasing concentrations of ML40. With increasing the ML40 concentration, the peak height of the compounds is decreased. Besides the main peak of each compound and the peak of ML40, a new peak can also be seen. Theoretically, it is the peak of the conjugate which usually cannot be completely separated with the peak of compound. According to the absorption spectra of the A3C223, A3C231, A4C238, A3C241, A4C241, A4C239, ZXF0337, ZXF0432, ZXF0519, ZXF0637A and their complexes obtained from the PDA detector, the new peaks were also proved to be compound ML40 complexes.

The binding constant is an important quantitative parameter for characterizing the interaction between the compounds and ML40. To obtain the calibration plots for each compound, increasing concentrations of the compounds were injected into the capillary, the peak heights being proportional to the concentrations. And the concentrations of each free compound were calculated from the calibration plot to obtain the values of r and from the slope of the Scatchard plot, K between ML40 and each compound was calculated (see Table 2).

Fig. 3 is the electropherograms of ZXF0416 and its mixture. The main peak of curve A is compound ZXF0416. While the

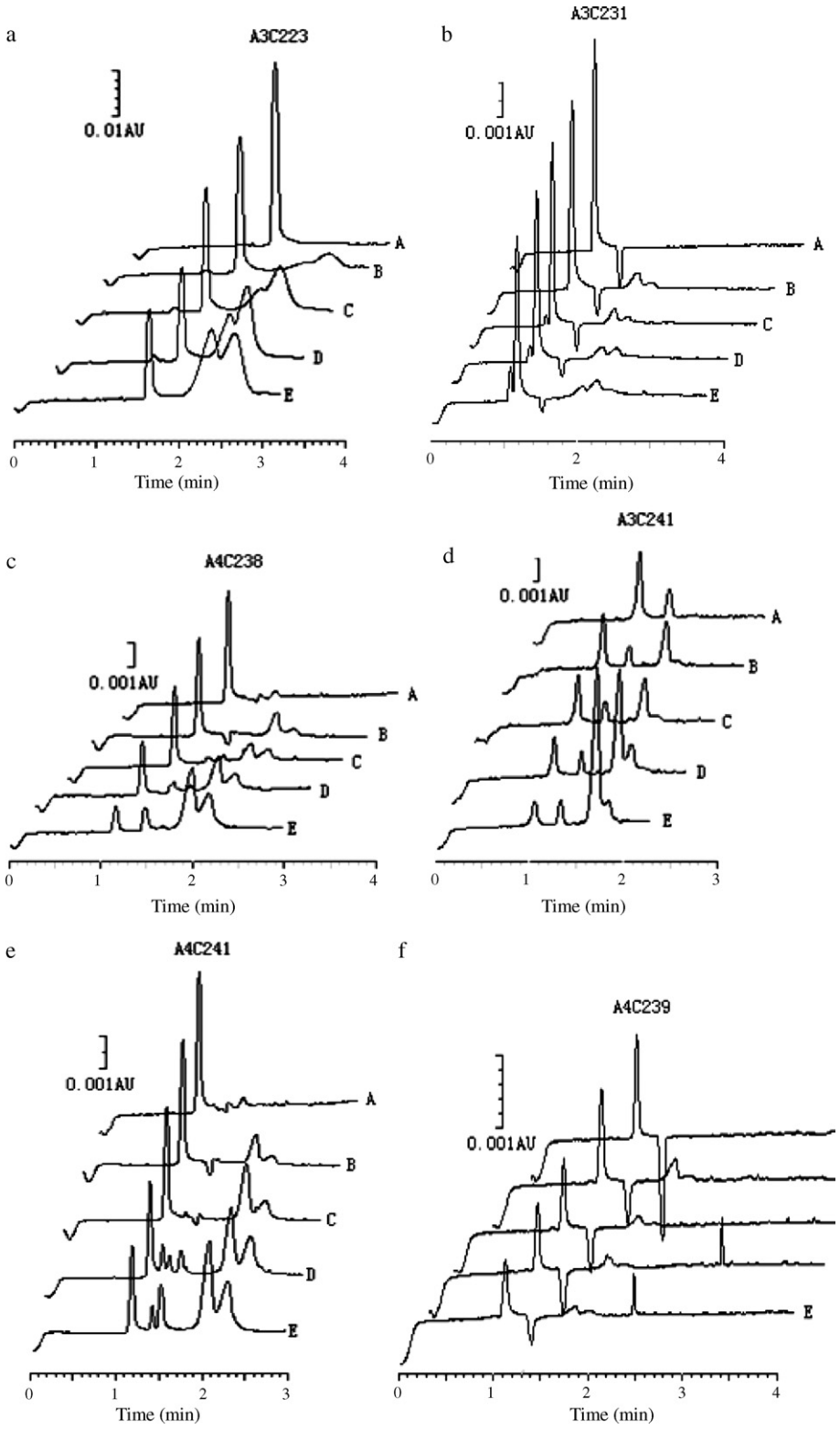


Fig. 2. Electropherograms of (a) A3C223-ML40 interaction, A3C223:ML40 (mol/mol) (A) 1:0; (B) 1:0.4; (C) 1:0.6; (D) 1:1.6; (E) 1:2.0. (b) A3C231–ML40 interaction, A3C231:ML40 (mol/mol) (A) 1:0; (B) 1:0.2; (C) 1:0.4; (D) 1:0.6; (E) 1:0.8. (c) A4C238–ML40 interaction, A4C238:ML40 (mol/mol) (A) 1:0; (B) 1:0.2; (C) 1:0.5; (D) 1:1.0; (E) 1:1.7. (d) A3C241–ML40 interaction, A3C241:ML40 (mol/mol) (A) 1:0; (B) 1:0.3; (C) 1:0.4; (D) 1:0.6; (E) 1:1.1. (e) A4C241–ML40 interaction, A4C241:ML40 (mol/mol) (A) 1:0; (B) 1:0.1; (C) 1:0.6; (D) 1:1.1; (E) 1:1.7. (f) A4C239–ML40 interaction, A4C239:ML40 (mol/mol) (A) 1:0; (B) 1:0.4; (C) 1:0.6; (D)1:0.8; (E) 1:1.2. (g) ZXF0337–ML40 interaction, ML40:ZXF0337 (mol/mol) (A) 1:0; (B) 1:4.1; (C) 1:8.3 (D) 1:12.4 (E) 1:16.6. (h) ZXF0432–ML40 interaction, ZXF0432:ML40 (mol/mol) (A) 1:0; (B) 1:0.2; (C) 1:0.5 (D) 1:0.8 (E) 1:0.9. (i) ZXF0519–ML40 interaction, ZXF0519:ML40 (mol/mol) (A) 1:0; (B) 1:0.1; (C) 1:0.4; (D) 1:0.6; (E) 1:0.7. (j) ZXF0637A–ML40 interaction, ZXF0519:ML40 (mol/mol) (A)1:0; (B) 1:0.2; (C) 1:0.7; (D) 1:0.9; (E) 1:1.2. The conditions used were: Beckman P/ACE MDQ capillary electrophoresis system. Injection: 0.5 p.s.i. for 5 s. Applied voltage: 15 kV. Capillary: capillary of 30.2 cm (effective length 20 cm) × 75 μm I.D. Observation by wavelength 214 nm.

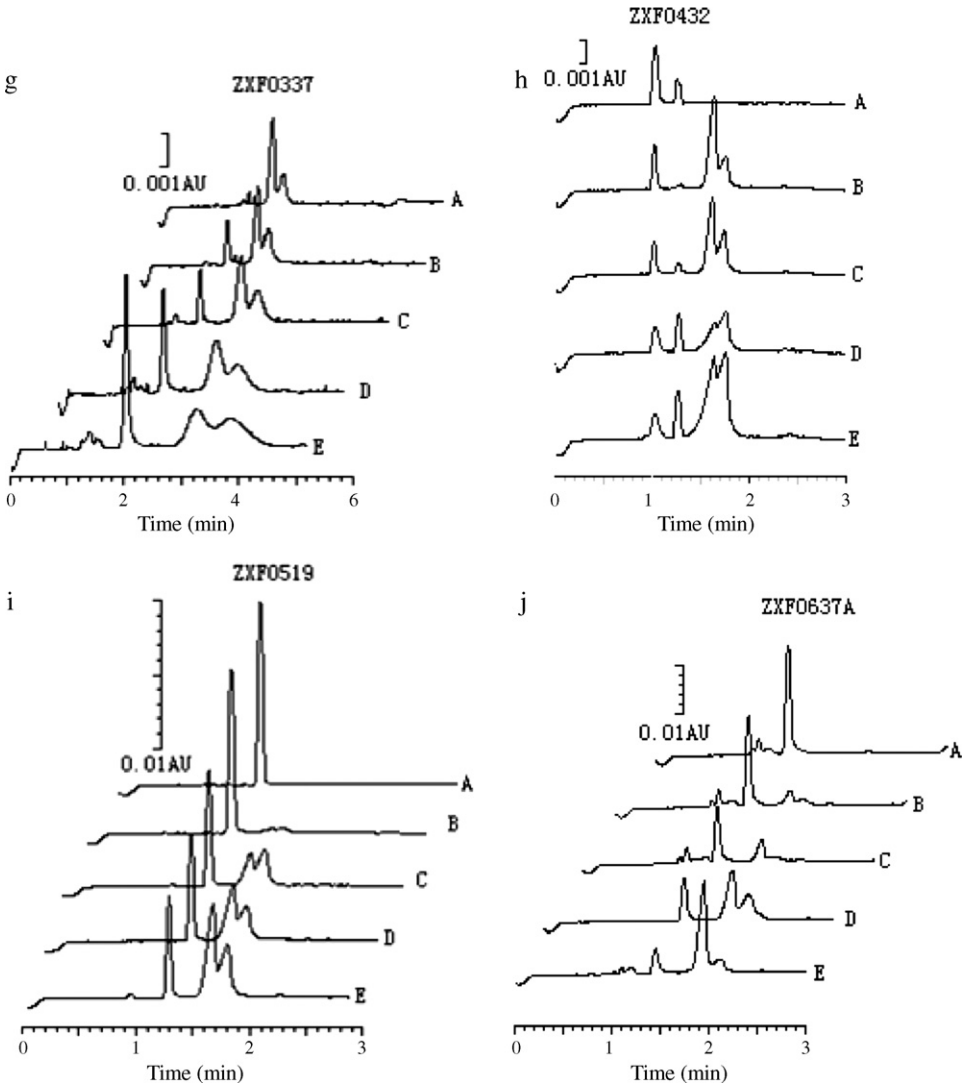


Fig. 2. (*Continued*).

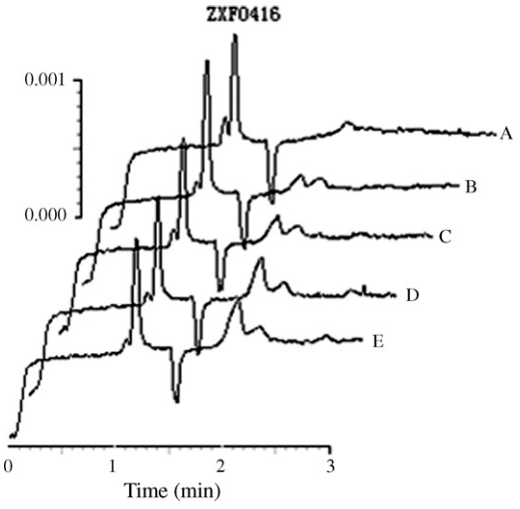
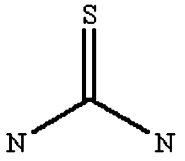


Fig. 3. Electropherograms of ZXF0416–ML40 interaction, ZXF0416:ML40 (mol/mol) (A) 1:0; (B) 1:0.5; (C) 1:1.0; (D) 1:1.8 (E) 1:2.2. The conditions used were: Beckman P/ACE MDQ capillary electrophoresis system. Injection: 0.5 p.s.i. for 5 s. Applied voltage: 15 kV. Capillary: capillary of 30.2 cm (effective length 20 cm) × 75 μm I.D. Observation by wavelength 214 nm.

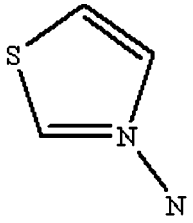
curves B–D shows the mixture containing a fixed concentration of ZXF0416 with an increasing concentration of ML40. It shows that the peak height of the corresponding compound does not change obviously with increasing the ML40 concentration. According to the absorption spectra from the PDA detector, UV of ZXF0416 and its mixtures had no obvious difference. No interactions were obtained. Compounds A3C112, A3C222, A3C232, A3C233, A4C236, A3C242, ZXF0437, ZXF0435, ZXF0436, ZXF0311, ZXF0319, ZXF0740, ZXF0636A, ZXF0636C, ZXF0636B, ZXF0640A, ZXF0640B, ZXF0636D, ZXF0710, ZXF0639B, ZXF0719A and ZXF0719B shared the same results.

Screening of synthetic compounds based on the concept of ligand/receptor interaction is an indispensable strategy to search for receptor inhibitors, and a number of strategies have been successfully applied. CCR4 is an important target for allergic inflammation diseases. The compounds, which have good CCR4-binding capacity, could be potential CCR4 antagonists. Compared with positive control S009, the compounds A3C223, A3C231, A4C238, A3C241, A4C241, A4C239, ZXF0337, ZXF0432, ZXF0519 and ZXF0637A interacted with ML40, while A3C112, A3C222, A3C232, A3C233, A4C236, A3C242, ZXF0437, ZXF0435, ZXF0436, ZXF0311, ZXF0319, ZXF0416, ZXF0740, ZXF0636A, ZXF0636C, ZXF0636B, ZXF0640A, ZXF0640B, ZXF0636D, ZXF0710, ZXF0639B, ZXF0719A and ZXF0719B had no binding to ML40.

The thiourea derivatives and C-arbonyl-2-aminothiazole derivatives were designed according the skeletal structures built by getting the coordinate of those key residues in the interaction between CCL22, CCL17 or CKLF1-C19 and CCR4. The compounds No. 1–No. 27 were thiourea derivatives while the compounds No. 28–No. 44 belonged to C-arbonyl-2-aminothiazole derivatives.



thiourea derivatives



C-arbonyl-2-aminothiazole derivatives

According to the binding constants calculated from the Scatchard plot, the C-arbonyl-2-aminothiazole derivatives were more hard being interacted with ML40 which could be attributed to the thiazole ring. The thiazole ring made the structure more rigid and limited the flexibility of the molecular structure, so the key sites were much harder to match the ML40 because of the difficulty of conformation changes. On the other hand, thiourea was a more open structure with greater flexibility and easier to interact.

4. Concluding remarks

The interactions between ML40 and synthetic compounds were studied using CZE in order to screen for the potential CCR4 antagonists for the first time. Forty-four compounds were designed according the skeletal structures built by getting the coordinate of those key residues in the interaction between CKLF1 and CCR4 and ten of them had the interaction with ML40. The binding constants of the compounds to ML40 were calculated and the binding constant of ZXF0432 was the largest among them [$(7.6334 \pm 0.1907) \times 10^4\,M^{-1}$]. Generally, the larger the binding constant was, the stronger the receptor inhibition activity would be. On the other hand, the binding constant of S009 as the positive control to ML40 was calculated to be $(9.9308 \pm 0.4090) \times 10^4\,M^{-1}$, basically the same as reported in the literature [37] which indicate this CZE method employed was reliable. Each run was completed within 4 min. Our results show that CZE provides a highly efficient, fast, quantitative, and sensitive method for studying the interactions between synthetic compounds and the target receptor CCR4. Furthermore, this method can be employed to screen a series of compounds with different targets in the drug discovery.

Acknowledgements

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