Full Paper

Synthesis and Structure-Activity Relationships of 3*H*-Quinazolin-4-ones and 3*H*-Pyrido[2,3-*d*]pyrimidin-4-ones as CXCR3 receptor antagonists

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CXC chemokine receptor-3 (CXCR3) is a G-protein coupled receptor (GPCR) predominantly expressed on activated T lymphocytes that promote Th1 responses. Previously, we described the 3*H*-quinazolin-4-one containing VUF 5834 (decanoic acid {1-[3-(4-cyano-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-ethyl}-(2-dimethylamino-ethyl)-amide) as a small-molecule CXCR3 antagonist with submicromolar affinity and as a lead structure for the development of CXCR3 antagonists. More recently, the related 3*H*-pyrido[2,3-*d*]pyrimidin-4-one compounds AMG 487 and NBI-74330 have been reported as nanomolar CXCR3 antagonists and these ligands are currently under clinical investigation. The aim of this study is to link the structure-activity relationship (SAR) of the previously published class of 3*H*-quinazolin-4-one containing CXCR3 ligands with these novel clinical candidates. From the modification of the lead structure VUF 5834 emerged the importance of the (4-fluoro-3-(trifluoromethyl)phenyl)acetyl and the 3-methylen-pyridine as substituents to improve the affinity at the human CXCR3 receptor, whereas other features are less important. The described molecules serve as tool to investigate the role of the CXCR3 receptor in various inflammatory conditions.

Keywords: Antagonist / Chemokine / CXCR3 / GPCR

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Introduction

Chemokines are a family of small proteins (70 – 80 amino acids) secreted by leukocytes or tissue cells that mediate cellular recruitment to sites of inflammation or injury [1]. Depending on the number and spacing of conserved cysteine residues, chemokines are classified into four major groups (CC, CXC, CX3C, and XC) [2, 3]. The IFN-γ-inducible CXC chemokines, CXCL9, CXCL10, and CXCL11 interact with CXC chemokine receptor-3 (CXCR3) to exert their functions [4]. Like all the chemokine receptors, CXCR3 belongs to the superfamily of the G-protein coupled receptors (GPCR) [5]. Activation of CXCR3 via G-proteins of the G_i-type leads to an increase of intracellular calcium and cellular migration [6, 7]. Several studies have shown that levels of CXCL9, CXCL10, and CXCL11 are elevated in various chronic inflammatory diseases such as rheumatoid arthritis [8, 9], multiple sclerosis [10], transplant rejection [11], hepatitis C infected liver [12], atherosclerosis [13], chronic skin reactions [6, 14, 15], and chronic obstructive pulmonary disease [16] and are related to the infiltration of CXCR3-positive T-cells. Consequently, it is believed that CXCR3 antagonists provide a therapeutic approach in chronic inflammatory and neoplastic diseases [17 – 19].

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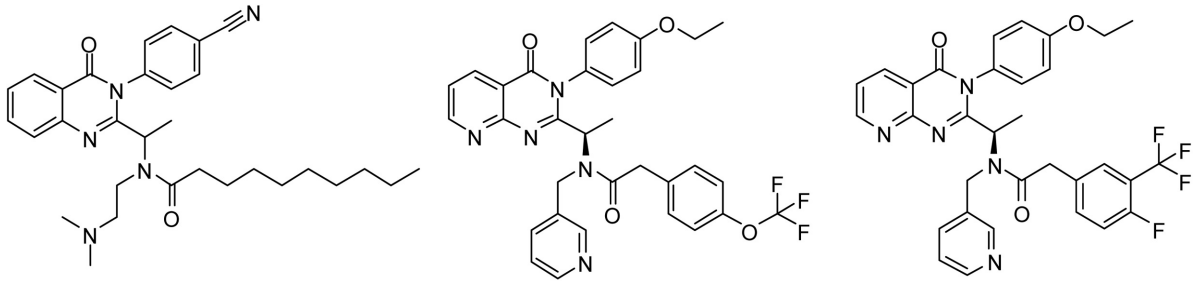
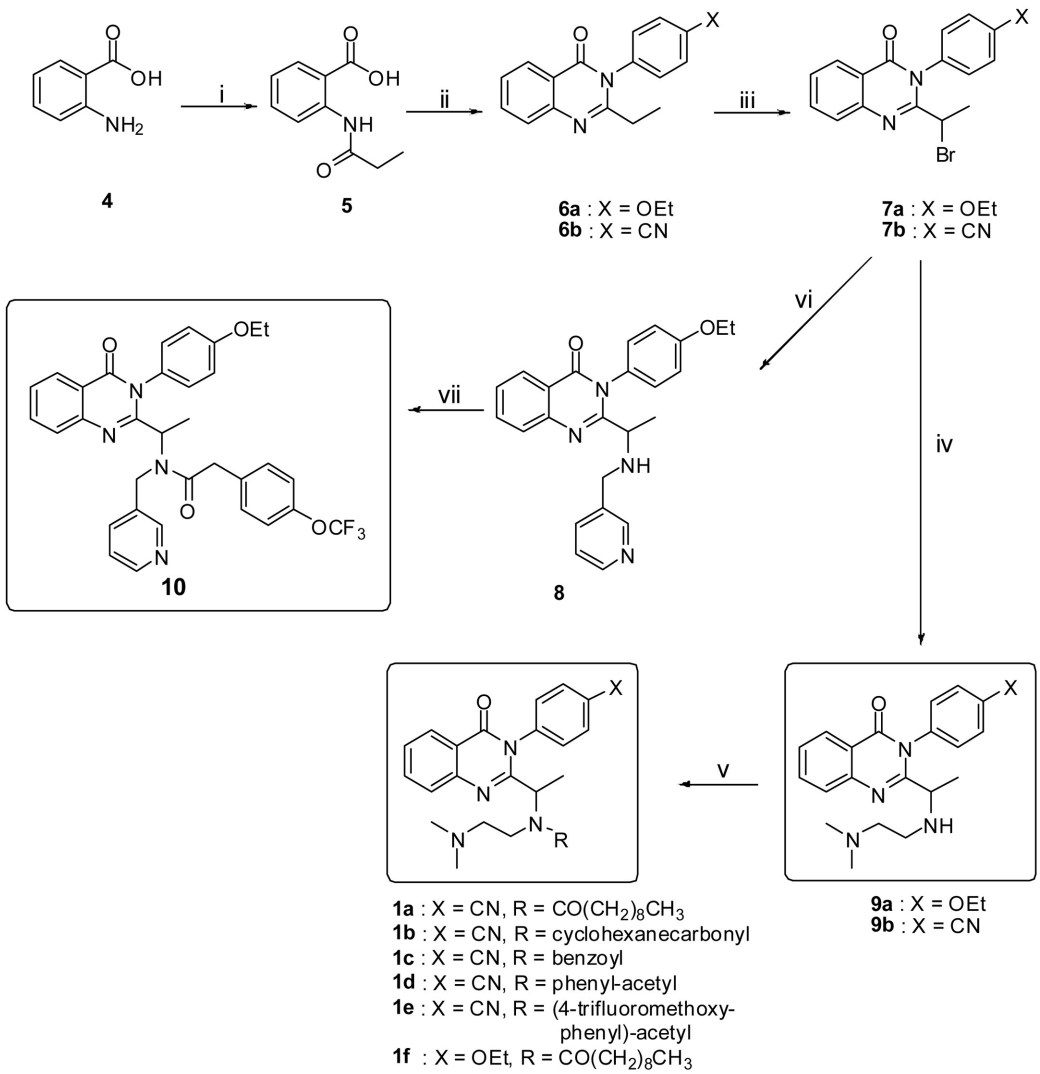


1a
VUF 5834

2
AMG 487

3
NBI-74330

Figure 1. Structures of reference compounds VUF 5834 **1a**, AMG 487 **2**, and NBI – 74330 **3**.



4

5

6a : X = OEt
6b : X = CN

7a : X = OEt
7b : X = CN

10

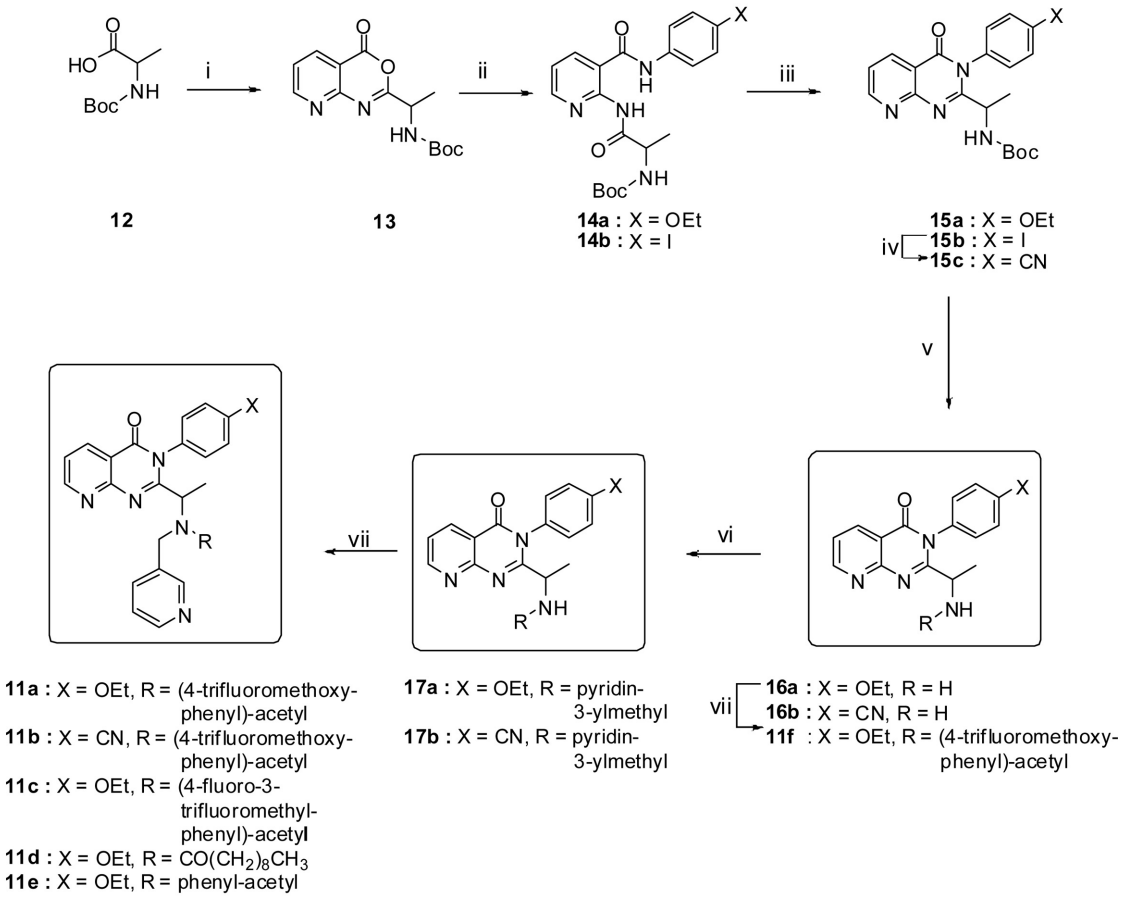
8

9a : X = OEt
9b : X = CN

1a : X = CN, R = $CO(CH_2)_8CH_3$
1b : X = CN, R = cyclohexanecarbonyl
1c : X = CN, R = benzoyl
1d : X = CN, R = phenyl-acetyl
1e : X = CN, R = (4-trifluoromethoxy-phenyl)-acetyl
1f : X = OEt, R = $CO(CH_2)_8CH_3$

Reagents and conditions: i) propionyl chloride, DMF, 400C; ii) *p*-phenetidine or *p*-amino-benzonitrile, PCl_3, toluene, reflux; iii) Br_2, CH_3COONa, CH_3COOH, 40°C; iv) $H_2N(CH_2)_2N(CH_3)_2$, EtOH, reflux; v) acid chloride, NEt_3, dioxane or carboxylic acid, EDCI, DCM, DMF; vi) 3-(aminomethyl)pyridine, NEt_3, DMF, DMF ; vii) carboxylic acid, EDCI, DCM.

Scheme 1. General synthesis of 3*H*-quinazolin-4-one analogues.



Reagents and conditions: i) *iso*-butylchloroformate, NMM, DCM, -20°C; ii) 4-ethoxy-phenylamine or 4-iodo-phenylamine, -20°C; iii) *iso*-butylchloroformate, NMM, DCM, −20°C; iv) Zn $(CN)_2$, $Pd(P(Ph)_3)_4$, DMF, 80°C; v) TFA, DCM, rt; vi) 3-pyridinecarboxaldehyde, $Na(CH_3COO)_3BH$, DCE; vii) acid chloride, NEt_3, dioxane or carboxylic acid, EDCI, DCM, DMF or carboxylic acid, EDCI, HOBT, DIPEA, DCM.

Scheme 2. General synthesis of 3*H*-pyrido[2,3-*d*]pyrimidin-4-one analogues.

Several classes of CXCR3 antagonists have recently been reported in scientific literature [20 – 25]. We [24] and others [26] have previously described VUF 5834, the decanoic acid {1-[3-(4-cyano-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-ethyl}-(2-dimethylamino-ethyl)-amide **1a**, as a submicromolar CXCR3 antagonist and we have continued to explore the SAR of this series. During our studies, Amgen Inc. announced that the structural derivative AMG 487 (**2**) was under clinical investigation [27], while scientists from Neurocrine Biosciences Inc. described pharmacological evaluation of patent compound NBI-74330 (**3**) [28]. These ligands are the most potent CXCR3 ligands reported to date, showing affinity values for the human CXCR3 receptor in the nanomolar range. In this study, we describe the SAR that links the previously published 3*H*-quinazolin-4-one compound VUF5834 **1a** and these novel 3*H*-pyrido[2,3-*d*]pyrimidin-4-one containing ligands **2** and **3**. For the structures of reference compounds VUF 5834 **1a**, AMG 487 **2**, and NBI −74330 **3** see Fig. 1.

Chemistry

The 3-phenyl-3*H*-quinazolin-4-one scaffold was constructed as reported earlier (Scheme 1) [24]. The reaction of 2-amino-benzoic acid with propionyl chloride afforded 2-propionylamino-benzoic acid **5** and treatment of this intermediate with phosphorus trichloride and 4-ethoxy-analine or 4-amino-benzonitrile afforded compounds **6a** and **6b**, respectively, in moderate yields. Intermediates **7a** and **7b** were obtained in good yields via reaction with bromine and were used for the subsequent reaction with the commercially available *N*,*N*-dimethylethylene diamine (leading to **9a** and **9b**) or 3-picolylamine (leading to **8**). Reaction of **9a** and **9b** with decanoyl, cyclohexanecarbonyl, benzoyl, or phenylacetyl chloride afforded compound **1a – d** and **1f** whereas compounds **1e** and **10** were

obtained via coupling of **8** or **9a**, respectively, with (4-trifluoromethoxy-phenyl)-acetic acid and using EDCI as activating agent.

The 3*H*-pyrido[2,3-*d*]pyrimidin-4-one derivatives **11a–f** were prepared according to Scheme 2 [26]. Boc-D-alanine was activated with *iso*-butylchloroformate under basic conditions at −20°C and then allowed to react with 2-aminonicotinic acid leading to **13**. Because of the poor stability of **13** [29], the crude material was immediately reacted with either 4-ethoxy-phenylamine or 4-iodo-phenylamine leading to compound **14a** and **14b**, respectively. Subsequent ring closure afforded compounds **15a** and **15b** in a moderate yield.

With the aim to obtain the *para*-cyano analogue (in our hand the best substituent of the 3-phenyl-3*H*-quinazolin-4-one series [24]) of reference compound **2**, intermediate **15b** was treated with zinc cyanide using tetrakis(triphenylphosphine)palladium as catalyst [30], resulting in **15c** in acceptable yield. The boc protecting group of **15a** and **15c** was cleaved off using trifluoroacetic acid and the amines **16a** and **16b** were obtained in excellent yield. HPLC analysis using chiral columns showed that the first three steps result in racemization and loss of optical activity of the material. Key intermediates **17a** and **17b** were obtained via reductive amination with pyridine-3-carbaldeyde. Final compounds **11a–c** and **11f** were obtained by coupling the appropriate amine with the corresponding carboxylic acids [31] whereas for compounds **11d** and **11e** the corresponding acid chlorides were used. Reference ligands **2** and **3** have been reported as enantiopure compounds [32]. In our hands, the synthesis route described in this patent, results in the racemic mixtures (compounds **11a** and **11c**).

Pharmacology

The affinity for the human CXCR3 of all compounds was determined by the displacement of [^{125}I]CXCL10-binding to membranes from HEK-293 cells stably expressing the human CXCR3 receptor (see Experimental, Section 4, for details).

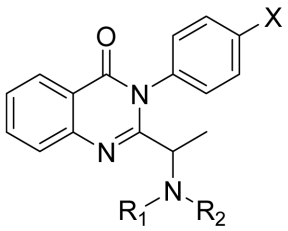
Results and discussion

In this study, we set out to link the SAR of the previously described 3*H*-quinazolin-4-one and the more recent 3*H*-pyrido[2,3-*d*]pyrimidin-4-one CXCR3 ligand classes. In the series of the 3*H*-quinazolin-4-one analogues (**1a–f, 9b, 10,** Table 1), proper decoration of the amide group is required to achieve appreciable affinity for the receptor. Previously, we found that the decanoyl chain of **1a** was necessary to obtain micromolar affinity values at CXCR3. Indeed, the absence of the decanoyl chain as in amine **9b** or in the presence of alternative lipophilic acetamide moieties such as cyclohexanecarbonyl **1b**, benzoyl **1c**, or phenylacetyl **1d** resulted in a complete loss of affinity (Table 1). Inspired by the Amgen patent application [32], we subsequently synthesized the 4-trifluoromethoxy-phenyl-acetyl compound **1e** which showed a threefold increase in CXCR3 affinity (Table 1) compared to our lead structure **1a**. Considering that the phenyl-acetyl derivative **1d** does not have affinity for the CXCR3 receptor, the affinity of the 4-trifluoromethoxy-phenylacetyl derivative **1e** is striking. Clearly, the 4-trifluoromethoxy substituent is having an enormous effect on the CXCR3 affinity and it seems that this part of the ligands is able to have very specific interaction with the binding site of the receptor.

Next, the influence of the *para*-substitution on the phenyl ring in position 3 of the 3*H*-quinazolin-4-one scaffold was investigated. We previously described the *para*-cyano moiety (as in **1a**) as the best substituent for CXCR3 binding and reported it to be substantially better than a methoxy group in this position [24]. Considering the *para*-ethoxyphenyl group in reference compound **2** [32], we synthesized the *para*-ethoxy substituted analogue for the 3*H*-quinazolin-4-one series and the resulting ligand **1f** showed a twofold better affinity compared to the *para*-cyano-substituted lead compound **1a**. The differences in affinity between the lower affinity of the *para*-methoxy-phenyl derivative and the improved *para*-ethoxyphenyl derivative, again illustrates the fine optimization work by Amgen scientists. The subsequent replacement of the *N*,*N*-ethylendiamine group at R_1 with a pyridin-3-ylmethyl afforded the analogue **10**. With a pK_i value of 7.5, this compound is the most potent CXCR3 antagonist belonging to the 3*H*-quinazolin-4-one series. Moreover, the development of **10** nicely illustrates the key role of both the R_1 and R_2 substituents at the amino function.

Next, we explored if the SAR of the 3*H*-quinazolin-4-ones could be transferred to the new 3*H*-pyrido[2,3-*d*]pyrimidin-4-ones scaffold. Comparing the two series (Tables 1 and 2) shows that the introduction of an additional nitrogen atom in the heterocyclic scaffold did not affect the CXCR3 affinity (compare **10** with **11a**). However, the different amino substituents at R_1 and R_2 again had dramatic effects on the CXCR3 affinity. Compound **11d** shows that the decanoyl chain is tolerated, as it is for the 3*H*-quinazolin-4-one series, although only a submicromolar affinity is obtained. In addition, the unsubstituted phenylacetyl analogue **11e** did not have affinity for the CXCR3 receptor, while the slightly more functionalized 4-trifluoromethoxy-phenylacetyl group, as in reference compound **2**, significantly improves affinity. Again, a

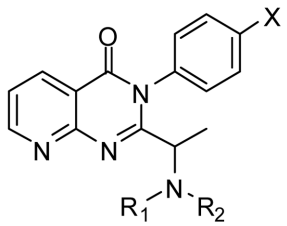
Table 1. The pK_i values of the 3*H*-quinazolin-4-one analogues as determined by the displacement of [^{125}I]CXCL10 binding to the human CXCR3 receptor.



Compound N°	X	R_1	R_2	pK_i[a)]
1a	CN	$CH_2CH_2N(CH_3)_2$	$CO(CH_2)_8CH_3$	6.26 ± 0.25
9b	CN	$CH_2CH_2N(CH_3)_2$	H	<5
1b	CN	$CH_2CH_2N(CH_3)_2$	cyclohexanecarbonyl	<5
1c	CN	$CH_2CH_2N(CH_3)_2$	benzoyl	<5
1d	CN	$CH_2CH_2N(CH_3)_2$	phenyl-acetyl	<5
1e	CN	$CH_2CH_2N(CH_3)_2$	(4-(trifluoromethoxy)-phenyl)acetyl	6.71 ± 0.04
1f	OCH_2CH_3	$CH_2CH_2N(CH_3)_2$	$CO(CH_2)_8CH_3$	6.56 ± 0.04
10	OCH_2CH_3	pyridin-3-ylmethyl	(4-(trifluoromethoxy)-phenyl)acetyl	7.45 ± 0.13

[a)] Binding experiments were carried out on cell-membranes fractions from HEK-293 cells stably expressing the human CXCR3 receptor. The compounds were tested for their ability to displace [^{125}I]CXCL10 binding. The values are represented as the mean ± S.E.M. of at least three independent experiments.

Table 2. The pK_i values of the 3*H*-pyrido[2,3-*d*]pyrimidin-4-one analogues as determined by the displacement of [^{125}I]CXCL10 binding to the human CXCR3 receptor.



Compound N°	X	R_1	R_2	pK_i[a)]
11a	OCH_2CH_3	pyridin-3-ylmethyl	(4-(trifluoromethoxy)-phenyl)acetyl	7.39 ± 0.08
11b	CN	pyridin-3-ylmethyl	(4-(trifluoromethoxy)-phenyl)acetyl	7.39 ± 0.05
11c	OCH_2CH_3	pyridin-3-ylmethyl	(4-fluoro-3-(trifluoromethyl)phenyl)-acetyl	8.13 ± 0.12
11d	OCH_2CH_3	pyridin-3-ylmethyl	$CO(CH_2)_8CH_3$	6.47 ± 0.09
11e	OCH_2CH_3	pyridin-3-ylmethyl	phenyl-acetyl	<5
11f	OCH_2CH_3	H	(4-(trifluoromethoxy)-phenyl)acetyl	5.34 ± 0.19
16a	OCH_2CH_3	H	H	<5
17a	OCH_2CH_3	pyridin-3-ylmethyl	H	<5

[a)] The binding experiments were carried out on cell-membranes fractions from HEK-293 cells stably expressing the human CXCR3 receptor. The compounds were tested for their ability to displace [^{125}I]CXCL10 binding. The values are represented as the mean ± S.E.M of at least three independent experiments.

properly substituted phenyl ring is a key determinant for CXCR3 binding.

Compound **11c** has a fivefold higher affinity for CXCR3 than **11a,** meaning that (4-fluoro-3-trifluoromethyl-phenyl)-acetyl is the best substituent at R_2 for the 3*H*-pyrido[2,3-*d*]pyrimidin-4-ones series. These results validate recent disclosures by Amgen Inc. and Neurocrine Biosciences Inc. [27, 28]. The presence of both a proper acetamide substituent and the pyridin-3-ylmethyl group are necessary for high CXCR3 affinity. The absence of one of the two (compounds **17a** and **11f**) or both (**16a**) causes a dramatic loss of affinity. Nevertheless, **11f** with (4-trifluoromethoxy-phenyl)-acetyl substituent retains a micromolar affinity for the receptor.

Finally, the influence of the *para*-substituent on the phenyl ring in position 3 of the 3*H*-pyrido[2,3-*d*]pyrimidin-4-ones was investigated. Compound **11b** was synthesized to determine the effect of *para*-cyano substitution. For this particular scaffold, the *para*-ethoxy derivative **11a** and *para*-cyano derivative **11b** do not show significant differences in CXCR3 affinity.

Conclusions

This study presents the synthesis and initial SAR of CXCR3 antagonists of the 3*H*-quinazolin-4-one and 3*H*-pyrido[2,3-*d*]pyrimidin-4-one series. Currently, we are evaluating these compounds as tools for targeting CXCR3 in a variety of inflammatory models. Moreover, the structural insights obtained may be used in the design of novel CXCR3 antagonists.

We thank Dr. Smoluch for conducting (HR) MS measurements, Dr. De Kanter for NMR analysis, and Dr. Orru for his constructive help with chiral HPLC analysis.

Experimental

Chemistry

General procedures

The solvents were dried according to standard procedures. All reactions were performed under an atmosphere of dry nitrogen. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC-200 (200 MHz; Bruker Bioscience, Billerica, MA, USA) spectrometer. Flash column chromatography was carried out with J. T. Baker silica gel (J. T. Baker, Deventer, The Netherlands). Analytical HPLC-MS analyses were conducted using a Shimadzu LC-8A preparative liquid chromatograph pump system with a Shimadzu SPD-10AV UV-Vis detector set at 254 nm, with the MS detection performed with a Shimadzu LCMS-2010 liquid chromatograph mass spectrometer (Shimadzu, Tokyo, Japan). The analyses were performed using the following two conditions. Condition *I*: an Xbridge (C18) 5 μm column (100 mm × 4.6 mm), with the following two solvents: solvent A: 10% buffer pH 8 – 90% H_2O; solvent B: 10% aqueous buffer pH 8 – 90% acetonitrile. Flow rate = 2.0 mL/min; gradient from 5% to 90% B in 10 min, then 5 min at 90% B, then 15 min at 5% B. The total run time was 30 min. The buffer-pH 8 is prepared with ammonia bicarbonate in H_2O (0.4% w/v) adjusted to pH 8 with ammonia hydroxide. Condition *II*: Xbridge (C18) 5 μm column (100 mm × 4.6 mm), with the following two solvents: solvent A: 0.1% formic acid – 99.9% H_2O; solvent B: 0.1% formic acid/99.9% acetonitrile. Flow rate = 2.0 mL/min. Gradient from 5% to 90% B in 10 min, then 5 min at 90% B, then 15 min at 5% B. The total run time was 30 min. Purities calculated are based on RP HPLC-UV peak surface area of the compounds. Chiral HPLC analyses were performed with a CHIRAL CEL OD-H column (4.6 mm × 250 mm) DAIC 14325, using as eluants 0.05% diethylamine in hexane/isopropyl alcohol (95 : 5). Flow rate = 1.1 mL/min, T = 30°C. High Resolution Electron Impact Ion Mass Spectra (HREIMS) were recorded on a Finnigan MAT 900 mass spectrometer (Thermo Electron Corporation, Bremen, Germany).

*2-Propionylamino-benzoic acid **5***

Anthranilic acid **4** (68.60 g, 0.50 mol) was dissolved in *N,N*-dimethylformamide (250 mL). Propionyl chloride (47.60 mL, 0.55 mol) was added dropwise at such a rate that the temperature of the mixture remained below 40°C. The product began to precipitate after about one half of the acid chloride was added, and the suspension was stirred vigorously at room temperature for an additional three hours after the addition was completed. The resulting mixture was poured into water (2.0 L) and stirred for an additional two hours. The precipitated product was collected by filtration, washed with water and dried in a vacuum oven at 400C under reduced pressure to afford 73.03 g (76%) of white crystals .^{1}H-NMR ($CDCl_3$) δ: 1.25 (t, *J* = 7.6 Hz, 3H), 2.55 (q, *J* = 7.6 Hz, 2H), 6.75 (brs, 1H), 7.07 – 7.14 (m, 1H), 7.55 – 7.59 (m, 1H), 8.02 – 8.14 (m, 1H), 8.72 – 8.76 (m, 1H), 10.98 (s, 1H).

*3-(4-Ethoxyphenyl)-2-ethylquinazolin-4(3H)-one **6a***

To a suspension of 2-propionylamino-benzoic acid (3.00 g, 15.52 mmol) and *p*-phenetidine (2.41 g, 15.52 mmol) in toluene was added dropwise phosphorus trichloride (0.61 mL, 6.98 mmol). The resulting suspension was heated to reflux for 8 h. After cooling to room temperature, a saturated sodium carbonate solution was added and the mixture was stirred vigorously until all solid was dissolved. The solvent was removed in vacuum and the resulting solid was collected by filtration, rinsed with water, dried, and recrystallized from isopropyl alcohol to afford the desired compound as a white solid. Yield: 2.19 g (48%). ^{1}H-NMR ($CDCl_3$) δ: 1.20 (t, *J* = 7.4 Hz, 3H), 1.42 (t, *J* = 7.0 Hz, 3H), 2.44 (q, *J* = 7.4 Hz, 2H), 4.10 (q, *J* = 7.0 Hz, 2H), 7.01 – 7.23 (m, 4H), 7.39 – 7.58 (m, 1H), 7.62 – 7.89 (m, 2H), 8.22 – 8.26 (m, 1H).

*4-(2-Ethyl-4-oxoquinazolin-3(4H)-yl)benzonitrile **6b***

Analogous to the preparation of **6a**, using *p*-amino-benzonitrile (2.22 g, 15.52 mmol). Yield: 1.50 g (35%). ^{1}H-NMR ($CDCl_3$) δ: 1.18 (t, *J* = 7.4 Hz, 3H), 2.34 (q, *J* = 7.4 Hz, 2H), 7.30 – 7.55 (m, 3H), 7.60 – 7.95 (m, 4H), 8.21 – 8.29 (m, 1H).

*2-(1-Bromo-ethyl)-3-(4-ethoxy-phenyl)-3H-quinazolin-4-one **7a***

A mixture of **6a** (2.23 g, 7.56 mmol), anhydrous sodium acetate (756 mg) and glacial acetic acid (10 mL) was warmed 40°C. A solution of bromine (7.56 mmol) in acetic acid (2 mL) was added over a period of 3 h. After the addition was completed the mixture was poured into water (150 mL) and stirred at room temperature for about 2 h. The precipitate product was isolated by filtration, washed with warm water to remove the trace of the acetic acid, then rinsed with a small amount of isopropyl alcohol and dried to provide the desired compound **7a** as a solid. Yield: 2.71 g (96%). ^{1}H-NMR ($CDCl_3$) δ: 1.44 (t, *J* = 7.0 Hz, 3H), 2.02 (d, *J* = 6.7 Hz, 3H), 4.08 (q, *J* = 7.0 Hz, 2H), 4.61 (q, *J* = 6.7 Hz, 1H), 6.90 – 7.25 (m, 3H), 7.30 – 7.55 (m, 2H), 7.75 – 7.80 (m, 2H), 8.24 – 8.28 (m, 1H).

4-[2-(1-Bromo-ethyl)-4-oxo-4H-quinazolin-3-yl]-benzonitrile ***7b***

Analogous to the preparation of **7a**, using **6b** (2.08 g, 7.56 mmol). Yield: 2.34 g (88%). ^{1}H-NMR ($CDCl_3$) δ: 2.02 (d, *J* = 6.6 Hz, 3H), 4.40 (q, 1H, *J* = 6.6 Hz), 7.25 – 7.35 (m, 1H), 7.45 – 7.65 (m, 1H), 7.70 – 7.95 (m, 5H), 8.20 – 8.24 (m, 1H).

2-[1-(2-Dimethylamino-ethylamino)-ethyl]-3-(4-ethoxy-phenyl)-3H-quinazolin-4-one ***9a***

A mixture of **7a** (2.82 g, 7.57 mmol) and *N*,*N*-dimetylethylendiamine (1.31 mL, 12.11 mmol) in ethanol (100 mL) was heated to reflux for 18 h. The ethanol was removed *in vacuo* and the concentrate dissolved in chloroform and washed with saturated aqueous sodium bicarbonate (3 × 50 mL) and the combined organic extracts dried over sodium sulfate, filtered, and concentrated. The crude material was purified by flash chromatography on silica gel eluting with methanol/dichloromethane (1 : 9) to afford the desired compound as white solid. Yield: 1.48 g (52%). ^{1}H-NMR ($CDCl_3$) δ: 1.24 (d, *J* = 6.4 Hz, 3H), 1.43 (t, *J* = 6.9 Hz, 3H), 2.20 (s, 6H), 2.35 – 2.75 (m, 4H), 3.48 (q, *J* = 6.4 Hz, 1H), 4.07 (q, *J* = 6.9 Hz, 2H), 7.15-7.25, (m, 4H), 7.40 – 7.55 (m, 1H), 7.55-7.80 (m, 2H), 8.22 – 8.26 (m, 1H).

4-{2-[1-(2-Dimethylamino-ethylamino)-ethyl]-4-oxo-4H-quinazolin-3-yl}-benzonitrile ***9b***

Analogous to the preparation of **9a**, using **7b** (2.68 g, 7.56 mmol). Yield: 1.28 g (47%). ^{1}H-NMR ($CDCl_3$) δ: 1.23 (d, *J* = 6.6 Hz, 3H), 2.22 (s, 6H), 2.30 – 2.59 (m, 4H), 3.31 (q, *J* = 6.6 Hz, 1H), 7.22 – 7.50 (m, 3H), 7.70 – 7.86 (m, 4H), 8.20 – 8,24 (m, 1H). ^{13}C-NMR ($CDCl_3$) δ: 20.87, 44.69, 45.12 (2 C), 54.93, 58.83, 113.55, 117.52, 120.42, 126.88, 127.04, 127.22 (2C), 129.71, 133.60, 133.66, 134.87, 140.66, 147.13, 158.59, 161.92. HREIMS *m*/*z*: 361.18876 (calcd. for $C_{21}H_{23}N_5O$, 361.1903). Anal. RP-HPLC *I*: t_R = 10.86 min (purity 100%), *II*: t_R = min 9.57 (purity 97%), MS (ESI) *m*/*z* 362.1 $[M+H]^+$.

3-(4-Ethoxy-phenyl)-2-{1-[(pyridin-3-ylmethyl)-amino]-ethyl}-3H-quinazolin-4-one ***8***

A solution of 3-(aminomethyl)pyridine (0.11 mL, 1.07 mmol) and triethylamine (0.15 mL, 1.07 mmol) in DMF (50 mL) was stirred for 0.5 h at room temperature. Then **7a** (0.40 g, 1.07 mmol) was added and the solution was stirred overnight. The reaction mixture was concentrated, dissolved in water and extracted with DCM. The organic layer was washed with water and a saturated aqueous sodium carbonate, then dried, concentrated, and purified via flash chromatography (DCM/MeOH, 9.5 : 0.5) to afford the desired compound as 170 mg (40%) of pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (d, *J* = 6.6 Hz, 3H), 1.40 (t, *J* = 7.0 Hz, 3H), 3.48 (q, *J* = 6.6 Hz, 1H), 3.40 – 3.80 (m, 2H), 4.00 (q, *J* = 7.00 Hz, 2H), 6.75 – 7.25, (m, 5H), 7.45 (m, 1H), 7.55 – 7.80 (m, 3H), 8.22-8.26 (m, 1H), 8.35 – 8.50 (m, 2H).

[1-(4-Oxo-4H-pyrido[2,3-d][1,3]oxazin-2-yl)-ethyl]-carbamic acid tert-butyl ester ***13***

N-(*tert*-butoxycarbonyl)-D-alanine (5.00 g, 26.43 mmol) was dissolved in dry DCM (350 mL). *N*-methylmorpholine (7.30 mL, 66.07 mmol) was added and the reaction mixture was allowed to stir for 20 min. *Iso*-butylchloroformate (6.9 mL, 52.85 mmol) was added and the reaction flask was cooled to – 20°C. Next, 2-aminonicotinic acid (3.65 g, 26.43 mmol) was added and the reaction mixture was allowed to warm up to room temperature, stirred overnight, and then refluxed for 2.5 h. The suspension was cooled to room temperature, filtrated, and concentrated under reduced pressure. The raw product was used for the next step without any purification.

{1-[3-(4-Ethoxy-phenylcarbamoyl)-pyridin-2-ylcarbamoyl]-ethyl}-carbamic acid tert-butyl ester ***14a***

Compound **13** (7.70 g, 26.43 mmol) as crude material was dissolved in dry DCM (250 mL) cooled to – 20°C. 4-Ethoxyphenylamine (3.27 mL, 26.43 mmol) was added and stirred for 3 h. The reaction mixture was allowed to warm up to room temperature overnight, and then washed with 1.0 N hydrochloric acid, saturated aqueous sodium bicarbonate, and brine. The organic phase was dried over sodium sulphate, filtered, and concentrated in vacuum. The raw product was used for the next step without further purification.

{1-[3-(4-Iodo-phenylcarbamoyl)-pyridin-2-ylcarbamoyl]-ethyl}-carbamic acid tert-butyl ester ***14b***

Analogous to the preparation of **14a**, using 4-iodo-phenylamine (5.79 g, 26.43 mmol). The raw product was used for the next step without further purification.

{1-[3-(4-Ethoxy-phenyl)-4-oxo-3,4-dihydro-pyrido[2,3-d]pyrimidin-2-yl]-ethyl}-carbamic acid tert-butyl ester ***15a***

A solution containing the crude **14a** (6.69 g, 15.62 mmol) in 50 mL DCM and *N*-methylmorpholine (0.86 mL, 7.81 mmol) was stirred for 20 min at room temperature, then cooled to – 20°C. *Iso*-butylchloroformate (1.01 mL, 7.81 mmol) was added dropwise. The solution was stirred for 3 h and subsequently allowed to warm to room temperature overnight. The reaction mixture was washed with 1.0 N hydrochloric acid, saturated aqueous sodium bicarbonate, and brine. The organic phase was dried over sodium sulphate, filtered, concentrated in vacuum to give a dark yellow oil. This material was dissolved in a small amount of DCM. While stirring, petroleum ether was added. The resulting precipitate was collected by filtration, washed with petroleum ether, and dried to afford **15a** as a pale yellow solid. Yield: 3.47 g (64%). ^{1}H-NMR ($CDCl_3$) δ: 1.17 (d, *J* = 7.00 Hz, 3H), 1.29 – 1.50 (m, 12H), 4.06 (q, *J* = 7.00 Hz, 2H), 4.61 – 4.66 (m, 1H), 5.75 – 5.79 (m, 1H), 7.01 – 7.44 (m, 5H), 8.56-8.58 (m, 1H), 8.93 – 8.95 (m, 1H).

{1-[3-(4-Iodo-phenyl)-4-oxo-3,4-dihydro-pyrido[2,3-d]pyrimidin-2-yl]-ethyl}-carbamic acid tert-butyl ester ***15b***

Analogous to the preparation of **15a**, using **14b** (6.40 g, 15.62 mmol). Yield: 3.80 g (59%) of a pale yellow solid. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (d, *J* = 6.77 Hz, 3H), 1.36 (s, 9H), 4.45 – 4.54 (m, 1H), 5.60 – 5.73 (m, 1H), 6.97 – 7.01 (m, 2H), 7.40 – 7.47 (m, 1H), 7.86 – 7.96 (m, 1H), 8.56 – 8.58 (m, 1H), 8.96 – 8.97 (m, 1H).

{1-[3-(4-Cyano-phenyl)-4-oxo-3,4-dihydro-pyrido[2,3-d]pyrimidin-2-yl]-ethyl}-carbamic acid tert-butyl ester ***15c***

To a solution containing **15b** (0.50 g, 1.02 mmol) and zinc cyanide (0.17 g, 1.52 mmol) in DMF (30 mL) was added tetrakis(triphenylphosphine)palladium (0) (0.24 g, 0.2 mmol). The reaction mixture was heated to 80°C for 5 h, diluted with ethyl acetate and filtered trough a pad of celite. The filtrate was concentrated

under reduced pressure. The residue was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate (1 × 50 mL). The aqueous layer was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were concentrated.

The crude material was purified by flash chromatography (ethyl acetate/hexane, 1 : 1) to afford **15c** as white solid. Yield: 0.24 g (60%). ^{1}H-NMR ($CDCl_3$) δ: 1.25 (d, *J* = 6.8 Hz, 3H), 1.31 (s, 9H), 4.42 – 4.44 (m, 1H), 5.53 – 5.56 (m, 1H), 7.34 – 7.94 (m, 5H), 8.53 – 8.55 (m, 1H), 8.93 – 8.95 (m, 1H).

2-(1-Amino-ethyl)-3-(4-ethoxy-phenyl)-3H-pyrido[2,3-d]-pyrimidin-4-one ***16a***

Compound **15a** (2.16 g, 5.27 mmol) in DCM (25 mL) was treated with trifluoroacetic acid (8.50 mL) and stirred for 4 h at room temperature. The reaction was extracted with hydrochloric acid 1M (3 × 50 mL), combined water layers were made basic with aqueous NH_4OH solution (25%, pH 9-10) and extracted with DCM (3 × 150 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to afford 1.64 g of **16a** in quantitative yields as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (d, *J* = 6.6 Hz, 3H), 1.44 (t, *J* = 7.0 Hz, 3H), 1.81 (s, 2H), 3.78 (q, *J* = 6.6 Hz, 1H), 4.07 (q, *J* = 7.0 Hz, 2H), 7.00 – 7.17 (m, 4H), 7.40 – 7.42 (m, 1H), 8.55 – 8.57 (m, 1H), 8.95 – 8.97 (m, 1H). ^{13}C-NMR ($CDCl_3$) δ: 14.61, 22.77, 49.57, 63.71, 115.65, 115.86, 123.55, 124.27, 128.58, 129.03, 132.35, 140.65, 152.15, 156.50, 159.56, 164.54. HREIMS *m/z*: 310.14254 (calcd. for $C_{17}H_{18}N_4O_2$, 310.1430). Anal. RP-HPLC *I*: t_R = 10.08 min (purity 99%), *II*: t_R = min 10.44 (purity 100%). MS (ESI) *m/z* 311.1 $[M+H]^+$. Chiral HPLC: I enantiomer: t_R = 14.00 min, II enantiomer: t_R = 22.00 min.

4-[2-(1-Amino-ethyl)-4-oxo-4H-pyrido[2,3-d]pyrimidin-3-yl]-benzonitrile ***16b***

Analogous to the preparation of **16a**, using **15c** (2.06 g, 5.27 mmol). Yield: 1.53 g (100%) of a white solid. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (d, *J* = 6.6 Hz, 3H), 2.39 (brs, 2H), 3.62 (q, *J* = 6.6 Hz, 1H), 7.23 – 7.70 (m, 3H), 7.70 – 7.87 (m, 2H), 8.51 – 8.53 (m, 1H), 8.96 – 8.98 (m, 1H).

3-(4-Ethoxy-phenyl)-2-{1-[(pyridin-3-ylmethyl)-amino]-ethyl}-3H-pyrido[2,3-d]pyrimidin-4-one ***17a***

To a solution containing **16a** (3.60 g, 11.60 mmol) in dichloroethane (150 mL) was added 3-pyridinecarboxaldehyde (1.21 mL, 12.76 mmol) followed by sodium triacetoxyborohydride (3.69 g, 17.40 mmol). The reaction was allowed to stir at room temperature overnight. The mixture was diluted with DCM and washed with 1.0 M ammonium hydroxide (1 × 150 mL). The organic phase was dried over magnesium sulfate, filtered, and concentrated to afford an yellow oil that was purified by flash chromatography (DCM/MeOH, 9.5 : 0.5). A white powder was isolated. Yield: 3.58 g (77%). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (d, *J* = 6.6 Hz, 3H), 1.41 (t, *J* = 7.0 Hz, 3H), 2.53 (brs, 1H), 3.42 – 3.80 (q, *J* = 6.6 Hz, 1H), 3.42 – 3.80 (m, 2H), 4.02 (q, *J* = 7.0 Hz, 2H), 6.80 – 7.24 (m, 5H), 7.41 – 7.43 (m, 1H), 7.61 – 7.63 (m, 1H), 8.41 – 8.43 (m, 2H), 8.59 – 8.61 (m, 1H), 8.97 – 8.99 (m, 1H). ^{13}C-NMR ($CDCl_3$) δ: 14.56, 21.32, 48.77, 53.27, 63.68, 115.45, 115.58, 115.99, 122.28, 123.19, 127.46, 128.62, 128.88, 134.83, 135.52, 136.77, 148.36, 149.44, 156.12, 157.51, 159.53, 162.67, 164.66. HREIMS *m/z*: 386.16152 (calcd. for $C_{23}H_{23}N_5O_2$ – CH_3, 386.1617). Anal. RP-HPLC *I*: t_R = 10.75 min (purity 97%), *II*: t_R = 10.63 min (purity 97%), MS (ESI) *m/z* 402.1 $[M+H]^+$.

4-(4-Oxo-2-{1-[(pyridin-3-ylmethyl)-amino]-ethyl}-4H-pyrido[2,3-d]pyrimidin-3-yl)-benzonitrile ***17b***

Analogous to the preparation of **17a**, using **16b** (3.38 g, 11.60 mmol) Yield: 2.52 g (57%) of a white powder. ^{1}H-NMR ($CDCl_3$) δ: 1.34 (d, *J* = 6.61 Hz, 3H), 3.35 (q, *J* = 6.61 Hz, 1H), 3.62 – 3.88 (m, 2H), 7.15 – 7.32 (m, 2H), 7.33 – 7.35 (m, 1H), 7.54 – 7.56 (m, 1H), 7.69 – 7.71 (m, 2H), 7.84 – 7.86 (m, 1H), 8.49 – 8.51 (m, 2H), 8.59 – 8.61 (m, 1H), 9.00 – 9.02 (m, 1H).

Decanoic acid {1-[3-(4-cyano-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-ethyl}-(2-dimethylamino-ethyl)-amide ***1a***

To one equivalent of **9b** (0.36 mg, 1.00 mmol) and one equivalent of triethylamine (0.14 mL, 1.00 mmol) dissolved in 25 mL dioxane was added one equivalent of decanoyl-chloride (0.21 mL, 1.00 mmol). The resulting mixture was stirred at room temperature for 18 h and concentrated under reduced pressure. The residue was dissolved in dichloromethane and washed with saturated aqueous Na_2CO_3 solution, then twice with water. The organic phases were dried over anhydrous Na_2SO_4 and evaporated. The crude material was purified by flash chromatography with ethyl acetate/methanol (9 : 1) to afford the desired compound as free bases. Yield 0.18 g (35%). ^{1}H-NMR ($CDCl_3$) δ: 0.83 – 0.85 (m, 3H), 1.20 – 1.22 (m, 16H), 1.34 (d, *J* = 6.9 Hz, 3H), 1.37 – 1.49 (m, 2H), 1.86 – 2.19 (m, 7H), 3.30 – 3.50 (m, 2H), 4.65 (q, 0.2H), 5.18 (q, *J* = 6.9 Hz, 0.8H), 7.33 – 7.83 (m, 7H), 8.19 – 8.22 (m, 1H). ^{13}C-NMR ($CDCl_3$) δ: 13.94, 16.35, 22.48, 22.23, 29.10, 29.27 (2C), 31.67, 30.09, 41.141, 45.33, 45.61 (2C), 50.58, 59.69, 113.36, 117.76, 120.66, 126.87, 127.27, 127.44, 129.33, 130.10, 133.28, 133.38, 134.66, 140.14, 146.78, 155.28, 161.89. HREIMS *m/z*: 515.32438 (calcd. for $C_{31}H_{41}N_5O_2$, 515.3260). Anal. RP-HPLC *I*: t_R = 15.80 min (purity 95%), *II*: t_R = 15.43 min (purity 100%), MS (ESI) *m/z* 516.3 $[M+H]^+$.

Cyclohexanecarboxylic acid {1-[3-(4-cyano-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-ethyl}-(2-dimethylamino-ethyl)-amide ***1b***

Analogous to the preparation of **1a**, using **9b** and cyclohexanoyl chloride (0.13 mL, 1.00 mmol). Yield 0.36 g (77%). ^{1}H-NMR ($CDCl_3$) δ: 1.21 – 1.56 (m, 6H), 1.63 – 1.89 (m, 8H), 2.12 – 2.36 (m, 8H), 3.41 – 3.59 (m, 2H), 1.86 – 2.19 (m, 7H), 5.24 (q, *J* = 6.7 Hz, 1H), 7.34 – 7.83 (m, 7H), 8.19 – 8.22 (m, 1H).^{13}C-NMR ($CDCl_3$) δ: 16.23, 25.40, 25.52 (2C), 25.61, 41.21, 41.64, 45.66 (2C), 50.18, 60.28, 113.31, 117.84, 120.67, 126.89, 127.40, 129.50, 130.13, 133.26, 133.60, 134.69, 140.10, 146.85, 155.35, 161.88, 176.36. HREIMS *m/z*: 471.26212 (calcd. for $C_{28}H_{33}N_5O_2$, 471.2634). Anal. RP-HPLC *I*: t_R = 13.19 min (purity 99%), *II*: t_R = 11.22 min (purity 100%), MS (ESI) *m/z* 472.2 $[M+H]^+$.

N-{1-[3-(4-Cyano-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-ethyl}-N-(2-dimethylamino-ethyl)-benzamide ***1c***

Analogous to the preparation of **1a**, using **9b** and benzoyl chloride (0.12 mL, 1.00 mmol).

Yield: 0.10 g (22%). ^{1}H-NMR ($CDCl_3$) δ: 1.47 (d, *J* = 6.8 Hz, 3H), 1.87(s, 6H), 2.01 – 2.45 (m, 2H), 3.42 – 3.57 (m, 2H), 5.21 – 5.25 (m, 1H), 6.75 – 7.14 (m, 8H), 7.15 – 7.95 (m, 4H), 8.12 – 8.25 (m, 1H). ^{13}C-NMR ($CDCl_3$) δ: 18.66, 44.33 (2C), 57.13, 52.35 58.40, 114.85 (2C), 119.75, 122.05, 127.87, 128.93 (2C), 129.92, 130.28, 130.32, 130.67, 131.64, 133.32, 133.81, 134.33, 134.71, 136.57, 141.03, 147.72, 156.22, 177.85 HREIMS: the compound is not eligible for detecting the mass ion peak. Anal. RP-HPLC *I*: t_R = 12.39 min

(purity 100%), *II*: t_R = 12.09 min (purity 100%), MS (ESI) *m/z* 466.1 $[M+H]^+$.

N-{1-[3-(4-Cyano-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-ethyl}-N-(2-dimethylamino-ethyl)-2-phenyl-acetamide ***1d***

Analogous to the preparation of **1a**, using **9b** and phenylacetyl chloride (0.13 mL, 1.00 mmol). Yield: 0.19 g (40%). ^{1}H-NMR ($CDCl_3$) δ: 1.37 (d, *J* = 7.0 Hz, 3H), 1.77-2.62 (m, 9H), 3.33-4.66 (m, 3H), 5.18 (q, *J* = 7.0 Hz, 1H), 7.12 – 7.97 (m, 7H), 8.21 – 8.23 (m, 1H). ^{13}C-NMR ($CDCl_3$) δ: 16.23, 40.14, 42.52, 45.64 (2C), 51.01, 59.73, 113.31, 117.76, 120.70, 126.88, 127.02, 127.35, 127.90, 128.03, 128.62, 128.76, 129.30, 130.06, 133.32, 133.81, 134.33, 134.71, 140.00, 146.72, 155.10, 161.85, 171.20. HREIMS *m/z*: 479.23178 (calcd. for $C_{29}H_{29}N_5O_2$, 479.2321). Anal. RP-HPLC *I*: t_R = 12.83min (purity 98%), *II*: t_R = 12.31 min (purity 99%), MS (ESI) *m/z* 480.2 $[M+H]^+$.

Decanoic acid (2-dimethylamino-ethyl)-{1-[3-(4-ethoxy-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-ethyl}-amide ***1f***

Analogous to the preparation of **1a**, using **9a** (0.38 g, 1.00 mmol) and decanoylchloride (0.21 mL, 1.00 mmol). Yield: 0.23 g (42%). ^{1}H-NMR ($CDCl_3$) at 27°C δ: 0.78 – 0.81 (m, 3H), 1.05 – 1.29 (m, 18H), 1.33 – 1.50 (m, 4H), 2.11 – 2.38 (m, 8H), 3.10 – 3.17 (m, 0.4H), 3.53 – 3.57 (m, 1.6H), 3.98 (q, *J* = 7.0 Hz, 2H), 4.80 (q, *J* = 7.2 Hz, 0.8H), 5.13 (q, *J* = 7.2 Hz, 0.2H), 6.84 – 7.15 (m, 3H), 7.25 – 7.77 (m, 4H), 8.05 – 8.28 (m, 1H). Peak splitting due to the presence of rotamers, as confirmed by ^{1}H-NMR (DMSO) measurements at 90°C. HREIMS *m/z*: 534.35434 (calcd. for $C_{32}H_{46}N_4O_3$, 534.3570). Anal. RP-HPLC *I*: t_R = 16.67 min (purity 100%), *II*: t_R = 16.44 min (purity 100%), MS (ESI) *m/z* 535.3 $[M+H]^+$.

Decanoic acid {1-[3-(4-ethoxy-phenyl)-4-oxo-3,4-dihydro-pyrido[2,3-d]pyrimidin-2-yl]-ethyl}-pyridin-3-ylmethyl-amide ***11d***

Analogous to the preparation of **1a**, using **17a** (0.40 g, 1.00 mmol) and decanoyl chloride (0.21 mL, 1.00 mmol). Yield: 0.22 g (40%). ^{1}H-NMR ($CDCl_3$) at 27°C δ: 0.80 – 0.82 (m, 3H), 1.10 – 1.97 (m, 20H), 2.23 – 2.30 (m, 2H), 4.01 (q, *J* = 7.0 Hz, 2H), 4.78 – 5.22 (m, 2.4H), 5.30 (q, *J* = 7.2 Hz, 0.6H), 6.54 – 6.59 (m, 0.3H), 6.90 – 8.19 (m, 8H), 8.42 – 8.55 (m, 1.7H), 8.90 – 9.01 (m, 1H). Peak splitting due to the presence of rotamers, as confirmed by ^{1}H-NMR (DMSO) measurements at 90°C. HREIMS Not eligible for detecting the ion peak mass. Anal. RP-HPLC *I*: t_R = 15.06 min (purity 96%), *II*: t_R = 16.28 min (purity 96%), MS (ESI) *m/z* 556.3 $[M+H]^+$.

N-{1-[3-(4-Ethoxy-phenyl)-4-oxo-3,4-dihydro-pyrido[2,3-d]pyrimidin-2-yl]-ethyl}-2-phenyl-N-pyridin-3-ylmethyl-acetamide ***11e***

Analogous to the preparation of **1a**, using **17a** and phenylacetyl chloride (0.13 mL, 1.00 mmol). Yield: 0.04 g (8%). ^{1}H-NMR ($CDCl_3$) at 27°C δ: 1.20 (d, *J* = 7.2 Hz, 2H), 1.40 (t, *J* = 6.9 Hz, 3H), 2.40 – 2.99 (m, 0.4H), 3.45-3.55 (m, 1.6H), 4.03 (q, *J* = 6.9 Hz, 2H), 4.35 – 5.17 (m, 2H), 5.35 (q, *J* = 7.2 Hz, 1H), 6.85 – 7.50 (m, 11H), 7.50 – 7.75 (m, 1H), 8.25 – 8.60 (m, 3H), 8.75 – 8.92 – 9.04 (m, 1H). Peak splitting due to the presence of rotamers, as confirmed by ^{1}H-NMR (DMSO) measurements at 90°C. HREIMS *m/z*: 519.22551 (calcd. for $C_{31}H_{29}N_5O_3$, 519.2270). Anal. RP-HPLC *I*: t_R = 12.16 min (purity 100%), *II*: t_R = 13.22 min (purity 100%), MS (ESI) *m/z* 520.2 $[M+H]^+$.

N-{1-[3-(4-Cyano-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-ethyl}-N-(2-dimethylamino-ethyl)-2-(4-trifluoromethoxy-phenyl)-acetamide ***1e***

To one equivalent of **9b** (0.36 mg, 1.00 mmol) and one equivalent (4-trifluoromethoxy-phenyl)-acetic acid (0.22 g, 1 mmol) dissolved in 25 mL dichloromethane and two drops *N,N*-dimethylformamide, one equivalent of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.19 g, 1.00 mmol) was added. The reaction mixture was stirred overnight, then basified with sodium bicarbonate solution and extracted with dichloromethane (3 × 50 mL). The organic layers were collected, dried over sodium sulfate concentrated, and purified by flash chromatography (DCM/methanol, 9.5 : 0.5) to afford the desired compound as free base. Yield: 0.26 g (47%). ^{1}H-NMR ($CDCl_3$) δ: 1.36 (d, *J* = 7.0 Hz, 3H), 1.88 – 2.48 (m, 9H), 3.34 – 4.74 (m, 3H), 5.13 (q, *J* = 7.0 Hz, 1H), 7.07 – 7.40 (m, 4H), 7.44 – 7.91 (m, 7H), 8.21 – 8.23 (m, 1H) ^{13}C-NMR ($CDCl_3$) δ: 16.22, 39.39, 42.80, 45.66 (2C), 51.25, 59.93, 113.41, 117.67, 120.69, 121.01, 126.95, 127.29, 128.12, 129.49, 130.26, 131.61 (2C), 132.18, 133.10, 133.32, 133.42, 133.81, 134.75, 135.04, 140.68, 148.00, 155.41, 161.80, 170.96. HREIMS *m/z*: 563.21360 (calcd. for $C_{30}H_{28}F_3N_5O_3$, 563.2144). Anal. RP-HPLC *I*: t_R = 14.01 min (purity 95%), *II*: t_R = 13.73 min (purity 100%), MS (ESI) *m/z* 564.2 $[M+H]^+$.

N-{1-[3-(4-Ethoxy-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-ethyl}-N-pyridin-3-ylmethyl-2-(4-trifluoromethoxy-phenyl)-acetamide ***10***

Analogous to the preparation of **1e**, using **8** (0.40 g, 1.00 mmol) and (4-trifluoromethoxy-phenyl)-acetic acid. Yield: 0.35 g (59%). ^{1}H-NMR ($CDCl_3$) at 27°C δ: 1.16 (d, *J* = 7.1 Hz, 2H), 1.32-1.41 (m, 5H), 2.56 (dd, J_1 = 16.1 Hz, J_2 = 40.0 Hz, 0.8H), 3.21 – 3.78 (m, 1.2H), 3.53 – 4.06 (m, 2.5H), 4.48 (dd, J_1 = 14.8 Hz, J_2 = 46.4 Hz, 0.6H), 4.79 – 5.02 (m, 1.6H), 5.34 (q, *J* = 7.1 Hz, 0.3H), 6.55 – 5.85 (m, 0.5H), 6.91 – 7.21 (m, 9H), 7.33 – 7.56 (m, 3H), 7.64 – 7.83 (m, 1.5H), 8.11 – 8.46 (m, 2H). Peak splitting due to the presence of rotamers, as confirmed by ^{1}H-NMR (DMSO) measurements at 90°C. HREIMS *m/z*: 602.21506 (calcd. for $C_{33}H_{29}F_3N_4O_4$, 602.2141). Anal. RP-HPLC *I*: t_R = 14.53 min (purity 100%), *II*: t_R = 15.36 min (purity 100%), MS (ESI) *m/z* 603.1 $[M+H]^+$.

N-{1-[3-(4-Cyano-phenyl)-4-oxo-3,4-dihydro-pyrido[2,3-d]pyrimidin-2-yl]-ethyl}-N-pyridin-3-ylmethyl-2-(4-trifluoromethoxy-phenyl)-acetamide ***11b***

Analogous to the preparation of **1e**, using **17b** (0.40 g, 1.00 mmol) and (4-trifluoromethoxy-phenyl)-acetic acid. Yield: 0.42 g (72%). ^{1}H-NMR ($CDCl_3$) δ: 1.27 (d, 3H, *J* = 7.2 Hz), 3.50 – 3.72 (m, 2H), 5.03 – 5.13 (m, 3H), 7.00 – 7.19 (m, 5H), 7.23 – 7.49 (m, 3H), 7.85 – 7.99 (m, 3H), 8.48 – 8.56 (m, 3H), 8.99 – 9.02 (m, 1H). ^{13}C-NMR ($CDCl_3$) δ: 16.23, 39.81, 46.69, 52.39, 114.13, 116.24, 117.47, 121.05, 122.77, 123.54 (2C), 129.39, 130.15 (3C), 130.35 (2C), 132.41, 132.98, 133.20, 133.77, 134.10, 136.81, 139.34, 147.20, 148.87, 156.40, 157.07, 161.48, 161.88, 172.77. HREIMS *m/z*: 584.17947 (calcd. for $C_{31}H_{23}F_3N_6O_3$, 584.1784). Anal. RP-HPLC *I*: t_R = 12.77 min (purity 100%), *II*: t_R = 13.70 min (purity 100%), MS (ESI) *m/z* 585.1 $[M+H]^+$.

N-{1-[3-(4-Ethoxy-phenyl)-4-oxo-3,4-dihydro-pyrido[2,3-d]pyrimidin-2-yl]-ethyl}-N-pyridin-3-ylmethyl-2-(4-trifluoromethoxy-phenyl)-acetamide ***11a***

4-(Trifluoromethoxy phenyl)-acetic acid (0.44 g, 2.00 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride EDCI (0.38 g, 2.00 mmol), 1-hydroxybenzotriazole hydrate HOBT (0.27 g, 2.00 mmol) and diisopropylethylamine (DIPEA) (2.27 mL) were stirred in DCM (70 mL). After 30 min, **17a** (0.40 g, 1.00 mmol) was added and the reaction mixture was stirred overnight, then extracted with 1 M HCl (3 × 50 mL), basified with ammonia hydroxide 25% solution and extracted with DCM (3 × 200 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to obtain the crude material that was purified by flash chromatography (EtOAc/EtOH, 9.5:0.5) to afford the desired compounds as free base. Yield: 0.33 g (54%).^{1}H-NMR ($CDCl_3$) at 27°C δ: 1.25 (d, J = 7.2 Hz, 0.9 H), 1.36 (t, J = 6.9 Hz, 3H), 1.43 (d, J = 7.2 Hz, 2.1H), 2.70 (dd, J_1 = 16.0 Hz, J_2 = 33.6 Hz, 0.6H), 3.59 (dd, J_1 = 15.7 Hz, J_2 = 13.5 Hz, 1.4H), 4.03 (q, J = 6.9 Hz, 2H), 4.57 (dd, J_1 = 14.8 Hz, J_2 = 107.3 Hz, 0.6 H), 5.05 – 5.12 (m, 0.3 H), 5.18 (dd, J_1 = 18.4 Hz, J_2 = 2.9 Hz, 1.4H), 5.37 (q, J = 7.2 Hz, 0.7H), 6.70 – 6.74 (m, 0.3H), 6.85 – 7.24 (m, 8.5H), 7.36 – 7.47 (m, 2H), 7.61 – 7.66 (m, 0.6H), 8.17 – 8.23 (m, 0.6H), 8.42 – 8.56 (m, 2H), 8.93 – 9.04 (m, 1H). Peak splitting due to the presence of rotamers, as confirmed by ^{1}H-NMR (DMSO) measurements at 90°C. HREIMS *m/z*: 603.20902 (calcd. for $C_{32}H_{28}F_3N_5O_4$, 603.2093). Anal. RP-HPLC *I*: t_R = 13.38 min (purity 99%), *II*: t_R = 14.65 min (purity 99%), MS (ESI) *m/z* 604.2 $[M+H]^+$.

N-{1-[3-(4-Ethoxy-phenyl)-4-oxo-3,4-dihydro-pyrido[2,3-d]pyrimidin-2-yl]-ethyl}-2-(4-trifluoromethoxy-phenyl)-acetamide ***11f***

Analogous to the preparation of **11a**, using **16a** (0.31 g, 1.00 mmol) and (4-trifluoromethoxy-phenyl)-acetic acid. Yield: 1.64 g (27%). ^{1}H-NMR ($CDCl_3$) δ: 1.31 (d, J = 6.8 Hz, 3H), 1.43 (t, J = 7.0 Hz, 3H), 3.53 (s, 2H), 4.04 (q, J = 7.0 Hz, 2H), 4.88 (q, J = 6.8 Hz, 1H,), 6.84 (d, 1H, J = 7.94 Hz), 6.99 – 7.46 (m, 8H), 8.58 – 8.61 (m, 1H,), 8.94 – 8.97 (m, 1H).^{13}C-NMR ($CDCl_3$) δ: 14.60, 20.01, 42.21, 46.84, 63.67, 115.63, 115.69, 116.27 (2C), 121.01, 122.47, 127.07, 129.04, 129.26 (2C), 130.48, 133.18, 137.02, 155.85, 156.96, 159.79, 162.33, 162.43, 169.74. HREIMS *m/z*: 512.16459 (calcd. for $C_{26}H_{23}F_3N_4O_4$, 512.1671). Anal. RP-HPLC *I*: t_R = 13.30 min (purity 99%), *II*: t_R = 14.87 min (purity 98%), MS (ESI) *m/z* 513.1 $[M+H]^+$.

N-{1-[3-(4-Ethoxy-phenyl)-4-oxo-3,4-dihydro-pyrido[2,3-d]pyrimidin-2-yl]-ethyl}-2-(4-fluoro-3-trifluoromethyl-phenyl)-acetamide ***11c***

Analogous to the preparation of **11a**, using **17a** (0.40 g, 1.00 mmol) and (4-fluoro-3-trifluoromethyl-phenyl)-acetic acid (0.44 g, 2.00 mmol). Yield: 0.11 g (19%).^{1}H-NMR ($CDCl_3$) at 27°C δ: 1.25 (d, J = 7.2 Hz, 2H), 1.39 (t, J = 7.0 Hz, 3H), 1.57 (d, J = 7.2 Hz, 1H), 2.30-2.95(m, 0.6H), 3.57 (dd, J_1 = 16.1 Hz, J_2 = 17.9 Hz, 1.4H), 4.03 (q, J = 7.0 Hz, 2H), 4.20 – 5.23 (m, 2H), 5.05 – 5.12 (m, 0.3H), 5.33 (q, J = 7.2 Hz, 1H), 6.94 – 7.61 (m, 10H), 8.18 – 8.57 (m, 3H), 8.20 – 8.95 (m, 1H). Peak splitting due to the presence of rotamers, as confirmed by ^{1}H-NMR (DMSO) measurements at 90°C. HREIMS *m/z*: 605.20450 (calcd. for $C_{32}H_{27}F_4N_5O_3$, 605.2050). Anal. RP-HPLC *I*: t_R = 13.26 min (purity 99%), *II*: t_R = 14.45 min (purity 99%), MS (ESI) *m/z* 606.1 $[M+H]^+$.

Pharmacology

^{125}I-CXCL10 binding to human CXCR3 receptor

Cell membranes from HEK293 cells stably expressing the human CXCR3 receptor were prepared as follows. Cells were washed with cold PBS, detached using cold PBS containing 1 mM EDTA and centrifuged twice at 1500*g* for 10 min at 4°C. The pellet was resuspended in cold membrane buffer (15 mM Tris pH 7.5, 1 mM EGTA, 0.3 mM EDTA, 2 mM $MgCl_2$) and homogenized by 10 strokes at 1100 – 1200 rpm using a Teflon-glass homogenizer. The membranes were subjected to two freeze-thaw cycles using liquid nitrogen and centrifuged at 40 000 g for 25 min at 4°C. The pellet was rinsed with cold Tris-sucrose buffer (20 mM Tris pH 7.4, 250 mM sucrose), resuspended in the same buffer and frozen in liquid nitrogen. Protein concentration was determined using a Bio-Rad protein assay (BioRad, USA). Membranes were incubated in 96-well plates in binding buffer (50 mM HEPES pH 7.4, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 100 mM NaCl, 0.5% BSA) with approximately 50 pM ^{125}I-CXCL10 (PerkinElmer Life and Analytical Sciences, Boston, MA) and increasing concentrations of antagonists for 2 h at RT. Subsequently, membranes were harvested via filtration through Unifilter GF/C plates (PerkinElmer Life and Analytical Sciences) pretreated with 1% polyethylenimine and washed three times with wash buffer (50 mM HEPES pH 7.4, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 500 mM NaCl). Radioactivity was measured using a MicroBeta (PerkinElmer Life and Analytical Sciences) counter. Binding data were analyzed using Graphpad Prism.

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