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Synthesis and structure–activity relationship of 3-phenyl-3*H*-quinazolin-4-one derivatives as CXCR3 chemokine receptor antagonists

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Abstract—A series of 3-phenyl-3*H*-quinazolin-4-ones have been synthesized and tested for affinity and activity at the chemokine CXCR3 receptor. The most potent compound (**1d**) has been evaluated using radioligand binding and calcium mobilization assays and is considered a useful tool for further characterization of the CXCR3 receptor.

Chemokines constitute a superfamily of small secreted proteins that attract and activate a variety of cell types. They are classified by structure according to the number and spacing of conserved cysteine residues into four major groups (CC, CXC, CX3C and XC).[1,2] Chemokine receptors are defined by their ability to signal on binding of one or more members of the chemokine superfamily of chemotactic cytokines. All of them belong to the family of G protein-coupled receptors (GPCRs).[3,4]

The chemokine receptor CXCR3 is mainly expressed on activated T cells and natural killer cells and binds CXCL9 (monokine induced by IFN-γ; Mig), CXCL10 (IFN-γ-inducible protein 10; IP10) and CXCL11 (IFN-γ-inducible T cell α-chemoattractant; I-TAC/IP9).[5–9] Exposure of cells expressing CXCR3 to CXCL9, CXCL10 and CXCL11 leads to an increase of intracellular calcium via activation of G_i proteins.[10] Increased expression of CXCR3-targeting chemokines is observed in chronic inflammatory diseases (rheumatoid arthritis,[11,12] multiple sclerosis,[13] hepatitis C-infected liver,[14] atherosclerosis[15] and chronic skin reactions[10,16,17]) and is associated with the infiltration of CXCR3-positive T-cells.

The involvement of CXCR3 in the pathophysiology of the Th1-type diseases suggests that CXCR3 may serve as a new molecular target for anti-inflammatory therapies.[18,19]

For further characterization of the receptor and for validating CXCR3 as a viable drug target, the development of small, nonpeptidergic CXCR3 ligands is essential. In patent literature, it has been suggested that decanoic acid (2-dimethylamino-ethyl)-{1-[3-(4-fluoro-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-y]-ethyl}-amide (**1c**) (Fig. 1)

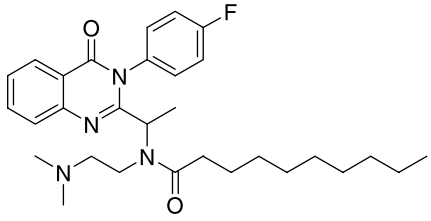

Figure 1. Structure of decanoic acid (2-dimethylamino-ethyl)-{1-[3-(4-fluoro-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-y]-ethyl}-amide (**1c**).

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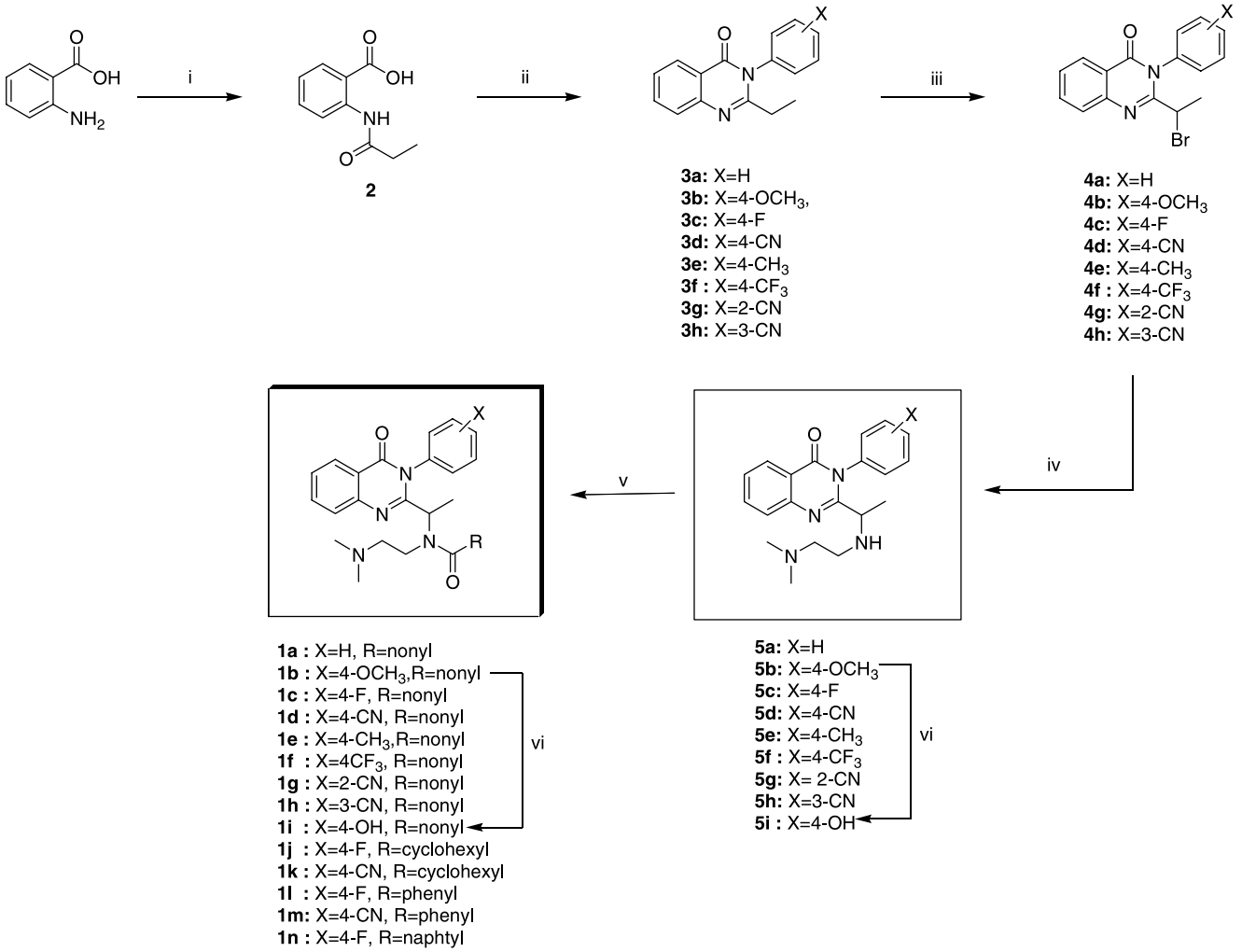
is a CXCR3 ligand with micromolar affinity.[20] We set out to explore the SAR of this compound. More specifically, the role of the nonyl chain of lead structure (**1c**) was investigated. In addition, the effect of the substituent at the 3-phenyl ring on the biological activity was evaluated.[21]

The 3-phenyl-3-*H*-quinazolin-4-ones **1a**–**n** were prepared according to Scheme 1. Reaction of 2-amino-benzoic acid with propionyl chloride gave 2-propionyl-amino-benzoic acid (**2**) (76% yield). Treatment of this intermediate with phosphorus trichloride and the appropriate substituted aniline gave compounds **3a**–**h** in moderate to good yields (35–75%). Reaction with bromine gave the intermediates **4a**–**h** (60–85% yields) that were used for the subsequent reaction with the commercially available *N*,*N*-dimethyl-ethylene-diamine. The crude oils were readily purified by flash chromatography giving the corresponding key derivatives **5a**–**h** (5–65% yields). Reaction with decanoyl, cyclohexanecarbonyl, benzoyl or naphthalene-1-carbonyl chloride afforded compound **1a**–**h** and **1j**–**n** (10–55% yields). The methoxy group of **5b** and **1b** was converted to a hydroxy moiety by treatment with boron tribromide[22,23] to give compounds **5i** and **1i**, respectively, in low isolated yields (10–15%).

Compounds **1a**–**n**, **5a**–**f** and **5i** were tested for their ability to displace [^{125}I]CXCL10 from HEK-293 cells expressing the human CXCR3 receptor.[24] HEK293–CXCR3 cells were grown at 5% CO_2 at 37 °C in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum, 250 mg/mL G-418, 50 IU of penicillin per mL, and 50 mg of streptomycin per mL. These cells were seeded in poly-L-lysine-coated 48-well plates. After 24 h, binding was performed on whole cells for 4 h at 4 °C using approximately 70 pM ^{125}I-labelled CXCL10 (Perkin–Elmer Life Science, Boston, USA) in binding buffer (50 mM HEPES [pH 7.4], 1 mM $CaCl_2$, 5 mM $MgCl_2$, 0.5% BSA) containing increasing concentrations of the indicated compounds. After incubation, cells were washed three times with ice-cold binding buffer supplemented with 0.5 M NaCl. Subsequently, cells were lysed and counted in a Wallac Compugamma counter.

In patent literature, compound **1c** has been reported as CXCR3 antagonist in the micromolar range.[20] We con-



Scheme 1. Reagents: (i) CH_3CH_2COCl, DMF; (ii) PCl_3, substituted anilines, toluene; (iii) Br_2, CH_3COOH, CH_3COONa; (iv) $H_2N(CH_2)_2N(CH_3)_2$, EtOH, reflux; (v) RCOCl, Et_3N, 1,4-dioxane; (vi) BBr_3, MeOH.

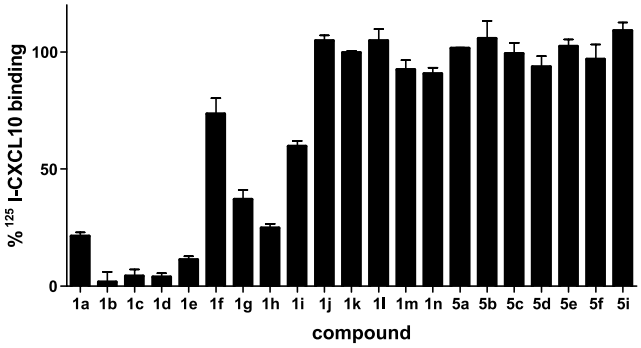


Figure 2. Displacement of [^{125}I]-CXCL10 binding to CXCR3. The binding experiments were carried out on HEK-293 cells expressing the human CXCR3 receptor. The compounds **1a**–**n**, **5a**–**f** and **5i** (10 μM) were tested for their ability to displace [^{125}I]CXCL10. Error bars show standard error of the mean.

firmed this result in our binding assay where **1c** showed an IC_{50} value of 3.2 μM. The importance of the nonyl chain of **1c** was investigated by its removal (**5a**–**i**) or substitution with different hydrophobic moieties (**1j**–**n**). These modifications result in a total loss of affinity (Fig. 2), suggesting an important role of the flexible aliphatic chain.

The role of the *para*-fluoro atom of the phenyl ring in the lead compound **1c** was also studied (Table 1). The presence of appropriate substituents is required for affinity at CXCR3, since the unsubstituted analogue (**1a**) is five times less potent (IC_{50} = 15 μM) than the lead compound (**1c**). Substitutions with different electronic and lipophilic properties resulted in ligands with altered affinities for the CXCR3 receptor. The *para*-methoxy and *para*-methyl derivatives (**1b** and **1e**) showed the same degrees of affinity as the prototype, **1c**, whereas the *para*-cyano derivative, **1d**, was threefold more potent than **1c**. In fact within this series **1d** is the only compound to show submicromolar affinity. Shifting the cyano substituent from the *para*- to the *ortho*- or *meta*-position caused a large decrease in CXCR3 affinity (IC_{50} = 10 μM for **1g** and 5.8 μM for **1h**, respectively).

Table 1. IC_{50} values of the compounds **1a**–**i** for their displacement of [^{125}I]CXCL10 binding to CXCR3

Compounds	R	X	IC_{50}[a] (μM)
1a	Nonyl	H	15 (10–20)
1b	Nonyl	4-OCH_3	3.3 (2.2–4.4)
1c	Nonyl	4-F	3.2 (2.3–4.2)
1d	Nonyl	4-CN	0.93 (0.74–1.1)
1e	Nonyl	4-CH_3	3.1 (2.0–4.1)
1f	Nonyl	4-CF_3	>100
1g	Nonyl	2-CN	10 (8.6–12)
1h	Nonyl	3-CN	5.8 (5.1–6.5)
1i	Nonyl	4-OH	>100

[a] The binding experiments were carried out on HEK-293 cells expressing the human CXCR3 receptor. The compounds were tested for their ability to displace [^{125}I]CXCL10. The values are represented as the mean and the interval of the IC_{50} (μM) values of at least three independent experiments.

Replacement of the 4-fluoro atom of **1c** by a 4-CF_3 (**1f**) or 4-OH (**1i**) group is not allowed for activity.

Compound **1d** has the highest affinity for CXCR3 in the [^{125}I]CXCL10 binding assay and was therefore tested in a [Ca^{2+}] mobilization assay. In this assay, CXCL11-induced increases in [Ca^{2+}] were quantified by monitoring the fluorescence of Fluo-4 AM-loaded HEK293–CXCR3 cells, using an automated NOVOstar microplate reader. Cells were loaded in Hanks' balanced salt solution containing 20 mM HEPES, 2.5 mM Probenecid, 2 μM Fluo-4 AM and 0.02% Pluronic F-127 [pH = 7.4]. Thereafter they were washed three times and fluorescence was measured (1 data point/s, excitation 485 nm, emission 520 nm) for 10 s to calculate the mean basal value. Cells were preincubated for 10 min with the indicated compounds (10 μM). After injection of the agonist CXCL11 (10 nM) fluorescence was recorded for 50 s. To determine the maximal fluorescence of the system, cells were exposed to Triton X-100 (0.25% [v/v]). Basal and maximal values determined for each well were used to normalize the data. Cells preincubated with compounds **1d** and **5d** show comparable baseline fluorescence to nontreated cells. Results were expressed as percentage of the maximal fluorescence induced by Triton X-100.

Stimulation of the human CXCR3 receptor with 10 nM CXCL11 resulted in a rapid increase of intracellular [Ca^{2+}], which returned thereafter to baseline levels (Fig. 3). Preincubation of the cells with 10 μM of **1d**, 10 min prior to chemokine stimulation completely abrogated the CXCL11-induced [Ca^{2+}] increase, while **5d** had no effect (Fig. 3).These data demonstrate that compound **1d** can efficiently block CXCR3-mediated calcium release.

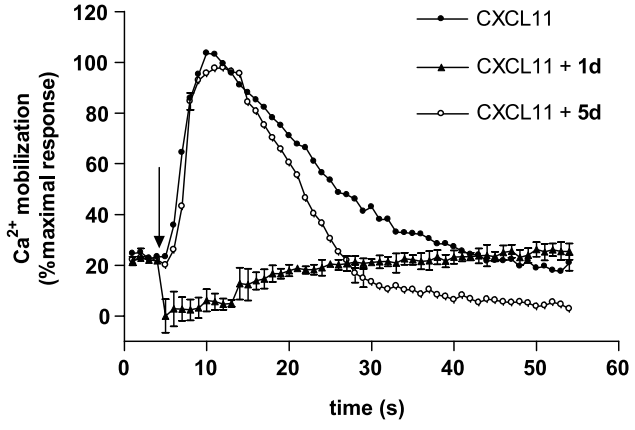


Figure 3. Compound **1d** abrogates CXCR3-mediated [Ca^{2+}] mobilization. Effect of compounds **1d** and **5d** (10 μM) on the CXCL11 (10 nM) induced Ca^{2+} mobilization in HEK-293–CXCR3 cells. Compound were added 10 min prior to chemokine stimulation (indicated by the arrow). The maximal response is defined as maximal Ca^{2+} concentration induced by CXCL11.The experiment is representative of two independent experiments performed in triplicate. Error bars show standard error of the mean.

In conclusion, we have described the SAR of a series of 3-phenyl-3*H*-quinazolin-4-one derivatives for their action as CXCR3 antagonist. Compound **1d** has the highest affinity for the human CXCR3 receptor and is able to effectively inhibit CXCR3 mediated calcium mobilization in transfected HEK-293 cells. Currently we are evaluating **1d** in other biological assays. Moreover, compound **1d** might be useful for future lead optimization programmes.

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