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I. Discovery of a novel series of CXCR3 antagonists. Multiparametric optimization of *N,N*-disubstituted benzylamines

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ABSTRACT

N,N-Disubstituted benzylamine derivatives have been identified as CXCR3 antagonists. Compounds were optimized to improve affinity and selectivity, to increase metabolic stability in human and mouse liver microsomes, to increase Caco-2 permeability. Optimization was supported by monitoring physico-chemical properties using both experimental and computational means. Several compounds with double-digit nanomolar CXCR3 affinity, favorable selectivity, microsomal stability, Caco-2 permeability and human hepatocyte clearance have been identified.

CXCR3 is an inflammatory chemokine receptor which is predominantly expressed on activated immune cells such as CD4+ T-cells, effector CD8+ T cells and innate-type lymphocytes.[1]

CXCR3 is selectively activated by three interferon-inducible chemokines, CXCL9 (also termed as MIG), CXCL10 (IP-10) and CXCL11 (ITAC). Activation of CXCR3 by these endogenous agonists elicits intracellular Ca^{2+} mobilization via phospholipases C (PLC) and activation of both mitogen-activated protein kinase (MAP-kinase) and PI3-kinase. These intracellular events finally result in stimulation of lymphocyte migration and proliferation. CXCR3 plays a key role in selective recruitment of activated immune cells to the site of inflammation. At the site of inflammation the recruited Th1 and CTL cells release IFN-gamma that stimulates epithelial cells and macrophages to further release of CXCR3 agonists that leads to a persistent inflammatory activation. Clinical evidence has shown significant overexpression of CXCR3 receptor and/or its endogenous agonists (CXCL10, CXCL11) in multiple autoimmune or inflammatory diseases.[2]

Blocking the activation of CXCR3 by antagonists represents a possible approach for the treatment of diseases such as COPD, psoriasis, organ transplant rejection, coeliac disease, inflammatory bowel disease (IBD), type-1 diabetes and other neuroinflammatory diseases.[3]

Our objective was to find an orally active CXCR3 antagonist. According to our knowledge no small molecule CXCR3 antagonist is in clinical development.[4] **AMG-487** (**1**, Fig. 1) has formerly reached phase II studies where its development was abandoned owing to the lack of efficacy in psoriasis.[2a,5]

The CXCR3 antagonist program was started by a FLIPR-based high throughput screening[6] of 500,000 compounds in Sanofi's proprietary collection followed by the radioligand binding (IP-10) test[7] as secondary assay. The hit-to-lead process identified compound **2a** as a Lead compound (Fig. 1).

Compound **2a** exhibited satisfactory activity in FLIPR in human cell lines (IC_{50} = 74 nM) and its binding value was also promising (IC_{50} = 403 nM vs IC_{50} of **1** = 121 nM). The *S*-enantiomer of **2a** showed higher activity (IC_{50} = 154 nM vs 790 nM of the *R*-enantiomer). Other parameters like *c*log*P*,[8] solubility and penetrability (Caco-2: 56.2 nm/s)[9] were also acceptable therefore our efforts have to be focused on the improvement of activity, the increase of metabolic stability[10] (53% and 52% of remaining parent compound on human and mouse liver microsomes) and the increase of selectivity (radioligand displacement over 65% at 10 μM for

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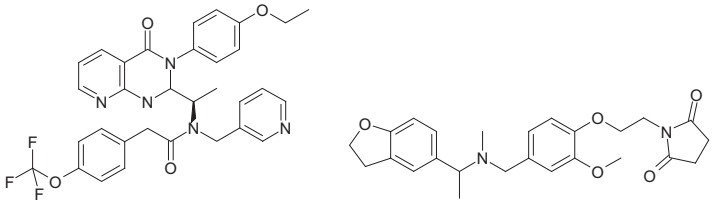
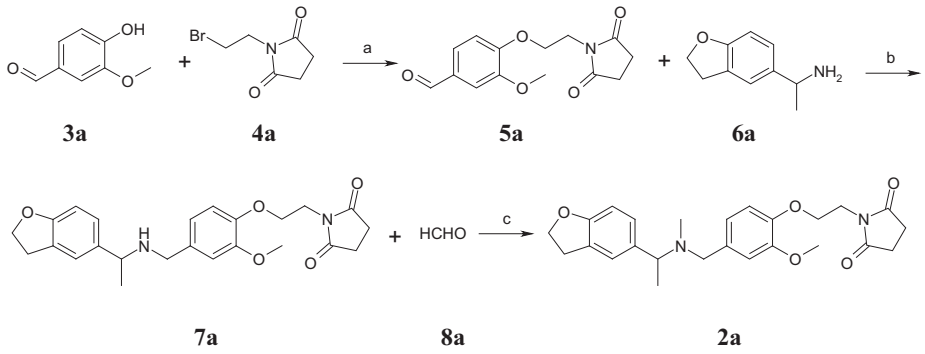
0960-894X/



1 (AMG-487) **2a**

Figure 1. Structure of **1** (**AMG-487**) and Lead compound (**2a**).



3a **4a** **5a** **6a**

7a **8a** **2a**

Scheme 1. Synthesis of Lead compound **2a**. Reagents and conditions: (a) acetonitrile, K_2CO_3, reflux; (b) $NaBH(OAc)_3$, AcOH, THF, rt; (c) $NaBH(OAc)_3$, AcOH, THF, rt.

adrenergic alpha 1A, 2A, histamine H1, muscarinic M2 and mu-opioid receptors).

In the hit-to-lead process the compounds were synthesized on solid phase applying a combinatorial chemistry method that allowed rapid progress. On the other hand, solution phase synthesis was preferred in the lead optimization (Scheme 1) so to facilitate scale-up. The synthesis was started with the alkylation of vanillin (**3a**) by the *N*-(bromo-ethyl) succinimide (**4a**). This reaction resulted in aldehyde **5a** that was reacted with amine **6a** to yield the secondary amine **7a**. Its reductive amination with formaldehyde (**8a**) led to the *N*,*N*-disubstituted benzylamine **2a**.[11]

First the optimal linker to the succinimide group was investigated. Various substitutions of the benzylic group were tested (Table 1). In the synthesis according to Scheme 1 aldehydes (A-CHO, **5**) formed from aromatic aldehydes (**3**) and *N*-(bromoalkyl)-succinimide derivatives (**4**) were used. Here and later on IP-10 radioligand binding[7] was used to measure CXCR3 affinity. (cAMP functional assay was performed for selected compounds to check antagonistic effect). Microsomal metabolism[10] was also monitored.

An interchange of the relative *ortho*-substituents at the benzyl group (**2b**) drastically reduced the activity. The *meta*-substitution (**2e** and **2f**) also led to very weak activity. Similarly, the replacement of the 2 carbon chain connecting the succinimide ring and *O*-atom at *para*-position of the benzyl group with either shortened (**2c**) or lengthened (**2d**) chain resulted in weakly active compounds.

It was also investigated if the *O*-ethyl chain linking the succinimide moiety and the benzyl group can be made more rigid in order to achieve higher activity. A conformational analysis[12] of this part of the molecule revealed that the *O*-ethyl linker can adopt various low energy conformations. The lowest energy conformation shown on Figure 2 does not seem to offer the possibility of rigidifying either by anellating a ring to the central phenyl ring or by introducing double bonds or rings in the chain. On the other hand, some of the conformers whose energy does not exceed the minimum energy by more than 20 kJ/mol are compatible with rigidified structures **2g** and **2h** in Table 1. However, these compounds exhibited considerably lower binding affinity than does **2a**. Thus affinity improvement by rigidifying the *O*-ethyl linker could not be achieved.

Structure–activity studies in the hit-to-lead process revealed that the cyclic 2,5-dioxo-pyrrolidine (i.e., succinimide ring) moiety at the end of the *O*-ethyl linker is required for the activity (activity was completely lost with 2-oxo-pyrrolo group—data not shown). Replacing a CH_2 group of the succinimide ring by NH or N-Me groups or *S*-atom (hydantoine, *N*-methyl-hydantoine and dioxo-thiazolidine ring, respectively) the activity increased significantly, but the metabolic stability did not improve or even decreased (Table 1, **9a–c**). The replacement of the *O*-ethyl linker by NH-ethyl linker reduced the activity (**9d**). When the methoxy group on the central phenyl group was replaced by methyl group or chlorine atom the activity increase was accompanied by a decrease in metabolic stability (**9e**, **9f**).

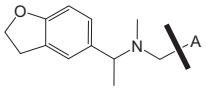
The effect of the basicity of the central *N*-atom and the substitution of the neighboring *C*-atom was then investigated (Table 2). Methyl substitution of the aliphatic *C*-atom of the benzylic group or the replacement of the central *N*-atom by *O*-atom dramatically reduced the activity (data not shown).

Other substituents on the benzylic group, namely the 3-methoxy and 4-[2-(2,5-dioxopyrrolidin-1-yl)ethoxy] groups were kept fixed in these studies. The synthesis was performed according to Scheme 1 with varying benzofuranylamines **6** (leading to different R^1 and R^2) and aldehydes **8** (to introduce R^3 see Table 2) while aldehyde **5a** was kept constant.

Secondary benzylamines were found to have improved metabolic stability but their activity was weaker than that of the Lead (*cf.* compounds **7a** with IC_{50} = 607 nM and Lead **2a** with IC_{50} = 403 nM in Table 2) Double methyl substitution of the *C*-atom adjacent to the *N*-atom further diminished the activity (compound **7b**, IC_{50} = 780 nM).

Converting the secondary benzylamine into tertiary benzylamine is beneficial for the activity. Interestingly, the effect of the

Table 1
Exploration of the substituents, linker and hetero rings on the right hand side of **2a**



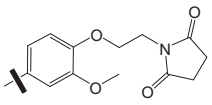
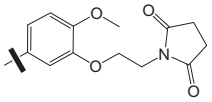
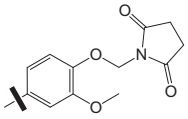
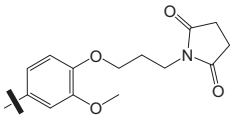
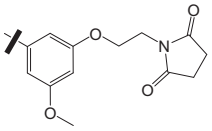
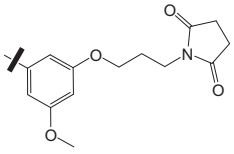
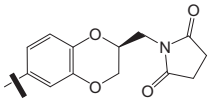
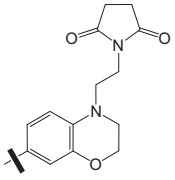
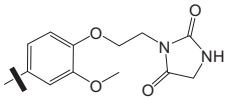
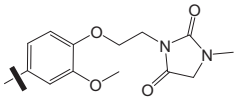
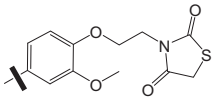
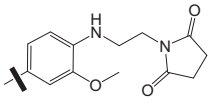
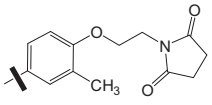
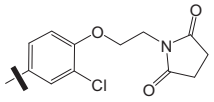
Compound	Structure (A)	hCXCR3[a] (IC_{50} nM or inhibition)	Met. stab.[b] (h/m; %)
AMG-487	See Figure 1	121	77/70
2a		403	53/52
2b		18% at 10 μM	N.D.
2c		8% at 10 μM	N.D.
2d		39% at 10 μM	N.D.
2e		4% at 10 μM	N.D.
2f		15% at 10 μM	N.D.
2g		36% at 10 μM	N.D.
2h		7% at 10 μM	N.D.
9a		240	60/55
9b		202	28/26
9c		120	4/7
9d		443	N.D.
9e		230	29/19

Table 1 (*continued*)

Compound	Structure (A)	hCXCR3[a] (IC_{50} nM or inhibition)	Met. stab.[b] (h/m; %)
9f		187	14/17

N.D. means not determined.
[a] Displacement of ^{125}I-labeled IP-10 from human recombinant CXCR3 receptors.[7]
[b] Human/mouse liver microsomes.[10]

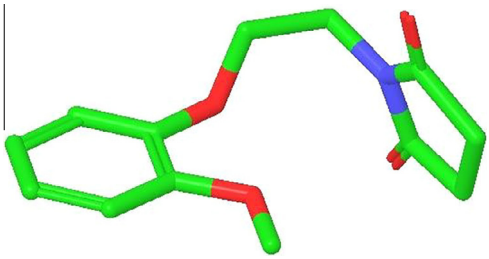


Figure 2. Minimum energy conformation of the model compound corresponding to a fragment of **2a**.

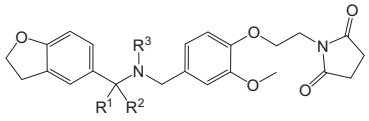
substituents on the *N*-atom (R^3) and on the neighboring *C*-atom (R^1 and R^2 in Table 2) was found to be interdependent. N-substitution is beneficial and the affinity increases with increasing the size of the substituent (*cf.* **2a** and **2i**). However, increasing the size of the *C*-substituent of the secondary benzylamines (i.e. without N-substitution, R^3 = H) tends to decrease the affinity (*cf.* **7a**–**f**). On the other hand, C-substitution of the tertiary amines resulted in affinities comparable to compounds substituted on the *N*-atom only. As these phenomena were observed for various substituents on both atoms, it is reasonable to assume that the substitutions partially exert their effects on the affinity through altering the conformational preference of the compounds. In order to examine this assumption conformational analysis of model compounds in Table 3 was performed.[12] It was found that *Model 3* (*N*-substituted) and *Model 4* (*N*- and *C*-substituted) adopt highly similar shape in their lowest energy conformation as it is shown in Figure 3.

This bent conformation does not correspond to the lowest energy for *Model 1* (unsubstituted) and *Model 2* (alpha-substituted). The excess energy of a similar bent conformation with respect to the minimum energy conformations is 4 and 6 kJ/mol for *Model 1* and *2*, respectively. This is in line with the observed order of affinities and also with their relative magnitude. (We note that ~6 kJ/mol free energy difference corresponds to a 10 fold difference in the population of states.)

Summarizing the results so far, tertiary benzylamines were identified whose activity can be modulated by substitutions on the *N*-atom and on the neighboring *C*-atom. The effect of the substituents can be rationalized by the conformational preference of the compounds. Double digit nanomolar CXCR3 compounds were found by appropriately chosen substituents (**2i**, **2k**). Although their activities were significantly better than **AMG-487**, these tertiary amines are metabolically unstable (Table 2).

The effect of the variation of the tertiary amine *N*-substituent on the activity and metabolic stability was then further investigated (Table 4). The introduction of hydroxalkyl-group decreased the IC_{50} below 100 nM, but the metabolic stability decreased (**10a**, **10b**). Similar effects were observed with keto group (**10d**) and with cyclohexyl group (**10e**). The basic aminoethyl group somewhat increased the human metabolic stability but the activity was also significantly reduced (**10c**). Neither aromatic nor heteroaromatic groups gave satisfactory activity and metabolic stability simultaneously (**10f**–**i**). Introduction of aliphatic (**10j**) and aromatic acids (**10k**) resulted in compounds with somewhat lower than optimal activity but with appropriate metabolic stability. They have,

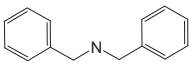
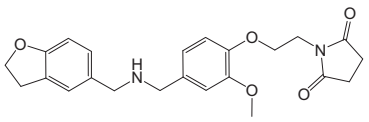
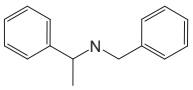
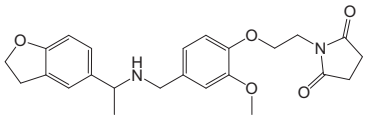
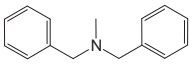
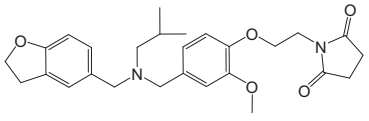
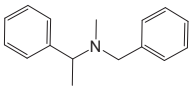
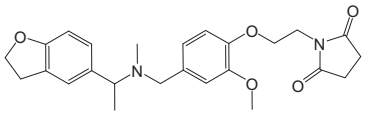
Table 2
Exploration of the substitutions around the central nitrogen atom



Compound	R^1	R^2	R^3	hCXCR3[a] (IC_{50} nM)	Met. stab.[b] (h/m; %)
2a	Me	H	Me	403	53/52
2i	Me	H	*i*-Bu	55	2/9
2j	Et	H	Et	110	5/5
2k	Et	H	*i*-Bu	38	19/19
2l	*n*-Pr	H	Me	110	9/8
2m	*i*-Bu	H	Me	330	N.D.
2n	H	H	*i*-Bu	70	7/11
7a	Me	H	H	607	61/62
7b	Me	Me	H	780	N.D.
7c	Et	H	H	1060	N.D.
7d	*n*-Pr	H	H	1200	N.D.
7e	*i*-Bu	H	H	1800	N.D.
7f	*c*-Penthyl	H	H	3260	N.D.
7g	H	H	H	290	61/67

N.D. means not determined.
[a] Displacement of ^{125}I-labeled IP-10 from human recombinant CXCR3 receptors.[7]
[b] Human/murine liver microsomes.[10]

Table 3
Model compounds used in the conformational analysis

Model compound	Name	Representative CXCR3 ligand	hCXCR3[a] (IC_{50} nM)	Compound
	*Model 1*		290	**7g**
	*Model 2*		607	**7a**
	*Model 3*		70	**2n**
	*Model 4*		403	**2a**

[a] Displacement of ^{125}I-labeled IP-10 from human recombinant CXCR3 receptors.[7]

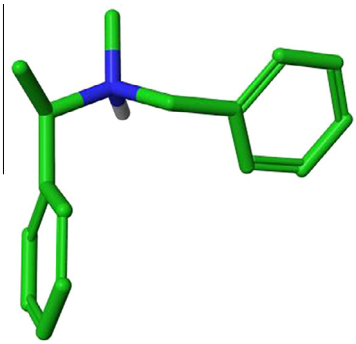


Figure 3. Minimum energy conformation of *Model 4* of Table 3 (*N*-atom protonated).

however, both low Caco-2 permeability[9] and low oral bioavailability (F) in mice[13] (Table 4).

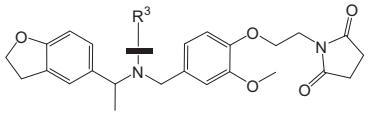
In order to facilitate the design of compounds with appropriate ADME parameters relationships were sought between various easily computed parameters (like log*D*, log*P*, polar surface area)[8] and measured permeability and metabolism values. It was found that permeability tends to increase with log*D* corresponding to pH = 7.4. This is in line with the general observation that absorption increases linearly with lipophilicity, at least in a certain range of the latter.[14]

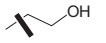
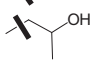
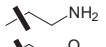
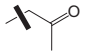
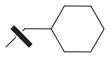
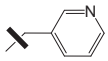
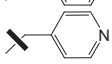
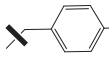
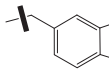
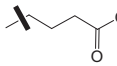
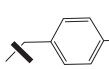
Figure 4a shows, that a log*D* >1 is required to achieve appropriate absorption (P_{app} >20 nm/s). On the other hand, metabolic stability exhibits a slight decrease with the increasing lipophilicity that sets a limit to log*D*. Figure 4b suggests that it is advantageous to keep logD around 3 or below. These two limits of log*D* >1 set by permeability and log*D* <3 set by metabolic stability were used as guidelines in the design of new compounds.

It is important to note that both permeability and metabolism studies discussed above are restricted to compounds with terminal succinimide. It was found, that when succinimide was replaced by *N*-methyluracil then both the log*D*-permeability and the log*D*-metabolism relationships changed; higher log*D* values were calculated at the same pH which resulted in higher permeability and lower metabolic stability. The lower number of compounds with *N*-methyluracil does not allow defining a log*D* range where both permeability and metabolism are expected to be acceptable, however, available data suggest that such a range exists but it is smaller and shifted towards higher values with respect to that of the succinimide derivatives.

Compounds with free carboxyl group and log*D* between 1 and 3 were designed in order to retain activity while to improve ADME

Table 4
Exploration of substitution of the central *N*-atom



Compound	R^3	hCXCR3[a] (IC_{50} nM)	Met. stab.[b] (h/m; %)
2a	CH_3	403	53/52
10a		82	3/4
10b		77	2/0
10c		2200	48/13
10d		202	2/0
10e		52	35/27
10f		190	19/29
10g		130	41/42
10h		97	24/19
10i		340	28/24
10j		210 Caco-2 = 0[c]; *F* = 2%[d]	100/95
10k		120 Caco-2 = 0[c]; *F* = 9%[d]	61/63

[a] Displacement of ^{125}I-labeled IP-10 from human recombinant CXCR3 receptors.[7]
[b] Human/mouse liver microsomes.[10]
[c] Permeability on Caco-2 cell assay.[9]
[d] Bioavailability in mice.[13]

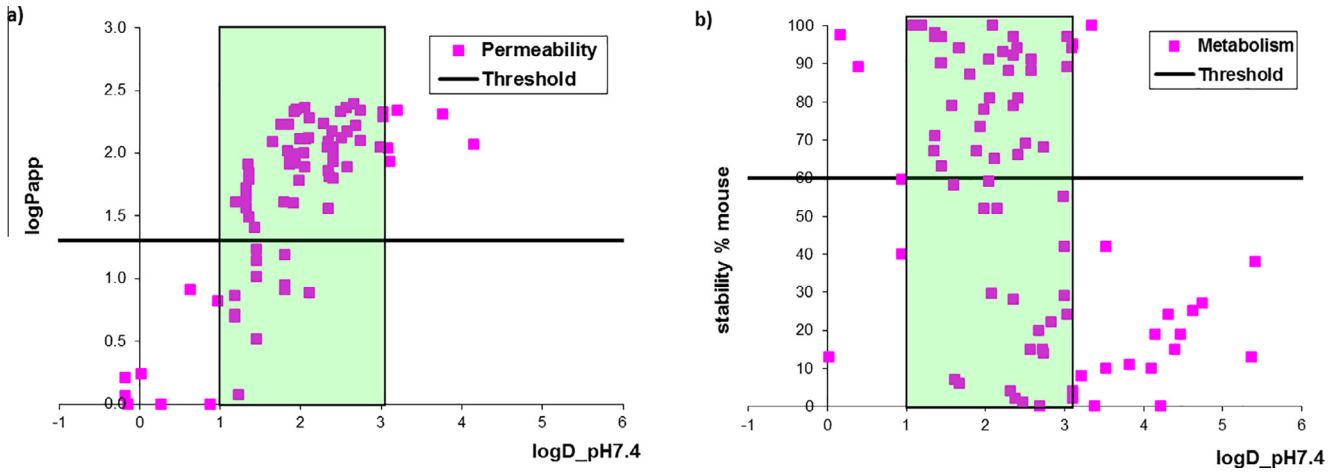
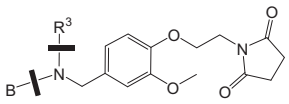
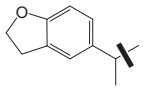
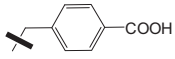
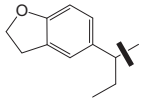
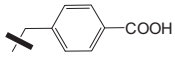
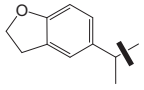
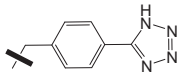
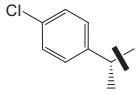
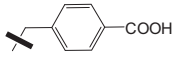
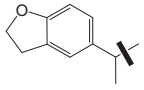
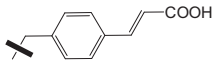
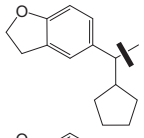
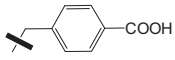
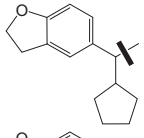
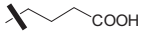
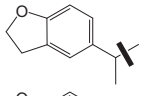
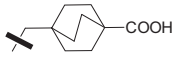
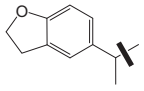
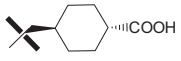


Figure 4. (a) Caco-2 permeability (P_{app} in 10^{-7} cm/s) and (b) metabolic stability (%) in mouse liver microsomes vs. calculated log*D* at pH = 7.4. Acceptable permeability and metabolic stability are most probably achievable in the shaded region.

Table 5
Simultaneous optimization of three parameters (activity, metabolic stability and permeability)



Compound	B	R^3	hCXCR3[a] (IC_{50} nM)	*c*log*D*[b]	Met.stab.[c] (h/m; %)	Caco-2[d] (nm/s)
10k			120 *F* = 9%	1.45	61/63	0
11a			160	2.0	60/78	14.6
11b			126	1.9	51/77	2
11c			345	2.1	98/100	37
11d			138	1.81	84/87	13.4
11e			4200	3.0	52/N.D.	N.D.
11f			382	1.67	92/94	32.1
11g			225	1.6	71/79	65.6
11h			60 *F* = 17.3%	1.2	98/92	9.8

N.D. means not determined.
[a] Displacement of ^{125}I-labeled IP-10 from human recombinant CXCR3 receptors.[7]
[b] Calculated Log*D*.[8]
[c] Human/mouse liver microsomes.[10]
[d] Permeability on Caco-2 cell assay.[9]

Table 6
Optimization of cyclohexanecarboxylic acids

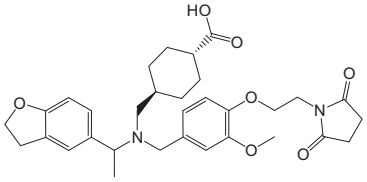
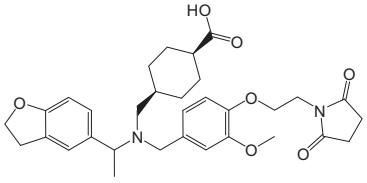
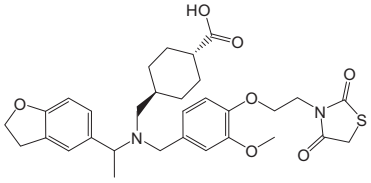
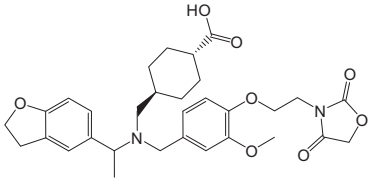
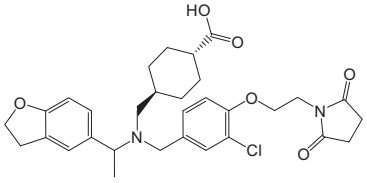
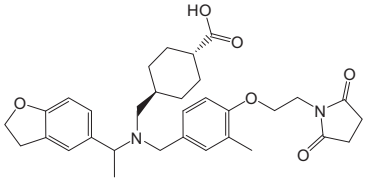
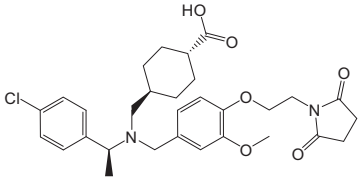
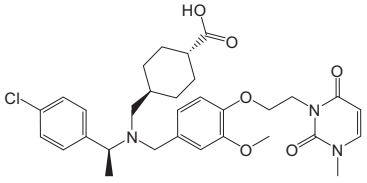
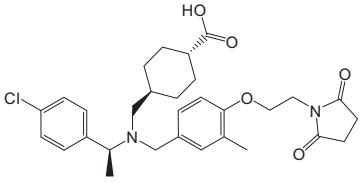
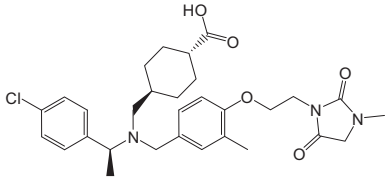
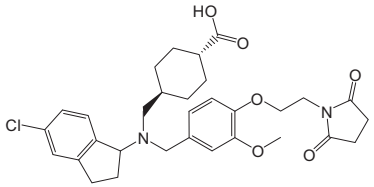
Compound	Structure	hCXCR3[a] (IC_{50} nM)	Met.stab.[b] (h/m; %)	CaCo-2[c] (nm/s)	Developability[d]
11h		60	98/92	9.8	*F* = 17.3% M2 = 54%
12		760	N.D.	N.D.	
13		37	73/73	29.3	M2 = 72% MOP = 94%
14		55	88/93	11.6	pH >6.5 chem. unstable
15		35	56/92	39.5	M2 = 98%
16		44	70/87	22.2	*F* = 4.1 M2 = 99% MOP = 78%
17		19	91/91	52.8	*F* = 13.3% MOP = 74%
18		76	62/76	80.1	*F* = 26.7% HEP = 0.159
19		38	73/87	195.2	*F* = 93.5% HEP = 0.138 α1A = 71% M2 = 97%

Table 6 (*continued*)

Compound	Structure	hCXCR3[a] (IC_{50} nM)	Met.stab.[b] (h/m; %)	CaCo-2[c] (nm/s)	Developability[d]
20		99	63/100	157.3	*F* = 63.3% HEP = 0.234
21		24	66/78	89.8	α1A = 52% MOP = 63% M2 = 0%

N.D. means not determined.

[a] Displacement of ^{125}I-labeled IP-10 from human recombinant CXCR3 receptors.[7]

[b] Human/mouse liver microsomes.[10]

[c] Permeability on Caco-2 cell assay.[9]

[d] *F*: Absolute oral bioavailability in mice.[13] Radioligand displacement on different receptors at 10 μM (M2: muscarinic; α1A: adrenergic; MOP: mu opioid); HEP: human hepatocyte clearance.[15]

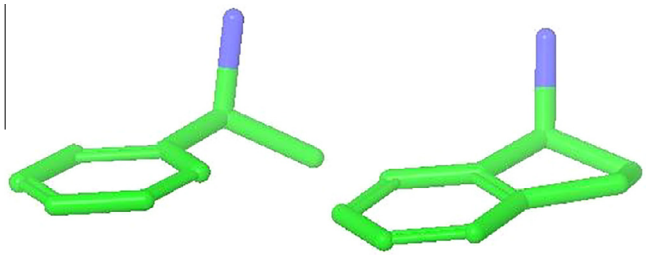


Figure 5. Minimum energy conformations of 1-phenylethyamine and indan-1-ylamine.

parameters and ultimately oral bioavailability (Table 5). Increasing the number of *C*-atoms in the substituents neighboring to the *N*-atom was beneficial to the permeability but decreased the activity (**11a**, **11e**, **11f**). The bioisosteric replacement of the carboxylic group by tetrazole (**11b**) did not improve the properties. The cinnamic acid derivative (**11d**) had slightly better permeability and it was further improved with bicyclo[2,2,2]octanecarboxylic acid (**11g**). This latter, however, showed decreased activity, just as the compound obtained with the replacement of benzofuran by 4-chlorophenyl group (**11c**). The introduction of the cyclohexanecarboxylic acid moiety (**11h**) advantageously changed all investigated parameters; activity and permeability increased while metabolism decreased. These improvements associated with higher bioavailability in mice (*F* = 17.3%).

It was found during the further investigation of compounds possessing a cyclohexane carboxylic acid group (Table 6) that the *trans* isomer (**11h**) is ten-fold more active than the *cis* isomer (**12**). Therefore, the *trans* isomer was used in all subsequent studies. In an attempt to improve oral bioavailability the succinimide group was replaced first by dioxo-thiazolidine (**13**) and then by dioxo-oxazolidine (**14**). Both activity and permeability improved, but **13** showed some affinity towards mu-opioid (MOP) and muscarin (M2) receptors, while **14** was found to be chemically unstable above pH = 6.5 (ring opening occurred). The introduction of a chlorine atom or a methyl group instead of the methoxy group on the benzyl group was also accompanied by the appearance of selectivity issues (**15** and **16**). Replacement of the benzofuran moiety by chlorophenyl (**17**) was highly beneficial for the activity (IC_{50} = 19 nM), but affinity towards opiate receptor appeared and the oral bioavailability decreased (*F* = 13.3%). The oral bioavailability improved by replacing the succinimide ring of this compound by *N*-methyluracil (**18**), but the human hepatocyte clearance (HEP; mL/h/10^6 cells) became too high.[15] The methoxy—methyl replacement on the benzyl group of **17** led to excellent oral bioavailability in mice (**19**, *F* = 93.5%), but it was associated with selectivity problems and somewhat higher than desirable human hepatocyte clearance. Changing the succinimide group of this latter compound to *N*-methylhydantoin (**20**) corrupted both oral bioavailability and hepatocyte clearance.

The introduction of 5-chloroindanyl group in place of the benzofuranylethyl moiety led to significant improvement in permeability while the compounds retained advantageous activity (*cf.* **21** and **11h**). The preservation of activity by this new structural element is in line with the conformational analysis performed to understand the coupled effect of substituents on the *N*- and adjacent *C*-atoms (see above). These calculations suggested that the binding conformation is bent and the C—N bond is close to being perpendicular to the aromatic ring of the terminal group (e.g. phenylethyl or benzofuranylethyl) as it is shown in Figure 5. The direction of the alpha methyl group of this conformation fits well to the direction of the corresponding bond in the minimum energy structure of 1-indanamine. This suggests that closing the alpha-methyl substituent in a ring and thus forming an indane-amine is in line with the bioactive conformation.

Compound **21** exhibited mild affinity towards the adrenergic alpha 1A and mu opiate receptors (it has no effect on muscarin M2 receptor). It is worth mentioning that the 6-chloroindane derivative showed a two order of magnitude lower affinity towards the CXCR3 receptor than did the 5-chloroindane compound (data not shown).

As a first step in 'fine-tuning' the structure of **21** (Table 7) the activity of the *cis* isomer (**22**) was measured. The former had a 25-fold higher binding affinity than did the latter. This is in line with the relative activity of **11h** and **12** (see above). The separation of the racemic **21** resulted in a highly active *S*-enantiomer (**24**, IC_{50} = 13 nM) and a 15 fold less active *R*-enantiomer (**23**). In addition, **24** showed better selectivity and higher metabolic stability. Since the final step in the synthesis of **24** is the hydrolysis of the ester function (Scheme 2) the activity of the **25** ester was also measured and was found lower by almost two orders of magnitude, so it did not work as a prodrug either.

Table 7
Fine-tuning of the compound **21**

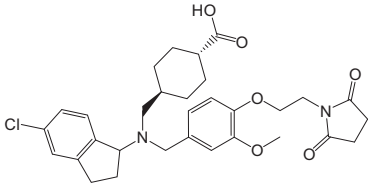
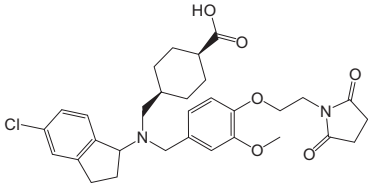
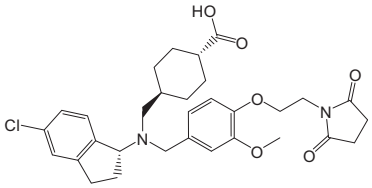
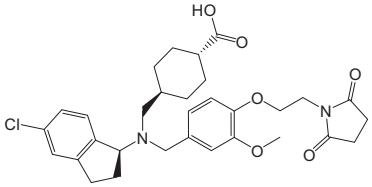
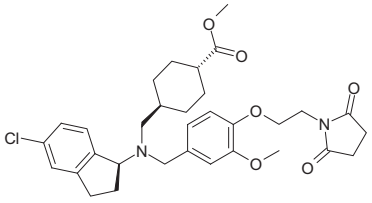
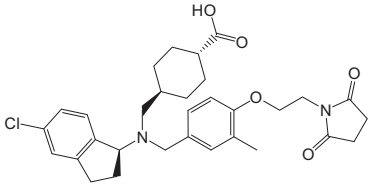
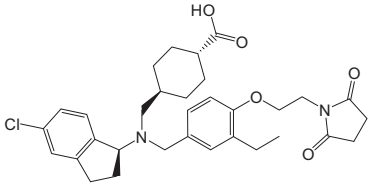
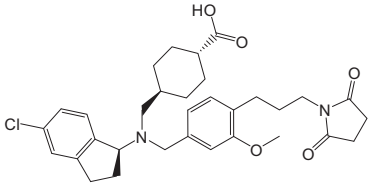
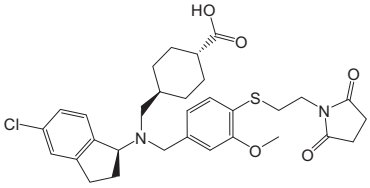
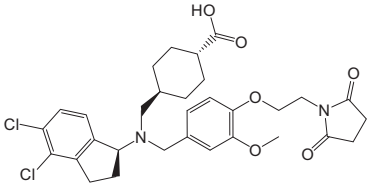
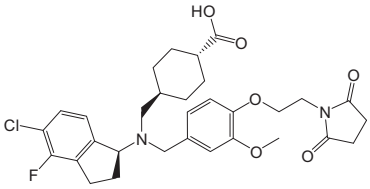
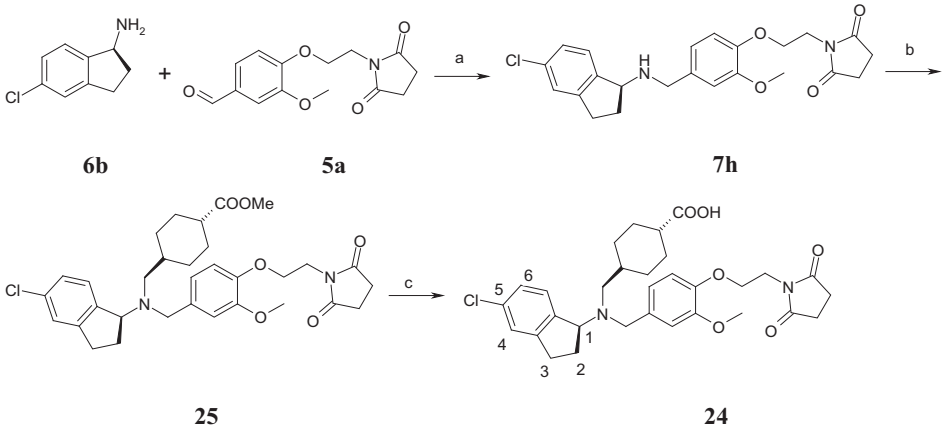
Compound	Structure	hCXCR3[*] (IC_{50} nM)	Met. stab.[*] (h/m; %)	Caco-2[*] (nm/s)	Developability[*]
AMG-487	See Figure 1	121	77/70	251	
21		24	66/78	89.8	α1A = 52% MOP = 64% M2 = 0%
22		1660	N.D.	N.D.	
23		200	54/55	66.2	
24		13	70/79	75.2	*F* = 24% HEP = 0.112 α1A = 25% MOP = 43%
25		840	N.D.	N.D.	
26		53	78/91	81.2	*F* = 69% HEP = 0.090 M1 = 82% M2 = 92% MOP = 87%
27		176	94/96	80.2	HEP = 0.069 M2 = 37%
28		1000	36/24	165.3	

Table 7 (*continued*)

Compound	Structure	hCXCR3* (IC_{50} nM)	Met. stab.* (h/m; %)	Caco-2* (nm/s)	Developability*
29		23	15/3	72.4	
30		25	75/81	57	HEP = 0.083 M2 = 0%
31		26	73/81	29.7	*F* = 47% HEP = 0.031 M2 = 16%

* See Table 6 for the abbreviations used.



Scheme 2. Synthesis of compound **24**. Reagents and conditions: (a) $NaBH(OAc)_3$, THF, rt; (b) *trans* 4-formylcyclohexanecarboxylic acid methyl ester (**8b**), $NaBH(OAc)_3$, THF, rt. (c) HCl, dioxane, 60 °C.[16]

Replacing the methoxy group on the central benzyl moiety by methyl- (**26**) or ethyl-group (**27**) the affinity towards the CXCR3 receptor decreased. The replacement of the *O*-atom in the 4th position on the central benzyl moiety by CH_2-group (**28**) decreased the affinity by two orders of magnitude. By contrast, the affinity was retained with an S-atom (**29**) instead of the *O*-atom, however, the metabolic stability was significantly reduced. These results suggest that the lone-pair present on both the *O*- and S-atoms but missing on the CH_2-group contributes advantageously to the activity. Owing to the expected lower metabolic stability of the compound containing S-atom, the properties are optimal with the *O*-atom. Exploring the possible substitutions on the indane-ring it was found that the activity only tolerates substitution in the 4th position in addition to the 5-chloro-substituent: 4-Cl (**30**) and 4-F (**31**) derivatives both exhibit slightly weaker affinities than does compound **24**.

In summary, a new series of potent CXCR3 antagonists was discovered. Starting with an HTS campaign, the Lead compound was extensively optimized for potency, in vitro metabolic stability, permeability and pharmacokinetic profile. Calculated and measured physico-chemical properties together with conformational analysis supported the optimization. Several compounds have highly advantageous profile and they are currently subject to further evaluation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.10.035. These data include MOL files and InChiKeys of the most important compounds described in this article.

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6. Bioactivity was tested using engineered CHO cells expressing the human CXCR3A coupled to a chimeric G-protein (Gαi4qi4). In the FLIPR instrument, following basal readings, IP-10 is added and fluorometric images are read. IC_{50}'s are the mean values ($n = 2$) with SD of ~15%.
7. The CXCR3 binding affinity was measured by displacement of ^{125}I-CXCL10 (IP-10) from human recombinant CXCR3 receptors generated by transfection of Flp-In-CHO host cells with plasmid construct of pCDA5-FRT-TO_IRES-Gαi4qi4_DEST. IC_{50}'s are the mean values ($n = 2$) with SD of ~13%. See Ref. 17 for detailed assay protocol.
8. Calculated log*P*, log*D*, PSA were determined by ACD/Log*D* Suite. Version 8, Advanced Chemistry Development, Inc.,Toronto, ON, Canada.
9. The Caco-2 cell assay was carried out in 24 and 96-well plates (Millipore). After verification of cell monolayer integrity, high and low permeability controls and test compound were added. Samples from both apical (A) and basal (B) sides were taken 2 h later. Compound transport was measured under four different conditions: A-B flux, pH 6.5/7.4 (0% or 0.5% BSA in A and 5% BSA in B); A-B flux and B-A flux pH 7.4/7.4 (0.5% BSA in A and B respectively). The amount of compound in each compartment was measured by LC/MS. Caco-2 value of the rapidly transported compounds was P_{app} >20 nm/s (=20 × 10^{-7} cm/s).
10. Metabolic stability of test compounds was performed using human and mouse liver microsomes in presence of NADPH at 37 °C. Parent compound loss was followed using LC–MS–MS technique. Classification: low: <40%; medium: between 40% and 60%; high: >60%.
11. Recently published low molecular weight CXCR3 inhibitors were different from our tertiary amine library; see: Wijtmans, M.; de Esch, I. J. P.; Leurs, R. In *Chemokine Receptors as Drug Targets*; Smit, M. J., Ed.; Wiley-VCH, 2011; p 302. Chapter 13.
12. *MacroModel, version 9.9*; Schrödinger, LLC: New York, NY, 2011.
13. Absolute oral bioavailability was measured following a single oral (10 mg/kg) and intravenous (3 mg/kg) administration to male Balb/c mouse (4 and 4 animals for individual profiles in each animal or using spare sampling in 24 mice, and 3 mice per time point for mean profiles).
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15. Intrinsic hepatic clearance (HEP) was determined using primary or cryopreserved human hepatocytes and compound concentration of 5 μM, in vitro Cl_{int} value is mL/h/10^{-6} cells. Classification (mL/h/10^{-6} cells): low: <0.04; intermediary: between 0.04 and 0.12; high: >0.12.
16. (*S*)-5-Chloroindan-1-ylamine (**6b**) and *trans* 4-formylcyclohexanecarboxylic acid methyl ester (**8b**) were commercially available materials.
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