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# Optimization of a series of quinazolinone-derived antagonists of CXCR3

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ABSTRACT

The evaluation of the CXCR3 antagonist AMG 487 in clinic trials was complicated due to the formation of an active metabolite. In this Letter, we will discuss the further optimization of the quinazolinone series that led to the discovery of compounds devoid of the formation of the active metabolite that was seen with AMG 487. In addition, these compounds also feature increased potency and good pharmacokinetic properties. We will also discuss the efficacy of the lead compound **34** in a mouse model of cellular recruitment induced by bleomycin.

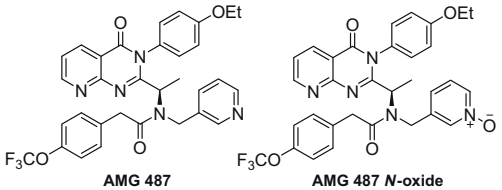


Chemokine receptors and their ligands play an important role in mediating cell trafficking. CXCR3 is a chemokine receptor primarily expressed on activated $CD4^+$ T cells with the Th1 phenotype.[1,2] The ligands for CXCR3, Mig (CXCL9), IP10 (CXCL9), and ITAC (CXCL11), mediate migration of CXCR3-expressing cells and are found at increased concentrations in inflamed tissue from patients suffering from IBD, MS, rheumatoid arthritis, and transplant rejection.[3–15] Furthermore, recent reports point to potential roles for CXCR3 and its ligands in viral infection[16] and tumor metastasis.[17] Therefore, several groups[18–25] including ours have been interested in discovering small molecules inhibitors of CXCR3.

We previously reported[18] the optimization efforts that led to the discovery of the CXCR3 antagonist AMG 487 from a series of quinazolinone derivatives identified by high-throughput screening. The evaluation of AMG 487 in the clinic was complicated by the discovery of significant circulating levels of a pyridine-*N*-oxide active metabolite[26] (Table 1). This active metabolite is approximately four fold more potent than AMG 487 in the in vitro cell migration assay in response to ITAC in the presence of human plasma. In this Letter, we report our efforts towards the identification of compounds with improved CXCR3 potency relative to AMG 487 and devoid of any major active metabolite formation.

The compounds reported in this Letter were synthesized following our previously reported route[18] (Scheme 1). The oxazinones **1** were obtained by addition of the appropriate 2-amino benzene

**Table 1**




| Compound | $^{125}$I -IP10 binding in buffer $IC_{50}$[a,b] (μM) | ITAC migration in plasma $IC_{50}$[a,c] (μM) |
|---|---|---|
| AMG 487 | 0.005 | 0.24 |
| AMG 487 *N*-Oxide | 0.004 | 0.058 |

[a] Values are means of three experiments, standard deviation is ±30%.
[b] Displacement of $^{125}$I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 28 for assay protocol.
[c] ITAC mediated migration of PBMC in the presence of 100% human plasma. See Ref. 28 for assay protocol.

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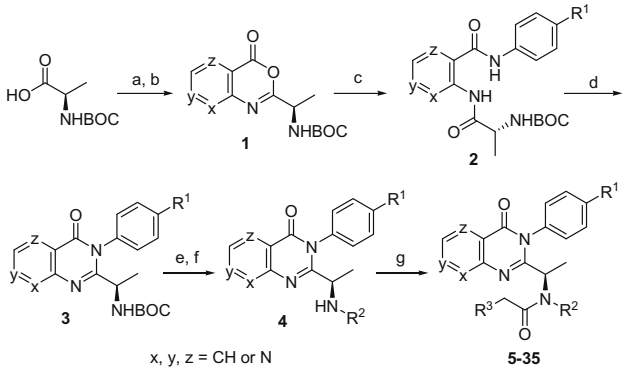
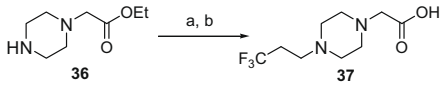


**Scheme 1.** Reagents and conditions: (a) isobutylchloroformate, NMM, DCM, −25 °C, 1 h; (b) 2-amino benzene or pyridine carboxylic acids, −25 °C, 12 h; (c) anilines, DCM, −25 °C, 2 h; (d) isobutylchloroformate, DCM, −25 to −15 °C, 6 h, ~30% four steps; (e) TFA, DCM, rt, 2 h, >90%; (f) for compounds **5–32**, aldehydes or ketones, $Na(OAc)_3BH$, $ClCH_2CH_2Cl$, rt, 2 h, ~80%; For compounds **33–35**, ethylsulfonylethene, TEA, MeOH, reflux, 90%; (g) acetic acids, EDC, HOBt, DMF, rt, 1 h, 50–90%.

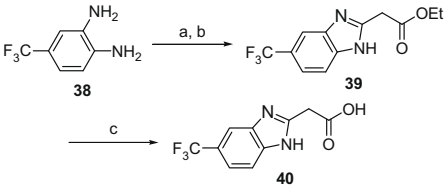
or pyridine carboxylic acids to a solution of the Boc-protected amino acids pretreated with isobutylchloroformate. Ring opening with the appropriate anilines provided bisamides **2**, which upon treatment with isobutylchloroformate afforded the Boc-protected amines **3**.

After the removal of the Boc group with TFA, the resulting primary amines were alkylated using one of two methods. For the synthesis of compounds **5–32**, the primary amines were alkylated using reductive amination with various aldehydes or ketones. For compounds **33–35**, Michael addition of ethylsulfonylethene to the primary amines afforded the alkylated secondary amines **4**. The secondary amines **4** were finally acylated using various substituted acetic acids to yield compounds **5–35**.

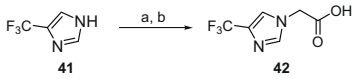
The syntheses of the heterocyclic-acetic acids **37, 40, 42**, and **44** used in the synthesis of compounds **6–9**, respectively, are shown in Scheme 2–5. Piperazine acetic acid **37** was prepared from compound **36** in two steps (Scheme 2) via alkylation with



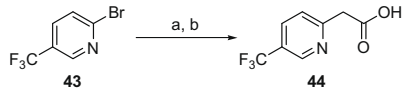
**Scheme 2.** Reagents and conditions: (a) 3,3,3-trifluoro-1-iodopropane, potassium carbonate, DMF, 100 °C, 1.5 day, 80%; (b) LiOH, MeOH/THF/water, 25 °C, 2 h, 90%.



**Scheme 3.** Reagents and conditions: (a) malonic acid mono ethyl ester, EDC, DCM, 25 °C, overnight; (b) HOAc, 70 °C, 12 h, 50% for two steps; (c) LiOH, MeOH/THF/water, 25 °C, 2 h, 90%.



**Scheme 4.** Reagents and conditions: (a) *t*-butyl bromoacetate, potassium carbonate, DMF, 25 °C, 12 h, 80%; (b) TFA, DCM, rt, 2 h, >90%.



**Scheme 5.** Reagents and conditions: (a) 2-*t*-butoxy-2-oxoethyl zinc chloride, $Pd(PPh_3)_4$, THF, 120 °C, microwave 5 min, 40%; (b) TFA, DCM, rt, 2 h, >90%.

3,3,3-trifluoro-1-iodopropane at 100 °C under basic conditions and subsequent ester hydrolysis using lithium hydroxide.

Benzimidazole acetic acid **40** was synthesized in three steps (Scheme 3). Diaminobenzene **38** was condensed with malonic acid mono ester to afford the amide, which was cyclized in acetic acid to give benzimidazole ester **39**. Hydrolysis of the ester afforded acid **40**.

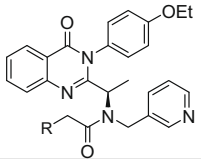
Imidazole acetic acid **42** was prepared via alkylation of 4-trifluoromethylimidazole (**41**) with *t*-butyl bromoacetate, followed by conversion of the *t*-butyl ester to the acid using TFA (Scheme 4).

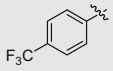
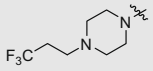
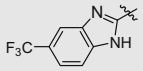
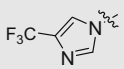
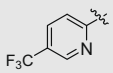
Pyridyl acetic acid **44** was synthesized via palladium catalyzed coupling[27] of the Reformatsky reagent with 5-trifluoromethyl-2-bromopyridine **43** and subsequent ester hydrolysis (Scheme 5).

The lead optimization was primarily guided by $^{125}$I-IP10 ligand displacement assay in buffer[28] and ITAC migration assay in plasma.[28] $IC_{50}$ of a compound in the $^{125}$I-IP10 ligand displacement assay reflects the compound's intrinsic binding affinity to the receptor, while $IC_{50}$ of a compound in the ITAC migration assay in plasma reveals the compound's antagonistic activity and its non-specific plasma protein binding affinity.

The quinazolinone core was used for initial SAR studies, instead of the 8-azaquinazolinone core as in AMG 487, because quinazolinone derivatives and their corresponding 8-azaquinazolinone compounds are equally potent[18] and using readily accessible quinazolinone derivatives could speed up optimization. Modifications of the acetamide moiety are shown in Table 2 and 3. As previously reported,[18] the trifluoromethyl group of the acetamide moiety in compound **5** and AMG 487 is a desirable substituent since it significantly improves potencies and microsomal stability.

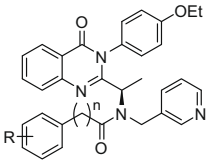
**Table 2**
Modification of phenyl acetamide phenyl ring



| Compd | R | $^{125}$I -IP10 binding in buffer $IC_{50}$[a,b] (μM) | ITAC migration in plasma $IC_{50}$[a,c] (μM) |
|---|---|---|---|
| **5** |  | 0.006 | 0.35 |
| **6** |  | 0.69 | ND |
| **7** |  | 0.21 | ND |
| **8** |  | 0.007 | 0.45 |
| **9** |  | 0.013 | 0.50 |

[a] Values are means of three experiments, standard deviation is ±30%.
[b] Displacement of $^{125}$I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 28 for assay protocol.
[c] ITAC mediated migration of PBMC in the presence of 100% human plasma. See Ref. 28 for assay protocol.

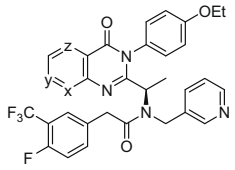
**Table 3**
Substitutions of phenyl acetamide phenyl ring



| Compd | *n* | R | $^{125}$I -IP10 binding in buffer $IC_{50}$[a,b] (μM) | ITAC migration in plasma $IC_{50}$[a,c] (μM) |
|---|---|---|---|---|
| **5** | 1 | 4-$CF_3$ | 0.006 | 0.35 |
| **10** | 2 | 4-$CF_3$ | 2.09 | ND |
| **11** | 1 | 3-$CF_3$ | 0.011 | ND |
| **12** | 1 | 4-$OCF_3$ | 0.007 | 0.14 |
| **13** | 1 | 4-CN | 0.021 | ND |
| **14** | 1 | 4-$SO_2$Me | 0.77 | ND |
| **15** | 1 | 3,4-F | 0.04 | ND |
| **16** | 1 | 3,4,5-F | 0.008 | ND |
| **17** | 1 | 3,5-$CF_3$ | 0.007 | 0.017 |
| **18** | 1 | 3-F-4-$OCF_3$ | 0.009 | 0.22 |
| **19** | 1 | 3-F-4-$CF_3$ | 0.002 | 0.075 |
| **20** | 1 | 4-F-3-$CF_3$ | 0.003 | 0.020 |

[a] Values are means of three experiments, standard deviation is ±30%.
[b] Displacement of $^{125}$I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 28 for assay protocol.
[c] ITAC mediated migration of PBMC in the presence of 100% human plasma. See Ref. 28 for assay protocol.

**Table 4**
Quinazolinone and azaquinazolinone cores



| Compd | x | y | z | $^{125}$I -IP10 binding in buffer $IC_{50}$[a,b] (μM) | ITAC migration in plasma $IC_{50}$[a,c] (μM) |
|---|---|---|---|---|---|
| **20** | CH | CH | CH | 0.003 | 0.020 |
| **21** | N | CH | CH | 0.005 | 0.023 |
| **22** | CH | N | CH | 0.032 | ND |
| **23** | CH | CH | N | 0.11 | ND |
| **24** | N | CH | N | 0.042 | ND |

[a] Values are means of three experiments, standard deviation is ±30%.
[b] Displacement of $^{125}$I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 28 for assay protocol.
[c] ITAC mediated migration of PBMC in the presence of 100% human plasma. See Ref. 28 for assay protocol.

Therefore, when we modified the acetamide moiety, we always tried to keep trifluoromethyl group in the region. In Table 2, we examined the replacement of the phenylacetamide phenyl group of compound **5** with various heterocycles. The imidazole (**8**) and pyridine (**9**) were similar in potency to the phenyl derivative (**5**), while other replacements yielded loss of potency. Overall, the examined heterocyclic replacements did not provide any improvement in potency and other modifications in the area provided significant improvement (see below), so the heterocyclic replacements were not adopted for further studies.

The substitutions of the phenylacetamide phenyl ring and the linker between the carbonyl and the phenyl ring were also explored (Table 3). The methylene between the carbonyl and the phenyl seems to be optimal, because the ethylene linker (**10**) significantly decreases the potency and removal of the methylene also results in dramatic loss in potency.[18] In order to investigate whether the improvement in potency observed with the trifluoromethyl group (**5**) is due to its electron-withdrawing ability, the cyano (**13**) and methyl sulfone (**14**) derivatives were evaluated. Compounds **13** and **14** yielded decreases in potency. Therefore, the data suggests that the electron-withdrawing ability of trifluoromethyl group cannot fully account for the improvement in potency afforded by the trifluoromethyl group. It is known that fluorine can have several kinds of interactions with proteins, such as hydrophobic, stacking, edge-on, and multipolar interactions.[29,30] Therefore, it was hypothesized that fluorine atoms in the phenylacetamide area may have favorable interactions with the receptor and additional fluorine atoms in the region may provide additional improvement on potency. It was rewarding to see that analogs with additional fluorine indeed improved potency in several cases, such as in compounds **16** versus **15**, **19** versus **5**, and **20** versus **11**. The 3-trifluoromethyl-4-fluorophenyl acetamide moiety in compound **20** is one of the best in the area and it was used for further SAR studies.

After optimization of the phenylacetamide moiety, the correlation between the quinazolinone core and other azaquinazolinone cores was re-examined. It was encouraging to observe that, as previously noted,[18] quinazolinone **20** and the corresponding 8-azaquinazolinone compound **21** are equally potent (Table 4). Other azaquinazolinones (**22**–**24**) are much less potent, with the 5-azaquinazolinone (**23**) being the least potent. In order to increase polarity, we chose to use the 8-azaquinazolinone core for further optimization.

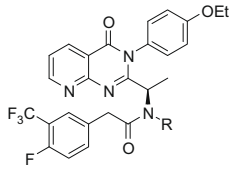
The amide *N*-alkyl moiety was studied with 8-azaquinazolinone core and 3-trifluoromethyl-4-fluorophenylacetamide moiety in the molecules (Table 5). As previously reported, this area tolerated many changes.[18] Alkoxy ethyl, amino ethyl and various heterocyclic methyl all produced compounds with good potencies. 3-Pyridyl methyl group as in AMG 487 was the optimal moiety discovered in the early optimization efforts. Table 5 shows other potent modifications discovered later and a couple modifications (**26** and **30**) provided compounds that are more potent than the compound (**21**) with 3-pyridyl methyl. This data, in combination with the previously reported data, suggest that polar groups are preferred in the area, but the position of the heteroatoms is not critical and many types of polar groups are tolerated in this region. We eventually decided to use the ethylsulfone-ethyl moiety (**33**), because of the improvement in potency and metabolic stability. In addition, compounds with the ethylsulfone moiety avoid major active metabolite formation as demonstrated by our in vitro metabolism studies.

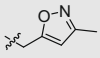
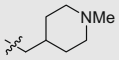
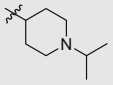
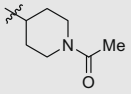
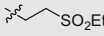
Substitutions of the 3-*N*-phenyl ring on the quinazoline core were studied extensively in the early lead optimization.[18] It was discovered that small substituents such as ethoxy or halogen atoms at the *para* position afforded compounds with good potencies. Deethylation of the ethoxy group in compound **33** was one of the metabolic routes observed in vitro, so in order to decrease this metabolism, 3,3,3-trifluoroethoxy and cyano were introduced to the *para* position of the 3-*N*-phenyl moiety (Table 6). The compound with the 3,3,3-trifluoroethoxy group (**34**) is as potent as the ethoxy analog (**33**). However, the cyano derivative (**35**) is not as potent as the ethoxy derivative (**33**), even though in the early optimization the cyano and ethoxy analogs have similar potencies.[18] Apparently, with changes in the core, the acetamide, and *N*-alkyl moieties, the cyano group is not as desirable as ethoxy group for potency.

Compound **34** was chosen for further evaluation. It was found that it is a potent antagonist of cell migrations mediated by CXCR3 in response to IP10, ITAC or MIG (Table 7).

The ability of compound **34** to inhibit the CXCR3 receptor across several species was demonstrated using several $^{125}$I-IP10 displacement assays (Table 7). The rat, mouse, dog, and rhesus monkey receptors were evaluated and **34** displayed similar activity in these species as in the human.

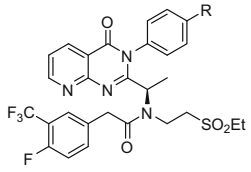
**Table 5**
Modification of the amide *N*-alkyl moiety



| Compd | R | $^{125}$I -IP10 binding in buffer $IC_{50}$[a,b] (μM) | ITAC migration in plasma $IC_{50}$[a,c] (μM) |
|---|---|---|---|
| **21** |  | 0.005 | 0.023 |
| **25** |  | 0.002 | 0.059 |
| **26** |  | 0.002 | 0.010 |
| **27** |  | 0.005 | 0.080 |
| **28** |  | 0.002 | 0.020 |
| **29** |  | 0.002 | 0.020 |
| **30** |  | 0.001 | 0.005 |
| **31** |  | 0.006 | 0.131 |
| **32** |  | 0.005 | 0.162 |
| **33** |  | 0.001 | 0.022 |

[a] Values are means of three experiments, standard deviation is ±30%.
[b] Displacement of $^{125}$I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 28 for assay protocol.
[c] ITAC mediated migration of PBMC in the presence of 100% human plasma. See Ref. 28 for assay protocol.

**Table 6**
Substitutions of 3-*N*-phenyl ring

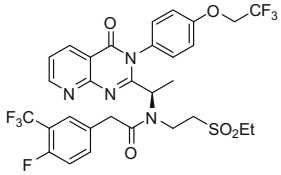


| Compd | R | $^{125}$I -IP10 binding in buffer $IC_{50}$[a,b] (μM) | ITAC migration in plasma $IC_{50}$[a,c] (μM) |
|---|---|---|---|
| **33** | –OEt | 0.001 | 0.022 |
| **34** | $-OCH_2CF_3$ | 0.001 | 0.015 |
| **35** | –CN | 0.011 | 0.115 |

[a] Values are means of three experiments, standard deviation is ±30%.
[b] Displacement of $^{125}$I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 28 for assay protocol.
[c] ITAC mediated migration of PBMC in the presence of 100% human plasma. See Ref. 28 for assay protocol.

The pharmacokinetic properties of **34** were evaluated across several species (Table 8). In mouse, dog, and cyno, the compound has low to moderate clearance and decent to good oral bioavailability. This compound also demonstrated low to moderate

**Table 7**
Activities of compound **34** in human and other species



**34**

| Assay ($IC_{50}$, μM) | In buffer | In plasma |
|---|---|---|
| Human $^{125}$I -IP10 binding[a,b] | 0.001 | 0.006 |
| Human $^{125}$I -ITAC binding[a,b] | 0.001 | ND |
| Human IP10 migration[a,c] | 0.0004 | ND |
| Human ITAC migration[a,c] | 0.0008 | 0.015 |
| Human MIG migration[a,c] | 0.0006 | ND |
| Rat $^{125}$I -IP10 binding[a,b] | 0.004 | ND |
| Mouse $^{125}$I -IP10 binding[a,b] | 0.003 | ND |
| Dog $^{125}$I -IP10 binding[a,b] | 0.005 | ND |
| Rhesus $^{125}$I -IP10 binding[a,b] | 0.002 | ND |

[a] Values are means of three experiments, standard deviation is ±30%.
[b] Displacement of $^{125}$I-labeled IP10 from the CXCR3 receptor expressed on PBMC. See Ref. 28 for assay protocol.
[c] ITAC mediated migration of PBMC in the presence of 100% human plasma. See Ref. 28 for assay protocol.

**Table 8**
Pharmacokinetic profile of compound **34**

| Parameter | Rat | Mouse | Dog | Cyno |
|---|---|---|---|---|
| CL (L/h/kg)[a] | 3.9 | 0.78 | 0.45 | 0.87 |
| MRT (h)[a] | 0.65 | 1.1 | 2.5 | 1.6 |
| Vdss (L/kg)[a] | 2.5 | 0.8 | 1.1 | 1.4 |
| $F_{po}$ (%)[b] | 26 | 84 | 25 | 14 |

[a] Following iv dosing in rat and mouse at 0.5 mg/kg and dog and cyno at 1 mg/kg.
[b] Following po dosing in rat and mouse at 2 mg/kg, dog at 2.5 and cyno at 5 mg/kg.

inhibition toward CYP 1A2, 3A4, 2C9, and 2D6 and it is selective against other chemokine receptors in MDS receptor screen.

The in vivo efficacy of **34** was studied in a mouse model of cellular recruitment induced by bleomycin. In this mouse model study, bleomycin was introduced in the lungs of mice via intra-tracheal instillation after tracheostomy.[31] Compound **34** and AMG

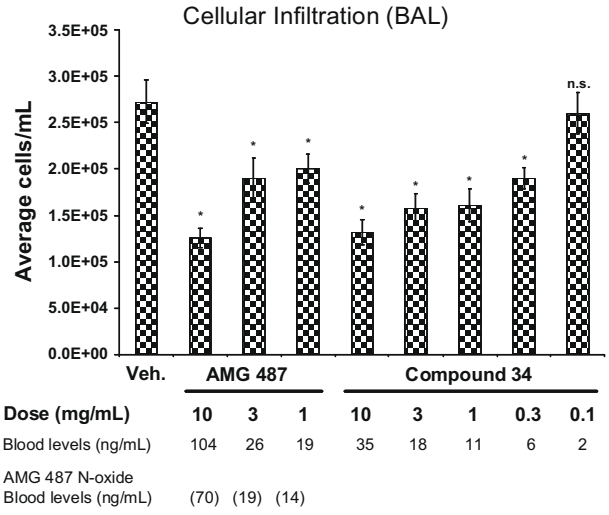



**Figure 1.** Evaluation of AMG 487 and compound **34** in a mouse bleomycin model of cellular recruitment. * $p$ <0.01 as determined by Student's $t$-tests.

487 were each dosed subcutaneously via Alzet osmotic mini-pump[32] for six days after the bleomycin challenge. Six days after bleomycin challenge, a bronchoalveolar lavage (BAL) was performed and the number of cells collected in the BAL were counted using a hemocytometer. As shown in Figure 1, compound **34** was able to achieve the same degree of inhibition of cell infiltration as AMG 487 with lower blood levels. If the blood level of the *N*-oxide of AMG 487 is also taken into consideration, the potency of compound **34** is even more impressive. AMG 487 *N*-oxide is more potent than AMG 487 (Table 1). At a blood concentration of 35 ng/mL, compound **34** provided the same inhibition of cell infiltration as AMG 487 with a blood concentration of 104 ng/mL and its *N*-oxide at a concentration of 70 ng/mL.

In summary, further optimization of the quinazolinone-derived CXCR3 antagonists led to the discovery of compounds that avoid the formation of the pyridine-*N*-oxide active metabolite seen with AMG 487 in the clinic. Compound **34** was selected for extensive evaluation. This compound displays increased potency in vitro and in vivo compared to AMG 487 and possesses good pharmacokinetic profile across several species. This compound represents a valuable tool in the exploration of the role of CXCR3 receptor in mediating autoimmune and other diseases.

## References and notes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