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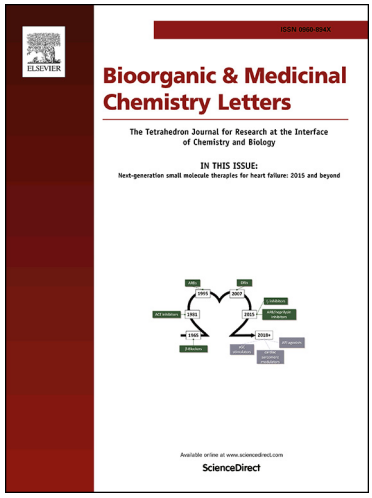

Discovery of a novel azetidine scaffold for colony stimulating factor-1 receptor (CSF-1R) Type II inhibitors by the use of docking models

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Discovery of a novel azetidine scaffold for colony stimulating factor-1 receptor (CSF-1R) Type II inhibitors by the use of docking models

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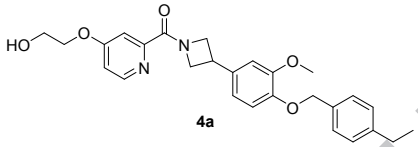

4a

CSF-1R IC50 = 9.1 nM

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ABSTRACT

We report the discovery of a novel azetidine scaffold for colony stimulating factor-1 receptor (CSF-1R) Type II inhibitors by using a structure-based drug design (SBDD) based on a docking model. The work leads to the representative compound **4**a with high CSF-1R inhibitory activity (IC_{50} = 9.1 nM). The obtained crystal structure of an azetidine compound with CSF-1R, which matched our predicted docking model, demonstrates that the azetidine compounds bind to the DFG-out conformation of the protein as a Type II inhibitor.

Colony-stimulating factor-1 receptor (CSF-1R or cFMS) is a type III receptor tyrosine kinases that is generally expressed at myeloid cells in the mononuclear phagocyte system and at progenitor cells in bone marrow.[1] It binds to CSF-1, known as macrophage colony-stimulating factor (M-CSF).[2] Also, interleukin-34 (IL-34) has recently been reported as another ligand.[3] The binding to CSF-1 and IL-34 activates the receptor kinase through a process of dimerization and subsequent autophosphorylation. The activation stimulates differentiation, proliferation, survival and migration of monocyte-macrophage lineage cells.[1,4] Also, macrophages produce inflammatory mediators such as interleukin and lymphokine, and lead to differentiation, proliferation and activation of a variety of immune cells. These immune cells are also involved in the pathological conditions of rheumatoid arthritis (RA) and multiple sclerosis.

In addition, this signaling promotes differentiation of osteoclastic precursors into mature osteoclasts.[4] Osteoclasts, whose formation requires the presence of a receptor activator of nuclear factor ligand kB (RANK) and CSF-1, promote bone destruction and bone absorption and play a key role in osteoporosis as well as deformity in RA. Therefore, inhibition of CSF-1R signaling is expected to be therapeutic in RA. This hypothesis is supported by the biological studies conducted with op/op mice which are missing endogenous CSF-1. In addition, a number of CSF-1R inhibitors with in vivo anti-inflammatory efficacy have been reported in the literature, including Ki20227,[5] GW2580,[6a-c] JNJ-28312141,[7] Arry382,[8] BLZ945,[9] AZD7507,[10] and PLX-3397.[11]

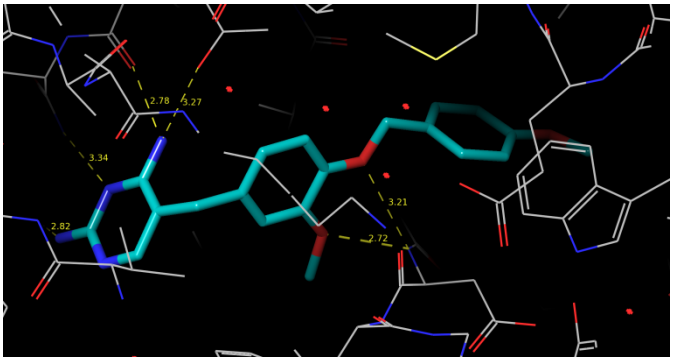
Functional states of a typical protein kinase seem to be characterized by the conformation of the DFG (Asp-Phe-Gly) motif, which are conserved among most of protein kinases and located in the activation loops. The majority of inhibitors, which are well known as Type I inhibitors, target the ATP-binding site of the kinase with the DFG-in conformation as its active state. On the other hand, Type II inhibitors (e.g., imatinib and sorafenib) induce the DFG-out conformations, and occupy an additional hydrophobic pocket created by this rearrangement.[12] With more favorable kinase selectivity and slower off-rates, Type II inhibitors are expected to possess several advantages over Type I inhibitor.

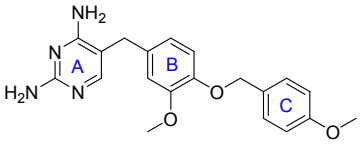
We have discovered a new class of azetidine compounds as selective CSF-1R Type II kinase inhibitors and our work led to the identification of **JTE-952**,[13] which is a clinical candidate with cellular and in vivo potency. Herein, we report the discovery of this azetidine scaffold.

Although several selective CSF-1R inhibitors have been reported, **GW2580** is one of the most selective inhibitors for CSF-1R.[6a-d] It shows no inhibition of 186 other kinases, except for TrkA. The co-crystal structure of **GW2580** with CSF-1R showed that **GW2580** is a typical Type II inhibitor, which binds to the DFG-out state protein, as shown in **Fig. 1**.[6b,c, 14] Ring A binds to the hinge region of the kinase protein with four hydrogen bonds. The rings A and B form an L-shape conformation through methylene. The two oxygen atoms of the methoxy and benzyloxy groups on ring B make the bidentate hydrogen bonding network to the backbone amide NH of G796, and ring C occupy the hydrophobic pocket in the DFG-out state.

We hypothesized that the unique L-shape conformation and the bidentate hydrogen bonds are important features for Type II inhibitors of CSF-1R. We planned to alter the hinge binding substructure to increase the activity while maintaining the bidentate hydrogen bonds, which are composed of methoxy and benzyloxy groups, for the Type II inhibitor.

Fig. 1 The co-crystal structure of **GW2580** and CSF-1R reported in US2004/0002145.[a]





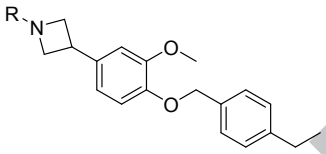
GW2580

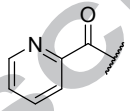
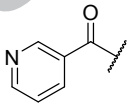
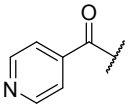
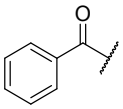
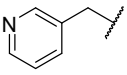
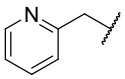
[a] Nitrogen, oxygen, and sulfur atoms are shown in blue, red, and orange, respectively. Hydrogen bonds between the compound and protein are shown as yellow dashed lines.

Initially, we expected that the binding of saturated rings such as azetidine, pyrrolidine, and piperidine directly to ring B could produce the L-shaped conformation similar to **GW2580**. We anticipated that the azetidine structure would be the most promising. The azetidine scaffold particularly overlapped with the **GW2580** active conformation without a collision against the protein (data not shown in this report), while the pyrrolidine and piperidine compounds did collide with the protein. Therefore, we focused our search on compounds based on the azetidine structure with the methoxy and benzyloxy substituents on ring B to preserve the kinase selectivity.

We began exploring azetidine analogues by using pyridine rings to identify the appropriate position of the hydrogen bond acceptor that would interact with the hinge region since it is well known that kinase hinge binders must have at least a hydrogen bond acceptor. Compounds **1a-d, 2a-b,** and **3a-b** were synthesized and tested in the CSF-1R enzyme assay (**Table 1**). In this study, we used the 4-ethyl-benzyloxy substituent instead of 4-methoxy-benzyloxy because our preliminary studies showed that this substituent had equivalent activity and greater chemical stability. The 2-picolylamide analogue (**1a**) showed the best activity, which was equivalent to **GW2580**, making it the optimum compound among the amide analogues (**1b-d**). Surprisingly, the benzoyl substituent, not the pyridine ring, showed medium activity. The pyridylmethyl analogues (**2a-b**) and the direct pyridine rings molecules (**3a-b**) had little or no activity. Thus, the amide analogues did not require the pyridine ring, but rather, a planar structure and the carbonyl seemed to serve as a hydrogen bond acceptor. Also, to verify the structural determinants, we prepared and evaluated pyrrolidine analogues instead of the azetidine ring. These compounds did not show activity as we had predicted (data not shown in this report).

Table 1. CSF1R Enzyme Assay [a] IC_{50} Values for compounds **1a-d**, **2a-b**, and **3a-b**.



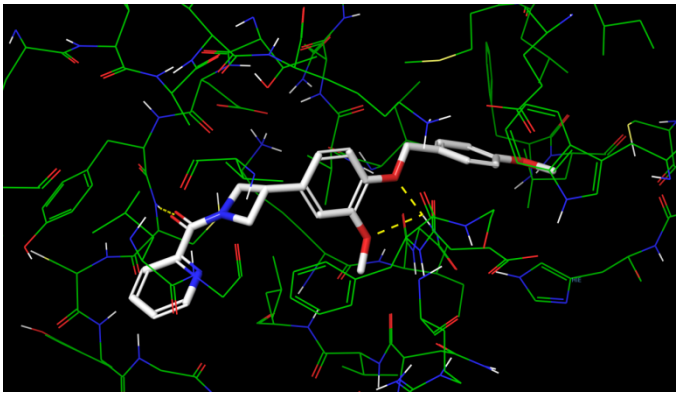
comp	R	CSF1R IC_{50}
1a		62 nM
1b		510 nM
1c		1100 nM
1d		430 nM
2a		>3000 nM
2b		>3000 nM
3a		>3000 nM
3b		1400 nM

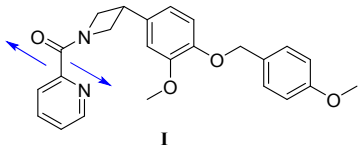
[a] Human CSF-1R (amino acids 538 – 972) encompassing the tyrosine kinase domain (obtained in-house) was used. For details, see the Supporting Information.

To gain insights into the mechanism of action, we modeled the binding mode of compound **I** based on the co-crystal structure of **GW2580**. The result of this docking study is shown in **Fig. 2**.[14,15] We were surprised to observe that the acceptor for hydrogen-bonding to the backbone NH of Cys666 was not the N-atom in pyridine, but rather the O-atom in C=O. Also, the conformation

of the 2-picolyl - azetidine amide moiety was perfectly planar and the position between C=O vs. C=N in the pyridine was anti (*i.e.,* anti-planar conformation). We thought that an electrostatic repulsion interaction between the C=O and the N-atom in pyridine was the main factor. Therefore, contrary to our assumption, the N-atom in pyridine was required, but not as a hydrogen bond acceptor, but rather for stabilizing the anti-planar conformation of the 2-picolyl-amide structure.

Fig. 2 The Docking Model of compound **I**.[a]





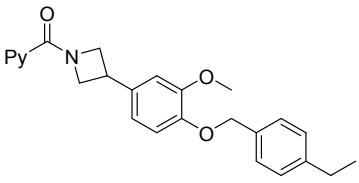
I

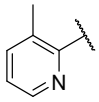
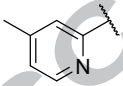
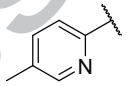
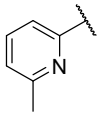
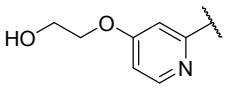
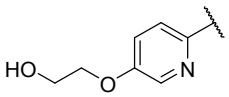
The electrostatic repulsion interaction

[a] Nitrogen, oxygen, and sulfur atoms are shown in blue, red, and orange, respectively. Hydrogen bonds between the compound and protein are shown as yellow dashed lines.

Next, we investigated the optimal direction to orient the compound toward the outside of the protein to increase the enzyme inhibition. From our docking model, we predicted that we could locate substituents at the 4-, 5-, 6-positions on the pyridine ring toward the outside of the protein and the 4-position specifically would be the best position. To confirm this idea, we synthesized compounds **1e-h** and **4a-b** and tested them in the CSF-1R enzyme assay (**Table 2**). Initially, we examined the methyl group on the pyridine ring. The IC_{50} values of the 4-methyl analogue (**1f**, IC_{50} = 41 nM) was best and also the 5-methyl compound (**1g**, IC50 = 86 nM) showed inhibition of the 10^{-8} M order, whereas the 6-methyl (**1h**, IC50 = 145 nM) and 3-methyl (**1e**, IC50 = 2216nM) derivatives led to reduced activity. To confirm the best position, we also introduced ethylene glycol unit at the 4- and 5-positions. We predicted that the alcohol unit would increase activity and hydrophilicity by extending the molecule toward the outside of the protein. The 4-(hydroxy-ethoxy)-pyridine derivative **4a** showed the best IC_{50} (9.1 nM). 5-(Hydroxyethoxy)-pyridine derivative **4b** led to approximately a 3-fold decrease in biochemical activity (IC_{50} = 25 nM) compared to **4a**. These results were consistent with our prediction based on the docking model.

Table 2. CSF1R Enzyme Assay [a] IC_{50} Values for compounds **1e-h** and **4a-b**



comp	Py	CSF1R IC_{50}
1e		2216 nM
1f		41 nM
1g		86 nM
1h		145 nM
4a		9.1 nM
4b		25 nM

[a] Human CSF-1R (amino acids 538 – 972) encompassing the tyrosine kinase domain (obtained in-house) was used. For details, see the Supporting Information.

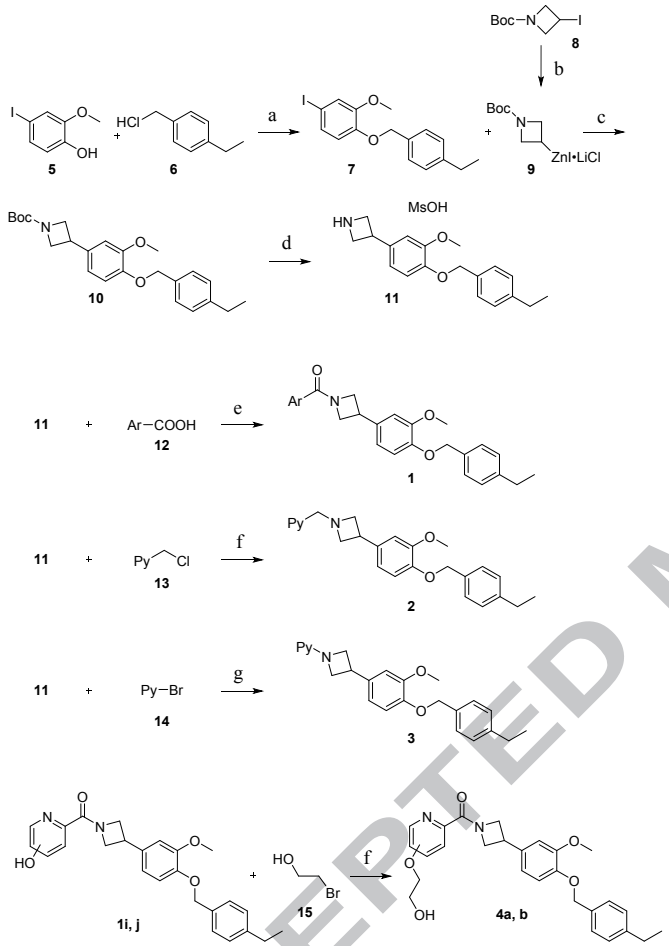
The syntheses of compounds in this study were accomplished as shown in **Scheme 1**.

The syntheses of **1a-j**, **2a-b**, **3a-b**, and **4a-b** were started from 4-iodo-2-methoxyphenol **5**. The phenol **5** was benzylated with 4-ethyl-benzylchloride **6** in the presence of K_2CO_3 in *N,N*-dimethylformamide (DMF) to give 1-benzyloxy-4-iodo-2-methoxybenzene derivatives **7**. Negishi coupling of **7** with azetidinyl zincate reagent **9** using bis(triphenylphosphine)palladium (II) chloride or $Pd(OAc)_2$ and Sphos (dicyclohexyl-(2'6'-dimethoxy-[1,1'-biphenyl]-2-yl)phosphine) as the catalyst provided the *N*-Boc-3-phenyl-azetidine **10**. The yields using the catalyst of $Pd(OAc)_2$ and Sphos were higher than with bis(triphenylphosphine)palladium (II) chloride. The azetidinyl zincate was prepared by N-Boc-3-iodo-azetidine **8** and zinc in the presence of lithium chloride in *N,N*-dimethylacetamide (DMA). Removal of the Boc protective group from **10** with methanesulfonic acid (MsOH) in ethanol or 2-propanol gave azetidine methanesulfonic acid salt **11** as a key intermediate. In this deprotection, the usage of HCl as an acid provided lower yields compared to MsOH because of opening of the azetidine ring by HCl (the results are not shown in this report). Condensation of **11** with the corresponding carboxylic acid **12**

afforded the azetidine-amide derivatives **1**. Alkylation of **11** with the corresponding pyridylmethyl chloride **13** afforded **2**. Pd-mediated amination, using $Pd(OAc)_2$ and BINAP as catalyst and Cs_2CO_3 as base, of **11** with the corresponding pyridyl halide **14** led to **3**.

4a-b were obtained by alkylations of **1i, j** with 2-bromoethanol in the presence of K_2CO_3 in DMF.

Scheme 1.

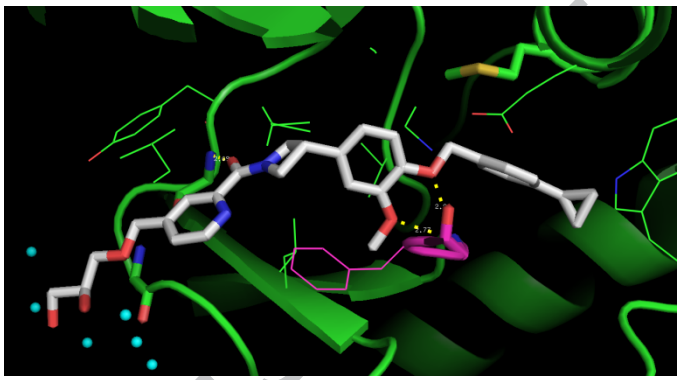


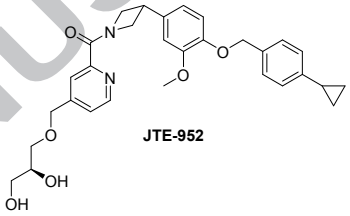
Reagents and conditions: (a) K_2CO_3, DMF, rt; (b) Zn, dibromoethane, LiCl, DMA, rt; (c) $PdCl_2(PPh_3)_2$ or $Pd(OAc)_2$ and Sphos, DMA, rt; (d) MsOH, EtOH or IPA, 80 °C; (e) WSC•HCl, HOBt•H_2O, Et_3N, DMF, rt; (f) K_2CO_3, DMF, 80 °C; (g) $Pd(OAc)_2$, BINAP, Cs_2CO_3, 1,4-dioxane, 80 °C.

To confirm our design, we attempted to obtain crystal structures of our azetidine series compounds in complex with CSF-1R. We obtained a crystal structure at 1.8Å resolution of **JTE-952**,[16] which is our clinical candidate and is described in detail in our next report. The enzyme-bound bioactive conformation was almost the same as our calculated result, as shown **Fig. 3**.[14] Compound **JTE-952** binds to the protein in a DFG-out state with the bidentate hydrogen bonding network of methoxy and benzyloxy and the backbone NH of Asp796. 4-Cyclopropylbenzyl substituent binds to a lipophilic pocket which is formed by the DFG-out conformational change of the protein. In the hinge region, the backbone NH of Cys666 forms a hydrogen bond to the C=O of the 2-picolyl–azetidine amide substructure, which assumes the anti-planar conformation between C=O vs. C=N in the pyridine. Also, the substituent at the 4-position on the pyridine ring is directed toward the outside of the protein and is stabilized by a hydrogen bond network with bulk water molecules.

The crystal structure indicated that we attained our aim of generating a novel scaffold for a CSF-1R Type II inhibitor.

Fig. 3 The co-crystal structure of **JTE-952** with CSF-1R.[a]





[a] Nitrogen, oxygen, and sulfur atoms are shown in blue, red, and orange, respectively. DFG(Asp-Phe-Gly) motif are shown in pink. Hydrogen bonds between the compound and protein are shown as yellow dashed lines.

In conclusion, we have described the discovery of a new class azetidine compounds as CSF-1R Type II inhibitors. We designed the azetidine scaffold based on the crystal structure of **GW2580**. We synthesized and assayed the azetidine compounds and also obtained the crystal structure of our compound and the CSF-1R protein. Our results allowed us to identify a novel scaffold with great therapeutic potential. Further efforts to improve cellular activity and in vivo efficacy will be described in the next report.

Acknowledgments

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14. The molecular image was generated with PyMOL (PyMOL Molecular Graphics System; DeLano Scientific: San Carlos, CA).
15. The docking model was generated with Schrödinger Suite 2017. Details were described in the Supporting Information.
16. The co-crystal structure coodinate has been deposited in Prtein Data Bank (PDB code 6IG8). For details, see the Supporting Information.

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Graphical Abstract

Discovery of a novel azetidine scaffold for colony stimulating factor-1 receptor (CSF-1R) Type II inhibitors by the use of docking models

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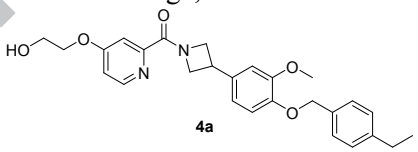
Kazutaka Ikegashira*, Taku Ikenogami, Takayuki Yamasaki, Yasunori Hase, Takayuki Yamaguchi, Koji Inagaki, Satoki Doi, Tsuyoshi Adachi, Yoshihisa Koga, Hiromasa Hashimoto



4a

CSF-1R IC50 = 9.1 nM

- A novel series of azetidine scaffold were identified as colony stimulating factor-1 receptor (CSF-1R) Type II inhibitors.
- Compound **4a** showed high CSF-1R inhibitory activity (IC_{50} = 9.1nM).
- A co-crystal structure of an azetidine compound with CSF-1R was obtained.