Discovery of BPR1R024, an Orally Active and Selective CSF1R Inhibitor that Exhibits Antitumor and Immunomodulatory Activity in a Murine Colon Tumor Model

Kun-Hung Lee, Wan-Ching Yen, Wen-Hsing Lin, Pei-Chen Wang, You-Liang Lai, Yu-Chieh Su, Chun-Yu Chang, Cai-Syuan Wu, Yu-Chen Huang, Chen-Ming Yang, Ling-Hui Chou, Teng-Kuang Yeh, Chiung-Tong Chen, Chuan Shih, and Hsing-Pang Hsieh*

ABSTRACT: Colony-stimulating factor-1 receptor (CSF1R) is implicated in tumor-associated macrophage (TAM) repolarization and has emerged as a promising target for cancer immunotherapy. Herein, we describe the discovery of orally active and selective CSF1R inhibitors by property-driven optimization of BPR1K871 (9), our clinical multitargeting kinase inhibitor. Molecular docking revealed an additional nonclassical hydrogen-bonding (NCHB) interaction between the unique 7-aminoquinazoline scaffold and the CSF1R hinge region, contributing to CSF1R potency enhancement. Structural studies of CSF1R and Aurora kinase B (AURB) demonstrated the differences in their back pockets, which inspired the use of a chain extension strategy to diminish the AURA/B activities. A lead compound BPR1R024 (12) exhibited potent CSF1R activity (IC50 = 0.53 nM) and specifically inhibited protumor M2-like macrophage survival with a minimal effect on antitumor M1-like macrophage growth. In vivo, oral administration of 12 mesylate delayed the MC38 murine colon tumor growth and reversed the immunosuppressive tumor microenvironment with the increased M1/M2 ratio.

INTRODUCTION

Immuno-oncology has emerged as a novel and important approach toward the treatment of cancer through the stimulation of T-cell responses by immune checkpoint inhibitors (ICIs) such as programmed cell death 1 (PD-1), programmed death ligand 1 (PD-L1), or T-lymphocyte-associated antigen 4 (CTLA4) blocking antibodies.1 Recently, however, many other cell types in the tumor microenvironment (TME) have been identified as mediating immunity through different mechanisms, and protein kinases are implicated in all of them.2 This has stimulated a search for novel immunokinase inhibitors.

Colony-stimulating factor-1 receptor (CSF1R or FMS) is a type of class III receptor tyrosine kinase (RTK), which also includes stem-cell factor receptor (c-KIT), FMS-like tyrosine kinase-3 (FLT3), and platelet-derived growth factor receptor (PDGFR) α/β.3 Activation of CSF1R occurs upon binding one of its ligands, CSF1 or IL-34, followed by switching the conformation of its intracellular kinase domain and then stimulating autophosphorylation for signal transduction.4

CSF1R plays an integral role in the differentiation and survival of macrophages.4,5 Tumor-associated macrophage (TAM) infiltration is correlated with a poor prognosis in most tumor types.6,7 Numerous studies have found that TAMs promote tumor progression and contribute to an immunosuppressive TME through the
recruitment of CD4+ regulatory T-cells, which blunt the response of CD8+ cytotoxic T cells.8,9 There are two phenotypes of TAM, M1 and M2, which have anti- and protumor functions, respectively.10−12 Depletion of the M2 TAMs results in TME reprogramming that unleashes T-cell responses and elicits antitumor immunity.13 CSF1R signal blockade repolarizes M2 TAMs toward M1 TAMs in the TME and thereby constitutes another immunotherapeutic approach distinct from T-cell ICIs.14−17 There are a number of CSF1R inhibitors and monoclonal antibodies in different stages of clinical development (Figure 1).18−22 For example, pexidartinib (1) is a dual CSF1R and c-KIT inhibitor approved by the US FDA in 2019 for the treatment of tenosynovial giant cell tumors (TGCT or PVNS), a rare disease with CSF1R overexpression;23,24 DCC-3014 (2), a highly specific CSF1R inhibitor, is in phase II clinical trials for TGCT and advanced malignant neoplasm;25 and edicotinib (3) and BLZ945 (4) are selective CSF1R inhibitors, which have been used for the treatment of CSF1R-dependent tumor cells.15,26,27 However, in addition to the use of CSF1R inhibitors as single agents, they can also be used in combination with other ICIs. The combinations of the highly selective CSF1R inhibitors DCC-3014 (2) and ARRY-382 (5) with avelumab and pembrolizumab, respectively, have shown great promise as methods to induce tumor suppression through the multifactorial modulation of immune cells.28 Many preclinical CSF1R inhibitors have also been reported in the literature.29−33 For example, Ki20227 (6), a 6,7-dimethox-
yquinoline-based derivative with CSF1R and vascular endothelial growth factor receptor 2 (VEGFR2) activities, was studied for the treatment of bone cancer and autoimmune disease, and 7, which suppressed the production of protumorigenic cytokines and inhibited the migration and invasion of macrophages in vitro. However, the poor kinase selectivity of 7 precluded its further development. Gratifyingly, an exquisitely selective CSF1R inhibitor, IACS-9439 (8), was recently reported to delay tumor growth or improve immunity in two animal models.

BPR1K871 (9) is a multitargeting kinase inhibitor developed by us and received U.S. IND approval in 2017. BPR1K871 (9) was demonstrated to potently inhibit CSF1R, Aurora A (AURA), and Aurora B (AURB) kinases by in-house Kinase-Glo assays with IC50 values of 19, 22, and 13 nM, respectively. However, AURA/B kinases are involved in mitotic arrest and their inhibition could have a cytotoxic effect on normal cells. Furthermore, the poor oral bioavailability of BPR1K871 (9) necessitated its intravenous (IV) administration. Herein, we describe the discovery of a series of orally active CSF1R inhibitors with diminished inhibition of AURA/B. Commencing from the structure of BPR1K871 (9), physicochemical property-driven optimization was employed to improve pharmacokinetics and kinase selectivity. Through in-depth analyses of the scaffold, linker, and terminal moieties, a lead compound BPR1R024 (12) was identified and further characterized for its target specificity of M2-like macrophages and antitumor immunity in the MC38 murine colon tumor model.

### DESIGN

Our strategy to optimize the pharmacokinetics and kinase selectivity of BPR1K871 (9) comprises a three-stage approach (Figure 2): (i) optimization of drug-like properties and improvement of CSF1R selectivity; (ii) attenuation of AURA/B inhibition; and (iii) improvement of pharmacokinetics. First, BPR1K871 (9) harboring an excessive number of rotatable bonds (NRB) is in violation of Veber’s rule, which may account for its poor oral bioavailability. Accordingly, we shortened the flexible side chains of BPR1K871 (9) to reduce the NRB and replaced the thiazole ring with a phenyl group. Following a structure−activity relationship (SAR) study, we installed an N,N-dimethylamino group at the 7-position of the quinazoline ring to obtain 10, which exhibited conserved anti-CSF1R potency but reduced AURA/B activities and improved oral bioavailability compared to BPR1K871 (9). Second, through structure-based exploration of the AURA/B back pocket, we introduced bulky substituents and elongated the terminal side chain to diminish AURA and AURB activities. Third, to further improve pharmacokinetics, we substituted the benzyl group at the terminal moiety with a pyridyl group. These modifications led us to identify BPR1R024 (12) as our lead candidate.

### RESULTS AND DISCUSSION

#### Optimization of Drug-like Properties and Improvement of CSF1R Selectivity

To address the poor oral
bioavailability of BPR1K871 (9, F = 0%), we first replaced its flexible side chain with fluoro or methoxy groups to reduce the NRB from 11 to 7 to afford orally active compound 13 and its analogue 14 (Table 1). However, 13 was poorly active against CSF1R, and 14 was poorly selective for it. We further modified compound 14 to yield 15 by incorporating a 1,4-diaminobenzene linker. Both 14 and 15 showed similar (100 nM) potency against CSF1R. To our delight, the substitution of 1,4-diaminobenzene (15) dramatically diminished its AURA/B activities; therefore, 15 was selected for further optimization.

To understand the differences in the binding modes of the compounds incorporating the two types of linkers (formula I and II), we carried out molecular modeling studies of 14 and 15. Type II kinase inhibitors can be regarded as incorporating three structural moieties (Figure 3A): the scaffold moiety, which is implicated in hinge binding (an essential hydrogen-bonding interaction with the specific residue located in the hinge region, which is Thr664 and Cys666 for CSF1R); 23,44 a terminal moiety, which interacts with the kinase back pocket; and a linker moiety, which connects the scaffold and terminal moieties. The binding mode of 14 with CSF1R is shown in Figure 3B. Modeling studies indicated that the quinazoline scaffold of 14 formed a hinge interaction with the backbone of Cys666, while the urea moiety formed other hydrogen-bonding interactions with the side chains of Asp796, Glu633, and Met637, respectively. It is known that Asp796 is part of the Asp-Phe-Gly (DFG) motif, a key modulator affecting the active/inactive form of kinases in the activation loop. Glu633, a highly conserved residue on the αC helix, is involved in the binding of most type II kinase inhibitors. On the other hand, a binding mode analysis of 15 indicated that its binding conserved the key hydrogen-bonding interactions (with C666, D796, and E633), despite the decrease of NRB and the absence of heteroatom involvement (Figure 3C). Superimposition of 14 and 15 showed that both analogues occupied the same space in the binding pocket, especially the quinazoline and urea moieties (Figure 3D). Intriguingly, a methoxy group at the 7-position of the quinazoline scaffold was unexpectedly discovered to participate in an additional nonclassical hydrogen-bonding (NCHB) interaction with CSF1R. In the case of 14 and 15, the key methoxy group binds to the backbone of Gly669 and Cys666 in the hinge region of CSF1R (Figure 3B,C). These findings prompted us to investigate whether the NCHB at the 7-position of the quinazoline scaffold contributed to CSF1R inhibitory activity.

We undertook a SAR investigation of scaffold moieties with various substituents or side chains at the 4, 6, or 7-positions to optimize interactions with CSF1R (Table 1). First, we examined the role of nitrogen at the 4-position of the quinazoline ring in 15. Replacement of this nitrogen atom with an oxygen atom afforded compound 16 (IC_{50} = 76 nM), which was roughly twice as potent against CSF1R compared to 15. A similar trend was observed for 10 (incorporating an oxygen atom) vs 20 (with a nitrogen atom). Second, we performed structural modifications of 16 on the 7-position of the quinazoline. The introduction of a fluoro group (17) greatly weakened CSF1R inhibitory activity (IC_{50} = 753 nM), whereas the incorporation of nonsubstituted amino (18), N-methylamino (19), N,N-dimethylamino (10), or N-pyrrolidinyl (21) groups resulted in increased potency (IC_{50} = 58, 44, 21, and 63 nM, respectively). These data suggest that electron-donating groups (EDGs) such as alkoxy or amino groups are critical for CSF1R activity and that the increased potencies could be due to the enhancement of the NCHB interactions with increasing numbers of methyl groups. The introduction of dissubstituted methoxy groups at the 6- and 7-positions (22) conserved CSF1R activity (IC_{50} = 30 nM) but increased AURA/B inhibition. Finally, moving the N,N-dimethylamino group from the 7- (10) to the 6-position of the quinazoline (23) halved the CSF1R potency, and moving the pyrrolidinyl group to the 6-position (24) eliminated it.
Table 2. SAR Exploration of Substituent Effects on the Terminal Phenyl Group
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| comp. | R       | enzyme activity assay | cell-based assay |
|-------|---------|------------------------|------------------|
|       |         | CSF1R IC50/nM | AURA inh. @1 μM | AURB inh. @0.1 μM | Ba/F3-CSF1R EC50/nM | M-NFS-60 EC50/nM |
| 10    | H       | 21                  | 40%              | 61%              | 66                   | 92               |
| 25    | 2-Cl    | 15.4                | 43%              | 79%              | 66                   | 145              |
| 26    | 2-Cl    | 10.5                | 71%              | 82%              | 20                   | 55               |
| 27    | 4-Cl    | 16.4                | 70%              | 50%              | 18.4                 | 25               |
| 28    | 2-OMe   | 30                  | 24%              | 53%              | 116                  | 144              |
| 29    | 2-OMe   | 11.6                | 46%              | 79%              | 65                   | 108              |
| 30    | 4-OMe   | 14.1                | 23% (>10 μM)b    | 34% (222 nM)b    | 23                   | 23               |
| 31    | 3-CF3   | 64                  | 35%              | 60%              | 45                   | NDc               |
| 32    | 3-CF3   | 64                  | 46%              | 37%              | 49                   | NDc               |
| 33    | 3-Cl, 4-OMe | 11.1            | 53%              | 44%              | 43                   | 53               |
| 34    | 3-Cl, 6-OMe | 36              | 70%              | 41%              | 86                   | 141              |

The IC50 values and inhibition ratio of CSF1R, AURA, and AURB were performed using the in-house Kinase-Glo assay; pexidartinib and VX-680 were used as controls for the CSF1R and AURA/B inhibition test, and all data are expressed as the mean of at least two independent experiments and are mostly within 15% error margins. IC50 value. ND, not determined.

Figure 4. Schematic illustration of the binding model of 26 (orange) and 27 (light pink) to AURB kinase (PDB: 4C2V, light green) or CSF1R kinase (PDB: 3LCO, light blue). (A) Molecular docking of 26 into AURB. Hydrogen-bonding (green) and anion-π interactions (brown) are shown. (B) The hydrophobic Interactions of 26 with the back pocket of AURB. Ile142 (cyan) is highlighted at the end of the back pocket to show the limited space inside the back pocket. (C) Superimposition of molecular docking of 26 (orange) and 27 (light pink) into AURB; fewer interactions are associated between compound 27 and the residues in the back pocket compared to compound 26. (D) Binding of 27 in CSF1R along with the superimposition of AURB. The schematic illustration depicts the conformation differences between CSF1R and AURB. A-loop, activation loop; αC, αC helix.
Table 3. SAR Exploration at the Terminal Moiety

| comp. | n  | R            | enzyme activity assay | cell-based assay | clogP | LipE |
|-------|----|---------------|-----------------------|------------------|-------|------|
|       |    |               | CSF1R IC₅₀/nM | AURB inh.@1 μM | AURB inh.@0.1 μM | Ba/F3-CSF1R EC₅₀/nM | M-NFS-60 EC₅₀/nM |        |
| 10    | 0  | H             | 27        | 40%            | 61%             | 66             | 92             | 4.3     |
| 30    | 0  | p-OMe         | 14.1      | 23%            | 34%             | 23             | 23             | 4.3     |
| 35    | 1  | H             | 16.3      | 7%             | 15%             | 85             | 58             | 4.3     |
| 36    | 2  | H             | 15.4      | 35%            | 29%             | 82             | 162            | 4.7     |
| 37    | 1  | p-SO₂NH₂      | 16.3      | 2%             | 62%             | 662            | 1090           | 3.1     |
| 38    | 1  | p-CN          | 50        | 5%             | ND              | 14.1           | 23             | 4.2     |
| 39    | 1  | p-CF₃         | 91        | 16%            | 25%             | 152            | 97             | 5.2     |
| 11    | 1  | p-F           | 8.3       | 5%             | (>10 μM)*       | 27%            | 46             | 4.3     |
| 40    | 1  | p-OMe         | 12.9      | 6%             | 29%             | 91             | 81             | 4.3     |
| 41    | 1  | 3,4-dimethoxy | 83        | 4%             | ND              | 133            | ND*            | 4.3     |
| 42    | 1  | pentfluorour   | 18.6      | 3%             | 34%             | 108            | ND*            | 5.4     |

*The IC₅₀ values and inhibition ratio of CSF1R, AURA, and AURB were performed using the in-house Kinase-Glo assay; pexidartinib and VX-680 were used as controls for the CSF1R and AURA/B inhibition test, and all data are expressed as the mean of at least two independent experiments and are mostly within 15% error margins. **IC₅₀ value. ND, not determined. †Predicted by Discovery Studio 2020. LipE = pIC₅₀ − clogP.

Terminal phenyl groups could attenuate AURA/B inhibition. Thus, we explored the substituent effects on the terminal phenyl group to further increase the CSF1R selectivity. Table 2 summarizes these results (compounds 25–34). To validate the inhibitory activities of these compounds in cells, we evaluated their effect on the growth of two CSF1R-dependent mouse cell lines in vitro: Ba/F3-CSF1R, an artificial cell transfected with human CSF1R expressing the kinase domain of CSF1R; and M-NFS-60, a mouse myelogenous leukemia cell line. Our data suggested that compounds with either chloro (25–27) or methoxy groups (28–30) at different positions possessed similar CSF1R inhibitory potencies (10.5–30 nM) as well as potent double-digit nM inhibition of cellular activities in Ba/F3-CSF1R and M-NFS-60 cells. Notably, the AURA and AURB inhibitory potencies of compound 30 were about half (23% at 1 μM for AURA, 34% at 0.1 μM for AURB) those of 10 (40% at 1 μM for AURA, 61% at 0.1 μM for AURB). The introduction of an ortho-substituent (25 and 28) decreased the AURA potency due to its steric hindrance, consistent with our previous docking studies. In addition, compounds bearing substituents at the para-position exhibited lower AURB inhibitory activity compared to those bearing substituents at the ortho- and meta-positions. Similar results were obtained for 32 bearing a trifluoromethyl group at the 4-position of a terminal moiety, which exhibited lower potency against AURB than its 3-trifluoromethyl analogue 31. Nevertheless, both trifluoromethyl analogues (31 and 32) were about 5-fold less potent against CSF1R than the chloro analogues (26 and 27). We also evaluated the potency of disubstituted analogues, including 3-chloro-4-methoxy (33) and 3-chloro-6-methoxy (34). However, the introduction of meta-chloro substituents to these compounds (to give 33 and 34) led to increased inhibition of AURA. Collectively, through an in-depth evaluation of the effects of variously substituted terminal phenyl groups, we identified compound 30 as a potential lead for further development. To better understand how substituents at the para and meta-positions influence inhibition of AURB, we conducted additional molecular docking studies. Analysis of the binding between 26 and AURB (4C2V) revealed several hydrogen-bonding interactions between 26 and Ala173, Lys122, and Ala233 residues of AURB, along with an anion-π interaction with E141, a conserved residue on the αC helix (Figure 4A). The chloro group at the terminal phenyl group of 26 extended into the back pocket, which consists of a cluster of highly hydrophobic residues, including Leu124, Leu138, Ile142, Met156, and Leu168 (Figure 4B). Ile142 (Figure 4, cyan) located at the αC helix was found to obstruct the end of the back pocket, limiting the size of substituents that could be accommodated in it. Docking results demonstrated that compound 26 (bearing a meta-chloro substituent, shown in orange) could be accommodated in the pocket of AURB. In addition, the chloro substituent contributed to a number of hydrophobic interactions with the alkyl side chains of Leu138, Ile142, and Leu168 (Figure 4B). On the contrary, the steric hindrance between the para-chloro group of 27 and Ile142 precluded alignment of 27 into the back pocket, resulting in the loss of the interactions within the back pocket (Figure 4C). Superimposition of CSF1R and AURB shows the conformational differences in the activation loop (A-loop) of CSF1R and AURB, the latter of which has a narrower space between its αC helix and A-loop (Figure 4D). Due to the larger cleft in CSF1R, 27 could be accommodated, and the interactions with the αC helix of CSF1R were maintained, but this would be impossible with AURB (Figure 4D).

The SAR (Table 2) and modeling (Figure 4) studies suggest that the introduction of a sterically bulky substituent at either the ortho- or para-position of the terminal phenyl ring decreased AURA and AURB inhibition. To further diminish the AURA/B...
activities, we adopted a chain extension strategy to increase the steric hindrance in the back pocket. The phenyl moiety of 10 (n = 0) was replaced with benzyl (35, n = 1) and homobenzyl groups (36, n = 2). As seen in Table 3, both compounds 35 and 36 exhibited potent CSF1R inhibitory activity with IC50 values of 16.3 nM and 15.4 nM, respectively. In particular, 35 showed much weaker activities against both AURA (7% at 1 μM) and AURB (15% at 0.1 μM) compared to 36 (35% at 1 μM for AURA, 29% for AURB at 0.1 μM). Thus, further SAR investigation was focused on the benzyl groups (n = 1).

A set of derivatives of 35 bearing various substituents at the para-position of the benzyl moiety including p-sulfonamide (37), p-cyano (38), p-trifluoromethyl (39), p-fluoro (11), p-methoxy (40), 3,4-dimethoxy (41), and pentafluoro (42) groups were synthesized. Four of these analogues (11, 37, 40, and 42) exhibited strong potency against CSF1R (IC50 from 8.3 to 18.6 nM, Table 3), whereas the other three (38, 39, and 41) were 2 or 3-fold less potent against CSF1R compared to 10. More importantly, compared to 10 (62% at 0.1 μM) and 30 (34% at 0.1 μM), these derivatives did not exhibit significant inhibitory activity of AURA at 1 μM, except for 37 (62% at 0.1 μM), and AURB inhibitory activity was also greatly reduced (low to 27% of inhibition at 0.1 μM).

Calculated partition coefficient (clogP) and lipophilic efficiency (LipE) values were also calculated for selected analogues (Table 3) to further assess their pharmacokinetic properties. Compounds 11, 30, 35, and 40 had the best LipE values of about 3.5 to 3.6, and 11, 30, and 40 were selected for PK studies in rats. However, all three compounds (11, 30, and 40) showed moderate systemic drug exposure and poor oral bioavailability (F) (Table 5).

**Improvement of Pharmacokinetics.** We speculated that the poor oral bioavailability of these compounds could be due to their low water solubility—a consequence of their hydrophobic terminal groups. Accordingly, we sought to introduce more hydrophilic heteroaromatic rings into the structure, inspired by the side chains of pexidartinib (1) and Ki20227 (6). A set of pyridine analogues (12 and 43–45) were synthesized, and their CSF1R enzymatic and cellular inhibitory activities were evaluated. Compounds 12, 44, and 45 showed potent CSF1R inhibition in both the enzymatic and cellular assays (Table 4).

Interestingly, while the pyridine analogues (12, 43, and 45) and compounds bearing terminal phenyl groups (36 and 39–40, Table 3) were equally potent inhibitors of CSF1R, the pyridine analogues exhibited weaker AURA and AURB inhibitory activities. Also, compound 46 bearing the same side chain as Ki20227 (6) showed 6-fold less CSF1R inhibitory activity (IC50 = 141 nM) compared to its analogue 47 (IC50 = 24 nM). The difference in potency of these two compounds was attributed to the steric bulk of the additional methyl group at the benzylic
position of the thiazole ring in compound 46; the removal of this methyl group restored CSF1R potency (47). Replacement of the thiazole ring with a N-methyl-3-pyrazolyl ring led to a 20-fold loss of the CSF1R activity (48). In addition, we examined the inhibitory activities of pexidartinib (1) and Ki20227 (6) against CSF1R and AURB/B. In our investigation, Ki20227 (6) also exhibited moderate AURB activity with 47% inhibition at 6 μM. The data suggest that our compounds incorporating the privileged 7-aminoquinazoline scaffold were more selective for CSF1R over AURB/B.

Based on these results, pyridine analogues 12, 44, and 45 were selected for PK evaluation in rats (Table 5). Each compound was dissolved in DMA/PEG400 (1:4) and dosed at 5 mg/kg (IV) and 20 or 25 mg/kg (PO). Compound 12 exhibited the highest systemic drug exposure with the dose-normalized area under curve (DNAUC) values of 3635 ng/mL·h by the IV route and 362 ng/mL·h by the PO route. The oral bioavailability of 12 was about F = 10%. In an attempt to improve its oral bioavailability, 12 was converted to a mesylate salt (12-MsOH) and formulated in 30% 2-hydroxypropyl-β-cyclodextrin (HP-β-CD). These modifications resulted in a 3-fold increase in oral bioavailability of 12 (F = 35%). Based on the above findings, 12 was moved forward for further characterisation.

Kinase Profiling. To explore the selectivity of 12, it was assessed against a panel of 403 nonmutant kinases at a concentration of 1 μM (Figure 5) using KINOMEScan technology. The profiling revealed that 12 possessed a superior target selectivity with a score S(10) of 0.017. Only 7 kinases, including CSF1R, c-KIT, PDGFRβ, PDGFRα, DDR1, VEGFR2, and PAK3, were inhibited more than 90% (Table S2). Because KINOMEScan does not require ATP participation, results obtained from this method may be different from those obtained by the ATP competition assay. Thus, the radioisotope filtration binding assay HotSpot was introduced to evaluate the inhibitory activity of 12 against these 7 kinases along with FLT3 and AURB as described in Table 6.54

| kinase | IC50 (nM) | selectivity |
|--------|----------|-------------|
| CSF1R  | 0.53     |             |
| c-KIT  | 1.1      | 2.1x        |
| PDGFRα | 52.7     | 48x         |
| PDGFRβ | 31.5     | 59x         |
| DDR1   | 59.7     | 115x        |
| FLT3   | 64.3     | 121x        |
| AURB   | 226      | 426x        |
| PAK3   | >9000    | >15 000x    |
| VEGFR2 | >9000    | >15 000x    |

Table 5. PK Profile of the Promising Compounds in Rats

| comp. | IV (dose: 5 mg/kg) | PO (dose: 20 or 25 mg/kg) |
|-------|-------------------|---------------------------|
|       | t1/2 (h) | CL (mL/(min·kg)) | Vm (l/kg) | DNAUC(0→inf) (ng/mL·h) | Tmax (h) | DNAUC(0→inf) (ng/mL·h) | F % |
| 11    | 3.3      | 12.2             | 1.4       | 1225                  | 24.2     | 56                | 0.5  | 89 | 7.3 |
| 12    | 2.9      | 4.5              | 0.4       | 3635                  | 8.7      | 24                | 5.5  | 362 | 10.0 |
| 12-MsOH | 2.4    | 8.9              | 1.0       | 1687                  | 11.6     | 68                | 0.5  | 588 | 34.9 |
| 30    | 1.6      | 5.6              | 0.7       | 2842                  | 4.0      | 10.6             | 2.7  | 91  | 3.2 |
| 40-HCl| 20.6     | 16.5             | 9.0       | 1252                  | 6.7      | 1.7              | 6.0  | 15  | 1.6 |
| 44    | 10.5     | 5.6              | 1.5       | 1578                  | 2.4      | 21                | 3.2  | 164 | 10.4 |
| 45    | 1.2      | 24.7             | 1.4       | 767                   | 4.8      | 35                | 2.3  | 236 | 30.7 |

*1/2, half-life; CL, clearance; Vm, steady state distribution volume; DNAUC(0→inf), dose-normalized area under curve from zero to time infinity; DNCmax, dose-normalized maximum plasma concentrations; Tmax, time to Cmax; and F, oral bioavailability. *DNAUC(0→inf) = AUC(0→inf)/dose.

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| PDGFRβ | 31.5     | 59x         |
| DDR1   | 59.7     | 115x        |
| FLT3   | 64.3     | 121x        |
| AURB   | 226      | 426x        |
| PAK3   | >9000    | >15 000x    |
| VEGFR2 | >9000    | >15 000x    |

*The experiments were performed using the HotSpot kinase assay according to the manufacturer’s instructions;55 also, the control compound, staurosporine, was tested in a 10-μM mode with 4-fold serial dilution starting at 20 mM. Relative to IC50 of CSF1R.

Evaluation of In Vitro Cellular Activity of 12. The inhibition of CSF1R phosphorylation by 12 was studied in two
different cell lines (RAW264.7, a murine macrophage, and THP-1, a human acute monocytic leukemia with a high level of CSF1R expression) by western blotting, as shown in Figure 6. Compound 12 significantly suppressed the CSF1R signal in a dose-dependent manner, at concentrations of approximately 50−75 and 1−10 nM in RAW264.7 and THP-1 cells, respectively. In addition, to further understand whether our CSF1R inhibitor could affect cellular downstream signal transduction, we evaluated the inhibitory effect of 12 on LPS-induced TNF-α production in RAW264.7 cells. The study indicated that 12 exhibited similar potency with pexidartinib (1) to inhibit CSF1/CSF1R signaling-mediated TNF-α production (Figure 6C).

The target specificity of 12 was evaluated using murine bone marrow-derived macrophages (BMDM) that are polarized into the M1-like or M2-like phenotype. It is well-known that treatment with CSF1 drives macrophage polarization into M(CSF1) cells, which exhibit an M2-like phenotype and require CSF1R for survival (Figure 7A).14,55,56 In contrast, macrophages treated with CSF2 (also known as GM-CSF) would be polarized into M1-like phenotype M(CSF2) and are independent of CSF1R for growth. As seen in Figure 7B, 12 was associated with dose-dependent growth inhibition of the CSF1-induced M2-like macrophage (IC50 = 31 nM) but had little effect on CSF2-induced M1-like macrophage growth at the same concentration (IC50 = 5.4 μM, Figure 7C). Notably, 12 was a more potent inhibitor of CSF1-induced M2-like macrophage growth than pexidartinib (1, IC50 = 56 nM, Figure 7C). Collectively, these results demonstrate that 12 is a potent and specific inhibitor, with more than 200-fold specificity targeting CSF1/CSF1R signaling.

**Evaluation of In Vivo Immunomodulatory Activity of 12.** The immunomodulatory activity of 12 was evaluated in a subcutaneously inoculated murine colon tumor MC38 syngeneic model.22,36 MC38 tumor expresses a high level of CSF1 and high prevalence of TAM and is utilized to assess macrophage and immune homeostasis in vivo.57 Six-week-old male C57BL/6 mice were treated orally with 12·MsOH at 100 mg/kg twice a day (BID) with a five-days-on and two-days-off (FOTO) treatment schedule for two weeks. After the last day of treatment (day 15), tumor tissues from control and treated animals were harvested to evaluate the effect of 12·MsOH on...
the tumor immune microenvironment by flow-cytometry analysis. As seen in Figure 8A, 12·MsOH was associated with a 2.5-fold decrease in the percentage of M2 TAMs (CD206+ CD11b+ F4/80+) and a 3-fold increase in the percentage of M1 TAMs (CD86+ CD11b+ F4/80+). This modulation of the TME by 12·MsOH was further confirmed by a pronounced increase in the ratio of M1 TAMs to M2 TAMs macrophages and cytotoxic CD8+ T-cells to M2 TAMs within the tumor (Figure 8B).

**Figure 8.** In vivo immunomodulatory activity of 12·MsOH in the MC38 syngeneic model. (A) Effect of 12·MsOH on tumor-associated M1 and M2 cell populations; (B) Effect of 12·MsOH on the intratumoral M1/M2 ratio and M2/CD8+ ratio. (C) Effect of 12·MsOH on tumor growth and body weight changes. Mean ± standard error of the mean (SEM), n = 5 to 6 mice per group. *: p < 0.01 vs vehicle control by the nonparametric t-test.

**Scheme 1.** Syntheses of Building Blocks 51, 54a−c, and 57a−m.

Reagents and conditions: (a) CH2Cl2/N,N-dimethylformamide (DMF) (2:1), rt, 10 h, 90%; (b) pyridine, CH2Cl2, −30 °C to rt, overnight, 43–92%; (c) CoCl2·7H2O, NaBH4, Boc2O, EtOH, 0 °C to rt, overnight; (d) 4 N HCl in 1,4-dioxane, rt, 63–83% over 2 steps; (e) H2NOH·HCl, NaOH, EtOH/water (5:1), reflux, 0.5 h; (f) Zn, EtOH/AcOH (3:1), 60 °C, 1 h, 42–65% over 2 steps; and (g) 53, N,N-diisopropylethylamine (DIPEA), CH2Cl2, −60 to −20 °C, 0.5 h, 30–99%.

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Reagents and conditions: (a) formamidine acetate, EtOH, reflux, 82−95%; (b) 2 M dimethylamine in tetrahydrofuran (THF), ethylene glycol monomethyl ether (EGME), sealed tube, 140 °C, 94%; (c) (i) SOCl₂, DMF, PhMe, reflux, 90−98% or (ii) POCl₃, PhMe, reflux, 92−93%; (d) isopropanol (IPA), reflux, 87−98%; (e) 50a, CH₂Cl₂, rt, 81−83%; (f) 51, K₂CO₃, DMF, rt, 80−95%; and (g) (i) benzenophenone imine, t-BuXPhos Pd G3, Cs₂CO₃, THF, reflux; then H₂NOH·HCl, MeOH, rt, 1 h, 64% over 2 steps, (ii) 2 M methylene in THF, Brettphos Pd G3, Cs₂CO₃, THF, reflux, 73−81% or (iii) pyrrolidine, Ruphos Pd G3, Cs₂CO₃, THF, reflux, 68−88%; and (h) 37% HCHO solution, NaBH₃CN, AcOH, 78%.

(TGI) value of 59%. No severe body weight loss was observed during the treatment period (Figure 8C). Taken together, these data indicate that 12 is an effective immunomodulatory agent capable of inhibiting the tumor growth by remodeling the tumor microenvironment.

CHEMISTRY

For the syntheses of urea derivatives 10−48, three types of building blocks (51, 54a−c, and 57a−n) were first prepared as described in Scheme 1. The reaction of p-aminophenol (49) with phenyl isocyanate (50a) afforded urea 51. Anilines 52a−c were treated with p-nitrophenyl chloroformate (53) to afford p-nitrophenyl carbamates 54a−c. The reduction of aromatic nitriles 55a−c in the presence of sodium borohydride, Boc anhydride, and catalytic cobalt(II) chloride followed by the removal of Boc groups under acidic conditions provided 56a−c. The condensation of 55d−e with hydroxylamine hydrochloride followed by reduction with zinc gave amines 56d−e; 56f−m are commercially available. The treatment of amines 56a−n with p-nitrophenyl chloroformate (53) afforded the corresponding carbamates 57a−n.

For the synthesis of 6- or 7-substituted quinazolines 15−24, as depicted in Scheme 2, cyclization of commercially available anthranilic acids 59a−e with formamidine gave quinazolines 60a−e, which were chlorinated with SOCl₂ or POCl₃ to yield chloro derivatives 61a−f. Nucleophilic aromatic substitution (SNAr) of the corresponding chloro derivatives with phenol 51 under basic conditions afforded O-linked ureas 16−17, 21, whereas SNAr with 1,4-diaminobenzene under acid-catalyzed conditions followed by urea formation with isocyanate 50a gave N-linked ureas (15 and 20). The introduction of the amino groups was performed by Buchwald−Hartwig cross-coupling of bromoquinazolines (62 and 63) to synthesize 18, 19, 21, 23, 24, and 64. Coupling with benzenophenone imine using a third-generation t-BuXphos precatalyst followed by imine cleavage in the presence of hydroxylamine hydrochloride gave 18; coupling with pyrrolidine using a third-generation Ruphos precatalyst gave 21 and 24; and coupling with methylene using a third-generation Brettphos precatalyst gave 19 and 64. However, coupling with dimethylamine using Ruphos, t-BuXphos, or Xphos as a catalytic ligand failed to yield 23, which could be alternately obtained by reductive amination of aniline 64 with formaldehyde in good yield.

The synthesis of 7-(dimethylamino)quinazolines (10−12 and 25−48) is depicted in Scheme 3. Our attempts to produce 65 by SNAr of 61f with p-aminophenol (49) were unsuccessful, presumably due to the strongly electron-donating effect of the dimethylamino group. Instead, an O-selective catalytic coupling in the presence of CuI and 1,10-phen proceeded in good yield to obtain 65 as a key intermediate, which was reacted with the corresponding isocyanates 50a−j to give ureas 10, 25−31, and 35−36. Similarly, treatment with corresponding carbamates 54a−c and 57a−m obtained ureas 11−12, 31−33, 36−40, and 42−48. The addition reaction of 65 with an isocyanate prepared in situ by Curtius rearrangement from the commercial carboxylic acid 66 afforded urea 41.
CONCLUSIONS

In summary, we have described the discovery of BPR1R024 (12), an orally available CSF1R inhibitor exhibiting potent CSF1R inhibitory activity (IC\textsubscript{50} = 0.53 nM) and superior target selectivity against a panel of 403 kinases. BPR1R024 (12) was developed from BPR1K871 (9) by a series of physicochemical property-driven optimizations to improve the pharmacokinetics via NRB reduction, LipE calculation, hydrophilic heteroaromatic rings substitution, and salt composition and formulation modification. Structural studies reveal the differences in the back pockets of CSF1R and AURB kinases—the latter of which is restricted in size by key residue Ile142, which triggers a chain extension leading to the diminished AURA and AURB activities. Serendipitously, our molecular docking studies reveal that the unique 7-aminoquinazoline scaffold provides additional NCHB interactions with the CSF1R hinge region, contributing to the potency enhancement. The biological data demonstrate that the 7-aminoquinazolines show the increased CSF1R kinase selectivity over AURB and VEGFR2, compared to the conventional 6,7-dimethoxy quinolines or quinazolines. BPR1R024 (12) displayed strong cellular inhibition of CSF1R phosphorylation by Western blotting analysis. Moreover, BPR1R024 (12) specifically inhibits M(CSF1) macrophages with an IC\textsubscript{50} value of 24 nM and minimally affects M1-like macrophage growth in the murine bone marrow-derived macrophage (BMDM) assay. In vivo, treatment with BPR1R024 mesylate (12·MsOH) increased the ratio of M1 to M2 TAMs and demonstrated the antitumor efficacy (TGI = 59%) in the MC38 murine colon tumor model. Efforts to further demonstrate the preclinical potential of BPR1R024 (12) are currently underway.

EXPERIMENTAL SECTION

General Methods for Chemistry. All commercial chemicals and solvents are of reagent grade and were used without further purification unless otherwise stated. All reactions were monitored by thin-layer chromatography (TLC) using Merck 60 F254 silica gel aluminum plates, which were detected visually under UV irradiation (254 nm) or by staining with a solution of basic potassium permanganate or acidic p-anisaldehyde. Flash column chromatography was carried out using silica gel (Merck Grade 9385, 230–400 mesh). \textsuperscript{1}H, \textsuperscript{13}C, and \textsuperscript{19}F NMR spectra were recorded with Varian Mercury-300, Varian Mercury-400, Bruker AVANCE III 400, or Bruker AVANCE III 600 NMR spectrometers. Data for NMR spectra were analyzed with Mnova software (Mestrelab Research). Chemical shift (\(\delta\)) was reported in ppm and referenced to solvent residual signals as follows: DMSO-\textsubscript{d}6 at 2.50 ppm, CDCl\textsubscript{3} at 7.26 ppm, CD3OD at 3.31 ppm for \(\textsuperscript{1}H\) NMR; DMSO-\textsubscript{d}6 at 39.5 ppm, CDCl\textsubscript{3} at 77.0 ppm, for \(\textsuperscript{13}C\) NMR; and PhF at \(-113.15\) ppm for \(\textsuperscript{19}F\) NMR. Splitting patterns are indicated as follows: s = singlet; d = doublet; t = triplet; q = quartet; quint = quintet; dd = doublet of doublets; dt = doublet of triplets; td = triplet of doublets; tt = triplet of triplets; sq = quartet of doublets; dd = doublet of doublets of doublets; br = broad; app = apparent; m = multiplet, wherein a secondary AA’XX’ pattern (a group of 6 peaks) resulted from para-disubstituted phenyl groups was specifically assigned as AAXX’; Coupling constants (\(J\)) were given in Hertz (Hz). Low-resolution mass spectra (MS) data were measured with Agilent MSD-1100 series or Agilent Infinity II 1290 LC/MS (ESI) systems. High-resolution mass spectra (HRMS) data were measured with Sciex TripleTOF 6600.
The reaction mixture was stirred at reflux for 10 h. The precipitate was filtered off, washed with excess CHCl₃, and dried under vacuum to afford title compound as a white solid. HRMS (ESI) for \( \text{C}_{24}\text{H}_{23}\text{FN}_{5}\text{O}_{2}\) 
- \( \text{F} = 10.1\text{Hz}, \text{C} = 7.4\text{Hz} \), 1.2\text{Hz} \), 1H), 6.88 (d, \( \text{J} = 9.2\text{Hz}, 1\text{H} \), 8.00 (d, \( \text{J} = 8.3\text{Hz}, 1\text{H} \), 7.54–7.50 (AA’X’, 2H), 7.46 (d, \( \text{J} = 9.4\text{Hz}, 2.5\text{Hz} \), 1H), 7.22–7.18 (AA’XX’, 2H), 6.93 (t, \( \text{J} = 6.0\text{Hz}, 1\text{H} \), 6.83 (d, \( \text{J} = 2.5\text{Hz}, 1\text{H} \), 4.44 (d, \( \text{J} = 6.0\text{Hz}, 2\text{H} \), 3.20 (s, 6H), 2.34 (s, 3H). Purity: 99.2%.

1-(3-Chlorophenyl)-3-(5-(2-fluoroquinazolin-4-yl)-4,2-yl)ethyl)thiazol-2-yl)urea (13). The title compound was synthesized according to our previous publication.

1-(3-Chlorophenyl)-3-(5-(2-methoxyquinazolin-4-yl)-4,2-yl)ethyl)thiazol-2-yl)urea (14). The title compound was synthesized according to our previous publication.

1-(1-(4-(4,2-yl)-2-(2-methoxyquinazolin-4-yl)-4,2-yl)-2-(methoxyquinazolin-4-yl)oxy)phenyl)-3-phenylurea (15). Step 1: a mixture of 61a (300 mg, 1.54 mmol) and 1,4-diaminobenzene (500 mg, 4.62 mmol) in IPA (5 mL) was heated to refflux for 4 h. The resulting mixture was cooled to rt and concentrated to dryness. The residue was suspended in a stirred biocatalyst solution of diethyl ether and aqueous NaHCO₃. The mixture was filtered, washed with water and diethyl ether, and then dried under vacuum to afford N-(1-(methoxyquinazolin-4-yl)-benzene-1,4-diamine (355 mg, 87%) as a tan solid, which was used in the next step without further purification.

Step 2: a mixture of 61a (150 mg, 0.771 mmol), N1 (194 mg, 0.848 mmol), and K₂CO₃ (266 mg, 1.93 mmol) in DMF (2 mL) was stirred at rt for 4 h. The resulting mixture was filtered and washed with excess CHCl₃ to afford 61b (61 mg, 81%) as a white solid.

1-(1-(4-(4,2-yl)-2-(2-methoxyquinazolin-4-yl)-4,2-yl)-2-(methoxyquinazolin-4-yl)oxy)phenyl)-3-phenylurea (16). General Procedure for the Preparation of Compounds 16–17, 22, and 62–63. A suspension of 61a (150 mg, 0.771 mmol), N1 (194 mg, 0.848 mmol), and K₂CO₃ (266 mg, 1.93 mmol) in DMF (2 mL) was stirred at rt for 4 h. The resulting mixture was filtered and washed with Et₂O to afford crude product, which was triturated with EtOOAc to afford 16 (261 mg, 88%) as a white solid.

1-(1-(4-(4,2-yl)-2-(2-methoxyquinazolin-4-yl)-4,2-yl)-2-(methoxyquinazolin-4-yl)oxy)phenyl)-3-phenylurea (17). Following the general synthetic procedure as described for 16, chloro 61b was used to obtain 17 (55 mg, 81%) as a white solid.
reaction was cooled to rt, filtered through a pad of Celite, and concentrated in vacuo to give 1-(4-((7-(diphenylmethylene)amino)-quinazolin-4-yl)oxy)phenyl)-3-phenylurea as a crude, which was used in the next step without further purification. To a suspension of the crude in MeOH was added hydroxylamine HCl salt. The mixture was stirred at rt for 1 h, resulting in a clear yellow solution. 1 N NaOH was added to the solution until the color disappeared. The mixture was evaporated, and the residue was purified by flash column chromatography with MeOH/CH$_2$Cl$_2$ (1:50) to afford 1B (54 mg, 63% over 2 steps) as a white solid. 1H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.73 (s, 1H), 8.68 (s, 1H), 8.37 (s, 1H), 8.00 (d, $J = 8.9$ Hz, 1H), 7.54–7.49 (AA’X’, 2H), 7.49–7.44 (m, 2H), 7.32–7.25 (m, 2H), 7.20–7.14 (AA’X’, 2H), 7.04 (dd, $J = 8.9, 2.2$ Hz, 1H), 6.97 (tt, $J = 7.4, 1.2$ Hz, 1H), 6.83 (d, $J = 2.2$ Hz, 1H), 6.53 (s, 2H). 13C NMR (150 MHz, DMSO-d$_6$) $\delta$ 165.7, 154.4, 154.1, 153.7, 152.6, 146.9, 139.7, 137.0, 128.8, 124.4, 121.8, 119.3, 118.6, 110.2, 101.6, 99.2, 92.4. HRMS (ESI) for C$_{16}$H$_{13}$NO$_{2}$ $\text{[M + H]}^+$, calcd: 272.1155, found: 272.1149. Purity: 99.4%. 1H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.77 (s, 1H), 8.70 (s, 1H), 8.54 (s, 1H), 7.56 (s, 1H), 7.55–7.52 (AA’X’, 2H), 7.48–7.45 (m, 2H), 7.38 (s, 1H), 7.31–7.27 (m, 2H), 7.24–7.20 (AA’X’, 2H), 6.97 (tt, $J = 7.2, 1.2$ Hz, 1H), 3.98 (s, 3H). 13C NMR (151 MHz, DMSO-d$_6$) $\delta$ 165.0, 155.7, 152.6, 152.3, 150.0, 148.8, 146.7, 139.7, 137.2, 128.8, 122.4, 121.8, 119.3, 118.2, 109.7, 106.7, 100.7, 56.1, 56.0. HRMS (ESI) for C$_{15}$H$_{13}$NO$_{3}$Na $\text{[M + Na]}^+$, calcd: 439.1377, found: 426.1383. Purity: 99.6%. 1H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.87 (s, 1H), 8.70 (s, 1H), 8.54 (s, 1H), 7.55–7.52 (AA’X’, 2H), 7.48–7.45 (m, 2H), 7.38 (s, 1H), 7.31–7.27 (m, 2H), 7.24–7.20 (AA’X’, 2H), 6.97 (tt, $J = 7.2, 1.2$ Hz, 1H), 3.98 (s, 3H). 13C NMR (151 MHz, DMSO-d$_6$) $\delta$ 165.0, 155.7, 152.6, 152.3, 150.0, 148.8, 146.7, 139.7, 137.2, 128.8, 122.4, 121.8, 119.3, 118.2, 109.7, 106.7, 100.7, 56.1, 56.0. HRMS (ESI) for C$_{15}$H$_{13}$NO$_{3}$Na $\text{[M + Na]}^+$, calcd: 439.1377, found: 426.1383. Purity: 99.6%.

### JMC14900

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**1-(4-((7-Dimethylamino)quinazolin-4-yl)oxy)phenyl)-3-phenylurea (23).** To a solution of 63 (56 mg, 0.145 mmol) in MeOH (2 mL) and AcOH (2 mL) were added 37% formaldehyde (43 µL, 0.581 mmol) and NaBH$_4$CN (183 mg, 0.291 mmol) in 3 portions. The mixture was stirred for 4 h. The resulting reaction was quenched with aqueous NaHCO$_3$ and extracted with EtOAc. The organic layer was washed with 2% aqueous NaHCO$_3$ and brine, dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo. The residue was purified by preparative column chromatography with MeOH/CH$_2$Cl$_2$ (1:1) to afford 23 (68 mg, 88% yield) as a white solid.

**1-(2-Chlorophenyl)-3-(4-((7-(dimethylamino)quinazolin-4-yl)oxy)phenyl)-3-phenylurea (25).** Following the general synthetic procedure as described for 10, isocyanate 50d was used to obtain 25 (18 mg, 73%) as a white solid.

**1-(3-Chlorophenyl)-3-(4-((7-(dimethylamino)quinazolin-4-yl)oxy)phenyl)-3-phenylurea (26).** Following the general synthetic procedure as described for 10, isocyanate 50d was used to obtain 26 (24 mg, 68%) as a white solid.

**1-(4-((7-(diphenylmethylene)amino)-quinazolin-4-yl)oxy)phenyl)-3-phenylurea (21).** To a suspension of 62 (100 mg, 0.230 mmol), Cs$_2$CO$_3$ (112 mg, 0.345 mmol), Ruphos Pd G3 (9.6 mg, 0.011 mmol), and Ruphos (10.7 mg, 0.023 mmol) in degassed THF (3.5 mL) was added pyridinylidine (24 µL, 0.287 mmol) under an Ar atmosphere. The reaction mixture was heated to reflux for 1 h. The resulting reaction was cooled to rt, filtered through a pad of Celite, and concentrated in vacuo. The residue was purified by column chromatography with MeOH/CH$_2$Cl$_2$ (1:50) to afford 21 (104 mg, 88%) as a white solid.

**1-(2-Chlorophenyl)-3-(4-((7-(dimethylamino)quinazolin-4-yl)oxy)phenyl)-3-phenylurea (25).** Following the general synthetic procedure as described for 10, isocyanate 50d was used to obtain 25 (18 mg, 73%) as a white solid.
1H), 8.45 (s, 1H), 8.41 (d, J = 9.2 Hz, 1H), 7.59 (br d, J = 8.0 Hz, 1H), 7.56−7.50 (m, 3H), 7.33−7.29 (m, 2H), 7.23−7.19 (AA′XX, 2H), 6.88 (d, J = 2.4 Hz, 1H), 3.12 (s, 6H). C24H24N5O3 [M + H]+, calcd: 434.1784, found: 434.1802. Purity: 98.9%.

Scheme 2A. Following the general synthetic procedure as described for 10, isocyanate 50f was used to obtain 29 (49 mg, 91%) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 8.74 (s, 1H), 8.45 (s, 1H), 8.11 (d, J = 9.2 Hz, 1H), 7.53−7.49 (AA′XX, 2H), 7.39−7.35 (AA′XX, 2H), 7.30 (d, J = 9.2, 2.6 Hz, 1H), 7.26−7.19 (AA′XX, 2H), 6.89−6.85 (m, 3H), 3.72 (s, 3H), 3.12 (s, 6H). C24H21F3N5O2 [M + H]+, calcd: 439.1877, found: 439.1911. Purity: 99.0%.

Scheme 2B. Following the general synthetic procedure as described for 10, isocyanate 50g was used to obtain 30 (157 mg, 80%) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 8.69 (s, 1H), 8.52 (s, 1H), 8.45 (s, 1H), 8.11 (d, J = 9.2 Hz, 1H), 7.69−7.63 (m, 4H), 7.56−7.50 (m, 3H), 7.33−7.29 (m, 2H), 7.23−7.19 (AA′XX, 2H), 6.88 (d, J = 2.4 Hz, 1H), 3.12 (s, 6H). C24H23ClN5O3 [M + H]+, calcd: 439.1877, found: 439.1911. Purity: 99.0%.

Scheme 3A. Following the general synthetic procedure as described for 10, isocyanate 50h was used to obtain 31 (59 mg, 89%) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 8.97 (s, 1H), 8.88 (s, 1H), 8.45 (s, 1H), 8.11 (d, J = 9.2 Hz, 1H), 7.69−7.63 (m, 4H), 7.56−7.50 (m, 3H), 7.33−7.29 (m, 2H), 7.23−7.19 (AA′XX, 2H), 6.88 (d, J = 2.6 Hz, 1H), 3.12 (s, 6H). C24H21F3N5O2 [M + H]+, calcd: 439.1877, found: 439.1911. Purity: 99.0%.

Scheme 3B. Following the general synthetic procedure as described for 10, isocyanate 50i was used to obtain 32 (37 mg, 72%) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 8.97 (s, 1H), 8.88 (s, 1H), 8.45 (s, 1H), 8.11 (d, J = 9.2 Hz, 1H), 7.69−7.63 (m, 4H), 7.56−7.50 (m, 3H), 7.33−7.29 (m, 2H), 7.23−7.19 (AA′XX, 2H), 6.88 (d, J = 2.6 Hz, 1H), 3.12 (s, 6H). C24H21F3N5O2 [M + H]+, calcd: 439.1877, found: 439.1911. Purity: 99.0%.

Scheme 4A. Following the general synthetic procedure as described for 11, carbamate 54a was used to obtain 33 (33 mg, 77%) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 8.76 (s, 1H), 8.67 (s, 1H), 8.45 (s, 1H), 8.11 (d, J = 9.2 Hz, 1H), 7.67 (d, J = 2.6 Hz, 1H), 7.53−7.49 (AA′XX, 2H), 7.30 (d, J = 9.2, 2.5 Hz, 1H), 7.28 (d, J = 9.0, 2.6 Hz, 1H), 7.21−7.17 (AA′XX, 2H), 7.09 (d, J = 9.0 Hz, 1H), 6.88 (d, J = 2.5 Hz, 1H), 3.81 (s, 3H), 3.12 (s, 6H). C24H21F3N5O2 [M + H]+, calcd: 439.1877, found: 439.1911. Purity: 99.0%.

Scheme 4B. Following the general synthetic procedure as described for 11, carbamate 57a was used to obtain 39 (29 mg, 60%) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 8.75 (s, 1H), 8.69 (s, 1H), 8.45 (s, 1H), 8.41 (d, J = 9.2 Hz, 1H), 7.74−7.69 (m, 3H), 7.67 (d, J = 2.6 Hz, 1H), 7.53−7.49 (AA′XX, 2H), 7.30 (d, J = 9.2, 2.6 Hz, 1H), 7.28 (d, J = 9.0, 2.6 Hz, 1H), 7.21−7.17 (AA′XX, 2H), 7.09 (d, J = 9.0 Hz, 1H), 6.88 (d, J = 2.5 Hz, 1H), 3.81 (s, 3H), 3.12 (s, 6H). C24H21F3N5O2 [M + H]+, calcd: 439.1877, found: 439.1911. Purity: 99.0%.

13C NMR (100 MHz, DMSO-d6) δ 165.9, 154.3, 153.9, 153.3, 152.7, 149.6, 146.9, 137.0, 133.5, 124.1, 122.3, 120.8, 120.1, 119.3, 118.3, 116.0, 113.1, 106.0, 103.8, 56.2, 39.8. HRMS (ESI) for C24H21F3N5O3 [M + H]+, calcd: 446.1844, found: 446.1859. Purity: 98.6%.
1H), 8.43 (s, 1H), 8.10 (d, J = 9.2 Hz, 1H), 7.71 (t, J = 4.8 Hz, 1H), 7.50 (d, J = 9.2 Hz, 1H), 7.48 (t, J = 4.8 Hz, 1H), 7.14 (d, J = 5.5 Hz, 1H)
°C NMR (101 MHz, DMSO-d6) δ 165.9, 156.3, 155.3, 153.9, 153.3, 148.0, 146.4, 137.8, 135.4, 132.8, 124.1, 122.7, 118.8, 115.9, 106.0, 103.9, 40.3, 39.8, 23.7. HRMS (ESI) for C23H23N4O4 [M + H]+: calcd: 429.2034, found: 429.2061. Purity: 99.86%.

1-(4-(Dimethylamino)quinazolin-4-yl)-3-(3-(thiazoxy)pyridin-3-yl)methylurea (45). Following the general synthetic procedure as described for 11, carbamate 57b was used to obtain 45 (158 mg, 76%) as a white solid.

Following the general synthetic procedure as described for 11, carbamate 57e was used to obtain 47 (33 mg, 73%) as a white solid.

Purity: 99.6%.

1-(4-(Dimethylamino)quinazolin-4-yl)-3-(3-(thiazoxy)pyridin-3-yl)methylurea (45). Following the general synthetic procedure as described for 11, carbamate 57e was used to obtain 47 (33 mg, 73%) as a white solid.

Following the general synthetic procedure as described for 11, carbamate 57e was used to obtain 47 (33 mg, 73%) as a white solid.

Purity: 99.6%.

1-(4-(Dimethylamino)quinazolin-4-yl)-3-(3-(thiazoxy)pyridin-3-yl)methylurea (45). Following the general synthetic procedure as described for 11, carbamate 57e was used to obtain 47 (33 mg, 73%) as a white solid.

Following the general synthetic procedure as described for 11, carbamate 57e was used to obtain 47 (33 mg, 73%) as a white solid.

Purity: 99.6%.
Discovery Studio 2020 with default parameters. The decision of the best pose was according to the lowest binding energy of the compound. The docking result was shown as the cartoon model processed by Discovery Studio 2020.

**In Vitro Kinase Activity Assay.** In Vitro CSF1R ATP Kinase-Glo Activity Assay. The recombinant GST-CSF1R (residues LS34-C972, NP_052022.2) containing kinase domain was expressed in Sf9 insect cells. The CSF1R ATP Kinase-Glo assay was carried out in 96-well plates at 30 °C for 180 min and tested the compound in a final volume of 50 μL, including the following components: 25 mM Tris–HCl pH 7.4, 4 mM MnCl₂, 10 mM MgCl₂, 0.01% bovine serum albumin (BSA), 0.5 mM Na₂VO₄, 0.02% Triton X-100, 8 mM ATP, 2 mM (25,35)-1,4-bis(sulanybutane-2,3-diol (DTT)) and 6 μM poly(Glu,Tyr) 4:1 peptide, and 400 ng recombinant GST-CSF1R proteins. Following incubation, 50 μL of Sx diluted Kinase-Glo Plus Reagent (Promega, Madison, WI) was added, and the mixture was incubated at 25 °C for 20 min. A 70 μL of an aliquot of each reaction mixture was transferred to a black microliter plate, and the luminescence was measured on a Wallac 1420 multilabel counter (PerkinElmer).

**In Vitro AURA and AURB ATP Kinase-Glo Activity Assay.** The AURKA ATP Kinase-Glo and AURKB ATP Kinase-Glo assays for the black microliter plate, and the luminescence was measured on a Wallac and then the cells were primed with LPS (1 μg/mL) for 24 h. The supernatants were collected and then measured using the mouse TNF-α ELISA kit.

**In Vitro Rat Microsomal Stability Assay.** The incubation mixture, in 74 mmol potassium phosphate buffer (pH 7.4), contained: microsomal proteins, 0.5 mg/mL; NADPH, 3 mMol; MgCl₂, 3 mMol; test compound, 1 μM. Incubation was carried out, in triplicate, aerobically at 37 °C with constant shaking on a temperature-controlled heating block. The reaction was started by the addition of NADPH after preincubating the reaction mixture (without NADPH) for 10 min at 37 °C. Control incubation without NADPH was performed as described above. At 0, 5, 10, 20, and 30 min after the start of the reaction, an aliquot of the incubation mixture was taken from each incubation and mixed with ice-cold acetonitrile to terminate the reaction. Before analysis, the sample was precipitated by centrifugation at room temperature. The remaining supernatant was analyzed for the concentration of each compound to determine the metabolic rate.

**Pharmacokinetics.** Male Sprague Dawley rats (300–400 g) were obtained from BioLASCO Taiwan Co., Ltd. (Ilan, Taiwan). The animal studies were performed according to NHRI institutional animal care and committee-approved procedures. Rats were surgically prepared with a jugular-vein cannula 1 day before dosing. Rats were fasted overnight (for approximately 18–20 h) before dosing. Water was available *ad libitum* throughout the experiment. Food was provided at 4 h after dosing. A single 5.0 and 20/25 mg/kg dose of a compound, as a PEG400/DMA (80/20, v/v) or 30% HP-β-CD solution for both IV and oral PEG400/DMA (80/20, v/v) or 30% HP-β-CD solution, was separately administered to rats. The groups consisted of three rats each route and a total of 6 for intravenously (IV) and oral gavage (PO), respectively. Each rat received 1 or 10 mL of the dosing solution/kg of body weight of dosing solution by intravenous injection and by gavage, respectively. At 0 (before dosing), 2, 5 (IV only), 15, and 30 min and at 1, 2, 4, 6, 8, and 24 h after dosing, a blood sample (0.15 mL) was collected from each rat through the jugular-vein cannula and stored in ice (0–4 °C). Immediately after collecting the blood sample, 150 mL of physiological saline (containing 30 Units of heparin per mL) was injected into the rat through the jugular-vein cannula. Plasma was separated from the blood by centrifugation (14 000 g for 15 min at 4 °C in a Beckman Model Allegra 6R centrifuge) and stored in a freezer (−80 °C). All samples were analyzed for the parent drug by liquid chromatography–mass spectrometry (LC–MS)/MS. Plasma concentration data were analyzed with a noncompartmental method.

**Bone Marrow-Derived Macrophage Assay.** Femur and tibia bones from 6 to 8-week-old male C57BL/6 mice were harvested. Bone marrow was flushed with cold PBS containing 2% heat-inactivated fetal bovine serum (FBS) and passed through a 21-gauge needle several times to dissociate the cells. Thereafter, the cell suspension was filtered through a 70 μm cell strainer to remove cell clumps, bone, hair, and other cell/tissue materials. The cells were incubated with NH₄Cl solution to remove red blood cells and resuspended in cold PBS plus 2% heat-inactivated FBS. Aliquots of the cell suspension were cultured in DMEM medium containing 10% FBS supplemented with either 10 ng/mL recombinant mouse CSF1 (R&D Systems) or 10 ng/mouse CSF2 (R & D Systems) for 7 days to obtain CSF1- or CSF2-induced bone marrow-derived macrophages (BMDM), M(CSF1), or M(CSF2), respectively. To determine the effect of 12% on the growth of M(CSF1) and M(CSF2) in vitro, cell suspensions were seeded onto a 96-well plate (5,000 cells per well) and incubated with a drug-containing medium at various concentrations for 3 days in a humidified incubator (37 °C, 5% CO₂). Cell proliferation was measured using a WST-8 assay kit (Cayman Chemical) as per the manufacturer’s instructions. Absorbance at 450 nm was measured using a microplate reader.

**Animal Studies.** Male C7BL/6 mice between 6 and 7 weeks of age were used. MC38 murine colon cancer cells were prepared at 10⁵ cells per mouse in 100 μL of culture medium and implanted subcutaneously into the left flank region of mice with a 25/5-gauge needle. Tumor cells were detected as free of *Mycoplasma* sp prior to injection into animals. Treatment was initiated after randomization when the average tumor size reached approximately 90–100 mm³. Animals received either vehicle control (30% HP-β-CD) or 12-MsOH at 100 mg/kg twice a day (BID) with a five-days-on and two-days-off (FOTO) schedule.
treatment schedule for 2 weeks. Tumor growth was measured with an electronic caliper, and volumes were calculated as \( V = \frac{4}{3} \pi \times W \times H \times \frac{W}{2} \).

Tumor size and animal body weight were measured once a week after tumor cell inoculation. The nonparametric t-test was used for statistical evaluation between treatment and control. The level of statistical significance was set at \( p < 0.05 \). % Tumor growth inhibition (% TGI) = \[ 1 - \frac{\Delta V}{\Delta C} \times 100 \], where \( \Delta V = \) difference of average tumor volume on the measured day and day 0 of treated groups, and \( \Delta C = \) difference of average tumor volume on the measured day and day 0 of control groups. The uses and experimental procedures in animals were approved by the Institutional Animal Care and Use Committees (IACUCs) of the National Health Research Institutes. All animals received humane care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals”.

Flow-Cytometry Analysis. At the end of the 2-week treatment, tumor samples from vehicle control and 12-MOS-treated animals were harvested. Non-necrotic tissues were carefully removed from the tumors followed by digestion with 0.1% collagenase III in DMEM medium for 30 min at 37 °C with agitation every 10 min. The digestion was terminated by adding 10% FBS containing PBS. The single-cell suspension was filtered through a 40 μm filter after red blood cell lysis. Aliquots of the cell suspension were preincubated with the mouse Fc receptor blocker for 10 min before staining with appropriate anti-mouse antibody conjugate for Cd45, Cd3, Cd4, Cd8, Cd11b, Cd86, and F4/80 (all antibody conjugates from BioLegend) at 4 °C for 30 min followed by two washes in 1% BSA containing buffer and analyzed by flow cytometry to quantify the accumulation of infiltrating immune cells into the tumor. For analysis of Cd206, the cells were fixed using the Cyto X/Cytoperm kit (BD Biosciences) after surface marker staining and then stained with anti-mouse Cd206 antibody.

**ASSOCIATED CONTENT**

1. Supporting Information
   The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c01006.
   Supplementary PK data; kinase profiling data; synthetic procedure of the intermediates; HPLC trace for the lead compound; and synthesized compound NMR spectra (PDF)
   Molecular formula strings (CSV)

**AUTHOR INFORMATION**

**Corresponding Author**

Hsing-Pang Hsieh — Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 350, Taiwan, ROC; Department of Chemistry, National Tsing Hua University, Hsinchu City 300, Taiwan, ROC; Biomedical Translation Research Center, Academia Sinica, Taipei City 115, Taiwan, ROC; orcid.org/0000-0002-1332-055X; Phone: +886-37-206-166; Email: hphsieh@nhri.edu.tw, alexhsieh@gate.sinica.edu.tw

**Authors**

Kun-Hung Lee — Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 350, Taiwan, ROC; Department of Chemistry, National Tsing Hua University, Hsinchu City 300, Taiwan, ROC; Biomedical Translation Research Center, Academia Sinica, Taipei City 115, Taiwan, ROC

Wan-Ching Yen — Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 350, Taiwan, ROC

Wen-Hsing Lin — Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 350, Taiwan, ROC

Pei-Chen Wang — Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 350, Taiwan, ROC

You-Liang Lai — Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 350, Taiwan, ROC

Yu-Chieh Su — Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 350, Taiwan, ROC

Chun-Yu Chang — Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 350, Taiwan, ROC

Cai-Syun Wu — Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 350, Taiwan, ROC

Ling-Hui Chou — Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 350, Taiwan, ROC

Teng-Kuang Yeh — Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 350, Taiwan, ROC

Chiu-Tong Chen — Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 350, Taiwan, ROC

Chuan Shih — Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 350, Taiwan, ROC

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**ABBREVIATIONS**

aa, amino acid; AcOH, acetic acid; A-loop, activation loop; AURA, Aurora A kinases; AURB, Aurora B kinases; BID, twice a
day; BMDM, bone marrow-derived macrophages; BSA, bovine serum albumin; c-KIT, stem-cell factor receptor; clogP, calculated partition coefficient; CSF1, colony-stimulating factor-1; CSFIR, colony-stimulating factor-1 receptor; CTLA4, T-lymphocyte-associated antigen 4; DDR1, discoidin domain receptor 1; DFG, Asp-Phe-Gly; DMA, N,N-dimethylecetamide; DMF, N,N-dimethylformamide; DNAUC, dose-normalized area under curve; DPPA, diphenylphosphoryl azide; DTT, (25,35)-1,4-bis(sulfanyl)butane-2,3-diol; EGME, ethylene glycol monomethyl ether; EtOH, ethanol; ETV6, ETS variant transcription factor 6; F, oral bioavailability; FBS, fetal bovine serum; FLT3, FMS-like tyrosine kinase-3; HP-β-CD, 2-hydroxypropyl-β-cyclodextrin; FOTO, five-days-on, two-days-off; IC50, half maximal inhibitory concentration; ICIs, immune checkpoint inhibitors; IL-34, interlukin-34; IND, investigational new drug; IPA, isopropanol; IV, intravenous administration; LipE, lipophilic efficiency; MS3A, 3 A molecular sieves; MsOH, methanesulfonic acid; NCHB, nonclassical hydrogen bonding; Lipt, lipophilic e

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