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# Arylcarboxyamino-substituted diaryl ureas as potent and selective FLT3 inhibitors

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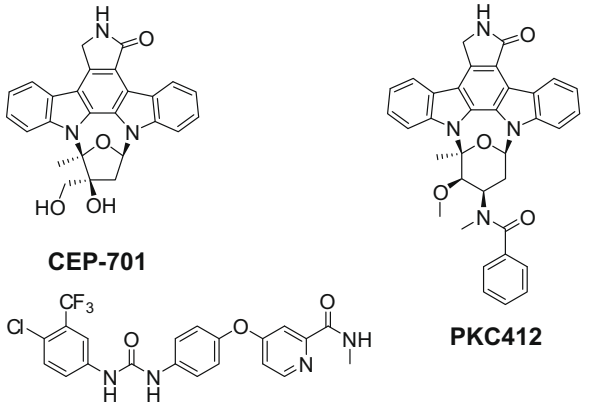
ABSTRACT

A series of diaryl ureas with an amide substitution at the 4-position was prepared and found to be potent and selective FLT3 inhibitors with good oral bioavailability and efficacy in a tumor xenograft model.



FMS-related tyrosine kinase 3 (FLT3) is a transmembrane tyrosine kinase that is expressed on dendritic cells, immature hematopoietic progenitors and also on some mature myeloid and lymphoid cells.[1] Activation of FLT3 is found to play a significant role in maturation and proliferation of these cell types. In acute myeloid leukemia (AML), FLT3 is expressed in myeloid cells in approximately 90% of the patients and this kinase is responsible for survival and proliferation of leukemic blasts.[2] Activating mutations of FLT3 are present in approximately one-third of all AML patients and are associated with adverse clinical outcome. FLT3 internal tandem duplications (ITDs) are associated with a poor prognosis in AML. Inhibition of the kinase activity of FLT3 using small molecule inhibitors has the potential to reduce leukemic blasts and hence control the disease in AML patients.[3]

A number of small molecule inhibitors of FLT3, such as CEP-701, PKC-412, MLN-518, sunitinib (SU-11248), and sorafenib (BAY-43-9006) have been evaluated in preclinical as well as clinical setting.[4,5] These FLT3 inhibitors are found to be less than optimal because of lack of sufficient potency or sub-optimal oral bioavailability or lack of adequate tolerability at efficacious doses. This Letter describes a novel class of FLT3 inhibitors that are highly potent, significantly more selective, and orally bioavailable with desirable efficacy and tolerability in animals.

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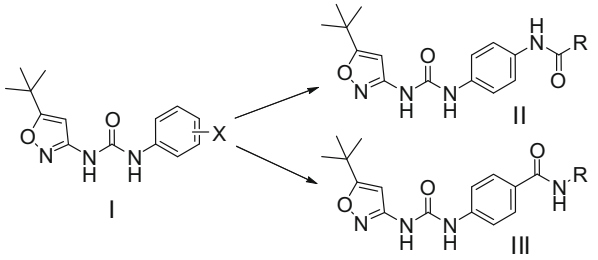
Profiling of small kinase focused libraries using KinomeScan™, a novel kinase screening platform to evaluate the binding affinity of small molecules to the active site of a large number of kinases[6], led us to identify urea derivatives, I, as potent FLT3 inhibitors. Evaluation of the effect of X on the FLT3 inhibitory activity indicated that compounds with an amino-carbonyl moiety at the para-position, like in II, have improved cellular activity over the carboxamides III. This Letter describes the SAR generated for the amide–urea series culminating in the identification of compounds **13 (AB460)** and **14 (AB530)**, which were found to be potent and selective FLT3 inhibitors with excellent pharmacokinetic properties and efficacy in a human tumor xenograft model in mice.

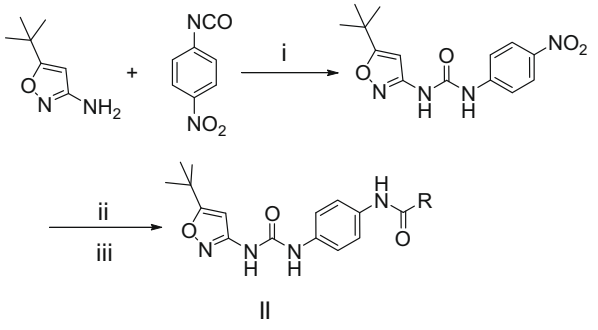


The amide (NHCO)–urea series of compounds, II, were prepared by condensing 3-amino-5-*t*-butyl-isoxazole with *p*-nitrophenyl isocyanate, followed by reduction of the nitro group to the corresponding amine and coupling of the amine with a variety of carboxylic acids (Scheme 1). The commercially unavailable carboxylic acid **1** was prepared as shown in Scheme 2. 2-Amino benzothiazole was condensed with ethyl bromopyruvate to give ethyl imidazo[2,1-*b*][1,3]benzothiazole-2-carboxylate, which was saponified to give **1**. The amide (CONH)-urea series of compounds, III, was prepared using the general synthetic sequence shown in Scheme 3. 5-*t*-Butyl-3-isoxazole isocyanate was condensed with 4-aminobenzoic acid, which was coupled with a number of commercially available amines to yield III.

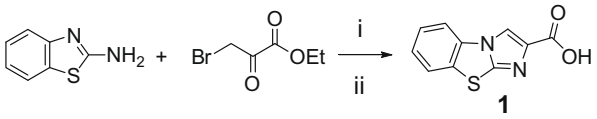
The binding affinity was measured for the catalytic domain (amino acids 592–969) of FLT3. The compounds which had desired binding affinity for the enzyme were tested for their ability to inhibit the proliferation of MV4-11 cells derived from AML patients.

Amide analogs of II derived from alkyl or substituted alkyl carboxylic acids were in general less potent in binding to the enzyme as shown for compounds **2** and **3** in Table 1.

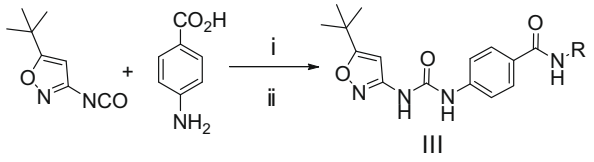
Therefore, the chemistry efforts focused on amide analogs derived from aryl carboxylic acids, which bound to FLT3 with high potency. A large number of aryl amides were prepared and the examples in Table 1 highlight the observed SAR. The phenyl and naphthyl amides, **4** and **5**, were potent in binding to the enzyme, with the naphthyl amide showing nearly 10-fold increased potency



**Scheme 1.** Reagents and conditions: (i) toluene, 100 °C, 97%; (ii) Pd/C, THF, water, >99%; (iii) RCOOH, CDI, DMF, 65–75%.



**Scheme 2.** Reagents and conditions: (i) EtOH, 90 °C, 28%; (ii) LiOH, THF, water, 80%.



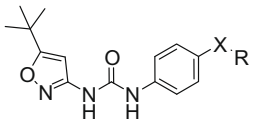
**Scheme 3.** Reagents and conditions: (i) DMA, 90 °C, 84%; (ii) $RNH_2$, HATU, DMA, 10–24%.

for inhibiting MV4-11 proliferation. Among the three positional isomers of the pyridyl amides, **6** with the 2-pyridyl substitution inhibited the cell proliferation significantly more potently than the other isomers, **7** and **8** (1.6 nM vs 159 nM and 132 nM). Introducing a hydroxyl group on the pyridine ring gave the two isomers, **9** and **10**. Compound **10** was significantly more potent in cells with sub-nanomolar $IC_{50}$ for inhibiting cell proliferation. It is not clear if the slight difference in intramolecular hydrogen bonding pattern contributes to the unexpected increase in cell penetration and potency of **10** over **9**. The 2-substituted benzothiophene analog, **11**, also inhibited MV4-11 cell proliferation with sub-nanomolar $IC_{50}$. Since the 2-pyridyl amide was superior to the corresponding 3- and 4-isomers, we prepared **12** and **13 (AB460)**, two isoquinoline amides and found that, of the two, **13** was significantly more potent than **12** in the cell proliferation assay. Comparison of the cell potency of **4** vs **5** and **11** and of **6** vs **13** and **12** allows one to point out that adding hydrophobicity at this position increases cell penetration and that the topology of the added hydrophobicity makes a significant difference to the cell penetration while having little effect on enzyme binding. Adding additional hydrophobicity through the imidazo-benzothiazole ring of **14 (AB530)** was not detrimental and retained the binding and cell potency. The amides **10**, **13** and **14** were found to be the most potent FLT3 inhibitors as measured by their enzyme binding affinity and ability to inhibit proliferation of MV4-11 cells. A hydrogen bond donor heteroatom at the ortho-position of the carboxamide linkage appears to impart better cell penetration (compound **4** vs **6** and **10**; compound **5** vs **11**, **13** and **14**). Compounds **13** and **14** appear to be more potent in the MV4-11 cell proliferation assay compared to their enzyme binding affinity. The likely reasons for this observation include changes in enzyme conformation from in vitro system to cells. It is also worth noticing that the cell proliferation assay was carried out in the presence of 0.5% serum protein. The $IC_{50}$ for the inhibition of proliferation decreased significantly for all compounds in the presence of 10% serum protein.

A number of amide analogs of the type III (compounds **15**–**18**) were prepared and found to have potent enzyme binding affinity but significantly lower potency in inhibiting cell proliferation.

Every compound was screened initially at 10 μM against a panel of at least 40 kinases[7] using the KinomeScan™ platform. A subset of the compounds was tested further to obtain a dose–response and $K_d$ for the kinases in the panel. In general, the amides of Type II were very selective with potent binding affinity restricted to the Type III RTK family of kinases, that is, FLT3, KIT, CSF1R, RET, PDGFRα and PDGFRβ. In particular, compounds 13 and 14 were found to be highly selective in a panel of 402 kinases with $K_d$ of

**Table 1**
The SAR of FLT3 inhibitors



| Compd | X | R | FLT3 $K_d$[a] (nM) | MV4-11 $IC_{50}$[a] (nM) |
|---|---|---|---|---|
| **2** | NHCO | Benzyl | 510 | 1090 |
| **3** | NHCO | 4-Piperidyl | 240 | 10,500 |
| **4** | NHCO | Phenyl | 0.98 | 30 |
| **5** | NHCO | 2-Naphthyl | 0.75 | 3.1 |
| **6** | NHCO | 2-Pyridyl | 0.76 | 1.6 |
| **7** | NHCO | 3-Pyridyl | 15 | 159 |
| **8** | NHCO | 4-Pyridyl | 10.1 | 132 |
| **9** | NHCO | 6-Hydroxy, 2-pyridyl | 2.5 | 46.7 |
| **10** | NHCO | 3-Hydroxy, 2-pyridyl | 0.74 | 0.71 |
| **11** | NHCO | 5-Methyl, 2-benzo[*b*]thienyl | 0.77 | 0.85 |
| **12** | NHCO | 1-Isoquinolyl | 0.74 | 2.8 |
| **13 (AB460)** | NHCO | 3-Isoquinolyl | 1.1 | 0.29 |
| **14 (AB530)** | NHCO | 2-Imidazo[2,1-*b*][1,3]benzothiazolyl | 1.6 | 0.44 |
| **15** | CONH | Phenyl | 7.4 | 101 |
| **16** | CONH | 2-Pyridyl | 8.15 | 104 |
| **17** | CONH | 3-Pyridyl | 25 | 199 |
| **18** | CONH | 4-Pyridyl | 38.4 | 414 |

[a] Each experiment was run in duplicate and the values shown are the average of the two; for **10**, **13** and **14**, the values are average of three experiments.

**Table 2**
The selectivity profile of compounds **10**, **13** and **14**

| Compd | FLT3 $K_d$[a] (nM) | KIT $K_d$[a] (nM) | RET $K_d$[a] (nM) | CSF1R $K_d$[a] (nM) | PDGFRα $K_d$[a] (nM) | PDGFRβ $K_d$[a] (nM) |
|---|---|---|---|---|---|---|
| **10** | 0.74 | 0.96 | 4.7 | 4.4 | 1 | 2.6 |
| **13 (AB460)** | 1.1 | 1.8 | 7.3 | 8.5 | 9.9 | 7 |
| **14 (AB530)** | 1.6 | 7.5 | 3 | 10 | 4.3 | 2.2 |

[a] The values are an average of three experiments.

~1–2 nM for FLT3, ~1–10 nM for KIT, RET, CSF1R, and PDGFRα/β, >200 nM for VEGFR2 and other kinases (Table 2).

Among the known FLT3 inhibitors, CEP-701, PKC-412 and sunitinib were found to bind tightly to a large number of kinases in this panel, while sorafenib bound tightly to moderate number of kinases and MLN-518 bound to a small number of kinases like compounds **13** and **14**.[8]

Compounds **10**, **13** and **14**, three of the more potent analogs in inhibiting cell proliferation, were tested for their PK profile upon oral administration in nude mice, the species used for assessing in vivo efficacy (Fig. 1). At an oral dose of 10 mg/kg of compounds **13** and **14**, a desirable Cmax (1.31 μM and 2.36 μM respectively), AUC (4.25 μM h and 13.20 μM h respectively), and $T_{1/2}$ (2.2 h and 2.3 h, respectively) were observed, indicating that the compounds are orally available in nude mice for testing their ability to inhibit tumor growth. Compound **10** was poorly bioavailable with significantly lower Cmax, AUC and $T_{1/2}$ values.

The oral efficacy of compounds **13** and **14** was evaluated in a xenograft model in athymic nude mice with a subcutaneous implant of a pellet of MV4-11 cells (Fig. 2). When the tumor grew to a size of ~160 $mm^3$, the animals were dosed orally once daily with 10 mg/kg of **13** and **14** for 28 days. The daily dosing was stopped and the animals were observed between days 29–56.

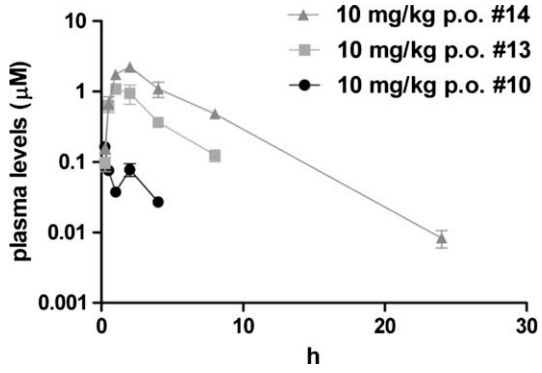



**Figure 1.** Pharmacokinetic profile of compounds **10**, **13**, and **14**.

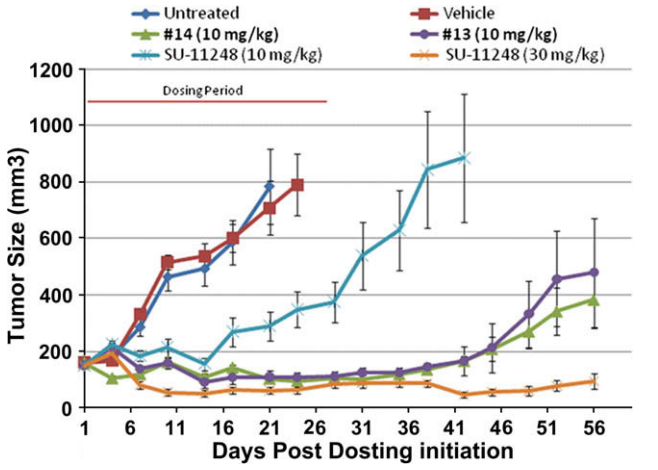



**Figure 2.** Antitumor activity of **13**, **14** and sunitinib (SU-11248) in a xenograft model in nude mice ($n$ = 10 animals per group).

Sunitinib, a positive control, was dosed orally at 10 and 30 mg/kg once a day for 28 days. Compounds **13** and **14** were effective in shrinking the tumor to ~90 $mm^3$ during the dosing period and this tumor shrinking effect persisted between days 29–36 when the animals did not receive the drugs. The tumor started growing back between days 36–56. The tumor grew rapidly in the untreated and vehicle treated animals. At 10 mg/kg po dose, the efficacy of sunitinib in shrinking the tumor in this model was modest, while at 30 mg/kg po dose it was superior to **13** and **14**. The effect of **13** and **14** on the body weight of treated animals, a measure of the compounds' tolerability, was minimal with <5% change in this parameter with either compound.

In conclusion, profiling of a focused library of diarylurea-based kinase inhibitors led us to discover potent and selective FLT3 kinase inhibitors. Optimization of the substituents on one of the aromatic rings helped us identify a series of urea–amide compounds like **13** and **14** that were found to bind to FLT3 kinase with subnanomolar potency and with exquisite selectivity in a panel of 402 kinases when compared to reported potent FLT3 inhibitors, CEP-701, PKC-412, MLN-518, sunitinib and sorafenib. Compounds **13 (AB460)** and **14 (AB530)** were found to have good oral availability in rats (data not shown) and mice with potent tumor growth inhibitory activity in a mouse xenograft model.

## Acknowledgements

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7. In the early stages of this lead optimization campaign, the panel consisted of 40 kinases, which grew steadily in size during lead optimization and currently has 402 kinases. Compounds **10**, **13** and **14** have been screened against 402 kinases.
8. The kinase binding affinity profile for PKC-412, sunitinib, sorafenib and MLN-518 have been published in Ref. 6 above. The profile for CEP-701 will be published in a manuscript submitted for publication.