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Finding the perfect spot for fluorine: Improving potency up to 40-fold during a rational fluorine scan of a Bruton's Tyrosine Kinase (BTK) inhibitor scaffold

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ABSTRACT

A rational fluorine scan based on co-crystal structures was explored to increase the potency of a series of selective BTK inhibitors. While fluorine substitution on a saturated bicyclic ring system yields no apparent benefit, the same operation on an unsaturated bicyclic ring can increase HWB activity by up to 40-fold. Comparison of co-crystal structures of parent molecules and fluorinated counterparts revealed the importance of placing fluorine at the optimal position to achieve favorable interactions with protein side chains.

The introduction of fluorine atoms into biologically active molecules has enjoyed tremendous success in small molecule drug discovery, and recent reviews have highlighted the large number of fluorinated compounds approved by the FDA for medical use.[1,2] The replacement of a C—H bond with a C—F bond often increases metabolic stability and improves membrane permeability.[3] Furthermore, it has been appreciated recently that the introduction of fluorine into small molecules can result in higher binding affinities.[4,5] To this end, 'fluorine scans' have been occasionally applied in order to identify fluorine substitutions that increase inhibitor affinity without significantly compromising physical properties. Notable example of this approach include Diederich's pioneering work exploring the impact of fluorine groups on a series of thrombin inhibitors[6] and the importance of fluorine substitution for Merck's DPP-4 inhibitors.[7]

We had previously described the optimization of a series of BTK inhibitors for the treatment of Rheumatoid Arthritis (RA), which led to the discovery of RN486 (**1a**, Fig. 1).[8,9] In this contribution, we detail our efforts to utilize a rational fluorine scan to further optimize this chemical scaffold starting from prototype molecules such as **1b**. This effort led to the identification of **1a** and several other inhibitors that were suitable candidates for advanced preclinical evaluation. It also revealed an unusual structure–activity relationship, in which a single fluorine substitution increased in vitro efficacy by up to 40-fold.

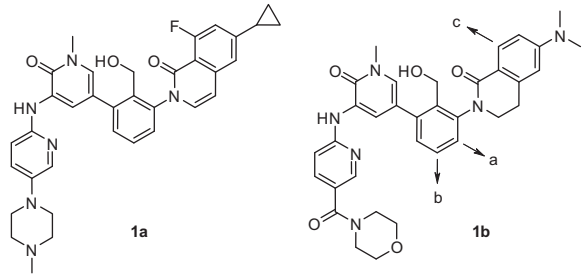

Figure 1. RN486 (**1a**) and lead scaffold **1b**.

Compound **1b** was described previously as a selective BTK inhibitor with reasonable biological activity (human whole blood (HWB) IC_{50} of 100 nM) and poor aqueous solubility (0.6/2/3 μg/mL in water, SIF and SGF).[9] In addition to improving the aqueous

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solubility of our inhibitors, we also sought to further improve their biological activity, as more potent inhibitors may be expected to have lower effective doses. Inspired by the various examples in Roche programs where fluorine scans have resulted in improved binding affinity, we decided to adopt this strategy to examine our chemical scaffold. Potentially favorable interactions between fluorine and proteins include the following: (1) F interacting with the LUMO of carbonyl groups[6] (2) F substitutions strengthening the interaction of adjacent aromatic C–H groups with protein residues;[10] (3) F forming electrostatic interactions with polarized groups;[11] (4) F engaging in hydrophobic interactions.[12]

Given the availability of co-crystal structures and knowledge of protein–ligand interactions involving fluorine, we decided to only investigate fluorine substitutions that could result in favorable interactions with the BTK protein. When the crystal structure of **1b** bound to BTK (1.7 Å resolution) was examined, three vectors were identified as potential candidates (Fig. 2).

Three matched pairs of fluorinated and non-fluorinated analogues (**2a**–**b**, **3a**–**b**, **4a**–**b**, Fig. 3) were prepared in order to explore the potential of fluorination at these vectors to improve potency (Fig. 3, note that in some cases the comparison was between analogues with methyl and ethyl groups in the solvent exposed region, which did not impact activity). These analogs were tested simultaneously in repeated human whole blood (HWB) assays for meaningful differentiation as their potent biochemical activity was beyond the dynamic range of our assay. As a consequence, it cannot be ruled out that potential changes in protein binding upon fluorination could contribute to activity difference in HWB assays, despite that previous work has shown a good correlation between biochemical and HWB potency for closely related compounds. Substitution at vector a (**2a** = 1600 nM, **2b** = 3700 nM) or b (**3a** = 190 n M, **3b** = 200 nM) did not result in any noticeable change in the HWB potency, whereas fluorination at vector c gave 7-fold boost in activity (**4a** = 280 nM, **4b** = 40 nM). It should be noted that the matched pair comparison for vector c was initially performed on the unsaturated isoquinolone scaffold instead of the dihydroisoquinolone scaffold.

Encouraged by the initial data resulting from fluorination at position c, we prepared inhibitor **5b**, which features the fluorine substitution on the partially saturated dihydroisoquinolone scaffold. To our surprise, the fluorinated analogue **5b** has a minimal increase in HWB activity (**5a** = 60 nM, **5b** = 50 nM). We hypothesized that the activity increase for **4b** might be specific to the isoquinolone ring system, although we did not have a rationale at the time. Therefore, **6a** and **6b** on the isoquinolone ring scaffold with a cyclopropyl group in place of the dimethylamino substituent were prepared. Astonishingly, **6b** was reproducibly found to be approximately 40-fold more potent than **6a** in the HWB assay

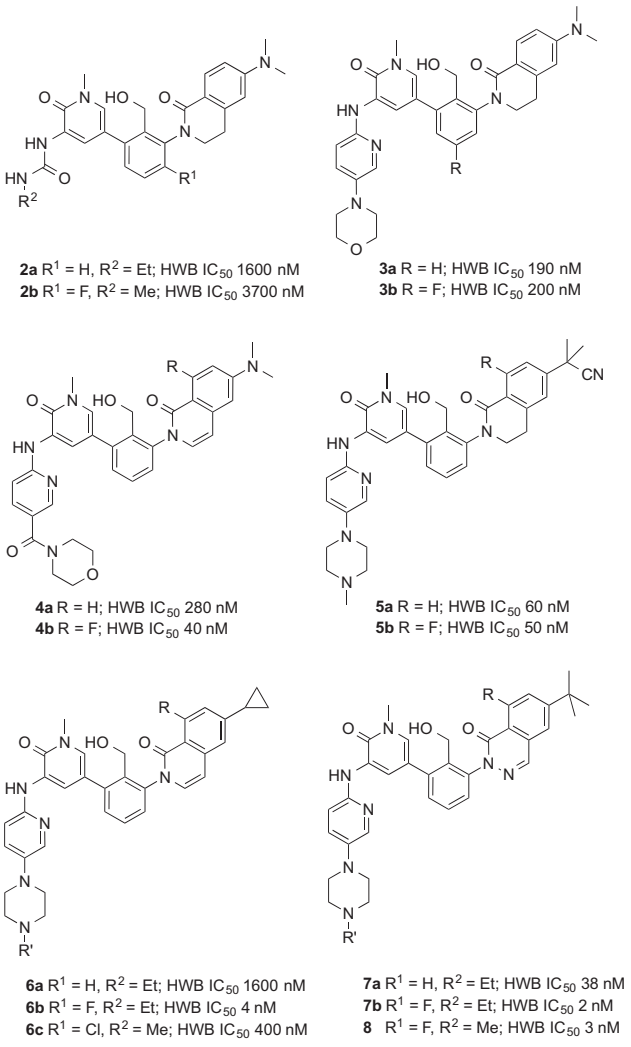

Figure 3. BTK inhibitor fluorinated and non-fluorinated matched pairs.

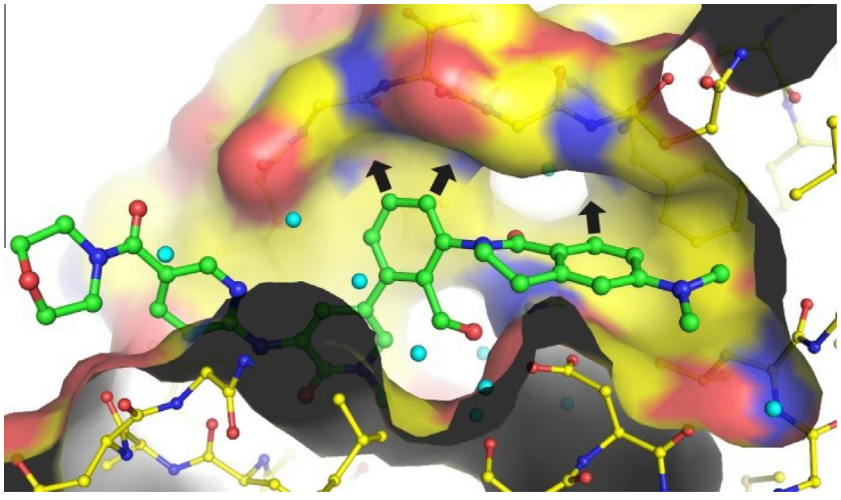

Figure 2. Co-crystal structure of compound **1b** and BTK (pdb code: 4RFY). Vectors explored with fluorine substitution are highlighted with black arrows.

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(**6a** = 160 nM, **6b** = 4 nM). It should be noted that this increase in potency was significantly more pronounced than of the potency improvement imparted by fluorination in the **4a**-**b** matched pair.

In order to understand the dramatically different impact of fluorination on these closely related scaffolds, we obtained co-crystal structures with BTK for **4b** (1.17 Å resolution), **5b** (2.5 Å) and **6b**[9] (1.95 Å) (Fig. 4). An overlay of the structures showed that the fluorine atom in **6b** sits most deeply in the binding pocket toward F413, while the fluorine in **5b** lies furthest away from this residue. The electronegative fluorine atom in **6b** is within van-der-Waals distance of the primary amine of K430, a conserved water molecule, and the aromatic hydrogen at the ortho position of F413. On the other hand, the fluorine atom in **5b** is relatively distant from these features and cannot form such favorable interactions. The fluorine atom in **4b** is within bonding distance of these groups (i.e., F413), but does not seem to be optimally positioned. Overall,

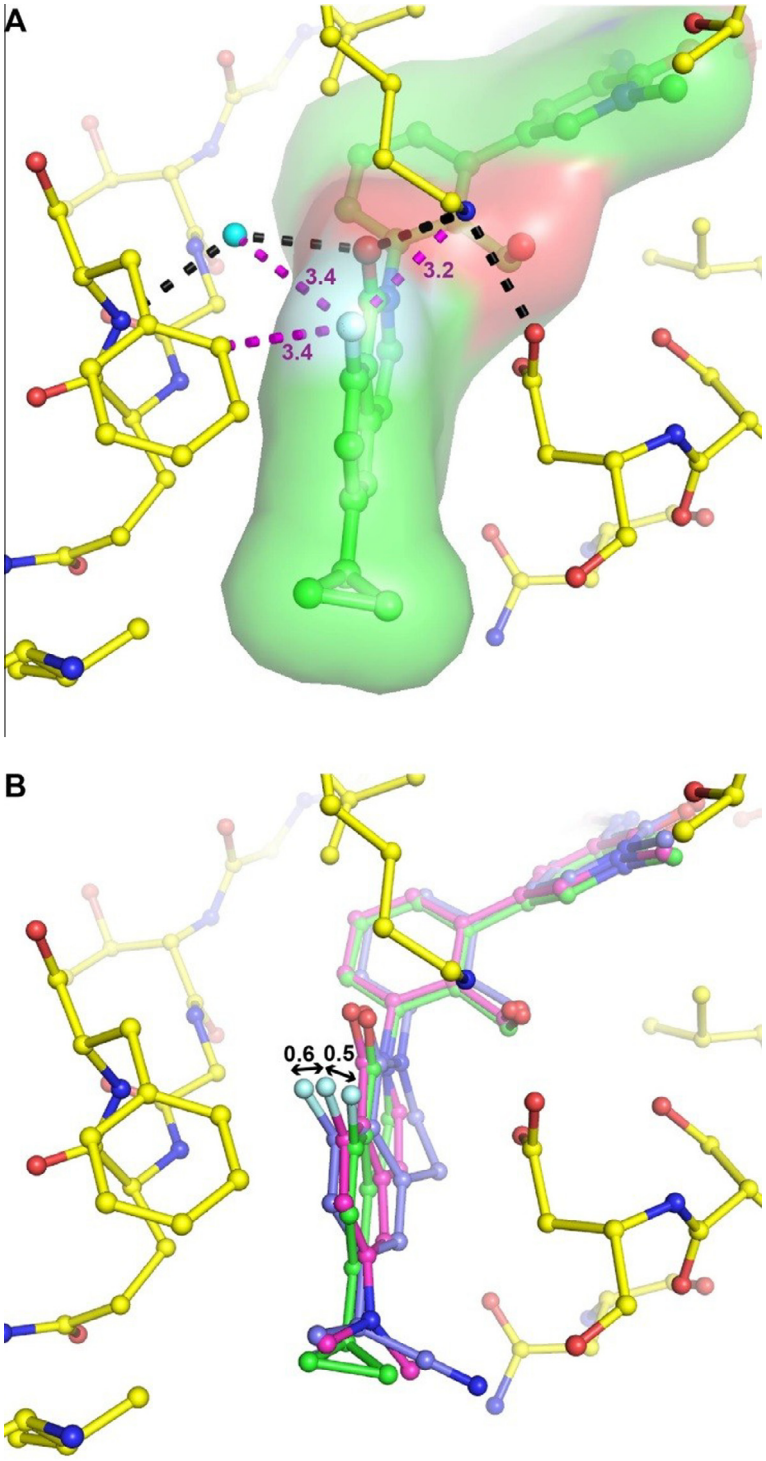

Figure 4. (A) Structure of **6b** highlighting the interactions of the isoquinolone fluorine and carbonyl groups. (B) Overlay of the structures of **4b** (pdb code: 4RFZ), **5b** (pdb code: 4RG0) and **6b**.

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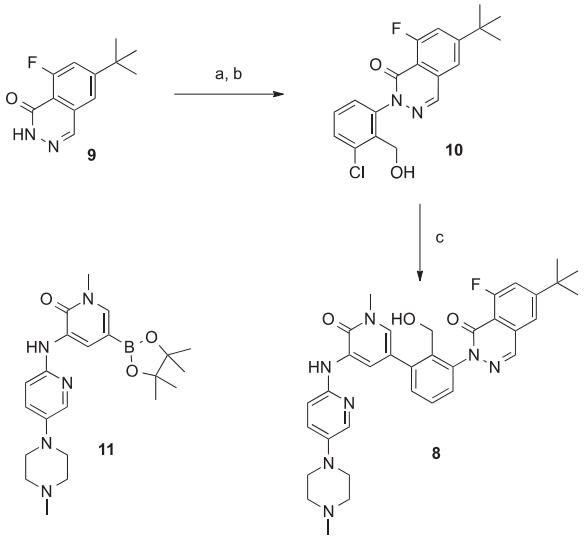
these structural observations and the apparent requirement for multiple simultaneous interactions of the fluorine group are consistent with our biological data.

The favorable interactions and impact of halogenation at this position appear to be quite unique to fluorine. Chlorine substitution negatively affected the biological activity of analogs in this series, and **6c** was found to be one hundred fold less active than the fluorinated counterpart **6b**.

The remarkable impact of fluorination on series **6** relative to series **5** deserved further comment. Recent publications have highlighted the role of synergy in structure–activity relationships in various systems, and we believe that this is an interesting example of substituent synergy[13–16] involving a fluorine atom. The hydrophobic cyclopropyl and dimethylcyano groups at the para position are anchored in a well-defined liphophilic pocket that strongly contributes to potency of these analogs. In the case of **6b**, the isoquinolone ring system is situated to place the both the cyclopropyl group and fluorine atom in an optimal position. Addition of the fluorine atom in this series does not require any reduction of the optimal hydrophobic interactions of the cyclopropyl group, and the full, mutually reinforcing, impact of the fluorine atom can be realized. In the case of scaffold **5**, it does not seem to be possible to benefit from the positive interactions of the fluorine group with the protein while preserving the interactions of the dimethylcyano functionality. While other effects of the fluorine group (e.g., influencing the solvation of the adjacent carbonyl group) are clearly possible, these explanations do not appear to be as consistent with overall structure–potency relationships observed in this series of inhibitors.



Scheme 1. Synthesis of inhibitor **7b**. Reagents and conditons: (a) 2-chloro-6-fluorobenzaldehyde, Cs_2CO_3, 60 °C, 4 h, 68.2%. (b) $NaBH_4$, *i*-PrOH, CH_2Cl_2, 4 °C, 0.5 h, 65.5%. (c) **11**, K_2CO_3, PCy_3, $Pd(dba)_2$, dioxane, water, 88 °C, 1.5 h, 83.5%.

This rationale suggests that other unsaturated bicyclic systems could also benefit from fluorine substitution at vector c. To test this hypothesis, pair **7a**-**b** were prepared and profiled in the HWB assay. We were pleased to discover that the fluorinated analogue **7b** was approximately 20-fold more active than **7a** (HWB IC_{50} of **7a** = 38 nM, **7b** = 2 nM). Further optimization and profiling led to the identification of the methyl analogue **8** (HWB IC_{50} of 3 nM) as a new, exceptionally potent BTK inhibitor with desirable properties for preclinical studies.

Additional research teams appear to have tested and recognized the importance of this fluorophilic vector c. In particular, patent applications from several research groups included incorporation of fluorine group on related scaffolds that would potentially place fluorine atoms at this hotspot.[17–19]

This work demonstrates that a rational fluorine scan can be used as a strategy to improve biological activity, even on synthetically challenging scaffolds. Careful examination of co-crystal structures reduced the number of novel fluorinated analogues pursued in this effort. The recent significant advances in synthetic fluorine chemistry facilitate employment of this strategy in medicinal chemistry.[20–22] An apparent synergetic substitution of a bicyclic ring system resulted in the identification of matched pairs in which the fluorinated analogs were dramatically more potent than their non-fluorinated analogs. These findings continue to impact discovery efforts targeting inhibitors of Bruton's Tyrosine Kinase (BTK).

The BTK inhibitors described were synthesized in a convergent manner as shown in Scheme 1. A C–N coupling or aromatic substitution of an isoquinolone, dehydroquinolone, or pthalazinone to a dihalobenzaldehyde or protected dibromobenzyl alcohol was followed by a Suzuki–Miyaura coupling as described previously. Reduction or deprotection afforded the final inhibitors.[23,24] For example, the synthesis of **8** proceeded from aromatic substitution of pthalazinone **9** with 2-chloro-6-fluorobenzaldehyde followed by reduction with $NaBH_4$ to produce alcohol **10**. This intermediate could be coupled with boronic ester **11** to give **8** as final product.

Phthalazinone **9** can be prepared in a four-step sequence starting from commercial chemical **12**. Lithiation and quenching with DMF generated an aldehyde that could be converted to the corresponding acetal **13**. An additional directed deprotonation followed by addition of *N*-flurorobenzenesulfonimide generated the desired flouroaromatic. Simultaneous removal of the acetal and oxazole protecting groups provided **14**, which could be converted to **9** by treatment with hydrazine hydrate (Scheme 2).

Acknowledgment

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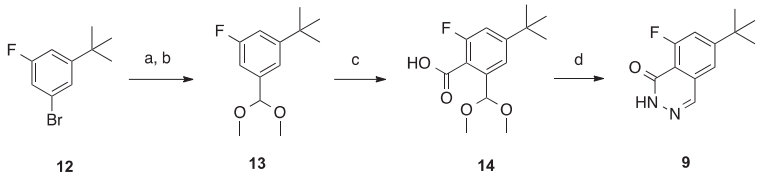

Scheme 2. Preparation of pthalazinone **9**. Reagents and conditions: (a) *i*-PrMgCl, *n*-BuLi, DMF, THF, −10 °C, (b) trimethyl orthoformate, toluenesulfonic acid, rt, 1 h, 98.5% in two steps. (c) *sec*-BuLi, CO_2, THF, −70 °C to 10 °C, 55.3%. (d) hydrazine hydrate, acetic acid, ethanol, 80 °C, 86%.

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