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PII: S0960-894X(16)30200-1
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.02.074
Reference: BMCL 23628

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 21 January 2016
Revised Date: 22 February 2016
Accepted Date: 24 February 2016

Please cite this article as: Stauffer, F., Cowan-Jacob, S.W., Scheufler, C., Furet, P., Identification of a 5-[3-phenyl-(2-cyclic-ether)-methylether]-4-aminopyrrolo[2,3-d]pyrimidine series of IGF-1R inhibitors, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.02.074

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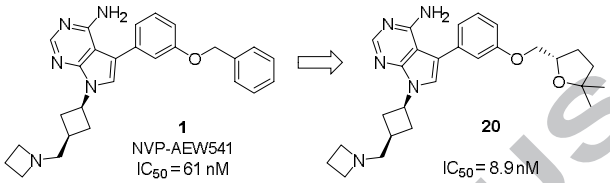

Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

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ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
IGF-1R inhibitor
IGF-1R X-ray co-crystal structure
Structure-guided design

ABSTRACT

We report structure-guided modifications of the benzyloxy substituent of the Insulin-like Growth Factor-1 Receptor (IGF-1R) inhibitor NVP-AEW541. This chemical group has been shown to confer selectivity against other protein kinases but at the expense of a metabolism liability. X-ray crystallography has revealed that the benzyloxy moiety interacts with a lysine cation of the IGF-1R kinase domain via its ether function and its aromatic π-system and is nicely embedded in an induced hydrophobic pocket. We show that 1,4-diethers displaying an adequate hydrophobic and constrained shape are advantageous benzyloxy replacements. A single digit nanomolar inhibitor (compound **20**, IC_{50} = 8.9 nM) was identified following this approach.

The first report on the role of IGF-1R in human cancers appeared almost three decades ago.[1] IGF-1 and IGF-2 growth factors, the two IGF-1R ligands, play a critical role in the development and maintenance of normal tissues but are also associated with inhibition of cell death, regulation of cell proliferation and protein synthesis in cancer cells. Consequently, inhibition of the IGF-1R signaling pathway has been considered a valid cancer therapeutic approach despite the complication of a potential selectivity issue due to the interplay with the closely related insulin receptor (InsR).[2] Somehow disappointing clinical results were obtained with several antibodies targeting IGF-1R but small molecule IGF-1R tyrosine kinase inhibitors, such as OSI-906 (linsitinib), showed the potential to mitigate some limitations of the antibodies.[3] However, none of the candidates targeting IGF-1R has successfully completed clinical evaluation yet. Current and future studies will reveal the most suitable schedules, combination partners and subpopulations to fully exploit the potential of IGF-1R inhibitors in cancer therapy.

NVP-AEW541 (compound **1**) is an ATP-site directed IGF-1R inhibitor showing some level of selectivity against the InsR at the cellular level. The compound displays good selectivity in enzymatic assays involving a panel of representative protein kinases.[4] The corresponding phenol debenzylated product (compound **2**) maintains a potent inhibition of IGF-1R (IC_{50} = 0.42 μM) but is a broader spectrum kinase inhibitor, indicating the importance of the benzyloxy substituent to achieve selectivity (Table 1). Investigation by LC-MS-MS of the metabolism of NVP-AEW541 in human liver microsomes indicated that compound **2** was among the two predominant metabolites (uncorrected peak area relative %). Modification of the benzylic metabolic soft spot could redirect the metabolism path thereby preventing the formation of the promiscuous kinase inhibitor compound **2**. However, a simple substitution at the benzylic position with a methyl group in compound **3** (IC_{50} = 3.7 μM) compromised IGF-1R affinity. Thus, we had to resort to another approach to try to solve this metabolism issue.

Table 1. Selection of kinases inhibited by compound **2** versus compounds **1**, **17** and **20**.

kinases	IC_{50} for **2**, μM[a]	IC_{50} for **1**, μM[a]	IC_{50} for **17**, μM[a]	IC_{50} for **20**, μM[a]
IGF-1R	0.42	0.06	0.08	0.009
InsR	3.8	1.0	0.93	0.10
EphB4	0.005	> 10	2.7	0.69
Ret	0.008	3.3	4.4	0.97
Jak1	0.26	> 10	> 10	> 10
KDR	0.44	7.1	6.6	> 10
Lck	0.69	5.6	8.9	7.4
Her1	2.3	> 10	> 10	> 10
PI3Kβ	0.23	> 9	> 9	> 9
PI3Kα	1.4	> 9	> 9	> 9

[a]Values are measured in duplicate.

Compound **1** was co-crystallized with the kinase domain of IGF-1R and the X-ray structure of the complex was solved at 2.20 Å resolution (Fig. 1). The structure revealed that compound **1** binds in the active site of the kinase making the canonical bidentate hydrogen bonds with the hinge segment of the ATP pocket. Interestingly, the structure indicated that the inhibitor disrupts the salt bridge between residues K1033 and E1050 present in the active conformation of the kinase,[5] causing a small

movement of helix C and a change in the side chain conformation of the DFG motif residue F1154. These alterations of the kinase active conformation create a small hydrophobic pocket filled by the benzyloxy group, conferring high IGF-1R selectivity to the inhibitor, as represented in Fig.1. The benzyloxy phenyl ring makes hydrophobic contacts in this pocket with helix C residues M1054, F1047, DFG motif residue F1154 and P-loop residue F1010. In addition, the side chain amino group of residue K1033 makes two polar stabilizing interactions with the benzyloxy moiety: a hydrogen bond is established with the ether oxygen atom while a π-cation interaction is formed with the phenyl ring.

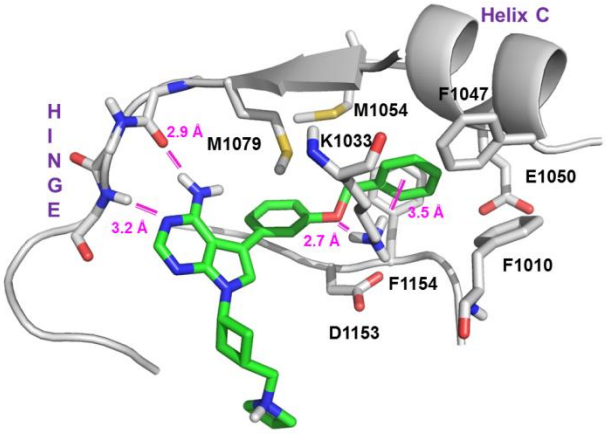


Figure 1. X-ray co-crystal structure of IGF-1R in complex with NPV-AEW541 (**1**) (ATP site). The hydrogen bond and π-cation interactions with K1033 are indicated by magenta lines (PDB code: 5HZN).

In our search for suitable replacements of the benzyloxy group of **1**, we wanted to preserve the favorable interactions observed in the X-ray structure. As indicated by the drop in potency compared to **1** of compounds **2** and **4**, the benzyl deleted and saturated analogs, respectively, both the hydrophobic contacts and the π-cation interaction are important. By keeping an ether linkage to maintain the hydrogen bond with K1033, we envisioned replacing the π-cation interaction by a second hydrogen bond with this lysine residue. In fact, modeling suggested that this could be achieved by replacing the benzyloxy moiety by a 1,4-diether group. Such a group preferentially adopts a gauche conformation,[6] which allows the two ether oxygens to chelate the amino group of K1033. This is illustrated in Fig.2 with a binding model for compound **5**, the simplest analogue synthesized to probe the design concept. Besides the favorable structure-activity progression obtained with additional analogues discussed in the next section, the concept could be validated by a co-crystal structure of the (R)-enantiomer tetrahydropyrane derivative **6** in complex with the highly homologous kinase domain of the insulin receptor.[7] As shown in Fig.3, the chelation of the lysine cation by the 1,4-diether system is clearly observed in this structure. In addition to a potency (IC_{50} = 0.080 μM) close to that of the original compound **1**, when incubated in human liver microsomes compound **6** did not reveal compound **2** as a detectable metabolite by LC-MS-MS. According to our model, the (S)-enantiomer of **6**, compound **7,** was also able to chelate the lysine cation while fitting the induced hydrophobic pocket with favorable contacts. Consistent with the model, **7** showed similar activity (IC_{50} = 0.13 μM).

The chelation of the lysine is productive but additional hydrophobic interactions are needed to drive the potency of a ligand extending in the induced hydrophobic pocket. As shown by the shortest 2-hydroxy ethoxy derivative (compound **8**, IC_{50} = 7.9 μM), absence of a hydrophobic extension was detrimental to potency. Since the benzyloxy pocket is induced by the ligand, one could not exclude that this pocket would adapt, at least partially, to the hydrophobic moiety that is filling it. This is why a broad exploration was performed to find the optimal appended hydrophobic group. A better affinity was obtained by introducing an alkyl substituent on the hydroxyl group of compound **8** and the increase in affinity went together with the increase in size of the linear alkyl chain from methyl (compound **4**, IC_{50} = 2.0 μM) to ethyl (compound **9**, IC_{50} = 0.63 μM) to propyl (compound **10**, IC_{50} = 0.31 μM). However, the shape of the alkyl substituent was also relevant for the optimal filling of the induced pocket. The branched isopropyl derivative (compound **11**, IC_{50} = 0.26 μM) was slightly more potent and introduction of an extra methyl to give the tert-butyloxyethyl derivative was optimal (compound **12**, IC_{50} = 0.16 μM). However, bridging the two methyl groups of compound **11** to give a cyclopentyl ring yielded a less potent compound (**13**, IC_{50} = 0.49 μM). The isopentyloxyethoxy derivative (compound **14**, IC_{50} = 0.37 μM) was also less potent than compounds **11** and **12** despite a larger hydrophobic substituent.

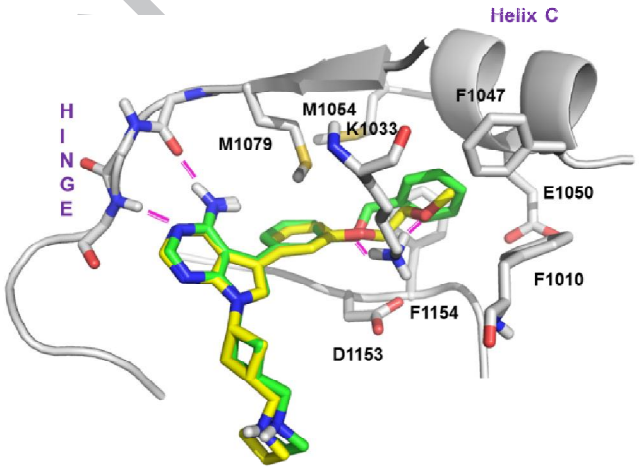


Figure 2. Binding model of compound **5** in X-ray structure of IGF-1R in complex with **1** (ATP site). The two hydrogen bonds with K1033 formed by the 1,4-diether group are indicated by magenta lines.

The analogs of compound **4** bearing a substituent with a smaller ring such as cyclobutyl (compound **15**, IC_{50} = 1.9 μM) or cyclopentyl (compound **16**, IC_{50} = 0.84 μM) maintained significant activity though compound **16** was marginally less potent than compound **4**. Based on our 1,4- diether chelator concept, we introduced an oxygen atom in the 5-membered ring of **16** to obtain the two tetrahydrofuran derivatives: compounds **17** ((S)-enantiomer, IC_{50} = 0.080 μM) and **18** ((R)-enantiomer, IC_{50} = 0.11 μM). Encouraged by the gain in potency obtained, we introduced a gem-dimethyl group at position 5 of the tetrahydrofuran ring to mimic the tert-butyl group of compound **12** in a constrained manner. This modification allowed a further increase of potency, the resulting (R)-enantiomer compound **19** (IC_{50} = 0.060 μM) being less active than the (S)-enantiomer compound **20** (IC_{50} = 0.0089 μM). This (S)-5,5-dimethyltetrahydro-furan-2-ylmethoxy substituent turned out to be most efficient among the series of 1,4-diether groups investigated in filling the induced hydrophobic pocket. As exemplified in Table 1, both compounds **17** and **20** show a favorable kinase selectivity profile similar to that of compound **1** which is much better than that of the unsubstituted compound **2**.

The optimized compound **20** which is one log unit more potent on target than compound **17** shows relatively greater reduction of the activity on the other kinases and therefore can be considered to be more selective than compound **17**. The (S)-5,5-dimethyltetrahydro-furan-3-ylmethyl group showed the same potential as the benzyl starting point to confer general kinase selectivity to compound **2**. However, this group did not substantially improve the hERG inhibition liability of the parent compound **1** (IC_{50} = 0.42 vs 0.13 μM in a hERG dofetilide competition binding assay) while compound **17** showed a reduced inhibition (IC_{50} = 3.6 μM) possibly linked to a decreased lipophilicity as compared to compound **1** (cLogP: 3.0 vs 4.4, respectively). Compound **20**, in addition to have a good water solubility, displayed a high permeability at neutral pH, and above, in a parallel artificial membrane permeability assay and showed no interference at 10 μM with 3 major human cytochrome P450 drug metabolizing isoforms (2D6, 2C9, 3A4).

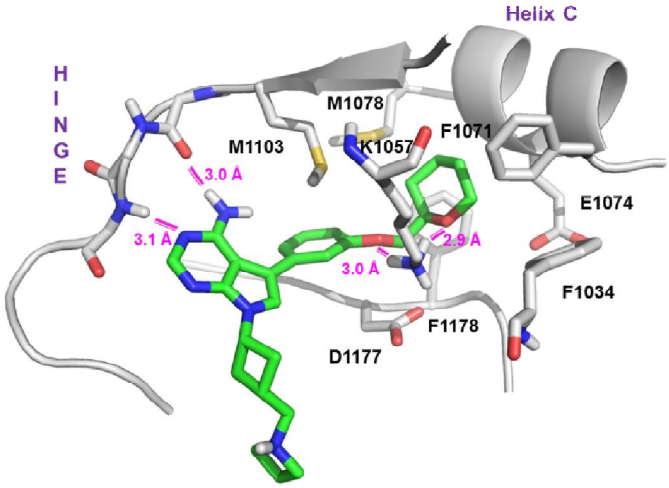


Figure 3. X-ray co-crystal structure of Ins-R in complex with compound **6** (ATP site). The two hydrogen bonds with K1057 formed by the 1,4-diether group are indicated by magenta lines (PDB code: 5HHW).

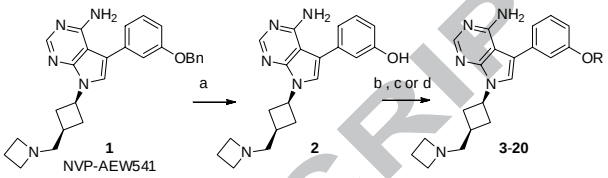
Table 2. Biochemical inhibition of IGF-1R for compounds **1–20**.

Compounds	R substituents	IGF-1R Inhibition IC_{50}, μM[a]
1	Benzyl	0.061
2	H	0.42
3	(rac)-1-phenylethyl	3.7
4	cyclohexylmethyl	0.55
5	methoxyethyl	2.0
6[b]	(R)-tetrahydropyran-2-ylmethyl	0.080
7[b]	(S)- tetrahydropyran-2-ylmethyl	0.13
8	hydroxyethyl	7.9
9	ethoxyethyl	0.63
10	propyloxyethyl	0.31
11	isopropyloxyethyl	0.26
12	tert-butyloxyethyl	0.16
13	cyclopentyloxyethyl	0.49
14	isopentyloxyethyl	0.37
15	cyclobutylmethyl	1.9
16	cyclopentylmethy	0.84
17	(S)-tetrahydrofuran-2-ylmethyl	0.080
18	(R)-tetrahydrofuran-2-ylmethyl	0.110
19	(R)-5,5-dimethyltetrahydro-furan-2-ylmethyl	0.060
20	(S)-5,5-dimethyltetrahydro-furan-2-ylmethyl	0.0089

[a]Geometric mean of duplicates.

[b]Separated from the racemic mixture by chiral preparative HPLC (>99.9%ee)

Synthesis of compounds **3-4**, **6-7** and **10-20** was achieved by standard Mitsunobu reaction of compound **2** with the suitable commercially available alcohol or (S&R)-(5,5-dimethyltetrahydrofuran-2-yl)methanol described in the literature.[8] Palladium on charcoal catalyzed hydrogenolysis of compound **1**[9] yielded compound **2**. Compound **8** was obtained subsequently by tert-butyl deprotection of compound **12** in aqueous TFA. Compounds **5** and **9** were obtained by alkylation of compound **2**, after deprotonation with NaH, with the corresponding commercial bromo reagent (Scheme 1).



Scheme 1. Reagents and conditions: (a) Pd-C 10%, H_2, 0.15 M in THF-MeOH 1:1, rt, 24 h, 96%; (b) ROH (1.1-1.3 eq.), DIAD (1.45-1.6 eq.), PPh_3 (1.45-1.6 eq.), 0.2 M in THF, 15-23 h, rt, 17-63%; (c) Only for compound **12** to obtain compound **8**: 0.9 M 49% aqueous TFA, rt, 18.5 h, 61%; (d) Compound **5** and **9**: 60% NaH in oil (1 eq.), 0.2 M in DMF, rt, 10 min then RBr (1 eq.), rt, 60-80 min, 27-42%.

In conclusion, we have shown that the benzyloxy group of NVP-AEW541 can be advantageously replaced by a 2-(cyclic-ether)-methylether moiety allowing redirection of the metabolism to avoid formation of the unwanted pankinase inhibitor compound **2,** while maintaining an adequate kinase selectivity and on target potency. The further optimization of the series as IGF-1R inhibitors is discussed in the following letter.[10]

Acknowledgments

The authors would like to thank Reiner Aichholz, Werner Gertsch, Jérome Dayer for human liver microsome metabolic investigations and Christoph Mura and Christian Ragot for their excellent technical assistance.

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