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'Click' synthesis of a triazole-based inhibitor of Met functions in cancer cells

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ABSTRACT

The use of Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition permitted the synthesis of a new compound that is able to inhibit the HGF-induced scattering of MDCK (epithelial cells) and in vitro tumorigenesis of H1437 (non-small-cell lung cancer) and GTL-16 (human gastric carcinoma). In agreement with biochemical and biological results, docking studies within the ATP binding site of Met suggested for the new synthesized compound a binding mode similar to that of the active compound Triflorcas previously reported.

Cell scattering and anchorage-independent growth are important biological processes underlying pathological events involved in the progression of malignant tumor and they are induced by hepatocyte growth factor (HGF, also known as scatter factor). HGF binds to its receptor Met which belongs to the tyrosine kinases receptors (RTK) family. Following its activation, the Met RTK triggers a number of signaling pathways that regulate specific biological events during development[1] and regenerative processes,[2] but also during tumorigenesis.[3] Moreover, in the course of tumor evolution, oncogenic Met confers oncogene addiction, drug and/or radiotherapy resistance.[4] For these reasons, Met and its ligand HGF have become leading candidates for molecular targeted cancer therapies. Several small-molecule Met inhibitors have been reported so far and some of them reached the clinical trial phase; examples of these include Pfizer's PF-2341066, Sugen's SU11274 and ARQ-197.[5] In this scenario, we recently reported the synthesis of a compound (Triflorcas, Fig. 1) that targets cancer cells dependent on oncogenic Met and impairs survival, anchorage-independent growth, and in vivo tumorigenesis without showing side effects.[6]

This finding, associated with the interesting results that we have recently obtained with the use of 1,2,3-triazole as new useful scaffold for the inhibition of tyrosine kinases,[7] stimulated our curiosity in exploring the replacement of the amide bond of Triflorcas with a 1,2,3-triazole ring (Fig. 1). The bioisosterism between amide group and 1,2,3-triazole ring is well known,[8] and docking simulations confirmed the same orientation of Triflorcas and the newly designed compound within the ATP binding site of Met. In this Letter, we report on the convergent synthesis of a Triflorcas analogue, having 1,2,3-triazole as a linkage of imidazo[2,1-*b*]benzothiazol-2-ylphenyl and phenyl-*N*-phenetyl acetamide moieties. The synthesis is based on a Cu(I)-catalyzed 1,3-dipolar cycloaddition[9] as the key step.

The synthetic plan is resumed in Scheme 1 and is based on the exploitation of 1,3-dipolar cycloaddition between **6** and **8**. Alkyne **6** has been prepared starting from compound **3**[6] by selective reduction of the ester group with $LiBH_4$ and subsequent Dess–Martin oxidation to give the aldehyde **5**. The use of Bestmann-Ohira reagent (**9**)[10] permitted the obtainment of the alkyne **6** in 84% yield. The basic conditions of the Bestman-Ohira homologation endanger the configuration of the stereocenter and prevents the preparation of the enantiopure alkyne derivative. This fact doesn't constitute a relevant drawback because of the identical biological activity of Triflorcas and its enantiomeric form (*ent*-1, Table 1), as previously reported.[6] The azide **8** has been prepared starting from compound **7**[6] with NaN_3 by formation of the corresponding diazonium salt. Cycloaddition reaction of alkyne **6** with azide **8** in THF/H_2O (4:1), in the presence of a catalytic amount of $CuSO_4 \cdot 5H_2O$/sodium ascorbate[11], gave selectively the desired 1,4 compound **2**: the regiochemistry of the reaction was assessed by a NOESY experiment.

We have previously shown that compounds can be efficiently screened and/or biologically validated for their inhibitory properties towards Met-triggered biological responses by using cell

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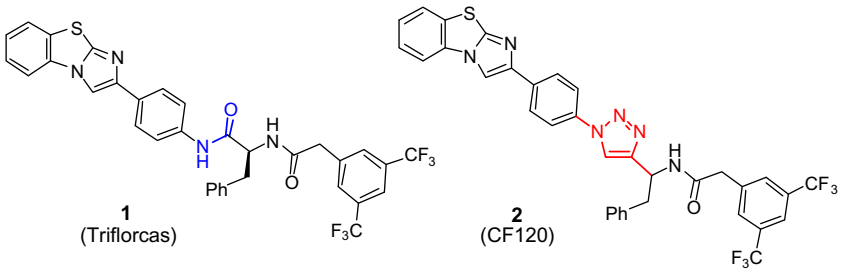
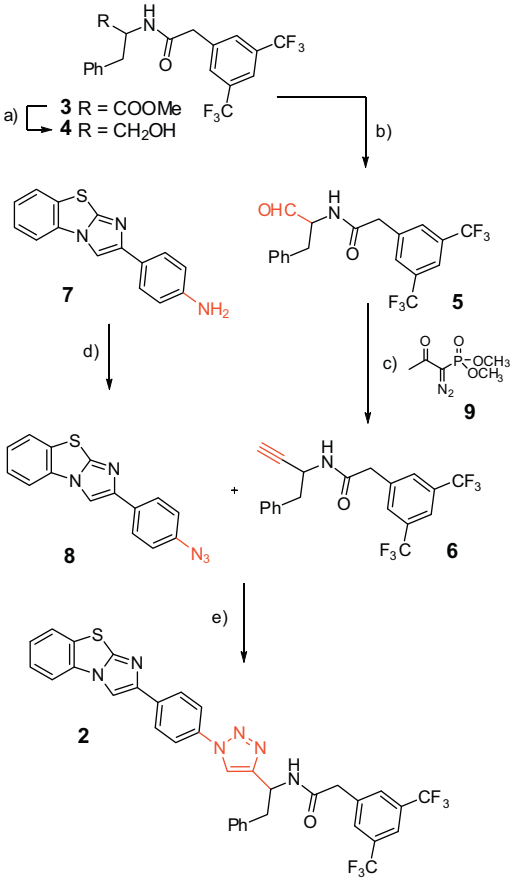


Figure 1. Structures of Triflorcas (**1**) and its triazole-based analogue (**2**).



Scheme 1. Synthesis of **2**. Reagents and conditions: (a) $LiBH_4$, dry THF, rt, 6 h; (b) Dess–Martin periodinane, dry CH_2Cl_2, rt, 4 h; (c) Bestmann-Ohira reagent (**9**), K_2CO_3, dry MeOH, rt, 16 h; (d) H_2SO_4, $NaNO_2$, NaN_3, H_2O, rt, overnight; (e) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, THF/H_2O, rt, 6 h.

Table 1
Inhibition of HGF-induced scattering. SU11274 was used as a reference Met inhibitor.

	IC_{50} (μM)	
	MDCK	GTL-16
Triflorcas (**1**)	0.2	0.8
ent-**1**	0.2	1.19
2	0.6	8.5
SU11274	0.2	0.23[a]

[a] Ref.[6]

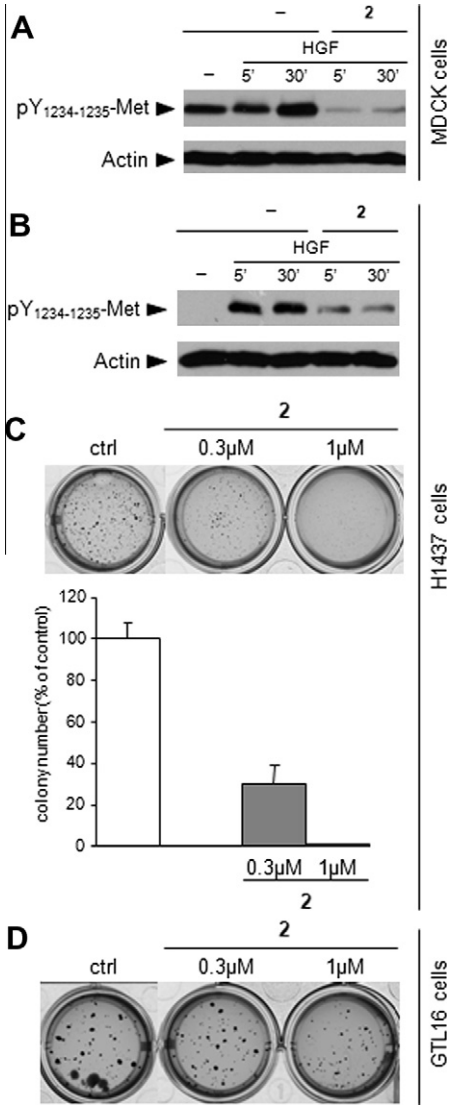


Figure 2. Compound **2** impairs Met-phosphorylation and anchorage-independent growth in cancer cells. (A and B) HGF-induced Met phosphorylation is impaired in the presence of compound **2** (10 μM) in MDCK (A) and H1437 (B) cells. Actin protein levels were used as loading controls. (C and D) Anchorage-independent growth of H1437 (C) and GTL16 (D) cells is impaired by compound **2** in a dose dependent manner. Quantification for H1437 cells in shown in C bottom panel.

scattering assays.[12] These studies were performed with MDCK epithelial cells, which acquire a 'scattered phenotype' after stimulation with Met ligand HGF. This response to HGF can be impaired by Met-targeting inhibitors. We have found that our triazole-based analogue impairs Met-triggered cell scattering. By evaluating the

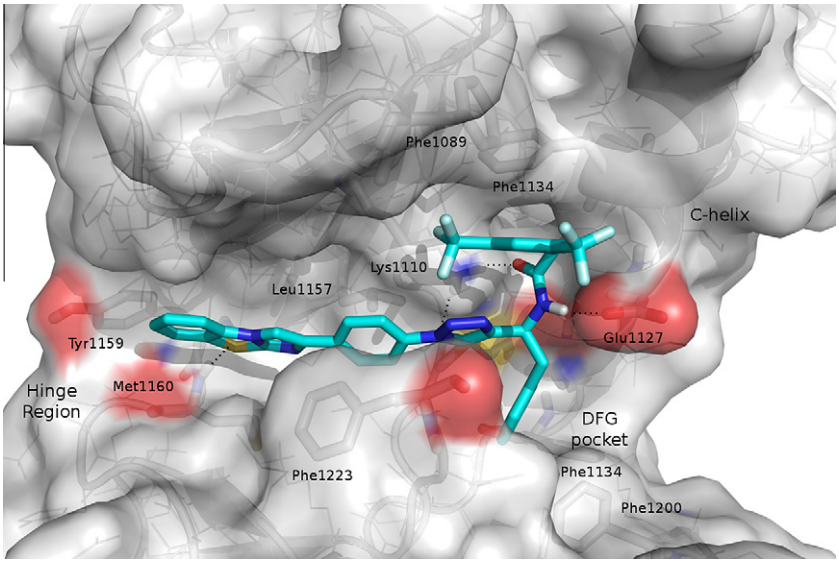


Figure 3. Binding mode of compound **2** within the ATP binding site of Met.

IC_{50} of our analogue, we have observed that its range of action was similar to that of Triflorcas (Table 1). No toxic effects have been observed at biologically active concentrations. We next biochemically evaluated the ability of compound **2** to interfere with Met activation by following the phosphorylation levels of two Tyrosine residues located in its kinase domain, namely Tyr1234 and Tyr1235. HGF-induced Met-phosphorylation was reduced by compound **2** in both MDCK and H1437 (harboring point mutations at the amino acid residue R988C) cells compared to controls (Fig. 2A and B). Tumorigenicity of cancer cells can be assessed in vitro by performing soft agar assays. We found that compound **2** inhibits anchorage-independent growth of H1437 cells in a dose dependent manner (Fig. 2C). In human GTL-16 gastric carcinoma cells, Met gene amplification leads to high Met protein levels, constitutive Met activation, and 'Met-addiction'. We found that compound **2** was efficient in blocking also GTL-16 anchorage-independent growth, with a range of action comparable to that of Triflorcas (Table 1, Fig. 2D). On the basis of these results, we can ascertain that our triazole-based analogue **2** efficiently inhibits Met-triggered biological activities, such as cell scattering and in vitro tumorigenicity.

Docking studies[13] were conducted on compound **2** in order to hypothesize the way, at the molecular level, by which the inhibitor is able to interfere with Met. As a result, the binding mode of compound **2** is similar to that previously found for Triflorcas.[6]

In detail, the benzothiazole ring is bound to the hinge region, where it's involved in a weak hydrogen bond interaction with the NH backbone of Met1160 by means of the S atom. Moreover, it establishes hydrophobic contacts with Tyr1159. The central aromatic ring makes lipophilic interactions with the side chains of Leu1157 and Phe1223 (π-stacking). The –NH–CO– portion is involved in H-bonds with Glu1127 and Lys1110 in the same way as the triazole ring is stabilized by polar interactions with Lys1110. Furthermore, the benzyl ring occupies the DFG pocket establishing Van der Waals contacts with Phe1134 and Phe1200, while the 3,5-bis(trifluoromethyl)benzyl fragment makes favorable hydrophobic interactions with Phe1089 and Phe1124 (Fig. 3).

The synthesis of a new compound that is able to interfere with two distinct biological responses by Met signaling, namely scattering and in vitro tumorigenesis, has been reported. The replacement of the amide bond with 1,2,3-triazole ring resulted in a good maintenance of the inhibitory activity of the lead compound (Triflorcas) as supported by docking simulation. The synthesis took advantage of the useful Cu(I)-catalyzed cycloaddition and the Bestmann-Ohira alkynylation reaction. 1,2,3-triazole ring appears an interesting scaffold in the preparation of inhibitors of Met signaling. Altogether, these studies are a further demonstration of the bioisosterism between 1,2,3-triazole and amide and they encourage the use of Cu(I)-catalyzed Huisgen cycloaddition to approach a convergent synthesis of analogs of amide containing drugs.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.078.

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