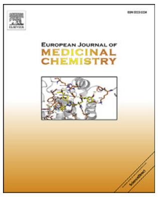
European Journal of Medicinal Chemistry 115 (2016) 191–200

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech



Research paper

Design and synthesis of novel benzo[*d*]oxazol-2(3H)-one derivatives bearing 7-substituted-4-enthoxyquinoline moieties as c-Met kinase inhibitors

Dong Lu [a, 1], Aijun Shen [b, 1], Yang Liu [a], Xia Peng [b], Weiqiang Xing [a], Jing Ai [b], Meiyu Geng [b, **], Youhong Hu [a, *]

[a] *State Key Laboratory of Drug Research, Department of Medicinal Chemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, China*

[b] *Division of Anti-tumor Pharmacology, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Rd, Shanghai 201203, China*

ARTICLE INFO

Article history:
Received 12 November 2015
Received in revised form
9 March 2016
Accepted 10 March 2016
Available online 12 March 2016

Keywords:
c-Met
Benzo[d]oxazol-2(3H)-one-quinolone
Molecular hybridization
Anti-cancer

ABSTRACT

Analysis of the results of studies of docking **1** and **7a** with c-Met kinase led to the identification of benzo[*d*]oxazol-2(3H)-one-quinolone derivatives as potential inhibitors of this enzyme. A molecular hybrid strategy, using a 4-ethoxy-7-substituted-quinoline core and a benzo[*d*]oxazol-2(3H)-one scaffold, was employed to design members of this family for study as inhibitors of the kinase and proliferation of EBC-1 cells. Most of the substances were found to display good to excellent c-Met kinase inhibitory activities. The results of a structure–activity relationship (SAR) study led to the discovery of benzo[*d*]oxazol-2(3H)-one-quinolone **13**, which has IC_{50} values of 1 nM against c-Met kinase and 5 nM against proliferation of the EBC-1 cell line.

1. Introduction

c-Met, encoded by Met proto-oncogene, is a receptor tyrosine kinase (RTK) whose binding with the hepatocyte growth factor (HGF) induces several complex signaling pathways that result in cell proliferation, migration and invasion [1–4]. Aberrant c-Met activation, mutation, amplification and translocation play important roles in cancer formation, progression, dissemination, as well as drug resistance [5–9]. Moreover, elevations of both c-Met and HGF are also associated with poor clinical outcomes for cancer patients [10,11]. As a result, c-Met kinase has received considerable attention as an attractive target for cancer treatment.

During the last decade, the utilization of a structure-based drug design approach [12] has led to the discovery of a number of small molecules, which possess various pharmacophores, such as triazolopyridazines [13], triazolotriazines [14], quinolones [15–20] and quinoxalines [21], that have c-Met inhibitory activities. Furthermore, a significant number of c-Met inhibitors are in clinic trails at the current time (Fig. 1).

The benzo[*d*]oxazol-2(3H)-one ring system is an important skeletal feature of molecules that have a diverse array of biological properties including anti-inflammatory, cytotoxicity, anti-tumor and neurological activities [22–26]. More pertinent to the current study is the report by workers at the Merck company that although benzo[*d*]oxazol-2(3H)-one **1** (Fig. 2A) is a modest c-Met inhibitor ($IC_{50} = 1.6$ μM) [27], it has poor cellular inhibitory activity against c-Met dependent EBC-1 cell proliferation. Based on observations made in a previous investigation of pyridazinone-quinoline derivatives, we speculated that the greatly lowered cellular activity of **1** is a consequence of the presence of the polar carbamate group [28], which is not compatible with the hydrophobic hinge pocket of the enzyme. We speculated that replacement of the carbamate by the more hydrophobic quinoline moiety (as in **2b** in Fig. 2A) would enhance both hydrogen bonding (with Met1160) and hydrophobic interactions with c-Met and lead to an improvement of cellular activity (Fig. 2A). Importantly, a quinoline moiety serving as a motif to promote hinge binding is prevalent in a number of known c-Met

---

* Corresponding author.
** Corresponding author.
E-mail addresses: mygeng@simm.ac.cn (M. Geng), yhhu@simm.ac.cn (Y. Hu).
[1] DL and AS equally contributed to this work.

http://dx.doi.org/10.1016/j.ejmech.2016.03.027

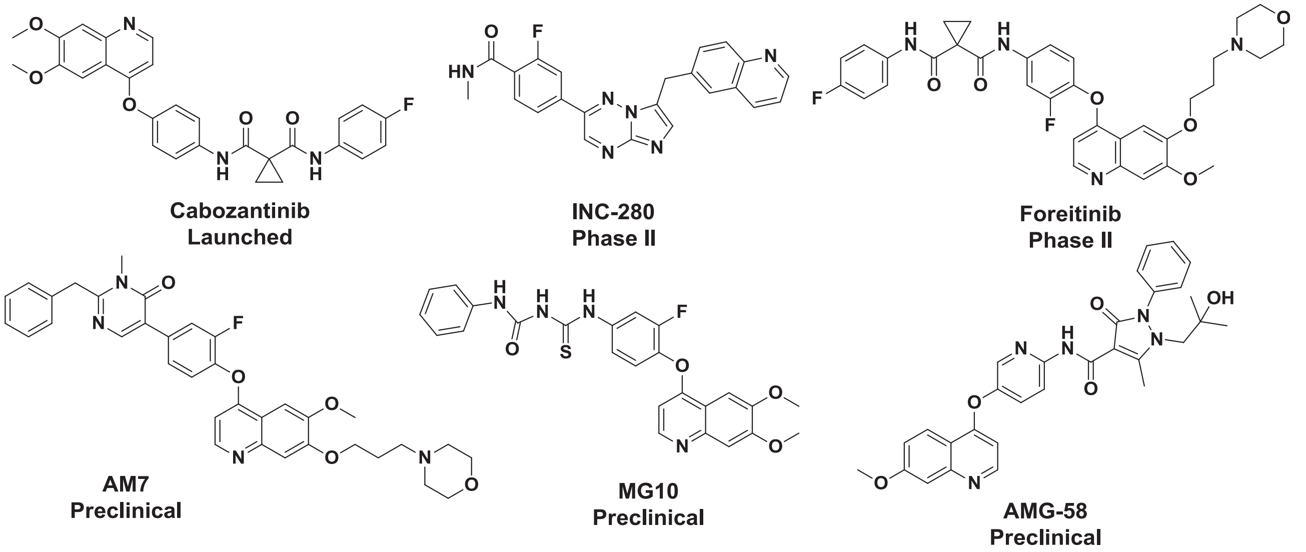


Fig. 1. Representative small-molecule c-Met inhibitors with quinoline pharmacophores.

inhibitors (Fig. 1).

Based on the proposal made above, substances containing the benzo[d]oxazol-2(3H)-one-quinolone scaffold were designed by using a molecular hybridization strategy to be novel c-Met inhibitors (Fig. 2A). The results of studies in which **1** and **7a** are docked with c-Met kinase revealed that benzo[*d*]oxazol-2(3H)-one moiety in **7a** exactly matches the binding model established using **1**. Specifically, **7a** exists in a “U” shape conformation in the ATP binding pocket of c-Met kinase where it is sandwiched between Met 1211 and Tyr1230 (Fig. 2B). In addition, the quinoline ring nitrogen in **7a** forms a hydrogen bond with Met1160 instead of a carbonyl group, and the substituent at C7 of the quinoline moiety appears to extend into the solvent exposed region of the protein. SAR studies were explored using selected benzo[*d*]oxazol-2(3H)-one and quinolone ring substituted analogs of **7a** (see Tables 1 and 2). The results of this effort demonstrated that the benzo[*d*]oxazol-2(3H)-one-quinolone **13** is a potent c-Met inhibitor and it displays anti-proliferation activity against EBC-1 cells.

2. Results and discussion

2.1. Chemistry

The routes employed to synthesize benzo[d]oxazol-2(3H)-one-quinolones **7a**–**7g** are outlined in Scheme 1. 6-Methylbenzo[d]oxazol-2(3H)-one, prepared from commercially available 2-amino-5-methylphenol [27], was subjected to N-alkylation with 2-bromoethoxy-(*t*-butyl-dimethylsilane) to form the corresponding N-alkylated derivative **3**. Removal of the TBS group in **3** generated alcohol **4**, which was subjected to Buchwald cross coupling with 7-substituted-4-chloroquinolines **6** to form the target benzo[d]oxazol-2(3H)-one-quinolones **7a**–**7g**. Intermediates **6c**–**6g** used in these routes were generated by O-alkylation reactions of 4-chloroquinoline-7-ol [29] followed by amination reactions with secondary amines.

The methods used to prepare benzo[d]oxazol-2(3H)-one-quinolones **7h**–**7i**, **11**, **12** and **13** are outlined in Scheme 2. 4-Chloro-7-methoxyquinoline or 4-chloro-7-bromoquinoline [30] were reacted with 2-*tert*-butoxyethanol to produce the corresponding O-ethoxy derivatives **9**, which were transformed to the respective alcohols **10**. Mesylation of the hydroxyl group in **10** was followed by N-alkylation reactions with substituted benzo[*d*]oxazol-2(3H)-ones **8** [27] to produce benzo-oxazole substituted quinolones **11**. Alternatively, **7h**–**7i** and **12a**–**12c** were generated by Suzuki or Buchwald cross coupling reactions of the corresponding bromo-substituted analogs **11**. Finally, the pyrazole derivative **13** was prepared by using a double Suzuki reaction of **11k** with 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole.

2.2. SAR Development and lead generation

An evaluation of the inhibitory activities of the benzo[d]oxazol-2(3H)-one-quinolones **7a**–**i** against c-Met kinase and proliferation of EBC-1 cells was carried out. The results of this SAR exploration, focusing on the effects of quinolone C7 substituents are given in Table 1. The findings show that these substances display excellent inhibitory activities against c-Met kinase with IC_{50} values in the 3.4–11.0 nM range. The C7 substituents on the quinolone ring, which modeling studies suggested would extend into the solvent exposed region of the protein, have an obvious effect on the cellular activity as determined by using the EBC-1 cell proliferation assay. In line with this reasoning, the results (Table 1) show that introduction of hydrophilic substituents leads to a dramatic improvement in proliferation inhibition. For example, **7d**–**7g** containing more hydrophilic C7 substituents have cellular activities with IC_{50} values in the 120–330 nM range. In addition, **7d** in which the carbamate moiety in **1** is replaced by a quinoline moiety is a more potent cell proliferation inhibitor. Furthermore, **7g** which contains a four carbon linker, is less potent than **7e** and **7f**, indicating that a linker length of two or three carbons is optimal for inhibition. Also, **7h** possessing an amino side chain has a more potent cellular inhibitory activity than does **7e** which contains an alkoxy side chain. Finally, introduction of highly water soluble N-methylpyrazole substituents, produces **7i**, which displays the most potent cell proliferation inhibitory activity ($IC_{50} = 35$ nM) of substances in this family. Notably, **7i** has the nearly the same anti-proliferation inhibitory activity as does Crizotinib and a 10 fold lower ratio of cellular to enzymatic IC_{50} values than this reference substances.

An additional SAR study was carried out to explore the effects of substituents on the benzo[*d*]oxazol-2(3H)-one moiety in the benzo[d]oxazol-2(3H)-one-quinolones. The results (Table 2) show that **7a**, **11b**, **11d** and **12b**, each of which contains a C6 substituent, exhibit better inhibitory activities against c-Met kinase than do **11a**,