European Journal of Medicinal Chemistry 115 (2016) 416–425

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

Substituted quinazolinones as kinase inhibitors endowed with anti-fibrotic properties

Giovanni Marzaro [a, *], Ignazio Castagliuolo [b], Giulia Schirato [b], Giorgio Palu' [b], Martina Dalla Via [a], Adriana Chilin [a], Paola Brun [b]

[a] Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Via Marzolo 5, 35131 Padova, Italy
[b] Department of Molecular Medicine, University of Padova, Via Gabelli 63, 35121 Padova, Italy

ARTICLE INFO

Article history:
Received 11 January 2016
Received in revised form
17 March 2016
Accepted 18 March 2016
Available online 19 March 2016

Keywords:
Quinazolinones
Tyrosine kinases
Multi kinase inhibitors
Fibrosis

ABSTRACT

Some new 3-substituted quinazolinones were synthesized and evaluated as inhibitors of kinases involved in fibrogenic process. The compounds were tested against a panel of both tyrosine and serine–threonine kinases. The profile of selectivity of some representative compounds was investigated through molecular docking studies. The most interesting compounds were also evaluated *in vitro* as potential agents for the treatment of fibrotic diseases. Quinazolinone derivatives reduced proliferation and expression of genes involved in the fibrogenic process in hepatic stellate cells (HSCs) and intestinal subepithelial myofibroblasts (ISEMFs). Furthermore some compounds downregulated phosphorylation of p38MAPK. Our findings provide evidences that 3-substituted quinazolinones target multiple essential pathways of the fibrogenic process.

1. Introduction

Protein kinases are ubiquitous enzymes devoted to the regulation of almost all cellular events. The kinases catalyze the transfer of a phosphate group from the ATP to specific substrate eventually leading to transduction and propagation of cellular signal [1]. Deregulated activity, mutation or over-expression of these enzymes have been correlated to cancer [2], chronic inflammatory disorders [3], diabetes [4], cardiovascular diseases [5] and hypertension [6]. According to the targeted amino acids, kinases are commonly grouped in two major families: the tyrosine kinases (TKs) and the serine–threonine kinases (STKs). Among the TKs, the epidermal growth factor receptor (EGFR), the type 2 vascular endothelial receptor (VEGFR2 or KDR), the type 1 fibroblast growth factor receptor (FGFR1), and the cytoplasmic enzymes Abl1 and Src play crucial roles in cell proliferation as well as in cancer onset and progression [7]. On the other hand, the phosphatidylinositol-3 kinase (PI3K) is mainly a lipid kinase that, along with the mammalian target of rapamycin (mTOR), is included in the STK family. Indeed, by phosphorylation of serine or threonine containing proteins the mTOR/AKT/PI3K pathway controls several cellular functions including inflammatory responses and cancer development [8].

Remarkably, TKs and STKs -induced intracellular signals are important modulators of fibrogenic process in lung, liver, pancreas, heart, and gut. Fibrosis can occur during tissue repair or inflammation as a result of persistent activation of fibrogenic cells, which leads to aberrant extracellular matrix (ECM) deposition and progressive substitution of the normal parenchyma by scar tissue [9]. For instance, persistent liver injury and unrestrained inflammatory cascade lead to EGFR-mediated proliferation and migration of hepatic stellate cells (HSCs), the cellular population involved in the deposition of ECM in the liver [10]. In addition, release of specific growth factors triggers the synergistic activation of a number of protein kinase pathways that serve different biological roles related to fibrogenesis [11]. Inhibition of KDR by neutralizing monoclonal antibody ameliorated carbon tetrachloride induced hepatic fibrosis in mice not only by suppressing the neovascularisation but also by reducing the α1(I)-procollagen mRNA expression in HSCs [12]. Likewise, preliminary randomized double-blind clinical trials

Abbreviations: CAN, cerium ammonium nitrate; CD, Crohn's Desease; COL1A1, prepro-alpha 1 collagen; DMF, dimethylformamide; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; FGFR1, type 1 fibroblast growth factor receptor; FN1, fibronectin 1; HSC, hepatic stellate cells; ISEMF, intestinal subepithelial myofibroblasts; KDR, type 2 vascular endothelial receptor; mTOR, mammalian target of rapamycin; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositol-3 kinase; STK, serine–threonine kinases; TFA, trifluoroacetic acid; TIMP1, tissue inhibitor of metalloproteinase 1; TK, tyrosine kinases; VEGFR2, type 2 vascular endothelial receptor.

* Corresponding author.
E-mail address: giovanni.marzaro@unipd.it (G. Marzaro).

http://dx.doi.org/10.1016/j.ejmech.2016.03.053
0223-5234/

indicate that sirolimus and everolimus, two mTOR inhibitors, induce remission in refractory Crohn's Disease (CD) by decreasing the number of intestinal subepithelial myofibroblasts (ISEMFs) and the expression of pro-fibrotic cytokines [13–15]. Currently, the pharmacological care in patients with tissue fibrosis relies on corticosteroids and immunosuppressant drugs but fibrosis-related consequences remain a major causes of morbidity and mortality [16].

The pivotal role of kinases as targets for novel drugs is clearly demonstrated by the huge amount of ATP-mimic kinase inhibitors, mainly TK inhibitors, developed in the last two decades [17]. The ATP-mimic kinase inhibitors belong to different chemical classes of compounds. However they share a common pharmacophore [17] that is generally composed by: *i*) a scaffold (mainly a nitrogen containing heterocycle) able to interact through an H-bond with the kinase *hinge region*; *ii*) a lipophilic moiety (mainly an aromatic or an heteroaromatic system) that occupy a pocket opened by the so called *gatekeeper* residue; *iii*) a spacer between the heterocycle and the hydrophobic moiety; *iv*) solvent exposed residues. As part of our novel bioactive compounds discovery projects [18–20] and due to our experience in quinazoline compounds [18,19,21], we decided to investigate whether it was possible to develop novel quinazolinone-based kinase inhibitors able to restrain the activation of fibrogenic cells. Quinazolinone compounds are endowed with a number of biological activities comprising antiviral [22], antitubercular [23], antimicrobial [24], antitubulin [25], antifolate [26], anticonvulsivant [27], anti-inflammatory [28], antifibrotic [29] and anticancer [30–32] properties. Their kinase inhibitory activity however has not been extensively explored.

Herein we report the synthesis and the preliminary evaluation of several 3-substituted quinazolinones against a panel of kinases mainly involved in the fibrogenic process. The binding mode of several compounds with the target kinases was investigated by means of molecular docking studies. The anti-fibrotic activity of the compounds was assessed *in vitro*.

2. Results and discussion

2.1. Chemistry

The general structure of the novel compounds is reported in Fig. 1A.

The 3-substituted quinazoline compounds are structurally related to another well known class of kinases inhibitors, the 4-anilinoquinazolines [34,35] (Fig. 1B), as demonstrated by the superimposition with erlotinib in its binding conformation with EGFR (Fig. 1C and D). According to our previous studies [19], the 6 and 7 positions of the quinazolinone scaffold were functionalized with dimethoxy functions or with fused dialkoxy rings, namely a dioxane and a dioxolane ring. The 3 position of the quinazolinone was substituted with several lipophilic moieties (2-bromopyridine, biphenyl, halophenyl) linked to the quinazolinone nitrogen with a bridge of variable sizes and chemical properties (methylene, 2-hydroxyethylene, 2-oxoethylene). According to the type of substitution at the positions 6 and 7, the newly synthesized compounds were grouped in three classes (Table 1). All the compounds have been synthesized starting from the appropriate aniline derivatives through quinazoline intermediates, taking advantage of an already reported synthetic strategy [36] (Scheme 1).

Briefly, anilines **16a-c** were protected as carbamates, submitted to condensation with hexametylenetetramine in TFA under microwave irradiation and then to aromatization with potassium ferricyanide in hydroalcoholic KOH at reflux. The obtained quinazoline **18a-c** were oxidized with CAN in acetic acid to quinazolinones **19a-c** [18], which were finally condensed with the suitable halobenzyl derivatives, aryloxyrane or haloacetophenones and NaH in DMF under microwave irradiation give the final products **1**–**15**.

2.2. Kinase screening

To outline the profile of activity/selectivity, all the synthesized compounds were preliminarily screened for their ability to counteract the kinase activity of a selected panel of kinases (both TKs and STKs) involved in fibrosis [37–43]. Thus, in this study the synthesized compounds have been tested at 1 μM against a panel of six tyrosine kinases and two serine–threonine kinases. Vatalanib (PTK787/ZK-22258, a poly-tyrosine kinase inhibitor endowed with anti-fibrotic and anti-neoplastic activities) [44,45] has been used as positive control. The results of the screening are summarized in Table 2.

Many compounds inhibited the activity of KDR or EGFR, though to a lower extent than vatalanib. The majority of these compounds (**6**, **10**, **11**, and **14**) were dual KDR and EGFR inhibitors, whereas compound **1** was a dual EGFR/PDGFRβ (Platelet-Derived Growth Factor Receptor β-isoform) inhibitor. Moreover compounds **7** and **11** were also active against one of the tested STKs. All the compounds were inactive against the cytoplasmic kinases Abl1 and Src and the receptor kinase FGFR1.

2.3. Molecular docking

To rationalize the activity profile of some representative compounds, molecular modelling studies were performed. In particular we focused on compounds **1** (active against EGFR; inactive against KDR), **11** (active against KDR, EGFR and PI3K; inactive against mTOR) and **7** (active against mTOR; moderately active against PI3K).

As depicted in Fig. 2A, compound **1** was expected to interact through the N^1-quinazoline nitrogen and the pyridine nitrogen with the hinge region residue M793 and with the gatekeeper T790

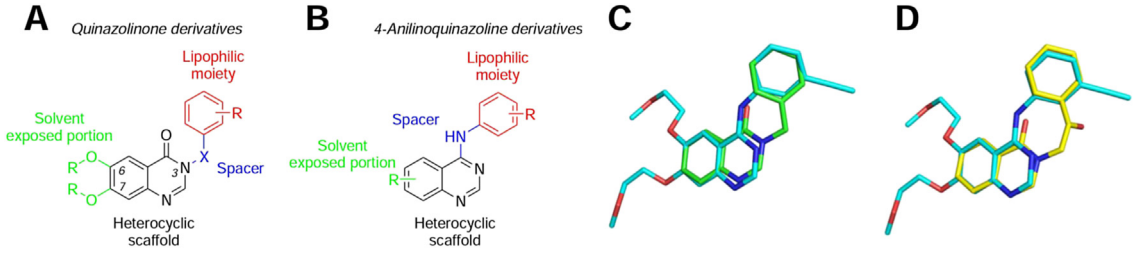

Fig. 1. (A) General structure of quinazolinone derivatives. (B) General structure of 4-anilinoquinazoline kinase inhibitors. (C) Superimposition of the 3-benzylquinazolin-4-one scaffold (green carbon sticks) and erlotinib (cyan carbon sticks). (D) Superimposition of the 3-(benzoylmethyl)quinazolin-4-one scaffold (yellow carbon sticks) and erlotinib (cyan carbon sticks). The tridimensional structure of erlotinib was extracted by the crystallographic complex with EGFR (PDB ID: 1M17) [33]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)