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Research paper

Novel click modifiable thioquinazolinones as anti-inflammatory agents: Design, synthesis, biological evaluation and docking study

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ABSTRACT

Click chemistry was used to synthesize a new series of thioquinazolinone molecules equipped with propargyl moiety,1,2,3-triazolyl and isoxazolyl rings. Our design was based on merging pharmacophores previously reported to exhibit COX-2 inhibitory activities to a thioquinazolinone-privileged scaffold. The synthesized compounds were subjected to *in vitro* cyclooxygenase COX-1/COX-2 and 15-LOX inhibition assays. Compounds **2c, 3b, 3h, 3j,** and **3k** showed COX-2 inhibition with IC_{50} (μM) 0.18, 0.19, 0.11, 0.16 and 0.17 respectively. These values were compared to celecoxib (IC_{50} 0.05 μM), diclofenac (IC_{50} 0.8 μM) and indomethacin (IC_{50} 0.49 μM) reference drugs. They also showed 15-LOX inhibition with IC_{50} (μM) 6.21, 4.33, 7.62, 5.21 and 3.98 respectively. These values were compared with Zileuton (IC_{50} 2.41 μM) and Meclofenamate sodium (IC_{50} 5.64 μM) as positive controls. These compounds were further challenged by PMA-induced THP-1 differentiation assay where compounds **2c** and **3j** inhibited monocyte to macrophage differentiation efficiently with IC_{50} values of 4.78 μM and 5.63 μM, respectively, compared to that of diclofenac sodium (4.86 μM). On the other hand, **3h** demonstrated a significantly increased potency compared to diclofenac in this assay ($IC_{50} = 0.13$ μM). The same compounds exhibited significant *in vivo* anti-inflammatory effect as indicated by the formalin-induced rat-paw edema test. Docking experiments of compounds **2c, 3b, 3h, 3j,** and **3k** into COX-2 binding pocket have been conducted, where strong binding interactions have been identified and effective overall docking scores have been recorded. Their drug-likeness has been assessed using *Molinspiration*, *Molsoft* and *Pre-ADMET* software products.

1. Introduction

Arachidonic acid (AA) is the biological precursor for a plethora of inflammatory mediators that are produced by two metabolic pathway key enzymes, namely: cyclooxygenase (COX) and lipooxygenase (LOX) [1–3]. COX-1 and COX-2 are responsible for converting AA to the hydroxyendoperoxide PGH2, which is further metabolized to prostaglandins (PGs), prostacyclin (PGI2) and thromboxane A2 (TXA2) [1–3]. COX-1 is constitutively active and synthesizes prostaglandins in the context of gastrointestinal tract cytoprotection, regulation of platelet aggregation and renal function maintenance [1–5]. COX-2 expression is induced by pro-inflammatory stimuli and it generates inflammatory signaling PGs [1–3,5,6]. On the other hand, LOX converts AA to hydroperoxyeicosatetraenoic acids (HPETE), subsequently to hydroxyeicosatetraenoic acids (HETE) and then to the leukotrienes (LTs) [1,7–9]. Pathogenesis of several inflammatory conditions, like osteoarthritis, rheumatoid arthritis, psoriasis, COPD and multiple sclerosis; is associated with induction of PGs and LTs [1,7–9]. Of note, selective COX-2 inhibitors gained much popularity in the late 1990s and early 2000s as anti-inflammatories that exhibited minimal GIT side effects [10]. However clinical practice proved them to

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be associated with myocardial infraction and cardiovascular thrombotic events [11–13]. Moreover, blocking only COX-2 would shunt the inflammatory pathway towards more production of LTs and hence, leads to more untoward side effects such as asthma [7–9]. As such, having a dual COX-2/LOX inhibitor would be a more judicious alternative to produce an efficacious anti-inflammatory with a better safety profile.

Quinazolines are privileged scaffolds [14–16] that display a wide range of biological activities such as analgesic [17–19], anti-inflammatory [17–19], antimicrobial [20–22], anticonvulsant [23,24] and anticancer [25–27] activities. Proquazone [28] (Structure **I**, Fig. 1a) and Fluproquazone [29] (structure **II**, Fig. 1a) are anti-prostaglandin quinazolines that are used to alleviate inflammatory symptoms in rheumatoid arthritis. Moreover, recently reported quinazolinones and thioquinazolinones either modified with small heterocyclic motif at C-2 [30] (structure **III**, Fig. 1a) or substituted on Sulfur atom with acetic acid hydrazide [31](structure **IV**, Fig. 1a), showed strong anti-inflammatory properties. Furthermore, Converso et al. reported the synthesis of thioquinazolinone-S-acetamide derivative (Structure **V**, Fig. 1a) that exhibited a strong inhibition of checkpoint kinase 1 (Chk1) ($IC_{50} = 1.3\ \mu M$) which should sensitize cancerous cells to DNA damaging agents without negatively affecting non-tumor cells [32]. Based on the structural information of the latter 5 structures, we reasoned that thioquinazolinone scaffold would be an appropriate skeleton to build on to reach potential active anti-inflammatory agents.

The discovery of the ability of Cu(I) to catalyze azide alkyne Huisgen cycloaddition (CuAAC) reaction, reported independently by the Sharpless-Fokin [33] and the Meldal groups [34] in 2002, represented a major breakthrough that revolutionized the applications of 1,2,3-triazole and illustrated a distinct example of click reactions [35]. Click reactions are broad in scope, high in yield, stereospecific (giving only 1,4-disubstituted derivative with Cu(I) catalysis), and carried out in aqueous or green solvents with minimal and/or inoffensive byproducts [35]. 1,4-disubstituted 1,2,3-triazoles are stable to extreme redox and pH conditions, which highlights their aromatic stabilization [36–38]. Their dipole moment is 5.06 D, which enables them to be involved in efficient hydrogen bonding and/or π-π stacking with biological targets [36–38]. Over and above, they are non-trivial bioisosteres of the amide group [37]. All of these features prompted Nobel Laureate K. Barry Sharpless to describe 1,2,3-triazoles as aggressive pharmacophores [37]. Several anti-inflammatory disubstituted 1,2,3-triazoles have been reported such as compound IV in Fig. 1a, which exhibited better *in vivo* anti-inflammatory activity than indomethacin in rat carrageenan-induced foot, paw edema model and displayed selective COX-2 inhibitory activity [39].

The toolbox for click reactions has been recently extended to include the cycloaddition reaction of nitrile oxides with alkynes to give Isoxazoles [40]. Such reaction shares many advantages of the CuAAC such as rapid kinetics, regioselectivity (giving only 3,5-disubstituted products) and synthetic ease [40]. Besides, isoxazoles, similar to 1,2,3-triazoles, represent a non-classical bioisostere of amide bond and have a capability of engaging in hydrogen bonding and/or π-π stacking with biological targets [40,41]. Of particular interest is the presence of isoxazole pharmacophore in the selective COX-2 inhibitor Valdecoxib (Fig. 1a).

Given the need for a dual selective COX-2/LOX inhibitor that is capable of maintaining the anti-inflammatory benefit without any untoward GI and asthma side effects, and in light of the above-mentioned facts; we decided to synthesize new thioquinazolinone molecules equipped with 1,2,3-triazolyl and isoxazolyl rings (Fig. 1b), via click chemistry, for evaluation as anti-inflammatory agents.

2. Results and discussion

2.1. Chemistry

The synthetic strategy to prepare the target compounds is illustrated in Scheme 1. 2-Mercapto-3-substituted phenyl-quinazolin-4(3*H*)-ones **1 (a–d)** were prepared by refluxing different

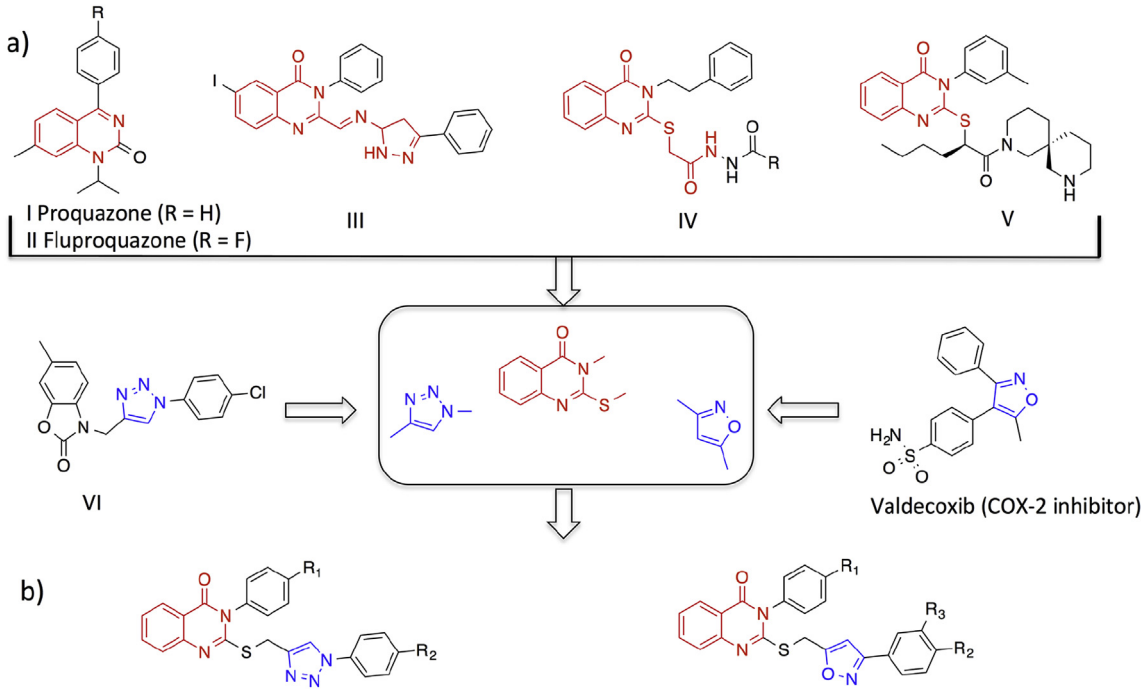

Fig. 1. Rationale for the design of the target compounds. a) Representative examples of some reported anti-inflammatory and anticancer compounds carrying quinazolinone scaffold, 1,2,3-triazolyl and isoxazolyl pharmacophores. b) Structure of the target compounds.