European Journal of Medicinal Chemistry 111 (2016) 103–113

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

Synthesis, antiproliferative activities, and computational evaluation of novel isocoumarin and 3,4-dihydroisocoumarin derivatives

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ARTICLE INFO

Article history:
Received 28 October 2015
Received in revised form
26 January 2016
Accepted 28 January 2016
Available online 30 January 2016

Keywords:
Isocoumarin
3,4-Dihydroisocoumarin
Castro–Stephens reaction
Antiproliferative

ABSTRACT

A series of novel isocoumarin derivatives were synthesized using Castro–Stephens cross-coupling. Moreover, novel 3,4-dihydroisocoumarin derivatives were obtained by catalytic hydrogenation of the corresponding isocoumarin precursors. The antiproliferative activity of all compounds was evaluated *in vitro* in different tumor cells. Furthermore, docking calculations were performed for the kallikrein 5 (KLK5) active site to predict the possible mechanism of action of this series of compounds. Theoretical findings indicate that the 3,4-dihydroisocoumarin derivative **10a** forms hydrogen bonds with Ser190 and Gln192 residues of KLK5. This derivative was the most active compound in the series with potent antiproliferative activity and high selectivity index (SI > **378.79**) against breast cancer cells (MCF-7, $GI_{50} = 0.66\ \mu g\ mL^{-1}$). This compound represents a promising matrix for developing new antiproliferative agents.

1. Introduction

Cancer is a significant public health problem and a major cause of death in humans [1]. Despite many efforts to fight cancer, successful treatment of certain types of tumors continues to be challenging because of their aggressiveness, the complex mechanisms underlying malignant cell metastasis, chemoresistance, and the lack of selectivity of some drugs [2]. Thus, the development of new, safe, and effective anticancer agents through the synthesis of simple small molecules is necessary.

Isocoumarins, including their 3,4-dihydro derivatives, are isolated from a variety of natural sources and have diverse biological activities such as antifungal, antimicrobial [3], antiallergic [4], immunomodulatory [5], enzyme inhibitory [6–8], antiangiogenic [9], and antioxidant properties [10]. The different biological activities of the natural compounds belonging to this class are thought to be because of the large structural variety found among these compounds [11].

Several natural and synthetic coumarins or 3-substituted- 3,4-dihydroisocoumarins have significant cytotoxic and antitumor activities. For instance, cytogenin **1**, a 3-hydroxymethyl coumarin, first isolated in 1990 from *Streptoverticillium eurocidicum*, was the first natural isocoumarin that showed anticancer activity against experimental tumor cells and human cancer cells [12]. NM-3 **2**, a synthetic analogue of cytogenin in phase I of clinical tests, potentiates the antineoplastic effects of other chemotherapeutic agents and inhibits angiogenesis [13]. 3-Arylisocoumarin **3** has potential anticancer and antimicrobial activity [14]. The compound 185322 **4**, an inhibitor of microtubule assembly, induces mitotic arrest and apoptosis of multiple myeloma cells [15]. The chiral 3-methyl-3,4-ochratoxin A **5**, a mycotoxin isolated from *Aspergillus ochraceus*, shows nephrotoxic, hepatotoxic, carcinogenic, and teratogenic properties [16]; further, the chiral 3-pentyl-3,4-dihydrocoumarin **6** has cytotoxic properties [3,7,8] (Fig. 1).

Typically, homophthalic acid derivatives are used as starting materials in the synthesis of isocoumarins and 3-substituted-3,4-dihydrocoumarins [17,18]. The recently developed cross-coupling reactions catalyzed by transition metals have facilitated the development of new methods for the synthesis of isocoumarins and 3,4-dihydroisocoumarins [11,19,20]. A cross-coupling reaction

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http://dx.doi.org/10.1016/j.ejmech.2016.01.051
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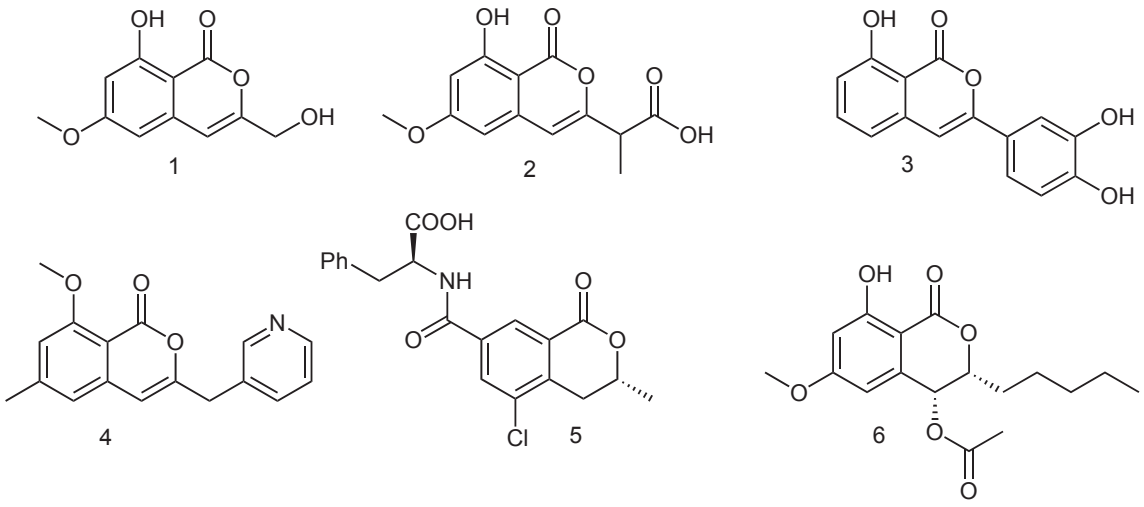


Fig. 1. Representative examples of biologically active isocoumarin and 3,4-dihydroisocoumarin.

known as the Castro–Stephens reaction [21], catalyzed by Cu (I) has been successfully used to synthesize these compounds. Wang and coworkers modified the methodology of this reaction, making it a one-pot reaction [22].

On the basis of the previously described biological activities of this class of compounds, we designed and synthesized a series of 3-substituted isocoumarin derivatives and a series of 3-substituted 3,4-dihydroisocoumarin derivatives using a modified Castro–-Stephens coupling reaction. The antiproliferative activity of the new compounds was evaluated *in vitro* against some tumor cell strains, and we performed theoretical investigations for a potential molecular target.

2. Results and discussion

2.1. Chemistry

To synthesize novel isocoumarin derivatives, we used a one-pot reaction between a terminal alkyne and a derivative of the 2-halobenzoic acid. The first step was the acquisition of some simple terminal alkynes (Scheme 1). Most of the alkynes were obtained by modification of both the commercially available propargyl alcohol (**7a**) and 4-pentyn-1-ol (**7b**). The choice of different alkynes governed a structural variability in the final products.

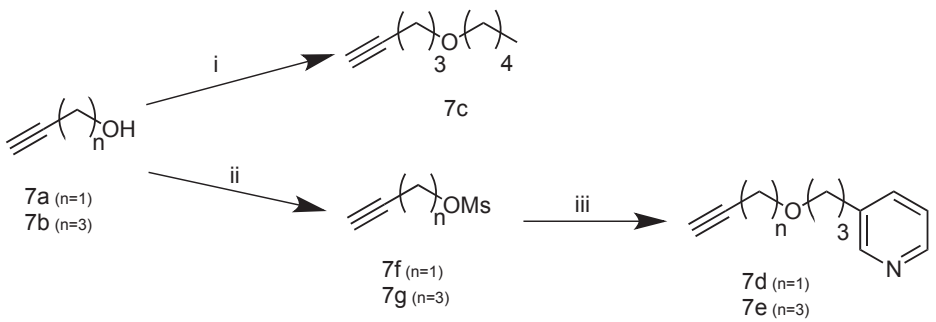
After obtaining the desired alkynes, we synthesized the isocoumarin derivatives in a one-pot reaction catalyzed by Cu (I) and *trans*-4-hydroxy-L-proline as a ligand. The presence of an amino acid ligand is thought to inhibit the formation of phthalides, which are common byproducts in these kinds of reactions. We did not detect these byproducts during our experiments. All the planned isocoumarins were obtained successfully (Scheme 2). The benzoic acid derivatives 2-iodobenzoic acid (**8a**) and 2-bromo-5-methoxybenzoic acid (**8b**) are commercially available.

The observed yields of the compounds **9a** (54%), **9b** (51%), **9c** (73%), and **9d** (69%) suggest that the size of the alkyne chain influences the performance of the reaction. Compounds **9e** and **9f** were obtained in moderate to good yields (65% and 71%, respectively). Compound **9c** was subjected to a subsequent mesylation reaction, which resulted in an intermediate **9i**. Subsequently, **9i** reacted with both the commercial 1-phenyl-1*H*-tetrazole-5-thiol and 5-phenyl-1*H*-tetrazole, thus resulting in compounds **9g** and **9h** (Scheme 3). We did not detect any isomer for compound **9h**.

The obtained isocoumarins were then submitted to Pd/C-catalyzed hydrogenation under pressure, generating their respective 3,4-dihydroisocoumarins as racemic mixtures (**10**) in good to high yields (Scheme 4). The compounds bearing the substituent *O*-3-propylpyridine, **9e** and **9f**, did not react under the catalytic hydrogenation conditions used. The results of the catalytic hydrogenation are shown in Table 1.

2.2. Antiproliferative studies

We examined the antiproliferative activities of compounds from series (**9a**–**h**, **10a**–**d**, and **10g**–**h**) according to the methodology



Scheme 1. Reagents and conditions: (i) **7b** (1.0 equiv), NaH (3.0 equiv), 1-iodopentane (3.0 equiv), THF, 0 °C-r.t., 16 h (**7c**: 87% yield); (ii) **7a** or **7b** (1.0 equiv), Et_3N (1.5 equiv), MsCl (1.7 equiv), CH_2Cl_2, −40 °C, 1 h (**7f**: 97% yield, **7g**: quant.); (iii) **7f** or **7g** (1.0 equiv), NaH (3.0 equiv), 3-pyridinepropanol (1.5 equiv), THF, 0 °C-r.t., 16 h (**7d**: 79% yield, 7e: 83% yield).