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Research paper

Synthesis of new 4-phenylpyrimidine-2(1*H*)-thiones and their potency to inhibit COX-1 and COX-2

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ABSTRACT

Several new 4-phenylpyrimidine-2(1*H*)-thiones have been prepared and investigated for their potencies to inhibit COX-1 and COX-2 enzymes, and COX-2 expression in THP-1 cells. Structure-activity-relationships and physicochemical parameters are discussed. Pharmacophore screening and docking studies were carried out for the most active compound.

1. Introduction

A single 4-phenylpyrimidine-2(1H)-thione has already been investigated for its analgesic, anticonvulsant and anti-inflammatory activities and was found more potent than prednisolone as anti-inflammatory agent [1]. Since the anti-inflammatory activity was described as an inhibition of plasma-PGE$_2$ and protection against carrageenan-induced oedema, it seemed to be interesting to investigate whether this activity is related to COX inhibition. Therefore, we prepared a series of similar compounds and determined their ability to inhibit COX-1 and COX-2 enzymes, and COX-2 expression in THP-1 cells.

2. Chemistry

The already investigated 3,4-dihydro-4-methyl-6-phenylpyrimidine-2(1*H*)-thione (**2a**) can be prepared by the reaction of 4-phenylbut-3-en-2-one (**1a**) with thiourea [2]. Alternatively, a reaction of 4-phenylbut-3-en-2-one (**1a**) with ammonium thiocyanate leads to compound **2a**, too [3]. We applied this method to prepare a series of new pyrimidinethiones **2b-2j** with various substituents at the aromatic moiety and for the synthesis of a single non-aromatic derivative **2k** to investigate the influence of the substitution patterns on the biological activity. For the preparation of compounds **2a-2j**, 4-phenylbut-3-en-2-one (**1a**) or substituted 4-arylbut-3-en-2-ones **1b-1j** were used as starting materials. The latter were prepared by aldol condensation of substituted benzaldehydes with acetone following reported procedures [4,5]. The non-aromatic pyrimidinethione **2k** was prepared from 5-methylhexen-2-one (**1k**) (Scheme 1). Evidence of structure was achieved by NMR spectroscopy. The signal of C-2 in ^{13}C NMR spectra appears at 173–175 ppm, a typical value for such thiourea

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Abbreviations: BCS, biopharmaceutics classification system; CH_3OH, methanol; COX, cyclooxy-genase; DMSO, dimethylsulfoxide; EDTA, ethylenediaminetetra-acetic acid; EIA, enzyme immuno assay; ELISA, enzyme linked immunosorbent assay; GAPDH, glycerinaldehyde-3-phosphate dehydrogenase; GOLD, genetic optimization for ligand docking; LPS, lipopolysaccharide; MR, molar refractivity; MW, molecular weight; PDB, protein data base; PGE$_2$, prostaglandin E$_2$; PGHS, prostaglandin H synthase; PMA, phorbol-12-myristate-13-acetate; QED, quantitative estimate of drug-likeness; RMSD, root-mean-square deviation of atomic position; SAR, structure activity relationship; TLC, thin layer chromatography; TRIS, tris(hydroxymethyl)-aminomethane; tPSA, topological polar surface area.

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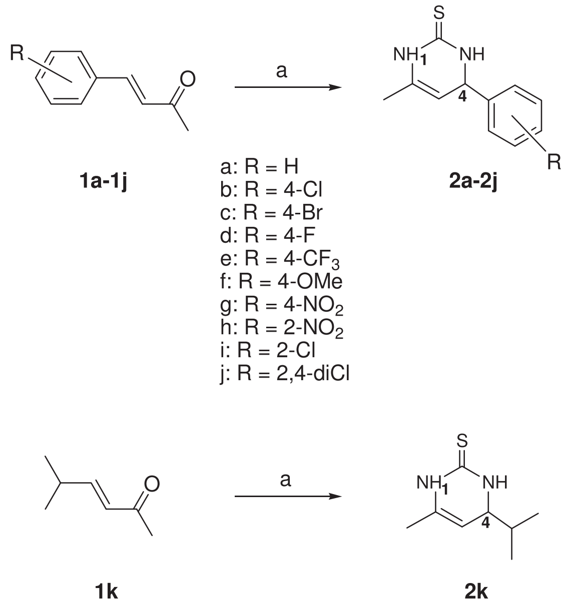

Scheme 1. Preparation of compounds **2a**–**2j**. Reagents and conditions: (a) ammonium thiocyanate, benzene, cyclohexanol, reflux 4–6 h.

structures [6]. The NH protons appear at about 8.8 and 9.7 ppm in ^{1}H NMR spectra and show long range couplings in HMBC spectra to the carbonyl carbon C-2. Furthermore, we observed w-couplings in COSY spectra of compounds **2** between both NH protons, between H-1 and H-5 and between H-5 and H-3 (Fig. 1).

3. COX-inhibition

The assays were performed in 96 well plates with purified PGHS-1 (COX-1) from rat seminal vesicles and purified PGHS-2 (COX-2) from sheep placental cotyledons (both Cayman Chemical Company, Ann Arbor, MI, USA) as previously described [7,8]. The concentration of PGE_2 was determined using a competitive PGE_2 EIA Kit (Enzo Life Sciences, Ann Arbor, MI, USA). NS-398 (Cayman Chemical Company) and indomethacin (MP Biochemicals) were used as positive controls.

For gene expression assays monocytic THP-1 cells (ECCAC, Lot No. 07I001, Sigma Aldrich) were differentiated to macrophages with 12 nM PMA for 48 h in 24-well plates, followed by incubation with test compounds, DMSO as calibrator and quercetin or dexamethasone as positive controls for NF-κB1 and COX-2 gene expression inhibition, respectively. Afterwards cells were stimulated with LPS and incubated for another three hours, except the well treated with DMSO (all Sigma Aldrich). RNA was extracted with GenElute Mammalian Total RNA Miniprep Kit (Sigma Aldrich) and reverse transcription was carried out with High Capacity cDNA Reverse Transcription Kit (Life Technologies). Relative expression was quantified via real-time PCR with TaqMan probes for NF-κB1 (NM_003998) and COX-2 (NM_000963) against endogenous control GAPDH (predesigned TaqMan assay) via ΔΔCt-method [9].

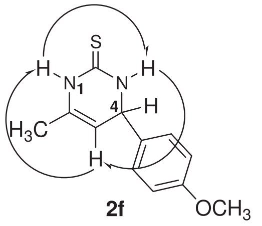

Fig. 1. W couplings in COSY spectrum of compound **2f**, marked as arrows.

4. Molecular modeling

The tested compounds were analyzed *in-silico* with two target-based methods: Pharmacophore modeling and docking. Pharmacophore models are a three-dimensional array of physicochemical features that represent the interacting functionalities of a given molecule with its target [10]. These features represent common interactions such as hydrogen bonds (H-bonds), ionic interactions, and hydrophobic interactions [11]. In principle, compounds that map a pharmacophore model, and therefore could interact in a similar manner as already known ligands, have an increased probability to be active against a specific target.

In this study, pharmacophore modeling was performed in LigandScout (version 3.02). Using this program, either a structure- or ligand-based modeling approach can be employed for the generation of pharmacophore models. In the structure-based approach, the interaction patterns of a ligand-target complex are used to calculate an initial model. In the ligand-based modeling, common pharmacophore features are extracted from three-dimensionally aligned known active molecules. These initial models can then be refined. In this study, all models were calculated based on the binding mode of co-crystallized inhibitors, because multiple ligand-target complexes were available in the Protein Data Bank [12]. If a part of the ligand fulfills the feature-specific distances, angles, and electronic requirements in relation to the protein binding site [11], a feature is placed on it. The resulting and refined 3D pharmacophore model can then be used for virtual screening. For this purpose, a 3D multi-conformational library of molecules is generated with OMEGA [13,14]. During virtual screening, LigandScout calculates a pharmacophore for each conformer and matches it to the query pharmacophore with a pattern-matching based alignment algorithm [15]. A Kabsch algorithm is used to minimize the feature distances between the query model and the conformers. Each match is evaluated with a geometrical fit value, which is calculated based on the RMSD between the fitting molecule and the pharmacophore model.

Furthermore, molecular docking was used to fit the compounds into the empty binding pocket of the target. The quality of the resulting binding poses is evaluated with a so-called docking score, which estimates the binding free energy [16]. To investigate the experimentally observed SAR, all measured compounds were screened against a previously reported pharmacophore model collection for COX-1 and COX-2 [17]. This process helped to compare the binding site interactions of the investigated compounds with the interaction patterns of well-known COX-inhibitors such as indomethacin that have been co-crystallized with the two COX isoforms. To further elucidate the binding mechanism of the novel active compound, all investigated structures were docked into the binding site of COX. The docking poses confirmed the crucial interactions from the pharmacophore model and also illustrated, why structurally closely related molecules did not display any biological activity.

5. Physicochemical properties

Physicochemical parameters are today considered to have a crucial role in the selection process of drug candidates during the early stages of drug discovery [18,19]. Most orally active drugs have a set of properties that fall in a defined physical and chemical 'drug-like space' [20–22]. For this reason an assessment of drug-likeness