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Original Article

Design and Synthesis of New Biarylhydrazides Possessing an Azido Pharmacophore as Selective COX-2 Inhibitors

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Abstract

A group of 1,3-biarylhydrazide derivatives possessing a COX-2 azido pharmacophore at the Para- position of the C-1 phenyl ring in conjunction with a N-3 phenyl or substituted-phenyl ring (4-F,4-Cl,4-OMe) were designed and synthesized based on nucleophilic substitution reaction. A molecular modelling study of these compounds showed that the designed molecules were well bound with the active site of COX-2 enzyme and the N3 pharmacophore was well oriented into the COX-2 secondary pocket. Thus, we expect the compounds show good potency and selectivity on COX-2 inhibition.

Keywords: Azido Pharmacophore , COX-2 inhibitors, Hydrazide Moiety

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1. Introduction

The use of non-steroidal anti-inflammatory drugs (NSAIDs) for the treatment of inflammation and pain is often accompanied by adverse gastrointestinal and renal side effects. Their anti-inflammatory activity results from inhibition of cyclooxygenase (COX), which catalyzes the bioconversion of arachidonic acid to prostaglandins. Nowadays, it is well established that there are at least two COX isozymes, COX-1 and COX-2. The COX-1 is responsible for the physiological production of prostaglandins while the COX-2 is responsible for the elevated production of prostaglandins during the inflammation (Vane et al., 1998; Steinmeyer, 2000; Jang et al., 2004; Li et al., 2004; Hamaya et al., 2010). Thus, selective inhibition of COX-2 over COX-1 is useful for the treatment of inflammation and inflammation-associated disorders with reduced gastrointestinal toxicities when compared with NSAIDs. Recently many selective COX-2 inhibitors have been reported including Celebrex (1) and Vioxx (2), the first COX-2 inhibitors on the market. The recent withdrawal of some diary heterocyclic selective COX-2 inhibitors due to the adverse cardiovascular side effects clearly delineates the need to explore and evaluate new structural ring templates possessing COX inhibitory activity (Mason et al., 2006) .

Recently we reported investigations of two novel classes of compounds possessing an acyclic 1,3-diarylprop-2-en-1-one (3) (Zarghi et al., 2006) and 1,3-diarylurea (4) (Zarghi et al., 2008) structural templates that exhibited high selective COX-2 inhibition. As part of our ongoing program to design new types of selective COX-2 inhibitors, we report the synthesis for a group of 1,3- biarylhydrazide derivatives possessing a COX-2 N3 pharmacophore.

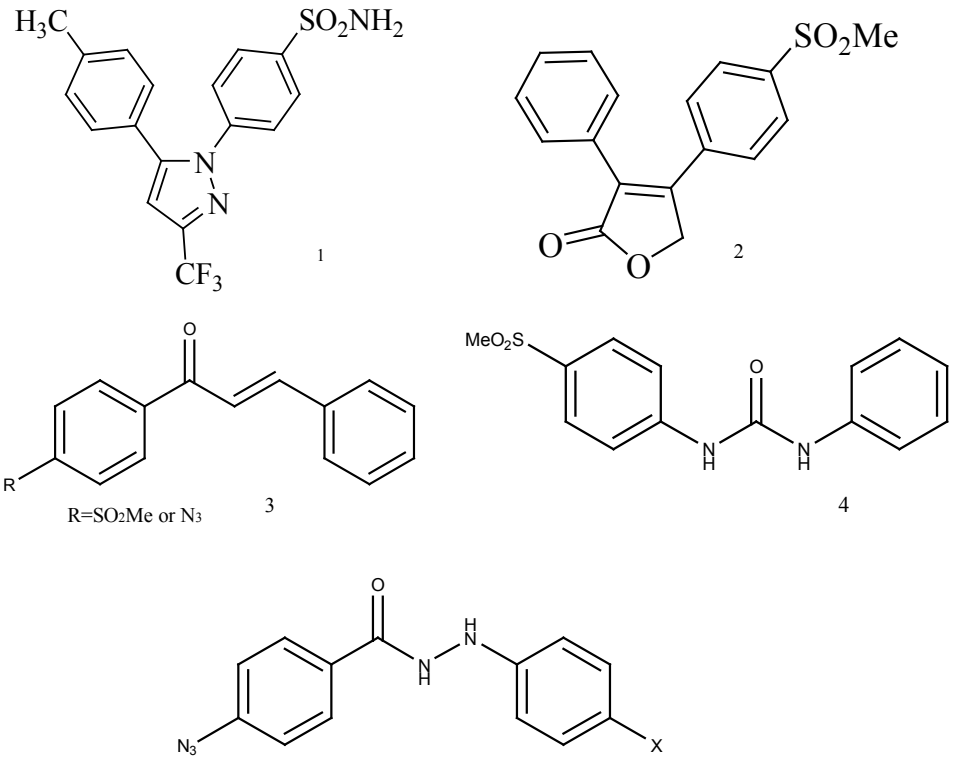


Figure 1. Some representative examples of COXIBs (celecoxib and rofecoxib), and some novel acyclic structural templates (scaffolds) that exhibit cyclooxygenase-1/2 inhibition

2. Materials and Methods

Chemistry

The target of 1,3- biarylhydrazide derivatives (7a–d) were synthesized via the route outlined in 1. A one-step nucleophilic substitution reaction was used to prepare the target 1,3-biarylhydrazides in which an azido substituent was attached to the C-1 phenyl ring (7a–d). The triethylamine catalyzed condensation of a para-azidobenzoylchloride (5) with a para-substituted-phenylhydrazine (6a–d) afforded the 1,3- biarylhydrazides (7a–d) in moderate to high yield (40–80%) as illustrated in Fig. 1. The 4-azidobenzoylchlorid precursor (5) was prepared by treating of 4-aminobenzoic acid with thionylchloride under dry condition. Diazotization of 4-aminobenzoic acid with sodium nitrite, followed by reaction of the diazonium salt with sodium azide according to a previously reported method, (Visser et al., 1995) afforded the para-azido benzoyl chloride.

Experimental

All chemicals and solvents used in this study were purchased from Merck AG and Aldrich Chemical. Melting points were determined with a Thomas–Hoover capillary apparatus (Zarghi et al., 2006).

General procedure for the synthesis of 4-azido-N'-(4-substitutedphenyl)benzohydrazide (7a-d) A 4-substituted phenyl hydrazine (1mmol) was dissolved in a minimum amount of dry DMF (3-5ml). A little amount of triethylamine (2-3ml) was then added. After that a solution of 4-azidobenzoylchloride in DMF was added drop wise to the stirred solution under dry condition at 0 □ C, then the mixture was allowed to stir at room temperature. After stirring for 2 hours, water was added to the reaction mixture and the solid product was collected on a filter, washed with water and was recrystallized from ethanol. The physical data for 7a–d are listed below:

4-azido-N'-phenylbenzohydrazide (7a)
Yield, 62%; yellow crystals; mp 115-117 ºC
4-azido-N'-(4-fluorophenyl)benzohydrazide (7b)
Yield, 40%; pale yellow crystals; mp 112-113 ºC
4-azido-N'-(4-chlorophenyl)benzohydrazide (7c)
Yield, 75%; pale brown crystals; mp 130-131 ºC
4-azido-N'-(4-methoxyphenyl)benzohydrazide (7d)
Yield, 80%; pale yellow crystals; mp 120-122 ºC

Molecular modeling (docking) studies

Docking studies were performed using vina software. The coordinates of the X-ray crystal structure of the selective COX-2 inhibitor SC-558 bound to the murine COX-2 enzyme was obtained from the RCSB Protein Data Bank (1c_2) and hydrogens were added. The ligand molecules were constructed using the Builder module and were energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/mol Å. The energy minimized ligands were superimposed on SC-558 in the PDB file1cx2 after which SC-558 was deleted. The purpose of the docking is to search for favorable binding configuration between the small flexible ligands and the rigid protein. Protein residues with atoms greater than 7.5 Å from the docking box were removed for efficiency. These docked structures were very similar to the minimized structures obtained initially. The quality of the docked structures was evaluated by measuring the intermolecular energy of the ligand-enzyme assembly (Llorens et al., 1999).

3. Results and Discussion

A group of 1,3-biarylhydrazides (7a–d), possessing an azido (N3) substituent on the C-1 phenyl ring, were synthesized. In this study, the substituents on the N-3 phenyl ring were simultaneously varied (H, F, OMe, and Cl) to determine the combined effects of steric and electronic substituent properties upon COX-1 and COX-2 inhibitory potency and selectivity .It has been reported that replacement of His513

in COX-1 by Arg513 in COX-2 plays a key role in the hydrogen bond network of the COX-2 binding site. Access of ligands to the secondary pocket of COX-2 is controlled by histidine (His90), glutamine (Gln192), and tyrosine(Tyr355), and interaction of Arg513 with the bound drug is a requirement for time-dependent inhibition of COX-2 (Garavito and DeWitt, 1999). Recently we exploited, for the first time, the amino acid Arg513 to design selective COX-2 inhibitors having a dipolar azide (N3) pharmacophore that can undergo an electrostatic (ion–ion) interaction with Arg513 in the COX-2 secondary pocket (Praveen Rao et al., 2003; Uddin et al., 2005; Aneja et al., 2011). An investigation of the 7b compound 4-azido-N'-(4-fluorophenyl) benzohydrazide docked in the COX-2 active site (Fig 2) shows that it binds to the primary binding site so that the para-azido substituent on the C-1 phenyl ring is oriented in the vicinity of the secondary pocket present in COX-2. The linear dipolar azido substituent, as proposed, is involved in an ion–ion (electrostatic) interaction with Arg513. The distance between the terminal N-atom of the azido substituent and the NH2 of Arg513 is about 5.61 A ˚ , whereas the distance between the N2-atom of the azido substituent and the other hydrogen of this amino acid is about 4.18 A ˚. The C=O is almost close to (distance = 5.8 Å) OH of Ser 530. In addition, the N-3 phenyl ring is very close to phenyl ring of Phe 518 which may engage in hydrophobic interaction. It was interesting to note that, the para-fluoro substituent of N-3 phenyl ring was forming a hydrogen bond with amide hydrogen (NH) of Gly526 (distance = 5.4 Å). Overall, docking studies of these compounds showed that the designed molecules were well docked at the active site of COX-2 enzyme and the N3 pharmacophore was well oriented into the COX-2 secondary pocket . The purity of all compounds was determined by TLC using several solvent systems and the structures of the final compounds were confirmed by IR, NMR and Mass spectra. The yields of final products were 40-80%.

COX-2 isozyme. Hydrogen atoms of the amino acid residues have been removed to improve clarity.

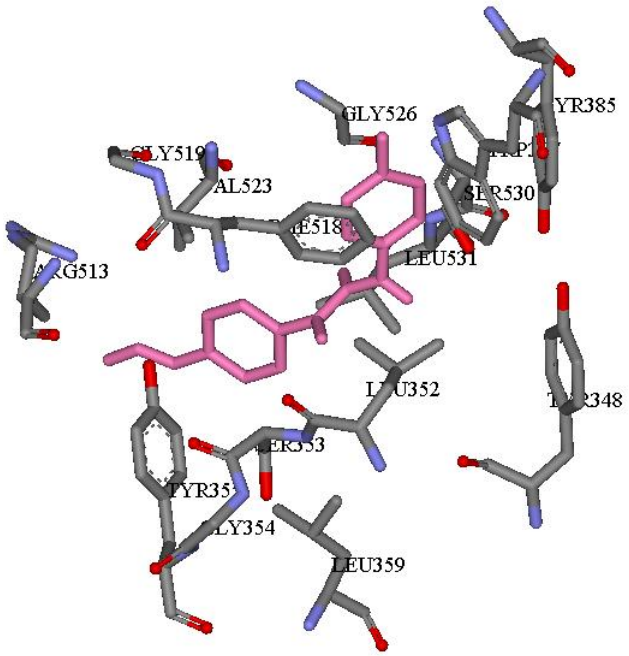
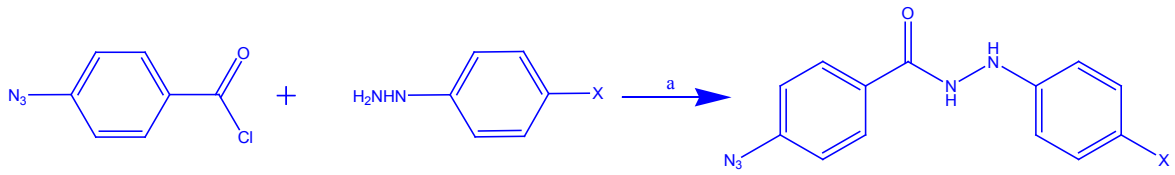


Figure 2. Compound 7b 4-azido-N'-(4-fluorophenyl)benzohydrazide in the active site of murine



5

6a,x=H
6b,x=F
6c,x=Cl
6d,x=OMe

7a,x=H
7b,x=F
7c,x=Cl
7d,x=OMe

Scheme 1. Reagents and conditions: (a) dry DMF, Et3N,0º C to rt, 2h

4. Conclusion

This study indicates that (i) a new class of acyclic 1,3-biarylhydrazides can be prepared via a simple one-step nucleophilic substitution reaction, (ii) the hydrazide moiety can be a suitable scaffold to design COX-2 inhibitors, (iii) the azido (N3) moiety can be a suitable COX-2 pharmacophore .

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