

Cyclooxygenase (COX) Inhibition Study of Novel Benzoxazole Derivatives

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Synthesized novel series of Benzoxazole derivatives, for example, Salts of Methyl-2-amino benzoxazole-5-carboxylate, Methyl-2-(substituted amido) benzoxazole-5-carboxylates, 2-Amino benzoxazole-5-carbohydrazide, Tosylate salt of 2-Amino benzoxazole-5-carbohydrazide, 2-(2-Amino benzoxazole-5-carbonyl)-N-phenylhydrazine carboxamide, Amide derivatives of 2-Amino benzoxazole-5-carbohydrazide, 2-Amino-N' (substituted benzylidene) benzoxazole-5-carbohydrazides and investigated their ability to inhibit human cyclooxygenase (COX-2) enzyme. The IC₅₀ values of the compounds determined are comparable with that of reference standard Celecoxib. Therefore these series of compounds may serve as excellent candidates for selective COX-2 inhibition.

Keywords: COX-2 inhibition, Novel Benzoxazole derivatives, anti-inflammatory.

1. Introduction

Cyclooxygenase (COX) also known as prostaglandin – endoperoxide synthase is an enzyme that is responsible for the formation of important biological mediators called Prostanoids, including prostaglandins, Prostacyclins and thromboxane. COX is the central enzyme in the biosynthetic pathway to Prostanoids from Arachinodic acid.

There are two distinct forms of Cyclooxygenase. They are, COX-1(involved in normal cellular homeostasis) and COX-2 (responsible for biosynthesis of Prostaglandins under acute inflammatory conditions). COX-2 is believed to be target enzyme for the anti-inflammatory activity of non-steroidal anti-inflammatory drugs.

Pharmacological inhibition of COX by non-steroidal anti-inflammatory drugs (NSAID) can provide relief from the symptoms of inflammation and pain.

Benzoxazole compounds show anti-inflammatory, muscle relaxant, anti-bacterial and anti-histaminic properties [1]. In the literature different derivatives of Benzoxazole were reported earlier as COX inhibitors [2]. Treatment of inflammation with steroids is associated with severe side effects leading, at times, to heart, liver and kidney damages [3]. As Benzoxazole compounds are classified as non steroidal antiinflammatory drugs (NSAID), there is always a search to design and synthesize new derivatives of Benzoxazole which can be used for different Pharmacological properties.

The present work involves study of Cyclooxygenase (COX) inhibition property of new benzoxazole compounds which were less toxic and chemically more stable. The study has been carried out using COX-2 Inhibitor Screening Kit which offers a rapid, simple, sensitive, and reliable test suitable for high-throughput screening of COX-2 inhibitors. The assay is based on the fluorometric detection of Prostaglandin G₂, the intermediate product generated by the COX enzyme.

2. Materials and Methods:

Screening kit used:

COX-2 inhibitor screening kit (Fluorometric), 100 assays.

Make : Biovision, USA.

Kit contents :

COX Assay Buffer , COX Probe (in DMSO) , COX Cofactor (in DMSO) , Arachidonic Acid, NaOH , COX-2(Human Recombinant) , Celecoxib, COX-2 inhibitor (in DMSO).

Dimethyl sulfoxide (Molecular biology grade).

Test samples :

Series	New Benzoxazole Compounds
A	Methyl-2-Amino benzoxazole carboxylate Mesylate
Series	Methyl-2-Amino benzoxazole carboxylate Tosylate.
B series	Methyl-2-Benzamido benzoxazole-5-carboxylate.
	Methyl-2-(Phenyl sulfonamido) benzoxazole-5-carboxylate
C series	2-Amino benzoxazole-5-carbohydrazide
	2-Amino benzoxazole-5-carbohydrazide Tosylate
	2-(2-Amino benzoxazole-5-carbonyl)-N-phenylhydrazine carboxamide
D series	N ¹ -(2-Amino benzoxazole-5-carbonyl)-4-methyl benzene sulfonohydrazide
	2-Amino-N ¹ -benzoyl benzoxazole-5-carbohydrazide
E series	2-Amino-N ¹ -(4-Hydroxy benzylidene) benzoxazole-5-carbohydrazide
	2-Amino-N ¹ -(3,4,5-Trimethoxy benzylidene) benzoxazole-5-carbohydrazide
	2-Amino-N ¹ -(4-Chloro benzylidene) benzoxazole-5-carbohydrazide

Experimental Procedure:

Preparation of reagents

COX Assay Buffer:

Ready to use. Equilibrated to room temperature before use. Stored at -20°C.

COX Probe in DMSO:

Ready to use. Warmed by placing in a 37°C bath for 1 – 5 minutes in DMSO solution before use.

COX Cofactor in DMSO:

Ready to use. Warmed by placing in a 37°C bath for 1 – 5 minutes in DMSO solution before use. Just before use, diluted COX Cofactor 1:200 by adding 2 µL COX Cofactor to 398 µL of COX Assay Buffer. Mixed well by pipetting up and down.

Arachidonic Acid (In EtOH, 50 µL):

Ready to use. Equilibrated to room temperature before use. Stored at -20°C. Just before use, prepared Arachidonic Acid solution: 5 µL of supplied Arachidonic Acid + 5 µL NaOH. Vortex briefly to mix.

Diluted Arachidonic Acid, NaOH solution 1:10 with 90µL sterile Vortex briefly to mix. Diluted Arachidonic Acid, NaOH is stable for at least 1 hour on ice.

NaOH (500 µL):

Ready to use. Equilibrated to room temperature before use. Stored at -20°C.

COX-2, Human Recombinant (lyophilized, 100 U):

Reconstituted in 110 µL of sterile ddH₂O. Stored at -70°C. To be used within two months. For short-term storage (~ 2 weeks), COX-2 can be stored at -20°C. Keep in ice while in use.

Celecoxib, COX-2 inhibitor (in DMSO):

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw DMSO solution before use.

Samples preparation:

Dissolved test compound in DMSO and prepared 1, 5,10,25,50 µg/ml concentrations. If any undisclosed particles were observed, filtered and used.

Diluted test compound 10x with COX Assay Buffer for the desired test concentration before use. (Used different concentrations of test compounds, if effective concentration is unknown.)

Added 10 µL test sample (S), Inhibitor Control (2 µL Celecoxib + 8 µL COX Assay Buffer), Enzyme Control (COX Assay Buffer) and Solvent control (Optional) to desired wells.

Added 80 µL reaction mixture into each well (Assay Buffer (76 µL), COX Probe (1 µL), Diluted COX Cofactor (2 µL) and COX-2 enzyme (1 µL)).

Added 10 µL of diluted Arachidonic Acid, NaOH solution into each well with a multi-channel

pipette to initiate the reactions at the same time.

Measured immediately fluorescence at Ex/Em = 535/587 nm on a microplate reader at 25°C. Choose two points (T_1 and T_2) in the linear range of the plot and obtained the corresponding fluorescence values (RFU_1 and RFU_2).

Calculations :

Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU ($RFU_2 - RFU_1$) values by the time $\Delta T(T_2 - T_1)$. Calculate the % of inhibition as below,

$$\% \text{ Relative Inhibition} = \frac{\text{Slope of EC} - \text{Slope of S}}{\text{Slope of EC}} \times 100$$

The concentrations of each compound are used to draw the graph taking concentration ($\mu\text{g/ml}$) on X-axis and %inhibition found on Y -axis. From the graph 50% inhibition concentration (IC_{50}) is calculated.

3. Results:

Series	Compound	IC_{50} ($\mu\text{g/ml}$)
A Series	Methyl-2-Amino benzoxazole carboxylate Mesylate	16.4
	Methyl-2-Amino benzoxazole carboxylate Tosylate.	11.5
B series	Methyl-2-Benzamido benzoxazole-5-carboxylate.	30.7
	Methyl-2-(Phenyl sulfonamido) benzoxazole-5-carboxylate	25.8
C series	2-Amino benzoxazole-5-carbohydrazide	26.3
	2-Amino benzoxazole-5-carbohydrazide Tosylate	22.8
	2-(2-Amino benzoxazole-5-carbonyl)-N-phenylhydrazine carboxamide	27.1
D series	N ¹ -(2-Amino benzoxazole-5-carbonyl)-4-methyl benzene sulfonohydrazide	19.6
	2-Amino-N ¹ -benzoyl benzoxazole-5-carbohydrazide	21.9
E series	2-Amino-N ¹ -(4-Hydroxy benzylidene) benzoxazole-5-carbohydrazide	22.3
	2-Amino-N ¹ -(3,4,5-Trimethoxy benzylidene) benzoxazole-5-carbohydrazide	33.5
	2-Amino-N ¹ -(4-Chloro benzylidene) benzoxazole-5-carbohydrazide	31.2
Celecoxib		13.4

4. Discussion and conclusion:

The data on COX-2 inhibitory activity of compounds of A series presented in the above table, compound Methyl-2-amino benzoxazole carboxylate Tosylate showed inhibition with IC_{50} value of 11.5 ($\mu\text{g/ml}$) and other compound, Methyl-2-amino benzoxazole carboxylate Mesylate showed moderate inhibition with IC_{50} value of 16.4 ($\mu\text{g/ml}$).

The data on COX-2 inhibitory activity of compounds of B series presented in the above table, compound Methyl-2-(phenyl sulfonamido) benzoxazole-5-carboxylate showed inhibition with IC_{50} value of 25.8 ($\mu\text{g/ml}$) and other compound, Methyl-2-benzamido benzoxazole-5-carboxylate showed inhibition with IC_{50} value of 30.7 ($\mu\text{g/ml}$).

The data on COX-2 inhibitory activity of compounds of C series presented in the above table, compound 2-amino benzoxazole-5-carbohydrazide Tosylate showed inhibition with IC_{50} value of 22.8 ($\mu\text{g/ml}$) and other compounds, 2-(2-amino benzoxazole-5-carbonyl)-N-phenylhydrazine carboxamide and 2-amino benzoxazole-5-carbohydrazide and showed inhibition with IC_{50} value of 27.1 ($\mu\text{g/ml}$) and 26.3 ($\mu\text{g/ml}$) .

The data on COX-2 inhibitory activity of compounds of D series presented in the above table, compound N^1 -(2-amino benzoxazole-5-carbonyl)-4-methyl benzene sulfonohydrazide showed inhibition with IC_{50} value of 19.6 ($\mu\text{g/ml}$) and other compound, 2-amino- N^1 -benzoyl benzoxazole-5-carbohydrazide showed inhibition with IC_{50} value of 21.9 ($\mu\text{g/ml}$).

The data on COX-2 inhibitory activity of compounds of E series presented in the above table, compound 2-amino- N^1 - (4-hydroxy benzylidene) benzoxazole-5-carbohydrazide showed inhibition with IC_{50} value of 22.3 ($\mu\text{g/ml}$) and other compounds, 2-amino- N^1 - (4-chloro benzylidene) benzoxazole-5-carbohydrazide and 2-amino- N^1 - (3,4,5-trimethoxy benzylidene) benzoxazole-5-carbohydrazide showed inhibition with IC_{50} value of 31.2 ($\mu\text{g/ml}$) and 33.5 ($\mu\text{g/ml}$) respectively.

The COX inhibitory activity of the compounds is compared with standard Celecoxib with IC_{50} value 13.4 ($\mu\text{g/ml}$). Methyl-2-amino benzoxazole carboxylate Tosylate with IC_{50} 11.5 ($\mu\text{g/ml}$) has shown comparable COX inhibitory activity with Celecoxib.

From the above results it can be safely concluded that all new benzoxazole derivatives synthesized have shown moderate to good COX-2 inhibition activity and complies with the objective of the study to synthesize COX inhibitors.

References

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