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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME NOVEL DIARYL IMIDAZOLES AS ANTI-INFLAMMATORY AGENTS

Dushyanth Reddy Vennapu and Palled M. S.*

Department of Pharmaceutical Chemistry, K.L.E.S's College of Pharmacy, Belgaum -10.

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***Corresponding Author**
Dr. Palled M. S.
Department of Pharmaceutical Chemistry, K.L.E.S's College of Pharmacy, Belgaum -10.

ABSTRACT

The synthesis and pharmacological activity of a new series of diarylimidazoles as potent selective COX–2 inhibitor were described. QSAR based approach to screen molecules using Schrodinger Maestro program to optimize the lead compounds against the murine cyclooxygenase receptor. The best fit ligand molecules based on the dock score were synthesized characterized and evaluated for their in-vivo anti-inflammatory activity using carrageenan induced paw oedema.

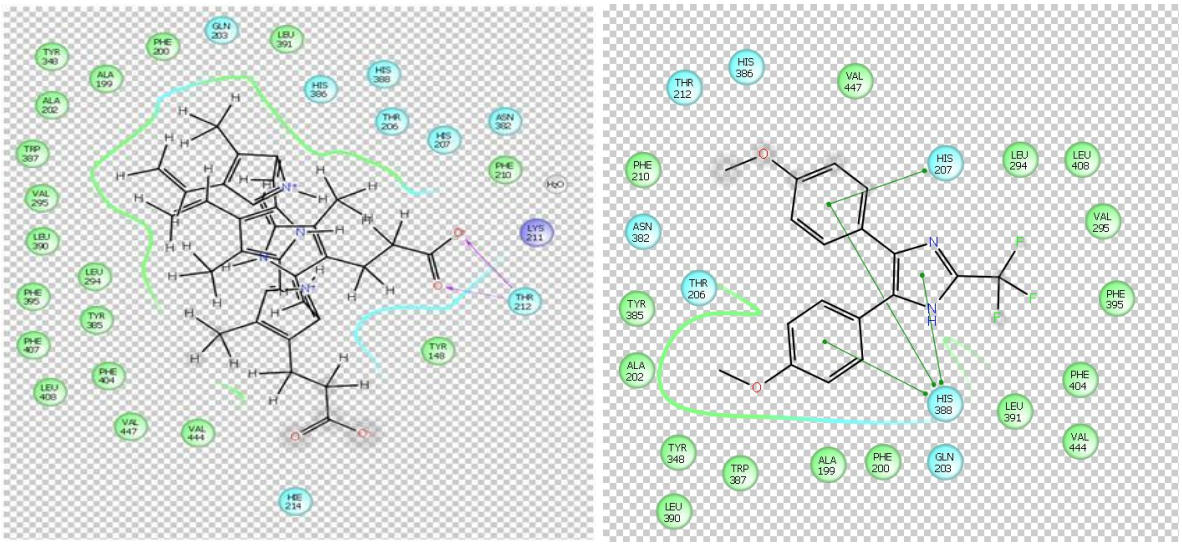
KEYWORDS: 1, 5 - diarylimidazoles, selective COX – 2 inhibitor, carrageenan induce paw oedema.

INTRODUCTION

Inhibition of Cyclooxygenase (COX), one of the key enzymes in arachidonic acid (AA) cascade is the main mechanism by which non-steroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory activity. The therapeutic use of NSAIDs, especially in chronic diseases, has revealed their association with well-known side effects at the gastrointestinal level (mucosal damage, bleeding) and less frequently, at the renal level.[1] After the discovery a decade ago of two COX iso forms, it was recognized that selective inhibitors of the inducible form (COX – 2, Cytokine inducible, and expressed mainly in inflammatory cells) could provide anti-inflammatory agents devoid of the undesirable effects associated with classical non selective NSAIDs. The inhibition of COX – 1, the form constitutively present in many tissues such as stomach, kidney and platelets, by non-selective NSAIDs may be responsible for the secondary effects associated with their use. Although modification of established non-selective agents, such as lengthening the carboxyl side chain of indomethacine, have been strategies for the design of COX – 2 selective inhibitors, the main effort has been addressed to the diarylheterocycles class, based an early known anti-inflammatory drugs, such as

Flumizole.[1] As new approach to diarylheterocycle class COX – 2 inhibitors we report here the pharmacological studies of new 1,5 – diarylimidazoles in which defined combination of substituent's confers appropriate polarity and charge distribution for good activity.

Molecular Docking Simulations Using Schrodinger Maestro Program



Interaction of co-crystallized ligand and standard flumizole on to the active site of receptor

All the docking simulations were done on chain-1 of 4RUT murine cyclooxygenase receptor using Flumizole as reference standard (ligand) to the optimized receptor pocket.

Molecular Docking: Molecular modeling is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Ligand protein alignment is a key factor in drug binding and pharmacological efficacy. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between Ligand and protein using scoring functions. Murine Cyclooxygenase with PDB code 4RUT was used for docking simulation.

Experimental section: Docking simulation was done with the help of SCHRODINGER MASTERO 10.3 Drug Discovery workbench. The 2D structures of compounds were drawn in Chem.-Draw and converted to 3D in Ligand preparation and compounds were filtered by specifying options for screening like Remove molecules that have a molecular weight of greater than 650. Remove molecules with too many H-bond acceptor and donor atoms,

acceptor groups> 3, donor groups> 3. Energy minimization: Was done by choosing a force field MMFFs.

Protein preparation: The co-crystallized structure of Murine Cyclooxygenase with PDB code 4RUT was selected from Protein Data Bank basing on the factors like RMSD, Resolution factor. A typical PDB structure file consists only of heavy atoms, can contain waters, cofactors, and metal ions, and can be multimeric. The structure generally has no information on bond orders, topologies, or formal atomic charges. Terminal amide groups can also be misaligned; because the X-ray structure analysis cannot usually distinguish between O and NH2 all these parameters were refined using SCHRODINGER MASTERO 10.3.

Rigid docking g on Murine Cyclooxygenase (PDB id: 4RUT1)

Compound	Dock score	Remarks
Flumizole	-46.02	
Comp-1	-55.09	Docks at the same site of native ligand
Comp-2	-62.41	Docks at the same site of native ligand
Comp-3	-53.20	Docks at the same site of native ligand
Comp-4	-57.98	Docks at the same site of native ligand
Comp-5	-55.14	Docks at the same site of native ligand

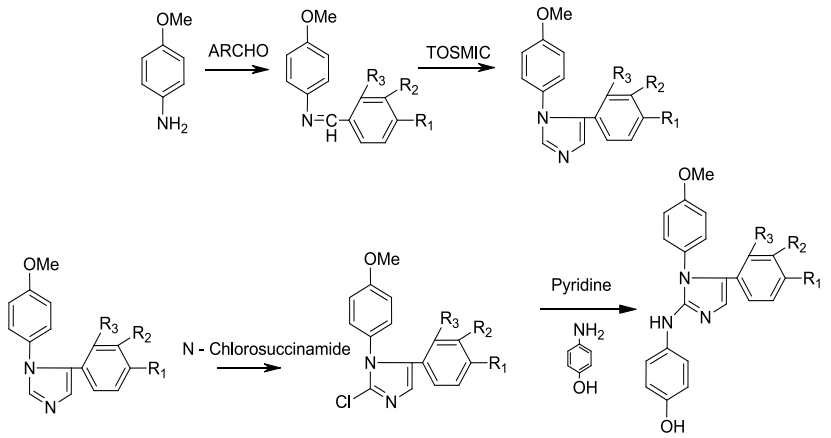
EXPERIMENTAL

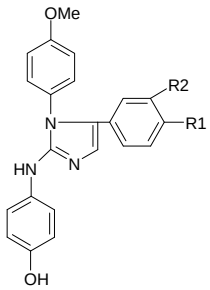
Melting points were uncorrected and were obtained in open capillary tubes in paraffin bath. TLC checking was done on glass plates coated with silica gel – G and spotting was done using iodine. IR spectra were recorded on NICOLET FT-IR instrument.^{1}HNMR was taken at IISc Bangalore. The structures of all the synthesized compounds were established on the basis of MP, chemical tests, TLC, UV, IR and NMR.

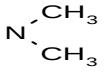
Protein preparation and Ligand Preparation using Schrodinger

The molecules were drawn using Schrodinger Maestro Elements, the 3D conformers were generated, the molecules were refined using OPLS energy minimization protocol, further the molecules were screened for Lig-Prep protocol, the prepared ligands were used in docking. The Crystal structure of protein 4RUT was retrieved from RCS-PDB directly in to the Schrödinger were by pre-processing parameters say addition of hydrogen, generating conformers for the co-crystallized ligands, removing water which don't have any interaction with protein, later on glide grid was generated using co-crystallised ligand.

Synthetic scheme





Sr. No.	Comp.	R1	R2	% Yield	M.P (^{0}C)	Solubility in water
1	**Comp – 1**	**OH**	**H**	69	105	Insoluble
2	**Comp – 2**	OCH_3	H	60	118	Insoluble
3	**Comp – 3**	OCH_3	OCH_3	58	120	Insoluble
4	**Comp – 4**		**H**	55	101	Insoluble
5	**Comp – 5**	H	Cl	62	115	Insoluble

Method of preparation of Schiff Bases

To an equimolar mixture of 4-methoxy aniline (0.1 mol) and aryl aldehyde (0.1 mol) in 25 ml of methanol was added, few drops of acetic acid and few drops of glacial acetic acid and the mixture was refluxed for a period of 3 to 4 hours. The reaction mixture was cooled and poured into cold water. The solid separated was filtered and the same was crystallized from methanol.

Method of synthesis of 1, 5 diaryl imidazoles

A mixture of compound I (115 mmol), tosylmethylisocyanide (172 mmol), anhydrous potassium carbonate (229 mmol) in methanol (40 ml) and dioxane (16 ml) was refluxed for 2 to 4 hours. The solvent was removed and the residue was dissolved in dichloromethane/brine mixture. The organic layer was separated and the aqueous phase was extracted with dichloromethane. The combined organic phases were dried over magnesium sulphate and concentrated. The residue obtained was recrystallized from methanol.

Synthesis of 4-chloro 1, 5-diaryl imidazoles

A mixture of compound II (86mmol), N-chlorosuccinimide (90mmol), and chloroform (35 ml) was refluxed for 18 hours. The solvent was removed and the residue was redissolved in 40 ml dichloromethane and washed with 25 ml of 1N HCl, 1N NaOH and brine. The organic

phase was dried over anhydrous magnesium sulphate and was concentrated. The product was crystallized from ethanol.

Synthesis of 1H-imidazol-2-ylamino) phenol derivative: (III)

A mixture of compound III (0.01 mmol), 4- amino phenol (0.01 mmol) in methanol (50 ml) and drops of hydrochloric acid was refluxed for a period of 8 to 10 hours. The refluxed product was cooled overnight in vacuum desiccators. The product was crystallized from ethanol.

SPECTRAL DATA

Analytical data of 1-comp

4-((5-(4-hydroxyphenyl)-1-(4-methoxyphenyl)-1H-imidazol-2-yl)amino)phenol eluted from column chromatography Etoac/Hexane (10/90, v/v). H^1 NMR(300MHz Chloroform-*d*) (δ ppm): 3.132 (3.9 (1H, -NH Ar.), 6.5 (11H, Aromatic). 6.95 – 6.89 (m, 3H), 6.78 – 6.71 (m, 2H), 4.90 (s, 1H), 3.80 (s, 2H). IR (neat, cm^{-1}), 2926 (NH Stretch, Aromatic Amino.), 3424 (OH Phenolic), 1349 (C-N Stretch.), 1200 (C-O Stretch).

Analytical data of 2-comp

4-((1,5-bis(4-methoxyphenyl)-1H-imidazol-2-yl)amino)phenol eluted from column chromatography Etoac/Hexane (10/90, v/v). H^1 NMR (300MHz Chloroform-*d*) 6.5 (11H, Aromatic) 6.91 – 6.82 (m, 3H), 6.73 – 6.66 (m, 2H), 3.87 (s, 2H), 3.80 (s, 2H). IR (KBr) cm^{-1}: 2915 (NH Stretch, Aromatic Amino.), 3403 (OH Phenolic), 11349 (C-N Stretch), 1198 (C-O Stretch).

Analytical data of 3-comp

4-((5-(3,4-dimethoxyphenyl)-1-(4-methoxyphenyl)-1H-imidazol-2-yl)amino)phenol eluted from column chromatography Etoac/Hexane (20/80, v/v). H^1 NMR (300MHz Chloroform-*d*) (δ ppm): 3.132 (9 H, 3 ($-OCH_3$), 3.9 (1H, -NH Ar.), 6.5 (11H, Aromatic). IR (KBr) cm^{-1}: 2926 (NH Stretch, Aromatic Amino.), 3410 (OH Phenolic), 1349 (C-N Stretch.), 1242 (C-O Stretch).

Analytical data of 4-comp

4-((5-(4-(dimethylamino)phenyl)-1-(4-methoxyphenyl)-1H-imidazol-2-yl)amino)phenol eluted from column chromatography Etoac/Hexane (70/30, v/v). H^1 NMR (300MHz Chloroform-*d*) (δ ppm): 6.90 – 6.81 (m, 3H), 6.82 – 6.75 (m, 2H), 6.73 – 6.66 (m, 2H), 3.80

(s, 2H), 2.90 (s, 4H).3.9 (1H, -NH Ar.), 6.5 (11H, Aromatic).IR (KBr) cm^{-1}: 2925 (NH Stretch, Aromatic Amino.), 3406 (OH Phenolic), 1349 (C-N Stretch.), 1237 (C-O Stretch.).

Analytical data of 5-comp

4-((5-(3-chlorophenyl)-1-(4-methoxyphenyl)-1H-imidazol-2-yl)amino)phenol eluted from column chromatography Etoac/Hexane (50/50, v/v). H^1 NMR (300MHz Chloroform-*d*) (δ ppm): 3.9 (1H, -NH Ar.), 6.5 (11H, Aromatic) 6.91 – 6.84 (m,2H), 6.73 – 6.66 (m, 2H), 3.80 (s, 2H).IR (KBr) cm^{-1}: 2931 (NH Stretch, Aromatic Amino.), 3425 (OH Phenolic), 1349 (C-N Stretch.), 1203 (C-O Stretch.).

PHARMACOLOGICAL STUDIES

All the synthesized compounds were subjected to in vivo anti-inflammatory activity i.e. carrageenan induced rat hind paw edema technique. Albino rats weighing 100-200 gram were used for the experiment and indomethacine taken as standard and surprisingly all the synthesized compounds shown very good anti-inflammatory activity.

PHARMACOLOGICAL SCREENING

ANTI-INFLAMMATORY ACTIVITY

MATERIALS

Oedema was produced using type IV lambada carrageenan from sigma laboratories. Foot volumes were measured in a plethysmograph by water displacement. The instrument was calibrated before performing the experiment using standard calibrated probe number and standard drug used ibuprofen was procured from sun pharmaceutical industry.

Acute anti-inflammatory method

Carrageenan induced rat hind paw oedema

The method of winter et al (Winter et al 1962)was used with slight modification. The apparatus used for the measurement of rat paw volume was that of Butle et al, modified by Singh and Gosh. The animals were divided into 9 groups of 5 animals each one group served as control, another group served as a standard (ibuprofen) and the rest of the groups were used for the test drugs.

The rats were dosed orally at 300mg/kg body weight, including the control and ibuprofen. Test compounds and standard drug were suspended 0.5% of sodium carboxyl methylcellulose

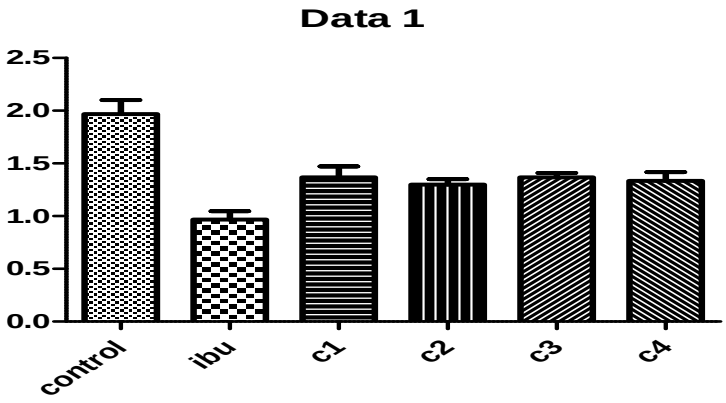
mucilage, which was used as a vehicle. For the control group a solution of 1% of carrageenan was used as an inflammatory agent.

Food was withdrawn overnight with adequate water before the experiment. The drugs were dosed orally with the help of oral catheter. After thirty minutes drug administration, according to the technique of Winter et al (1963) 0.1 ml of 1% carrageenan in normal saline was injected into the subplanter region of left hind paw. The volume of the injected paw was measured with a plethysmograph. By water displacement method at zero hour immediately after injecting carrageenan the same procedure was repeated at 1 hr, 2 hr and 4 hr. The difference between 0 hour and subsequent readings was taken as actual oedema volume.

Sr. No.	Drug (300mg/kg)	Mean Paw Oedema Volume (ml) ± SE				% inhibition after 3 hour
		O hr	1 hr	2 hr	3 hr	
1.	Control	0.133±0.0516	2.02±0.172	2.60±0.261	2.57±0.301	-
2.	Indomethacine	0.0833±0.0753	1.03±0.242	1.68±0.182	1.15±0.243	50
3.	Comp.1	0.117±0.0408	1.60±0.424	1.98±0.145	1.47±0.314	30
4.	Comp.2	0.133±0.0516	1.48±0.214	1.62±0.138	1.35±0.315	34
5.	Comp.3	0.100±0.0	1.32±0.133	1.60±0.200	1.35±0.105	30
6.	Comp.4	0.117±0.0408	1.38±0.194	1.82±0.232	1.45±0.442	32

NA-P < 0.05 – Non significant, ** P < 0.01 – Significant, (ANOVA followed by Dunnet ‘t’ test)

Effect of synthesized compound and ibuprofen and carrageenan induced rat paw oedema by oral administration



Graph showing % inhibition on Carrageenan-induced Rat Paw Oedema

Ibuprofen was used as the standard drug for Anti-inflammatory activity, compounds synthesized have shown the maximum Anti-inflammatory activity when compared with

control but are not comparable to standard Ibuprofen. The results were calculated by taking mean ±SE and finding the 'P' values.

RESULTS AND DISCUSSION

The synthesized imidazole derivative compounds resemble to some of COX-2 inhibitory agents like flumizole, Celecoxib and Rofocoxib. Hence it was thought of carrying out anti-inflammatory screening by carrageenan induced paw edema method by using water displacement plethysmography. The screened compounds have shown good anti-inflammatory activity.

CONCLUSION

The compound could be synthesized from readily available p-methoxy aniline. The compounds were found to be active and shown good anti-inflammatory activity. Molecular docking provided basic insights in designing newer leads for anti-inflammatory agents, which were confirmed for their pharmacological efficiency using in-vivo studies.

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