4th International Electronic Conference on Medicinal Chemistry

1-30 November 2018

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Design, synthesis and biological evaluation of new pyridine/bipyridine carbonitriles and some related compounds interfering with arachidonic acid pathway as potential anti-inflammatory agents

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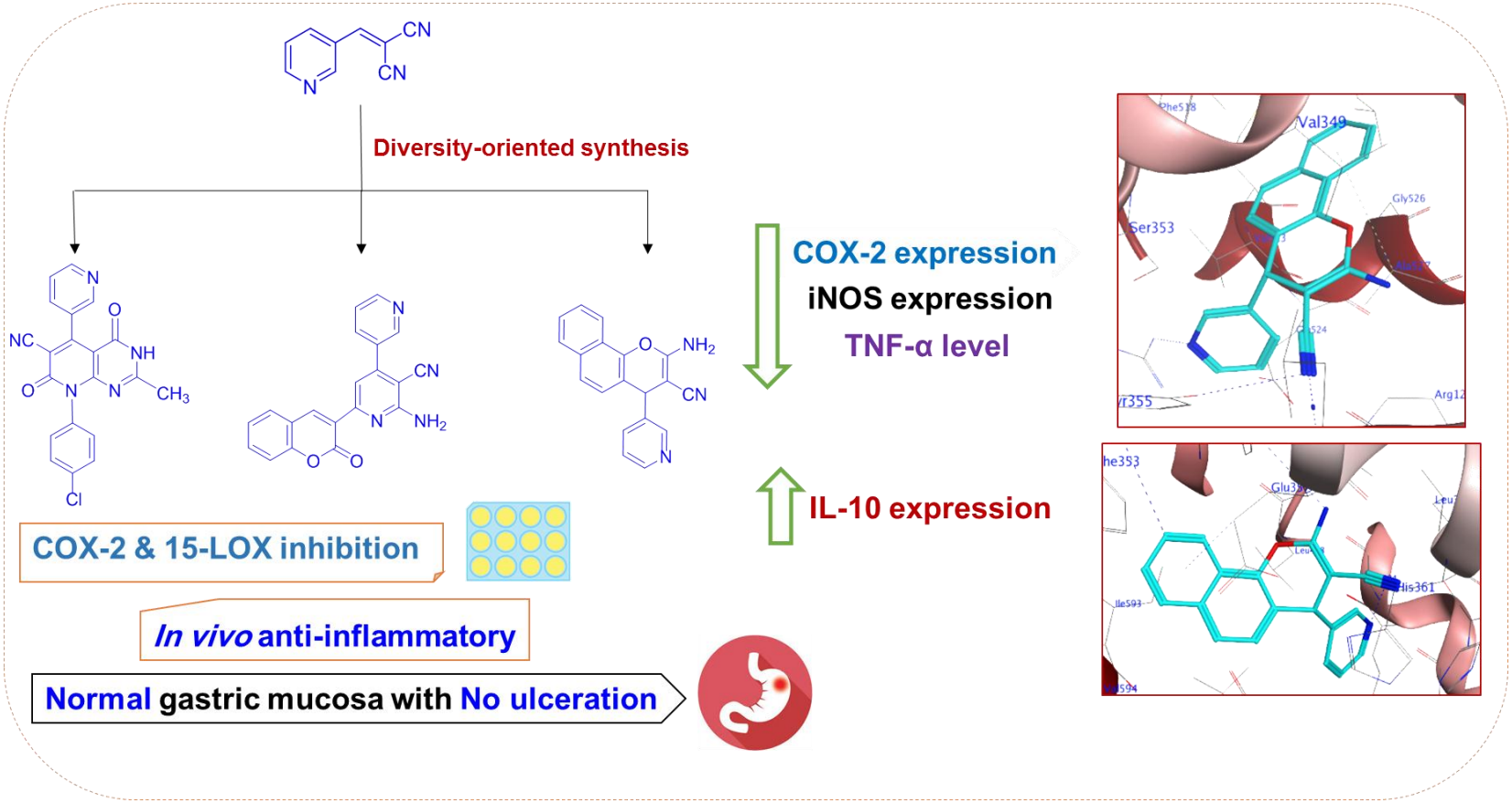
Design, synthesis and biological evaluation of new pyridine/bipyridine carbonitriles and some related compounds interfering with arachidonic acid pathway as potential anti-inflammatory agents

Graphical Abstract



Abstract:
Management of inflammation constitutes an unmet medical need. Thus, there is a rising demand for safer and efficacious anti-inflammatories. Two pathways correlated to the arachidonic acid cascade have been recognized, namely cyclooxygenase and lipoxygenase pathways. Emerging approaches for the treatment of inflammation have shifted towards simultaneously targeting multiple enzymes in the ARA cascade through combination therapy and multi-target inhibitors, to circumvent the risks associated with single pathway inhibition. Based on these premises, it was rationalized to synthesize some pyridine/bipyridine carbonitrile derivatives and some related compounds, to be explored for their anti-inflammatory activity.
In vitro assay results revealed that 5 compounds showed significant COX-2 inhibitory potential. 15-LOX inhibitory activities of the test compounds were also assessed. Three compounds showed significant *in vivo* anti-inflammatory activity (higher % inhibition of edema than celecoxib). Moreover, histopathological examination revealed that they showed superior gastrointestinal safety profile. Some compounds reduced the expression levels of pro-inflammatory enzymes (COX-2 and iNOS) while increased that of anti-inflammatory cytokine (IL-10) in LPS-stimulated monocytes. They also restored normal TNF-α titers. Docking of the most active compounds into COX-2 and 15-LOX active sites showed similar binding pattern to those of the cocrystallized ligands.

Keywords: Inflammation; Cyclooxygenase-2; 15-Lipoxygenase; Pyridine; Docking

Introduction

- **Inflammation** is a normal reaction to infection and injury. It encompasses the recruitment of the immune system components to neutralize invading pathogens, repair injured tissues, and promote wound healing. Yet, during chronic or over activation of the immune system, nitric oxide synthase (iNOS) is stimulated by which nitric oxide (NO) and pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukins are released.
- **Highly-networked disorders** such as inflammation can benefit from complex treatment that modulates multiple targets.
- In the field of anti-inflammatories, both non-selective and selective COX inhibitors provides a model for the limitations of using **single-target-based drugs** in treating a complex disease.
- Severe side effects of 'Coxibs' indicate that inhibition of any of the arachidonic acid (AA) biosynthetic pathways could switch the metabolism to the other. Thus, it is believed that **dual inhibitors of COX-2 and LOX** will consequently shut off the production of mediators of inflammation from AA pathway.

Introduction

- Moreover, **Diversity-oriented synthesis (DOS)** recently emerged as a new synthetic approach to meet the challenge of synthesizing structurally diverse small molecule collections. It is defined as the deliberate, simultaneous and efficient synthesis of more than one target compound in a diversity-driven approach. It aims at efficiently **interrogating wide areas of chemical space simultaneously**; this may include known bioactive regions of chemical space as well as unexplored ones. This will ultimately increase the possibilities of identifying some hits/leads.
- The **reagent-based approach to skeletal diversity** is a branching synthetic strategy which involves a short series of divergent, complexity generating reactions from a common starting material to produce a collection of compounds with distinct molecular skeletons (**Figure 1**).
- In our study, reagent-based skeletal diversity is achieved via the use of a **pluripotent functional group** where exposure of a given molecule to different reagents results in different reactions occurring at the same part (functional group) of the molecule.

Introduction

- Pyridine nucleus represents an important scaffold in drug discovery due to its diversified biological activities. Hence, 2-(Pyridin-3-ylmethylene)malononitrile moiety has been selected as a common intermediate amenable for diversity- oriented synthesis (**Figure 1**).
- Based on these premises, it was rationalized to synthesize some pyridine/bipyridine carbonitrile derivatives and some related compounds, substituted or fused to other heterocyclic/aromatic rings, to be explored for their anti-inflammatory activity. The final target compounds comprise pyrido[2,3-*d*]pyrimidine (**A**), 1,2,4-triazolo[1,5-*a*]pyridine (**B**), 4-pyridinyl chromene-3-carbonitrile (**C**) and 3,4'-bipyridine-3'(,5')-(di)carbonitrile (**D**) skeletons. Several compounds carrying these molecular frameworks are reported to possess significant anti-inflammatory properties as exemplified in **Figure 1**.

Introduction

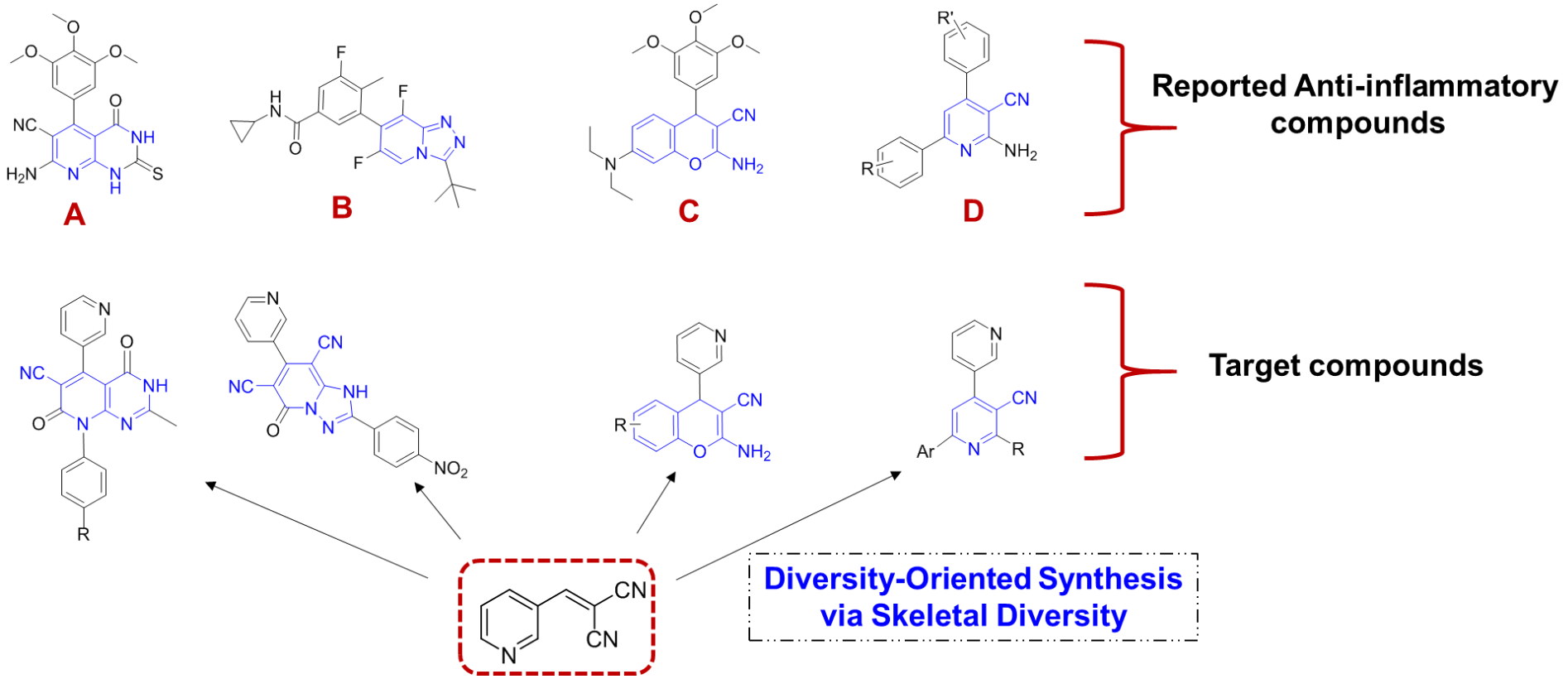
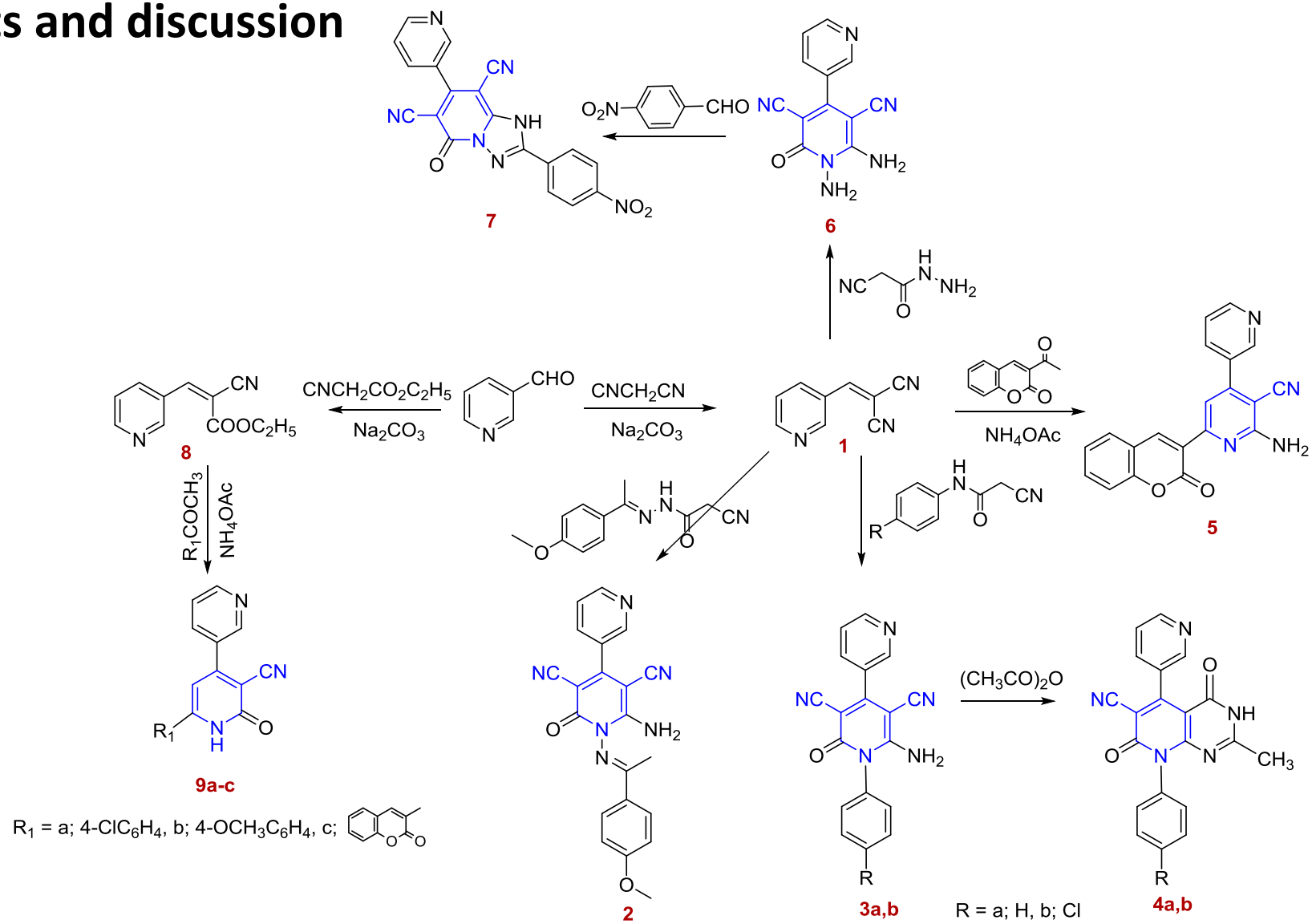


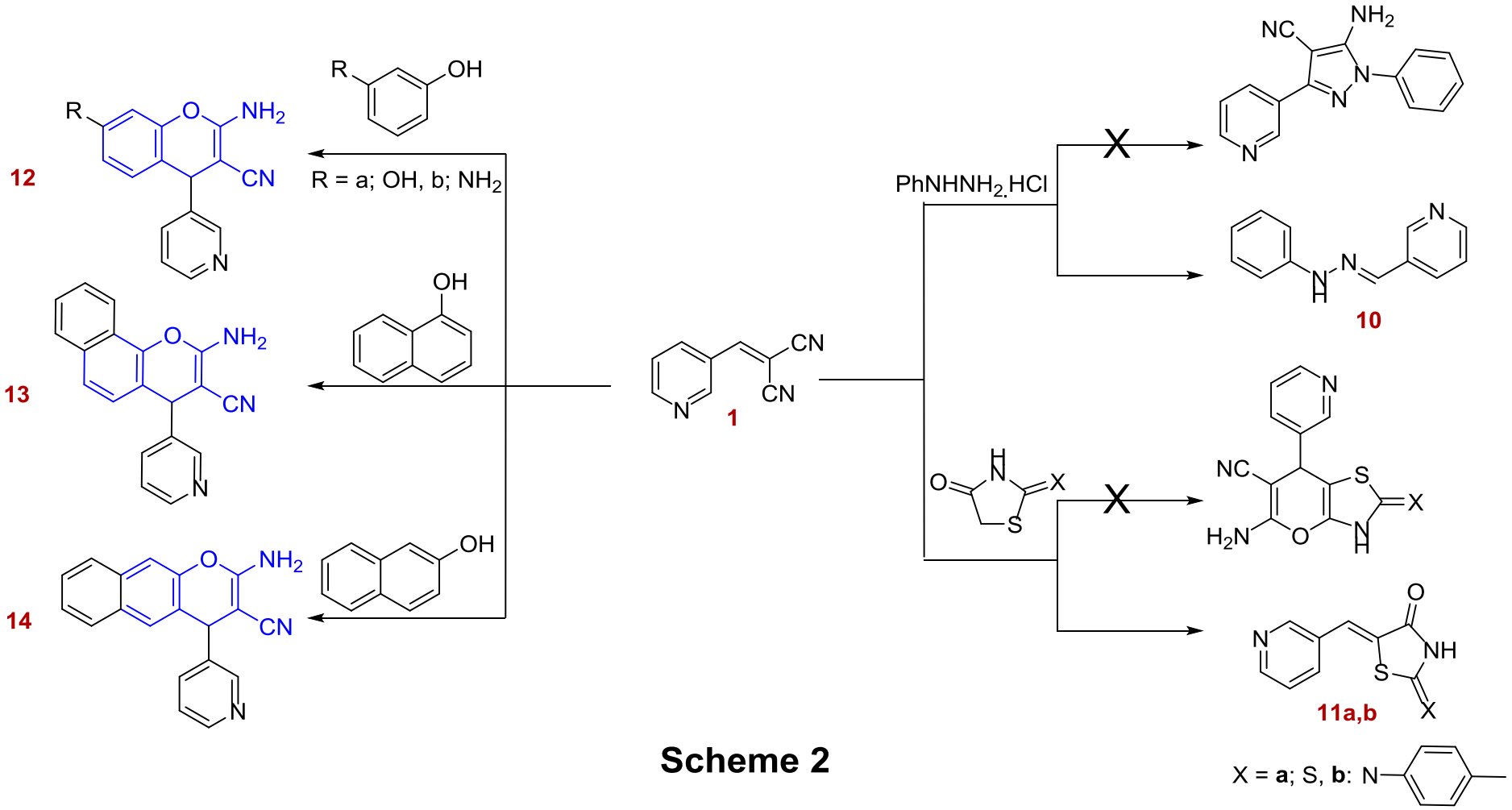
Figure 1. Design of the target compounds

Results and discussion



Scheme 1

Results and discussion



Scheme 2

Results and discussion

Table 1. ***In vitro*** **COX-1/2 and 15-LOX enzymes inhibition assays**

Code	IC_{50} (μM)			SI (COX-1/COX-2)
	COX-1	COX-2	15-LOX	
Celecoxib	15.1	0.049	-	**308**
Diclofenac Na	4.91	0.36	-	**13**
1	8.32	0.21	2.96	**39**
2	8.92	0.14	3.54	**63**
3a	7.65	0.27	2.67	**28**
3b	12.74	0.10	6.34	**127**
4a	10.98	0.11	6.29	**99**
4b	10.42	0.11	5.74	**94**
5	13.54	0.11	7.21	**123**
6	10.52	0.11	4.75	**95**
7	10.33	0.11	5.61	**93**
8	6.87	0.29	2.09	**23**
9a	12.62	0.10	4.89	**126**
9b	5.87	0.42	2.54	**13**
9c	8.67	0.19	3.42	**45**
10	9.23	0.34	5.24	**27**
11a	7.54	0.31	3.07	**24**
11b	11.41	0.10	6.21	**114**
12a	5.98	0.34	2.37	**17**
12b	7.86	0.29	3.11	**27**
13	13.41	0.10	5.33	**134**
14	6.97	0.31	2.97	**22**
Zileuton	-	-	2.43	**-**
Quercetin	**-**	**-**	**3.34**	**-**

Results and discussion

Figure 2. *In vivo* anti-inflammatory activities of selected compounds in formalin-induced rat paw edema bioassay (acute inflammation model)

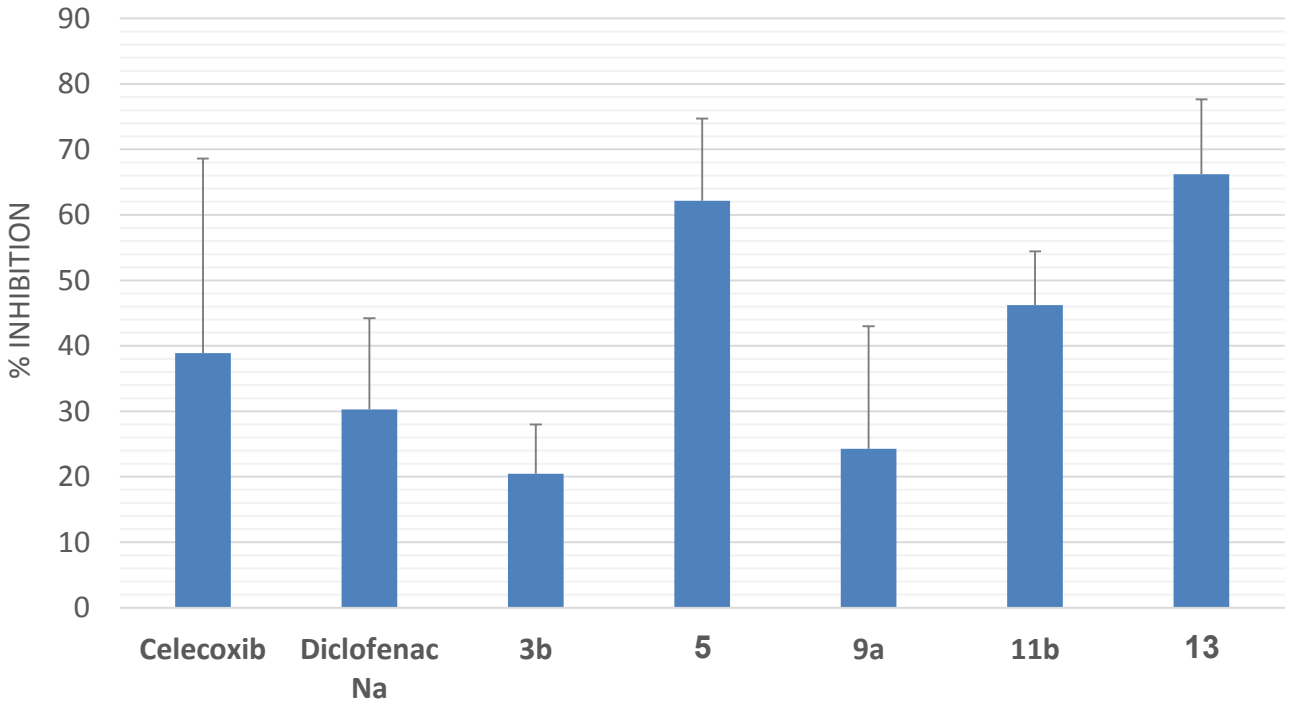


Table 2. ED_{50} (µmol/kg) of the most active compounds

Code	ED_{50} (µmol/kg)
Celecoxib	115.90
Diclofenac Na	163.45
5	146.91
13	56.12

Results and discussion

Histopathological Examination

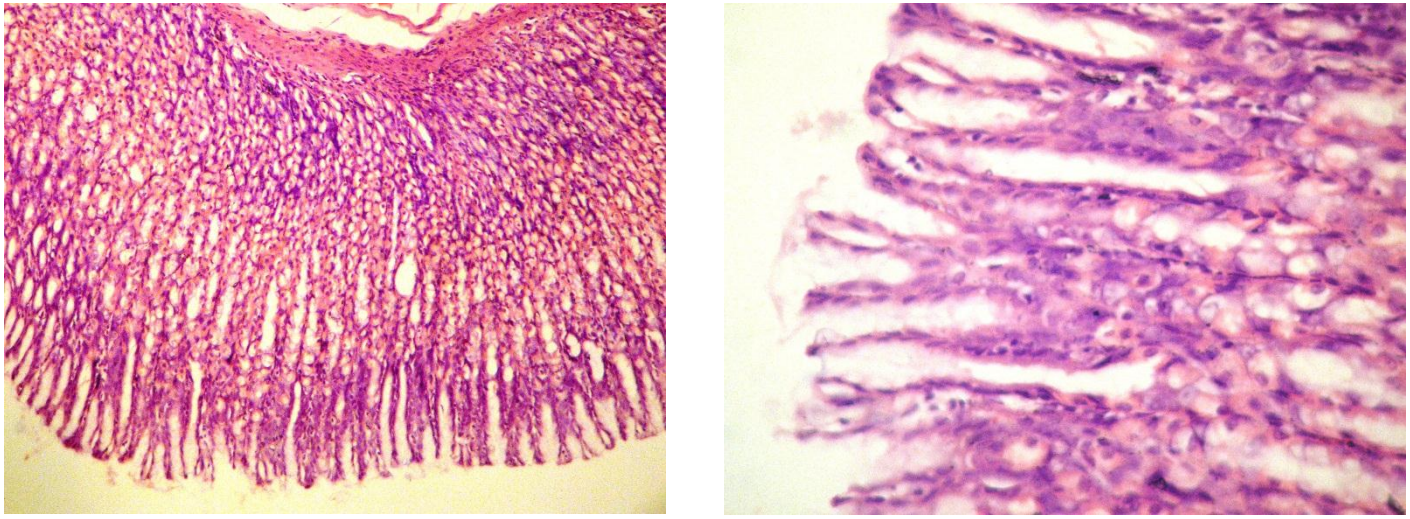


Figure 3. Histopathological Examination (x100-left) and (x400-right)

Histopathological examination revealed that compounds showed superior gastrointestinal safety profile (normal gastric mucosa with no ulceration).

Results and discussion

Code	EC_{100}	EAIC
4a	20.29±0.72[d]	103.42±0.86[e]
4b	32.188±0.58[b]	**47.07±4.24[a]**
5	6.761±0.96[f]	123.15±0.45[f]
13	**57.088±1.08[a]**	67.97±5.65[d]
Celecoxib	22.77±2.7[c]	47.76±0.35[b]
Diclofenac	14.17±1.8[e]	57.72±0.76[c]

Table 3. EC_{100} and EAIC (effective anti-inflammatory concentration) values (µmol/ml) of the tested compounds

Table 4. TNF-α level (pg/ml) in compounds-treated LPS-stimulated monocytes

Code	TNF-α
4a	95.13±5.75[a]
4b	**87.17±4.87[a]**
5	96.46±0.88[b]
13	**84.51±4.4[a]**
Celecoxib	148.67±5.31[c]
Diclofenac	107.96±5.3[b]
LPS (Induced)	272.15±2.1[d]
Control (untreated)	81.773±0.23[a]

Results and discussion

Table 5. Relative expression levels of pro-inflammatory enzymes (COX-2 and iNOS) and anti-inflammatory cytokine (IL-10) in compounds-treated LPS-stimulated monocytes

Code	COX-2	iNOS	IL-10
4a	8.69±0.0002^{d}	4.924±0.002^{c}	0.09±0.001^{b}
4b	**0.42±0.1^{a}**	**0.654±0.1^{a}**	**0.256±0.01^{a}**
5	17.27±0.0009^{e}	28.84±0.02^{d}	0.029±0.003^{c}
13	**0.288±0.1^{a}**	**0.417±0.1^{a}**	**0.0296±0.003^{c}**
Celecoxib	0.72±0.001^{b}	1.972±0.0008^{b}	0.285±0.07^{a}
Diclofenac	1.812±0.009^{c}	27.792±0.02^{d}	0.098±0.001^{b}
LPS (Induced)	22.24±1.76^{e}	30.987±0.017^{e}	0.0023±0.0001^{d}

All values are expressed as mean±SEM. Different letters in the same column are significantly different at $p<0.05$.

Results and discussion

Molecular docking

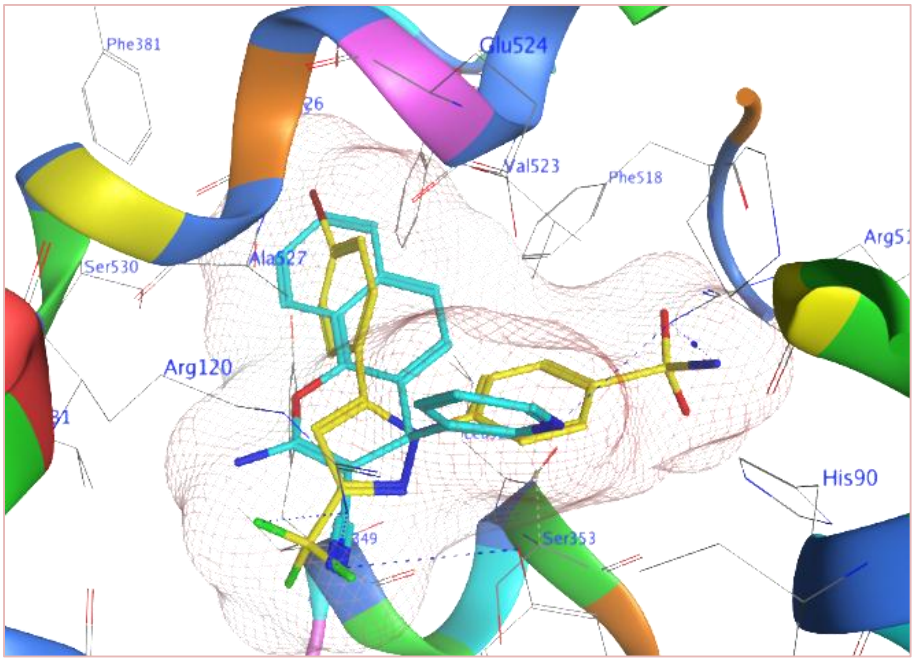


Figure 4. 3D View of the complex of 15 (cyan) docked in COX-2 & overlaid over S58 (yellow) (PDB ID:1CX2) using MOE 2016.0802.

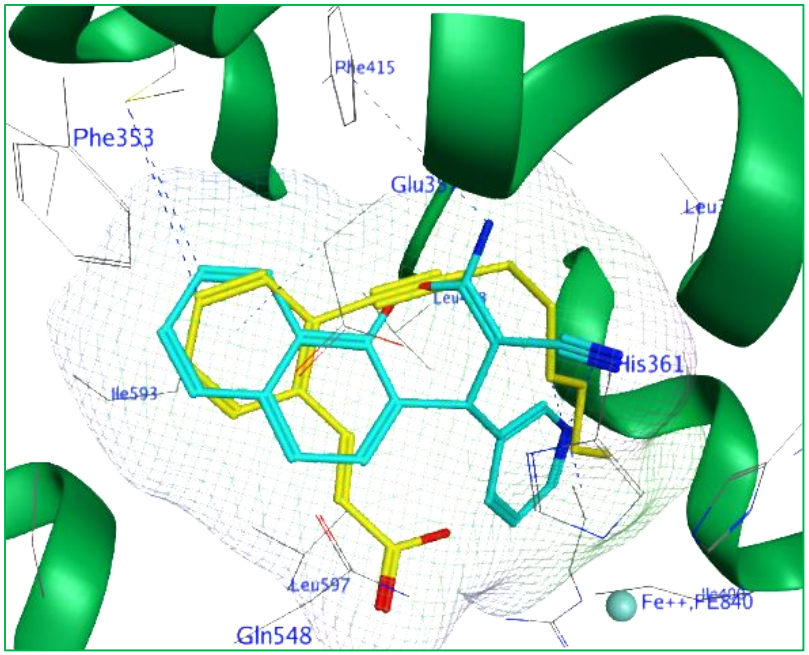


Figure 5. 3D View of the complex of 15 (cyan) docked in 15-LOX & overlaid over RS7 (yellow) (PDB ID:1LOX) using MOE 2016.0802.

Conclusions

- In our search for new potential dual COX/LOX inhibitors acting as anti-inflammatory leads with minimal ulcerogenic liability, a new series of pyridine derivatives were designed and synthesized via diversity-oriented synthesis approach.
- Biological screening results revealed that compounds **3b, 4a,b, 5-7, 9a, 11b** & **13** showed significant COX-2 inhibitory potential with IC_{50} values of 0.1-0.11 µM, compared to 0.049 µM for the reference celecoxib.
- 15-LOX inhibitory activities of the test compounds were also assessed (IC_{50} values 2.09-7.21 µM, compared to 3.34 µM for the reference quercetin).
- Compounds **5** & **13** showed significant *in vivo* anti-inflammatory activity (62 & 66% edema inhibition and ED_{50} of 147 & 56 µmol/kg, respectively).

Conclusions

- Moreover, histopathological examination revealed that they showed superior gastrointestinal safety profile (normal gastric mucosa with no ulceration).
- Compounds **4b** & **13** reduced the expression levels of pro-inflammatory enzymes (COX-2 and iNOS) while compound **4b** markedly increased that of anti-inflammatory cytokine (IL-10) in LPS-stimulated monocytes.
- Compounds **4a,b, 5** & **13** restored TNF-α titer to the normal level of the control untreated cells.
- Docking of the most active compound **5** into **COX-2** and **15-LOX** active sites showed similar binding pattern to those of the cocrystallized ligands.
- These findings could provide guidance to further chemical modifications and optimization for the development of new multi-target anti-inflammatory agents.