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ORIGINAL RESEARCH

Docking Study, Synthesis, and Anti-Inflammatory Potential of Some New Pyridopyrimidine-Derived Compounds

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Background and Purpose: Because of gastrointestinal irritation and kidney toxicity associated with non-steroidal anti-inflammatory drugs and the cardiovascular problems of Coxibs use, developing novel anti-inflammatory agents with reduced toxicity and improved selectivity remains a major challenge. Depending on our previous work, a novel series of pyridopyrimidinones **IIIa-i** has been synthesized via reaction of 6-amino-2-thioxo-2,3-dihydro-1*H*-pyrimidin-4-one (**I**) and phenyldiazenyl aromatic aldehydes (**IIa-i**). All the new constructed compounds were fully characterized by elemental and spectral analysis.

Methods: The target compounds **IIIa–i** were investigated for their potential towards COX inhibition, anti-inflammatory properties using carrageenan induced edema model in rat paw, and the ulcer indices of the most active members.

Results: The ethyl pyridopyrmidinone-benzoates **IIIf, IIIg** and **IIIh** showed superior inhibitory activity of carrageenan induced edema to celecoxib. Furthermore, the pyridopyrimidinones **IIId, IIIf, IIIg**, and **IIIi** exerted improved COX-2 inhibitory activity (IC_{50} = 0.67–1.02 μM) comparing to celecoxib (IC_{50} = 1.11 μM). Moreover, the gastric ulcerogenic potential assay of compounds **IIIf–h** revealed their lower ulcerogenic liability than indomethacin with comparable effect to celecoxib.

Conclusion: Virtual docking investigation of the most active candidates **IIId, IIIf, IIIg** and **IIIi** in the active site of COX-2 enzyme showed that these compounds implied interaction and binding motif similar to the cocrystallized ligand bromocelecoxib.

Keywords: cyclooxygenase inhibitors, anti-inflammatory activity, ulcerogenic effects, tricyclic pyridopyrimidines

Introduction

Inflammation is a cellular reaction to any harmful stimuli and conditions like tissue damage and infection.[1–3] This physiological response includes the delivery of blood components to the local infection site or injury triggering vasodilation and increased vascular permeability.[4–7] NSAIDs (Non-steroidal anti-inflammatory drugs) are clinically indicated for relieving fever, pain, and inflammation by suppressing cyclooxygenase (COX) enzymes.[8,9] Cyclooxygenases (COXs) are key enzymes responsible for transforming arachidonic acid, which is released on affected tissues by the effect of phospholipase A2 to various prostaglandins.[10,11] The major two isoforms of cyclooxygenase are COX-1 and 2.[12] The constitutive COX-1 mediates the formation of diverse cytoprotective prostaglandins, which are responsible for lining the gastric mucosa, inducing platelet aggregation and preserving homeostasis.[13,14] However, the inducible COX-2 is accountable for synthesizing of pain and inflammatory mediating prostaglandins.[15,16]

Conventional NSAIDs non-selectively inhibit COX-1 and 2 isozymes, therefore their administration is associated with gastrointestinal side effects.[17–19] Therefore, selective COX-2 inhibitors were developed to achieve enhanced safety profile on gastric mucosa. Nonetheless, certain members such as rofecoxib and valdecoxib have been associated with

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increased probability of myocardial infarction incidences as well as hypertensive actions.[20] In this regard, discovery of selective COX-2 blockers for management of pain and inflammation with diminished side actions emerged as an urgent medical need.

The pyridine nucleus has been incorporated in many core structures of several anti-inflammatory agents.[21–23] For example, the trifluoromethanesulfonamide pyridine (**1**) (Figure 1) was reported as COX-2 inhibitor with higher COX-2 selectivity index (SI = 15.35) than celecoxib (SI =7.46).[24] Moreover, Chung et al reported a set of different tricyclic chromeno-pyridines as promising anti-inflammatory agents.[25] As a representative example, compound **2** exerted potent anti-inflammatory effect through reducing the formation of PGE_2 at 10–20 mg/kg dose. Furthermore, the pyridoacyl-sulfonamide derivative **3** was described as COX-2 inhibitor with IC_{50} equal to 5.6 μM,[26] and it strongly inhibited PGE_2 (IC_{50} = 0.15 μM) in a comparable potency to celecoxib (IC_{50} = 0.10 μM).

On the contrary, a number of pyrimidine-based small molecules have been reported as potent anti-inflammatory candidates.[27] As an example, the hexahydroimidazo[1,2-*c*]pyrimidine **4** was documented to possess 34.3% anti-inflammatory activity at 50 mg/kg dose.[28] Furthermore, Keche et al reported a series of pyrimidine-diarylurea conjugates such as compound **5**, which demonstrated higher inhibitory properties against interleukin IL-6 (96%) and proactive kinase TNF-ά (78%) than that exhibited by dexamethasone against interleukin IL-6 (86%) and TNF-ά (72%).[29] Several

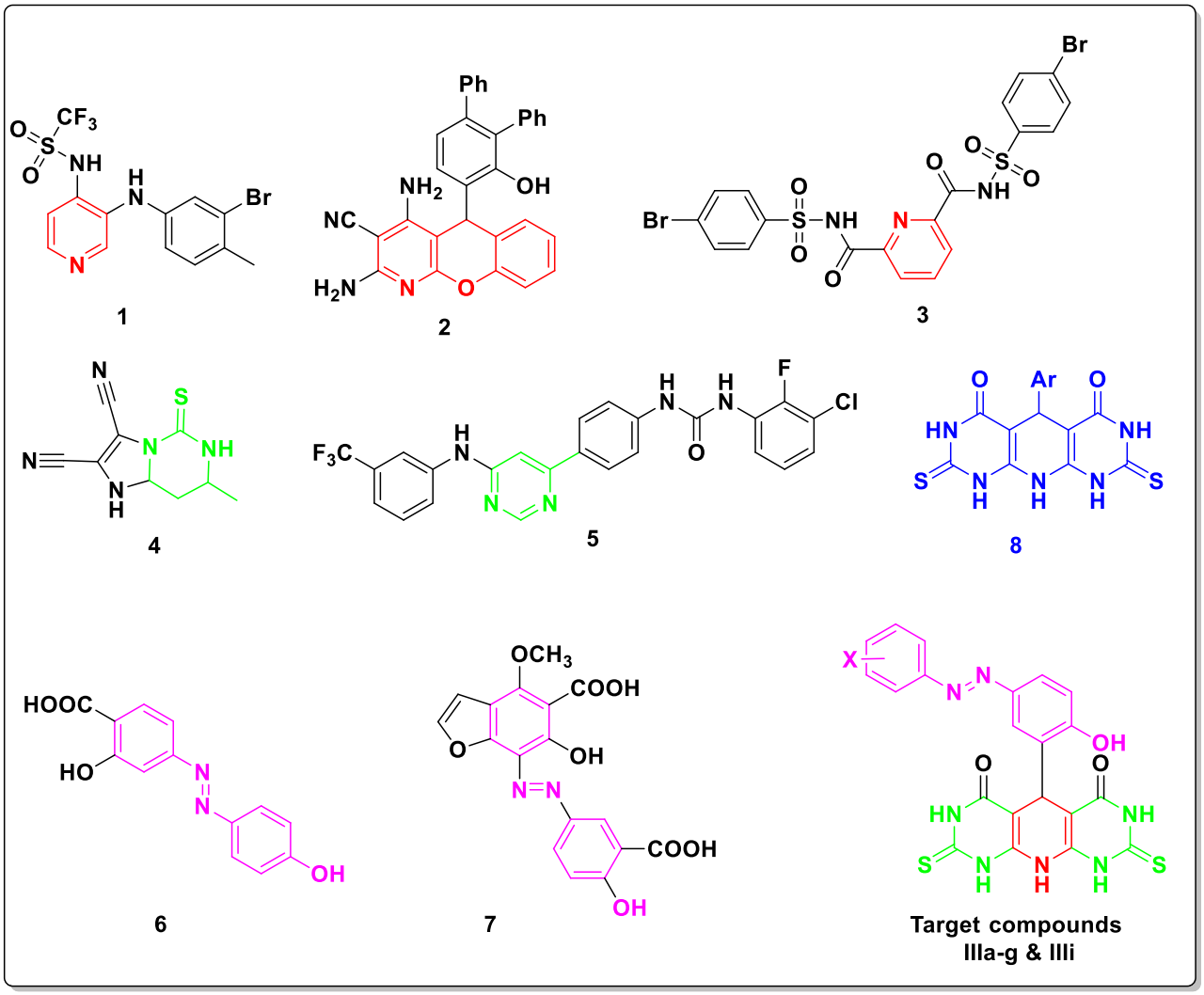


Figure 1 Representative examples of previously identified anti-inflammatory pyridines (**1–3**), pyrimidines (**4, 5**), azo containing derivatives (**6,7**), tricyclic pyridopyrimidine (**8**), and target compounds **IIIa–i.**

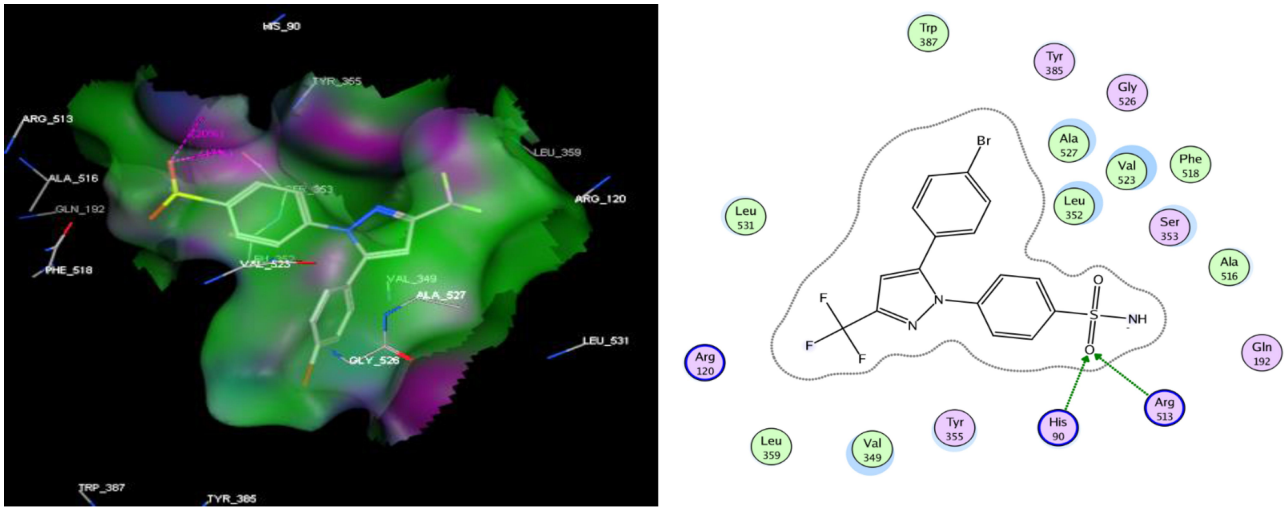


Figure 2 The 2D (right panel) and 3D (left panel) putative binding mode of SC-558.

4-(phenyldiazenyl)phenol containing compounds **6** and **7** exhibited anti-inflammatory activity in case of inflammatory bowel disease and ulcerative colitis.[30–33] A variety of tricyclic pyridodipyrimidinones **8** was identified and elicited promising anti-inflammatory activities with low incidence of gastric ulcer.[34,35]

According to reported mentioned studies and in continuation to our former studies for identification of selective COX-2 blockers,[12,19,36–44] further derivatives of pyridodipyrimidinone scaffold **IIIa–i** have been prepared and biologically investigated for their potential anti-inflammatory properties. The target molecules have been designed via conjugation of the privileged tricyclic pyridodipyrimidinone with 4-(phenyldiazenyl) phenol structural feature in a single chemical entity in an attempt to achieve selective COX-2 blocking activity along with favorable anti-inflammatory activity and minimized gastric side effects (Figure 1).

Results and Discussion

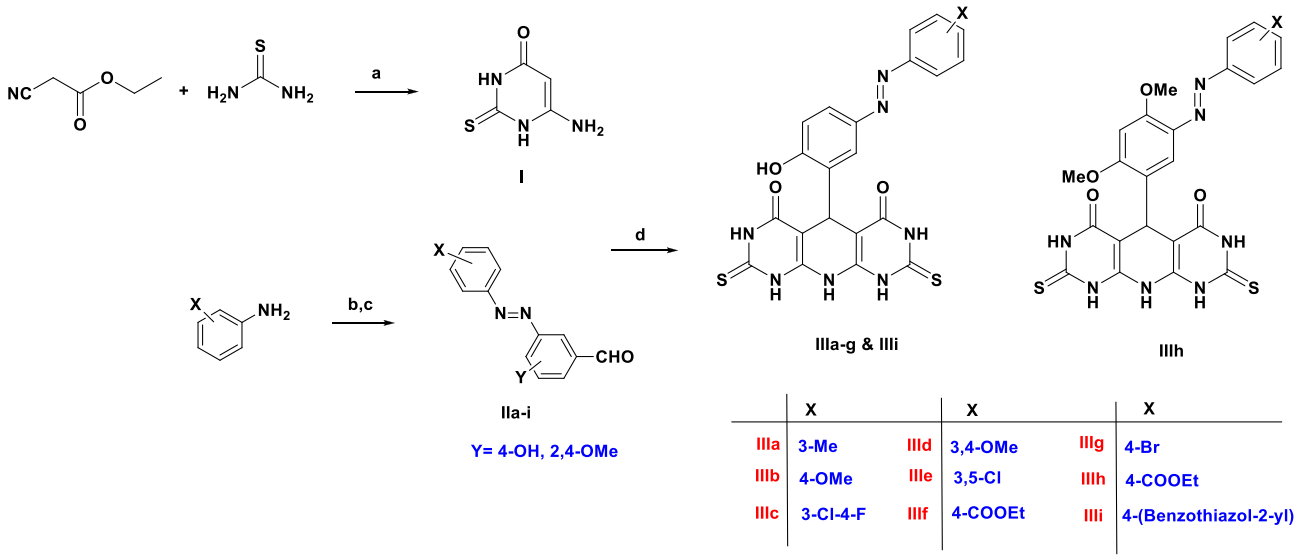
Chemistry

The key building block, pyrimidine-4-one **(I)** and phenyldiazenyl aromatic aldehydes **IIa–i**, were chemically synthesized as shown in Scheme 1. Condensation of the ethyl cyanoacetate ester with thiourea in presence of sodium ethoxide as a strong base afforded compound **I** in quantitative yield.[45] On the other hand, phenyldiazenyl aldehydes **IIa–i** were achieved through diazotization of various anilines followed by treatment with aromatic aldehydes under basic conditions.[46–48] The synthesis of the target pyridopyrimidinones **IIIa–i** was accomplished in 66–93% yield by treatment of compound **I** with various phenyldiazenyl aromatic aldehydes in CH_3OH containing a catalytic amount of HCl adopting the reported method.[34] In this reaction, the acidic polar solvent favors the reaction progress by the creation of a 6-imino that directs to higher nucleophilic character of carbon five, causing reaction to occur on the aromatic aldehydic carbonyl (Scheme 2). The chemical structures of newly prepared compounds **IIIa–i** have been elucidated via different spectroscopic and elemental analysis. ^{1}HNMR data for the novel compounds **IIIa-i** is shown in Table 1.

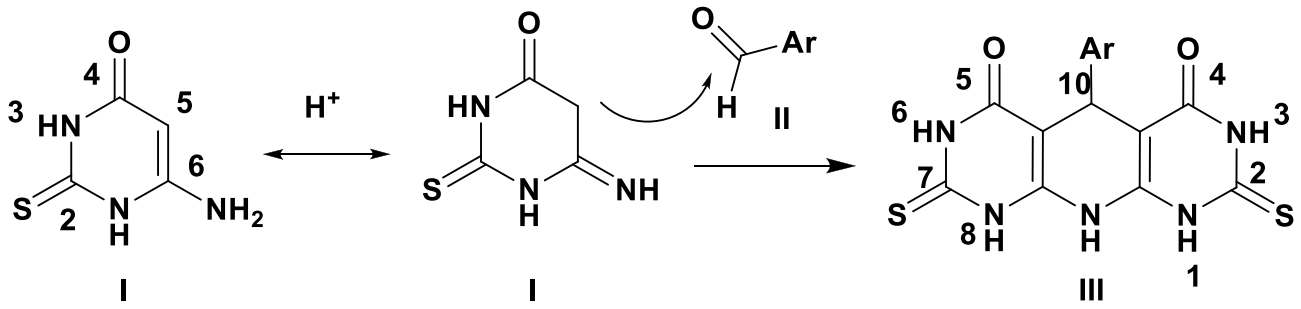
Pharmacological Activity

Assay of COX Inhibition

The newly prepared azo molecules **IIIa–i** were investigated for their COX blocking action – in terms of IC_{50} – by enzyme immunoassay (EIA) utilizing ovine COX-1/2 assay kit. Furthermore, SI (selectivity index) was assessed as IC_{50} against COX-1/IC_{50} against COX-2 applying positive standard celecoxib. As shown in Table 2, the obtained data pointed out that the pyridopyrimidinones **IIIa–i** exhibited modest to moderate blocking activity to COX-1 (IC_{50} = 3.25–11.23 μM), and favorable suppressing activity for COX-2 (IC_{50} = 0.67–4.78 μM). While the pyridopyrimidinone **IIId**,



Scheme 1 The synthetic pathway of molecules IIIa–i. Reagents and conditions: a) NaOC2H5, C2H5OH, reflux, 6h, 99%; b) NaNO2, HCl, 0 oC, 2 h; c) Aromatic aldehyde, NaOH, stirring, 0 oC, 12 h; d) conc. HCl, methanol, rt, 7h, 66–93%.



Scheme 2 The reasonable mechanism for compound III formation.

possessing 3,4-dimethoxyphenyl, was the highest COX-2 blocker (IC_{50} = 0.67 μM), **IIIf** emerged as the best selective member to COX-2 (SI = 11.82) being superior to celecoxib.

The nature of substitution pattern on both proximal and distal phenyl rings of pyridopyrimidinone had a substantial role in modulating compound's selectivity and activity against COX-2. The hydroxybenzaldehyde derived pyridopyrimidinones **IIId** (COX-2, IC_{50} = 0.67 μM), **IIIi** (COX-2, IC_{50} = 0.69 μM), **IIIf** (COX-2, IC_{50} = 0.95 μM), and **IIIg** (COX-2, IC_{50} = 1.02 μM), elicited distinct inhibition for COX-2 in comparable pattern to celecoxib (IC_{50} = 1.11 μM). Upon comparing the activity of 4-ethyl carboxylate derivatives **IIIf** and **IIIh**, it was evident that appendage of hydroxyl group (**IIIf**; COX-2, IC_{50} = 0.95 μM, SI = 11.82) on the proximal phenyl is advantageous than 2,4-dimethoxy substitution (**IIIh**; COX-2, IC_{50} = 2.43 μM, SI = 3.23) for achieving better COX-2 suppressive activity and selectivity. Moreover, it was found that replacing the 3-chloro-4-fluorophenyl of **IIIc** (COX-2, IC_{50} = 3.87 μM) with 3,4-dimethoxyphenyl led to 5.8-fold improvement in activity (**IIId**; COX-2, IC_{50} = 0.67 μM). Of special significance, pyridopyrimidinones **IIIf** (SI = 11.82) and **IIIg** (SI = 9.02) displayed remarkable selectivity for COX-2 outperforming that observed for celecoxib (SI = 6.61).

In vivo Anti-Inflammatory Action

Animal and ethics: we used in this study adult male Wister albino rats weighing 150–180g. Before any experimental study, rats are given 14 days to acclimate. The rats were kept in a controlled environment with access to water and food. All assays and practical animal studies had been done in Nahda university and adapting rules for care of animals in lab in

Table 1 NMR Data of the Novel Compounds **IIIa-l**

Compound No.	^{1}HNMR	^{13}CNMR
IIIa	δ 2.41 (s, 3H, CH_3), 4.71 (s, 1H, pyridine), 6.63–6.70 (m, 3H, 2NH, H-3\'), 7.00–7.20 (m, 2H, H-4\', 5\'), 7.30–7.63 (m, 2H, H-2\', 6\'), 7.67–7.75 (m, 2H, H-4\', 2\'), 11.56–11.75 (s, 3H, 3NH), 12.40 (s, 1H, OH).	δ 21.4, 29.7, 78.6, 115.6, 120.2, 120.9, 122.0, 124.2, 125.2, 129.6, 131.4, 139.2, 145.4, 152.7, 153.3, 160.7, 163.1, 175.0.
IIIb	δ 3.77 (s, 3H, OCH_3), 5.29 (s, 1H, pyridine), 6.82–6.91 (m, 5H, H-3\', 3\', 5\', 2NH), 7.54–7.57 (m, 2H, H-6\' 4\'), 7.80 (d, *J* = 8.4 Hz, 2H, H-2\', 6\'), 11.58–11.62 (m, 3H, 3NH), 12.14 (s, 1H, OH).	δ 29.4, 56.5, 90.5, 113.9, 115.8, 121.9, 123.9, 125.9, 125.8, 144.7, 145.5, 153.9, 161.8, 164.3, 166.9, 174.5.
IIIc	δ 5.31 (s, 1H, pyridine), 6.68–6.89 (m, 4H, H-3\', 5\', 2NH), 7.11–7.21 (m, 2H, H-4\', 6\'), 7.59 (s, 1H, H-6\'), 7.83 (s,1H, H-2\'), 11.49–11.90 (s, 3H, 3NH), 12.01 (s, 1H, OH).	δ 29.7, 91.0, 115.8, 117.9, 121.2, 121.2, 122.3, 123.4, 125.4, 126.9, 145.1, 149.3, 153.36, 160.0, 162.8, 168.0, 173.0.
IIId	δ 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 5.29 (s, 1H, pyridine), 6.82–6.85 (m, 4H, H-3\', 5\', 2NH), 7.11–7.16 (m, 2H, H-2\', 6\'), 7.54–7.57 (m, 2H, H-6\' H-4\'), 11.92–11.97 (m, 3H, 3NH), 12.03 (s, 1H, OH).	δ 31.2, 55.9, 56.2, 90.5, 111.6, 115.6, 115.8, 115.9, 121.5, 123.9, 125.7, 145.2, 145.7, 146.7, 151.3, 154.0, 162.8, 166.5, 173.7.
IIIe	δ 5.31 (s, 1H, pyridine CH), 6.61–6.67 (m, 3H, H-3\', 2NH), 6.87 (d, *J* = 8.4 Hz, 1H, H-5\'), 7.65–7.71 (m, 2H, H-4\', H-6), 7.77 (s, 1H, H-6\'), 7.96 (s,1H, H-2\'), 11.52–11.88 (s, 3H, 3NH), 12.02 (s, 1H, OH).	δ 29.7, 89.0, 115.8, 121.1, 121.8, 122.4, 125.5, 129.3, 135.3, 145.1, 153.3, 154.3, 160.6, 163.1, 173.0
IIIf	δ 1.35 (t, *J* = 6.8 Hz, 3H, CH_3), 3.36 (q, J = 6.8 Hz, 2H, CH_2), 5.31 (s, 1H, pyridine), 6.63–6.89 (m, 3H, H-3\', 2NH), 7.65–7.68 (m, 2H, H-4\', 6\'), 7.88 (d, *J* = 8.4 Hz, 2H, H-3\', 5\'), 8.11 (d, *J* = 8.4 Hz, 2H, H-2\', 6\'), 11.90–11.91 (s, 3H, 3NH), 12.06 (s, 1H, OH).	δ, 14.6, 29.7, 61.4, 90.7, 115.8, 121.6, 122.6, 123.2, 125.7, 130.8, 131.0, 145.5, 153.3, 155.3, 160.5, 163.1, 165.7, 172.9.
IIIg	δ, 5.30 (s, 1H, pyridine), 6.63–6.68 (m, 4H, H-3\', 4\', 2NH), 7.62–7.65 (m, 3H, H-6\' 3\, 5\), 7.73 (d, 1H, *J* = 8 Hz, H-2\', H-6), 11.92–12.01 (m, 3H, 3NH), 12.06 (s, 1H, OH).	δ 29.7, 78.6, 115.7, 121.2, 123.8, 124.4, 125.6, 126.8, 132.8, 145.2, 151.5, 153.3, 159.8, 163.1, 175.0.
IIIh	δ 1.33 (t, *J* = 7.2 Hz, 3H, CH_3), 3.73 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 4.33 (q, *J* = 7.2 Hz, 2H, CH_2), 4.75 (s, 1H, pyridine CH), 6.42–6.56 (m, 3H, H-3\', 2NH), 7.53 (s, 1H, H-6\'), 8.12 (d, *J* = 8.4 Hz, 2H, H-3\', 5\'), 8.13 (d, *J* = 8.4 Hz, 2H, H-2\', 6\'), 10.37 (s, 1H, NH), 11.68 (s, 2H, 2NH).	δ, 14.6, 19.0, 56.4, 56.5, 61.3, 85.7, 101.2, 115.6, 122.4, 125.2, 130.6, 130.9, 153.2, 156.6, 165.1, 167.3, 168.7, 174.9.
IIIi	δ 5.35 (s, 1H, pyridine CH), 6.89–7.48 (m, 6H, H-3\',4\',3\', 4\', 2NH), 7.53–7.77 (m, 3H, H-6\' 3\,5\'), 7.69 (d, 1H, *J* = 8 Hz, H-2\', 6\'), 8.13–8.23 (m, 2H, H-2,6\'), 11.92–12.01 (m, 3H, 3NH), 12.06 (s, 1H, OH).	δ 29.7, 90.8, 115.8, 121.3, 122.8, 123.4, 123.8, 125.8, 126.21, 127.2, 134.3, 135.1, 145.5, 153.3, 154.0, 154.0, 160.1, 166.6, 166.8, 173.0.

accordance with NIH Guidelines for the Care and Use of Laboratory Animals. The design of the current work was authorized from Nahda University ethical committee, Beni-Suef, Egypt (NUB-059-019).

Carrageenan induced edema model in rat paw was used to evaluate the anti-inflammatory activity of the target candidates **IIIa–i** and celecoxib was chosen as the positive control.

The pyridopyrimidinones were orally administrated in a dose of 50 mg/kg nearly earlier persuading inflammation through SC (subcutaneous injection) of carrageenan. Inhibition of carrageenan induced inflammation was estimated by measuring the changes of paw dimensions after 1, 3 and 5 h (Table 3). The obtained findings showed that the pyridopyrimidinones **IIIf** and **IIIh** had superior anti-inflammatory properties than celecoxib at the three examined

Table 2 In vitro COXs Inhibitory Action of Molecules **IIIa–i**

Compound No.	IC_{50} (μM)[a]		SI[b]
	COX-1	COX-2	
IIIa	7.88 ± 0.14	4.78 ± 0.08	1.65
IIIb	6.86 ± 0.12	2.51 ± 0.09	2.73
IIIc	10.52 ± 0.24	3.87 ± 0.07	2.72
IIId	4.00 ± 0.09	**0.67** ± 0.02	5.97
IIIe	7.52 ± 0.11	2.11 ± 0.07	3.56
IIIf	11.23 ± 0.27	**0.95** ± 0.01	11.82
IIIg	9.20 ± 0.22	1.02 ± 0.03	9.02
IIIh	7.84 ± 0.16	2.43 ± 0.07	3.23
IIIi	3.25 ± 0.07	**0.69** ± 0.02	4.71
Celecoxib	7.34 ± 0.18	1.11 ± 0.04	6.61

Notes: [a]IC_{50}: compound concentration required to produce 50% inhibition of COX-1 or COX-2 for means of three determinations, bold figures refer to submicromolar; [b]SI = IC_{50} (COX-1)/ IC_{50} (COX-2).

Table 3 In vivo Anti-Inflammatory Activities of Compounds **IIIa–i**

Compound No.	Anti-Inflammatory Activity % (AI)[a]		
	1 h	3 h	5 h
IIIa	0.95 ± 0.10* (34%)	0.83 ± 0.12* (41%)	0.85 ± 0.06*(39%)
IIIb	1.00 ± 0.11* (31%)	0.95 ± 0.12 (32%)	0.90 ± 0.14 (36%)
IIIc	1.10 ± 0.08 (24%)	1.00 ± 0.10 (29%)	0.65 ± 0.05*** (54%)
IIId	1.05 ± 0.10 (28%)	0.95 ± 0.12 (32%)	1.05 ± 0.13 (55%)
IIIe	1.05 ± 0.12 (28%)	0.95 ± 0.09 (32%)	0.78 ± 0.08** (45%)
IIIf	0.60 ± 0.12***(52%)	0.58 ± 0.11***(59%)	0.48 ± 0.14*** (66%)
IIIg	1.08 ± 0.09 (26%)	0.65 ± 0.13***(54%)	0.60 ± 0.15*** (57%)
IIIh	0.49 ± 0.05***(67%)	0.58 ± 0.11***(63%)	0.58 ± 0.17***(57%)
IIIi	1.10 ± 0.14 (24%)	0.80 ± 0.15**(43%)	0.68 ± 0.13** (52%)
Control	1.45 ± 0.15 (0%)	1.58 ± 0.15 (0%)	1.33 ± 0.10 (0%)
Celecoxib	0.83 ± 0.09**(43%)	0.80 ± 0.11** (43%)	0.65 ± 0.10*** (54%)

Notes: [a]The presented values are the average of triplicate experiments ± SEM, Significance levels *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ as compared to the control group.

time intervals. In addition, the target compound **IIIg** exhibited higher anti-inflammatory potential than displayed by celecoxib after 3 and 5 hours.

Close inspection of the results listed in Table 3 underscored that the pyridopyrimidinone derivatives substituted with ethyl ester **IIIf** (Anti-inflammatory (AI) % = 52–66) and **IIIh** (AI % = 57–67) displayed higher activity for inhibition of edema than those congeners containing either electron donating (**IIId**; AI % = 28–55) or electron withdrawing (**IIIc**; AI % = 24–54) groups, particularly after 1 and 3 hours. In harmony with the COX-2 inhibitory assessment, the 4-benzothiazol-2-yl containing pyridopyrimidinone **IIIi** exerted superior in vivo anti-inflammatory activity than its corresponding methoxy derivative **IIIb**. Moreover, introducing bromine on compound **IIIg** augmented the anti-inflammatory activity (3 h; AI % = 54, 5 h; AI % = 57).

The ethyl acetoxy group in compounds IIIf and IIIh has important role in vivo activity anti-inflammatory activity also bromine at for position 4 in compound IIIg. Also, the ethyl acetoxy group in IIIf and IIIh increases absorption so fast onset of action consequently high anti-inflammatory activities 52% and 67% respectively but bromine in IIIg delay onset of action firstly in compared with IIIf and IIIh.

Table 4 Gastric Ulcerogenic Effect of Compounds **IIIf–h**

Comp. No.	Ulcer Number	Ulcer Index	Relative Ulcerogenicity to Celecoxib
IIIf	3.75 ± 0.15	3.75 ± 0.11	1.25
IIIg	6.75 ± 0.07	4.75 ± 0.14	1.58
IIIh	6.50 ± 0.11	5.25 ± 0.13	1.75
Celecoxib	3.25 ± 0.17	3.00 ± 0.09	1
Indomethacin	14.25 ± 0.31	22.5 ± 0.21	7.5

Ulcerogenic Liability

The top three active pyridopyrimidinones **IIIf–h** were further evaluated for their gastric ulcerogenic liability in rats (Table 4). The ulcerogenicity liability of the investigated pyridopyrimidinones was compared with both COX-1 inhibitor (indomethacin) and COX-2 inhibitor (celecoxib). Interestingly, compound **IIIf** had the lowest ulcerogenic effect, which might be attributed to its potential selectivity for COX-2 (SI = 11.82). Additionally, all of the tested candidates showed lower ulcerogenic action than the standard indomethacin.

Molecular Docking Studies

To acquire insights about the underlying mechanism of action of the newly investigated pyridopyrimidinones, virtual docking of the highly selective COX-2 inhibitors **IIId, IIIf, IIIg** and **IIIi** within the active binding site of COX-2 enzyme was studied. The co-crystal structure of COX-2 complex with SC-558, a selective COX-2 blocker, was acquired from PDB (protein data bank: 1CX2),[49] and the virtual docking was conducted using MOE (Molecular Operating Environment; 2010). Validation of docking protocol had been performed by redocking the ligand bromocelecoxib (SC-558) into COX-2 active site with root mean standard deviation (RMSD) of 1.1524, and showed an energy score (S) of −11.93 kcal/mol. The ligand sulphonyl group was engaged in two hydrogen bonds with the receptor amino acids His90 and Arg513 (Figure 2, Table 5).

Interestingly, compound **IIIf** showed profitable fitting with COX-2 with superior docking score (S = −13.89 kcal/mol) to bromocelecoxib (S = −11.93 kcal/mol). Furthermore, **IIIf** displayed two HB interactions through its carbonyl oxygen with Arg120 (2.65 A°) as well as azo moiety with His90 (3.25 A°) (Figure 3; Table 5). Also, the pyridopyrimidinone **IIIg** exhibited score energy (S) of −11.20 kcal/mol, and was able to form three HB interactions with Arg120, His90 and Tyr355 (Figure 3).

Conclusion

Novel derivatives of pyridopyrimidinones **IIIa–i** were prepared and assessed in vitro and in vivo for their COXs and carrageenan induced edema anti-inflammatory activities, respectively. Preliminary screening of the target compounds disclosed that the pyridopyrimidinone **IIIf** possessing ethyl acetate had the best activity with potent edema inhibition in percent = 52% after one hour, 59% after three hours and 66% after five hours. Moreover, certain members among this focused library were identified to be selective COX-2 inhibitors. Particularly, **IIId, IIIf, IIIg** and **IIIi**, exerted superior inhibition for COX-2 (IC_{50} = 0.67–1.02 μM) than celecoxib (IC_{50} = 1.11 μM). Ulcerogenic accountability of compounds **IIIf–h** exhibited their comparable activity to celecoxib along with less ulcerogenic effect than indomethacin. It was noted that the lipophilic group (ethyl ester or benzothiazol-2-yl) containing compounds **IIIf, IIIh** and **IIIi** elicited superior anti-inflammatory effect and better selectivity for COX-2 than other derivatives. Overall, combining the privileged pyrido-dipyrimidinone scaffold with diphenylazo structural feature in single molecule with appropriate hydrophobic substitution pattern may represent a promising core structure for further design of potent anti-inflammatory agents with minimized gastric side effects.

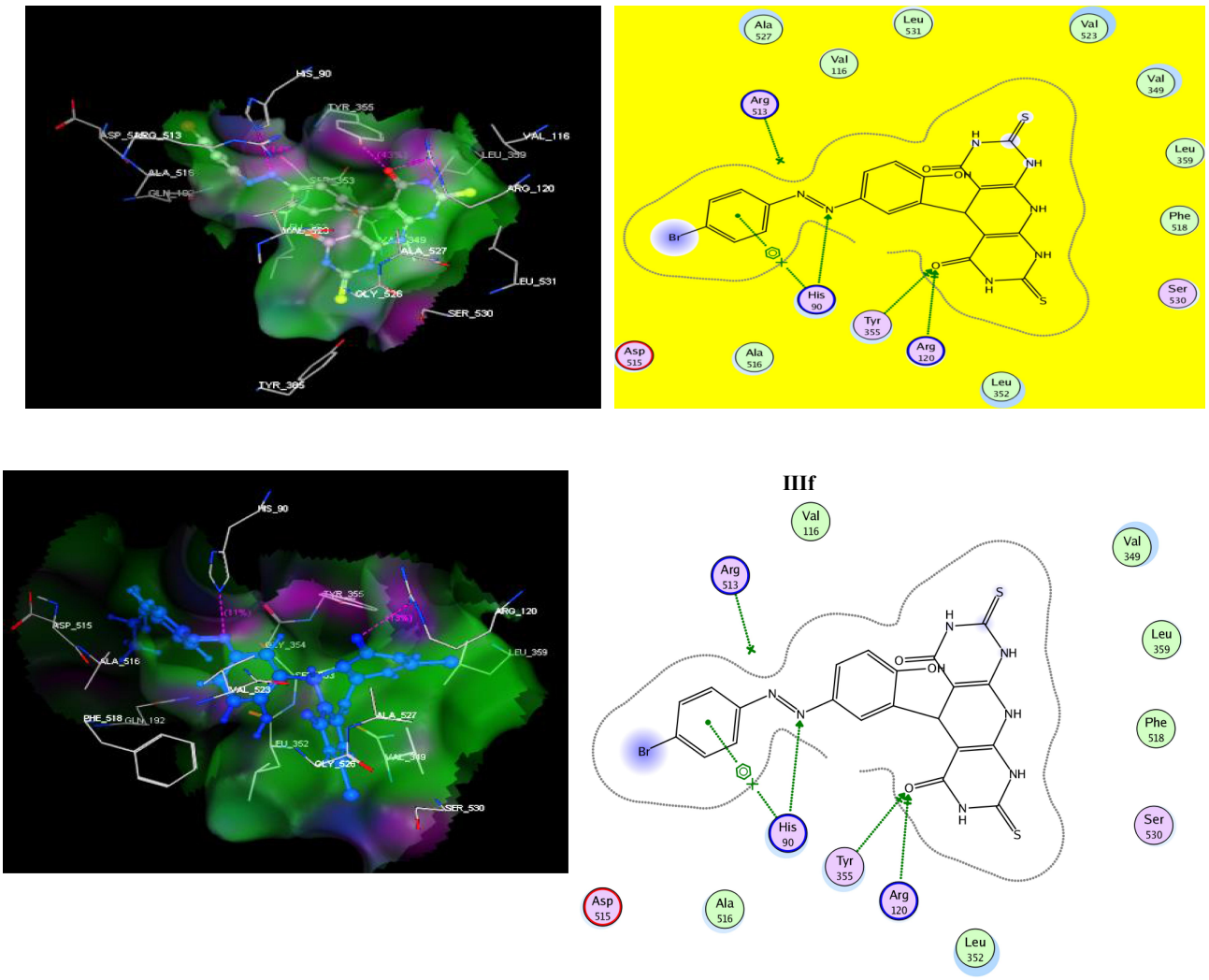


Figure 3 The 2D (right panel) and 3D (left panel) putative binding mode of compounds **IIIf** and **IIIg.**

Experimental

Chemistry

Melting points were determined with Thomas-Hoover capillary apparatus and uncorrected. Infrared (IR) spectra of the new compounds were detected utilizing FT-IR spectrometer (Nicolet 550 Series II Magna) as films on NaCl plates, and expressed in wave number (cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker Avance III 400 MHz in deuterated dimethyl sulfoxide (DMSO-d_6). Chemical shifts were measured in ppm (δ scale), and the coupling constant (*J*) values were expressed in Hertz (Hz). Mass spectra were recorded using Hewlett Packard 5988 spectrometer. Elemental microanalyses for N, C, and H were measured utilizing Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA), at Cairo University (Micro analytical unit), Egypt, and all analyzed compounds were within ± 0.4% of the assessed values. Thin layer chromatography (TLC) was carried out utilizing silica gel plates (Germany, MERCK 60F 254, 0.25 mm), a mixture of chloroform /methanol (9.5:0.5 mL) as eluent and visualized with UV lamp. All chemicals and reagents were commercially purchased and used directly without purification. 6-Aminopyrimidinone derivative **I** and the aldehyde derivatives **IIa**–**i** were prepared adopting the reported methods.[45,50,51]

Table 5 The Virtual Docking Data of Compounds **IIId, IIIf, IIIg, IIIi** and **SC-558**

Compound No.	Affinity Kcal/mol	No. of HBs	Distance (A°) from Main Residue		Functional Group
IIId	−13.95	5	2.97 3.20 2.99 3.28 2.97	Tyr355 His90 Ser353 Ser530 Tyr385	C=O OH OH OCH_3 OCH_3
IIIf	−13.89	2	2.65 3.25	Arg120 His90	C=O N=N
IIIg	−11.20	3	2.68 2.15 2.98	Arg120 His90 Tyr355	C=O N=N C=O
IIIi	−14.52	3	3.11 2.87 3.09	Arg120 His90 Tyr355	C=O N=N C=O
SC-558	−11.93	2	2.41 2.30	Arg513 His90	$-SO_2$ $-SO_2$

Synthesis of Pyridopyrimidinones IIIa–i

6-Amino-2-thioxo-2,3-dihydro-*1H*-pyrimidin-4-one (**I**) (2.86 g, 20 mmol), appropriate aromatic aldehyde (**IIa–i**) (10 mmol), and conc. hydrochloric acid (5 mL) in methanol (30 mL) were stirred at room temperature (rt) for 7 h. The precipitated product was collected, washed with cold ethyl alcohol, dried and crystallized from DMF to afford the target molecules **IIIa–i** in pure forms.

5-(2-Hydroxy- 5-(m-Tolyldiazenyl) Phenyl)−2,8- Dithioxo-2, 3, 5, 8, 9, 10-Hexahydropyrido[2,3-D:6,5d′]Dipyrimidine-4,6–(1H,7H)-Dione (IIIa)

Yield (85%), yellowish white crystals, m.p. > 300 °C; IR (cm^{-1}): 1649 (C=O), 3223(NH), 3415(OH); ^{1}H NMR δ 2.41 (s, 3H, CH_3), 4.71 (s, 1H, pyridine), 6.63–6.70 (m, 3H, 2NH, H-3\), 7.00–7.20 (m, 2H, H-4\, 5\), 7.30–7.63 (m, 2H, H-2\, 6\), 7.67–7.75 (m, 2H, H-4\, 2\), 11.56–11.75 (s, 3H, 3NH), 12.40 (s, 1H, OH); ^{13}C NMR δ 21.4, 29.7, 78.6, 115.6, 120.2, 120.9, 122.0, 124.2, 125.2, 129.6, 131.4, 139.2, 145.4, 152.7, 153.3, 160.7, 163.1, 175.0; EIMS (m/z) 491 (M^+, 19.44%). Elemental analysis of $C_{22}H_{17}N_7O_3S_2$: C, 53.76; H, 3.49; N, 19.95. Found: C, 53.40; H, 3.56; N, 20.03.

5-(2-Hydroxy-5-(4-Methoxyphenyl)Diazenyl) Phenyl)−2,8-Dithioxo-2, 3, 5, 8, 9, 10-Hexahydropyrido[2,3-D:6,5d′]dipyrimidine-4,6–(1H,7H)-Dione (IIIb)

Yield (80%), greyish white crystals;; m.p. > 300°C; IR (cm^{-1}): 1669 (C=O), 3257(NH), 3419 (OH); ^{1}H NMR δ 3.77 (s, 3H, OCH_3), 5.29 (s, 1H, pyridine), 6.82–6.91 (m, 5H, H-3\, 3\, 5\, 2NH), 7.54–7.57 (m, 2H, H-6\, 4\), 7.80 (d, J = 8.4 Hz, 2H, H-2\, 6\), 11.58–11.62 (m, 3H, 3NH), 12.14 (s, 1H, OH); ^{13}C NMR δ 29.4, 56.5, 90.5, 113.9, 115.8, 121.9, 123.9, 125.9, 125.8, 144.7, 145.5, 153.9, 161.8, 164.3, 166.9, 174.5; EIMS (m/z) 507 (M^+, 24.52%). Elemental analysis of $C_{22}H_{17}N_7O_4S_2$: C, 52.06; H, 3.38; N, 19.32. Found: C, 52.00; H, 3.50; N, 19.53.

5(5-((3-Chloro-4-Fluorophenyl) Diazenyl)-2-Hydroxyphenyl)−2, 8- Dithioxo-2, 3, 5, 8, 9, 10-Hexahydropyrido [2,3-D:6,5d′]Dipyrimidine-4,6–(1H,7H)-Dione (IIIc)

Yield (66%), whitish grey crystals, m.p. > 300°C; IR (cm^{-1}): 1641 (C=O), 3040(ArH), 3150(NH), 3422(OH); ^{1}H NMR δ 5.31 (s, 1H, pyridine), 6.68–6.89 (m, 4H, H-3\, 5\, 2NH), 7.11–7.21 (m, 2H, H-4\, 6\), 7.59 (s, 1H, H-6\), 7.83 (s,1H, H-2\), 11.49–11.90 (s, 3H, 3NH), 12.01 (s, 1H, OH); ^{13}C NMR δ 29.7, 91.0, 115.8, 117.9, 121.2, 121.2, 122.3, 123.4,

125.4, 126.9, 145.1, 149.3, 153.36, 160.0, 162.8, 168.0, 173.0; EIMS (m/z) 529 (M^+, 29.39%), 67 (100%). Elemental analysis of $C_{21}H_{13}ClFN_7O_3S_2$: C, 47.59; H, 2.47; N, 18.50. Found: C, 47.50; H, 2.45; N, 18.54.

5-(5-((3,4-Dimethoxyphenyl-2-Diazenyl)-2-Hydroxyphenyl)−2, 8- Dithioxo-2, 3, 5, 8, 9, 10-Hexahydropyrido[2,3-D:6,5d']Dipyrimidine-4,6-(1H,7H)-Dione (IIId)

Yield (80%), yellow crystals, m.p. > 300°C; IR (cm^{-1}): 3419 (OH), 3215 (NH), 1667 (C=O); ^{1}H NMR δ 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 5.29 (s, 1H, pyridine), 6.82–6.85 (m, 4H, H-3\, 5\, 2NH), 7.11–7.16 (m, 2H, H-2\, 6\), 7.54–7.57 (m, 2H, H-6\, H-4\), 11.92–11.97 (m, 3H, 3NH), 12.03 (s, 1H, OH); ^{13}C NMR δ 31.2, 55.9, 56.2, 90.5, 111.6, 115.6, 115.8, 115.9, 121.5, 123.9, 125.7, 145.2, 145.7, 146.7, 151.3, 154.0, 162.8, 166.5, 173.7; EIMS (m/z) 537 (M^+, 32.07%). Elemental analysis of $C_{23}H_{19}N_7O_5S_2$: C, 51.39; H, 3.56; N, 18.24. Found: C, 51.55; H, 3.45; N, 18.03.

5-(5-((3,4-Dichlorophenyl-2-Diazenyl)-2-Hydroxyphenyl)−2, 8- Dithioxo-2, 3, 5, 8, 9, 10-Hexahydropyrido[2,3-D:6,5d'] Dipyrimidine-4,6-(1H,7H)-Dione (IIIe)

Whitish yellow crystals (yield 75%), m.p. > 300°C; IR (cm^{-1}); 3416 (OH), 3180 (NH), 1652 (C=O); ^{1}H NMR δ 5.31 (s, 1H, pyridine CH), 6.61–6.67 (m, 3H, H-3\, 2NH), 6.87 (d, J = 8.4 Hz, 1H, H-5\), 7.65–7.71 (m, 2H, H-4\, H-6\), 7.77 (s, 1H, H-6\), 7.96 (s,1H, H-2\), 11.52–11.88 (s, 3H, 3NH), 12.02 (s, 1H, OH); ^{13}C NMR δ 29.7, 89.0, 115.8, 121.1, 121.8, 122.4, 125.5, 129.3, 135.3, 145.1, 153.3, 154.3, 160.6, 163.1, 173.0; EIMS (m/z) 545 (M^+, 20.55%). Elemental analysis of $C_{21}H_{13}Cl_2N_7O_3S_2$: C, 46.16; H, 2.40; N, 17.94. Found: C, 46.00; H, 2.56; N, 18.23.

Ethyl (E)-4-((3-(4,6-Dioxo-2,8-Dithioxo-1,2,3,4,5,6,7,8,9,10-Decahydropyrido[2,3-D:6,5-D']Dipyrimidin-5-Yl)-4-Hydroxyphenyl)Diazenyl)Benzoate (IIIf)

Yield (82%), yellowish white crystals, m.p. > 300°C; IR (cm^{-1}): 1686 (C=O), 3229(NH), 3451(OH); ^{1}H NMR δ, 1.35 (t, J = 6.8 Hz, 3H, CH_3), 3.36 (q, J = 6.8 Hz, 2H, CH_2), 5.31 (s, 1H, pyridine), 6.63–6.89 (m, 3H, H-3\, 2NH), 7.65–7.68 (m, 2H, H-4\, 6\), 7.88 (d, J = 8.4 Hz, 2H, H-3\, 5\), 8.11 (d, J = 8.4 Hz, 2H, H-2\, 6\), 11.90–11.91 (s, 3H, 3NH), 12.06 (s, 1H, OH); ^{13}C NMR δ, 14.6, 29.7, 61.4, 90.7, 115.8, 121.6, 122.6, 123.2, 125.7, 130.8, 131.0, 145.5, 153.3, 155.3, 160.5, 163.1, 165.7, 172.9; EIMS (m/z) 549 (M^+, 18.52%). Elemental analysis of $C_{24}H_{19}N_7O_5S_2$: C, 52.45; H, 3.48; N, 17.84. Found: C, 52.55; H, 3.52; N, 18.01.

(E)-5-(5-((4-Bromophenyl) Diazenyl)-2-Hydroxyphenyl)-2.8-Dithioxo-2, 3, 5,8,9,10-Hexahydropyrido [2,3-D:6,5-D'] Dipyrimidine-4,6(1H,7H)-Dione (IIIg)

Yield (75%), yellowish white crystals, m.p. > 300°C; IR (cm^{-1}): 1657 (C=O), 3185(NH), 3413(OH); ^{1}H NMR δ, 5.30 (s, 1H, pyridine), 6.63–6.68 (m, 4H, H-3\, 4\, 2NH), 7.62–7.65 (m, 3H, H-6\, 3\, 5\), 7.73 (d, 1H, J = 8 Hz, H-2\, H-6), 11.92–12.01 (m, 3H, 3NH), 12.06 (s, 1H, OH); ^{13}C NMR δ 29.7, 78.6, 115.7, 121.2, 123.8, 124.4, 125.6, 126.8, 132.8, 145.2, 151.5, 153.3, 159.8, 163.1, 175.0; EIMS (m/z) 556 (M^+, 15.28%). Elemental analysis of $C_{21}H_{14}BrN_7O_3S_2$: C, 45.53; H, 2.54; N, 17.62. Found: C, 45.33; H, 2.50; N, 17.60.

Ethyl (E)-4-((5-(4,6-Dioxo-2,8-Dithioxo-1,2,3,4,5,6,7,8,9,10-Decahydropyrido[2,3-D:6,5-D']Dipyrimidin-5-Yl)-2,4-Dimethoxyphenyl)Diazenyl)Benzoate (IIIh)

Yield (70%), yellowish white crystals, m.p. > 300°C; IR (cm^{-1}): 1645 (C=O), 3425(NH),; ^{1}H NMR δ 1.33 (t, J = 7.2 Hz, 3H, CH_3), 3.73 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 4.33 (q, J = 7.2 Hz, 2H, CH_2), 4.75 (s, 1H, pyridine CH), 6.42–6.56 (m, 3H, H-3\, 2NH), 7.53 (s, 1H, H-6\), 8.12 (d, J = 8.4 Hz, 2H, H-3\, 5\), 8.13 (d, J = 8.4 Hz, 2H, H-2\, 6\), 10.37 (s, 1H, NH), 11.68 (s, 2H, 2NH); ^{13}C NMR δ, 14.6, 19.0, 56.4, 56.5, 61.3, 85.7, 101.2, 115.6, 122.4, 125.2, 130.6, 130.9, 153.2, 156.6, 165.1, 167.3, 168.7, 174.9; EIMS (m/z) 593 (M^+, 8.69%). Elemental analysis of $C_{26}H_{23}N_7O_6S_2$: C, 52.61; H, 3.91; N, 16.52. Found: C, 52.51; H, 4.03; N, 16.43.

(E)-5-(5-((4-(Benzo[D]thiazol-2-Yl)Phenyl)Diazenyl)-2-Hydroxyphenyl)-2,8-Dithioxo-2,3,5,8,9,10-Hexahydropyrido [2,3-D:6,5-D']Dipyrimidine-4,6(1H,7H)-Dione (IIIi)

Yield (93%), whitish yellow crystals, m.p. > 300°C; IR (cm^{-1}): 1172(C=S), 1651 (C=O), 3181(NH), 3419 (OH);

^{1}H NMR, δ 5.35 (s, 1H, pyridine CH), 6.89–7.48 (m, 6H, H-3\',4\',3\', 4\', 2NH), 7.53–7.77 (m, 3H, H-6\', 3\',5\'), 7.69 (d, 1H, J = 8 Hz, H-2\', 6\'), 8.13–8.23 (m, 2H, H-2,6\'), 11.92–12.01 (m, 3H, 3NH), 12.06 (s, 1H, OH); ^{13}C NMR δ 29.7, 90.8, 115.8, 121.3, 122.8, 123.4, 123.8, 125.8, 126.21, 127.2, 134.3, 135.1, 145.5, 153.3, 154.0, 154.0, 160.1, 166.6, 166.8, 173.0; EIMS (m/z) 610 (M^+, 6.46%). Elemental analysis of $C_{28}H_{18}N_8O_3S_3$: C, 55.07; H, 2.97; N, 18.35. Found: C, 55.38; H, 3.12; N, 18.74.

Pharmacological Activity Studies

All utilized procedures in the pharmacological evaluation were carried out as described earlier. Colorimetric assay of COXs,[20] anti-inflammatory activity (in-vivo),[40] ulcerogenic liability[52] were cited in the Supplementary Materials.

Molecular Docking

The virtual docking study was performed by utilizing the x-ray crystal structure of COX-2 enzyme (pdb code: 1CX2).[28] Ligand and protein preparation (3D protonation for the amino acid side chain of enzyme, addition of hydrogen atoms, and deletion of all water of crystallization away from the active site) was performed using MOE software (version 2010, Chemical Computing Group Inc., QC, Canada). The pyridopyrimidinones compounds were sketched in their three-dimensional (3D) structures by Chemo-Draw, protonated, and subjected to energy minimization. Molecular docking of these compounds has been applied, amino acid interactions were examined, and the hydrogen bond lengths were recorded.

Statistical Analysis

The significant difference for groups was measured utilizing one-way ANOVA followed by Dunnett's test. Significant differences are at *P >0.05, **P > 0.01 and ***P >0.001, and GraphPad Prism software (version 5) was used for statistical tests (version 5).

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Disclosure

The authors report no conflicts of interest in this work.

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