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Research Article
A Potent Cyclooxygenase-2 Inhibitor for Synthesized Pyrimidine and Thiazolopyrimidine Derivatives

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Abstract
Ten pyridine and pyrimidine and thiazolopyrimidine derivatives (1-10) were synthesized and screened as analgesic, anticonvulsant and antiparkinsonian agent before. Herein, all the target compounds showed anti-inflammatory activity. The active compounds showed selective inhibitory activity towards COX-2 enzyme as revealed by the *in vitro* enzymatic assay. All the tested compounds proved to have superior gastrointestinal (GI) safety profiles as compared to indomethacin, when tested for their ulcerogenic effects.

Key words: Pyridine derivatives, thiazolopyrimidine, anti-inflammatory activities, COX-2 inhibitors

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) recognized as the most important class of clinically used agents for the treatment of pain and inflammatory manifestations associated with a number of pathological conditions. However, long term treatments with NSAIDs associated with numerous side effects such as gastrointestinal mucosal damage, bleeding, intolerance and renal toxicity (Sontag, 1986; Flower, 2003; Allison *et al*., 1992). Consequently, extensive research work oriented towards improving their pharmacological profile that led to the discovery of multiple isoforms of cyclooxygenase (COX) that are differently regulated (Chandrasekharan *et al*., 2002; Song *et al*., 1999). The discovery of the inducible isoform of cyclooxygenase enzyme (COX-2) spurred the search for anti-inflammatory agents free of the undesirable effects associated with old classical NSAIDs. A novel class of selective COX-2 inhibitors has been discovered. Amongest this class, celecoxib (Fig. 1) was shown to be a potent and gastrointestinal (GI) safe anti-inflammatory agent. Chemically it is pyrazole containing, diaryl-heterocyclic template that is known to selectively inhibit COX-2 (Palomer *et al*., 2002).

Several compounds containing pyrazole moity were also reported to exhibit anti-inflammatory activity with acceptable safty margines (Tsuji *et al*., 1998; Beers *et al*., 1997). Recently, it was worth to mentioned, benzene sulfonamides (Bekhit *et al*., 2008), pyrimidines (Venu *et al*., 2008), imidazoles (Salimi *et al*., 2007) and thiazolidinones (Hu *et al*., 2013) are other important pharmacodynamic heterocyclic nuclei which when incorporated into different heterocyclic templates, have been reported to possess excellent potent anti-inflammatory activity. In view of these observations and in continuation of our previous work in pyridine and pyrimidine chemistry, some heterocyclic compounds containing the pyridine derivatives; thiazolopyrimidine moiety were synthesized and tested their anti-inflammatory activities.

MATERIALS AND METHODS

Chemistry: All the tested compounds were confirmed by physical and spectroscopic evidences according to the previously reported procedures (Amr *et al*., 2005).

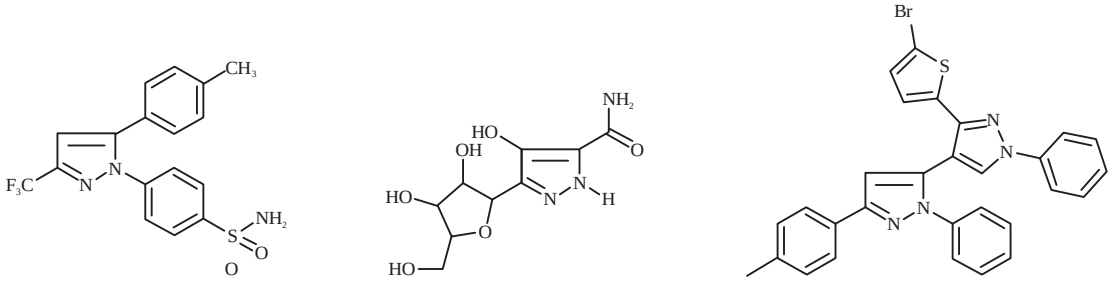
Pharmacological activities

Experimental animals: All animals were obtained from National Research Center, Cairo, Egypt, Giza, Egypt and were acclimatized for 10 days under standard housing conditions (24±1°C; 45-55% RH with 12:12 h light/dark cycle). The animals had free access to rat food and water. The animals were habituated to laboratory conditions for 48 h prior to the experimental protocol to minimize any nonspecific stress. Animals were maintained under standard conditions in the animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Cotton pellet-induced granuloma bioassay: The experimental method which was used in cotton pellet-induced granuloma bioassay has been adopted from Bekhit *et al.* (2008).

Carrageenan-induced rat paw edema: The experimental method which was used in carrageenan-induced rat paw edema has been adopted from Di Rosa and Willoughby (1971).

Human COX-1 and COX-2 enzymatic assay: Human COX-1 and COX-2 activities were determined as described by Wakitani *et al.* (1998).



Celecoxib Pyrazofurin 3-(5-bromothiophen-2-yl)-1-phenyl-4-(1-phenyl-3-p-tolyl-1H-pyrazol-5-yl)-1H-pyrazole

Fig. 1: Structures of celecoxib and reported active pyrazole derivatives

Ulcerogenic effects: All target compounds were evaluated for their ulcerogenic potential in rats (Abouzeit-Har *et al*., 1982).

Human, rat and dog microsomal COX assays: The experimental method which was used in human, rat and dog microsomal COX assay bioassay has been adopted from Chan *et al*. (1999).

Acute toxicity: The oral acute toxicity of compounds was investigated using male mice (20 g) according to previously reported methods (Verma *et al*., 1994; Litchfield and Wilcoxon, 1949). The animals were divided into groups of six mice each. The compounds were given orally, suspended in 1% gum acacia, in doses of 1, 10, 100, 200, 250 and 300 mg kg^{-1}. The mortality percentage in each group was recorded after 24 h. Additionally, the test compounds were investigated for their parenteral acute toxicity in groups of six mice each as reported earlier (Bekhit and Fahmy, 2003). The compounds, or their vehicle propylene glycol (control), were given by intraperitoneal injection in doses of 10, 25, 50, 75 and 100 mg kg^{-1}. The percentage survival was followed up to seven days (Bekhit and Fahmy, 2003).

Statistical analysis: Results are expressed as Mean±SEM. Differences between vehicle control and treatment groups were tested using one-way analysis ANOVA, followed by multiple comparisons by the Dunnett's test. A value of $p \leq 0.005$ was considered statistically significant. Dose-response curves for percent protection and ulceration were fitted by a four-parameter logistic function using a nonlinear least-squares regression.

RESULTS AND DISCUSSION

Chemistry: Herein a series of spiroalkanones, pyrimidine and thiopyrimidine heterocyclic derivatives 1-10 (Fig. 2) were synthesized and illustrated by physical, chemical and spectroscopic evidences before and screened as analgesic, anticonvulsant and antiparkinsonian agents (Amr *et al*., 2005). In this study, we report the activities of these compounds as antiinflammatory agents.

Pharmacological activities: A series of substituted pyrimidines synthesized and screened for selective COX-2 inhibitors (Tietz *et al*., 2013). Many thiazolopyrimidines was

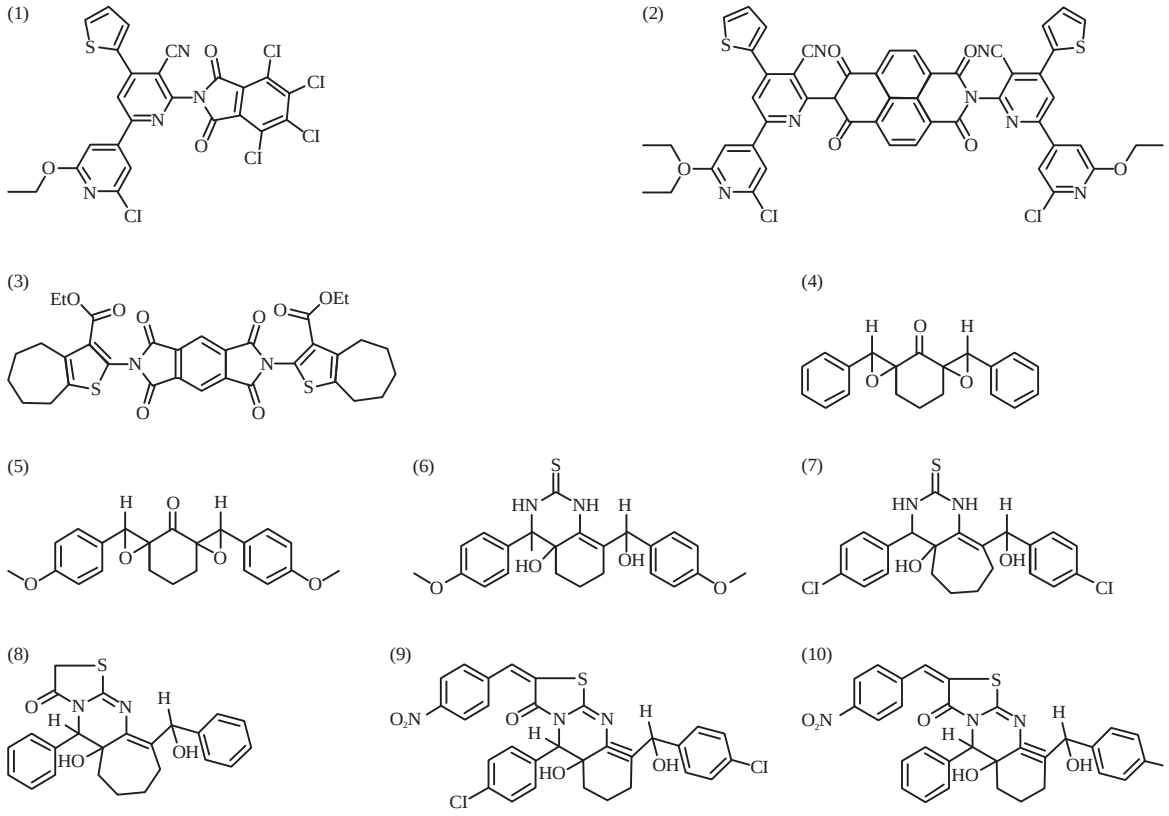


Fig. 2: Chemical structure of the tested compounds (1-10)

Table 1: Anti-inflammatory activity (ED_{50} mmol) and ulcerogenic activity

Test compounds	ED_{50} (μmol)	Ulceration (%)
1	1.83±0.0001	0.00
2	1.77±0.0003	5.44
3	1.98±0.0002	0.00
4	1.88±0.0002	0.00
5	1.85±0.0002	0.00
6	1.81±0.0002	0.00
7	1.61±0.0001	7.43
8	1.56±0.0001	8.45
9	1.67±0.0001	6.23
10	1.71±0.0002	6.18
Indomethacin	9.568±0.00078	100

Values were calculated from the mean values of data from three separate experiments and presented as Mean±SEM. All results are significant different from control values at $p \leq 0.005$. All results are significant different from reference standard values at $p \leq 0.005$

Table 2: Effects of tested compounds on carrageenan-induced rat paw edema (mL), percentage protection and activity relative to indomethcin

Test compounds	Increase in paw edema (mL)±SEM[a,b]	Protection (%)
Control	0.988±0.0006	0
Indomethacin	0.25±0.0005	74.49
1	0.19±0.0007	80.77
2	0.16±0.0002	83.81
3	0.31±0.0001	68.62
4	0.24±0.0003	75.71
5	0.23±0.0004	76.72
6	0.17±0.0004	82.79
7	0.13±0.0003	86.84
8	0.12±0.0001	87.85
9	0.14±0.0002	85.83
10	0.16±0.0004	83.81

Values were calculated from the mean values of data from three separate experiments and presented as Mean±SEM. All results are significant different from control values at $p \leq 0.005$. All results are significant different from reference standard values at $p \leq 0.005$

synthesised and have potent anti-inflammatory activities and some of them showed potent antipyretic activities (Myakushkene *et al*., 1999). On the other hand, some of pyrimidine derivatives were synthesized and have been potent anti-inflammatory activities (Sondhi *et al*., 2001, 1999, 2000).

Pharmacological activities

Anti-inflammatory activities: Determination of the antiiflammatory activities via two animal models the firest was determintining the ED_{50} that induce antiinflammatory activities using cotton pellet-induced granuloma bioassay (Table 1), all the tested compounds showed excellent anti-inflammatory activity and the order of activity was 8 (ED_{50} = 1.56 μmol), 7 (ED_{50} = 1.61 μmol), 9 (ED_{50} = 1.67 μmol), 10 (ED_{50} = 1.71 μmol), 2 (ED_{50} = 1.77 μmol), 6 (ED_{50} = 1.81 μmol), 1 (ED_{50} = 1.83 μmol), 5 (ED_{50} = 1.85 μmol), 4 (ED_{50} = 1.88 μmol) and 3 (ED_{50} = 1.98 μmol). All tested compounds were more active than indomethacin (ED_{50} = 9.568 μmol).

The second one involving determination of increasing in paw edema by using carrageenan-induced rat paw edema (Table 2). All the tested compounds showed excellent protection against carrageenan-induced rat paw edema and the decending order of activity was 8 (protection, 87.85%), 7 (protection, 86.84%), 9 (protection, 85.83%), 10 (protection, 83.81%), 2 (protection, 83.81%), 6 (protection, 82.79%), 1 (protection, 80.77%), 5 (protection, 76.72%), 4 (protection, 75.71%) and 3 (protection, 68.62%). All tested compounds were more active than indomethacin (protection, 74.49%). The results obtained by carrageenan-induced rat paw edema considered as a good evidence toll that confirmed the results obtained by the cotton pellet-induced granuloma bioassay.

Ulcerogenic activities: Compounds 1 (ulceration, 0.00%), 3 (ulceration, 0.00%), 4 (ulceration, 0.00%), 5 (ulceration, 0.00%) and 6 (ulceration, 0.00%) was devoided from ulcerogenic activities, while other showed low ulcerogenic activities. Compounds 2 (ulceration, 5.44%), 7 (ulceration, 7.43%), 8 (ulceration, 8.45%), 9 (ulceration, 6.23%) and 10 (ulceration, 6.18%) causing minor ulceration while indomethacin causing 100% ulceration. It was oserved that as the antiinflammatory activities increases the ulcerogenic activities increases (Table 1).

***In vitro* human COX-2a and COX-1b enzymes inhibitory activities:** *In vitro* human COX-2a and COX-1b enzymes inhibitory activities of compounds revealed that the tested compounds inhibited both COX-2a and COX-1b enzymes but greatly on COX-2a enzyme more than COX-1b enzyme (Table 3). The selectivity ratio COX-2/COX-1 was determined and was in the following desceniding selectivity order 8 (COX-2/COX-1 selectivity ratio: 1058.333), 7 (COX-2/COX-1 selectivity ratio: 921.429), 9 (COX-2/COX-1 selectivity ratio: 835.294), 10 (COX-2/COX-1 selectivity ratio: 835.000), 2 (COX-2/COX-1 selectivity ratio: 890.476), 6 (COX-2/COX-1 selectivity ratio: 792.000), 1 (COX-2/COX-1 selectivity ratio: 748.148), 5 (COX-2/COX-1 selectivity ratio: 731.034), 4 (COX-2/COX-1 selectivity ratio: 740.000) and 3 (COX-2/COX-1 selectivity ratio: 825.806). Compounds 8, 7, 9, 10, 2, 6, 1 and 5 were more active than celecoxi compound 4 equally active to celecoxib while, compound 3 less active than celecoxib (COX-2/COX-1 selectivity ratio: 333.333).

Table 3: *In vitro* human COX-2a and COX-1b enzymes inhibitory activities of compounds

Test compounds	COX-2 IC_{50}^{c} (μmol)	COX-1 IC_{50}^{c} (μmol)	Approximate selectivity ratio COX-2/COX-1
Indomethacin	2.63±0.0005	0.26±0.0001	0.099
Celecoxib	0.30±0.0004	100.00±2.43	333.333
1	0.27±0.0006	202.00±2.58	748.148
2	0.21±0.0004	187.00±2.39	890.476
3	0.31±0.0007	256.00±3.45	825.806
4	0.30±0.0008	222.00±2.36	740.000
5	0.29±0.0009	212.00±3.67	731.034
6	0.25±0.0005	198.00±1.40	792.000
7	0.14±0.0002	129.00±4.63	921.429
8	0.12±0.0001	127.00±2.44	1058.333
9	0.17±0.0003	142.00±2.57	835.294
10	0.20±0.0005	167.00±3.48	835.000

Values were calculated from the mean values of data from three separate experiments and presented as Mean±SEM. All results are significant different from control values at $p \leq 0.005$. All results are significant different from reference standard values at $p \leq 0.005$

Table 4: Effect of compounds on human, dog and rat microsomal COX activities

	IC_{50} nM		
Compounds	Human	Dog	Rat
Celecoxib	89	112	132
1	40	65	88
2	29	48	59
3	78	90	156
4	65	87	134
5	51	77	96
6	33	53	71
7	19	25	36
8	10	21	32
9	21	32	39
10	24	37	43

Values were calculated from the mean values of data from three separate experiments and presented as Mean±SEM. All results are significant different from control values at $p \leq 0.005$. All results are significant different from reference standard values at $p \leq 0.005$

Table 5: Acute toxicity of compounds (1-10)

Test compounds	LD_{50} (mg kg^{-1})
1	3265.764
2	2531.532
3	2341.211
4	4576.798
5	3421.643
6	2438.543
7	4576.453
8	4323.000
9	2341.456
10	5342.678

Values were calculated from the mean values of data from three separate experiments and presented as Mean±SEM. All results are significant different from control values at $p \leq 0.005$

Effect of compounds on human, dog and rat microsomal COX activities: In human, dog and rat kidney microsome preparations (COX-1), celecoxib was substantially less potent than all the tested compounds and the order was as follow 8, 7, 9, 10, 2, 6, 1, 5, 43 and celecoxib (Table 4).

Acute toxicity: All tested compounds showed high LD_{50} mainly above 2 g kg^{-1}. These high LD_{50} ensure high sfety and go theraputic windows (Table 5).

CONCLUSION

Carfull examination of the relation between chemicall structure and apharmacological activities cualminated on the following assumptions.

Structure activity relationship;

- Cyclheptenes fused to heterocyclinc ring (compounds 8 and 7) system essential for higher antiinflammatory activities and greatest COX-2b selective inhibition
- Cyclhexenes fused to heterocyclinc ring (compounds 9 and 10) system were less active than cyclheptene ones
- Polycyclic fused ring systems showed moderate antiinflammatory activities with greatest COX-2b selective inhibition
- Chlorine atoms and small size molecules showed lower antiinflammatory

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