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Synthesis and anti-inflammatory activity of sulfonamides and carboxylates incorporating trimellitimides: Dual cyclooxygenase/carbonic anhydrase inhibitory actions

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ABSTRACT

Trimellitimides **6–21** were prepared and investigated *in vivo* for anti-inflammatory and ulcerogenic effects and *in vitro* for cytotoxicity. They were subjected to *in vitro* cyclooxygenase (COX-1/2) and carbonic anhydrase inhibition protocols. Compounds **6–11** and **18** exhibited anti-inflammatory activities and had median effective doses (ED_{50}) of 34.3–49.8 mg kg^{-1} and 63.6–86.6% edema inhibition relative to the reference drug celecoxib (ED_{50}: 33.9 mg kg^{-1} and 85.2% edema inhibition). Compounds **6–11** and **18** were weakly cytotoxic at 10 μM against 59 cell lines compared with the reference standard 5-fluorouracil (5-FU). Compounds **6–11** had optimal selectivity against COX-2. The selectivity index (SI) range was > 200–490 and was comparable to that for celecoxib [COX-2 (SI) > 416.7]. In contrast, compounds **12**, **13**, and **16–18** were nonselective COX inhibitors with a selectivity index range of 0.92–0.25. The carbonic anhydrase inhibition assay showed that sulfonamide incorporating trimellitimides **6–11** inhibited the cytosolic isoforms hCA I and hCA II, and tumor-associated isoform hCA IX. They were relatively more susceptible to inhibition by compounds **8**, **9**, and **11**. The K_I ranges were 54.1–81.9 nM for hCA I, 25.9–55.1 nM for hCA II, and 46.0–348.3 nM for hCA IX.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are extensively used in the treatment of inflammatory disorders [1]. NSAIDs inhibit cyclooxygenase (COX-1/COX-2) isozymes, which convert arachidonic acid to prostaglandins (inflammatory eicosanoids) [1–3]. The COX-1 isozyme maintains gastric and renal integrity. Its inhibition accounts for the stomach and kidney side effects of NSAIDs [1b,2b,2c]. Cyclooxygenase (COX-2) isozyme produces the inflammatory mediator prostaglandin. Its inhibition is responsible for the therapeutic effects of NSAIDs [1b,2a-b,2d-e]. A major challenge in the discovery and development of pharmaceuticals is to develop a drug with anti-inflammatory properties but without the toxic side effects associated with the currently used NSAIDs [1a,4,5].

COX-2 isozyme is overexpressed in human gastric, hepatocellular, breast, colon, lung, ovarian, and prostate cancers [6–10]. COX-2 inhibitors may diminish the incidence and risk of these cancers. Therefore, COX-2 isozyme is a potential cancer therapy target [6,8,9]. For example, celecoxib as COX-2 inhibitor (Fig. 1; **A**) has been investigated for its anti-inflammatory and antitumor activities [7,9,11].

Carbonic anhydrases (CA) are zinc metalloenzymes (ZBGs) present in almost all living organisms [12–14]. There are fifteen CA isoforms. Their major function is to catalyze the reversible hydration of carbon dioxide to bicarbonate and protons. This process is involved in several crucial pathophysiological pathways [12–14] like glaucoma [15], epilepsy [16], obesity [17], and cancers [18]. For this reason, it is challenging to find isoform-selective inhibitors. However, it has been reported that various classes of inhibitors have these properties [19].

Certain CA inhibitors like celecoxib with antitumor or anti-inflammatory effects have dual COX-2 and hCA II inhibitory activity [18,20–27]. Numerous studies on novel multitarget, polyfunctional

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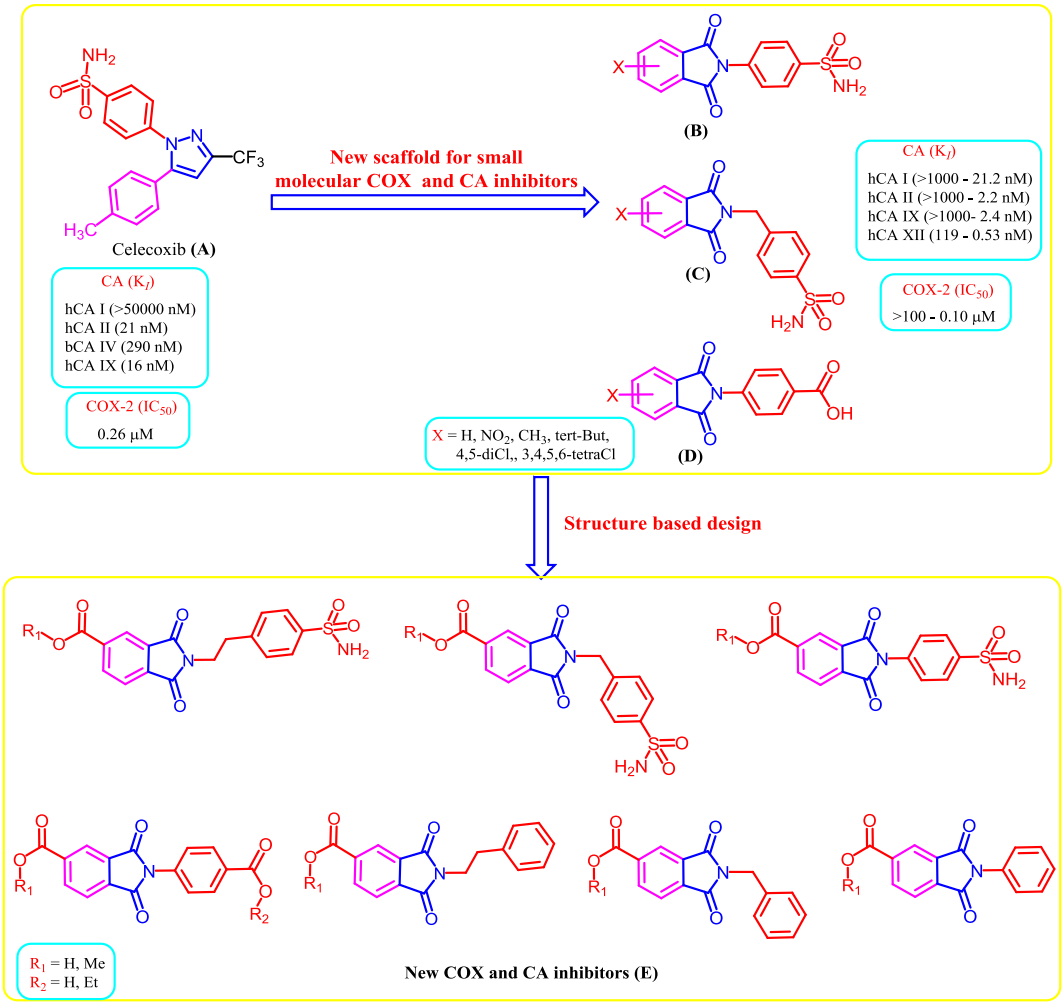


Fig. 1. Reported examples of selective COX-2 inhibitors and CA inhibitors (**A–D**) and the designed trimellitimides (**E**).

medications reported that they were used to treat bacterial infections, cancers, inflammation, and neurological disorders [19b,20–22,24,26,28–30]. Hence, the synthesis of new dual COX and CA inhibitors is a priority in drug discovery (Fig. 1). Sulfonamides and carboxylates are versatile inhibitors with multiple mechanisms (Fig. 1; celecoxib **A**, **B**, **C**, and **D**) [31–33].

In addition, unique derivatives of cyclic imides incorporating sulfonamides, carboxylic acids, and halides have received considerable attention because they presented with anti-inflammatory, antitumor, and antihyperlipidemic properties [34–36]. We recently reported the synthesis and activity of *N*-substituted cyclic imides as potent carbonic anhydrase and COX-2 inhibitors (Fig. 1; **B**, **C**, and **D**) [19b–19c,33a–33c,34b,37–42].

Here, we report the synthesis of trimellitimide derivatives with sulfamoyl or carboxylate moieties. We studied the *in vivo* anti-inflammatory and ulcerogenic activities and *in vitro* cytotoxicity of these zinc metalloenzymes. Moreover, these compounds were tested against COX-1/2 and the CA isoforms cytosolic hCA I and II, membrane-bound hCA IV, and tumor-associated hCA IX (Fig. 1; **E**).

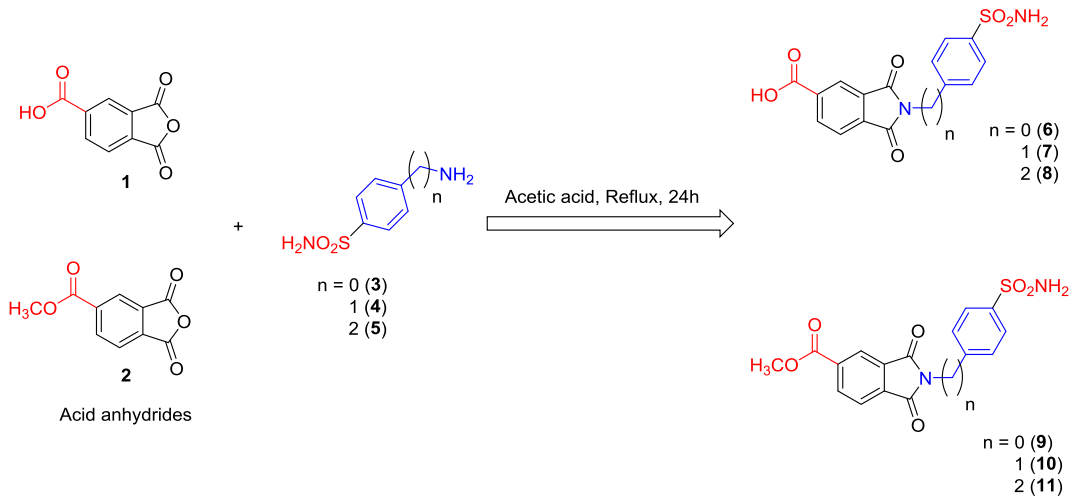
These cyclic imides (Fig. 1; **E**) were designed and evaluated as COX-1/2 and CA inhibitors in order to: (i) study their *in vivo* anti-inflammatory and ulcerogenic effects and *in vitro* cytotoxicity; (ii) elucidate their molecular mechanisms using *in vitro* COX-1/2 and carbonic anhydrase (CA) inhibition assays; (iii) describe the COX-1/COX-2 inhibition structure–activity relationships (SAR) with molecules incorporating variously substituted trimellitimides; and (iv) compare the efficacies of 4-aminoethylbenzenesulfonamide and 4-aminomethylbenzenesulfonamide with those of benzenesulfonamide and benzoic acid in terms of inhibition of COX-1/2 and the carbonic anhydrase isoforms CA I, II, IV, and IX in compounds incorporating the same scaffold (i.e., trimellitic acid and its methyl ester).

2. Results and discussion

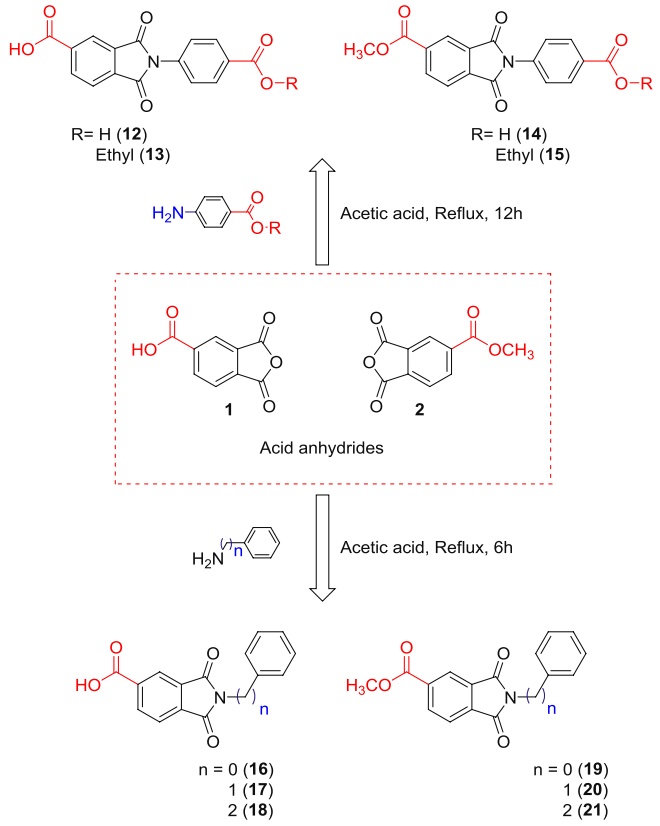
2.1. Chemistry

The lead molecules used to design and synthesize the COX-2 inhibitors and CAIs were sulfanilamides incorporating different imide moieties [33a,33b,37–42]. Here, we prepared compounds incorporating carboxylate and sulfonamide moieties. The syntheses of trimellitimide derivatives incorporating both sulfamoyl and carboxylate **6–11** are depicted in Scheme 1. Trimellitic acid anhydride **1** or **2** was heated with 4-aminobenzenesulfonamide (**3**), 4-(aminomethyl)benzenesulfonamide (**4**), or 4-(2-aminoethyl)benzenesulfonamide (**5**) in acetic acid in the presence of anhydrous sodium acetate [33a,33b,34–41]. The imides **6–11** were generated in reasonable yields (Scheme 1).

Compounds **12–21** had carboxylic acid and methyl carboxylate moieties and were synthesized with aromatic amines like 4-aminobenzoic acid, ethyl 4-aminobenzoate, aniline, benzylamine, and phenethylamine and the trimellitic acid anhydrides **1** and **2** to give the corresponding acid imides **12–21** (Scheme 2) [43,44]. The structures of the isolated products **6–11** and **12–21** were verified from their spectral analyses.



Scheme 1. Synthesis of trimellitimides incorporating *N*-benzenesulfonamides **6–11**.



Scheme 2. Synthesis of trimellitimides incorporating aromatic amines **12–21**.

2.2. Biological activity

2.2.1. In vivo anti-inflammatory studies

Compounds **6–21** and the reference drug celecoxib were subjected to *in vivo* anti-inflammatory activity screening with a conventional rat carrageenan-induced foot paw edema model. The percent edema inhibition was measured after 2 h of carrageenan treatment (Table 1) at which time the maximum inhibition of carrageenan-induced edema was attained [5,45].

Most of the tested compounds reduced edema by 20.8–86.6% (Table 1). A major exception was compound **15,** which was relatively latent and mitigated edema by only 10.5%. The carboxylic acid derivatives were generally more active than their corresponding methyl ester derivatives. For example, compounds **6**, **7,** and **8** showed the highest anti-inflammatory activities with 79.0%, 83.2%, and 86.6% edema reduction, respectively. These efficacies were comparable to that for celecoxib (85.2% edema inhibition). Conversion of the carboxylic acids **6**, **7,** and **8** into their corresponding methyl esters **9**, **10,** and **11** slightly decreased their anti-inflammatory activity levels (66.3%, 70.9%, and 75.3% edema inhibition, respectively). The dual carboxylic acid compound **12** showed higher anti-inflammatory activity than its ester analogs **13–15** (50.8%, 29.4%, 38.6%, and 10.5% edema inhibition, respectively). Compound **18**, which incorporates one carboxylic moiety at the 5-position of the trimellitimide core, presented with 63.6% edema inhibition. In contrast, its ester derivative (compound **21**) provided only 22.6% edema inhibition. The trimellitimides incorporating sulfonamide fragments (compounds **6–11**) had the greatest anti-inflammatory activity levels among all tested products. Compounds **7** and **8** were the most potently anti-inflammatory (83.2% and 86.6% edema reduction, respectively). These products had similar efficacies to celecoxib (85.2% edema reduction). The trimellitimide-like compound **18** incorporating a phenethyl moiety showed higher anti-inflammatory activity (63.6% edema inhibition) than their corresponding analogs compound **16** (31.0% edema reduction) and compound **17** (35.3% edema reduction). Isoindole-1,3-diones containing a sulfonamide moiety like compounds **6** and **9** had stronger anti-inflammatories potency than those with carboxylic or ester fragments at the same positions (compounds **12, 13,** and **14**). These results indicate that the sulfonamide moiety is strongly influential at the COX receptor binding site. The anti-inflammatory activities of compounds **6**, **7**, **8**, **10**, **11**, **18**, and celecoxib were studied using three graded doses as shown in Table 2.

2.2.2. Ulcerogenicity

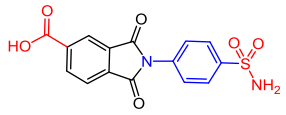
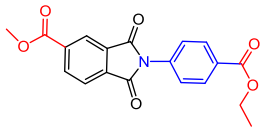
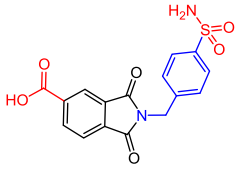
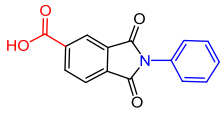
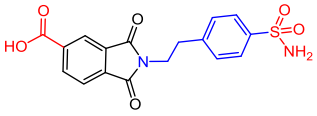
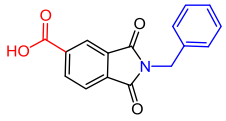
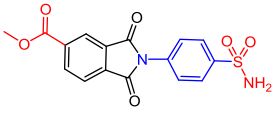
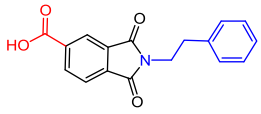
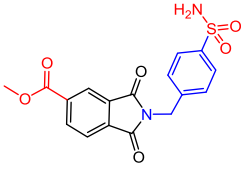
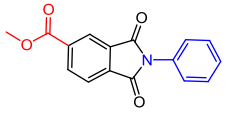
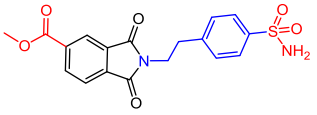
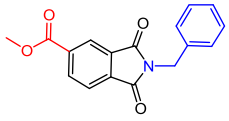
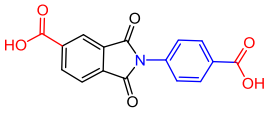
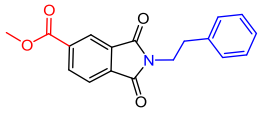
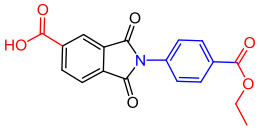
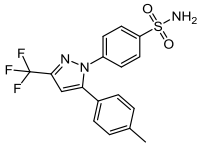
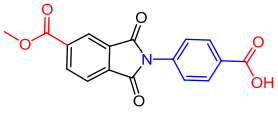
The ulcerogenic activity levels of compounds **6**, **7**, **8**, **10**, **11**, **18**, celecoxib, and diclofenac were determined by a previously reported method [5,46]. Compounds **6**, **7**, and **8** were as ulcerogenic as celecoxib but less ulcerogenic than diclofenac. On the other hand, compounds **10** and **11** showed negligible ulcerogenic activity relative to compound **18,** which had the highest ulcerogenic effect of all tested compounds (Table 3).

2.2.3. Cytotoxicity

In vitro cytotoxicity evaluations of certain compounds are shown in Table 4. These substances were selected by the National Cancer Institute (Bethesda, MD, USA) on the basis of the structural variations.

Compounds **6–11** and **18** were used in single doses of 10 μM in a full NCI 59 cell line panel assay. The source tissues were the tumor subpanels leukemia, non-small cell lung, colon, CNS, melanoma, ovarian,

Table 1
Results of anti-inflammatory activity of the tested compounds against carrageenan induced rat paw edema in rats.

Comp No.		Mean %[a] increase in paw weight ±SEM[b]	% Inhibition of paw edema from control group	Comp No.		Mean %[a] increase in paw weight ±SEM[b]	% Inhibition of paw edema from control group
6		38.7±0.5	79.0±0.7	**15**		152.3±1.1	10.5±0.2
7		30.9±0.4	83.2±0.6	**16**		127.3±0.9	31.0±0.1
8		26.6±0.3	86.6±0.8	**17**		119.3±1.0	35.3±0.4
9		62.1±0.6	66.3±0.4	**18**		67.1±0.5	63.6±0.5
10		53.6±0.3	70.9±0.4	**19**		146.1±1.2	20.8±0.1
11		45.5±0.7	75.3±0.6	**20**		134.5±1.1	27.1±0.3
12		90.7±0.6	50.8±0.3	**21**		142.7±1.3	22.6±0.1
13		130.2±1.4	29.4±0.1	**Celecoxib**		27.3±0.4	85.2±0.3
14		113.3±1.1	38.6±0.3				

[a]Result of control group (%): 166.5 ± 1.5.
[b]Significant difference from control and celecoxib-treated group using unpaired Student's "t" test $p < 0.05$.

renal, prostate, and breast cancers [28,35b,47]. Table 4 presents the % tumor cell growth inhibition (GI %) caused by the test compounds. The positive cytotoxic effects (PCE) of compounds **6–11** and **18** at 10 μM were 4/59, 1/59, 3/59, 7/59, 3/59, 4/55, and 2/55, respectively, while 5-fluorouracil (5-FU) had a PCE of 55/59 (Table 4).

2.2.4. COX-1/2 inhibition

The molecular mechanisms of compounds **6–21** were evaluated in terms of COX-1 and COX-2 inhibition using an ovine COX-1/COX-2 assay kit (Catalog No. 560101, Cayman Chemicals Inc., Ann Arbor, MI, USA). The IC_{50} (μM) and the selectivity indices (SI; IC_{50} (COX-1)/IC_{50} (COX-2)) are listed in Table 5 [3,34,48]. The COX-1/COX-2 assay showed that the IC_{50} values of celecoxib for COX-1 and COX-2 were > 50 μM and 0.12 μM, respectively, and the SI of COX-2 was > 416.7. Table 5 shows that molecules incorporating a benzenesulfonamide fragment like compounds **6–11** are extremely potent COX-2 inhibitors ($IC_{50} \cong 0.10–0.25$ μM and SI ≅ 490 − > 200). This efficacy is comparable to that for their COX-1 inhibition ($IC_{50} \cong 37.2–49.0$ μM).

Table 2
Results of anti-inflammatory activity of compounds **6**, **7**, **8**, **11**, **18**, and celecoxib against carrageenan induced rat paw edema in rats at three graded doses.

Compound No.	Dose (mg/kg)	% Inhibition of paw edema from control group	ED_{50} (mg/kg)
6	40	55.1	40.1
	59	79.0	
	80	85.0	
7	40	61.2	35.4
	61	83.2	
	80	89.3	
8	40	65.0	34.3
	63	86.6	
	80	93.5	
10	40	49.1	40.4
	66	75.3	
	80	82.0	
11	40	45.0	45.1
	50	70.9	
	80	79.0	
18	40	35.2	49.8
	68	63.6	
	80	71.0	
Celecoxib	40	68.6	33.9
	65	85.2	
	80	91.9	

Table 3
Ulcerogenic potential of the tested compounds **6**, **7**, **8**, **11**, **18**, and celecoxib in mice.*

Compound No.	Average number of ulcers	Mean sum of lengths of elongated ulcer (mm ± SEM)
Control	0.0	0.0
Celecoxib	5	5.8 ± 0.72
Diclofenac	10	7.7 ± 0.88
6	8	7.2 ± 0.87
7	6	6.1 ± 0.82
8	5	5.9 ± 0.63
10	4	3.7 ± 0.25
11	3	1.4 ± 0.18
18	11	8.1 ± 0.91

* Significantly less than the celecoxib and diclofenac sodium treated group using unpaired Student's "t" test $p < 0.05$.

On the other hand, compounds **12–21**, which lack any sulfonamide moiety, are nonselective COX-1/2 inhibitors and their SI range is 0.25–1.11.

The IC_{50} values of the most potent inhibitors, compounds **6**, **7**, **8**, **9**, **10,** and **11** were 0.14 μM, 0.13 μM, 0.10 μM, 0.25 μM, 0.25 μM, and 0.2 μM, respectively. Their SIs were 265.7, 346.9, 200, 200, 490.0, and 250.0, respectively. These values are comparable to those for celecoxib (IC_{50} = 0.12 μM; COX-2 (SI) > 416.7). Trimellitimides with both sulfonamide and carboxylic groups like compounds **6–8** (COX-2 (SI) 265.7–490.0) are more potent than their corresponding ester derivatives like compounds **9–11** (COX-2 (SI) > 200.0 − > 250.0). These molecules may interact differently with the COX-2 binding pocket. The presence of two carboxylic groups (compound **12**) results in non-selective inhibition with relatively higher COX-1 inhibitory activity (IC_{50} = 11.6 μM) and lower COX-2 inhibitory activity (IC_{50} = 30.4 μM). Introduction of two ester groups (compound **15)** diminished COX-1/2 inhibition ($IC_{50} \geq 50$ μM). Compound **18** has one carboxylic group and the *N*-phenethyl substituent. It had very low COX-2 inhibition (IC_{50} = 40.1 μM) and very high COX-1 inhibition (IC_{50} = 9.9 μM) relative to its analogs **8** and **11** ((SI) > 490.0 and > 250.0, respectively). Therefore, the sulfonamide fragment is essential for binding with the COX-2 receptor pocket. Compounds **14**, **19**, **20**, and **21** were the weakest COX-1/2 inhibitors (SI = 1.02 > 1.11).

The structure–activity relationships (SAR) for COX-1/2 inhibition with compounds **6–21** (Table 5) indicated the following. (i) COX-2 may have been inhibited by **6–11**. Their IC_{50} (μM) range was 0.10–0.25, which is comparable to that for celecoxib

Table 5
In vitro cyclooxygenase (COX-1/COX-2) enzyme inhibition assay and calculated selectivity indices.

Compound No.	IC_{50} (μM)[a]		SI[b]
	COX-1	COX-2	
6	37.2	0.14	265.7
7	45.1	0.13	346.9
8	49.0	0.10	490.0
9	> 50	0.25	> 200.0
10	> 50	0.25	> 200.0
11	> 50	0.20	> 250.0
12	11.6	30.4	0.38
13	30.0	32.5	0.92
14	> 50	48.8	1.02
15	> 50	> 50	> 1.0
16	35.9	44.0	0.82
17	35.9	44.0	0.82
18	9.9	40.1	0.25
19	> 50	47.9	> 1.04
20	> 50	47.9	> 1.04
21	> 50	45.0	> 1.11
Celecoxib	> 50	0.12	> 416.7

[a] IC_{50} value is the compound concentration required to produce 50% inhibition of COX-1or COX-2 for means of two determinations using an ovine COX-1/COX-2 assay kit (catalog no. 560101, Cayman Chemicals Inc., Ann Arbor, MI, USA) and deviation from the mean is < 10% of the mean value.
[b] Selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

Table 4
Antitumor activity of trimellitimides derivatives **6–11** and **18** presented as growth inhibition percentages (GI %) over 59 subpanel tumor cell lines.

Compound No.	59 cancer cell lines assay in one dose 10.0 μM concentration: GI%	
	PCE*	Most sensitive cell lines*
6	4/59	***Leukemia*** (HL-60(TB): 14.0%), ***NSC Lung Cancer*** (NCI-H522: 15.0%), ***Renal Cancer*** (TK-10: 11%, UO-31: 15%).
7	1/59	***Renal Cancer*** (UO-31: 16%).
8	3/59	***Leukemia*** (HL-60(TB): 11.0%), ***NSC Lung Cancer*** (HOP-92: 17.0%), ***Renal Cancer*** (UO-31: 15%).
9	7/59	***Leukemia*** (HL-60(TB): 11.0%), ***NSC Lung Cancer*** (HOP-92: 12%, NCI-H522: 16%), ***Colon Cancer*** (HT29: 11%), ***CNS Cancer*** (SNB-75: 11%), ***Renal Cancer*** (TK-10: 16%, UO-31: 16%).
10	3/59	***NSC Lung Cancer*** (A549/ATCC: 11%, NCI-H522: 11%), ***Renal Cancer*** (UO-31: 12%).
11	4/59	***Leukemia*** (SR: 11%), ***Colon Cancer*** (HCC-2998: 11%), ***Renal Cancer*** (UO-31: 17%), ***Breast Cancer*** (MDA-MB-468: 11%).
18	2/59	***NSC Lung Cancer*** (NCI-H522: 11%), ***Renal Cancer*** (UO-31: 13%).

PCE: Positive cytotoxic effect which is ratio between number of cell lines with percentage growth inhibition > 10% and total number of cell lines.
* PCE of standard drug 5-FU = 55/59 and GI% range of 17–100%.

(IC_{50} = 0.12 μM). (ii) Both the 5-carboxylic acid and the 5-methyl ester moieties of the isoindole-1,3-dione derivatives incorporating benzenesulfonamide (compounds **6–11)** are potentially effective COX-2 inhibitors but the non-sulfonamide derivatives **12–21** are not. Therefore, the sulfonamide fragment (SO_2NH_2) is necessary for COX-2 inhibition. (iii) The trimellitimide with a 4-(2-aminoethyl)benzenesulfonamide moiety (compound **8**; IC_{50} = 0.1 μM) had the strongest COX-2 inhibition activity. On the other hand, the derivatives with 4-(aminomethyl)benzenesulfonamide and 4-aminobenzenesulfonamide moieties (compounds **7** (IC_{50} = 0.13 μM) and **6** (IC_{50} = 0.14 μM), respectively) demonstrated the importance of the spacer group for COX-2 inhibition. (iv) Replacement of the sulfonamide fragments (-SO_2NH_2) in compounds **6**, **7**, and **8** (IC_{50} = 0.14 μM, 0.13 μM, and 0.10 μM, respectively) with hydrogen atoms in compounds **16**, **17**, and **18** increased COX-1 inhibition (IC_{50} = 35.9 μM, 35.9 μM, and 9.9 μM, respectively) and decreased COX-2 inhibition (IC_{50} = 40.1–44.0 μM). (v) Conversion of the sulfonamide fragment (-SO_2NH_2) into a carboxylic acid (–COOH) or ethyl carboxylate (–$COOCH_2CH_3$) moiety incorporating the same 5-carboxylphthalimide scaffold (compounds **12** and **13**) increased COX-1 inhibition (IC_{50} = 10.0 μM and 30.0 μM, respectively) and decreased COX-2 inhibition (IC_{50} = 30.4 μM and 32.5 μM, respectively). (vi) Other non-sulfonamide and non-carboxylic trimellitimides had significantly different activity levels. Compounds **19**, **20**, and **21** were much weaker inhibitors than compounds **6–11**. In brief, the most potent trimellitimide scaffolds were the 5-carboxyphthalimides and 5-methylcarboxyphthalimides **6–11**. They had higher selective COX-2 inhibition levels (SI ≥ 200–490.0) than celecoxib (SI ≥ 416.7).

2.2.5. Carbonic anhydrase inhibition

Compounds **6–21** and the reference standard acetazolamide (**AAZ**) were screened for their CA inhibitory activity by a stopped-flow CO_2 hydrase assay [49]. The isoforms hCA I and II (cytosolic), hCA IV, and the tumor-associated hCA IX were tested. The results are shown in Table 6.

The following structure–activity relationship (SAR) was inferred from the inhibition data shown in Table 6.

(i) The slow cytosolic isoform hCA I was inhibited by a medium–high nanomolar range (K_I = 54.1–276.5 nM) of the sulfonamides. The linker between the trimellitimide scaffold and the sulfonamide in **6–11** modulated the inhibition profile. The methylene chain in compound **7** did not significantly affect the inhibition profile. However, further chain elongation as in compound **8** doubled K_I. For compounds **9–11,** the length of the methylene linker was critical because one carbon atom in the spacer reduced inhibition efficacy by five times. Compounds **12–21** did not inhibit this isoform.

(ii) The dominant physiological isoform hCA II was inhibited by compounds **6–11** in the medium nanomolar range (K_I = 25.9–82.9 nM). The methylene linker was not essential for inhibition profile modulation. Esterification of the carboxylic groups in compounds **9–11** nearly doubled (compounds **10** and **11)** and tripled (compound **9)** inhibition efficacy against this isoform. Compounds **12–21** did not inhibit hCA II.

(iii) The membrane-bound hCA IV was inhibited by compounds **6–11** in the high nanomolar to micromolar range (K_I = 316.6 nM-7.0 μM). Compounds **9–11** with trimellitimide carboxylic ester scaffolds showed better inhibition profiles than compounds **6–8** with trimellitimide carboxylic acid scaffolds. The methylene linker for this isoform is critical in determining inhibition potency. One carbon atom in the spacer between the trimellitimide scaffold and the sulfonamide resulted in the highest inhibition efficacy. The hCA IV isoform was not inhibited by compounds **12–21**.

(iv) The second membrane-bound, tumor-associated isoform, hCA IX, was inhibited in the high nanomolar to micromolar range by all compounds with sulfonamide groups (**6–11**) except **9**, which inhibited it in the medium nanomolar range (K_I = 46.0 nM). Unlike hCA IV, the introduction of a linker in compounds **6–11** had various effects on the hCA IX inhibition profile. Inhibition potency decreased with a one-carbon methylene linker (compound **7**, K_I = 2,857.7 nM; compound **10,** K_I = 1,271.3 nM). In contrast, further chain elongation as in compounds **8** and **11** increased inhibition potency (K_I = 348.3 nM (**8**); K_I = 185.6 nM (**11**)). Compounds **12–21** did not inhibit this isoform.

Table 6
In vitro CA I, II, IV and IX inhibition with compounds **6–21** and acetazolamide (AAZ) as standard, by a stopped-flow, CO_2 hydrase assay [49].

Compound No.	K_I (nM)*			
	hCA I	hCA II	hCA IV	hCA IX
6	175.8	82.9	1176.1	1161.2
7	178.5	58.0	340.4	2857.7
8	81.9	55.1	7006.9	348.3
9	65.8	25.9	536.1	46.0
10	276.5	35.8	316.6	1471.3
11	54.1	26.9	3206.2	185.6
12	> 10000	> 10000	> 10000	> 10000
13	> 10000	> 10000	> 10000	> 10000
14	> 10000	> 10000	> 10000	> 10000
15	> 10000	> 10000	> 10000	> 10000
16	> 10000	> 10000	> 10000	> 10000
17	> 10000	> 10000	> 10000	> 10000
18	> 10000	> 10000	> 10000	> 10000
19	> 10000	> 10000	> 10000	> 10000
20	> 10000	> 10000	> 10000	> 10000
21	> 10000	> 10000	> 10000	> 10000
AAZ	250	12.1	74	25.2

* Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5–10% of the reported values).

3. Conclusion

The trimellitimide compounds **6–21** were synthesized and screened for anti-inflammatory activity and cytotoxicity. They were also evaluated for the inhibition of COX-1/COX-2 and the carbonic anhydrase isoforms hCA I, hCA II, hCA IV, and hCA IX. The results showed that benzenesulfonamide derivatives were stronger selective COX-2 inhibitors than the non-sulfonamide products incorporating the same scaffolds. Compounds **6–11** were the most potent COX-2 inhibitors (IC_{50} = 0.14 μM, 0.13 μM, 0.10 μM, 0.25 μM, 0.25 μM, and 0.20 μM) and were comparable to celecoxib (IC_{50} = 0.12 μM). Only trimellitimides bearing sulfonamide moieties inhibited the cytosolic isoforms hCA I and hCA II. Therefore, these are candidates for preclinical evaluation in the treatment of glaucoma and other eye diseases involving these enzymes. Moreover, the tumor-associated hCA IX isoform was susceptible to inhibition by compounds **8**, **9** and **11** whose K_I were in the range of 46.0–348.3 nM.

4. Experimental

4.1. Chemistry

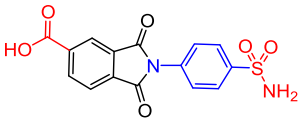
Melting points (uncorrected) were recorded on a Barnstead 9100 electrothermal melting apparatus (APS Water Services Corporation, Van Nuys, CA, USA). IR spectra were recorded on a FT-IR Perkin-Elmer spectrometer (PerkinElmer Inc., Waltham, MA, USA). The ^{1}H NMR and ^{13}C NMR were measured in DMSO-d_6 and $CDCl_3$, respectively, on Bruker 500 and 125 MHz instruments, respectively (Bruker, Billerica, MA, USA), using TMS as an internal standard. Chemical shifts were

reported in δ ppm. Mass spectra were recorded on an Agilent 6320 Ion Trap mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). C, H, and N were analyzed at the Research Centre of College of Pharmacy, King Saud University, Saudi Arabia. The results were within ± 0.4% of the theoretical values. Compounds **12**, **13**, **16**, **17**, and **18** were prepared according to a previously reported procedure [43,44,50].

*4.1.1. General procedure for the synthesis of trimellitimides **6–8** (Scheme 1)*

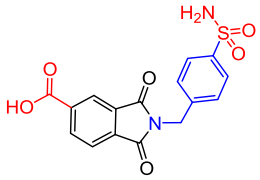
Benzenesulfonamide (5.0 mmol), anhydrous sodium acetate (0.69 g, 5.0 mmol), and the appropriate acid anhydrides (5.0 mmol) in glacial acetic acid (15 mL) were heated with refluxing for 24 h. After cooling the reaction mixture, the precipitate was filtered, washed with water, dried, and re-crystallized using the appropriate solvents.

*4.1.1.1. 1,3-Dioxo-2-(4-sulfamoylphenyl)isoindoline-5-carboxylic acid (**6**).*



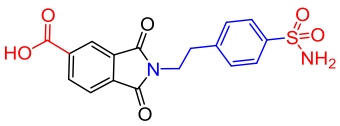
M.P. > 300 °C, 87% yield (CH_3OH); IR (KBr, cm^{-1}) ν: 3400 (OH), 1775, 1730 (C═O), 1300, 1210 (O═S═O); ^1H NMR (500 MHz, DMSO-d_6): δ 7.50 (s, 2H), 7.69–7.71 (d, 2H, *J* = 8.5 Hz), 7.99–8.01 (d, 2H, *J* = 8.5 Hz), 8.07–8.08 (d, 1H, *J* = 8.0 Hz), 8.32 (s, 1H), 8.42–8.43 (d, 1H, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 123.98, 124.30, 126.86, 127.97, 132.33, 134.92, 135.10, 136.02, 138.11, 143.85, 166.44, 166.47; $C_{15}H_{10}N_2O_6S$: *m/z* (346).

*4.1.1.2. 1,3-Dioxo-2-(4-sulfamoylbenzyl)isoindoline-5-carboxylic acid (**7**).*



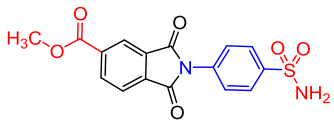
M.P. > 300 °C, 83% yield (CH_3CH_2OH); IR (KBr, cm^{-1}) ν: 3451 (OH), 1784, 1711 (C═O), 1360, 1190 (O═S═O); ^1H NMR (500 MHz, DMSO-d_6): δ 4.88 (s, 2H), 7.35 (s, 2H), 7.52–7.54 (d, 2H, *J* = 8.0 Hz), 7.78–7.79 (d, 2H, *J* = 8.5 Hz), 8.01–8.03 (d, 1H, *J* = 8.0 Hz), 8.25 (s, 1H), 8.37–8.39 (d, 1H, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 41.20, 123.72, 124.14, 126.42, 128.30, 132.57, 135.42, 135.87, 136.82, 140.73, 143.68, 166.28, 167.41, 167.44; $C_{16}H_{12}N_2O_6S$: *m/z* (3 6 0).

*4.1.1.3. 1,3-Dioxo-2-(4-sulfamoylphenethyl)isoindoline-5-carboxylic acid (**8**).*



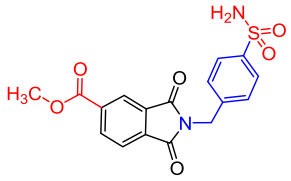
M.P. > 300 °C, 89% yield (CH_3OH); IR (KBr, cm^{-1}) ν: 3398 (OH), 1778, 1718 (C═O), 1330, 1210 (O═S═O); ^1H NMR (500 MHz, $CDCl_3$/DMSO-d_6): δ 3.00–3.03 (t, 2H, *J* = 7.5 Hz), 3.84–3.87 (t, 2H, *J* = 7.5 Hz), 7.23 (s, 2H), 7.36–7.37 (d, 2H, *J* = 8.0 Hz), 7.72–7.75 (t, 3H, *J* = 8.0 Hz), 8.26 (s, 1H), 8.30–8.32 (d, 1H, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$/DMSO-d_6): δ 34.07, 38.83, 122.68, 123.86, 126.26, 129.42, 131.54, 132.41, 135.21, 142.70, 142.86, 167.92, 168.07; $C_{17}H_{14}N_2O_6S$: *m/z* (374).

*4.1.1.4. Methyl 1,3-dioxo-2-(4-sulfamoylphenyl)isoindoline-5-carboxylate (**9**).*



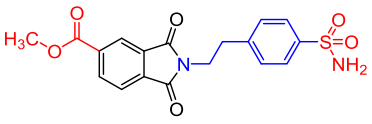
M.P. 283–285 °C, 76% yield (CH_3OH/CH_2Cl_2); IR (KBr, cm^{-1}) ν: 3180 (NH), 1776, 1711 (C═O), 1333, 1199 (O═S═O); ^1H NMR (500 MHz, $CDCl_3$/DMSO-d_6): δ 3.95 (s, 3H), 7.62–7.63 (d, 2H, *J* = 8.5 Hz), 7.70 (s, 1H), 7.98–8.00 (d, 2H, *J* = 8.5 Hz), 8.05–8.06 (d, 2H, *J* = 6.5 Hz), 8.39 (s, 1H), 8.43–8.45 (d, 1H, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 53.03, 118.81, 124.29, 126.91, 127.21, 132.14, 134.74, 135.18, 135.88, 135.97, 143.81, 164.95, 165.94, 169.21; $C_{16}H_{12}N_2O_6S$: *m/z* (360).

*4.1.1.5. Methyl 1,3-dioxo-2-(4-sulfamoylbenzyl)isoindoline-5-carboxylate (**10**).*



M.P. 216–218 °C, 79% yield (CH_3OH); IR (KBr, cm^{-1}) ν: 3231 (NH), 1790, 1736 (C═O), 1309, 1119 (O═S═O); ^1H NMR (500 MHz, DMSO-d_6): δ 3.92 (s, 3H), 4.86 (s, 2H), 7.21 (s, 2H), 7.46–7.47 (d, 2H, *J* = 8.0 Hz), 7.78–7.80 (d, 2H, *J* = 8.0 Hz), 7.96–7.98 (dd, 1H, *J* = 8.0, 6.0 Hz), 8.30 (s, 1H), 8.36–8.38 (dd, 1H, *J* = 7.5, 6.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 41.23, 53.06, 123.94, 123.96, 126.47, 128.34, 132.37, 135.44, 135.57, 135.69, 140.24, 143.84, 164.99, 166.96; $C_{17}H_{14}N_2O_6S$: *m/z* (374).

*4.1.1.6. Methyl 1,3-dioxo-2-(4-sulfamoylphenethyl)isoindoline-5-carboxylate (**11**).*

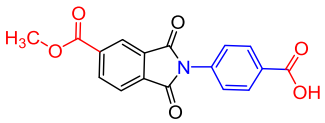


M.P. 182–184 °C, 81% yield (CH_3CH_2OH); IR (KBr, cm^{-1}) ν: 3222 (NH), 1792, 1732 (C═O), 1343, 1119 (O═S═O); ^1H NMR (500 MHz, $CDCl_3$/DMSO-d_6): δ 3.00–3.03 (t, 2H, *J* = 7.5 Hz), 3.86–3.89 (t, 2H, *J* = 7.5 Hz), 3.91 (s, 3H), 7.34–7.37 (t, 3H, *J* = 8.0 Hz), 7.74–7.75 (d, 3H, *J* = 8.0 Hz), 7.91–7.93 (d, 1H, *J* = 8.0 Hz), 8.23 (s, 1H), 8.32–8.34 (d, 1H, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$/DMSO-d_6): δ 33.98, 38.75, 53.10, 123.67, 123.81, 126.16, 126.33, 129.91, 131.46, 135.42, 135.60, 142.42, 142.85, 165.04, 167.29; $C_{18}H_{16}N_2O_6S$: *m/z* (388).

*4.1.2. General procedure for the synthesis of trimellitimides **12–21** (Scheme 2)*

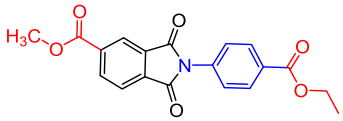
The appropriate aromatic amines (5.0 mmol), anhydrous sodium acetate (0.69 g, 5.0 mmol), and an acid anhydride (5.0 mmol) in glacial acetic acid (15 mL) were heated with refluxing for 6–12 h. After cooling the reaction mixture, the precipitate was filtered, washed with water, dried, and re-crystallized using the appropriate solvents.

*4.1.2.1. 4-(5-(Methoxycarbonyl)-1,3-dioxoisoindolin-2-yl)benzoic acid (**14**).*



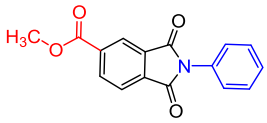
M.P. 295–297 °C, 80% yield (CH_3CH_2OH); IR (KBr, cm^{-1}) ν: 3404 (OH), 1791, 1745 (C=O); ^1H NMR (500 MHz, $CDCl_3$/DMSO-d_6): δ 3.95 (s, 3H), 7.58–7.95 (d, 2H, J = 8.5 Hz), 8.07–8.10 (q, 3H, J = 7.5, 5.0 Hz) 8.36–8.37 (d, 1H, J = 5.0 Hz), 8.43–8.45 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 53.18, 124.07, 124.36, 126.94, 130.29, 130.63, 132.32, 135.38, 135.75, 135.81, 135.92, 165.05, 166.06, 167.09; $C_{17}H_{11}NO_6$: m/z (M + 1, 326).

*4.1.2.2. Methyl 2-(4-(ethoxycarbonyl)phenyl)-1,3-dioxoisoindoline-5-carboxylate (**15**).*



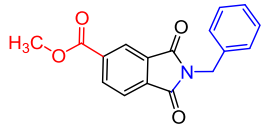
M.P. 151–152 °C, 95% yield (CH_3OH/CH_2Cl_2); IR (KBr, cm^{-1}) ν: 1798, 1756 (C=O); ^1H NMR (500 MHz, $CDCl_3$/DMSO-d_6): δ 1.35–1.38 (t, 3H, J = 7.5 Hz), 3.95 (s, 3H), 4.32–4.36 (q, 2H, J = 7.0 Hz), 7.60–7.61 (d, 2H, J = 8.0 Hz), 8.06–8.11 (q, 3H, J = 8.5, 7.5 Hz), 8.39 (s, 1H), 8.44–8.45 (d, 1H, J = 8.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$/DMSO-d_6): δ 14.53, 53.13, 61.21, 124.18, 124.31, 126.90, 129.70, 130.11, 132.25, 135.30, 135.82, 135.94, 136.08, 164.99, 165.40, 165.96; $C_{19}H_{15}NO_6$: m/z (3 5 3).

*4.1.2.3. Methyl 1,3-dioxo-2-phenylisoindoline-5-carboxylate (**19**).*



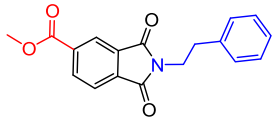
M.P. 207–209 °C, 94% yield (CH_2Cl_2); IR (KBr, cm^{-1}) ν: 1787, 1726 (C=O); ^1H NMR (500 MHz, $CDCl_3$): δ 4.03 (s, 3H), 7.43–7.48 (q, 3H, J = 7.5, 6.5 Hz), 7.53–7.56 (t, 2H, J = 7.5 Hz), 8.05–8.06 (d, 1H, J = 7.5 Hz), 8.50–8.51 (d, 1H, J = 7.5 Hz), 8.61 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 52.94, 123.85, 124.88, 126.49, 128.37, 129.21, 131.41, 131.99, 135.06, 135.77, 136.01, 165.15, 166.34, 166.36; $C_{16}H_{11}NO_4$: m/z (2 8 1).

*4.1.2.4. Methyl 2-benzyl-1,3-dioxoisoindoline-5-carboxylate (**20**).*



M.P. 105–107 °C, 96% yield (CH_2Cl_2); IR (KBr, cm^{-1}) ν: 1777, 1718 (C=O); ^1H NMR (500 MHz, $CDCl_3$): δ 4.00 (s, 3H), 4.88–4.89 (d, 2H, J = 5.0 Hz), 7.24–7.32 (m, 3H), 7.42–7.43 (d, 2H, J = 7.5 Hz), 7.88–7.90 (m, 1H), 8.36–8.38 (m, 1H), 8.44 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 41.91, 52.87, 123.41, 124.45, 127.99, 128.70, 128.74, 132.36, 135.45, 135.62, 136.02, 165.20, 167.05, 167.10; $C_{17}H_{13}NO_4$: m/z (295).

*4.1.2.5. Methyl 1,3-dioxo-2-phenethylisoindoline-5-carboxylate (**21**).*



M.P. 117–118 °C, 93% yield (CH_2Cl_2); IR (KBr, cm^{-1}) ν: 1778, 1717 (C=O); ^1H NMR (500 MHz, $CDCl_3$): δ 3.00–3.03 (t, 2H, J = 7.5 Hz), 3.95–3.98 (t, 2H, J = 7.5 Hz), 4.00 (s, 3H), 7.21–7.30 (m, 5H), 7.90–7.91 (d, 1H, J = 8.0 Hz), 8.40–8.41 (d, 1H, J = 7.5 Hz), 8.46 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 34.49, 39.56, 52.85, 123.28, 124.30, 126.73, 128.59, 128.83, 132.28, 135.35, 135.37, 135.55, 137.79, 165.22, 167.15, 167.19; $C_{18}H_{15}NO_4$: m/z (309).

4.2. Biological evaluation

4.2.1. Anti-inflammatory activity

The anti-inflammatory activity was evaluated with an *in vivo* carrageenan-induced rat paw edema model [45]. This assay is conventional for acute inflammation determinations. Rats were divided into six groups with five rats per group. Each was injected intraperitoneally with equimolar doses (equivalent to 0.170 M kg^{-1}) of the test compounds and the reference drug (celecoxib). One hour after drug administration, the rats were subcutaneously injected with 0.05 mL of a 1% carrageenan solution in saline (prepared 24 h before use) into the subplantar tissue of the right hind paw. The left hind paw of each rat received a subplantar injection of 0.05 mL normal saline. Two hours after the injections, the rats were killed by cervical dislocation and both of their hind paws were excised at the tibiotarsic articulation and weighed. The weight difference between the right and left paws was recorded for each rat. The percentage weight increases of the carrageenan-injected paws relative to the control paws were calculated. The percentage reductions in edema relative to the control group were used as activity measures. Compounds **6**, **7**, **8**, **10**, **11**, **18**, and celecoxib were tested in three graded doses to determine their ED_{50}.

4.2.2. Potential ulcerogenicity

Ulcerogenicity was determined according to the method of Adami et al. [46]. The rats were divided into four groups with five rats each. Compounds **6**, **7**, **8**, **10**, **11**, **18**, celecoxib, and diclofenac sodium were administered orally to the rats in equimolar doses (equivalent to 0.170 M kg^{-1}) daily for 5 d. The animals were sacrificed by ether overdose 10 h after the last treatment. The stomachs were removed, rinsed with saline, opened along the greater curvature, and inspected under a hand lens (x10 magnification). The number and total length of the ulcers for each animal were recorded. The means were calculated and taken as the ulcer indices.

4.2.3. In vitro COX-1/COX-2 inhibition assay

The colorimetric COX (ovine) inhibitor screening assay kit (catalog no. 560101, Cayman Chemicals Inc., Ann Arbor, MI, USA) was used according to the manufacturer's instructions to determine the relative abilities of the test compounds and reference drugs to inhibit COX-1/COX-2 isozymes [3,34,48].

4.2.4. Carbonic anhydrase inhibition

The SX.18MV-R stopped-flow instrument (Applied Photophysics, Oxford, UK) was used to assay the inhibition of various CA isozymes [49]. Phenol Red (0.2 mM) was the indicator. The absorbance was 557 nm. Hepes 10 mM (pH 7.4) was the buffer and 0.1 M Na_2SO_4 or 0.1 M $NaClO_4$ was used to maintain a constant ionic strength. At this concentration, these anions are not inhibitory [53]. The CA-catalyzed CO_2 hydration reaction then proceeded for 5–10 s. Saturated aqueous CO_2 solutions at 25 °C were used as substrates. Stock 10-mM inhibitor solutions were prepared using a 1:1 v/v DMSO:water solvent mixture. Dilutions up to 0.01 nM were prepared using the aforementioned assay buffer. At least seven inhibitor concentrations were used to determine the inhibition constant. Inhibitor- and enzyme solutions were mixed and preincubated for 10 min at room temperature before the assay to allow the E-I complex to form. Triplicate experiments were conducted for each inhibitor concentration. The values reported in the present study are the means of these measurements. The inhibition constants were obtained by the nonlinear least-squares method using the Cheng-Prusoff equation as reported earlier [39,42]. They represent the means of ≥3 different determinations.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.11.033.

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