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RESEARCH ARTICLE

Fluorine bearing sydnones with styryl ketone group: synthesis and their possible analgesic and anti-inflammatory activities

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Abstract

In continuation of structure activity relationship studies, a panel of fluorine containing sydnones with styryl ketone group 4-[1-oxo-3-(substituted aryl)-2-propenyl]-3-(3-chloro-4-fluorophenyl)sydnones **2a–i**, was synthesized as better analgesic and anti-inflammatory agents. The title compounds were formed by condensing 4-acetyl-3-(3-chloro-4-fluorophenyl)sydnone with various substituted aryl aldehydes, characterized by spectral studies and evaluated at 100 mg\kg b.w., p.o. for analgesic, anti-inflammatory and ulcerogenic activities. Compounds **2c** and **2e** showed good analgesic effect in acetic acid-induced writhing while none showed significant activity in hot plate assay in mice. In carrageenan-induced rat paw oedema test, compound **2c** and **2f** exhibited good anti-inflammatory effect at 3rd h, whereas compounds **2c**, **2e**, **2d**, **2g** and **2h** showed activity in croton oil induced ear oedema assay in mice. Compounds **2c** and **2e** were less ulcerogenic than ibuprofen in rats, when tested by ulcer index method. Compounds with electron attracting substituents such as **2c** and **2e** were found to be promising in terms of the ratio of efficacy and adverse effect. These compounds generally exhibited better activity than those of earlier series signifying fluorine substitution.

Keywords: 3-(3-chloro-4-fluorophenyl)sydnone, styryl ketone, hot plate assay, writhing test, paw oedema, ear oedema, ulcerogenicity

Introduction

Structure activity relationship (SAR) is one of the tools in drug design and development with which a medicinal chemist can explore the biological activity better by effecting structural variation in a panel of compounds. We undertook SAR studies of 4-[1-oxo-3-(substituted aryl)-2-propenyl]-3-(3-different substituted phenyl) sydnones as analgesic and anti-inflammatory agents on the basis that molecules containing both the structural features of sydnone and styryl ketone would be better acting, because these compounds have been reported separately in literature to exhibit analgesic and anti-inflammatory activities[1–6]. As a part of this, we already reported 3-(4-chlorophenyl) and 3-(4-methylphenyl) substituted compounds of the said series for analgesic and anti-inflammatory activities[7,8]. In continuation with it, we synthesized 3-(3-chloro-4-fluorophenyl) substituted compounds of the above series i.e., 4-[1-oxo-3-(substituted aryl)-2-propenyl]-3-(3-chloro-4-fluorophenyl)sydnones **2a–i**, and screened them for analgesic and anti-inflammatory activities. The findings of the study are presented in this paper.

Sydnones containing fluorine were planned to synthesize in an anticipation of good pharmacological profile because fluorine, when present, can alter the biological properties of a molecule. It affects lipophilicity thereby enhancing absorption, transport, recognition and interaction of the molecule with the biological target *in vivo*, thus increasing intrinsic activity, chemical and metabolic stability[9]. In testimony of this, over 150 fluorinated drugs are there in the market today making up to ~20% of all pharmaceuticals. Some of the fluorinated molecules which made their way to clinic include the antidepressant fluoxetine, the cholesterol-lowering atorvastatin,

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the anticancer agent 5-fluorouracil, the general anaesthetic halothane, the anti-inflammatory drug flumethasone and the antibacterial ciprofloxacin[10,11]. Therefore, recently there is an increased interest in the synthesis of fluorinated compounds as pharmaceuticals.

Methods

The analytical reagent grade chemicals were used without further purification. Carrageenan was obtained from Sigma (St. Louis, MO), croton oil from Paras perfumers (Delhi, India) and pentazocine from Pharma Impex Laboratories (P) Ltd., (Kolkata, India). Embiotic Laboratories (P) Ltd., (Bengaluru, India) gifted acetyl salicylic acid (ASA), ibuprofen and indomethacin. The completion of reaction and purity of products were monitored by thin layer chromatography using silica gel 60 F_{254} pre-coated aluminium sheets (Merck, Darmstadt, Germany) and benzene-ethyl acetate 8.5:1.5 by volume as mobile phase. The spots were visualized by iodine vapour.

Instrumentation

Melting points were determined in Dolphin open capillary tube apparatus (Mumbai, India) and are uncorrected. Ultra Violet-Visible (UV-Vis) spectra were taken on Systronics V530 (Ahmedabad, India), Infrared (IR) spectra on ThermoNicolet 200 FT-IR (Madison, WI) by potassium bromide pellet technique and mass spectra (MS) on Finnegan-Mat1020 (electron impact, 70 ev) (Bremen, Germany). Proton nuclear magnetic resonance (^{1}H-NMR) spectra in deuterochloroform were recorded on BrukerAC200F (200 MHz) (Rheinstetten, Germany), whereas ^{13}C-NMR spectra were taken at 50 MHz. Chemical shifts were measured on δ scale in parts per million downfield to tetramethylsilane. Peak multiplicities are indicated as s (singlet), d (doublet), m (multiplet) and br (broad); coupling constant (*J*) values are given in Hz. Eddy's Hot Plate (Kshitij International, Ambala, India) and digital micrometer (Digitrik mark II, NSK, Japan) were used in biological activities.

Animals

Adult healthy Albino mice (20–25 g b.w.) and Sprague-Dawley rats (100–150 g b.w.) of either sex, bred at the animal house facility of HSK College of Pharmacy, Bagalkote, were used. The animals were maintained on standard pellet diet and free access to water and housed in polypropylene cage (4 per cage) on paddy husk bedding under laboratory conditions at 22 ± 3°C temperature, 60 ± 10% humidity and 12-h light/dark cycle. They were acclimatized for 7 days. The food was withdrawn 18 h before the experiment, but free access to water was allowed. The experiments were performed on randomly formed groups during the light phase of the cycle and the animals were used for once experiment only. The test compounds and standard drugs were administered p.o., as aqueous suspension in 0.5% sodium caboxymethyl cellulose (Sod CMC). The animals in the vehicle control group were administered 0.5% Sod CMC 10 mL/kg b.w. p.o. All efforts were made to minimize animal suffering and to reduce the number of animals used. The guidelines of institutional animal ethics committee constituted as per committee for the purpose of control and supervision of experiments on animals, Ministry of Environment and Forestry, Government of India, were followed.

Statistics

The data of animal experiments were expressed as mean ± standard deviation (SD) and analyzed by Graph-pad Prism (San Diego, CA). Statistical differences between the treatments and the control were tested by analysis of variance, followed by Dunnett's multiple comparison test. Data with p value < 0.05 was considered significant.

Synthesis of 2a–g: 4-[1-oxo-3- (4-N, N-dimethylamino phenyl)-2-propenyl]-3-(3-chloro-4-fluoro phenyl) sydnone 2f

A mixture of 4-acetyl-3-(3-chloro-4-fluorophenyl) sydnone **1** (2.6 g, 0.01 mol), sodium hydroxide aqueous solution (0.6 g, 0.015 mol, 3 mL) and ethanol (95%, 20 mL) was cooled (5–10°C) and to this was added 4-N,N-dimethylaminobenzaldehyde (2 g, 0.012 mol) while being stirred. The reaction mixture was stirred further for 1 h. The precipitate obtained was filtered washed with cold water and re-crystallised from ethanol (95%) and ethyl acetate (1:1) to give the title compound (1.67 g, 0.0043 mol, 43%): mp 109–110°C; IR cm^{-1} 1755 (C=O, sydnone), 1660 (C=O, styryl ketone); ^{1}H-NMR δ 3.08 (s, 6H, $N(CH_3)_2$), 6.74–6.77 (m, 3H, ArH and olefinic αH), 7.36–7.49 (m, 2H, ArH), 7.78–7.83 (m, 4H, ArH and olefinic βH); ^{13}C-NMR δ 40.06, 106.03, 111.01, 118.2, 121.48, 124.2, 125.11, 131.97, 133.26, 134.20, 136.50, 154.39, 157.66, 162.77, 168.61, 190.31; MS *m/z* 387.59 (M^+).

Remaining compounds were prepared similarly using respective aryl aldehydes.

*4-[1-oxo-3-(phenyl)-2-propenyl]-3-(3-chloro-4-fluorophenyl) sydnone 2a IR cm^{-1} 1753 (C=O, sydnone), 1662 (C=O, styryl ketone); ^{1}H-NMR δ 6.*73 (d, 1H, *J* = 10.00, olefinic αH), 7.2–7.61 (m, 5H, ArH), 7.64–7.72 (m, 4H, ArH and olefinic βH); ^{13}C-NMR δ 106.31, 117.12, 120.83, 121.45, 125.16, 128.22, 128.63, 131.37, 135.48, 135.68, 145.36, 158.96, 167.35, 187.53; MS *m/z* 344.63 (M^+).

*4-[1-oxo-3-(2-furyl)-2-propenyl]-3-(3-chloro-4-fluorophenyl) sydnone 2b IR cm^{-1} 1765 (C=O, sydnone), 1676 (C=O, styryl ketone); ^{1}H-NMR δ 6.*92 (d, 1H, *J* = 11.76, olefinic αH), 7.03 (t, 1H, *J* = 11.76, furyl-4H), 7.26–7.42 (m, 2H, ArH), 7.73–7.96 (m, 3H, ArH, furyl-3H, and olefinic βH), 8.25 (d, 1H, *J* = 7.05, furyl-5H); ^{13}C-NMR δ 105.18, 112.68, 113.93, 117.26, 120.88, 121.25, 125.37, 127.48, 131.24, 135.68,

143.78, 152.26, 159.15, 166.83, 187.28; MS *m/z* 334.46 (M^+).

4-[1-oxo-3-(4-cholrophenyl)-2-propenyl]-3-(3-chloro-4-fluorophenyl)sydnone 2c IR cm^{-1} 1763 (C=O, sydnone), 1671(C=O, styryl ketone); ^{1}*H-NMR δ 6.*81 (d, 1H, *J* = 10.52, olefinic αH), 7.28–7.43 (m, 2H, ArH), 7.6 (d, 2H, *J* = 5.26, ArH), 7.73–7.82 (m, 4H, ArH and olefinic βH); ^{13}C-NMR δ 105.37, 117.32, 120.93, 121.35, 124.98, 128.98, 129.17, 131.48, 133.52, 135.73, 145.68, 159.47, 167.28, 186.79; MS *m/z* 379.18 (M^+).

4-[1-oxo-3-(3,4,5-trimethoxyphenyl)-2-propenyl]-3-(3-chloro-4-fluorophenyl)sydnone 2d IR cm^{-1} 1752 (C=O, sydnone), 1657 (C=O, styryl ketone); ^{1}*H-NMR δ 3.*93 (s, 9H, OCH_3), 6.82 (d, 1H, *J* = 10.52, olefinic αH), 6.87 (s, 2H, ArH), 7.21–7.38 (m, 2H, ArH), 7.81 (t, 2H, *J* = 10.52, ArH and olefinic βH); ^{13}C-NMR δ 55.97, 61.00, 104.16, 105.48, 117.32, 120.86, 121.74, 125.53, 126.84, 132.10, 136.21, 138.63, 153.46, 159.28, 166.58, 187.33; MS *m/z* 434.53 (M^+).

4-[1-oxo-3-(4-nitrophenyl)-2-propenyl]-3-(3-chloro-4-fluorophenyl)sydnone 2e IR cm^{-1} 1755 (C=O, sydnone), 1667 (C=O, styryl ketone); ^{1}*H-NMR δ 7.*38 (d, 1H, *J* = 11.56, olefinic αH), 7.42–7.56 (m, 2H, ArH), 7.76 (m, 1H, ArH), 7.93 (d, 1H, *J* = 11.56, olefinic βH), 8.69 (d, 2H, *J* = 5.78, ArH), 8.93 (d, 2H, *J* = 5.78, ArH); ^{13}C-NMR δ 106.15, 117.28, 120.92, 121.36, 124.18, 125.38, 129.13, 130.96, 135.57, 141.46, 145.37, 147.14, 158.84, 167.35, 187.46; MS *m/z* 389.49 (M^+).

4-[1-oxo-3-(2-nitrophenyl)-2-propenyl]-3-(3-chloro-4-fluorophenyl)sydnone 2 g IR cm^{-1} 1759 (C=O, sydnone), 1662 (C=O, styryl ketone); ^{1}*H-NMR δ 6.*98 (d, 1H, *J* = 11.56, olefinic αH), 7.32–7.48 (m, 2H, ArH), 7.78–8.21 (m, 4H, ArH), 8.30–8.33 (m, 2H, ArH and olefinic βH); ^{13}C-NMR δ 105.82, 117.30, 121.18, 121.63, 123.97, 125.46, 127.48, 129.00, 131.35, 134.68, 135.74, 145.56, 147.98, 159.12, 165.94, 188.20; MS *m/z* 389.52 (M^+).

Synthesis of 2h and 2i: 4-[1-oxo-3-(4-hydroxy-3-methoxy phenyl)-2- propenyl]-3-(3-chloro-4-fluorophenyl)sydnone 2 h

To the suspension of compound **1** (2.6 g, 0.01 mol) and vanillin (1.8 g, 0.012 mol) in 20 mL ethanol (95%) was passed dry hydrogen chloride gas for 0.5 h under cooling (5°C). The reaction mixture was left overnight at room temperature and poured into cold water. The separated precipitate was filtered, washed, dried in air and re-crystallised from ethanol (95%) to give the title compound (2.18 g, 0.0056 mol, 56%): mp 158–160°C; IR cm^{-1} 1758 (C=O, sydnone), 1660 (C=O, styryl ketone); ^{1}H-NMR δ 3.97 (s, 3H, OCH_3), 5.98 (s, br, 1H, OH), 6.79–6.88 (m, 2H, ArH and olefinic αH), 7.32–7.68 (m, 4H, Ar-H), 7.76–7.78 (m, 2H, ArH and olefinic βH); ^{13}C-NMR δ 55.93, 105.73, 112.08, 117.32, 120.96, 121.48, 123.31, 125.83, 128.36, 131.89, 136.15, 146.10, 148.63, 149.76, 159.38, 166.48, 188.56; MS *m/z* 390.50 (M^+).

Compound **2i** was prepared similarly using 2-hydroxyquinolin-3-carboxaldehyde.

4-[1-oxo-3-(2-hydroxy-3-quinolinyl)-2-propenyl]-3-(3-chloro-4-fluorophenyl)sydnone 2i IR cm^{-1} 1757 (C=O, sydnone), 1672 (C=O, styryl ketone); ^{1}*H-NMR δ 6.*76 (d, 1H, *J* = 12.30, olefinic αH) 7.27–7.39 (m, 2H, ArH), 7.57–7.8 (m, 2H, ArH and olefinic βH), 7.81–8.14 (m, 4H ArH), 8.42 (s, 1H, ArH), 11.47 (s, br, 1H, OH); ^{13}C-NMR δ 106.18, 117.61, 121.13, 122.24, 124.30, 125.26, 125.60, 127.31, 127.86, 128.63, 130.75, 131.89, 136.12, 136.65, 145.74, 146.58, 159.38, 166.37, 174.92, 187.64; MS *m/z* 411.48 (M^+).

Acute toxicity

Mice and rats were divided into groups of four each and the test compounds were administered p.o. to different groups in increasing dose levels of 250, 500, 750 and 1000 mg/kg b.w. They were observed continuously for 3 h, for neurological (ptosis, drowsiness, gait, tremors and convulsions), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and general behavioural profiles and then every 30 min for next 3 h and finally for lethality after 24 h[12].

Analgesic activity

It was assessed in mice by chemically as well as thermally induced pain using acetic acid-induced writhing[13] and hot plate assay[14], respectively.

Acetic acid-induced writhing

A sensitivity test was carried out a day prior to drug administration wherein the mice were injected 0.2–0.25 mL of 0.6% acetic acid i.p. Mice showing writhing within 30 min were chosen for study. Groups of six mice each were dosed with the test compounds or with ASA at a dose of 100 mg/kg b.w. p.o., 1 h before the i.p. injection of 0.6% acetic acid (10 mL/kg b.w.). After 5 min of acetic acid injection, they were observed for 20 min and the total number of writhes was recorded. The percent reduction of the number of writhes was calculated.

$$\%\,\text{Reduction} = \frac{\left(\begin{array}{c}\text{Mean number of writhes}\\ \text{in Control Group} - \text{Mean number}\\ \text{of writhes in Treated Group}\end{array}\right)}{\begin{array}{c}\text{Mean number of}\\ \text{writhes in Control Group}\end{array}} \times 100$$

Hot plate assay

Groups of six mice each were administered with the test compounds at a dose of 100 mg/kg b.w. p.o. or with pentazocine (5 mg/kg b.w. p.o.) 1 h before the test. The animals were placed on hot plate maintained at 55 ± 1°C and the basal reaction time taken to cause a discomfort (licking of paw or jumping response whichever appeared first) was recorded at zero min. Cut-off period of 15 s was established to prevent damage to the

paws. The reaction time in seconds was re-investigated at 30, 60, and 120 min. The activity was expressed as percent protection.

$$\%\,\text{Protection} = \frac{\left(\begin{array}{c}\text{Average reaction time}\\ \text{in Control Group} - \text{Average reaction}\\ \text{time in Treated Group}\end{array}\right)}{\begin{array}{c}\text{Average reaction}\\ \text{time in Control Group}\end{array}} \times 100$$

Anti-inflammatory activity

Acute systemic and local anti-inflammatory activities were carried out by carrageenan-induced paw oedema in rats[15] and croton oil induced ear oedema in mice[16], respectively.

Carrageenan-induced paw oedema

Groups of six rats were dosed at 100 mg/kg b.w. p.o. with the test compounds or ibuprofen 1 h before 0.05 mL of a 1% suspension of carrageenan in saline was injected into the sub plantar region of the right hind paw. An equal volume of saline was injected into the other hind paw and served as control. Paw volumes were measured by plethismograph immediately after carrageenan injection and again after 1, 2, 3 and 5 h later. Data were reported as percent oedema inhibition.

$$\begin{array}{l}\%\,\text{Edema}\\ \text{inhibition}\end{array} = \frac{\left(\begin{array}{c}\text{Average Paw volume in}\\ \text{Control Group} - \text{Average Paw volume}\\ \text{in Treated Group}\end{array}\right)}{\begin{array}{c}\text{Average Paw volume}\\ \text{in Control Group}\end{array}} \times 100$$

Topical ear oedema

Groups of six mice received topical application (5 mg/ear) of test compounds or indomethacin dissolved in dimethyl sulfoxide (DMSO) on the anterior surface of the right ear while 0.05 mL croton oil (4 parts of croton oil and 96 parts of DMSO) was instantly applied on the posterior surface of the same ear. Control animals received an equivalent volume of DMSO. The ear thickness was measured by digital micrometer 4 h after challenge of croton oil to assess an increase in oedema. The % inhibition of oedema was calculated using the following relation.

$$\%\,\text{Inhibition} = \frac{\left(\begin{array}{c}\text{Average ear thickness in}\\ \text{Control Group} - \text{Average ear thickness}\\ \text{in Treated Group}\end{array}\right)}{\begin{array}{c}\text{Average ear thickness}\\ \text{in Control Group}\end{array}} \times 100$$

Ulcerogenic assay

Groups of six rats were administered test compounds or ibuprofen at a dose of 100 mg/kg b.w. p.o. After 4 h, the rats were sacrificed, the stomachs removed, opened along the greater curvature and observed for gastric lesions on the mucosa. The lesion index for each group was determined by counting the number of lesions (x) in each of five size classes (y) which were defined as y = 1 (pinpoint lesion), y = 2 (lesions < 1 mm diameter), y = 3 (lesions 1–2 mm diameter), y = 4 (lesions 2–4 mm diameter) and y = 5 (lesions >4 mm diameter).[17]

$$\text{Lesion index} = \sum_{i=1}^{5} x_i y_i$$

Results and discussion

The starting material, 4-acetyl-3-(3-chloro-4-fluorophenyl)sydnone **1**, was prepared by the action of glacial acetic acid on 3-(3-chloro-4-fluorophenyl)sydnone in presence of phosphorous pentoxide[18]; the 3-(3-chloro-4-fluorophenyl)sydnone itself was synthesized by literature protocol from 3-chloro-4-fluoroaniline[19]. The IR spectrum of compound **1** showed sydnone C=O and acetyl C=O stretching at 1772 and 1671 cm^{-1}, respectively, and in ^{1}H-NMR spectrum, it showed a singlet at δ 2.54 corresponding to three hydrogens of $COCH_3$ and a multiplet at 7.61–7.89 accounting three aromatic protons (data not shown). Claisen-Schmidt reaction of compound **1** with different aryl aldehydes in presence of either alkali or acid afforded the title compounds **2a–i** (Figure 1). The UV-Vis spectra of compounds **2a–i** exhibited considerable bathochromic shifts due to π-π* (343–601 nm) in comparison to 321 nm for compound **1** indicating the extension of conjugation due to formation of α,β-unsaturated ketone. In their IR spectra, compounds **2a–i** showed sydnone C=O and styryl ketone stretching at 1753–1765 cm^{-1} and 1660–1676 cm^{-1}, respectively. Compounds **2a–i** in their ^{1}H-NMR spectra displayed, apart from other hydrogens, doublets due to α protons of α,β-unsaturated ketone moiety at δ 6.73–6.98, whereas β protons were seen merged with aromatic protons at δ 7.64–8.33 in most instances. The ^{13}C-NMR spectra of compounds **2a–i** exhibited α-carbons of α,β-unsaturated ketone at δ 121.36–127.86, β-carbons at δ 146.10–159.47, carbons of styryl ketone at δ 186.79–190.31, the sydnone C=O at δ 165.94–168.61 and sydnone C-4 at δ 105.18–106.31 amidst other carbons. The mass spectra of compounds **2a–i** showed the M^+ ion peak at their respective *m/z* values which are consistent with their molecular weight. The physical data of compounds **2a–i** are presented in Table 1.

After 24 h of administration up to 1000 mg/kg b.w., compounds **2a–i** produced no mortality in both rats and mice in acute toxicity testing. But few changes in the behavioural response like alertness, touch and restlessness were noticed. There were no significant changes in neurological and autonomic profiles. Hence, 1/10th of the maximum tolerated dose i.e., 100 mg/kg b.w. was chosen for the pharmacological studies.

Compound **2b**, **2e** and **2c** showed good activity with 46, 44 and 40% reduction in writhes, respectively, in writhing test (Table 2). The presence of chloro and nitro groups at *para* position enhanced the activity. The furyl analogue was equiactive to that of phenyl. The presence of trimethoxy group on the phenyl ring sustained activity, whereas N, N-dimethylamino group at *para* position did not. It is noteworthy that compounds **2a–i** were

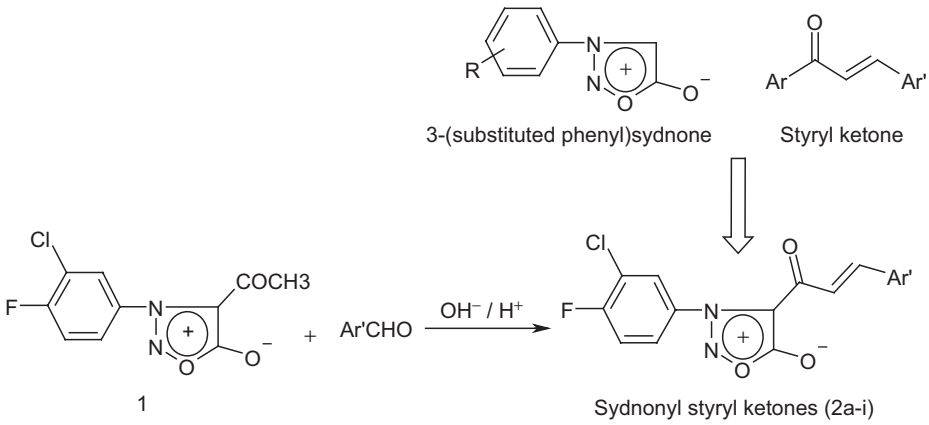
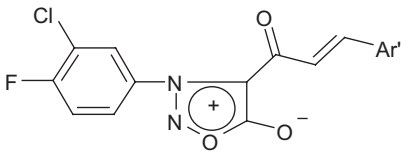
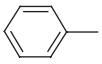
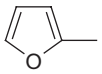
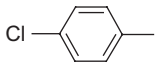
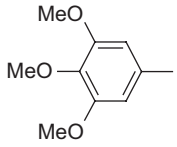
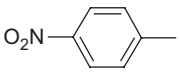
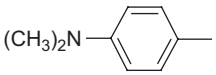
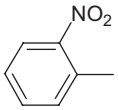
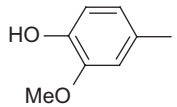
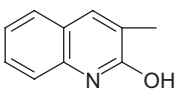


Figure 1. Design and synthesis of title compounds.

Table 1. Physical data and yields of compounds 2a–i.



Comp	Ar′	Mol. Formula	Mol. Wt.	Yield (%)	Mp (°C)	UV-Vis Abs. (nm)*
2a		$C_{17}H_{10}N_2O_3ClF$	344.5	48	151–152	346, 246
2b		$C_{15}H_8N_2O_4ClF$	334.5	37	133–135	344, 246
2c		$C_{17}H_9N_2O_3Cl_2F$	379	41	134–135	343, 249
2d		$C_{20}H_{16}N_2O_6ClF$	434.5	42	119–121	397, 309, 241
2e		$C_{17}H_9N_3O_5ClF$	389.5	45	137–139	358, 245
2f		$C_{19}H_{15}N_3O_3ClF$	387.5	43	109–110	344, 245
2g		$C_{17}H_9N_3O_5ClF$	389.5	42	139–141	601, 247
2h		$C_{18}H_{12}N_2O_5ClF$	390.5	56	158–160	356, 246
2i		$C_{20}H_{11}N_3O_4ClF$	411.5	47	207–209	393, 290, 244

*In $CHCl_3$.

found to be more active than the corresponding 4-methyl analogues reported by us earlier[8]. It is reported that intraperitoneal administration of an agent that irritates the serous membrane such as acetic acid results in the release of prostaglandins like PGE_2 and $PGF_{2\alpha}$ and sympathomimetic system mediators in the peritoneal fluid[20]. It is speculated that the activity of the test compounds could be due to inhibition of synthesis of prostaglandins and or sympathomimetic system mediators. In hot plate assay, centrally acting analgesics increase the reaction time in laboratory animals[21]. Since none of the tested compounds significantly increase the reaction time in animals in hot plate test (Table 2), it can therefore be concluded that compounds **2a–i** do not possess centrally mediated analgesic effect.

The hind paw inflammation oedema produced by carrageenan is a biphasic event and the agents which act on the first stage inhibit the chemical mediators such as histamine, serotonin, and bradykinin, while the second stage of the oedema is related to the arachidonic acid metabolites since it is inhibited by aspirin, indomethacin, and other COX inhibitors[22]. In paw oedema test, compounds **2a–i** displayed very weak anti-inflammatory activity at 1 and 2 h than the corresponding 4-chloro analogues reported by us[7] indicating none of the compounds **2a–i** is inhibiting the synthesis or action of chemical mediators. Nitric oxide (NO) is known to inhibit the adhesion of leucocytes to the endothelium, preventing adhesion cascade and reduce inflammation in initial stages[23]. The weak and slow release of NO by sydnones[24] may be responsible for the weak activity shown by these compounds in the early stage of inflammation. At 3 h, compounds **2a–i** showed significant activity with **2c** and **2f** showing highest activities with 56 and 55% oedema inhibition followed by **2e**, **2h** and **2d** with 49, 45 and 43% oedema inhibition, respectively (Table 3). These compounds showed more activity than the 4-chloro counterparts[7] highlighting the effect of fluorine substitution. The good activity shown by these compounds in the second phase of inflammation may be attributed to more availability of these compounds at the site of action due to increased lipophilicity by fluorine substitution. They may act by inhibiting either COX and or lipoxygenase enzyme thereby preventing the formation of inflammatory prostaglandins and or leukotrienes from the arachidonic acid cascade. At 5th h, only compound **2c** and **2f** showed significant activity with 20 and 18% oedema inhibition, respectively. The chloro and N,N-dimethylamino substitution at *para* position seems to favour the activity.

The good systemic anti-inflammatory activity displayed by title compounds and the paucity of data on local anti-inflammatory activity of sydnone derivatives prompted us to screen compounds **2a–i** for topical anti-inflammatory activity. It is known that the oedematous inflammation induced by croton oil treated ears is related to the activation of phopholipaseA2, which releases arachidonic acid from the cell membrane that in turn, is metabolized to proinflammatory prostaglandins and leukotrienes[25]. Reduction in the thickness of the croton oil induced ear oedema by any agent is an index of its topical anti-inflammatory activity. Compound **2c**, **2e**, **2d**, **2g** and **2h** exhibited significant topical anti-inflammatory activity with 27, 25, 24, 23 and 22% oedema inhibition, whereas standard drug indomethacin showed 69% oedema inhibition (Table 3). Here also, the chloro derivative displayed good activity. However, the topical anti-inflammatory activity of these compounds was not pronounced compared to systemic activity. Again, their topical anti-inflammatory activity could be due to inhibition of COX and or lipoxygenase enzymes.

The major drawback of non-steroidal anti-inflammatory drugs is their propensity to cause gastric ulceration[26], and the three active compounds in the series **2c**, **2e** and **2f** were tested for this effect. The compounds with electron attracting substituents such as **2c** and **2e** showed ulcerogenic index of 11.12 and 14.38, respectively, that was less than that of ibuprofen (Table 4). From this study, compounds **2c** and **2e** have emerged to be the most promising in terms of the ratio of efficacy and adverse

Table 2. Analgesic activity of compounds 2a–i.

	Hot plate assay				Acetic acid writhing assay
	Mean reaction time ± SD, sec (% protection)				Mean no. of writhes ± SD
Comp	0 min	30 min	60 min	120 min	(% reduction)
2a	3.87 ± 0.04 (2)	4.14 ± 0.05 (3)	4.15 ± 0.07 (4)	4.10 ± 0.09 (3)	47 ± 05** (35)
2b	3.83 ± 0.41 (3)	3.95 ± 0.10 (2)	3.97 ± 0.12 (0)	3.89 ± 0.17 (2)	46 ± 08** (36)
2c	3.82 ± 0.36 (3)	3.89 ± 0.24 (3)	4.16 ± 0.18 (4)	3.97 ± 0.14 (1)	40 ± 11** (44)
2d	3.78 ± 0.24 (4)	3.97 ± 0.12 (1)	3.93 ± 0.08 (1)	3.95 ± 0.23 (1)	48 ± 10** (33)
2e	3.79 ± 0.31 (4)	4.01 ± 0.13 (0)	4.15 ± 0.17 (4)	3.74 ± 0.16 (6)	44 ± 05** (39)
2f	3.90 ± 0.41 (1)	3.85 ± 0.19 (5)	3.73 ± 0.16 (6)	3.91 ± 0.20 (2)	58 ± 06* (19)
2g	3.95 ± 0.52 (0)	3.87 ± 0.14 (4)	3.94 ± 0.10 (1)	3.83 ± 0.17 (4)	55 ± 08** (23)
2h	3.85 ± 0.07 (2)	3.98 ± 0.09 (1)	4.23 ± 0.05 (6)	3.88 ± 0.21 (3)	53 ± 07** (26)
2i	3.92 ± 0.24 (1)	3.86 ± 0.05 (4)	4.08 ± 0.31 (2)	3.97 ± 0.17 (1)	52 ± 06** (28)
Pentazocine	3.94 ± 0.04 (0)	7.23 ± 0.21** (80)	7.89 ± 0.13** (98)	7.38 ± 0.14** (85)	—
ASA	—	—	—	—	29 ± 09** (60)
Control	3.96 ± 0.03	4.02 ± 0.38	3.98 ± 0.03	3.99 ± 0.05	72 ± 07

*$p < 0.05$, ** $p < 0.01$ when compared to control.

Table 3. Anti-inflammatory activity of compounds 2a–i.

	Carrageenan-induced paw oedema inflammation				Croton oil induced ear oedema inflammation
	Mean oedema vol. ± SD, mL (% oedema inhibition)				Mean ear thickness ± SD, mm (% oedema inhibition)
Compound	1 h	2 h	3 h	5 h	
2a	0.34 ± 0.04 (–)	0.43 ± 0.02 (04)	0.44 ± 0.03** (17)	0.42 ± 0.04 (–)	0.156 ± 0.002* (14)
2b	0.33 ± 0.02 (03)	0.42 ± 0.01 (06)	0.43 ± 0.05** (19)	0.40 ± 0.02 (–)	0.153 ± 0.004* (16)
2c	0.30 ± 0.01 (11)	0.31 ± 0.03* (31)	0.23 ± 0.02** (56)	0.31 ± 0.03** (20)	0.133 ± 0.006** (27)
2d	0.31 ± 0.04 (09)	0.37 ± 0.05 (18)	0.30 ± 0.03** (43)	0.36 ± 0.03 (07)	0.138 ± 0.003* (24)
2e	0.32 ± 0.05 (06)	0.36 ± 0.04 (20)	0.27 ± 0.04** (49)	0.35 ± 0.04 (10)	0.136 ± 0.005* (25)
2f	0.30 ± 0.02 (11)	0.33 ± 0.04 (26)	0.24 ± 0.01** (55)	0.32 ± 0.05* (18)	0.147 ± 0.002* (19)
2g	0.34 ± 0.03 (–)	0.40 ± 0.02 (11)	0.38 ± 0.03** (28)	0.41 ± 0.04 (–)	0.132 ± 0.004* (23)
2h	0.32 ± 0.03 (06)	0.34 ± 0.03 (24)	0.29 ± 0.03** (45)	0.36 ± 0.02 (07)	0.142 ± 0.004* (22)
2i	0.33 ± 0.05 (03)	0.41 ± 0.01 (08)	0.41 ± 0.05** (22)	0.41 ± 0.05 (–)	0.158 ± 0.003* (13)
Ibuprofen	0.28 ± 0.03* (18)	0.26 ± 0.04** (42)	0.16 ± 0.06** (70)	0.27 ± 0.03** (31)	—
Indomethacin	—	—	—	—	0.056 ± 0.002** (69)
Control	0.34 ± 0.01	0.45 ± 0.04	0.53 ± 0.04	0.39 ± 0.02	0.182 ± 0.006 (–)

*$p < 0.05$, **$p < 0.01$ when compared to control.

Table 4. Ulcerogenicity of selected compounds.

Compound	Lesion index (±SD)
2c	11.12 (1.93)**
2e	14.38 (2.56)*
2f	21.68 (2.15)*
Ibuprofen	19.16 (4.28)*
Control	4.23 (0.76)

*$p < 0.05$, **$p < 0.01$ when compared to control.

effect; **2c** being the best. This could be due to selective inhibition of COX-2 by them.

Conclusion

The 4-[1-oxo-3-(substituted aryl)-2-propenyl]-3-(3-chloro-4-fluoro phenyl)sydnones prepared as a part of our ongoing SAR study showed good analgesic activity in acetic acid-induced writhing but failed to show appreciable activity in hot plate test suggesting their action is through peripheral mechanism. These compounds also exhibited systemic as well as topical anti-inflammatory activities. In general, the title compounds showed more activity than earlier series of compounds prepared by us suggesting the favourable effect of fluorine on activity. The mechanism of the effects exerted by the compounds covered in the study is under progress that probably would direct future course of action.

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Declaration of interest

The authors report no conflicts of interest.

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