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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF CERTAIN MESOIONIC SYDNONE COMPOUNDS CONTAINING CHALCONE MOIETY

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ABSTRACT: In order to have antibacterial, analgesic and anti-inflammatory activity in the same molecule, 4-[1-oxo-3- (substituted aryl)-2-propenyl]-3-(4-chlorophenyl) sydnones were synthesized by condensing 4-acetyl-3-(4-chlorophenyl)sydnone with various substituted aryl aldehydes and characterized by spectral studies; 4-acetyl-3-(4-chlorophenyl)sydnone itself, was prepared by acetylation of 3-(4-chlorophenyl) sydnone. The newly synthesized compounds were evaluated for antibacterial and anti-inflammatory activities by cup plate and carrageenan induced rat paw edema methods respectively. Some of the compounds showed promising antibacterial and anti-inflammatory activities.

KEYWORDS: Synthesis, sydnone, chalcone, antibacterial, anti-inflammatory

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INTRODUCTION

Over the years, mesoionic compounds have generated lot of interest among synthetic chemists due to their peculiar dipolar, mesoionic character and associated biological activities. Sydnones, being mesoionic compounds, are 1, 2, 3-oxadiazolium-5-olates and their chemistry has been widely studied [1, 2]. A large number of sydnone compounds have been synthesized with a varied biological interest such as antimicrobial [3], anti-inflammatory [4], analgesic and antipyretic [5], nitric oxide donor [6], free radical scavenging [7] and anticancer [8] activities.

Chalcones are 1, 3-diaryl-2-propen-1-ones and are natural or synthetic compounds belonging to the flavonoid family and have been reported for a battery of biological activities [9]. Synthetic chalcones have been shown to exhibit good anti-inflammatory and analgesic activities [10]. Some of the fluorinated chalcones have also been reported to possess promising antimicrobial activity [11].

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Bacterial infections are rampant in developing countries such as India, due to poor public hygiene and sanitation and are often associated with inflammation and pain. Such infections are presently treated separately by antimicrobial and anti-inflammatory and analgesic agents. It could be advantageous if, both these conditions are treated by a single molecule that has antimicrobial and anti-inflammatory activities. In the present study, an attempt is made to develop the compounds containing both sydnone and chalcone moieties anticipating good antibacterial and anti-inflammatory activities, since both sydnones and chalcones have been reported for antibacterial, analgesic and anti-inflammatory activities.

MATERIALS AND METHODS

The chemicals used for synthesis were of laboratory reagent and rest were analytical reagent grade. They were used without further purification. Melting points were determined in an open capillary tube and are uncorrected. IR spectra were recorded on Thermonicolet 200

FT-IR spectrometer by KBr pellet technique. ^{1}H-NMR spectra in $CDCl_3$ were recorded on Bruker AC 200 (200 MHz) spectrometer using TMS as internal standard. Mass spectra were recorded on Finnigan-Mat 1020 instrument (ei, 70 ev). The progress of the reactions and the purity of products were monitored by TLC.

Animals

Sprague–Dawley rats of either sex weighing between 100 and 150 g, housed at temperature 22 ± 3 °C, humidity (60 ± 10%) and 12 h light/dark cycle maintained on standard diet and water *ad libitum* were used. They were acclimatized to laboratory condition for a period of 10 days. The experiments were performed during the light phase of the cycle and animals were used for once experiment only. All efforts were made to minimize animal suffering and to reduce the number of animals used. The study protocol was approved by Institutional Animal Ethics Committee (IAEC).

Statistics

The results are presented as mean ± S.D. The statistical significance of the differences for the comparison between the treated groups and the control was carried out using ANOVA, followed by Dunnett's multiple comparison tests. P-values of less than 0.05 ($p<0.05$) were considered indicative of significance. All the statistical analysis was done using Graph-pad Prism software.

Synthesis of 4-acetyl-3-(4-chlorophenyl) sydnone 2

To a suspension of phosphorous pentoxide (17 g, 0.12 mol) in 100 ml of benzene was added 3-(4-chlorophenyl)sydnone **1** (7.7 g, 0.04 mol). To the stirred mixture while refluxing, glacial acetic acid (2.3 ml, 0.04 mol) was added drop wise over a period of 10 min and the stirred reaction mixture was refluxed for 5 h. After cooling to room temperature, the benzene was decanted and the black residue was extracted twice with 20 ml benzene. Combined extract and decantate were evaporated to dryness and recrystallised from alcohol to give **2** (yield 38%): mp 123-125 °C; IR cm^{-1} 1785 (C=O, sydnone), 1660 (COCH3); ^{1}H-NMR δ ppm 2.53 (s, 3H, $COCH_3$), 7.04-7.43 (m, 4H, Ar-H)

Synthesis of 3a-g: 4-[1-oxo-3-(4-chlorophenyl) -2-propenyl]-3-(4-chlorophenyl) sydnone 3c

To the cooled (5-10 °C) mixture of **2** (0.175g, 0.0075 mol), sodium hydroxide aqueous solution (0.4g, 0.01 mol, 0.2ml) and ethanol (2ml) was added 4-chlorobenzaldehyde (1.4g, 0.01 mol) while being stirred. The reaction mixture was stirred for 1h. The precipitate obtained was filtered washed thoroughly with cold water and recrystallised from ethanol and ethyl acetate (1:1) to give **3c**. IR cm^{-1} 1730 (C=O, sydnone), 1654 (C=O, styryl ketone); ^{1}H-NMR δ ppm 7.41-7.88 (m, 10H, Ar-H and olefinic).

4-[1-oxo-3- (phenyl)-2-propenyl]-3-(4-chlorophenyl) sydnone 3a:

IR cm^{-1} 1749 (C=O, sydnone), 1673 (C=O, styryl ketone); ^{1}H-NMR δ ppm 7.06-7.95 (m, 11H, Ar-H and olefinic).

4-[1-oxo-3- (2-furyl)-2-propenyl]-3-(4-chlorophenyl) sydnone 3b:

IR cm^{-1} 1750 (C=O, sydnone), 1664 (C=O, styryl ketone); ^{1}H-NMR δ ppm 6.89-7.76 (m, 9H, Ar-H and olefinic).

4-[1-oxo-3- (3, 4, 5-trimothoxyphenyl)-2-propenyl]-3-(4-chlorophenyl) sydnone 3d:

IR, cm^{-1} 1753 (C=O, sydnone), 1675 (C=O, styryl ketone); ^{1}H-NMR, δ ppm 3.92 (s, 9H, OCH_3), 7.11-7.60 (m, 8H, Ar-H and olefinic).

4-[1-oxo-3- (4-nitrophenyl)-2-propenyl]-3-(4-chlorophenyl) sydnone 3e:

IR cm^{-1} 1758 (C=O, sydnone), 1657 (C=O, styryl ketone); ^{1}H-NMR δ ppm 7.11-7.98 (m, 10H, Ar-H and olefinic).

4-[1-oxo-3-(4-N, N-dimethylaminophenyl)-2-propenyl]-3-(4-chlorophenyl) sydnone 3f:

IR cm^{-1} 1755 (C=O, sydnone), 1660 (C=O, styryl ketone); ^{1}H-NMR δ ppm 3.07 (s, 6H, N $(CH_3)_2$), 6.65-7.87 (m, 10H, Ar-H and olefinic).

4-[1-oxo-3- (2-nitrophenyl)-2-propenyl]-3-(4-chlorophenyl) sydnone 3g:

IR cm^{-1} 1755 (C=O, sydnone), 1662 (C=O, styryl ketone); ^{1}H-NMR δ ppm 7.25-7.88 (m, 10H, Ar-H and olefinic).

Synthesis of 3h and 3i: 4-[1-oxo-3-(4-hydroxy-3-methoxyphenyl)-2-propenyl]-3-(4-chlorophenyl)sydnone 3h

Into a suspension of vanillin (0.18g, 0.0012mol) and **2** (0.25g, 0.001mol) in 2ml ethanol, dry hydrogen chloride gas was passed 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 and recrystallised from ethanol to give **3h**. 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, 1H, OH), 6.92-6.96 (d, 1H, olefinicαH), 7.16-7.71 (m, 8H, Ar-H and olefinicβH); MS *m/z* 372.43 (M^+)

4-[1-oxo-3-(2-hhydroxy-3-quinolinyl)-2-propenyl]-3-(4-chlorophenyl)sydnone 3i:

IR cm^{-1} 1754 (C=O, sydnone), 1668 (C=O, styryl ketone); ^{1}H-NMR δ ppm 5.95 (s, 1H, OH), 7.12-7.98 (m, 11H, Ar-H and olefinic).

Antibacterial activity

The compounds **3a-i** were screened for preliminary antibacterial activity by cup plate method [12] at 50, 100 and 250µg/ml concentrations against *Staphylococcus aureus* NCIM 2602, *Bacillus subtilis* ATCC 6633 (Gram-positive) and *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 13311 (Gram-negative) grown on nutrient agar medium and the diameter of zone of inhibition was measured; norfloxacin at 100µg/ml concentration was employed as standard. The test compounds were dissolved in minimum quantity of DMSO to get 50, 100 and 250µg/ml concentrations and 200 µl of the solutions was added to cups for testing. Norfloxacin solution in DMSO was prepared to get 100 µg/ml concentration and tested at 200 µl. In case of solvent control only 200 µl DMSO was added to cups. The activity was expressed as relative % inhibition (considering the activity of standard as 100%) as

Relative % Inhibition = 100 (X – Y) / (Z – Y)

where, X, Y and Z are zone of inhibition by test compound, solvent and standard respectively.

Acute toxicity [13]

The rats fasted overnight were divided into groups of four each and the compounds **3a-i** were administered po, as a suspension in 0.5% sodium caboxymethyl cellulose to different groups in an increasing dose levels of 250, 500, 750 and 1000 mg/kg b.w. The rats were then observed continuously for 3 h for general behavioral, neurological and autonomic profiles and then every 30 min for next 3 h and finally for lethality after 24 h.

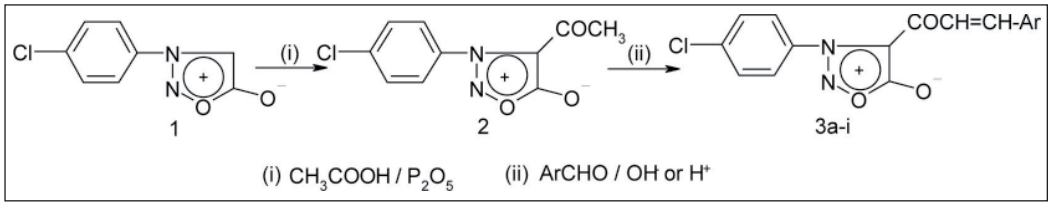
Anti-inflammatory activity

It was done in rats by carrageenan induced paw edema method [14]. Rats fasted overnight were divided into different groups comprising six in each group. The acute inflammation was induced by sub plantar injection of 0.05 ml freshly prepared 1% suspension of carrageenan in the right hind paw of the rats and paw volume was measured by mercury displacement in a plethysmograph at 0, 1, 2, 3 and 5 h after carrageenan injection. Test groups were administered with compounds **3a-i** 100 mg/kg po and the standard group with ibuprofen 100 mg/kg po in 0.5% sodium caboxymethyl cellulose one h before injection of carrageenan. The percentage inhibition of edema was calculated.

RESULTS AND DISCUSSION

The starting material 3-(4-chlorophenyl) sydnone **1** was synthesized as per the protocol described in the literature [2]. Acetylation of **1** by glacial acetic acid in presence of phosphorous pentoxide afforded 4-acetyl 3-(4-chlorophenyl) sydnone **2**. IR spectrum of **2** exhibited a band at 1671 cm^{-1} attributing to C=O stretching of acetyl group and a signal at δ 2.53 accounting acetyl protons in addition to four aromatic hydrogens at δ 7.04-7.43 in its ^{1}H-NMR spectrum. The compounds, 4-[1-oxo-(3-substituted aryl)-2-propenyl]-3-(4-chlorophenyl) sydnones **3a-i** were prepared employing Claisen-Schmidt reaction, by condensing **3** with different substituted aryl aldehydes in presence of either alkali or acid (Scheme 1).

SCHEME 1:



The compounds **3a-i** in their IR spectra showed bands at 1730-1758 cm^{-1} and 1654-1675 cm^{-1} due to sydnone C=O and styryl ketone C=O stretching respectively. These compounds in their ^{1}H-NMR spectra exhibited the protons attached to the carbon atoms of α, β unsaturated ketone moiety at 7.2-7.8, that were seen merged with aromatic protons. The mass spectrum of **3h** showed the M^+ ion peak at *m/z* 372.43. The physical data of compounds **3a-i** are presented in Table 1.

At 50 and 100 µg/ml, the newly synthesized compounds showed moderate antibacterial activity. Only compounds **3c** and **3e** showed the activity comparable to standard at 250 µg/ml against both Gram-positive and Gram-negative organisms, indicating the electron attracting substituents like chloro and nitro at *para* position is essential for activity (Table 2).

No death were seen after 24 h following doses up to 1000 mg/kg b.w. but there were few changes in the behavioral response like alertness, touch response and restlessness in acute toxicity testing of **3a-i**. Therefore, 1/10th of the maximum tolerated dose *i.e.*, 100 mg/kg b.w. was chosen for anti-inflammatory activity. Compounds **3c-h** showed highly significant ($p<0.01$) anti-inflammatory activity at the end of 2 and 3 h. Compound **3c** and **3f** showed highest *i.e.*, 49% and 51% edema inhibition respectively at the end of 3 h (Table 3). It seems that, the chloro and N, N-dimethylamino substituents at *para* position of the phenyl ring augmented the activity. Nitric oxide (NO) has been reported to inhibit the leucocytes adhesion to endothelium at initial stages of inflammation, preventing adhesion cascade thus reducing inflammation [15, 16]. Since sydnones are weak and slow releasers of NO (6), that may, in part, explain the initial weak anti-inflammatory activity exhibited by these compounds. Some sydnone derivatives have also been shown to be less ulcerogenic than NSAIDs [17]. It could be presumed that, the anti-inflammatory activity exhibited at later stages by these compounds may be due to more selective inhibition of cyclooxygenase-2 (COX-2) than COX-1.

Table 1: Physical data and yields of compounds 3a-i

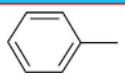
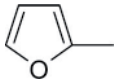
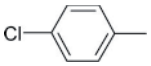
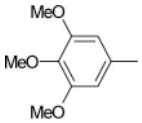
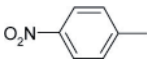
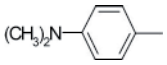
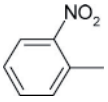
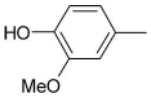
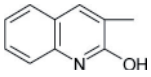
Comp	Ar	Mol. Formula	Mol. Wt.	Yield (%)	mp (°C)
3a		$C_{17}H_{11}N_2O_3Cl$	326.5	45	112-114
3b		$C_{15}H_9N_2O_4Cl$	316.5	41	118-120
3c		$C_{17}H_{10}N_2O_3Cl_2$	361	48	100-101
3d		$C_{20}H_{17}N_2O_6Cl$	416.5	37	79-81
3e		$C_{17}H_{10}N_3O_5Cl$	371.5	44	106-107
3f		$C_{19}H_{16}N_3O_3Cl$	369.5	50	74-75
3g		$C_{17}H_{10}N_3O_5Cl$	371.5	42	113-115
3h		$C_{18}H_{13}N_2O_5Cl$	372.5	47	204-206
3i		$C_{20}H_{12}N_3O_4Cl$	393.5	48	260-262

Table 2: Antibacterial activity of compounds 3a-i

Comp	Zone of Inhibition, mm (% Relative Inhibition)											
	Staph. aureus			*B. subtilis*			*E. coli*			*S. typhi*		
	50 µg/ml	100 µg/ml	250 µg/m	50 µg/m	100 µg/ml	250 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml
3a	9 (50)	12 (66.7)	15 (83.3)	11 (55)	15 (75)	17 (85)	8 (29.6)	16 (59.3)	20 (74)	7 (30.4)	12 (52.2)	15 (65.2)
3b	11 (61.1)	15 (83.3)	16 (88.9)	11 (55)	16 (80)	18 (90)	10 (37)	17 (63)	22 (81.5)	9 (39.1)	13 (56.5)	18 (78.3)
3c	10 (55.5)	16 (88.9)	19 (105.5)	10 (50)	17 (85)	20 (100)	12 (44.4)	18 (66.7)	25 (92.6)	11 (47.8)	16 (69.5)	21 (91.3)
3d	7 (38.9)	11 (61.1)	14 (77.8)	8 (40)	13 (65)	15 (75)	5 (18.5)	12 (44.4)	16 (59.3)	4 (17.4)	10 (43.5)	13 (56.5)
3e	10 (55.5)	15 (83.3)	18 (100)	11 (55)	15 (75)	19 (95)	9 (33.3)	16 (59.3)	23 (85.2)	8 (34.8)	14 (60.9)	20 (87)
3f	6 (33.3)	10 (55.5)	15 (83.3)	6 (30)	11 (55)	16 (80)	7 (30.4)	14 (51.8)	19 (70.4)	6 (26)	11 (47.8)	15 (65.2)
3g	9 (50)	13 (72.2)	16 (88.9)	10 (50)	14 (70)	17 (85)	8 (29.6)	15 (55.5)	18 (66.7)	8 (34.8)	12 (52.2)	14 (60.9)
3h	8 (44.4)	13 (72.2)	17 (94.4)	8 (40)	14 (70)	17 (85)	6 (22.2)	12 (44.4)	16 (59.3)	7 (30.4)	13 (56.5)	18 (78.3)
3i	8 (44.4)	11 (61.1)	13 (72.2)	9 (45)	12 (60)	15 (75)	7 (30.4)	15 (55.5)	18 (66.7)	6 (26)	13 (56.5)	17 (74)
Std.		18 (100)			20 (100)			27 (100)			23 (100)	
DMF	--	--	--	--	--	--	--	--	--	--	--	--

Table 3: Anti-inflammatory activity of compounds 3a-i

Comp	% Edema Inhibition(±SD)			
	1h	2h	3h	5h
3a	06(03)	11(02)	13(02) *	03(01)
3b	09(02)	13(00) *	17(01) **	05(00)
3c	15(03)	33(01) **	49(01) **	13(03)
3d	12(02)	26(01) **	40(00) **	18(02) **
3e	17(00)	31(03) **	43(02) **	15(01) *
3f	20(01)	38(02) **	51(01) **	20(00) **
3g	12(03)	15(01) **	21(01) **	10(01)
3h	15(03)	24(00) **	40(02) **	07(03)
3i	06(04)	11(03)	15(02) **	02(02)
Std.	18(02)*	42(00) **	70(02) **	31(01) **
Control	--	--	--	--

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

CONCLUSION

The present study demonstrated the mesoionic sydnones having styrylketone moiety, 4-[1-oxo-(3-substitutedaryl)-2-propenyl]-3-(4-chlorophenyl)sydnones, possess preliminary antibacterial and anti-inflammatory activities. However, the profile of activity shown by these compounds was not in the expected level. Further work in this direction can lead to the identification of a lead compound that could be optimized to get potent compounds.

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CONFLICT OF INTEREST STATEMENT:

None

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