

Synthesis, Antibacterial and Analgesic Activities of 4-[1-Oxo-3-(substituted aryl)-2-propenyl]-3-(4-methylphenyl) sydnones

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Abstract: A series of sydnone derivatives containing styryl ketone moiety, 4-[1-oxo-3-(substituted aryl)-2-propenyl]-3-(4-methylphenyl)sydnones (**6a-i**) has been synthesized and characterized by spectral data. The synthesized compounds have been screened for preliminary antibacterial activity by cup plate method and analgesic activity by the method of acetic acid induced writhing in mice. The chloro and nitro derivatives showed good antibacterial activity against both gram positive and gram negative bacteria while the chloro and furyl derivatives exhibited highest analgesic effect among the series.

Keywords: Synthesis, Sydnone, Styryl ketone, Antibacterial, Analgesic

Introduction

Sydnones are unique, dipolar heteroaromatic members of the general class mesoionic compounds and chemically they are 1, 2, 3-oxadiazolium-5-olates¹. A large number of sydnone derivatives have been synthesized with biological interest²⁻⁵ and reported to possess a wide spectrum of biological activities such as antiviral⁶, antimicrobial⁷, anti-inflammatory⁸, analgesic⁸, anthelmintic⁸, antitumor⁹, free radical scavenging¹⁰ and nitric oxide donor¹¹ activities. The potential value of sydnones as biologically active substances is found in their planar aromatic character, their relatively small size and variation in electron density around the ring. It is thought that the ionic resonance structures of sydnones promote significant interactions with biological molecules. Synthetic compounds containing styryl ketone pharmacophore such as chalcones¹², dehydrogingerone analogs¹³ and benzylidene amino coumarins¹⁴ have been reported to exhibit analgesic and anti-inflammatory activities.

Prompted by these observations, we thought it worth to synthesize compounds containing both sydnone and styryl ketone moieties in the same molecule with an intention of having good biological activities. The present communication describes the synthesis, antibacterial and analgesic activities of 4-[1-oxo-3-(substituted aryl)-2-propenyl]-3-(4-methylphenyl) sydnones.

Experimental

General

The chemicals used were of analytical reagent grade. Melting points were determined in an open capillary and are uncorrected. IR spectra were recorded as KBr disc on Thermo Nicolet 200 FT-IR spectrometer. ¹H NMR spectra in CDCl₃ were obtained on Bruker AC 200 (200 Mhz) spectrometer using TMS as internal standard. Mass spectra were taken on Finnigan-Mat 1020 instrument (*i.e.*, 70 eV). The progress of reactions and the purity of products were analyzed by thin layer chromatography (TLC).

Animals

Albino mice of either sex weighing between 20 and 25 g, maintained on standard diet, water *ad libitum* were used and the usage of animals was approved and permitted by Institutional Animal Ethics Committee (IAEC).

Statistics

The values expressed were mean \pm SD of a triplicate result. Data were analyzed by one-way ANOVA (analysis of variance) followed by Dunnett multiple comparison test using Graph-Pad prism software. The results were considered significant at $p < 0.05$.

Synthesis of *N*-nitroso (4-methylphenyl)glycine(3)

Ethyl chloroacetate (73 g, 0.6 mol), *p*-toluidine (53 g, 0.5 mol) and anhydrous sodium acetate (50 g, 0.6 mol) in 120 mL of alcohol were refluxed for 6 h. The reaction mixture was left overnight at room temperature and poured into ice cold water, a precipitate of *N*-(4-methylphenyl)glycine ethyl ester (**1**) was obtained (84 g, 0.43 mol, 87%, m.p. 48-49 °C). To **1** (84 g, 0.43 mol) sodium hydroxide (20 g, 0.5 mol) in 225 mL of water was added and the mixture was refluxed for 0.5 h. After cooling, the reaction mixture was acidified to pH 2 using hydrochloric acid under cooling. The precipitated *N*-(4-methylphenyl) glycine (**2**) was filtered and washed with cold water (14.5 g, 0.09 mol, 21%, m.p. 115-117 °C). A solution of sodium nitrite (6.3 g, 0.09 mol) in water (20 mL) was added to **2** (14.5 g, 0.09 mol) in water (60 mL) at 0 °C during 0.5 h. Further stirring for an additional 2 h resulted in a clear solution which was acidified with hydrochloric acid. The precipitated **3** was washed with cold water, dried (12.5 g, 0.06 mol, 67%, m.p. 102-104 °C) and recrystallised with methanol.

Synthesis of 3-(4-methylphenyl) sydnone (4)

Acetic anhydride (25 mL) was added to **3** (12.5 g, 0.06 mol). The reaction mixture was left overnight at room temperature (18 h) and poured into cold water. The separated **4** was filtered, dried (9 g, 0.05 mol, 83%) and recrystallised using alcohol. M.p 139-141 °C; IR, cm⁻¹ 1753 (C=O, sydnone), 3139 (C-4 of sydnone C-H stretch); ¹H NMR, δ ppm 2.48 (s, 3H, CH₃), 6.67 (s, 1H, sydnone C-4H) 7.34-7.61 (m, 4H, Ar-H)

Synthesis of 4-acetyl-3-(4-methylphenyl)sydnone (5)

To a suspension of phosphorous pentoxide (21.3 g, 0.15 mol) in 125 mL of dried benzene, in a two necked flask with a reflux condenser equipped with calcium chloride guard tube was

added **4** (8.9 g, 0.05 mol). The mixture was heated to reflux while being stirred. Glacial acetic acid (2.9 mL, 0.05 mol) was added drop wise through a dropping funnel over a period of 10 min. The stirred reaction mixture was heated for 5 h. After cooling to room temperature, the benzene was decanted and the remaining black residue was extracted twice with 20 mL benzene. Combined extract were evaporated to dryness to yield **5** (5.6 g, 0.025 mol, 51%) and it was recrystallised from aqueous alcohol. M.p 82-84 °C; IR, cm^{-1} 1759 (C=O, sydnone), 1668 (COCH₃); ¹H-NMR, δ ppm 2.50 (s, 3H, CH₃), 2.56 (s, 3H, COCH₃), 7.33-7.63 (m, 4H, Ar-H)

General procedure for synthesis of 6a-g

Synthesis of 4-[1-oxo-3-(3,4,5-trimethoxy phenyl)-2-propenyl]-3-(4-methylphenyl) sydnone (6d)

A mixture of **5** (0.22 g, 0.001 mol), sodium hydroxide aqueous solution (0.06 g, 0.0015 mol, 0.3 mL) and ethanol (95%, 2 mL) was cooled (5-10 °C) and to this was added with 3,4,5-trimethoxybenzaldehyde (0.3 g, 0.0015 mol) while being stirred. The reaction mixture was stirred further for 1 h. The precipitated **6d** was filtered washed thoroughly with cold water and recrystallised from ethanol (95%) and ethyl acetate (1:1) mixture, Yield 63%. M.p. 116-118 °C; IR, cm^{-1} 1753 (C=O, sydnone), 1675 (C=O, styryl ketone); ¹H NMR, δ ppm 2.46 (s, 3H, CH₃), 3.92 (s, 9H, OCH₃), 6.8 (d, 1H, α olefinic), 7.81 (d, 1H, β olefinic), 7.11-7.6 (m, 6H, Ar-H). **6c**: ¹H NMR, δ ppm 2.49 (s, 3H, CH₃), 6.76 (d, 1H, α olefinic), 7.39-7.63 (m, 9H, Ar-H and β olefinic) **6a**: MS, m/z 306 (M⁺). Rests of the compounds were synthesized in the similar fashion.

General procedure for Synthesis of 6h-i

Synthesis of 4-[1-oxo-3-(4-hydroxy-3-methoxyphenyl)-2-propenyl]-3-(4-methylphenyl) sydnone (6h)

Into the suspension of **5** (0.22 g, 0.001 mol) and vanillin (0.15 g, 0.001 mol) in 2 mL of ethanol (95%) dry hydrogen chloride gas was passed for 0.5 h under cooling condition (5 °C). The reaction mixture was left overnight at room temperature and poured into cold water. The separated **6h** was filtered, washed, dried in air and recrystallised from ethanol (95%). Yield 55%. M.p 211-213 °C; IR, cm^{-1} 1760 (C=O, sydnone), 1649 (C=O, styryl ketone); ¹H NMR, δ ppm 2.45 (s, 3H, CH₃), 3.93 (s, 3H, OCH₃), 7.0-7.7 (m, 9H, Ar-H and olefinic), 9.89 (s, 1H, OH). Compound **6i** was synthesized in the similar fashion.

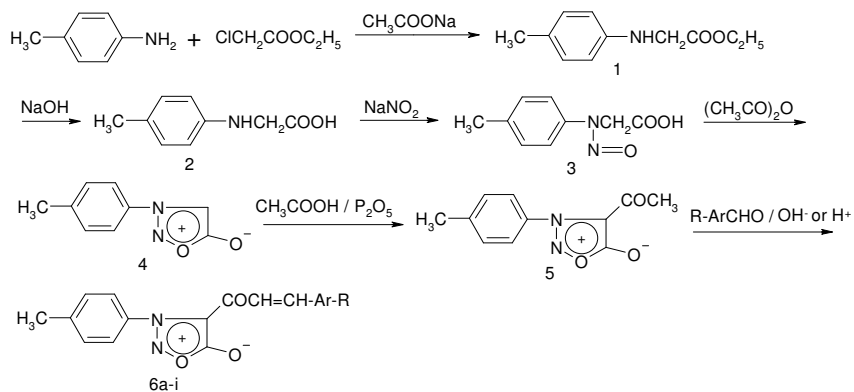
Biological activity

The compounds **6a-i** were screened for preliminary antibacterial activity by Cup Plate method¹⁵ at 10, 20 and 50 μg in DMSO against *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive) and *Escherichia coli*, *Salmonella typhi* (Gram-negative) grown on nutrient agar medium and the diameter of zone of inhibition was taken as a measure of antibacterial activity; norfloxacin at 20 μg concentration was employed as standard drug. The newly synthesized compounds were tested for acute toxicity studies in mice¹⁶ and then screened for analgesic activity by acetic acid induced writhing in mice¹⁷ at 100 mg/kg b.w.; aspirin at 100 mg/kg b.w. was used as standard drug and percentage inhibition of writhes was calculated to assess the analgesic activity.

Results and Discussion

3-(4-Methylphenyl) sydnone (**4**) was prepared according to reported method¹⁸ wherein, 4-toluidine was condensed with ethyl chloroacetate in presence of anhydrous sodium acetate to give ethyl ester of *N*-(4-methylphenyl) glycine (**1**), which upon hydrolysis with sodium hydroxide

afforded *N*-(4-methylphenyl) glycine (**2**); this on nitrosation with sodium nitrite and hydrochloric acid yielded *N*-nitroso-*N*-(4-methylphenyl) glycine (**3**). Thus obtained **3** was cyclo-dehydrated using acetic anhydride to **4**. Treatment of **4** with glacial acetic acid in presence of phosphorous pentoxide¹⁹ afforded 4-acetyl 3-(4-methylphenyl) sydnone **5**. The compounds, 4-[1-oxo-(3-substituted aryl)-2-propenyl]-3-(4-methylphenyl)sydrones **6a-i** were synthesized employing Claisen-Schmidt reaction by treating **5** with different aryl aldehydes in presence of either base or acid (Scheme 1). The physical data of compounds **6a-i** are presented in Table 1.



Scheme-1

Table 1. Physical data and yields of 4-[1-oxo-3- (substituted aryl)-2-propenyl]- 3-(4-methylphenyl) sydrones (**6a-i**).

Compd	Ar	R	Mol Formula	Mol. Wt.	Yield, %	m.p, °C
6a		H	C ₁₈ H ₁₄ N ₂ O ₃	306	62	137-139
6b		H	C ₁₆ H ₁₂ N ₂ O ₄	296	57	128-130
6c		4-Cl	C ₁₈ H ₁₃ N ₂ O ₃ Cl	340.5	60	134-136
6d		3,4,5-OCH ₃	C ₂₁ H ₂₀ N ₂ O ₆	396	63	116-118
6e		4-NO ₂	C ₁₈ H ₁₃ N ₃ O ₅	351	58	138-140
6f		4-N(CH ₃) ₂	C ₂₀ H ₁₉ N ₃ O ₃	349	62	181-182
6g		2-NO ₂	C ₁₈ H ₁₃ N ₃ O ₅	351	56	122-124
6h		4-OH, 3-OCH ₃	C ₁₉ H ₁₆ N ₂ O ₅	352	55	211-213
6i		2-OH	C ₂₁ H ₁₅ N ₃ O ₄	373	61	241-242

IR spectrum of **4** exhibited a strong band at 1753 cm^{-1} due to sydnone ketone and at 3139 cm^{-1} for C-4 of sydnone C-H stretch. The ^1H NMR spectrum of **4** showed a singlet at δ 6.67 for H atom at C-4 of sydnone. The signals at δ 2.48 and δ 7.34-7.61 can be accounted for methyl and aromatic protons respectively.

A band at 1668 cm^{-1} and absence of band at 3139 cm^{-1} exhibited by compound **5** in IR spectrum confirmed the acetylation at C-4 sydnone. The ^1H NMR spectrum of **5**, showed the signal at δ 2.53 and absence of signal at δ 6.67 confirming the acetyl protons at C-4 of sydnone.

The IR spectra of **6a-i** showed the bands due to C=O of sydnone and styryl ketone at $1753\text{-}1760\text{ cm}^{-1}$ and $1649\text{-}1675\text{ cm}^{-1}$ respectively. In ^1H NMR spectra, these compounds showed the doublets due to the protons attached to the carbon atoms of α , β unsaturated ketone moiety at 7.2-7.8, but in most of the cases, were observed merging with aromatic protons. The mass spectrum of **6a** showed the M^+ ion peak at m/e 306 confirming its molecular weight.

All the compounds showed moderate antibacterial activity against both Gram-positive and Gram-negative organisms at 10 and 20 μg concentrations. At 50 μg , compounds **6c** and **6e** exhibited good activity against *Staph. Aureus* and *B. subtilis*, while **6c** and **6i** showed good activity against *E. coli* and **6e** and **6i** possessed good activity against *S. typhi*. Compounds having electron withdrawing substituents such as chloro and nitro at *para* position, for example, **6c** and **6e** showed good activity against both Gram-positive and Gram-negative organisms (Table 2).

Table 2. Antibacterial activity data of **6a-i**.

Compound	Zone of Inhibition, mm											
	<i>Staph. aureus</i>			<i>B. subtilis</i>			<i>E. coli</i>			<i>S. typhi</i>		
	10 μg	20 μg	50 μg	10 μg	20 μg	50 μg	10 μg	20 μg	50 μg	10 μg	20 μg	50 μg
6a	2	6	8	3	7	9	1	9	12	--	5	8
6b	4	7	8	4	8	10	5	12	14	3	9	12
6c	6	8	9	5	9	11	8	16	19	5	12	13
6d	1	5	7	3	6	9	4	10	13	2	8	12
6e	5	7	10	6	9	11	6	11	17	4	10	15
6f	--	4	5	2	5	7	2	8	10	3	6	9
6g	3	7	8	5	9	10	5	10	16	3	8	13
6h	2	7	7	4	8	9	3	9	14	--	6	11
6i	--	3	6	1	5	8	7	13	18	6	10	15
Norfloxacin		12			14			21			17	

No deaths were seen in acute toxicity study of **6a-i** after 24 h of administration of doses up to 1000 mg/kg b.w. But there were few changes in the behavioral response like alertness, touch response and restlessness at 1000 mg/kg b.w. Therefore, $1/10^{\text{th}}$ of the maximum tolerated dose *i.e.*, 100 mg/kg b.w. was chosen for the pharmacological studies.

Compounds **6a-6e** and **6g** showed highly significant analgesic activity ($p < 0.01$) while **6f** and **6i** showed significant activity ($p < 0.05$) against control. Compounds **6b** and **6c** showed highest activity among the series (Table 3). The presence of chloro group at *para* position of the phenyl group that comes from aldehyde enhanced the activity. Trimethoxy group when present on the phenyl ring sustained activity while *N,N*-dimethylamino group at *para* position did not sustain the activity.

Table 3. Analgesic activity data of **6a-i**.

Compound	Mean No. of Writhes (\pm SD)	% Reduction from Control (\pm SD)
6a	54 (06)	25(01)**
6b	46 (05)	36(02)**
6c	47 (09)	35(02)**
6d	53 (11)	26(04)**
6e	52 (06)	28(01)**
6f	59 (07)	18(00)*
6g	55 (05)	24(02)**
6h	61 (10)	15(03)
6i	58 (08)	19(01)*
Aspirin	29 (09)	60(02)**
Control	72 (07)	--

* $p < 0.05$, ** $p < 0.01$

Conclusion

In the present work 4-[1-oxo-3- (substituted aryl)-2-propenyl]-3-(4-methylphenyl) sydnonones were conceived and synthesized with an augmented biological activity. The compounds having electron-withdrawing substituents like chloro and nitro at *para* position exhibited more antibacterial activity. The furyl and *para* chloro phenyl analogs showed good analgesic activity. Thus, 3-(substituted phenyl)sydnone derivatives possessing substituted styrylketone moieties appear to be another interesting source of antibacterial and analgesic compounds. However, the difference in activity profile with structural modifications provides further scope to explore the sydnonones possessing styryl ketone moiety for better bio-activity.

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