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ORIGINAL ARTICLE

A facile microwave assisted one pot synthesis of novel xanthene derivatives as potential anti-inflammatory and analgesic agents

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KEYWORDS

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PASS prediction

Abstract Microwave assisted irradiation of resorcinol and substituted aryl aldehydes using sulfamic acid as catalyst afforded novel 9-aryl-9*H*-xanthene-3,6-diol derivatives (**1a–f**) in good yields. The newly synthesized compounds which were previously selected on the basis of PASS prediction were tested for anti-inflammatory activity using carrageenan-induced rat paw edema and analgesic activity using acetic acid induced writhing and formalin-induced paw edema in mice along with the estimation of gastric ulcerogenicity index. Compounds **1e** and **1f** exhibited significant anti-inflammatory and analgesic activities as compared to standard drug. The study also revealed that compounds (**1a–f**) showed minimum or no ulcerogenicity in mice as that of the standard drug.

1. Introduction

Inflammation is a defensive but exaggerated local tissue reaction in response to exogenous or endogenous insult. It is a complex phenomenon, comprising of biochemical as well as immunological factors. It is recognized by heat, redness, tumor and pain. Traditionally most treatment of inflammation is with non-steroidal anti-inflammatory drugs (NSAID's). The most important mechanism of anti-inflammatory action of NSAIDs is considered to be primarily by inhibition of prostaglandin synthesis (Hunashal et al., 2011). However long usage of NSAID's and selective cyclooxygenase-2 (COX-2) inhibitors give unacceptable side effects such as gastric ulcer (Bush and Imani, 1991) with NSAID's and cardiac toxicity with coxibs. So there is a constant need for discovery of novel and safer anti-inflammatory drugs.

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Xanthene functionality is a key structural element of many biologically active compounds. Xanthene derivatives have been reported to possess various pharmacological properties such as antibacterial (Hideo, 1981), antiviral (Lambert et al., 1997), anti-inflammatory (Poupelin et al., 1978) and CCR1 antagonist (Naya et al., 2003). Xanthenes are of great interest in the lead optimization process of drug discovery. Some evidence has surfaced that suggests the xanthene core structure also may be useful in the design and development of pharmacological agents (Ornstein et al., 1998; Goodell et al., 2006). Thus synthesis of xanthene derivatives is of immense interest. As a result of our investigation using PASS (prediction of activity spectra for substances) studies, it was found that 9-aryl xanthene moiety do possess some anti-inflammatory activity influenced by the substitution pattern of these molecules. The compounds chosen for synthesis and activity determination were selected on the basis of their best prediction values as anti-inflammatory and analgesic as well as ease of their synthesis.

Reported synthetic methodologies involve harsh conditions or longer reaction time leaving considerable scope for development of further clean, facile and efficient process for the synthesis of these important molecules. The organic reactions taking place in aqueous media have recently attracted much attention in synthetic chemistry, not only because water is one of the most abundant, cheapest and environmentally friendly solvents but also because it exhibits unique reactivity and selectivity, which is different from those observed in conventional organic solvents (Demko and Sharpless, 2001; Li, 2005). Apart from this, to overcome the limitations from the catalysis point of view, we started to search for new catalysts having high catalytic activity, easy availability and short reaction time involving simple work-up procedure, and sulfamic acid attracted our attention as it is known to catalyze a number of organic transformations. Recently, sulfamic acid has emerged as a promising solid acid catalyst for acid catalyzed reactions, such as functional group protections and deprotections and the synthesis of isoamyl acetate and polymeric ethers. Moreover, some important organic transformations, including the Beckmann rearrangement (Wang et al., 2004 and Singh et al., 2004) and Bignelli condensations (Li et al., 2003) have been performed successfully in the presence of sulfamic acid. As a continuation of our research devoted to the development of green route methods (Thomas et al., 2009, 2010), herein we report an efficient and convenient method for microwave assisted synthesis of some novel 9-aryl xanthenes derivatives by condensation of aldehydes with resorcinol in the presence of sulfamic acid as catalyst followed by screening for *in vivo* anti-inflammatory and analgesic activities.

2. Experimental

2.1. Activity prediction

In an effort to optimize biological profile, a wide structural diversity of xanthenes possessing the 9-aryl moiety as a salient feature of the xanthene core have been virtually designed, synthesized and investigated for the foresaid pharmacological activity. In order to accelerate search for New Chemical Entities (NCEs), an internet version of the PASS (prediction of biological activity spectra) (http://www.ibmh.msk.su/PASS/Ref.html) was used to predict the anti-inflammatory and analgesic action of different 9-aryl xanthene derivatives.

The technique of PASS is based on the analysis of SARs for the training set currently including about 46,000 drugs, drug candidates and lead compounds whose biological activities are determined experimentally. The set of MNA (multilevel neighborhood atoms) descriptors are generated on the basis of structural formulas presented in the MOL-file (SDF-file) form. Since MNA descriptors are generated for each compound de novo, new descriptors can be obtained upon presentation of a novel structural feature in the compound under study.

Based on the statistics of MNA descriptors for active and inactive compounds from the training set, two probabilities are calculated for each activity: Pa—probability of compound being active and Pi—probability of compound is being inactive. Being probabilities, the Pa and Pi values vary from 0.000 to 1.000 and in general Pa + Pi < 1, since these probabilities are calculated independently.

The PASS predictions can be interpreted and used in a flexible manner-

(i) Only activities with Pa > Pi are considered as possible for a particular compound.
(ii) If Pa > 0.7—the substance is very likely to exhibit activity in experiment, but chance of the substance being analog of a known pharmaceutical agent is also high.
(iii) If 0.5 < Pa < 0.7—the substance is likely to exhibit the activity in the experiment, but the probability is less and the substance is unlike known pharmaceutical agents.
(iv) If Pa < 0.5—the substance is unlikely to exhibit the activity in the experiment. However if the presence of this activity is confirmed in the experiment, the substance might be an NCE.

2.1.1. Selection of possible cognition enhancers

Prediction of biological activity spectra was made for about 200 virtually designed variedly substituted structures from the theoretical calculations on the basis of PASS prediction. The compounds screened were the structures formed from the various combinations of R (H, 2-OH, 4-OH, 3-OCH_3-4-OH, 4-Cl and 4-NO_2) groups in the hydroxylated xanthene moiety. On the basis of prediction results from the database analysis, potential xanthenes were selected for testing their anti-inflammatory and analgesic activities. The compounds screened above were further subjected to 'Lipinski rules of five'. The MIPC (mol inspiration property calculator) program has been utilized (www.molinspiration.com) for calculating the Lipinski descriptors. The log *P* values and the associated parameters for the compounds under consideration have been provided in Table 1 and the probabilities of the test compounds for being active (Pa) are provided in Table 2. On the basis of the above-mentioned criteria, the chosen compounds were synthesized and experimentally tested as anti-inflammatory and analgesic agents.

2.2. Materials and reagents

All research chemicals were purchased from Across organics (NY, USA), Sigma–Aldrich (St. Loius, Missiouri, USA) and

Table 1 Log*P* values and the associated parameters for the compounds under consideration.

Compound	MLog*P*	TPSA	N atoms	M_w
1a	3.985	49.69	22	290.318
1b	3.925	69.918	23	306.317
1c	3.506	69.918	23	306.317
1d	3.324	79.152	25	336.343
1e	4.663	49.69	23	324.763
1f	3.944	95.514	25	335.315

Compound	nON	nOHNH	nViolations	Nrotb
1a	3	2	0	1
1b	4	3	0	1
1c	4	3	0	1
1d	5	3	0	2
1e	3	2	0	1
1f	6	2	0	2

Table 2 The probabilities of being active Pa of the test compounds on the basis of PASS prediction.

Compound	P_a	P_i
1a	0.469	0.060
1b	0.436	0.078
1c	0.467	0.061
1d	0.599	0.020
1e	0.396	0.019
1f	0.318	0.077

used as such for the reactions. Melting point (m.p.) was determined by Veego melting point apparatus (VMP PM, 32/1104) and is uncorrected. Reactions were monitored by thin layer chromatography carried out using pre-coated silica gel plates (E. Merck and Co., Darmstadt, Germany). Infrared (IR) spectra (KBr) were recorded on a FTIR Spectrophotometer (Shimadzu 8400S) with Diffuse Reflectance Attachment. ^{1}H-NMR and ^{13}C-NMR spectra were obtained using NMR Spectrophotometer (Bruker Avance II 400 MHz NMR) with Dimethyl Sulphoxide as solvent. Chemical shifts were expressed in δ ppm relative to tetramethylsilane (TMS) as internal standard. A mass spectrum was obtained on a Hewlett Packard model GCD-1800A Electron Impact mass spectrometer at 70 eV ionizing beam and using direct insertion probe. Elemental analyses of the new compounds (C, H and N) were performed with a Perkin Elmer 2400-CHN Elemental Analyzer instrument and the results were within ±0.4% of the theoretical values.

2.3. Synthesis

2.3.1. Synthesis of 9-aryl-9H-xanthene-3,6-diol derivatives

Resorcinol (2 mM) was reacted with various aromatic aldehydes (1 mM) and sulfamic acid (0.04 mM) as a catalyst in required quantity of water in a two necked round bottom flask (RBF). The mixtures were irradiated in a microwave attached with reflux condenser and constant stirring to avoid risk of high pressure development (Make-Raga's Scientific) at 350 W (110 °C) till the completion of the reaction. The reaction was monitored by TLC (*n*-Hexane:Ethyl Acetate 1:1). After completion of the reaction, the reaction mixture was cooled to room temperature and ice was added along with stirring. The mixture was extracted with ethyl acetate two to three times in a separating funnel and the organic layer was evaporated to dryness. The crude products on recrystallisation from hot ethanol gave the pure 9-aryl xanthene derivatives (**1a–f**). The synthesized compounds were characterized by their melting point and spectral data (IR, ^{1}H-NMR). Tables 3 and 4 represents structure, properties and spectral data of synthesized 9-aryl xanthenes derivatives.

*2.3.1.1. 9-Phenyl-9H-xanthene-3,6-diol (**1a**).* Buff colored powder; Yield 77.34%; m.p. 172–175 °C. IR (KBr): ν/cm^{-1} = 3475–3224 (OH), 3091–3029 (CH), 1610, 1514, 1429 (ring C=C), 1076 (ether C–O); 840, 748, 702 (CH). ^{1}H-NMR (DMSO-d_6): δ ppm = 5.15 (b.s, 2H, -OH), 5.76 (s, 1H, CH), 6.21–7.10 (m, 11H, Ar-H), 6.32 (s, 1H), 6.90 (d, 2H), 7.08 (s, 1H), 7.10 (s, 2H). EIMS (70 eV, m/z) 290 (M+). MW: 290.31 Anal. Calcd for $C_{19}H_{14}O_3$: C, 78.61; H, 4.86. Found: C, 78.34; H, 4.98.

*2.3.1.2. 9-(2-Hydroxyphenyl)-9H-xanthene-3,6-diol (**1b**).* Yellow powder; Yield 78.52%; m.p. 132–134 °C. IR (KBr): ν/cm^{-1} = 3463–3205 (OH), 3151–3037 (CH), 1606, 1508, 1454 (ring C=C), 1076 (ether C–O); 837, 756 (CH). ^{1}H-NMR (DMSO-d_6): δ ppm = 5.43 (s, 3H, -OH), 5.63 (s, 1H, CH), 6.21–6.86 (m, 10H, Ar-H), 6.27 (s, 2H), 6.49 (d, 2H), 6.86 (d, 2H). EIMS (70 eV, *m/z*) 306 (M+). MW: 306.31 Anal. Calcd for $C_{19}H_{14}O_4$: C, 74.50; H, 4.61. Found: C, 74.69; H, 4.68.

*2.3.1.3. 9-(4-Hydroxyphenyl)-9H-xanthene-3,6-diol (**1c**).* Yellow powder; Yield 80.62%; m.p. 129–131 °C. IR (KBr): ν/cm^{-1} = 3292–3178 (OH), 3129–3076 (CH); 1604, 1510, 1431(ring C=C), 1076 (ether C–O), 844, 577 (CH).

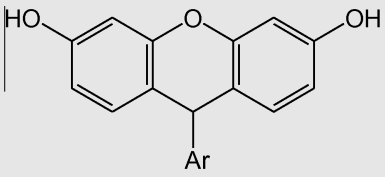
^{1}H-NMR (DMSO-d_6): δ ppm = 5.48 (s, 1H, -OH), 5.69 (s, 2H, -OH), 5.77 (s, 1H, CH), 6.13–6.82 (m, 6H, Ar-H), 6.37 (s, 2H), 6.53 (d, 2H), 6.63 (d, 2H), 6.82 (d, 2H). EIMS (70 eV, m/z) 306 (M+). MW: 306.31 Anal. Calcd for $C_{19}H_{14}O_4$: C, 74.50; H, 4.61. Found: C, 74.76; H, 4.54.

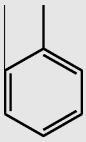
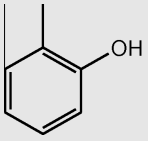
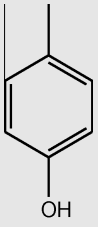
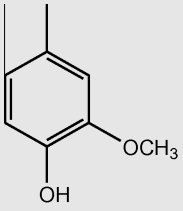
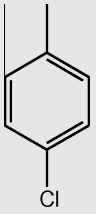
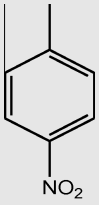
*2.3.1.4. 9-(4-Hydroxy-3-methoxyphenyl)-9H-xanthene-3,6-diol (**1d**).* Orange crystals; Yield 76.54%; m.p. 199–202 °C. IR (KBr): ν/cm^{-1} = 3259–3190 (OH), 3156–3028 (CH); 1604, 1512, 1427 (ring C=C), 1076 (ether C–O), 844, 767 (CH). ^{1}H-NMR (DMSO-d_6): δ ppm = 3.59 (s, 3H, OCH3), 5.23 (s, 2H, -OH), 5.48 (s, 1H, -OH), 5.73 (s, 1H, CH), 6.15–6.98 (m, 9H, Ar-H), 6.35 (s, 2H), 6.37 (s, 2H), 6.94 (d, 2H). EIMS (70 eV, m/z) 336 (M+). MW: 336.34 Anal. Calcd for $C_{20}H_{16}O_5$: C, 71.42; H, 4.79. Found: C, 71.56; H, 4.68.

*2.3.1.5. 9-(4-Chlorophenyl)-9H-xanthene-3,6-diol (**1e**).* Yellow crystals; Yield 82.00%; m.p. > 225 °C. IR (KBr): ν/cm^{-1} = 3510–3328 (OH), 3192–3045 (CH); 1612, 1496, 1431 (ring C=C), 1087 (ether C–O), 844, 767 (CH); 725 (C-l).

^{1}H-NMR (DMSO-d_6): δ ppm = 5.28 (s, 2H), 5.45 (s, 1H), 6.21–6.68 (m, 6H, Ar-H), 6.24 (s,2H), 6.67,6.68 (d,2H), 6.90, 6.93 (d, 2H), 7.18,7.21 (d, 2H). EIMS (70 eV, m/z) 324 (M+), 326 (M + 2H). MW: 324.76 Anal. Calcd for $C_{19}H_{13}ClO_3$: C, 70.27; H, 4.03. Found: C, 70.02; H, 3.88.

Table 3 Chemical structures and properties of 9-aryl xanthenes (**1a–f**).



Compounds	−Ar	Yield (%) (Reaction time)	Rf value[a]	Melting point (°C)[b] (Recrys. solvent)	Solubility	Molecular formula (Molecular weight)	Analysis (%) Calcd/Found C	H	N	M^+ 70 eV EI
1a		77.34 (10 min)	0.33	172–175 (Ethanol)	Ethanol	$C_{19}H_{14}O_3$ (290.31)	78.61 78.34	4.86 4.98	–	290
1b		78.52 (10 min)	0.37	132–134 (Ethanol)	Ethanol	$C_{19}H_{14}O_4$ (306.31)	74.50 74.69	4.61 4.68	–	306
1c		80.62 (10 min)	0.38	129–131 (Ethanol)	Ethanol	$C_{19}H_{14}O_4$ (306.31)	74.50 74.76	4.61 4.54	–	306
1d		76.54 (10 min)	0.40	199–202 (Ethanol)	Ethanol	$C_{20}H_{16}O_5$ (336.34)	71.42 71.56	4.79 4.68	–	336
1e		82.00 (8 min)	0.42	> 225 (Ethanol)	Ethanol	$C_{19}H_{13}ClO_3$ (324.76)	70.27 70.02	4.03 3.88	–	326
1f		82.74 (8 min)	0.46	> 225 (Ethanol)	Ethanol	$C_{19}H_{13}NO_5$ (335.31)	68.06 68.32	3.91 4.14	4.18 4.16	335

[a] Stationary phase: silica gel G, mobile phase: *n*-hexane:ethyl acetate (1:1), iodine vapors as visualizing agent.
[b] All melting points were uncorrected.

*2.3.1.6. 9-(4-Nitrophenyl)-9H-xanthene-3,6-diol (**1f**).* Yellow crystals; Yield 82.74%; m.p. > 225 °C. IR (KBr): ν/cm^{-1} = 3321–3228 (OH), 3116–3078 (CH); 1604, 1512, 1431 (ring C=C), 1345 (symmetric NO), 1091 (ether C-7 O), 844 (Symmetric NO), 744, 702, 628 (CH). ^{1}H-NMR (DMSO-d_6): δ ppm = 5.38 (s, 2H), 5.63 (s, 1H), 6.30–6.73 (m, 6H, Ar-H), 6.30 (s, 2H), 6.83,6.88 (d, 2H), 7.30, 7.33 (d,2H), 8.18, 8.21 (d,2H). ^{13}C-NMR (DMSO-d_6, 62.9 MHz) δ 173.5, 155.3,

Table 4 ^{1}H-NMR spectral data of the synthesized compounds.

Compound No.	^{1}H-NMR δ (ppm)
1a	5.15 (b.s, 2H, -OH), 5.76 (s, 1H, CH), 6.21–7.10 (m, 11H, Ar-H), 6.32 (s,1H), 6.90 (d, 2H), 7.08 (s, 1H)
1b	5.43 (s, 3H, -OH), 5.63 (s, 1H, CH), 6.21–6.86 (m, 10H, Ar-H), 6.27 (s, 2H), 6.49 (d, 2H), 6.86 (d,2H)
1c	5.48 (s, 1H, -OH), 5.69 (s, 2H, -OH), 5.77 (s, 1H, CH), 6.13–6.82 (m, 6H, Ar-H), 6.37 (s, 2H), 6.53 (d, 2H), 6.63 (d, 2H), 6.82 (d, 2H)
1d	3.59 (s, 3H, OCH_3), 5.23 (s, 2H, -OH), 5.48 (s, 1H, -OH), 5.73 (s, 1H, CH), 6.15–6.98 (m, 9H, Ar-H), 6.35 (s, 2H), 6.37 (s, 2H), 6.94 (d, 2H)
1e	5.28 (s, 2H), 5.45 (s, 1H), 6.21–6.68 (m, 6H, Ar-H), 6.24 (s,2H), 6.67,6.68 (d,2H), 6.90, 6.93 (d, 2H), 7.18,7.21 (d, 2H)
1f	5.38 (s, 2H), 5.63 (s, 1H), 6.30–6.73 (m, 6H, Ar-H), 6.30 (s, 2H), 6.83,6.88 (d, 2H), 7.30, 7.33(d,2H), 8.18,8.21(d,2H)

147.0, 146.1, 139.7, 131.0, 130.1, 122.4, 121.1, 113.5, 103.6 EIMS (70 eV, m/z) 335 (M+). MW: 335.31 Anal. Calcd for $C_{19}H_{13}NO_5$: C, 68.06; H, 3.91; N, 4.18. Found: C, 68.32; H, 4.14; N, 4.00.

2.4. Pharmacology

2.4.1. Experimental animals

Wistar rats (150–220 g) and Swiss albino mice (20–25 g) of either sex were obtained from National Toxicological Centre (NTC) Pune and Serum India Ltd. Pune (India) respectively for the experimental studies. The animals were housed in polypropylene cages at a room temperature (25 ± 2 °C) with 12 h of light and dark cycles and had free access to standard pellet diet and water *ad libitum*. On the day before the treatments, food was withdrawn, but the animals were allowed free access of water. The allocation of animals to different groups was randomized and the experiments were carried out under blind conditions. Animals used in the present study were cared for as per guidelines of the Committee for the purpose of control and supervision on experiments on animals (CPCSEA), Dept. of Animal Welfare, Govt. of India procedures involving animals and their care were conducted in conformity with international laws and policies and animal studies accepted by the Institutional Animal Ethics Committee **(IAEC Reg. No. DYPIPSR/IEAC/08-09/P-26).**

2.4.2. Acute toxicity determination

The acute toxicity study for the test drugs was carried out in mice according to the OECD guidelines (OECD-423, 2004). Albino mice of either sex weighing between 20 and 25 g were divided into six groups of six animals each. Animals were starved for 24 h with water *ad libitum* prior to test. On the day of the experiment, test drugs were administered at graded doses up to 2000 mg/kg, p.o. and animals were monitored individually and continuously for 30 min, 2 h and up to 24 h to detect any changes in the autonomic or behavioral responses and also for tremors, convulsions, excessive salivation, diarrhea, lethargy, sleep and coma. The animals were then monitored for any mortality for the following 14 days. As there was no toxic reaction or mortality observed, the test drugs were found to be devoid of any toxicity up to a maximum dose of 2000 mg/kg body weight.

2.4.3. Anti-inflammatory activity

In vivo anti-inflammatory activity was evaluated by carrageenan induced rat paw edema assay model (Winter et al., 1962). Rats were divided into different groups each consisting of 8 animals and administered with 0.5% sodium CMC (10 ml/kg p.o.) as control group, reference standard indomethacin (10 mg/kg p.o.) and test compounds **1a–f** were administered at the dose of 30, 60 and 90 mg/kg p.o. One hour after dosing, the rats were challenged by a sub plantar injection of 0.05 ml of 1% solution of carrageenan (Sigma, St. Louis, Missouri, USA) in sterile distilled water which was administered to the left hind footpad of each animal. The total increase in edema volume is measured by mercury displacement technique with the help of the plethysmometer (UGO Basile, Italy) at 1, 2, 3, 4, 5 and 6 h after carrageenan treatment. The percentages of the inhibition in the treated animals versus control group were calculated at each 60 min interval by using the following formula:

$$\%\text{Inhibition} = \text{Ec} - \text{Et}/\text{Ec} \times 100 \quad (1)$$

Ec = Mean of edema volume of control group.
Et = Mean of edema volume of test group.

2.4.4. Gastric ulcerogenicity

Animals were subjected to acute ulcerogenic effect after administration of control group 0.5% sodium CMC (10 ml/kg p.o.), test drugs (30, 60 and 90 mg/kg p.o.) and standard Indomethacin (10 mg/kg p.o.). Twenty four hours after the dosing, rats under deep ether anesthesia were sacrificed so that the stomach could be removed, opened along the curvature and cleaned gently by dipping in saline. Then the stomachs were examined for lesions under a dissecting microscope. Stomachs exhibiting one or more ulcers were considered positive (Hafez et al., 2010).

2.4.5. Analgesic activity

2.4.5.1. Acetic acid induced writhing in mice (Koster et al., 1959). The mice were fasted overnight and provided water *ad libitum* and divided into different groups each consisting of eight animals. Mice were administered with 0.5% sodium CMC (control, 10 ml/kg, p.o.), Aspirin (300 mg/kg, p.o.) and Indomethacin (10 mg/kg p.o.) as reference standards and test compounds (**1a–f**) at the dose of 150 and 300 mg/kg. The number of writhing episodes was counted for 30 min following acetic acid injection (0.6% v/v). A significant reduction in the number of writhings by any treatment as compared to the number of writhings in control animals was considered a positive analgesic response. The analgesic activity was expressed as a percentage change from writhing controls.

2.4.5.2. Formalin-induced paw edema bioassay. The antinociceptive effect was also evaluated by this sub-acute inflammatory model. The mice were fasted overnight and pro-

vided water *ad libitum* and divided into different groups each consisting of 5 animals. After administration of the test drugs and subplantar route of formalin 0.02 ml (1%), the time (seconds) which each mice spent licking was observed. Mice were administered with 0.5% sodium CMC (10 ml/kg p.o.) as control group, pentazocin (10 mg/kg i.p.) and Indomethacin (10 mg/kg p.o.) as reference standards and test compounds (**1a–f**) at the dose of 150 mg/kg p.o. and 300 mg/kg p.o. The amount of time spent licking the injected paw was timed, and was considered as indicative of pain. The first of the nociceptive response normally peaked 5 min after formalin injection and the second phase 15–30 min after formalin injection, representing the neurogenic and inflammatory pain responses, respectively (Hunskaar and Hole, 1987). The animals were pretreated with samples 1 h before being challenged with buffered formalin, and the responses were observed for 30 min.

2.4.6. Statistical analysis

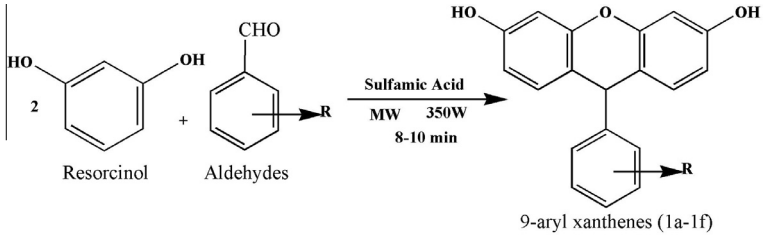
All values in the texts are expressed as means ± standard error of the mean (SEM) for n observations, where n represents number of animals studied. The results were analyzed by one way analysis of variance (ANOVA) followed by Dunnett's test. '*p*' values more than 0.05 was considered as non significant, less than 0.05 as significant and less than 0.01 as extremely significant compared with control group.

3. Results and discussion

3.1. Chemistry

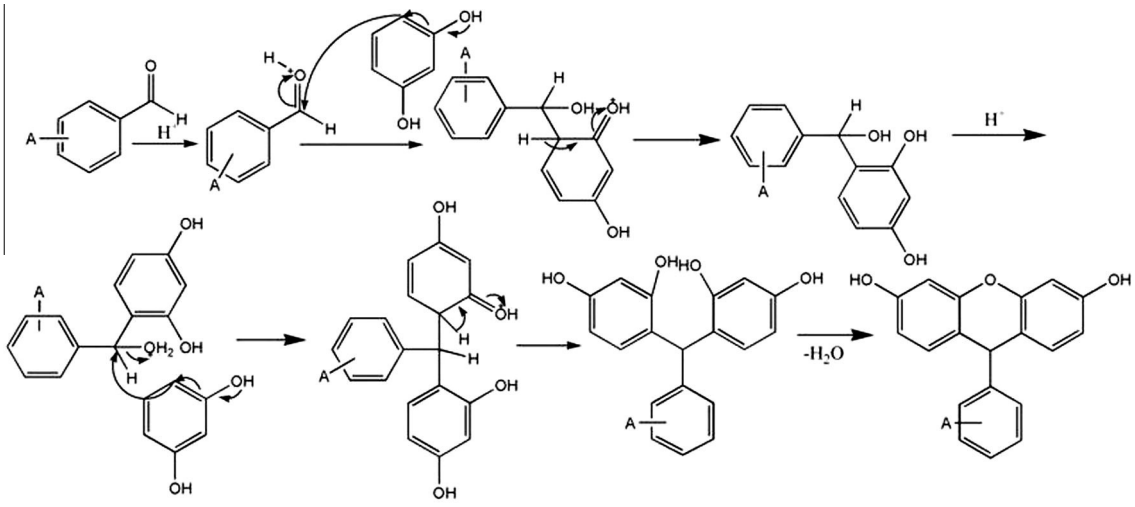
The use of sulfamic acid as a catalyst to bring about the condensation of resorcinol with different aryl aldehydes afforded the final products (**1a–f**) in good yields. The best results were obtained using 0.04 mmol of the catalyst. Using lower amounts of the catalyst resulted in lower yields, while higher amounts of the catalyst did not affect the reaction rate and yields. However in the absence of catalyst, the yield of the product was found to be very low wherein starting materials remained intact along with some undesired impurities. Another advantage of using sulfamic acid as a catalyst is in its ability to provide conditions which are mild enough not to damage moieties such as methoxy substituent which often undergoes cleavage in strongly acidic reaction conditions.

The synthetic route for the synthesis of compounds (**1a–f**) with plausible mechanism is outlined in Schemes 1 and 2. Based on the synthetic method for fluorescein synthesis, resorcinol which undergoes electrophilic reactions same as phenol was selected for the xanthene synthesis. The presence of two hydroxyl groups present in resorcinol strongly activates the ortho and para positions of the benzene ring. The ortho–ortho position with respect to each hydroxyl group is sterically hindered for bulkier substitution and hence the possible



R= H (1a), 2-OH (1b), 4-OH (1c), 3-OCH_3-4-OH (1d), 4-Cl (1e), 4-NO_2 (1f)

Scheme 1 Synthesis of 9-aryl xanthene derivatives by microwave method.



Scheme 2 Reaction mechanism.

Table 5 Carrageenan-induced paw edema and incidence of gastric ulcers in rats.

Compound (n = 8)	Dose (mg/kg p.o.)	Dose (mg/kg p.o.)						% Animals with ulcers
		Edema volume (ml) mean ± SEM (% inhibition)						
		60 min	120 min	180 min	240 min	300 min	360 min	
Control	10	0.45 ± 0.027	0.57 ± 0.033	0.64 ± 0.046	0.76 ± 0.045	0.93 ± 0.062	0.87 ± 0.052	–
Indo	10	0.15 ± 0.009** (66.67)	0.14 ± 0.008** (75.43)	0.12 ± 0.008** (81.25)	0.098 ± 0.006** (87.11)	0.091 ± 0.004** (90.22)	0.09 ± 0.005** (89.66)	7/8 (87.5)
1a	30	0.44 ± 0.035 (2.22)	0.54 ± 0.040 (5.26)	0.59 ± 0.035 (7.81)	0.66 ± 0.049 (13.15)	0.70 ± 0.059* (24.73)	0.63 ± 0.028* (27.58)	0/8 (0)
	60	0.43 ± 0.035 (4.44)	0.52 ± 0.041 (8.77)	0.55 ± 0.034 (14.06)	0.64 ± 0.042 (15.78)	0.63 ± 0.055* (32.25)	0.57. ± 0.029** (34.48)	0/8 (0)
	90	0.42 ± 0.037 (6.66)	0.49 ± 0.039 (14.03)	0.49 ± 0.036* (23.43)	0.57 ± 0.033* (25.0)	0.55 ± 0.049** (40.86)	0.50 ± 0.024** (42.52)	1/8 (12.5)
1b	30	0.43 ± 0.029 (4.44)	0.53 ± 0.038 (7.01)	0.57 ± 0.030 (10.93)	0.64 ± 0.045 (15.78)	0.62 ± 0.049* (33.33)	0.57 ± 0.029** (34.48)	0/8 (0)
	60	0.42 ± 0.031 (6.66)	0.52 ± 0.039 (8.77)	0.54 ± 0.035 (15.62)	0.60 ± 0.042* (21.05)	0.55 ± 0.042** (40.86)	0.51. ± 0.027** (41.37)	0/8 (0)
	90	0.41 ± 0.033 (8.88)	0.48 ± 0.038 (15.79)	0.51 ± 0.035* (20.31)	0.56 ± 0.040* (26.31)	0.49 ± 0.031** (47.31)	0.47 ± 0.022** (45.97)	0/8 (0)
1c	30	0.42 ± 0.035 (6.66)	0.52 ± 0.039 (8.77)	0.55 ± 0.035 (14.06)	0.60 ± 0.031* (21.05)	0.50 ± 0.020** (46.23)	0.48 ± 0.024** (44.82)	0/8 (0)
	60	0.41 ± 0.028 (8.88)	0.50 ± 0.032 (12.28)	0.51 ± 0.022 (20.31)	0.56 ± 0.019* (26.31)	0.45 ± 0.029** (51.61)	0.44. ± 0.018** (49.42)	0/8 (0)
	90	0.40 ± 0.028 (11.11)	0.49 ± 0.035 (14.03)	0.50 ± 0.018* (21.87)	0.51 ± 0.022* (32.39)	0.42 ± 0.016** (54.83)	0.41 ± 0.019** (52.87)	0/8 (0)
1d	30	0.40 ± 0.030 (11.11)	0.50 ± 0.039 (12.28)	0.53 ± 0.035 (17.18)	0.58 ± 0.048* (23.68)	0.48 ± 0.031** (48.38)	0.46 ± 0.029** (47.12)	1/8 (12.5)
	60	0.39 ± 0.031 (13.33)	0.48 ± 0.039 (15.78)	0.48 ± 0.026* (25.0)	0.51 ± 0.038* (32.89)	0.42 ± 0.022** (54.83)	0.41. ± 0.021** (52.87)	1/8 (12.5)
	90	0.38 ± 0.025 (15.55)	0.47 ± 0.038 (17.54)	0.46 ± 0.025* (28.12)	0.46 ± 0.029** (39.47)	0.38 ± 0.022** (59.13)	0.36 ± 0.020** (58.62)	2/8 (25)
1e	30	0.42 ± 0.029 (6.66)	0.48 ± 0.039 (15.78)	0.48 ± 0.024* (25.0)	0.43 ± 0.039** (43.42)	0.39 ± 0.028** (58.06)	0.37 ± 0.020** (57.47)	2/8 (25)
	60	0.39 ± 0.028 (13.33)	0.46 ± 0.032 (19.29)	0.41 ± 0.022** (35.93)	0.36 ± 0.029** (52.63)	0.34 ± 0.016** (63.44)	0.33 ± 0.019** (62.06)	4/8 (50)
	90	0.40 ± 0.025 (11.11)	0.44 ± 0.034* (22.80)	0.36 ± 0.027** (43.75)	0.33 ± 0.022** (56.57)	0.30 ± 0.016** (67.74)	0.29 ± 0.019** (66.66)	4/8 (50)
1f	30	0.41 ± 0.032 (9.75)	0.49 ± 0.035 (14.03)	0.50 ± 0.034* (21.87)	0.54 ± 0.045** (28.94)	0.42 ± 0.020** (54.83)	0.38 ± 0.031** (56.32)	1/8 (12.5)
	60	0.40 ± 0.035 (11.11)	0.46 ± 0.041 (19.29)	0.46 ± 0.031* (28.12)	0.48 ± 0.029** (36.84)	0.36 ± 0.016** (61.29)	0.34. ± 0.019** (60.91)	1/8 (12.5)
	90	0.37 ± 0.034 (17.77)	0.45 ± 0.039* (21.05)	0.43 ± 0.027* (32.81)	0.42 ± 0.029** (44.73)	0.32 ± 0.021** (65.59)	0.30 ± 0.018** (65.51)	2/8 (25)

Each value is the mean ± SEM of eight rats. Statistical analysis by one way ANOVA followed by Dunnett's test.
Control: 0.5% sodium CMC solution in distilled water (10 ml/kg p.o.).
Indo: Reference standard Indomethacin (10 mg/kg p.o.).
1a: 9-Phenyl-9*H*-xanthene-3,6-diol.
1**b**: 9-(2-Hydroxyphenyl)-9*H*-xanthene-3,6-diol.
1c: 9-(4-Hydroxyphenyl)-9*H*-xanthene-3,6-diol.
1d: 9-(4-Hydroxy-3-methoxyphenyl)-9*H*-xanthene-3,6-diol.
1e: 9-(4-Chlorophenyl)-9*H*-xanthene-3,6-diol.
1f: 9-(4-Nitrophenyl)-9*H*-xanthene-3,6-diol.
* $p < 0.05$, compared with control.
** $p < 0.01$, compared with control.

substitution is at the para position. The condensation of resorcinol with different substituted aldehydes gave 75–85% yield irrespective of the reactivity factors involved with benzaldehyde substituted with electron donating groups like the hydroxy group and the electron withdrawing groups like the nitro, chloro substituted aldehydes. Apart from this, the presence of water was found in this case to strongly enhance the reaction rates which may be attributed to the presence of a polar group compound in the reaction enabling some degree of solubility in water.

The series of 9-aryl-9*H*-xanthene-3,6-diol derivatives with the xanthenes nucleus synthesized by the microwave method

Table 6 Acetic acid writhing and formalin tests of 9-aryl xanthene derivatives (150 mg/kg) in mice.

Compound (n = 5)	Dose (mg/kg p.o.)	Total no. of writhings (Mean ± SEM) (% inhibition)	Time spent with licking (sec) (Mean ± SEM) (% inhibition)	
			Phase I (0–10 min)	Phase II (15–30 min)
Control	10	40.0 ± 2.19	161.2 ± 2.42	131.0 ± 3.05
Indomethacin	10	9.40 ± 0.60** (76.5)	121.66 ± 4.41** (24.53)	70.33 ± 3.83** (46.67)
Pentazocin	10 (i.p.)	–	82.67 ± 4.10** (48.72)	54.33 ± 2.96** (58.53)
Aspirin	300	15.40 ± 1.30** (61.5)	–	–
1a	150	16.8 ± 1.40** (58.0)	152.2 ± 1.98 * (5.58)	94.80 ± 3.29** (27.63)
1b	150	35.2 ± 3.16(12.0)	156.8 ± 1.68(2.72)	118.8 ± 2.33(9.31)
1c	150	32.8 ± 1.85(18.0)	151.0 ± 1.66 * (5.95)	118.4 ± 5.68(9.61)
1d	150	17.8 ± 1.49** (55.5)	151.2 ± 1.56 * (6.20)	103.6 ± 1.72** (20.91)
1e	150	14.2 ± 1.38** (64.5)	150.8 ± 2.37 * (6.45)	90.40 ± 2.71 ** (31.0)
1f	150	30.2 ± 2.67*(24.5)	154.6 ± 1.77(4.09)	117.4 ± 3.95* (10.38)

Each value is the mean ± SEM of five mice. Statistical analysis by one way ANOVA followed by Dunnett's test.
Control: 0.5% sodium CMC solution in distilled water (10 ml/kg p.o.).
1a: 9-Phenyl-9*H*-xanthene-3,6-diol.
1**b**: 9-(2-Hydroxyphenyl)-9*H*-xanthene-3,6-diol.
1c: 9-(4-Hydroxyphenyl)-9*H*-xanthene-3,6-diol.
1d: 9-(4-Hydroxy-3-methoxyphenyl)-9*H*-xanthene-3,6-diol.
1e: 9-(4-Chlorophenyl)-9*H*-xanthene-3,6-diol.
1f: 9-(4-Nitrophenyl)-9*H*-xanthene-3,6-diol.
* $p < 0.05$, compared with control.
** $p < 0.01$, compared with control.

Table 7 Acetic acid writhing and formalin tests of 9-aryl xanthene derivatives (300 mg/kg) in mice.

Compound (n = 5)	Dose (mg/kg p.o.)	Total no. of writhings (Mean ± SEM) (% inhibition)	Time spent with licking (sec) (Mean ± SEM) (% inhibition)	
			Phase I(0–10 min)	Phase II(15–30 min)
Control	10	41.4 ± 1.80	164.60 ± 2.06	132.2 ± 2.51
INDO	10	9.8 ± 0.73** (76.32)	123.0 ± 1.15** (25.27)	71.0 ± 3.05** (46.29)
Pentazocin	10 (i.p.)	–	85.0 ± 2.30** (48.35)	56.33 ± 2.33** (57.39)
Aspirin	300	14.4 ± 1.26** (65.21)	–	–
1a	300	15.0 ± 1.14** (63.76)	152.8 ± 5.41* (7.16)	93.4 ± 2.78** (29.34)
1b	300	34.6 ± 2.69(16.42)	159.4 ± 1.86(3.16)	123.2 ± 2.87(6.80)
1c	300	32.4 ± 2.12* (21.73)	154.8 ± 2.26(5.95)	121.8 ± 2.26(7.86)
1d	300	16.6 ± 1.56** (59.90)	151.8 ± 1.77* (7.77)	99.2 ± 2.45** (24.96)
1e	300	13.6 ± 1.12** (67.14)	151.4 ± 2.69* (8.01)	88.4 ± 2.54** (33.13)
1f	300	29.8 ± 2.67* (28.01)	154.0 ± 2.34(5.22)	116.2 ± 4.29* (12.10)

Each value is the mean ± SEM of five mice. Statistical analysis by one way ANOVA followed by Dunnett's test.
Control: 0.5% sodium CMC solution in distilled water (10 ml/kg p.o.).
1a: 9-Phenyl-9*H*-xanthene-3,6-diol.
1**b**: 9-(2-Hydroxyphenyl)-9*H*-xanthene-3,6-diol.
1c: 9-(4-Hydroxyphenyl)-9*H*-xanthene-3,6-diol.
1d: 9-(4-Hydroxy-3-methoxyphenyl)-9*H*-xanthene-3,6-diol.
1e: 9-(4-Chlorophenyl)-9*H*-xanthene-3,6-diol.
1f: 9-(4-Nitrophenyl)-9*H*-xanthene-3,6-diol.
* $p < 0.05$ compared with control,
** $p < 0.01$.

was characterized by the presence of strong band at 1072–1091 cm^{-1} for the ether linkage formed, considered to be a strong confirmation for formation of xanthene nucleus. Another piece of evidence for cyclization is the appearance of singlet signal equivalent to 1 proton in the ^{1}H-NMR spectrum between 5.9 and 6.1 ppm (C-9, CH) which represents the formation of the xanthenes nucleus. The mass spectra and elemental analysis further confirmed the assigned structures. The physical and spectroscopic data of the synthesized compounds are given in Tables 3 and 4.

3.2. Pharmacology

All the newly synthesized 9-aryl xanthene derivatives were tested for *in vivo* anti-inflammatory, analgesic, and ulcerogenic activities.

3.2.1. Anti-inflammatory activity

Carrageenan induced paw edema model is used to study and evaluate the effects of acute inflammation involving various types of inflammatory mediators like histamine, serotonin, bradykinin and prostaglandin (Vinger et al., 1987). The anti-inflammatory activity of synthesized derivatives (**1a–f**) were assessed from their ability to inhibit the paw edema induced by carrageenan in mice and activity was expressed as "mean increase in paw volume ± SEM", in terms of millimeters (mm) and percentage inhibition in paw volume by different doses of the compounds. As observed from the results in Table 5, the compounds (**1a–f**) possessed significant anti-inflammatory activity at second phase (at a time interval between 180 and 360 min) at a dose of 30, 60 and 90 mg/kg dose p.o.

Edema produced by carrageenan is a biphasic event in which the involvement of the cyclooxygenase products of arachidonic acid metabolism and the production of reactive oxygen species are well established. (Smith et al., 1974). If the inhibition is more effective in the first phase of carrageenan-induced edema, it indicates that the anti-inflammatory effect is taking place through the inhibition of the pathway mediated by is histamine and serotonin. In addition, if the inhibition is more effective in the second phase of carrageenan-induced edema, it indicates that the anti-inflammatory effect is taking place through the inhibition of the pathway mediated by prostaglandin (PG) (Holsapple et al., 1980). According to this literature information, it might be stated that the synthesized xanthene derivatives (**1a–f**) exhibit their anti-inflammatory effect through the PG mediated mechanism (Table 5).

3.2.2. Ulcerogenic activity

In order to determine the extent of these side effects, the compounds were further tested for ulcerogenicity. The ulcerogenic liabilities of compounds **(1a–f)** were either absent or less than that of indomethacin at all three graded doses. The results indicating% inhibition of inflammation and% of animals found ulcerogenic has been shown in Table 5.

3.2.3. Analgesic activity

3.2.3.1. Acetic acid-induced writhing test (Gonzalez et al., 2001). Acetic acid induces pain by increasing fluids of PGE2 and PGF2α (Deraedt et al., 1980) at the peritoneal receptors. It has been postulated that acetic acid acts indirectly by inducing the release of endogenous mediators, which in turn stimulates the nociceptive neurons that are sensitive to non-steroidal anti-inflammatory drugs and narcotics (Bentley et al., 1983; Collier et al., 1968). In the acetic acid induced writhing test the analgesic activity of the compounds are expressed as "mean increase in latency after drug administration ± SEM" relative to control and percentage inhibition in writhing reflex. Although the derivatives 1b, 1c showed some activity, it is not significant statistically. Among the remaining, the derivatives **1f**, **1a**, **1d** and **1e** exhibited significant activity. In the acetic acid-induced writhing test, derivative **1e** exhibited higher analgesic activity than aspirin with percentage inhibition values 64.50%, 67.14%, respectively, at 100 and 150 mg/kg dose level.

3.2.3.2. Formalin induced model. In the formalin test, pretreatment of mice with the 9-aryl xanthene derivatives (**1a–f**) at the doses of 150 and 300 mg/kg had significant effect during the first phase of the test (0–5 min) and the second phase (15–30 min) (Tables 6 and 7) except derivative **1b** which was not having significant activity at both the phases. The formalin test assesses the behavioral response to injection of dilute formation into the paw of an animal. The formalin-induced pain test defines two distinct periods of response, i.e. early response (tonic pain response) and late phase (inflammatory pain response). The response consists of licking and elevation (lifting) of the injected paw, flinching, and also protection of the paw from full pressure when walking or resting (Porro and Cavazzuti, 1993). Drugs that act primarily on the CNS inhibit both phases equally while peripherally acting drugs inhibit the late phase (Tjolsen et al., 1992). The ability of 9-aryl xanthenes derivatives to have an effect on the second phase indicates that the 9-aryl xanthenes contain an active analgesic principle acting peripherally and not through CNS as shown by indomethacin and pentazocin.

4. Conclusion

- In summary, 9-aryl-9*H*-xanthene-3,6-diol derivatives were synthesized by microwave assisted synthesis using sulfamic acid as a catalyst and water as a solvent with satisfactory yields. Compared to other conventional methods, the reported route of synthesis provided several advantages such as mild reaction conditions, shorter reaction times thus supporting to the cause of Green Chemistry.
- On the basis of anti-inflammatory and analgesic studies it was observed that test compounds **1e** and **1f** showed comparable activity as compared to standard drugs.
- Results also showed that the synthesized 9-aryl xanthenes derivatives are less ulcerogenic as compared to standard drug indomethacin.
- In conclusion, the findings of the above study are necessary as they shed light onto a series of novel 9-aryl xanthenes as lead compounds having anti-inflammatory and analgesic effects which can be further exploited in the search of New Chemical Entities (NCE's).

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