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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, docking studies and molecular iodine catalyzed synthesis of benzo[*a*]xanthen-one derivatives as *hyaluronidase* inhibitors

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Abstract A series of novel benzo[*a*]xanthen-11(12*H*)-one derivatives **4a–m** were designed and subjected to docking studies. The title compounds were synthesized from 3-aryl-4-formylsydnones **1a–m**, β-naphthol and dimedone in presence of molecular iodine as a catalyst and evaluated for their in vitro inhibitory effects on the *hyaluronidase*.

Keywords Sydnone · Dimedone · Molecular iodine · Molecular docking · Hyaluronidase · Anti-inflammatory activity

Introduction

Xanthenes and benzoxanthenes are the most important classes of biodynamic heterocycles and hence their synthesis has received much attention especially in the field of medicinal and pharmaceutical chemistry (Lambert et al., 1997, Cao et al., 2007, Djoufack et al., 2010, Niu et al., 2012, Tao et al., 2008, Zelefack et al., 2009). Sydnones (Browne and Harrity, 2010; Stewart, 1964; Baker and Ollis, 1957) are the most prominent five-member non benzenoid hetero-aromatic compounds. They are the well-defined class of mesoionic compounds and have oxadiazole skeleton exhibiting aromaticity through a net separation of formal positive and negative charges. Since their discovery in 1935, sydnones have attracted significant attention from a wide variety of researchers due to the interesting structural and physicochemical properties. Their derivatives have been recognized to exhibit a wide spectrum of bioactivities including antibacterial (Mohamed et al., 2004), anticancer (Christopher and Charles, 2003) and anti-inflammatory (Wagner and Hill, 1974) etc.

During drug development of non-steroidal anti-inflammatory drugs (NSAIDs) it was believed that serotonin was a possible mediator for inflammation. Consequently, serotonin was used as a lead for anti-inflammatory agents and on these bases the anti-inflammatory drug *Indomethacin* was developed. So also sydnone appended to dihydropyrimidines (DHPMs) have been found to show anti-inflammatory properties (Tegginamath et al., 2013) (Fig. 1). Structure activity relationship (SAR) for the above drug development results and the structures of various class of clinically established NSAID's confirm to a broadly accepted pharmacophore (Fig. 2) which suggests four important structural requirements for anti-inflammatory action on *hyaluronidase* enzyme *viz*., i) aromatic ring **A** and naphthalene nucleus **C** (help in crossing biological membrane) fused with six member heterocyclic unit i.e., pyran **B** (required for bioactivity) ii) Pyran is further attached to a nitrogen containing aromatic/heterocyclic unit **D** (pharmacophore). Based on the above observations, the lead molecules were designed in this report (Fig. 3).

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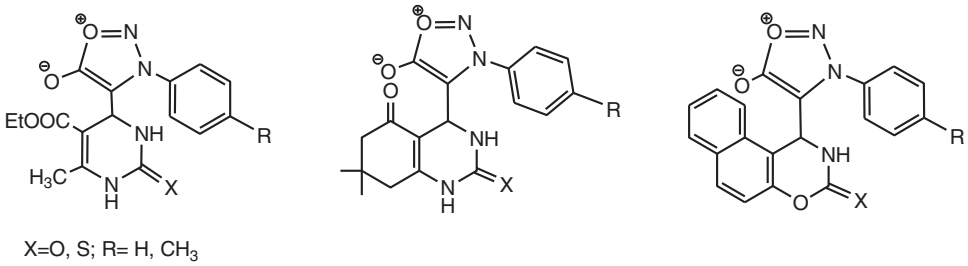
Fig. 1 DHPMs containing sydnone



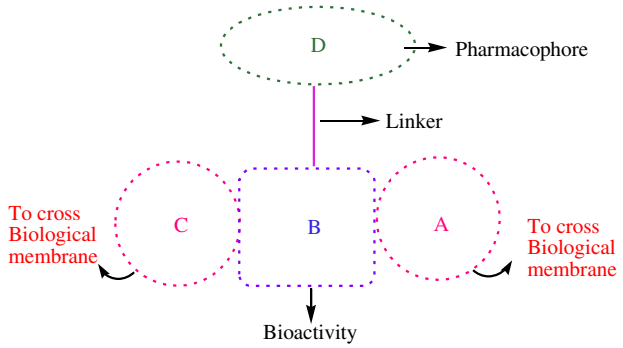


Fig. 2 SAR of anti-inflammatory drug

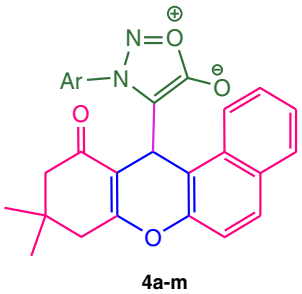


Fig. 3 Newly designed xanthenes containing sydnone

After designing the molecules based on the SAR studies, we planned to carry out the docking. Molecular docking study is a well-established technique to determine the interaction of two molecules and find the best orientation of ligand that would form a complex with overall minimum energy (Thangasamy et al., 2014). *Hyaluronidase* is one of the most important enzymes in the development of many diseases that degrade hyaluronic acid (HA). HA is ubiquitous component of the extracellular matrix of vertebrates which is a linear polysaccharide composed of a repeating unit with the structure [D-Glucuronic acid (1-β-3) *N*-acetyl-D-glucosamine (1-β-3)]. HA can form highly viscous solution and thereby influence the properties of this matrix. It has been implicated in many biological processes such as fertilization, embryonic development, cell migration and differentiation, inflammation, growth including metastasis of tumor cells. The activity of *hyaluronidase* is increased during chronic inflammatory conditions including inflammatory joint disease (Foschi, 1990; Laurent and Fraser, 1992). Therefore, *hyaluronidase* is one of the drug targets to exhibit the anti-inflammatory property and hence our efforts were concentrated to develop *hyaluronidase* inhibitors to maintain the HA level which may lead to the development of new class of therapeutics of NSAIDs.

All the newly designed molecules **4a–m** were docked within the "active site" of the *hyaluronidase* (*hyaluronate lyase* PDB ID: 1W3Y) enzyme with different orientations. The protein structure files and the ligand molecules were drawn by using Surflex-Dock program interfaced with Sybyl-X 2.0. The acting forces of this binding mode are mainly hydrogen bonding, electrostatic forces, Vander Waal's forces, and hydrophobic interaction due to non-polar residue interaction. Docking results were followed by in vitro *hyaluronidase* inhibition analysis.

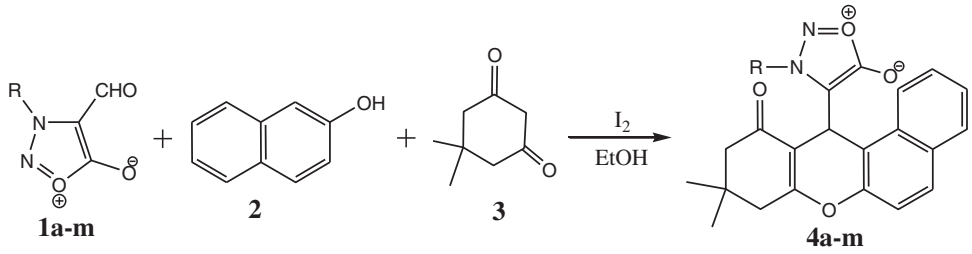
Results and discussion

Chemistry

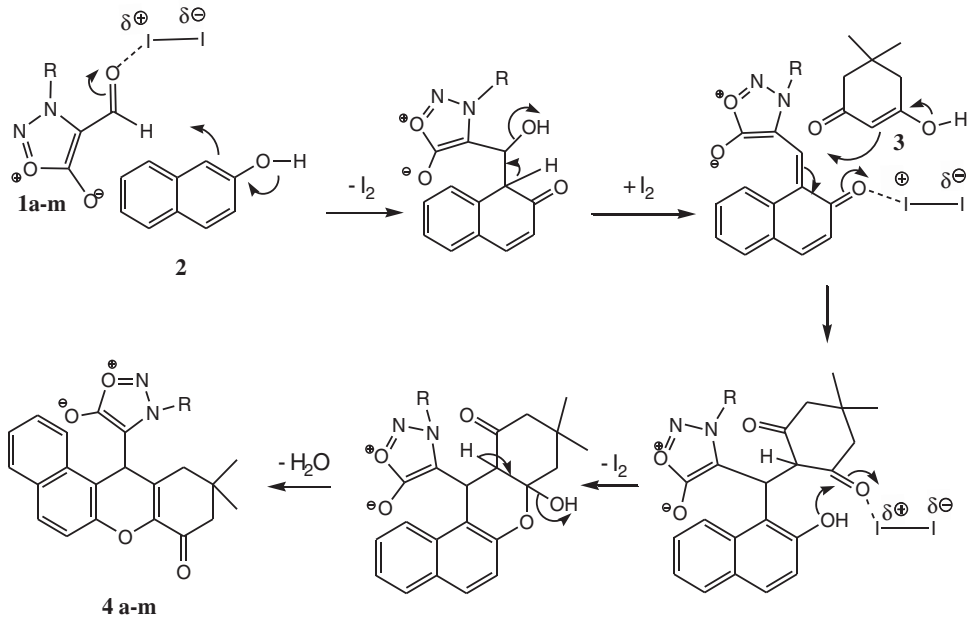
One-pot three component synthetic route to target compounds is outlined in Scheme 1. This involves the condensation of 3-aryl-4-formylsydnones **1a–m** with 2-naphthol and dimedone using molecular iodine as catalyst at 90 °C in ethanol to give 9,10-dihydro-12-(3-arylsydnon-4-yl)-9,9-dimethyl-8*H*-benzo[*a*]xanthen-11(12*H*)-one derivatives **4a–m**. Scheme 2 depicts the plausible mechanism of formation of title compounds **4a–m** under the influence of molecular iodine as catalyst.

The structures of synthesized compounds **4a–m** were confirmed by infrared (IR), ^{1}H & ^{13}C NMR, MS and elemental analyses. In case of IR spectral studies, the compounds have shown strong adsorption band for carbonyl of sydnone and xanthene rings at 1715–1739 and 1672–1689 cm^{-1} respectively. ^{1}H NMR spectral studies exhibit a singlet for six protons in the range 1.00–1.11 ppm. due to two methyl groups and a singlet for one proton present on pyran ring at 3.28–3.41 ppm. Further, two singlets for each two protons in the range of 2.96–2.49 and 2.54–2.05 ppm. were observed due to CH_2 groups of xanthene. The aromatic protons in all compounds appeared as multiplets in the range 6.76–7.99 ppm. In case of ^{13}C NMR spectral study, the

Scheme 1 Synthetic route for the title compounds **4a–m**. Where **a**, R = –C_6H_5; **b**, R = 4–CH_3–C_6H_4; **c**, R = 4–OCH_3–C_6H_4; **d**, R = 4–Cl–C_6H_4; **e**, R = 4–Br–C_6H_4; **f**, R = 4–NO_2–C_6H_4; **g**, R = 3–CH_3–C_6H_4; **h**, R =2 –CH_3–C_6H_4; **i**, R = 3–Cl–C_6H_4; **j**, R = 2–Cl–C_6H_4; **k**, R = 2–OCH_3–C_6H_4; **l**, R = 3–OCH_3–C_6H_4; **m**, R = –C–$(CH_3)_3$



Scheme 2 Plausible mechanism for the formation of benzoxanthene derivatives **4a–m** showing the catalytic role of molecular iodine



numbers of signals are in consistent with number of magnetically nonequivalent carbon atoms in the molecule and in mass spectra of all the synthesized compounds showed the molecular ion peaks at their respective *m/z* values.

Docking studies

All the inhibitors were docked into the binding site of *hyaluronidase* and the energy scores of the inhibitors are shown in Table 1 where a precise correlation can be found between docking studies and in vitro activity results. A complete overview of receptor – inhibitor binding interactions is presented in Figs. 4 and 5. They vividly represent the interaction model of the most potent inhibitors **4f** and **4k** with *hyaluronidase* (*hyaluronate lyase*), respectively in which both the inhibitors are suitably situated at the enzyme binding site and there are various interactions between them and the binding region of the enzyme. Compound **4f** showed four hydrogen binding interactions at the active site of the enzyme (PDB ID: 1W3Y) with the amino acids such as ARG336, and ASN349. Among the four hydrogen bond interactions, oxygen atom of sydnone interacts with hydrogen of AGR336 (–O ----- H–ARG336; 1.95 & 1.92 Å), nitrogen atom of sydnone ring makes a hydrogen bonding interaction with H atom of ARG336 (N ------ H–ARG336; 2.69 Å), and oxygen atom present at fifth position (carbonyl) makes the hydrogen bonding interaction with H atom of ARG336 (C–O -----H–ARG336; 2.42 Å) and remaining hydrogen bond interaction was raised from the interaction of carbonyl oxygen group of xanthene ring (pyran) with H atom of ASN349 (C=O -----H–ASN349; 2.10 Å). Another compound **4k** also has shown three hydrogen bonding interactions with the enzyme. The first hydrogen bonding was due to the interaction of H atom of ASN290 with nitrogen atom of sydnone (N -----H-ASN290; 2.50 Å). Remaining two interactions were due to oxygen atom of xanthene carbonyl with H atoms of ARG462 (C=O -----H-ARG462; 1.73 Å) and H atom of TYR408 (C=O -----H– TYR408; 2.28 Å). The C score (consensus score) values indicated the summary of all four interactions between the inhibitors and the enzyme. Crash score revealed the penetration into the binding site which was in

Table 1 Surflex docking score (kcal/mol) of the compounds **4a–m**

Compounds	C score[a]	Crash score[b]	Polar score[c]	D score[d]	PMF score[e]	G score[f]	Chem score[g]
4a	4.73	−0.70	1.71	−2674.24	−84.68	−202.41	−38.83
4b	4.85	−0.73	1.26	−2664.21	−90.07	−212.33	−38.41
4c	5.36	−2.23	0.68	−3119.85	−55.62	−252.49	−26.75
4d	4.91	−1.06	1.10	−2908.13	−97.61	−255.69	−37.74
4e	5.44	−1.89	0.01	−3744.14	−60.23	−260.16	−28.66
4f	**6.39**	**−1.06**	**1.62**	**−2819.74**	**−102.47**	**−229.90**	**−37.97**
4g	5.32	−1.40	1.84	−3046.88	−82.60	−231.38	−33.50
4h	4.07	−0.51	0.99	−3638.24	−70.60	−188.08	−30.63
4i	6.11	−0.88	1.38	−2535.01	−88.27	−235.11	−29.73
4j	3.99	−1.28	0.54	−2326.51	−103.34	−232.72	−30.95
4k	**5.65**	**−1.12**	**1.93**	**−2691.52**	**−117.04**	**−225.52**	**−37.51**
4l	5.14	−1.64	0.96	−3636.93	−110.64	−261.43	−37.92
4m	4.86	−1.29	1.46	−2644.67	−89.14	−237.05	−41.64
Indomethacin	4.29	−0.84	2.09	−2010.66	−56.00	−145.66	−26.98

[a] C score (Consensus score) integrates a number of popular scoring functions for ranking the affinity of ligands bound to the active site of a receptor and reports the output of total score

[b] Crash score revealing the inappropriate penetration into the binding site. Crash scores close to 0 are favorable. Negative numbers indicate penetration

[c] Polar indicating the contribution of the polar interactions to the total score. The polar score may be useful for excluding docking results that make no hydrogen bonds

[d] D score for charge and Vander Waals interactions between the protein and the ligand

[e] PMF score indicating the Helmholtz free energies of interactions for protein-ligand atom pairs (potential of mean force, PMF)

[f] G score showing hydrogen bonding, complex (ligand–protein), and internal (ligand–ligand) energies

[g] Chem score points for H-bonding, lipophilic contact, and rotational entropy, along with an intercept term

The bold values indicate that the results are impressive

favor of the compounds under analysis. The compounds **4f** and **4k** showed better hydrogen bonding and favorable internal energies than the other compounds including standard drug *Indomethacin*. These compounds are attributed to the lipophilicity of the sydnone moiety fused with xanthene (pyran) due to which better Vander Waal's interaction was achieved between ligand and the receptor. Molecular docking study revealed that majority of the docked compounds exhibited H-bonds and this fact prompted us to carry out in vitro *hyaluronidase* inhibition analysis. (Fig. 6)

In vitro *hyaluronidase* inhibition (anti-inflammatory activity) of title compounds

The docking results were further substantiated by in vitro anti-inflammatory activity using inhibition of *hyaluronidase* enzyme as a model. From Table 2 it can be concluded that the synthesized molecules **4f** and **4k** have shown 86–88 % growth inhibition which was almost equal to the standard Indomethacin at 100 µg concentration. However, the compounds **4a–b**, **4h–i** have exhibited 52–65 % growth inhibition at the 100 µg concentration. The remaining compounds **4c–e**, **4g**, **4l** and **4m** with large aromatic moieties have shown least activity. The standard deviation for percentage inhibition of *hyaluronidase* enzyme at 10, 50, and 100 µg concentrations were found to be 32.15, 59.86, and 96.44 respectively (Fig. 7).

Conclusions

In summary, a series of novel 9,10-dihydro-12-(3-arylsydnon-4-yl)-9,9-dimethyl-8*H*-benzo[*a*]xanthen-11(12*H*)-one derivatives **4a–m** were designed and synthesized using molecular iodine as a catalyst. Molecular docking results against *hyaluronidase* (*hyaluronate lyase*) were substantiated by in vitro inhibition analysis. It is interesting to note that synthesized compounds showed moderate to potent inhibition. These results indicate that the compounds reported in this paper are useful for further studies exhibiting the anti-inflammatory activity.

Experimental protocols

Melting points were determined in open capillaries and are uncorrected. Progress of the reaction checked by Aluchrosep

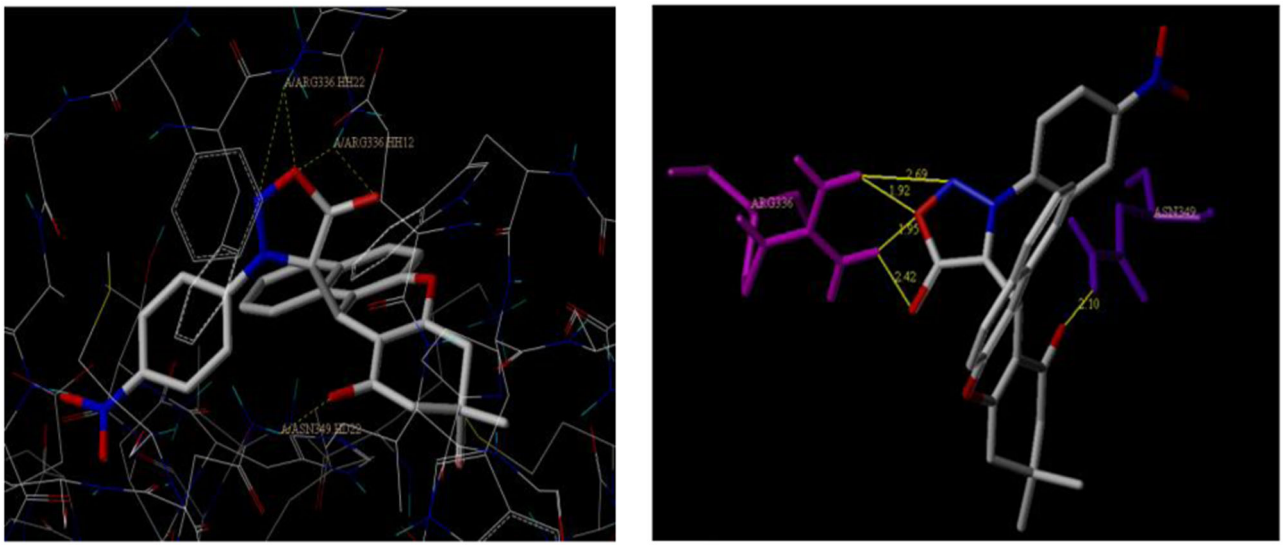


Fig. 4 Docked view of compound **4f** at the active site of enzyme *hyaluronate lyase*

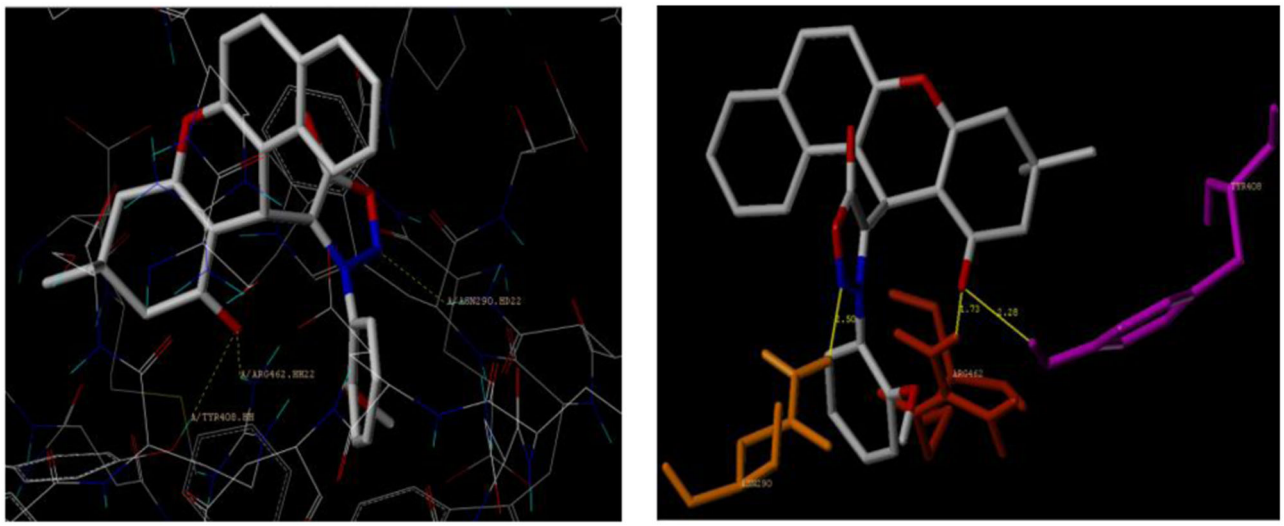


Fig. 5 Interaction of **4k** at the binding site of enzyme *hyaluronate lyase*

Silica Gel 60/UV 254 thin layer chromatography (TLC) on silica gel coated plates in ethyl acetate and hexane (3:7, 30 %) as eluent. IR spectra were recorded in a KBr disc matrix using an IMPACT-410 Nicolet (USA). ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 300 MHz FT NMR spectrometers with (DMSO-d_6) and $CDCl_3$ as solvent and tetramethylsilane was used as internal standard. The mass spectra were recorded on Shimadzu gas chromatography–mass spectrometry instrument operating at 70 eV spectrometer and elemental analyses were carried out using Heraus CHN rapid analyzer. All the solvents and reagents were of analytical grade and are purchased from Sigma-Aldrich and E-Merck used without further purification.

General procedure for the preparation of title compounds

3-Aryl-4-formylsydnone **1a**–**m** was prepared by formylation of 3-arylsydnone using *N*-methylformanilide in $POCl_3$ (Thoman et al., 1964). Iodine (0.2 mmol) in absolute ethanol (6 ml) was added to a mixture of 3-aryl-4-formylsydnone (1 mmol), β-naphthol **2** (1 mmol), and dimedone **3** (1.2 mmol). The mixture was stirred at 90 °C for 3–4 h. After completion (TLC), the reaction mixture was quenched into crushed ice. The crude product formed was filtered, washed and dried. Further recrystallized in ethanol to get pure title compounds **4a**–**m**.

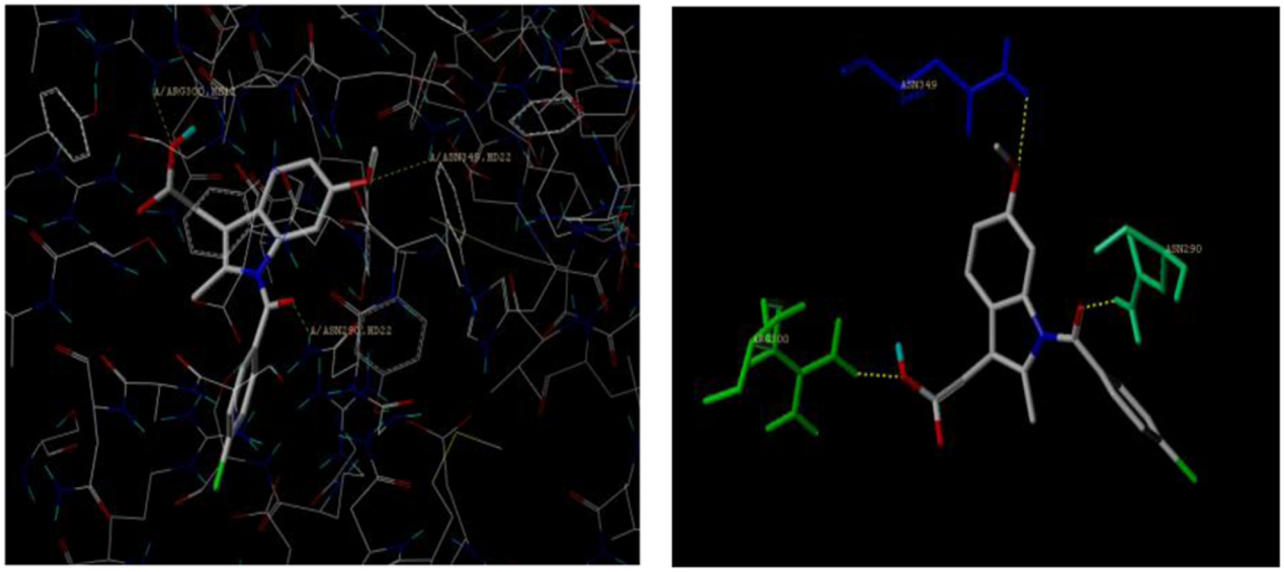


Fig. 6 Docked image of standard drug Indomethacin with *hyaluronate lyase*

*9,10-Dihydro-12-(3-phenylsydnon-4-yl)-9,9-dimethyl-8*H-*benzo[*a*]xanthen-11(12*H*)-one (**4a**)*

Yellow solid, recrystallized from ethanol, yield: 82 %. m.p.: 196–197 °C; IR (KBr) cm^{-1}: 1716 (sydnone C=O), 1669 (xanthene C=O). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.86–7.21 (11H, m, Ar–H), 4.25 (1H, s, pyran C_4–H), 2.49 (2H, s, CH_2), 2.08 (2H, s, CH_2), 1.01 (6H, s, -$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 200.02 (xanthene C=O), 175.90 (sydnone C=O), 172.52 (C–O), 166.03, 160.02 (Ar–C), 155.22 (Ar–C), 146.02 (Ar–C), 140.01 (Ar–C), 134.54 (Ar–C), 129.21 (Ar–C), 127.66 (Ar–C), 127.46 (Ar–C), 126.02 (Ar–C), 125.91 (Ar–C), 122.53 (Ar–C), 118.54 (Ar–C), 115.20 (Ar–C), 112.51 (Ar–C), 108.58, 106.02 (Ar–C), 102.54 (Ar–C), 100.08 (sydnone C_4), 55.70, 34.61, 32.50 (pyran C_4), 30.54, 27.02 (–$CH_3)_2$; Anal. calcd. for $C_{27}H_{22}N_2O_4$: C, 74.02; H, 5.08; N, 6.45. Found C, 73.96; H, 5.06; N, 6.39 %. MS (EI, 70 eV) *m/z*: 438.5 (M + 1).

*9,10-Dihydro-12-(-3-*p*-tolylsydnon-4-yl)-9,9-dimethyl-8*H-*benzo[*a*]xanthen-11(12*H*)-one (**4b**)*

Brown color solid, recrystallized from ethanol, yield: 76 %. m.p.: 172–173 °C; IR (KBr) cm^{-1}: 1724 (sydnone C=O), 1677 (xanthene C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 –7.01 (10H, m, Ar–H), 3.34 (1H, s, pyran C_4–H), 2.77 (2H, s, CH_2), 2.51 (2H, s, CH_2), 2.37 (3H, s, –CH_3), 1.08 (6H, s, –$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 200.45 (C=O), 177.42 (C=O), 175.27 (C–O), 162.94, 154.05 (Ar–C), 152.19 (Ar–C), 139.47 (Ar–C), 137.92 (Ar–C), 135.20 (Ar–C), 134.18 (A–C), 131.18 (Ar–C), 129.25 (Ar–C), 128.31 (Ar–C), 126.67 (Ar–C), 125.35 (Ar–C), 123.24 (Ar–C), 122.90 (Ar–C), 118.75 (Ar–C), 115.46 (Ar–C), 114.51, 108.68 (Ar–C), 101.77 (sydnone C_4), 55.09, 43.22, 32.88 (pyran C_4), 27.90 (–$CH_3)_2$, 25.63 (–CH_3); Anal. calcd. for $C_{28}H_{24}N_2O_4$: C, 74.39; H, 5.41; N, 6.24. Found C, 74.32; H, 5.35; N, 6.19 %. MS (EI, 70 eV) *m/z*: 452.5 (M + 1).

Table 2 Results of in vitro *hyaluronidase* inhibition of title compounds **4a–m**

Compounds	Concentration (μg)		
	10	50	100
4a	10.12	51.27	60.42
4b	11.92	53.14	65.73
4c	09.98	14.96	24.94
4d	09.62	15.42	27.99
4e	17.21	19.45	31.17
4f	**21.47**	**51.60**	**86.76**
4g	09.18	16.03	25.63
4h	12.75	53.14	62.86
4i	11.45	49.43	64.98
4j	05.56	11.42	22.92
4k	**29.14**	**50.16**	**88.43**
4l	09.16	15.76	27.87
4m	09.42	14.56	28.31
Indomethacin	32.15	59.86	96.44

The bold values indicate that the results are impressive.

*9,10-Dihydro-12-(3-*p*-methoxyphenyl-sydnon-4-yl)-9,9-dimethyl-8*H*-benzo[*a*]xanthen-11(12*H*)-one (**4c**)*

Pale yellow color solid, recrystallized from ethanol. Yield: 65 %. m.p.: 156–157 °C; IR (KBr) cm^{-1}: 1722 (sydnone

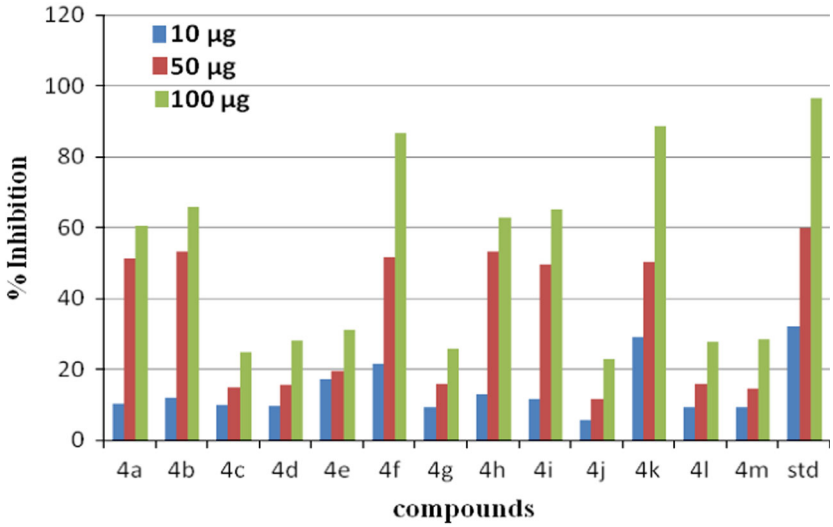


Fig. 7 Bar graph of in vitro *hyaluronidase* inhibition of compounds **4a–m**

C=O), 1673 (xanthene C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99–7.42 (6H, m, Ar–H), 7.21 (2H, d, Ar–H), 7.08 (2H, d, Ar–H), 4.38 (1H, s, pyran C_4–H), 3.29 (3H, s, OCH_3), 2.49 (2H, s, CH_2), 2.48 (2H, s, CH_2), 1.50 (6H, s, $-(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 200.53 (C=O), 177.00 (C=O), 174.80 (C–O), 163.01, 153.91 (Ar–C), 138.91 (Ar–C) 137.21 (Ar–C), 135.48 (Ar–C), 134.64 (A–C), 129.59 (Ar–C), 128.67 (Ar–C), 127.65 (Ar–C), 126.27 (Ar–C), 125.98 (Ar–C), 123.48 (Ar–C), 123.26 (Ar–C), 118.00 (Ar–C), 114.89, 109.39 (Ar–C), 101.31 (sydnone C_4), 64.37 ($-OCH_3$), 55.82, 50.52, 42.92, 32.49 (pyran C_4), 28.19 ($-CH_3)_2$, 27.64; Anal. calcd. for $C_{28}H_{24}N_2O_5$: C, 71.84; H, 5.21; N, 6.07. Found C, 71.78; H, 5.16; N, 5.98 %. MS (EI, 70 eV) *m/z*: 468.5 (M + 1).

*12-(3-(*p*-Chlorophenylsydnon-4-yl)-9,10-dihydro-9,9-dimethyl-8*H*-benzo[*a*]xanthen-11(12*H*)-one (**4d**)*

Yellow solid, recrystallized from ethanol, yield: 77 %. m.p.: 197–198 °C; IR (KBr) cm^{-1}: 1729 (sydnone C=O), 1678 (xanthene C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (2H, d, Ar–H), 7.70 (2H, d, Ar–H), 7.68–7.05 (6H, m, Ar–H), 3.32 (1H, s, pyran C_4–H), 2.92 (2H, s, CH_2), 2.54 (2H, s, CH_2), 1.11 (6H, s, $-(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 196.09 (C=O), 175.24 (C=O), 171.38 (C–O), 166.56, 161.24 (Ar–C), 157.31 (Ar–C), 155.63 (Ar–C), 140.92 (Ar–C), 138.67 (Ar–C), 132.79 (Ar–C), 129.99 (Ar–C), 127.82 (Ar–C), 125.62 (Ar–C), 123.46 (Ar–C), 122.90 (Ar–C), 120.82 (Ar–C), 114.46, 113.67 (Ar–C), 110.34 (Ar–C), 109.29 (Ar–C), 100.37 (sydnone C_4), 54.43, 42.75, 33.19 (pyran C_4), 31.92, 28.2 ($-CH_3)_2$, 27.12; Anal. calcd. for $C_{27}H_{21}N_2ClO_4$: C, 68.51; H, 4.53; N, 5.98. Found C, 68.57; H, 4.48; N, 5.92 %. MS (EI, 70 eV) *m/z*: 474.1 (M + 2), 472.1 (M + 1).

*12-(3-(*p*-Bromophenylsydnon-4-yl)-9,10-dihydro-9,9-dimethyl-8*H*-benzo[*a*]xanthen-11(12*H*)-one (**4e**)*

Dark brown color, recrystallized from ethanol. Yield: 72 %. m.p.: 174–175 °C; IR (KBr) cm^{-1}: 1715 (sydnone C=O), 1666 (xanthene C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (2H, d, Ar–H), 7.73 (2H, d, Ar-H), 7.72–7.23 (6H, m, Ar–H), 3.28 (1H, s, pyran C_4–H), 2.73 (2H, s, CH_2), 2.49 (2H, s, CH_2), 1.07 (6H, s, $-(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.03 (C=O), 176.19 (C=O), 172.38 (C–O), 164.12, 162.88 (Ar–C), 159.40 (Ar–C), 154.45 (Ar–C), 141.12 (Ar–C), 140.49 (Ar–C), 134.52 (Ar–C), 129.59 (Ar–C), 128.68 (Ar–C), 126.01 (Ar–C), 124.52 (Ar–C), 122.23 (Ar–C), 121.79 (Ar–C), 115.03, 113.74 (Ar–C), 111.23 (Ar–C), 107.12 (Ar–C), 101.23 (sydnone C_4), 50.38, 40.61, 33.16 (pyran C_4), 31.23, 28.20 ($-CH_3)_2$; Anal. calcd. for $C_{27}H_{21}N_2BrO_4$: C, 62.76; H, 4.17; N, 5.55. Found C, 62.68; H, 4.09; N, 5.41 %. MS (EI, 70 eV) *m/z*: 518.1 (M + 2), 516.1.

*9,10-Dihydro-12-(-3-(*p*-nitrophenylsydnon-4-yl)-9,9-dimethyl-8*H*-benzo[*a*]xanthen-11(12*H*)-one (**4f**)*

Pale yellow color solid, recrystallized from ethanol. Yield: 52 %. m.p: 132–133 °C; IR (KBr) cm^{-1}: 1715 (sydnone C=O), 1666 (xanthene C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (2H, d Ar–H), 7.84 (2H, d, Ar–H), 7.46–7.03 (6H, m, Ar–H), 3.37 (1H, s, pyran C_4–H), 2.70 (2H, s, CH_2), 2.55 (2H, s, CH_2), 1.09 (6H, s, $-(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.62 (C=O), 176.56 (C=O), 174.19 (C–O), 161.87, 154.91 (Ar–C), 139.20 (Ar–C) 136.46 (Ar–C), 135.94 (Ar–C), 132.16 (A–C), 130.73 (Ar–C), 129.02 (Ar–C), 128.34 (A–C), 126.98 (Ar–C), 124.43 (Ar–C), 122.61 (Ar–C), 121.65 (Ar–C), 119.00 (Ar–C), 113.76, 108.49

(Ar–C), 102.73 (sydnone C_4), 66.51 (–OCH_3), 57.52, 49.68, 31.76 (pyran C_4), 30.91, 28.63 (–CH_3)$_2$, 27.94; Anal. calcd. for $C_{27}H_{21}N_3O_6$: C, 67.09; H, 4.42; N, 8.77. Found C, 67.07; H, 4.38; N, 8.69 %. MS (EI, 70 eV) *m/z*: 483.5 (M + 1).

*9,10-Dihydro-12-(3-*m*-tolylsydnon-4-yl)-9,9-dimethyl-8*H*-benzo[*a*]xanthen-11(12*H*)-one (**4g**)*

Pale brown color solid, recrystallized from ethanol, yield: 72 %. m.p.: 182–183 °C; IR (KBr) cm^{-1}: 1727 (sydnone C=O), 1682 (xanthene C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49–7.03 (10H, m, Ar–H), 3.39 (1H, s, pyran C_4–H), 2.96 (2H, s, CH_2), 2.51 (2H, s, CH_2), 2.42 (3H, s, –CH_3), 1.02 (6H, s, –$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 197.72 (C=O), 177.58 (C=O), 174.62 (C–O), 160.21, 153.14 (Ar–C), 150.54 (Ar–C), 1742.67 (Ar–C), 139.69 (Ar–C), 136.82 (Ar–C), 133.49 (A–C), 130.82 (Ar–C), 129.61 (Ar–C), 127.73 (Ar–C), 127.02 (Ar–C), 124.87 (Ar–C), 122.23 (Ar–C), 121.34 (Ar–C), 116.86 (Ar–C), 115.73 (Ar–C), 112.87, 110.24 (AR–C), 103.72 (sydnone C_4), 53.51, 41.16, 31.97 (pyran C_4), 28.64 (–CH_3)$_2$, 27.03 (–CH_3); Anal. calcd. for $C_{28}H_{24}N_2O_4$: C, 74.37; H, 5.41; N, 6.22. Found C, 74.32; H, 5.35; N, 6.19 %. MS (EI, 70 eV) *m/z*: 452.2 (M + 1).

*9,10-Dihydro-12-(3-*o*-tolylsydnon-4-yl)-9,9-dimethyl-8*H*-benzo[*a*]xanthen-11(12*H*)-one (**4h**)*

Pale brown color solid, recrystallized from ethanol. Yield: 72 %. m.p.: 144–145 °C; IR (KBr) cm^{-1}: 1732 (sydnone C=O), 1689 (xanthene C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–6.76 (10H, m, Ar–H), 3.41 (1H, s, pyran C_4–H), 2.84 (2H, s, CH_2), 2.66 (2H, s, CH_2), 2.45 (3H, s, –CH_3), 1.10 (6H, s, –$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.02 (C=O), 176.09 (C=O), 174.49 (C–O), 161.16, 154.81 (Ar–C), 151.31 (Ar–C), 140.57 (Ar–C), 138.39 (Ar–C), 136.67 (Ar–C), 134.50 (A–C), 132.59 (Ar–C), 130.19 (Ar–C), 129.42 (Ar–C), 127.61 (Ar–C), 124.91 (Ar–C), 123.84 (Ar–C), 121.13 (Ar–C), 117.25 (Ar–C), 116.43 (Ar–C), 113.64, 109.97 (AR–C), 106.04 (sydnone C_4), 56.61, 40.19, 30.96 (pyran C_4), 28.42 (–CH_3)$_2$, 26.32 (–CH_3); Anal. calcd. for $C_{28}H_{24}N_2O_4$: C, 74.39; H, 5.43; N, 6.27. Found C, 74.32; H, 5.35; N, 6.19 %. MS (EI, 70 eV) *m/z*: 452.2 (M + 1).

*12-(3-(*m*-Chlorophenylsydnon-4-yl)-9,10-dihydro-9,9-dimethyl-8*H*-benzo[*a*]xanthen-11(12*H*)-one (**4i**)*

Yellow solid, recrystallized from ethanol. Yield: 75 %. m.p.: 177–178 °C; IR (KBr) cm^{-1}: 1726 (sydnone C=O), 1678 (xanthene C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68–7.09 (10H, m, Ar–H), 3.39 (1H, s, pyran C_4–H), 2.72 (2H, s, CH_2), 2.49 (2H, s, CH_2), 1.07 (6H, s, –$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.64 (C=O), 176.64 (C=O), 172.61 (C–O), 164.16, 160.49 (Ar–C), 156.12 (Ar–C), 153.01 (Ar–C), 141.84 (Ar–C), 133.14 (Ar–C), 131.67 (Ar–C), 130.13 (Ar–C), 126.62 (Ar–C), 124.13 (Ar–C), 124.68 (Ar–C), 121.29 (Ar–C), 120.72 (Ar–C), 115.79, 114.67 (Ar–C), 111.42 (Ar–C), 107.81 (Ar–C), 102.19 (sydnone C_4), 55.49, 40.57, 32.26 (pyran C_4), 30.74, 28.34 (–CH_3)$_2$, 26.63; Anal. calcd. for $C_{27}H_{21}N_2ClO_4$: C, 68.62; H, 4.51; N, 6.01. Found C, 68.57; H, 4.48; N, 5.92 %. MS (EI, 70 eV) *m/z*: 474.1 (M + 2), 472.1 (M+).

*12-(3-(*o*-Chlorophenylsydnon-4-yl)-9,10-dihydro-9,9-dimethyl-8*H*-benzo[*a*]xanthen-11(12*H*)-one (**4j**)*

Yellow color solid, recrystallized from ethanol. Yield: 71 %. m.p.: 162–163 °C; IR (KBr) cm^{-1}: 1729 (sydnone C=O), 1672 (xanthene C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74–7.05 (10H, m, Ar–H), 3.36 (1H, s, pyran C_4–H), 2.79 (2H, s, CH_2), 2.51 (2H, s, CH_2), 1.11 (6H, s, –$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 196.77 (C=O), 174.56 (C=O), 172.94 (C–O), 162.19, 161.85 (Ar–C), 155.81 (Ar–C), 152.07 (Ar–C), 140.46 (Ar–C), 132.87 (Ar–C), 131.98 (Ar–C), 130.02 (Ar–C), 125.31 (Ar–C), 124.74 (Ar–C), 124.08 (Ar–C), 122.34 (Ar–C), 120.97 (Ar–C), 114.80, 114.12 (Ar–C), 111.09 (Ar–C), 108.26 (Ar–C), 101.53 (sydnone C_4), 56.34, 41.61, 31.71 (pyran C_4), 30.82, 28.73 (–CH_3)$_2$, 26.93; Anal. calcd. for $C_{27}H_{21}N_2ClO_4$: C, 68.60; H, 4.53; N, 5.98. Found C, 68.57; H, 4.48; N, 5.92 %. MS (EI, 70 eV) *m/z*: 474.1 (M + 2), 472.1 (M+).

*9,10-Dihydro-12-(3-(*o*-methoxyphenylsydnon-4-yl)-9,9-dimethyl-8*H*-benzo[*a*]xanthen-11(12*H*)-one (**4k**)*

Dark yellow color solid, recrystallized from ethanol. Yield: 62 %. m.p.: 137–138 °C; IR (KBr) cm^{-1}: 1734 (sydnone C=O), 1674 (xanthene C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (1H, d Ar–H), 7.88 (1H, d, Ar–H), 7.81 (1H, d, Ar–H), 7.47 (1H, t, Ar–H), 7.39 (1H, t, Ar–H), 7.23 (1H, d, Ar–H), 7.09 (1H, d, Ar–H), 7.06 (1H, d, Ar–H), 7.03 (1H, d, Ar–H), 6.81 (1H, d, Ar–H), 4.29 (1H, s, pyran C_4–H), 3.77 (3H, s, OCH_3), 2.76 (2H, s, CH_2), 2.51 (2H, s, CH_2), 1.04 (6H, s, –$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 200.06 (C=O), 177.49 (C=O), 174.15 (C–O), 162.48, 151.81 (Ar–C), 139.06 (Ar–C) 137.75 (Ar–C), 135.67 (Ar–C), 133.15 (A–C), 130.79 (Ar–C), 128.16 (Ar–C), 127.90 (Ar–C), 126.73 (Ar–C), 124.36 (Ar–C), 123.79 (Ar–C), 122.05 (Ar–C), 117.86 (Ar–C), 115.62, 108.46 (Ar–C), 102.94 (sydnone C_4), 65.86 (OCH_3), 56.31, 49.79, 41.64, 31.32 (pyran C_4), 28.97 (–CH_3)$_2$, 26.95; Anal. calcd. for $C_{28}H_{24}N_2O_5$: C, 71.82; H, 5.21; N, 6.03. Found C, 71.78; H, 5.16; N, 5.98 %. MS (EI, 70 eV) *m/z*: 468.5 (M + 1).

*9,10-Dihydro-12-(3-(*m*-methoxyphenylsydnon-4-yl)-9,9-dimethyl-8*H*-benzo[*a*]xanthen-11(12*H*)-one (**4l**)*

Yellow color solid, recrystallized from ethanol. Yield: 67 %. m.p: 155–156 °C; IR (KBr) cm^{-1}: 1731 (sydnone C=O), 1677 (xanthene C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (1H, d, Ar–H), 7.83 (1H, d, Ar–H), 7.80 (1H, d, Ar–H), 7.46 (1H, t, Ar–H), 7.39 (1H, t, Ar–H), 7.18 (1H, d, Ar–H), 7.12 (1H, d, Ar–H), 7.07 (1H, d, Ar–H), 7.02 (1H, d, Ar–H), 6.76 (1H, d, Ar–H), 4.32 (1H, s, pyran C_4–H), 3.79 (3H, –OCH_3, s), 2.78 (2H, s, CH_2), 2.61 (2H, s, CH_2), 1.07 (6H, s, –$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 200.16 (C=O), 176.49 (C=O), 173.43 (C–O), 162.79, 153.47 (Ar–C), 139.03 (Ar–C) 137.68 (Ar–C), 136.17 (Ar–C), 132.15 (A–C), 129.61 (Ar–C), 128.94 (Ar–C), 127.37 (Ar–C), 127.03 (Ar–C), 124.24 (Ar–C), 123.92 (Ar–C), 122.47 (Ar–C), 119.84 (Ar–C), 113.76, 108.64 (Ar–C), 102.85 (sydnone C_4), 66.53 (–OCH_3), 57.29, 51.37, 40.18, 30.96 (pyran C_4), 28.71 (–$CH_3)_2$, 26.59; Anal. calcd. for $C_{28}H_{24}N_2O_5$: C, 71.84; H, 5.19; N, 6.06. Found C, 71.78; H, 5.16; N, 5.98 %. MS (EI, 70 eV) *m/z*: 468.5 (M + 1).

*12-(3-(*p*-t-Butylphenylsydnon-4-yl)-9,10-dihydro-9,9-dimethyl-8*H*-benzo[*a*]xanthen-11(12*H*)-one (**4m**)*

Dark yellow color solid, recrystallized from ethanol. Yield: 73 %. m.p.: 149–150 °C; IR (KBr) cm^{-1}: 1739 (sydnone C=O), 1679 (xanthene C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (1H, d, Ar–H), 7.59 (1H, d, Ar–H), 7.57 (1H, d, Ar–H), 7.48 (1H, d, Ar–H), 7.30 (1H, t, Ar–H), 7.21 (1H, t, Ar–H), 7.16 (1H, d, Ar–H), 7.09 (1H, d, Ar–H), 7.05 (1H, d Ar–H), 6.98 (1H, d Ar–H), 3.92 (1H, s, pyran C_4–H), 2.95 (2H, s, CH_2), 2.07 (2H, s, CH_2), 1.38 (9H, s, –$(CH_3)_3$), 1.10 (6H, s, –$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.6 (C=O), 176.31 (C=O), 174.56 (C–O), 160.68, 154.78 (Ar–C), 152.35 (Ar–C), 141.51 (Ar–C), 136.71 (Ar–C), 133.49 (Ar–C), 131.78 (Ar–C), 130.67 (Ar–C), 129.96 (Ar–C), 126.28 (Ar–C), 125.23 (Ar–C), 123.83 (Ar–C), 122.79 (Ar–C), 120.46 (Ar–C), 118.58 (Ar–C), 114.32, 110.87 (Ar–C), 102.68 (sydnone C_4), 52.87, 45.60, 41.79, 32.8 (–$(CH_3)_3$), 30.8, 28.3 (–$CH_3)_2$, 27.90 (pyran C_4); Anal. calcd. for $C_{31}H_{30}N_2O_4$: C, 71.82; H, 5.21; N, 6.03. Found C, 71.78; H, 5.16; N, 5.98 %. MS (EI, 70 eV) *m/z*: 468.5 (M + 1).

Pharmacological evaluation

Molecular docking

The crystal structure used was that of *hyaluronidase* (*hyaluronate lyase*) in complex with palmitoyl-vitamin C (PDB ID: 1W3Y) for the docking studies obtained from the Protein Data Bank. The enzyme for docking was prepared by adding polar hydrogen atom with Gasteiger-Hückel (Gasteiger and Marsili, 1980) charges and water molecules were removed. The 3D structure of the ligands was generated by the SKETCH module implemented in the SYBYL program (Tripos Inc., St. Louis, USA) and its energy-minimized conformation was obtained with the help of Tripos force field using Gasteiger - Hückel charges and molecular docking was performed with Surflex-Dock program that is interfaced with Sybyl-X 2.0 (Tripos International, 2012). Other miscellaneous parameters were assigned with the default values given by the software. In the present work the default parameters of Surflex Docking program were used for the molecular docking of ligands. To find the correct conformations of the ligands and to obtain minimum energy structures ligands were allowed to be flexible. At the end of docking, the best conformations of the ligands were analyzed for their binding interactions.

Hyaluronidase inhibition (anti-inflammatory) assay (Ling et al., 2003)

The assay medium consisting of 3-5U hyaluronidase (from Sigma–Aldrich, Bangalore) in 100 µl of 20 mM sodium phosphate buffer (pH 7.0) with 77 mM sodium chloride, 0.01 % bovine serum albumin (BSA) was pre-incubated with different concentrations (10, 50 and 100 µg) of the test compound for 15 min at 37 °C. The assay was commenced by adding HA (100 µl, Sigma–Aldrich, Bangalore; 0.03 % in 300 mM sodium phosphate, pH 5.35) to the incubation mixture and incubated for further 45 min at 37 °C. The undigested hyaluronic acid was precipitated with acid albumin solution (1 ml) made up of 0.1 % BSA in 24 mM sodium acetate and 79 mM acetic acid, (pH 3.75). After standing at room temperature for 10 min, the absorbance of the reaction mixture was measured at 600 nm. The absorbance in absence of enzyme was used as reference value for maximum inhibition. The inhibitory activity of test compound was calculated as the percentage ratio of absorbance in presence of test compound vs. absorbance in the absence of enzyme. The enzyme activity was checked by control experiment run simultaneously, in which the enzyme was pre-incubated with DMSO (5 µl) followed by the assay procedures described above. Samples were tested in the range of 5 µg, 10 µg and 100 µg in the reaction mixture. Indomethacin was used as reference standard.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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