WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 9, 922-930 Research Article ISSN 2278 – 4357

DESIGN, MOLECULAR DOCKING, SYNTHESIS, & ANTI-CANCER EVALUATION OF NOVEL XANTHENE DERIVATIVES

Dr. Sujit Kumar Mohanty[1]*, Anuradha Khuntia[1], B. Sandhyarani[2], Yashaswini[1], B. Likitha[1], C. I. Muzammil[1], Sneha[1], Harshitha[1], P. Samuel Shine[1]

[1]Dept. of Pharmaceutical Chemistry, Creative Educational Society's College of Pharmacy, Chinnatekur, Kurnool-518218.

[2]Dept. of Pharmaceutical Chemistry, Raghu College of Pharmacy, Visakhapatnam-531162.

Article Received on
30 June 2018,

Revised on 20 July 2018,
Accepted on 09 August 2018

DOI: 10.20959/wjpps20189-12250

***Corresponding Author**
Dr. Sujit Kumar Mohanty
Dept. of Pharmaceutical Chemistry, Creative Educational Society's College of Pharmacy, Chinnatekur, Kurnool-518218.

ABSTRACT

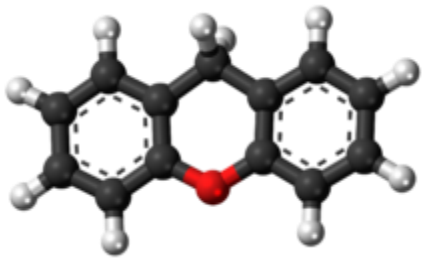
A series of seven new Xanthene derivatives were synthesized by reacting appropriate amines with ester of fluorescein. Different derivatives were synthesized using unique techniques. Purity of synthesized compound was checked by TLC and structures were elucidated by their IR, ^{1}H NMR and Mass data. The synthesized compounds were screened for anti-cancer activities using Hela cell line (*Cervical Cancer).* All the products showed good binding scores (Insilco) with respective receptors. Moreover, Compound SDC, SAN & SHH, showed potential in-vitro cytotoxicity activity when compared to standard drugs.

KEYWORDS: Xanthene derivatives, Hela cell line, Insilco, in-vitro cytotoxicity.

INTRODUCTION

Xanthene is a yellow organic heterocyclic compound having chemical formula $C_{13}H_{10}O$. It is soluble in diethyl ether. Its melting point is 101-102^{o}C and its boiling point is 310-312^{0}C. Xanthene is used as a fungicide and it is also a useful intermediate in organic synthesis. Derivatives of xanthene are commonly referred to collectively as xanthenes and among other uses are the basis of a class of dyes which includes fluorescein[1], eosins and rhodamines.[2] Xanthene dyes tend to be fluorescent, yellow to pink to bluish red, brilliant dyes. Many xanthene dyes can be prepared by condensation of derivates of phthalic anhydride with

derivates of resorcinol or 3-aminophenol. Xanthenes are frequently occurring motifs in a natural products[3] and have been used as versatile synthons due to the inherent reactivity of the inbuilt pyran ring.[4]



Xanthenes and xanthene derivatives exhibit anti-cancer[5], anti-oxidant[6], anti-inflammatory and potential analgesic activites.[7] Xanthenes are rare in natural plants; most of them are synthesized or arise as microbial metabolite. To date, xanthenes has only been isolated from two plant families, Fabaceae and Compositae.[8,9]

Experimental

Melting points of the synthesized compounds were determined in open capillary tubes and were uncorrected. IR spectra were recorded on Shimadzu FTIR Spectrophotometer with KBr pellets. Mass Spectra were recorded on GCMS QD 5000 Shimadzu. H1NMR Spectra was recorded on Bruker AV- 500 MHz, using DMSO as solvent. TLC was carried out using pre coated Silica gel plates. All the chemicals and solvents used were of LR grade and obtained from SD fine Chem. Limited. The test compounds were synthesized by the following procedures.

1. *Synthesis of fluorescein (3)*

A mixture containing phthalic anhydride powder (5gm, 0.01mol) and resorcinol (7.5gm, 0.01mole) in Round Bottom Flask with 75% H_2SO_4 (2-3 drops) was heated for 3-4 hours, at 180^0C on water bath, until a semisolid mixture was obtained. Then it was cooled to room temperature and the solidified product was dissolved in dilute sodium hydroxide solution. Then it was neutralized by hydrochloric acid to get fluorescien precipitation. Finally the product was washed with cold water. Melting point was 124-125^0C.

2. *Synthesis of methyl 2-(3,6-dihydroxy-9H-xanthen-9-yl)benzoate (4)*

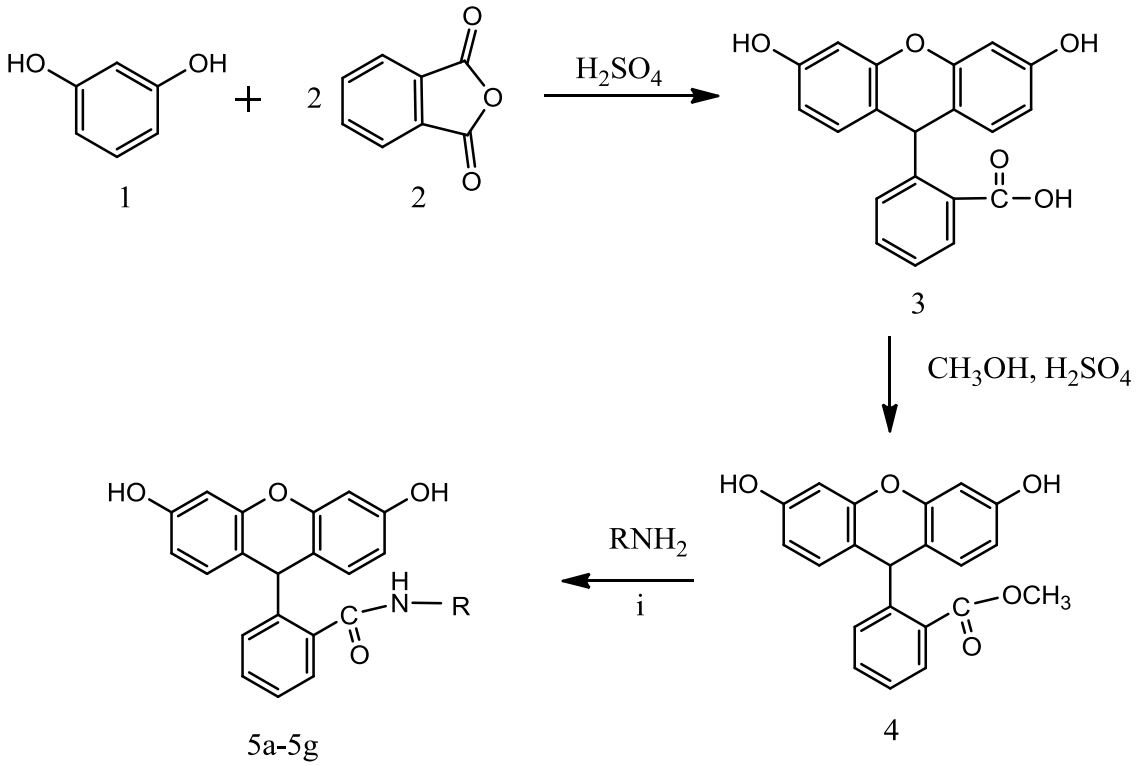
A mixture containing fluorescein (8gm) and methanol (10ml) with 75% H_2SO_4 (2-3 drops) was refluxed for 4 hours. Then the excess alcohol was distilled off and allowed to cool. The

residue was poured to 25ml of water in a separating funnel and rinsed. Then the lower ester layer was separated.

3. *Synthesis of 5a-5e (General Procedure)*

2 ml of appropriate amines were heated for 5 minutes with methyl 2-(3, 6-dihydroxy-9H-xanthen-9-yl) benzoate (4), then refluxed for 20-30 minutes. Then 5 ml of ethanol was added and again refluxed for 5-6 hours.

SCHEME-I



Reaction conditions:
i:ester and amine in ethanol reflux 4-5 hrs.
a:aniline,b:phenyl hydrazine,c:2,4-DNP,d:o-amino phenol,e:hydrazine hydrate
f:2,4-dichloro phenol,g:p-amino phenol.

Docking Studies

Docking is a method that predicts the preferred orientation and binding affinity between two given molecules. Docking is usually used to predict the binding orientation of the drug candidates to their protein targets in order to predict the affinity and activity of the molecule. Hence docking plays a key role in the design of drugs. To increasing the biological as well

as pharmaceutical importance of molecular docking, considerable efforts have been made towards improving the methods used to predict docking.[10, 11]

All the compounds were docked to 1j4x protein receptor obtained from Protein data bank. All the synthesized compounds were found to have good negative binding scores proving their drug likeliness property.

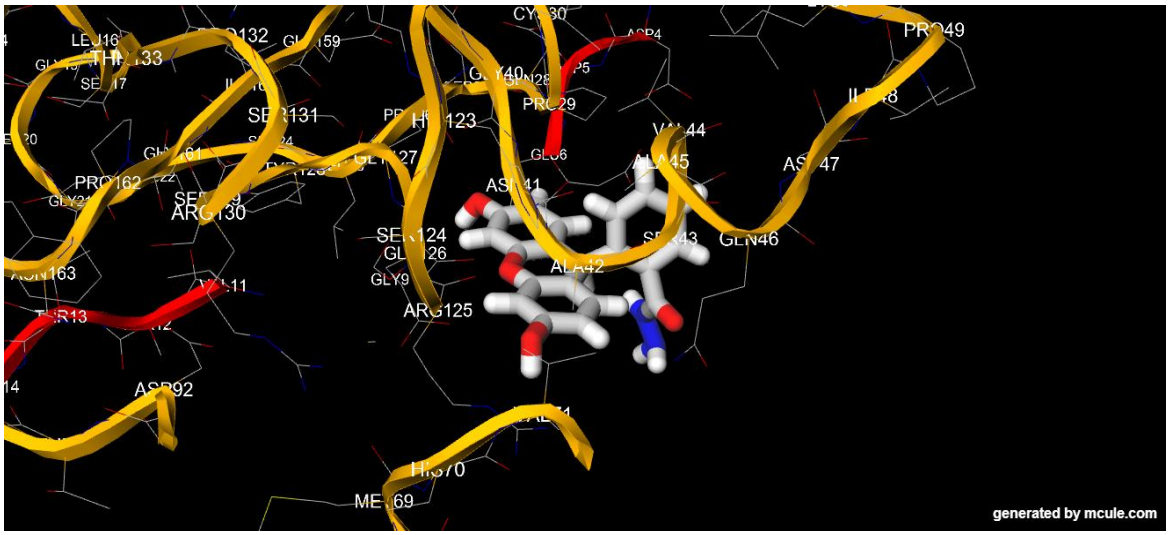


Figure 4: Docking of SHH.

Analytical Data

SAP: 2-(3, 6 dihydroxy-9H-xanthen-9-yl)-N-(2-hydroxyphenyl) benzamide.

M.P:1074.92, Yield: 20%, IR (KBr cm^{-1}): 3313.6 (NH str.), 2987.1 (Aro. str.), 1717.4 (C=O), 1221.5 (C-O str. ether). H^1NMR (DMSO): δ3.72 (s, 3H, OH) δ9.75 (s, H, NH), δ6.9 - δ7.2 (m, 4H, ArH), MS (m/z):424

SDN: 2-(3, 6-dhydroxy-9H-xanthen-9-yl)-N-(2,4-dinitrophenyl)benzohydrazide).

M.P: 1076.21, Yield: 32%, IR (KBr cm^{-1}): 3322.4 (NH), 2920.8 (Ar CH str.), 1647.4 (C=O), 1218.1(C-O str. (ether), 1333.6 and 1514.0 (N-O str.), H^1NMR (DMSO): δ5.0(s, 1H, OH), δ2.0 (d, 2H, NH) δ8.11 (m, 4H, Ar-H), δ9.11 (m, 2H, Ar-H), MS (m/z):514.

SAN: 2-(3,6- dihydroxy – 9H-xanthen-9-yl)-N-phenylbenzamide.

M.P: 963.2, Yield: 25%, IR (KBr cm^{-1}): 3404.9 (OH), 1600.7 (NH), 2924.4 (Ar CH str.), 1718(C=O), 1116.8(C-O str. ether), H^1NMR (DMSO): δ3.72(s, 1H, OH), δ3.52 (s, 1H, NH), δ6.7 (m, 3H, Ar-H), δ7.41-7.43 (m, 3H, Ar-H), MS (m/z):409.

SHH: 2-(3,6 dihydroxy-9H-xanthen-9-yl)benzohydrazide.
M.P: 952, Yield: 42%, IR (KBr cm^{-1}): 3326.06 (NH), 1196.7 (C=O), 2924.9 (Ar CH str.) 1117.66 (C-O str. ether), H^1NMR (DMSO): δ5.6 (s, 2H, OH), δ1.8(s, 1H, NH) δ2.3 (m, 2H, NH2), δ7.2 (m, 2H, Ar-H), MS (m/z):348.

SPH: 2-(3,6-dihydroxy-9H-xanthen-9-yl)-N-phenylbenzohydrazide.
M.P: 952, Yield: 31%, IR (KBr cm^{-1}): 3335. (NH), 1696.7 (C=O), 2924.9 (Ar CH str.) 1180.6 (C-O str. ether), H^1NMR (DMSO): δ3.6 (s, 1H, OH), δ7.8(s, 1H, NH) δ6.8 (m, 2H, Ar-H), δ7.2 (m, 2H, Ar-H), MS (m/z): 424.

SDC: N-(2,4-dichlorophenyl)-2-(3,6-dihydroxy-9H-xanthen-9-yl)benzohydrazide.
M.P: 1096.21, Yield: 52%, IR (KBr cm^{-1}): 3320.4 (NH), 2924.8 (Ar CH str.), 1647.4 (C=O), 1218.1(C-O str. (ether), 1333.6 and 1517.0 (N-O str.), H^1NMR (DMSO): δ5.0(s, 1H, OH), δ2.0 (d, 2H, NH) δ8.01 (m, 4H, Ar-H), δ9.1 (m, 2H, Ar-H), MS (m/z):493.

SPA: 2-(3,6-dihydroxy-9H-xanthen-9-yl)-N-(3-hydroxyphenyl)benzamide.
M.P:1074.92, Yield: 20%, IR (KBr cm^{-1}): 3313.6 (NH str.), 2987.1 (Aro. str.), 1717.4 (C=O), 1241.5 (C-O str. ether). H^1NMR (DMSO): δ3.71 (s, 3H, OH) δ9.55 (s, H, NH), δ6.9 - δ7.2 (m, 4H, ArH), MS (m/z):425.

Anti cancer activity

The cell line used for the study were HeLa (human procured from NCCS, Pune).The cell lines were maintained in 96 wells micro titer plate containing MEM media supplemented with 10% heat inactivated fetal calf serum (FCS), containing 5% of Cisplatin in presence of 5% CO_2 at 37ºC for 48-72 hours.

Invitro growth inhibition effect of test compound was assessed by calorimetric or spectrophotometric determination of conversion of MTT into "Formazan blue" by living cells. The supernatant was removed from the plate and fresh MEM solution was added and treated with different concentrations of extract or compound appropriately diluted with DMSO. Control group contains only DMSO. In your study, 10, 20, 25, 30 and 50ul of the stock solution (10mg / ml prepared in DMSO) were added to respective wells containing 100ul of the medium. So, the final concentrations were 10, 20, 25, 30 and 50ug / ml. After 48hrs incubation at 37ºC in a humidified atmosphere of 5% CO_2, stock solution of MTT was added to each well (20µl, 5mg per ml in sterile PBS) for further 4 hr incubation. The

supernatant carefully aspirated, the precipitated crystals of "Formazan blue' were solubilised by adding DMSO (100µl) and optical density was measured at wavelength of 570 nm by using LISA plus. The results represent the mean of five readings of the concentration at which the OD of treated cells was reduced by 50% with respect to the untreated control.[12]

Formula

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of test compound} \times 100}{\text{Mean OD at control}}$$

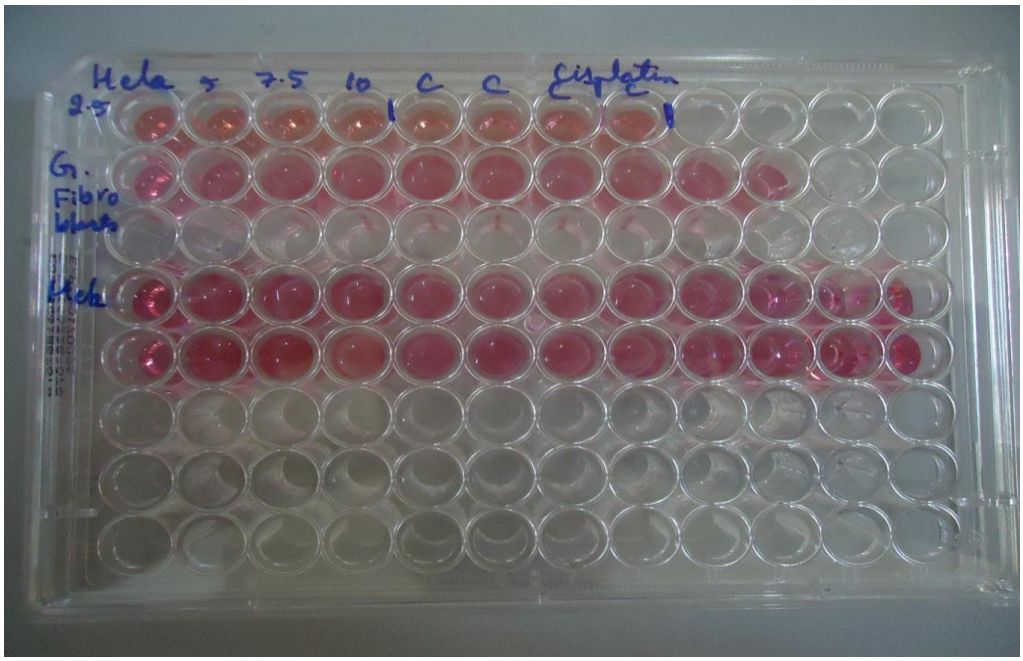


Fig: Anticancer activity of standard (Cisplatin) on Hela celline.

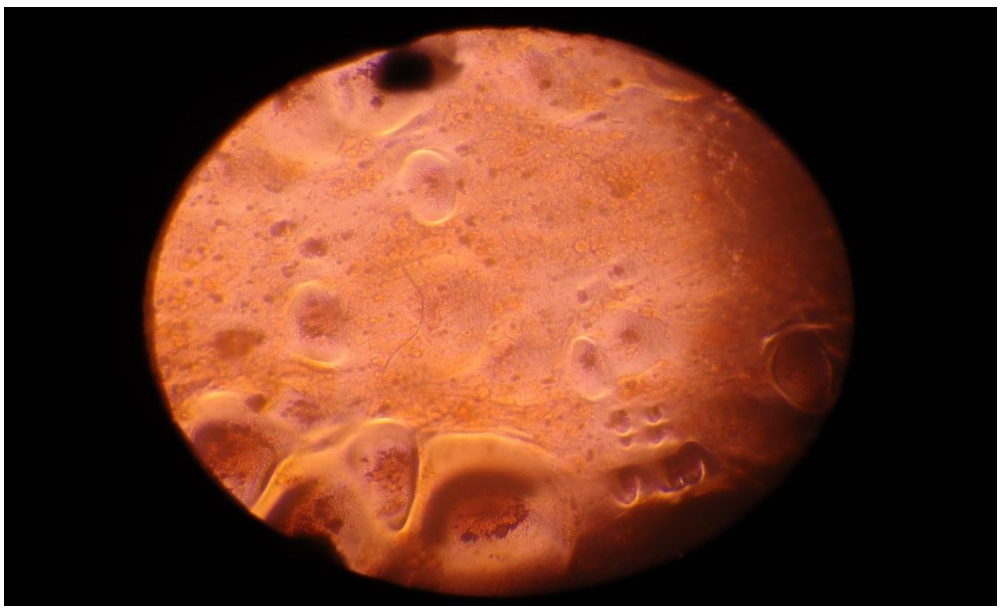


Fig: Anticancer activity of SDC (10ug).

RESULT AND DISCUSSION

Results for anti-cancer activity.

S.No.	Sample	Concentration (µg/ml)	Absorbance (nm)	Results as observed	IC50 (µg)
1.	SAP	10	1.990	No lysis	
2.		20	1.532	No lysis	
3.		25	1.327	No lysis	
4.		30	1.298	No lysis	
5.		50	0.864	No lysis	
	SDN				
6		10	2.350	No lysis	
7		20	2.113	No lysis	
8		25	1.992	No lysis	
9		30	1.682	No lysis	
10		50	1.003	No lysis	
11	SAN	10	0.576	<50% lysis	20ug
12		20	0.404	50% lysis	
13		25	0.356	>50% lysis	
14		30	0.204	>50% lysis	
15		50	0.141	>50% lysis	
16	SHH	10	0.515	<50% lysis	20ug
17		20	0.413	50% lysis	
18		25	0.248	>50% lysis	
19		30	0.233	>50% lysis	
20		50	0.212	>50% lysis	
21	SPH	10	0.119	No lysis	
22		20	1.011	No lysis	
23		25	0.812	No lysis	
24		30	1.601	No lysis	
25		50	0.726	No lysis	
26	SDC	10	0.119	50% lysis	10ug
27		20	0.011	>50% lysis	
28		25	1.812	>50% lysis	
29		30	1.621	>50% lysis	
30		50	0.525	>50% lysis	
31	SPA	10	1.980	No lysis	
32		20	1.532	No lysis	
33		25	1.027	No lysis	
34		30	0.278	No lysis	
35		50	1.864	No lysis	
36	Control	00	0.806	No lysis	

Results for anti-cancer activity (Positive control)

Sample	Concentration	Absorbance (nm)	Results as observed	IC50 (µg)
Cisplatin	**2.5**	0.196	<50% lysis	7.5
	5	0.182	<50% lysis	
	7.5	0.176	**50% lysis**	
	10	0.136	**>50% lysis**	
Control	**00**	0.332	No lysis	

Cell line - Hela

IC_{50} is half maximal inhibitory concentration - it is the half maximal (50%) inhibitory concentration (IC) of a substance (50% IC, or IC_{50})

CONCLUSION

The overriding purpose of this study was to determine the relative importance of several novel xanthene derivatives, we have aimed to synthesize, characterize and evaluate the anti cancer activity of hybrid molecules containing xanthene nucleus. For this purpose a series of xanthene derivatives were designed by the available literature search. Then the reaction conditions were optimized and Insilco docking were carried out to predict the affinity of these molecules towards the target protein. After confirming the possible biological activity the selected compounds were synthesized step by step along with their purifications.

To confirm the structures, they were exposed to analytical studies like IR, H^1NMR, etc. All the spectral studies were done from Centralized Sophisticated Instrument Facilities (CSIF) & Interdisciplinary Institute of Indian System of Medicine (IIISM), SRM University, Kattankulathur.

Then after getting satisfactory interpretations the biological activity was carried out to prove the potency. In this regard anti-cancer studies were successfully carried out from Maratha Mandal's Institute of Dental Science and Reasearch Center, Belgaum with good results.

A set of seven compounds were synthesized, characterized and subjected to anti cancer activities (against 1j4x.pdb). Moreover all the compounds possess good molecular binding activity against target protein receptor. All the compounds were found to have good affinity and maximum binding score (-10.7) with the target receptor. Compound SDC, SAN & SHH showed good anti-cancer activity on HeLa cell line when compared to standard drugs. Hence, these compounds can be of lead importance in the development of pharmaceutical drugs.

REFERENCES

1. Wu XL, Tian M, He H.Z, Sun W, Li JL, Shi Z. Synthesis and biological applications of two novel fluorescent proteins-labeling probes. Med. Chem. Lett, 2009; 19(11): 2957-9.
2. Beija, M.; Afonso, C. A. M.; Martinho, J. M. G. Synthesis and applications of Rhodamine derivatives as fluorescent probes. Chem .Soc. Rev, 2009; 38(8): 2410-33.
3. Hatakeyma, N., Ochi, H., Numata, S., Takano. A new route to substituted 3-methoxycarbonyl dihydropyrans. enantioselective synthesis of (–) - methyl elenolate; J. Chem. Soc. Chem. Commun, 1988; 1202-1204.
4. Song, G.; Wang, B.; Luo, H.; Yang, L. Catal. Commun, 2007; 8: 673.
5. Chatterjee, S., Iqbal, M., Kauer, J. C., Mallamo, J. P., Senadhi, S.; Mallya, S. Xanthene derived potent nonpeptidic inhibitors of recombinant human calpain I. Bioorg. Med. Chem. Lett, 1996; 6(13): 1619-22.
6. Nishiyama, T.; Sakita, K.; Fuchigami, T.; Fukui, T. Antoixidant activities of fused heterocyclic compounds, xanthenes-2, 7-diols with BHT or catecholskeleton. Polym. Degrad. Stab, 1998; 62(3): 529-34.
7. Hafez, H. N., Hegab, M. I., Ahmed Farag, I. S., Gazzar, A. B. A. A facile regioselective synthesis of novel *spiro*-thioxanthene and *spiro*-xanthene-9′, 2-[1,3,4]thiadiazole derivatives as potential analgesic and anti-inflammatory Bioorganic & medicinal chemistry letters, 2008 18(16): 4538-4543.
8. Cao, J. Q.; Yao, Y.; Chen, H.; Qiao, L.; Zhou, Y. Z.; Pei, Y. H. Chin. Chem. Lett, 2007; 18: 303.
9. Thangadurai, D.; Ramesh, N.; Viswanathan, M. B.; Prasad, D. X. Fitoterapia, 2001; 72: 92.
10. Lengauer T, Rarey M. Computational methods for biomolecular docking. Current opinion in structural Biology, Jun 1996; 6(3): 402-6.
11. Kitchen DB, Decornez H, Furr JR, Bajorath J. "Docking and scoring in virtual screening for drug discovery: methods and applications". Nature Reviews. Drug Discovery, Nov 2004; 3(11): 935-49.
12. 'Cell and Tissue Culture for Medical Research '.Alan Dolly, J. Brayan Griffiths, Jhon Willey and Son's.