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SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIINFLAMMATORY ACTIVITY OF SOME NEW 4-ARYL-8-ARYLIDENE-5,6-DIHYDRO-2-IMINO-6, 6-DIMETHYL-4H,7H-[3,1] BENZOTHIAZINES

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ABSTRACT

Claisen-Schmidt condensation of 4,4-dimethyl cyclohexanone (1) with different aromatic aldehydes yields 2,6-diarylidene-4,4-dimethyl cyclohexanone (2a-g), which on cyclocondensation with thiourea in presence of potassium hydroxide yielded title compounds (3a-g). Their structures were characterized by UV, IR, ^{1}H NMR and Mass spectra. The newly synthesized compounds were screened *in-vivo* for anti-inflammatory activity by carrageenan induced paw edema test at 200 mg/kg p.o. The compound 3b exhibited anti-inflammatory activity comparable to standard drug diclofenac.

Keywords: Synthesis, Benzothiazines, Anti-Inflammatory Activity, Carrageenan, Diclofenac.

INTRODUCTION

Inflammation is a physiological, local tissue response or immunological reaction of the organism to different stimuli such as infection, trauma, irritation and any foreign substance in order to eliminate or limit the spread of injurious agent [1]. Carrageenan- induced paw edema method is extensively used for determining the acute phase of inflammation. The inflammation consists of two phases. In the first acute phase cyclooxygenase catalysed products such as histamine, 5-hydroxy tryptamine and bradykinin are produced due to carrageenan administration [2]. In the second phase prostaglandins, neutrophils and arachidonic acid metabolites are produced [3]. The current existing drugs such as opiates and NSAIDS are producing many adverse effects [4] hence there is a need for the search of new drugs which is essential and valuable in the treatment of inflammation.

Benzothiazine derivatives exhibiting a wide variety of biological activities such as anti-inflammatory [5], anti-rheumatic [6], analgesic [7], anti-microbial [8], anti-proliferative [9] and anti-convulsant [10] are well documented in the literature. In continuation of the efforts in search of potent molecules possessing anti-inflammatory activity, novel 4-aryl-8-arylidene-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1]benzothiazines (3a-g) have been synthesized and screened for their anti-inflammatory activity which is reported in this paper.The synthesis pathway of title compounds is given in the Scheme 1.

MATERIALS AND METHODS

Melting points of all synthesized compounds were determined in open capillary tubes on an electro thermal apparatus and are uncorrected. The progress of the reaction and purity of the compounds was checked by TLC on silica gel coated aluminium plates (Merck) as adsorbent and the spots were observed by iodine chamber method. The absorption maxima of the synthesized compounds were recorded in methanol. The IR spectra were recorded on a

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BRUKER ATR-IR spectrophotometer in the range of 4000-400 cm^{-1} .^{1}HNMR spectra were recorded on INOVA (400 MHz) NMR spectrometer using $CDCl_3$ as solvent and TMS as an internal standard (chemical shifts in δ ppm). Mass spectra were recorded on a VG Autospec MS using ESI mode positive ion trap detector.

The drugs and chemicals were purchased from Sigma-Aldrich, India. All other chemicals and solvents were obtained from local firms (India) and were of highest purity and analytical grade.

EXPERIMENTAL

The reactant and product melting points were different from each other. It clearly indicates the formation of new chemical entities. All synthesized compounds were observed in a single spot which confirms the purity and completion of reaction. The IR, ^{1}HNMR and Mass spectral values confirm the structure of the synthesized compounds.

SYNTHESIS PROCEDURES

General procedure for the synthesis of 2,6-diarylidene-4,4-dimethylcyclohexanone (2a-g) [11]: A mixture of 10% sodium hydroxide, ethyl alcohol, 4, 4-dimethylcyclohexanone (0.01 mol) and aromatic aldehyde (0.02 mol) was stirred at 20-25˚C for 2h. Later the reaction mixture was kept in an ice chest over night. The product was filtered, washed with ice cold water followed by ice cold ethanol, dried and recrystallized from DMF.

General procedure for the synthesis of 4-aryl-8-arylidene-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1] benzothiazines (3a-g) [12]: A mixture of 2, 6-diarylidene cyclohexanone derivative (0.01 mol), thiourea (0.015 mol) and potassium hydroxide (0.01 mol) dissolved in 10 ml of water was refluxed in isopropyl alcohol for 16 h. Later the solvent was removed under reduced pressure and the residue obtained was treated with ice cold water, filtered, dried and recrystallized from ethanol. The physical data of the compounds were given in the table 1.

PHARMACOLOGICAL EVALUATION

The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Bharat Institute of Technology (Pharmacy), Hyderabad, Andhrapradesh with CPCSEA Registration No: 1015/c/06/CPCSEA.

Acute anti-inflammatory studies

Animals

Albino Wistar rats of either sex weighing 200-250 g, were placed in polypropylene cages with paddy husk as bedding in a controlled room temperature 24±2 ^oC, relative humidity 30-70 % and provided with food and water *ad libitum* were used for acute oral toxicity study.The animals were starved for 24 h before experimentation but fed with tap water throughout. All studies were carried out by using six rats in each group.

Acute oral toxicity studies

All the synthesized compounds (3a-g) were tested for acute toxicity study [13] by following OECD guidelines 420 after obtaining ethical clearance from animal ethical committee. Albino Wistar rats were (180–220 g) fasted for 24 h and divided into 3 groups of six animals each. The test compounds, suspended in sodium carboxy methyl cellulose (CMC) solution (0.5%) were administered orally in doses of 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg. None of the test compound had shown toxicity even at 2000 mg/kg, p.o. No signs of toxicity, weight variation, mortality were observed during 48 h of observation. Thus the cut off LD_{50} was $1/10^{th}$ of 2000 mg/kg i.e. 200 mg/kg was selected for each test compound.

Carrageenan induced rat paw edema method

Edema was induced by sub plantar injection of 0.1 ml of 1% freshly prepared suspension of carrageenan diluted in normal saline into the right hind paws of the rats. The volume of the carrageenan injected paws was measured after 1, 2, 3, 4 and 5h of induction of inflammation using a plethysmometer. Increase of paw edema thickness was calculated according to the method described by Winter *et al.,* [14]. The test groups received the synthesized compounds (200 mg/kg), the standard group received diclofenac (100 mg/kg), and the control animals received the vehicle only alone (0.5% w/v sodium CMC, 0.5 ml/100 g) p.o. The data obtained was subjected to statistical analysis by One-Way Analysis of Variance (ANOVA) followed by student's t-test. All the compounds showed significant difference when compared with control ($P \leq 0.001$). The results are presented in table 2.

% Reduction of inflammation was calculated by using the formula:

$$\%\ \text{red (reduction of inflammation)} = \frac{Vc - Vt}{Vc} X\ 100$$

Where,

Vt = Edema volume in the drug treated group

Vc = Edema volume in the control group

RESULTS AND DISCUSSION

The physical constants and characterization data of all the synthesized compounds reveals their successful synthesis. Anti-inflammatory activity of all the synthesized compounds (3a-g) was screened by carrageenan induced rat paw edema method against the standard drug diclofenac. Therefore from the overall findings it can be concluded that the title compounds substituted with strong electron withdrawing group showed excellent anti-inflammatory activity.

All the test compounds were shown 35 to 69% inhibitions of edema after 5h from the time of administration (Figure-1) while compounds 3f and 3g showed less activity even after 5h at a dose of 200 mg/kg.

Standard drug diclofenac shows 78% of inhibition after 5h from the time of administration. Among the test compounds, compound 3b was more active which shows 69% reduction of inflammation after 5h. Compounds 3a, 3c, 3d and 3e showed moderate activity when compared to the standard drug. Statistically significant results were observed for compounds 3b and 3c with P<0.001 and P<0.01 respectively using one way ANOVA, followed by student's t-test where P<0.001, P<0.01 and P<0.05 was considered statistically significant.

The structures of new compounds prepared during the present investigation have been authentically established by their ATR-IR, ^{1}HNMR and mass spectral studies. In the following section the spectral studies of some selected compounds were dealt.

Spectral data of 4-Phenyl-8-benzylidene-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1]benzothiazine (3a): UV (λ_{max} nm) 274; ATR-IR (ν_{max} cm^{-1}): 3262 –imine NH stretching; 3160 -cyclic NH stretching; 1025 -C-N stretching; 1543, 1462 -C=C stretching, 1602 -C=N stretching , 2920 -C-H stretching, ^{1}HNMR δppm 400 MHz, $CDCl_3$: δ0.9 (s, $(CH_3)_2$, 6H), δ1.7 (d, CH_2, 2H), δ1.9 (d, CH_2, 2H), δ4.9 (s, -CH-S, 1H), δ8.2 (s, imine, 1H), δ6.8 (s, cyclic NH, 1H), δ7.2-7.5 (m, ArH, 10H), δ8.0 (s, bezylic H, 1H), MS-m/z 361(M+).

Spectral data of 4-(4-Chlorophenyl)-8-(4-Chlorobenzylidene)-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1]benzothiazine (3b): UV (λ_{max} nm) 287; ATR-IR (ν_{max} cm^{-1}): 3259 -imine NH stretching; 3154 -cyclic NH stretching; 1007-C-N stretching; 1571,1480 -C=C stretching; 1614-C=N stretching; 2989 -C-H stretching; ^{1}HNMR δppm 400 MHz, $CDCl_3$: δ0.9 (s, $(CH_3)_2$, 6H), δ1.6 (d, CH_2, 2H), δ1.8 (d, CH_2, 2H), δ4.9 (s,-CH-S, 1H), δ6.6 (s, cyclic NH, 1H), δ7.1-7.4 (m, ArH, 8H, benzylic H, 1H), δ7.7 (s, imine H, 1H), MS-m/z 429(M+).

Spectral data of 4-(4-Methoxyphenyl)-8-(4-ethoxybenzylidene)-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1]benzothiazine (3c): UV (λ_{max} nm) 282; ATR-IR (ν_{max} cm^{-1}): 3498 –imine NH stretching; 3149 -cyclic NH stretching; 1023-C-N stretching; 1563, 1504 -C=C stretching; 1601 -C=N stretching; 1053 -C-O-C stretching; 2947-C-H stretching; ^{1}HNMR δppm 400 MHz, $CDCl_3$: δ0.9 (s, $(CH_3)_2$, 6H), δ1.7 (d, CH_2, 2H), δ1.8 (d, CH_2, 2H), δ3.8 (s, OCH_3, 6H), δ4.8 (s,-CH-S, 1H), δ8.2 (s, imine, 1H), δ6.7 (s, cyclic NH, 1H), δ6.9-7.3 (m, ArH, 8H), δ7.9 (s, bezylic H, 1H). MS-m/z 421(M+).

Spectral data of 4-(2,5-Dimethoxyphenyl)-8-(2,5-dimethoxybenzylidene)-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1] benzothiazine (3d): UV (λ_{max} nm) 273; ATR-IR (ν_{max} cm^{-1}): 3191-imine NH stretching; 3101-cyclic NH stretching; 1023 -C-N- stretching; 1572, 1486 -C=C stretching; 1616 -C=N stretching; 2920, 2825 -C-H stretching; ^{1}HNMR δppm 400 MHz, $CDCl_3$: δ0.9 (s, $(CH_3)_2$, 6H), δ1.9 (d, CH_2, 2H), δ2.1(d, CH_2, 2H), δ4.8 (s,-CH-S, 1H), δ8.0 (s, imine, 1H), δ6.4(s, cyclic NH, 1H), δ6.8 (s, bezylic H, 1H), δ7.0-7.5 (m, ArH, 6H),3.8 (s, 6H, 2×OCH_3), 4.3 (s, 6H, 2×OCH_3), MS-m/z 481(M+).

Spectral data of 4-(3,4,5-Trimethoxyphenyl)-8-(3,4,5-trimethoxybenzylidene)-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1] benzothiazine (3e): UV (λ_{max} nm) 276; ATR-IR (ν_{max} cm^{-1}): 3271 –imine NH stretching; 3128-cyclic NH stretching; 1021 -C-N stretching; 1564 &1452 -C=C stretching; 1597 -C=N stretching; 2944, 2834 -C-H stretching; ^{1}HNMR δppm 400 MHz, $CDCl_3$: δ1.0 (s, $(CH_3)_2$, 6H), δ1.8 (d, CH_2, 2H), δ2.0 (d, CH_2, 2H), δ4.6 (s,-CH-S, 1H), δ7.9 (s, imine, 1H), δ6.5 (s, cyclic NH, 1H), δ6.7 (s, bezylic H, 1H). δ6.9 (s, ArH, 2H), δ7.2 (s, ArH, 2H), 3.9 (s, 12H, 4×OCH_3), 4.0 (s, 6H, 2×OCH_3), MS-m/z 484(M+1).

Spectral data of 4-(4-Ethoxyphenyl)-8-(4-ethoxybenzylidene)- 5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1]benzothiazine (3f): UV (λ_{max} nm) 276; ATR-IR (ν_{max} cm^{-1}): 3301 –imine NH stretching; 3176 -CyclicNH stretching; 1028 -C-N stretching; 1512,1443 -C=C stretching; 1603 -C=N stretching; 1063 -C-O-C stretching; 2912, 2880 -C-H stretching;^{1}HNMR δppm 400 MHz, $CDCl_3$: δ0.9 (s, $(CH_3)_2$, 6H), δ1.4 (t, $(CH_3)_2$, 6H), δ1.9 (d, CH_2, 2H), δ2.1 (d, CH_2, 2H), δ2.6 (q, $(CH_2)_2$, 4H), δ4.9 (s, -CH-S, 1H), δ8.0 (s, imine, 1H), δ6.4 (s, cyclic NH, 1H), δ7.2-7.5 (m, ArH, 8H), δ6.8 (s, bezylic H, 1H), MS-m/z 450 (M+1).

Spectral data of 4-(4-N,N-dimethylphenyl)-8-(4-N,N-dimethylbenzylidene)-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1]benzothiazine(3g): UV (λ_{max} nm) 281; ATR-IR (ν_{max} cm^{-1}): 3258 –imine NH stretching; 3159 -cyclic NH stretching; 1089 -C-N stretching; 1569, 1480 -C=C stretching; 1615 -C=N stretching; 2964, 2881 -C-H stretching; ^{1}HNMR δppm 400 MHz, $CDCl_3$: δ0.9 (s, $(CH_3)_2$, 6H), δ1.7 (d, CH_2, 2H), δ1.9 (d, CH_2, 2H), δ1.2 (s, $(CH_3)_4$, 12H), δ4.9 (s, -CH-S, 1H), δ6.5 (s, cyclic NH, 1H), δ7.2-7.9 (m, ArH, 8H and imine H, 1H), δ6.7 (s, bezylic H, 1H), MS-m/z 447(M+).

Table 1. Physical data of the compounds (3a-g)

Compound. no.	Ar	Mol. formula	Mol. weight	M.P (˚C)	$R_f$value	% Yield
3a	Phenyl	$C_{23}H_{24}N_2S$	360.5	148-150	0.45	70
3b	*p*-Chloro phenyl	$C_{23}H_{22}Cl_2N_2S$	429.4	284-286	0.48	84
3c	*p*-Methoxy phenyl	$C_{25}H_{28}N_2O_2S$	420.5	240-242	0.43	88

3d	*o,m*-Dimethoxy phenyl	$C_{27}H_{32}N_2O_4S$	480.6	242-244	0.46	60
3e	*o,m,p*-Trimethoxy phenyl	$C_{29}H_{36}N_2O_6S$	482.5	286-288	0.53	90
3f	*p*-Ethoxy phenyl	$C_{27}H_{32}N_2O_2S$	448.6	205-206	0.60	70
3g	*p*-Dimethylamino phenyl	$C_{27}H_{34}N_4S$	446.6	140-142	0.54	65

Table 2. Anti-inflammatory activity of synthesized compounds (3a-g)

Compound	1h		2h		3h		4h		5h	
	Mean±SEM	% red	Mean±SEM	% red	Mean±SEM	% red	Mean±SEM	%red	Mean±SEM	% red
Control	1.73±0.004		1.97±0.002		1.93±0.006		1.87±0.007		1.84±0.005	
Standard	1.24±0.015***	28	1.10±0.054***	44	0.84±0.010**	56	0.54±0.004***	71	0.40±0.002***	78
3a	1.49±0.15	14	1.43±0.13	27	1.26±0.1	35	1.00±0.11	47	0.81±0.043	56
3b	1.29±0.17***	25	1.27±0.2**	36	1.12±0.16*	42	0.75±0.18**	60	0.57±0.023**	69
3c	1.30±0.15**	25	1.25±0.19**	37	0.96±0.13*	50	0.83±0.04*	56	0.73±0.65*	60
3d	1.51±0.21*	13	1.40±0.21*	29	1.23±0.24	36	1.10±0.25	41	0.93±0.04	49
3e	1.58±0.11	9	1.60±0.1	19	1.38±0.15	28	1.15±0.16	39	0.88±0.25	52
3f	1.43±0.07**	8	1.75±0.10	11	1.55±0.07	20	1.40±0.07	25	1.20±0.12	35
3g	1.68±0.07	3	1.80±0.08	9	1.48±0.07	23	1.29±0.07	31	1.04±0.46	43

N=6, Values are Mean±SEM; values are compared with control group.
P<0.001***, P<0.01** and P<0.05* was considered statistically significant.

Scheme1. Schematic representation of synthesized compounds (3a-g)

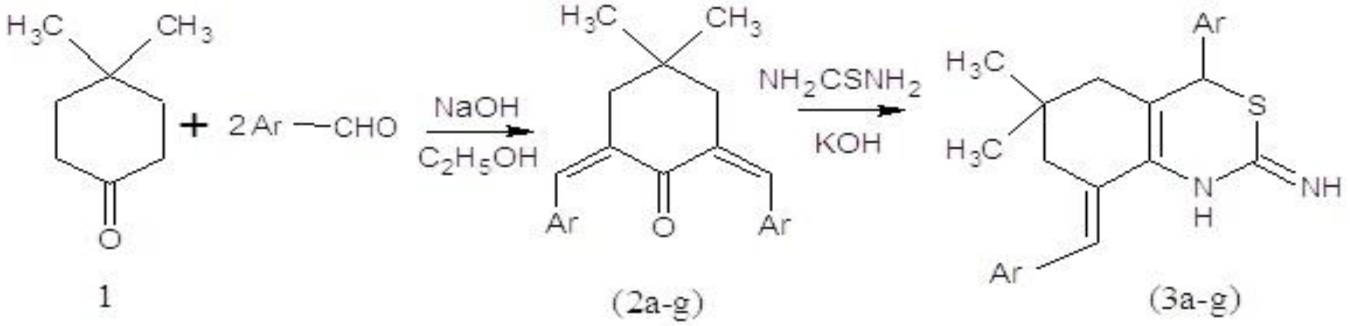
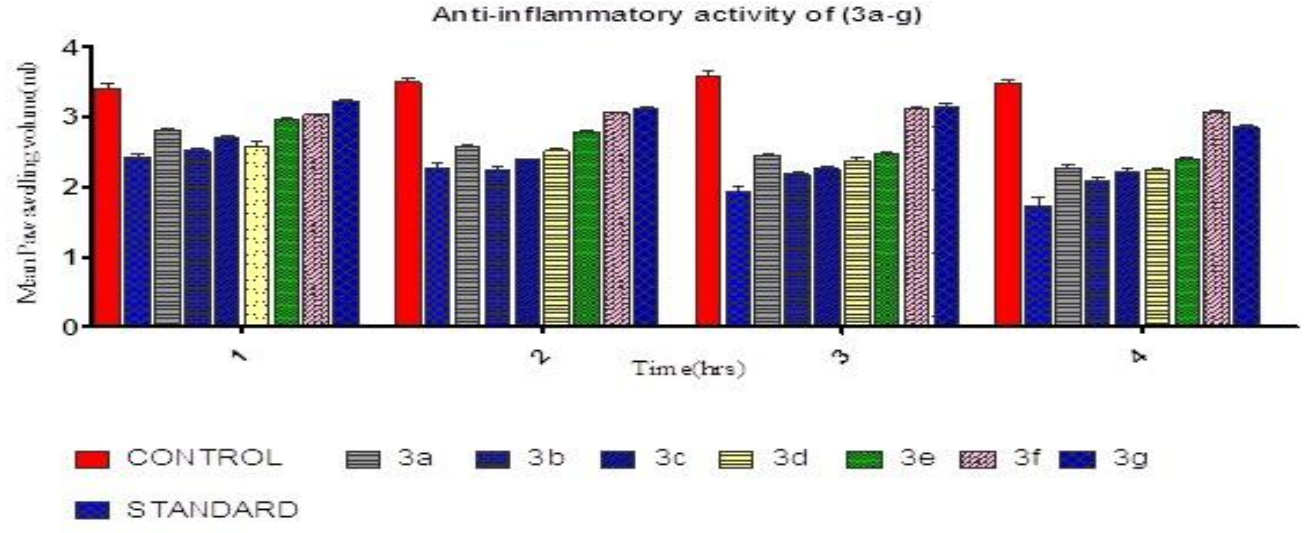


Figure 1. Graphical representation of anti-inflammatory activity of compounds (3a-g)



CONCLUSION

New 3, 1-benzothiazine derivatives were synthesized and evaluated for the anti-inflammatory activity. Some of these derivatives were shown to be fairly effective. The others have exhibited slight inhibition of inflammation. The thiazine derivatives reduced the carrageenan induced paw edema in rats. It may be due to inhibition of cyclooxygenase which activates prostaglandin synthesis followed by prevention of inflammatory mediators release. While considering all the newly synthesized compoundsof this series together, we may conclude that, presence of chloro substituent has shown better anti-inflammatory activity than other compounds.

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REFERENCES

1. Harsh M. Text book of pathology, 5th edition, Jitendar P Vij, Jaypeebrothers medical publishers (p) ltd, EMCA House, New Delhi 110002. India, 2005, 133-153.
2. Di Rosa M, Willoughby DA. Screening for anti-inflammatory drugs. *J Pharm Pharmacol*., 23(4), 1971, 297-298.
3. Salvemeni D, Wang ZQ, Bourdon D M, Stern M K, Currie M G, Manning P T. Evidence of peroxynitrate involvement in the carraeenan-induced rat paw edema. *Eur J Pharmacol*., 303(3), 1996, 217-220.
4. Ahmadiani A, Fereidoni M, Semnanion S, Kamalinejad M, Saremi S. Antinociceptive and anti-inflammatory effects of Sambucusebulus rhizome extracts in rats. *J Ethanopharmacol*., 61(3), 1998, 229-235.
5. Bozsing D, Sohar P, Gigleer G and Kovacs G. Synthesis and pharmacological study of new 3,4-dihydro-2H,6H-pyrimido-[2,1-b][1,3] thiazines. *Eur. J. Med. Chem.*, 31, 1996, 663.
6. Hiroharu M, Nobuhiro O, Masahiko M. Novel Methotrexate Derivatives Bearing a Benzoxazine or Benzothiazine Moiety. *J. Med. Chem.*, 40(1), 1997, 105-111.
7. Haider F, Haider Z. The synthesis and antimicrobial screening with spectral analysis of some 1,3-thiazines. *J. Chem. Pharm. Res*., 4(4), 2012, 2263-2267.
8. Banda G, Hipparagi SM, Ramjith US and Jacob CM. The Synthesis of thiazine derivatives of fluoro, chlorobenzimidazole by the microwave induced reaction and screened for their antibacterial and analgesic activity. *Int J Res Pharam Sci*., 2(3), 2012, 146 -158.
9. Joanna M. Synthesis, antiproliferative and antifungal activities of some 2-(2,4-dihydroxyphenyl)-4H-3,1-benzothiazines.*Bioorganic & Medicinal Chemistry*, 14, 2006, 2613-2619
10. Tadeusz SJ. Synthesis and biological activity of certain novel derivatives of 1H-pyrrolo [1,2-][1,3] thiazine. *Actapolaniae drug research*, 60(1), 2003, 67-74.
11. Vogel AI. Text Book of Practical Organic Chemistry, 4th ed. ELBS, London, 1986, 796.
12. Harode K and Sharma T C. Indian J Chem., 27B, 1988, 1144.
13. OECD Guidelines for testing of chemicals. Acute oral toxicity-fixed dose procedure. 420, modified, adopted 23rd Mar. 2006.
14. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med, *Pro SocBiol Med*, 11, 1962, 544-547.