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Research Article

SCREENING OF IN -VITRO ANTI-INFLAMMATORY ACTIVITY OF SOME NEWLY SYNTHESIZED 1,3-THIAZINE DERIVATIVES

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ABSTRACT

1,3-Thiazines are one of the N and S containing heterocyclics having prominent activities like Antibacterial, Antifungal, Antitubercular, Antihelmintic Antitumoral, Antihistaminic, Anti-inflammatory activities etc. In the present study various 1, 3-Thiazine derivatives were synthesized by reacting acetanilide derivatives with substituted aryl aldehydes to give chalcones (A & E) which are then cyclized by reacting with thiosemicarbazide to give 2-hydrazinyl 1,3-thiazine derivatives (B & F). The latter compounds were treated with substituted aryl aldehydes or ketones to give 2-arylidene hydrazinyl 1,3-thiazine derivatives (C & G). These derivatives (C & G) were refluxed with Glycine in ethanol / Vilsmeir-Hack reagent (DMF: $PoCl_3$) giving 2-substituted Imidazolidin-4-one 1, 3-Thiazine derivatives (D_{1-4}) and 2-substituted pyrazolyl 1, 3-Thiazine derivatives (H_{1-4}) respectively. All the derivatives were spectrally characterized and screened for *In-vitro* Antihelmintic activity. All compounds have shown significant activity when compared with standard.

Keywords: Vilsmeir-Hack reagent, anti-inflammatory activity, thiazines.

INTRODUCTION

Thiazines[1] are six membered heterocyclics that contain in their structure a nitrogen atom and a sulfur atom. Thiazines are very useful units in the fields of medicinal and pharmaceutical chemistry and have been reported to exhibit a variety of biological activities. Chemicals that include thiazines are also used for dyes and insecticides. Depending on the relative position of Nitrogen, Sulphur atoms and the cycle oxidation grade three positional isomers are possible they are 1, 2- thiazines, 1, 3- thiazines and 1, 4-thiazines. The current work is going to be done on 1, 3-Thiazines. Structure of 1, 3-thiazines possesses an N-C-S linkage that is believed to be very useful units in the fields of medicinal and pharmaceutical chemistry. 1,3-thiazines and its derivatives have been reported to exhibit a variety of biological activities like Antibacterial[2], Antifungal[3], Antitubercular[4], Anti-inflammatory[5], Analgesic[6], Sedative-hypnotic[7], Immunosuppressive agents[8], Herbicidal[9].General synthesis involves synthesis of chalcones by Claisen Scmidth condensation reaction[10] followed by cyclo condensation of chalcones in the presence of thiosemicarbazide giving 1, 3-Thiazines. The constitution of derivatives has been supported by elemental analysis, IR, NMR and Mass spectral data.

MATERIALS AND METHODS

All the melting points were determined using open capillary tubes in scientific melting point apparatus and are uncorrected. IR spectra of synthesized compounds were scanned by using Bruker ALPHA- T model. NMR spectra of synthesized derivatives were recorded on Bruker AMX model at 400 MHz, Mass spectra of synthesized derivatives were recorded on Agilent 1100 Series LC-MSD. Progress, purity of

reaction and intermediates were analyzed using TLC method.

Synthesis of 3-(4-(dimethyl amino) phenyl)-N-(4-nitrophenyl) acryl amide (A)
To equimolar mixture (0.01 moles) of Nitro acetanilide and benzaldehyde in ethanol (50 ml), 2% NaOH solution (1 ml) was added and stirred for 10 hrs at room temperature and then refluxed for 6 hrs on water bath. The excess solvent was distilled off under vacuum and poured into ice cold water, filtered and recrystallized from ethanol.

Synthesis of 6-(4-(dimethylamino)phenyl)-2-hydrazinyl-N-(4-nitrophenyl)-6H-1,3-thiazin-4-amine (B)
To equimolar mixture (0.008 moles) of compound **1** and Thiosemicarbazide in ethanol (30 ml) was refluxed on water bath for 6 hrs. The reaction mixture was concentrated under vacuum, cooled and poured into ice cold water. The solid separated was filtered, dried and recrystallized from ethanol.

Synthesis of 2-(2-arylidenehydrazinyl)-6-(4-(dimethylamino) phenyl)-N-(4-nitrophenyl)-6H-1, 3-thiazin-4- amine (C)
To equimolar mixture (0.01 moles) of compound **2** add hydrazine hydrate in ethanol (20 ml) and refluxed for 2 hrs. Then the solution was kept in refrigerator for 2 to 3 hrs and then filtered, dried and recrystallized from ethanol.

Synthesis of 3-(4^1-(4-nitrophenylamino)-6 ^{1} -(4-(dimethylamino) phenyl) - 6H-1,3-thiazin-2 ^{1} -ylamino)-2- substituted imidazolidin-4-one derivatives (D_{1-4})[11]
To compound **3** 0.7 gm (0.001 moles) in ethanol (50 ml) and glycine 0.07 gm (0.001 moles) with pinch of zinc chloride is refluxed on water bath for about 8 hrs. The separated solid was recrystallized from methanol: chloroform (6:4).
The characterization data of **(D_{1-4})** were shown in **Table no:1**

Synthesis of 5-(4-chlorophenyl)-3-oxo-N-phenylpent-4-enamide (E)
To mixture of Acetoacetanilide (0.01 moles) and benzaldehyde (0.01 moles) in ethanol (50 ml), 2% NaOH solution (1 ml) was added and stirred for 10 hrs at room temperature and then refluxed for 6 hrs on water bath. The excess solvent was distilled off under vacuum and poured into ice cold water, filtered and recrystallized from ethanol.

Synthesis of 6-(4-chlorophenyl)-2-hydrazino-6H-1, 3-thiazin-4-yl-N-phenylacetamide (F)
To a mixture (0.006 moles) of compound **1** and Thiosemicarbazide (0.006 moles) in ethanol (30 ml) was refluxed on water bath for 6 hrs. The reaction mixture was concentrated under vacuum, cooled and poured into ice cold water. The solid separated was filtered, dried and recrystallized from ethanol.

Synthesis of 2-(2-arylidene hydrazinyl)-6-(4-chlorophenyl)-6H-1, 3-thiazin-4-yl-N-phenylacetamide (G)
To a mixture of compound **2** (0.01 moles) add hydrazine hydrate (0.01 moles) in ethanol (20 ml) and refluxed for 2 hrs. Then the solution was kept in refrigerator for 2 to 3 hrs and then filtered, dried and recrystallized from ethanol.

Synthesis of 2-(2^1-(3-(substituted)-4-formyl-2.3-dihydro-(pyrazol-1-yl)-6^1-(4-chlorophenyl)-6H-1, 3-thiazin-4^1-yl)-N-phenyl acetamide derivatives (H_{1-4})
To the mixture of compound 3 add 1 ml of Vilsmeir-Hack reagent (prepared from 10 ml[0.13 moles]) of DMF and 1.1 ml [0.012 moles] of $PoCl_3$ stirred continuously at 60-65 ^oC for 3 hrs and then poured into ice cold water. The solid was separated out on neutralization with $NaHCO_3$ solution, Filtered and washed with water. Recrystallized with Chloroform.
The characterization data of (H_{1-4}) were shown in **Table no: 2**

Spectral data of synthesized derivative compounds

3-(4-(4-nitrophenylamino)-6-(4-(dimethyl amino)phenyl)-6H-1,3-thiazin-2-ylamino)-2-(furan-2-yl)-imidazolidin-4-one *(D_1)*
IR: (KBr pellet, υ in cm-1): 2987.32 (str, CH), 2313.78 (str, CSC), 1714.97 (str, C=O), 1584.53 (str, NO), 1474.92 (str, ArC=C), 1170.11 (str, CN).
^{1}H NMR: (DMSO-d6, δ in ppm): 7.975 (2H, s, Ar-H), 7.566 (1H, s, CH), 6.590 (2H, d, Ar-H), 2.957 (6H, s, CH_3), 2.505 (2H, s, NH).

3-(4-(4-nitrophenylamino)-6-(4-(dimethyl amino)phenyl)-6H-1,3-thiazin-2-ylamino)-2-(4-hydroxyphenyl)- imidazolidin-4-one *(D_2)*
IR :(KBr pellet, υ in cm-1): 3664.91 (str, OH), 3355.95 (str, NH), 2987.97 (str, Ar CH), 2803.52 (str, CH), 2315.36 (str, CSC), 1583.32 (str, C=N), 1432.51 (str, ArC=C), 1162.19 (str, CN).
^{1}H NMR: (DMSO-d6, δ in ppm): 7.953 (2H, s, Ar-H), 4.231 (1H, s, NH), 3.410 (2H, s, CH_2).

^{13}C NMR : (DMSO-d6, δ in ppm) : 186 (C=O), 154.20 (Ar-C), 143.50(Furan-C), 38-40 (aliphatic, C)

3-(4-(4-nitrophenylamino)-6-(4-(dimethyl amino))-6H-1,3-thiazin-2-ylamino)-2-(4-(dimethylamino)phenyl)-imidazolidin-4-one *(D_3)*

IR :(KBr pellet, υ in cm-1): 3168.58 (str, Ar CH), 2902.30 (str, CH), 2315.10 (str, CSC), 1578.72 (str, C=N), 1442.41 (str, ArC=C), 1167.76 (str, CN).

^{1}H NMR: (DMSO-d6, δ in ppm): 7.952 (2H, d, Ar- H), 6.588 (1H, d, NH), 4.207 (1H, s, NH), 2.506 (6H, s, CH_3).

3-(4-(4-nitrophenylamino)-6-(4-(dimethyl amino)phenyl)-6H-1,3-thiazin-2-ylamino)-2-(4-bromophenyl)- imidazolidin-4-one *(D_4)*

IR :(KBr pellet, υ in cm-1): 3179.78 (str, Ar CH), 2921.34 (str, CH), 1516.51 (str, C=N) 1439.85 (str, ArC=C), 1162.11 (str, CN).

^{1}H NMR: (DMSO-d6, δ in ppm): 7.539(2H, d, Ar-H), 6.795 (2H, d, Ar-H), 3.369 (1H, s, NH), 2.506(1H, s, CH), 2.038 (12H, s, CH_3).

2-(2-(3-(2-amino-4-chlorophenyl)-4-formyl-1H-pyrazol-1-yl)-6-(4-chlorophenyl)-6H-1,3-thiazin-4-yl)-N-phenylacetamide *(H_1)*

IR :(KBr pellet, υ in cm-1): 3415.36 (str, Ar NH), 2982.74 (str, Aldehyde CH), 2900.51 (str, Methylene CH), 2314.02 (str, CSC), 1667.62 (str, amide C=O), 1464.12 (str, ArC=C), 1075.69 (str, Ar-Cl), 695.03 (C-S).

^{1}H NMR: (DMSO-d6, δ in ppm): 11.573 (1H, s, Aldehyde C=O), 7.728 (2H, d, Ar-H), 2.528 (1H, d, CH), 1.994 (2H, d, CH_2).

2-(6-(4-chlorophenyl)-2-(4-formyl-3-(4-nitro phenyl)-1H-pyrazol-1-yl)-6H-1,3-thiazin-4-yl)-N-phenylacetamide *(H_2)*

IR :(KBr pellet, υ in cm-1): 3486.23 (str, NH), 3295.29 (str, ArC-H), 2980.92 (str, methylene C H); 2899 (str, C- H), 2312.34 (str, CSC), 1659.67 (str, C=O conjugated with α,β-unsaturated ketone), 1596.67 (str, NO), 689.44 (C-S), 689.15(C-S).

^{1}H NMR: (DMSO-d6, δ in ppm): 8.167 (1H, d, NH), 7.670 (2H, d, Ar H), 3.869 (1H, s, CH), 2.501 (2H, d, CH_2).

2-(2-(3-(4-aminophenyl)-4-formyl-1H-pyrazol-1-yl)-6-(4-chlorophenyl)-6H-1,3-thiazin-4-yl)-N-phenylacetamide *(H_3)*

IR :(KBr pellet, υ in cm-1): 3293.06 (str, NH), 2980.24 (str, Ar CH), 2899.65 (str, Aldehyde CH), 2311.37 (str, CSC), 1651.94 (str, C=O conjugated with α,β-unsaturated ketone), 1441.60 (str, ArC=C), 689.15(C-S).

2-(6-(4-chlorophenyl)-2-(4-formyl-3-(2-hydroxyphenyl-)1H-pyrazol-1-yl)-6H-1,3-thiazin-4-yl)-N-phenylacetamide *(H_4)*

IR :(KBr pellet, υ in cm-1): 3157.32 (str, NH), 2984.96 (str, methylene CH), 2888.14 (str, aldehyde CH), 2312.00 (str, CSC), 1585.15 (str, C=N), 1479.94 (str, ArC=C), 1164.34 (str, CN).

^{1}H NMR: (DMSO-d6, δ in ppm): 11.473 (1H, s, CHO), 7.954 (1H, s, NH), 7.547 (2H, d, Ar-H), 1.238 (1H, s, CH_2).

^{13}C NMR: (DMSO-d6, δ in ppm): 189 (Aldehyde, C), 159 (Acetamide, C), 128 (Ar, C) , 39.60 (aliphatic , C).

***In-vitro* Anti-inflammatory activity**

Membrane stabilization test[12]

Preparation of red blood cells (RBCs) suspension

Fresh whole human blood (10 ml) was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and the supernatant carefully removed with a sterile pipette, washed three times with equal volume of isotonic saline (resuspended in an equal volume of isotonic saline and centrifuged again). The process was repeated three times until the supernatants were clear. The volume of blood was measured and reconstituted as 10% v/v suspension with isotonic saline.

Heat induced hemolytic

The reaction mixture (3 ml) consisted of 1.5 ml of test sample solution and 1.5 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. **Diclofenac** was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent Inhibition of hemolysis was calculated by the formula mentioned below

% Inhibition of hemolysis = abs (control) – abs(sample)/ abs(control) × 100

or

% inhibition of haemolysis = 100 × (1- abs (sample)/ abs (control))

Synthesized derivatives were evaluated for anti inflammatory activity with appropriate method at concentrations 100, 250, 500 and 1000 µg/ml. **Diclofenac** has been taken as standard. Saline is taken as Control. The results were expressed as Mean ± SEM and statistically analyzed by one way ANNOVA followed by Dunnett's multiple Comparison Test with level of significance set at $P < 0.05$. **Table 3 and figure 1** shows Anti-inflammatory activity of synthesized compounds D_{1-4} and **Table 4 and figure 2** shows Anti-inflammatory activity of synthesized compounds H_{1-4}.

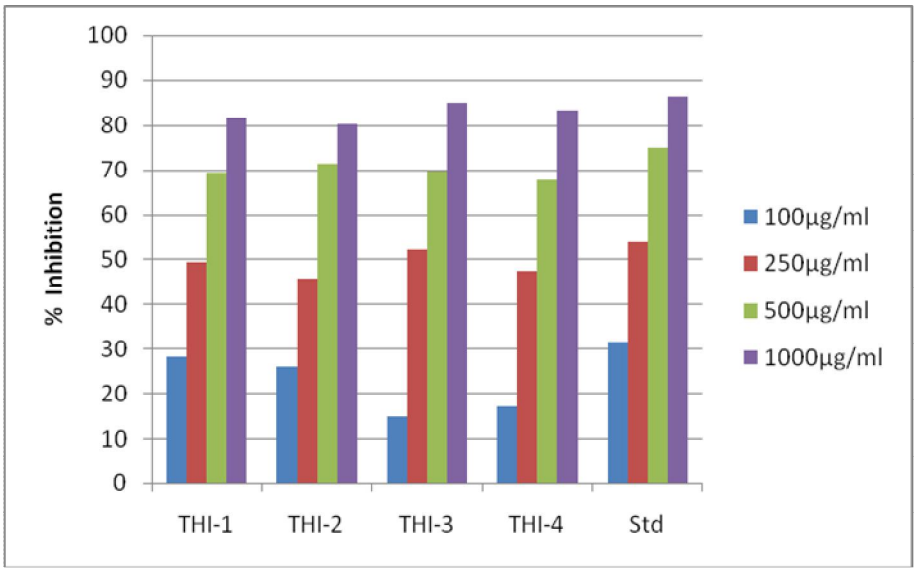


Fig. 1: % Inhibition of synthesized compounds D1-4

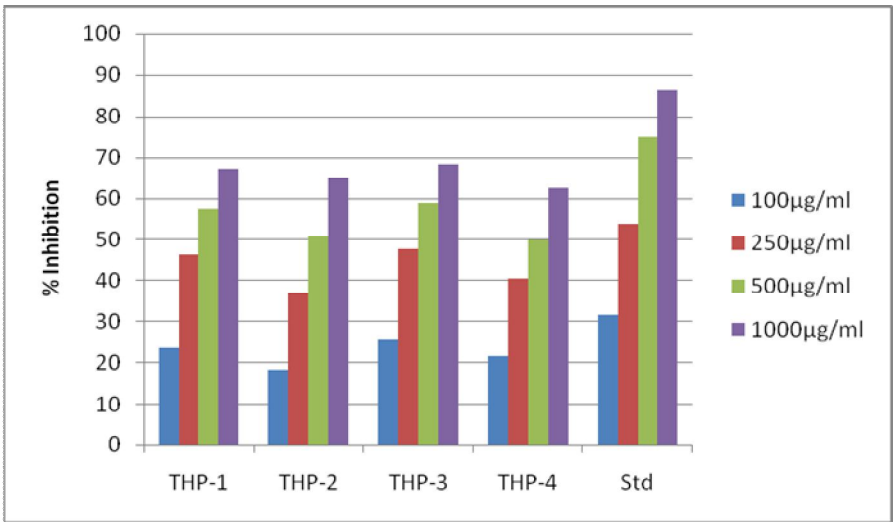
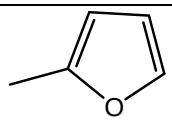
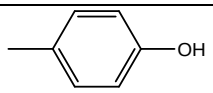
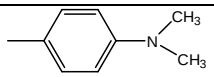
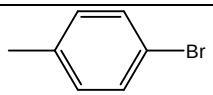


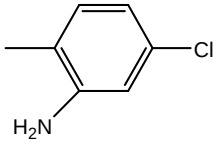
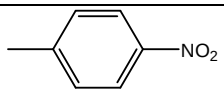
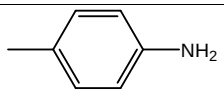
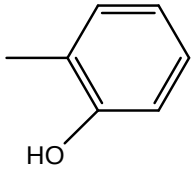
Fig. 2: % Inhibition of synthesized compounds H1-4

Table 1: Characterization data of D1-4

Cpd. Code	R	Mol. Formulae	Mol. Wt.	M.P (^{0}C)	% Yield (w/w)	Recrystallisation Solvent	R_f Value*
D 1		$C_{25}H_{25}N_7O_4S$	519.58	75-78	76.15	CH_3OH:$CHCl_3$(6:4)	0.85
D 2		$C_{27}H_{27}N_7O_4S$	545.61	68-71	81.76	CH_3OH:$CHCl_3$(6:4)	0.60
D 3		$C_{29}H_{32}N_8O_3S$	572.68	60-65	73.56	CH_3OH:$CHCl_3$(6:4)	0.86
D 4		$C_{27}H_{27}BrN_6O_2S$	579.51	72-74	66.06	CH_3OH:$CHCl_3$(6:4)	0.81

*Mobile Phase = Ethyl acetate: Chloroform (7:3)

Table 2: Characterization data of H 1-4

Cpd. code		Mol. Formulae	Mol. Wt.	M.P (^{0}C)	% Yield (w/w)	Recrystallization Solvent	R_f Value*
H 1		$C_{33}H_{26}Cl_2N_5O_2S$	627.56	85- 89	56.96	$CHCl_3$	0.73
H 2		$C_{28}H_{20}ClN_5O_4S$	558.01	90-93	48.83	$CHCl_3$	0.81
H 3		$C_{28}H_{22}ClN_5O_2S$	528.02	110 - 113	67.53	$CHCl_3$	0.89
H 4		$C_{28}H_{21}ClN_4O_3S$	529.01	100 - 103	74.69	$CHCl_3$	0.65

*Mobile Phase = Ethyl acetate: Chloroform (7:3)

Table 3: Anti-inflammatory activity of synthesized compounds D1-4

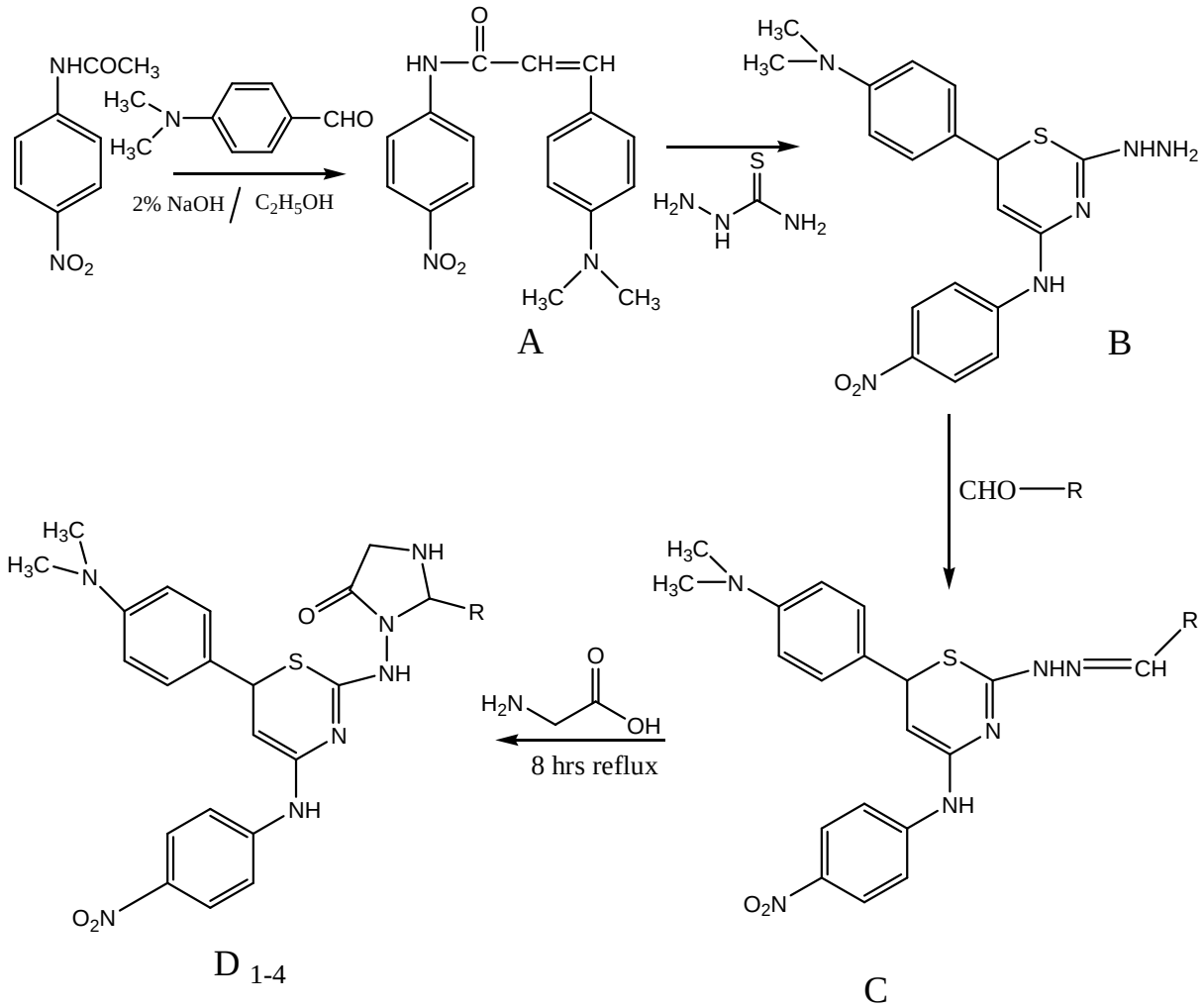
Compound Code	Percentage Inhibition values ± S.E.M				
	100µg/ml (%)	250µg/ml (%)	500µg/ml (%)	1000µg/ml	(%)
D_1	28.07±0.81	49.42±0.53	69.28±0.54	81.62±0.66	
D_2	25.77±0.77	45.66±0.62	71.43±0.83	80.28±0.55	
D_3	14.90±0.74	52.02±1.21	69.54±0.89	84.91±0.41	
D_4	16.98±0.95	47.44±0.65	67.75±1.09	83.32±0.29	
Std.	31.54±0.21	53.69±0.32	74.87±0.36	86.31±1.14	

Note: Results are average of Triplicate experiments. S.E.M = Standard Error Mean

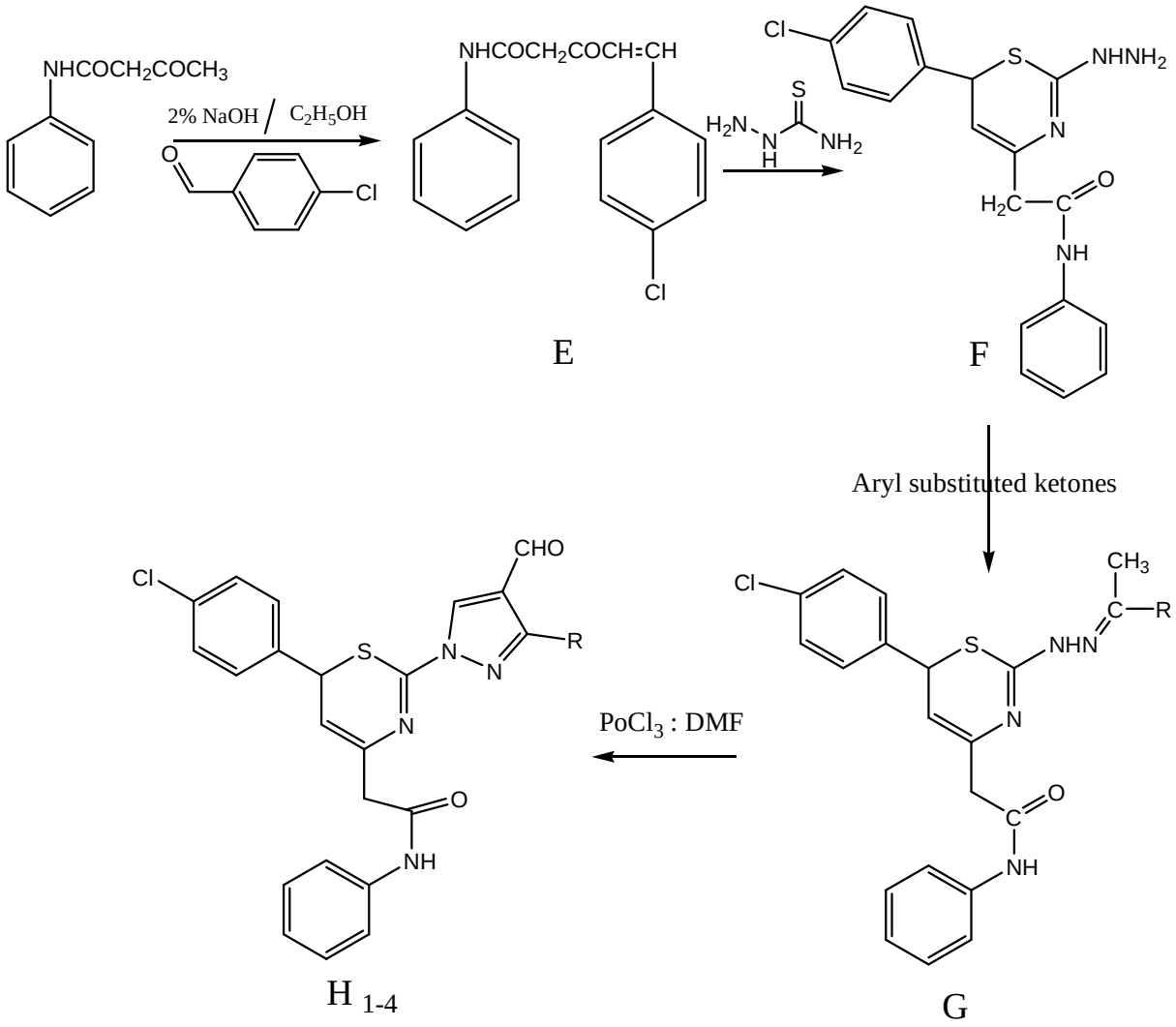
Table 4: Anti-inflammatory activity of synthesized compounds H1-4

Compound Code	Percentage Inhibition values ± S.E.M				
	100µg/ml (%)	250µg/ml (%)	500µg/ml (%)	1000µg/ml	(%)
H_1	23.77±0.12	46.20±0.34	57.45±0.21	67.10±0.23	
H_2	18.18±0.79	36.70±1.12	50.82±0.35	64.82±0.58	
H_3	25.54±0.86	47.66±0.76	58.72±0.43	68.21±0.31	
H_4	21.32±0.88	40.45±0.41	49.83±0.64	62.39±0.87	
Std.	31.54±0.21	53.69±0.32	74.87±0.36	86.31±1.14	

Note: Results are average of Triplicate experiments. S.E.M = Standard Error Mean



Scheme 1: Synthesis of 3-(41-(4-nitrophenylamino)-6 1 - (4-(dimethylamino) phenyl) - 6H-1,3-thiazin-2 1 -ylamino)-2- substituted imidazolidin-4-one derivatives (D1-4)



Scheme 2: synthesis of 2-(2^{I}-(3-(substituted)-4-formyl-2,3-dihydro-(pyrazol-1-yl)-6^{I}-(4-chlorophenyl)-6H-1,3-thiazin-4^{I}-yl)-N-phenyl acetamide derivatives (H_{1-4})

CONCLUSION

Novel derivatives containing 1,3-Thiazine nucleus have been synthesized and spectrally analyzed. These derivatives were subjected to In-vitro Anti-inflammatory activity, which revealed that all compounds have shown dose dependent significant activity when compared with standard drug.

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