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SYNTHESIS OF SOME NOVEL 2-(N-SUBSTITUTED AMINO)-4, 5, 6, 7-TETRAHYDRO-BENZO[*b*] THIOPHENE-3-CARBOXYLIC ACID ETHYL ESTER DERIVATIVES AND IT'S ANTI-MITOTIC AND ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT
Synthesis of some novel 2-(N-substituted amino)-4, 5, 6, 7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid ethyl ester derivatives was carried and evaluated by spectral studies. The compounds (2a-2d) were screened for preliminary cytotoxic evaluation on germinating seeds of Vigna *radiate* (mung bean) for rapid and inexpensive screening of drugs exhibiting cytotoxic properties. Aspirin was used as a standard drug. The compounds 2a and 2c have shown good anti-mitotic activity at 5 mg/ml as par with aspirin at 2 mg/ml. The compounds (2a-2d) were screened for their anti-inflammatory activity. Ibuprofen was used as standard. All the synthesized compounds and standard were used at 100 µg/ml concentration. The compound 2d having 4-amino phenyl group showed maximum activity (72.72 %).
Key Words: Benzo [*B*] Thiophene derivatives, anti-inflammatory, anti-mitotic activity.

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INTRODUCTION

Thiophenes and its derivatives are an important class of heterocyclic compounds consisting of a flat five membered ring with a sulphur atom. Specifically, 2-amino substituted thiophenes were reported to possess a wide spectrum of biological properties such as antimicrobial (1-4), antifungal (5), analgesic (6), anti-inflammatory (7), antioxidant, antitumor (8-9), anticancer (10) and local anesthetic activity (11).
2-amino substituted thiophene derivatives were Reported to possess anti-inflammatory activity which is believed to inhibit the arachidonic acid binding site of cyclooxygenase enzyme and suppress the biosynthesis of pro-inflammatory agents. Some thiophene derivatives were reported to possess antidepressant, sedative and analgesic activities (12).
So far various new thiophene derivatives have been synthesized and screened in laboratories for anti-mitotic and anti-inflammatory activities (13).
Extensive literature review gave us an impetus to focus on the synthesis of some novel 2-(N-substituted amino)-4, 5, 6, 7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid ethyl ester derivatives and evaluate for anti-mitotic and anti-inflammatory activity using *in-vitro* methods.

EXPERIMENTALS

Synthesis some novel 2-(N-substituted amino)-4, 5, 6, 7-tetrahydro-benzo[*b*]thiophene-3-carboxylicacid ethyl ester derivatives is given in fig-1.

Synthesis of 2-amino-3-carbethoxy-4,5,6,7-tetrahydro-benzo[*b*]thiophene

Cyclohexanone (0.04 mol, 3.75 g), ethylcyanoacetate (0.04 mol, 4.25 ml) and sulphur (0.04 mol, 1.28 g) in ethanol (40 ml) were taken in a round bottomed flask and warmed with stirring meanwhile diethylamine (0.0386 mol, 4 ml) was added drop by drop (maintaining the reaction at a temperature 40 ^{0}C) until sulphur went into solution completely. After completion of the reaction, the solid gets separated out. The resultant precipitated solid was filtered, dried and recrystallized from chloroform.

Synthesis of 2-benzoylamino-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid ethyl ester: (2a)

2-Amino-3-carbethoxy-4,5,6,7-tetrahydro-benzo[*b*]thiophene **(1)** (0.004 mol, 1 g) and dry pyridine (10 ml) were taken in a 100 ml two necked round bottomed flask and kept for stirring on a magnetic stirrer at 0 ^{0}C. Benzoyl chloride (0.0026 mol, 0.306 ml) was added drop by drop for about 30-40 minutes .The progress of the reaction was checked by using TLC. After completion of the reaction, the reaction mixture was poured into a beaker containing crushed ice. The precipitated solid was filtered, dried and then recrystallized using ethanol.

Synthesis of 2-acetylamino-4, 5, 6, 7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid ethyl ester: (2b)

2-Amino-3-carbethoxy-4,5,6,7-tetrahydro-benzo[*b*]thiophene **(1)** (0.002 mol, 0.5 g) and dry pyridine (10 ml) were taken in a 100 ml two necked round bottomed flask and kept for stirring on a magnetic stirrer at 0 ^{0}C. Acetyl chloride (0.0023 mol, 0.17 ml) was added drop by drop for about 30-40 minutes. The progress of the reaction was checked by using TLC. After completion of the reaction, the reaction mixture was poured into a beaker containing crushed ice. The precipitated solid was filtered, dried and then recrystallized using ethanol.

Synthesis of 2-(4-nitro-benzoylamino)-4, 5, 6, 7-tetrahydro-benzo[*b*]thiophene-3-carboxylicacid ethyl ester: (2c)

4-Nitrobenzoic acid was taken in a two necked round bottomed flask fitted with a reflux condenser. Phosphorusoxychloride was added to this and heated at 115 ˚C for two hours. The excess of phosphorusoxychloride was distilled off and the crude acid chloride was collected.

Reaction of compound (1) with p-nitro benzoyl chloride

2-Amino-3-carbethoxy-4,5,6,7-tetrahydro-benzo[*b*]thiophene **(1)** (0.0017 mol,0.4 g) and dry pyridine(10 ml) were taken in a 100 ml two necked round bottomed flask and kept for stirring on a magnetic stirrer at 0 ^{0}C. p-Nitro benzoyl chloride (0.0119 mol, 2.22 g) was added. The progress of the reaction was checked by using TLC. After completion of the reaction, the reaction mixture was poured into a beaker containing crushed ice. The precipitated solid was filtered, dried and then recrystallized using ethanol.

Synthesis of 2-(4-amino-benzoylamino)-4, 5, 6, 7-tetrahydro-benzo[*b*] thiophene-3-carboxylic acid ethyl ester: (2d)

2-Amino-3-carbethoxy-4,5,6,7-tetrahydro-benzo[*b*]thiophene **(1)** (0.002 mol,0.5 g) and dry pyridine(10 ml) were taken in a 100 ml two necked round bottomed flask and kept for stirring on a magnetic stirrer at 0 ^{0}C. P-Amino benzoyl chloride (0.0030 mol, 0.36 ml) was added drop by drop for about 30-40 minutes. The progress of the reaction was checked by the TLC. After completion of the reaction, the reaction mixture was poured into a beaker containing crushed ice. The precipitated solid was filtered, dried and then recrystallized using ethanol.

SCHEME

Synthesis of some novel 2-(N-substituted amino)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid ethyl ester derivatives:

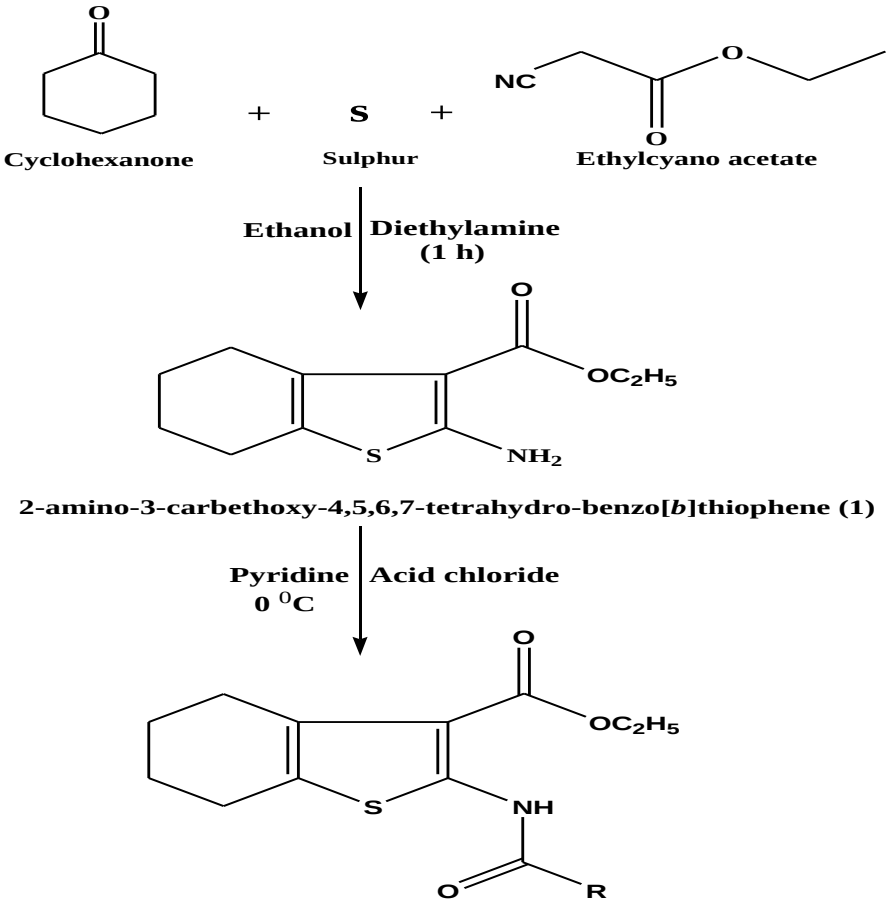


Fig-1 Scheme of synthesis of some novel 2-(N-substituted amino)-4, 5, 6, 7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid ethyl ester derivatives

Anti-Mitotic Activity

Mung beans (weighing 47.82 ± 1.50 mg) used in this study were obtained from the local market. They were soaked in tap water in the control group or in a drug solution in the test group for 6 h. The water or the drug solution was drained and the seedlings were kept moist (either with tap water or the drug solutions in covered petridish) until the radicles in the control group had grown to 1.0 – 1.5 cm (time 0, T_0). At T_0, the weight of seedlings, % germination, length of radical were recorded both in the control and test group. The seedlings were maintained at room temperature under moist conditions for an additional period of 48 h (T_{48}). All the parameters recorded at T_0 were again measured at T_{48}. The change in weight and gain in radicle length between T_0 and T_{48}were calculated. The seeds that did not germinate were simply weighed and no other parameters could be measured on these seeds (14).

In-vitro anti-inflammatory activity of 2-(N-substituted amino)-4, 5, 6, 7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid ethyl ester derivatives

The synthesized compounds were screened for anti-inflammatory activity using inhibition of albumin denaturation technique reported by Mizushima and Kobayashi with slight modification. The standard drug and test compounds were dissolved in minimum quantity of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solution was less than 2.5 %. Test solution (1 ml) containing different concentrations of drug was mixed with 1 ml of 1mM albumin solution in phosphate buffer and incubated at 27° ±1° C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60° ± 1° C in water bath for 10 min. After cooling, the turbidity was measured at 660 nm (UV-Visible Spectrophotometer SL-159, Elico India Ltd.). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average is taken. The Ibuprofen was used as a standard reference (15-18).

The percentage inhibition of denaturation was calculated by using following formula.

$$\%\ of\ inhibition = \left[\frac{V_t}{V_c} - 1\right] 100$$

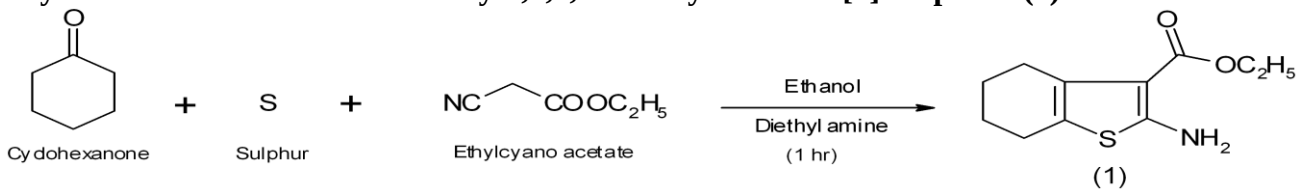
Where, Vt = Mean absorbance of test sample

Vc = Mean absorbance of control

RESULTS AND DISCUSSION

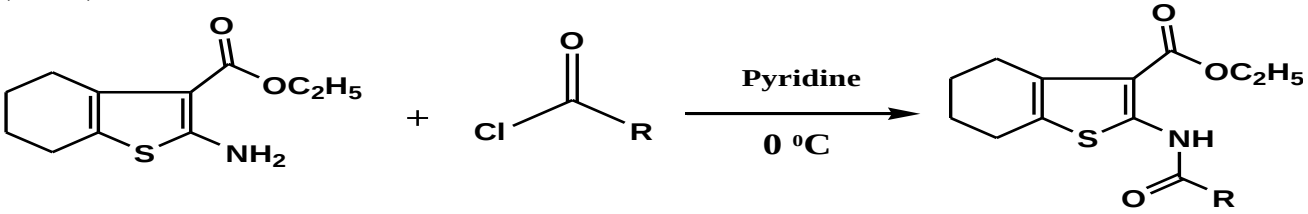
A series of 2-(N-substituted amino)-4, 5, 6, 7-tetrahydro-benzo[*b*]thiophene-3-caroxylic acid ethyl esters (2a-2d) have been synthesized using the appropriate synthetic procedures.

STEP I: Synthesis of 2-amino-3-carbethoxy-4,5,6,7-tetrahydro-benzo[*b*]thiophene (1)



The synthesis of 2-amino-3-carbethoxy-4, 5, 6, 7-tetrahydro-benzo[*b*]thiophene **(1)** has been carried out reacting cyclohexanone (0.04 mol, 3.75 g), ethylcyanoacetate (0.04 mol, 4.25 ml) and sulphur (0.04 mol, 1.28 g) in ethanol (40 ml) in a round bottom flask with stirring. Diethylamine (0.0386 mol, 4 ml) was added drop by drop (at 40 ^{0}C) until sulphur went into solution completely, continuing the stirring for 1 hour. The reaction mixture was then cooled to room temperature. Obtained precipitated solid was filtered, dried and recrystallized from chloroform.

STEP II: Synthesis of 2-(N-substituted amino)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-caboxylic acid ethyl esters: (2a-2d)



(1) **(2a-2d)**

Following the above reported method, compound **(1)** was treated with acid chloride in the presence of dry pyridine to obtain compounds **(2a-2d)**. Compound (1) was taken in dry pyridine and stirred at 0 ^{0}C. Acid chloride was added drop wise for about 30-40 minutes to the stirred solution. After completion of the reaction, excess pyridine was distilled off and the reaction mixture was worked out by pouring onto crushed ice. The precipitated solid was filtered, dried and then recrystallized using chloroform.

The compound **(1)** was treated with various acid chlorides, in dry pyridine to produce the title compounds. IR spectra showed prominent single peak at (3500-3200 Cm^{-1}), which are characteristic of secondary amino group indicating the formation of expected product.

The ^1H NMR (400 MHz, $CDCl_3$) of compound 2a (phenyl derivative) showed peaks at δ 1.43-1.39 triplet for three protons - $O-CH_2-CH_3$, δ 1.82-1.80 triplet for four protons $2CH_2$ at 5,6, δ 2.70-2.67 triplet for two protons CH_2 at 4, δ 2.82-2.80 triplet for two protons CH_2 at 7, δ 4.40-4.35 quardlet for two protons $-O-CH_2-CH_3$, δ 7.53-7.50 triplet for two protons at $3^1,5^1$, δ 7.59-7.56 triplet for one proton at 4^1, δ 8.03-8.01 doublet for two protons at $2^1,6^1$, δ 12.32 singlet for one proton – NH indicating the formation of 2-benzoylamino-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid ethyl ester (**2a**).

The ^1H NMR (400 MHz, $CDCl_3$) of compound 2b (methyl derivative) showed peaks at δ 1.40-1.36 triplet for three protons $-O-CH_2-CH_3$, δ 1.78 singlet for four protons $2CH_2$ at 5,6, δ 2.25 singlet for three protons $-NH-CO-CH_3$, δ 2.65-2.62 triplet for two protons CH_2 at 4, δ 2.77-2.75 triplet for two protons CH_2 at 7, δ 4.35-4.29 quardlet for two protons $-O-CH_2-CH_3$, δ 11.25 singlet for one proton –NH indicating the formation of 2-acetylamino-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid ethyl ester (**2b**).The mass spectrum of **2b** showed molecular ion peak [M^+] at 267. [M+1] peak at 268 as the base peak characteristic to its structure.

The ^1H NMR (400 MHz, $CDCl_3$) of compound 2c (4-nitro phenyl derivative) showed peaks at δ 1.44-1.40 triplet for three protons $-O-CH_2-CH_3$, δ 1.82 singlet for four protons $2CH_2$ at 5, 6, δ 2.72-2.69 triplet for two protons CH_2 at 4, δ 2.83-2.80 triplet for three protons CH_2 at 7, δ 4.42-4.36 quardlet for two protons $-O-CH_2-CH_3$, δ 8.19-8.17 doublet for two protons at $3^1,5^1$, δ 8.38-8.36 doublet for two protons at $2^1,6^1$, δ 12.55 singlet for one proton –NH indicating the formation of 2-(4-nitro-benzoylamino)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid ethyl ester (**2c**).

The mass spectrum showed molecular ion peak [M^+] at 345. [M+1] peak at 346 as the base peak characteristic to its structure indicating the formation of 2-(4-amino benzoylamino)-4, 5, 6, 7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethyl ester **(2d).**

Anti-mitotic activity of some novel 2-(N-substituted amino)-4, 5, 6, 7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid ethyl ester derivatives.

The title compounds **(2a-2d)** were screened for preliminary cytotoxic evaluation on germinating seeds of Vigna *radiate* (mung bean) for rapid and inexpensive screening of drugs exhibiting cytotoxic properties. Aspirin was used as a standard reference drug. Various parameters measured at T_0 and T_{48} are % germination, change in radical length, change in weight were reported in tables-7, 8, 9 for evaluating the cytotoxicity. The compounds **(2a-2d)** were evaluated for in vitro anti-mitotic activity. The compounds **2a** and **2c** have shown good anti-mitotic activity at **5 mg/ml** as par with aspirin at **2 mg/ml** (Table 1 and 2)**.**

Table-1 In-*vitro* anti-mitotic activity of some novel 2-(N-substitutedamino)-4, 5, 6, 7-etrahydrobenzo[*b*] thiophene-3-carboxylic acid ethyl ester derivatives

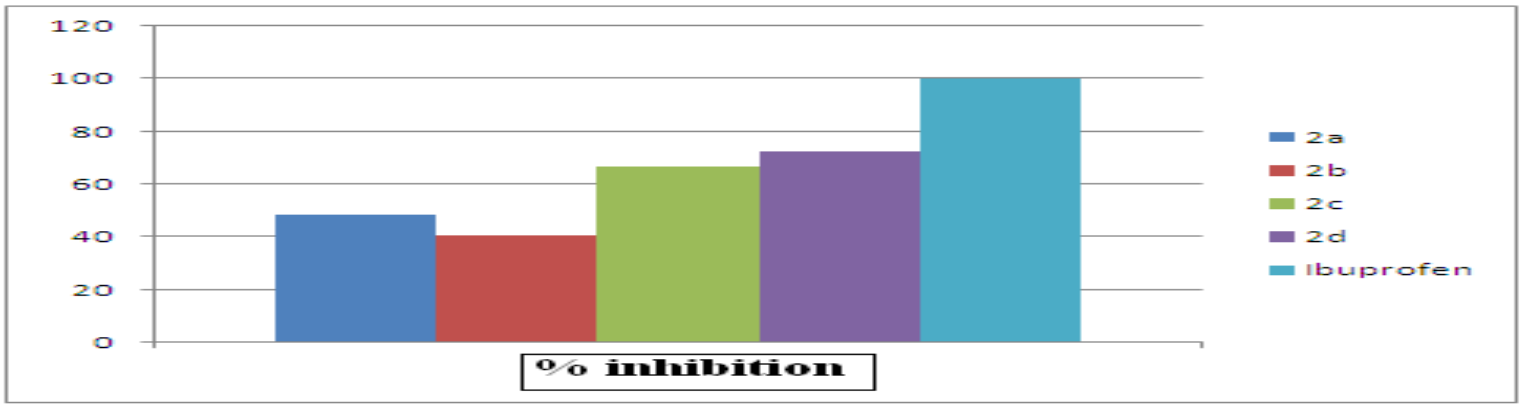
Name of the drug	% of germination at T_0 of different drug Concentrations					
	0.5 mg/ml	1 mg/ml	1.5 mg/ml	2 mg/ml	2.5 mg/ml	5 mg/ml
Aspirin	20	-	-	10	-	-
2a	80	75	65	75	65	25
2b	80	70	60	55	45	35
2c	75	70	75	70	60	30
2d	85	75	65	55	50	35
Control	90					

Table-2 In-*vitro* anti-mitotic activity of some novel 2-(N-substituted amino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic acid ethyl ester derivatives

Compound name	Difference in MRL between T_0 and T_{48}						% Length gained					
	0.5 mg/ml	1 mg/ml	1.5 mg/ml	2 mg/ml	2.5 mg/ml	5 mg/ml	0.5 mg/ml	1 mg/ml	1.5 mg/ml	2 mg/ml	2.5 mg/ml	5 mg/ml
Aspirin	1.29	-	-	0.74	-	-	40.56	-	-	23.27	-	-
2a	2.81	2.42	2.08	1.78	1.4	0.71	88.36	76.1	65.4	55.97	44.02	22.32
2b	2.85	2.46	2.15	1.89	1.5	0.89	89.62	77.35	67.61	59.43	47.16	27.98
2c	2.79	2.33	2.13	1.82	1.4	0.75	87.73	73.27	66.98	57.23	44.02	23.58
2d	2.75	2.48	2.29	1.93	1.51	0.83	86.47	77.98	72.01	60.69	47.48	26.1
Control	3.18						100					

In-vitro anti-inflammatory activity of 2-(N-substituted amino)-4, 5, 6, 7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid ethyl ester derivatives

The title compounds **(2a-2d)** were screened for their anti-inflammatory activity. Ibuprofen was used as standard. All the synthesized compounds and standard were used at 100 µg/ml concentration. The synthesized compounds **(2a-2d)** were compared with standard drug Ibuprofen. The derivatives **(2a-2d)** showed inhibition ranging from 40.9-72.72%. The compound **2d** having 4-amino phenyl group showed maximum activity (72.72 %). The compound **2c** having 4-nitro phenyl group showed good activity (66.68 %). The other compounds **2a** and **2b** having phenyl and methyl groups showed moderate activity (48.5 % and 40.9 %) respectively in comparison to standard drug Ibuprofen (Fig-2).



% Anti-inflammatory activity Vs Compound

Fig-2 *In-vitro* anti-inflammatory activity of some novel 2-(N-substituted amino)-4, 5, 6, 7-tetrahydro benzo[*b*]thiophene-3-carboxylic acid ethyl ester derivatives

The compounds **(2a-2d)** were evaluated for in vitro anti-inflammatory activity. Ibuprofen was used as reference standard. The compound **2d** having 4-amino phenyl group showed maximum activity (72.72 %) when compared to all other compounds.

CONCLUSION

2-amino-3-carbethoxy-4,5,6,7-tetrahydro-benzo[*b*]thiophene **(1)** was treated with different acid chlorides under synthetic conditions to give corresponding amide derivatives **(2a-2d)** the title compounds in good yields. All the compounds synthesized were characterized by physical (R_f values, Melting point, Molecular weight, Molecular formula) and spectral data (^{1}H NMR, IR, Mass spectra).The title compounds were screened for anti-mitotic activity. The obtained anti-mitotic results were analyzed statistically. All compounds **(2a-2d)** showed dose dependent inhibitory effect on % germination, radical length, mean weight when compare with control. The compound **2a** showed good anti-mitotic

activity at **5 mg/ml** when compared to all other compounds. The title compounds were screened for anti-inflammatory activity. The obtained anti-inflammatory results were analyzed statistically. All compounds **(2a-2d)** showed inhibitory effect on % denaturation of albumin when compared with standard. Apart from these **2d** and **2c** showed good anti-inflammatory activity at **100 µg/ml** concentration as par with ibuprofen. This acts as a lead for further optimization.

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