

SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL SCREENING OF 2-METHYL-1H-INDOLE-3-CARBOXYLIC ACID [2-(2-SUBSTITUTED-PHENYL)-4-OXO-THIAZOLIDIN-3-YL]-AMIDES DERIVATIVES.

Manish Rapolu¹, Kumanan R.¹, Duganath N.², Srinivasa Murthy M.¹,
Nazeer Ahmed¹, Subramanyam S.³

¹Vignan Institute of Pharmaceutical Sciences, Deshmukhi, Nalgonda Dist, Andhra Pradesh, India - 508284

²Department of Pharmaceutical Chemistry, OTRI campus, JNTUA, Anantapur, Andhra Pradesh - 515001

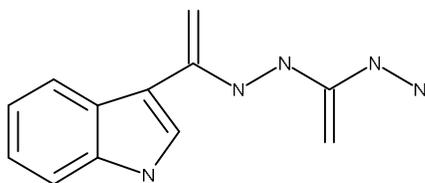
³Bharat Institute of Technology (Pharmacy), Ibrahimpatnam, R.R.Dist, Andhra Pradesh, India - 501 510

ABSTRACT:

A series of Schiff bases 2-methyl -1-H indole-3-carbohydrazide V(a-e) and N-benzylidene -2-methyl -1-H indole-3-carbohydrazide VI (a-e) were synthesized and evaluated for their anti-inflammatory by the carragenan induced paw oedema method and dextran induced oedema method. Analgesic activity by using hot plate latency and acetic acid induced writhing test. Most of the Schiff bases having two hydroxyl groups and one methoxy group on the phenyl ring showed excellent anti-inflammatory and analgesic activity.

Keywords: 2-methyl-1-H-indole-3-carboxylic acid, thiazolidine-3-yl amide, anti-inflammatory, analgesic activity

INTRODUCTION



1H-Indole-3-carbohydrazide

Carbohydrazide is used as an oxygen scavenger in water treatment for boilers. It is an alternative to the hazardous and potentially carcinogenic hydrazine.

Carbohydrazide reacts with oxygen to make water and nitrogen and urea. It also passivates metals and reduces metal oxides. Since these carbohydrazides are used much in other fields and not so popularly used in the field of lead discovery. Further the compounds containing

indole derivatives have been reported to exhibit antifungal [1-7], antibacterial [1-4,8,9], antiphage [2], antiproliferative [10], optimal inhibitory [11], anticholinergic [12], antiviral [13], antitumor [14], antiinflammatory [15] and antihypertensive [16] activities and also as plant growth regulators [17,18]. The agents containing the indolyl moiety should be of therapeutic value [19] in treating disorders resulting from hypo and hyperdopaminergic activity without side effects.

Thus, in the present study a effort has been carried out to synthesize some novel carbohydrazides and evaluation of some possible pharmacological activities.

MATERIALS AND METHODS

Open capillary method was adopted for determining melting points for the compounds synthesized using the below mentioned procedures. The purity if the compounds were

determined using Thin layer Chromatography. Thin layer Chromatography was performed using silica gel G (thin layer chromatography grade) and the spots was observed using employing iodine chamber destructive method.

Chemicals and reagents

Ethyl acetate (manufacturer), glacial acetic acid, methanol, phenyl hydrazine, hydrazine hydrate, benzaldehyde, piperidine, salicylaldehyde, chlorobenzaldehyde, thioglycolic acid, dimethylformamide, furfuraldehyde.

Synthesis

Procedure for Synthesis of Ethyl-2-methyl-1H-indole-3-carboxylate (III)

A mixture of Ethylacetoacetate (II) (6.3ml,0.05mol) and glacial acetic acid (3ml,0.05mol) was placed in the flat bottom flask were refluxed in methanol(25ml) and Add (5ml, 0.05mol) slowly during first 1hr. Continue the stirring for further 1hr. Pour the reaction mixture into a 50ml beaker and stir vigorously while it solidifies. Then add sufficient water and the solid was filtered, dried and recrystallize with ethanol to obtain compound (III). The yield was 60% and melting point was 150°C. The purity of the compound was confirmed by Thin Layer Chromatography. Further the compound was subjected for spectral studies.

Procedure for Synthesis of 2-methyl-1H-indole-3-carbohydrazide (IV)

Ethanol solution of Compound (III) (10gm 0.05mol) was refluxed with hydrazine hydrate(2.5gm,0.05mol) for 3hrs at 70°C. The reaction mixture was allowed to cool and poured over crushed ice. The solid was filtered dried and recrystallize with ethanol to obtain compound (IV). The yield of compound III was found to be 57% with an melting point of 145°C. The purity of the compound was confirmed by Thin Layer Chromatography. Further the compound was subjected for spectral studies.

Procedure for synthesis of benzylidene-2-methyl-1H-indole-3-carbohydrazide derivatives (Va-Ve)

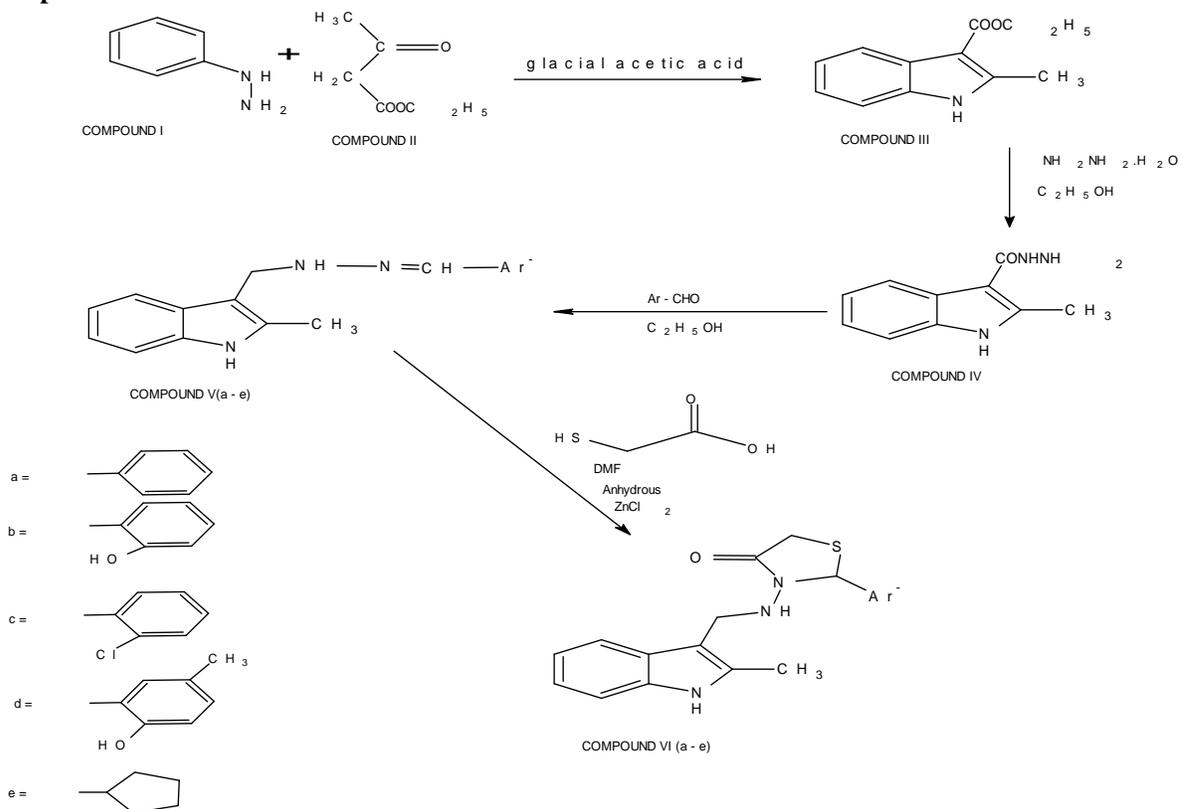
Compound (IV) {2.11gms (0.01mol)} and various aldehydes (0.015mol) in absolute ethanol (50ml) in presence of 2 drops of piperidine for 2hrs. The mixture was then cooled by pouring into 100ml of chilled water. The solid was filtered, dried and recrystallize with suitable solvents to obtained compound (Va-e). The purity of the compound was confirmed by Thin Layer Chromatography. Further the compound was subjected for spectral studies. The physical properties of these synthesized compounds are represented in table no. 1

Procedure for Synthesis of 1H-indole-3-carboxylic acid thiazolidine derivatives (VI a-e):

Compound (Va-e) (0.005mol) was treated with thioglycolic acid (0.005mol) in dimethylformamide (15ml) containing a pinch of anhydrous ZnCl₂ was refluxed for 6hrs. The resultant solution was cooled to room temperature and poured over crushed ice, was allowed to settle down overnight filtered and dried. The crude product was recrystallized using methanol to obtain compound (VI a-e) respectively.

The compounds synthesized using the above mentioned procedures were subjected for physical and spectral analysis like HNMR, MASS, and FT-IR spectroscopy.

The scheme for the synthesis of the above mentioned procedure is represented in Fig. No. 1.

SCHEME:**Fig No.1. Schematic representation for synthesizing compound VI(a-e)****Experimental Methods****Acute Toxicity Studies**

The acute oral toxic study was done according to the OECD guidelines 423. The animals were monitored for the behavioural changes, weight variation, toxicity and death rate.

Animals

Albino mice weighing 200–250 g, supplied by M/s. B.N. Ghosh & co., Calcutta, India, were placed in cages with wire-net floors in a controlled room temperature 29°C, relative humidity 60–70% and provided with food and water ad libitum. The animals were deprived of food for 24 h before experimentation but allowed free access to tap water throughout. All studies were carried out by using six rats in each group.

Analgesic Activity**Acetic acid induced writhing method²⁰**

The writhing test described by Koster et al. (1959) was adopted. A total of 42 mice divided into seven groups (n=6) were used and treated as follows; group 1 served as control and received vehicle alone (3% V/V tween-80 10 ml/kg) p.o., groups 2 to 6 received 200 mg/kg p.o. of the synthesized compounds like VIa, VIb, VIc, VI d and VIe

respectively, while group 7 received 100 mg/kg of acetyl salicylic acid (standard drug) p.o. Ten ml/kg of 0.7% aqueous solution of acetic acid were given to all mice i.p. 30 min later. Each mouse was placed in a transparent observation cage and abdominal constriction resulting from injection of acetic acid for the period of 20 minutes was counted. Results were presented as percent inhibition of analgesia, calculated as the reduction in the number of writhes between control animals and those pre-treated with either the synthesized compounds or acetyl salicylic acid⁷².

Hot plate method²¹

Experiments were carried out according to method described by Adzu et al., (2001). Mice that showed nociceptive responses within 20s when placed on hot plate maintained at $55 \pm 0.5^\circ\text{C}$ were selected and grouped into seven groups of (n=6). Group 1 served as control and received vehicle

alone (3% V/V tween-80 10 ml/kg) p.o: groups 2-6 received 200mg/kg p.o. of the synthesized compounds like VIa, VIb, VIc, VI d and VIe while group 7 received 100mg/kg of acetyl salicylic acid p.o. Each mouse was placed singly on the hot plate and the latency to exhibit thermal stimulus were determined at 0min, 30min, 60min and 120min before and after the treatment. Licking of paws and jumping were the parameters evaluated s the thermal stimulus. Sixty seconds was taken as the cut-off time to avoid mouse tissue damage⁷³.

Analgesic activity was expressed as mean percent maximal effect calculated as % MPE = Post-drug latency—Pre-drug latency/cut-off time-Pre-drug latency.

Anti-inflammatory activity

Animals

Male albino Wister rats weighing 200–250 g, supplied by Ms. B.N. Ghosh & co., Calcutta, India, were placed in cages with wire-net floors in a controlled room temperature 29°C, relative Humidity 60–70% and provided with food and water ad libitum. The animals were deprived of food for 24 h before experimentation but allowed free access to tap water throughout. All studies were carried out by using six rats in each group.

Carrageenan Induced Rat Paw Edema Method

Oedema was induced by sub planter injection of 0.1 ml of 1% freshly prepared suspension of carrageenan into the right hind paws of the rats of four groups of six animals each. The volume of the injected and contra-lateral paws were measured 1, 3 and 5 h after induction of inflammation using a plethysmometer according to the method described by Winter et al. (1962) The test groups received the synthesized compounds (200mg/kg), the standard group received phenylbutazone (100 mg/kg), and the control animals received the vehicle only alone (3% V/V tween-80 10 ml/kg) p.o. All the treatments were given intraperitoneally 30 min prior to the injection of carrageenan except for the synthesized compounds .Increase of paw oedema thickness was calculated²¹.

Dextran induced rat paw oedema²²

In this model oedema was induced by sub planter injection of 0.05 ml of freshly prepared 1% solution of dextran into the right hind paw of the rats. Group division of the animals and the

treatment of test, standard and control animals were the same as the carrageenan model⁷⁴.

For comparison purpose, the volume of oedema at various prefixed time intervals was measured. The difference between paw volumes of the treated animals was measured and the mean oedema volume was calculated.

Percentage reduction in oedema volume was calculated by using the formula,

$$\text{Percentage reduction} = \frac{V_o - V_t}{V_o} \times 100$$

Where, V_o = Volume of the paw of control at time 't'.

V_t = Volume of the paw of drug treated at time 't'.

From the data obtained, the mean oedema volume and percentage reduction in oedema was calculated.

The results are expressed as mean \pm S.E.M. Dennett's t-test was used to verify the statistical significance at $p < 0.05$ between the treated and control groups.

RESULTS AND DISCUSSION

The compounds synthesized using the above scheme was subjected for TLC, elemental analysis, spectral studies like MASS, HNMR, and FTIR spectral studies and their results are discussed below.

The melting point and the percentage yield of the compounds VI (a-e) are represented in Table No.1. Thin layer chromatography of the compounds showed single spot with the R_f value of 0.40, 0.43, 0.44, 0.55, and 0.46 respectively for compounds VI (a-e).

Spectral datas of 2-methyl- 1H-indole-3-carboxylic acid (4-oxo-2-phenyl-thiazolidin-3-yl)-amide (VI a):

The compound VI (a) synthesized using the above mentioned procedure showed characteristic peaks for FTIR at 3417cm^{-1} (NH – Str 2° anine), 3010cm^{-1} (Ar CH-str), 1610cm^{-1} (C=O str). HNMR δ 2.5 (3H, S, CH₃), δ 3.6(2H, S, CH₂), δ 5.4(1H,S, CH), δ 6.5-7.0(9H, M, Ar-H), δ 8.4 (1H, S, N-H), δ 10.3 (1H, S, N-H). The molecular ion peak M^+ was obtained at 351.

Spectral data of 2-methyl -1H-indole-3-carboxylic acid [2-(2-hydroxy-phenyl)- 4-oxo-thiazolidin-3-yl] - amide (VI b):

The compound VI (a) synthesized using the above mentioned procedure showed characteristic peaks for FTIR at 3474cm^{-1} (OH –str), 3375cm^{-1} (NH – Str 2° amine), 1604cm^{-1} (C=O str). HNMR values are δ 2.5 (3H, S, CH₃), δ 3.5 (2H, S, CH₂), δ 5.4 (1H, S, CH), δ 6.7-7.0 (9H, M, Ar-H), δ 8.0 (1H, S, N-H), δ 10.3 (1H, S, N-H). The mass spectrum showed the M⁺ at 367 which confirms the molecular weight as 367.

Spectral data of 2-methyl -1H-indole-3-carboxylic acid [2-(2-chloro-phenyl)-4-oxo-thiazolidin-3-yl] (VI c):

Compound VI c showed FT-IR characteristic peaks at 3398cm^{-1} (2° amine N-H Str), 3058cm^{-1} , 1601cm^{-1} (C=O Str). HNMR peaks at δ 2.5 (3H, S, CH₃), δ 3.6 (2H, S, CH₂), δ 5.3 (1H, S, CH), δ 6.8-7.5 (8H, M, Ar-H), δ 8.4 (1H, S, N-H), δ 10.1 (1H, S, N-H).

Spectral data of 2-methyl -1H-indole-3-carboxylic acid -[2-(3-hydroxy-4-methoxy-phenyl)- 4-oxo-thiazolidin-3-yl]-amide (VI d):

The FTIR spectrum of compound VI d showed characteristic peaks at 3474cm^{-1} (OH Str), 3375cm^{-1} (2° amine N-H Str) and 2847.11cm^{-1} (C=O Str). HNMR peaks at δ 2.4 (3H, S, CH₃), δ 3.9 (2H, S, CH₂), δ 5.3 (1H, S, CH), δ 6.5-7.3 (7H, M, Ar-H), δ 5.6 (1H, S), δ 8.3 (1H, S), and δ 10. (1H, S).

Spectral data of 2-methyl -1H-indole-3-carboxylic acid (4-oxo- 2-furan-thiazolidin-3-yl) (VI e).

The FTIR spectra for compound VI e showed 3256cm^{-1} (2° amine N-H Str), and 3059cm^{-1} (Ar-C-H Str). HNMR peaks at δ 2.5 (3H, S, CH₃), δ 3.6 (2H, S, CH₂), δ 5.2 (1H, S, CH), δ 6.5-7.3 (7H, M, Ar-H), δ 8.4 (1H, S), and δ 10. (1H, S).

The above spectral values confirm the structure of the synthesized compounds and the mass spectral values also corresponds to their calculated molecular weight.

Pharmacological activity

The above mentioned compounds were subjected for various pharmacological activities and the data's of the compounds are discussed here.

Acute toxicity studies

There were no deaths of rat's upto 3000mg/kg b.w. of all the synthesized compounds VIa, VIb, VIc, VI d & VI e within short and long term outcome of the limit dose test up and down procedure. However, showed some behavioral signs of toxicity include irritation, restlessness, tachypnoea, anorexia, bilateral narrowing of the eyelids and abnormal posture (which was characterised by tugging of the head in-between the hind limbs) at 3000mg/kg b.w. The LD₅₀ was calculated to be greater than 3000mg/kg b.w. oral route.

According to Clarke and Clarke, (1997) substances with LD₅₀ of 1000mg/kg body weight/oral route are regarded as being safe or of low toxicity²³. In the present study, acute toxicity study of all the synthesized compounds VI a, VI b, VI c, VI d & VI e showed that no mortality of rats occurred, at a limit dose of 3000 mg/kg and 5mg/kg body weight given orally. This is an indication that the all the synthesized compounds have low acute toxicity when administered orally.

Analgesic Activity

Analgesic activity of all the compounds were carried out using acetic acid induced writhing and hot-plate latency in mice model. The results of which are presented below.

Acetic Acid Induced writhing method

The result of the acetic acid induced writhing method was represented in Fig.No.2. The % inhibitions were 8.63%, 42.47%, 31.19%, 57.52% and 11.7% respectively for compound VIa, compound VI b, compound VI c, compound VI d and compound VI e respectively with significance value less than 0.05. Compound VI b, and compound VI d inhibited the writhing response almost to the same degree as Aspirin (65. 03% inhibition) at 100mg/kg.

The results suggests that the synthesized Indole derivatives fused with Thiazolidine-4-ones derivatives significantly inhibited the writhing response induced by acetic acid in mice, which suggested that the these compounds displayed central (Through writhing test) analgesic effects. The compounds also showed inhibitory effects on writhing response induced by acetic acid, which suggested that the Indole derivatives fused with Thiazolidine-4-ones acted like non-steroidal anti-

inflammatory drugs such as acetylsalicylic acid and the analgesic effect may attribute to its anti-inflammatory action.

Hot-plate latency test in mice

The result of the Hot-plate method was represented in Fig.No.3. The percentage protections of the animal by the compounds from the thermal stimuli were comparable to that of Aspirin. The latencies were found to be 29.88, 43.52, 33.18, 38.63 and 34.81 for compound VIa, compound Vib, compound Vic, compound Vid and compound VIe respectively. Compound Vib & Vid showed the latency time almost to the same degree as aspirin (48.50).

The results showed that the compounds significantly increased the pain threshold to hot-plate in Mice, which suggested that the extract displayed peripheral analgesic effect. Compounds Vib & Vid showed maximum % protection in hot-plate test, which suggested that the compound acted like non-steroidal anti-inflammatory drugs such as acetylsalicylic acid and the analgesic effect, may attribute to its anti-inflammatory action.

Thus based on the above two models it could be concluded that the compounds Vib and Vid possess potent analgesic activity than other compounds and was in par with the analgesic activity of the standard drug.

Anti-inflammatory activity

The anti-inflammatory activity was performed only for compounds VI b and VI d based on the results of analgesic activity. The anti-inflammatory activity was performed using carragennan induced paw oedema method and dextran induced oedema method, and the results are discussed below.

Carrageenin Induced Paw-Oedema Method

The results of the anti-inflammatory effect of the 100mg/kg of compounds Vib&Vid on carragenin induced oedema in rat's right hind paws are presented in Fig No 4. There was a gradual increase in oedema paw volume of rats in the control (carragennin treated). However, in the test groups, treated with compound Vib and compound Vid both showed a significant reduction in the oedema paw volume. The significant anti-inflammatory effect induced by compounds

appeared at 1h and progressively increased. The compounds Vib & Vid showed a maximum inhibition of 17.3% &30.52% respectively at 3rd hour. The anti-inflammatory effect induced by Phenyl butazone progressively increased and reached a maximum (38.94%) at 3rd hour.

Dextran Induced Oedema Method

The results of the anti-inflammatory effect of the 100mg/kg of compounds Vb&Vib on dextran induced oedema in rat's right hind paws are presented in Fig No 4. There was a gradual increase in oedema paw volume of rats in the control (dextran treated). However, in the test groups, treated with compound Vib and compound Vid both showed a significant reduction in the oedema paw volume. The significant anti-inflammatory effect induced by compounds appeared at 1h and progressively increased. The compounds Vib & Vid showed a maximum inhibition of 16.44% & 31.8% respectively at 3rd hour. The anti-inflammatory effect induced by Phenyl butazone progressively increased and reached a maximum (38.99%) at 3rd hour.

The present study establishes the anti-inflammatory activity of the compound Vib and Vid. The compounds effectively suppressed the inflammation produced by hem. eenan. It is evident that hem. eenan induced oedema is commonly used as an experimental animal model of acute inflammation and it is believed to be biphasic of which the first phase is mediated by release of histamine and serotonin in the early phase followed by kinin release and then by prostaglandin in the later phase.

The effect of synthesized compounds Vib & Vid on the acute phase of inflammation was observed in the dextran-induced paw oedema test. Dextran-induced rat paw oedema is a suitable experimental animal model for evaluating the anti-inflammatory effect of chemical products and it is believed to be biphasic (Winter et al., 1962). The first phase (1 h) involves the release of histamine and serotonin and the second phase (over 1 h) is due to the release of prostaglandin-like substances. Based on this, the second phase may be explained by an inhibition of cyclooxygenase. Thus it can be said that compounds Vib & Vid reduced the oedema produced by dextran which is known to be

mediated by both histamine and serotonin. 100 mg/kg doses of synthesized compounds Vb & Vib showed anti-inflammatory effects at all hours after dextran injection. But the most significant anti-inflammatory response occurred at the third hour, reduction in paw oedema by, 16.44% and 31.8%, respectively for compound Vib & Vid. The standard (phenylbutazone) showed a maximum response at third hour and % inhibition is recorded to be 38.99%.

Conclusion

As per the results of the present study, it could be concluded that, the presence of phenyl group at the 1st position of the pyrazolinone nucleus which is attached to the 1,8-naphthyridine through azo linkage at the 2nd position may be contributing to the anti-inflammatory effect. The presence of methyl group at the 3rd position of the pyrazolinone ring in compound Vib may be enhancing the activity hence it is more effective over Vid. Furthermore works has to be carried out on the toxicological profile and to optimize the structure of this moiety for more potent, safer and effective analgesic and anti-inflammatory drug.

References

- [1] Dantia, A., Sehgal, V. and Upreti, M., (1995), *Phosphorus, Sulfur, Silicon Relat. Elem.*, 105 93-99.
- [2] Varvaresou, A., Tsantili-Kakoulidou, A., Siatra-Papastaikoudi, T. and E. Tiligada, (2000), *Arzneim Forsch*, 50 pg 48-54.
- [3] Mohan, J. and Kataria, (1996), *S Indian Chem J., Sect B* 35, pg 456-458.
- [4] Palluotto, F., Carotti, A., Casini, G., Ferappi, M., Rosata, A., Vitali, C. and Campagna, F., (1999), *Farmaco*, 54, No. 3, pg 191-194.
- [5] Dzurilla, M., Ruzinsky, M., Kutschy, P., Tewari, J. and Kovacik, V., (1999), *Collect. Czech. Chem. Commun*, 64. pg 1448-1456.
- [6] Kutsy, P., Dzurilla, Takasugi, M. and Sabova, A., (1999), *Collect Czech. Chem. Commun*, 64. pg 348-362.
- [7] Kutsy, P., Dzurilla, M., Takasugi, M., Toerock, M., and Achbergerova, I. "New Synthesis of Indole Phytoalexins and Related Compounds". *Tetrahedron*, 54 (1998), pg3549-3566.
- [8] Khan, M.H., Twari, S., Begum, K. and Nizamuddin, (1998), *Indian Chem J., Sect B*, 37, pg1075-1077.
- [9] Machia, M., Mamera, C., Nencetti, S., Rossello, A., Broccali, G. and Limonta, D., (1996), *Farmaco*, 5.1 pg75-78.
- [10] Garuti, L., Roberti, M., Rossi, T., Castelli, M. and Malagoli, M., (1998), *Eur. J. Med. Chem. Chim. Ther.* 33. pg 43-46.
- [11] Draheim, S. A., Bach, N. J., Dillard, R. D., Berry, D. R. and Carlson, D. G., (1996), *J. Med. Chem.*, 39. pg5159-5175.
- [12] Battagba, S., Boldrini, E., Settimo, F.D., Dondio, G. and Motta, C. L. (1999), *Eur. J. Med. Chem. Chim. Ther.* 34. pg93-106.
- [13] Zotova, S.A., Korneeva, T.M., Shvedov, V.I., Fadaeva, N.I. and Leneva, I. A., (1995), *Pharm Chem. J.*, 29. pg 57-59.
- [14] Lai G. and Anderson, W.K., (2000), *Tetrahedron*, 56. pg2583-2590.
- [15] Amir, M., Dhar, N. and Tiwari, S.K., (1997), *Indian J. Chem. Sect B*. 36. pg96-98
- [16] Grasso, S., Molica, C., Monforte, A.M., Monforte, P. and Zappala, M., (1995), *Farmaco.*, 50. pg113-118.
- [17] Katayama, M. and Gautam, R.K., (1996), *Biosci. Biotechnol. Biochem.*, 60. pg755-759.
- [18] Katayama, M., Kato, K., Kimoto, H. and Fujii, S., (1995), *Experientia*, 51. pg721-724.
- [19] Mewshaw, R. E., Webb, M. B., Marquis, K.L., Mc Gaughey, G.B. and Shi, X., (1999), *J. Med. Chem.*, 42. pg2007-2020.
- [20] Mekin, T., Gulcin, C., Bulent G., and Bilge, H., (1996), *Pharm. Biol.* 32 (3). pg194-197.
- [21] Adzu B, Amos S, Wambebe C, Gamaniel K, (2001), *Fitoterapia* 72, pg344-350.
- [22] Maiti, Subodh Kumar, (1998), Carrier activated sludge process (CASP) – a new branch in wastewater treatment technology. In *Advances in wastewater treatment technologies* (ed. RK Trivedy), Enviromedia, Karad, pg. 81-85.
- [23] Clarke, Ronald V. Introduction. In Clarke, Ronald V., (1997), "Situational Crime Prevention: Successful Case Studies" 2nd Ed. pg1-43.

Figures:

Table No.1 The melting point and the percentage yield of the compounds VI (a-e).

Compound no	Molecular formula	R1	R2	R3	Molecular weight	Percentage yield	Melting Point °C
VIa	C ₁₉ H ₁₇ N ₃ O ₂ S	H	H	H	385.87	64%	230°c
VIb	C ₁₉ H ₁₇ N ₃ O ₃ S	OH	H	H	367.42	55%	154°c
VIc	C ₁₉ H ₁₆ ClN ₃ O ₂ S	Cl	H	H	385.87	69%	196°c
VIId	C ₂₀ H ₁₉ N ₃ O ₄ S	H	OH	OCH ₃	397.45	62%	212°c
VIe	C ₁₇ H ₁₅ N ₃ O ₃ S	H	H	O	341.08	65%	180°c

Fig.No.2. Graphical representation of effect of compounds VIa , VIb, VIc, VIId, VIe and standard drug in acetic acid-induced writhing test on mice

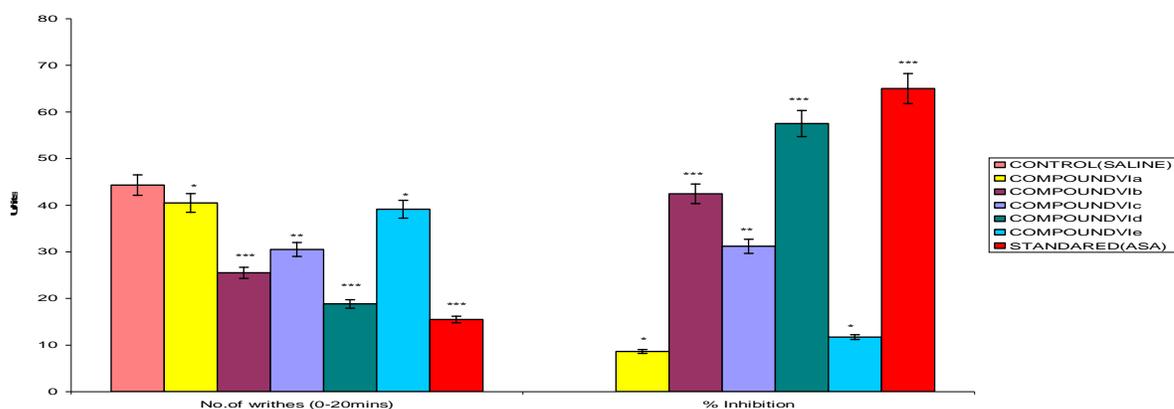


Fig.No.3. Graphical representation of effect of the compounds VIa, VIb , VIc, VIId, VIe and standard drug in Hot-plate latency test on mice

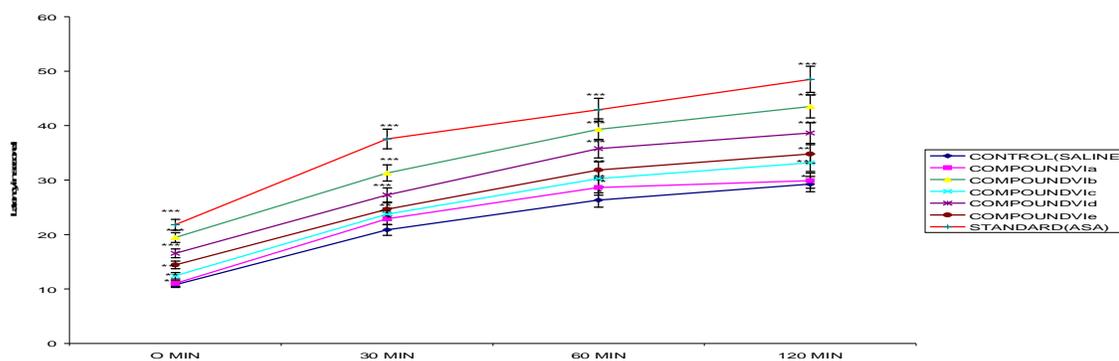
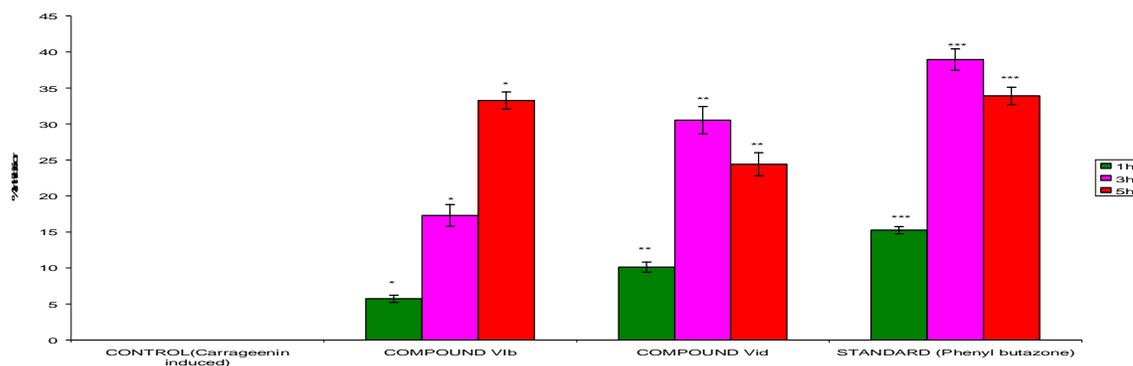
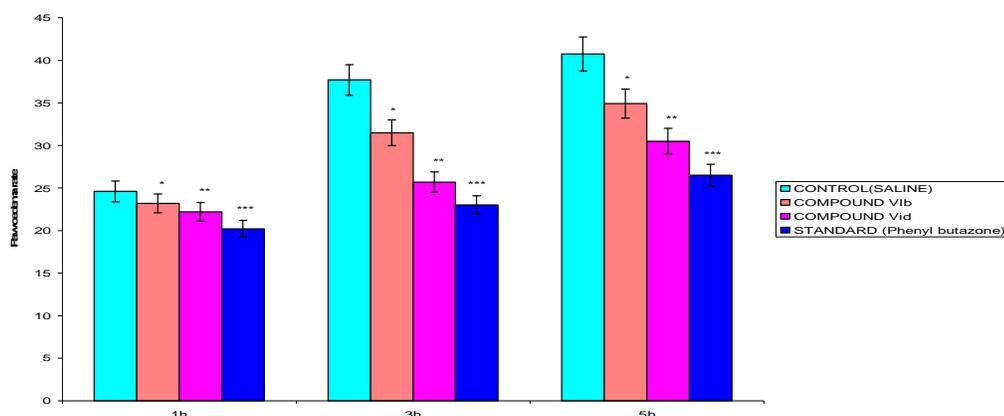


Fig.No.4. Graphical representation of Percentage Inhibition of the oedema by compounds Vb & VIb and standard drug in Carrageenin induced paw-oedema method



***p<0.001; ** p< 0.01; *p< 0.05

Figure 5.18 Graphical representation of Percentage Inhibition of the oedema by compounds Vb & VIb and standard drug in Dextran induced paw-oedema method.



***p<0.001; ** p< 0.01; *p< 0.05