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SYNTHESIS AND ANALGESIC ACTIVITY OF [1,3,4]-THIADIAZOLE-[1,3-DIONE]-ISOINDOLE DERIVATIVES

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Analgesic, Ibuprofen, Isoindole, Mice, Thiadiazole

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ABSTRACT: Background: During recent years, there has been a large investigation on different classes of thiadiazole compounds, many of which were found to possess an extensive spectrum of pharmacological activities. This study was undertaken to synthesize and evaluate the analgesic activity of [1,3,4]-thiadiazole-[1,3-dione]-isoindole derivatives. **Methods:** The purity of the synthesized compounds was checked by performing thin layer chromatography and determining melting points. All the synthesized compounds were subjected to spectral analysis such as FT-IR and UV-visible spectroscopy. The acute toxicity study was performed using the 'up and down method' while the *in-vivo* analgesic activity was evaluated using 'Eddy's hot plate method in mice, taking ibuprofen as standard. Data analysis was done using one-way ANOVA followed by Dunnett's *t*-test. **Results:** The melting point values of each of the synthesized compounds were found to be between 145-238°C, and the molecular weight values were found to be between 204-496 gm. Spectral analysis of all the synthesized compounds has shown satisfactory results. R_f values of all the synthesized compounds were found to be different and supporting the fact of formation of new compounds and also the purity of the compounds. The LD_{50} value of the compounds ($A_5$1-$A_5$4) was less than 1750mg/kg. When compared to ibuprofen, two of the compounds showed good analgesic activity. **Conclusions:** The results suggest that such compounds exert an analgesic effect. Therefore, promising results can be expected from future investigations of these compounds.

INTRODUCTION: Nitrogen-containing hetero-cycles have been the desired targets for synthesis over many years because of their structural diversity and biological importance. Among these, phthalimides have been reported to possess herbicidal, insecticidal and anti-inflammatory properties.

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Organic synthetic chemists consider phthalimide as a very important subunit for preparing a wide range of biologically active molecules. Phthalimide falls under an important class of drugs exhibiting anxiolytic, antimicrobial, antibacterial, antituber-culosis, anticancer, hypolipidemic, analgesic, anti-proliferative, acetylcholinesterase inhibitors and inhibitor of human neuronal nitric oxide synthase [1]. The cyclic imides, especially phthalimides and their derivatives, are a core structure of numerous natural products and designed pharmaceutical molecules. They are also used in the treatment of acquired immunodeficiency syndrome (AIDS), leprosy and other diseases.

Anthelmintic activity has also been reported from phthalimide derivatives of amino acid analogues. Apart from these, dyes, pesticides and the polymer industries employ phthalimide derivatives in a variety of products as synthetic intermediates. There is a growing interest in the usefulness of phthalimides and the synthesis of many types of alkaloids and pharmacophores utilize phthalimides as starting materials and intermediates [2, 3].

Phthalimide is an imido derivative of phthalic acid, and an imide is a functional group consisting of two carbonyl groups bound to nitrogen. Phthalimides are an interesting class of compounds among bicyclic non-aromatic nitrogen heterocycles. Phthalimide is considered as nitrogen analogues of anhydrides or as diacyl derivatives of ammonia. The chemical core of phthalimides (-CO-N(R)-CO-) arelipophilic can therefore easily cross biological membranes *in-vivo* and showing different pharmacological activities [4].

Phthalimide and N-substituted phthalimides are an important class of compounds because they possess important biological activities, including anti-inflammatory activity, analgesic activity and hypolipidemic activity and also it is used in organic synthesis and other industrial fields such as in drugs synthesis. Acetylenicphthalimides have been reported to have anticholinergic and anti-Parkinsonian activity. Phthalimides derivative and their analogues have potential in a number of areas such as aminopeptidase inhibition, anticonvulsants activity and promotion of tumor necrosis factor-alpha (TNF α) production. Many synthetic polymers use imide in their preparation. These polymers are used as an insulating coating in electrical equipment and plastic heat-resistant glass fiber. Imides have also found application as plant growth regulators, and some of them are useful as herbicides. Some of such imides are employed as inhibitors against mammalian, plant, bacterial and fungal copper-containing amine oxidases. They are also employed as prevulcanization inhibitors used in sulphur-cured rubber polymer systems [5].

Phthalimide easily donates the proton and form water-soluble salts with stronger bases because it is highly acidic in nature. Phthalimides are oxidative stable, heat retardant, solvent resistant and have superior mechanical properties.

The relative acidity of the NH group, which is a direct consequence of the presence of the two carbonyl groups, is the reason for the specific reactivity of imides. It is also observed that the metal complexes are more active than the free organic ligand. Chelation reduces the polarity of the metal ion and enhances the lipophilicity or hydrophobicity of the metal chelate, which favours its permeation through a microbial cell wall. The metal chelates may also disturb the respiration process of the microbial cells and thus protein synthesis and further growth of the micro-organism is hindered. Though the co-ordination of aliphatic tertiary amino nitrogen is not sterically favored, the high electron density available on the tertiary amino nitrogen favors its coordination to a metal ion where there is a possibility for chelation.

The identifiable structural features for their activity of phthalimide and N-substituted phthalimides are hydrophobic aryl ring, a hydrogen bonding domain, an electron-donor group, another distal hydrophobic site [6]. The phthalimide moiety serves as a 'protected' form of ammonia. The phthalimide carbonyls increase the acidity of the nitrogen and thus allowing formation of its conjugate base. Most importantly, the phthalimide carbonyls protect the nitrogen from 'over alkylation' thus preventing the formation of quaternary ammonium salts [7]. N-benzoyl phthalimide resembles both classical benzodiazepines and barbituric acid structures. It consists of a tricyclic hydrophobic structure comparable to that of benzodiazepines. Size and tridimentional structure of benzodiazepines and phthalimide backbones are similar [8].

Heterocyclic moieties can be found in a large number of compounds that display biological activity. The biological activity of the compounds is mainly dependent on their molecular structure. 1,3,4-thiadiazoles are very interesting compounds due to their important applications in many pharmaceuticals, biological and analytical fields. Schiff bases-bimolecular condensation products of primary amines with aldehydes- represent valuable intermediates in organic synthesis and, at the same time, compounds with various applications. Schiff bases resulted from aromatic aldehydes *ortho*-substituted with a hydroxyl group have initially aroused the researchers interest because of their ability to act as bidentate ligands for transition

metal ions [9, 10]. Schiff bases are an important class of compounds due to their flexibility, structural similarities with natural biological substances and also due to presence of imine (N=CH-) which imports in elucidating the mechanism of transformation and racemization reaction in biological system [11]. These novel compounds could also act as valuable ligands whose biological activity has been shown to increase on complexation. Derivatives of 1, 3, 4-thiadiazoles have been recognized as molecules with potential antimicrobial utility [12]. In accordance with the availability of the earlier drugs having thiadiazole nucleus for the chemotherapy of bacterial diseases, we aimed to synthesize the molecules having thiadiazole nucleus with more potency. Among the wide variety of heterocycles that have been explored, developing pharmaceutically important molecules like thiadiazole containing Schiff bases have played an important role in heterocyclic chemistry. Thiadiazole derivatives have occupied a unique position in medicinal chemistry. The naturally occurring B_6-vitamins pyridoxine, pyrodoxal, pyridoxamine, and codecarbaxylase contain a thiadiazole nucleus. In addition to this, many naturally occurring and synthetic compounds containing the thiadiazole scaffold possess interesting pharmacological properties. Among them, 2-amino-3-cyanopyridines have been identified as IKK-inhibitors. Besides, they are important and useful intermediates in preparing a variety of heterocyclic compounds [13].

Aromatic five-membered nitrogen heterocycles have been potential targets of investigations by several research groups owing to their interesting biological activities and medicinal properties. Among these, the 1,2,4-triazole scaffold constitutes the core moiety of several therapeutically active compounds as antimicrobial [14], analgesic [15], antiviral [16], antioxidant [17], anti-inflammatory [18], and anticancer [19] agents. Furthermore, 1,3,4-thiadiazoles and 1,3,4-oxadiazoles are also important classes of azoles endowed with significant biological properties as there are several examples in the literature, including antifungal [20, 21], anti-inflammatory [22, 23], antimicrobial [24, 25], antiviral [26, 27,] and anticancer [28, 29] activities. Additionally, many investigations showed that the clubbing of two or three heterocyclic units may significantly potentiate the antimicrobial activities [30, 31]. In addition, Schiff bases have been the focus of numerous studies due to their wide spectrum of biological activities. Moreover, azomethine Schiff bases linkages, as attractive connecting units that could bind two pharmacophores to generate innovative bi-functional drugs, have rapidly emerged as one of the most challenging and attractive topics in drug design for constructing novel bioactive molecules [32].

The ability of 1, 3, 4-thiadiazole heterocyclic compounds to undergo various chemical reactions has made them important for molecule planning because of their privileged structure, which has enormous biological potential. Two examples of compounds containing the 1, 3, 4-thiadiazole unit currently used in clinical medicine are: acetazola-mide and methazolamide as carbonic anhydrase inhibitors [33].

Thiadiazole is a 5-membered ring system containing hydrogen-binding domain, sulfur atom, and two-electron donor nitrogen system that exhibit a wide variety of biological activity. Theyoccur in four isomeric forms in nature *viz*. 1,2,3-thiadiazole;1,2,5-thiadiazole; 1,2,4-thiadiazole; and 1,3,4-thiadiazole [34] **Fig. 1**. The synthesis of novel 1,3,4-thiadiazole derivatives and investigation of their chemical properties and biological behavior has accelerated in the last two decades. In recent years, the number of scientific studies with these compounds has increased considerably.

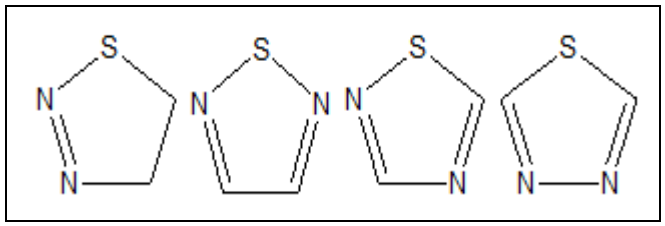

FIG. 1: ISOMERIC FORMS OF THIADIAZOLE

MATERIALS AND METHODS:

Materials: Phthalic anhydride, benzene, 1,2-dichloroethane, m-chlorophenyl isocyanate, aceto-nitrile, aniline, and 4-aminophenol were purchased from Merck Specialities Pvt. Ltd, Mumbai, India. Thionyl chloride, diethyl ether, and 2,4-dinitro-phenylhydrazine were purchased from Universal Laboratories Pvt. Ltd, Mumbai, India. Ethanol, methanol, and acetone were purchased from Shenzhen Esun Industrial Co. Ltd, Shenzhen, China. Thiosemicarbazide, sulphuric acid, formal-

dehyde, silica gel G, ibuprofen, and normal saline were purchased from Trial Biotech Ltd, S d fine-chem Ltd. (Kolkata, India), Xilong Scientific Co. Ltd. (Guangdong, China), Nebula Chemical Co. Ltd. (Shandong, China), Bhavishya Pharmaceuticals Pvt. Ltd. (Hyderabad, India) and Cadila Pharmaceuticals Ltd. (Secunderabad, India) respectively. The following apparatus was used apart from common laboratory equipment: Melting point apparatus and vacuum pump manufactured by Remi Instruments Ltd. (Mumbai, India), hot air oven manufactured by B.S. Trading (Kolkata, India), UV-Visible spectrophotometer (UV–SPECORD® 50 PLUS-232H1004), Fourier transform infrared (FT-IR) spectrophotometer (Bruker αE), analgesiometer manufactured by H.L. Scientific Industries (Ambala, India).

Methods:

Synthesis of the Desired Compounds: The scheme of the synthesis is given in **Fig. 2**.

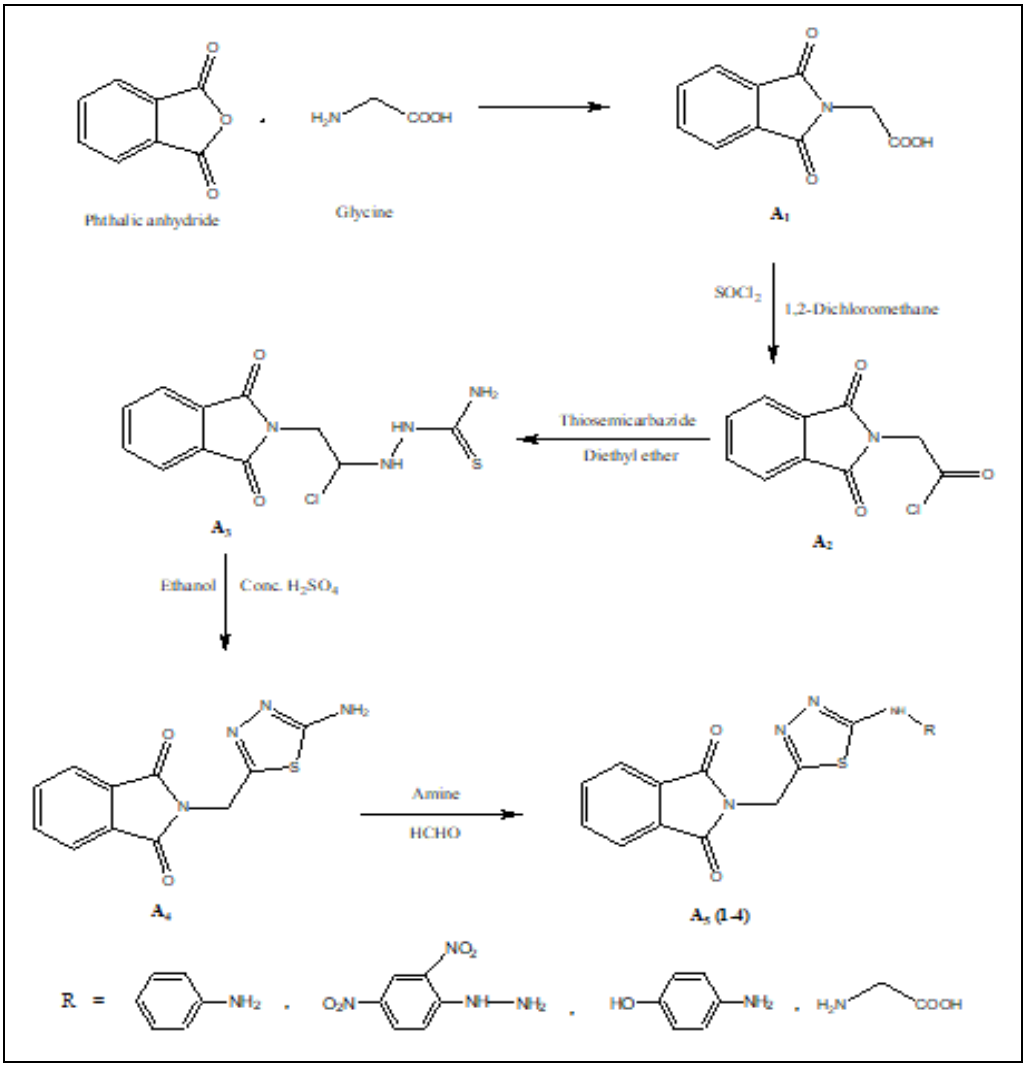

FIG. 2: SCHEME OF SYNTHESIS

Synthesis of 2-(1, 3-dioxoisoindolin-2yl)-acetic acid (A_1): Equimolar quantities of phthalic anhydride and glycine and distilled water were taken in a round bottom flask. The above was mixed well and heated on a heating mantle, and refluxed for two and half hours. After reflux, the hot mixture was transferred and kept in a beaker at room temperature overnight. The crystalline product, which was found to be insoluble in benzene and chloroform but soluble in hot water, was collected by filtered and dried to remove moisture. The unreacted raw materials were removed by repeatedly shaking with 50ml portions of benzene A.R. and then with 50ml portions of chloroform A. R. filtered, dried, and recrystallized from hot water.

The pure product was collected by filtration and dried to remove moisture.

Synthesis of 2-(1,3-dioxoisoindolin-2yl)acetyl chloride (A_2): [35] In a round-bottom flask equipped with condenser and drying tube, was added solution of 2-(1,3-dioxoisoindolin-2yl) acetic acid (0.1 mol) in anhydrous 1,2-dichlorethane and thionyl chloride (0.2 mol). The mixture was refluxed for 3 h. The solvent and the excess thionyl chloride were removed by reduced pressure distillation.

Synthesis of 1-(2-(1,3-dioxoisoindolin-2-yl) acetyl) thiosemicarbazide (A_3): [36] Thiosemicarbazide (0.03 mol) was dissolved in 36ml ether. 2-(1,3-dioxoisoindolin-2yl)acetyl chloride (0.03 mol diluted in 3.6ml ether) was added into the mixture drop by drop. After evaporating ether, the final mixture was washed until the smell of test compound disappeared and then recrystallized from ethanol.

Synthesis of 2-((5-amino-1, 3, 4-thiadiazol-2-yl)-methyl)Isoindoline-1,3-dione (A_4): [37] 15ml of 50% sulphuric acid was added to 1-(2-(1,3-dioxoisoindolin-2-yl)acetyl)thiosemicarbazide and refluxed at 110-150°C for 6 hours. The mixture was washed with distilled water for a couple of times. The precipitate was filtered, washed with distilled water, and recrystallized from ethanol.

Synthesis of Substituted Derivatives (A_5 1-$A_5$4): [38] A methanolic solution of 1-(2-(1,3-dioxoiso-indolin-2-yl)acetyl) thiosemicarbazide was charged into a three neck flask equipped with a stirred and dropping funnel. The solution was stirred to dissolve it completely. To this solution, formal-dehyde (7ml, 37%) was added dropwise during 15-20 minutes. The resulting mixture was stirred during half an hour to complete the reaction of formaldehyde and to yield methanol derivative. To this reaction mixture, the methanolic solution of amine (0.01mol) was added dropwise with stirring in about half an hour at 30 °C and refluxed for 2 h at 65-70 °C. It was allowed to cool and poured in ice water. The solid obtained was filtered off, washed thoroughly with hot water and air-dried.

Melting Point (MP) Determination: Melting points of all synthesized compounds were determined in open capillaries using Thiele's tube method [39].

Thin Layer Chromatography (TLC): In this method, one or more compounds were spotted on a thin layer of adsorbent coated on a chromato-graphic plate (made of silica gel GF). The spot was kept at least 2 cm above the base of the plate, and the spotting area was not immersed in the mobile phase in the development tank. The development chamber was lined inside with filter paper moistened with the mobile phase so as to saturate the atmosphere and the plates were kept vertically. After the development of TLC plate, the spots were visualized by keeping the TLC plates in a tank with few iodine crystals at the bottom. For detection of colorless spots, the plates were observed under UV chamber, at 254 nm (short λ) or at 365 nm (long λ) and bright spots were seen under a dark background then the R_f (Retardation factor) values for all synthesized compounds were calculated by using following formula [40]

R_f = Distance travelled by solute / Distance travelled by solvent front

Infra-Red Spectroscopy (IR): The infrared spectra of all synthesized compounds were recorded by Bruker αE FT-IR spectrophotometer.

Ultraviolet/Visible Spectroscopy: Ultraviolet, visible spectroscopy analysis of the synthesized compounds was carried out in UV–SPECORD® 50 PLUS-232H1004 UV-visible spectrophotometer.

Pharmacological Evaluation: Various pharmaco-logical evaluations were performed for the synthesized compounds to check their potential pharmacological activities. All experimental protocols involving laboratory animals were approved by the University Animal Ethical Committee bearing CPCSEA registration number 1574/PO/Re/11/CPCSEA and approval number AdtU/IAEC/2017/007.

Selection of Experimental Animals: Adult either sex Swiss albino mice weighing 20-25 gm were taken. They were obtained from the animal house of the university. The mice were grouped and housed in polyacrylic cages (38 cm × 23 cm × 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature of 25±2 °C and dark/light cycle of 14/10 hours). They were allowed free access to a standard dry pellet diet (Hindustan Lever, Kolkata,

India; starch – 66%, casein – 20%, fat – 8%, vitamins – 2%, salt – 4%) and water *ad libitum*. The mice were acclimatized to laboratory conditions for 7 days before the commencement of the experiment. OECD Guidelines 423 (Annexure 2d) was followed during carry out the acute toxicity study. All procedures described were reviewed and approved by the University Animal Ethical Committee.

Acute Toxicity Study:

Up and Down Method: [41, 42] A limit test can be used efficiently to identify chemicals that are likely to have low toxicity. The limit test is a sequential test that uses a maximum of five animals. A test dose of up to 2000 mg/kg, or exceptionally 5000 mg/kg may be used. The procedure for testing at 2000 mg/kg and 5000 mg/kg are slightly different.

The main test consisted of a single ordered dose progression in which animals were dosed, one at a time, at 48 h intervals. The first animal received a dose a step below the level of the best estimate of the LD_{50}. If the animal survived, the dose for the next animal was increased to a factor of 3.2 of the original dose; if it died, the dose for the next animal was decreased by a similar dose progression. Each animal was observed carefully for up to 48 hours before making a decision on whether and how much to dose the next animal. Dosing was stopped when one of these criteria was satisfied, at which time an estimate of LD_{50} and a confidence interval were calculated for the test based on the status of all animals at termination.

Parameters of Observation: Observation included changes in skin, eyes, mucous membranes, respiratory, circulatory, autonomic nervous systems, central nervous systems, and behaviour patterns. Attention was also directed to observe tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

Assessment of *in-vivo* Analgesic Activity:

Analgesic Effect in Mice using Eddy's Hot Plate Method: [43, 44]

Experimental Design:

Group 1: Normal saline solution [Control group]

Group 2: Ibuprofen (100 mg/kg, p.o.) [Standard group]

Group 3-6: Test compounds (50 mg/kg, p.o and 100 mg/kg, p.o. each) [Test group]

Procedure:

- The mice were weighed and numbered.
- The basal reaction-time was taken by observing hind paw licking or jump response (whichever appeared first) in animals when placed on the hot plate maintained at constant temperature (55°C).
- Normally animals showed a response in 6-8 seconds. A cut-off period of 15 seconds was observed to avoid damage to the paws.
- The standard drug and the test sample were orally administered to the grouped animals and the reaction time of animals on the hot plate was noted down at 0.5 h, 1 h, 2 h, and 3 h after the drug administration.
- As the reaction time increased with ibuprofen, 15 seconds is taken as maximum analgesia, and the animals were removed from the hot plate to avoid injury to paws.

Statistical Method: The results are expressed as mean ± S.E.M. n=3. The criterion for statistical significance was fixed at $p<0.05$ as compared to control group. Data was evaluated using One Way Analysis of Variance (ANOVA) followed by Dunnett's *t* test.

RESULTS AND DISCUSSION: In the present study, various 1, 3, 4-thiadiazole fused with phthalamide derivatives were synthesized as depicted in the scheme. The synthetic sequences employed in our laboratories for the preparation of series 2-((5-amino-1, 3, 4-thiadiazol-2-yl)methyl) isoindoline-1,3-dione ($A_5$1-$A_5$4) were prepared in good yield by the reaction of the corresponding1,3-dione withamine in the presence of formaldehyde.

All the synthesized final compounds were first purified by successive recrystallization using appropriate solvents. The purity of the synthesized compounds was checked by performing thin layer chromatography and determining melting points. The melting point value of each of the synthesized compounds was found to be different in comparison to their respective parent compounds which imply the fact of formation of new

compounds. Melting points of all the final products were compared with the melting points of the intermediates.

The preliminary qualitative analysis (melting point) ascertained the formation of new products. The melting point values of each of the synthesized compounds were found to be in between 145-238 °C. Individually all the compounds showed a clear difference in melting points, which indicates that the compounds must have different structural arrangements and they must have different arrangements of atoms or configurations amongst themselves. In the preliminary qualitative analysis, all the compounds that showed higher melting points was may be due to the presence of polar group and may be because the molecules were held together by dipole-dipole interaction. All the synthesized compounds were verified for their purity, and it was found that all of them melted over a range of not more than 3 °C temperatures of their individual melting temperature.

R_f values of all the synthesized compounds were found to be different and supporting the fact of formation of new compounds and also the purity of the compounds **Table 1**. It was evident that out of all the synthesized compounds, almost all the compounds were polar except a few. The synthesized compounds were examined to obtain their molecular weight, and the values were found to be in between 204-469 gm.

TABLE 1: PHYSICAL DATA OF THE SYNTHESIZED COMPOUNDS

Compd.	Mol. formula	Mol. wt. (g)	Color	Melting point (°C)	% yield (%w/w)	R_f	Solubility
A_1	$C_{11}H_8O_4$	204.17	White	190-193	82	0.66	Water
A_2	$C_{11}H_7ClO_3$	222.62	White	185-187	86	0.63	Acetone
A_3	$C_{12}H_{12}ClN_3O_2S$	297.76	White	198-200	89	0.72	10% sulfuric acid
A_4	$C_{12}H_9N_3O_2S$	259.28	White	201-203	85	0.78	Methanol
$A_5$1	$C_{19}H_{16}N_4O_2S$	364.42	Yellowish white	235-238	80	0.54	Acetone
$A_5$2	$C_{19}H_{16}N_4O_3S$	364.42	Yellow	224-227	82	0.61	Water
$A_5$3	$C_{19}H_{15}N_7O_6S$	469.43	Light Brown	145-147	84	0.58	Acetone
$A_5$4	$C_{15}H_{14}N_4O_4S$	346.36	White	189-202	86	0.69	Methanol

All the synthesized compounds were subjected to spectral analysis such as FT-IR and UV-visible to confirm the structures. Spectral analysis of all the synthesized compounds has shown satisfactory results. All the spectral data of the synthesized compounds are shown in table **Table 2**.

TABLE 2: SPECTRAL DATA OF SYNTHESIZED COMPOUNDS ($A_5$1-$A_5$4)

Compound	IUPAC Name	Wavenumber in cm^{-1} (FT-IR)	λ-max (nm)
$A_5$1	2-[(5-{[(phenylamino) methyl]amino}-1,3,4-thiadiazol-2-yl) methyl]-1*H*-indene-1,3(2*H*)-dione	1712.91 (C=O); 1603.10 (C=C, Ar); 3067.53 (C-H, CH_2); 1498.53, 1553.72 (C=N); 688.03(C-S-C); 3396.82(N-H, Amine) ;737.16, 782.10 (Ar. ring vib)	207
$A_5$2	2-{[5-({[(4-hydroxyphenyl)amino]methyl}amino)-1,3,4-thiadiazol-2-yl]methyl}-1*H*-indene-1,3(2*H*)-dione	1737.39 (C=O); 1602.04, 1496.47 (C=C, Ar); 3120.39 (C-H, CH_2) ; 1518.44(C=N); 689.56 (C-S-C); 3204.33 (N-H, Amine);3447.09 (O-H, Phenol); 1066.82, 1092.88(C-N, Alkyl)	220
$A_5$3	2-{[5-({[(2,4-dinitrophenyl)amino]methyl}amino)-1,3,4-thiadiazol-2-yl]methyl}-1*H*-indene-1,3(2*H*)-dione	1741.14 (C=O); 1613.96, 1489.51 (C=C, Ar); 3033.74 (C-H, CH_2); 1584.37 (C=N); 691.47 (C-S-C); 3305.14 (N-H, 2^0Amine); 1498.51 ($C-NO_2$).	234
$A_5$4	2-{[({5-[(1, 3-dioxo-2,3-dihydro-1*H*-inden-2-yl)methyl]-1,3,4-thiadiazol-2-yl}amino)methyl] amino}acetic acid	1724.15 (C=O); 1622.53 (C=C, Ar); 3050.30 (C-H, CH2);1650.30 (C=N); 696.13 (C-S-C); 3305.14 (N-H, 2^0Amine);1735.49C=O(Carboxylic); 3292.97(st),1448.70(ben) O-H(Carboxylic); 749.68, 786.02(Ar. ring vib)	278

Various functional groups of the synthesized desired derivatives were identified by analyzing their IR spectra. As evident from the IR spectra, for compound $A_5$1**,** peak at1712 cm^{-1} might be due to the presence of a C=O group; methylene group (C-H, -CH_2) had possibly displayed a stretching band

at 3514 $cm^{-1,}$ and the presence of C-S-C might have resulted in the peak at 688 cm^{-1}. The appearance of peak might be seen for N-H(Amine) group of $A_5$2**,** at 3204 cm^{-1}, in addition to the that O-H(Phenol) had possibly displayed a stretching band at 3447 cm^{-1} while C-S-C might have resulted in the peak at689 cm^{-1}, and 1496cm^{-1} and 1602 cm^{-1} might be due to C=C (aromatic) bond. For compound $A_5$3, peak at 1741cm^{-1} might be due to the presence of a C=O group, and the presence of N-H (secondary amine group) gave a peak at 3305$cm^{-1,}$ while C-NO_2 might have given the peak at 1498cm^{-1}. In the case of compound $A_5$4, O-H group might have resulted in a peak at 3292$cm^{-1,}$ and C-H (CH_2) might have given the peak at 3050cm^{-1} **Fig. 3**, **Fig. 4**, **Fig. 5** and **Fig. 6**.

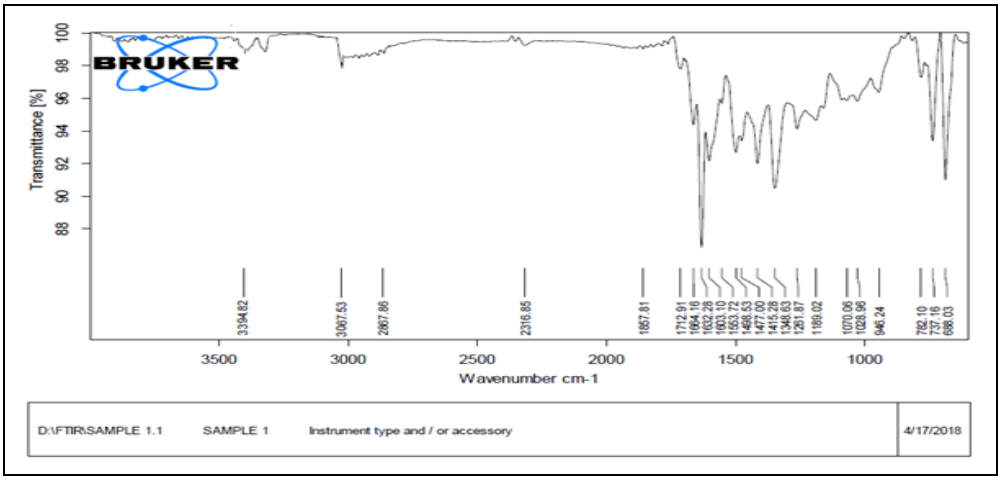

FIG. 3: FT-IR SPECTRUM OF COMPOUND $A_5$1

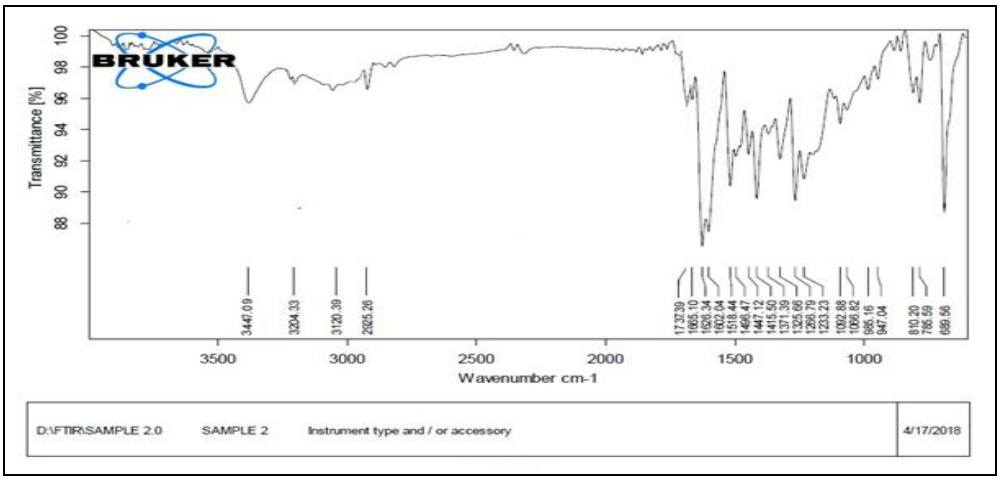

FIG. 4: FT-IR SPECTRUM OF COMPOUND $A_5$2

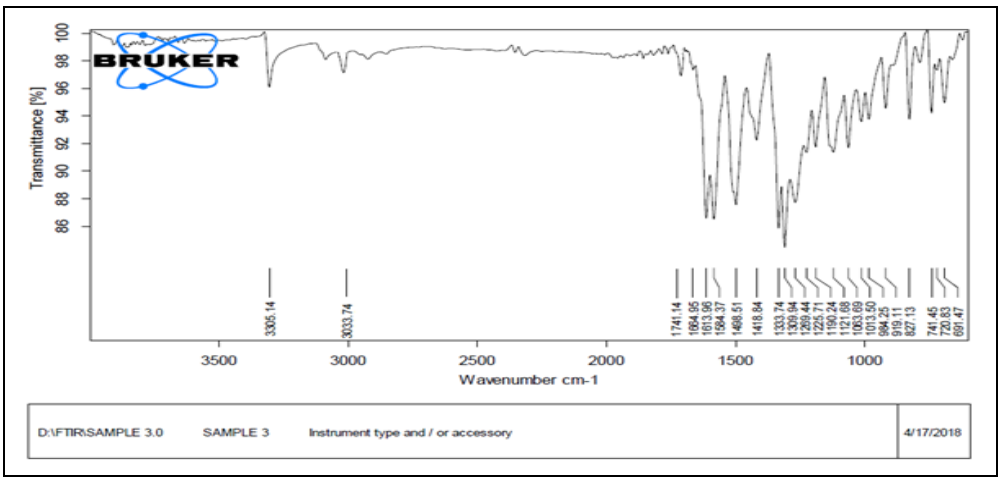

FIG. 5: FT-IR SPECTRUM OF COMPOUND $A_5$3

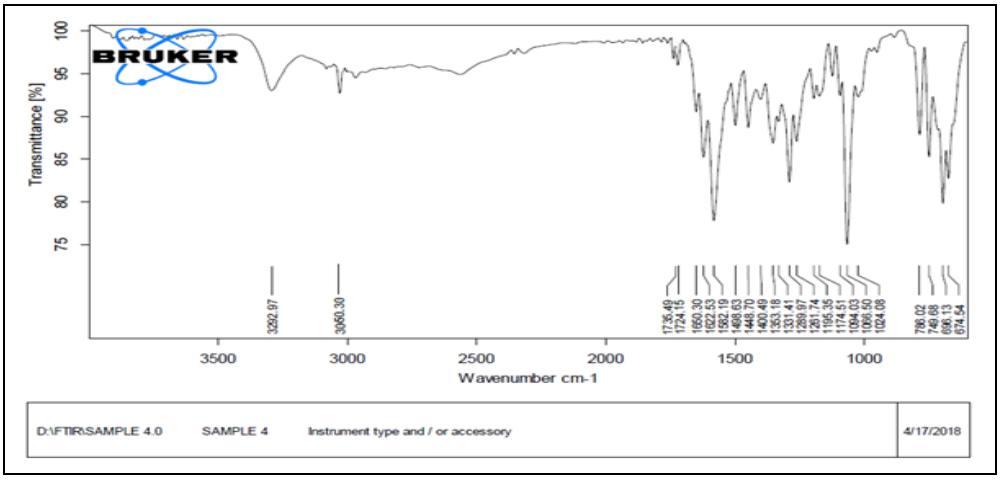

FIG. 6: FT-IR SPECTRUM OF COMPOUND $A_5$4

The UV-Visible spectral analysis of the compounds $A_5$1, $A_5$2, $A_5$3 and $A_5$4 showed the λ-max values to be 207nm, 220nm, 234nm and 278nm respectively.

According to OECD guidelines, the LD_{50} value of the compounds ($A_5$1-$A_5$4) were less than 1750 mg/kg so the limit test was not performed further and the main test of the compound was started **Table 3**, **Table 4**, **Table 5**, **Table 6,** and **Table 7**. In the hot plate analgesic test, it can be observed that compounds $A_5$3 and $A_5$2 exhibit an interesting profile of analgesic activity in comparison to ibuprofen. While compounds $A_5$1 and $A_5$4 gave a moderate response. However, the activity was less than that of the standard drug **Fig. 7**.

TABLE 3: ACUTE TOXICITY STUDY FOR SYNTHESIZED COMPOUNDS

Compounds	Animals	Dose (mg/kg/p.o.)	Log dose	X or O	LD_{50}
$A_5$1	1	175	2.2430	O	LD_{50} is Less than 1750 mg/kg
	2	550	2.7404	O	
	3	1750	3.2430	X	
$A_5$2	1	175	2.2430	O	LD_{50} is Less than 1750 mg/kg
	2	550	2.7404	O	
	3	1750	3.2430	X	
$A_5$3	1	175	2.2430	O	LD_{50} is Less than 1750 mg/kg
	2	550	2.7404	O	
	3	1750	3.2430	X	
$A_5$4	1	175	2.2430	O	LD_{50} is Less than 1750 mg/kg
	2	550	2.7404	O	
	3	1750	3.2430	X	

X= Response; O= No response

TABLE 4: DETERMINATION OF LD_{50} VALUES FOR $A_5$1 IN MICE (MAIN TEST)

Step	Animals	Dose (mg/kg/p.o.)	Log dose	X or O	LD_{50}
1	1st	100	2.000	O	Maximum likelihood
2	2nd	200	2.3010	O	calculation be completed, LD_{50}
3	3rd	400	2.6020	O	is 1000 mg/kg
4	4th	500	2.6989	O	
5	5th	1000	3.000	X	

X= Response; O= No response

TABLE 5: DETERMINATION OF LD_{50} VALUES FOR $A_5$2 IN MICE (MAIN TEST)

Step	Animals	Dose (mg/kg/p.o.)	Log dose	X or O	LD_{50}
1	1st	100	2.000	O	Maximum likelihood calculation
2	2nd	200	2.3010	O	be completed, LD_{50} is 1250
3	3rd	400	2.6020	O	mg/kg
4	4th	500	2.6989	O	
5	5th	1000	3.000	X	
6	6th	1250	3.0969	X	

X= Response; O= No response

TABLE 6: DETERMINATION OF LD_{50} VALUES FOR $A_5$3 IN MICE (MAIN TEST)

Step	Animals	Dose (mg/kg/p.o.)	Log dose	X or O	LD_{50}
1	1st	100	2.000	O	Maximum likelihood calculation be completed, LD_{50} is 1250 mg/kg
2	2nd	200	2.3010	O	
3	3rd	400	2.6020	O	
4	4th	500	2.6989	O	
5	5th	1000	3.000	X	
6	6th	1250	3.0969	X	

X= Response; O= No response

TABLE 7: DETERMINATION OF LD_{50} VALUES FOR $A_5$4 IN MICE (MAIN TEST)

Step	Animals	Dose (mg/kg/p.o.)	Log dose	X or O	LD_{50}
1	1st	100	2.000	O	Maximum likelihood calculation be completed, LD_{50} is 1000 mg/kg
2	2nd	200	2.3010	O	
3	3rd	400	2.6020	O	
4	4th	500	2.6989	O	
5	5th	1000	3.000	X	

X= Response; O= No response

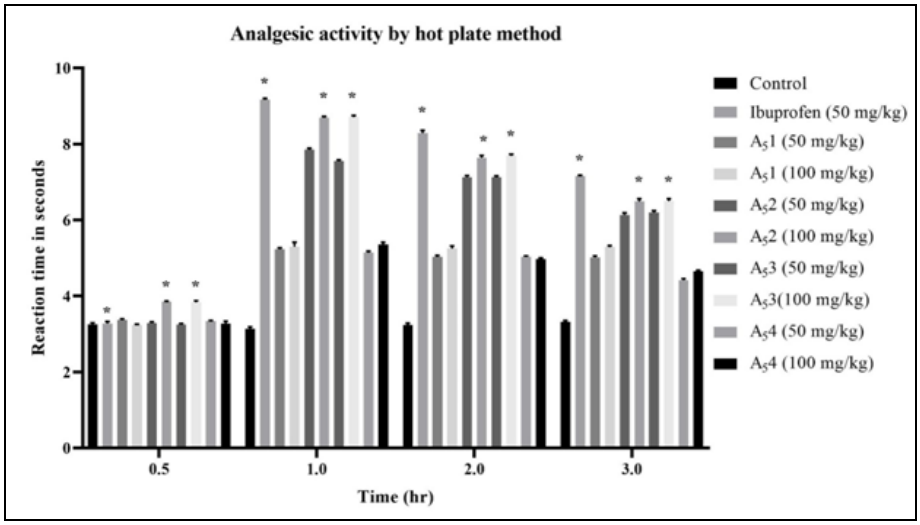

FIG. 7: EVALUATION OF ANALGESIC ACTIVITY BY HOT PLATE METHOD. All values are given in mean ± SEM, n=3. *p<0.05 as compared to control (ANOVA followed by Dunnett's *t* test).

CONCLUSION: Therefore, the results suggest that such compounds exert their pharmacological effects. In conclusion, it can be said that this offers a future promise to investigators in the field of medicinal chemistry for a search of analgesic agents containing [1, 3, 4]-thiadiazole-[1, 3-dione]-isoindole derivatives.

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