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Research Article

SYNTHESIS AND CHARACTERIZATION OF NOVEL 1, 3, 4- THIADIAZOLE DERIVATIVES AND SCREENING FOR CERTAIN BIOLOGICAL ACTIVITIES

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ABSTRACT
In the present study 5-aryl N-phenyl-1, 3, 4- thiadiazole 2-amino derivatives (PM1-PM10) were synthesized by the oxidative cyclization of substituted phenyl thiosemicarbazone using $FeCl_3$ as catalyst. The chemical structures of the synthesized compounds were confirmed by IR, H^1NMR and CHNS analysis. These compounds were screened for antibacterial, anti inflammatory, anti diabetic, anti oxidant, anxiolytic and locomotor activities. N-phenyl 5-(2, 3, 4-trimethoxyphenyl)-1, 3, 4 – thiadiazole-2-amine showed maximum antibacterial and anti inflammatory activities, N,5-diphenyl 1, 3,4 – thiadiazole -2-amine showed maximum antioxidant and anxiolytic activity, N-phenyl 5-(2, 4-dimethoxyphenyl)-1, 3, 4 –thiadiazole-2-amine showed maximum anti diabetic activity and N-phenyl 5-(3- bromophenyl)-1, 3, 4 –thiadiazole-2-amine showed maximum anxiolytic activity.

Keywords: 1,3,4 – thiadiazole, antibacterial activity, anti inflammatory activity, anti diabetic activity.

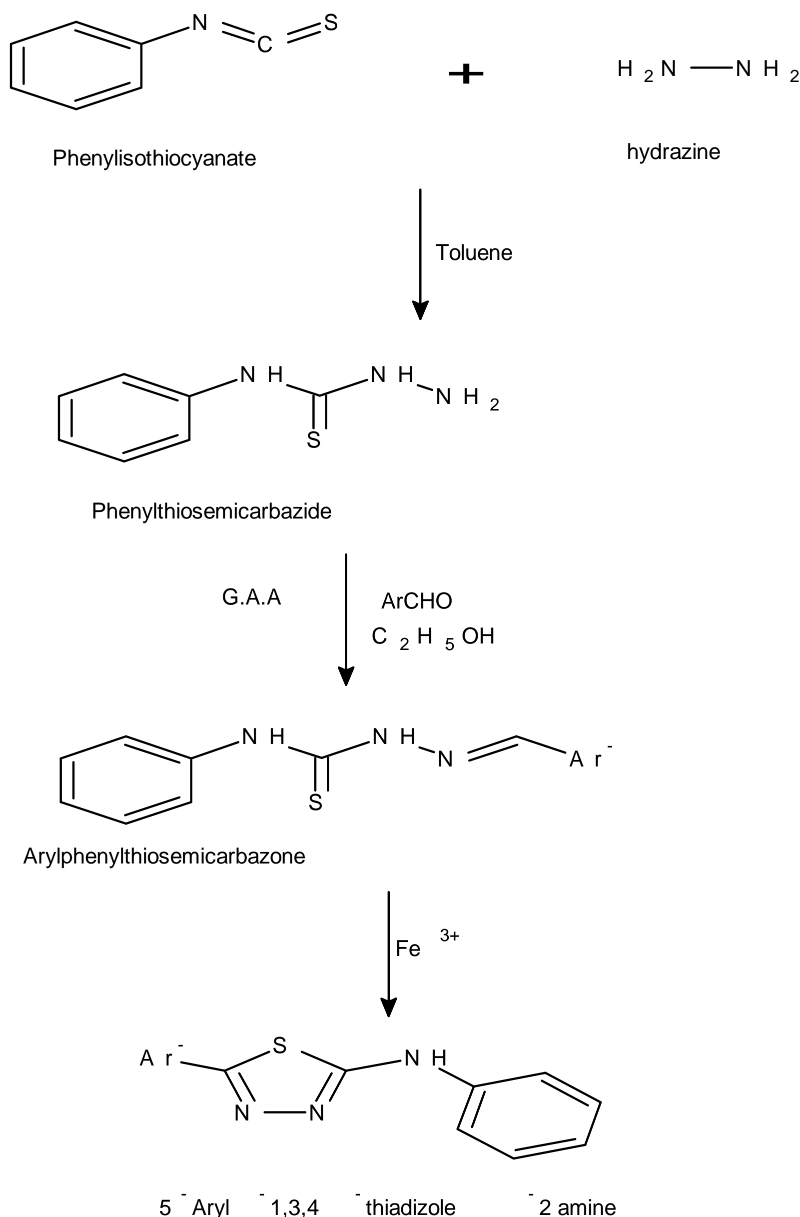
INTRODUCTION
The biological activity of compounds mainly depends on their molecular structure. Heterocyclic moieties can be found in a large number of compounds which display large number of biological activity. Thiadiazole is a versatile moiety that exhibits a wide variety of activity due to the presence of N=C-S moiety in the ring.They have become an important class of heterocycles of great interest of researches because of their broad types of biological activity. Many drugs containing 1,3,4-thiadiazole nucleus like acetazolamide(1), butazolamide(2), sulfamethazole(3) are available in market. In addition other analogues have found to be used as dye, pesticides, lubricants and conducting polymers[1].
Literature reviews showed that the thiadiazole nuclei have antimicrobial[2], anticonvulsant[3], anti-inflammatory[4], anticancer[5], antidepressant, radio protective, antioxidant[6], antileishmanial and antidiabetic[7] activities.

EXPERIMENTAL
SYNTHESIS
All the regents used were of LR grade obtained from Spectrum, Otto and Chemco. Melting points were recorded by using melting point apparatus (Veego, model no.VMP-D), thin layer chromatography was performed on silica gel G and R_f value was calculated. IR were obtained on Shimadzhu FT-IR Affinity 1, using KBr discs. H1NMR and CHNS analysis done at SAIF, STIC Cochin.

- General procedure for the preparation of thiosemicarbazide[8]
  Mix phenyl isothiocyanate(25mmol) and hydrazine hydrate (25mmol) in the presence of toluene (20ml). Keep the reaction mixture under stirring for one hour at room temperature. Filter the solid product and wash with ice cold Toluene. Recrystalize from ethanol.
- General procedure for the preparation of aldehyde phenyl thiosemicarbazone by microwave irradiation[8]

Mix aldehyde (0.0084M) and phenyl thiosemicarbazide(0.0084M)in the presence of ethanol (50ml) and added few drops of glacial acetic acid and submit to microwave irradiation for 20-40min at 280W. Filter the solid product obtainedand wash with ice cold ethanol several times.

- Preparation of 2- aminophenyl-5-aryl-1,3,4-thiadiazole derivatives
  Suspend thiosemicarbaone(0.015) in a mixture of warm water and of alcohol (4:6). To this add ferric chloride (0.045mol) in 100ml water quantitatively slowly with constant stirring and heat the reaction mixture at 80 – 90^0 c for 2 hours. Filter the solution while hot. Add 100 ml citrate buffer containing citric acid (0.033mol) and sodium citrate (0.015mol). Neutralize the resulting mixture with aqueous ammonia (10%) to pH 7. Filter the separatthiadiazole, dry and recrystalizewithan appropriate solvent.



Scheme for the synthesis of 1,3,4-thiadiazole derivatives

BIOLOGICAL ACTIVITIES

Screening for anti bacterial activity,[9]

The newly synthesized compounds were tested for their preliminary antibacterial activity against different microorganisms representing gram positive bacteria (*Bacillus subtilis, Staphylococcus epidermidis*) and gram negative bacteria(*Pseudomonas aeroginosa, E. coli*) by disc diffusion method using ciprofloxacin as standard.

The antibacterial screening was carried out in a laminar air flow unit and all types of precautions were strictly maintained to avoid any type of contamination during the test. Ultraviolet light was switched on for half an hour before working in the laminar hood to avoid any accidental contamination. Petri dishes and other glassware were sterilized in the autoclave at 121°C temperature and at a pressure of 15 lbs/sq inch for 15 minutes. Micropipette tips, culture media, cotton, forceps, blank disks, and so forth, were also sterilized. In disc diffusion method bacterial inoculum is prepared and inoculated into the entire surface of solid agar plate with a sterile cotton- tipped swab to form an even lawn. The paper disc 6mm in diameter impregnated with diluted test drug solution(500μg/ml in ethanol) was placed on the surface of each of agar plates using a sterile pair of forceps. The forceps were sterilized using flame. The plates were incubated for 2 - 3 days at 20 -25 ^{0}C and observed without opening them and the zone of inhibition was measured.

SCREENING FOR ANTIDIABETIC ACTIVITY

By *in vitro* α amylase inhibitory method,[10]

Porcine pancreatic α amylase (PPA) was used for the preliminary screening of α amylase inhibitors from the compounds. The inhibition assay was performed using the chromogenic dinitro salicylic acid method. A mixture of 500μl of test (100μg/ml)[44] and 500μl of α amylase solution prepared in 0.02M sodium phosphate buffer (pH 6.9 with 0.0006M NaCl) was incubated at 25^{0}C in a BOD incubator for 10 minutes. 500μl of preincubated 1% starch solution in 0.02M phosphate buffer of pH 6.9 was added to the above mixture. The reaction mixture was then incubated at 25^{0}C for 10 minutes. The reaction was stopped by adding 1.0ml dinitro salicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 minutes and then cooled to room temperature. The reaction mixture was then diluted by adding 10ml distilled water and the absorbance was measured at 540nm. The control reaction representing 100% enzyme activity did not contain any compound. The α amylase inhibitory activity was calculated according to the equation

Percentage inhibition = $(A_c\text{-}A_s)/ A_c *100$

A_c - Absorbance of control, A_s - Absorbance of standard

SCREENING FOR ANTI INFLAMMATORY ACTIVITY

In vitro protein denaturation method[11],

A solution of 0.2 % w/v of Bovine Serum Albumin(BSA) was prepared in tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Test drug of 100μg/ml concentration were prepared using ethanol as solvent. 50μl of each test drug was transferred to test tubes using micropipette. 5ml of 0.2% w/v BSA was added to the test tubes. The control consists of 5 ml of 0.2%w/v BSA solution and 50μl of alcohol. Diclofenac 100μg/ml is used as standard. The test tubes were heated at 72^{0}C for 5 minutes and then cooled for 10 minutes. The absorbances of these solutions were determined using UV-VIS spectrophotometer at a wavelength of 660nm.

Percentage inhibition = $(A_c - A_t)/A_c *100$

A_c : absorbance of control

A_t : absorbance of test

SCREENING FOR ANTIOXIDANT ACTIVITY

In vitro antioxidant activity by H_2O_2 scavenging method[12],

A solution of H_2O_2 (20mM) was prepared in phosphate buffer saline (pH 7.4). 1ml of test sample and standard (ascorbic acid) in a concentration of 100μg/ml in ethanol were added to 2ml of H_2O_2 solution in phosphate buffer saline. The absorbance was measured at 230nm after 10minutes

Percentage of H_2O_2 scavenged = $(A_c\text{-}A_t)/A_c*100$

ANIMALS USED FOR THE STUDY

Albino rats (wistar strain) were used to carry out the activities. The animals had free access to standard commercial diet and water *ad libitum*and were housed in cages under standard laboratory conditions i.e., 12:12 hour light or dark cycle at 25±2^{0}C. The experiments were carried out as per the guidelines of CPCSEA, New Delhi, India. and approved by the Institutional Animal Ethical Committee (IAEC) (Reg. No: PCP/2015/IAEC/1776/05).

ACUTE TOXICITY TEST[13]

Acute toxicity studies of the synthesized compounds were carried out using OECD/OCED guideline 423. Healthy young adult non pregnant female albino rats were used for this study. Animals were fasted prior to dosing (food

but not water should be withheld overnight). Following the period of fasting, the animals were weighed and the test substance administered orally at different dose levels(5,50,300 & 2000mg/kg) and the animals were tested for their mortality.

SCREENING FOR ANXIOLYTIC ACTIVITY

In vivo anti anxiety using hole board apparatus[14]

By placing a rat on the hole board apparatus induces anxiety in rats as it is exposed to a new environment. The anxiogenic agents will reduce the number of head poking where as anxiolytic agents will increase the number of head poking. The hole board apparatus consists of a square frame, frame stand and hole board plate. The frame is equipped with 32 IR cells, out of which 16 cells are on X- axis and 16 cells are on Y-axis. The instrument control panel will display number of beam brakers by animal on all axis and total of all in hole board mode. The hole board plate consist of 16 holes of 3cm diameter.
Swiss albino rat (150-250g) were taken and divided into 12 groups each group comprised of 6 animals. 60mg/kg of test drugs PM1-PM10 were administered orally 60 minutes before test and standard group was treated with diazepam (2mg/kg) intra peritoneally 30 minutes before test. The control group was treated with vehicle 60 minutes before the test. At the start of the test, rat was placed in the edge of the board. The number of head poking into the holes during a 5 minutes period was taken as the measurement.

LOCOMOTOR ACTIVITY[15]

The locomotor activity can be easily studied with the help of IR actimeter. The actimeter consists of a square frame, frame stand and hole board plate. The frame is equipped with 32 IR cells,out of which 16 cells are on X-axis and 16 cells are on Y axis. The instrument control panel will display number of beam brakes by animal on all axis and total of all in hole board mode and actimeter mode.
Swiss albino rat weighing between 150-250g were divided into 12 groups, each group comprising of six animals. Each animal was placed individually and the basal activity score of all the animals were recorded after 30 and 60 minutes of drug treatment. Dose of drug given was 60mg/kg orally. Diazepam at a dose of 2mg/kg was given as standard intra peritoneally. The activity on each rat was tested for 10 min. Finally percentage decrease in locomotor activity was calculated.

Statistical analysis

Values are represented as mean ± standard error mean for groups of six animals. The results were analysed by one way analysis of variance (ANOVA) followed by post hoc Dunnett's multiple comparison test.

RESULTS AND DISCUSSION

All 1,3,4- thiadiazole derivatives were synthesized and structures were determined by IR,H^1NMR and CHNS analysis and all the compounds were evaluated for antibacterial, *in vitro* anti inflammatory, *in vitro* anti diabetic activity, *in vitro* anti oxidant, in vivo anxiolytic activity and *in vivo* locomotor activities.

SPECTRAL DATAS

PM1 ; IR(KBr) 3032.23(Ar C-H), 1602.91(SCN), 1571.09(C=N), 1500,1444.75(Ar C-H), 1333.83,1275.97(C-N), 769.3(C-S-C); H^1NMR(δ,ppm) 7.874-7.012 (m, 10 H, Ar-H),10.552(s,1H, NH) CHNS ANALYSIS(Cal/Ana)C(66.40%/64.02%)H(4.3% /3.47%)N(16.60%/16.07%)S(12.64%/12.08%)
PM2; IR(KBr)3053.45(Ar C-H), 1600.2(SCN), 1574.95(C=N), 1454.39(Ar C=C), 1326.12(C-N), 743.59(C-S-C),695.37(C-Cl);H^1NMR 7.507-7.295(m,9H,Ar-H); CHNS ANALYSIS(CAL/ANA)C(58.43%/57.79%),H(3.47%/2.17%),N(14.6%/14.40%), S(11.13%/10.53%)
PM3; IR(KBr) 3053.45(Ar C-H), 1600.2(SCN), 1574.95(C=N), 1454.39(Ar C=C), 1326.12(C-N), 743.59(C-S-C), 695.37(C-Cl);H^1NMR 7.507-7.295 (m, 9 H, Ar-H); CHNS ANALYSIS (CAL/ANA)C(58.43%/57.79%), H(3.47%/2.17%), N(14.6%/14.40%), S(11.13%/10.53%)
PM4; IR(KBr) 3417.04(N-H) 3048.62(Ar C-H), 1642.46(SCN), 1599.06(C=N), 1599.06(Ar C=C), 1349.26(C-N), 749.38 (C-S-C), 642.32 (C-Cl); H^1NMR 8.087-7.041 (m, 10 H, Ar-H), 10.608(s,1H, NH) CHNS ANALYSIS (CAL/ANA) C(58.43%/41.65%), H(3.47%/1.00%), N(14.61%/9.73%), S(11.13%/7.81%)
PM5; IR(KBr) 3289.74(N-H), 3026.44(Ar C-H), 2917.46(C-H), 1606.77(SCN), 1518(C=N), 1404.24(Ar C-H), 1332.87(C-N), 702.12(C-S-C); 7.646-7.001 (m, 8H, Ar-H), 10.457(s,1H, NH), 3.852(s, 3H, OCH3), 3.825(s, 3H,OCH3 ; C(61.34%/59.91%), H(4.79%/2.94%), N(13.42%/12.91%), S(10.22%/9.48%)
PM6; IR (KBr) 3054.41(Ar C-H), 2886.60(C-H), 1640.53 (SCN), 1587.48(C=N), 1498.75(Ar C=C), 1239.32(C-N), 749.38(C-S-C); H^1NMR 7.525-6.776 (m, 7 H, Ar-H),3.850 (s,3H, OCH3), 3.824(s, 3H, OCH3), 3.3485 (s,3H, OCH3) CHNS ANALYSIS (CAL/ANA) C(59.47%/43.70%),

H(4.95%/2.94%), N(12.24%/10.56%), S(10.22%/6.34%)
PM7; IR(KBr) 3290.70(N-H), 2903(C-H), 1598.09 (C=N), 1404.24(Ar C=C), 1265.36(C-N), 767.70(C-S-C); H[1]NMR 7.684-7.010(m, 8 H, Ar-H), 10.769(s,1H, NH), 3.874(s, OCH3), 3.729(s, OCH3); CHNS ANALYSIS(CAL/ANA) C(61.34%/58.18%), H(4.79%/3.99%), N(13.42%/12.70%), S(10.22%/8.80%)
PM8; IR(KBr) 3190.40(N-H), 3049.59 (Ar C-H), 2895.28(C-H), 1608.70(SCN), 1569.16(C=N), 1451.50(Ar C=C), 1230.64(C-N), 749.38(C-S-C); H[1]NMR 7.658-6.562 (m, 9H, Ar-H), 10.353(s,1H, NH), 2.981(s, CH3), 2.879(s, CH3);CHNS ANALYSIS C(64.86%/62.35%), H(5.40%/4.62%), N(18.92%/18.17%), S(10.81%/9.59%)
PM9; IR (KBr) 3080.45 (Ar C-H), 2923.25(C-H), 1622.20,1602.91(SCN), (C=N), 1493.93,1455.35(Ar C-H), 1236.42(C-N), 766.74(C-S-C); H[1]NMR 7.678-7.050 (m, 9 H, Ar-H), 10.490(s,1H, NH), 2.154 (s, CH3); CHNS ANALYSIS (CAL/ANA) C(67.42%/65.95%), H(4.87%/3.43%), N(15.73%/10.11%), S(11.99%/15.64%);
PM10; IR(KBr) 3251.16(N-H) 3053.45(Ar C-H), 1622.20 (SCN), 1572.05(C=N), 1433.17(Ar C=C), 1256.68(C-N), 739.73(C-S-C), 501.51(C-Br); H[1]NMR 8.04-7.050 (m, 9 H, Ar-H), 10.626(s,1H, NH) ; CHNS ANALYSIS (CAL/ANA) C(50.60%/49.13%), H(3.01%/1.85%), N(12.65%/12.01%), S(9.6%/8.17%)

ANTIBACTERIAL ACTIVITY

All the synthesized compounds have been screened for their anti bacterial activity by disc diffusion method by measuring zone of inhibition in mm. 10μg Ciprofloxacin was used as standard.The compounds were screened for their antibacterial activity againstGram positive bacteria*[Bacillus subtilis (*NCIM No. 2063), *Staphylococcus epidermidis*(NCIM No. 2493)]Gram negative bacteria[*Pseudomonas aerogenosa* (NCIM No. 5029), *E. coli* (NCIM No. 293)].

Compounds PM2, PM3, PM4, PM5, PM6, PM7, PM9, and PM10 showed activity against gram positive bacteria *Bacillus subtilis.* Compounds PM5, PM6, showed good antibacterial activity against *Bacillis subtilis*, compounds PM2, PM3 PM4, PM7, PM9, PM10 showed moderate activity against *Bacillus subtilis*the other thiadiazole derivatives did not show any anti bacterial activity. The synthesized thiadiazole derivatives did not show any antibacterial activity against *Staphylococcus epidermidis*, or gram negative bacterias *Pseudomonas aeroginosa*, *E. coli*etc.

2,4 dimethoxy substituted compound showed maximum antibacterial activity followed by 2,3,4- trisubstituted compound.

In vitro ANTI-INFLAMMATORY ACTIVITY

The synthesized thiadiazole derivatives were tested for anti inflammatory activity at a concentration of 100μg/ml by *in vitro* protein denaturation method using bovine serum albumin. The result obtained is shown in **Table 3**.

4-bromophenyl (PM3), 2,3,4-trimethoxy phenyl(PM6), 2-methyl phenyl(PM9) and 3-bromophenyl(PM10) derivatives of 5-aryl N-phenyl 1, 3, 4 – thiadiazole-2- amine derivatives showed significant anti inflammatory activity compared to standard drug diclofenac (100μg/ml). phenyl (PM1),4-chloro phenyl(PM2), 2-chloro phenyl(PM4), 2,4dimethoxy phenyl(PM5), 3,4 dimethoxy phenyl (PM7),N,N-dimethyl amino phenyl (PM8) do not have any effect on protein denaturation. 2,3,4-Trimethoxy phenyl derivative showed maximum inhibition of heat induced protein denaturation of 43.55% which is comparable to standard drug diclofenac which showed 54.01% of inhibition of heat induced protein denaturation.

Inflammation is the response of living tissues to injury. It involves a complex mechanism of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair. Denaturation of protein is one of the main causes of inflammation. Several anti- inflammatory drugs have shown the ability to inhibit thermally induced protein denaturation in dose dependent manner. The ability of thiadiazole derivative to bring down thermal denaturation of protein is possibly a contributing factor for its anti inflammatory activity.

In vitro ANTIDIABETIC ACTIVITY

Thiadiazole derivatives synthesized were tested for anti diabetic activity by *in vitro* α amylase inhibitory method. The result obtained is shown in the **Table 4**.

Phenyl (PM1),4 chloro phenyl(PM2), 2,4 dimethoxy phenyl (PM5), 3,4-dimethoxy phenyl(PM7) and 3- bromophenyl (PM10) derivatives of 5-aryl N-phenyl 1, 3, 4 – thiadiazole-2- amine showed significant antidiabetic activity.

Among which compounds PM1, PM5, PM7 showed very good α amylase inhibitory activity, were as compounds PM2 and PM10 showed significant α- amylase inhibitory activity. 4-bromo phenyl (PM3), 2 chlorophenyl (PM4), 2, 3, 4- trimethoxy phenyl, N,N dimethyl amino

phenyl(PM8) 2-methyl phenyl(PM9) derivatives do not have any α- amylase inhibitory activity.
α amylase inhibitors are also called as starch blockers since it prevents or slows the absorption of starch into the body, mainly by blocking the hydrolysis of 1,4-glycosidic linkage of starch and other oligosaccharides into maltose and other simple sugars. By the α-amylase inhibitory activity the rate of digestion of carbohydrate and subsequent digestion of glucose is reduced. Thus blood sugar level will be reduced. So the α- amylase inhibitory activity 1,3,4 thiadiazole derivatives might play a role in diabetes treatment.

In vitro ANTIOXIDANT ACTIVITY

Thiadiazole derivatives synthesized were tested for antioxidant activity by H_2O_2scavenging method. The result obtained is given in the **Table 5**.
Phenyl(PM1), 4-bromo phenyl (PM3), N,N dimethyl phenyl(PM8) derivatives of 5-aryl N-phenyl 1,3,4- thiadiaole-2-amine showed significant H_2O_2scavenging activity compared to standard ascorbic acid which showed 28.28% H_2O_2scavenging activity, compounds 2,4 dimethoxy phenyl(PM5), 3,4 dimethoxy phenyl (PM7) showed moderate activity and compound3-bromo phenyl(PM10) derivative showed little activity. 4-chloro phenyl (PM2), 2-chlorophenyl (PM4), 2,3,4 trimethoxy phenyl(PM6),2-methyl phenyl(PM9) derivatives donot have hydrogen peroxide scavenging activity. PM1 showed maximum hydrogen peroxide scavenging activity.

ANXIOLYTIC ACTIVITY USING HOLE BOARD APPARATUS

Thiadiazole derivatives synthesized were tested for anti anxiety activity using hole board apparatus. The result obtained is shown in the **Table 6**
Anxiolytic activity of PM1-PM10 was studied using hole board apparatus. Placing the rat on the hole board apparatus will induce anxiety in rats as it is exposed to a new environment. The anxiogenic agents will reduce the number of head poking where as anxiolytic agents will increase the number of head poking. Diazepam showed significant increase in head poking and it has a significant anxiolytic activity. Phenyl substitutued derivative (PM1) and 3-bromosubstituted derivative (PM10) of thiadiazole showed significant increase in head poking compared to control and has anxiolytic activity. All other compounds except PM5 showed decrease in the number of head poking and hence have anxiogenic activity, and PM5 has the effect same as that of the control.

In vivo LOCOMOTOR ACTIVITY

The sedative effect of the novel thiadiazole derivatives are tested by observing the locomotor activity of rats administered with the synthesized compound in digital actophotometer. Depending upon the CNS depressant action of the drug the animals will show reduced locomotor activity.
The locomotor activity was significantly reduced in standard diazepam (2mg, i.p) and thiadiazole derivatives PM1-PM10 except PM5 showed significant decrease in locomotor activity. It may be due to the CNS depressant activity of the drug. 3-bromosubstituted derivatives showed maximum sedative activity were as 4-bromosubstituted derivative showed minimum sedative activity. Locomotor activity is an index of alertness. Reduction indicates that it must possess a sedative effect. It may be due to the CNS depressant activity of the drug.

CONCLUSION

The objective of the study was to synthesize 5-aryl N-phenyl-1, 3, 4- thiadiazole-2-amino derivatives (PM1-10). All the synthesized compounds were characterized by their physicochemical properties like melting point, R_f value, solubility and IR and H^1NMRspectral data and CHNS analysis. All these confirmed the structure of the synthesized compounds.
Synthesized compounds were screened for antibacterial activity by disc diffusion method, *in vitro* anti inflammatory activity by protein denaturation method, *in vitro* anti diabetic activity by α amylase inhibitory activity method, *in vitro* anti oxidant activity by H_2O_2 scavenging method, in vivo anxiolytic activity using hole board apparatus and *in vivo* locomotor activity using actophotometer.
N-phenyl 5-(2, 3, 4-trimethoxyphenyl)-1, 3, 4 –thiadiazole-2-amine showed maximum antibacterial and anti inflammatory activities, N,5-diphenyl 1, 3,4 – thiadiazole -2-amine showed maximum antioxidant and anxiolytic activity, N-phenyl 5-(2, 4-dimethoxyphenyl)-1, 3, 4 –thiadiazole-2-amine showed maximum anti diabetic activity and N-phenyl 5-(3-bromophenyl)-1, 3, 4 –thiadiazole-2-amine showed maximum anxiolytic activity.
Thus from the present study, it can be concluded that the synthesized 5-aryl N-phenyl-1, 3, 4-thiadiazole-2-amino derivatives are biologically active and that they can potentially developed into useful anti bacterial, anti inflammatory, antioxidant, anti diabetic or sedative agent that can prompt future researcher to choose this nucleus to synthesize a series of other

derivatives containing wide varieties of substitution.

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Table 1: physicochemical properties

S. No	Molecular formula	M. F	State	Colour	M.P	Yield	R_f
PM1	$C_{14}H_{11}N_3S$	253	Crystalline	Reddish Brown	187^{0}C	61.05% w/w	0.59
PM2	$C_{14}H_{10}N_3SCl$	287.5	Amorphous	Cream	230^{0}C	30%w/w	0.3019
PM3	$C_{14}H_{11}N_3SBr$	332	Amorphous	Yellow	180^{0}C	35%w/w	0.2968
PM4	$C_{14}H_{10}N_3SCl$	287.5	Amorphous	Brown	216^{0}C	32%w/w	0.3548
PM5	$C_{16}H_{15}N_3SO_2$	313	Solid powder	Red wine	184^{0}C	62%w/w	0.3
PM6	$C_{17}H_{17}N_3SO_3$	343	Crystallie solid	Dark red wine	160^{0}C	64%w/w	0.298
PM7	$C_{16}H_{15}N_3SO_2$	313	Solid powder	Pink	175^{0}C	64.5%w/w	0.3016
PM8	$C_{16}H_{16}N_3S$	296	Solid powder	Dark green	185^{0}C	63.4%w/w	0.31
PM9	$C_{15}H_{13}N_3S$	267	Solid powder	Brown	124^{0}C	124^{0}C	0.2881
PM10	$C_{14}H_{10}N_3SBr$	332	Solid powder	Brown	189^{0}C	124	0.2982

Table 2: Antibacterial activity of PM1-PM10

S. No.	Sample code	Zone of inhibition in mm			
		Bacillus subtilis	***Staphylococcus epidermidis***	***Pseudomonas aerogenosa***	***E. coli***
1	Standard	38 mm	40mm	25 mm	20 mm
2	PM1	-	-	-	-
3	PM2	10 mm	-	-	-
4	PM3	12 mm	-	-	-
5	PM4	9 mm	-	-	-
6	PM5	19 mm	-	-	-
7	PM6	16 mm	-	-	-
8	PM7	8 mm	-	-	-
9	PM8	-	-	-	-
10	PM9	10 mm	-	-	-
11	PM10	12 mm	-	-	-

Table 3: Percentage inhibition of protein denaturation by PM1-PM10

Test	Percentage of inhibition
Standard	54.01%
PM1	No significant result
PM2	No significant result
PM3	24.20%
PM4	No significant result
PM5	No significant result
PM6	43.55%
PM7	No significant result
PM8	No significant result
PM9	33.98%
PM10	31.27%

Table 4: Percentage inhibition of α amylase inhibitory activity by PM1-PM10

Test drug	% of inhibition
PM1	52.14%
PM2	38.31%
PM3	No significant result
PM4	No significant result
PM5	64.36%
PM6	No significant result
PM7	56.55%
PM8	No significant result
PM9	No significant result
PM10	36.11%

Table 5: Hydrogen peroxide scavenging activity of PM1-PM10

Test drug	% of H_2O_2 scavenged
Ascorbic acid	28.28%
PM1	26.22%
PM2	No significant result
PM3	18.21%
PM4	No significant result
PM5	9.3%
PM6	No significant result
PM7	6.3%
PM8	15.08%
PM9	No significant result
PM10	0.1214%

Table 6: *in vivo* anxiolytic activity of PM1-PM10 using hole board apparatus

Test group	Number of head poking during a 5 minutes test period
Control(propylene glycol)	7 ± 0.3651
Standard (diazepam, 2mg/kg)	14.67 ± 0.4944**
PM1(60mg/kg)	9 ± 0.3651**
PM2(60mg/kg)	5 ± 0.3651
PM3(60mg/kg)	6 ± 0.3651
PM4(60mg/kg)	5.67 ± 0.4216
PM5(60mg/kg)	7.167 ± 0.4773
PM6(60mg/kg)	5.33 ± 0.4944
PM7(60mg/kg)	2.67 ± 0.33
PM8(60mg/kg)	3.167 ± 0.3073
PM9(60mg/kg)	5 ± 0.3651
PM10(60mg/kg)	9 ± 0.2582**

Each value represent Mean ± SEM, n = 6, ** = $p < 0.01$
(one way ANOVA followed by Dunnett's 't' test)

Table 7: Effect of PM1-PM10 in locomotor activity in rats (actophotometer) in different time intervals

Group	Treatment	PHOTOCELL COUNTS			
		30 minutes	Percentage of inhibition	60 minutes	Percentage of inhibition
I	Control (propylene glycol)	247.33±1.994	--	247.5 ± 0.7638	--
II	Diazepam (2mg/kg)	135.53± 1.167**	45.20	100.167±1.195**	59.23
III	PM1 (60mg/kg)	146 ± 1.713**	40.96	127.83 ± 2.136**	48.35
IV	PM2 (60mg/kg)	145 ± 1.414**	41.37	121.83 ± 1.537**	50.77
V	PM3 (60mg/kg)	208 ± 1.826**	15.90	169.167 ± 2.167**	31.65
VI	PM4 (60mg/kg)	190.67 ± 2.231**	22.90	140.67 ± 1.406**	43.16
VII	PM5 (60mg/kg)	204.67 ± 1.333	17.25	286 ± 1.238	Not significant result
VIII	PM6 (60mg/kg)	204.33 ± 1.406**	17.25	141.6 ± 1.333**	42.78
IX	PM7 (60mg/kg)	150.83 ± 1.537**	39.02	130.5 ± 1.55**	47.27
X	PM8 (60mg/kg)	232.33 ± 1.647**	6.06	134.33 ± 1.687**	45.72
XI	PM9 (60mg/kg)	194.5 ± 1.688**	21.36	133.5 ± 1.821**	46.06
XII	PM10 (60mg/kg)	201.5 ± 2.045**	18.53	121.167 ± 1.447**	51.04

Each value represent Mean ± SEM, n = 6, ** = $p < 0.01$ (one way ANOVA followed by Dunnett's 't' test)

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