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SYNTHESIS, CHARACTERIZATION AND ANTI-INFLAMMATORY ACTIVITY OF ANALOGUES OF 1, 3, 4- THIADIAZOLE

RESEARCH ARTICLE

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ABSTRACT

A *new series of novel analogues of 1, 3, 4- thiadiazole were synthesized. These analogues were identified on the basis of melting point range, Rf values, IR, ^1H NMR and mass spectral analysis. The analogues were screened for anti-inflammatory activity. The analogues exhibited significant to moderate anti-inflammatory activity.*

Keywords: *Thiadiazole, analogues, anti-inflammatory activity.*

INTRODUCTION

Thiadiazole contains the five-membered diunsaturated ring structure having molecular structure formula $C_2 H_2 N_2 S$ containing two carbon atoms, two hydrogens, two nitrogens and one sulphur. The ending azole designates a five membered ring system with two or more heteroatoms, one of which is Nitrogen. Thiadiazoles are associated with diverse biological activity probably by virtue of –N=C-S- grouping. Literature reveals that compounds having thiadiazole nucleus have wide spectrum of pharmacological activities such as antimicrobial[1], antitubercular[2], ulcerogenic [3], anti-inflammatory[4], analgesic[5], CNS depressant[6], anticonvulsant[7], anticancer[8], antioxidant[9], antiviral[10], antiepileptic [11] properties etc**.** Research in this area is still unexplored, therefore the present study is directed towards the synthesis of newer analogues of 1, 3, 4- thiadiazole with good yield and enhance anti-inflammatory activity.

MATERIALS AND METHODS

All the chemicals procured from Central Drug House (P.) Ltd, New Delhi. The melting points were determined in open glass capillaries and were uncorrected. Thin Layer Chromatography using silica gel G (E. Merck) plates were used to access the reaction and purity of synthesized compounds. The IR spectra were recorded on Perkin-Elmer FTIR/FTFIR system in KBr pellets and noted the absorption levels (cm^{-1}) were listed. ^1H NMR spectra were run on Bruker DPX400 in DMSO-d_6 as solvent and TMS as an internal standard. The Mass spectra were recorded on EI ionization mode on a JEOL JMS600H EI mass spectrometer.

STEP 1: Synthesis of N-phenyl thiosemicarbazide

- **From aromatic amines**

Aniline (0.01mol) was dissolved in ethanol and ammonia (25ml). Carbon disulfide (0.01mol) was added drop wise and stirred for 30 minutes. To this mixture, hydrazine hydrate (0.01 mol) was added, Reaction mixture was refluxed on water bath for 9-12 hrs. Completion of reaction was checked by TLC. After reaction, reaction mixture was allowed to cool to room temperature, kept overnight in freezing condition to get solid product. Separated solid product was filtered and dried. Recrystalized from ethanol-water mixture (4:1 ratio) to yield white shining crystals. Yield: 62.33% W/W.

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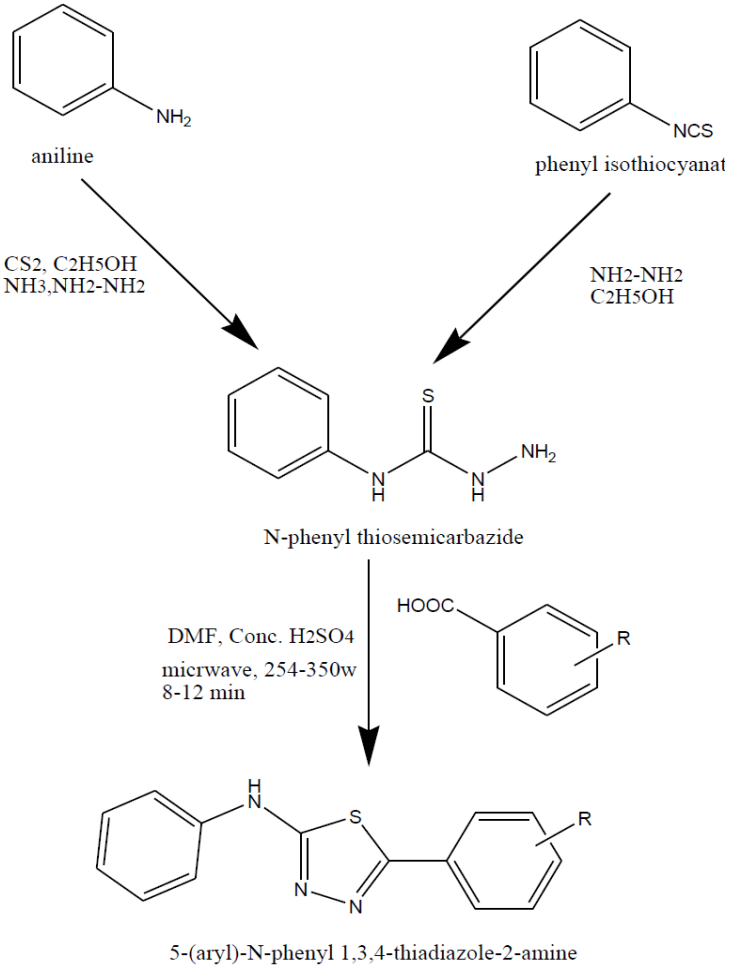
➢ **From phenylisothiocyanate**

Phenylisothiocyanate (0.01mol), hydrazine hydrate (0.01mol) in ethyl alcohol (25ml) were taken and subjected to microwave irradiation for 6-12 minutes at 245-350W power. In between, the completion of reaction was checked by TLC. After that reaction, mixture was slowly poured into crushed ice and kept overnight. Separated solid was filtered, washed with water and dried. Solid was then purified by recrystallisation from ethanol-water mixture (4:1 ratio) to yield desired compounds.

STEP 2: Synthesis of 5-aryl-N-phenyl-1,3,4-thiadiazole-2-amine.

N-phenyl thiosemicarbazide (0.01mol), aromatic acid (0.01mol) and sulfuric acid in DMF (25ml) were taken and subjected to microwave irradiation for 6-12 minutes at 245-350W power. In between, the completion of reaction was checked by TLC. After that reaction mixture was slowly poured into crushed ice and kept overnight. Separated solid was filtered, washed with water and dried. Solid was then purified by recrystallisation from ethanol-water mixture (4:1 ratio) to yield desired compounds [TD1-TD8] respectively.



Anti-Inflammatory activity [12][13]

Carageenan induced rat paw oedema method

Male Wistar rats (175-200g) were starved overnight, being allowed access to water. The animals were divided into 4 groups of 1 animal each. The first group was treated as a vehicle control receiving 1% CMC sodium. Group 2 received Ibuprofen (200 mg/ kg) orally as positive control and group 3 and 4 received test compound TD1 orally at doses of 100 and 200 mg/ kg body weight.After 60 minutes of treatment, 0.1ml 1% carageenan was injected into sub planar region of right hind paw and left hind paw served as the control. The displacement volume of both right and left paw were measured 3 hrs after the induction of oedema using plethysmometer. The readings were recorded.

The percentage inhibition of paw oedema was calculated by using the formula,
Percentage inhibition = 100[1-(Dtest/ Dcontrol)]
Where Dtest = Difference in paw volume in the drug-treated group.
Dcontrol = Difference in paw volume in the control group.
The result was expressed as percentage inhibition of oedema over the untreated group.(Table).

RESULTS AND DISCUSSION

The melting points of all synthesized compounds were found in open capillary tubes and readings were uncorrected. The structures of the synthesized compounds were supported by physical data and following spectral analysis.

N-phenyl thiosemicarbazide
MF: $C_7H_9N_3S$, MW: 167.23 AMU, m.p: 120-123 ^{0}C, Rf: 0.77 (ethanol : ethyl acetate ;9:1), IR(υ cm^{-1}) : 3414.32 (NH-NH_2), 3051.37 (aromatic C-H str),1648.11(primary amino N-H str), 1591.27 (N-H bend), 1490.97, 1425.40 (aromatic C=C ring str), 1233.87 (C=S), 755.38 (C_6H_5), LC-MS: *m/z* 167 (M^+).

5-(4-chlorophenyl)-N-phenyl-1,3,4-thiadiazole-2-amine [TD1]
MF: $C_{14}H_{10}ClN_3S$, MW: 287.77 AMU, m.p: 233-235 ^{0}C, Rf: 0.86 (ethyl acetate:n Hexane; 1:1), IR(υ cm^{-1}): 3051.39 (aromatic C-H str), 1573.91 (N-H bend), 1490.97 (C6H5), 1425.40 (aromatic C=C ring str), 1321.24 (secondary aromatic C-N str), 1111.00 (N-N=C), 1091.71(C-Cl str), 1014.56 (N-N str), 852.54(C_6H_4), 761.88 (C_6H_5), 682.80 (C-S-C), ^{1}H NMR(DMSO- *d6*) δ: 13.201- singlet, -NH (1H)7.563-7.953- m, Ar (9H), LC-MS: *m/z* 287.66 (M^+).

5-(4-nitrophenyl)-N-phenyl-1,3,4-thiadiazole-2-amine [TD2]
MF: $C_{14}H_{10}N_4O_2S$, MW: 298.327 AMU, m.p: 230-232 ^{0}C, Rf: 0.87 (ethyl acetate: n Hexane; 1:1), IR(υ cm^{-1}) : 3035.71 (aromatic C-H str), 1686.41(asymmetric aromatic NO2 str),1604.99 (N-H bend, 1426.19 (aromatic C=C ring str), 1349.58(symmetric aromatic NO2 str), 1310.55 (secondary aromatic C-N str), 1109.23 (N-N=C), 1014.04 (N-N str),887.97(aromatic nitro C-N str), 800.45(C_6H_4), 715.31 (C_6H_5), 671.45 (C-S-C), ^{1}H NMR(DMSO- *d6*) δ: 13.692- singlet, -NH (1H)8.163-8.342- m, Ar (9H), LC-MS: *m/z* 298.3 (M^+).

4(5-(phenyl amino)-1, 3, 4-thiadiazole-2-yl) phenol [TD3]
MF: $C_{14}H_{11}N_3OS$, MW: 269.329 AMU, m.p: 168-171 ^{0}C, Rf: 0.75 (ethyl acetate: n Hexane; 1:1), IR(υ cm^{-1}) : 3380.88 (O-H str), 3085.72 (aromatic C-H str), 1600.57(N-H bend),1574.46, 1487.82,1455.51, 1418.21 (aromatic C-C ring str), 1298.57 (secondary aromatic C-N str), 1200.14(phenolic C-O str), 1181.76 (N-N=C), 1032.53 (N-N str), 771.51(C_6H_4), 746.84 (C_6H_5), 686.08 (C-S-C), 606.83(O-H out of plane bend), LC-MS: *m/z* 269.3 (M^+).

5-(3-aminophenyl)-N-phenyl-1, 3, 4-thiadiazole-2-amine [TD4]
MF: $C_{14}H_{12}N_4S$, MW: 268.34 AMU, m.p: 165-167 ^{0}C, Rf: 0.79 (ethyl acetate: n Hexane; 1:1), IR(υ cm^{-1}) : 3030.31 (aromatic C-H str), 1598.29(N-H bend), 1545.71,1477.21,1442.96 (aromatic C=C ring str), 1310.49 (secondary aromatic C-N str), 1252.29(primary aromatic C-N str) 1100.88 (N-N=C), 1011.31 (N-N str), 825.13(C_6H_4), 745.00 (C_6H_5), 692.84 (C-S-C) LC-MS: *m/z* 268.34 (M^+).

3(5-(phenyl amino)-1, 3, 4-thiadiazole-2-yl) phenol [TD5]
MF: $C_{14}H_{11}N_3OS$, MW: 269.329 AMU, m.p: 163-165 ^{0}C, Rf: 0.73 (ethyl acetate: n Hexane; 1:1), IR(υ cm^{-1}): 3186.16(O-H str), 3030.85 (aromatic C-H str), 1598.17 (N-H bend), 1546.99 (C_6H_5), 1470.98,1442.65 (aromatic C=C ring str), 1310.00 (secondary aromatic C-N str), 1251.65, 1170.15(phenolic C-O str), 1189.39 (N-N=C),1024.77 (N-N str), 833.26(C_6H_4), 743.88 (C_6H_5), 692.53 (C-S-C), 603.93(out of plane O-H bend), LC-MS: *m/z* 269.32 (M^+).

N-phenyl-5-p tolyl-1,3,4-thiadiazole-2-amine [TD6]
MF: $C_{15}H_{13}N_3S$, MW: 267.357 AMU, m.p: 143-145 ^{0}C, Rf: 0.76 (ethyl acetate: n Hexane; 1:1), IR(υ cm^{-1}) : 2970.43 (aliphatic C-H str), 1601.49 (N-H bend), 1501.35 (C6H5), 1455.72, 1417.97 (aromatic C=C ring str), 1280.17 (secondary aromatic C-N str), 1115.69 (N-N=C), 771.92(C_6H_4), 747.50 (C_6H_5), 686.95 (C-S-C) ^{1}H NMR(DMSO- *d6*) δ: 12.794- singlet, -NH (1H)6.981-7.846- m, Ar (9H)2.371- s, -CH3(3H), LC-MS: *m/z* 267.35 (M^+).

5-(5-(phenyl amino)-1,3,4-thiadiazole-2-yl) benzene 1,2,3 triol [TD7]
MF: $C_{14}H_{11}N_3O_3S$, MW: 301.328 AMU, m.p: 167-169 ^{0}C, Rf: 0.78 (ethyl acetate: n Hexane; 1:1), IR(υ cm^{-1}) : 3393.43 (secondary aromatic N-H str) 3030.51 (aromatic C-H str), 3242.74(O-H str), 1601.11 (N-H bend), 1496.41, 1455.68, 1422.31(aromatic C=C ring str), 1299.00 (secondary aromatic C-N str), 1200.42(phenolic C-O str), 1082.15(N-N=C), 1058.84 (N-N str), 893.34(C_6H_3), 745.58 (C_6H_5), 687.88 (C-S-C), 606.94 (O-H out of plane bend), LC-MS: *m/z* 301.3 (M^+).

2(5-(phenyl amino)-1,3,4-thiadiazole-2-yl) phenol [TD8]
MF: $C_{14}H_{11}N_3OS$, MW: 269.329 AMU, m.p: 231-233 ^{0}C, Rf: 0.8 (ethyl acetate: n Hexane; 1:1), IR(υ cm^{-1}) : 3186.18(O-H str), 3031.01 (aromatic C-H str), 1598.87 (N-H bend), 1548.49,1495.67, 1471.68 (aromatic C=C ring str), 1310.95 (secondary aromatic C-N str), 1252.13(phenolic C-O str), 1189.61 (N-N=C), 1024.73 (N-N str), 743.78(C_6H_4), 692.41 (C-S-C), 603.93(O-H out of plane bend), LC-MS: *m/z* 269.32 (M^+).

Carrageenan induced rat paw oedema method in *Albino rats* was used for screening anti-inflammatory activity of the analogues. The analogues TD1 was selected and evaluated for anti-inflammtory activity at doses of 100 and 200 mg/kg body weight. Ibuprofen was used as standard drug at a dose of 200 mg/kg body weight. Control group was given 1% CMC. The test analogues TD1 showed comparable anti-inflammatory activity as that of Ibuprofen. Test analogues given orally gave good result and it showed better absorption from GIT. The results obtained was shown in table.

Anti-inflammatory activity screening of analogues TDI

GROUP	DOSE mg/kg	DIFFERENCE IN PAW VOLUME AFTER 3 Hrs	% INHIBITION
CONTROL	-	0.078	
TEST 1	100	0.037	52.16
TEST 2	200	0.023	70.51
+ CONTROL (Ibuprofen)	200	0.018	76.92

Graphical representation of % anti-inflammatory activity of analogues TDI

CONCLUSION

The research work was oriented towards the finding of newer analogues of 1, 3, 4- thiadiazole with enhance anti-inflammatory activity. The different analogues were synthesized. Synthesized analogue TD1 showed very good anti-inflammatory activity against previously reported analogues of 1, 3, 4- thiadiazole.

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