Research Article

# Synthesis of Some Newer Derivatives of Thiadiazole as Anti-Inflammatory and Analgesic Agents

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**ABSTRACT**

5-phenyl-1,3,4-thiadiazol-2-amine (1), on reaction with substituted indolaldehyde in presence of glacial acetic acid give N-((2-substituted indol-3-yl)methylene)-5-phenyl-1,3,4-thiadiazol-2-amine (2-6). The compounds 2-6 when treated with acetyl chloride in presence of triethylamine undergo cycloaddition to produce 4-(2-substituted indol-3-yl)-1-(5-phenyl-1,3,4-thiadiazol-2-yl)azetidin-2-one (7-11). The final products 4-(2-substituted indol-3-yl)-1-(5-phenyl-1,3,4-thiadiazol-2-yl)-3((phenylamino)methyl)azetidin-2-one (12-16) have been synthesized by reaction of compounds 7-11 with formaldehyde and aniline. All the newly synthesized compounds are evaluated for their anti-inflammatory activity as well as analgesic activity. The newly synthesized compounds have been characterized by elemental (C, H, N) and spectral (IR, $^1$HNMR and Mass) analysis.

**Keywords**: Thiadiazole, Indole, Azetidinone, Anti-inflammatory activity, Analgesic activity.

## INTRODUCTION

As a part of surge of interest in heterocycles that have been exploxed for developing pharmaceutically important molecules, thiadiazoles, indole and azetidinones have played an important role in medicinal chemistry. Thiadiazole exhibit a plethora of bioactivities viz. antimicrobial[1,2], anti-inflammatory[3,4], analgesic[5,6] etc. Indole derivative also possess anticancer[7], analgesic[8,9], anti-inflammatory[10] and antihelminthic[11] activities. Condensed azetidinone derivatives are reported to possess interesting pharmacological properties such as anti-inflammatory[12], analgesic[13], antimicrobial[14-16] activities etc. Keeping these observations in view and in continuation to the earlier work on the synthesis of thiadiazole, indole and azetidinone heterocycles and as a part of the continuing programme in this area, a series of new derivatives of thiadiazole having indole and azetidinone moiety have been prepared. Their anti-inflammatory and analgesic activities have also been evaluated and some of them show promising results.

## EXPERIMENTAL

All reagents and solvents were of analytical grade and used directly. Reactions were routinely performed in oven-dried borosil glassware. The melting points of compounds were determined in open capillaries with the help of thermonic melting point apparatus and were uncorrected. The homogeneity of all newly synthesized compounds was routinely checked by thin layer chromatography (TLC) on silica gel G plates and spots were located by using iodine chamber. Elemental analysis (C, H, N) of all the synthesized compounds were determined by perkin-Elmer 2400 elemental analyzer, and results were found within the ± 0.4% of theoretical values. The IR spectra were recorded on a Beckman Acculab-10 spectrometer ($\nu$ max in $cm^{-1}$) and the $^1$H NMR spectra were recorded by Brucker DPX-300 MHz using $CDCl_3$ as solvent. Mass spectra were determined on VG-70-S instrument. The animal research study was approved by the animal ethical committee (CPCSEA). The synthesis of the target compounds was accomplished according to the reaction sequence illustrated in Scheme 1.

### Synthesis of 5-phenyl-1,3,4-thiadiazol-2-amine (1)

Benzoic acid (0.1 mol) and thiosemicarbazide (0.1 mol) in phosphorous oxychloride (30 ml) were refluxed gently for 30 min and cooled followed by careful addition of water (90 ml). The separated solid was filtered and suspended in water and basified with aqueous potassium hydroxide followed by filtration, drying and crystallization from mixture of DMF and ethanol.

**General procedure for the preparation of N-((2-substituted indol-3-yl)methylene)-5-phenyl-1,3,4-thiadiazol-2-amine (2-6)**

To a solution of 5-phenyl-1,3,4-thiadiazol-2-amine (1) (0.1 mol) in methanol (50 ml), substituted indolaldehyde (0.1 mol) was added in presence of glacial acetic acid (2 ml). The reaction mixture was refluxed for about 10 h. The excess of solvent was distilled off at reduced pressure and the solid thus obtained was recrystallized from acetone to yield compounds 2-6.

**General procedure for the preparation of 4-(2-substituted indol-3-yl)-1-(5-phenyl-1,3,4-thiadiazol-2-yl)azetidin-2-one (7-11)**

In DMF (50 ml) solution of compounds 2-6 (0.1 mol), triethylamine (0.2 mol) and acetyl chloride (0.2 mol) were added dropwise at 0-5$^0$C. The reaction mixture was stirred for about 5-8 h. The completion of the reaction was checked by TLC. The precipitated amino hydrochloride filtered off. The filtrate was concentrated under reduced pressure and poured into cold ice water. The product so obtained was recrystallized from methanol to yield compounds 7-11.

**General procedure for the preparation of 4-(2-substituted indol-3-yl)-1-(5-phenyl-1,3,4-thiadiazol-2-yl)-3((phenyl amino) methyl) azetidin-2-one (12-16)**

To a mixture of compounds 7-11 (0.01 mol) in methanol (20 ml), formaladehyde (0.02 mol) and in this solution aniline was added in dropwise manner and mixture was refluxed for 4-6 h. The completion of the reaction was checked by TLC. The excess of methanol was distilled off. The obtained solid residue was washed with petroleum ether (40-60$^0$C) and recrystallized from acetone to give compounds 12-16. The physical and analytical data of all the newly synthesized compounds are given in table-1 and IR, $^1$HNMR and Mass spectral data are given in table-2.

**Pharmacological evaluation**

**Anti-inflammatory activity**

Preliminary study at all the three tested dose (25, 50, 100 mg/kg) were compared with standard drug, phenyl butazone. These compounds were administered either by oral or intraperitoneal route. Rats of either sex weighing 60-130 were divided into groups of 6 animals each. A freshly prepared suspension of carrageenin (1.0% in 0.9% saline) 0.05 ml, was injected under the planter aponeurosis of right paw of the rat by the method of Winter et al. [17] . One group was kept as control and the animals of other group were pretreated with the test drugs given orally 1 h before the carrageenin injection. The volume of foot was measured before one and 3 h after carrageenin treatment with the help of a Plethysmometer. The mean increase of paw volume in each group was measured and percentage anti-inflammatory activity was calculated according to the formula given below-

**Percentage of inhibition of oedema = $(1-V_t/V_c) \times 100$**

Where $V_t$ and $V_c$ are the volumes of oedema in drug treated and the control groups.

**Analgesic activity**

Acetic acid writhing test was performed on mice by following the method of Davis at el [18] . Test compounds were given to the animals at the dose of 50 mg/kg, 30 min later the animals were injected inter peritoneally with 0.25 ml/mouse of 0.5% acetic acid. The mean number of writhes for each experimental groups and percentage decrease compared with the control group was calculated after 60 min.

**RESULTS AND DISCUSSION**

All the newly synthesized compounds 1-16 were tested in vivo in order to evaluate their anti inflammatory and analgesic activity. These compounds were screened for their anti-inflammatory and analgesic activities at a dose of 50 mg/kg p.o. exhibited substantive anti-inflammatory activity of varying degree from 8.7-38.3 and analgesic activity of varying degree 6.8-35.5 are given in table-3. The characteristic feature of this series is substituted phenyl moiety at second position of indole nucleus. It was observed that compound 16 showed maximum anti-inflammatory 38.3% inhibition of oedema and inhibition of 35.5% of writhes. This compound showed better anti-inflammatory and analgesic activities than standard drug phenyl butazone at the three graded doses of 25,50 and 100 mg/kg p.o.

**CONCLUSION**

1. The azetidinones showed better anti-inflammatory and analgesic activity than parent compounds.
2. Furthermore the substitution with phenyl group having chloro group at 2,6 position showed better activities than other groups.

**ACKNOWLEDGEMENT**

We are thankful to SAIF Punjab University, Chandigarh India for spectral and analytical analysis of newly synthesized compounds. We are also thankful to Department of Microbiology LLRM Medical College Meerut UP for their anti-inflammatory activity.

**Table 1: Physical and analytical data of the compounds[1-16]**

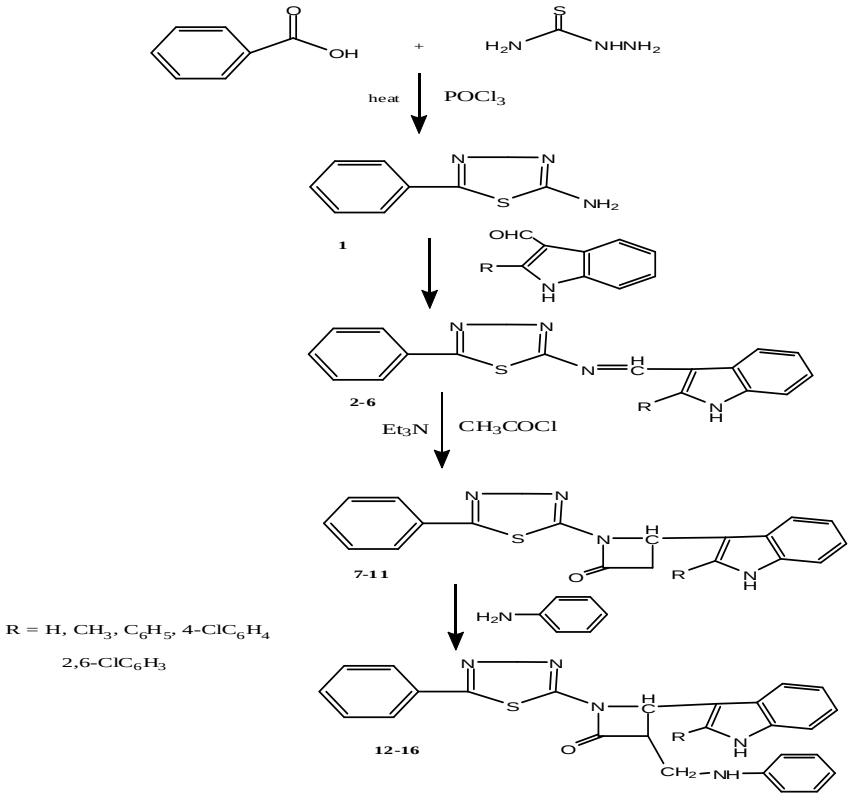
| Compounds | R | Recrystalization solvent | Yield% | M.P ($^0$c) | Mol. Formula | Analysis % found (calculated) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | C | H | N |
| 1 | - | D.M.F. | 85 | 153 | $C_8H_7N_3S$ | 54.23 (54.22 | 3.95 3.98 | 23.70 23.71) |
| 2 | H | Acetone | 82 | 161 | $C_{17}H_{12}N_4S$ | 67.04 (67.08 | 3.96 3.97 | 18.43 18.41) |
| 3 | $CH_3$ | D.M.F. | 80 | 173 | $C_{18}H_{14}N_4S$ | 67.92 (67.90 | 4.41 4.43 | 17.58 17.60) |
| 4 | $C_6H_5$ | Ethanol | 78 | 186 | $C_{23}H_{16}N_4S$ | 72.63 (72.61 | 4.26 4.24 | 14.75 14.73) |
| 5 | 4-$ClC_6H_4$ | Methanol | 79 | 198 | $C_{23}H_{15}ClN_4S$ | 66.56 (66.58 | 3.65 3.64 | 13.52 13.50) |
| 6 | 2,6-$ClC_6H_3$ | D.M.F. | 78 | 185 | $C_{23}H_{14}Cl_2N_4S$ | 61.46 (61.48 | 3.13 3.14 | 12.46 12.47) |
| 7 | H | Methanol | 76 | 214 | $C_{19}H_{14}N_4OS$ | 65.86 (65.88 | 4.04 4.07 | 16.14 16.17) |
| 8 | $CH_3$ | Ethanol | 74 | 236 | $C_{20}H_{16}N_4OS$ | 66.68 (66.65 | 3.43 4.47 | 15.55 15.54) |
| 9 | $C_6H_5$ | Acetone | 75 | 221 | $C_{25}H_{18}N_4OS$ | 71.09 (71.07 | 4.30 4.29 | 13.24 13.26) |
| 10 | 4-$ClC_6H_4$ | Methanol | 73 | 243 | $C_{25}H_{17}ClN_4OS$ | 65.74 (65.71 | 3.76 3.75 | 12.23 12.26) |
| 11 | 2,6-$ClC_6H_3$ | D.M.F. | 70 | 252 | $C_{25}H_{16}Cl_2N_4OS$ | 61.14 (61.11 | 3.26 3.28 | 11.43 11.40) |
| 12 | H | Acetone | 68 | 276 | $C_{26}H_{21}N_5OS$ | 69.18 (69.16 | 4.67 4.69 | 15.50 15.51) |
| 13 | $CH_3$ | Methanol | 63 | 262 | $C_{27}H_{23}N_5OS$ | 69.62 (69.65 | 4.97 4.98 | 15.07 15.04) |
| 14 | $C_6H_5$ | Acetone | 62 | 246 | $C_{32}H_{25}N_5OS$ | 72.87 (72.84 | 4.76 4.78 | 13.23 13.27) |
| 15 | 4-$ClC_6H_4$ | Ethanol | 60 | 266 | $C_{32}H_{24}ClN_5OS$ | 68.34 (68.38 | 4.32 4.30 | 12.47 12.46) |
| 16 | 2,6-$ClC_6H_3$ | D.M.F. | 57 | 298 | $C_{32}H_{23}Cl_2N_5OS$ | 64.45 (64.43 | 3.87 3.89 | 11.73 11.74) |

**Table 2: Spectral data of compounds[1-16]**

| Compound No. | $[M]^+$ m/z | IR (KBr) ν max in $Cm^{-1}$ | $^1$H-NMR ($CDCl_3$+$DMSOd_6$)δ in ppm |
|---|---|---|---|
| 1 | 177.23 | 3345 ($NH_2$), 3032 (C-H aromatic), 1680 (C=N), 1530 (C-C of aromatic ring), 1061 (C-S-C), 1021 (N-N) | 8.57 (s, 2H, $NH_2$ exchangeable with $D_2O$), 7.13 -8.15 (m, 5H, Ar-H) |
| 2 | 304.37 | 3322 (NH), 3038 (C-H aromatic), 1680 (C=N), 1538 (C-C of aromatic ring), 1061 (C-S-C), 1024 (N-N) | 8.87 (s, 1H, NH of indole exchangeable with $D_2O$), 8.40 (s, 1H, N=CH), 7.13 - 8.16 (m, 10H, Ar-H), |
| 3 | 318.40 | 3334 (NH), 3037 (C-H aromatic), 1680 (C=N), 1536 (C-C of aromatic ring), 1061 (C-S-C), 1029 (N-N) | 8.89 (s, 1H, NH of indole exchangeable with $D_2O$), 8.43 (s, 1H, N=CH), 7.17-8.14 (m, 9H, Ar-H), 3.52 (s, 3H, $CH_3$) |
| 4 | 380.46 | 3328 (NH), 3039 (C-H aromatic), 1680 (C=N), 1531 (C-C of aromatic ring), 1060 (C-S-C), 1027 (N-N) | 8.90 (s, 1H, NH of indole exchangeable with $D_2O$), 8.44 (s, 1H, N=CH), 7.13-8.15 (m, 14H, CH-Ar) |
| 5 | 414.91 | 3326 (NH), 3036 (C-H aromatic), 1684 (C=N), 1535 (C-C of aromatic ring), 1065 (C-S-C), 1028 (N-N), 766 (C-Cl) | 8.87 (s, 1H, NH of indole exchangeable with $D_2O$), 8.43 (s, 1H, N=CH), 7.11-8.14 (m, 13H, CH-Ar) |
| 6 | 449.36 | 3328 (NH), 3037 (C-H aromatic), 1680 (C=N), 1531 (C-C of aromatic ring), 1061 (C-S-C), 1024 (N-N), 760 (C-Cl) | 8.93 (s, 1H, NH of indole exchangeable with $D_2O$), 8.45 (s, 1H, N=CH), 7.13-8.15 (m, 12H, CH-Ar) |
| 7 | 346.41 | 3325 (NH), 3032 (C-H aromatic), 1688 (C=N), 1670 (C=O), 1532 (C-C of aromatic ring), 1063 (C-S-C), 1023 (N-N) | 8.85 (s, 1H, NH of indole exchangeable with $D_2O$), 8.44 (s, 1H, N=CH), 7.12-8.16 (m, 10H, CH-Ar), 3.85 (s, 2H, $CH_2$ of azetidinone) |
| 8 | 360.43 | 3324 (NH), 3039 (C-H aromatic), 1680 (C=N), 1675 (C=O), 1531 (C-C of aromatic ring), 1061 (C-S-C), 1025 (N-N) | 8.92 (s, 1H, NH of indole exchangeable with $D_2O$), 8.47 (s, 1H, N=CH), 7.15-8.13 (m, 7H, CH-Ar), 3.83 (s, 2H, $CH_2$ of azetidinone), 3.55 (s, 3H, $CH_3$) |
| 9 | 422.50 | 3323 (NH), 3036 (C-H aromatic), 1683 (C=N), 1678 (C=O), 1535 (C-C of aromatic ring), 1060 (C-S-C), 1024 (N-N) | 8.90 (s, 1H, NH of indole exchangeable with $D_2O$), 8.45 (s, 1H, N=CH), 7.13-8.15 (m, 14H, CH-Ar), 3.80 (s, 2H, $CH_2$ of azetidinone) |
| 10 | 456.95 | 3330 (NH), 3039 (C-H aromatic), 1688 (C=N), 1676 (C=O), 1537 (C-C of aromatic ring), 1065 (C-S-C), 1027 (N-N), 767 (C-Cl) | 8.89 (s, 1H, NH of indole exchangeable with $D_2O$), 8.48 (s, 1H, N=CH), 7.14-8.16 (m, 13H, CH-Ar), 3.82 (s, 2H, $CH_2$ of azetidinone) |
| 11 | 491.39 | 3328 (NH), 3038 (C-H aromatic), 16805(C=N), 1674 (C=O), 1533 (C-C of aromatic ring), 1064 (C-S-C), 1024 (N-N), 763 (C-Cl) | 8.92 (s, 1H, NH of indole exchangeable with $D_2O$), 8.46 (s, 1H, N=CH), 7.13-8.14 (m, 12H, CH-Ar), 3.85 (s, 2H, $CH_2$ of azetidinone) |
| 12 | 451.54 | 3323 (NH), 3037 (C-H aromatic), 1682 (C=N), 1671 (C=O), 1534 (C-C of aromatic ring), 1060 (C-S-C), 1023 (N-N) | 8.93 (s, 1H, NH of indole exchangeable with $D_2O$), 8.44 (s, 1H, N=CH), 7.14-8.16 (m, 15H, CH-Ar), 3.89 (s, 2H, $CH_2$ of azetidinone), 3.63 (s, 2H, $CH_2$) |
| 13 | 465.57 | 3329 (NH), 3031 (C-H aromatic), 1687 (C=N), 1677 (C=O), 1538 (C-C of aromatic ring), 1065 (C-S-C), 1022 (N-N) | 8.91 (s, 1H, NH of indole exchangeable with $D_2O$), 8.45 (s, 1H, N=CH), 7.12-8.15 (m, 14H, CH-Ar), 3.87 (s, 2H, $CH_2$ of azetidinone), 3.66 (s, 2H, $CH_2$), 3.35 (s, 3H, $CH_3$) |
| 14 | 527.64 | 3325 (NH), 3036 (C-H aromatic), 1689 (C=N), 1678 (C=O), 1535 (C-C of aromatic ring), 1063 (C-S-C), 1026 (N-N) | 8.87 (s, 1H, NH of indole exchangeable with $D_2O$), 8.41 (s, 1H, CH-Ar), 7.14-8.13 (m, 19H, CH-Ar), 3.86 (s, 2H, $CH_2$ of azetidinone), 3.65 (s, 2H, $CH_2$) |
| 15 | 562.08 | 3324 (NH), 3030 (C-H aromatic), 1682 (C=N), 1670 (C=O), 1530 (C-C of aromatic ring), 1060 (C-S-C), 1021 (N-N), 765 (C-Cl) | 8.89 (s, 1H, NH of indole exchangeable with $D_2O$), 8.43 (s, 1H, CH-Ar), 7.15-8.15 (m, 18H, CH-Ar), 3.88 (s, 2H, $CH_2$ of azetidinone), 3.67 (s, 2H, $CH_2$) |
| 16 | 596.53 | 3328 (NH), 3032 (C-H aromatic), 1680 (C=N), 1674 (C=O), 1531 (C-C of aromatic ring), 1061 (C-S-C), 1024 (N-N), 760 (C-Cl) | 8.88 (s, 1H, NH of indole exchangeable with $D_2O$), 8.42 (s, 1H, CH-Ar), 7.11-8.12 (m, 17H, CH-Ar), 3.85 (s, 2H, $CH_2$ of azetidinone), 3.68 (s, 2H, $CH_2$) |

**Table 3: Anti–inflammatory and analgesic activity data of compounds[1-16]**

| Compound No. | Dose (mg/kg p.o.) | Antiinflammatory activity % oedema inhibition relative to control | Analgesic activity % decrease of writhes in 60 min after treatment relative to control |
|---|---|---|---|
| 1 | 50 | 8.7 | 6.8 |
| 2 | 50 | 9.8 | 10.2 |
| 3 | 50 | 10.9 | 9.7 |
| 4 | 50 | 12.3 | 11.9 |
| 5 | 50 | 11.6 | 13.2 |
| 6 | 50 | 12.9 | 10.9 |
| 7 | 50 | 13.7 | 13.2 |
| 8 | 50 | 14.1 | 14.3 |
| 9 | 50 | 16.8 | 15.9 |
| 10 | 50 | 17.2 | 15.7 |
| 11 | 50 | 20.5 | 19.9 |
| 12 | 50 | 21.4 | 22.1 |
| 13 | 50 | 23.7 | 24.4 |
| 14 | 50 | 26.4 | 25.2 |
| 15 | 50 | 35.6 | 33.9 |
| 16 | 25<br>50<br>100 | 16.8<br>38.3<br>69.2 | 15.9<br>35.7<br>70.3 |
| Phenylbutazone | 25<br>50<br>100 | 17.6<br>36.3<br>65.6 | 18.4<br>34.1<br>68.8 |




SCHEME 1

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