INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN BIOLOGY AND MEDICINE
ISSN: 2455-944X
https://darshanpublishers.com/ijcrbm/ijcrbmindex.html
Volume 7, Issue 1 - 2022

Original Research Article

DOI: http://dx.doi.org/10.22192/ijcrbm.2022.07.01.003

Synthesis and Biological evaluation of some novel Thiadiazole derivatives

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Abstract

Heterocyclic compounds as cyclic compounds having as ring members atoms of at least two different elements, e.g. quinoline, 1,2-thiazole, bicyclo[3.3.1]tetrasiloxane. In simple terms, cyclic compounds wherein the ring incorporates carbon and other elements like oxygen, nitrogen and / or sulphur are known as heterocyclic compounds. Such compounds can contain either one ring or may be composed of two or more rings fused together. A plethora of natural and synthetic molecules that are essential for life are known to contain several of the heterocylic systems.
The objective of the present investigation was to develop newer anti-inflammatory molecules based on 1,3,4-thiadiazole scaffold. It was accomplished by cyclizing thiosemicarbazide and benzoic acid to thiadiazole nucleus and reacting with aldehydes to form Schiffs base. The synthesized compounds presented anti-antiinflammatory activity comparable to that of the standard drug.
The basis of the present investigation was a continuous thirst for development of new treatments for available pathological conditions. The objective of the present investigation was to synthesize and evaluate the anti-inflammatory potential of some newer 1,3,4-thiadiazole compounds.

Keywords: 1,3,4-thiadiazole, anti-inflammatory, Heterocyclic, cyclic compounds, analgesic activities

Introduction

Heterocyclic systems are produced indigenously by plants and animals for energy and other metabolic activities. Thiadiazole is a heterocyclic compound containing two nitrogen atom and one sulfur atom. It is a five membered ring. The 1, 3, 4-Thiadiazole was for the first time described by Fischer in 1882 but the true nature of the ring system was explained and demonstrated in 1890 by Freund and Kuh. They occur in nature in four isomeric forms as (a) 1,2,3-thiadiazole; (b) 1,2,5-thiadiazole; (c) 1,2,4-thiadiazole and (d) 1,3,4-thiadiazole.

The importance of 1,3,4-thiadiazole nucleus in antimicrobial, anti-inflammatory and analgesic activities, led us to envision the synthesis of newer compounds with anti-inflammatory activity.

Materials and Methods

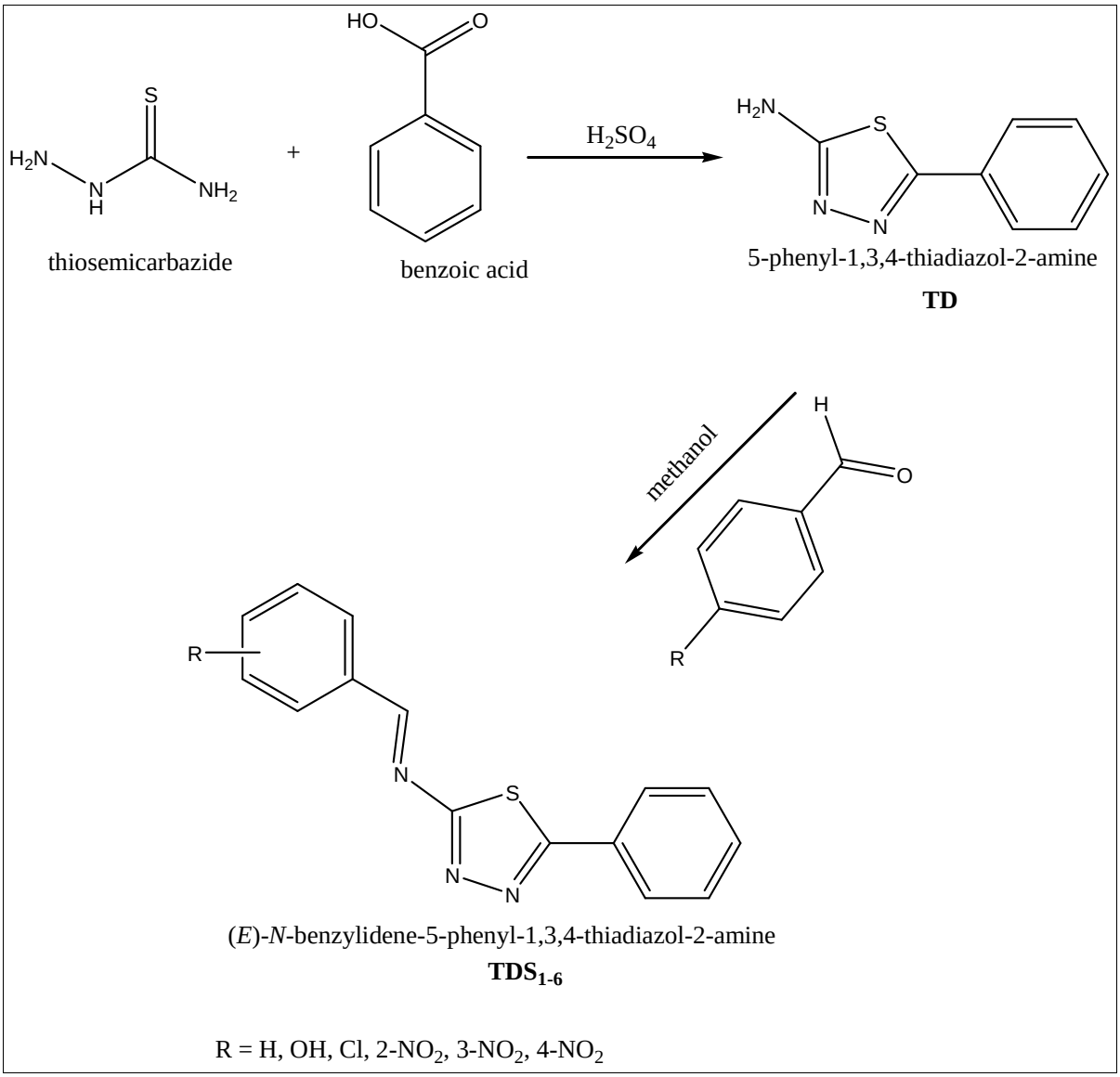
In the present investigation a few novel 1,3,4-thiadiazole derivatives would be synthesized and their potential to inhibit the mediators of inflammation would be studied. The current section presents the detailed information regarding the protocols used for the synthesis and characterization as well as the evaluation of inflammatory activity. All the chemical and reagents used were of analytical or laboratory grade and all the reactions were monitored for completion by thin layer chromatography (TLC) employing silica gel G as stationary phase, different solvent systems as mobile phase and iodine vapors as detecting agent. Melting points of the compounds were determined in open capillary tube by Melting Point Apparatus and are reported uncorrected.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker NMR instrument using tetra-methyl silane (TMS) as the internal standard. Infrared Spectra were recorded by Bruker FT-IR spectrophotometer. The absorbance (optical density) of the biological activity mixtures was recorded using Labtronics, LT-2201 UV-Visible spectrophotometer.

Synthesis of 5-phenyl-1,3,4-thiadiazol-2-amine.

Synthesis of 1,3,4-thiadiazoles

The synthesis of the thiadiazole derivatives were carried out by modifying the procedure reported by Jatav et al[8] and Deep et al[37]. The scheme of synthesis is reported in Figure 4.1. It involves a two step procedure wherein in the first step the thiadiazole nucleus is synthesized and in the second step Schiffs base compounds are prepared.



The synthesis of 2-amino-5-phenyl-1,3,4-thiadiazole was carried out by modification of the reported method[8]. Equimolar quantity of benzoic acid (0.1 mol) and thiosemicarbazide (0.1 mol) were taken in 30mL of concentrated sulphuric acid and heated at temperature maintained at 80-90°C on a water bath for 7-8 h. The reaction mixture was cooled, poured on to crushed ice and neutralized with ammonia. The crude product that precipitated out was filtered, washed with distilled water, dried and recrystalised from hot water. The completion of reaction was monitored by TLC using Chloroform: Benzene: Glacial acetic acid (3: 1: 1) as the solvent system.

Synthesis of N-(substituted benzylidene)- 5-phenyl-1,3,4-thiadiazol-2-amine.

N-(substituted benzylidene)- 5-phenyl-1,3,4-thiadiazol-2-amine compounds were synthesized according to the method reported by Deep et al[37]. Equimolar quantity of 2-amino-5-phenyl-1,3,4-thiadiazole (0.02 mol) and aromatic aldehydes (0.02 mol) were taken in a round bottomed flask. To it 30 mL of methanol was added and the mixture was heated at 60-70°C on water bath for 4 h. A few drops of glacial acetic acid were added to catalyze the reaction. The completion of the reaction was monitored by TLC using Chloroform: Benzene: Glacial acetic acid (3: 1: 1) as the solvent system. The excess of methanol was removed under reduced pressure using rotary vacuum evaporator and the crude product obtained was recrystallized from methanol.

Evaluation of *in vitro* anti inflammatory activity

Though a number of methods are available for *in vitro* evaluation of anti inflammatory activity, only two methods were considered for the present work.

Inhibition of albumin denaturation

Preparation of Phosphate Buffer Saline (PBS)

A solution of PBS was prepared by dissolving an accurately weighed quantity of 8 g NaCl, 0.2 g KCl, 1.44 g disodium hydrogen phosphate and 0.24 g potassium dihydrogen phosphate in deionized water to produce 1 L of solution. The technique of inhibition of albumin denaturation reported previously was used with slight modifications. The volume of each component of the reaction mixture was reduced to half its volume. The synthesized molecules were individually dissolved in DMSO and appropriately diluted to prepare solutions of 100, 200, 300, 400 and 500 µg/mL concentration. A solution of 1% BSA in deionized water was prepared for the test. The reaction vessel was filled with 200 µL of BSA, 1400 µL of PBS and 1000 µL of the test solutions. Ibuprofen solution (1 µg/mL) was used in the positive control and distilled water was used in the negative control vessels instead of test solution. The reaction mixtures were incubated at 37°C for 15 min and then heated at 70°C for 5 min. The mixtures were then allowed to cool to room temperature and the absorbance of constituent of each vessel were analyzed in UV-Visible spectrophotometer at 660 nm. The inhibition of percent denaturation of albumin was determined using the following formula:

$$\% \text{ Denaturation inhibition} = (1-D/C) \times 100\%$$

Where D is the absorbance reading of the test sample, and C is the absorbance reading without test sample (negative control).

Antiprotease action

Preparation of Tris-HCl buffer

An accurately weighed quantity of 121.44 g of Tris was dissolved in 800 mL of distilled water. The pH of the solution was adjusted to 7.0 by addition of appropriate volume of concentrated HCl and the final volume of the solution was made up to 1 L with distilled water. The technique of antiprotease action reported by Oyedepo *et al* and Sakat *et al* was used with slight modifications. The reaction mixture was prepared with 0.06 mg trypsin, 1 mL 20 mM Tris-HCl buffer (pH 7.0) and 1 mL test sample of different concentrations (100 - 500 µg/mL). The mixture was incubated at 37°C for 5 min followed by the addition of 1 mL of 0.8% w/v solution of casein in water. The mixture was incubated additionally for 20 min. In order to stop the reaction, 2 mL of 70% perchloric acid was added to the mixture. The turbid suspension obtained after the reaction was centrifuged and the absorbance of the supernatant was recorded at 210 nm against buffer as blank. The percentage inhibition of protease inhibitory activity was calculated by the following formula:

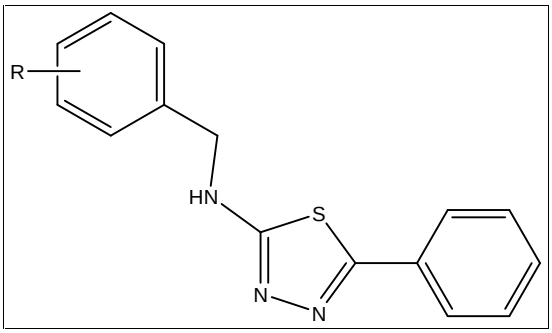
Percentage inhibition =

(Abs control –Abs sample) x 100/ Abs control

Results and Discussion

The synthesis was carried out by utilizing the optimized scheme. Six derivatives of 1,3,4-thiadiazole were prepared and characterized by using TLC, IR, NMR, mass and result of the yield, melting point and solubility of the synthesized compounds are presented in the following sections.

Physicochemical data of TD and TDS_{1-6}



Compound	R	Molecular Formula	Rf value	Yield (%)	Melting Point (°C)
TD	-	$C_8H_7N_3S$	0.62	79.4	246-249
TDS_1	H	$C_{15}H_{11}N_3S$	0.54	67.3	218-222
TDS_2	OH	$C_{15}H_{11}N_3OS$	0.58	69.1	153-156
TDS_3	Cl	$C_{15}H_{10}ClN_3S$	0.71	72.6	170-174
TDS_4	2-NO_2	$C_{15}H_{10}N_4O_2S$	0.69	71.8	192-195
TDS_5	3-NO_2	$C_{15}H_{10}N_4O_2S$	0.74	68.7	191-194
TDS_6	4-NO_2	$C_{15}H_{10}N_4O_2S$	0.77	70.2	192-194

Structural Confirmation of Synthesized Compounds

The structural confirmation of the synthesized compounds was done by interpretation of the IT, Mass and 1H NMR spectra of the compounds. The IR spectrum is an indicator of the relevant functional groups present in the compounds, while the mass spectrum is a representation of the molecular mass of the compounds. The proton NMR spectrum indicates the number and position of the hydrogen atoms present in the molecule.

The anti-inflammatory action of the synthesized compounds was evaluated using two of the well established *in vitro* methods viz., antiprotease activity and inhibition of albumin denaturation. The results are presented in table 5.3 and 5.4 respectively.

Inhibition of albumin denaturation by test compounds

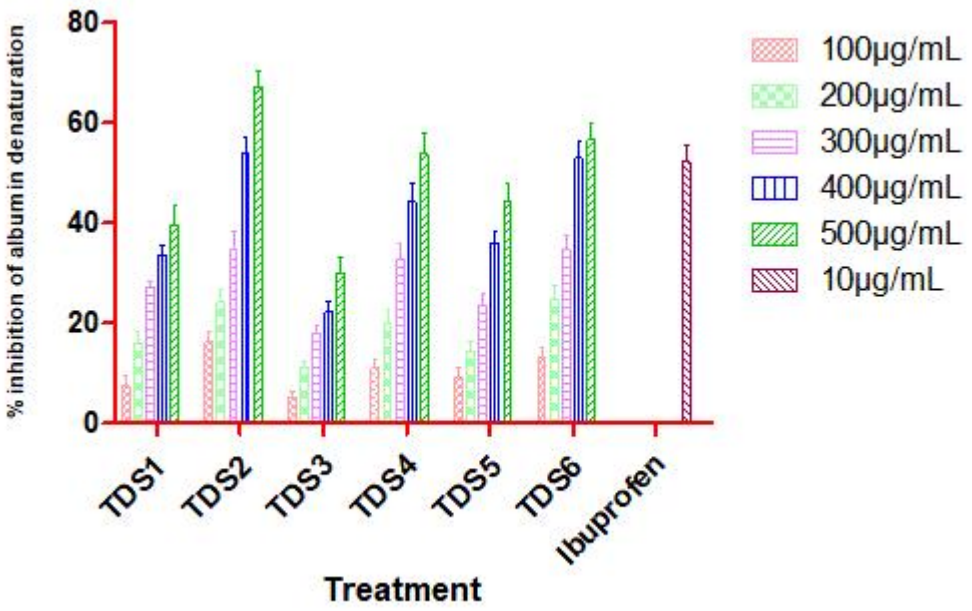
Treatment	Inhibition of albumin denaturation (%)					
	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL	10 µg/mL
TDS1	7.5±2.291	16.1±2.156	27.3±1.194	33.5±2.167	39.6±3.869	ND
TDS2	16.36±2.165	24.35±2.314	34.64±3.821	53.83±3.292	67.36±2.998	ND
TDS3	5.29±1.163	11.24±1.196	18.06±1.695	22.37±2.068	30.06±3.162	ND
TDS4	11.01±1.659	20.18±2.617	32.86±3.009	44.29±3.616	53.93±4.209	ND
TDS5	9.39±2.002	14.37±2.099	23.63±2.228	36.17±2.069	44.36±3.754	ND
TDS6	13.37±1.657	24.97±2.623	34.69±3.034	53.05±3.165	56.64±3.194	ND
Ibuprofen	ND	ND	ND	ND	ND	52.28±3.261

ND-Not Determined; n=5; Values are Mean±SD

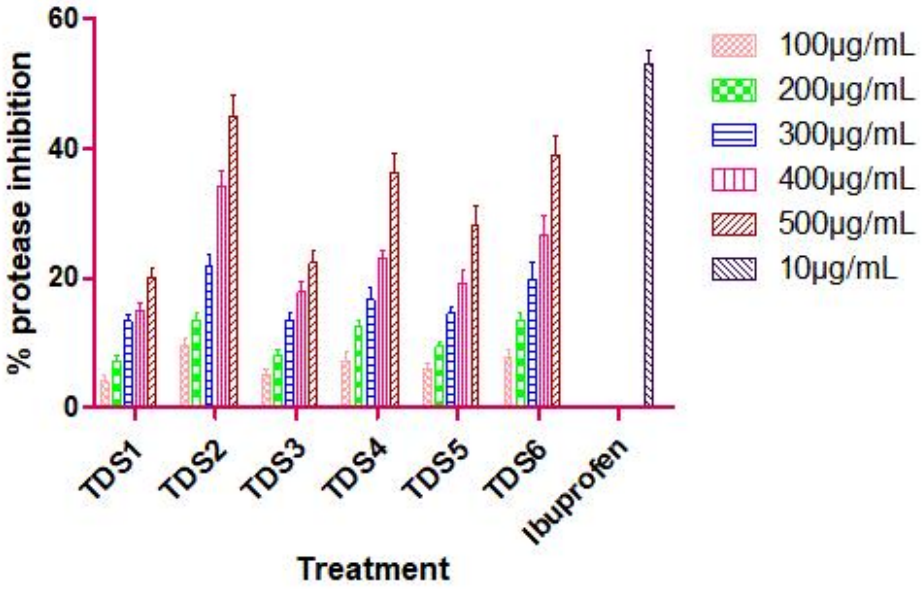
Percent inhibition of protease action by test compounds

Treatment	Inhibition of Protease Action (%)					
	10 µg/mL	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL
Ibuprofen	53.17±2.146	ND	ND	ND	ND	ND
TDS1	ND	4.12±0.899	7.21±1.033	13.43±1.066	15.08±1.066	20.18±1.523
TDS2	ND	9.63±1.033	13.64±1.168	21.82±2.011	34.22±2.333	45.03±3.211
TDS3	ND	5.18±0.911	8.14±0.833	13.57±1.011	17.91±1.633	22.35±2.033
TDS4	ND	7.29±1.333	12.46±0.933	16.66±2.066	23.11±1.333	36.22±3.011
TDS5	ND	6.04±0.933	9.47±0.666	14.55±1.033	19.28±2.011	28.24±2.988
TDS6	ND	7.95±1.011	13.50±1.066	19.87±2.666	26.77±3.033	38.96±3.033

ND-Not Determined; n=5; Values are Mean±SD



Comparison of albumin denaturation inhibitory action



Comparison of protease inhibitory action

The thiadiazoles represent a class of heterocyclic compounds with vivid variety of pharmacological potential. The synthesis of the 1,3,4-thiadiazole derivatives was accomplished in two steps with synthesizing the thiadiazole nucleus as the first step and formation of Schiffs base as the final step. The physicochemical characteristics were evaluated and the spectral characters of the compounds were studied. The formation of 1,3,4-thiadiazole from thiosemicarbazide iniates with a nucleophilic attack of the nitrogen electron pair of thiosemicarbazide to the carboxylic acid sp^2 carbon, followed of dehydration of the intermediary. The sulfur atom electron pair attacks the carbonyl causing cyclization and the intermediary formed is then dehydrated. Finally, an electron migration produces the aromatic heterocycle.

The Schiffs base formation occurs via nucleophilic attack of the amine nitrogen to the electrophilic carbonyl carbon of the aldehyde. This leaves the nitrogen deprotonated, with the NH electrons pushing the away the oxygen atom from the carbonyl group leading to the formation of a imine bond (C=N). A molecule of water is being displaced during this process.

All the compounds exhibited peaks of N-N stretching (1000-1100 cm^{-1}), C-S-C stretching (600-700 cm^{-1}), aromatic C-H stretching (3000-3200 cm^{-1}). The vibrations of O-H stretching (3300-3500 cm^{-1}), C-O stretching (1670-1340 cm^{-1}), C-N stretching (1215-1260 cm^{-1}) and C-Cl (820-850 cm^{-1}) stretching were also found in the corresponding compounds.

The ^{1}HNMR spectra of the compounds presented peaks at 6.6-7.9 corresponding to aromatic CH and 8.1-8.2 corresponding to the imine hydrogen (N=CH) in all the compounds. Peak of OH was obtained at 5.1 in TDS_2. The mass spectra of the compounds were found to contain peaks due to fragmentation of the molecules. All the compounds presented a distinct and prominent molecular ion peak along with isotopic peaks.

Protein denaturation has been significantly correlated with the occurrence of the inflammatory response and may lead to various inflammatory diseases including arthritis. It has been said that tissue injury might be due to denaturation of the protein constituents of cells or of intercellular substance. Hence, the ability of the test compounds to inhibit the denaturation of protein signifies obvious potential for anti-inflammatory activity. It has also been reported that leukocytes protease have an important role in the development of tissue damage during inflammatory reactions and significant level of protection could be provided by protease inhibitors. Hence the inhibition of protease action by test compounds signifies its role as anti-inflammatory molecules.

All the compounds exhibited dose dependent inhibition of albumin denaturation with TDS_2 having the highest capacity to cause the inhibition (67.36±2.998 %) at the concentration of 500µg/mL. The antiprotease action was also dose dependent and TDS2 at 500µg/mL was able to inhibit (45.03±3.211 %) of protease activity. The position of the nitro group on the benzylidine ring was found to affect the anti-inflammatory action. A meta substituted nitro group decreased the antiprotease as well as inhibition of albumin denaturation action whereas the ortho and para substituted nitro group had better action with para substitution exhibiting the highest of the three.

The synthesized compounds were characterized for the physicochemical properties such as melting point, colour and solubility. All the compounds were dark brown in colour and were obtained in 67-80% yields using the optimized reaction conditions. The compounds were insoluble in water, methanol and hexane while soluble in chloroform and ethanol. All the compounds exhibited peaks of N-N stretching (1015-1045 cm^{-1}), C-S-C stretching (640-655 cm^{-1}), aromatic C-H stretching (3026-3099 cm^{-1}). The vibrations of O-H stretching (3300-3400 cm^{-1}), C-O stretching (1670-1340 cm^{-1}), C-N stretching (1215-1260 cm^{-1}) and C-Cl (820-850 cm^{-1}) stretching were also found in the corresponding compounds. The ^{1}HNMR spectra of the compounds presented peaks at 6.6-7.9 corresponding to aromatic CH and 8.1-8.2 corresponding to the imine hydrogen (N=CH) in all the compounds. Peak of OH was obtained at 5.1 in TDS_2.

All the compounds presented a distinct and prominent molecular ion peak along with isotopic peaks. The compounds were evaluated for *in vitro* anti-inflammatory potential using albumin denaturation and inhibition of protease action methods. All the compounds exhibited dose dependent inhibition of albumin denaturation with TDS_2 having the highest capacity to cause the inhibition (67.36±2.998 %) at the concentration of 500µg/mL. The antiprotease action was also dose dependent and TDS2 at 500µg/mL was able to inhibit (45.03±3.211 %) of protease activity.

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How to cite this article:
Gopal Krishna Sharma, Deepak Kumar Basedia, B. K. Dubey. (2022). Synthesis and Biological evaluation of some novel Thiadiazole derivatives. Int. J. Curr. Res. Biol. Med. 7(1): 17-24.
DOI: http://dx.doi.org/10.22192/ijcrbm.2022.07.01.003