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Evaluation of Antioxidant Activity of [1,2,4]Triazolo[3,4-b][1,3,4]Thiadiazole Derivatives from 2,4-Dichlorophenyl Acetic Acid.

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ABSTRACT

In the present study a series of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole derivatives were synthesised from 2,4-dichlorophenyl acetic acid. Structures of synthesized derivatives were characterized and confirmed by IR and NMR. The synthesized compounds were screened for *in-vitro* antioxidant activity by DPPH method. The results of this investigation revealed that the synthesized compounds **5a, 5b, 5d** and **5f** showed excellent antioxidant activity compared to standard.

Keywords: Triazole, Thiadiazole, DPPH, Antioxidant activity, 2,4-Dichlorophenyl acetic acid.

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INTRODUCTION

Oxidation is a chemical reaction characterized by transfer of an electron from electron rich to electron deficient entity. Phenolic antioxidants inhibit or prevent oxidative stress in biological systems. Free radicals are one of the main causes of many pathological conditions such as those that cause several degenerative [1] and chronic diseases [2].

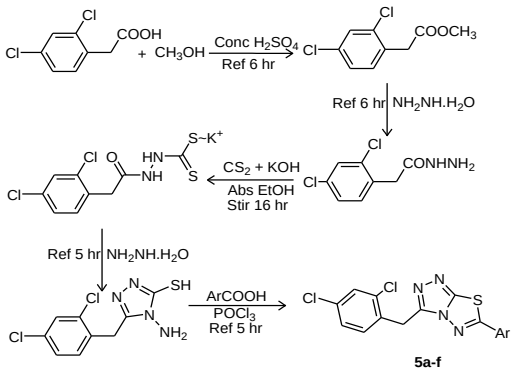
In chemical synthesis five membered heterocyclic moieties are important targets because of their prominent biological activities. In the last few decades, the chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable interest toward their synthetic and effective biological importance. 1,2,4-Triazole moieties have been integrated into a wide range of therapeutically interesting drug candidates including antimicrobial, anti-inflammatories, CNS stimulants, sedatives, antianxiety agents [3-5] and and antimycotics such as voriconazole, fluconazole, intraconazole[6-7]. There are marketed drugs containing the 1,2,4-triazole group viz. Triazolam, Etizolam, Alprazolam and Furacylin[8].

1,3,4-thiadiazole nucleus gives antidepressant, anticancer, and antimicrobial activity[9-11]. Likewise attached triazolo-thiadiazole nucleus is associated with a broad spectrum of biological activities and possess N-C=S pharmacophoric moiety [12]. Thus, the design and synthesis of triazol-thiadiazole derivatives are the future direction of medicinal chemistry for the researchers working in this field.

Herein, we describe the synthesis and antioxidant activity of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole derivatives from 2,4-dichlorophenyl acetic acid. All the synthesized compounds have been supported by their spectral data and screened for their antioxidant activity.

MATERIAL AND METHOD

Chemicals employed for the synthetic work were purchased from Central Drug House (CDH) Ltd, Merck India and Sigma Aldrich as LR grade. Melting points were determined in open capillary tubes on a digital auto melting point apparatus. Reaction progress was monitored by thin layer chromatography on silica gel-G coated TLC plates and spots detected under UV light and iodine chamber. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker Avance II 400 NMR spectrometer using deuterated $CDCl_3$ as solvent and are expressed in parts per million (δ, ppm) downfield from tetramethylsiliane (TMS) as an internal standard. IR spectra of synthesised compounds were recorded on Perkin Elimer IR 4000-400 (V_{max} in cm^{-1}) Spectrophotometer by using KBr pellets method.



Scheme:1 Synthesis of [1,2,4]triazolo[3,4,b][1,3,4]thiadiazole derivatives form 2,4-dichlorophenyl acetic acid

Table 1: Physicochemical properties of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole derivatives (5a-f)

Compound	Ar	Molecular formula	% Yield	M.p. (°C)	R_f
5a	3,4-$CH_3OC_6H_3$	$C_{18}H_{14}Cl_2N_4O_2S$	89.0	123	0.67
5b	4-$NO_2C_6H_4$	$C_{16}H_9Cl_2N_5O_2S$	96.0	118°C	0.72
5c	4-$CH_3OC_6H_4$	$C_{17}H_{12}Cl_2N_4OS$	87.9	143°C	0.76
5d	2,4- $CH_3OC_6H_3$	$C_{18}H_{14}Cl_2N_4O_2S$	70.5	140	0.66
5e	4-$C_2H_5OC_6H_4$	$C_{18}H_{14}Cl_2N_4OS$	69.2	138	0.61
5f	2-HOC_6H_4	$C_{16}H_{10}Cl_2N_4OS$	65.0	136	0.50

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EXPERIMENTAL

Synthesis of ester (1)

2,4-Dichlorophenyl acetic acid (0.1 Mol) was taken in 32 ml of methanol and 1.1 ml of H_2SO_4 was refluxed for 5-6 h. The completion of the reaction was checked on silica gel G coated TLC plates using chloroform: methanol (9:1) as an eluent and observed under ultraviolet light. The solution was cooled and product obtained was collected by vacuum distillation and recrystallized from methanol.

Synthesis of acid hydrazide (2)

A mixture of ester **1** (0.1 mol) and hydrazine hydrate 99% (0.11 mol) in 50 ml methanol was heated under reflux for 5-6 h. The completion of the reaction was monitored on silica gel G TLC coated plates using ethyl acetate and petroleum ether (1:1) as an eluent and observed under ultraviolet lamp. The reaction mixture was left overnight and solid obtained was collected by flask evaporator and recrystallized from methanol [13].

Synthesis of potassium dithiocarbazinate (3)

A solution of 50 ml of alcoholic potassium hydroxide (0.03 mol) was cooled in an ice bath and compound **2** (0.016 mol) was added with stirring. Then, carbon disulphide (0.025 mol) was added in small portions under constant stirring. The reaction mixture was agitated continuously for 16 h at room temperature. The precipitated potassium dithiocarbazinate was filtered, washed with ethanol, dried and directly used for the next step without further purification [14].

Synthesis of 4-amino-5-(2,4-dichlorobenzyl)-4H-1,2,4-triazole-3-thiol (4)

The above potassium dithiocarbazinate **3** was mixed with water (8 ml) and hydrazine hydrate 99% (0.02mol) and refluxed for 4-5 h. During the progress, homogeneous reaction mixture which turned green with evolution of hydrogen sulphide gas was obtained. The reaction product was cooled to room temperature and diluted with water and precipites were obtained by addition of dilute acetic acid. The purity was checked by TLC using silica gel G plate with toluene: ethyl acetate: formic acid (5:4:1) as solvent system and observed under ultraviolet lamp and iodine chamber [14].

Synthesis of 3-(2,4-dichlorobenzyl)-6-aryl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5)

An equimolar mixture of compound **4** and appropriate aromatic acids **(5a-f)** in 10 ml phosphorus oxychloride ($POCl_3$) was refluxed for 5 h. The reaction mixture was cooled to room temperature and poured onto crushed ice with stirring. Finally, to remove the excess of phosphorus oxychloride powdered potassium carbonate and the required amount of potassium hydroxide solution were added till the pH of the mixture was raised to 8. The solid was collected by vacuum distillation, dried and recrystallized from methanol [15].

Spectral Analysis

3-(2,4-dichlorobenzyl)-6-(3,4-dimethoxyphenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (5a):

IR (KBr), ν cm^{-1}: 2928 (C-H str, Ar), 1606 (C=N str), 1509 (C-N str) 1260 (N-N=C str), 773 (C-Cl str), 697 (C-S-C str), 1030 (-OCH_3). ^{1}HNMR ($CDCl_3$) δ (ppm): 7.6 (m, 3H, Ar-H), 6.8 (m, 3H, Ar-H), 4.3 (s, 2H, CH_2), 4.0-4.1 (s, 3H, CH_3), 3.8 (s, 3H, CH_3).

3-(2,4-dichlorobenzyl)-6-(4-nitrophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (5b):

IR (KBr), ν cm^{-1}: 2928 (C-H str, Ar), 1600 (C=N str), 1512, 1351 (-NO_2 str), 1502 (C-N str) 1234 (N-N=C str), 760 (C-Cl str), 668 (C-S-C str). ^{1}HNMR ($CDCl_3$) δ (ppm): 7.5 (m, 5H, Ar-H), 7.2 (m, 3H, Ar-H), 4.0 (s, 2H, CH_2).

3-(2,4-dichlorobenzyl)-6-(4-methoxyphenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (5c):

IR (KBr), ν cm^{-1}: 2926 (C-H str, Ar), 1605 (C=N str), 1520 (C-N str) 1237 (N-N=C str), 769 (C-Cl str), 685 (C-S-C str), 1030 (-OCH_3). ^{1}HNMR ($CDCl_3$) δ (ppm): 7.6 (m, 5H, Ar-H), 6.9 (m, 3H, Ar-H), 4.0 (s, 2H, CH_2), 3.8 (s, 3H, CH_3).

3-(2,4-dichlorobenzyl)-6-(2,4-dimethoxyphenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5d):

IR (KBr), ν cm^{-1}: 2930 (C-H str, Ar), 1600 (C=N str), 1534 (C-N str) 1233 (N-N=C str), 1030 (-OCH_3), 753 (C-Cl str), 689 (C-S-C str). ^{1}HNMR ($CDCl_3$) δ (ppm): 7.2 (m, 3H, Ar-H), 6.9 (m, 3H, Ar-H), 4.4 (s, 2H, CH_2), 4.1 (s, 3H, CH_3), 3.3 (s, 3H, CH_3).

3-(2,4-dichlorobenzyl)-6-(4-ethoxyphenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5e):

IR (KBr), ν cm^{-1}: 2909 (C-H str, Ar), 1611 (C=N str), 1517 (C-N str) 1236 (N-N=C str), 763 (C-Cl str), 691 (C-S-C str), 1040 (-OC_2H_5). ^{1}HNMR ($CDCl_3$) δ (ppm): 7.1 (m, 5H, Ar-H), 6.6 (m, 3H, Ar-H), 4.1 (s, 2H, CH_2), 3.9 (s, 2H, CH_2), 2.9 (s, 3H, CH_3).

2-[3-(2,4-dichlorobenzyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl]phenol (5f):

IR (KBr), ν cm^{-1}: 3360 (OH, Ar-OH), 2930 (C-H str, Ar), 1601 (C=N str), 1516 (C-N str) 1229 (N-N=C str), 760 (C-Cl str), 695 (C-S-C str), ^{1}HNMR ($CDCl_3$) δ (ppm): 7.4 (m, 5H, Ar-H), 7.1 (m, 3H, Ar-H), 5.2 (s, 1H, OH), 4.4 (s, 2H, CH_2).

Antioxidant Activity

Antioxidant activity of synthesized [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole derivatives (**5a-f**) were carried out by DPPH method using ascorbic acid as reference standard and 3 µg/ml concentration of DPPH in methanol were used. The dilutions of synthesized compounds (100, 200, 300, 400 and 500µg/ml) and standard drug was made in volumetric flask by using methanol. Methanol (1 ml) with DPPH solution (1 ml) was used as blank. DPPH (1 ml) solution was mixed with 1 ml of sample solution and standard solution separately. These mixed solutions were kept in dark for 30 min and then optical density was measured at 517 nm. The percentage inhibition was calculated using the formula given below:

$$\text{Percent inhibition of DPPH activity} = \frac{A - B}{A}$$

Table 2: Antioxidant activity of [1,2,4]triazolo[3,4-b][1,3,4] thiadiazole derivatives.

Compound	Percentage Inhibition				
	100µg/ml	200µg/ml	300µg/ml	400µg/ml	500µg/ml
5a	86.14	86.82	86.45	87.16	85.81
5b	88.17	89.52	89.18	89.86	89.79
5c	29.25	25.67	27.02	31.41	22.29
5d	87.11	87.25	88.90	89.11	88.90
5e	45.21	45.35	45.80	46.09	46.55
5f	88.29	89.44	89.60	89.83	89.80
Ascorbic acid	86.82	87.53	88.17	88.10	89.52
Blank	0.296	0.296	0.296	0.296	0.296

RESULTS AND DISCUSSION

The structures of the synthesized compounds (**5a-f**) were ascertained on the basis of their consistent IR and NMR spectral characteristics. The antioxidant activity of the synthesized compounds and the standard ascorbic acid was assessed on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical activity. DPPH assay was carried out for compounds 5a-f at different concentrations (100 to 500 µl/ml). The percentage inhibition was measured for all the synthesized compounds. From the results showed in **Table 2** it was found that **5a, 5b, 5d** and **5f** showed excellent antioxidant activity.

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CONCLUSION

Synthesized compounds were screened for *in-vitro* antioxidant assay and possess significant activity. The results revealed that the test compound is electron donor and could react with free radical chain reaction and can be selected for further studies.

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