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Synthesis, Antimicrobial and Antioxidant Evaluation of Novel 5, 6-dihydro-3-(substituted phenyl)[1,3,4]thiadiazine-7-one Derivatives

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Abstract

A series of 1, 2, 4-triazole derivatives (1-17, Scheme 1) was synthesized and evaluated for their antimicrobial and antioxidant potential. Results of antimicrobial screening of the synthesized compounds indicated that most of the synthesized compounds showed good activity as compare to the norfloxacin as antibacterial and fluconazole as antifungal standard drugs. Compounds 7 and 12 were found to be most effective against various strains used for the antimicrobial activity like *S. aureus, B. subtilis*, *E. coli*, *Candida albicans* and *A. niger* using tube dilution method having MIC 3.12 µg/ml for *S. aureus* and 06.25 µg/ml for rest strains. All the synthesized compounds also evaluated for antioxidant activity by DPPH method and results revealed that the compounds 8 and 16 showed antioxidant activity comparable to the positive control ascorbic acid.

Keywords: Triazoles derivatives; Anti-microbial; Anti-oxidant

Introduction

The incidence of systemic microbial infections has been increased severely due to increased of immune compromised hosts. Also, the increasing predominance of microbial resistance to huge antibiotic is becoming foremost apprehension. Consequently, the expensive treatment, toxicities and drug resistance pose new conundrum insisting constant renewed efforts in the development of new classes of antimicrobial with more specific action [1]. 1,2,4-Triazoles have attracted considerable attention in the fields of medicine and agrochemical research as well as in materials science, due to their unique structures and properties. 1,2,4-Triazole and its derivatives belong to a class of exceptionally active compounds possessing many pharmacological properties.1,2,4-Triazole and its derivatives are an important class of compounds which possess diverse agricultural, industrial and biological activities including anti-microbial Palekar et al. [2], sedative anticonvulsant Li et al. [3], anticancer Hou et al. [4], anti-inflammatory Ayse et al. [5], antitubercular Jadhav et al. [6]. In recent years, the synthesis of these heterocyclic compounds has received considerable attention. This wide range of applications has been covered by more than sixty papers in the literature, many in the form of patents.

Experimental

Starting material was obtained from commercial sources and were used without further purification. Reaction progress was observed by thin layer chromatography. Melting points were determined in open capillary automated melting point apparatus and are uncorrected. ^{1}H NMR spectra were determined by Bruker Avance II 400 MHz NMR spectrometer using DMSO-d6 solvent and are expressed in parts per million (δ, ppm). NMR data are given as multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) and number of protons. IR spectra were recorded on Brucker 12060280, software OPUS 7.2.139.1294 ATR spectrophotometer in a KBr disc. The physicochemical characteristics of synthesized compounds are presented in Table 1. Aryl acid, ethyl esters (2a-q) and their hydrazides (3a-q). These compounds were obtained from different aryl/aroyl acids (1a-q) by the method reported in the literature [7].

Synthesis of potassium dithiocarbazinate (4a-q)

Potassium hydroxide (0.03 mol) was dissolved in absolute ethanol (50 mL). The solution was cooled in an ice bath and acid hydrazide (3a-q; 0.02 mol) was added with stirring. To this, carbon disulfide (0.025 mol) was added in small portions with constant stirring. The reaction mixture was stirred continuously for 12 h at room temperature. The precipitated potassium dithiocarbazinate was collected by filtration, washed with anhydrous ether and dried in vacuum. The potassium salt thus obtained was used in the next step without further purification.

4-amino-5-substituted-3-mercapto-(4H)-1,2,4-triazoles (5a-c)

A suspension of potassium dithiocarbazinate derivatives (4a-q; 0.02 mol) and hydrazine hydrate (99%, 0.04 mol) in water (50 mL) was refluxed for 10-15 h with occasional shaking. The color of the reaction mixture changed to light green with evolution of hydrogen sulfide gas. A homogenous mixture was obtained during the reaction process. The reaction mixture was cooled to room temperature and diluted with cold water (20 mL). On acidification with dil. HCl the required triazole was precipitated as white precipitate. It was filtered, washed with cold water, dried and recrystallized from ethanol. The compound was found pure in TLC analysis using benzene as solvent system.

Synthesis of 5,6 -dihydro-3-(substituted phenyl) [1,2,4] triazolo (3,4b)[1,3,4]thiadiazine-7-one (1-17)

An equimolar mixture (0.01 mol) of 4-amino-5-substituted-3-mercapto-(4*H*)-1,2,4-triazoles (**5a-q**)and aromatic acids in chloroacetyl chloride (10 mL) was refluxed for 5 h. After completion of reaction, the reaction mixture was cooled to room temperature and then poured onto crushed ice with stirring. The mixture was allowed to stand overnight and a solid mass separated out was filtered, treated with aq. ammonia and washed with cold water. It was dried and crystallized from ethanol.

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Comp.	Mol.Formula	Mol.Wt.	Rf Value*	M.pt.	%Yield
1	CHNOS	248	0.63	180-182	70.59
2	CHNOS	246	0.56	172-174	56.29
3	CHNOS	322	0.43	156-158	62.23
4	CHNO4S	293	0.59	98-100	65.30
5	CHClNOS	266	0.34	120-122	68.45
6	CHNOS	247	0.39	136-138	56.10
7	CHNOS	277	0.51	154-156	54.20
8	CHClNOS	300	0.24	143-145	75.23
9	CHNOS	322	0.28	196-198	46.53
10	CHNOS	246	0.34	103-105	51.23
11	CHNOS	232	0.39	165-167	46.25
12	CHClNOS	300	0.42	158-160	64.25
13	CHNOS	277	0.46	104-106	61.35
14	CHClNOS	266	0.28	123-125	56.36
15	CHNO	262	0.37	174-176	59.19
16	CHNOS	246	0.49	126-128	71.06
17	CHNOS	277	0.51	135-137	67.89

* TLC mobile phase:* Benzene

Table 1: The Physicochemical properties of the synthesized compounds of 5,6 -dihydro-3-(substituted phenyl) [1,2,4] triazolo (3,4b)[1,3,4]thiadiazine-7-one.

Pharmacology

***In vitro* evaluation of antimicrobial activity:** The antimicrobial activity was performed against Gram-positive bacteria: *Staphylococcus aureus* (MTCC 2901), *Bacillus subtilis* (MTCC 2063), and Gram-negative bacterium *Escherichia coli* (MTCC 1652), and several fungal strains: *Candida albicans* (MTCC 227), and *Aspergillus niger* (MTCC 8189) using tube dilution method [8]. Dilutions of test and standard compounds were prepared in double strength nutrient broth - I.P. (bacteria) or Sabouraud dextrose broth - I.P [9]. The samples were incubated at 37°C for 24 h (bacteria), at 25°C for 7 d (*A. Niger*) and at 37°C for 48 h (*C. albicans*) and the results were recorded in terms of minimum inhibitory concentration (MIC). MIC was defined as the lowest conc. of compound that inhibited visible growth of microbes after incubation at 35°C for 24 h. for bacteria and 48 h for fungi. Synthesized compounds compared to the standard drugs norfloxacin as antibacterial and fluconazole as antifungal.

***In vitro* evaluation of antioxidant activity:** Free radical scavenging activity of synthesized compounds against stable free radical 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH), was detrmined spectrophotometrically using UV-Visible spectrophotometer. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it gets reduced. Following the reduction, its deep violet colour in methanol bleached to yellow, and shows a significant absorption decrease at 517 nm. Fifty milliliters of various concentrations (25, 50, 75 and 100) µg/ml of the compounds dissolved in methanol was added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min. incubation period at room temperature, the absorbance was read against a blank at 517 nm [10].

$$\%I = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where,

$A_{control}$=absorbance of the control reaction

A_{sample}=absorbance of the test compounds

Compounds concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted as inhibition percentage against compound concentration (Figure 1). Tests were carried out in triplicate and ascorbic acid was used as a positive control. Standard curve was plotted for different concentrations of ascorbic acid.

Results and Discussion

A series of 5,6-dihydro-3-(substituted phenyl)[1,2,4]triazolo(3,4-b) [1,3,4]thaidiazine-one (1-17) was as outlined in Scheme 1. At first, substituted aromatic acid was reacted with ethanol in the presence of sulphuric acid and the resultant product was treated with hydrazine hydrate to yield substituted hydrazide. The later on treatment with carbon disulphide and potassium hydroxide and later again with hydrazine hydrate yielded substituted triazole and later the product on treatment chloroacetyl chloride yielded title products (1-17). The structures of synthesized compounds were characterized by IR and ^{1}H NMR. In all the cases the TLC of the product showed the single spot confirming the chromatogram for only one product. The physical constants of the title compounds are given in Table 2. Spectral details of synthesis of *5,6 -dihydro-3-(substituted phenyl) [1,2,4] triazolo (3,4b) [1,3,4]thiadiazine-7-one*.

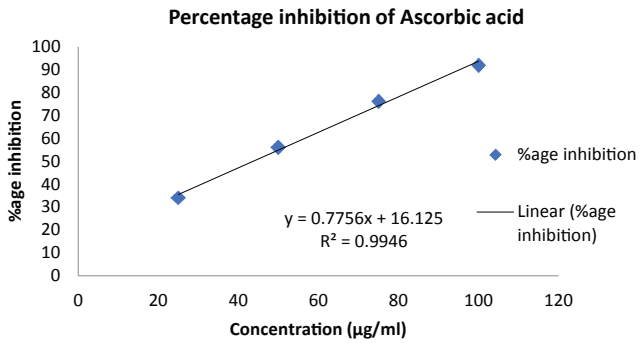
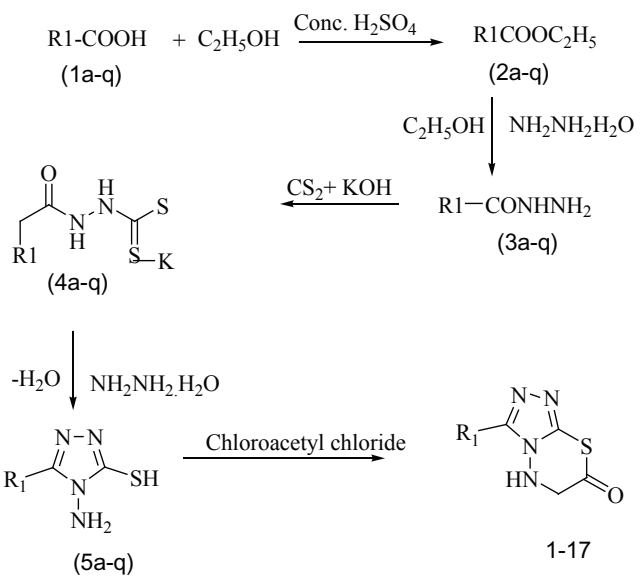


Figure 1: Result of percentage inhibition of ascorbic acid.



Scheme 1: Synthesis of 5,6 -dihydro-3-(substitutedphenyl) [1,2,4] triazolo (3,4b)[1,3,4]thiadiazine-7-one.

Comp.	Antibacterial			Antifungal	
	MIC	MIC	MIC	MIC	MIC
	12.50	06.25	06.25	12.50	12.50
	12.50	06.25	03.12	06.25	06.25
	12.50	12.50	06.25	12.50	12.50
	12.50	12.50	06.25	12.50	12.50
	25.00	12.50	12.50	12.50	12.50
	12.50	12.50	06.25	12.50	12.50
	06.25	**06.25**	**03.12**	**06.25**	**12.50**
	12.50	06.25	06.25	12.50	12.50
	12.50	12.50	06.25	12.50	12.50
	06.25	12.50	06.25	12.50	12.50
	25.00	12.50	12.50	12.50	12.50
	06.25	**06.25**	**03.12**	**06.25**	**12.50**
	12.50	12.50	12.50	12.50	12.50
	12.50	06.25	06.25	06.25	12.50
	12.50	12.50	12.50	12.50	12.50
	12.50	06.25	06.25	12.50	12.50
	25.00	12.50	12.50	12.50	25.00
Std	**01.56***	**01.56***	**01.56***	**01.56****	**01.56****

*Norfloxacin, ** Fluconazole

Table 2: Antimicrobial activity (MIC in µg/ml) of synthesized synthesis of 5,6-dihydro-3-(substituted phenyl)[1,2,4]triazolo(3,4-b)[1,3,4]thiadiazine-7-one.

Compound 1 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 2987 (C-H str., aromatic), 1707 (C=O str., carbonyl),1251 (C-O str., ester), 1660 (C=C str., aromatic ring),1567 (C-C str., aromatic in ring),3467 (O-H str.) 1063 (C-N str.),3331 (N-H str.,hydrazide). ^{1}H NMR (DMSO) δ; 7.71-7.90 (m, 4H, Ar-H), 2.187 (t,1H,NH2),1.239 (d,2H,CH2),4.14 (s,H,OH).

Compound 2 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 3000 (C-H str., aromatic),1717 (C=O str., carbonyl),1251 (C-O str., ester),1649 (C=C str., aromatic ring),1541 (C-C str., aromatic in ring), 1070 (C-N str.),3335(N-H str.).

Compound 3 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 3034 (C-H str., aromatic),1731 (C=O str., carbonyl),1257 (C-O str., ester),1681 (C=C str., aromatic ring),1571 (C-C str., aromatic in ring), 1033 (C-O str., ether), 1061(C-N str.),3407(N-H str.).

Compound 4 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 3046 (C-H str., aromatic),1730 (C=O str., carbonyl),1256 (C-O str., ester),1689 (C=C str., aromatic ring),1571 (C-C str., aromatic in ring), 1020 (C-O str., ether), 1104(C-N str.),3369(N-H str.), 3478 (OH str.).

Compound 5 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 3097 (C-H str., aromatic),1748 (C=O str., carbonyl),1680 (C=C str., aromatic ring),1571 (C-C str., aromatic in ring),1108(C-N str.),3368(N-H str.), 745 (C-Cl str. halide).

Compound 6 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 3064 (C-H str., aromatic),1730 (C=O str., carbonyl),1680 (C=C str., aromatic ring),1571 (C-C str., aromatic in ring),1125(C-N str.),3368(N-H str.), 1076(C-N str.).

Compound 7 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 3063 (C-H str., aromatic),1724 (C=O str., carbonyl),1690 (C=C str., aromatic ring),1570 (C-C str., aromatic in ring), 1101 (C-N str.),3368 (N-H str.,) 1018(C-N str.), 1506 (N-O str., nitro). ^{1}H NMR (DMSO) δ; 7.414-7.7.978(m, 4H, Ar-H), 2.188 (t,1H,NH),1.239 (d,2H,CH2).

Compound 8 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 3087 (C-H str., aromatic),1734 (C=O str., carbonyl),1662 (C=C str., aromatic ring),1567 (C-C str., aromatic in ring), 1100 (C-N str.),3365 (N-H str.,) 1007(C-N str.), 1506 (N-O str., nitro) 759 (C-Cl str.,). ^{1}H NMR (DMSO) δ; 7.001-7.991(m, 4H, Ar-H), 2.183 (t,1H,NH),1.241 (d,2H,CH2).

Compound 9 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 3042 (C-H str., aromatic),1709 (C=O str., carbonyl),1691 (C=C str., aromatic ring),1549 (C-C str., aromatic in ring),1104(C-N str.),3484(N-H str.), 1062(C-N str.), 1015 (C-O str.,ether).

Compound 10 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 3020 (C-H str., aromatic),1720 (C=O str., carbonyl),1671 (C=C str., aromatic ring),1561 (C-C str., aromatic in ring),1097(C-N str.),3457(N-H str.), 1013(C-N str.).

Compound 11 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 3067 (C-H str., aromatic),1716 (C=O str., carbonyl),1669 (C=C str., aromatic ring),1548 (C-C str., aromatic in ring), 1082 (C-N str.),3364 (N-H str.,) 1029(C-N str.). ^{1}H NMR (DMSO) δ; 7.693-7.956(m, 6H, Ar-H), 2.180 (t,1H,NH),1.237 (d,2H,CH2).

Compound 12 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 3045 (C-H str., aromatic),1728 (C=O str., carbonyl),1666 (C=C str., aromatic ring),1588 (C-C str., aromatic in ring), 1093 (C-N str.),3344 (N-H str.,), 819 (C-Cl str.,). ^{1}H NMR ppm (DMSO- d6) 7.009-7.917(m, 4H, Ar-H), 2.185 (t,1H,NH),1.245 (d,2H,CH2).

Compound 13 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 3015 (C-H str., aromatic),1730 (C=O str., carbonyl),1664 (C=C str., aromatic ring),1560 (C-C str., aromatic in ring), 1105 (C-N str.),3317 (N-H str.,) 1013(C-N str.), 1507 (N-O str., nitro).

Compound 14 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 3049 (C-H str., aromatic),1707 (C=O str., carbonyl),1683 (C=C str., aromatic ring),1561 (C-C str., aromatic in ring), 1083 (C-N str.),3370 (N-H str.,),843 (C-Cl str., halide).

Compound 15 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 3063 (C-H str., aromatic),1718 (C=O str., carbonyl),1674 (C=C str., aromatic ring),1561 (C-C str., aromatic in ring),1108(C-N str.),3493(N-H str.), 1063(C-N str.), 1014 (C-O str.,ether). ^{1}H NMR (DMSO) δ; 7.409-7.630(m, 4H, Ar-H), 2.182 (t,1H,NH),1.232 (d,2H,CH2).

Compound 16 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 3097 (C-H str., aromatic),1720 (C=O str., carbonyl),1679 (C=C str., aromatic ring),1574 (C-C str., aromatic in ring),1007(C-N str.),3483(N-H str.), 993(C-N str.). ^{1}H NMR (DMSO) δ; 7.130-7.958(m, 4H, Ar-H), 2.185(t,1H,NH),1.486 (d,2H,CH2). 0.954 (d,3H,CH3).

Comp	Conc. (µg/ml)				
	25	50	75	100	IC
	12.56	28.56	53.69	68.23	74.39
	22.26	35.42	45.26	63.95	77.58
	13.56	26.35	38.59	68.39	80.27
	13.46	35.59	53.26	76.39	68.93
	08.39	35.95	43.56	65.29	78.24
	15.65	28.25	45.62	68.65	77.06
	23.56	46.42	58.39	76.80	60.64
	30.65	**54.89**	**70.64**	**89.38**	**47.77**
	12.45	26.56	32.59	69.36	80.96
	19.25	27.64	55.28	67.52	73.03
	26.14	41.10	61.25	72.36	62.16
	06.25	25.10	41.03	69.84	79.67
	10.62	22.08	37.40	68.08	81.98
	06.72	27.28	50.92	64.27	78.49
	23.25	42.56	58.29	69.35	65.12
	32.14	**53.26**	**67.36**	**86.36**	**48.74**
	09.25	26.15	42.36	57.36	87.74
Ascorbic acid	**34.02**	**56.22**	**76.14**	**92.01**	**43.78**

Table 3: Percentage inhibition and IC value of the synthesized 5,6-dihydro-3-(substituted phenyl)[1,2,4]93,4-b)[1,3,4]thiadiazine-7-one derivatives.

Compound 17 ATR (cm^{-1})

IR v_{max} cm^{-1}(KBr): 3044 (C-H str., aromatic),1754 (C=O str., carbonyl),1674 (C=C str., aromatic ring),1565 (C-C str., aromatic in ring), 1104 (C-N str.),3313 (N-H str.,) 1017(C-N str.), 1504 (N-O str., nitro).

Antimicrobial activity

Among the synthesized compounds most of the compounds showed good activity as compared to the Norfloxacin as antibacterial and Fluconazole as antifungal standard drugs. Compound 7 and 12 were found to be most effective against various strains used for the antimicrobial activity like *S. aureus, B. subtilis*, *E. coli*, *Candida albicans* and *A. niger* using tube dilution method having MIC 3.12 µg/ml for *S. aureus* and 06.25 µg/ml for rest strains.

Antioxidant activity

The results of antioxidant activity showed that few synthesized compounds exhibit considerable antioxidant activity. Compounds 8 and 16 showed antioxidant activity comparable to the positive control ascorbic acid (Table 3).

Conclusion

In order to develop novel antimicrobial and anticancer compounds, a series of 1,2,4-triazole derivatives was synthesized and characterized by physiochemical and spectral means. The synthesized compounds were evaluated for their antimicrobial and antioxidant potentials. Compounds 7 and 12 were found to be most effective against various strains used for the antimicrobial activity like *S. aureus, B. subtilis*, *E. coli*, *Candida albicans* and *A. niger* using tube dilution method having MIC 3.12 µg/ml for *S. aureus* and 06.25 µg/ml for rest strains. Compounds 8 and 16 showed antioxidant activity comparable to the positive control ascorbic acid.

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