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Original Article

Synthesis, characterization and antimicrobial activity of 1,3,4-thiadiazole bearing 2-phenylacetominophenone derivatives

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ABSTRACT

In the present study 1,3,4-thiadiazole bearing 2-phenylacetominophenone derivatives were synthesized and antimicrobial activities of different derivatives were checked with various microbial stains. Various intermediates was synthesized and characterized in between time-to-time by chromatographic and spectral methods. Substituted benzaldehyde and sodium cyanide were the starting material and finally formed the 2-phenylacetophenone 2-(1,3,4-Thiadiazole)-2-thiol (e), while in the intermediate steps 1, 2-diphenylethan-1-one (a) reflex with sulphuric acid to get 1,2-diphenylbutane-1,3-dione (b). 1,3,4-Thiadiazole derivative have been synthesize from Schiff base of the corresponding hydrazine by using ethanol with reflex. Then carbon disulfide with potassium hydroxide to give 2-oxa-1, 2 diphenylethylidene 2-hydrazine potassium carbon disulfide (d) and this d finally treated with sulfuric acid to get the title compound. The structures of all the synthesized compound was established on the basic of elemental, chromatographic and spectral analysis. The synthesized compound was evaluated for their antimicrobial activity. The compound most active be against *Escherichia coli* and *staphylococcus aureus.*

Key Words: *Thiadiazole, Schiff base, antimicrobial, Escherichia coli and staphylococcus aureus.*

INTRODUCTION

Thiazoles, thiadiazole, indole, oxadiazole and pyrrole are some of the most important classes of heterocyclic compounds due to their in string Heterocyclic chemistry; are one of the most fascinated areas in the field of biological activities.[1] During last few decades, new infectious disease have appeared and already controlled old ones have re-emerged[2] for instance, most of the bacteria and fungi have reported to developed resistance to most of the drugs by modifying their gene sequence and pharmacokinetic differentials. Inspite of the critical need for new antimicrobial agent the development of these agents are declining[3] In this contest

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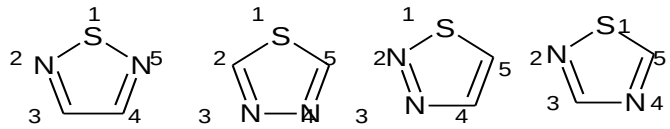
solution toward encouraging and facilitating the development of new antimicrobial agent are necessary. Therefore the synthesis of the novel antimicrobial drugs with modification from its basic structure is fundamental need, so that it can sense the growth of microbial activity.[4] Consequently, intense efforts in antimicrobial drug discovery still need to develop more promisingly, economically and effectively for their use in the clinical arena.[,5].

A literature survey has revealed that 1,3,4-thiadiazole derivatives, as an important five-member ring heterocyclic which possesses a broad spectrum of biological activity such as anticancer,[6,7,8] antioxidant,[9,10] anti-inflammatory,[11] antimicrobial,[12,13,14,15] antibacterial,[16,17] and antitubercular.[18] Surprisingly, due to the presence of (S-C=N) toxophoric units in substituted thiadiazole, they show wide range of pharmacological activities. Having relatively high aromaticity and the inductive effect of sulphur atom in 1,3,4-thiadiazole ring behave as a slightly weak base, substituted *thiadiazole* might have the optimum antimicrobial activity because of its easy metabolism by biochemical reactions and their reports shows increased lipid solubility. Thiadiazole is a five member ring system containing sulphur and nitrogen atom with two double bonds, to give an aromatic ring having molecular formula $C_2H_2N_2S$. It occurs in four isomeric form 1,2,5-thiadiazole(1), 1,3,4-thiadiazole(2), 1,2,3-thiadiazole(3), and 1,2,4-thiadiazole(4).The numbering of monocyclic azoles system begins with the heteroatom that is in the highest group in the periodic table and with the element of lowest atomic weight in that group.[19]



(1) (2) (3) (4)

EXPERIMENTAL

Synthesis of 1, 2-diphenyethan-1-one: In a 500 ml round bottom flask take 65ml of rectified spirit, 50ml of (47.5ml, 0.47mol) pure benzaldehyde & 2.5ml 1% solution of sodium cyanide (96-98%). Attach a reflex condenser and boil the mixture gently for half an hour. Cool the content of the flask in an ice bath. Filter the crude benzoin; which was white or pale yellow in color and soluble in ethanol; mp is about 180-120^{0}C, molecular weight-212.2, % yield 80%, R_f value-o.6 (Mobile phase) ethanol: benzene, IR (KBr) cm^{-1}:1241(C-O), 1650(C=O), 1550(C-H),

Synthesis of 1, 2-diphenybuthan-1, 3 dione: A three –necked 250 ml flask fitted with a thermometer, dropping funnel and magnetic stirring bar was charged with 4-chlorobenzoic acid (40g, 0.26mol) and anhydrous methanol (100ml); to this solution concentrated sulfuric acid (16ml) was added drop wise through a dropping funnel. The solution was heated to reflex for 6h. Cooled to room temperature and poured into an equal volume of water. The % yield was -75%, R_f value-0.5, mp-140-143^{0}C and Mol. wt-254.23. IR (KBr) cm^{-1}:1240 C-O), 1040(N-N).

Synthesis of 2-oxa -1, 2-diphenylethylidone hydrazine: A three necked 250 ml flask fitted with a thermometer, dropping funnel and magnetic stirrer was charged with 2(37.6g 0.22mol), anhydrous ethanol (80ml) and hydrazine hydrate (40ml, 80%). The solution was heated at reflux temperature for 8h.The reaction mixture was then cooled to 20^{0}C; a white solid precipitated. The crude product was recrystallized from anhydrous ethanol. The % yield was found 68.34%, MP.165-167^{0}C, R_f value -0.7, and molecular wt.-244.4.

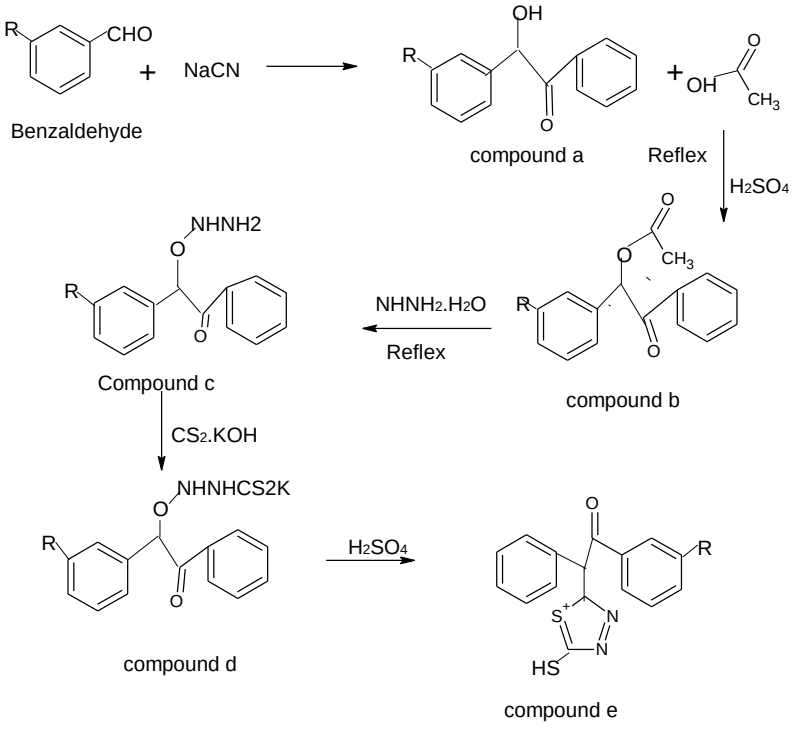
Synthesis of 2-oxa-1,2diphenylethylidene hydrazine potassium carbon disulfide: A three-necked 250 ml flask fitted with a thermometer, dropping funnel and a magnetic stirring bar was with KOH (6.1g, 0.11 mol), anhydrous ethanol (12oml) and (14.6g, 0.09 mol) .The mixture was stirred to obtain a homogenous solution and then CS_2 (6.9g) was added slowly through a dropping funnel. The reaction system was stirred at room temperature for an additional 6h, filtered and resulting solid was used for the next step without further purification. The % yield was -68%, mp.-165-167^{0}C, R_f value-0.7, and mol. wt-242.98. IR (KBR) cm^{-1}:1241(C-O), 1030(N-N) and 1300(C-S).

Synthesis of 2- phenylacetophenone 2-(1, 3, 4-thiadiazole-2-thiol): A three-necked 250 ml flask fitted with a thermometer, dropping funnel and magnetic stirring bar was first charged with sulfuric acid (80ml, 98%) and then cooled on an ice both with slowly added into it. After completion of the addition, the reaction mixture was kept below 0^{0}C

and stirred for and the 5h. The mixture was poured into ice water. The % yield was -84%, mp-260-265^{o}C, R_f value-0.8, mol. wt.-332. IR (KBr) CM^{-1}:1530(C-H), 1650(C=C), 1818(C=O), 1300(C-N), 1300(N-N), C=N(1610), 2600(H-S). 1HNMR (400 MHz) (CDCl3) δ (ppm): 6.91 (d, 12H, Ar\\H), 5.50 (s, 2H, CH_2).13C NMR (DMSO-d6) δ: 149.4, 142.6, 127.9, 121.7.

Synthesis of 2- chlorophenylacetophenone 2-(1, 3, 4-thiadiazole-2-thiol): The % yield was 71.56%, mp.-143-145 ^{o}C, Rf valu-0.3, molecular wt-371, 1HNMR (400 MHz) (CDCl3) δ (ppm): 7.7 (d, 4H, Ar\\H), 8.0 (d, 4H,Ar\\H).13C NMR (DMSO-d6) δ: 149.4, 142.6, 127.9, 121.7. . Anal. Cal. for $C_{16}H_{15}N_2OS_2$ (332.24): C, 53.85; H, 2.58; N, 17.94: S, 40.1. Found: C, 53.85; H, 2.58; N, 17.94; S, 40.1%.

Synthesis of 2-bromo phenylacetophenone 2-(1, 3, 4-thiadiazole-2-thiol): The % yield was 56.76%, mp.-167-169 ^{0} C, R_f value-0.45, molecular wt-404,1HNMR (400 MHz) (CDCl3) δ (ppm): 7.7 (d, 4H, Ar\\H), 8.0 (d, 4H,Ar\\H).13C NMR (DMSO-d6) δ: 149.4, 142.6, 127.9, 121.7. . Anal. Cal. for $C_{16}H_{15}N_2OS_2$ (332.24): C, 53.85; H, 2.58; N, 17.94: S, 40.1. Found: C, 53.85; H, 2.58; N, 17.94; S, 40.1%.



R=NO_2,Cl

Scheme R=Chlorobenzaldehyde R=Nitrobenzaldehyde

ANTIMICROBIAL ACTIVITY

Disk diffusion test: The **disk diffusion test**, or **agar diffusion test**, or **Kirby–Bauer test** (disc-diffusion antibiotic susceptibility test, disc-diffusion antibiotic sensitivity test, KB test), is an antibiotic sensitivity test. It uses **antibiotic discs** to test the extent to which bacteria are affected by those antibiotics. In this test, wafers containing antibiotics are placed on an agar plate where bacteria have been placed, and the plate is left to incubate. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible.[20] This is called a zone of inhibition. Once the zone diameter is measured it must be compared to a database of zone standards to determine if the bacterium being studied is susceptible, moderately susceptible, or resistant to the antibiotic in question.

A pure bacterial culture is suspended in a buffer, standardized to turbidity, and swabbed uniformly across a culture plate. A filter-paper disk impregnated with the compound to be tested is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be highest next to the disk and will decrease as the distance from the disk increases. If the compound is effective

against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. This along with the rate of antibiotic diffusion are used to estimate the bacteria's susceptibility to that particular antibiotic. In general, larger zones correlate with smaller minimum inhibitory concentration (MIC) of antibiotic for that bacteria. Inhibition produced by the test is compared with that produced by known concentration of a reference compound. This information can be used to choose appropriate antibiotics to combat a particular infection.[21]

Preparation: All aspects of the Kirby– Bauer procedure are standardized to ensure consistent and accurate results. Because of this, a laboratory must adhere to these standards. The media used in Kirby–Bauer testing must be Mueller-Hinton agar at only 4 mm deep, poured into either 100 mm or 150 mm Petri dishes. The pH level of the agar must be between 7.2 and 7.4.

Disc diffusion assay: Antimicrobial activity was assessed using a disc diffusion method [21]. Petri plates were prepared with 20 mL of sterile MHA. In the begging, the test cultures were swabbed on the top of the solidified media and then they were allowed to dry for 10 mines. The tests were conducted at 1000µg/disc and the loaded discs were sited on the surface of the medium and left for 30 mins at room temperature for the diffusion of compounds. The negative control was prepared using the respective solvent and Sulphamethiazole (10µg/disc) was used as a positive control. All the plates were incubated for 24 h at 37 °C for bacteria and48 h at 27 °C for fungi. Finally, a zone of inhibition was recorded in millimeters and the whole experiment was repeated twice.

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration assay of the title compound e1, e2 and e3 was accomplished by following the standard method for antibacterial [28]. For the antimicrobial assay the necessary concentrations (1000, 500, 250, 125, 62.5, 31.25 and15.62µg/mL) of the compound were dissolved in 2% DMSO, and diluted.

RESULT AND DISCUSSION

In the present scheme, the different derivative of thiadiazole were synthesized with good % yield .The reaction sequence for the titled compound is outline in scheme1.The various intermediate and final compounds were synthesized and characterized appropriately at each step and the antimicrobial activities of the synthesized derivatives were performed by Zone of Inhibition method and results are shown in the table 2-3.

Table-1: Physical properties of synthesized compounds

SN	Comp code	Mol. Formula	Mol.wt	MP/BP	Solubility	Rf value	Practical yield
1	A	$C_{14}H_{12}O_2$	212.2	140-143	Ethanol	0.5	80.2%
2	B	$C_{16}H_{14}O_3$	254.5	165-167	Ethanol	0.7	75.76%
3	C	$C_{14}H_{14}O_2N_2$	242.89	260-262	ethanol	0.8	68.34%
4	D	$C_{15}H_{13}O_2N_2S_2K$	332	198-200	Ethanol	0.5	84.00%
5	E	$C_{16}H_{12}ON_2S_2$	325	170-173	Ethanol	0.7	68.45%
6	F	$C_{16}H_{12}O_2N_2S_2Cl$	371	205-208	ethanol	0.4	71.02%
7	G	$C_{16}H_{12}ON_2S_2Br$	404	225-227	ethanol	0.5	80.2%

Table 2: Antibacterial activity of synthesized compounds against *Escherichia coli*.

Compound code	Concentration					
	1ug|ml	50ug/ml	100ug/ml	200ug/ml	500ug/ml	APP.MICug/ml
e1	-	-	+	+	++	200
e2	-	+	++	++	+++	200
e3	_	-	-	+	++	500
sulphamethazine	-	++	+++	+++	+++	100

Symbol (-),no inhibition;(+),weakly active;(++),moderately active;(+++),highly active.

Table 3: Antibacterial activity of synthesized compounds against *S. aureus.*

Compound code	Concentration					
	1ug|ml	50ug/ml	100ug/ml	200ug/ml	500ug/ml	APP.MICug/ml
e1	-	-	+	+	++	200
e2	-	+	++	++	+++	200
e3	_	-	+	++	+++	500
sulfamethazine	-	++	+++	+++	+++	100

Symbol(-),no inhibition;(+),weakly active;(++),moderately active;(+++),highly active.

CONCLUSION

The development of thiadiazole bearing phenylacetophenone system has new potentially active antibacterial agent. The result of *in vitro* antimicrobial studies also showed that the compound having deactivate group (electron withdrawing group like NO_2, Cl, Br) were most active against *E. coli* and *S. aureus.*

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