Synthesis and Antibacterial Activity of 2-Cinnamyl-5-Substituted-1,3,4-Oxadiazole, 1,3,4-Thiadiazoles and 5-Cinnamyl-3-Substituted-1,2,4-Triazoles

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(NJC)

(Received on 1/8/2006) (Accepted for publication on 18/2/2007)

Abstract

In this work the synthesis of 2-cinnamyl-5-substituted-1,3,4-oxadiazoles, 1,3,4-thiadiazole and 5-cinnamyl-3-substituted-1,2,4-triazoles was achieved. Ethyl cinnamate was refluxed with hydrazine hydrate in ethanol to give the corresponding acid hydrazides, which then treated with ammonium thiocyanate to give thiosemicarbazide. The thiosemicarbazide cyclized to 5-amino-2-cinnamyl-1,3,4-thiadiazole and 5-cinnamyl-1,2,4-triazole-3-thiol with sulfuric acid and sodium hydroxide solution respectively.

The acid hydrazide was treated with substituted benzaldehyde to yield hydrazone, which then treated with PbO_2 to give 2-cinnamyl-5-aryl/phenyl-1,3,4-oxadiazoles. 2-Cinnamyl-1,3,4-oxadiazole was obtained from acid hydrazide by its reaction with formic acid followed by PbO_2. Acid hydrazide was converted to substituted thiosemicarbazode by the reaction with phenyl isothiocyanate.

The synthesized conpounds (1, 2, 5, 6, 9, 11 and 13) were tested against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas*.

The structures of the synthesized compounds were established by physical and spectral means.

الخلاصة

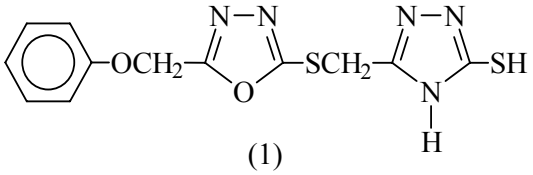
تم في هذا البحث تحضير 2-سينناميل-5-معوضات-1،3،4-اوكسادايازول و 1،3،4-ثايادايـازول و 5-سينناميل-3-معوضات-1،2،4-ترايازول. حولت سينامات الاثيل الى هيدرازيد حامض الـسينناميك مـن خلال مفاعلته مع الهيدرازين المائي في الايثانول. تم مفاعلة هيدرازيد الحامض مع ثايوسـيانات الامونيـوم ليعطي ثايوسيميكاربازيد. اعطت مفاعلة الثايوسيميكاربازيد مع حامض الكبريتيك 5-امينـو-2-سـينناميل-1،3،4-ثايادايازول ومع هيدروكسيد الـصوديوم 5-سـينناميل-1،2،4-تراي‌ـازول-3-ثـايول. حـضرت الهيدرازونات من خلال مفاعلة هيدرازيد الحامض مع البنزالديهايد او معوضاته. وتم مفاعلتة الهيـدرازونات

مع PbO_2 ليعطي 2-سينناميل-5-اريل/فنيل-1،3،4-اوكساديازول. كما تم تحضير 2-سـينناميل-1،3،4-اوكساديازول من خلال مفاعلة هيدرازيد الحامض مع حامض الفورميك ثم PbO_2 .

اختبـرت فعاليـة المركبـات(1،2، 5، 6، 9، 11، 13) تجـاه البكتريـا *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas* . شخـصت المركبـات المحـضرة بالطرق الفيزياوية والطيفية.

Introduction

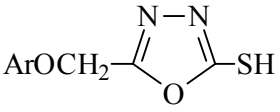
The synthesis of substituted 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles, the biological activities and some of their uses were investigated and they show various applications as antibacterial and antifungal properties[1,2] such as compound (1)[3]. Other triazoles act as blood glucose level regulator[4].



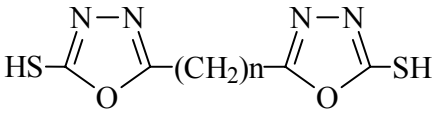
(1)

Some 1,3,4-oxadiazole act as plant growth regulator[5], and act against tuberculosis[6]. 1,3,4-Oxadiazole and 1,3,4-thiadiazole and 1,2,4-triazole derivatives are thermally stable[7].

The importance of these heterocyclic compounds draw the attention of many research works to the synthesis of the above mentioned diazoles. 1,3,4-Oxadiazoles were synthesized from the reaction of acid hydrazide with carbon disulfide in ethanolic potassium hydroxide as compound (2)[8] and (3)[9].



(2)



(3)
n = 3,4,8

Thiosemicarbazides were oxidized by mercuric oxide, lead oxide or iodine/potassium iodide to substituted 1,3,4-oxadiazoles[10,11].

Microwave assisted reaction of acid hydrazide with carboxylic acid in presence of phosphorous oxychloride gave 2,5-disubsituted-1,3,4-oxadiazole[12].

While 1,3,4-thiadiazoles were synthesized from substituted thiosemicarbazides by their reaction with concentrated sulfuric acid[13] or phosphoric acid as in synthesis of 5-(3-indolyl methyl)-2-amino-1,3,4-thiadiazole and 5-(2-methyl-3-indolyl methyl)-2-anilino-1,3,4 thiadiazole[14]. Whereas substituted 1,2,4-triazoles were synthesized from substituted thiosemicarbazide by their reaction with sodium hydroxide, for example 1-(3-methoxy benzoyl) thiosemicarbazide was treated with 2N NaOH to give 5-(3-methoxy phenyl)-1,2,4-triazole-3-thione[15].

In the present work the synthesis of substituted 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles were investigated (Scheme 1).

Experimental

All chemicals were purchased from Flucka and BDH Chemical Ltd. The melting points were measured on an Electrothermal 9300 Engineering LTD and were uncorrected. IR spectra were recorded on Infrared Spectrophotometer Model Tensor 27, Bruker Co., Germany, using KBr discs. UV spectra were recorded on Shimadzu Double-Beam Spectrophotometer UV-210 A using chloroform as a solvent.

Cinnamic acid hydrazide (1):

A mixture of ethyl cinnamate (1.76 g, 0.01 mole) and hydrazine hydrate (10 ml, 0.2 mole) in absolute ethanol (100 ml) was refluxed for 3 hours. The solvent was evaporated to give the hydrazide as oil, yield 75%, Table (2).

Substituted thiosemicarbazide (2):

A mixture of acid hydrazide (1) (3.24 g, 0.02 mole), ammonium thiocyanate (4.56 g, 0.06 mole), hydrochloric acid (8 ml) in absolute ethanol (50 ml) was refluxed for 22 hrs. The solvent was evaporated and the residue poured on crushed ice with stirring, the solid formed, filtered dried and recrystallized from ethanol, Tables (1, 2).

5-Cinnamyl-1,2,4-triazole-3-thiol (3):

A mixture of substituted thiosemicarbazide (2) (2.30 g, 0.01 mole) and 1% aqueous sodium hydroxide (15 ml) was refluxed for 3 hours, the mixture was treated with charcoal and the charcoal then removed by hot filtration. The solution was acidified by 10% hydrochloric acid with cooling, the precipitate filtered, and recrystallized from ethanol, Tables (1, 2).

2-Cinnamyl-5-amino-1,3,4-thiadiazole (4):

Concentrated sulfuric acid (10 ml) was added to substituted thiosemicarbazide (2) (1.15 g, 0.005 mole). The mixture was heated on water bath at 90 °C with stirring for 2 hours. The mixture then poured onto ice-water and neutralized with concentrated ammonium hydroxide solution with cooling, the formed precipitate filtered, washed with cold water, dried and recrystallized from benzene, Table (1, 2).

1-Acyl-4-phenyl thiosemicarbazide (5):

A mixture of acid hydrazide (1) (3.24 g, 0.02 mole), phenyl isothiocyanate (2.7 g, 0.02 mole), concentrated hydrochloric acid (8 ml) in ethanol (30 ml) was refluxed for (10) hours, the solvent was evaporated and the residue poured on crushed ice, the solid then filtered, dried and recrystallized from ethanol, Tables (1, 2).

Hydrazones (6-9):

Benzaldehyde/substituted benzaldehyde (0.01 mole), acid hydrazide (1) (1.62 g, 0.01 mole) in ethanol (20 ml) was refluxed for (2) hours. The solvent was condensed and the precipitate filtered and

recrystallized from benzene, Tables (1, 2).

1-Acyl-2-formyl hydrazine (10):

A mixture of acid hydrazide (1) (1.62 g, 0.01 mole) formic acid (0.46 g, 0.01 mole) in ethanol (20 ml) was refluxed for 3 hours. The mixture was cooled and the solid filtered, dried and recrystallized from ethanol, Tables (1, 2).

Cyclization of 1-acyl-2-formyl hydrazine and hydrazones to substituted 1,3,4-oxadiazoles (11-15):

To a homogenous solution of hydrazones (6-10) (0.01 mole) in glacial acetic acid, PbO_2 (2.39 g, 0.01 mole) was added the mixture then stirred with mechanical stirrer at 25 °C for 1 hr. The reaction mixture was diluted with ice-water and left to stand for 24 hours. The precipitate was filtered and recrystallized from benzene, Tables (1, 2).

The Biological Activity Test:

In the present work the following bacteria were used, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas*. The procedure of Bauer[16] was used in sensitivity test as five colonies of the above mentioned bacteria which were transferred to the nutrient broth. The medium was incubated at 37 °C for 15-16 hours, and the diluted with normal saline and then (0.1) ml of this medium was transferred to the nutrient agar and distributed on the surface of the Petri-dishes, then left for about 30 minutes at 37 °C in the incubation.

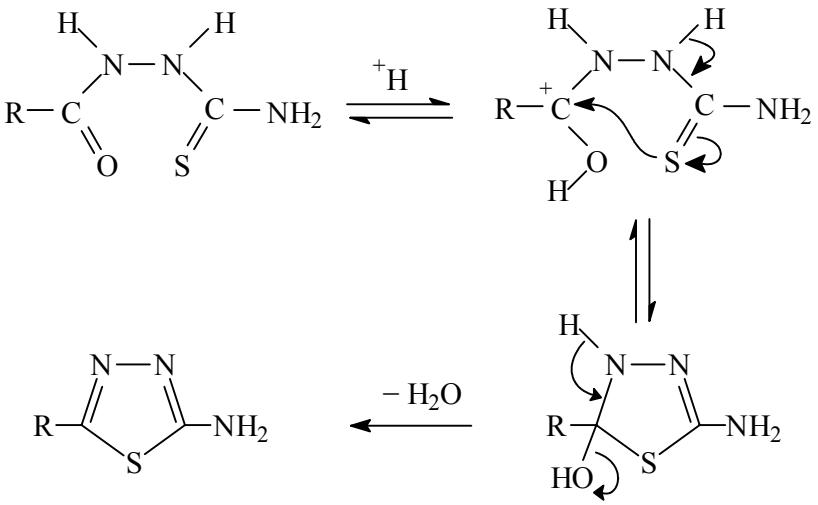
To determine the inhibitory effect, filter paper discs were saturated with different concentrations of solutions for tested compounds (1, 2, 5, 6, 9, 11, 13) in DMSO and were distributed on the surface of the agar medium and then incubated for (15-16 hours).

The antibiotic Ampicillin, Tetracycline and Vancomycine were used for *Staphylococcus aureus* and *Pseudomonas*, Table (3).

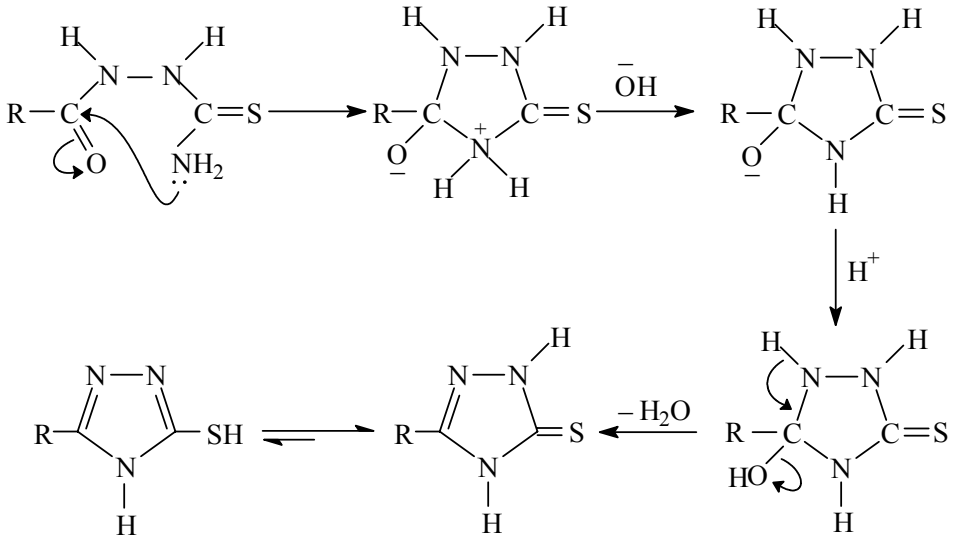
Results and Discussion

The synthesis of substituted 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles is reported. The ethyl cinnamate was treated with hydrazine hydrate in ethanol give acid hydrazide (1), the hydrazide show absorption ν cm^{-1} at 1660 (C=O), 1614 (CH=CH) and 3340 (N-H).

The hydrazide then converted to thiosemicarbazide (2) by its reaction with ammonium thiocyanate / hydrochloric acid, the thiosemicarbazide (2) then transferred to 2-substituted-1,3,4-thiadiazole-5-thiol and 5-substituted-1,2,4-triazole-3-thiol by their reaction with sulfuric acid and sodium hydroxide solution respectively, through the following mechanisms:



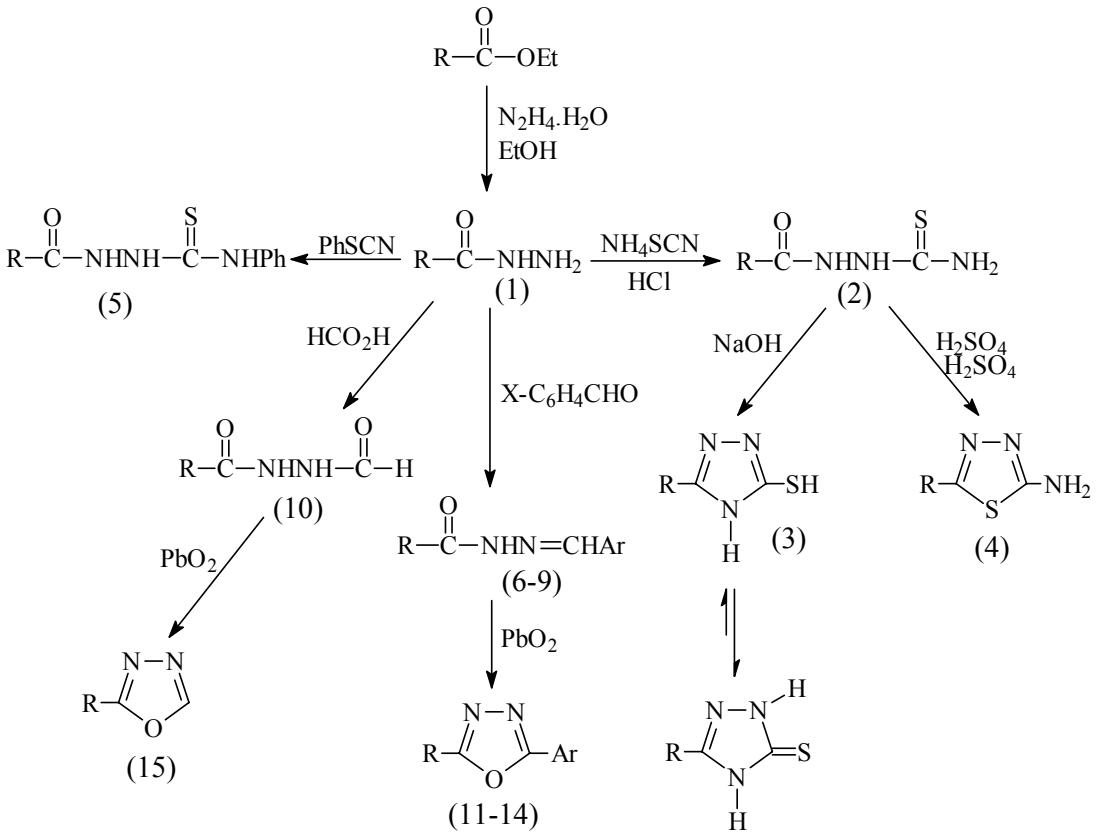
Mechanism (1)



Mechanism (2)

The 1,2,4-triazole (3) show absorption ν cm^{-1} at 1625 (CH=CH), 1206 (C=S), while 1,3,4-thiadiazole (4) show absorption at ν cm^{-1} 1619 (CH=CH) and 3380 (N-H). In order to synthesis monosubstituted oxadiazole, acid hydrazide (1) was treated with formic acid to give 1-formyl-2-acyl hydrazine (10) which transferred to 2-cinnamyl-1,3,4-oxadiazole by its reaction with PbO_2. Compound (10) show absorption ν cm^{-1} at 1699 and 1647 (2C=O), 1620 (CH=CH), while compound (15) show absorption at 1614 (CH=CH), 1088 (C-O-C) and ν cm^{-1} 1656 (C=N). The acid hydrazide (1) was treated with benzaldehyde or substituted benzaldehyde to give hydrazones (6-9). Compound (6) show absorption ν cm^{-1} at 1663 (C=O), 1616 (CH=CH) and 3300 (N-H). Hydrazones (6-9) was then cyclized to 2,5-disubstituted-1,3,4-oxadiazoles (11-14) by PbO_2. Compound (11) show absorption at ν cm^{-1} 1624 (CH=CH) and 1197 (C-O-C) while compound (12) show absorption ν cm^{-1} at 3566 (O-H), 1640 (CH=CH), 1120 (C-O-C) and 1667 (C=N).

The hydrazide (1) then treated with phenyl isothiocyanate to give substituted thiosemicarbazide (5), ν cm^{-1} at 1698 (C=O), 1598 (CH=CH) and 1189 (C=S). The U.V spectra of the synthesized compounds (1-15) show higher absorption λ_{max} range from 298 to 242 nm and ε_{max} range from 4000 to 1101, Table (2).



R = PhCH=CH
X = H, 2-OH, 3-NO_2, Cl

Scheme (1)

Table (1): Physical data of compounds (2-15)

Comp. No.	Molecular formula	Yield (%)	m.p. (°C)	Color
2	$C_{10}H_{11}NO_3S$	55	197-199	White
3	$C_{10}H_9N_3S$	40	210-212	Pale brown
4	$C_{10}H_9N_3S$	52	> 300	White
5	$C_{16}H_{14}N_2OS$	65	164-166	Yellow
6	$C_{10}H_{14}N_2O$	65	160-162	Pale yellow
7	$C_{16}H_{14}N_2O_2$	70	206-208	White
8	$C_{16}H_{13}N_3O_3$	50	152-154	Dark yellow
9	$C_{16}H_{13}N_2OCl$	55	122-125	Pale orange
10	$C_{10}H_{10}N_2O_2$	60	151-154	White
11	$C_{16}H_{12}N_2O$	35	213-215	White
12	$C_{16}H_{12}N_2O_2$	40	> 280	White
13	$C_{16}H_{11}N_3O_3$	45	177-179	Yellow
14	$C_{16}H_{11}N_2OCl$	30	178-180	White
15	$C_{10}H_8N_2O$	40	71-73	White

Table (2): Spectral data of synthesized compounds (1-15)

Comp. No.	IR (KBr) ν cm^{-1}							UV, EtOH	
	C=O	C=C	N-H	C=S	C-O-C	C=N	O-H	λ_{max} (nm)	ε_{max}
1	1660	1614	3340	-	-	-	-	244	2800
2	1650	1609	3350	1280	-	-	-	268	1884
3	-	1625	3380	1206	-	1660	-	266	3311
4	-	1619	3382	-	-	1662	-	242	1101
5	1698	1598	3211	1189	-	-	-	250	4000
6	1663	1616	3300	-	-	-	-	298	4000
7	1669	1624	-	-	-	-	3600	256	4000
8	1698	1643	3314	-	-	-	-	252	4000
9	1670	1625	3320	-	-	-	-	250	4000
10	1699 1647	1620	3340	-	-	-	-	246	2474
11	-	1624	-	-	1197	-	-	286	4000
12	-	1640	-	-	1120	1667	3566	280	3311
13	-	1625	-	-	1075	1653	-	252	4000
14	-	1624	-	-	1089	1653	-	258	4000
15	-	1613	-	-	1088	1656	-	290	3763

Biological Activity:

Antibacterial activity of compounds (1, 2, 5, 6, 9, 11 and 13) was evaluated by agar plate diffusion technique against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas*.

The result indicate that some of the tested compounds were active against certain bacteria, the activity of these compounds shown in Figure (1) and Table (3).

Figure (1): The effect of some synthesized compounds against *Staph. aureus*

Table (3): The activity of compounds (1,2,5,6,9,11 and 13) as antibacterial

Comp. No.	Test organism*				
	Staph. aureus	*Strept. pyo.*	*Salmonella*	*E. coli*	*Pseudomonas*
1	S	S	S	S	S
2	R	R	S	R	R
5	S	R	S	R	R
6	R	R	R	R	R
9	S	R	R	S	R
11	R	R	R	R	R
13	R	R	R	R	R
Ampicillin	14 m				
Tetracycline	26 m				
Vancomycin	18 m				

R = resistance
S = sensitive

References

1. S.B. Bhavsar, D.B. Shinde and M.S. Shingare, ***Indian J. Chem.***, 1955, **34**, 70.
2. R.B. Pathak, U. Srivastava and S.C. Bahel, ***J. Indian Chem. Soc.***, 1982, **LIX,** 776.
3. M.A. Kuba'a, 1999, "Synthesis of new nitrofuran derivatives from agricultural waste", M.Sc. Thesis, University of Nahrain, College of Science, Baghdad-Iraq.
4. Agarwal, ***Indian J. Pharm. Sci***., 1991, **53**, 912.
5. Z. Ziyi, F. Xiaoming, L. Haixln, C. Maoqi and L. Youbang, ***Xouji Huaxue***, 1989, **9(4)**: 355.
6. D.N.R. Libermann and F. Grumbach, ***Congr. Intern. Biochem. Rsumes Communs.***,

1955, 3° Congr., Brusse **15**, 136.

7. C. Ainsworth, ***J. Am. Chem. Soc.***, 1965, **187,** 5800.
8. R.S. Sharma and S.C. Bahel, 1982, "Synthesis and antifungal activity of 5-substituted 1,3,4-oxadiazole and related compounds", Bokin Bobai, 10, 293; Chem. Abst., (1982), 97, 216081c.
9. A.O. Maslat, M. Abussad, H. Tashtoush and M. Al-Talib, ***Pol. J. Pharmacol.***, 2002, **54**, 55.
10. A. Amir and Shikha, ***J. Europ. Med. Chem.***, 2004, **39**, 535.
11. R.S. Sharma and S.C. Bahel, ***J. Indian Chem. Soc.***, 1982, **LIX,** 877.
12. K.M. Khan, Zia-Ullah, M. Roni, S. Perveen, S.M. Haider, A. Choudhany, A. Rehmn and U. Velter, ***Mendeleev Communication Electronic Version, Issue,*** 2004, **4**, 129.
13. S. Karakus and S. Rollas, ***IL Farmaco.***, 2002, **57**, 577.
14. A. Varvaresou, P.T. Siatra, T.A. Dalla and K. Tsantili, ***IL Farmaco***., 1998, **53**, 320.
15. L. Labanauskas, E. Udrenaite, P. Gaidelic and A. Brukstus, ***IL Farmaco.***, 2004, **59**, 255.
16. A.W. Bauer, W.A.M. Kirby, J.S. Sherris and M. Turk, ***Am. J. Clin. Pathol.***, 1966, **45**, 493.