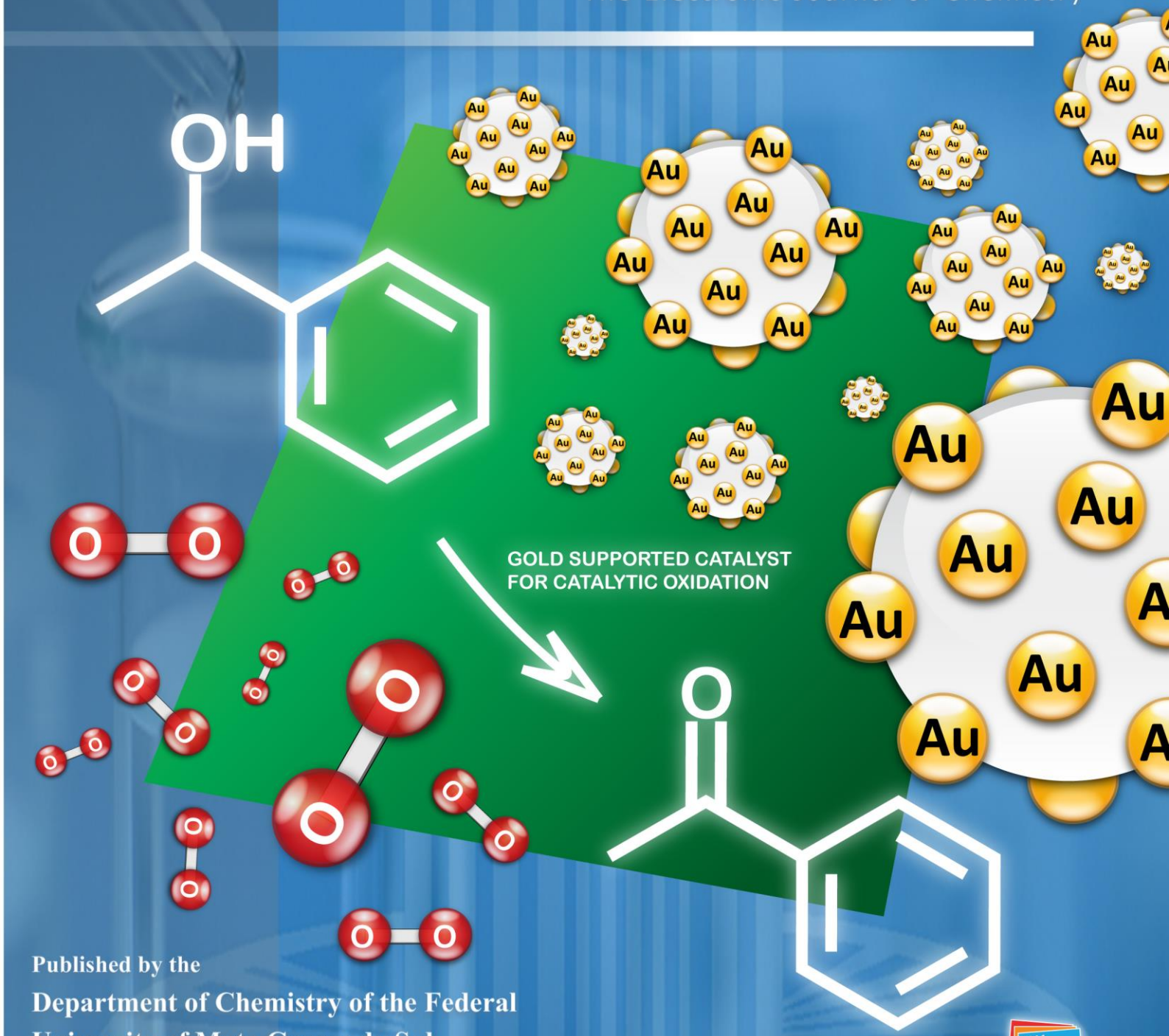
ISSN 1984-6428

APRIL-JUNE
2011
VOLUME 3
NUMBER 2

Orbital

The Electronic Journal of Chemistry



Published by the
Department of Chemistry of the Federal
University of Mato Grosso do Sul.
Campo Grande, BRAZIL

International Year of
CHEMISTRY
2011

UFMS

www.orbital.ufms.br

Orbital - Vol. 3 No. 2 - April-June 2011

Table of Contents

FULL PAPERS

ISSN 1984-6428
ONLINE
www.orbital.ufms.br

| Vol 3 | | No. 2 | | April-June 2011 |

Full Paper

Synthesis, characterization and application of novel bisazo reactive dyes on various fibers

*Divyesh R. Patel, Nikul S. Patel, Hemant S. Patel and Keshav C. Patel**

Synthetic Organic Chemistry Research Laboratory, Department of Chemistry, Veer Narmad South Gujarat University, Surat (Gujarat), India-395 007.

Received: 14 February 2010; revised: 15 February 2011; accepted: 05 May 2011. Available online: 05 September 2011.

ABSTRACT: *Ten hot brand bisazo reactive dyes (D_1 to D_{10}) have been synthesized by coupling bis(diazotised), 4,4'-methylene bis(2,6-dichloroaniline) (A) with various 5-sulfo anthranilo cyanurated coupling components (R) and their dyeing performance as reactive dyes has been assessed on silk, wool and cotton fibres. The purity of dyes was checked by TLC. The IR spectra and ^{1}H-NMR spectra prove the structure of newly reactive dyes. The percentage dye bath exhaustion on different fibres was reasonable good and acceptable. The dyes exhibited high levels of light, washing and rubbing fastness.*

Keywords: *4-4'-methylene bis(2,6-dichloroaniline); hot brand bisazo reactive dyes; silk; wool; cotton*

Introduction

Reactive dyes are colored compounds which contain one or two groups capable of forming covalent bond between a carbon atom or phosphorus atom of the dyes ion or molecules and an oxygen atom, nitrogen atom or sulfur atom of a hydroxyl, an amino or a mercapto group respectively, of the substrate [1]. These dyes are generally used on higher value clothes, which are normally mercerized [2].

Reactive dyes though late entry in to the field of synthetic dyes, very soon attained a commercial status. Several new reactive systems have been introduced from time to time, which covers the subject of innumerable patents and publication [3]. It was for the first time that dyeing has been done by chemical reaction between the dye and the fibre, enabling one to get assortment of bright, attractive shades of adequate

* Corresponding author. E-mail: divyeshpatel905@gmail.com

fastness with considerable ease of dyeing. It can also be easily understood that dyes with two reactive groups give a higher fixation yield than dyes with one reactive group for it one of the two dye-fibre bonds is hydrolyzed, one is still left for fixation [4, 5]. Reactive dyes are well known and applied for dyeing of different materials [6]. Among them triazine derivatives have an important place [7].

s-Triazine based chemicals have been applied variously in the manufacture of polymers, dyes, drugs, explosives, pesticides and commodity chemicals [8] as a consequence, theoretical and experimental studies on these chemicals have been widely carried out [9, 10] with the result that the s-triazine ring is known as an important conjugated heterocycle whose electronics properties are expected to show suitable differences from those of benzene due to the alternate replacement of -CH- group by nitrogen atoms [11].

s-Triazine plays an important role in synthesized dyes. The key compound of reactive dyes are a cyanuric chloride in synthesized dyestuff have two reactive groups in their structure which give high fixation yields, excellent wet fastness, brilliant shade and simple application techniques in textile printing. The advantage owing to the chloro triazine groups (s-triazine) is that due to the electrophilic property of the cyanuric group, a wide range of chromophores having good fastness to light, perspiration and chlorine.

Hot-Brand reactive dyes have been widely considered due to their fixation yield on various fibers [12].

Various bisazo reactive dyes (Figure 1 and Figure 2) have been reported earlier which shows good dyeing properties on silk, wool and cotton [13, 14].

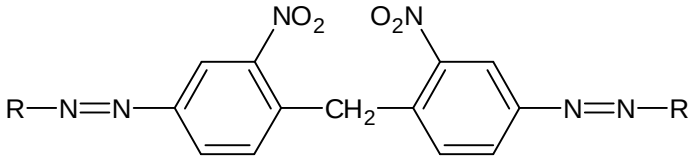


Figure 1. Cold brand bisazo reactive dyes. Where R = Various cyanurated coupling components.

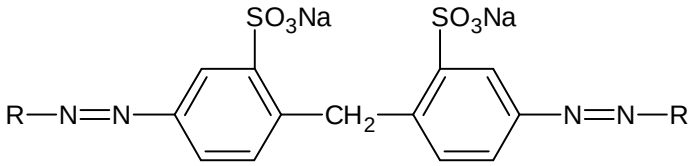


Figure 2. Hot brand bisazo reactive dyes. Where R = Various m-nitro anilino cyanurated coupling components.

In a continuation of our work we report here the synthesis of some hot-brand reactive dyes with a higher degree of activity synthesis and study of the dyeing

properties of the bisazo reactive dyes base on 4,4'-methylene bis(2,6-dichloroaniline). The reactive dyes of the following structure were prepared (Figure 3).

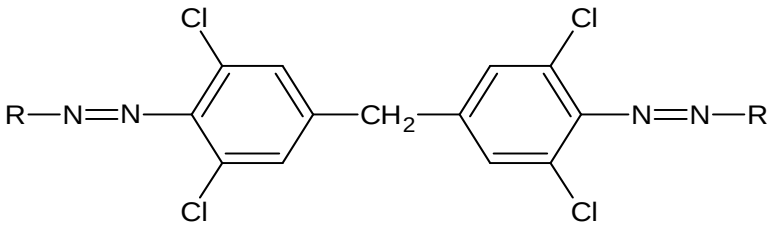


Figure 3. Hot brand bisazo reactive dyes. Where R = Various 5-sulfo anthranilo cyanurated coupling components.

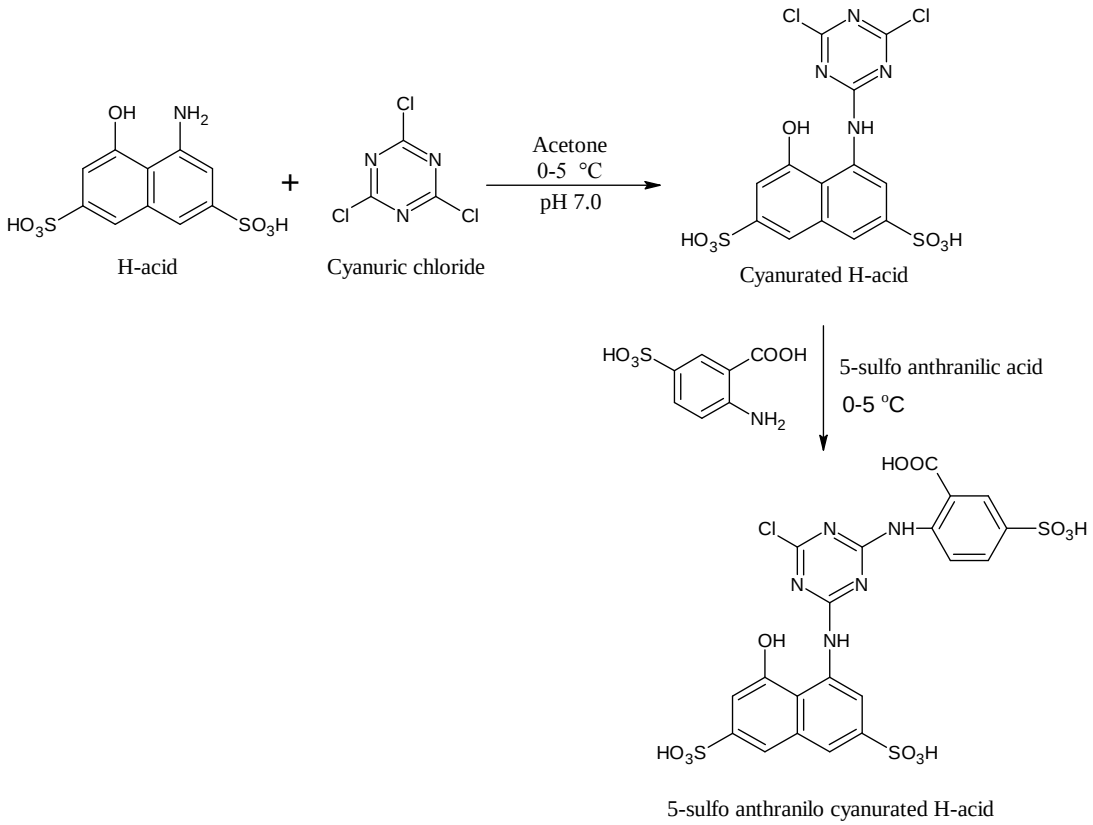
Material and Methods

General

All melting points are determined using a DSC 7, Perkin-Elmer (USA) Differential Scanning Caloriate (heating rate 5 °C/min, N_2 gas) digital melting apparatus and are uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer model-377 spectrophotometer instrument. Elemental microanalyses were performed on a LECI CHN-932 for C, H and N. Thin Layer chromatography was performed using silica-coated aluminum plates (60-F_{254}, Merck) [15]. ^{1}H NMR spectra were obtained with a Jeol JNM0-FX 200 at 300 MHz instrument, Using TMS as the internal standard and DMSO-d_6 as a solvent. Chemical shifts are given in δ ppm. Absorption spectra were obtained with Becman DB-GT grafting spectrometer instrument. Fastness to light was assessed in accordance with BS 1006-1978 [16]. Rubbing fastness was carried out with an Atlas Crock meter in accordance with AATCC TM 8-1961 [17] and the wash fastness test in accordance with ISO: 765-1979 [18]. The entire regents were purchased from Merck and Renkem, which were of G. R. grade and used without further purification. All crude products were isolated as solids and purified by a combination of column chromatography and recrystallization.

Chemistry

Preparation of 4-4' methylene bis(2,6-dichloroaniline) [19]: 2,6-dichloroaniline (14.8 g, 0.1 mole) was dissolved in water (125 mL) and 36.5% hydrochloric acid (25 mL) at 50 °C. The reaction mixture was then treated with 3% aqueous formaldehyde solution (35 mL). The temperature was maintained at 60 °C and stirred for an hour and neutralized with 10% sodium hydroxide solution, yellow precipitates obtained were filtered, washed with hot water, dried and recrystallised from acetic acid. Yield 84%, m.p. 273 °C. IR (KBr) 3420 cm^{-1}, 3300 cm^{-1} (N-H), 2850 cm^{-1} (C-H). ^{1}H NMR (DMSO): δ 8.2 (2H, s, NH_2), 3.45 (2H, s, CH_2), 7.05-7.15 (4H, m, Ar–H). Elemental analysis: Found C-46.40%; H-2.94% N-8.32% $C_{13}H_{10}N_2Cl_4$ (MF require C-46.45%; H-2.99%; N-8.37%).



Scheme 1. Preparation of 5-sulfo anthranilo cyanurated H-acid (R).

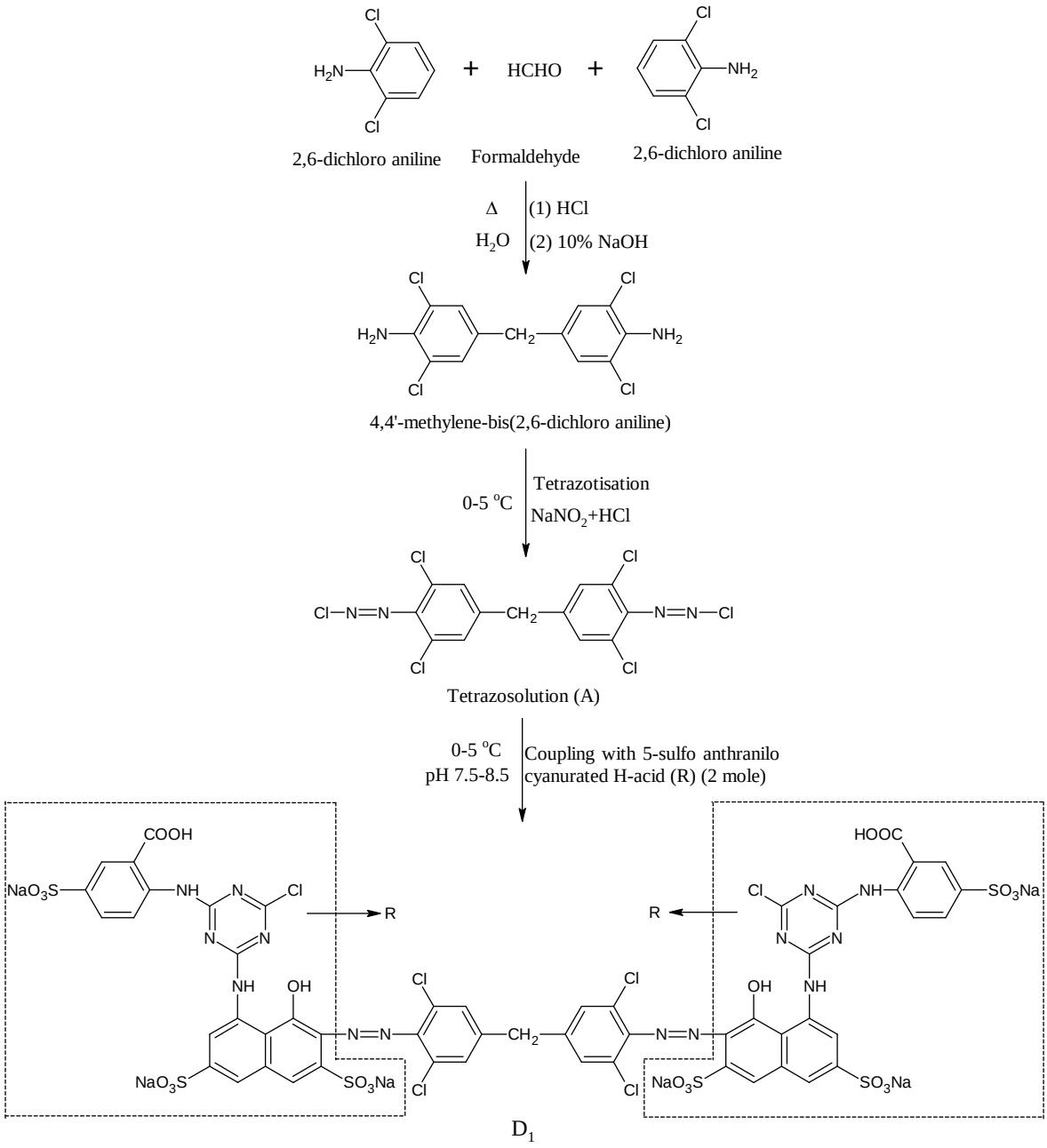
Tetrazotisation of 4-4'-methylene bis(2,6-dichloroaniline): 4-4'-methylene bis(2,6-dichloroaniline) (1.68 g, 0.005 mole) was suspended in H_2O (60 mL). Hydrochloric acid (10 mL) was added drop wise to this well stirred suspension. The mixture was gradually heated up to 70 °C till clear solution obtained. The solution was cooled to 0-5 °C in an ice bath. A solution of $NaNO_2$ (1.38 g) in H_2O (8 mL) previously cooled to 0 °C, was then added over a period of 5 minutes with stirring. The stirring was continued for an hour maintaining the same temperature, with positive test for nitrous acid with required amount of a solution of a sulphamic acid. The clear tetrazo solution (A) at 0-5 °C was used for subsequent coupling reaction.

Preparation of 5-sulpho anthranilo cyanurated H-acid (R)

(i) Cyanuration of H-acid: Cyanuric chloride (1.85 g, 0.01 mole) was stirred in acetone (50 mL) at a temperature below 5 °C for a period of an hour. A neutral solution of H-acid (3.19 g, 0.01 mole) aqueous sodium carbonate solution (10% w/v) was then added in small lots in about an hour, the neutral pH was maintained below 5 °C through this reaction. The reaction mass was then stirred at 0-5 °C for further four hours when a clear solution was obtained. The cyanurated H-acid solution thus formed was used for subsequent coupling reaction.

(ii) Condensation with 5-sulpho anthranilic acid: The ice-cooled and well-stirred

solution of cyanurated H-acid (4.67 g, 0.01 mole) was heated up to 40-50 °C for half an hour. To this 5-sulpho anthranilic acid (2.17 g, 0.01 mole) was added dropwise at same temperature during a period of 30 minutes maintaining the pH neutral, by simultaneous addition of sodium carbonate solution (1% w/v). After the addition was completed the stirring was continued for further 3 hours, to prepare a 5-sulpho anthranilo cyanurated H-acid (R). The resulting solution thus obtained was used for further coupling reaction.



Where R= Various 5-sulfo anthranilo cyanurated coupling components

Scheme 2. Synthesis of dye D_1

Preparation of dye (D_1): To a well-stirred solution of 5-sulpho anthranilo cyanurated H-acid (R) (6.48 g, 0.01 mole), a freshly prepared solution of tetrazo solution (A) (2.155

g, 0.005 mole) was added drop wise over a period of 10-15 minutes. The pH was maintained at 7.5 to 8.5 by simultaneous addition of sodium carbonate solution (10% w/v). During coupling the purple solution was formed. Stirring was continued for 3-4 hours, maintaining the temperature below 5 °C. The reaction mixture was heated up to 60 °C and sodium chloride (15 g) added until the colouring material was precipitated. It was stirred for an hour, filtered and washed with a small amount of sodium chloride solution (5% w/v). The solid was dried at 80-90 °C and extracted with DMF. The dye was precipitated by diluting the DMF-extract with excess of chloroform. A purple dye was then filtered, washed with chloroform and dried at 60 °C.Yield 86%.

Following the above procedure other reactive dyes D_2 to D_{10} were synthesized using 5-sulfo anthranilo cyanurated coupling components such as J-acid, N-phenyl-J-acid, Gamma-acid, K-acid, Chicago acid, Peri acid, Bronner acid, Tobias acid and Sulfo tobias acid. All the synthesized dyes were recorded in Table 1.

Results and Discussion

Spectral properties of dyes

The absorption maxima (λ_{max}) of the dyes D_1 to D_{10} were recorded in water and conc. H_2SO_4 and are shown in Table 4. The λ_{max} values are directly proportional to the electronic power, nature and position of the substituents in the naphthyl ring of the coupler moiety. The values of logε (molar extinction coefficient) are summarized in Table 3. All the values are in the range of 4.19-4.30, which indicates the dyes have high intensity of absorption.

Dye D_2 have λ_{max} is about 462 nm while D_3 have λ_{max} is about 482 nm. Here the introduction of phenyl ring which produce bathochromic effect and shifting the λ_{max} value. Here 20 nm shifting in absorption is observed. Dye D_4 have λ_{max} is about 458 nm while D_7 and D_8 have λ_{max} values are 435 nm and 440 nm respectively. Here the introduction of auxochrome like hydroxyl group which produce bathochromic effect and give rise to the 23 nm and 18 nm λ_{max} value in D_4 with respect to D_7 and D_8. Dyes D_1, D_5 and D_6 have same groups but the positions of the groups are different so the oscillation of electron is fast in D_5 and D_6 as compare to D_1. So dye D_1 possesses higher λ_{max} value as compare to D_5 and D_6. The shifting of 20 nm λ_{max} value in D_{10} as compare to D_9 due to the introduction of auxochrome like sulfonic acid group in D_{10}, which increase the λ_{max} value in D_{10} as compare to D_9.

Dyeing properties of dyes

All the dyes D_1 to D_{10} were applied at 2% depth on silk, wool and cotton fibres according to usual procedure [21] in the dye bath containing materials as listed in Table 2.

Table 1. Characterization data of dyes D_1 to D_{10}

Dye No.	Various 5-sulfo anthranilo cyanurated coupling Components (R)	Molecular Formula	Mol. Wt.	Yield (%)	% C Found / Req.	%H Found / Req.	%N Found / Req.	[a]R_f
D_1	H-acid	$C_{53}H_{26}O_{24}N_{14}S_6Na_6Cl_6$	1788	86	35.57 / 35.60	1.46 / 1.51	10.96 / 10.99	0.43
D_2	J-acid	$C_{53}H_{24}O_{24}N_{14}S_4Na_4Cl_6$	1577	84	40.32 / 40.35	1.52 / 1.54	12.42 / 12.46	0.39
D_3	N-phenyl J-acid	$C_{65}H_{32}O_{24}N_{14}S_4Na_4Cl_6$	1839	84	42.41 / 42.45	1.74 / 1.78	10.65 / 10.68	0.40
D_4	Gamma-acid	$C_{53}H_{24}O_{24}N_{14}S_4Na_4Cl_6$	1577	85	40.33 / 40.38	1.52 / 1.57	12.42 / 12.45	0.44
D_5	K-acid	$C_{53}H_{26}O_{24}N_{14}S_6Na_6Cl_6$	1788	80	35.57 / 35.61	1.46 / 1.48	10.96 / 11.00	0.46
D_6	Chicago acid	$C_{53}H_{26}O_{24}N_{14}S_6Na_6Cl_6$	1788	85	35.57 / 35.62	1.46 / 1.49	10.96 / 11.01	0.42
D_7	Peri acid	$C_{53}H_{24}O_{24}N_{14}S_4Na_4Cl_6$	1545	87	41.17 / 41.20	1.55 / 1.59	12.68 / 12.71	0.45
D_8	Bronner acid	$C_{53}H_{24}O_{24}N_{14}S_4Na_4Cl_6$	1545	83	41.17 / 41.19	1.55 / 1.58	12.68 / 12.70	0.40
D_9	Tobias acid	$C_{53}H_{24}O_{24}N_{14}S_4Na_4Cl_6$	1545	80	41.17 / 41.22	1.55 / 1.59	12.68 / 12.71	0.40
D_{10}	Sulpho tobias acid	$C_{53}H_{22}O_{24}N_{14}S_6Na_6Cl_6$	1749	84	36.36 / 36.41	1.26 / 1.32	11.20 / 11.24	0.46

[a]Determined by TLC using Toluene: Ethyl acetate (7.5: 2.5 v/v) solvent system on Silica gel-G F_{254} TLC plate.

Table 2. Dye-bath containing materials

Materials	For silk	For wool	For cotton
Fabric	2.0 g	2.0 g	2.0 g
Amount of dye	40 mg	40 mg	40 mg
Glauber's salt (20% w/v)	1.0 mL	1.5 mL	1.0 mL
Soda ash (10% w/v)	-	-	1.0 mL
Acetic acid (10% w/v)	1.0 mL	-	-
Formic acid (10% w/v)	-	1.5 mL	-
pH	3	3	8
MLR	1:40	1:40	1:40
Dyeing time	40 min	60 min	90 min
Dyeing temp.	60-80 °C	60-80 °C	60-80 °C
Total volume	80 mL	80 mL	80 mL

Infrared spectra of dyes

IR spectra [20] in general shows characteristic band at 3400-3430 cm^{-1} indicates the N-H and O-H stretching vibrations. The band at 3050-3085 cm^{-1} and 2870-2900 cm^{-1} indicates the C-H stretching vibrations. The strong band observed at 1700-1720 cm^{-1} indicates the C=O stretching vibration. The band at 1440-1445 cm^{-1} shows the C-N stretching vibration. The bands at 1180-1192 cm^{-1} and 1035-1050 cm^{-1} shows S=O asymmetric and symmetric stretching vibrations. The azo and chloro groups are confirmed at the 1370-1385 cm^{-1} and 760-780 cm^{-1} respectively. (IR and ^{1}H-NMR data

are summarized in Table 3).

Table 3. IR and ^{1}H-NMR spectra of dyes D_1 to D_{10}

Dye No.	IR (KBr): v_{max} (cm^{-1})	^{1}H-NMR (Chemical shift in δ ppm)
D_1	3400_{br} (O-H & N-H), 3050_m, 2890_m (C-H), 1700_s (C=O), 1440_s (C-N), 1375_m (N=N) 1190_s, 1045_s (S=O), 760_s (C-Cl).	3.45 (s, 2H, CH_2), 5.07 (s, 2H, 2OH), 9.02 (s, 2H, 2COOH), 9.96 (s, 2H, 2NH), 10.75 (s, 2H, 2NH), 7.82-8.01 (m, 16H, Ar-H).
D_2	3410_{br} (O-H & N-H), 3060_m, 2900_m (C-H), 1710_s (C=O), 1445_s (C-N), 1370_m (N=N), 1185_s, 1042_s (S=O),765_s (C-Cl).	3.45 (s, 2H, CH_2), 5.06 (s, 2H, 2OH), 9.05 (s, 2H, 2COOH), 9.92 (s, 2H, 2NH),10.76 (s, 2H, 2NH), 7.86-8.32 (m, 18H, Ar-H).
D_3	3420_{br} (O-H & N-H), 3060_m, 2900_m (C-H), 1720_s (C=O), 1440_s (C-N), 1375_m (N=N), 1192_s, 1050_s (S=O), 760_s (C-Cl).	3.46 (s, 2H, CH_2), 5.04 (s, 2H, 2OH), 9.02 (s, 2H, 2COOH), 10.75 (s, 2H, 2NH), 7.79-8.01 (m, 28H, Ar-H)
D_4	3420_{br} (O-H & N-H), 3070_m, 2890_m (C-H), 1725_s (C=O), 1445_s (C-N), 1380_m (N=N), 1180_s, 1035_s (S=O), 780_s.	3.44 (s, 2H, CH_2), 5.07 (s, 2H, 2OH), 9.02 (s, 2H, 2COOH), 10.02 (s, 2H, 2NH),10.75 (s, 2H, 2NH), 7.82-8.01 (m,18H, Ar-H)
D_5	3420_{br} (O-H & N-H), 3085_m, 2890_m (C-H), 1700_s (C=O), 1445_s (C-N), 1385_m (N=N), 1185_s, 1035_s (S=O), 780_s (C-Cl).	3.45 (s, 2H, CH_2), 5.07 (s, 2H, 2OH), 9.01 (s, 2H, 2COOH), 9.96 (s, 2H, 2NH), 10.79 (s, 2H, 2NH), 7.82-8.01 (m,16H, Ar-H).
D_6	3400_{br} (O-H & N-H), 3060_m, 2870_m (C-H), 1700_s (C=O), 1440_s (C-N), 1375_m (N=N), 1192_s, 1048_s (S=O), 770_s (C-Cl).	3.45 (s, 2H, CH_2), 5.08 (s, 2H, 2OH), 9.02 (s, 2H, 2COOH), 9.96 (s, 2H, 2NH), 10.78 (s, 2H, 2NH), 7.86-8.09 (m, 16H, Ar-H).
D_7	3420_{br} (N-H), 3055m, 2890_m (C-H), 1710_s (C=O), 1440_s (C-N), 1375_m (N=N), 1195_s, 1030_s (S=O), 755_s (C-Cl).	3.45 (s, 2H, CH_2), 9.02(s, 2H, 2COOH), 9.92 (s, 2H, 2NH), 10.62 (s, 2H, 2NH), 7.79-7.97 (m, 20H, Ar-H).
D_8	3420_{br} (N-H), 3050m, 2890_m (C-H), 1700_s (C=O), 1440_s (C-N), 1380_m (N=N), 1182_s, 1045_s (S=O), 760_s (C-Cl).	3.45 (s, 2H, CH_2), 9.03(s, 2H, 2COOH), 9.92 (s, 2H, 2NH), 10.64 (s, 2H, 2NH), 7.06-8.2 (m, 20H, Ar-H).
D_9	3400_{br} (N-H), 3080m, 2890_m (C-H), 1710_s (C=O), 1440_s (C-N), 1375_m (N=N), 1185_s, 1035_s (S=O), 765_s (C-Cl).	3.43 (s, 2H, CH_2), 9.01(s, 2H, 2COOH), 9.92 (s, 2H, 2NH), 10.81 (s, 2H, 2NH), 7.82-8.61 (m, 22H, Ar-H).
D_{10}	3430_{br} (N-H), 3050_m, 2870_m (C-H), 1700_s (C=O), 1440_s (C-N), 1375_m (N=N), 1192_s, 1048_s (S=O), 780_s C-Cl.	3.42 (s, 2H, CH_2), 9.00(s, 2H, 2COOH), 9.96 (s, 2H, 2NH), 10.78 (s, 2H, 2NH), 7.88-8.63 (m, 20H, Ar-H)

Abbreviations**:** IR: br-broad, m-medium, s-strong
^{1}H NMR: s, singlet; d, doublet; t, triplet; m, multiplet.

Exhaustion and fixation study

The percentage exhaustion [22] of 2% dyeing on silk ranges from 67-75%, for wool ranges from 65-72% and for cotton ranges from 65-73%. The percentage fixation [23] of 2% dyeing on silk fabric ranges from 84-92 %, for wool ranges from 85-93% and for cotton ranges from 85-92%.

All the dyes have good exhaustion value may be expected due to the diffusion of the dye molecule within the fabric proceed rapidly under dyeing condition. Also the introduction of triazine molecule in to the dye improves the exhaustion and fixation value (Table 4).

Fastness properties

All the dyes show generally fair to very good light fastness properties on cotton and wool and moderate to very good for silk. The washing and rubbing fastness for good to excellent fastness on silk, wool and cotton (Table 5).

Table 4. Exhaustion and fixation data of the dyes D_1 to D_{10}

Dye No.	Shade on dyed fibre	λ_{max} nm (H_2O)	λ_{max} nm (H_2SO_4)	Logε (H_2O)	% Exhaustion			% Fixation		
					S	W	C	S	W	C
D_1	Purple	535	520	4.30	75.30	70.90	71.55	91.63	93.08	91.54
D_2	Yellow	462	455	4.20	73.50	68.82	67.65	88.43	89.35	84.99
D_3	Orange	482	472	4.27	70.60	70.47	68.72	85.69	91.52	86.57
D_4	Light yellow	458	445	4.21	69.55	65.55	74.72	89.14	87.71	88.99
D_5	Light purple	522	512	4.24	67.97	66.12	69.45	91.94	85.44	88.55
D_6	Light purple	525	508	4.30	72.60	71.10	69.57	84.02	88.60	87.67
D_7	Greenish yellow	435	410	4.30	69.35	68.00	71.82	85.80	84.55	90.49
D_8	Light yellow	440	425	4.29	75.45	65.27	70.52	90.12	91.91	85.78
D_9	Light yellow	432	418	4.26	69.65	68.40	65.17	89.73	86.94	84.38
D_{10}	Reddish yellow	452	420	4.22	71.23	67.23	72.58	90.25	88.45	87.28

Abbreviations: S-Silk, W-Wool, C-Cotton.

Table 5. Fastness properties data of the dyes D_1 to D_{10}

Dyes No.	Light fastness			Wash fastness			Rubbing fastness					
							Dry			Wet		
	S	W	C	S	W	C	S	W	C	S	W	C
D_1	6	5-6	6	4	4	3	4-5	3	4	3-4	3	4-5
D_2	3-4	5	4	4	4-5	4-5	4	4	3	5	5	4
D_3	4-5	5	4-5	4-5	3	3	4	3-4	5	3	4-5	3
D_4	4	4-5	5	4-5	4	3-4	5	5	3-4	4	5	4
D_5	3	4	5-6	3-4	3	4	3	4	3	3-4	4-5	3-4
D_6	5-6	6	4	5	4	4	4	3	4-5	3-4	5	3
D_7	4	5	4	3	3-4	5	4-5	3-4	4-3	3	3	3-4
D_8	4-5	4	4	4-5	5	3	3	3	3	4	4	5
D_9	3	5	4-5	4	4-5	4-5	3-4	5	4	5	3-4	4-5
D_{10}	5-6	6	6	3	3	4	4	3-4	3	3	4	3

Abbreviations: S-Silk, W-Wool, C-Cotton.
Light fastness: 1-poor, 2-slight, 3-moderate, 4-fair, 5-good, 6-very good.
Wash & Rubbing fastness: 1-poor, 2-fair, 3-good, 4-very good, 5-excellent.

Conclusion

A series of bisazo reactive dyes containing 4,4'-methylene-bis(2,6-dichloro aniline) coupling moiety have been synthesized by conventional method and their colour properties examined by application on silk, wool and cotton fibres. These dyes give yellow to purple hues depending on the coupling components used. The exhaustion and fixation values of all the dyes are very good and show good fastness properties. These dyes are also used as a substitute for benzidine dyes which shows dangerous carcinogen properties.

Acknowledgments

The authors are thankful to Veer Narmad South Gujarat University, Surat, for research facilities. SAIF, Chandigarh for ^{1}H NMR spectra and Atul Limited, Valsad for providing dyeing facilities, fastness tests and important chemicals.

References and Notes

[1] Zollinger, H.; Color Chemistry, 2nd ed. Weinheim: VCH-Wiley, 1991.
[2] Shah, K. M.; Hand Book of Synthetic Dyes and Pigments, Vol. I, 1st ed. Mumbai: Multi-Tech Publishing Co, 1994.
[3] Siegel, E.; In: Venkataraman, K.; Editor. The Chemistry of Synthetic Dyes, Vol. I, New York: Academic Press: 1972.
[4] Bredereck, K.; Schumacher, C. *Dyes and Pigments* **1993**, 21, 23.
[5] Masaki, M.; Ulrich, M.; Zollinger, H. *Journal of Society of Dyers and Colourists* **1988**, 104, 425.
[6] Warnig, M; Hallas, G.; Editor. The Chemistry and Application of Dyes, London: Plenum, 1990.
[7] Konstantinova, T.; Petrova, P. *Dyes and Pigments* **2002**, 52, 115.
[8] Zhan, Z.; Mullner, M.; Lercher, J. A. *Catal. Today* **1996**, 27, 167.
[9] Korkin, A. A.; Bartlett, R. J. *J. Am. Chem. Soc*. **1996**, *118*, 12244.
[10] Stringfield, T. W.; Shephered, R. E. *Inorg. Chim. Acta*. **1999**, *292*, 225.
[11] Zheng, W.; Wong, N.; Zhon, G.; Liang, X.; Jinshan, L.; Tian, A. *New J. Chem.* **2004**, *28*, 275.
[12] Cassela, C. A.; G. P. 1,794,297 **1959** (CA. 58:43253).
[13] Mehta, J. R.; Patel, S. K.; Patel, K. C. *Colourage* **2008**, *10*, 85.
[14] Patel, D. R.; Patel, J. A.; Patel, K. C. *J. Saudi Chem. Soc.* **2009**, *13*, 279.
[15] Fried, B; Sherma, J.; Thin layer Chromatography Techniques and Application. New York and Basel: Marcel-Dekker. Inc., 1982.
[16] Standard Test Method. BS 1006, (U.K.) 1978: ISO 105, (India) 1994.
[17] AATCC Test Method 1961, 8.
[18] Indian Standard. ISO: 1979, 765.
[19] Nafziger, J. L.; US Pat. 4,554,378 **1985**; Cruros, Z.; Ger.Offen. 2,108,402 **1971**.

[20] Colthup, N. B.; Daly, L. H.; Wiberley, S. E.; Introduction to Infrared and Raman Spectroscopy, 3rd ed. New York: Academic Press, 1991.

[21] Shenai, V. A.; Chemistry of Dyes and Principles of Dyeing, Mumbai: Sevak Publication, 1973.

[22] Iyer, V.; Gurjar, A. M.; Bhyrappa, K. S. *Indian J. Fibre and Text*. Res. **1993**, 18, 120.

[23] Venkatraman, K.; Ed. The Analytical Chemistry of Synthetic Dyes, New York: Wiley, 1977.

ISSN 1984-6428
ONLINE
www.orbital.ufms.br

| Vol 3 | | No. 2 | | April-June 2011 |

Full Paper

Synthesis and antimicrobial evaluation of spiro compound containing 1,2,4-triazole and isatin

*Visha P. Modi, Darshan H. Jani and Hasmukh S. Patel**

Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar-388 120, Gujarat, India.

Received: 07 October 2010; revised: 20 January 2011; accepted: 23 April 2011. Available online: 12 September 2011.

ABSTRACT: *The synthesis of 6′-Phenyl-4′-thioxo-3′-(4H-1,2,4-triazole-4-yl)-3′,4′-dihydrospiro [Indoline-3,2′-[1,3,5]oxadiazine]-2-one (**5a-h**) is carried out by two a step reaction, beginning with acid catalyzed condensation of 4H-1,2,4-triazole-4-amine (**1**) with Indoline -2,3-dione (**2**), also called isatin, to obtain 3-(4H-1,2,4-triazole-4-yl-imino)indoline-2-one (**3**) that on reaction with 4-(substituted)-cyclohexa-1,5-diene carbonyl isothiocyanate (**4a-h**) in the presence of anhydrous $ZnCl_2$ gives targeted compounds. The compounds thus obtained, are characterized by IR, ^1H NMR, ^{13}C NMR, and elemental analysis data. The Compounds have been screened for their antimicrobial activity.*

Keywords: *isatin; 4H-1,2,4-triazole-4-amine; 4-R-benzoyl isothiocyanate; antimicrobial screening*

Introduction

The manifold pharmaceutical potential of triazole and oxadiazinone, an antibacterial, antimicrobial, antiviral and anticonvulsant agent is well documented. Spiro [Indole-oxadiazones] are endowed with various pharmacological activities e.g. anti-inflammatory, fungistatic, bacteriostatic and anticonvulsing. A large number of synthetic protocols leading to these compounds are reported in literature [1].

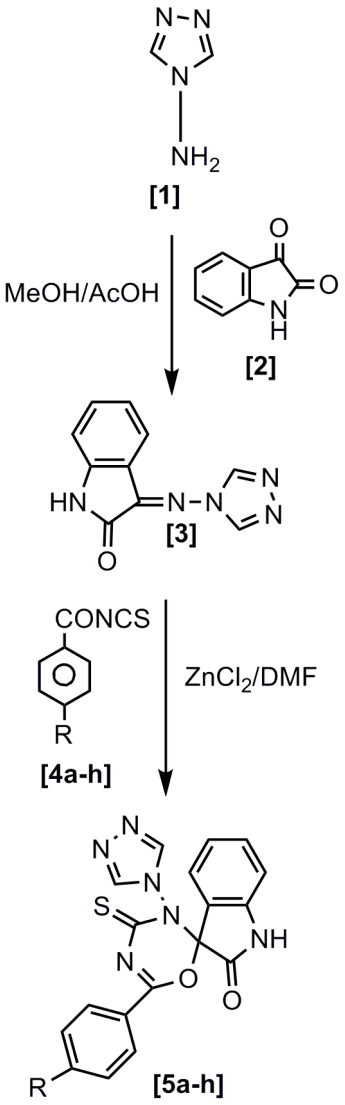
1, 2, 4-triazoles and their derivatives also, are found to possess anticonvulsant [2, 3], antifungal [4-6], anticancer [7-10], anti-inflammatory [11-13] and antibacterial [14-17] activities. The synthesis of these heterocycles has received considerable attention in recent years [18-21]. In present work we are going to develop new compound with

* Corresponding authors: drhspatel786@yahoo.com

potential biological activity.

Isatin derivatives have recently drawn considerable attention of researchers worldwide due to their wide applications as anti-HIV, anti tubercular [22], anti plasmodical [23], anticonvulsant [24], sedative and hypnotic [25] agents. Triazole and another class of azole group is a versatile pharmacophore, possessing diverse pharmacological properties [26, 27]; such as herbicidal [28], antitumor [29], antipsychotic [30], anticoagulant [31], antimicrobial [32] and antagonist [33].

The important biological activities of isatin and triazole derivatives as discussed above impelled us to take up the synthesis of these new combinational heterocycles which are likely to have augmented diverse biological activity. We report here the synthesis of Spiro compounds containing 1,2,4–triazole and isatin and their biological activity.



R= -H, -CH_3, -Cl, -Br, -OCH_3, -NH_2, -OH, -NO_2

Scheme 1

Material and Methods

General

All the chemicals used were of laboratory grade. The triazole and isatin were procured from local market. Melting points were taken in open capillary method and were uncorrected. Purity of the compounds was checked on silica gel TLC plates of 2 mm thickness using chloroform and pet-ether as solvent system. Elemental analysis (%C, H, N) was carried out by Perkin Elmer 2400 CHN elemental analyzer. IR spectra were recorded in KBr pellets on Nicolet 760D spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker Avance DPX 400 spectrometer in $CDCl_3$. ^{13}C NMR spectra were recorded on Bruker Avance DPX 100 spectrometer.

General Procedure

Synthesis of 3-(4H-1, 2, 4-triazole-4-ylimino) indolin-2-one: An equimolar mixture of 4H-1,2,4-triazole-4-amine and isatin were refluxed in methanol (40 mL) in presence of catalytic amount (2-3 drops) of glacial acetic acid for 3 h and then allowed to cool. Schiff bases thus obtained were filtrated from methanol and recrystallized from methanol to give **3** (Scheme 1) whose analytical results are: $C_{10}H_7N_5O$; mol.wt. 213.20, yield: 73%, yellowish brown crystal m.p. 195-197 °C; IR (KBr cm^{-1}) 1667 cm^{-1} (C=N of Schiff base), 1713 cm^{-1} (C=O of isatin), 1642 cm^{-1} (C=N of 1,2,4-triazole). ^{1}H-NMR($CDCl_3$): 8.66 (s, 1H, NH of isatin), 7.35-7.91 (4H, m, Ph.H of isatin), 8.42 (s, 1H, H of 1,2,4-triazole); ^{13}C-NMR: 14.3 (2C-triazole), 168.3 (C_2) (HN-C=O of isatin), 161.1 (C_3, C=N of Isatin), C-Ph. of isatin: 128.9 (C_4), 124.1 (C_5), 130.8 (C_6), 119.1 (C_7), 141.4 (C_8), 117.3 (C_9). Anal. calcd. (Found): C = 56.34 (56.35), H = 3.31 (3.30), N = 32.85 (32.84).

Synthesis of 6'-Phenyl -4'-thioxo-3'-(4H-1,2,4-triazole-4-yl)-3',4'-dihydrospiro [indoline-3,2'-[1,3,5]oxadiazine]-2-one ***(5a):*** A well stirred solution of **3** (Scheme 1) (0.01mole) in dry DMF containing pinch of anhydrous $ZnCl_2$ and benzoyl isothiocyanate (0.02mole) was refluxed for 12 h. Excess of solvent was distilled off under reduced pressure and the residual reaction mixture was cooled and poured into ice-cold water. The separated solid was filtered, washed and recrystallized from ethanol to yield **5a** (Scheme 1).The compound is characterized by IR and NMR data which are: $C_{18}H_{12}N_6O_2S$**;** mol.wt.-376.39; yield: 65%, light yellow crystals, m.p.: 258-262 °C, IR (KBr cm^{-1}) ν 1160 cm^{-1} (C=S), 1738 cm^{-1} (C=O of isatin), 1652 cm^{-1} (C=N). ^{1}H-NMR ($CDCl_3$): 7.48-7.96 (5H, m, Ph.H of Phenyl ring), 8.62(s, 1H, NH of isatin), 8.31 (s, 1H, H of 1, 2, 4-triazole), 7.12-7.41 (4H, m, Ph.H of isatin). ^{13}C-NMR: 144.9 (2C of triazole), 168.1 (C_2) (HN-C=O of isatin), 119.3 (C_3) (C of Spiro), C-Ph. of isatin: 128.7 (C_4), 136.1 (C_5), 127.7 (C_6), 115.5 (C_7), 141.2 (C_8), 132.9 (C_9), 185.1 (C=S), 156.3 (C=N), C-Ar: 135.8 (C_1), 125.8 (2C), 128.5 (2C), 130.8 (C_4). Anal. calc.(Found): C = 57.44 (57.44), H = 3.21 (3.22), N =

22.33 (22.31), S = 8.52 (8.53).

The compounds (**5b-h**) (Scheme1) were synthesized by similar method with minor changes in reflux time. The spectral data of these compounds are given below:

*4′-thioxo-6′-totyl-3′-(4H-1,2,4-triazole-4-yl)-3′,4′-dihydrospiro[Indoline-3,2′-[1,3,5] oxadiazin] - 2-one (**5b**):* $C_{19}H_{14}N_6O_2S$**;** mol.wt: 390.42, yield: 69%, colorless crystals m.p.: 275-276 °C, IR (KBr cm^{-1}) ν 1164 cm^{-1} (C=S), 1714 cm^{-1} (C=O of isatin), 1658 cm^{-1} (C=N). ^{1}H-NMR ($CDCl_3$): 7.44-7.98 (5H, m, Ph.H of totyl), 8.58 (s, 1H, NH of isatin), 8.27 (s, 1H, H of 1, 2, 4-triazole), 7.14-7.38 (4H, m, Ph.H of isatin), 2.34 (3H, m, CH_3). ^{13}C-NMR: 144.4 (2C of triazole), 168.6 (C_2) (HN-C=O of isatin), 118.9 (C_3) (C of Spiro), C-Ph. of isatin: 128.1 (C_4), 135.7 (C_5), 127.5 (C_6), 115.2 (C_7), 141.6 (C_8), 132.6 (C_9), 185.3 (C=S), 156.9 (C=N), C-Ar: 132.5 (C_1), 129.4 (4C), 140.1 (C_4), 21.5 (C-CH_3). Anal. calc. (Found): C = 58.45 (58.46), H = 3.61 (3.60), N = 21.53 (21.52), S = 8.21 (8.20).

*6′-(4-Chlorophenyl)-4′-thioxo-3′-(4H-1,2,4-triazole-4-yl)-3′,4′ dihydrospiro [indoline-3,2′-[1,3,5]-oxadiazin] -2-one (**5c**):* $C_{18}H_{11}ClN_6O_2S$; mol.wt.: 410.84, yield: 67%, colorless crystals, m.p.: 283-284 °C, IR (KBr cm^{-1}) ν 1168 cm^{-1} (C=S), 1734 cm^{-1} (C=O of isatin), 1628 cm^{-1} (C=N), 730 cm^{-1} (C-Cl). ^{1}H-NMR (CDCl3): 7.48-7.72 (4H, m, Ph.H of chlorophenyl), 8.62 (s, 1H, NH of isatin), 8.31 (s, 1H, H of 1, 2, 4-triazole), 7.12-7.41 (4H, m, Ph.H of isatin).^{13}C-NMR: 144.6 (2C of triazole), 168.3 (C_2) (HN-C=O of isatin), 119.1 (C_3, C of Spiro), C-Ph. of isatin: 128.4 (C_4), 136.3 (C_5), 127.8 (C_6), 115.7 (C_7), 141.3 (C_8), 132.7 (C_9), 185.7 (C=S), 156.1 (C=N), C-Ar: 133.3 (C_1), 129.2 (2C), 128.6 (2C), 135.7 (C_4). Anal. calc.(Found): C= 52.62 (52.61), H = 2.70 (2.71), N = 20.46 (20.47), S = 7.80 (7.81).

*6′-(4-Bromophenyl)-4′-thioxo-3′-(4H-1,2,4-triazole-4-yl)-3′,4′-dihydrospiro [indoline-3,2′-[1,3,5]-oxadiazin]-2-one (**5d**):* $C_{18}H_{11}BrN_6O_2S$; mol.wt.: 455.29, yield: 63%, colorless crystal, m.p.:267-268 °C, IR (KBr cm^{-1}) ν 1155 cm^{-1} (C=S), 1738 cm^{-1} (C=O of isatin), 1645 cm^{-1} (C=N), 570 cm^{-1} (C-Br). ^{1}H-NMR (CDCl3): 7.48-7.96 (5H, Ph.H of bromophenyl), 8.62 (s, 1H, NH of isatin), 8.31 (s, 1H, H of 1, 2, 4-triazole), 7.12-7.41 (4H, m, Ph.H of isatin). ^{13}C-NMR: 144.7 (2C of triazole), 168.5 (C_2) (HN-C=O of isatin), 119.5 (C_3, C of Spiro), C-Ph. of isatin: 128.8 (C_4), 136.4 (C_5), 127.6 (C_6), 115.3 (C_7), 141.7 (C_8), 132.4 (C_9), 185.5 (C=S), 156.7 (C=N), C-Ar: 134.6 (C_1), 131.3 (2C), 131.8 (2C), 125.1 (C_4). Anal. calc. (Found): C = 47.48 (47.48), H= 2.44 (2.42), N = 18.46 (18.47), S = 7.04 (7.02).

*6′-(4-methoxyphenyl)-4′-thioxo-3′-(4H-1,2,4-triazole-4-yl)-3′,4′-dihydrospiro [indoline-3,2′-[1,3,5]-oxadiazin]-2-one (**5e**):* $C_{19}H_{14}N_6O_3S$; mol.wt.: 406.42, Yield: 70%, white crystal, m.p.: 271 °C, IR (KBr cm^{-1}) ν 1160 cm^{-1} (C=S), 1724 cm^{-1} (C=O of isatin), 1654 cm^{-1} (C=N), 2835cm^{-1} (C-H of –OCH_3). ^{1}H-NMR (CDCl3): 7.48-7.96 (5H, Ph.H of methoxy phenyl), 3.84 (3H, m, OCH_3), 8.62 (s, 1H, NH of isatin), 8.31 (s, 1H, H of 1, 2, 4-

triazole), 7.12-7.41 (4H, m, Ph.H of isatin). ^{13}C-NMR: 55.6 (C-OCH_3), 144.2 (2C of triazole), 168.8 (C_2) (HN-C=O of isatin), 119.7 (C_3, C of Spiro), C-Ph. of isatin: 128.5 (C_4), 136.5 (C_5), 127.3 (C_6), 115.1 (C_7), 141.5 (C_8), 132.2 (C_9), 185.2 (C=S), 156.4 (C=N), C-Ar: 127.6 (C_1), 129.9 (2C), 114.6 (2C), 163.1 (C_4). Anal. calc. (Found): C = 56.16 (56.15), H = 3.47 (3.49), N = 20.68 (20.67), S = 7.89 (7.88).

6′-(4-aminophenyl)-4′-thioxo-3′-(4H-1,2,4-triazole-4-yl)-3′,4′-dihydrospiro [indoline-3,2′-[1,3,5]-oxadiazin]-2-one (***5f***)*:* $C_{18}H_{13}N_7O_2S$; mol.wt.: 391.41, yield: 65%, yellow crystals, m.p.:278-280 °C, IR (KBr cm^{-1}) ν 1167 cm^{-1} (C=S), 1726 cm^{-1} (C=O of isatin), 1633 cm^{-1} (C=N), 3270 cm^{-1} (C-NH_2 of amino phenyl). ^{1}H-NMR (CDCl3): 7.48-7.96 (5H, Ph.H of aminophenyl), 8.62 (s, 1H, NH of isatin), 8.31 (s, 1H, H of 1, 2, 4-triazole), 7.12-7.41 (4H, m, Ph.H of isatin), 6.13 (2H, s, NH_2).^{13}C-NMR: 144.3 (2C-of triazole), 168.7 (C_2) (HN-C=O of isatin), 119.2 (C_3) (C of Spiro), C-Ph. of isatin: 128.3 (C_4), 136.2(C_5), 127.2 (C_6), 115.9 (C_7), 141.4 (C_8), 132.5 (C_9), 185.6 (C=S), 156.8 (C=N), C-Ar: 125.5 (C_1), 126.7 (2C), 114.1 (2C), 150.4 (C_4). Anal. calc. (Found): C = 55.23 (55.22), H = 3.35 (3.36), N = 25.05 (25.06), S = 8.19 (8.20).

6′-(4-Hydroxyphenyl)-4′-thioxo-3′-(4H-1,2,4-triazole-4-yl)-3′,4′-dihydrospiro [indoline-3,2′-[1,3,5]-oxadiazin]-2-one (***5g***)*:* $C_{18}H_{12}N_6O_3S$; mol.wt.: 392.39, yield: 68%, gray crystals, m.p.: 281 °C, IR (KBr cm^{-1}) ν 1185 cm^{-1} (C=S), 1733 cm^{-1} (C=O of isatin), 1642 cm^{-1} (C=N), 3460 cm^{-1} (O-H). ^{1}H-NMR (CDCl3): 7.48-7.96 (5H, Ph.H of hydroxyphenyl), 8.62 (s, 1H, NH of isatin), 8.31 (s, 1H, H of 1, 2, 4-triazole), 7.12-7.41 (4H, m, Ph.H of isatin), 12.3 (1H, s, OH of hydroxyphenyl). ^{13}C-NMR: 144.8 (2C of triazole), 168.4 (C_2) (HN-C=O of isatin), 119.8 (C_3, C of Spiro), C-Ph. of isatin: 128.6 (C_4), 135.9 (C_5), 127.9 (C_6), 115.6 (C_7), 141.6 (C_8), 132.1 (C_9), 185.8 (C=S), 156.6 (C=N), C-Ar: 128.1 (C_1), 130.5 (2C), 116.1 (2C), 160.7 (C_4). Anal. calc. (Found): C = 55.10 (55.11), H = 3.08 (3.09), N = 21.42 (21.41), S = 8.17 (8.16).

6′-(4-Nitrophenyl)-4′-thioxo-3′-(4H-1,2,4-triazole-4-yl)-3′,4′-dihydrospiro [indoline-3,2′-[1,3,5]-oxadiazin]-2-one (***5h***)*:* $C_{18}H_{11}N_7O_4S$; mol.wt.: 421.39, yield: 68%, yellow crystals, m.p.: 263-264 °C, IR (KBr cm^{-1}) ν 1165 cm^{-1} (C=S), 1742 cm^{-1} (C=O of isatin), 1633 cm^{-1} (C=N), 1550-1570 (N=O of $-NO_2$). ^{1}H-NMR (CDCl3): 8.03-8.38 (4H, Ph.H of nitrophenyl), 8.68 (s, 1H, NH of isatin), 8.27 (s, 1H, H of 1, 2, 4-triazole), 7.08-7.34 (4H, m, Ph.H of isatin).^{13}C-NMR: 144.6 (2C-of triazole), 168.9 (C_2, HN-C=O of isatin), 119.9 (C_3, C of Spiro), C-Ph. of isatin: 128.7 (C_4), 136.1 (C_5), 127.8 (C_6), 115.4 (C_7), 141.2 (C_8), 132.7 (C_9), 185.3 (C=S), 156.7 (C=N), C-Ar: 127.4 (C_1), 123.8 (2C), 141.9 (2C), 150.3 (C_4). Anal. calc. (Found): C = 51.30 (51.31), H = 2.63 (2.62), N = 23.27 (23.25), S = 7.61 (7.60).

Bioassay

Zone of inhibition technique

Antimicrobial Screening:

Antimicrobial activity i.e. antibacterial and antifungal was screened by well or cup method [34] in nutrient agar and dextrose agar medium. Agar medium was sterilized by autoclaving at 15 psi and 120 °C for 20 min. The medium was poured in Petri dishes and left to solidify. These Petri dishes were inoculated with 0.2 mL suspension of organism by spread plate method [35].

Antibacterial activities of all the compounds were studied against gram positive bacteria and gram negative bacteria by agar cup plate method. Methanol system was used as control in this method. Under similar condition, *tetracycline* was used as a standard drug for comparison. The area of inhibition zone was measured in cm. The results of screening for the compounds **(5a-h,** Scheme 1) showed that against the entire microorganisms tested, the compounds **5c, 5e** and **5h** have activity comparable to the standard drugs used.

Table 1. Antibacterial Activity of compounds (**5a-h**)

Compounds	**Zone of inhibition (activity index)[std.]**				
	Gram + ve		**Gram –ve**		
	Bacilius subtilis	*Staphylococcus aureus*	*Klebsiella promioe*	*Salmonella typhi*	*E. coli*
5a	17 (0.65)	16 (0.66)	18 (0.69)	17 (0.68)	16 (0.61)
5b	17 (0.65)	18 (0.75)	17 (0.65)	16 (0.64)	17 (0.65)
5c	23 (0.88)	22 (0.91)	24 (0.92)	23 (0.92)	24 (0.92)
5d	14 (0.53)	12 (0.50)	11 (0.42)	13 (0.52)	12 (0.46)
5e	21 (0.80)	19 (0.79)	22 (0.84)	21 (0.84)	22 (0.84)
5f	13 (0.5)	11 (0.45)	12 (0.46)	11 (0.44)	13 (0.50)
5g	15 (0.57)	13 (0.54)	14 (0.53)	14 (0.56)	15 (0.57)
5h	22 (0.84)	20 (0.83)	23 (0.84)	21 (0.84)	22 (0.84)
Tetracycline	26	24	26	25	26

Activity index = Inhibition zone of compound/Inhibition zone of the standard drug

The Fungicidal activity was studied at 1000 ppm concentration in vitro. The antifungal activity of all the compounds (**5a-h**) was measured on plant pathogenic strains using a potato dextrose agar (PDA) medium. Such a PDA medium contained potato 200 g, dextrose 20 g, agar 20 g and 1 L water. Five day old cultures were employed. The compounds (1000 ppm) to be tested were suspended in a PDA medium and autoclaved at 121 °C for 20 min. at 15 atm. pressure. The product was poured into sterile Petri plates and the organisms were inoculated after cooling the Petri plates. The

percentage inhibition for fungi was calculated after five days using the formula given below:

Percentage of inhibition = 100 (X-Y)/X

Where, X= area of colony in control plate; and Y= area of colony in test plate.

Table 2. Antifungal Activity of Compound (**5a-h**)

Compounds	Zone of inhibition at 1000 ppm (%) (activity index)[std.]				
	Penicillium expansum	*Botrydepladia thiobromine*	*Nigrospora sp.*	*Trichothesium sp.*	*Rhizopus nigricum*
5a	14 (0.50)	13 (0.52)	13 (0.48)	15 (0.57)	15 (0.55)
5b	16 (0.57)	15 (0.60)	16 (0.59)	17 (0.65)	16 (0.59)
5c	24 (0.85)	23 (0.92)	22 (0.81)	24 (0.92)	23 (0.85)
5d	17 (0.60)	16 (0.64)	14 (0.51)	16 (0.61)	17 (0.62)
5e	21 (0.75)	22 (0.88)	20 (0.74)	21 (0.80)	22 (0.81)
5f	13 (0.46)	14 (0.56)	12 (0.44)	13 (0.50)	14 (0.51)
5g	11 (0.39)	12 (0.48)	11 (0.40)	12 (0.46)	11 (0.40)
5h	23 (0.82)	21 (0.84)	20 (0.74)	23 (0.88)	22 (0.81)
Amphotericin B	28	25	27	26	27

Activity index = Inhibition zone of compound/Inhibition zone of the standard drug

Table 3. Minimum inhibitory concentrations (ppm) of the compounds against bacteria

Compounds	Minimum inhibitory concentrations (ppm) of the compounds		
	Bacilius subtilis	*Staphylococcus aureus*	*E. coli*
5a	32	44	50
5b	34	39	40
5c	54	47	44
5e	40	38	37
5g	35	39	36
5h	44	47	45
Ciprofloxacin	0.4	0.6	0.5

Minimum Inhibitory Concentration

The minimal inhibitory concentration (MIC) was ascertained using serial tube dilution technique [36] by variation of compound concentration. The antibacterial and antifungal activity of the control, standard drug (ciprofloxacin) and compounds were screened for different bacterial strains such as *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* as well as fungus such as *Penicillium expansum*, *Botrydepladia thiobromine* and *Rhizopus nigricum*. All the compounds were found to be more potent

against bacterial as well as fungal strains with different MIC.

Table 4. Minimum inhibitory concentrations (ppm) of the compounds against fungus

	Minimum inhibitory concentrations (ppm) of the compounds		
Compounds	*Penicillium expansum*	*Botrydepladia thiobromine*	*Rhizopus nigricum*
5a	30	42	48
5b	32	37	39
5c	52	46	42
5e	38	36	36
5g	33	37	35
5h	42	45	42
Ciloquinol	0.8	1.2	0.8

Results and Discussion

In the present investigation, we aimed at synthesizing the derivatives of 6′-(4-(substituted) phenyl)-4′-thioxo-3′-(4H-1,2,4-triazole-4-yl)-3′,4′-dihydrospiro[indoline-3,2′-[1,3,5]oxadiazine]-2-one **(5a-h,** Scheme 1**)** through a two step process. For this purpose, Schiff base, 3-(4H-1, 2, 4-triazole-4-ylimino) indolin-2-*one* (**3**, Scheme 1) was prepared from acid catalyzed condensation of 4H-1, 2, 4-triazole-4-amine and isatin.

The ^{1}H-NMR of 4H-1,2,4-triazole–4-amine showed the resonance peak of -NH_2 group at δ 5.87 ppm which disappeared in the ^{1}H-NMR spectra of compound **3**, supporting the participation of this group in the Schiff base formation. This is further confirmed with the help of IR spectra. The resonance peak at δ 8.66 ppm due to –NH proton of isatin, further confirmed the formation of **3**. The ^{1}H-NMR and ^{13}C-NMR spectral data of compounds (**5a-h**) are already shown in experimental section. All the compounds showed appropriate NMR resonances for different kinds of protons and carbons. The results of elemental analysis provided further support for the structure of 6′-(4-(substituted)phenyl)-4′-thioxo-3′-(4H-1,2,4-triazole-4-yl)-3′,4′-dihydrospiro[indoline-3,2′-[1,3,5] oxadiazine]-2-one (**5a-h,** Scheme1).

The IR spectra have absorptions at 1160-1185 cm^{-1}(C=S), 1713-1742 cm^{-1} (C=O of isatin), 1633-1658 cm^{-1} (C=N), 1040 cm^{-1} (N-N of triazole), 3250-3300 cm^{-1} (C-H of triazole), 3030-3080 cm^{-1} (C-H of Ar.), 1475-1525 cm^{-1}(C=C of Ar.), 1575-1625 cm^{-1}(C-C of Ar.). Additional absorption band appear at 3460cm^{-1} (O-H), 2835cm^{-1} (C-H of –OCH_3), 730cm^{-1} (C-Cl), 570cm^{-1} (C-Br), 3270cm^{-1} (C-NH_2 of amino phenyl), 1550-1570 (N=O of –NO_2).

It is gratifying to note that IR and NMR spectral data and results of elemental analysis are in agreement with the formation of compounds depicted in Scheme 1. All the compounds were screened for their antibacterial and antifungal activity.

Biological Evaluation

Zone of inhibition technique

The antibacterial and antifungal activity of the compounds (**5a-h**) is shown in tables 1 and 2, respectively. The antibacterial activity was carried out against certain strains of bacteria. The results showed that the compounds synthesized are toxic against the gram positive bacteria (*Bacillus subtilis and staphylococcus aureus*) and gram negative bacteria (*E. coli, Salmonella typhi, and Klebsiella promioe*) at a concentration of 50 µg/mL by agar cup plate method in same solvent. The data in Table 1 indicate that most of the compounds show good biological activity. Further, when the substituents on the phenyl ring were changed, the biological activity showed significant changes. When the phenyl ring had an electron-donating group, the corresponding target compounds showed poor activity, but compounds having electron- attracting group at the phenyl ring gave better activity.

In addition, all the target compounds were screened for antifungal activities in vitro at 1000 ppm concentration. Plant pathogenic organisms used were: *penicilian expansum, Botrydepladia thiobromine, Nigrospora Sp., Trichothesium Sp. and Rhizopus nigricum*. The antifungal activity of these compounds are compared with that of *amphotericin B*.

As shown in Table 2, some of the compounds showed moderate antifungal activities. Substitution of different groups at the phenyl ring could enhance the fungicidal activities. As far as the relation between structure and activity is concerned the chloro, and nitro substituted compounds were found to display enhanced activity than the other substituent. This enhancement in antibacterial activity is rationalized.

Overall activity profile of compounds (**5a-h**) was found to be moderate. We have attempted to increase antimicrobial activities by fusing 4-R-benzoyl isothiocyanate moiety with 3-4H-(1, 2, 4-triazole – 4-yl)imino)indoline-2-one ring system in the present investigation.

Minimum inhibitory concentration

The antibacterial and antifungal activity of the compounds using standard drug ciprofloxacin and ciloquinol respectively screened against different bacterial strains and fungus as stated above. *Staphylococcus aureus* is the preliminary screening test organism of choice for several reasons. Being a systemic pathogen with an ability to develop antibiotic resistance more readily than any other bacteria, the laboratory animals can be readily infected. The inhibition of growth for these Gram-positive organisms produced by various concentrations of the test compounds were compared under identical conditions with the inhibition of growth for same organism by ciprofloxacin (a standard antibiotic showing resistance to the growth of organism).

Similarly, the inhibitions of the Gram-negative organism growth produced by the test compounds were compared with those for same concentrations of ciprofloxacin, which is a broad spectrum antibiotic. A standard volume (5 mL) of Luria broth medium (2%) to support the growth of the test organism was added to several labeled sterile stopper identical assay tubes. A solution of each test compound was prepared in DMSO and a series of dilutions was prepared. Concentrations tested were 0.25–100 ppm of the compounds under investigation, a broad-spectrum antibiotic. A control tube containing no test compound was also included. A 0.1 mL aliquot of the test organism from the overnight grown test cultures was added. All these operations were carefully performed under aseptic conditions. Assay tubes were incubated at 30 ^{o}C for 24 h. The resultant turbidities were measured using a Systronics spectrophotometer model no. 106. The minimum inhibitory concentration (MIC) of a test compound is the lowest concentration showing no visible turbidity. However, the final concentration of bacterial growth inhibition produced by a certain concentration of the test compound was calculated using the following relationship:

Percentage of inhibition = 100 (Tc-Tt)/Tc

Where Tc is the turbidity of the control and Tt is the turbidity of the specific treatment or the test compound.

The same procedure was apply for the Minimum inhibition concentration value of antifungal activity and the data for antibacterial and antifungal activity are summarized in Table 3 and Table 4, respectively.

It was observed that all the compounds shows the comparable antimicrobial activities of these compounds compare to the standard drug ciprofloxacin and ciloquinol and suggest the need for further investigation.

Conclusion

The reaction have introduced the group 1,2,4-triazole and 4-R-benzoyl isothiocyanate in isatin were performed successfully. Their structures were characterized by IR, ^{1}H NMR and ^{13}C NMR spectroscopy. Bioassays showed that the title compounds exhibit good biological and antifungal activities. The compounds **5c, 5e** and **5h** displayed encouraging results as antibacterial and antifungal agents. Data shows that compounds give similar activities with zone of inhibition and minimum inhibitory concentration. It will be interesting to probe in details the medicinal use of these compounds. Further investigations are in progress.

Acknowledgments

We are grateful to authorities of Department of Chemistry, for providing

Laboratory facilities to carry out the research work. Authors thank Sophisticated Instrumentation Centre for Applied Research & Training (SICART), Vallabh Vidyanagar, CVM, Gujarat, for providing the results of elemental analysis (CHN).

References and Notes

[1] D'hooge, M.; De Kimpe, N. *Tetrahedron* **2006**, *62*, 513.
[2] Kane, J. M.; Baron, B. M.; Dudley, M. W.; Sorensen, S. M.; Staeger, M. A.; Miller, F. P. *J. Med. Chem.* **1990**, *33*, 2772.
[3] Küçükgüzel, İ.; Küçükgüzel, Ş. G.; Rollas, S.; Ötük-Sanış, G.; Özdemir, O.; Bayrak, İ.; Altuğ, T.; Stables, J. P. *Il Farmaco* **2004**, *59*, 893.
[4] Rollas, S.; Kalyoncuoglu, N.; Sur-Altiner, D.; Yegenoglu, Y. *Pharmazie* **1993**, *48*, 308.
[5] Chollet, J. F.; Bonnemain, J. L.; Miginiac, L.; Rohr, O. *J. Pestic. Sci.* **1990**, *29*, 427.
[6] Murabayashi, A.; Masuko, M.; Niikawa, M.; Shirane, N.; Futura, T.; Hayashi, Y.; Makisumi, Y. *J. Pestic. Sci*. **1991**, *16*, 419.
[7] Gilbert, B. E.; Knight, V. *Antimicrob. Agents Chemother.* **1986**, *30*, 201.
[8] Holla, B. S.; Veerendra, B.; Shivananda, M. K.; Poojary, B. *Eur. J. Med. Chem.* **2003**, *38*, 759.
[9] Turan-Zitouni, G.; Sıvaci, M. F.; Kılıç, S.; Erol, K. *Eur. J. Med. Chem.* **2001**, *36*, 685.
[10] Bekircan, O.; Kucuk, M.; Kahveci, B; Kolaylı, S. *Arch. Pharm*. **2005**, *338*, 365.
[11] Wade, P. C.; Vogt, B. R.; Kissick, T. P.; Simpkins, L. M.; Palmer, D. M.; Millonig, R. C. *J. Med.Chem.* **1982**, *25*, 331.
[12] Gruta, A. K.; Bhargava, K. P. *Pharmazie* **1978**, *33*, 430.
[13] Modzelewska, B.; Kalabun, J. *Pharmazie* **1999**, *54*, 503.
[14] Malbec, F.; Milcent, R.; Vicart, P.; Bure, A. M. *J. Heterocycl. Chem.* **1984**, *21*, 1769.
[15] Milcent, R.; Vicart, P.; Bure, A. M. *Eur. J. Med. Chem.* **1983**, *18*, 215.
[16] Gülerman, N.; Rollas, S.; Kiraz, M.; Ekinci, A.C.; Vidin A. *Il Farmaco* **1997**, *52*, 691.
[17] Ikizler, A. A.; Johansson, C. B.; Bekircan, O.; Çelik, C. *Acta Polon. Pharm. Drug. Res*. **1999**, *56*, 283.
[18] Buscemi, S.; Vivona, N.; Caronna, T. *J. Org. Chem*. **1996**, *61*, 8379.
[19] Yoo, B. R.; Suk, M. Y.; Yu, Y-Man.; Hong, S-Gyn.; Jung, I. N. *Bull. Korean Chem. Soc*. **1998**, *19,* 358.
[20] Paulvannan, K.; Chen, T.; Hale, R. *Tetrahedron* **2000**, *56,* 8071.
[21] Catarzi, D.; Colotta, V.; Varano, F.; Cecchi, L.; Filacchioni, G.; Galli, A.; Costagli, C.; Carla, V. *J. Med. Chem*. **2000**, *43*, 3824.
[22] Sriram, D.; Yogeeswari, P.; Meena, K. *Pharmazie* **2006**, *61*, 129.
[23] Chiyanzu, I.; Clarkson, C.; Smith, P. J.; Lehman, J.; Rosenthal, P. J.; Chibale, K. *Bioorg. Med. Chem.* **2005**, *13*, 3249.
[24] Verma, M.; Pandey, S. N.; Singh, K. N.; Stables, J. P. *Acta Pharm*. **2004**, *54*, 49.

[25] Smitha, S.; Pandey, S. N.; Stables, J. P. *Acta Pharm.* **2002**, *335*, 129.
[26] Moustafa, O. S.; Ahmed, R. R. *Phosphorus, Sulfur, Silicon and Related Elements* **2003**, *178*, 475.
[27] Mahadevan, K. M.; Basavaray, K. M.; Mathias, D. A. P.; Vaidya, V. P. *Indian J. Chem*. **2005**, *44*, 853.
[28] Nizamuddin. N.; Khan, M. H.; Srivastava, M. K.; Tiwari, S. *Indian J. Chem.* **2000**, *39,* 853.
[29] Ganjee, A.; Yuan, M.; Queener, S. F. *J. Med. Chem.* **1998**, *41*, 4533.
[30] Markourtz, J. S.; Brown, C. S.; Moare, T. R. *Ann. Pharm. Cotha* **1999**, *33*, 73.
[31] Quan, M. L.; Liquw, A. Y.; Pruitty, J. R.; Carini, D. S.; Bostrom, L.; Harrison, K. R.; Knabb, M. *J. Med. Chem.* **1999**, *42*, 170287.
[32] Kedar, R. M.; Vidhale, N. N.; Chincholkar, M. M. *Orient J. Chem.* **1999**, *13*, 143.
[33] Zhang, H. Li.; Chung, J. C.; Costello, T. D.; Valcis, I.; Ward, R. *J. Org. Chem*. **1997**, *62*, 2466.
[34] Simmons, A. Practical Medical Microbiology, 14th Edn. Edinburg: Churchill Livingstone, 1996, Vol. 11, 163.
[35] Bisen, P. S.; Verma, K.; Hand Book of Microbiology, 1st Edn. New Delhi: CBS Publishers and Distributors, 1996.
[36] Pelczar, M. J.; Reid, R. D.; Chan, E. C. S. *Microbiology*, 4th edn. New Delhi: Tata McGraw–Hill, 1979.

ISSN 1984-6428
ONLINE
www.orbital.ufms.br

| Vol 3 | | No. 2 | | April-June 2011 |

Full Paper

Kinetics of phosphotungstic acid catalyzed oxidation of propan-1,3-diol and butan-1,4-diol by *N*-chlorosaccharin

Sanjay K. Singh[a*], *Raman Deep Kaur*[b], *Monika Arora*[c], *Harjeet Gujral*[d], *Gurpreet Kaur*[e]

[a]Department of Chemistry, Govt. T. R. S. College, Rewa 486001 (M.P.), India
[b]Dravidian University, Kuppam, (A.P.), India
[c]S. Govt. Science and Research College Jagraon, Punjab, India
[d]Govt. College Ropar Punjab, India
[e]B. B. S. Polytechnic College, Fathehgarh Saheb, Punjab, India

Received: 16 October 2010; revised: 10 June 2011; accepted: 18 April 2011. Available online: 24 September 2011.

ABSTRACT: *The kinetic studies of N-chlorosaccharin (NCSA) oxidation of propan-1,3-diol and butan-1,4-diol have been reported in presence of phophotungstic acid and in aqueous acetic acid medium. The reactions follow first-order in NCSA and one to zero order with respect to substrate and phosphotungstic acid. Increase in the concentration of added perchloric acid increases the rate of oxidation. A negative effect on the oxidation rate is observed for solvent whereas the ionic strength does not influence the rate of reaction. Addition of the reaction product, saccharin, exhibited retarding effect. Various activation parameters have been evaluated. The products of the reactions were identified as the corresponding aldehydes. A suitable scheme of mechanism consistent with the experimental results has been proposed.*

Keywords: *oxidation; kinetics; phosphotungstic acid; saccharin and propan-1,3-diol*

Introduction

N-Chlorosaccharin (NCSA) as an oxidant with two electron systems has now been acclaimed as another versatile oxidizing agent. NCSA is less hazardous and is easy to handle. The potentiality of this *N*-halo oxidant has remained largely unrealized. Relatively NCSA received a little attention towards the oxidation. *N*-chlorosaccharin has been employed as a mild versatile oxidant by different researchers [1-9]. Phosphotungstic acid (PTA) is efficient and eco-friendly catalyst in oxidation of organic compounds such as

* Corresponding authors: sanjaysinghchemtrs@gamil.com

aromatic amines [10], aromatic alcohols [11], allyl alcohols [12], styrene [13], oximes [14], benzhydrols [15] etc.

The extensive survey of current literature reveals that kinetic oxidation of polyhydroxy alcohols has been studied by various authors with ozone [16], pyridinium bromochromate [17], chloramine B [18], hexacyanoferrate (III) ion [19], BTMAB [20], and TFATB [21]. The aim of this work is to investigate the kinetics and mechanism of PTA catalyzed oxidation of propan-1,3-diol and butan-1,4-diol by NCSA in aqueous acetic acid medium in order to throw some light to the mechanistic aspect of the above title reaction.

Material and Methods

Materials

All the materials employed in this investigation were of analytical grade. The stock solution of synthesized NCSA [22, 23] was prepared by dissolving its sample in 100% acetic acid (BDH). The solutions used were standardized iodometrically. De-mineralized distilled water was used for preparing the solution of hypo, propan-1,3-diol, butan-1,4-diol and other reagents.

Kinetic measurements

The oxidation kinetic runs were performed under pseudo first-order condition. The experiments were carried out in a black coated stopper glass vessel to avoid any photochemical effect. A thermo-stated water bath was used to maintain the desired temperature within ± 0.1K. Requisite volumes of all reagents, except NCSA, were introduced into a reaction vessel and equilibrated at 308 K. A measured volume of NCSA, equilibrated separately at the same temperature, was rapidly poured into the reaction vessel. The progress of the reactions was monitored by periodically examining aliquots of the reaction mixture for unconsumed NCSA iodometrically using starch as the indicator.

Stoichiometry and product analysis

The stoichiometric results indicated consumption of 1 mol of diols consumes 1 mol NCSA as represented by the following empirical equation:

$$R\text{-}CH_2OH + C_6H_4COSO_2NCl + H_2O \rightarrow R\text{-}CHO + C_6H_4COSO_2NH + HCl$$

(R= $HO\text{-}CH_2CH_2$ for propan-1,3-diol and $HO\text{-}CH_2CH_2CH_2$ for butan-1,4-diol)

The corresponding aldehydes $HOCH_2CH_2CHO$, $HOCH_2CH_2CH_2CHO$ and saccharin found as the product of oxidation. These products were identified by forming their 2,4 dinitrophenylhydrazone (DNP). Which were characterized by their melting point (116 ^{0}C and 131 ^{0}C).

Results and Discussion

Oxidation kinetics has been carried out in binary solvent mixture of the acetic acid and water under the reaction condition [NCSA] << [diol] [H^+] [PTA] had the following kinetic feature.

Order with respect to [NCSA]

The order of the reaction with respect to the concentration of NCSA is determined by studying the rate of the reaction at different initial concentrations of the NCSA.

It is evident from the accompanying graph presented in Figure 1 that this reaction is first-order with respect to disappearance of the NCSA.

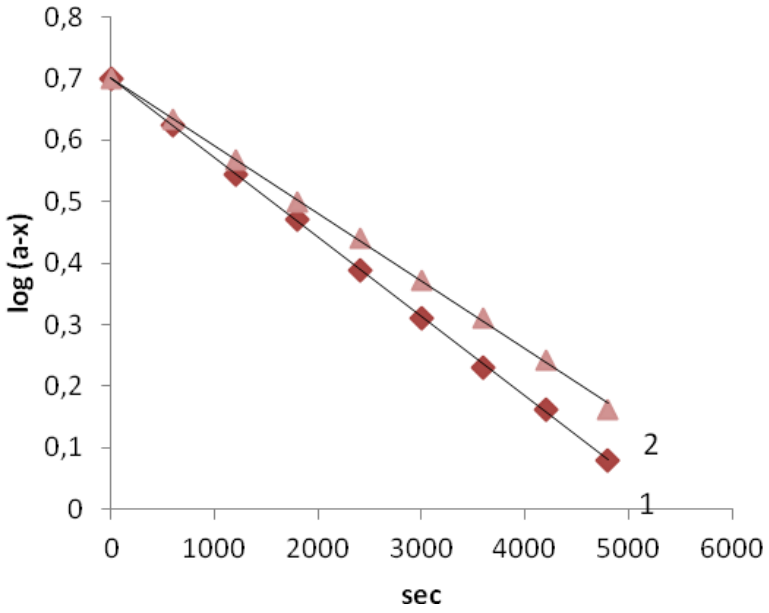


Figure 1. The plot of log (a-x) *versus* time. Conditions are given in Table 1.

Order with respect to [diols]

The plots of k_1 *vs* [diol] for each diol are initially linear passing through origin and tend to obtain limiting value, bending towards horizontal axis (Figure 2). This shows that order with respect to diols falls from 1 to zero at higher concentration of diols. This fact shows that the reactions exhibit 1 to zero order kinetics with respect to all diols indicating Michaelis-Menten kinetics.

Order with respect to [PTA]

Inspection of Figure 4, which is a plot of k_1 *versus* [PTA] demonstrate a kinetic evidence of complex formation and support the first-order dependence.

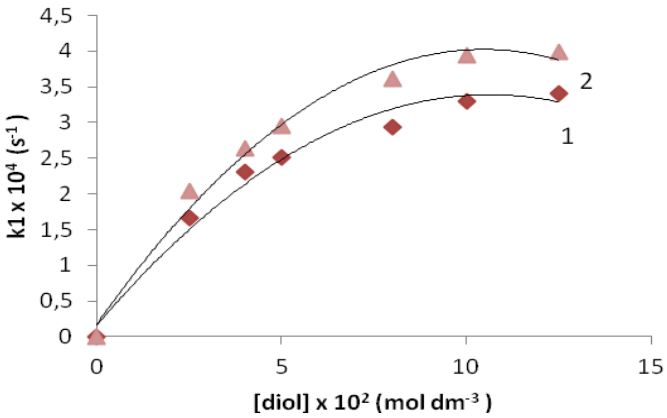


Figure 2. Dependence of k_1 on [diol]. Conditions are given in Table 1.

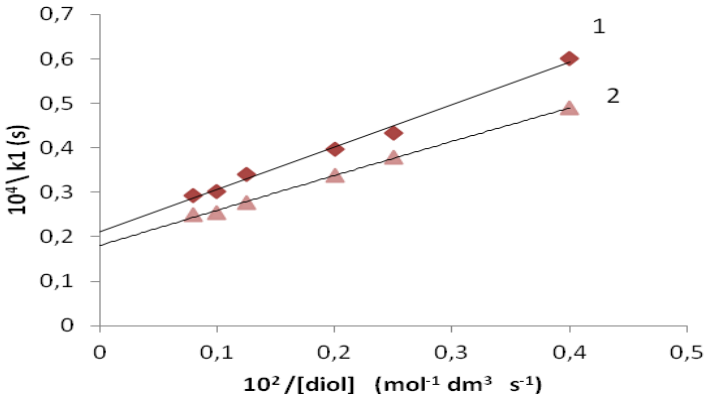


Figure 3. Double reciprocal plot. Conditions are given in Table1.

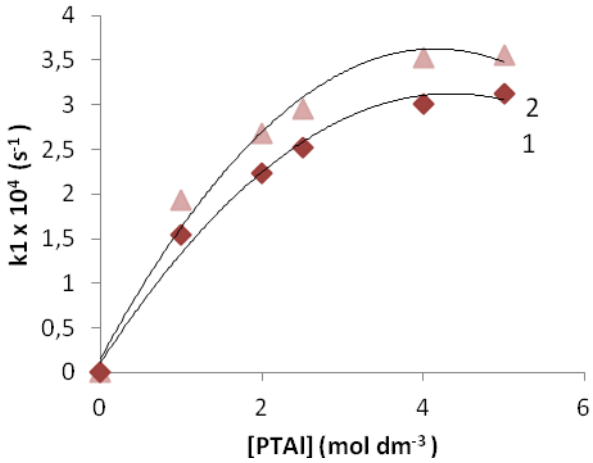


Figure 4. Dependence of k_1 on [PTA]. Conditions are given in Table 1.

Table 1. Effect of variation of reactants on pseudo first-order rate constant k_1 at 308K

10^2 [Substrate] (mol dm^{-3})	10^3 [NCSA] (mol dm^{-3})	$[H^+]$ (mol dm^{-3})	[PTA] (mol dm^{-3})	% HOAc - H_2O	k_1x10^4 (s^{-1})	
					Propan-1,3-diol (1)	Butan-1,4-diol (2)
2.5	2.5	0.15	2.5	30	1.662	2.045
4.0	2.5	0.15	2.5	30	2.302	2.638
5.0	2.5	0.15	2.5	30	2.519	2.959
8.0	2.5	0.15	2.5	30	2.934	3.615
10.0	2.5	0.15	2.5	30	3.302	3.942
12.5	2.5	0.15	2.5	30	3.412	3.987
5.0	1.0	0.15	2.5	30	2.524	2.957
5.0	2.0	0.15	2.5	30	2.527	2.964
5.0	4.0	0.15	2.5	30	2.563	2.972
5.0	5.0	0.15	2.5	30	2.576	2.993
5.0	2.5	0.10	2.5	30	2.201	2.642
5.0	2.5	0.20	2.5	30	2.897	3.625
5.0	2.5	0.25	2.5	30	3.024	3.964
5.0	2.5	0.30	2.5	30	3.402	4.084
5.0	2.5	0.40	2.5	30	3.954	4.612
5.0	2.5	0.50	2.5	30	4.602	5.216
5.0	2.5	0.15	2.5	10	2.953	3.821
5.0	2.5	0.15	2.5	20	2.752	5.534
5.0	2.5	0.15	2.5	40	2.201	2.516
5.0	2.5	0.15	2.5	50	1.864	2.871
5.0	2.5	0.15	1.0	30	1.542	2.104
5.0	2.5	0.15	2.0	30	2.234	2.686
5.0	2.5	0.15	4.0	30	3.012	3.524
5.0	2.5	0.15	5.0	30	3.124	3.562

Effect of variation of $[H^+]$

The catalyzed kinetics was observed by the addition of perchloric acid. On varying perchloric acid concentration there is an increase in reaction rate (Table 1). The plot of log k_1 versus log $[H^+]$ (Figure 5) gave a straight line with negative intercept, suggesting that acid plays a complex role in the reaction system.

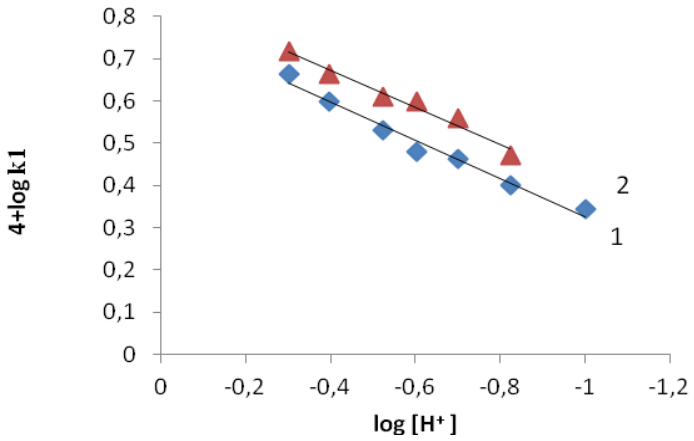


Figure 5. Dependence of k_1 on $[H^+]$. Conditions are given in Table 1.

Variation of dielectric constant, ionic strength, and saccharin

The effect of dielectric constant in reaction medium was studied by adding acetic

acid in the reaction medium at constant concentrations of other reactants. The rate of reaction decreases by increasing the proportion of acetic acid in the solvent medium. The effect of ionic strength has been studied by varying the concentration of neutral sodium perchlorate. It was found that there is no substantial change in the reaction rate on varying the ionic strength. Addition of saccharin (one of the reaction products), at constant NCSA and diol concentration, decreases the rate of reaction. This confirms that HOCl is the main oxidizing species. The retardation of reaction rate on the addition of saccharin suggests a pre-equilibrium step that involves a process in which saccharin is one of the products. If this equilibrium is involved in the oxidation process the retardation should be an inverse function of saccharin concentration.

Induced polymerization of acrylonitrile

The undertaken reactions failed to induce polymerization of acrylonitrile. This indicates the absence of free radical species during the reaction. Hence a free radical mechanism is ruled out.

Activation parameter

Activation parameters are believed to provide useful information regarding the environment in which chemical reactions take place. The effect of temperature on the reaction of propane-1,3-diol and butane-1,4-diol with NCSA were also studied. The value of energy of activation was calculated and the values of ΔS, ΔG were also computed. These values are summarized in Table 2 along with the other parameters.

Table 2. Thermodynamic parameters of diol-NCSA system

Diols	Ea (kJ mol^{-1})	A (s^{-1})	ΔH* (kJ mol^{-1})	ΔG* (kJ mol^{-1})	-ΔS* (JK^{-1} mol^{-1})
Propan-1,3-diol	59.752	1003528	39.5	88.57	185.4
Butan-1,4-diol	57.866	956482	36.8	88.29	188.7

Mechanism and rate law

The reaction is first-order with respect to NCSA. Individual kinetic runs are strictly first-order in NCSA. Further, the first-order rate coefficients do not vary with the initial concentration of the NCSA. The order with respect to the diol is one but tends to zero at higher concentration. Thus Michaelis-Menten type kinetics is observed with respect to diols.

The overall mechanism therefore involves the formation of an intermediate complex before equilibrium and slow disproportionation of the intermediate in the slow step. From the various relevant literatures the different probable steps involved in NCSA system may be summarized as follows:

$$NCSA + H_2O \underset{K_2}{\overset{K_1}{\rightleftharpoons}} HOCl + \text{saccharin (s)} \quad (1)$$

$$HOCl + H^+ \rightleftharpoons H_2\overset{\oplus}{O}Cl \quad (2)$$

$$NCSA + H^+ \rightleftharpoons NCSAH^+ \quad (3)$$

$$NCSAH^+ + H_2O \rightleftharpoons H_2\overset{\oplus}{O}Cl + \text{saccharin} \quad (4)$$

Therefore HOCl, H_2O^+Cl and $NCSAH^+$ are the possible oxidizing species in acidic medium. The retardation of reaction rate with the added saccharin to the reaction mixture rules out the possibility of $NCSAH^+$ as the reacting species. The reaction is acid dependent and it is justified to assume analogous to H_2O^+Br, $H2O^+Cl$ as the reacting species. At the same time experimental evidence indicates HOCl is also an oxidizing species.This leads to the postulation of the following overall mechanism and rate law.

$$RCH_2OH + PTA \overset{K_3}{\rightleftharpoons} C_1 \quad (5)$$

$$C_1 + HOCl \overset{K_4}{\rightleftharpoons} (X_1^{\#}) + H_2O \quad (6)$$

$$C_1 + H_2\overset{\oplus}{O}Cl \overset{K_5}{\rightleftharpoons} (X_2^{\#}) + H_2O \quad (7)$$

$$(X_1^{\#}) \xrightarrow{K_6} RCHO + HCl + PTA \quad (8)$$

$$(X_2^{\#}) \xrightarrow{K_7} RCHO + HCl + PTA + H^+ \quad (9)$$

(R= $HO\text{-}CH_2CH_2$ for propan-1,3-diol and $HO\text{-}CH_2CH_2CH_2$ for butan-1,4-diol)

On the basis of the aforementioned steps involved in the proposed mechanism and at steady state approximation condition, the final rate law is derived as;

$$k_1 = \frac{K_1\,[diol]\,[PTA](\,K_3\,K_4K_6 + K_2\,K_5\,K_7\,[H^+]\,)}{[S]+K_1+K_1K_3\,[diol]\,[PTA]+(K_1\,K_2\,[H^+]\,)\,(1+K_5\,[diol]\,[PTA]\,)} \quad (10)$$

On comparing the values of rate constants obtained in the case of two diol viz. Propan-1,3-diol and butan-1,4-diol; it is quite clear that the rate of complex formation of hypochlorite ester is in the following order, i.e.,

butan-1,4-diol > propan-1,3-diol

The above rate law equation explain fully well the experimental results obtained for the first-order kinetics, the plot of 1/[rate] *versus* 1/[substrate] give rise a straight line with positive intercept which gives the value of *k* and furnishes an evidence for the formation of complex between substrate and reactive species of the oxidant. Thus in both

the cases the rate determining step involves C-H bond fission. Thus degree of agreement again shows the validity of rate law and hence confirms the proposed reaction mechanism. The E_a value is the highest for the slowest. The value of enthalpy shows that reactions are enthalpy controlled.

Conclusion

The oxidation of diols by NCSA is a PTA catalyzed reaction. Phosphotungstic acid makes a ternary complex with substrate and oxidant. This ternary complex decomposes in a slow rate determining step and yields the corresponding aldehydes as product. The negative values of ΔS^* provided support for the formation of a rigid activated complex.

Acknowledgments

We are very thankful to Dr. V. J. Scaria for checking and correcting the language.

References and Notes

[1] Khan, M. U.; Tiwari, R. K.; Verma, J. K.; Gupta, H. D. *Oxid.Commun*. **1997**, *20*, 117.
[2] Singh, V. P.; Khan, M. U.; Chauhan, D. B. S.; Verma, J. K. *Oxid.Commun*. **1997**, *20*, 124.
[3] Khan, M. U.; Verma, J. K.; Parihar, S. S.; Dwivedi, H. P. *Oxid. Commun*. **1998**, *21*, 362.
[4] Khan, M. U.; Nigam, S. K.; Nigam, Gupta, O. P.; Tiwari, A. K. S.; Nigam, A. *Oxid. Commun.* **1999**, *22*, 259.
[5] Khan, S.; Khan, M. U.; Singh, S. K.; Gupta, H. D.; Singh, P. K. *Asian J. Chem.* **2003**, *15*, 595.
[6] Khan, S.; Nigam, S. K.; Khan, M. U.; Dwivedi, H. P.; Singh, P. K. *Asian J. Chem.* **2004**, *16*, 751.
[7] Nigam, S. K.; Khan, M. U.; Tiwari, S.; Dwivedi, H. P.; Singh, P. K. *Asian J. Chem.* **2004**, *16*, 755.
[8] Sharma, V., Sharma, K. V.; Bhagwat, V. W. *E-J. Chem.* **2008**, *5*, 598.
[9] Singh, S. K.; Khan, M. U.; Gupta, H. D.; Baghel, S. S. *Orbital Elec. J. Chem.* **2010**, *2*, 118.
[10] Firouzabadi, H.; Iranpoor, N.; Amani, K. *Green. Chem.* **2001**, *3,* 131.
[11] Firouzabadi, H.; Iranpoor, N.; Amani, K. *Synthesis* **2003**, 408.
[12] Kuznetsova, L. I.; Maksimovskaya, R. I.; Fedotov, M. A. *Inzvestiya Akademii Nauk SSSR.* **1985**, *3,* 537.
[13] Sharma, P.; Patel A. *Indian J. Chem.* **2009**, *48A,* 964.
[14] Jain, N.; Kumar, A.; Chauhan, S. M. S. *Tetrahedron Lett*. **2005**, *46,* 2599.
[15] Bharad, J. V.; Madje, B. R.; Ubale, M. B. *Int. J. Chem. Tech. Research* **2010**, *2*, 346.
[16] Zimin, Y. S.; Trikhanova, N. V.; Shamsuldinov, R. R.; Komissarov, V. D. *Reaction*

Kinetics and Catalysis Letters **1999**, *68*, 237.
[17] Rao, P. S. C.; Suri, D.; Kothari, S.; Banerji, K. K. *Int. J. Chemical Kinetics* **1999**, *30,* 285.
[18] Mohana, K. N.; Prasad, N.; Rangaswamy. *Bulgarian Chemical Communication,* **2006**, *38***,** 293.
[19] Behari, K.; Srivastava, R.; Veena. *J. Chem. Res.(S)* **2001,** 182.
[20] Goswami, G.; Kothari, S.; Banerji, K. K. *Proc. Ind. Acad. Sci.* **2001**, *113*, 43.
[21] Gosain, J.; Sharma, P. K. *Proc. Ind. Acad. Sci.* **2003**, *115*, 135.
[22] Chattaway, F. D. *J. Chem. Soc.* **1905**, *87*, 1884.
[23] Bacchawat, J. M.; Mathur, N. C. *Indian J. Chem*. **1971**, *9*, 1335.

ISSN 1984-6428
ONLINE
www.orbital.ufms.br

| Vol 3 | | No. 2 | | April-June 2011 |

Full Paper

Supported nano gold as a recyclable catalyst for green, selective and efficient oxidation of alcohol using molecular oxygen

Bashir Ahmad Dar[a,b] and Mazahar Farooqui[a]

[a]Post Graduate and Research Center Maulana Azad College Aurangabad India 431001
[b]Indian Institute Of Integrative Medicine(CSIR) Canal Road Jammu India 180001

Received: 07 October 2010; revised: 20 January 2011; accepted: 23 April 2011. Available online: 12 September 2011.

ABSTRACT: *The myth that gold cannot act as a catalyst has been discarded in view of recent studies, which have demonstrated the high catalytic efficiency of pure nano-gold and supported nano-gold catalysts. In recent years, numerous papers have described the use of supported nano-gold particles for catalysis in view of their action on CO and O_2 to form CO_2, as well as a variety of other reactions. Special emphasis is placed on the oxidation studies undertaken on model nano-Au systems. In this work a solvent free oxidation of 1-phenyl ethanol was carried out using gold supported on ceria-silica, ceria-titania, ceria- zirconia and ceria-alumina at 160 ^{0}C. Almost 88-97% conversion was obtained with >99% selectivity. Temperature screening was done from 70 to 160 ^{0}C.Catalysts were prepared by deposition co-precipitation method and deposition was determined by EDEX analysis.*

Keywords: *ceria-silica; ceria-titania; ceria-zirconia; ceria-alumina; gold supported catalyst; catalytic oxidation*

Introduction

As compared to their bulk counter parts, the tiny size of nano-materials instruct considerably different fundamental properties (e.g. adsorption properties, melting point, etc.) to these systems, which makes it applicable to several processes such as catalysis, chemical and bio-detectors, advanced drug delivery systems, enhanced computing systems and opto-electronics etc. In recent years there has been a mounting attention in nano-gold systems [1-6] since particle size is known to have an immense influence on the catalytic properties of gold [7]. Gold exhibits catalytic activity but its use as catalyst

* Corresponding authors: bashir_15_dar@yahoo.com and mazahar.farooqui@gmail.com

is very recent compared to the other metals such as metals those belonging to VIII and IB group (Cu, Ag) of the periodic table [8]. The gold catalyst supported on ceria-alumina promoted by presence of vanadia and molybdena exhibit a high and stable activity in complete oxidation of benzene at low temperature [9]. It has been reported that oxide supported gold has superior catalytic activity over the colloidal form [10]. The oxides like ceria have high oxygen storage capacity [11], which can be enhanced by doping it with some metals like Al and Sm etc. Gold nanoparticles supported on ceria promote the selective oxidation of oximes into the corresponding carbonyl compounds [12]. Therefore we decided to study oxidation of alcohol using oxide supported gold as a catalyst, since catalytic activity of oxide supported gold depends on various factors such as the method of preparation, the solid support, the pre-treatment etc. The catalytic property of gold/ metal oxide interface strongly depends on the nature and textual structure of the support [13]. The present study investigates the oxidation of 1-phenyl ethanol as a model using gold supported on mixed oxides as catalyst.

Ceria was chosen as main constituent of catalyst support for the reason that CeO_2 is having many beneficial properties in catalysis, such as improving the dispersion of surface metals, and store and release oxygen. The later property is expected to help minimizing the pyrophorocity of the metal supported on CeO_2 [14] the two unique features which are responsible for making CeO_2 a talented substance for exercise either as a support or as an active catalyst are (a) the Ce^{3+}/Ce^{4+} redox couple, with its ability to shift between CeO_2 and Ce_2O_3 under oxidizing and reducing environments, respectively, and (b) the ease of formation of labile oxygen vacancies [15]. However, the oxygen storage capacity of pure ceria is inadequate for practical uses. CeO_2 crystallizes in the fluorite structure in which each cerium ion is coordinated to eight oxygen neighbours, making CeO_2 more stable and the reduction of Ce (IV) to Ce (III) is unfavourable. To tackle this problem, one of the best approaches is replacing of another metal/metal oxide into the ceria lattice thus facilitating the formation of composite oxides and replacement of cerium ions by cations of different size and/or charge modifies ionic mobility within the lattice ensuing in the formation of a defective fluorite structured solid solution. Such modifications in the structure of ceria impart new properties to the catalysts, such as density, ionic conductivity and lattice parameters, improved thermal stability, high catalytic activity [16-18]. Supported metals are used in large scale in heterogeneous catalysis. The role of the support is to disperse the metal particles and preserve them from sintering. The supports widely used are SiO_2, Al_2O_3, TiO_2, Nb_2O_5, CeO_2 and ZrO_2. In addition to the dispersion of the metallic components the support can influence the electronic and catalytic properties of the supported metal particles by electron transfer or chemical bond formation. Metal support interactions have been extensively studied by using chemisorptions techniques after the discovery of the strong metal support

interaction (SMSI) effect by Tauster et al. The occurrence of SMSI has been well established for reducible support such as TiO_2 and Nb_2O_5 using chemisorption of hydrogen and carbon monoxide as probe molecules. Ceria supported metals have been investigated for metal support interactions and catalytic applications [19]. Despite large number of investigations, no clear picture seems to have emerged on metal-ceria interactions.

Material and Methods

Preparation of the samples

The first step was to perform a co-precipitation of the metal oxides from metal nitrate solutions. To accomplish this, a solution containing $Ce(NO_3)_3.6H_2O$ (9.314 g) and $SiO(NO_3)_2$ (3.6052 g) was treated with a NH_4OH solution at constant pH 9.0 and room temperature with constant mechanical stirring . The resulting precipitates were aged at the same temperature for 12 h, then filtered and washed until the removal of nitrate ions. The washed precipitates were dried at 100 ^{0}C and calcined under air at 500 ^{0}C for 5 h. The support prepared in this way was denoted as CS. Similarly we prepared ceria-titania (CT) using solution containing 8.6112 g $Ce(NO_3)_3.6H_2O$ and 3.7276 g $TiO(NO_3)_2$: ceria-zirconia (CZ) by using 7.3004 g $Ce(NO_3)_3.6H_2O$ and 3.915 g $ZrO(NO_3)_2$ and ceria-alumina (CA) by using 7.9169 g $Ce(NO_3)_3.6H_2O$ and 6.8432 g $Al(NO_3)_3.9H_2O$. The normal molar ration for all solid supports was 1:1.

To prepare the catalysts Au/CS, Au/CZ, Au/CT and Au/CA, aqueous solutions of $HAuCl_4.3H_2O$ were precipitated by adding 1 N aqueous NaOH at constant pH and temperature upon mixed metal oxides preliminary suspended in water by mechanical stirring. The resulting precipitate was aged at room temperature for 12 h, then filtered and washed carefully until complete elimination of Cl^- ions as detected by using silver nitrate as precipitating agent. The sample was dried at 80 ^{0}C and then calcined at 200 ^{0}C. The samples contained Au/CS = 1.01, Au/CZ = 1.15, Au/CT = 1.79 and Au/CA = 1.34 Wt% gold, as determined by SEM EDEX using instrument SEM Hitachi- S520, Japan; Oxford Link ISIS-300 UK with instrument operated at 98 eV resolution. The results were further confirmed by fluorescence X-ray crystallography using D8 – Advance, Bruker, Germany MultiRes-Vac34 method. So it may be concluded that maximum deposition of the metal is on ceria-titania and this may be the cause of highest percentage conversion by Au/CT as shown in Table 1. The metal loading on these supports depends on the surface area of support and the available sites. Thus ceria-titania avails it better than the other supports used

Catalytic tests

The reactant used for the present work was of analytical grade obtained from SD

fine chemicals. The reaction was carried out in two neck glass reactors fitted with a condenser in one neck whilst the other neck was closed by a rubber septum through which a syringe needle was passed to supply oxygen (O_2). Reactant (2 mL) was placed in the reactor without solvent, together with 50 mg of catalyst with constant stirring. Molecular oxygen was passed through syringe needle. The reaction was run for 4 h at 160-170 ^{0}C. The quantitative analysis was done by using HPLC C-R8A chromatopac shimadzu model SPD-10A with UV-Visible detector and LC 18. A Shimadzu column and methanol as eluent.

Results and Discussion

As a first stage of our work, we selected 1-phenylethanol as a model compound to study the activity of ceria based mixed oxide supported nano-gold catalyst for selective oxidation using molecular oxygen in solvent free conditions.

For the present work, temperature screening was done from 70 to 160 ^{0}C and no reaction was observed even up to 155 ^{0}C and therefore 160 ^{0}C is the threshold temperature for this reaction. The catalyst was recycled for many times and it was observed that there is little activity lost which was recovered by washing the catalyst with piranha solution [20]. Almost 97 % conversion is achieved by using these catalysts (Table 1) which is much better than previously reported [21-22].

Scheme 1

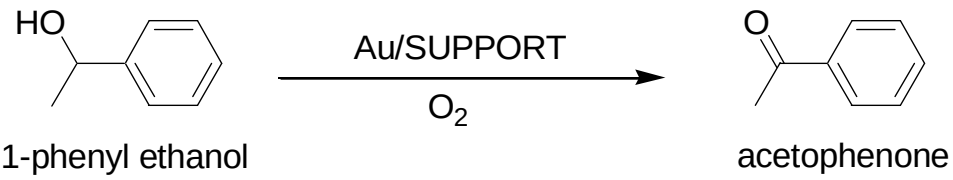


Table 1. Percentage conversion using catalyst over different supports

Catalyst	Time in hours	Percentage conversion	Selectivity %
Au/CS	4	92	>99
Au/ CT	4	97	>99
Au/ CZ	4	88	>99
Au/ CA	4	96	>99

Conclusion

Hence it can be concluded that nano gold supported on CeO_2-M_2O_3 is a suitable, efficient, and selective catalyst for the oxidation of organic compounds in aerobic conditions. The process is heterogeneous solvent free, and catalysts can be reused without decay. Over and above we can propose that the process is environment friendly, economic and thus green.

Acknowledgments

We are grateful to CSIR, New Delhi, for generous financial support to accomplish this work.

References and Notes

[1] Bolin, Z. R. J.; Angelici, R. J. *J. Am. Chem. Soc.* **2006**, *128*, 14460
[2] Bond, G. C.; Thompson, D. T. *Appl. Catal. A: Gen.* **2006**, *302*, 1.
[3] Thompson, D. T. *Top. Catal.* **2006**, *38*, 231.
[4] Chen, M. S.; Goodman, D. W. *Catal. Today* **2006**, *111*, 22.
[5] Kung, M. C.; Costello, C. K.; Kung, H. H. *Catalysis* **2004**, *17*,152.
[6] Hutchings, G. J.; Haruta, M. *Appl. Catal. A: Gen*. **2005**, *291*, 2
[7] Choudhary, T. V; Goodman, D. W. *Topics in Catalysis* **2002**, *21*, 13.
[8] Berndt, H.; Martin, A.; Pitsch I.; Prusse U.; Vorlop K. D. *Catal. Today* **2004**, *91*, 191.
[9] Andreeva, D.; Petrova P.; Sobczak, J. W.; Ilieva, L.; Abrashev, M. *Appl Cat. B: Env*. **2006**, *67*, 237.
[10] Mirescu, A.; PruBe, U. *Cat. Comm.* **2006**, *7*, 11.
[11] Donka, A.; Margarita, K.; Ivan I.; Lyuba, I.; Janusz, W. S.; Wojciech, L. *Catal. Today* **2010**, *10*, 101.
[12] Abdessamad, G.; Avelino, C.; Hermenegildo, G. *J. Catal.* **2009**, *268*, 350.
[13] Michela, V.; Jordi, L.; Marta, B.; Carla, D. L.; Alessandro, T. *J. Rare Earth* **2009**, *27*, 196.
[14] Elise, S.; Bickford, S. V.; Chunshan, S. *Prepr. Pap. Am. Chem. Soc., Div. Fuel Chem*. **2003**, *48*, 810.
[15] Trovarelli, A.; Leitenburg, C.; Dolcetti, G. *Chem. Tech*. **1997**, *27*, 32.
[16] Sugiura M. *Catal. Surveys Asia* **2003**, *7*, 77.
[17] Fallah, J. E.; Boujana, S.; Dexpert, H.; Kiennemann, A.; Majerus, J.; Touret, O.; Villain, F.; Normand, F. L. *J. Phys. Chem.* **1994**, *98*, 5522.
[18] Bernal, S.; Calvino, J. J.; Cauqui, M. A.; Gatica, J. M.; Larese, C.; Perez, O. J.; Pintado, J. M. *Catal. Today* **1999**, *50*, 175.
[19] Bunluesin, T.; Gorte, R. J.; Graham, G. W. *Appl. Catal. B: Environ*. **1997**, *14*, 105.
[20] Vijayender, B.; Sandro, C.; Claudio, S.; Bruno, S. *Thin Solid Films* **2010**, *518*, 3360.
[21] Gadi, R.; Gabriela, B.; Yoel, S. *Tetrahedron* **1999**, *55*, 6301.
[22] Giordano, L.; Tullio, P.; Alessandro, S.; Alessandra, S. *Tetrahedron Asymmetry* **2008**, *19*, 1363.

ISSN 1984-6428
ONLINE
www.orbital.ufms.br

| Vol 3 | | No. 2 | | April-June 2011 |

Full Paper

Mechanically-induced solvent-less synthesis of cobalt and nickel complexes of cimetidine

Adedibu C. Tella, Uche B. Eke, Aaron Y. Isaac and Christianah A. Ojekanmi*

Department of chemistry, P.M.B.1515, University of Ilorin, Nigeria

Received: 22 November 2010; revised: 17 March 2011; accepted: 04 May 2011. Available online: 26 September 2011.

ABSTRACT: Solvent-less synthesis of $[Co(CIM)_2](SO_4)$ and $[Ni(CIM)_2](OAC)_2$ by *grinding of* $CoSO_4$ *and* $Ni(CH_3COO)_2.4H_2O$ *with cimetidine without any solvent is described. The complexes have been characterized by elemental analysis, melting point, AAS, conductivity measurements, TLC, infrared and UV-Vis spectroscopies as well as X-ray powder diffraction. Cimetidine was found to be bidentate or tridentate ligand. Cobalt ion coordinate with cimetidine through the sulphur atom in the thiol group, nitrogen atom of imidazole ring and the nitrogen atom of the secondary amine to give an octahedral geometry with ligand acting as tridentate whereas nickel ion coordinates through the sulphur atom in the thiol group, nitrogen atom of imidazole ring to give tetrahedral structure with ligand acting as bidentate. X-Ray diffraction patterns of the complex were different from that of the ligand suggesting formation of coordination compounds. The method is quick and gives a quantatively yield, without the need for solvents or external heating. Clearly, it can present higher efficiency in terms of materials, energy and time compared to classical solution phase synthesis.*

Keywords: *solvent-less synthesis; cimetidine; X-Ray diffraction pattern; mechanochemistry; green chemistry*

Introduction

Due to problems of environmental pollution associated with solvent disposals and shrinking energy resources, there is a need to develop benign synthetic pathways which are simple and exhibit high atom economy [1]. Indeed the idea that "no reaction proceeds without solvent", ascribed to Aristotle, has guided much laboratory synthesis both historically and to present day. However, in recent years solvent-less chemistry has gained much currency in both academic and industrial laboratories [2]. For instance, one of the major targets in green chemistry is to limit the extensive use of solvents or even

* Corresponding authors: ac_tella@yahoo.co.uk

better to carry out the synthetic reaction in the absence of them [3, 4]. Solvent free or solvent less reactions (system in which neat reagents react together, in the absence of a solvent) are thought to occur in the solid phase [5]. The problem of releasing volatile organic compounds (VOCs), low yield and slow reaction can be avoided by this method. Solvent-free synthesis involves the use of mechanochemistry or melt-phase methods. Mechanochemical method ranges from simply grinding reactants with mortar and pestle to ball-milling process [6]. Melt-phase is the induction of the reactants thermochemically by the direct application of heat or the converse of other forms of energy into thermal energy. This helps to initiate and sustain the chemical reactions due to a rise in temperature. A lot of work has been done on solvent-free synthesis in metal-organic frameworks [7-9], but few report regarding metal-drug complexes appeared in literature [10]. Nichols et al. [11] reported mechanochemical synthesis of $[Ni(phenathroline)_3]^{2+}$. The complex was synthesized in only two minutes by manually grinding nickel nitrate with phenanthroline. Pichon et al. [7] described the first solvent less mechanochemical synthesis of a microporous metal-organic framework (MOF) $[Cu(INA)_2]$ (INA–isonicotinic acid). The reaction occurred within 10 minutes by simply grinding hydrated copper acetate $Cu(CH)_3CO_2.2H_2O$ and isonicotinic acid. The formation of a reaction product was indicated by a change in colour from green to blue and the characteristic odor of acetic acid, released as by-product. Balema et al. [12] obtained 98% *cis*-$PtCl_2(PPh_3)_2$ by grinding $PtCl_2$ and PPh_3 together in a ball-mill for one hour . The first solvent-free synthesis of metal-drug complexes was reported by Braga et al. [10]. Zinc and copper complexes of neuroleptic drug gabapentin were synthesized by Braga and co-workers by simply grinding of solid gabapentin and the inorganic salts of $ZnCl_2.2H_2O$ and $CuCl_2.2H_2O$. The structure of the product was characterized by comparing its melting point, ^1H NMR, ^{13}C NMR, and IR spectra with the authentic method. This method has a higher yield with low reaction time.

Cimetidine (CIM) is an imidazole derivative used as antihistamine H_2–receptor drug in the treatment of ulcer. It is the most commonly used H_2–blocker drugs in famotidine [13]. Cimetidine (2-Cyano-1-methyl-3-(2-[5-methyl-1H-imidazol-4yl]methylthio)guanidine), is also used in the treatment of patients with gastric and duodenal ulcers and other hypersecretory conditions [14]. It is a chelating agent. Its chelating ability is due to the presence of two electron-donating nitrogen and sulphur atoms separated by two carbon atoms [15, 16]. Metal complexes cimetidine are reported in the literature [17-20]. Most of these complexes have been synthesized by the traditional method using volatile organic compounds (VOCs) as solvents. In continuation of our studies of the synthesis of metal complexes of biologically active molecules in the absence of solvent [21], we report the synthesis of Co (II) and Ni (II) complexes of cimetidine without the use of solvent. To the best of our knowledge, mechanochemical

solvent induced synthesis of cimetidine metal complexes has not been reported in the literature.

Material and Methods

Materials and instrumentation

All reagents and chemicals were of analytical grade and used as obtained from Aldrich. Cimetidine was obtained as gift from Sam Pharmaceutical Ltd, Ilorin, Nigeria. Metal salts used include nickel acetate [$Ni(CH_3COO)_2.4H_2O$], cobalt sulphate. Infra-red Spectra were obtained from samples in the form of KBr pellets using a Pelkin Elmer FTIR spectrometer. Metal analyses were determined by atomic absorption spectroscopy with Pelkin-Elmer Spectrometer, model 3110. UV-Vis spectra were obtained on Aquamate v4.60 spectrophotometer. The analyses of carbon, hydrogen and nitrogen were carried out on a Pelkin-Elmer 204C microanalyser. Powder XRD analysis was performed on a Syntag PADS diffractometer at 294K using Cu Kα radiation (λ=1,54059Å) obtained at Energy Centre Obafemi Awolowo University, Ile-Ife, Nigeria.

Experimental Procedure

Synthesis of Co(CIM)$_2$](SO$_4$) complex: 2 mmol (0.504 g) of cimetidine and 1 mmol (0.281 g) of cobalt sulphate ($CoSO_4$) were carefully weighed into an agate mortar which has been washed and dried before use. The reactants were ground together for fifteen minutes to a fine paste. The reaction was monitored by TLC using chloroform/methanol (9:1) till no traces of reactants were found. The compound was purified by recrystallization from ethanol. The equation of the reaction is shown below (eq. 1).

Synthesis of [Ni(CIM)$_2$](OAc)$_2$.H$_2$O: 2 mmol (0.504 g) of cimetidine and 1 mmol (0.248 g) of nickel acetate [$Ni(CH_3COO)_2.4H_2O$] were weighed into agate mortar which has been washed and dried before use. The reactants were ground together for fifteen minutes. The reaction was monitored by TLC using chloroform/methanol (9:1) till no traces of reactants were found. The compound was purified by recrystallization from ethanol. The equation of reaction is shown below (eq.2).

$$MX_2.nH_2O + \text{Cimetidine} \xrightarrow[\text{fifteen min. room tempt. no solvent}]{\text{Grinding}} [M(CIM)_2]\,X_2\,H_2O \quad \text{or} \quad (1)$$

$$MX_2.nH_2O + \text{Cimetidine} \xrightarrow[\text{fifteen min. room tempt. no solvent}]{\text{Grinding}} [M(CIM)_2]\,X_2 \quad (2)$$

M = Cu, Ni $x_2 = (OAc)_2, SO_4$

Results and Discussion

The Co (II) and Ni (II) complexes of cimetidine were synthesized by reaction of metal salts with cimetidine by grinding.

The method produces in most cases 76-78% yields as shown in table within a shorter time of 15 minutes as compared to 50-60 % by classical solution phase which usually takes 3 hours for reaction to complete (17, 18, 20).

The complexes were characterized by AAS, conductivity, TLC, infrared, UV-Vis spectroscopy and XRPD. The complexes are generally soluble in methanol, ethanol, DMSO but insoluble in non-polar organic solvents. The physical properties of the various complexes are collected in Table 1. The complexes are also non-hygroscopic solids with melting points higher than the parent drug. The molar conductance values measured in methanol (10^{-3} M) for these complexes are 106 and 119 Ω^{-1} mol^{-1} cm^2 for the Co and Ni complexes respectively (Table 1). The results showed that the complexes are electrolytes because of the high conductivity values. The presence of free sulphate ions were estimated by means of $BaCl_2$ as corresponding barium salts. The test is positive which indicates that sulphate ions are outside the coordination sphere. The presence of acetate ions outside the coordination sphere was detected by addition of 1:1 (H_2SO_4:H_2O) to the Ni (II) complex which gave an odor of vinegar. Determination of stoichiometric ratio using job's method suggest a 1:2 metal to ligand stoichiometry for the complexes. Elemental analysis of the complexes for C, H, N are consistent with the proposed formulae as shown in Table 1. The physical properties and stoichiometric ratio data for the two complexes were in good agreement with that obtained for Pd (II) and Pt (II) cimetidine complexes [21]. The proposed structures of the complexes are shown in figures 6 and 7.

Table 1. Results of physical properties, melting point, conductivity, TLC and % metal

Ligand/mixture	Colour	m.p. (°C)	Conductivity $\Omega^{-1}mol^{-1}cm^2$	TLC R_f	Metal %	Yield %	CHN %Found (Calc)
Cimetidine (ligand)	White	142	0.7	0.64	-	-	**-**
$[Co(CIM)_2]SO_4$	Light pink	200	106	0.49	8.35	78	39.4, 4.6, 26.3 (38.4 , 4.9, 25.5)
$[Ni(CIM)_2](OAC)_2.H_2O$	Mint green	230	119	0.56	8.24	76	40.3, 6.0, 23.2 (41.2 , 5.7, 24.0)

UV-Visible spectroscopy results

The UV-Visible spectra and the assignments of the cimetidine ligand and its complexes are shown in figures 1-3 and Table 2.

The UV-Visible spectra of cimetidine in methanol present two absorption band

maxima at 256 and 296 nm assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions respectively within the organic ligand. The cobalt (II) complex exhibits 3 bands at 367, 585 and 850 nm. These bands are assigned to $^4T_{1g} \rightarrow {}^4T_{1g}(P)$, $^4T_{1g} \rightarrow {}^4A_{2g}$ and $^4T_{1g} \rightarrow {}^4T_{2g}$ transitions respectively nickel (II) complex exhibits 2 bands centered at 425 and 575 nm which may be tentatively assigned considering Td symmetry around the metal to $^3T_1(F) \rightarrow {}^3T_2(F)$ and $^3T_1(F) \rightarrow {}^3A_2(F)$ transitions respectively. All these characteristic bands observed in the UV-VIS. Spectra confirm octahedral configuration for Co (II) complex and tetrahedral configuration for Ni (II) complex [22].

These modes are in agreement with data obtained for Co (II)CIM and Ni (II)CIM synthesized in a solvent medium [19].

Table 2. UV-Visible spectra of cimetidine (CIM) metal complexes

Complexes/ligand	***Wavelength (nm)***	***Energies (cm^{-1})***	**Extinction (ε)**	***Assignment***
CIM	196	51020	266	$\pi \rightarrow \pi^*$
	241	41493	564	$\pi \rightarrow \pi^*$
$[Co(CIM)_2]SO_4$	367	27247	208	$^4T_{1g} \rightarrow {}^4T_{1g}(P)$
	585	12239	609	$^4T_{1g} \rightarrow {}^4A_{2g}$
	850	11764	281	$^4T_{1g} \rightarrow {}^4T_{2g}$
$[Ni(CIM)_2](OAc)_2 H_2O$	425	23529	288	$^3T_1(F) \rightarrow {}^3T_2(F)$
	575	17391	535	$^3T_1(F) \rightarrow {}^3A_2(F)$

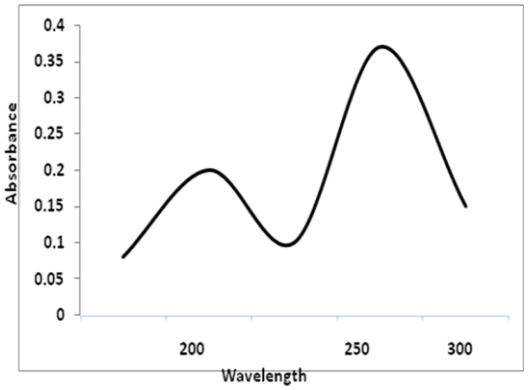


Figure 1. UV-visible spectra of cimetidine.

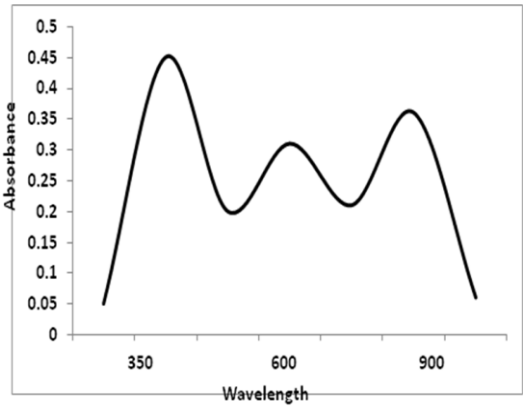


Figure 2. UV-visible spectra of $[Co(CIM)SO_4$.

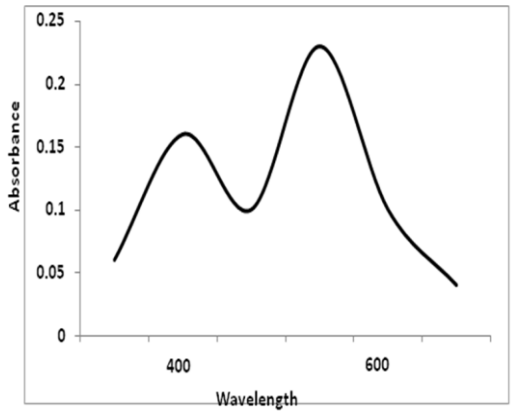


Figure 3. UV-visible spectra of $[Ni(CIM)_2](OAc)_2.H_2O$.

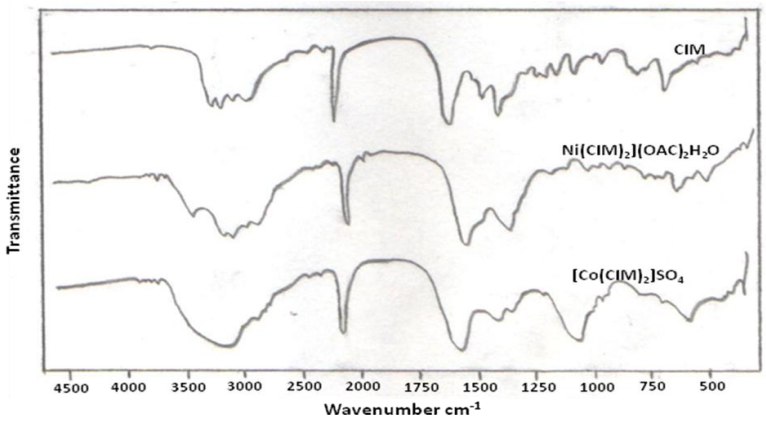


Figure 4. Infrared spectra of cimetidine and its complexes.

Table 3. Infrared spectra result for cimetidine (CIM) and its metal complexes

Ligand/Complex	' v(N-H)	v(C=N)	v(C=N-C=C)	v(C≡N)	v(C-S)	vM-N	vM-S
CIM (Ligand)	3225-3144	1592	1456	2176	797-678	-	-
$[Co(CIM)_2]SO_4$	3270	1598	1425	2171	618	453	312
$[Ni(CIM)_2](OAc)_2H_2O$	3227-3146	1588	1400	2174	877-677	458	315

Cimetidine has several potential donor atoms but due to the steric constraints, the ligand can provide a maximum of 3 donor atoms to any of the metal center. The donor atoms are sulphur atom in the thiol group, nitrogen atom of imidazole ring and the nitrogen atom of the secondary amine. The infra-red spectra of cimetidine and its complexes as shown in Figure 4 and Table 3 have been assigned mainly for those specific frequencies directly involved in complex formation. The vibrational band at 3225cm^{-1} assigned to vN-H in the ligand shifted higher wave number (3270 cm^{-1}) in $[Co(CIM)_2]SO_4$ complex; this is probably due to coordination of metal via nitrogen of N-H group in the complex. The observation of these bands at the same wavenumber in the IR of the Ni (II) complex suggests the inertness of this group toward coordination with nickel. The band at 1486 cm^{-1} corresponding to v(C=N-C=C) in the cimetidine is shifted to lower wave number 1400 cm^{-1} for Ni (II) complex and 1425 Co (II) complex, thus indicating coordination through v(C=N) of the imidazole group. Furthermore the v(C=N) stretching vibration in the ligand is shifted from 1592 cm^{-1} to 1598 cm^{-1} in the $Co(CIM)_2]SO_4$ and 1588 cm^{-1} in the $[Ni(CIM)_2](OAc)_2H_2O$. The changes in both bands v(C=N-C=C) and v(C=N) suggests the coordination of both metal ions through nitrogen of the imidazole group. The principle behind this phenomenon is due to the donation of the unpaired electrons from one of the nitrogen ones to the metal (II) ion. The absorption spectra of these complexes are similar to those of imitidine metal complexes synthesized in solvent

medium [17-19]. No significant change are observed in the band at 2187 cm^{-1} assigned to v(C≡N) of the nitriles group, indicating that this group is not involved in coordination of both complexes.

The first peak of 797-678 cm^{-1} due to v(C-S) was shifted to lower frequency of 618 cm^{-1} and the second to higher frequency 877 cm^{-1} in the complexes. The metal-sulphur and metal-nitrogen bands are indicated by the appearance of new bands of v(M-S) and v(M-N) stretching vibration observed around 310-315 cm^{-1} and 450-458 cm^{-1}, respectively, in the spectra of complexes. This is probably due to coordination of metal ion through the sulphur of the thiol group and nitrogen atom of the imidazole in the cimetidine. The presence of water of crystallization in nickel complex is revealed by broad band at 3475 cm^{-1} and the results of elemental analysis also supported the presence of water of crystallization.

Structure of the complexes

The spectroscopic results of this study revealed that coordination of cimetidine occurs through the sulphur atom of thiol, nitrogen atom of the imidazole group and nitrogen atom of secondary amine for $Co(CIM)_2]SO_4$ complex to give an octahedral geometry, with ligand acting as tridentate. This mode of coordination has also been found in copper (II) complexes of cimetidine in solution [18, 23].

On the other hand, for $[Ni(CIM)_2](OAc)_2H_2O$ complex, the coordination probably occurs through the nitrogen of the imidazole and sulphur atom of thiol to give tetrahedral geometry, ligand acting as bidentate. This mode of coordination is in agreement with the data obtained from the synthesis of $[Ni(CIM)_2](OAc)_2H_2O$ in solvent medium [19, 20, 24].

Evidence of formation of complex was demonstrated by comparing the powder X-Ray diffraction pattern of the cimetidine with that of $Co(CIM)_2]SO_4$ complex as shown in Figure 5.

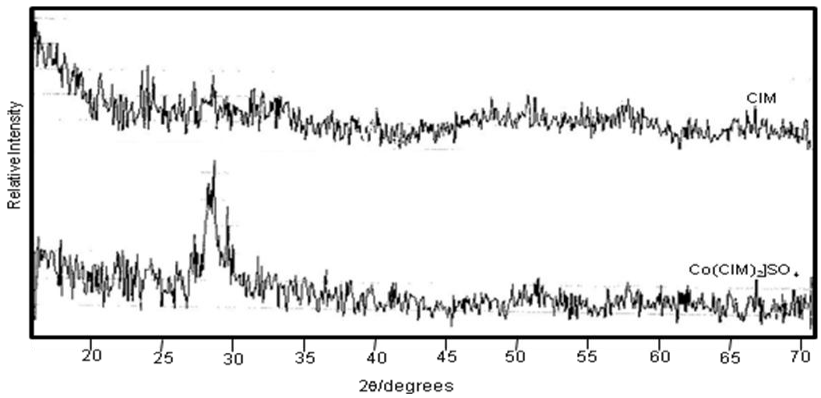


Figure 5. Powder X-Ray diffraction pattern of the cimetidine and $[Co(CIM)_2]SO_4$.

The X-Ray powder diffraction pattern of the mixture of cimetidine and

$Co(CIM)_2]SO_4$ was different from the diffraction patterns of the ligand (cimetidine). The peaks observed for the ligand (cimetidine) are $2\Theta^o$: (24.14, 28.09, 30.34, 31.02, 51.92, 58.65). The peaks present in the $[Co(CIM)_2]SO_4$ are $2\Theta^o$: (22.71, 23.58, 25.57, 32.77, 32.96). Some values observed for the ligand are absent in the $[Co(CIM)_2]SO_4$ which shows that complexation of cimetidine with cobalt might have occurred. The proposed structures of the complexes are shown in figures 6 and 7.

Proposed structures of cimetidine metal complexes

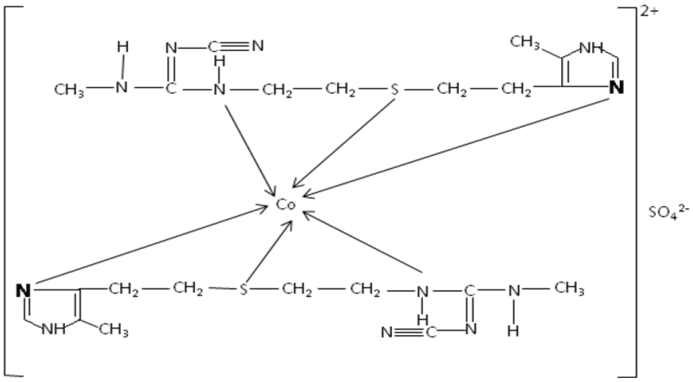


Figure 6. Proposed structure of $[Co(CIM)_2]SO_4$.

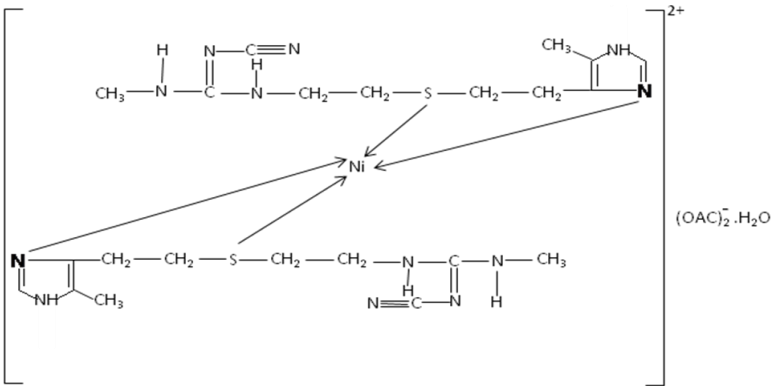


Figure 7. Proposed structure of $[Ni(CIM)_2](OAc)_2H_2O$.

Conclusion

Nickel and cobalt complexes of cimetidine drug have been synthesized simply by grinding solid cimetidine molecule and the inorganic salts of nickel and cobalt. The structure of the product formed was characterized by elemental analysis, AAS, conductivity, TLC, infrared, UV-Vis spectroscopy and XRPD. In all the complexes formed, the ligand coordinated through the nitrogens of the amine group, sulphur atom and nitrogen of the cyanide group. The ligand being tridendate is assumed to coordinate with cobalt ion forming an octahedral geometry. Two donors atom (sulphur atom of thiol group and nitrogen atom of the imidazole group) are involved in coordination of cimetidine with nickel ion. Nitrogen atom of the secondary amine is not involved in

coordination. For $[Ni(CIM)_2](OAc)_2H_2O$ complex, tetrahedral geometry is proposed, cimetidine acting as bidentate. Our spectroscopic data and mode of coordination are in agreement with synthesis of the complexes in solvent medium. In summary, we showed that cimetidine metal complexes can be prepared in high yields in shorter time during mechanically-induced solvent-less conditions. The mechanochemical technique appears to be convenient and extremely efficient experimental tool which opens a pathway to new processes where chemical transformations are performed in an environmentally benign manner.

Acknowledgments

We are grateful for financial support from University of Ilorin Senate research grant committee.

References and Notes

[1] Cave, G. V. W.; Raston, C. L.; Scott, J. L. *Chem. Commun.* **2001**, 2159.
[2] Bala, M. D.; Coville, N. J. *J. Organomet. Chem.* **2007**, *692*, 709.
[3] Mikhailanko, M. A.; Shakhtshneider, T. P.; Boldyrev, V. V. *J. Malaria Science* ***2004***, *39*, 5435.
[4] Rasmussen, M. O.; Axelsson, O.; Tanner, D. *Synth. Commun*. **1997**, *27*, 4021.
[5] Rottenberg, G.; Downie, A. P.; Raston, C. L.; Scott, J. L. *J. Am. Chem. Soc*. **2001**, *123*, 8701.
[6] Mashkouri, S.; Naimi-Jamal, M. R. *Molecules* **2009**, *14*, 474.
[7] Pichon, A.; Lazuen–Garey, A.; James, S. L. *Cryst. Eng. Comm*. **2006**, *8*, 211.
[8] Carson, C. G.; Hardcastle, K.; Schwartz, J.; Liu, X.; Hoffman, C.; Gerhardt, R. A.; Tannenbaum, R. *Eur. J. Inorg. Chem*. **2009**, 2338.
[9] Vuan, W.; Friscic, T.; Apperley, D.; James, S. L. *Angew. Chem. Int. Ed.* **2010**, *49*, 3916.
[10] Braga, D.; Grespions, F.; Maini, L.; Brescello, R.; Catairea, L. *Cryst. Eng. Comm*. **2008**, *10*, 469.
[11] Nichols, P. J.; Paston, C. L.; Steed, J. W. *Chem. Commun*. **2001**, 1062.
[12] Balema, V. P.; Wiench, J. W.; Prusky, M.; Percharsky, V. K. *Chem. Commun*. **2002**, 1606.
[13] Myers, D. *J. Exotic Pet. Medicine* **2006**, *15*, 150.
[14] Hussain, A.; Habib, S. S. (2007). *Pak. J. Physiol*. **2007**, *3*, 8.
[15] Penston, J.; Wormsley, G. *Adverse Drugs Exp*. **1986**, *1*, 192.
[16] Naveh, Y.; Weis, P.; Chung, H. R.; Bogden, J. D. *J. Nutri*. **1987**, *117*, 1576.
[17] Amigo, J. M.; Reventos, M. M.; Sancho, A.; Soto-Tuero, L.; Cantarero, A. *Zeitchrift fir Kristallographie* **1987**, *180*, 123.
[18] Bianucci, A. M.; Dermatin, F.; Monassero, Masciocchi, M.; Ganadu, M. L.; Naldini, L.; Panzarell, A. *Inorg. Chim. Acta* **1991**, *182*, 197.
[19] Abadia, A.; Sancho, A.; Soto, L.; Borras, J. *Transition Met. Chem.* **1986**, *11*, 8.

[20] Kanumfre, F.; Lima, E. M.; Scheidt, G.; Carneiro, P. I. B.; Rosso, N. D. *J. Braz. Chem. Soc.* **2010**, *21*, 800.

[21] Tella, A. C.; Isaac, A. Y.; Adeniran, R. A. *International Journal of Applied Chemistry* **2010**, *In Press*.

[22] Onoa, G. B.; Moreno, V.; Freisinger, E.; Lippert, B. *J. Inorg. Biochem.* **2002**, *89*, 237.

[23] Lever, A. B. Inorganic Electronic Spectroscopy. Amsterdam: Elsevier, 1984.

[24] Soto, L.; Borras, J.; Sancho, A.; Fuertes, A.; Miravitlles, C. *Act. Cryst.* **1985**, *41*, 1431.

ISSN 1984-6428
ONLINE
www.orbital.ufms.br

| Vol 3 | | No. 2 | | April-June 2011 |

Full Paper

Degradation dynamics of quinalphos on cabbage under subtropical conditions of Ludhiana, Punjab, India

Gurminder S. Chahil, Gurmail Singh, Urvashi Bhardwaj, Reenu Takkar, Raminderjit S. Battu and Balwinder Singh*

Pesticide Residue Analysis Laboratory, Department of Entomology, Punjab Agricultural University, Ludhiana, Punjab 141004, India

Received: 26 November 2010; revised: 20 March 2011; accepted: 23 Mach 2011. Available online: 27 September 2011.

ABSTRACT: *A study was undertaken to determine the disappearance trends of quinalphos residues on cabbage under subtropical conditions at Ludhiana. Single application of quinalphos was made @ 500 and 1000 g a.i. ha^{-1} on cabbage and samples were collected at intervals of 0 (1 h), 1, 3, 5, 7, 10 and 15 days after application. Average initial deposits of quinalphos on cabbage were found to be 0.41 and 0.75 mg kg^{-1} at single dose and double dosages, respectively. Residues of quinalphos dissipated below determination limit of 0.01 mg kg^{-1} in 7 and 10 days, respectively at single and double dose. The half-life of quinalphos on cabbage was observed to be 3.02 and 2.70 days for single and double dose, respectively. The study suggested a waiting period of 7 days for safe consumption of cabbage.*

Keywords: *quinalphos; residues; cabbage; persistence; waiting period*

Introduction

Cabbage (*Brassica oleracea var. capitata* L.) is an important cole crop of India. This is widely cultivated throughout the sub-tropical parts of north India, with annual production of 3.39 million tonnes [1]. In Punjab, the area under cabbage is 3.34 thousand hectares with a production of 73.23 thousand tonnes [2]. Cabbage is heavily attacked by many insect pests, resulting in severe loss of quality and production. The major insect pests responsible for crop losses are stem borer (*Hellula undalis*), diamond-back moth (*Plutella xylostella)*, leaf eating caterpillars and aphids [3, 4]. A number of organochlorine, organophosphate, carbamate and pyrethroid insecticides have been

* Corresponding authors: gurminderchahil77@yahoo.co.in

recommended against various insect pests of cabbage. Quinalphos, *O,O*-diethyl-*O*-quinoxalin-2-yl-phosphorothioate (Figure 1) is a selective insecticide, intensively used by the farmers in many parts of India as a crop protection measure . It is solid at low temperature having melting point 31.5 °C and is very toxic to mammals (LD_{50} 71 mg kg^{-1}) [5]. It is recommended (@ 500 g a.i. ha^{-1}) for the management of diamond-back moth and tobacco caterpillar on cabbage [6]. Accumulation of quinalphos residues in cabbage pose human health hazards as quinalphos is very toxic chemical [7]. Studies revealed that in India about 50-70% of vegetables are contaminated with insecticides residues [8], mainly due to harvesting the crops before the safe waiting period. Waiting period between the time of application and harvesting of vegetables reduced the risk of harmful effects of insecticides on consumer [9]. Therefore, the present investigation was carried out to study the dissipation of quinalphos on cabbage and soil, with the objective to estimate safe waiting period.

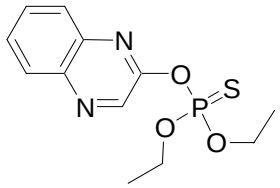


Figure 1. Structure of quinalphos.

Material and Methods

Chemicals and reagents

The certified reference standard of quinalphos (purity 99.2%) was obtained from Dr Ehrenstorfer, India. All the solvents used in this study were of analytical grade. Before use these were redistilled in all glass apparatus and their suitability was ensured by running reagent blanks along with actual analysis. Stock solution of quinalphos was prepared at 1000 µg mL^{-1} in hexane. Working standards ranging from 0.10 to 2.0 µg mL^{-1} were prepared by appropriate dilutions and stored at 4 °C.

Field experiment and collection of samples

The field experiment was conducted at Entomological Research Farm, Punjab Agricultural University, Ludhiana during 2009. Cabbage (var. Drum head) was raised according to recommended agronomic practices [5]. There were three replications for each treatment i.e. untreated control (T_1), Quinalphos 25 EC @ 500 g a.i. ha^{-1} (T_2) and Quinalphos 25 EC @ 1000 g a.i. ha^{-1} (T_3). Single application of quinalphos 25 EC was made @ 500 and 1000 g a.i. ha^{-1} on cabbage crop at 50 percent head formation using knapsack sprayer equipped with triple action nozzle. Water used to dissolve the formulation was @ 500 mL ha^{-1}. Untreated control plots were sprayed with water only.

The experiments were laid out in randomized block design (RBD). Analysis of cabbage samples were carried out on 0, 1, 3, 5, 7, 10 and 15 days after the application of insecticide. Soil samples were collected after 15 days of the application. The characteristics of field soil were sand 78.0%, slit 10.2%, clay11.8%, organic carbon 0.30%, EC 0.30 dsm^{-1} and pH 8.0.

Extraction and clean-up of residues

The cabbage samples were harvested from each plot, pooled together, packed in plastic bags and transported to laboratory for processing. A total of 2 kg sample were collected for each treatment. Samples were cut into small pieces, mixed in a waring blender and a representative 50 g chopped and macerated cabbage sample was dipped overnight into 100 mL acetone in an erlenmayer flask. The extract was filtered into 1 L separatory funnel along with rinsings of acetone. The filtrate in the separatory funnel was diluted with 600 mL brine solution and partitioned the contents into with dichloromethane (2 × 75 mL) and hexane (2 × 75 mL). Dichloromethane and hexane fractions were combined, dried over anhydrous sodium sulfate and treated with 500 mg activated charcoal powder for about 2-3 hours at room temperature. The clear extract so obtained was filtered through Whatman filter paper No.1, concentrated to near dryness and again added about 20 mL hexane and concentrated using rotary vacuum evaporator at 30 ^{o}C. Repeated the process to completely evaporate dichloromethane and the final volume was reconstituted to about 5 mL using hexane. A representative 50 g soil sample were taken for analysis and processed as per the method described above without any charcoal cleanup.

Estimation of residues

Gas chromatographic (GC) analysis was carried out on gas chromatograph (Shimadzu GC 2010) equipped with a FTD and capillary column (Rtx-5, 30.0 m × 0.25 mm ID, 0.25-µm film thickness). The column temperature was initially held at 190 °C for 2 min., raised to 220 °C at a rate of 10 °C min^{-1} and held for 10 min. Other conditions were: injector temperature 290 °C, detector temperature 300 °C, gas flow: nitrogen- 30 mL min^{-1} ; hydrogen -3.0 mL min^{-1} and zero air -145 mL min^{-1}. Under these operating conditions the retention time of quinalphos was found to be 9.98 min. The 2 µL extracts were injected onto the GC column in a pulsed splitless mode.

Confirmation of residues

The residues of quinalphos on cabbage were confirmed by gas chromatograph mass spectrometer (GC-MS) in selective ion monitoring (SIM) mode. The gas chromatograph (Shimadzu-QP 2010) with autoinjector, equipped with mass spectrometer and capillary column Rtx-5 Sil MS (30 m × 0.25 mm i.d. × 0.25 µm film thickness) was

used to verify the results. The system software used was GCMS solution version 2.5. The GC-MS operating conditions were: injector temperature 285 °C, oven initial temperature was 80 °C and held for 3.0 min, raised to 180 °C at a rate of 20 °C min^{-1} and held for 2 min, then ramped 5 °C min^{-1} to 260 °C, ion source temperature 200 °C, interface temperature was 290 °C. Helium was used as a carrier gas with a flow rate of 0.94 mL min^{-1}. The fragmentation of quinalphos produced selective m/*z* ions 118, 146, 156, 157 and 269. The treated samples also showed the presence of these ions, it confirms the presence of quinalphos.

Calculation of residues

The residue content was calculated by using the formula:

$$\text{Residues in ppm (µg/g)} = \frac{A_1 \times C \times V_1}{A2 \times W \times V_2}$$

Where,

A_1 = Peak area of the sample, in chromatogram

A_2 = Peak area of the standard, in chromatogram

V_1 = Total volume of the sample (mL)

C = Concentration of analytical standards in ng

W = Weight of sample (g)

V_2 = Injected volume of the sample (µL)

Recovery studies

Recovery assays were performed by spiking blank samples prior to extraction at three different concentration levels viz. 0.05, 0.10 and 0.15 mg kg^{-1}, with three replicates for each level. Recoveries were calculated by the formula that the actual measured concentrations were compared against the expected concentrations in the blank samples. Mean recoveries of quinalphos were found to be consistent and more than 80% (Table 1).

Results and Discussion

The results of dissipation of quinalphos in cabbage and soil are presented in Table 2 (Figure 2). Average initial deposit of quinalphos on cabbage were found to be 0.41 and 0.75 mg kg^{-1}, in recommended dose and double the recommended dose, respectively. About 40-43 % dissipation of quinalphos was observed after 24 hours of spraying. After 5 days, the residues further reduced to 0.10 and 0.20 mg kg^{-1}, respectively, in single dose and double dose. The residues of quinalphos dissipated below its determination limit of 0.01 mg kg^{-1} in 7 and 10 days at recommended and double the recommended

dosages, respectively. Soil samples collected 15 days after the spraying did not reveal the presence of quinalphos residue (Table 2). The half-life of quinalphos on cabbage were observed to be 3.02 and 2.70 days, respectively, in single and double dose. The residues were confirmed on GC-MS with m/*z* ratio 146. The identified ions were 118, 146, 156, 157 and 269 (Figure 3).

Table 1. Recovery of quinalphos on cabbage and soil

Substrate	Level of fortification (mg kg^{-1})	% Recovery*
Cabbage	0.15	90.44 ± 1.39
	0.10	88.67 ± 2.08
	0.05	88.33 ± 2.31
Soil	0.15	85.31 ± 1.42
	0.10	87.26 ± 2.65
	0.05	88.73 ± 3.09

* Mean ± SD of the three replication

Table 2. Residues of quinalphos (mg kg^{-1}) on cabbage and soil at different time intervals after application of quinalphos 25 EC @ 2000 mL and 4000 mL ha^{-1}

Days after application	Quinalphos @ 500 g a.i. ha^{-1}			Quinalphos @ 1000 g a.i. ha^{-1}		
	Replicates	Mean±S.D.	Dissipation (%)	Replicates	Mean±S.D.	Dissipation (%)
Before application	BDL BDL BDL	BDL	-	BDL BDL BDL	BDL	-
0 (1 hr after spray)	0.40 0.42 0.40	0.41 ± 0.01	-	0.75 0.73 0.76	0.75 ±0.02	-
1	0.25 0.22 0.22	0.23 ± 0.02	43.90	0.44 0.46 0.46	0.45±0.01	40.00
3	0.20 0.20 0.22	0.21 ± 0.01	48.78	0.38 0.38 0.35	0.37 ± 0.02	50.66
5	0.10 0.12 0.10	0.10 ± 0.01	75.61	0.21 0.20 0.20	0.20 ± 0.01	73.33
7	BDL BDL BDL	BDL	-	0.10 0.12 0.11	0.11 ± 0.01	85.33
10	BDL BDL BDL	BDL	-	BDL BDL BDL	BDL	-
15	BDL BDL BDL	BDL	-	BDL BDL BDL	BDL	-
Soil samples after 15 days	BDL BDL BDL	BDL	-	BDL BDL BDL	BDL	-
$T_{½}$ (days)		3.02			2.70	

BDL= Below determination limit of 0.01 mg kg^{-1}

In a persistence study of quinalphos on cabbage [4] the initial deposits of quinalphos was found to be 1.59 and 3.62 mg kg^{-1}, respectively, when quinalphos was applied @ 0.05 and 0.10 % concentration and calculated waiting period and half-life were

2.5 and 4.2 days and 1.3 and 1.3 days for lower and higher dosages, respectively. In another study [1] it was observed that following four applications of quinalphos @ 500 and 1000 g.a.i. ha^{-1}, the residues of quinalphos dissipated below determination limit of 0.01 mg kg^{-1} in 10 days, in both the treatments. The results were in conformity with findings of present work. On cauliflower curds the initial deposits of quinalphos was observed to be 2.28 and 3.94 mg kg^{-1} in single and double dose, respectively [10]. The variations in initial deposits reported by other researchers may be due to variation in insecticide formulation, growing season, variety of crop, method of analysis and weather conditions.

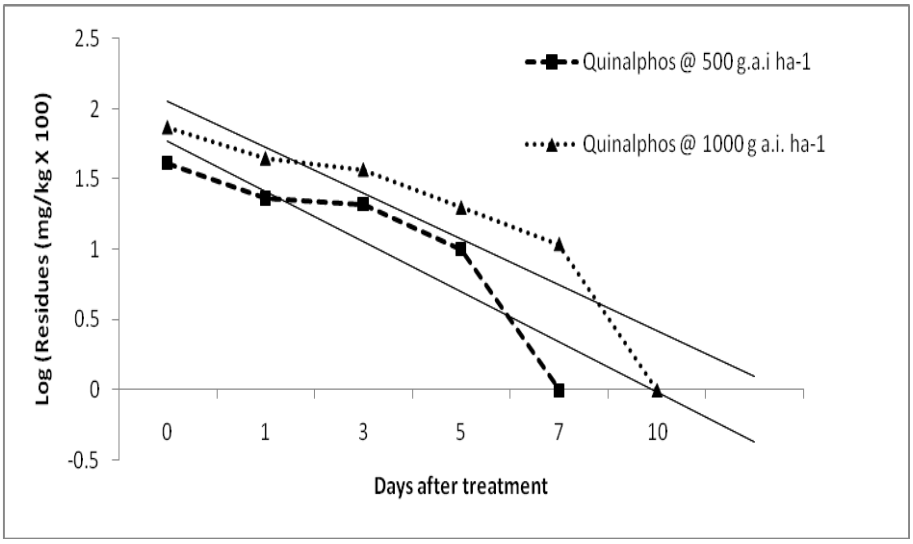
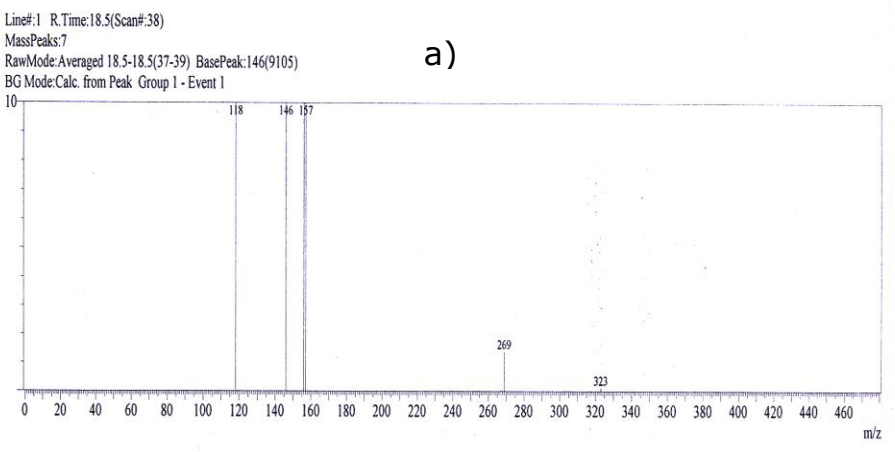


Fig.2 Semi-logarithm graph showing dissipation kinetics of quinalphos on cabbage. Regression equation y = -0.36 x + 2.13 (single dose) and y = -0.33 x + 2.38 (double dose).



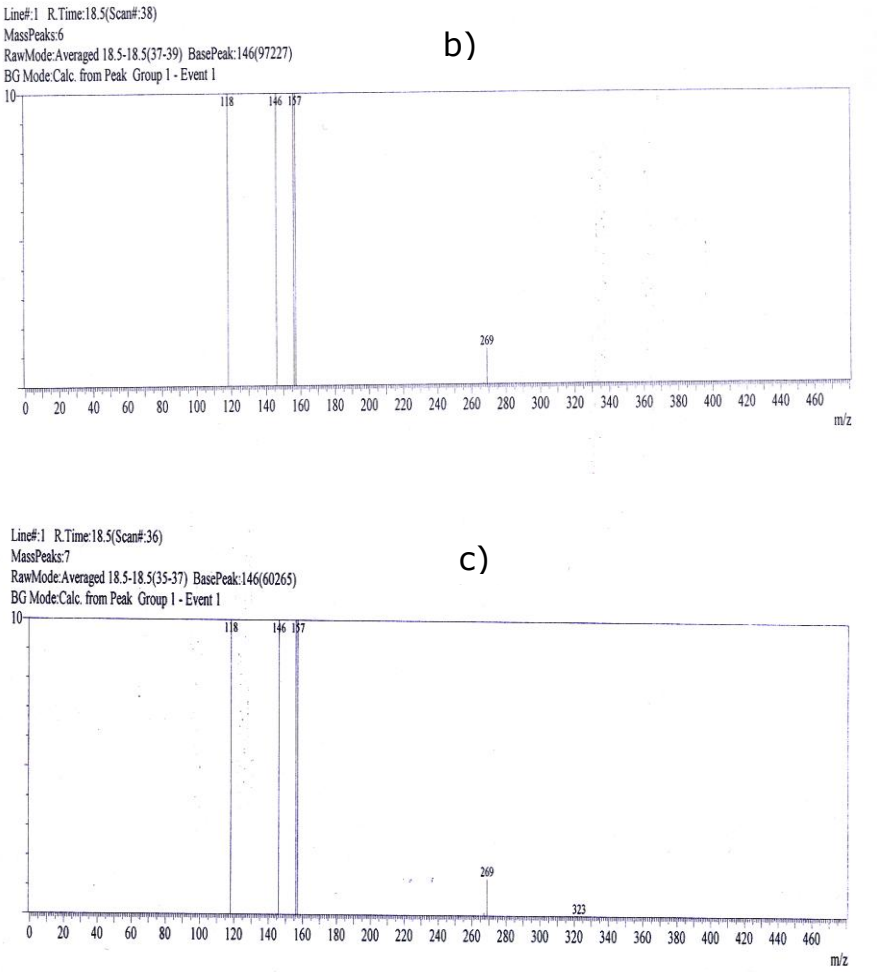


Figure 3. Mass spectra of quinalphos a) standard; b) Treated sample (0 day) single dose; c) Treated sample (0 day) double dose.

Conclusion

Quinalphos is intensively used by the farmers in northern India as plant protection in cabbage. The half life values of quinalphos were 3.02 and 2.70 days, in recommended and double the recommended dosages, respectively, which showed the persistence nature of the quinalphos. The safe waiting period 7.0 days is suggested for recommended dose of quinalphos on cabbage. Therefore, quinalphos could be used as a protective measure in cabbage without any risk of toxic residues to consumers if the safe waiting period is maintained after recommended application.

References and Notes

[1] Aktar, M. W.; Sengupta, D.; Purkait, S.; Chowdhury, A. *Environ. Monit. Assess.* **2010**, *163*, 369.

[2] Anonymous **2008**, www.indiastat.com

[3] Regupathy, A.; Habcebullah, B.; Balasubramanian, M. *Pesticides* **1985**, *19,* 53.

[4] Patel, B. A.; Shah, M. F.; Patel, B. K.; Patel, J. L.; Talati, J. G. *Pestic. Res. J.* **1999**,

11, 194.
[5] Anonymous **2011**, http://sitem.herts.ac.uk/aeru/footprint/en/Reports/576.htm
[6] Anonymous. In: *Package of Practices for Vegetables Crops*, Punjab Agricultural University, Ludhiana. 2009.
[7] Banerjee, K.; Upadhyay, A. K.; Adsule, P.; Pandurang, G.; Patil, S. H.; Oulkar, D. P. *Food Add. Contam.* **2006**, *23,* 994.
[8] Karanth, N. G. K. Abstract in Proceedings of the international workshop, CIRAD-FAO, 1-3 December 2000.
[9] Kumari, B.; Madan, V. K.; Singh, J*. Environ. Monit. Assess*. **2004,** *90,* 65.
[10] Jacob, S.; Verma, S. *Indian J. Entomol.* **1985,** *47,* 304.

ISSN 1984-6428
ONLINE
www.orbital.ufms.br

| Vol 3 | | No. 2 | | April-June 2011 |

Full Paper

Bacteriological studies of new substituted hydroxy -1, 3-propanediones and 4-methyl-5-chloroacetophenones

Pradip V. Tekade

Department of Chemistry, Jankidevi Bajaj College of science, Jamnalal Bajaj Marg, Civil lines, Wardha-442001-M.S. India

Received: 16 January 2011; revised: 06 May 2011; accepted: 18 June 2011. Available online: 27 September 2011.

ABSTRACT: *The titled compounds were screened for antibacterial activity against gram + & gram – bacteria such as Escherichia coli, Bacillus subtilis, Staphylococcus aureus, and Pseudomonas aeruginosa, Proteus vulgaris and Enterobacter aerogenes by using Muller Hinton Hiveg Agar No.2 MV-1084 (Hi-Media). All the screened compounds were found inactive against Bacillus subtilis, Enterobacter aerogenes and Escherichia coli. The produced compounds have shown average to good antibacterial activity.*

Keywords: *hydroxy-1,3-propanediones; antibacterial activity; chloroacetophenones*

Introduction

1, 3-Propanedione is used as a starting material for the synthesis of flavanones and their derivatives viz. flavones, isoxazolines, isoxazoles, pyrazolines and pyrazoles. These compounds showed diverse biological activities [1-6]; such as antimicrobial [7], antibacterial [8], insecticidal, antifungal, pharmaceuticals, antipyretic, antitubercular, antiflammatory and insect repellent properties. Chlorodiketones [9] are found to posses' antihelmenthic and antifungal activities. The synthesis of 1-(2'–hydroxy-4'-methyl-5'-chlorophenyl)–3-(2'–chlorophenyl)-1,3-propanedione has been reported [3] by Baker-Venkatraman transformation of 2-(2'-chlorobenzoyloxy)-4-methyl-5–chloroacetophenone.

The literature survey reveals that, much work has been done over many years for the study of antimicrobial activities of heterocyclic compounds on gram + and gram -

* Corresponding authors: pradiptekade@gmail.com

microorganisms and also antifungal activities on fungi. The synthesized new substituted β-diketone (**3a-c**) as well as substituted 2-hydroxy acetophenone (**1**) and 2-benzoyloxy acetophenones (**2a-c**) were not yet been studied for antimicrobial activities. Thus, it was thought of interest to study the antibacterial activities of these compounds against pathogenic organisms with the help of paper disc method and disc diffusion method

Screenings of following compounds were carried out against the bacteria such as *Escherichia coli*, *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris* and *Enterobacter aerogenes* by using Muller Hinton Hi-Veg Agar MV-1084 (Hi-Media). The following Compounds were tested.

2-hydroxy-4 -methyl-5-chloroacetophenone (**1**)

1-(2'–hydroxy-4'-methyl-5'-chlorophenyl)-3-(4'–chlorophenyl)-1,3-propanedione (**3a**)

1-(2'–hydroxy-4'-methyl-5'-chlorophenyl)-3-(2'–chlorophenyl)-1,3-propanedione (**3b**)

1-(2'–hydroxy-4'-methyl-5'-chlorophenyl)-3-(4'–methoxyphenyl)-1,3-propanedione(**3c**)

2-(2'-chlorobenzoyloxy)-4-methyl-5-chloroacetophenone (**2a**)

2-(4'-chlorobenzoyloxy)-4-methyl-5-chloroacetophenone (**2b**)

2-(4'-methoxybenzoyloxy)- 4-methyl- 5-chloroacetophenone (**2c**)

The newly substituted hydroxy-1,3-propanediones and 4-methyl-5-chloroacetophenone were synthesized by microwave irradiation [10] as well as by conventional methods [3] as described elsewhere. The structures of some compounds were confirmed on the basis of chemical properties, analytical data and spectral analysis (viz. IR, UV, PMR, ^{13}C, Mass fragmentation). Newly substituted 1-(2'-hydroxy aryl)-3-(aryl)-1,3-propanediones (**3a-c**) have been synthesized by base catalyzed Baker-Venkatraman transformation of 2-benzoyloxyacetophenone (**2a-c**). The chemicals used for the synthesis were of analytical reagent grade.

Material and Methods

Preparation of wet disc for antibacterial activity

Discs (6.25 mm) in a diameter from whatman filter paper were punched and batches of 100 in screw-capped bottles were dispersed and sterilized by dry heat at 140 ^{o}C for 60 minutes. DMSO solvent is used for the preparation of the solution so that 1 mL contains 100 times the amount of the compound required in the disc. 1 mL solution of

the compound was added to each bottle of 100 discs and as a whole of this volume is absorbed. It was assumed that each disc contains approximately 0.01 mL. Discs were sorted in wet conditions.

Cultural medium

The medium used throughout the experiment was Muller Hinton Hiveg MV-1084 (Indian Make) agar No. 2 and having following composition.

The media was prepared by suspending 36 g ingredients in 1 L distilled water. It was boiled to dissolve the medium completely and was sterilized by autoclave at 1.02 atm pressure at 121 ^{0}C temperature. It was cooled to about 50 ^{0}C and poured into sterile petri plates and allowed to solidify.

Table 1. Composition of Muller Hinton Hi-Veg. agar

Ingredients	g L^{-1}
Hiveg acid Hydrolysate	17.50
Hiveg Infusion	2.00
Starch soluble	1.50
Agar	17.00

Table 2. Method of preparation of media

Medium used	Muller Hinton Hiveg agar
Size (plate)	8.5 cm in diameter
Depth of agar	14 mm
Distance between 2 discs	2 cm
Diameter of antibiotic disc	6.25 mm in diameter

Test Procedure

The culture material was inoculated in a nutrient broth and kept at 37 ^{o}C for 24 hours incubation. The culture plate Muller Hinton Hiveg agar was dried until its surface was free from visible moisture. The inoculating material was then flooded on the surface of Muller Hinton Hiveg agar uniformly taking all aseptic precautions. The plate was dried again for up to 30 minutes without further delay and the compound discs were applied at adequate spacing (2 cm or more apart) to the surface of the plate with sterile fine-pointed forceps and gently press to ensure full contact with the medium and moistening of the disc. Control was run using plane DMF solvent for aseptic conditions. The plates were incubated at 36 ^{o}C for18-24 hours. After incubation degree of sensitivity to drugs is determined by measuring the visible clear areas of growth of free zones (zones of inhibition) produced by diffusion of antibiotics into the media from the discs.

Width of the zone of inhibition depends on: size of inoculums, nature of culture medium, presence of inhibitors, concentration of agar in the medium, thickness of the medium in the plate, condition and time of incubation, composition of antibiotic discs.

zones of inhibition are measured and reported. The results are cited in Table 3.

Results and Discussion

Table 3. Showing zones of inhibition

Comp. No.	*E. coli* Gram-	*S. aureus* Gram+	*P. vulgaris* Gram-	*P. aeruginosa* Gram-	*B. subtilis* Gram+	*E.aerogenes* Gram-
1	-	++	+++	-	-	-
2a	-	++	+	++	-	-
2b	-	+++	+++	++	-	-
2c	-	+++	++	+++	-	-
3a	-	++	++	+++	-	-
3b	-	++++	+++	-	-	-
3c	-	++	++	-	-	-

+++++: Very strongly active ≥ 20 mm, ++++: Strongly active ≥ 15 mm
+++ : Active ≥ 8 mm, ++ : Moderately active ≥ 5 mm
+ : Weakly active ≥ 3 mm, - : Inactive

From the results of screening against the bacteria *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, and Pseudomonas aeruginosa, Proteus vulgaris* and *Enterobacter aerogenes,* the following conclusions were drawn from the Table 3.

All the screened compounds were found inactive against *Bacillus subtilis, Enterobacter aerogenes* and *Escherichia coli.* The compound **3b** was found to have good activity *against Staphylococcus aureus.*

The compound 2-hydroxy-4 -methyl-5-chloroacetophenone (**1**) was inactive against *E. coli, P. aeruginosa*, *B. subtilis, E. aerogenes,* moderately active against S*. aureus* and active against *P. vulgaris*.

The compound 1-(2'–hydroxy-4'-methyl-5'-chlorophenyl)-3-(4'–chlorophenyl)-1,3-propanedione (**3a**) was inactive against *E. coli, B. subtilis, E. aerogenes, moderately* active against S*. aureus, P. vulgaris* and active against *P. aeruginosa*.

The compound 1-(2'–hydroxy-4'-methyl-5'-chlorophenyl)-3-(2'–chlorophenyl)-1,3-propanedione (**3b**) was inactive against *E. coli, B. subtilis, E. aerogenes, P. aeruginosa strongly* active against *S. aureus,* and active against *P. vulgaris*.

The compound 1-(2'–hydroxy-4'-methyl-5'-chlorophenyl)-3-(4'–methoxyphenyl)-1,3-propanedione(**3c**) was inactive against *E. coli, B. subtilis, E. aerogenes, P. aeruginosa* ,moderately active against S*. aureus,* and *P. vulgaris*.

The compound 2-(2'-chlorobenzoyloxy)-4-methyl-5-chloroacetophenone (**2a**) was inactive against *E. coli, B. subtilis, E. aerogenes*, moderately active against*, P. aeruginosa* S*. aureus,* and weakly active against *P. vulgaris*.

The compound 2-(4'-chlorobenzoyloxy)-4-methyl-5-chloroacetophenone (**2b**) was

inactive against *E. coli, B. subtilis, E. aerogenes*, moderately active against*, P. aeruginosa* and active against *P. vulgaris,* S*. aureus*.

The compound 2-(4'-methoxybenzoyloxy)-4-methyl-5-chloroacetophenone (**2c**) was inactive against *E. coli, B. subtilis, E. aerogenes*, moderately active against *P. vulgaris,* and active against *S. aureus and P. aeruginosa*.

Conclusion

The biocidal evaluation of synthesized compounds shows average to good antibacterial activity. The synthesized compound shows more activity against gram – bacteria.

Acknowledgments

The authors are thankful to University Grants Commission for sanctioning this research as a part of minor research project.

References and Notes

[1] Roushdi, J. M.; EI- Sehai, I. A.; Habib, N. S. *Egypt J. Pharm. Sci.* **1975**, *16*, 407.
[2] Duffin, G. F.; Kendall, J. D. J*. Chem. Soc*. **1954**, 408.
[3] Tekade, P. V.;Patil, K.N.; Bodkhe, P. S. *Asian J. Chem.* **2005**, *17*, 1340.
[4] Osman, A .M; Hassan, K. M.; El-Maghavang, M. A. *Ind. J. Chem*. **1976**, *14 B*, 282.
[5] Khalil, Z. H.; Yenni, A. S. *J. Ind. Chem. Soc.* **1981**, *58*, 168.
[6] Metzner, J.; Zamuka, J.; Heger, J. *Pharmazie* **1981**, *36*, 157.
[7] Ito, C; Itoigawa, M.;Furukawa, H.; Ichiishi, E.; Mukainaka, T.; Okuda, M.; Ogata, M.; Tokuda, H.; Nishino, H. *Cancer letters* **1999**, *142*, 49.
[8] Okud, T.; Kirampra, J.; Aziker, K. A. *Proc. Giter. Coll. Pharma*. **1955**, 208.
[9] Bodkhe, P. S. Synthesis of some new substituted 3-aroyl flavanoids, corresponding heterocycles and study of their biocidal effects, Ph.D. Thesis, S.G.B. Amravati University, Amravati 2002.
[10] Tekade, P. V.; Mahodaya, O. A.; Satone, A. K. Reflection *des* era- Journal of physical sciences, In Press.

ISSN 1984-6428
ONLINE
www.orbital.ufms.br

| Vol 3 | | No. 2 | | April-June 2011 |

Full Paper

Synthesis and evaluation of antimicrobial activity of novel 1,3,4-oxadiazole derivatives

P. B. Mohite[a] and V. H. Bhaskar[b]*

[a]Department of Pharmaceutical Chemistry, MES College of Pharmacy, Sonai, Ahmednagar, India, 414 105
[b] MP Patel College of Pharmacy, Kapadwanj, Gujrat, India-687 320

Received: 19 January 2011; revised: 18 June 2011; accepted: 20 June 2011. Available online: 28 September 2011.

ABSTRACT: *In attempt to find new pharmacologically active molecules, we report here the synthesis and in vitro antimicrobial activities of various novel 1,3,4-oxadiazole containing 5-phenyltetrazole. The Schiff bases were obtained by condensation 2-(5-phenyl-1H-tetrazol-1-yl)acetohydrazide with various aromatic aldehydes. Cyclocondensation of Schiff's bases with acetic anhydride results in 1,3,4-oxadiazole derivatives. The structures of the newly synthesized 1,3,4-oxadiazole were confirmed by FT-IR, ^{1}H NMR and mass spectral data. The antimicrobial activity was determined by MIC method. All the compounds exhibited weak to potent antimicrobial activity. Some derivatives bearing a methoxy group exhibited very good antimicrobial activity at conc. of 62.5 µg/mL.*

Keywords: *1,3,4-oxadiazole; tetrazole; antimicrobial activity*

Introduction

The earliest evidence of successful chemotherapy is from ancient Peru, where the Indians used bark from the cinchona tree to treat malaria. Modern chemotherapy has been dated to the work of Paul Ehrlich in Germany, who sought systematically to discover effective agents to treat trypanosomiasis and syphilis. Ehrlich postulated that it would be possible to find chemicals that were selectively toxic for parasites but not toxic to humans. Progress in the development of novel antibacterial agents has been great, but the development of effective, nontoxic antifungal and antiviral agents has been slow. Amphotericin B, isolated inthe1950s, remains an effective antifungal agent, although newer agents such as Fluconazole are now widely used. An antimicrobial is a substance

* Corresponding authors: mohitepb@rediffmail.com

that kills or inhibits the growth of microbes such as bacteria (antibacterial activity), fungi (antifungal activity) and viruses (antiviral activity). Any attempt to discuss the chemotherapeutic properties of heterocyclic compounds must, of necessity, be confined to a limited aspect of the subject. Therefore, the present discussion will be limited to monocyclic compounds with 5-membered ring. By definition, this includes not only compounds with a single 5-membered ring but also substances with two or more rings, one of which must be six membered. The polyene antibiotics, which apparently act by binding to membrane sterols, contain a rigid hydrophobic center and a flexible hydrophilic section. Structurally, polyenes are tightly packed rod sheld in rigid extension by the polyene portion. They interact with fungal cells to produce a membrane-polyene complex that alters the membrane permeability, resulting in internal acidification of the fungus with exchange of K^+ and sugars; loss of phosphate esters, organic acids, nucleotides; and eventual leakage of cell protein. In effect, the polyene makes a pore in the fungal membrane and the contents of the fungus leak out. Although numerous polyene antibiotics have been isolated, only amphotericin B is used systemically. Nystatin is used as a topical agent and primaricin as an ophthalmic preparation. A number of other agents interfere with the synthesis of fungal lipid membranes. These agents belong to a class of compounds referred to as imidazoles: miconazole, ketoconazole, clotrimazole, and fluconazole. These compounds inhibit the incorporation of subunits into ergosterol and may also directly damage the membrane. The development of antifungal agents has lagged behind that of antibacterial agents. This is a predictable consequence of the cellular structure of the organisms involved. This difficulty complicates experiments designed to evaluate the *in vitro* or *in vivo* properties of a potential antifungal agent [1-5]. Tetrazole derivatives possess broad spectrum of biological activity in both medicinal and pharmaceutical such as antibacterial, antifungal, antiviral, analgesic anti-inflammatory, antiulcer [6-13]. 1,3,4-Oxadiazoles show various biological activities and have been synthesized from different compounds. Many reaction schemes were followed for the synthesis of the ring and1, 3,4-oxadiazole ring showed diversity in biological activity. 1,3,4-Oxadiazole is popularly known for its antimicrobial, anti-inflammatory, pesticidal, antihypertensive activities *etc* [14-22]. It is well known that the synthesis of heterocyclic compounds tends to contain multi-structure in a molecule. The ring formation involves the condensation reaction. In each step, a water molecule is formed.

The challenge is to develop the ring system by incorporating the tetrazole nucleus into it through the proposed reaction scheme. In this study, it was planned to incorporate the oxadiazole ring system into tetrazole ring, as it has not been reported earlier. Synthesis of derivatives of 1,3,4-oxadiazoles from different benzaldehydes and Schiff bases. Characterization of the synthesized compounds along with their antimicrobial

activity on different strains of bacteria and fungi has been performed.

Material and Methods

General

All chemicals and solvents were purchased from Qualigens and were of AR-grade purity. All reactions are carried out at laboratory condition. Melting points were determined with open capillary and were uncorrected. FT-IR spectra were recorded on a Shimadzu FT-IR model 8010 spectrophotometer, ^{1}H NMR spectra were recorded in DMSO on a Varian Mercury FT-NMR model YH-300 instrument using TMS as internal standard. Mass spectra were recorded on GC-MS auto tune EI instrument.

General procedure for synthesis of compound 1: A mixture of phenyl tetrazole 5 g (0.03 mol) in methanol is prepared, stirred well to dissolve compound **1**.To this add 3.67 mL (0.03 mol) of ethyl chloroacetate drop wise with continuous stirring to get clear solution. Reflux the reaction mixture on water bath for about 2 hours. A solid residue was obtained by cooling at room temperature. The product was filtered, dried, recrystalised from warm ethanol.

General procedure for synthesis of compound 2: Ethyl (5-phenyl-1*H*-tetrazol-1-yl) acetate 9 g (0.03 mol) was condensed with 1.95 mL (0.03 mol) 99% hydrazine hydrate. Reflux the reaction mixture on water bath for about 5 hours. The solid residue of acetohydrazide was obtained by cooling. The product was filtered, dried, recrystalised from warm ethanol.

General procedure the synthesis of Schiff's bases (3a- h) [23]: 2-(5-phenyl-1*H*-tetrazol-1-yl) acetohydrazide (**1**) 2 g (0.009 mol) was refluxed with various aromatic aldehydes (0.009 mol) in the presence of sulphuric acid for 6 h. The reaction mixture was then poured into the crushed ice. The resultant solid was washed with distilled water, dried and recrystalised from ethanol.

General procedure for synthesis of 1,3,4-oxadiazole (4a-h): A mixture of compounds Schiff bases **3a-h** (0.01 mol) and acetic anhydride (5 mL) was refluxed for 2 h. The mixture was cooled, poured onto crushed ice and allowed to stand at room temperature overnight. The separated solid was washed with water, dried and recrystalised from ethanol.

Biological evaluation

Antimicrobial activity by minimum inhibitory concentration (MIC) method

The minimum inhibitory concentration (MIC) of the test substances against *Staphylococcus aureus, Escheshia coli*, *Candida albicans* and *Aspergillus niger* was determined by liquid broth method of two fold serial dilution technique [24-25]. In this

assay, the minimum concentration of each test substance required to inhibit the growth of microorganism was determined.

For this assay, a series of assay tubes were prepared containing uniform volume (1 mL) of sterile SD broth and equal volume of known concentration of test substance was added. The test substance in the first tube was serially diluted in twofold decreasing concentrations through the sixth tube and seventh tube was left without test substance as positive control. The tubes with the test substance i.e. from one to seventh were inoculated with 1 mL of inoculums ($1x10^6$ CFU per mL). The final concentration of test substance ranged from 1000 to 31.25 µg/mL. Solvent control and sterility controls were maintained in the experiment. The tubes were incubated at 28 ^{0}C for 48 h. Standard antibiotic, ampicillin and fluconazole were tested as standard drug at concentrations ranging from 100 to 3.12 µg/mL.

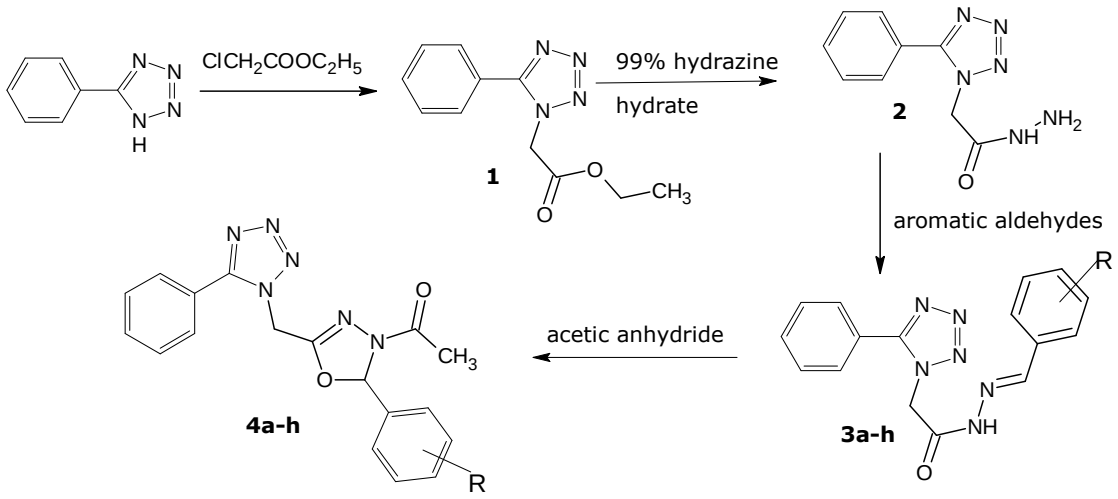
The tubes were inspected visually to determine the growth of the organism as indicated by turbidity (In fact, turbidity of the culture medium is indicative of the presence of a large number of cells), the tubes in which the antibiotic is present in concentration sufficient to inhibit fungal growth remain clear. In experimental terms the MIC is the concentration of the drug present in the last clear tube, i.e. in the tube having the lowest concentration in which growth is not observed.

Results and Discussion

Herein we have described the synthesis (Scheme 1), characterization and biological evaluation of some 1,3,4-oxadiazole containing 5-phenyltetrazole. All compounds were analyzed satisfactorily by CHN elemental analysis. All the IR and NMR spectral characteristics of different 1,3,4-oxadiazole are in good agreement with proposed structure and are shown in experimental section. The IR spectra of compounds **4a-h** shows absorption bands at 3057 due to (Ar-H str.), 1656 due to C=N ring stretch and 2376, 2247 due -NCH2. Similarly absorption also occurs at 1285(N-N=N-), 1108 and 1138 (tetrazole ring). The ^{1}H NMR spectra shows chemical shift at 7.10-8.10 due to aromatic protons, 6.30 (s, 1H CH of 1,3,4-oxadiazole), 3.98 (d, 2H CH2), 2.15(s, 3H,CH3).The results of spectral data are in good agreement with the structure of synthesized compounds. The physicochemical and spectral data of the compounds **4a-h** are described in tables 1 and 2.

The antimicrobial activity of all the synthesized compounds is shown in Table 3. All the compounds exhibited significant antibacterial and antifungal activities. Good antibacterial activity was observed in **4d, 4b, 4g** against *S. aureus*. Whereas compounds **4f, 4g, 4d** and **4e** showed noticeable activity against *E. coli*. Compound **4d, 4g, 4f** showed marked activity against *A. niger* and *C. albicans*. The compound **4f** bearing

methoxy group shows MIC at 62.5 µg/mL against *E. coli* and *C. albicans* and 125 µg/mL against *S. aureus* and *A. niger* respectively.



Scheme 1: Synthetic route of titled compounds.

Table 1. Physicochemical characterization of titled compounds

Sr. No	R	Mol. Form	Mol. Wt	M.P. (°C)	Yield (%)	C, H, N Calculated (found)		
						C %	H %	N %
1	H	$C_{18}H_{16}N_6O_2$	348	128-130° C	62%	62.06 (60.00)	4.63 (4.60)	24.12 (24.08)
2	2-Cl	$C_{18}H_{15}ClN_6O_2$	382	136-138° C	70%	56.48 (56.22)	3.95 (3.90)	21.95 (21.90)
3	4-Cl	$C_{18}H_{15}ClN_6O_2$	382	138-140° C	72%	56.48 (56.20)	3.95 (3.90)	21.95 (21.88)
4	4-Br	$C_{18}H_{15}BrN_6O_2$	427	144-146° C	68%	50.60 (50.10)	3.54 (3.48)	19.67 (19.58)
5	4-CH_3	$C_{19}H_{18}N_6O_2$	362	180-182° C	69%	62.97 (62.75)	5.01 (4.96)	23.19 (23.05)
6	4-OCH_3	$C_{19}H_{18}N_6O_3$	378	155-157° C	74%	60.31 (60.15)	4.79 (4.60)	22.21 (22.10)
7	3-NO_2	$C_{18}H_{15}N_7O_4$	393	188-190° C	68%	54.96 (54.80)	3.84 (3.75)	24.93 (24.78)
8	$(CH_3)_2$-N-	$C_{20}H_{21}N_7O_3$	391	180-182° C	55%	61.37 (61.20)	5.41 (5.30)	25.05 (24.98)

Table 2. Spectral Characterization of titled compounds

Sr.No.	R	IR (KBr) cm^{-1}	1H NMR(300.00 MHz) δ ppm	m/e ratio
4a	H	3057(Aromatic C-H), 2376,2247(-NCH_2),1656 (-C=N of 1,3,4oxadiazole),832(C-H def disubstituted benzene)	7.10- 8.10 (m, 10H, Ar), 6.30(s,1H,CH)3.98 (s, 2H, -CH_2),2.15(s,3H,CH_3)	348
4b	2-Cl	30570(Aromatic C-H), 2376,2247(-NCH_2),1652 (-C=N of 1,3,4oxadiazole),828(C-H def disubstituted benzene),776(C-Cl).	7.10- 8.10 (m, 9H, Ar), 6.28(s,1H,CH)3.88 (s, 2H, -CH_2),2.15(s,3H,CH_3)	383 [M+1]
4c	4-Cl	3048(Aromatic C-H), 2376,2247(-NCH_2),1658 (-C=N of 1,3,4oxadiazole),842(C-H def disubstituted benzene),776(C-Cl).	7.10- 8.10 (m, 9H, Ar), 6.28(s,1H,CH)3.90 (s, 2H, -CH_2),2.15(s,3H,CH_3)	383 [M+1]
4d	4-Br	3040(Aromatic C-H), 2376,2247(-NCH_2),1651 (-C=N of 1,3,4oxadiazole),838(C-H def disubstituted benzene),697(C-Br).	7.10- 8.10 (m, 9H, Ar), 6.24(s,1H,CH)3.85 (s, 2H, -CH_2),2.15(s,3H,CH_3)	427
4e	4-CH_3	3060(Aromatic C-H), 2376,2247(-NCH_2),1656 (-C=N of 1,3,4 oxadiazole),840(C-H def disubstituted benzene)	7.10- 8.10 (m, 9H, Ar), 6.42(s,1H,CH)3.80 (s, 2H, -CH_2),2.15(s,3H,CH_3)	362
4f	3-OCH_3	3045(Aromatic C-H), 2376,2247(-NCH_2),1648 (-C=N of 1,3,4 oxadiazole),832(C-H def disubstituted benzene),1165(-OCH_3).	7.10- 8.10 (m, 9H, Ar), 6.15(s,1H,CH)3.75 (s, 2H, -CH_2),2.15(s,3H,CH_3)	380 [M+2]
4g	3-NO_2	3048(Aromatic C-H), 2376,2247(-NCH_2),1645 (-C=N of 1,3,4 oxadiazole),835(C-H def disubstituted benzene),1564 (NO_2).	7.10- 8.10 (m, 9H, Ar), 6.24(s,1H,CH)3.70 (s, 2H, -CH_2),2.15(s,3H,CH_3)	393
4h	(CH3)2-N	3042(Aromatic C-H), 2376,2247(-NCH_2),1642 (-C=N of 1,3,4 oxadiazole),828(C-H def disubstituted benzene)	7.10- 8.10 (m, 9H, Ar), 6.26(s,1H,CH)3.94(s, 2H, -CH_2),2.15(s,3H,CH_3)	392 [M+1]

Table 3. *In vitro* antimicrobial activity of 1,3,4-oxadiazole derivatives

Compound	Minimum Inhibitory Conc.(MIC) in ug/ml			
	S. aureus	***E. Coli***	***A. niger***	***C. albicans***
4a	250	250	500	500
4b	125	250	250	125
4c	500	250	250	250
4d	62.5	125	125	250
4e	250	125	250	125
4f	125	62.5	125	62.5
4g	125	62.5	125	500
4h	500	250	250	125
Ampicillin	6.25	6.25	-	-
Fluconazole	-	-	6.25	6.25

Conclusion

Conclusively, a variety of 1,3,4-oxadiazole derivatives containing 5-phenyltetrazole have been successfully synthesized in appreciable yields by simple synthetic route from 5-phenyltetrazole and screened in vitro for their antimicrobial activities against both strains of Gram-positive and Gram-negative bacteria. Most of the compounds found to possess good antimicrobial activity at minimal inhibitory concentration.

Form the above evidence; it is clear that 1,3,4-oxadiazole derivatives can be used to discover bioactive synthetic products that may serve as leads for the development of new pharmaceuticals that address hither to unmet therapeutic needs. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of synthetic origin.

Acknowledgments

The authors are thankful to University of Pune, India, for providing spectral data, and Principal, MES College of Pharmacy, Sonai, for providing necessary facilities.

References and Notes

[1] Arthur, M.; Courvalin, P. *Antimicrob Agents Chemother.* **1993**, 37, 1563.
[2] Murray, B. *J. Infect. Dis.* **1991**, *163*, 1185.
[3] Gale, E. F.; Cundliffe, E.; Reynolds, P. E.; Richmond, M. H.; Waring, M. J. The Molecular Basis of Antibiotic Action, 2nd Ed. New York: John Wiley & Sons, 1981, 473.
[4] Foye, O. W. Principles of Medicinal Chemistry, 3rd Ed. Varghese Publishing House (P) Ltd, 1974, 676-736.
[5] Tripathi, K.; D. Essential of Medical Pharmacology, 5th Ed. Jaypee Brothers Medical Publishers (P) Ltda. 2003, 627-629.
[6] Silverstein, R. M.; Webster, X. F. Spectrometric Identification of Organic Compounds, 6th Ed. John Wiley & Sons (Asia) Pvt. Ltd, Singapore, 2005, 81-109.
[7] Mulwad, V. V.; Pawar, R. B.; Chaskar, A. C. *J. Korean Chem. Soc*.**2008**, *52*, 249.
[8] Rajasekaran, A.; Sankar, N.; Murugesh, A.; Kalasalingam, R. A.; *Archives of Pharmacal Research* **2006**, *29*, 535.
[9] Upadhayaya, R. S.; Jain, S.; Sinha, N.; Kishore, N.; Chandra, R.; Arora, S. K. *Eur. J. Med. Chem.* **2004**, *39*, 579.
[10] Bachar, S. C.; Lahiri, S. C. *Pharmazie* **2004**, *59*, 435.
[11] Ray, S. M.; Lahiri, S. C. *J. Ind. Chem. Soc*. **1990**, *67*, 324.
[12] Aliasghar, J.; Khalili D.; Clercq, E. D.; Salmi, C.; Brunel, J. M. *Molecules* **2007**, *12*, 1720.
[13] Adamec, J.; Waisser, K.; Kunes, J.; Kaustova, J. *Arch. Pharm*. **2005**, *338*, 385.

[14] Zhang, Z. Y.; Chu, C. H.; Hui, X. P. *Ind. J. Chem.* **2002**, *41B*, 2176.
[15] Singh,H.; Srivastava, M. K.; Singh, B. K.; Singh, S. K.; Dubey, G. *Ind. J. Chem*. **2001**, *40B*, 159.
[16] Mogilaiah, K.; Sakram, S.; *Ind. J. Chem.* **2004***, 43B*, 2014.
[17] Ajitha, M.; Rajnarayana, K.; Sangarapani, K. *Pharmazie* **2002**, *57*, 796.
[18] Bhat, K. S.; Karthikeyan, M. S.; Holla, B. S.; Shetty, N. S. *Ind. J. Chem*. **2004**, *43B*, 1765.
[19] Aboraia, A. S.; Rahman, H. M. R.; Mahfouz, N. M.; Gendy, A. E. L. *Bioorg. Med. Chem*. **2006**, *14*, 1236.
[20] Amir, M.; Shahani, S.; *Ind. J. Heterocycl. Chem*. **1998**, 8, 107.
[21] Axena, S.; Verma, M. *Ind. J. Pharm. Sci.* **1992**, *54*, 1.
[22] Mullican, D.; Wilson, M. W.; Cannor, D. T.; Kostlan, C. R.; Schirier, D. J.; Dyer R. D. *J. Med. Chem*. **1993**, *36*, 1090.
[23] Bhaskar, V. H.; Mohite, P. B. *Digest J. Nanomaterials and Biostructures* **2010**, *5*, 177.
[24] Indian Pharmacopoeia*;* Vol. II (P-Z); Published by the Controller of Publications, Delhi, 1996, A-100.
[25] Gibbons, S.; Birgit, O.; Jhonsen, I. *Fitoterapia* **2002**, *73,* 300.