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Synthesis of some biologically active pyrimidine and isoxazole derivatives

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Abstract : 4-(2,5-Dichloro-3-thienyl)-6-substitutedphenylpyrimidine-2(1*H*)-thione (2a-j), 3-(2,5-dichloro-3-thienyl)-5-substitutedphenyl-4,5-dihydroisoxazole (3a-j) have been synthesized by the reaction of (2*E*)-1-(2,5-dichloro-3-thienyl)-3-substitutedphenylprop-2-en-1-one (1) with thiourea and hydoxylamine hydrochloride respectively. These compounds have been screened for their antibacterial and antifungal activities against different microorganisms. The structures of novel synthesized compounds have been established on the basis of elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data.

Keywords : Thienyl, pyrimidine, isoxazole, antibacterial, antifungal.

Introduction

Over the years pyrazoles, isoxazoles and pyrimidines have emerged as interesting classes of five and six membered heterocycles with astonishingly wide range of applications in pharmaceutical chemistry. The increasing importance of pyrimidine and its derivatives as intermediates for the synthesis of biologically active[1] compounds has led to continuing development of new simple procedures for their synthesis. Pyrimidines being an integral part of DNA and RNA, play an essential role in several biological processes and have considerable chemical and pharmacological[2] importance. Diverse biological properties have been shown to be associated with numerous fused pyrimidines, including antiallergic, antibacterial[3], anti-inflmmatory, antiviral[4], antimicrobial[5–8] and plant bactericidal effects. Pyrimidine derivatives, a constituent unit of nucleobases are useful in drug discovery. Recently, it has been proved that pyrimidine and fused heterocyclic pyrimidine nucleus are potent antiviral agents.

Isoxazole nucleus can be found frequently in the structure of numerous naturally occurring and synthetic compounds with interesting biological and pharmacological properties. Additionally, isoxazole moiety displays a wide range of organic reactivities and could be used as a effective means of preparing new molecular scaffolds. Isoxazoles have been reported as useful synthons in organic synthesis. For instance, isoxazole possesses a broad spectrum of biological activities such as antiviral[9], antitubercular[10], analgesic, antiprotozoal, anticancer[11], mosquito larvicidal, HIV-1 inhibitors etc. Isoxazole also serves as an important building block for the synthesis of biologically active molecules and serves as a prodrug for an antiarthritic agent. In fact, Valdecoxib[12] is an isoxazole derivative, now widely used in the market as an anti-inflammatory[13] drug.

Results and discussion

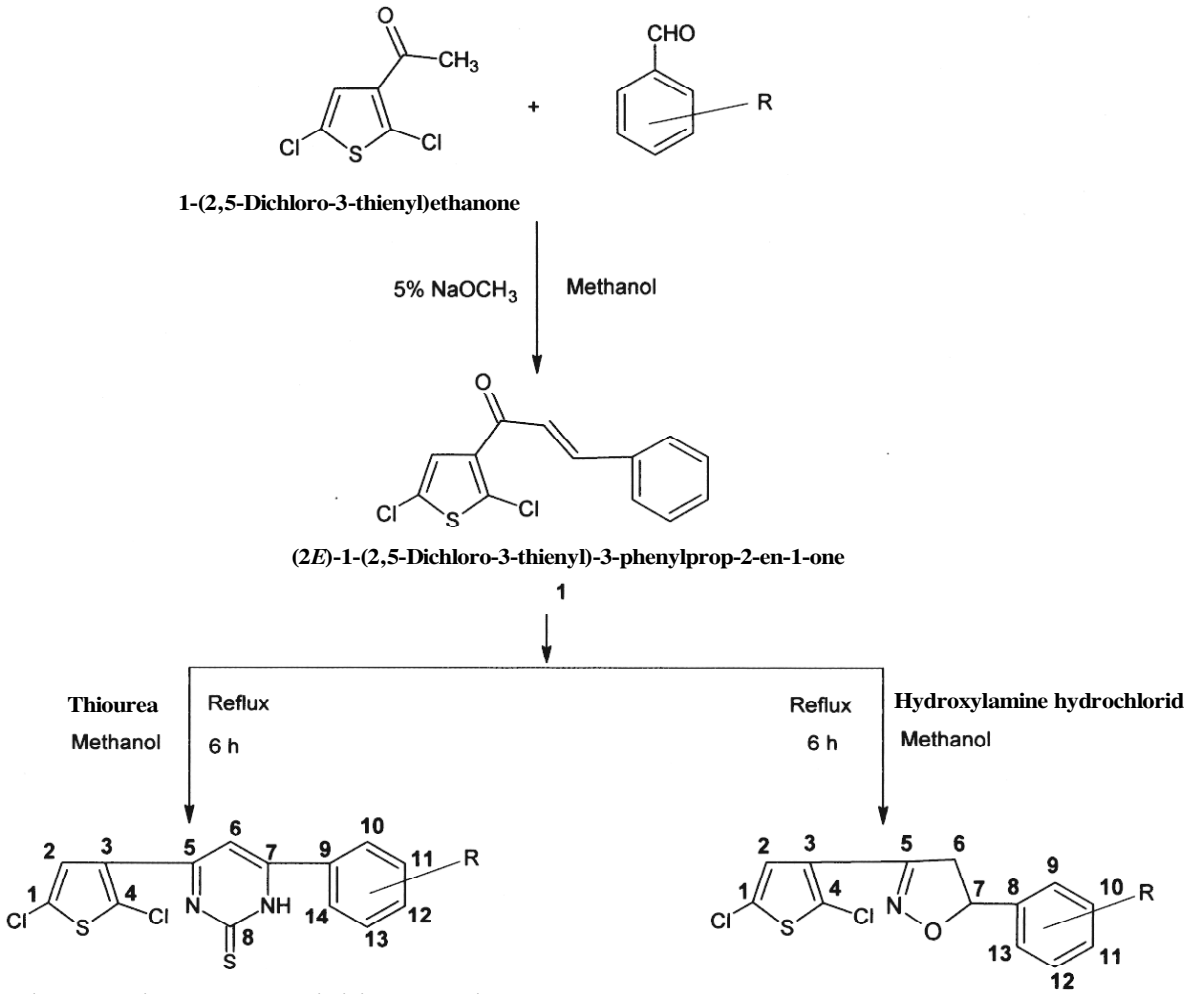
The starting compounds (2*E*)-1-(2,5-dichloro-3-thienyl)-3-substitutedphenylprop-2-en-1-one (**1**) have been synthesized by reaction of various substituted aldehydes and 1-(2,5-dichloro-3-thienyl)ethanone in presence of alkali. Further, on reaction with thiourea and hydroxylamine hydrochloride it gives **2a-j** and **3a-j**, respectively (Scheme 1, Table 1).

The structures were established through IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. In IR spectra of **2a-j**, significant bands appeared at 1554 cm^{-1} -C=N (pyrimidine ring) and 3186 cm^{-1} -NH (pyrimidine ring). ^{1}H NMR spectra of these compounds revealed signals at δ 9.92 (-NH pyrimidine ring) and δ 9.08 (-CH pyrimidine ring),

Table 1. Physical characterization data of compounds **2a-j** to **3a-j**

Compd.	Substituent R	Mol. formula (Mol. wt.)	Yield (%) (time/h)	M.p. (°C)	Required (Found) C	H	N
2a	-H	$C_{14}H_8Cl_2N_2S_2$ (339.26)	78 (6–8)	185–191	49.56 (49.53)	2.38 (2.34)	8.26 (8.27)
2b	4-Cl	$C_{14}H_7Cl_3N_2S_2$ (373.70)	74 (6–8)	100–110	44.99 (44.94)	1.89 (1.87)	7.50 (7.52)
2c	2-OH	$C_{14}H_8Cl_2N_2OS_2$ (355.26)	74 (6–8)	125–135	47.33 (47.30)	2.27 (2.24)	7.89 (7.89)
2d	4-OH	$C_{14}H_8Cl_2N_2OS_2$ (355.26)	78 (6–8)	170–180	47.33 (47.31)	2.27 (2.25)	7.89 (7.88)
2e	3-OH 4-OCH_3	$C_{15}H_{10}Cl_2N_2O_2S_2$ (385.28)	72 (6–8)	215–222	46.76 (46.74)	2.62 (2.59)	7.27 (7.26)
2f	4-OCH_3	$C_{15}H_{10}Cl_2N_2OS_2$ (369.28)	76 (6–8)	115–123	48.79 (48.75)	2.73 (2.70)	7.59 (7.60)
2g	3,4-di-OCH_3	$C_{16}H_{12}Cl_2N_2O_2S_2$ (399.31)	73 (6–8)	150–157	48.13 (48.09)	3.03 (2.97)	7.02 (7.08)
2h	3,4,5-tri-OCH_3	$C_{17}H_{14}Cl_2N_2O_3S_2$ (429.34)	75 (6–8)	95–105	47.56 (47.52)	3.29 (3.26)	6.52 (6.50)
2i	2-NO_2	$C_{14}H_7Cl_2N_3O_2S_2$ (384.26)	77 (6–7)	174–176	43.76 (43.73)	1.84 (1.80)	10.94 (10.93)
2j	3-NO_2	$C_{14}H_7Cl_2N_3O_2S_2$ (384.26)	75 (6–8)	145–154	43.76 (43.73)	1.84 (1.81)	10.94 (10.94)
3a	-H	$C_{13}H_9Cl_2NOS$ (298.18)	72 (6–8)	82–92	52.36 (52.31)	3.04 (2.98)	4.70 (4.74)
3b	4-Cl	$C_{13}H_8Cl_3NOS$ (332.63)	70 (6–8)	90–98	46.94 (46.91)	2.42 (2.40)	4.21 (4.25)
3c	2-OH	$C_{13}H_9Cl_2NO_2S$ (314.18)	77 (6–8)	66–78	51.61 (51.58)	2.89 (2.82)	4.46 (4.48)
3d	4-OH	$C_{13}H_9Cl_2NO_2S$ (314.18)	70 (6–8)	90–100	51.61 (51.57)	2.89 (2.86)	4.46 (4.45)
3e	3-OH 4-OCH_3	$C_{14}H_{11}Cl_2NO_3S$ (344.21)	72 (6–8)	75–88	48.85 (48.81)	3.22 (3.18)	4.07 (4.08)
3f	4-OCH_3	$C_{14}H_{11}Cl_2NO_2S$ (328.21)	71 (6–8)	64–71	51.92 (51.89)	2.99 (2.93)	4.27 (4.25)
3g	3,4-di-OCH_3	$C_{15}H_{13}Cl_2NO_3S$ (358.23)	73 (6–8)	77–81	50.29 (50.26)	3.66 (3.61)	3.91 (3.91)
3h	3,4,5-tri-OCH_3	$C_{16}H_{15}Cl_2NO_4S$ (388.26)	73 (6–8)	98–102	49.49 (49.44)	3.89 (3.82)	3.61 (3.63)
3i	2-NO_2	$C_{13}H_8Cl_2N_2O_3S$ (343.18)	72 (6–8)	135–140	45.50 (45.46)	2.35 (2.32)	8.16 (8.15)
3j	3-NO_2	$C_{13}H_8Cl_2N_2O_3S$ (343.18)	75 (6–8)	139–144	45.50 (45.47)	2.35 (2.33)	8.16 (8.15)

besides the other signals in their expected positions. The signals observed at δ 177.72, 124–127 in the ^{13}C NMR spectrum of **2f** were attributed to -C=S, thiophene ring respectively. In IR spectra of **3a-j**, significant bands ap-



No.	R	No.	R
a	-H	**f**	4-OCH_3
b	4-Cl	**g**	3,4-di-OCH_3
c	2-OH	**h**	3,4,5-tri-OCH_3
d	4-OH	**i**	2-NO_2
e	3-OH, 4-OCH_3	**j**	3-NO_2

Scheme 1

peared at 1597 for -C=N (isoxazole ring) and 2835 for -CH_2 (isoxazole ring). ^{1}H NMR spectra of these compounds revealed signals at δ 2.55 (-CH_2 isoxazole ring), and δ 3.55 (-CH isoxazole ring) and besides the other signals in their expected positions. The signals observed at δ 57.95, 103.55, 55.57, 122–125 in the ^{13}C NMR spectrum of **3f** were attributed to -OCH_3, -CH_2 (isoxazole), -CH (isoxazole), thiophene ring respectively.

Experimental

Reagents, instrumentation and measurements :

All chemicals used were of reagent grade and were

used as received without further purification. All the melting points were determind using open glass capillary and are uncorrected. The homogenecity of the compounds was checked by TLC (silica gel H, BDH, hexane : ethyl acetate 8 : 2).

Apparatus :

IR spectra (cm^{-1}) were recorded on a Shimadzu FT-IR spectrophotometer using KBr pellet method. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker 300 MHz and 75 MHz NMR instrument, using DMSO-d_6 as solvent and TMS as internal reference (chemical shifts in δ ppm). Mass spectra were obtained on a JMS-Tl00LC, Accu TOF Mass spectrometer (DART).

The elemental analysis were carried out using a Carlo Erba 1108 C, H and N analyzer. The results were found to be in good agreement with the calculated values.

Antimicrobial activity :

Following common standard strains were used for screening of antibacterial and antifungal activities : *E. coli, P. aeruginosa, S. aureus, S. pyogenus, C. albicans, A. niger, A. clavatus*. The strains were procured from Institute of Microbial Technology, Chandigarh. DMSO was used as diluents to get desired concentration of drugs to test upon Standard bacterial strains. Each synthesized drug was diluted obtaining 2000 µg/ml concentration, as a stock solution. In primary screening 1000, 500 and 250 µg/ml concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 200, 100, 50, 25, 12.5 and 6.250 µg/ml concentrations. The highest dilution showing at least 99% inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculum.

The comparative activities of the newly synthesized compound **2a-j** and **3a-j** and the control antibiotic Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Nystatin, Greseofulvin on bacterial and fungal strains respectively were summarized in Table 2. The compound **3j** exhibited the growth inhibition almost equal to the standard drug against all four bacterial strains. The compound **2g** exhibited the growth inhibition almost equal to standard drug against *S. aureus* and *S. pyrogenus* bacterial strains only. Even in case of the fungal activity assay the compound **3g** have shown the remarkable activity against *A. clavatus*. The other compounds have shown good to moderate activity against bacterial and fungal strains.

(2E)-1-(2,5-Dichloro-3-thienyl)-3-substitutedphenylprop-2-en-1-one (**1**) :

A mixture of 1-(2,5-dichloro-3-thienyl)ethanone (1.0 mol), various aromatic aldehydes (1.2 mol) and 5% $NaOCH_3$ solution in methanol (30 mL) was taken in a round bottom flask. The reaction mixture was stirred over night at room temperature. Progress of reaction was monitored by TLC (mobile phase : ethyl acetate : hexane 2 : 8). The solid thus obtained was filtered, washed with chilled methanol, and purified by recrystallization from methanol. Yield 60–71%.

4-(2,5-Dichloro-3-thienyl)-6-substitutedphenylpyrimidine-2(1H)-thione (**2a-j**) :

A mixture of (2*E*)-1-(2,5-dichloro-3-thienyl)-3-substitutedphenylprop-2-en-1-one (1.0 mol), thiourea (1.2 mol) and methanol was taken in a round bottom flask. The reaction mixture was reflux for 6–8 h in water bath. Progress of reaction was monitored by TLC (mobile phase : ethyl acetate : hexane 2 : 8). The reaction mixture was then cooled, poured into crushed ice and acidified with glacial acetic acid. The solid thus obtained was filtered, washed with water, dried and purified by recrystallization from acetone. Yield 72–76%.

3-(2,5-Dichloro-3-thienyl)-5-substitutedphenyl-4,5-dihydroisoxazole (**3a- j**) :

A mixture of (2*E*)-1-(2,5-dichloro-3-thienyl)-3-substitutedphenylprop-2-en-1-one (1.0 mol), hydroxylamine hydrochloride (1.2 mol) and methanol was taken in a round bottom flask. The reaction mixture was reflux for 6–8 h in water bath. Progress of the reaction was monitored by TLC (mobile phase : ethyl acetate : hexane 2 : 8). The reaction mixture was then cooled and poured

Table 2. Antimicrobial activity

Compd.	Minimum inhibition concentration						
	Antibacterial				Antifungal		
	E. coli MTCC 443	*P. aerugiosa* MTCC 1688	*S. aureus* MTCC 96	*S. pyogenus* MTCC 442	*C. albicans* MTCC 227	*A. niger* MTCC 282	*A. clavatus* MTCC 1323
2a	200	250	200	**125**	**250**	> 1000	> 1000
2b	500	250	250	250	1000	> 1000	> 1000
2c	250	500	500	500	1000	500	500
2d	250	500	500	250	500	1000	1000
2e	**100**	**100**	250	200	1000	**250**	**250**
2f	**125**	**62.5**	500	200	> 1000	> 1000	> 1000
2g	**62.5**	**100**	500	250	1000	500	500
2h	200	250	200	500	> 1000	1000	1000
2i	250	250	**125**	**100**	**250**	500	1000
2j	250	250	250	250	1000	**200**	**200**
3a	200	250	500	500	**250**	1000	1000
3b	200	250	500	500	**100**	500	500
3c	**125**	200	200	250	500	1000	1000
3d	250	**100**	**125**	200	250	1000	1000
3e	200	200	200	200	1000	500	500
3f	200	250	200	200	> 1000	500	1000
3g	500	200	500	500	> 1000	**200**	**250**
3h	**100**	200	500	500	500	1000	1000
3i	250	200	250	250	**250**	**200**	500
3j	250	200	250	250	1000	1000	1000
Gentamycin	0.05	1	0.25	0.5	–	–	–
Ampicillin	100	–	250	100	–	–	–
Chloramphenicol	50	50	50	50	–	–	–
Ciprofloxacin	25	25	50	50	–	–	–
Norfloxacin	10	10	10	10	–	–	–
Nystatin	–	–	–	–	100	100	100
Greseofulvin	–	–	–	–	500	100	100

into crushed ice with continuous stirring. The solid thus obtained was filtered, washed with water, dried and purified by recrystallization from acetone. Yield 71–80%.

Spectral data of 4-(2,5-dichloro-3-thienyl)-6-substitutedphenylpyrimidine-2(1H)-thione :

(**2e**) IR (KBr, cm^{-1}) : 831 (-Cl), 1186 (-C=S), 1554 (-C=N), 3186 (-NH); (**2f**) 835 (-Cl), 1006 (-OCH_3), 1128 (-C=S), 1589 (-C=N), 3097 (-NH).

^1H NMR spectral data of compound :

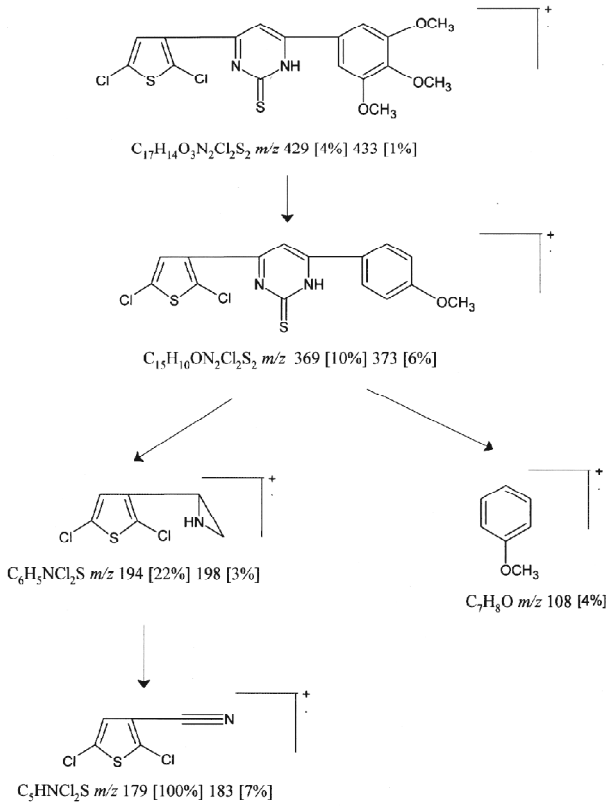
(**2f**) ^1H NMR (300 MHz, DMSO-d_6) : δ 3.11 (1H, s), 3.19 (1H, s), 7.19 (1H, s), 9.08 (1H, s), 9.92 (1H, s), 7.32–7.49 (4H, m).

^{13}C NMR spectral data of compound :

(**2f**) ^{13}C NMR (75 MHz, DMSO-d_6) : 124.26 (C_1), 127.29 (C_2), 127.69 (C_3), 125.92 (C_4), 174.61 (C_5), 177.72 (C_6), 174.61 (C_7), 123.08 (C_8), 137.39 (C_9), 131.71 (C_{10}), 128.43 (C_{11}), 132.32 (C_{12}), 128.66 (C_{13}), 128.79 (C_{14}).

Mass spectral data of compound :

(**2h**) M/S (*m/z*) : 429 (4%), 433 (1%), 369 (10%), 373 (6%), 194 (22%), 198 (3%), 179 (100%), 183 (7%), 108 (4%).



Mass fragmentation pattern of compound **2h**

Spectral data of 3-(2,5-dichloro-3-thienyl)-5-substitutedphenyl-4,5-dihydroisoxazole :

(**3f**) IR (KBr, cm^{-1}) : 827 (-Cl), 1014 ($-OCH_3$), 1597 (-C=N), 2999 ($-CH_2$), 3099 (-OH); (**3b**) 827 (-Cl), 1608 (-C=N), 2835 ($-CH_2$).

^1H NMR spectral data of compound :

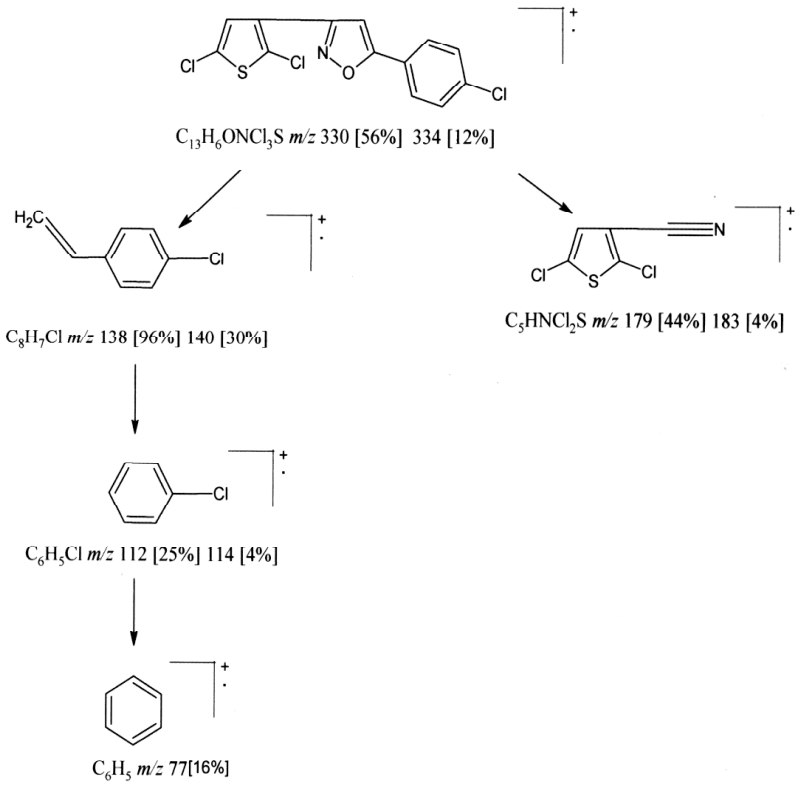
(**3f**) ^1H NMR (300 MHz, DMSO-d_6) : δ 2.16 (3H, s), 2.55 (2H, d, *J* 12.0 Hz, H-6, H-6′), 3.55 (1H, s), 6.90 (1H, s), 7.10–7.65 (3H, m), 11.55 (1H, s).

^{13}C NMR spectral data of compound :

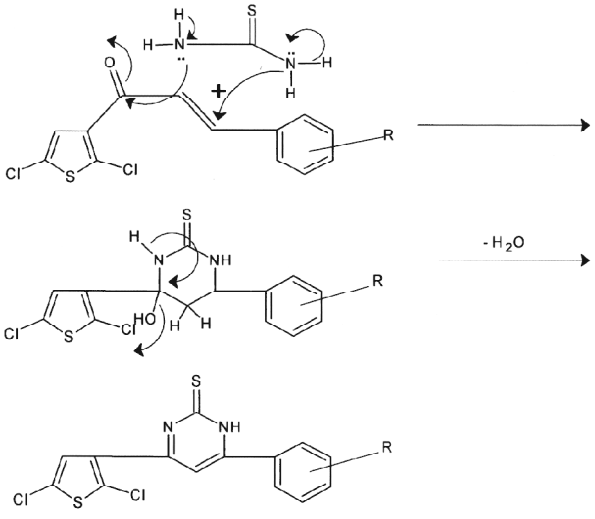
(**3f**) ^{13}C NMR (75 MHz, DMSO-d_6) : 122.50 (C_1), 125.09 (C_2), 125.20 (C_3), 123.00 (C_4), 148.62 (C_5), 13.55 (C_6), 55.57 (C_7), 135.42 (C_8), 127.27 (C_9), 133.61 (C_{10}), 145.44 (C_{11}), 127.12 (C_{12}), 127.27 (C_{13}), 57.95 (C_{14}).

Mass spectral data of compound :

(**3b**) M/S (*m/z*) : 330 (56%), 334 (12%), 179 (44%), 183 (4%), 138 (96%), 140 (30%), 112 (25%), 114 (4%), 77 (16%).

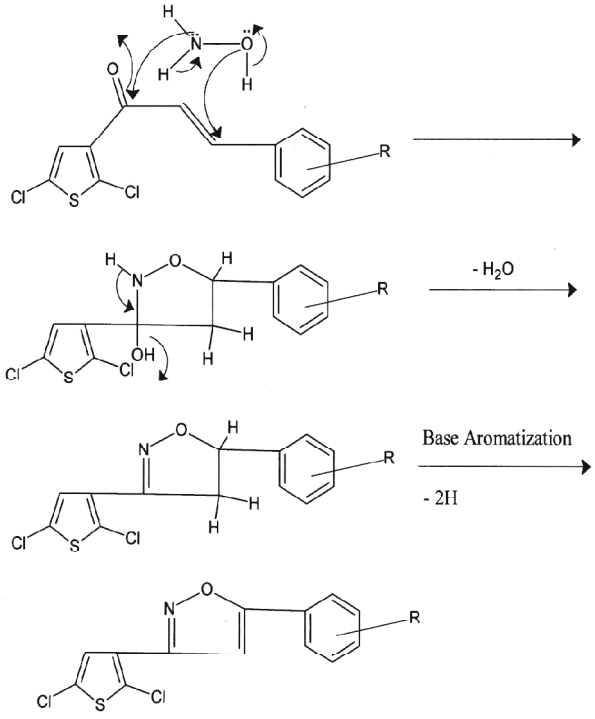


Mass fragmentation pattern of compound **3b**



2a-j

Reaction mechanism of formation of **2a-j**



3a-j

Reaction mechanism of formation of **3a-j**

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