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Chemical modification of PVA with four, five and seven heterocyclic compounds and study anticancer activity

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Abstract

Schiff bases [1]a,b were prepared from the reaction of 2-amino-5-mercapto-1,3,4-thiadiazole with aromatic aldehydes . In the present study a series of some four , five-and seven-membered heterocyclic compounds have been synthesized by the reaction of schiff bases [1]a,b with thioglycolic acid , chloroacetyl chloride , sodium azide or various anhydrides to give thiazolidinone [2]a , azetidinone [6]b , tetrazole [7]b and 1,3-oxazepine derivatives [14-16]b respectively , then compounds[2]a , [6,7]b and [14-16]b were reacted with Na_2CO_3 of distilled water as a solvent, then of $ClCH_2COOH$ was added to produce [3]a , [8,9]b and[17-19]b . The compounds [3]a , [8,9]b and [17-19]b reacted with $SOCl_2$ in the presence of Benzene to producing the compounds [4]a , [10,11]b and [20-22]b. Chemical modification of Poly(vinyl alcohol) were obtained by reaction of PVA with compounds [4]a , [10,11]b and [20-22]b using the Dimethyl formamide to produce compounds [5]a ,[12,13]b and [23-25]b. The structure of the synthesized compounds were set by their analytical and spectral data such as , FTIR spectra , ^{1}H-NMR. , UV-Vis Spectroscopy and Elemental analysis (CHNS) . Finally study antibacterial activity screened via two types of bacteria. Anticancer activity also examined for all modifier polyvinyl alcohol.

Key words: Azetidinone , Tetrazole , Thiazolidinone, 1,3-Oxazepine .

INTRODUCTION

Heterocyclic compounds consisting of four , five and seven- membered rings have gave more importance in the recent decades for industrial and medicinal reasons. Azetidinone derivatives are one of these compounds , they represent an important class of four –membered cyclic amides, commonly known as β-lactam , due to their antibacterial [1-4] , antifungal [2-4], antitubercular[3,4] , antianthelminic [5] and enzymetic activity [6], furthermore they found to inhibit cholesterol absorption [7] . Five membered heterocyclic like thiazolidinone derivatives and tetrazole have gained increasing according to their industrial and biological properties. Tetrazole for example, is well known to have antibacterial , anti-inflammatory [8-10] and fungicide [11] activities. They also have been examined for reducing uric acid [12] , Moreover , tetrazole derivatives are used to prepare epoxy resin which is a raw material of printed circuit boards [13]. On the other hand thiazolidinone are obriously important because of their wide use in medicaments as antihyperglycemic [14] , antitumor growth [15] , antifungal [16] , anticonvulsant [17] and inhipitor for CDC7 protein kinase agents [18]. Finally, Oxazepine is non-homologous seven member ring that containing of two heteroatom (Oxygen andNitrogen). Oxazepine and their derivatives have some significant biological pharmacological activities like enzyme inhibitors , analgesic , antidepressant and psychoactive drugs [19]. Polyvinyl alcohol is used in biomedical and pharmaceutical applications[20] and in industries due to the stellar chemical and physical properties , good chemical resistance, non-toxicity, good film formation capacity [21]. PVA is inert in the body and does not cause harmful immune responses due to the hydroxyl groups [22]

Modified of polyvinyl alcohol with these compounds four , five and seven- membered rings via applying different Strategies to get more active and less toxic might possess a good biological scope as anticancer agents.

EXPERIMENTAL

A-Materials

All the chemicals utilized in the work were provided from Sigma-Aldrich and BDH.

B – Instrumentation

Infrared spectra were recorded as KBr disc on SHIMADZU-FT-IR-8400 spectrometer.^{1}H-NMR spectra was recorded on Bruker 400 MHz , measurement were made at Central lab , Tahran University (Iran).

1-Preparation of Schiff bases [1]a,b [23]

Amixture of 2-amino-5-mercapto-1,3,4-thiadiazole (0.01mol) and dimethyl benzaldehyde , 3-nitro benzaldehyde (0.01mol) were stirred under reflux in absolute EtOH (15 ml.) 3 drops of glacial acetic acid for (8 hrs.). The mixture was allowed to cool at room temperature then filtered and dried , recrystallized from EtOH to gave colored crystals. (80% and 72%) , m.p = 220-222 ^{0}C and 216-218^{0}C, respectively.

2-Preparation of [2]a[23]

(0.001mol. , 0.09g) of mercapto acetic acid in dry benzene (30ml.) `was added quietly to (0.001mol.) of compound[1]a .The addendum continuous for (5min) with continued stirring then for about (3 hrs.) .Then reflux on a steam bath for (18 hrs.) spare solvent was evaporated and the residue was remedy with $NaHCO_3$, filtered and recrystallized with dioxan.

3-Preparation of [6]b[23]

(0.01mol.) of $ClCH_2COCl$ in (12ml.) of dioxan cooled at (0-5) C^o , then added (0.01mol.) of Et_3N in(12ml.) dioxane and Schiff base (0.01mol.) in (12ml.) of dioxane was tardily added and refluxed in water bath for (12hours) . When the reaction had finished - (reveal by thin layer chromatography) , the mixture teeming into ice-cold water to afford solid precipitate , then filtered , dried and recrystallization by benzene-ether(50-50).

4-Synthesis of [7]b[24]

Sodium azide (0.01mol) was added to a stirring solution of azomethine [1]b (0.01mol) in DMF (15ml.) , the mixture was refluxed for 4hrs. , then it was allowed to cool and the

precipitate was filtered , washed with water and recrystallized from petroleum ether. Elemental analysis of compound[7]b , Calcd: C%=35.17 H%= 1.62 N% =31.92 S%=20.84
Found: C%=35.28 H%= 1.71 N% =31.99 S%=20.97

5-Synthesis of 1,3-oxazepine derivatives [14-16]b[19]

Amixture of equimolar amounts (0.01mol) of Schiff base [1]b and different acid anhydrides(pyromellitic dianhydride , phthalic anhydride and maleic anhydride) in dry benzene (0.01mol) were reflux for 6hrs. The solvent, resulting crystalline solid, was removed and recrystallized from EtOH.
Elemental analysis of compound[16]b , Calcd: C%=42.85 H%= 2.19 N% =15.83 S%= 17.58
Found: : C%=42.96 H%= 2.29 N% =15.97 S%= 17.69

6-Synthesis of [3]a and [8,9,17,18,19]b [25]

A liquot (0.001mol.) of hot compound [2]a , [6,7,14,15,16]b mixed with (0.002 mol.) Na_2CO_3 in (15ml.) of solvent (distilled water) , then (0.001mol.) of $ClCH_2COOH$ was added.The solution refluxed for (3hr.) , acidified the solution by used conc. hydrochloric acid to PH= 2 after cooling. Filtered the product and washed with distilled water and recrystallized from absolute ethanol.
Elemental analysis of compound[8]b , Calcd: C%=38.75H%= 2.23 N% =13.91 S%= 15.90
Found: C%=38.69 H%= 2.11 N% =13.82 S%= 15.82
Elemental analysis of compound[18]b , Calcd: C%=48.30 H%= 2.54 N% =11..86 S%= 13.55
Found: C%=48.41 H%= 2.62 N% =11..98 S%= 13.67

7-Synthesis of [4]a and [10,11,20,21,22]b[26]

A mixture of compounds [3]a , [8,9,17,18,19]b (0.01mol) was mixed with $SOCl_2$ (0.01mol.) in dry benzene (10 ml.) then refluxed for(7 hr .) . The Amount of $SOCl_2$ and benzene separated under vacuum after cooling.

8-Chemical modification of polyvinyl alcohol[5]a and [12,13,23,24,25]b[27]

Poly vinyl alcohol (1mol.) was placed in (20 ml.) DMF and (1mol.) of compounds[4]a , [10,11,20,21,22]b , the mixture was frequent shaking for (3hr.)and refluxed for(2hr.). The product was poured into the water, washed with a little sodium bicarbonate washed with water then with ethanol the product purified by DMSO and precipitating from ethanol.

Biological Activity

All of synthesized compounds have been screened their antibacterial activities versus (*Bacillus cereus* and *Esherichia coli*) using cup-plate agar diffusion method [28] . The zone of inhibition measured in mm.. Penciline (50µg /ml) was utilized as a standard drug for antibacterial activity to compare with the activity of the synthesized compounds.

Cytotoxicity Assay

Preparation of Cell Lines for Cytotoxicity Assay [29] Six modifier PVA compound with different sizes and concentrations were screened for their anticancer activity and cytotoxicity by using cultured cells in micro titer plate (96wells). The assay was applied by the following steps:

Seeding , Incubation , Exposure Staining .

The absorbance was measured on a micro plate reader at 620 nm. The rate of inhibition of cell growth calculated according to [30]as follow equation:

$$\text{Inhibition rate} = \frac{mean\ of\ control - mean\ of\ treatment}{mean\ of\ control} \times 100$$

RESULTS AND DISCUSSION

Scheme (1) summarized the performed reactions in this work compound [1] a,b , [2]a , [6]b intended according to literatures[23]. The forming of these compounds were indicated by m.p. and FT-IR which assent with these literatures. .

Tetrazole [7]b was prepared by reaction of Schiff base[1]b with NaN_3 in dry dimethyl formamide. FTIR spectrum of [7]b showed demise of absorption stretching band of imine group with appearance of new absorption stretching band in the zone (1525 , 1350) cm^{-1} which are assigned to N=N and C-N stretching.

Scheme(2) a series of 1,3-oxazepine derivatives [14-16] b were prepared from substituted imine[1]b with different anhydrides: pyromellitic dianhydride , phthalic anhydride , maleic anhydride. FTIR of compound [15]b , figure (1) was proven from the disappearance of band due to C=N of schiff base and appearance of band at (1691 , 1710) cm^{-1} for carbonyl groups in oxazepine ring and bands around (1269 and 1114 cm^{-1}) belong to asymmetric and symmetric (C-O-C) band .

Compounds[3]a , [8,9]b and [17-19]b were synthesized by reaction compounds[2]a, [6,7]b , [14-16]b with ClCH2COOH in distilled water as a solvent in basic media. FTIR of [3]a , figure(2) displays [31] an absorption bands, (3400-2400) cm^{-1} for hydroxyl group, (3007) cm^{-1} for (C-H) aromatic, (2972-2818pl) cm^{-1} for (C-H) aliphatic, (1695) cm^{-1} for carboxylic group, (1622) cm^{-1} for azomethine and (1600) cm^{-1} for (C=C) aromatic , figure(3) ^{1}H-NMR (DMSO-d6) revealed signals: singlet signal at δ (2.06) ppm for proton of $N(CH3)_2$, singlet signal at δ (4.09) ppm for proton of CH_2 of thiazolidinone ring, (4.20) for (2H, SCH2), signal at δ(4.81)ppm for proton of (CH-N) , multiple peaks at δ(7.38-7.60) for aromatic protons and singlet peak at δ(11.6) for (1H, COOH). The ^{1}H-NMR spectrum of [19]b (DMSO-d6) , figure (4) revealed signals at δ(ppm)at : (3.94) for (2H, SCH2) ,doublet signal at δ(4.886) for (CH=CH) , singlet signal at δ(6.16) for proton of (CH-N) , multiple peaks at δ(6.59-7.87) for aromatic protons and singlet peak at δ(9.6) for (1H, COOH).

Compounds[4]a,[10,11]b and [20-22]b were synthesized by the condensation of compounds[3]a ,[8,9]b and [17-19]b with thionyl chloride in dry benzene . FT-IR spectrum of compound [21]b , figure(5) illustrated [26] the absence of band at (1683) $cm^{-1}$and (3400-2400) cm^{-1}due to (carbonyl, hydroxyl) group of carboxylic acid and presence of band at (1741) cm^{-1} related to acyl chloride.

Chemical modification of Poly (vinyl alcohol) [5]a, [12,13]b and[23-25]b were gained by reaction of PVA with compounds[4]a, [10,11]b and [20-22]b using the dimethyl form amide.FT-IR spectrum of compound [12]b , figure(6) explain the being of a large peak at 3419 cm^{-1}this peak is attached to the stretching of hydroxyl from the intermolecular and intermolecular hydrogen bonds, which

seen at 2908cm^{-1} and 2943 cm^{-1}respectively refer to the symmetric and asymmetric stretching vibration of C–H from alkyl groups , showed the disappearance of absorption band at (1761) cm^{-1} due to acyl chloride and apparition of absorption band at (1716) cm^{-1} caused by carbonyl of ester. The ^{1}H-NMR spectrum of compound [12]b , figure(7) , showed the signals: doublet peak at δ(1.26) ppm for (2H, CH2) and triplet peak at δ(1.37) ppm for (1H, CH) , singlet peak at δ(2.4) ppm for (1H,OH) , singlet peak at δ(3.86) ppm for (2H, SCH2), singlet peak at δ(4.24) ppm for (1H,CH-N) , singlet peak at δ(4.96) ppm for(2H,CH_2Cl) and multiple peaks at δ(7.32-7.62) ppm for aromatic protons . ^{1}H-NMR spectrum of [23]b , figure(8) , showed the following signals: doublet peak at δ(1.55) ppm for (2H, CH2) and triplet peak at δ(2.10) ppm for (1H, CH) , singlet peak at δ(3.85) ppm for (1H,OH) , singlet peak at δ(4.39) ppm for (2H, SCH2), singlet peak at δ(5.34) ppm for (1H,CH-N) and multiple peaks at δ(7.33-8.0) ppm for aromatic protons. The UV-Vis spectrum of compounds [12]b and [23]b , show the absorption peaks at[(332-510) , (330-404)] may refer to(π- π*) and (n-π*) respectively.

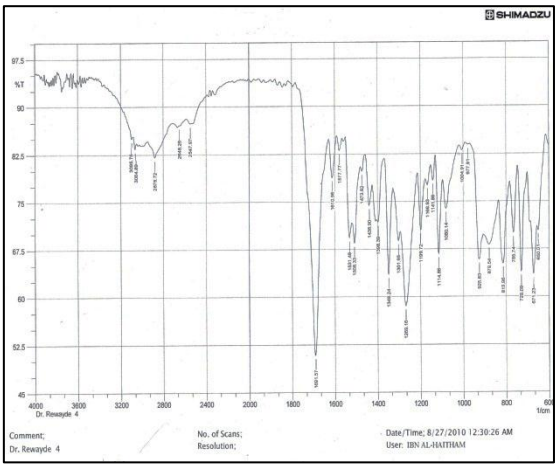


Fig. (1): FT-IR of[15]b

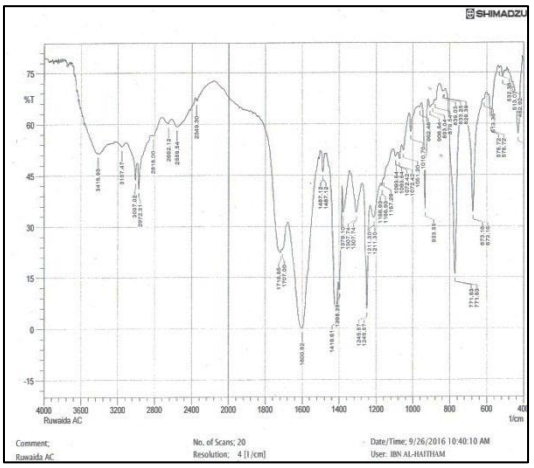


Fig. (2): FT-IR of [3]a

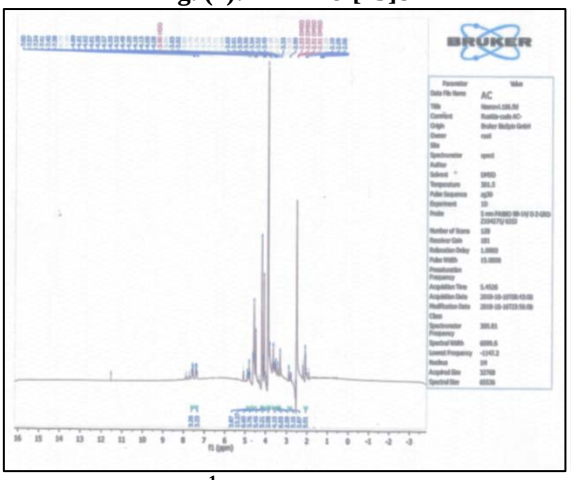


Fig. (3): ^{1}H-NMR of [3]a

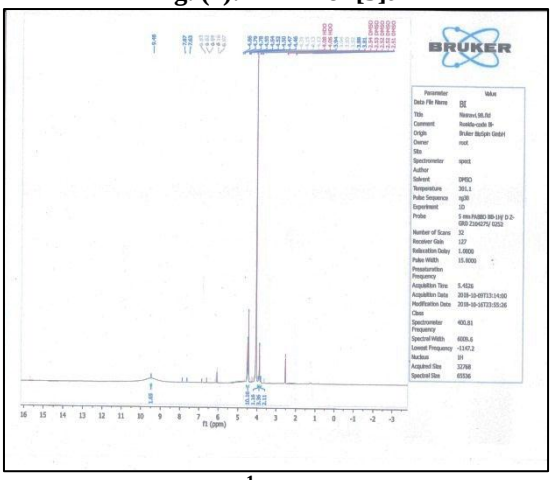


Fig. (4): ^{1}H-NMR of [19]b

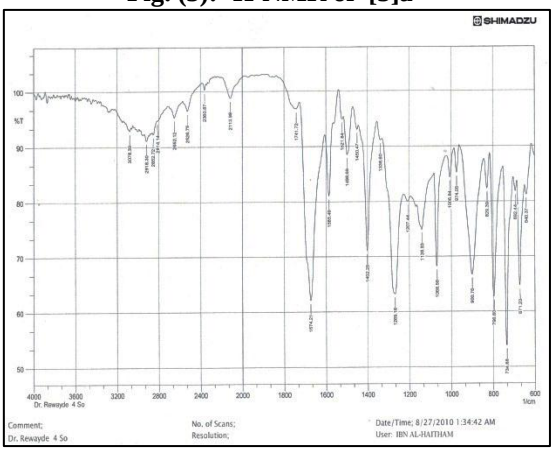


Fig. (5): FT-IR of [21]b

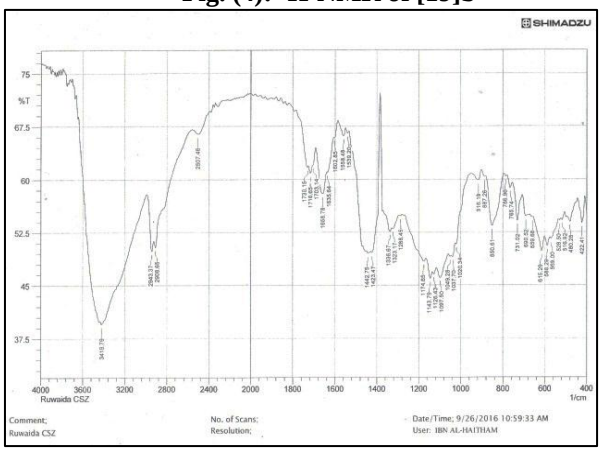


Fig. (6): FT-IR of [12]b

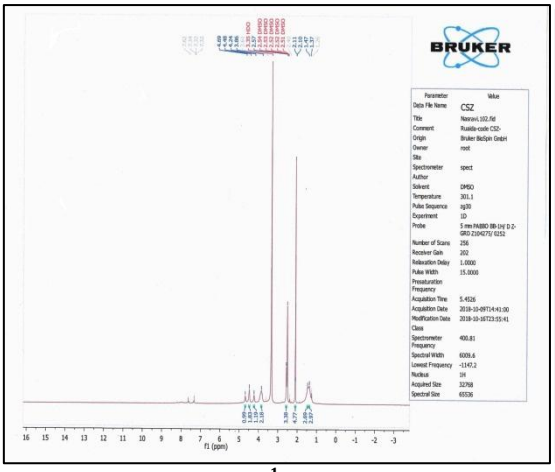


Fig. (7): ^{1}H-NMR of [12]b

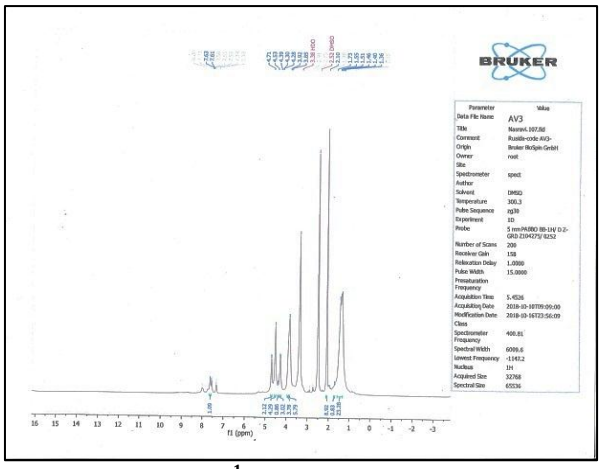


Fig. (8): ^{1}H-NMR of [23]b

Biological Activity

All the newly synthesized derivatives were screened for their in vitro antimicrobial activity against *Escherichia coli* , *Bacillus cereus* by measuring the zone of inhibition in mm . Result showed modified polymer [5]a,[12,13,24]b were high activity than [2]a ,[6,7,15]b and that compound$[12]_b$ exhibit high antibacterial activity with against *E .coli* and *Bacillus cereus*. Resalts of all compounds and their antibacterial activities listed in Table(1).

Anticancer activity

Six compounds modified polyvinyl alcohol were chosen for examend their anticancer activity in Bio-technology research center , Al-Nahrain University,Iraq. Two cell lines were used (mice intestines carcinoma cell line L20b and human pelvic rhabdomyosarcoma (RD) according to the mode described by Freshney[29] . Results are expressed in percentage . Compound$[5]_a$ exhibit high inhibition for two cell line L20b and(RD). All compounds except $[25]_b$ showed more than 50% inhibition for (RD).

Table (1) : The antibacterial activity of some synthesized compounds.

Comp.	*E .coli* (G-)	*Bacillus* ***cereus*****(G+)**
Penciline	16	22
DMSO	Nil	Nil
[2]a	10	12
[5]a	16	20
[6]b	15	16
[7]b	13	15
[12]b	30	20
[13]b	21	19
[15]b	14	13
[24]b	27	22

Table (2):The inhibition of cells growth of some synthesized compounds.

Comp.	**For(L20b)**	**For(RD)**
$[5]_a$	75.3%	67.8%
$[12]_b$	59.7%	63.9%
$[13]_b$	47.2%	50.3%
$[23]_b$	66.2%	55.0%
$[24]_b$	42.6%	54.4%
$[25]_b$	35.1%	44.3%

Table(3):FT-IR of [14,16]b

Comp. No.	**M.P**	**(C-H) arom.**	**(C=O)N**	**(C=O)O**	**(C=C)**	**(C-S)**
[14]b	186-188	3066	1693	1741	1585	671
[16]b	190-191	3024	1687	1728	1600	671

Table(4):FT-IR spectroscopy data of compounds$[8,9,17,18]_b$

Comp. No.	**M.P**	**(O-H)**	**(C-H) arom.**	**(C=O) carboxlic**	**(C=N)**	**(C=C)**
$[8]_b$	200-202	3400-2400	3007	1697	1610	1593
$[9]_b$	178-180	3400-2400	3012	1695	1612	1598
$[17]_b$	208-210	3300-2400	3057	1693	1612	1588
$[18]_b$	166-168	3400-2400	3061	1695	1627	1590

Table(5):FT-IR spectroscopy data of compounds[4]a and $[10,11,20,22]_b$

Comp. No.	**(C-H) arom.**	**(C=O)Cl**	**(C=N)**	**(C=C)**
[4]a	3008	1766	1622	1589
$[10]_b$	3008	1770	1620	1591
[11]b	3007	1760	1612	1590
$[20]_b$	3010	1753	1620	1581
$[22]_b$	3082	1766	1631	1587

Table(6):FT-IR spectroscopy data of polymers[5]a and [12,13,23,24,25]$_b$

Comp. No.	(O-H)	(C-H) aliph.	(C=O) ester	(C-O)	(C=C)
[5]a	3419	2943,2908	1714	1143	1600
[12]$_b$	3419	2943,2907	1716	1143	1590
[13]b	3313	2931,2908	1728	1139	1598
[23]$_b$	3381	2921,2910	1710	1139	1590
[24]$_b$	3292	2922,2852	1724	1135	1589
[25]$_b$	3338	2920,2852	1712	1141	1587

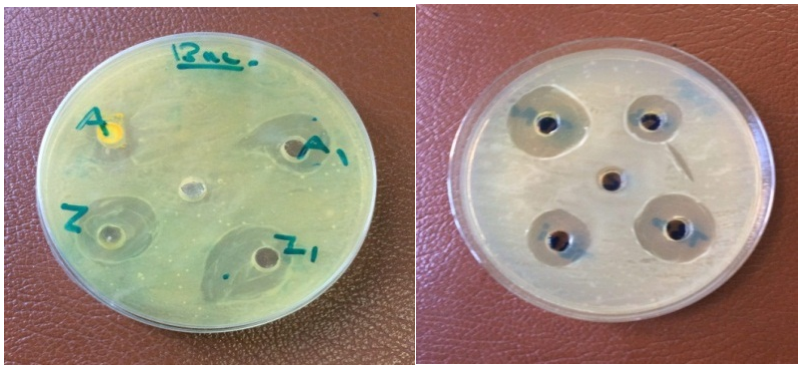


Figure (9)Antibacterial activities of compounds against *Bacillus cereus*

Figure (10) Antibacterial activities of compounds against *E.coli*

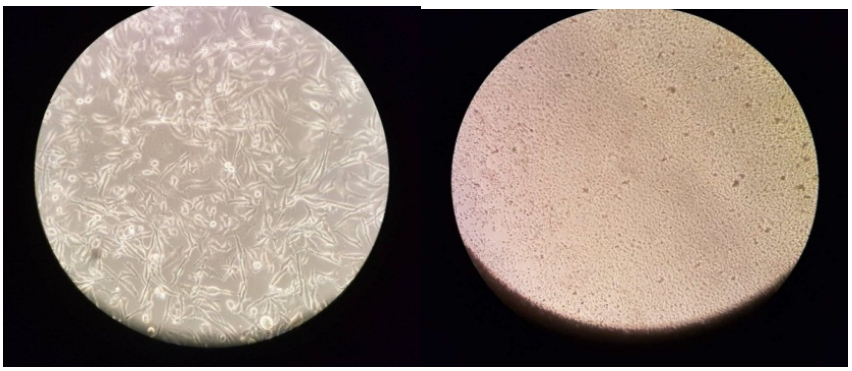
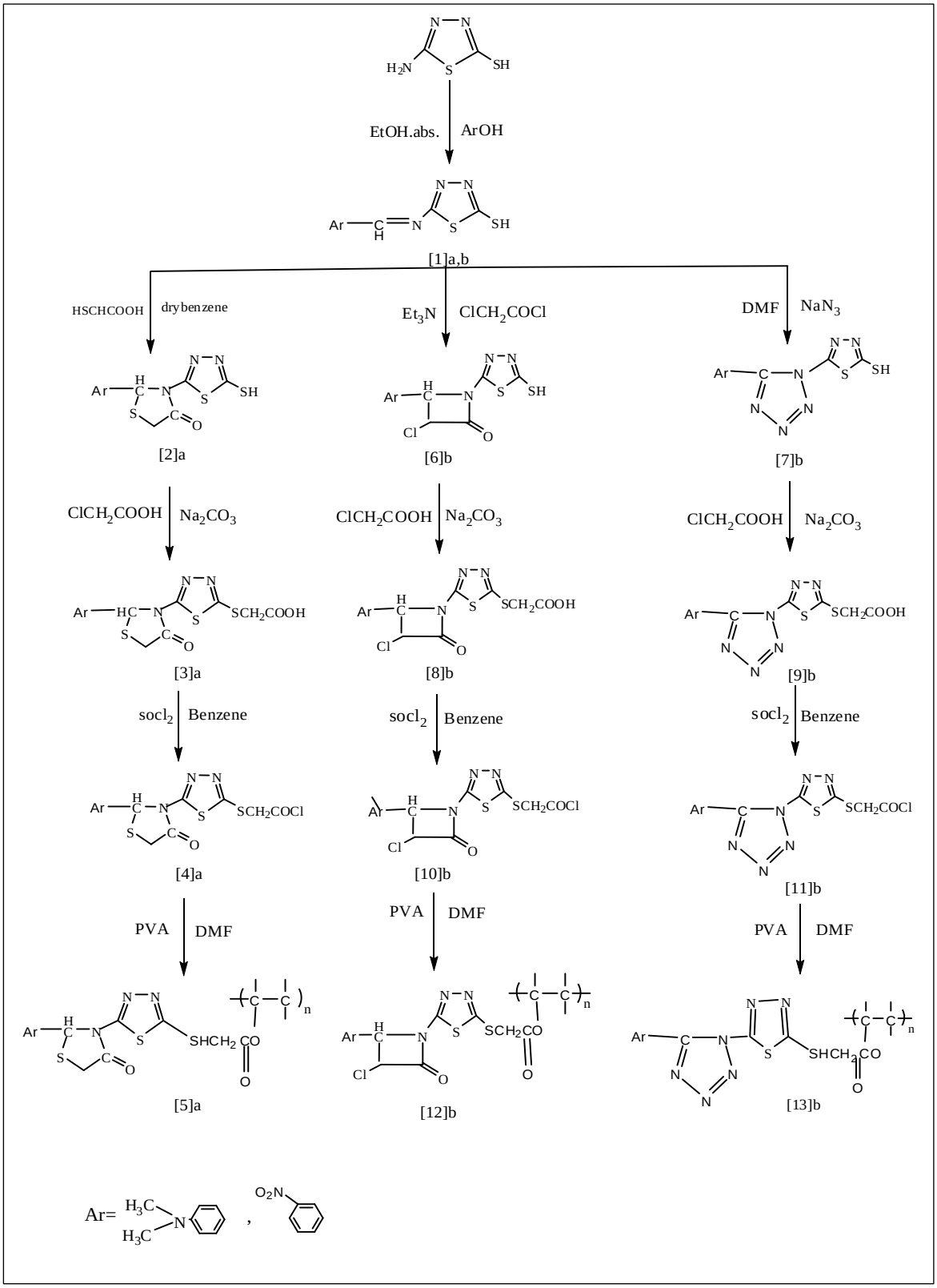
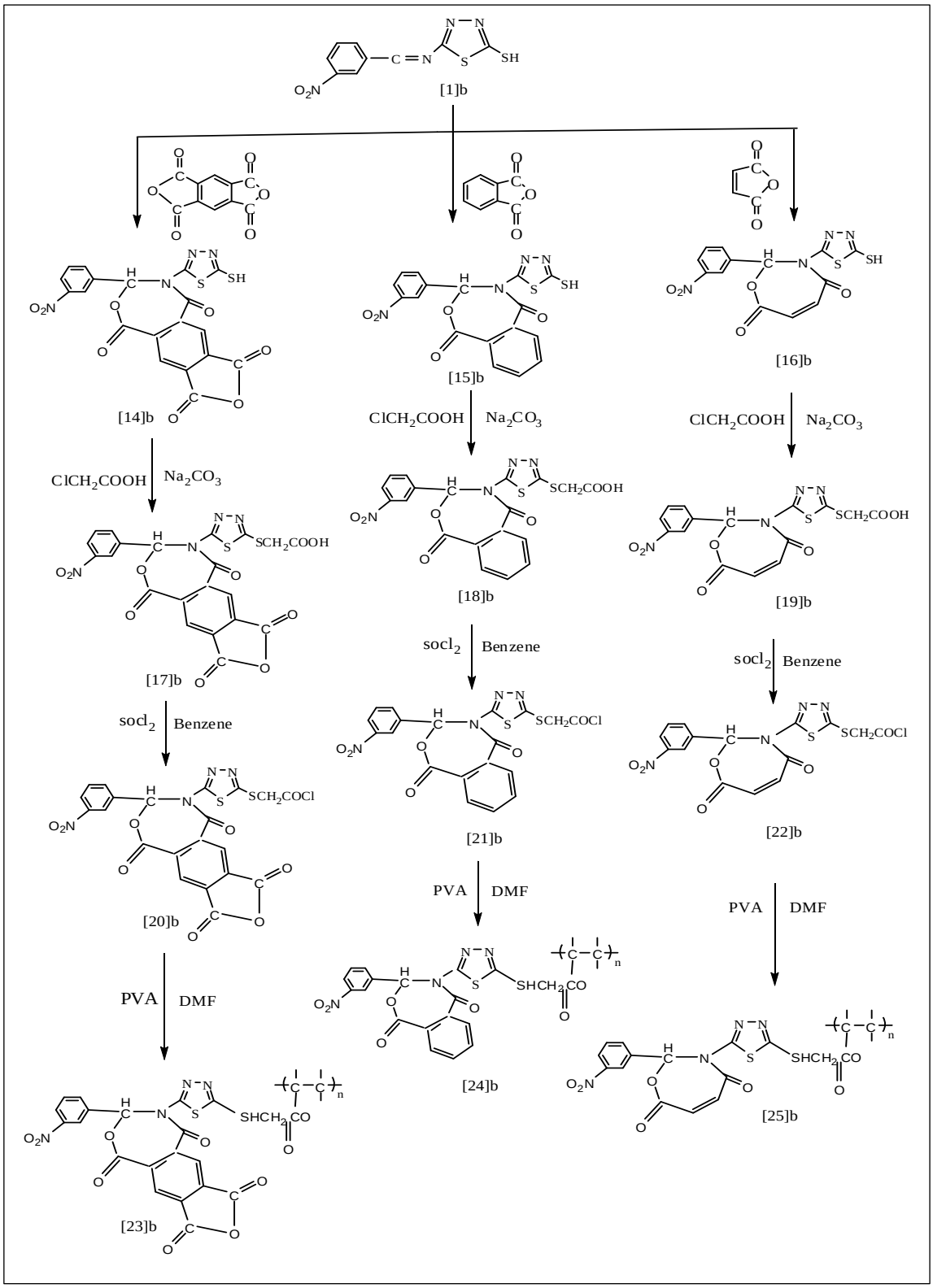


Fig.(11). Image of well


EtOH.abs.
ArOH
[1]a,b
HSCHCOOH
drybenzene
Et3N
ClCH2COCl
DMF
NaN3
[2]a
[6]b
[7]b
ClCH2COOH
Na2CO3
[3]a
[8]b
[9]b
socl2
Benzene
[4]a
[10]b
[11]b
PVA
DMF
[5]a
[12]b
[13]b
Ar=

Scheme(1)



Scheme(2)

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