ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis of Antifungal Chemical Compounds from Fluconazole with (Pharma- Chemical) Studying.

Nagham Mahmood Aljamali*

Assist. Professor, Chemistry Department, Faculty of Education for women., Kufa University, Iraq.

ABSTRACT

Several types from microbial (fungi) live on parts of body like skin ,some of them can cause infections of the body in (skin, mouth or vagina). The most of them cause skin infections which are the tinea group of fungi and *Candida*. For this reason , series fluconazole drug derivatives were synthesized to increasing resistance of antibiotics against fungi, like fluconazole - (thiadizole , oxadizole , formazan , enamine , anile , other).Fourteen fluconazole derivatives synthesized in this work through various reactions like ring closure of thiadizole or oxadiazole or fomazane reaction or via mannich reaction ,all of them investigated by many techniques like using (TLC) and techniques((FT.IR ,^{1}H.NMR ,^{13}C.NMR ,other analysis)) ,then studying, studying of physical characterization and other analytical studies like solubility in various solvents)) and they have been tested against fungi via bio studying .

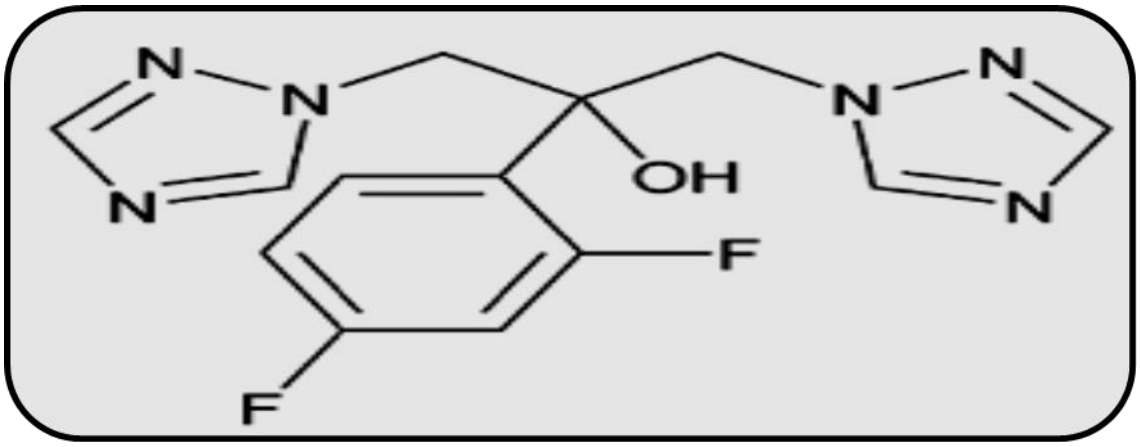
Keywords: fluconazole , antifungal , thiadizole, oxadizole, pharm , formazan.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Fluconazole drug is an antifungal medication which is given via mouth or by injection. Which is used to treatment many fungal infections like Candida infections in the vagina, mouth with bloodstream..



1.1. Fluconazole Structure

Fluconazole is triazole derivative designated in medical chemistry [1-3] as 2,4-difluoro-α,α1-bis(1H-1,2,4-triazol-1-ylmethyl) benzyl alcohol with an empirical formula of (($C_{13}H_{12}F_2N_6O$)) . Fluconazole drug is a triazole cycle , a white crystalline solid which is soluble in water, which is used in medicine to treatment fungal infections .It has high effect and a broad spectrum of fungi

A. It is used against Dermatophytes.
B. It is used against Candida with malassezia.
C. It is used against Cryptococcosis or coccidiodomycosis.
D. It is used against Invasive candidiasis.
E. It is used against Vaginal candidiasis.
F. It is used against Dermatomycosis.

There are types of fluconazole drugs capsules or tablet (as 50 mg, 150 mg and 200 mg) under trade name (Diflucan) , or injection by intravenous use (2 mg/ml) , which absorbed orally with or without food , then it will be distributed through 22 hours in body tissues.

Mechanism of Fluconazole action as antifungal[4-7] is a highly sensitive inhibitor of fungal (cyto chrome P450) dependent enzyme lanosterol 14-α-demethylase and hetero cycles which liked with drugs [3-9] . We know action of this enzyme which convert lanosterol to ergosterol.

EXPERIMENTAL & INSTRUMENTAL PART

FT-IR spectra were recorded by using (FT-IR 8300 Shimadzu) in the range (400-4000)cm^{-1} by using KBr disc .

^{1}H.NMR–spectra in DMSO– d6 solvent were carried out in Canada , ^{13}C.NMR–spectra in DMSO– d6 solvent were carried out in Canada , and other physical and analytical studies with biological studying in biology lab.

Methodology :

Synthesis of Compounds {1 - 3 }:

A mixture of (0.02 mole) of Fluconazole and (0.02 mole) of chloro ethyl acetate were reacted in presence of potassium carbonate to give compound {1} ,which (0.01mole) refluxed with semicarbazide (0.01mole) in presence of absolute ethanol for (4hrs) , the precipitate was filtered and dried then re

ISSN: 0975-8585

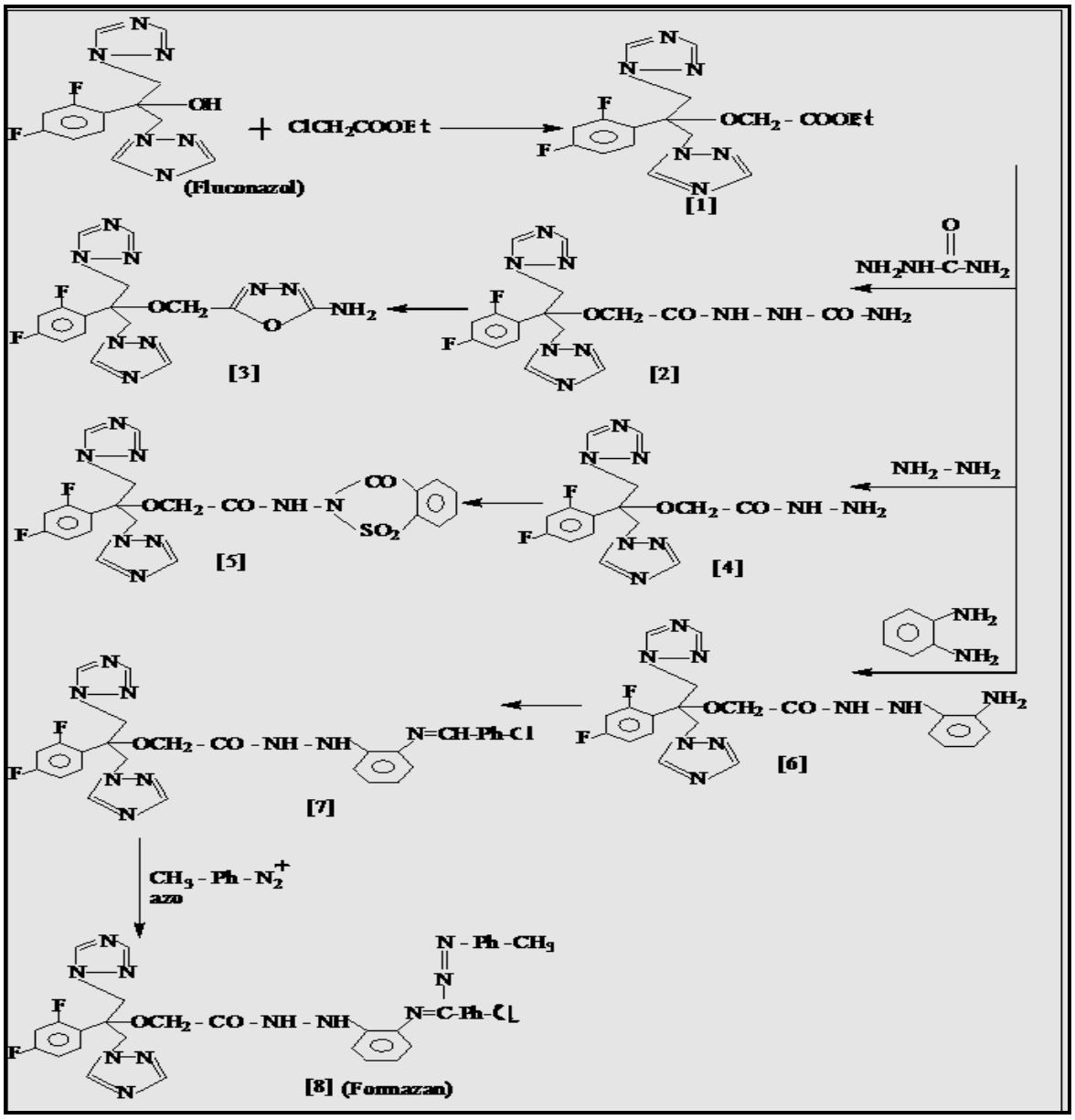
crystallized to give compound {2} ,which (0.01mole) heated in presence of (7 ml) of H_2SO_4 and absolute ethanol with refluxing for (9hrs), the precipitates filtered then re crystallized from ethanol to produce oxadizole- fluconazole derivative which acts compounds{3}.

Synthesis of compound { 4 , 5 } :

Compound {1} (0.01mole) was refluxed with (0.01mole) of hydrazine for (3hrs) , the precipitates filtered then dried to give compounds {4} , which (0.001 mole) refluxed for (5 hrs) with (0.00l mole) from sulphobenzoic anhydride in presence of acetone to yield compound {5} .

Synthesis of compound { 6 - 8 } :

Compound {1} (0.02mole) was refluxed with (0.02mole) of ortho-phenylene di amine for (3hrs) , the precipitates filtered then dried to give compounds {6} , which refluxed (0.001 mole) for (4 hrs) with (0.00l mole) of 4-chlorobenzaldehyde in presence of ethanol with drops of glacial acetic acid to yield compound {7} , which dissolved in basic ethanol then reacted with 4-methyl phenyl diazonium ,after (48 hr) gave formazan – fluconazole derivative acts compound {8}. .

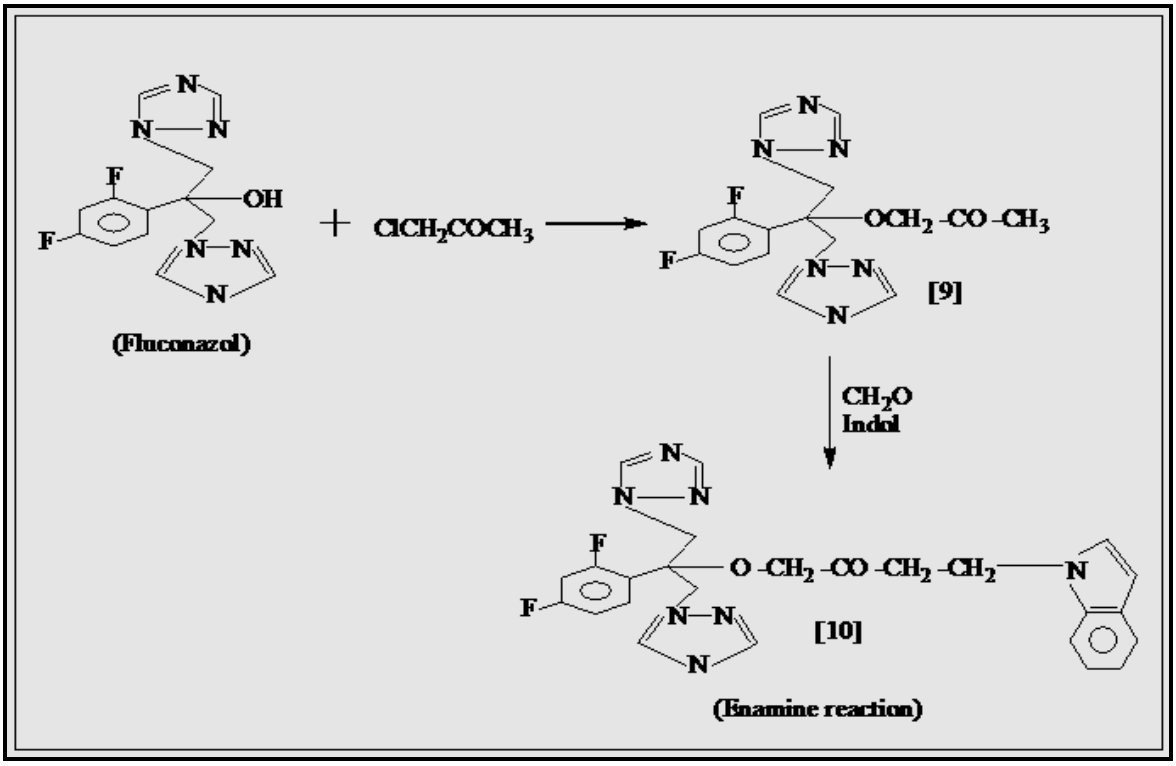


Scheme (1): Synthesis of Drugs Derivatives {1 – 8 }

ISSN: 0975-8585

Synthesis of Compounds {9 , 10 }:

A mixture of (0.01 mole) of Fluconazole and (0.01 mole) of acetone chloride were reacted in presence of potassium carbonate to give compound {9} ,which (0.01mole) reacted with formaldehyde (0.01mole) with (5 ml) of sulphuric acid after that added (0.01 mole) from indole in ice path for one hours after that , refluxed for 3 hrs , the precipitate was filtered and dried then re crystallized to give compound {10} ,which acts enamine - fluconazole derivative (mannich reaction).

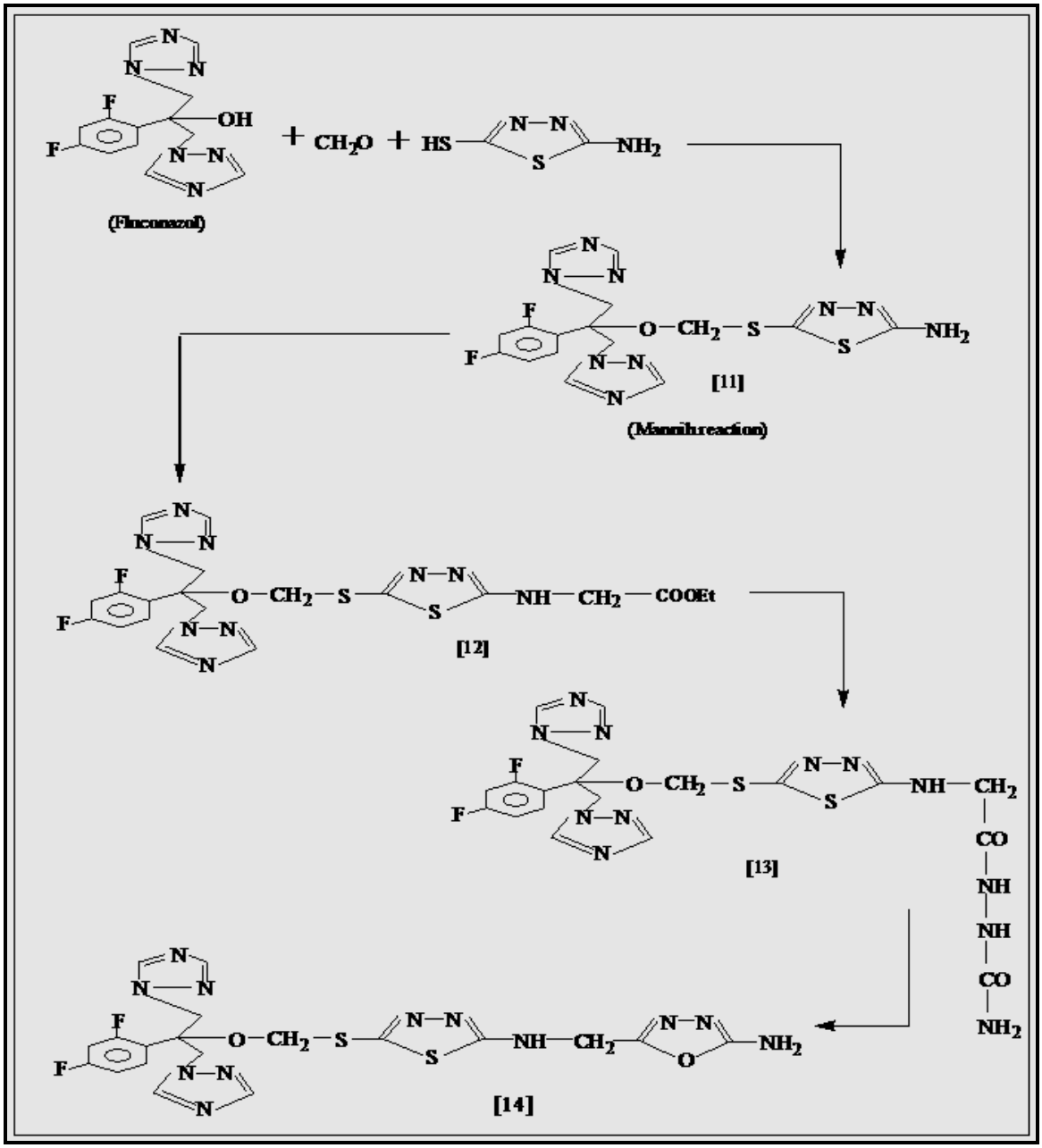


Scheme (2): Synthesis of Drugs Derivatives {9 , 10 }

Synthesis of Compounds { 11 - 14 }:

Fluconazole (0.01mole) reacted with formaldehyde (0.01mole) with (5 ml) of sulphuric acid after that added (0.01 mole) of 5-mercapto-2-amino-1,3,4-thiadiazole in ice path for (2 hrs) after that, refluxed for (2 hrs) , the precipitate was filtered and dried then re crystallized to give compound {11}, which (0.01mole) refluxed with (0.01mole) of chloro ethyl acetate for (3hrs) to produce precipitation which filtered and dried then re crystallized to yield compound{12}, which refluxed with (0.01mole) of semicarbazide in presence of ethanol for (4 hrs) to yield compound{13}, which refluxed for (5 hrs) with (7 ml) of sulfuric aid to produce compound {14}.

ISSN: 0975-8585



Scheme (3): Synthesis of Drugs Derivatives {11 – 14 }

RESULTS AND DISCUSSION

Many chemists prepared several drugs derivatives from various starting materials ,now, our paper involved synthesis of new series of fluconazole drugs as antifungal, fourteen compounds were synthesized via many reactions like (cyclization , mannich reaction, formazan reaction) , then all formatted compounds investigated by using many spectral techniques represented by ((FT.IR, H.NMR , ^{13}C.NMR)) and Studying of some physical and chemical properties, then all fluconazole derivatives tested with fungi :

Spectral Investigation :

The FT.IR-spectrum showed an absorption bands at (1721 -1726)cm^{-1} in compounds {1 , 12 } due to the carbonyl of ester group (-COO-) which disappeared and other bands appeared as a result of formation of new compounds ., while other bands are appeared at {(3296- 3215)cm^{-1} for (NH) amide and amine groups in compounds { 2 ,4,5,6,7, 8,13 } respectively , bands at (3300 -3392)cm^{-1} in compounds {2, 3, 4, 6, 13, 14

ISSN: 0975-8585

} due to the of amine group (NH_2). ,but compound {8 } appeared bands at (1471 , 1496)cm^{-1} for azo groups (-N=N-) and other bands like { (S-C) , (C-O-C) , (NH-NH), (C-Cl) ,….} in Table (1) .

Table (1): FT.IR- data (cm^{-1}) of formatted derivatives {1 - 14} .

Compounds	$I.R_{(KBr)}$ ((Only Important Groups))
{ 1 }	(C-O-C) ether: 1165 ., (-COO-) carbonyl of ester :1721
{ 2 }	(C-O-C) ether: 1181, (NH_2) amine :(3321 ,3300) ., (CO-NH)carbonyl of amide :1679 ., (NH-NH) :3254
{ 3 }	(C-O-C) ether: 1172 , (NH_2): 3382, 3367 ., (C=N) endo cycle :1609,
{ 4 }	(C-O-C) ether: 1181., (CO-NH)carbonyl of amide :1681., (NH-NH_2) :3291 ,3308
{ 5 }	(C-O-C) ether: 1187 ., (CO-NH)carbonyl of amide :1683, (NH):3215., (SO_2) : 1321.
{ 6 }	(C-O-C) ether: 1175, (NH_2) amine :(3372 ,3324) ., (CO-NH)carbonyl of amide :1686 ., (NH-NH) :3234
{ 7 }	(C-O-C) ether: 1185., (CO-NH)carbonyl of amide :1679 ., (NH-NH) :3226 ., (CH=N) imine group : 1625 ., (C-Cl) : 703 .
{ 8 }	(C-O-C) ether: 1153., (CO-NH)carbonyl of amide :1693 ., (NH-NH) :3254 (C=N): 1637 ., (C-Cl) : 718 , (N=N)azo : (1471 , 1496).
{ 9 }	(C-O-C) ether: 1181, (CO) ketone : 1713.
{ 10 }	(C-O-C) ether: 1179, (CO) ketone : 1719.
{11 }	(C-O-C) ether: 1170 , (NH_2) amine :(3341 ,3312) ., (CH_2-S): 1215 ., (C-S): 732.
{12 }	(C-O-C) ether: 1170 , (NH) amine :3296., (CH_2-S): 1207 ., (C-S): 745 .,(-COO-) carbonyl of ester :1726.
{13 }	(C-O-C) ether: 1162 , (NH) amine :3288., (CH_2-S): 1219 ., (C-S): 738 ., (NH_2) amine :(3390 ,3363) ., (CO-NH)carbonyl of amide :1682 ., (NH-NH) :3251
{14 }	(C-O-C) ether: 1166 , (NH) amine :3289., (CH_2-S): 1202 ., (C-S): 731 ., (NH_2) amine :(3392 ,3374)

3.1.2. The ^{1}H.NMR- Spectra: showed many peaks indicate to functional groups in organic compounds which disappearance in product compounds due to formatted derivatives like peaks at Б {Б (3.04 – 3.74) for proton of (-OCH_2-) in all formatted compounds {1-14} respectively .But compounds {3, 4, 6 , 11-14} showed signal for protons of (NH or NH_2) amine at Б (5.10 - 5.56) respectively .
Compounds {1 , 12} showed peaks at Б(3.16 – 4.0) for proton of (COO-C_2H_5) ethyl groups of ester , respectively .

Compound { 2 ,4 -8 , 13} showed signals at Б(10.0 - 10.44) proton of amide (NH-CO-)., and other signals in Table (2).

Table (2): H.NMR- data (Б - ppm) of drug derivatives {1-14}

Compounds	H.NMR ((Only Important Peaks))
{ 1 }	protons of (-OCH_2-) : (3.04)., (COO-C_2H_5): (3.5 – 4.0)
{ 2 }	protons of (-OCH_2-) :(3.10)., (CO –NH-NH-CO-NH_2)amide: (10.34 ,10.18 ,10.0) .
{ 3 }	protons of (-OCH_2-) : (3.12)., amine (NH_2) : 5.98
{ 4 }	protons of (-OCH_2-) :(3.05)., (CO –NH-)amide : 10.24 ., amine (NH_2) :5.96
{ 5 }	(protons of (-OCH_2-) :(3.11)., (CO –NH-)amide : 10.08 .
{ 6 }	(protons of (-OCH_2-) :(3.13)., (CO –NH-)amide : 10.15 ., (NH): 5.67 ., (NH_2) : 5.88 .
{ 7 }	(protons of (-OCH_2-) :(3.04)., (CO –NH-)amide : 10.11 ., (NH): 5.43 ., imine proton (CH=N) : 8.75
{ 8 }	protons of (-OCH_2-) :(3.17)., (CO –NH-)amide : 10.21 ., (NH): 5.81 ., (CH_3-) :0.97 .
{ 9 }	protons of (-OCH_2-CO-) :(3.46)., ,(CH_3-CO): 2.91 .
{ 10 }	protons of (-OCH_2-CO-) :(3.41)., ,(-CO- CH_2 - CH_2): (2.53- 2.98)
{11 }	protons of (-OCH_2-S-) :(3.74)., amine (NH_2) : 5.63
{12 }	protons of (-OCH_2-S-) :(3.62)., (-CH_2 -) : 1.03 ., (COO-C_2H_5) : (3.16 – 3.45)
{13 }	protons of (-OCH_2-S-) :(3.58)., (-CH_2 -) : 1.00 ., (CO –NH-NH-CO-NH_2)amide: (10.44 ,10.27 ,10.08) .
{14 }	protons of (-OCH_2-S-) :(3.51)., (-CH_2 -) : 1.01 ., amine (NH_2) : 5.96

ISSN: 0975-8585

The ^{13}C.NMR spectral data of some compounds appeared signals which was evidence to important functional groups in prepared compounds , it gave signals due to carbonyl of ester , carbonyl of amide , carbon of hetero cycles , carbon of phenyl ring , carbon of imine group (Schiff base) , carbon of ether and other functional group, all these peaks indicated to formation of new drugs derivatives , table (3) .

Table (3): ^{13}C.NMR- data of Compounds

Comp. No.	^{13}C.NMR-data ((only Important Peaks))
{1}	(60.01):(C , OCH_2)., (27.5 , 31.3): (C, Ethyl group) ,(171.1): (COO , ester) , (119 -138): (C , phenyl group). , (143 -156): (C , heterocycles) .
{2}	(56.31):(C , OCH_2)., (168.1 , 164.5): (CO-NH , amide) , (114 -139): (C , phenyl group). , (148 -157): (C , heterocycles) .
{3}	(61.08):(C , OCH_2)., (121 -132): (C , phenyl group). , (140 -158): (C , heterocycles) .
{4}	(62.4):(C , OCH_2)., (169.5): (CO-NH , amide) , (124 -134): (C , phenyl group). , (142 -155): (C , heterocycles) .
{5}	(59.22):(C , OCH_2)., (166.23 , 161.36): (CO-NH , amide) , (124 -140): (C , phenyl group). , (146 -158): (C , heterocycles) .
{6}	(60.571):(C , OCH_2)., (165.7): (CO-NH , amide) , (120 -142): (C , phenyl group). , (146 -155): (C , heterocycles) .
{7}	(57.63):(C , OCH_2)., (163.8): (CO-NH , amide) , (125 -144): (C , phenyl group). , (148 -159): (C , heterocycles) ., (101.8): (C , CH=N)imine .
{ 8 }	(58.45):(C , OCH_2)., (167.6): (CO-NH , amide) , (123 -145): (C , phenyl group). , (149 -155): (C , heterocycles) ., (111.2): (C , C=N)

Solvaition of Drug Derivatives in Various Solvents:

All drug derivatives were tested with types of solvents which have various polarity , the results of test of solubility are summarized in Table (4).

Table (4) : Interaction of Compounds in Different Solvents.

Drug Derivatives	Solvents					
	C_2H_5OH	CH_3OH	THF	Ether	Tuloun	Hexane
{ 1 }	+	+	-	-	-	-
{ 2 }	+	+	-	-	-	-
{ 3 }	+	+	-	-	-	-
{ 4 }	+	+	-	-	-	-
{ 5 }	+	+	-	-	-	-
{ 6 }	+	+	-	-	-	-
{ 7 }	+	+	-	-	-	-
{ 8 }	+	+	-	-	-	-
{ 9 }	+	+	-	-	-	-
{ 10 }	+	+	-	-	-	-
{11 }	+	+	-	-	-	-
{12 }	+	+	-	-	-	-
{13 }	+	+	-	-	-	-
{14 }	+	+	-	-	-	-

Solubility and interaction process of compounds according to properties of drug derivatives and type of its polarity (functional groups of derivatives) due to nature of groups in chemical compounds like hydroxyl group , carboxyl group , or any other group.

Properties of Drug Derivatives :

Some properties represented in R_f of Technique for following the reactions , type of products and products % , all data are showed in Table (5):

ISSN: 0975-8585

Table(5): Some Properties of Drug Derivatives

Derivatives	Products %	R_f	Type of Product
{ 1 }	70	0.62	Solid
{ 2 }	74	0.70	Solid
{ 3 }	72	0.72	Solid
{ 4 }	76	0.62	Solid
{ 5 }	72	0.76	Solid
{ 6 }	70	0.78	Solid
{ 7 }	72	0.70	Solid
{ 8 }	70	0.68	Solid
{ 9 }	76	0.62	Solid
{ 10 }	76	0.68	Solid
{ 11 }	78	0.60	Solid
{ 12 }	72	0.64	Solid
{ 13 }	76	0.62	Solid
{ 14 }	78	062	Solid

Microbial Assay :

Antifungal :

The biological activities of synthesized compounds have been studied for their antifungal by agar via biological methods[1] .,the stock solution (0.0 mol) was prepared through dissolving the derivatives in DMSO as a solvent and the solutions were serially diluted to find the minimum inhibitory concentration (MIC) in ($mgmL^{-1}$). Three types of fungi stains (***Aspergillus , Tricophyton , C. albicans***) were incubated for 24 h at 37◦C.

Antimicrobial activity[8-12] test were performed in triplicate, and the average was taken as the final reading.

The antibacterial activities were done at three concentration (1 , 3 , 5) mg/ml concentrations in DMSO solvent , but concentration (5 mg/ml) gave higher activity than other concentrations with three types of fungi.

Fungal Studying of Drug Derivatives:

All drug derivatives {1-14 } were screened against three types of fungi ,all results are described in tables (6) and figures (1 ,2) . The presence of heterocyclic ring like five membered ring , thiadiazole , oxadiazole are reported to posses antimicrobial effect increase the antimicrobial activity of the thiadiazole or oxadiazole , sulpho cycle derivatives.

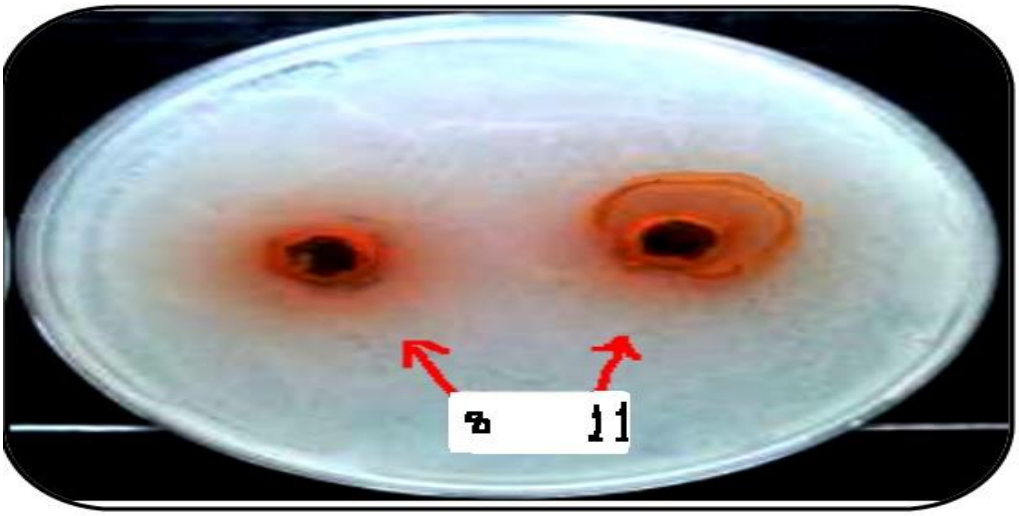
The antifungal results are listed in table (6)., which indicate that the results of antifungal tests which it was found to be potentially activity against all types of fungi ,which gave evident from the results that the biological activity of all compounds have high biological activity which inhibit the growth of fungi .

The drug derivatives {14 , 13, 12, 11 } have higher activity than other derivatives due to (oxadiazole nuclei , sulpho ring , thiadiazole , sulfur atom) in their structure[7-16] and formazan compound in {8 } which inhibit the fungal growth through formation of hydrogen bond with the active centers of the cell constituents resulting in the interference with the normal cell process.

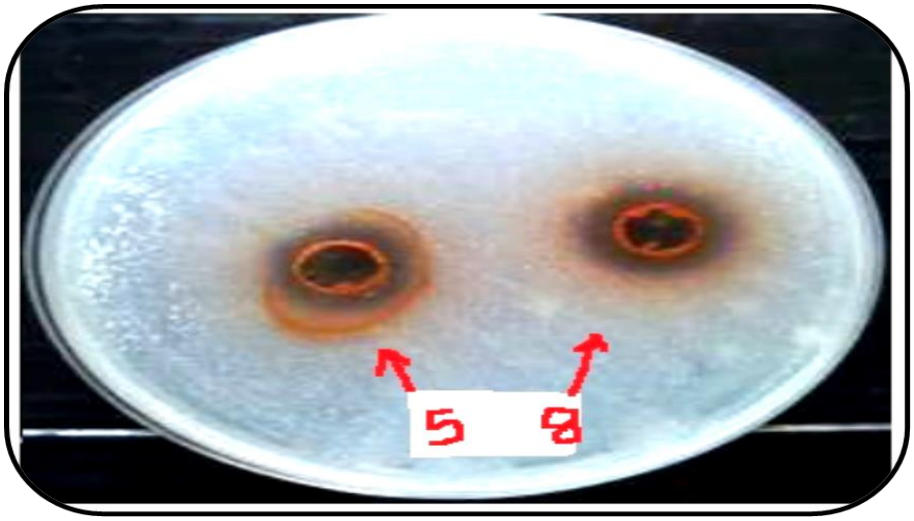
ISSN: 0975-8585

Table(6):Antifungal Activity of Drug Derivatives (Inhibition Zone in (mm)) and in Concentration (5 mg.ml^{-1})

Drug Derivatives	*Average (3 Concentrations)* *Aspergillus ((Funigatus))*	*Average (3 Concentrations)* *Tricophyton ((Funigatus))*	*Average (3 Concentrations)* *C. albicans*
{ 1 }	6	6>	6>
{ 2 }	8	10	8
{ 3 }	12	14	12
{ 4 }	8	6	6
{ 5 }	14	14	16
{ 6 }	10	12	10
{ 7 }	10	10	12
{ 8 }	16	18	18
{ 9 }	10	10	8
{ 10 }	12	10	10
{ 11 }	20	22	20
{ 12 }	24	26	24
{ 13 }	28	26	26
{ 14 }	30	30	26



Figure(1):Inhibition zone of derivative{8 ,11} against *Aspergillus*



Figure(2):Inhibition zone of derivative{8 ,5} against *C. albicans*

REFERENCES

[1] Mrunmayee P , Vilasrao J , Vithal M , Ashok K , "Synthesis and antifungal activity of some azetidinones " , International Journal of PharmTech Research , 2010 , 2,2 , 762-773.

[2] Amita V , Pathak Pratee K , Anjali T, Parjanya K , "Piperazine bridged 4-aminoquinoline 1,3,5- triazine derivatives: Design, Synthesis, characterization and antibacterial evaluation" , International Journal of ChemTech Research, 2016,9(4), 261-269.

[3] Barbara E, Brian S, Makoto M. J, Jose C , Kavitha D, Tao H, Jian Y, Tom G, Matthew B , Melinda M , Lauri A , Matthew H., " Variation in Outpatient Antibiotic Prescribing for Acute Respiratory Infections in the Veteran Population" ., Annals of Internal Medicine, 2015; 163 (2): 73 ., DOI: 10.7326/M14-1933.

[4] Emiley A , Eloe F, Arthur B, Jonathan C, Elliott F , Bing M, Anup M, Jacques R , Miriam H, Anne M, Christine B, Irina A, Patricia L , Claire M. F., "Functional Dynamics of the Gut Microbiome in Elderly People during Probiotic Consumption" ., M Bio, 2015; 6 (2): e00231-15 DOI:10.1128/mBio.00231-15.

[5] Bhave A , Chandrachood S , Deshpande R , Kashalkar R ," Efficient route to synthesize Triazoles using copper on carbon catalyst via click chemistry" ,International Journal of ChemTech Research, 2016,9(5),392-394.

[6] Rajasekaran S , Gopalkrishna R ., "Synthesis and Anti-Denaturation Activity of Some Substituted Quinazolinone Analogs " ., International Journal of ChemTech Research., 2012, 4(3) , 1207-1211.

[7] Tracey J , Diane R , Robert D, Timothy H, Riva T . ,"Effect of Lactobacillus rhamnosus LGG and Bifidobacterium animalis ssp. lactis BB-12, on health-related quality of life in college students affected by upper respiratory infections". ,British Journal of Nutrition, 2012; 1 DOI: 10.1017/S0007114512004138.

[8] McEvoy G , Drug information.. "American society of health-system pharmacists", J. Inc., 2006, Vol.5, No.1, pp. 91-96.

[9] Nagham M , "Synthesis and Biological Study of Hetero (Atoms and Cycles) Compounds", Der Pharma Chemica, 2016, 8(6):40-48.

[10] Nagham M. "Synthesis and chemical identification of macro compounds of (Thiazol and Imidazol)" . Res J Pharm Tech 2015;8(1):78-84

[11] Nagham M , Intisar O. "Synthesis of sulfur heterocyclic compounds and study of expected biological activity". Res J Pharm Tech 2015, 8(9): 1225-1242, DOI: 10.5958/0974-360X.2015.00224.3

[12] Nagham M , Saher M, Zainab M, Seena K. , "Microbial Studying of (Thiazole ,Oxadiazole, Thiadiazole)–Derivatives on Mouth and Teeth Bacteria", International Journal of Medical Research and Pharmaceutical Sciences, 2016, 3, 8, ,30-39 , DOI:10.5281/zenodo.61357 .

[13] Muthukumar C, Sabastiyan A , Ramesh M , Subramanian M and Yosuva M, "synthesis, physico-chemical characterization and antimicrobial studies On 7-diethylaminosalicyl-8- hydroxyquinoline and its metal complexes" ., International Journal of ChemTech Research, 2012,4(4).

[14] El-Sayed W, Flefe E, Morsy E, "Anticancer and antimicrobial activities of some synthesized pyrazole and triazole derivatives" ,Der Pharma Chemica, 2012, 4 (1):23-32.

[15] Maske P , Makhija S , "Synthesis and antibacterial activity of 5-chloro-N-cyclohexyl- 6-thio subtituted-nicotinamide derivatives" , Der Pharmacia Lettre, 2011, 3 (5)110-114.

[16] Mohamed A , Chhavi A ., "2, 4- Di substituted-5-Imino-1, 3, 4- Thiadiazole Derivatives: Synthesis and Biological Evaluation of Antiinflammatory Activities" ., International Journal of ChemTech Research , 2009 ,1, 4, 1200-1205.