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RESEARCH ARTICLE

USE OF 2-(1-(4-BROMOPHENYL) ETHYLIDENE)HYDRAZINECARBOTHIOAMIDE AND 2-(5-CHLORO-2-OXOINDOLIN-3-YLIDENE)HYDRAZINECARBOTHIOAMIDE IN THE SYNTHESES OF 2-THIOHYDANTOIN, PYRIMIDINE DERIVATIVES: EVALUATION OF THEIR ANTIMICROBIAL ACTIVITIES.

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Abstract

2-(1-(4-bromophenyl)ethylidene)hydrazinecarbothioamide **2**a and 2-(5-chloro-2-oxoindolin-3-ylidene)hydrazinecarbothioamide **2**b were used in the syntheses of new series of compounds. Acetylation of compounds **2**a,b with acetic anhydride afforded acetyl derivatives **3**a,b. Alkylation of **2**b with ethyl iodide afforded the corresponding 2-(5-chloro-2-oxoindolin-3-ylidene)-N-ethylhydrazinecarbothioamide **4.** Hydrazinolysis of **2**a with hydrazine hydrate afforded 1,2-bis(1-(4-bromophenyl)ethylidene)hydrazine **5**. Reaction of compounds **2**a,b with ethyl acetoacetate and/or ethyl chloroacetate lead to the formation of 1-(1-(4-bromophenyl)ethylideneamino)-6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one **6**, 3-(1-(4-bromophenyl)ethylideneamino)-2-thioxoimidazolidin-4-one **7**a and 5-chloro-3-(5-oxo-2-thioxoimidazolidin-1-ylimino)indolin-2-one **7**b respectively. Condensation of compounds **7**a,b with different aldehydes and ketones led to the formation of compounds **8**a,b, **9** and **10**. Acetylation of **7**b with acetic anhydride under reflux gave N- acetyl derivative **11**. Mannich base **12** was prepared by reaction of **7**b with secondary amines such as di phenyl amine and formaldehyde in ethanol. Characterization of the synthesized compounds were done by IR, ^{1}HNMR, ^{13}CNMR, mass spectroscopy and elemental analysis. The antimicrobial activity was evaluated against Gram- positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis*, Gram – negative bacteria: *Escherichia coli* and *Salmonella typhimurium*, Yeast: *Candida albicans* and Fungus: *Aspergillus fumigatus*the. The tested compounds recorded variable antimicrobial activities towards the used microorganism. Among the tested compounds, **9** showed the best activity against all the tested microorganisms.

Introduction:-

Thiohydantoins are sulfur analogues of hydantoins with one or both carbonyl group(s) replaced by a thiocarbonyl group.[1–5]. Thiohydantoins find important applications as medicinal (anticonvulsant drugs in the treatment of epilepsy [6,7], antiarrhythmic [8,9], antitumor [10] and anticancer [11] drugs), as agrochemicals (bactericides and

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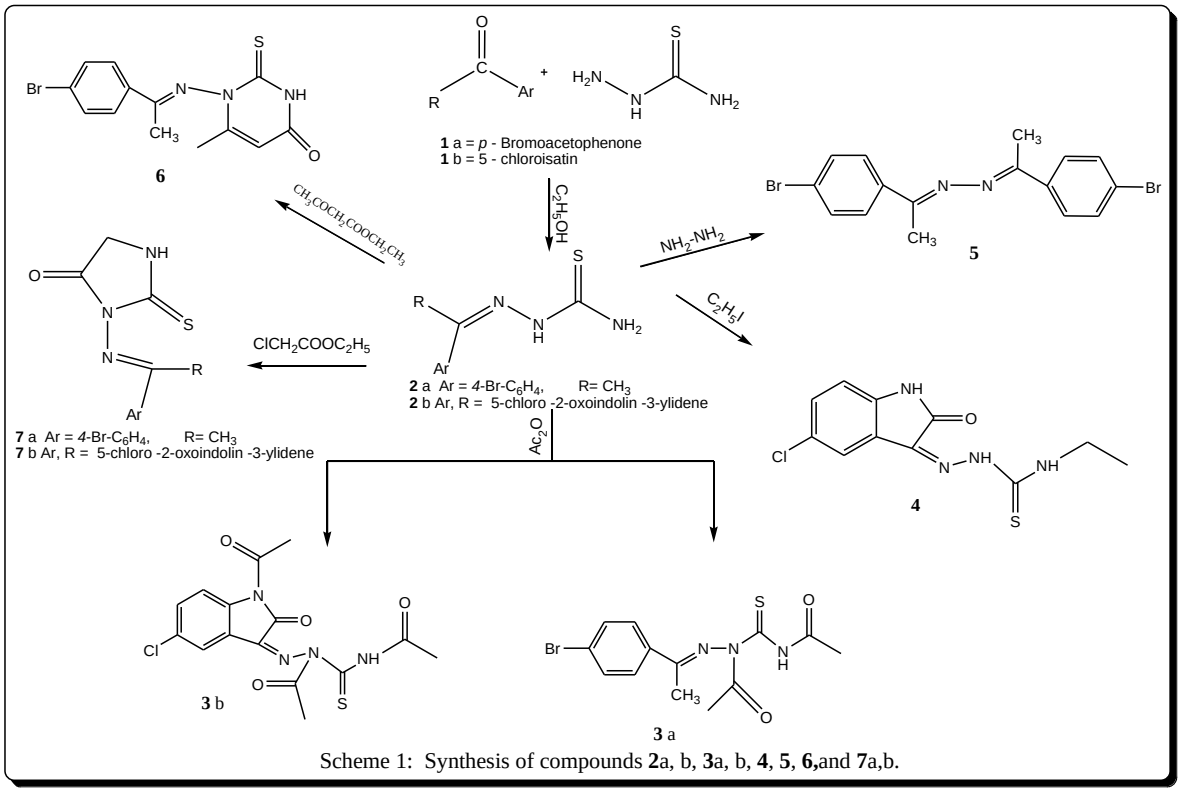
fungicides) [12]. Thiohydantoins are known for their uses as hypolipidemic [13] and antimutagenic [14]. In addition, thiohydantoin compounds are used as herbicides [15] and fungicides agents [16]. Recently, there has been interest in the search of new synthetic routes for the preparation of these type of compounds, via solution or solid state reactions [17-19]. This paper now reported the syntheses of thiohydantoins and pyrimidines using 2-(1-(4-bromophenyl)ethylidene)hydrazinecarbothioamide and 2-(5-chloro-2-oxoindolin-3-ylidene)hydrazinecarbothioamide as a key starting materials. The newley synthesized compounds were evaluated as antimicrobial agents using gram – positive bacteria, gram – negative bacteria, yeasts and Fungi.

Results and discussion:-

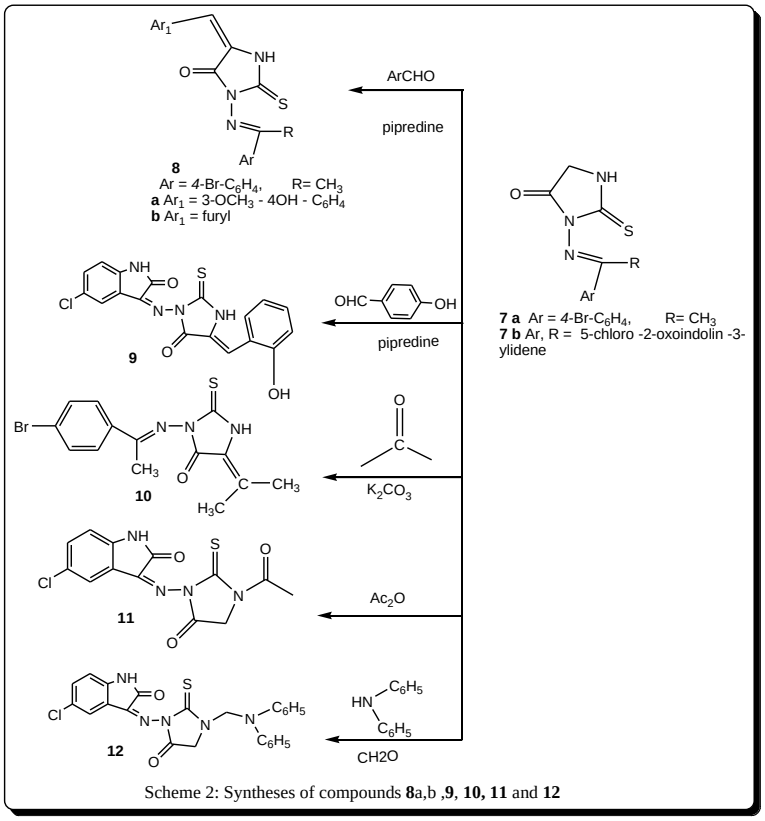
2-(1-(4-bromophenyl)ethylidene)hydrazinecarbothioamide **2**a and 2-(5-chloro-2-oxoindolin-3-ylidene)hydrazinecarbothioamide **2**b were prepared via condensation of 4- bromoacetophenone **1**a and/or 5-Chloroisatin **1**b with thiosemicarbazide under reflux in ethanol respectively [20]. Structures of **2**a, b were elucidated on the basis of elemental analysis, spectral data and chemical transformation. Thus, acetylation with acetic anhydride under reflux gave N-(1-acetyl-2-(1-(4-bromophenyl)ethylidene)hydrazinecarbonothioyl) acetamide **3**a and N-(1-acetyl-2-(1-acetyl-5-chloro-2-oxoindolin-3-ylidene)hydrazinecarbon thioyl)acetamide **3**b. Structures of **3**a,b were elucidated on the bases of spectral analysis, where, the infrared spectrum of compound **3**a showed the disappearance of the bands attributed to the (NH_2) group and appearance of the bands at 1705 cm^{-1} for (C=O) group and 3232 cm^{-1} for (NH) group. While the infrared spectrum of **3**b showed the appearance of the bands at 1753, 1715, 1704 cm^{-1} attributed to (C=O) groups. The ^{1}HNMR spectrum of **3**a showed signals at 11.68 ppm assigned to (NH) group, 2.183, 2.02 ppm for methyl groups of $2COCH_3$ and signal at 2.24 ppm for methyl group. The ^{1}HNMR spectrum of **3**b showed signals at 10.86 ppm for (NH) group and signals at 2.16, 2.10, 2.08 ppm for three methyl groups of $COCH_3$. The ^{13}CNMR spectrum of **3**b exhibited signals at 170.33, 170.14, 167.42, 167.16 ppm for (4C=O) groups and signals at 22.32, 22.15, 21.84 for ($3CH_3$) groups, in addition to the signals of C-aromatic. Mass spectrum of **3**a showed molecular ion peak at m/z 356, 355 (M^+, ^{79}Br) and 357 (M^+, ^{81}Br). While **3** b showed molecular ion peak at 380 and 382(M^+, ^{37}Cl). Treatment of **2**b with ethyl iodide in presence of dimethyl formamide under reflux and stirring led to the formation of the corresponding 2-(5-chloro-2-oxoindolin-3-ylidene)-N-ethylhydrazinecarbothioamide **4**. Structure of **4** was elucidated on the basis of spectral analysis, where, infrared spectrum showed the disappearance of (NH_2) group bands and appearance of bands at 3360, 3251, 3165 cm^{-1} for (3NH) groups , 1697 cm^{-1} for (C=O), 1620 cm^{-1} for (C=N) and 1380 cm^{-1} (C=S). The ^{1}HNMR spectrum showed signals at 10. 63, 10.55 ppm for (NH) groups, triplet signal at 1.13 ppm for (CH_3) and quartet signal at 3.73 ppm for (CH_2) group. Mass spectrum showed molecular ion peak at m/z 282 and 284 (M^+, ^{37}Cl). Hydrazinolysis of **2**a with hydrazine hydrate by fusion at 120-130 ^oC for 30 min, then adding ethanol and refluxing 2h, afforded the corresponding 1,2-bis(1-(4-bromophenyl)ethylidene)hydrazine **5**. Structure of **5** was elucidated on the basis of elemental analysis and spectral data, where, in the ^{1}HNMR spectrum signals at 10.26 ppm assigned to NH and 8.81 ppm assigned to NH_2 have been disappeared, and only signals at 2.00 ppm for methyl groups and 7.47-7.56 ppm for aromatic ring have been appeared. The ^{13}CNMR spectrum showed the disappearance of signals assigned to (C=S) and appearance of signals at 140.28 ppm for (2C=N), 139.04, 131.00, 126.77, 120.01ppm for the aromatic ring and signal at 11.19 ppm for ($2CH_3$) groups. Mass spectrum of **5** showed molecular ion peak at m/z 394, 393 (M^+, ^{79}Br) and 395 (M^+, ^{81}Br). Reaction of **2**a with ethyl acetoacetate led to the formation of 1-(1-(4-bromophenyl)ethylideneamino)-6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one **6**, structure of **6** was elucidated on the basis of spectral analysis where, IR spectrum showed band at 1678 cm^{-1} assigned to carbonyl group. The ^{1}HNMR spectrum displayed signal at 10.23 ppm assigned to (NH) group and two signals at 2.30 ppm and 2.25 ppm assigned to two methyl groups. The ^{13}CNMR spectrum showed signals at 197.59 ppm assigned to (C=S) group, signal at 179.44 ppm assigned to (C=O), 147.05 ppm (C=N), 27.15 and 14.25 ppm for methyl groups. Mass spectrum showed molecular ion peak at m/z 338, 337 (M^+, ^{79}Br) and 339 (M^+, ^{81}Br). Reaction of **2** a, b with ethyl chloroacetate in ethanol under reflux a afforded 3-(1-(4-bromophenyl)ethylideneamino)-2-thioxoimidazolidin-4-one **7**a and 5-chloro-3-(5-oxo-2-thioxoimidazolidin-1-ylimino)indolin-2-one **7**b (Scheme: 1).



Scheme 1: Synthesis of compounds **2**a, b, **3**a, b, **4**, **5**, **6,**and **7**a,b.

Structures of **7**a,b were elucidated on the bases of elemental analysis and spectral data, where, infrared spectrum of **7**a showed bands at 3384 cm^{-1} for (NH) group, 1704 cm^{-1} characteristic to (C=O), 1592 cm^{-1} for (C=N) and 1394 cm^{-1} for (C=S). While **7**b showed bands at 3392, 3274 cm^{-1} for (NH) groups, 1688, 1726 cm^{-1} for (2C=O), 1614 cm^{-1} for (C=N), 1412 cm^{-1} for (C=S). The ^{1}HNMR spectrum of **7**a showed signals at 11.99 ppm for (NH) group, 3.86 ppm singlet for (CH_2) group and 2.35 ppm singlet for methyl group, while **7**b showed signals at 12.31, 11.03 ppm for (2NH) groups and 3.96 ppm for (CH_2) group. The ^{13} CNMR spectrum of **7**a showed signals at 173.98 ppm (C=S), 164.69 ppm (C=O), 195.50 ppm (C=N), 32.90 ppm (CH_2) group and 14.51 ppm for methyl group. Mass spectrum of **7**a showed molecular ion peak at m/z 312, 311 (M^+, ^{79}Br) and 313 (M^+, ^{81}Br). While **7** b showed molecular ion peak at 294 and 296 (M^+, ^{37}Cl). Condensation of **7**a with different aromatic aldehydes (such as 4-hydroxy-3-methoxy benzaldehyde and furaldehyde) in presence of piperidine under reflux led to the formation of 3-(1-(4-bromophenyl)ethylideneamino)-5-arylidene-2-thioxoimidazolidin-4-one **8**a,b. Structures of **8**a,b were elucidated on the bases of elemental analysis and spectral data, where, Infrared spectrum of **8**a showed the presence of broad band at 3406-3356 cm^{-1} attributed to the presence of (OH), 3240 cm^{-1} for (NH), 1693 cm^{-1} for (C=O) group, 1612 cm^{-1} for (C=N) and 1408 cm^{-1} for (C=S). While **8**b showed bands at 3417 cm^{-1} for (NH), 1705 cm^{-1} for (C=O), 1597 cm^{-1} for (C=N) and 1384 cm^{-1} for (C=S). The ^{1}HNMR spectrum of **8**a showed the appearance of signal at 12.06 ppm characteristic to (OH), signal at 10.24 ppm for (NH) and they are exchangeable with D2O NMR and signal at 3.84 ppm for (OCH_3), while ^{1}HNMR spectrum of **8**b showed signal at 8.28 ppm characteristic to (NH) and it is exchangeable with D2O NMR, in addition to signals of (CH) aromatic and (CH) olefinic. The ^{13}CNMR spectrum of compound **8**a showed signals at 179.44 ppm for (C=S), 174.33 ppm for (C=O), 165 ppm for (C=N), 159.68 ppm for (C-$COCH_3$) and 147.03 ppm for (C-OH). The spectrum also showed signals characteristic to C-aromatic, signal at 114.36 ppm for (CH) aliphatic, 56.48 ppm for (OCH_3) and 14.86 ppm for (CH_3) group. Mass spectrum of **8**a showed molecular ion peak at m/z 446, 445 (M^+, ^{79}Br) and 447 (M^+, ^{81}Br), while mass spectrum of **8**b showed molecular ion peak at m/z 390, 389 (M^+, ^{79}Br) and 391 (M^+, ^{81}Br while condensation of compound **7**b with 2-hydroxy benzaldehyde in ethanol in presence of piperidine under stirring led to the formation of (Z)-5-chloro-3-((Z)-4-(2-hydroxybenzylidene)-5-oxo-2-thioxoimidazolidin-1-ylimino)indolin-2-one **9**. The structure of **9** was elucidated on the basis of elemental analysis and spectral data, where, infrared spectrum of **9** showed bands at 3214 cm^{-1} for (NH), 1720 and 1697 cm^{-1} for (2C=O), 1616 cm^{-1} for (C=N) and 1383 cm^{-1} for (C=S). The ^{1}HNMR spectrum of compound **9** showed the appearance of signals at 12.03, 11.30 and 9.11 ppm characteristic to OH and NH groups, signal at 8.10 for (C=CH). In addition to signals of (CH) aromatic. Mass spectrum of compound **9** showed molecular ion peak at m/z 398 and 400 (M^+, ^{37}Cl) [20-24]. Condensation of compound **7**a with acetone in

presence of anhydrous potassium carbonate led to the formation of 3-(1-(4-bromophenyl)ethylideneamino)-5-(propan-2-ylidene)-2-thioxoimidazolidin-4-one **10**. The structure of **10** was elucidated on the basis of elemental analysis and spectral data, where, infrared spectrum of compound **10** showed band at 3335 cm^{-1} for (NH), 1716 cm^{-1} for (C=O), 1605 cm^{-1} for (C=N) and 1388 cm^{-1} for (C=S). The ^{1}HNMR spectrum showed the appearance of signal at 10.26 ppm characteristic to (NH), signal at 2.50 for ($2CH_3$) and signal at 2.28 for (CH_3), in addition to signals of (CH) aromatic. Mass spectrum showed molecular ion peak at m/z 352, 351 (M^+, ^{79}Br) and 353 (M^+, ^{81}Br) [25]. Structure of **7**b also confirmed chemically, thus, acetylation with acetic anhydride under reflux gave 3-(3-acetyl-5-oxo-2-thioxoimidazolidin-1-ylimino)-5-chloroindolin-2-one **11**. Structure of **11** was elucidated on the basis of elemental analysis and spectral data, where, infrared spectrum of **11** showed bands at 3433 cm^{-1} (NH), 1685, 1716, 1766 (3C=O) groups, 1619 cm^{-1} (C=N) and 1403 cm^{-1} (C=S). The ^{1}HNMR spectrum showed signal at 12.06 for (NH) group and signal at 2.16 ppm for CH_3 of acetyl group. The ^{13}CNMR spectrum showed signals at 167.29, 170.22, 170.25 ppm attributed to three carbonyl groups and signal at 18.56 ppm for (CH_3), in addition to signals at 172.50 ppm for thiocarbonyl (C=S), 144.05 ppm for (C=N), 56.04 ppm for (CH_2) group and signals at 137.93, 130.21, 129.76,129.73, 132.94 and 117.29 for C- aromatic. Mass spectrum of compound **11** showed molecular ion peak at m/z 336 and 338 (M^+, ^{37}Cl). A mixture of compound **7**b, different secondary amines such as di phenyl amine and formaldehyde in ethanol was stirred to give 5-chloro-3-(3-((diphenylamino)methyl)-5-oxo-2-thioxoimidazolidin-1-ylimino)indolin-2-one **12** (Scheme: **2**). Structure of **12** was elucidated on the basis of elemental analysis and spectral data, where, infrared spectrum of **12** showed band at 3388 cm^{-1} for NH group. ^{1}HNMR spectrum of compound **12** showed signal at 12.20 ppm for (NH), 4.37, 5.23 ppm for ($3CH_2$) groups, in addition to the characteristic signals of (CH) aromatic. Mass spectrum of compound **12** showed molecular ion peak at m/z 475, 477 (M^+, ^{37}Cl) (Scheme: 2).



Scheme 2: Syntheses of compounds **8**a,b ,**9**, **10, 11** and **12**

Antimicrobial activity:-

The synthesized compounds in the investigation were evaluated for their antimicrobial activity [26,27]. The exanimated data are summarized in table: **1** which revealed that, most of compounds showed moderate to good inhibition zone. Compounds **2**a,b have low to moderate activity against *candida albicans* as yeast. Structure activity relationship of the tested compounds showed that, presence of oxoindolin, indole moieties and biologically active groups such as OH, OCH_3 with thiohydantoin nucleus enhanced the antimicrobial activity, where, reaction of **2**a with ethyl iodide gives **4** that increases the antimicrobial activity. Hydrazinolysis of **2**a gives **5** that increases the antibacterial activity against *Bacillus Subtilis* as gram positive bacteria, and increases the reactivity against yeast

from low to high activity. The reactivity on formation of thiohydantoin moiety differs with difference of attached group, where presence of oxoindolin moiety in **7**b increases reactivity against yeasts and fungi. Introducing OH, OCH_3 on thiohydantoin ring increases the activity as in compounds **8**a,b. Cyclization of **7**b to indol ring enhanced the reactivity as in compound **9**, where it is the most active compound against all the tested microorganisms. The minimum inhibitory concentration (MIC) of the biologically active compounds was measured by a two-fold serial dilution method. The results are depicted in Table **2**.

Table 1:- The Antimicrobial Activity Of The Tested Compounds.

	Mean* of zone diameter**,** nearest whole mm.											
Organism	**Gram - positive bacteria**				**Gram - negative bacteria**				**Yeasts and Fungi****			
	Staphylococcus aureus (ATCC 25923)		*Bacillus subtilis* (ATCC 6635)		*Salmonella typhimurium* (ATCC 14028)		*Escherichia coli* (ATCC 25922)		*Candida albicans* (ATCC 10231)		*Aspergillus fumigatus*	
Concentration Sample	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml
2a	-	-	-	-	-	-	-	-	10 L	7L		
2b	-	-	-	-	-	-	-	-	23 H	18 I	-	-
4	15 I	12 I	-	-	20 H	17 I	-	-	11 L	8 L	-	-
5	-	-	19 I	16 I	-	-	-	-	25 H	20 H	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-
7a	-	-	-	-	-	-	-	-	-	-	-	-
7b	-	-	-	-	-	-	-	-	21 H	18 I	20 H	15 I
8a	-	-	18 I	15 I	-	-	9 L	7 L	14 I	9 L	-	-
8b	-	-	11 L	8 L	-	-	-	-	20 H	16 I	-	-
9	17 I	14 I	23 H	19 H	23 H	20 H	14 I	10 I	38 H	35 H	41 H	35 H
10	-	-	-	-	-	-	-	-	18 I	15 I	29 H	27 H
Control #	**35**	**26**	**35**	**25**	**36**	**28**	**38**	**27**	**35**	**28**	**37**	**26**

* = Calculate from 3 values, ** = identified on the basis of routine cultural, morphological and microscopical characteristics, - = No effect, L: Low activity = Mean of zone diameter ≤ 1/3 of mean zone diameter of control, I: Intermediate activity = Mean of zone diameter ≤ 2/3 of mean zone diameter of control, H: High activity = Mean of zone diameter > 2/3 of mean zone diameter of control, #: Chloramphenicol in the case of Gram-positive bacteria, Cephalothin in the case of Gram-negative bacteria and cycloheximide in the case of fungi.

Table 2:- MIC of the tested compounds.

	MIC μg/ml					
Compound	**Gram - positive bacteria**		**Gram - negative bacteria**		**Yeasts and Fungi****	
	Staphylococcus aureus (ATCC 25923)	*Bacillus subtilis* (ATCC 6635)	*Salmonella typhimurium* (ATCC 14028)	*Escherichia coli* (ATCC 25922)	*Candida albicans* (ATCC 10231)	*Aspergillus fumigatus*
2a	-	-	-	-	-	-
2b	-	-	-	-	≤ 16	-
4	≤ 15	-	-	-	-	-
5	-	≤ 16	-	-	≤ 64	≤ 8
6	-	-	-	-	-	-
7a	-	-	-	-	-	-
7b	-	-	-	-	≤ 32	32
8a	-	≤ 32	-	-	-	-
8b	-	-	-	-	≤ 16	-
9	≤ 32	≤ 32	≤ 2	≤ 16	≤ 16	≤ 64
10	-	-	-	-	≤ 16	≤ 16

- = Not determined.

Experimental:-
Melting points were taken in open capillaries using electro thermal digital melting points apparatus and are uncorrected. IR spectra were recorded on NICOLET (iS50 FT-IR) spectrometer using KBr pellets. ^{1}H and ^{13}C NMR were recorded on a Bruker AS 850 TM NMR and chemical shifts were given with respect to TMS. Mass spectra were recorded on GC/MS with CI (chemical ionization) and a hewlette-packard MS Engine Thermospray and ionization by electron impact to (70 ev). Microanalysis was conducted using elemental analyzer 106.

Syntheses of 2-(substituted ylidene)hydrazinecarbothioamide 2a,b.:-
A mixture of **1**a, b (0.01 mol) and thiosemicarbazide (0.01mol) in ethanol 50 mL were heated under reflux for 4h. The solid formed after cooling was filtered off, washed with water , dried and purified by crystallization from ethanol to give compounds **2**a, b .

2-(1-(4-bromophenyl)ethylidene)hydrazinecarbothioamide 2a.:-
White solid, in 90% yield, mp 198-199 ºC (EtOH). IR (KBr) 3410(NH), 3236, 3198 (NH_2),1589 (C=N), 1392 (C=S) cm^{-1}. ^{1}H-NMR (DMSO-d6): 10.26 (s, 1H, NH), 8.31 (s, 2H, NH_2), 7.91 (d, 2H, 2CH), 7.54 (d, 2H, 2CH) and 2.28 (s, 3H, CH_3) ppm. MS: m/z (%) =272 (16) , 271(M^+, ^{79}Br, 87) and 273 (M^+, ^{81}Br, 97) , 256 (100). Anal. Calcd. For $C_9H_{10}N_3SBr$: C, 39.70; H, 3.67; N, 15.44; S, 11.76; Br, 29.41 Found. C, 39.82; H3.75; N15.36; S, 11.83; Br, 29.30.

2-(5-chloro-2-oxoindolin-3-ylidene)hydrazinecarbothioamide 2b:-
Yellow crystals, in 88% yield, mp 268-269 ºC (EtOH). IR (KBr) 342**2**, 331**8** (NH), 32**84**, 3221 (NH_2), 1697 (C=O), 160**9** (C=N), 138**9** (C=S) cm^{-1} . ^{1}H-NMR (DMSO-d6): 12.30 , 11.28 (s, 1H,2NH), 8.8-9.11 (s, 2H, NH_2), 7.75 (s, 1H, CH), 7.36 (d, 1H, CH), 6.92 (d, 1h, CH)ppm. . ^{13}C-NMR (DMSO-d6): 178.84 (C=S) , 162.48 (C=O), 141.04 (C=N), 130.91, 130.55, 126.62, 121.96, 120.73 and 112.61 C- aromatic. MS: m/z (%) = 254 (45), 256 (M^+, ^{37}Cl, 18), 226(100). Anal. Calcd. For $C_9H_7N_4SOCl$: C, 42.51; H, 2.75; N, 22.04; S,12.59; Cl, 13.77. Found. C, 42.48; H, 2.77; N, 22.07; S, 12.45; Cl, 12.98.

Syntheses of N-(1-acetyl-2-(1-(4-bromophenyl)ethylidene)hydrazinecarbonothioyl) acetamide 3a **and N-(1-acetyl-2-(1-acetyl-5-chloro-2-oxoindolin-3-ylidene)hydrazinecarbon thioyl)acetamide 3**b:-
A solution of **2**a, b (0.01 mol) in acetic anhydride (25 mL) were heated under reflux for 2 h, then cooled and the resulting solid was collected by filtiration, dreied and purefied by crystallization from prober solvent to give compounds **3**a, b.

Synthesis of N-(1-acetyl-2-(1-(4-bromophenyl)ethylidene)hydrazinecarbonothioyl) acetamide 3a:-
Pale yellow crystals, in 75% yeild, mp 205-207 ºC (EtOH). IR (KBr): 3232(NH), 1705 (2C=O), 1600 (CN) and 1389 (C=S) cm^{-1}. ^{1}H-NMR (DMSO-d6): 11.68 (s, 1H, NH), 7.54 (d, 2H, 2CH), 7.31 (d, 2H, 2CH), 2.24 (s, 3H, CH_3), 2.18 (s, 3H, CH_3) and 2.02 (s, 3H, CH_3) ppm. MS: m/z 356 (24), 355 (M^+, ^{79}Br, 25) and 357 (M^+, ^{81}Br, 4). Anal. Calcd. $C_{13}H_{14}N_3O_2SBr$. C, 43.82; H, 3.93; N, 11.79; S, 8.98; Br, 22.74. Found. C, 43.93; H, 3.82; N, 11.68; S, 8.89; Br, 22.83.

Synthesis of N-(1-acetyl-2-(1-acetyl-5-chloro-2-oxoindolin-3-ylidene)hydrazinecarbonthioyl)acetamide 3b:-
Yellow crystals , in 83% yield, mp 180-182 ºC (EtOH). IR (KBr): 3350 (NH), 1753, 1715, 1704 (C=O), 1616 (C=N) and 1407 (C=S) cm^{-1}. ^{1}H-NMR (DMSO-d6): 10.86 (s, 1H, NH), 7.6 (s, 1H, CH), 7.3 (d, 1H, CH), 6.85 (d, 1H, CH), 2.16 (s, 3H, CH_3), 2.10 (s, 3H, CH_3) and 2.08 (s, 3H, CH_3) ppm. ^{13}C-NMR (DMSO-d6): 173.23 (C=S), 170.33, 170.14, 167.42, 167.16 (C=O), 140.08 (C=N), 130.01, 129.84, 126.51, 124.05, 123.99, 117.8 C-aromatic, 22.32, 22.15 and 31.84 ($3CH_3$). MS: m/z (%), 380 (50), 382(M^+, ^{37}Cl, 18) and 268 (100). Anal. Calcd. $C_{15}H_{13}O_4N_4SCl$. C, 47.3; H, 3.42; N, 17.73; S, 8.42; Cl, 9.34. Found. C, 47.25; H, 3.51; N, 14.73; S, 8.42; Cl, 9.34.

Synthesis of 2-(5-chloro-2-oxoindolin-3-ylidene)-N-ethylhydrazinecarbothioamide 4:-
A mixture of **2**b (0.01 mol) and ethyl iodide (0.015 mol) in di methyl formamide (50 mL) was heated under reflux and stirring for 6h, the reaction mixture was cooled and poured into ice-water. The crude product obtained was filtered off, washed with water, dreid and purified by crystalization from ethanol to produce compound **4** as orange crystals, in 79% yield, mp 205-207 ºC (benzene). IR (KBr): 3360, 3251, 3165 (NH), 1697 (C=O), 1620 (C=N) and 1380 (C=S) cm^{-1}. ^{1}H-NMR (DMSO-d6): 10.63, 10.55 (s, 3H, 3NH), 7.31 (s, 1H, CH), 7.10 (d, 1H, CH), 6.85 (d,1H, CH), 3.73 (q, 2H, CH_2) and 1.13 (t, 3H, CH_3) ppm. MS: m/z 282 (40), 284 (M^+, ^{37}Cl, 17) and 180 (100). Anal.

Calcd. $C_{11}H_{11}N_4OSCl$. C, 46.80; H, 3.90; N, 19.85; S, 11.34; Cl, 12.58. Found. C, 46.92; H, 3.81; N, 19.97; S, 11.22; Cl, 12.44.

Synthesis of 1,2-bis(1-(4-bromophenyl)ethylidene)hydrazine 5:-

A mixture of **2**a (0.01 mol) and hydrazine hydrate (0.03 mol) was fused on hot plate at 100-120 ºC for ½ h, then adding ethanol (25 mL), the reaction mixture was heated under reflux for 2h, then poured into ice water and acidified with hydrochloric acid (1N). The crude product obtained was filtered off, washed with water, dreid and purified by crystalization from ethanol to give **5** as white crystals, in yield 78%, m.p 84-86 ºC. ^{1}H-NMR (DMSO-d6): 7.55 (d, 4H, 4CH), 7.47 (d, 4H, 4CH) and 2.00 (s, 6H, CH_3) ppm. ^{13}C-NMR (DMSO-d6): 140.82 (2C=N), 139.04, 131.00, 126.77, 120.01 C-aromatic ring and 11.19 ($2CH_3$) ppm. MS: m/z (%): 394 (33), 393 (M^+, ^{79}Br, 9), 395 (M^+, ^{81}Br, 8) and 55(100). Anal. Calcd. $C_{16}H_{14}N_2Br_2$. C, 48.85; H, 3.56; N, Br, 20.36. Found. C, 48.82; H, 3.62; N, 7.05; Br, 20.39.

Synthesis of 1-(1-(4-bromophenyl)ethylideneamino)-6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one 6:-

A mixture compound **2**a (0.01 mol), ethyl acetoacetate (0.01 mol) and fused sodium acetate (0.03 mol) in ethanol (50 mL) was heated under reflux for 6 h, the reaction mixture then cooled and poured into ice-water. The crude product obtained was filtered off, washed with water, dreid and purified by crystalization from hexane to give **6** as pale white crystals, in yield 69 %, m.p 122-124 ºC. IR (KBr): 3410 (NH), 1678 (C=O), 1585 (C=N) and 1393 (C=S) cm^{-1}. ^{1}H-NMR (DMSO-d6): 10.23 (s, 1H, NH), 7.88-7.51 (m, 5H, Ar-H, CH), 2.30 (s, 3H, CH_3), 2.25 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d6): 197.59 (C=S), 179.44 (C=O), 147.05 (C=N), 137.3, 132.18, 131.53, 130.63, 129.11, 123.139 C-aromatic and (C=C) pyrimidinn, 27.15 (CH_3) and 14.25 (CH_3). MS: m/z (%): 338 (4), 337 (M^+, ^{79}Br, 1) and 339 (M^+, ^{81}Br, 1). Anal. Calcd. $C_{13}H_{12}ON_3SBr$. C, 46.15; H, 3.55; N, 12.43; S, 9.47; Br, 23.67. Found. C, 46.23; H, 3.59; N, 12.38; S, 9.53; Br, 23.54.

Syntheses of 3-(1-(4-bromophenyl)ethylideneamino)-2-thioxoimidazolidin-4-one 7a and 5-chloro-3-(5-oxo-2-thioxoimidazolidin-1-ylimino)indolin-2-one 7 b:-

A mixture of **2**a, b (0.01mol) and ethyl chloroacetate (0.01mol) in ethanol (50 mL) in presence of fused sodium acetate (0.03 mol) was heated under reflux for 2h, then cooled and poured into water. The solid formed was filtered off, washed with water , dried and purified from a suitable solvent to give **7**a, b.

3-(1-(4-bromophenyl)ethylideneamino)-2-thioxoimidazolidin-4-one 7a:-

Pale yellow crystals in yield 75%, mp 173-175 ºC (EtOH). IR (KBr): 3384 (NH), 1704 (C=O), 1592 (C=N) and 1394 (C=S) cm^{-1}. ^{1}H-NMR (DMSO-d6): 11.99 (s, 1H, NH), 7.77 (d, 2H, 2CH), 7.63 (d, 2H, 2CH), 3.86 (s, 2H, CH_2) and 2.35 (s, 3H, CH_3) ppm. ^{13}C-NMR (DMSO-d6): 173.98 (C=S), 164.69 (C=O), 159.50 (C=N), 036.98, 131.47, 128.42, 123.43 C-aromatic, 32.90 (CH_2) and 14.51 (CH_3) ppm. MS: m/z (%): 312 (74), 311 (M^+, ^{79}Br, 311) and 313 (M^+, ^{81}Br, 99).Anal. CalCd. $C_{11}H_{10}N_3OSBr$. C,42.30; H, 3.20; N, 13.46; S, 10.25; Br, 25.64. Found. C, 42.19; H, 3.29; N, 13.57; S, 10.34; Br, 25.72.

5-chloro-3-(5-oxo-2-thioxoimidazolidin-1-ylimino)indolin-2-one 7 b:-

Orange crystals in yield 65%, %, mp 260-262 ºC (EtOH). IR (KBr): 3392, 3274 (NH), 1688, 1726 (2C=O), 1614 (C=N), 1411 (C=S) cm^{-1}. ^{1}H-NMR (DMSO-d6): 12.31 (s, 1H, NH), 11.30 (s, 1H, NH), 7.74 (s, 1H, CH), 7.30 (d, 1H, CH), 6.93 (d, 1H, CH) and 3.96 (S, 2H, CH_2). MS: m/z (%): 294 (100) and 296 (M^+, ^{37}Cl, 34).Anal. Calcd. $C_{11}H_7N_4O_2SCl$. C, 44.89; H, 2.38; N, 19.04; S, 10.88; Cl, 11.90. Found. C, 44.78; H, 2.45; N, 17.98; S, 10.96; Cl, 12.13.

Syntheses of 3-(1-(4-bromophenyl)ethylideneamino)-5-arylidene-2-thioxoimidazolidin-4-one 8a,b:-

A mixture of **7**a (0.01 mol), aromatic aldehydes such as (vanillin, furaldehyde) (0.01 mol) and piperidine (1mL) was fused on hot plate at 100-110 ^{o}C for 1/2h then ethanol (25 mL) was added and refluxed for 2h. The reaction mixture then cooled and acidified with diluted hydrochloric acid. The resulting solid was filtered off, washed with water, dried and purified by crystallization from (EtOH) to give **8**a, b.

3-(1-(4-bromophenyl)ethylideneamino)-5-(4-hydroxy-3-methoxybenzylidene)-2-thioxoimidazolidin-4-one 8a:-

Red crystals, in yield 87% (EtOH), m.p79-80 ^{o}C. IR (KBr): 3406-3356 (br.OH), 3240 (NH), 1693 (C=O) group, 1612(C=N) and 1408 (C=S) cm^{-1}. ^{1}H-NMR (DMSO-d6): 12.06 (br. s, 1H, OH, D2O exchangeable), 10.24 (s, 1H, NH, D2O exchangeable), 7.51-7.884 (m, 8H, Ar-H, CH olefinic), 3.84 (s, 1H, OCH_3) and 2.30 (s, 3H, CH_3) ppm. ^{13}C-NMR (DMSO-d6): 179.44 (C=S), 174.33 (C=O), 165 (C=N), 159.68 (C-$COCH_3$), 147.03 (C-OH), 137.36 (C of

thiohydantoin), 137.31, 131.81, 131.53, 129.11, 128.76, 123.78, 123.13, 116.39 C-aromatic, 114.36 (CH aliphatic), 56.48 (OCH_3) and 14.86 (CH_3) ppm. MS: m/z (%):446 (6), 445 (M^+, ^{79}Br,10) and 447 (M^+, ^{81}Br, 11) and 180 (100). Anal. Calcd. $C_{19}H_{16}O_3N_3SBr$. C,51.23; H, 3.59; N, 9.43; S, 7.19; Br, 17.97. Found. C, 51.19; H, 3.65; N, 9.55; S, 6.99; Br, 17.86.

Synthesis of 3-1-(4-bromophenyl)ethylideneamino)-5-(furan-2-ylmethylene)-2-thioxoimidazolidin-4-one 8b:- Brown crystals, in yield 65% (EtOH), m.p128-130 ^oC. IR (KBr): 3417 (NH), 1705 (C=O), 1597 (C=N) and 1384 (C=S) cm^{-1}. ^{1}H-NMR (DMSO-d6): 8.28 (s, 1H, NH, D2O exchangeable), 6.87-7.97 (m, 8H, Ar-H, thiophene, CH olefinic), 2.03 (s, 1H, CH_3) ppm. MS: m/z (%):390 (3.9), 389 (M^+, ^{79}Br, 6), 391 (M^+, ^{81}Br, 7) and 313 (100). Anal. Calcd. $C_{16}H_{12}O_2N_3SBr$. C, 49.23; H, 3.08; N, 10.76; S, 8.21; Br, 20.51. Found C, 49.31; H, 3.14; N, 10.66; S, 8.18; Br, 20.59.

Synthesis of 5-chloro-3-(4-(2-hydroxybenzylidene)-5-oxo-2-thioxoimidazolidin-1-ylimino)indolin-2-one 9.
A mixture of **7**b (0.01 mol), aromatic aldehydes such as (2-hydroxy benzaldehyde) (0.01 mol) and piperidine (1mL) was fused on hot plate at 100-110 ^oC for 1/2h then ethanol (25 mL) was added and refluxed with stirring for 2hr. The reaction mixture then cooled and acidified with diluted hydrochloric acid. The resulting solid was filtered off, washed with water, dried and purified by crystallization from (EtOH) to give **9** as red crystals, in yield 88% (EtOH), m.p 188-190 ^oC. IR (KBr): 3214 cm^{-1} (NH), 1720, 1697 (2C=O), 1616 (C=N) and 1383 (C=S) cm^{-1}.^{1}H-NMR (DMSO-d6): 12.03 (s, 1H, OH), 11.30 (s, 1H, NH), 9.11 (s, 1H, NH), 8.1 (s,1H, C=CH), 7.75-6.81 (m, 7H, Ar-H) ppm. MS: m/z (%): 398 (2) , 399 (M^+ + 1, 1), 400 (M^+, ^{37}Cl, 1) and 84(100). Anal.Calcd. $C_{19}H_{11}N_4O_3SCl$. C,60.00; H, 2.36; N, 14.73; S,8.42; Cl, 9.34. Found. C, 59.89; H, 2.45; N, 14.65; S, 8.49; Cl, 9.45.

Synthesis of 3-(1-(4-bromophenyl)ethylideneamino)-5-(propan-2-ylidene)-2-thioxoimidazolidin-4-one 10:-
A mixture of **7**a and acetone (25mL) was refluxed in presence of anhydrous potassium carbonate (0.03 mol) for 1 h, the resulting solid was filtered off, washed with water, dried and crystallized from ethanol to give compound **10**, as white crystals in yield 92%, m.p 238-240 ^oC. IR (KBr): 3335 (NH), 1716 (C=O), 1605 (C=N) and 1388 (C=S) cm^{-1} .^{1}H-NMR (DMSO-d6): 10.26 (s, 1H, NH), 7.90(d, 2H, 2CH), 7.55 (d, 2H, 2CH), 2.50 (s, 6H, $2CH_3$) and 2.28 (s, 3H, CH_3) ppm. MS: m/z (%):352 (20), 351 (M^+, ^{79}Br, 10), 353 (M^+, ^{81}Br, 14) and 71(100). Anal.Calcd. $C_{14}H_{14}ON_3SBr$. C, 47.72; H, 3.97; N, 11.93; S, 9.09; Br, 22.72. Found. C, 47.80; H, 3.89; N, 11.87; S, 8.98; Br, 22.66.

Synthesis of 3-(3-acetyl-5-oxo-2-thioxoimidazolidin-1-ylimino)-5-chloroindolin-2-one 11:-
A solution of **7**b (0.01 mol) in acetic anhydride (25 mL) were heated under reflux for 2 h, then cooled and the resulting solid was collected by filtiration, dreied and purefied by crystallization from benzene to give compounds **11** as yellow crystals, in yield 68%, m.p 148-150 ºC (EtOH). IR (KBr): 3433 (NH), 1685, 1716, 1766 (3C=O) groups, 1619 (C=N) and 1403 (C=S) cm^{-1}. ^{1}H-NMR (DMSO-d6): 12.06 (s, 1H,NH), 8.08 (d, 1H, CH), 7.61 (d, 1H, CH), 7.49 (s, 1H, CH), 4.37 (s, 2H, CH_2) and 2.16 (s, 3H, CH_3) ppm. ^{13}C-NMR (DMSO-d6): 172.50 (C=S), 170.25, 170.22, 167.29 (3C=O), 144.05 (C=N), 137.93, 130.21, 129.76, 129.73, 123.94, 117.29 C-aromatic, 56.04 (CH_2) and 18.56 (CH_3) ppm. MS: m/z (%):336 (100) and 338 (M^+, ^{37}Cl, 35). Anal.Calcd. $C_{13}H_9O_3N_4SCl$.C, 46.42; H, 2.67; N, 16.66; S, 9.52; Cl, 10.56. Found. C. 46.31; H, 2.75; N, 16.74; S, 9.45; Cl, 10.42.

Syntheses of 5-chloro-3-(3-((diphenylamino)methyl)-5-oxo-2-thioxoimidazolidin-1-ylimino)indolin-2-one 12.
To a solution of soluble **7**b (0.01mol) in 50 ml ethanol added a mixture of secondary amines (0.01mol) diphenyl amine and aqueous formaldehyde 37% (1.25mol), also dissolved in 10 mL ethanol , drop wise throw 30 min. And stirred at room temperature for 3h. Then refrigerated for 48 h to form crystals. The solid formed was filtered off and crystallized from ethanol to give compound **12**. Brown crystals in yield 75%, m.p. 154-156 ^oC. IR (KBr): 3388 (NH), 1721 (C=O), 1605 (C=N) and 1416 (C=S) cm^{-1}. ^{1}H-NMR (DMSO-d6): 12.20 (s,1H, NH), 7.22-6.78(m, 13 H, Ar-H), 5.23 (s, 2H, CH_2) and 4.37 (s, 2H,CH_2)ppm. MS: m/z (%):475 (1), 476 (M^+ +1, 2), 477 (M^+, ^{37}Cl, 0.5) and 407 (100). Anal.Calcd. $C_{24}H_{18}O_2N_5SCl$. C, 60.63; H, 3.78; N, 14.73; S, 6.73; Cl, 7.47. Found. C, 60.51; H, 3.89; N, 14.61; S, 6.84; Cl, 7.35.

Antimicrobial activity:-
Screening of antimicrobial activity was performed at a Microbiology Lab in Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. Antimicrobial activity of the newly synthesized compounds was determined *in vitro* by standardized disc – agar diffusion method. Cultures of the following microorganism were used in the test:Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6635), Gram – negative

bacteria: *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028), Yeast: *Candida albicans* (ATCC 10231) and Fungus: *Aspergillus fumigatus*.

Preparation of tested compound:-
The tested compounds were dissolved in dimethyl formamide (DMF) solvent and prepared in two concentrations; 100 and 50 mg/ml and then 10 µl of each preparation was dropped on disk of 6 mm in diameter and the concentrations became 1 and 0.5 mg/disk respectively. In the case of insoluble compounds, the compounds were suspended in DMF and vortexed then processed.

Testing for anti-bacterial and yeasts activity:-
Bacterial cultures were grown in nutrient broth medium at 30 °C. After 16 h of growth, each microorganism, at a concentration of 10^8 cells/mL, was inoculated on the surface of Mueller-Hinton agar plates using sterile cotton swab. Subsequently, uniform size filter paper disks (6 mm in diameter) were impregnated by equal volume (10 µl) from the specific concentration of dissolved compounds and carefully placed on surface of each inoculated plate. The plates were incubated in the upright position at 36ºC for 24 hours. Three replicates were carried out for each extract against each of the test organism. Simultaneously, addition of the respective solvent instead of dissolved compound was carried out as negative controls. After incubation, the diameters of the growth inhibition zones formed around the disc were measured with transparent ruler in millimeter, averaged and the mean values were tabulated.

Testing for anti-fungal activity:-
Active inoculum for experiments were prepared by transferring many loopfuls of spores from the stock cultures to test tubes of sterile distilled water (SDW) that were agitated and diluted with sterile distilled water to achieve optical density corresponding to $2.0x10^5$ spore/ml. inoculum of 0.1 % suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes then the same procedure was followed as described above.

Standard references:-
The antibiotic chloramphenicol was used as standard reference in the case of Gram – negative bacteria, Cephalothin was used as standard reference in the case of Gram – positive bacteria and cycloheximide was used as standard reference in the case of fungi.

Measurement of minimal inhibitory concentration (MIC):-
MIC values of the synthesized compounds were determined using agar dilution technique [28]. Each compound with high or intermediate antimicrobial effect shown in the disk diffusion test was further diluted with DMF to 25.6, 12.8, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, and 0.1 mg/ml respectively. The concentrations of the compounds became 256, 128, 64, 32, 16, 8, 4, 2, and 1 µg/ml respectively. Then 100 µl of each diluted compound was mixed with 10 ml of cooled (50 ºC) melted Mueller-Hinton agar and 10 µl of specific microbial culture (at concentration of 10^8 cells/mL) which were grown in nutrient broth medium for 16 h at 30 °C. then plated into 6 cm sterile Petri dish. Each dilution was prepared in duplication. Each concentration was prepared for 2 dishes. All plates were incubated at 33 ºC for 24 hours. MIC of each compound was measured from the plate with the lowest concentration with no visible growth of specific

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