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Synthesis and biological activity study of some novel Tri-Schiff's basses derived from 6-aminopenicillanic acid and Tri-amide containing three 1,3,4-thiadiazole units.

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الخُلاصّة

ان تفاعل المسيتيلين مع البروم بوجود مصدر ضوئي يولد الاشعة فوق البنفسجية ينتج سداسي بروميد المسيتيلين[2]. التحلل الحامضي للمركب [2] بوجود المورفلين يعطي ١,٣,٥–ثلاثي فورميل بنزين [3], و ينتج من تكاثف المركب [3] مع ثلاث مكافئات من ٦–امينو حامض البنسينلك المركب[4].

ان تفاعل ٤–ن–الكوكسي حامض البنزويك $[6]_n$ مع مركب ثايوسيميكاربازايد بوجود ثلاثي كلوريد الفسفوريل اعطى المركبات ٢–امينو–٥–(٤–ن–الكوكسي فنيل)–١,٣,٤–ثايادايازول $[7]_n$. ان تكاثف ثلاث مكافئات من ٢–امينو–٥–(٤–ن–الكوكسي فنيل)–١,٣,٤–ثايادايازول $[7]_n$ مع مكافئ واحد من ١,٣,٥–ثلاثي حامض الميسك [5] يعطي المركبات $[8]_n$.

كل المركبات المحضرة قد تم تشخيصها من خلال قياس درجات إنصهارها و بأستخدام مطيافية الأشعة تحت الحمراء, كما تم الأستعانة بمطيافية الأَشعة فوق البنفسجية في عملية التشخيص. و قد دلت النتائج المستحصلة على' صحة التراكيب المقترحة للمركبات المحضرة.

و أخيراً تم دراسة الفعالية البايولوجية لهذه المركبات المحضرة حيث كانت بعض المركبات لها فعالية بايولوجية و لم تمتلك مركبات اخرى هذه الفعالية, انظر الجدول (٧).

Abstract

Reaction of meistylene with bromine under UV-light afforded α,α,α',α',α'',α''-hexabromo mesitylene [2]. The reaction of compound [2] with morpholine and hydrochloric acid afforded 1,3,5-triformyl benzene [3]. Condensation of compound [3] with three equivalents of 6-aminopenicillanic acid afforded 1,3,5-tri[penicillanic acid]-6-N-trimesilydene [4].

The reaction of thiosemicarbazide and 4-n-alkoxy benzoic acid $[6]_n$ in $POCl_3$ afforded the corresponding 2-amino-5-(4-n-alkoxyphenyl)-1,3,4-thiadiazole $[7]_n$. condensation of 1,3,5-benzene tricarboxylic acid [5] with three equivalents of appropriate amino-thiadiazole $[7]_n$ afforded the corresponding Tri-amide compounds $[8]_n$. The characterization of the synthesized compounds

was elucidated by their melting points and different spectroscopic methods (UV, FT-IR) spectroscopy. The results are in agreement with the suggested structures. The synthesized compounds were tested ***in vitro*** for antimicrobial activity. The results obtained indicated that some of these compounds are more active than with others, Table (7).

Introduction

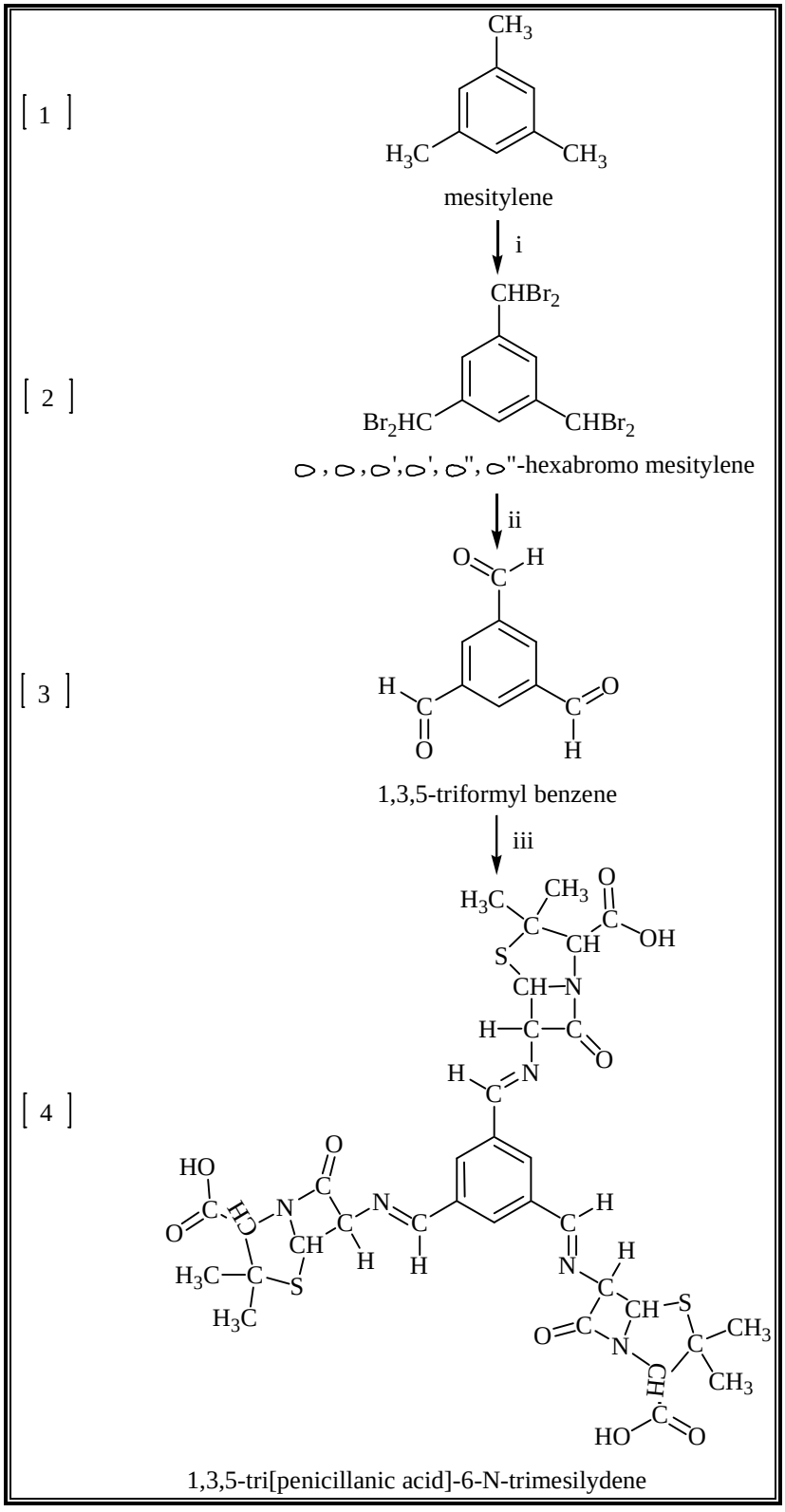
As a part of our continuous synthetic program using 6-aminopenicillanic acid as starting material, hoping to obtain novel penicillins that have one or more of the following advantages: (i) broader antimicrobial spectra, (ii) more favorable absorbance patterns and (iii) reduced undesirable side effect. In this work, we prepared a new penicillins derivatives [4] by the reaction of 6-aminopenicillanic acid with 1,3,5-triformyl benzene [3], Scheme (1).

Further more, the growing patent literature from the sixties demonstrates that the 1,3,4-thiadiazoles are becoming of great interest, this is primarily due to the large number of uses of 1,3,4-thiadiazoles in the most diverse areas, for example in drug synthesis, scintillation material, dyestuffs industry[1], photography[2], and corrosion inhibitors[3]. Numerous 1,3,4-thiadiazoles have been synthesized and reported to be biological versatile compounds having bactericidal[4], fungicidal[5], muscle relaxant[6] properties ….etc., therefore, we prepare various derivation of medicinally interest 1,3,4-thiadiazole[7, 8], synthesis of some new Tri-amide $[8]_n$ by reaction of 1,3,5-benzene tricarboxylic acid [5] with appropriate 2-amino-5-(4-n-alkoxyphenyl)-1,3,4-thiadiazole [7]n as shown in Scheme (2).

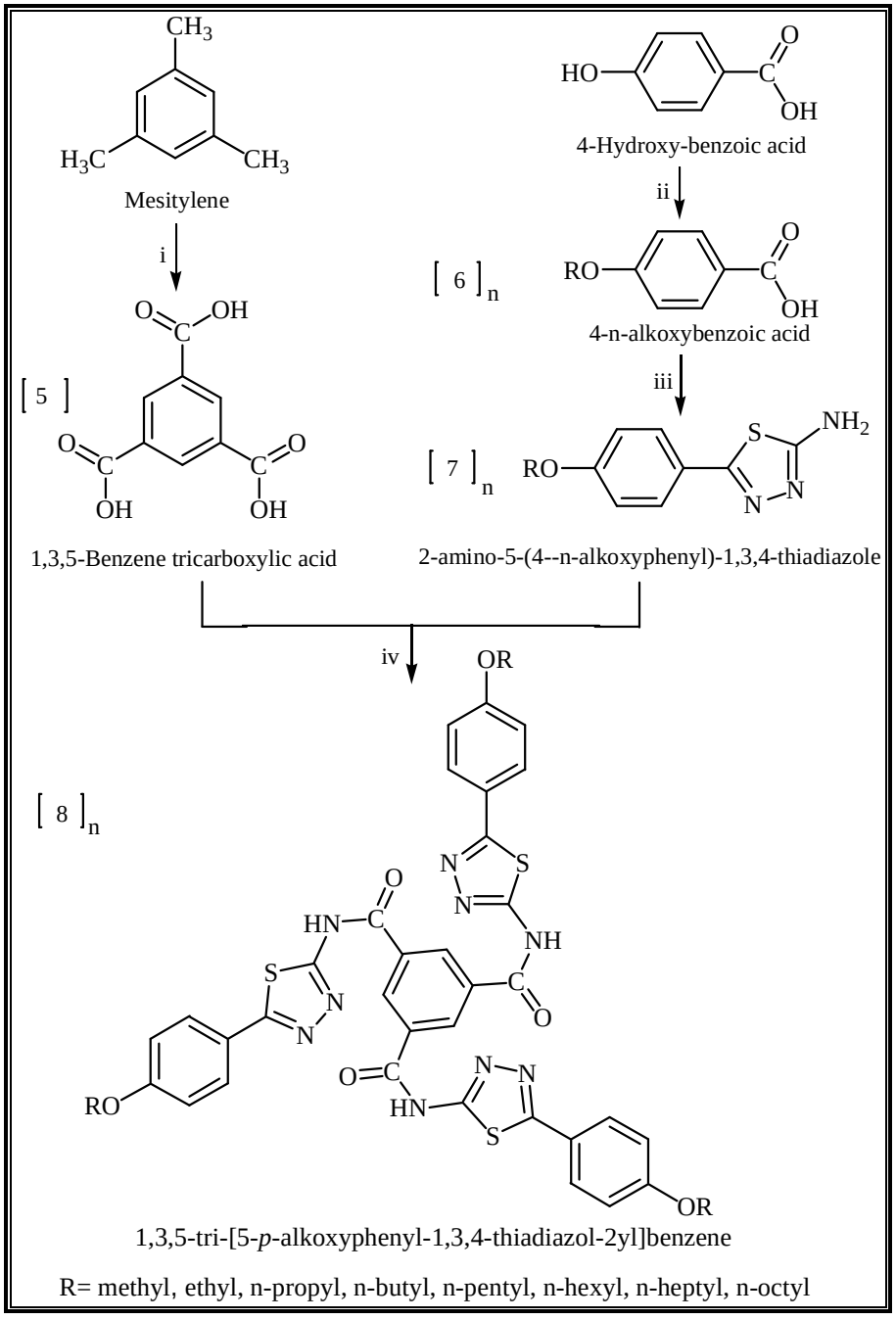
Material and Methods

Instruments

Melting points were measured using Gallen Kamp melting point apparatus and were uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1310 infrared spectrophotometer and FTIR – 8300 fourier transform infrared spectrophotometer SHIMADZU as potassium bromide disc. U.V-Visible Absorbance measurements were recorded on a Cintra 5 UV-Visible spectrophotometer.



Scheme (1) Reagents and Conditions: (i) Br_2, UV light, reflux (9) hrs.; (ii) Morpholine, reflux (4) hrs. at (40 ^{0}C); (iii) 6-aminopenicillanic acid, absolute ethanol, reflux (24) hrs..



Scheme (2) Reagents and Conditions: : (i) $KMnO_4$, Na_2CO_3, reflux (24) hrs; (ii) MeOH, KOH, appropriate alkyl halide, reflux over night; (iii) $POCl_3$, thiosemicarbazide, reflux (5) hrs.; (iv) $POCl_3$, reflux (7) hrs..

Synthesis

Preparation of α,α,α',α',α'',α''-hexabromo mesitylene [2]:

To a (100 mL) round bottom flask containing (23 mL) of mesitylene [1], (15 mL) of Bromine was added within (2) hours at 155°C, another (15 mL) of Bromine was added also within (6) hours at (170-180)°C. The addition of

Bromine was administrated under UV light. The mixture was then refluxed for another (1) hour at 170°C. After cooling to room temperature the mixture was filtered to give (80) % of the titled compound[9] [2], M.P. =183 ^{0}C.

Preparation of 1,3,5-triformyl benzene [3]:

A (2.97 g, 0.005 mole) of α,α,α',α',α'',α''-hexabromo mesitylene [2] was dissolved in (22 mL) of anhydrous morpholine. The solution was then refluxed for (4) hours by using water bath, after that the solution was poured into a (30 g) of concentrated hydrochloric acid (d = 1.19 g/mL) with ice. The solution was then filtered, and the precipitate was then dissolved again in (200 mL) boiled water. The solution was neutralized by potassium hydroxide, (60 % from the titled compound[9] [3] was formed by cooling to room temperature), M.P. =154^{0}C.

Preparation of 1,3,5-tri[penicillanic acid]-6-N-trimesilydene [4]:

A mixture of 6-aminopenicillanic acid (0.03 mole, 6.48 g) and 1,3,5-tri formyl benzene (0.01 mole, 1.62 g) [3] dissolved in (35 ml) of absolute ethanol. The mixture was then refluxed for (24) hours with stirring. After cooling to room temperature the solvent was evaporated, and the precipitate was recrystallized from ethanol to give the titled compound [4]. Yield (48) %, M.P. =(218-220) ^{0}C.

Preparation of 1,3,5-benzene tricarboxylic acid (trimesic acid)[10] [5]:

(0.05 mole, 6.5 g) of mesitylene [1] is added to a solution of (0.32 mole, 51.2 g) of potassium permanganate and (0.16 mole, 15 g) of sodium carbonate in (250 ml) of water and the mixture is heated under reflux (24) hours until the color of the permanganate has disappeared. The reaction mixture was filtered while still hot to get rid of MnO_2 precipitate. The cooled filtrate is acidified with sulphuric acid (20) %, the precipitated carboxylic acid is filtered off, washed with a little cold water and used without further purification. Yield (40) %, M.P. => 330 ^{0}C, Lit.,[10]> 330 ^{0}C.

Preparation of 4-n-alkoxybenzoic acid $[6]_n$:

4-Hydroxybenzoic acid (0.08 mole, 11.25 g) was dissolved in (75 mL) of methanol at 25°C with stirring, after the acid was dissolved, (0.2 mole, 13.12 g) of potassium hydroxide in (10 mL) of distilled water was added dropwise, the reaction mixture was heated to reflux and (0.1 mole) of appropriate alkyl halide was then added over (2) hours. The reaction mixture was refluxed over night, and then (50 mL) of methanol was removed by evaporation. The remained of reaction mixture was cooled at 25°C and then added to (250 ml) of distilled water. Hexane (25 mL) was added to extract organic impurities. After discarding

organic phase, the aqueous phase was heated to 40°C and neutralized with (20) % HCl. The product precipitated during the neutralization was collected by filtration and purified by recrystallization from ethanol. The physical properties of the prepared compounds $[6]_n$ are listed in Table (1).

Compound no.	R group	Yield (%)	M.P.(°C)
$[6]_1$	Methyl	70	184
$[6]_2$	Ethyl	75	196
$[6]_3$	n-propyl	68	145
$[6]_4$	n-butyl	75	147
$[6]_5$	n-pentyl	70	124
$[6]_6$	n-hexyl	55	105
$[6]_7$	n-heptyl	60	92
$[6]_8$	n-octyl	50	101

Table 1: The melting points of 4-n-alkoxybenzoic acid $[6]_n$

Preparation of 2-amino-5-(4-n-alkoxyphenyl)-1,3,4-thiadizole $[7]_n$

A mixture of appropriate 4-n-alkoxybenzoic acid $[6]_n$ (0.01 mole) and (0.01 mole, 0.91 g) of thiosemicarbazide with (5 mL) of phosphorus oxychloride was refluxed gently for (5) hours. After cooling (50 mL) of water was added, the mixture was then refluxed for (7) hours and filtrated, neutralized with potassium hydroxide. The precipitate was washed with water and recrystallized from (ethanol-water) to give the titled compounds $[7]_n$. The physical properties of 1,3,4-thiadiazole derivatives are listed in Table (2).

Compound no.	R group	Yield (%)	M.P.(C^0)
$[7]_1$	methyl	65	188
$[7]_2$	ethyl	64	190
$[7]_3$	n-propyl	60	195
$[7]_4$	n-butyl	65	200
$[7]_5$	n-pentyl	63	197
$[7]_6$	n-hexyl	52	180
$[7]_7$	n-heptyl	50	170
$[7]_8$	n-octyl	45	165

Table 2: The melting points of 2-amino-5-(4-n-alkoxyphenyl)-1,3,4-thiadizole $[7]_n$.

Preparation of 1,3,5-tri-[5-*p*-alkoxy phenyl-1,3,4-thiadiazol-2-yl]-benzene $[8]_n$:

A mixture of appropriate 2-amino-5-(4-n-alkoxyphenyl)-1,3,4-thiadizole $[7]_n$ (0.03 mole), 1,3,5-benzene tricarboxylic acid [5] (0.01 mole, 2.1 g), and phosphorus oxychloride (25 ml) was refluxed for (7) hours. The cold reaction mixture was poured on crushed ice and made basic by adding sodium bicarbonate solution. The separated product was filtered off and recrystallized from (chloroform - petroleum ether) to give the title compounds $[8]_n$. The physical properties of the synthesized compounds are listed in Table (3).

Compound no.	R group	Yield (%)	M.P.(C^0)
$[8]_1$	methyl	60	260
$[8]_2$	ethyl	61	270
$[8]_3$	n-propyl	50	275
$[8]_4$	n-butyl	55	278
$[8]_5$	n-pentyl	65	280
$[8]_6$	n-hexyl	60	268
$[8]_7$	n-heptyl	45	300
$[8]_8$	n-octyl	44	262

Table 3: The melting points of 1,3,5-tri-[5-*p*-alkoxy phenyl-1,3,4-thiadiazol-2-yl]-benzene $[8]_n$.

Results and Discussion

α,α,α',α',α'',α''-hexabromo mesitylene [2] was prepared by the free radical bromination under a source of UV light of refluxed mesitylene.

The structure of this compound was established by:

1- Melting point: The melting point of the compound [2] agrees with that reported in the literature[9] which is (183 ^oC).

2- Infrared spectroscopy: The FT-IR spectrum assures the formation of the compound [2] through the presence of the band at (3050 cm^{-1}) attributed to the (C-H) stretching band of aromatic ring and the peak at (1529 cm^{-1}) of (C=C) show that the aromatic ring of mesitylene is still found, and (2910 cm^{-1}) of (C-H) aliphatic and the C-Br vibration frequencies appeared at (536 cm^{-1}). The FT-IR spectrum of α,α,α',α',α'',α''–hexabromo mesitylene [2] is shown in Figure (2) and for mesitylene [1] in Figure (1).

3- Ultra-Violet-Visible spectrophotometer: The electronic spectrum of mesitylene [1], Figure (5) displayed band at (261 nm) assigned to (π-π^*) transition. The electronic spectrum of α,α,α',α',α'',α''–hexabromo mesitylene [2], Figure (6), showed the corresponding transition of (300 nm) which assigned to (π-π^*) transition, the shifting of the (π-π^*) in this

compound [2] with transition in compound [1] is because of the presence of the six bromine atoms.

The 1,3,5-triformyl benzene was prepared through the hydrolysis of α, α,α',α'',α''-hexabromo mesitylene in anhydrous morpholine, then this solution was hydrolyzed with concentrated hydrochloric acid.

The structure of 1,3,5-triformyl benzene was elucidated using melting point and (UV-Visible, FT-IR) spectroscopy. The melting point of 1,3,5-triformyl benzene is (154 ^{0}C) and this melting point agrees very well with that reported in the literature(9) . The FT-IR spectroscopic results assure the formation of 1,3,5-triformyl benzene through the disappearance of the peak at (2910 cm^{-1}) attributed to the (C-H) aliphatic stretching frequency and the presence of the (C=O) of aldehyde at (1718 cm^{-1}), the peak at (2727 cm^{-1}) for (C-H) stretching of aldehyde, and the peak of (3095 cm^{-1}) for the C-H aromatic stretching frequency. The electronic spectrum of 1,3,5-triformyl benzene [3], displayed band at (320 nm), assigned to (π-π^*) transition, Figure (7). All these evidences give us the prompt for the formation of 1,3,5-triformyl benzene. The FT-IR spectrum of 1,3,5-triformyl benzene is shown in Figure (3).

Tri-Schiff's base compound [4] was synthesized by refluxing of compound [3] with three equivalents of 6-aminopenicillanic acid in absolute ethanol. This compound was characterized by its melting point (218-220)^{0}C and (U.V., FT-IR) spectroscopy. The FT-IR spectrum assures the formation of Schiff's base through the disappearance of two absorption bands due to NH_2 stretching of 6-aminopencillanic acid, and the disappearance of the absorption band of C=O stretching frequency at (1718 cm^{-1}) for 1,3,5-triformyl benzene, and the appearance of stretching band at about (1649 cm^{-1}) which was assigned to C=N. The FT-IR spectrum of this compound is shown in Figure (4). The electronic spectrum of the synthesized compound [4], dissolved in DMSO, showed the corresponding transition at (269 and 324 nm) assigned to π-π^* and n-π^* transitions, respectively, Figure (8).

1,3,5-benzene tricarboxylic acid (trimesic acid) [5] was prepared in the easiest fashion by the oxidation of the three-methyl groups in mesitylene [1] using potassium permanganate. The structure of the acid was confirmed by its very high melting point (>330 ^{0}C), the melting point agrees well with that reported in the literature(11). Also by its IR spectrum, which displays a broad (O-H) stretching absorption in the region of (3260 cm^{-1}) as well as the carboxylic acid (C=O) absorption at (1690 cm^{-1}), Figure (9).

4-n-alkoxybenzoic acid $[6]_n$ was prepared by the refluxing of 4-hydroxybenzoic acid with different alkyl halides in strong basic media of potassium hydroxide. The melting point of the compounds above agree with that reported in the literature[11].

The reaction of appropriate 4-n-alkoxybenzoic acid, with thiosemicarbazide in the presence of phosphorus oxychloride under the condition reported previously[12], afforded 2-amino-5-(4-n-alkoxyphenyl)-1,3,4-

thiadiazoles $[7]_n$. The structures of these compounds were elucidated using melting point and infrared spectroscopy. The FT-IR spectra of 2-amino-5-(4-n-alkoxyphenyl)-1,3,4-thiadiazole $[7]_n$ give the evidence for the formation of the titled compounds through the disappearance of the two bands at (3550-2750)cm^{-1}, and (1687 cm^{-1}) attributed to the O-H stretching frequency, and C=O of 4-n-alkoxybenzoic acid together, with the appearance of the band at about (3300-3100)cm^{-1} which could be assignable to NH_2 group, asymmetric and symmetric stretching vibrations. Besides that, the band at about (1615-1600)cm^{-1} due to the C=N group stretching frequency is also observed, the C-O-C group stretching vibrations (asymmetric and symmetric) appear at (1250-1020)cm^{-1}, also the γ C-H bending of *P*-disubstituted appears at about (830 cm^{-1}). The spectroscopic data and charts of the synthesized 2-amino-5-(4-n-alkoxyphenyl)-1,3,4-thiadiazoles $[7]_n$ are shown in Figures (10 and 11) respectively. The spectroscopic data of the compounds $[7]_n$ are shown in Table (4).

Comp. no.	**R group**	**υ_{as} N-H**	**υ_{sy} N-H**	**υC-H aromatic**	**$\upsilon_{as,sy}$C-H aliphatic**	**υC=N**	**$\upsilon_{as,sy}$ C-O-C**
$[7]_1$	methyl	3281	3105	3105	2928 2858	1611	1252 1042
$[7]_2$	ethyl	3410	3277	3092	2924 2856	1612	1248 1056
$[7]_3$	n-propyl	3369	3105	3105	2928 2858	1614	1252 1024
$[7]_4$	n-butyl	3269	3105	3105	2926 2858	1612	1252 1024
$[7]_5$	n-pentyl	3241	3101	3101	2936 2864	1611	1254 1018
$[7]_6$	n-hexyl	3273	3099	3099	2961	1611	1252 1036
$[7]_7$	n-heptyl	3279	3113	3113	2951 2930	1609	1252 1051
$[7]_8$	n-octyl	3210	3105	3105	2928 2858	1611	1252 1042

Table (4): The characteristic FT-IR absorption bands of $[7]_n$.

The electronic spectra of 2-amino-5-(4-n-alkoxyphenyl)-1,3,4-thiadiazole $[7]_n$, Figure (14 and 15) showed an adsorption bands at about (300-312 nm) for all compounds which attributed to the (π-π^*) transition.

The reaction of trimesic acid chloride with appropriate amino-thiadiazole $[7]_n$ afforded the corresponding Tri amide compounds were established by using FT-IR spectra, Figure (12 and 13). The appearance of the absorption of the carbonyl group for amide group at (1680 cm^{-1}), as well as, the appearance of the absorption of C=N for 1,3,4-thiadiazole ring at (1600 cm^{-1}), is good evidence for the formation of amide group, The spectroscopic data of the compounds $[8]_n$ are shown in Table (5).

Comp. no.	R group	υ_{sy} N-H	υC-H aromatic	$\upsilon_{as,sy}$C-H aliphatic	υC=N	$\upsilon_{as,sy}$ C=O
$[8]_1$	methyl	3301	3091	2933 2868	1604	1645
$[8]_2$	ethyl	3282	3118	2937 2877	1606	1699
$[8]_3$	n-propyl	3243	3105	2995 2839	1602	1685
$[8]_4$	n-butyl	3242	3115	2931 2862	1610	1674
$[8]_5$	n-pentyl	3256	3182	2937 2866	1610	1695
$[8]_6$	n-hexyl	3290	3082	2937 2910	1606	1639
$[8]_7$	n-heptyl	3310	3205	2965 2883	1611	1698
$[8]_8$	n-octyl	3142	2977	2935 2814	1602	1702

Table 5: Show the characteristic FT-IR absorption bands of compounds $[8]_n$.

The electronic spectra of the synthesized Tri amide compounds $[8]_n$, dissolved in (DMSO) gave the (λ_{max}) absorption bands at about (310-312 nm) for all compounds which attributed to the (π-π^*). The (n-π^*) were forbidden, therefore not appear. The (λ_{max}) and the type of the electronic transitions of Tri amide compounds $[8]_n$ are listed in Table (6).

Compound no.	R group	λ_{max} (nm)
$[8]_1$	methyl	311
$[8]_2$	ethyl	312
$[8]_3$	n-propyl	311
$[8]_4$	n-butyl	310.5
$[8]_5$	n-pentyl	311
$[8]_6$	n-hexyl	311.5
$[8]_7$	n-heptyl	311.5
$[8]_8$	n-octyl	310.5

Table (6) show the electronic transitions and (λ_{max}) for compounds $[8]_n$

The (λ_{max}) for all Tri amide compounds are approximately constant because the difference between each compound in these series is methylene group and this group is of no effect on the (λ_{max}). The UV-Vis. Spectra of the Tri amide compounds are shown in Figure (16 and 17).

Biological activity

The antibacterial activity[13] of the synthesized compounds was determined ***in vitro*** using paper disc method (agar plate diffusion method) against two pathogenic microorganism[14] viz., ***staphylococcus aureus*** (Gram +ve) and ***Escherichia coli*** (Gram –ve). In this method, a standard 5mm diameter sterilized filter paper impregnated with the compound (1mg per 1ml DMSO and 1mg per 10ml DMSO for concentration (1) and (2), respectively) was placed on agar plate seeded with the test organism. The plates were incubated for 24 hours at 37 ^{0}C. The zone of inhibition of bacterial growth were measured in mm depending upon the diameter as shown in Table (7), and Figures (18) to (23). In addition the antifungal also determine as shown in Figure (24 and 25).

Compound No.	Compound No. *in Figure*	*staphylococcus aureus*	Escherichia coli	*Candida albicans*
6-APA	1	++	++	++
[5]	2	+	++	-
[3]	3	+	+	++
[4]	4	+++	+++	++++
$[7]_1$	5	+	+	
$[7]_2$	6	+	+	
$[7]_3$	7	+	+	
$[8]_1$	1	+	++	+
$[8]_2$	2	+	+	+
$[8]_3$	3	+	+	+
$[8]_4$	4	+	+	
$[8]_5$	5	+	+	
$[8]_6$	6	+	-	
$[8]_7$	7	+	+++	
$[8]_8$	8	+	+	

Table 7: Antibacterial activity of the synthesized compounds.

Key to symbols
Highly active = +++ (inhibition zone > 20mm)
Moderately active = ++ (inhibition zone 11-20mm)
Slightly active = + (inhibition zone 5-10mm)
Inactive = - (inhibition zone <5mm)

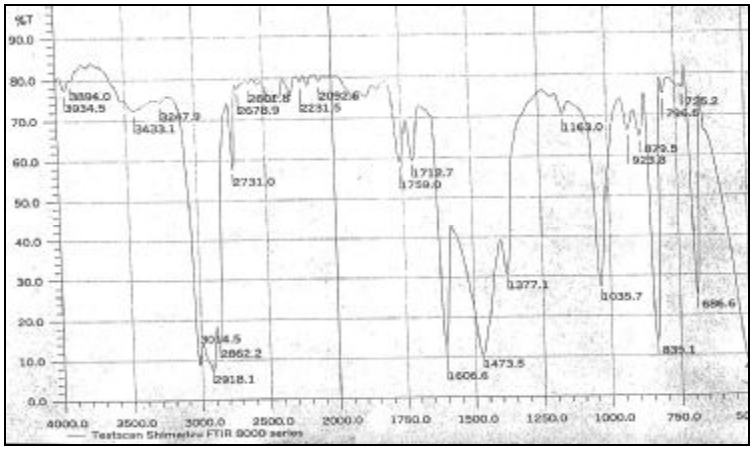


Figure 1: FT-IR spectrum of mesitylene [1].

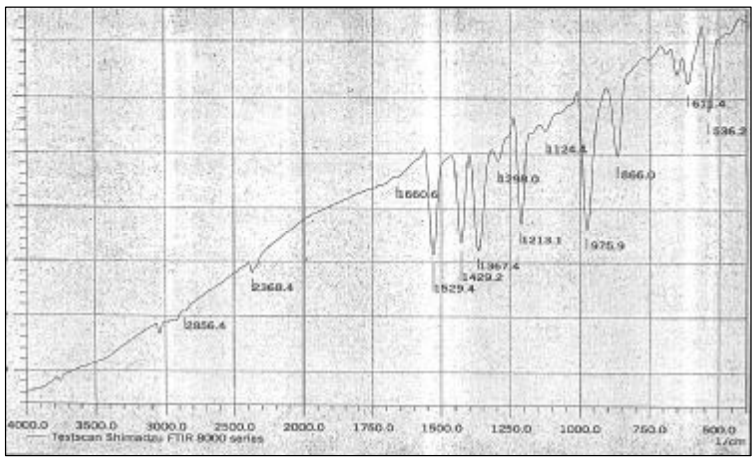


Figure 2: FT-IR spectrum of α,α,α',α',α",α"-hexabromo mesitylene [2].

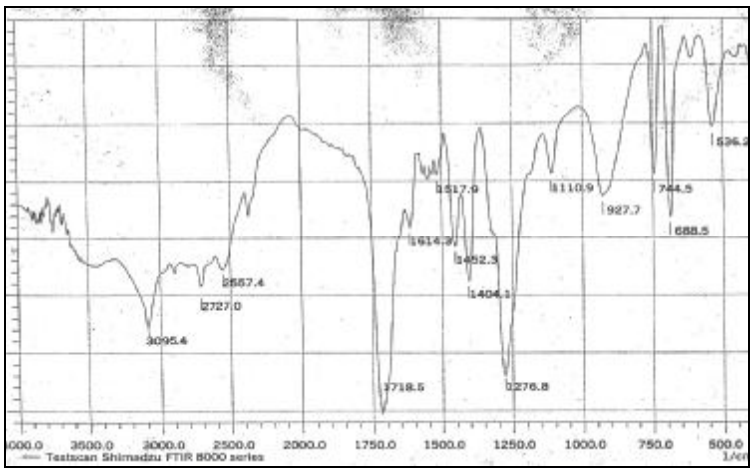


Figure 3: FT-IR spectrum of 1,3,5-triformylbenzene [3].

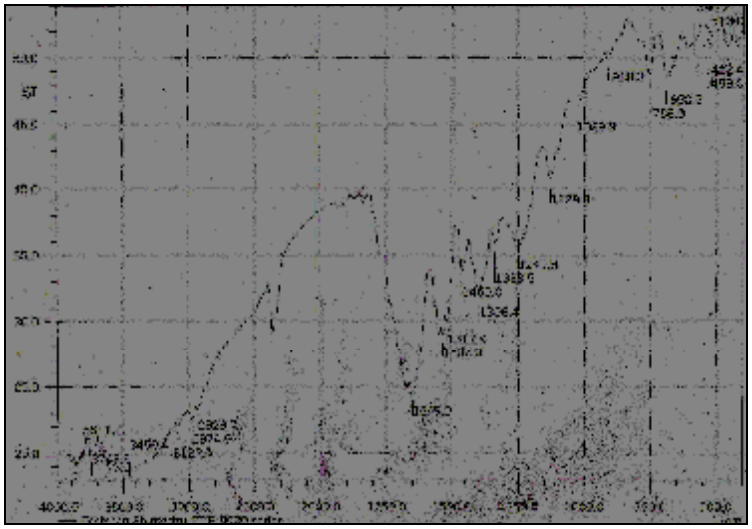


Figure 4: FT-IR spectrum of 1,3,5-tri[pencillinic acid]-6-N-trimesilydene[4].

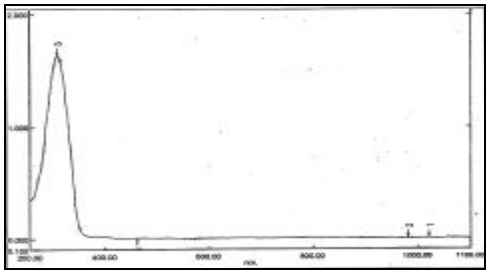


Figure5: Ultraviolet spectrum of mesitylene [1].

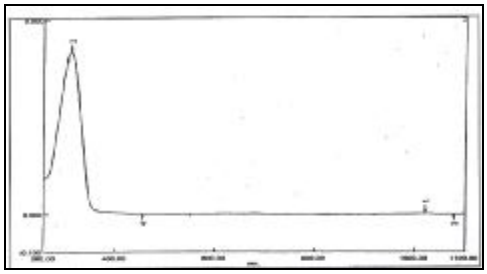


Figure 6: Ultraviolet spectrum of α,α,α',α',α'',α''-hexabromo mesitylene [2].

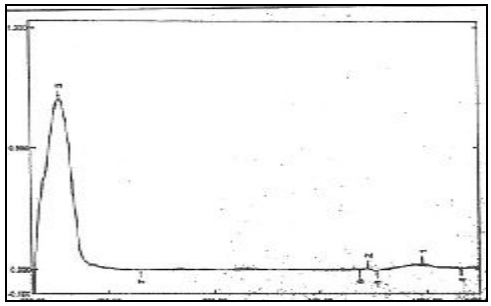


Figure 7: Ultraviolet spectrum of 1,3,5-triformyl benzene [3].

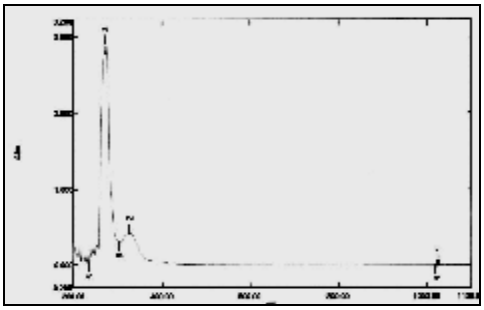


Figure 8: Ultraviolet spectrum of 1,3,5- tri[pencillanic acid]-6-N- trimesitydene [4]

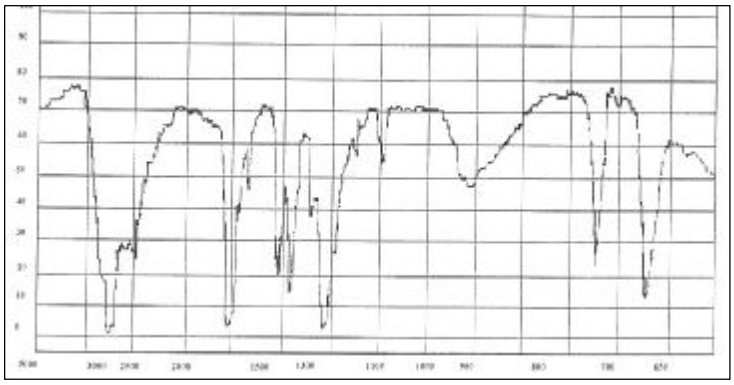


Figure 9: IR spectrum of 1,3,5-benzenetricarboxylic acid(trimesic acid) [5].

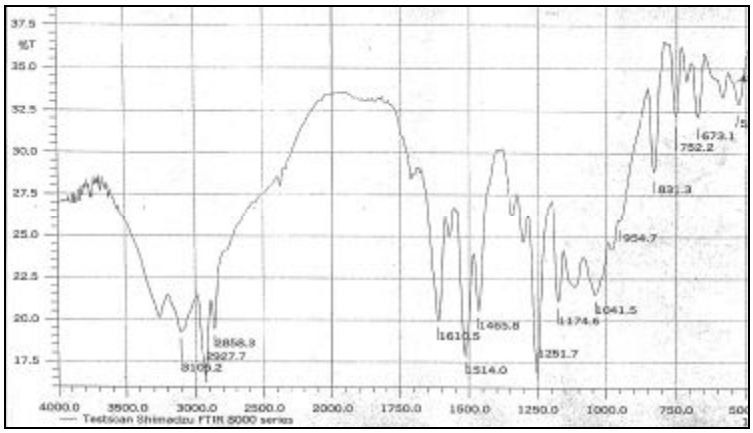


Figure 10: FT-IR spectrum of 2-amino-5-(4-methoxyphenyl)-1,3,4-thiadiazole $[7]_1$.

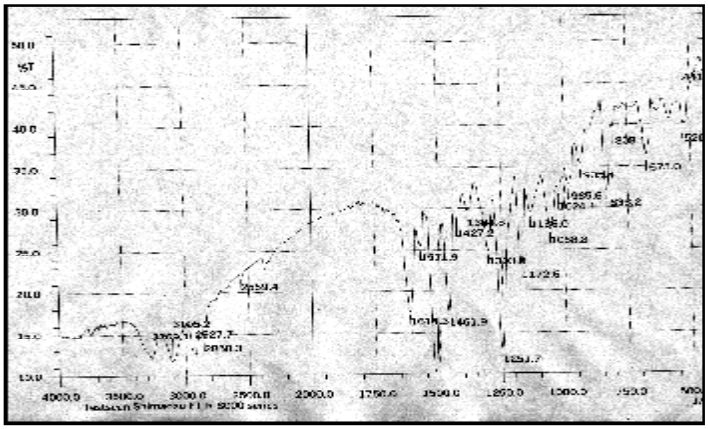


Figure 11: FT-IR spectrum of 2-amino-5-(4-propoxyphenyl)-1,3,4-thiadiazole $[7]_3$

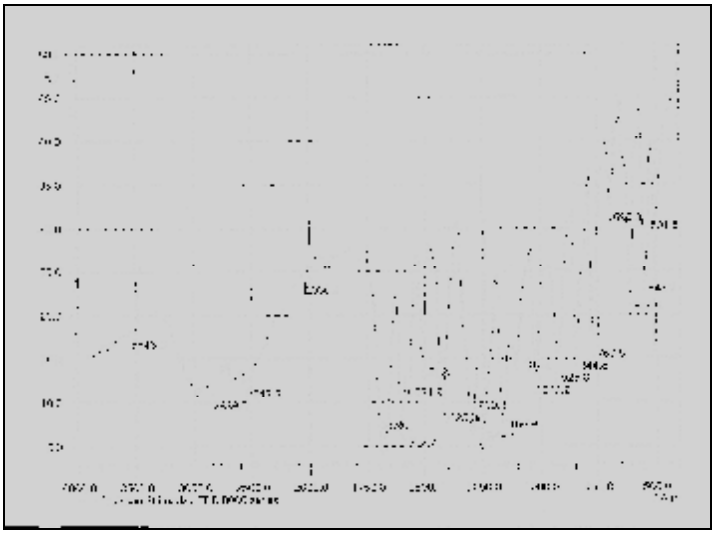


Figure 12: FT-IR spectrum of 1,3,5-tri-[5-*p*-propoxy phenyl-1,3,4-thiadiazol-2-yl]-benzene $[8]_3$

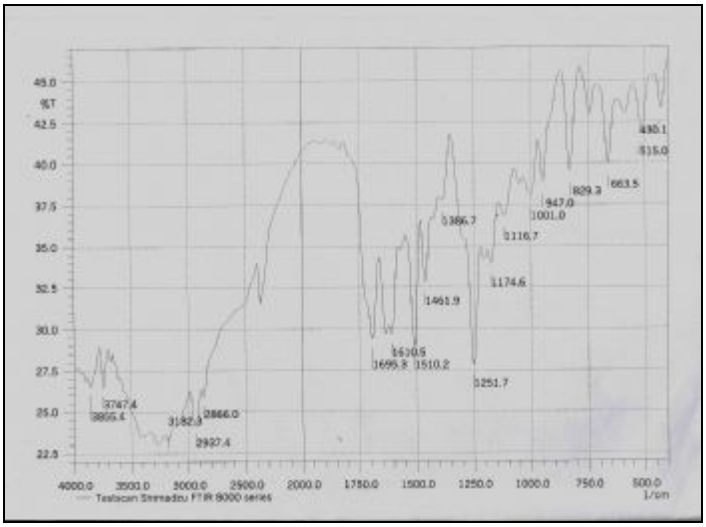


Figure 13: FT-IR spectrum of 1,3,5-tri-[5-*p*-pentoxy phenyl-1,3,4-thiadiazol-2-yl]-benzene $[8]_5$

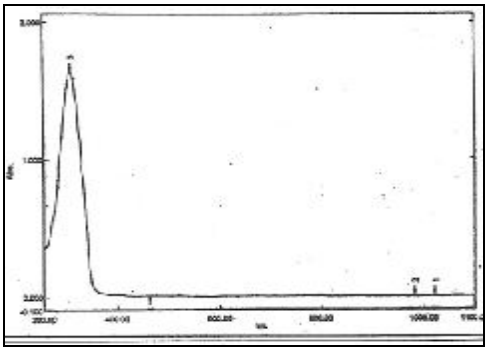


Figure 14: Ultraviolet spectrum of compound $[7]_1$

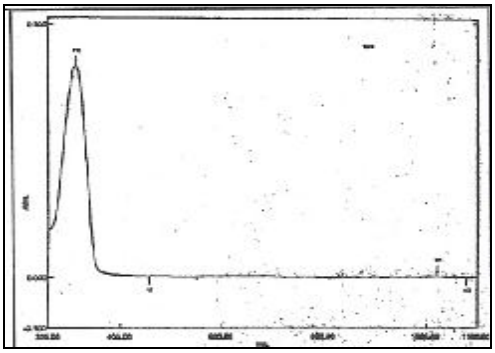


Figure 15: Ultraviolet spectrum of compound $[7]_2$.

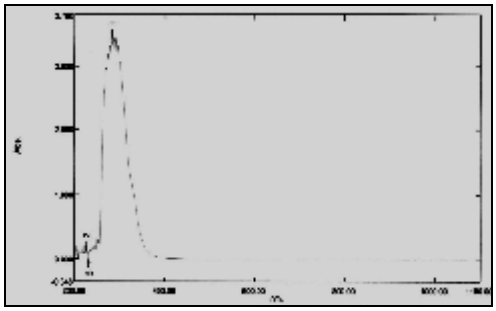


Figure 16: Ultraviolet spectrum of compound $[8]_1$

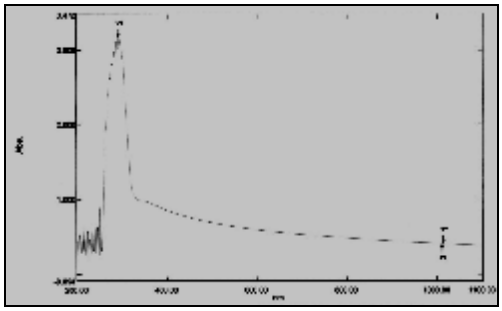


Figure 17: Ultraviolet spectrum of compound $[8]_2$.

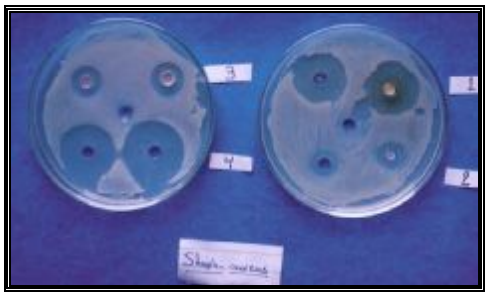
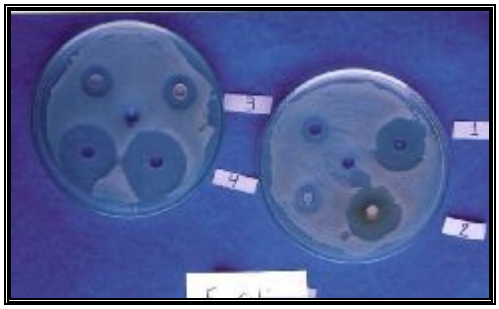


Figure 18: {1-Effect of 6-aminopencillanic acid, 2-Effect of [5], 3-effect of [3], 4-effect of [4]} on Staph.aureus.

Figure 19: {5-Effect of $[7]_1$, 6-effect of $[7]_2$, 7-effect of $[7]_3$}. On Staph.aureus.



Figure(20):{1-Effect of 6-aminopencillanic acid, 2-Effect of [5], 3-effect of [3], 4-effect of [4]}. on Esch.Coli.

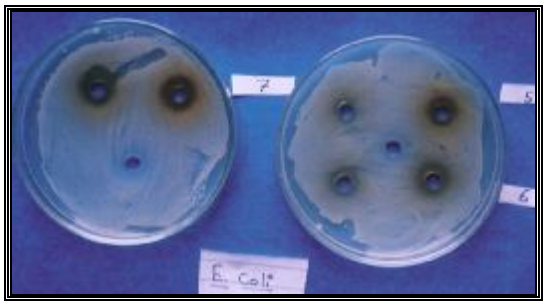


Figure 21: {5-Effect of $[7]_1$, 6-effect of $[7]_2$, 7-effect of $[7]_3$}. on Esch.Coli.

Figure 22:{1,2,3,4,5,6,7,8-Effect of $[8]_1$, $[8]_1$,$[8]_2$,$[8]_3$,$[8]_4$,$[8]_5$,$[8]_6$, $[8]_7$,$[8]_8$} on Staph.aureus

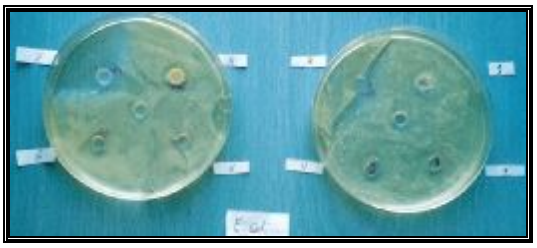


Figure 23: {1,2,3,4,5,6,7,8-Effect of $[8]_2$,$[8]_3$,$[8]_4$,$[8]_5$,$[8]_6$,$[8]_7$,$[8]_8$} on Esch.Coli.

Figure 24:{1-Effect of 6-amino pencillinic acid, 2-Effect of [5], 3-effect of [3], 4-effect of [4]}. on Candida albicans.

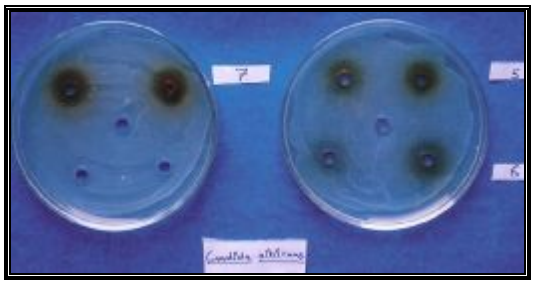


Figure 25: {5-Effect of $[8]_1$, 6-effect of $[8]_2$, 7-effect of $[8]_3$}. on Candida albicans.

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