Synthesis, Characterization and Microbial Study Via Some New Salbutamol's Derivatives

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Abstract

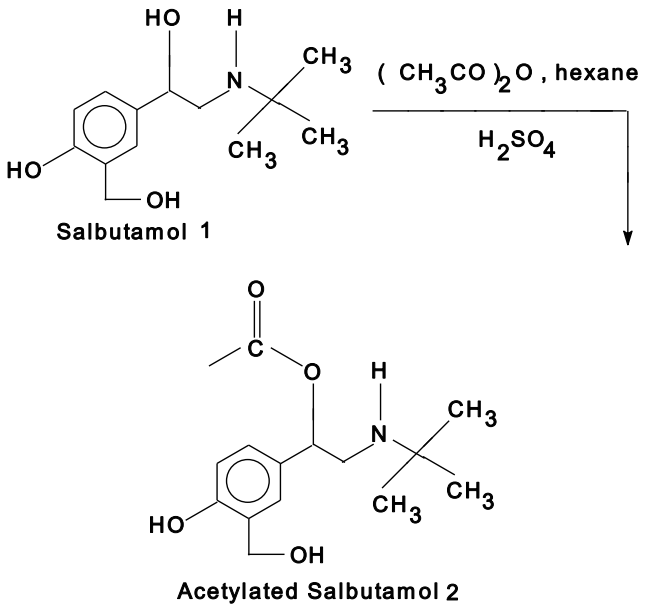
During this work, some new medical salbutamol's derivatives were prepared, characterized and their biological activity was determined. A simple and efficient procedure for obtaining 4-(1-oxoacetyl-2-tert-butylaminoethyl)-6-methanolyl phenol 2 (acetylated salbutamol 2) from a reaction between acetic anhydride and 4-(1-hydroxy-2-tert-butylaminoethyl)-6-methanolyl phenol 1 (salbutamol 1) in an acidic medium was reported. A coupling reaction between the diazonium salts of aniline derivatives with an acetylated salbutamol 2 gave the new corresponding azo derivatives in the selective yields. These compounds were characterized by various physical techniques like: FTIR spectra, UV-Visible spectra, determination of the total number of hydroxyl groups of an acetylated salbutamol 2, melting point and the evaluation of microbial activity in vitro against Gram $^{+}$Ve, Gram $^{-}$Ve bacteria and fungi. A potent antibacterial activity was noticed only for the following compounds:

2-(2′,4′-Dichloroazophenyl)-4-(1-oxoacetyl-2′′-tert-butylaminoethyl)-6-methanolyl phenol 3.
2-(2′,3-Dimethylazophenyl)-4-(1-oxoacetyl-2′′-tert-butylaminoethyl)-6-methanolyl phenol 5.
2-(5-Mercapto-1′,3,4′-thiadiazol-2′-azoyl)-4-(1-oxoacetyl-2′′-tert-butylaminoethyl)-6-methanolyl phenol 6.

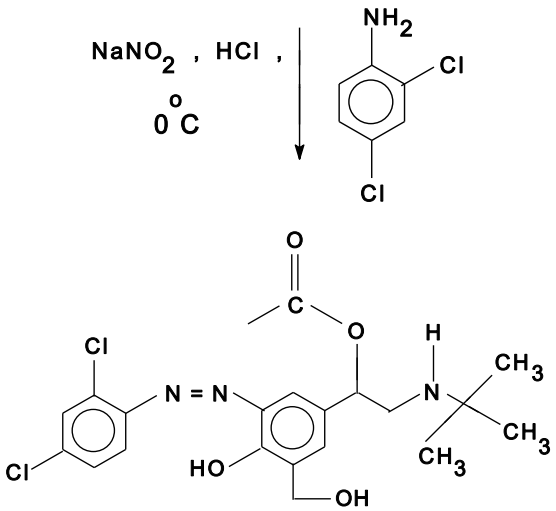
Keywords: Acetylated salbutamol, aniline derivatives, diazonium salt and microbial study.

Introduction

The drugs of amine's content are an important synthetic target for a wide variety of the medical agents and the agrochemicals [1-2]. Salbutamol is a secondary amine and the 2-sympathicomimetic drug, which was granted a marketing authorization in 1973. It is noticed for the treatment of a reversible airway obstruction in a bronchial asthma, a chronic bronchitis and an emphysema [3]. The diazonium salts that produced from using primary aromatic amine could be constant at low temperatures (0-5 ^{o}C), but it's dissociation would occur rapidly at high temperatures [4]. The diazonium salts are the active compounds for various purposes [5]. The drug's-traditional methods for the preparation of the N-methyl secondary amines have often resulted in the formation of the complexes mixtures of the unmethylated, the partially methylated and the permethylated products [6]. A particularly important is the development of the novel methods that can produce the amines with a reagent sensitive functional group [2].



4-(1-oxoacetyl-2-tert-butylaminoethyl)-6-methanolyl phenol 2.



2-(2′, 4′-Dichloroazophenyl)-4-(1-oxoacetyl-2′′-tert-butylaminoethyl)-6-methanolyl phenol 3.

Scheme (1) The reactions of the salbutamol's derivatives with the diazonium salts of the aniline derivatives.

The new azo compounds (4 – 8) were:
2-(4′-Aminoazophenyl)-4-(1-oxoacetyl-2′-tert-butylaminoethyl)-6-methanolyl phenol 4.
2-(2′,3-Dimethylazophenyl)-4-(1-oxoacetyl-2′′-tert-butylaminoethyl)-6-methanolyl phenol 5
2-(5-Mercapto-1′,3,4′-thiadiazol-2′-azoyl)-4-(1-oxoacetyl-2′′-tert-butylaminoethyl)-6-methanolyl phenol 6.
2-(β-Azonaphthyl)-4-(1-oxoacetyl-2′-tert-butylaminoethyl)-6-methanolyl phenol 7.
2-(4′-Aminoazobiphenyl)-4-(1-oxoacetyl-2′-tert-butylaminoethyl)-6-methanolyl phenol 8.

Experimental

Chemicals and instruments:

The reagents, salbutamol 1 and the solvents were of a standard grade and used without further purification. FTIR spectra were recorded on a Shimadzu IR prestige-21 spectrophotometer, KBr disc. UV-Visible spectra were recorded on a Shimadzu UV-Visible 160 Ultra-violet spectrophotometer. The melting points were determined using a Gallenkamp melting point apparatus and uncorrected.

Determination of the total-number of the hydroxyl groups of an acetylated salbutamol 2:

The hydroxyl groups were determined according to a modified reported method [7] as follows:

10 ml of a fresh mixture of (acetic anhydride: pyridine, 1:4, V/ V) was added once to an acetylated salbutamol 2 (0.1 gm, 0.36 mmole) in a round flask and shacked well. 10 ml of the same mixture was put in another round flask as a reference. The two flaks were stirred and refluxed for 45 minutes. They were cooled to room temperature, 20 ml of distilled water was added to each. The contents were stirred for 15 minutes at room temperature, then cooled to –5 ^{o}C for 15 minutes. Few drops of phenolaphthalene - indicator were added to each. The contents were titrated with 1N concentration of sodium hydroxide solution as follows:

Number of OH groups = Z / 1000×M.wt / wt.

Z = The exhausted volume of the reference - exhausted volume of a compound 2 in (ml).

M.wt = Molecular weight of compound 2 (281 gm / mole).

Wt. = Weight of compound 2 (0.1 gm).

Z = 18.9 ml – 18.1 ml = 0.8 ml.

Number of OH groups = 0.8/1000×281/0.1 = 2.248.

OH - Calculated value (practical value): 2.000 (2.248).

Typical Procedure

Synthesis of 4-(1-oxoacetyl-2′-tert-butylaminoethyl)-6-methanolyl phenol 2:

1.5 ml of acetic acid anhydride (1.62 gm, 16 mmole) and few drops of conc. sulfuric acid were added once to salbutamol 1 (0.43 gm, 1.8 mmole) at room temperature. The mixture was stirred and refluxed for 6 hrs, then set aside for 30 minutes at room temperature. 6 ml of distilled water was added. The mixture was shacked and filtered off. A solution of 10% sodium hydroxide was added to neutralize the mother - solution.

An organic layer was extracted with toluene (2×15 ml), dried over anhydrous magnesium sulfate and filtered off. The solvent was removed under reduced pressure. The residue was recrystallized from hexane (twice) to give a yellow product (0.41 gm, 81% yield), m.p = 220 – 222 ^{o}C.

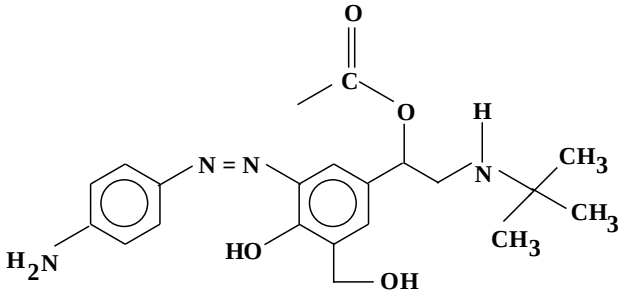
Synthesis of 2-(2′,4′-dichloroazophenyl)-4-(1-oxoacetyl-2′′-tert-butylaminoethyl)-6-methanolyl phenol 3:

(0.676 gm, 4.2 mmole) of 2,4-dichloroaniline was dissolved in 6 ml of 50% solution of hydrochloric acid at 0 ^{o}C. 3 ml of 20% solution of sodium nitrite was added dropwise to solution of a 2,4-dichloroaniline hydrochloride, while the temperature was kept at 0 ^{o}C to obtain the diazonium salt. (1.18 gm, 4.2 mmole) of 4-(1-oxoacetyl-2′-tert-butylaminoethyl)-2-methanolyl phenol 2 was dissolved in 4 ml of 10% solution of sodium hydroxide at 0 ^{o}C. The diazonium salt was added dropwise with stirring to the substrate at the same temperature. The mixture was left for 2 hrs, then 3 ml of 30% solution of hydrochloric acid was added, while a temperature was kept at 0 ^{o}C. Again, set aside the mixture for 1 hr at room temperature. The mixture was filtered off and washed with cold water. The product was dried at room temperature and recrystallized from ethanol to obtain a pale brown product (1.4 gm, 74% yield), m.p = 218 - 220 ^{o}C.

The new azo compounds (4 – 8) were synthesized in the same molar ratio and procedure of compound 3 as follows:

Synthesis of 2-(4′-aminoazophenyl)-4-(1-oxoacetyl-2′-tert-butylaminoethyl)-6-methanolyl phenol 4.

Recrystallization from methanol (twice) gave a deep brown product (1.2 gm, 71% yield), m.p = 240 – 242 ^{o}C.



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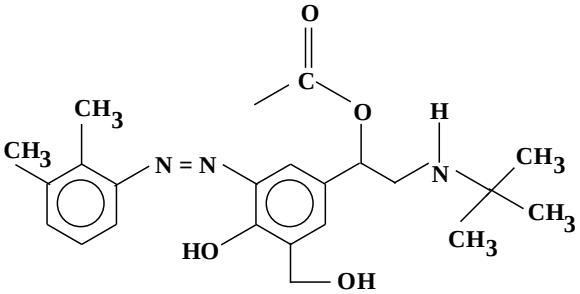
Synthesis of 2-(2′,3-dimethylazophenyl)-4-(1-oxoacetyl-2′′-tert-butylaminoethyl)-6-methanolyl phenol 5

Recrystallization from 90% ethanol gave a yellowish – orange product (1.1 gm, 68% yield), m.p = 184 - 186 ^{o}C.



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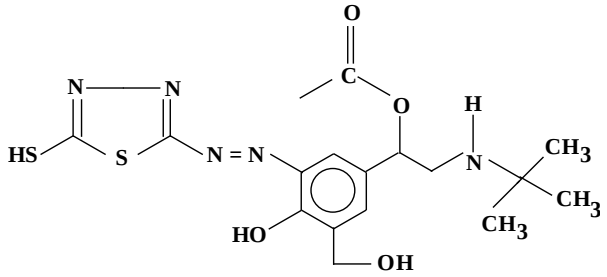
Synthesis of 2-(5-mercapto-1′,3,4′-thiadiazol-2′-azoyl)-4-(1-oxoacetyl-2′′-tert-butylaminoethyl)-6-methanolyl phenol 6

Recrystallization from chloroform gave an orange product (1.2 gm, 67% yield), m.p = 118 - 120 ^{o}C. A starting material of a 2-amino-5-mercapto-1,3,4-thiadiazol was prepared according to published method [8].



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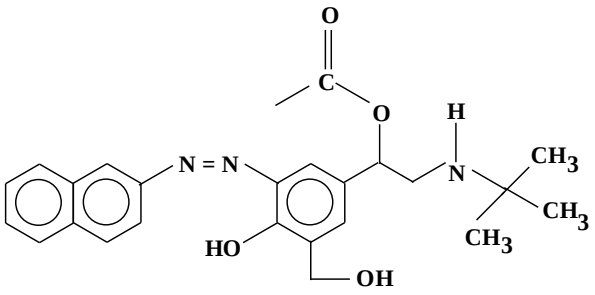
Synthesis of 2-(β-azonaphthyl)-4-(1-oxoacetyl-2′-tert-butylaminoethyl)-6-methanolyl phenol 7.

Recrystallization from mixture of (1:1, hexane: ethanol) gave a deep brown product (1.24 gm, 72% yield), m.p = 228 - 230 ^{o}C.



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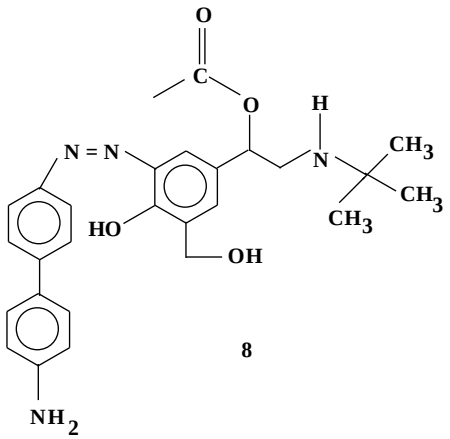
Synthesis of 2-(4′-aminoazobiphenyl)-4-(1-oxoacetyl-2′-tert-butylaminoethyl)-6-methanolyl phenol 8

Recrystallization from mixture of (1:1, hexane: ethanol) gave a deep brown product (1.28 gm, 64% yield), m.p = 220 - 222 ^{o}C.



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Results and Discussion

The present investigation describes synthesis, characterization and microbial activity of some new medical salbutamol's derivatives (Scheme (1), Tables (1-3) and Fig.(1)). A synthetic strategy is based on coupling reaction of the diazonium salts with an acetylated salbutamol 2 to get the new azo compounds (3 - 8).

Table (1)
Physical data for the new salbutamol's derivatives.

Comp. No.	*Molecular Formula*	*Color*	*Molecular Weight*	*Melting Point (^{o}C)*	*Electronic Spectra, Max. Wave Length (nm) / Ethanol Solvent*
2.	$C_{15}H_{23}NO_4$	Yellow	281	220 - 222	225, 300, 710
3.	$C_{21}H_{25}N_3O_4Cl_2$	Pale brown	453	218 - 220	234, 304, 739
4.	$C_{21}H_{28}N_4O_4$	Deep brown	400	238 - 240	212, 306, 417
5.	$C_{23}H_{31}N_3O_4$	Yellowish - orange	413	184 - 186	213, 332, 575
6.	$C_{17}H_{23}N_5O_4S$	Orange	393	118 - 120	213, 301, 415
7.	$C_{25}H_{29}N_3O_4$	Deep brown	435	228 - 230	214, 304, 627
8.	$C_{27}H_{32}N_4O_4$	Deep brown	476	220 - 222	237, 302, 720

The reaction between 4-(1-hydroxy-2-tert-butylaminoethyl)-6-methanolyl phenol 1 (salbutamol 1) and an excess of acetic anhydride in an acidic medium guides to obtain a starting material of a 4-(1-oxoacetyl-2-tert-butylaminoethyl)-6-methanolyl phenol 2 (acetylated salbutamol 2), (81% yield, in a molar ratio of 1: 8.9). salbutamol 1 has three different types of the hydroxyl groups and it was necessary to prove which one was acetylated. The acetylated salbutamol 2 was tested against (Lucas reagent: anhy. $ZnCl_2$ / conc.HCl) and 1% solution of ($FeCl_3$) to confirm the presenance or absence of primary – hydroxyl alcohol and phenolic – hydroxyl group respectively. The organic tests were showed that secondary hydroxyl alcohol of salbutamol 1 was acetylated only to get an acetylated salbutamol 2. The calculations of total - number of the hydroxyl groups were a decisive tool to confirm an endeavor of the presenance of two hydroxyl groups only in the molecular skeleton of an acetylated salbutamol 2 [7]. The results confirmed that the total-number of hydroxyl-practical value was (2.248) and so close to the total-number of a hydroxyl-calculated value which was (2.000). The process was repeated twice to have the same results. The FTIR spectra data were assisted and showed a band belongs to the stretching vibration of a phenolic-hydroxyl group in the region 3630 cm^{-1} (table 2). It was a significant that the aromatic compounds which undergo coupling are also the ones which under nitrosation, like nitrosonium ion (^{+}NO), but a diazonium ion (ArN_2^{+}) was very weakly electrophilic and capable to attack only the very reactive rings.

All the new compounds (4 - 8) were prepared in the same molar ratio and a procedure of a synthesis of 2-(2′,4′-dichloroazophenyl)-4-(1-oxoacetyl-2′′-tert-butylaminoethyl)-6-methanolyl phenol 3 (Scheme (1), Table (1)).

FTIR spectra:

The FTIR spectra data of the new compounds showed the bands of the stretching vibrations due to (phenolic – OH, secondary amine N - H, primary alcohol – OH, ketonic – C = O and N = N) groups in the regions (3630 – 3650 cm^{-1}), (3471 – 3591 cm^{-1}), (3190 – 3255 $cm^{-1)}$, (1715 – 1800 cm^{-1}) and (1562 – 1597 $cm^{-1)}$ respectively. A broad band was observed around (3190 - 3650 $cm^{-1)}$ due to an intermolecular hydrogen bonding of phenolic – OH and the primary alcohol - OH. The diagnostic bands were assigned and the bands positions are given in (Table (2)).

Table (2)
The FTIR spectral data (wave number in cm^{-1}) of the stretching vibration of the functional groups for the new salbutamol's derivatives.

Comp. No.	Functional groups					Other groups
	O – H Phenolic	N – H Sec. amine	O – H Primary alcohol	C = O Ketone	N = N Azo	
2	3630	3480	3252	1715	1562	C – $H_{aliph.}$, 2985; C – $H_{arom.}$, 3100
3	3650	3471	3217	1728	1577	C – $H_{aliph.}$, 2978; C – $H_{arom.}$, 3066; C – Cl, 813, 860
4	3645	3591	3205	1735	1597	C – $H_{aliph.}$, 2975; C – $H_{arom.}$, 3008; NH_2, 3522, 3444
5	3640	3525	3190	1739	1585	C – $H_{aliph.}$, 2931; C – $H_{arom.}$, 3060
6	3641	3475	3255	1775	1566	C – $H_{aliph.}$, 2874; C – $H_{arom.}$, 3050; S – H, 2360; C = N, 1624
7	3641	3591	3201	1775	1581	C – $H_{aliph.}$, 2893; C – $H_{arom.}$, 3050
8	3645	3591	3217	1800	1562	C – $H_{aliph.}$, 2895; C – $H_{arom.}$, 3060; NH_2, 3414, 3329

UV – Visible spectra:

Electronic absorption spectra of the new compounds (2 – 8) were measured in ethanol and characterized by the mainly bands (Table (1)). The first band at maximum wave length = 212 – 237 nm can be assigned to a medium energy of II – II* transition of aromatic ring, while a second band at maximum wave length = 301 – 332 nm is due to the low energy of II – II* transition. The bands in the range of maximum wave length = 415 – 739 nm of amine and hydroxyl group are assigned to an intermolecular charge transfer absorption involving the whole molecule, (Table (1)).

Microbial study:

Microbial study of the new compounds (2 – 8) were evaluated against representative G^{+}Ve, G^{-}Ve bacteria and fungicide according to agar plate method [9]. All the solutions were prepared freshly by dissolving in DMSO solvent to obtain a final concentration (0.5 mg / ml). The microbial results are given in (table 3). The diameter of inhibition zone in (mm unit) including a disc diameter was measured for each treatment. The compounds (3, 5 & 6) showed a moderate antimicrobial activity against G^{+}Ve bacteria (Staphylococcus aureus and Bacillus subtilis). All the new compounds (2 – 8) have not any antimicrobial activity against G^{-}Ve bacteria (Escherichia Coli, Klebsiella spp. Salmonella spp. and Pseudomonas spp.). All the new compounds (2–8) showed a moderate microbial activity against fungicide (Aspiringinussb) except compound 2.

Table (3)
Microbial study of the new salbutamol's derivatives.

Comp. No.	***Activity against G^{+} Ve bacteria ****	***Activity against G^{-} Ve bacteria *****	***Activity against Fungicide ******
	Staph.aureus, Bacillus subtillis	***Escher.Coli, Kleb.spp., Salm.spp., Pseu.spp.***	***Aspiringinussb***
2.	Nil, Nil	All Nil	Nil
3.	23, 19	All Nil	15
4.	Nil, Nil	All Nil	10
5.	19, 17	All Nil	13
6.	18, 16	All Nil	12
7.	Nil, Nil	All Nil	11
8.	Nil, Nil	All Nil	10

Note: A diameter around 6mm disc impregnated with the all prepared compounds.

* G^{+}Ve bacteria = Staphylococcus aureus and Bacillus subtilis respectively.

** G^{-}Ve bacteria = Escherichia Coli, Klebsiella spp., Salmonella spp., Pseudomonas spp.

*** Fungicide = Aspiringinussb.

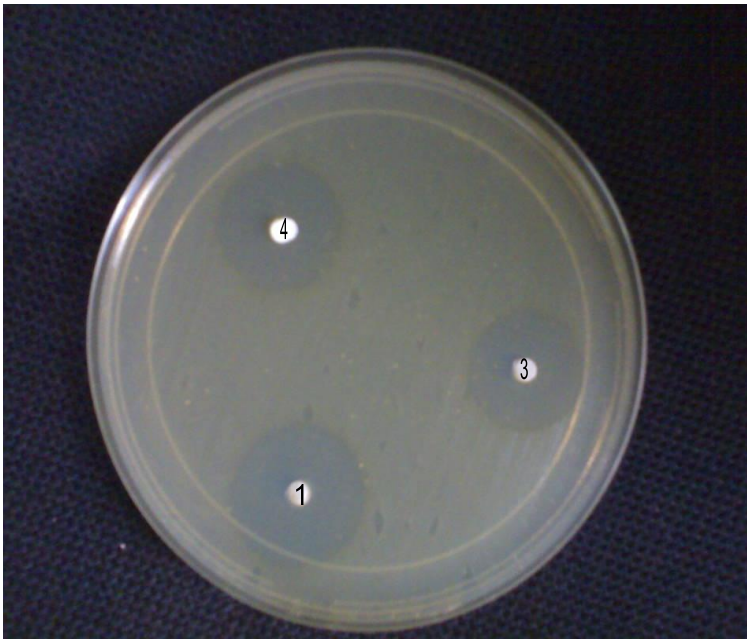

Fig.(1) Microbial activity of the new compounds: Numbers 1, 3 and 4 in (figure 1) mean the compounds 3, 5 and 6 respectively against G $^{+}$Ve bacteria (Staphylococcus aureus) only.

Conclusions

A present investigation furnished the good routes for a synthesis of one new acetylated salbutamol 2 and six new azo – acetylated salbutamol derivatives (3 - 8) in the selective yields by a coupling of the diazonium salts of an aromatic or a cyclic amine with an acetylated salbutamol 2 [one scheme, three tables and one figure]. This coupling is an electrophilic aromatic substitution in which a diazonium ion (ArN_2^+) is an attacking reagent. The organic tests, a FTIR, a UV-Visble spectra and the calculations of the determination's total number of the hydroxyl groups were done successfully to determine the structures of these new compounds (2 - 8). We found a microbial activity in a vitro against Gram $^{+}$Ve bacteria and a fungicide for the some new compounds (3, 5 and 6) only.

References

[1] Saadiyah A., Sanaa A., and Wasan A., "Synthesis, characterization and antimicrobial Activity study Via some new Schiff – bases for trimethoprim", J. Bagh. Scie., 2010, 7 (1), 614.

[2]Kurt A. and John S., "Facile preparation of N – methyl secondary amines by titanium (IV) isopropoxide – mediated reductive amination of carbonyl compounds", J. Chem. Soc. Perkin Trans., 1998, 1, 2527.

[3]Dutch SPC Ventolin ® (version date 2007), http://www.cbg–meb.n1/IB-teksten/h 12541 pdf.

[4]Haubrich H. and Galinke H., "Coupling activity of heterocyclic diazo compounds", J. Chem.Ber., 1960, 93, 397.

[5]Al – Badri H., M.Sc thesis, "Study and synthesis of new 2–amino–5–thiodiazole Derivatives", College of Science, University of Baghdad - Iraq, 2007.

[6]Sandier S. and Karo W., "Textbook, the organic functional group preparations", Academic press, New York, 2nd edn., 1983, 12 – I, 378 .

[7]Saadi M., Al – Rawi A. and Salma A., "Preparation and identification of oxidation Derivatives for salts and acids of bile for medical uses", J. Umm. Salamma, 2004, 1, 1.

[8]Daoud S., M.Sc. thesis, "Study and synthesis of some new thiadiazole", College of Science, University of Baghdad - Iraq, 2005.

[9]Agar, J.F. and Goldstien, F.W., "Disc susceptibility test in antibiotic in laboratory Medicine", Williams and Wilkins, 4th edn., 1996, 1.

الخلاصة

إنّ أهداف هذا البحث هوتحضير وتشخيص وتعيين الفعالية البايولوجية لبعض مشتقات السلبوتمول الدوائية الجديدة. لقد تمّ إيجاد طريقة كفوءة وبسيطة لتحضير المركب [4 – (1 – أوكسوأستيل – 2 – رابع بيوتيل أمين الاثيل) – 6 – ميثانوليل فينول 2] أو (أستيل السلبوتمول 2) من تفاعل حامض الخليك اللامائي في الوسط الحامضي مع المركب [4 – (1 – هيدروكسي – 2 – رابع بيوتيل أمين الاثيل) – 6 – ميثانوليل فينول 1] أو (السلبوتمول 1). إنّ تفاعل الازدواج بين مركبات أملاح الدايازونيوم لمشتقات الانيلين مع المركب (أستيل السلبوتمول 2) أعطى مركبات الازو الجديدة المقابلة وبمنتوج جيد. لقد تمّ تشخيص هذه المركبات الجديدة بوساطة مختلف التقنيات الفيزيائية مثل: طيف الاشعة تحت الحمراء، وطيف الاشعة المرئية وفوق البنفسجية، وحسابات تعيين عدد مجاميع الهيدروكسيل في المركب (أستيل السلبوتمول 2)، وتحديد درجة الانصهار، إضافة الى تعيين الفعالية البايولوجية ضد البكتيريا الموجبة والبكتيريا السالبة والفطريات، وأظهرتْ الدراسة وجود فعالية بايولوجية للمركبات الجديدة الآتية فقط:

2 – (2′،4′ – ثنائي كلورو آزوفنيل) – 4 – (1 – أوكسواستيل – 2′′ – رابع بيوتيل أمين الاثيل) – 6 – ميثانوليل فينول 3.

2 – (2′، 3 – ثنائي مثيل آزو فنيل) – 4 – (1 – أوكسواستيل – 2′′ – رابع بيوتيل أمين الاثيل) – 6 – ميثانوليل فينول 5.

2 – (5 – مركبتو – 1′، 3، 4′ – ثاياديازول – 2′ – أزويل) – 4 – (1 – أوكسواستيل – 2′′ – رابع بيوتيل أمين الاثيل) – 6 – ميثانوليل فينول 6.