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Reaction and Antibacterial Efficacy of Active Methylene Compounds with Coumarin Derivatives

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New coumarin derivatives namely (5-oxo-chromeno[3,2-c] dihydropyran derivatives 2a,b, 3a,b),(3 -acetyl-4-aryl -2-methyl-5-oxo-chromeno[3,2-c]2,3,4-trihydropyran 4a,b), (2-amino-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile 5a,b and 2-amino-5-oxo-3,4,4a,10b-tetrahydro-2H,5H-pyrano[3,2-c]chromene-3-carboxylic acid amide 6a,b) were synthesized starting from 3-arylidene chromen-2,4-dione derivatives. The structures of the obtained compounds were confirmed by analytical elemental analysis, IR, 1H-NMR and mass spectra. The new compounds were screened for their antibacterial activity .Compound 3a was the most active compound against gram positive and gram negative bacteria.

Key words: Active methylene, Coumarin derivatives, Biological activity.

Coumarin and coumarins derivatives are widely used in pharmaceutical and commercial applications [1-3]. Coumarins have a number of biological activities as anticoagulant, antimicrobial, anti-inflammatory, antioxidant, anticancer, antiviral, antimalarial [4-7]. Derivatives of 3-arylidene chromen-2, 4-dione 1a,b were synthesized following reported procedures [8] Many attempts were made to utilize compounds 1a,b as a starting materials for synthesizing some new fused heterocycles containing coumarin moiety.

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EXPERIMENTAL

Melting points were determined by an electro thermal melting point apparatus and are uncorrected .The reaction times were determined using the thin-layer chromatography (TLC) technique which was performed with fluorescent silica gel plates HF_{245} (Merck) and plates were viewed under UV 245 and 265 light . Silica gel (230-400 mesh) was used for flash chromatography separations. Elemental analysis were carried out by Micro analytical Unit, (Faculty of Science ,Cairo University) , IR (KBr) spectra were recorded on a Pye-Unicam infrared spectrophotometer SP 2000 (Faculty of Science , Fayoum University), The mass spectra were run by a Shimadzu-GC-MS-GP 1000 EX using the direct inlet system and Nuclear

magnetic resonance spectra were recorded on Varian Mercury 300MHz spectrometer using TMS as internal standard ;chemical shifts are recorded in δ units (National Center Researcher).

Synthesis of 5-oxo-chromeno [3,2-c] dihydropyran derivatives 2a,b and 3a,b

A mixture of **1a** (2.94g, 0.01mol.) and ethyl acetoacetate and/or ethyl cyanoacetate (0.01mole) was heated under reflux for 3hrs in solution of sodium methoxide. The reaction mixture was cooled, poured into ice and hydrochloric acid, filtered off and crystallized from propel solvent.

2a

Crystallized from dioxane as yellow crystals in 67% yield, m.p.209-211 ºC. Analysis for $C_{21}H_{14}O_7$ (M.wt. 378.33). Calculated % C 66.67, H 3.73 , Found % : C 66.32 , H 3.60 **,** IR (cm^{-1}) : 3087 due to ν_{CH} aromatic, 2989, 2891 due to ν_{CH} aliphatic, broad at 1732 , 1701 due to $\nu_{CO}$of δ-lactone and ketone .^{1}H-NMR (δ,ppm,DMSO-d_6): 2.46 ppm (s , 3H, CH_3)**,** 3.46-4.20 ppm (d,2H,2CH-pyran ring) **,** 5.87 ppm (s,2H,O-CH_2O), 6.51-7.80 ppm (m,7H, 2Ar-H).

2b

Crystallized from DMF as black crystals in 56% yield ,m.p.>360. Analysis for $C_{18}H_{12}O_6$ (M.wt. 324.06). Calculated % C 66.67 , H 3.73 , Found % C 66.44, H 3.85, IR (cm^{-1}) : 3099 due to ν_{CH} aromatic , 2940 , 2843 due to ν_{CH} aliphatic , broad at 1725, 1708 due to $\nu_{CO}$of δ-lactone and ketone .

3a

Crystallized from dioxane as yellow crystals in 54 % yield , m.p.232 ºC.Analysis for $C_{20}H_{12}O_8$ (M.wt. 380.05).Calculated % C 63.16 , H 3.18 , Found %: C 63.00, H 3.21 , IR (cm^{-1}) : 3459 due to ν_{OH}in carboxylic acid , 3080 , 3021 due to ν_{CH} aromatic , 2888 due to ν_{CH} aliphatic , broad band at 1742 and 1660 due to ν_{CO} of carboxylic acid, ketone and δ-lactone .

3b

Crystallized from DMF as black crystals in 61% yield, m.p.>300ºC. Analysis for $C_{17}H_{10}O_7$ (M.wt.326.26) ,Calculated % :C 62.58 , H 3.09, Found % C: 62.80 , H 2.98 , IR (cm^{-1}): 3455 due to ν_{OH}in carboxylic acid , 3048 due to ν_{CH} aromatic , 2956 due to ν_{CH} aliphatic , broad band at 1731 and 1710 due to $\nu_{CO}$of carboxylic acid , ketone and δ-lactone

Synthesis of 3-acetyl-4-aryl -2-methyl-5-oxo-chromeno [3,2-c]2,3,4-trihydropyran 4a,b

A mixture of 1a (2.94g, 0.01mol.) and acetyl acetone (0.01mole) was refluxed in boiling pyridine for 12hrs. The reaction mixture was cooled, poured into ice and hydrochloric acid, filtered and crystallized from propel solvent.

4a

Crystallized from methanol as brown crystals, in 88% yield ,m.p.>360 ºC .Analysis for $C_{22}H_{18}O_6$ (M.wt.378.13). Calculated % :C : 69.46 , H : 5.30, Found % : C 69.22 , H 5.18, IR(cm^{-1}) : 3001 due to ν_{CH} aromatic , 2973 , 2891 due to ν_{CH} aliphatic, broad band at 1722 due to ν_{CO} of δ-lactone and acetyl group MS(m/z%) : 378(100%)

4b

Crystallized from DMF as black crystals in 66% yield, m.p.>360 ºC. Analysis for $C_{19}H_{16}O_5$ (M.wt.324.12).Calculated % : C 69.93 , H 5.56, Found %: C 70.22 , H 5.69, IR (cm^{-1}) : 3046 due to ν_{CH} aromatic, 2865 , 2841 due to ν_{CH} aliphatic and band at 1735due to ν_{CO} of δ- lactone and acetyl group . ^{1}H-NMR (δ , ppm , DMSO-$d_6$1.73 ppm (s,3H,CH_3) , 2.25 (s,3H,$COCH_3$) , 3.53 (s,1H,CH-Ar), 5.88 (s,2H,O-CH_2-O), 6.50-7.72(m,7H,2Ar-H) .

Synthesis of 2-amino-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile 5a,b and 2-amino-5-oxo-3,4,4a,10b-tetrahydro-2H,5H-pyrano[3,2-c]chromene-3-carboxylic acid amide 6a,b

A mixture of **1a** (2.94g, 0.01mol.) and malonitrile (0.66g, 0.01mol.) was refluxed in boiling pyridine for 12hrs. The reaction mixture was cooled, poured into ice and hydrochloric acid, filtered and crystallized from propel solvent.

5a , 6a

Crystallized from methanol as orange crystals, m.p.250-253 ºC .IR (cm^{-1}) : 3084 due to ν_{CH} aromatic, 2913 due to ν_{CH} aliphatic, at 2197 cm^{-1} due to ν_{CN} , at 1721 and 1685 due to ν_{CO} of δ-lactone and amide. MS (m/e %) of 5a : M^+ 360(0.89%), MS (m/e %) of 6a : M^+ 382(0.93%)

5b , 6b

Crystallized from dioxane as black crystals, in 56 % yield , m.p. > 360 ºC .IR(cm^{-1}) : 3099 due to ν_{CH} aromatic, 2857 due to ν_{CH} aliphatic, at 2214 due to ν_{CN}, at 1725 and 1665 due to ν_{CO} of δ-lactone and amide .

Antibacterial Assay

New coumarin derivatives were tested for their antibacterial activity against 4 kinds of bacteria: two Gram-positive bacteria (*Bacillus subtilis* and *Streptococci*) and two Gram-negative bacteria (*Klebsiella pneumoniae* and *E. coli*).

Compounds **3a,b** showed excellent activity against all kinds of tested bacteria except *E.coli* , while compound **4a** exhibited moderate activity against *Bacillus subtilis* and good activity against *Klebsiella pneumoniae*. All other compounds showed no activity against Gram +ve and Gram – ve bacteria (Fig. 1).

Culture media used

Muller-Hinton agar medium	g/l
Beef extract powder	3.0
Casein hydrolase	17.5
Starch	1.5
Agar	17.0

Preparation of agar

Muller-Hinton agar (38 g) was suspended in one liter of distilled water, heated to dissolve the medium completely then sterilized by autoclaving at 121 ºC for 15 min.

Test Organisms

The Gram positive bacteria: *Bacillus subtilis* and *Streptococci.*

The Gram negative bacteria: *Klebsiella pneumoniae* and *Escherichia coli.*

Antibacterial test

The antibacterial activities of tested compounds were evaluated by the disc diffusion method[9,10] using sterile Whatman-No5 filter paper discs (11 mm diameter). Tested compounds were dissolved in N,N-dimethylformamide (DMF) . Filter paper discs (11 mm) were loaded with certain amount of the tested material (50µL) then left with care under hot air to complete dryness. Test plates were prepared by pouring 10 ml Muller-Hinton agar medium seeded with the test organism .The discs were deposited on the surface of agar plates along with control disc, which loaded only with used solvent. The discs were incubated at 5 ºC for 1 hr, to permit good diffusion. All the plates were then incubated for 24 hr at 37 ºC the zones of inhibition were measured .

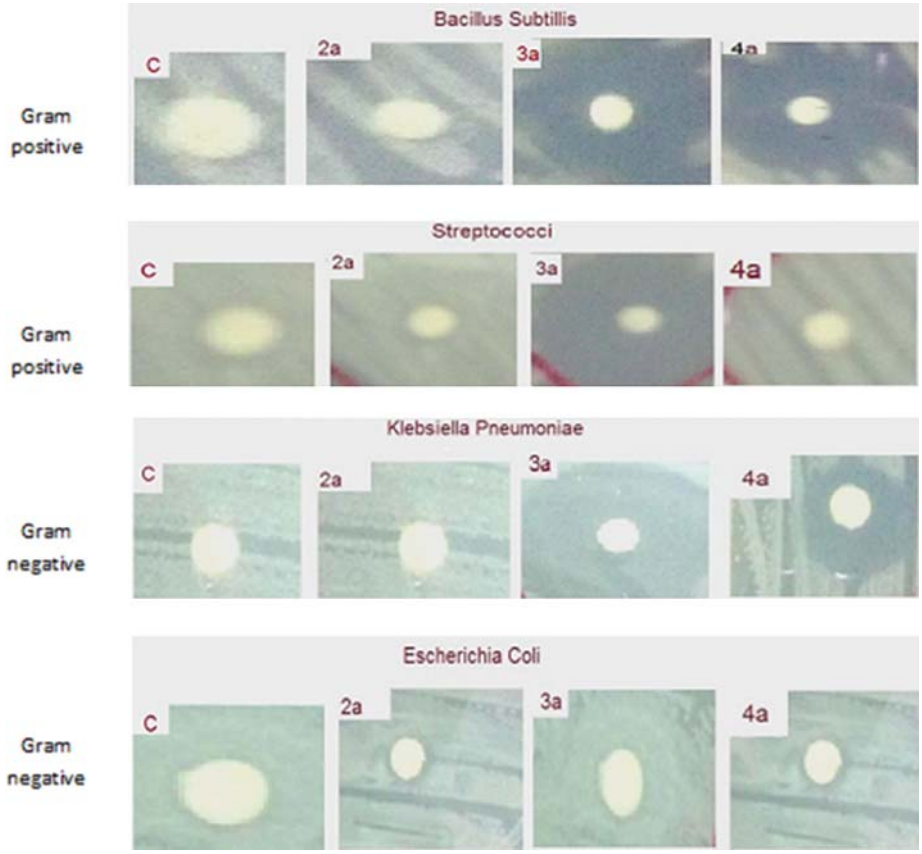
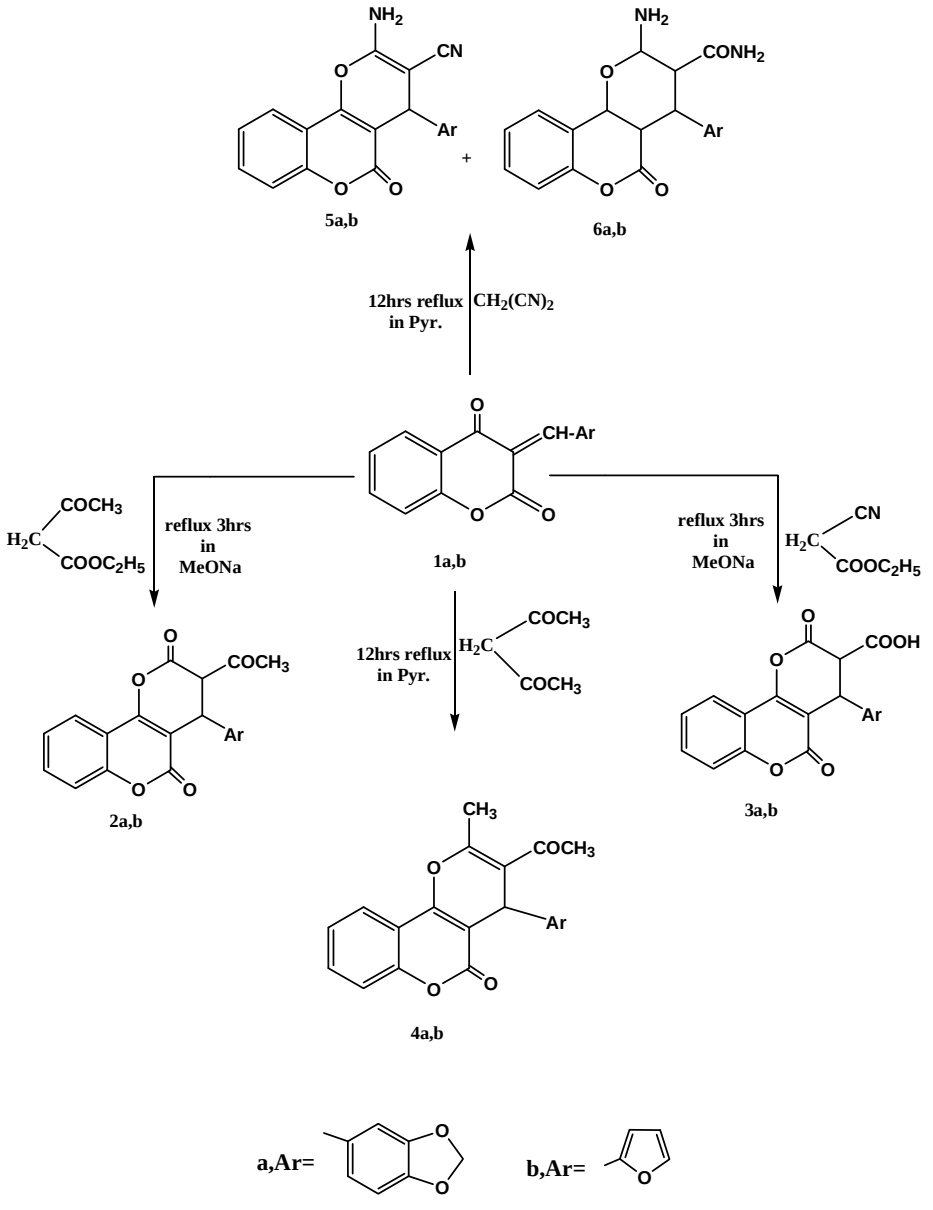


Fig. 1. Antibacterial activity of compounds 2a,b ,3a,b, and 4a

RESULTS AND DISCUSSION

In continuation of our research program[11-14], we synthesized and studied the coumarin nucleus that fused with other heterocyclic ring. We have synthesized 12 novel compounds of coumarin derivatives by using active methylene compounds as ethyl acetoacetate, ethyl cyanoacetate, acetylacetone and malonitrileave, 5-oxo-chromeno[3,2-c] dihydropyran derivatives (2a,b), (3a,b) were synthesized by refluxing 1a,b with ethyl acetoacetate/ethyl cyanoacetate respectively for 3h in solution of sodium methoxide. While compounds (4a,b), (5a,b and 6a,b) were prepared by reacted compounds 1a,b with acetyl acetone / malonitrile in boiling pyridine for 12h as shown in Scheme 1.



Scheme 1

The structures of these compounds 2a,b-6a,b were confirmed from elemental analysis and spectral data. The IR spectrum showed absorption bands at 1742-1665 cm^{-1} due to ν_{CO} of δ-lactone, ketones and amide. Compound 2a showed signal at δ 2.46 ppm (s,3H, CH_3), IR (cm^{-1}) of 3a showed bands at : 3459 due to ν_{OH} in carboxylic acid. The mass spectrum of compound 4a showed ion peak at m/e = 378(100%) corresponding to the molecular formula $C_{22}H_{16}O_6$. The ^{1}H-NMR (DMSO-d_6) spectrum of compound 4b showed signal at δ 1.73 ppm (s,3H,CH_3) , 2.25 (s,3H,$COCH_3$).Compounds 5a,b showed bands at 2197 and 2214 cm^{-1} respectively due to ν_{CN}. The mass spectrum of compounds 5a, 6a showed ion peak at m/e = 360 (6.23%), 382(0.93%) corresponding to the molecular formula $C_{20}H_{12}N_2O_5$, $C_{20}H_{16}N_2O_6$.

CONCLUSION

A novel coumarin derivatives were synthesized by reaction of 1a,b with active methylene compounds using appropriate synthetic method. All new compounds were characterized and screened for their in vitro antibacterial activity against four types of bacteria. 5-oxo-chromeno [3, 2-c] dihydropyran derivatives demonstrated the best activity against 3 types of bacteria.

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