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A Convenient One-Step Synthesis and 3D QSAR Pharmacophore modeling of 2-Iminochromene and Chromenopyridine Derivatives for Antimicrobial Evaluation

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ABSTRACT

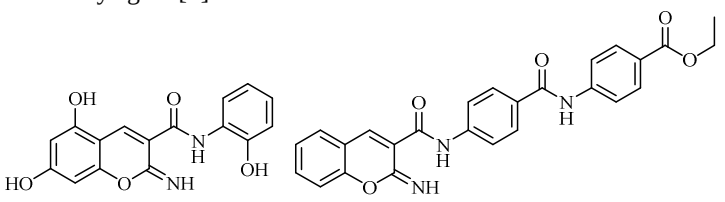
*Cynoactanilides **1a**,**b** were reacted with o-hydroxyaldehydes under different conditions. Thus, cyclocondensation of **1a**,**b** with salicylaldehyde, 2-hydroxynaphthal-dehyde and 7-hydroxy-5-methoxy-coumarin-6-carboxaldehyde in ethanolic ammonium acetate afforded the corresponding 2-iminochromene derivatives **3**,**7** and **9**, respectively. On the other hand, repeating the same reaction in presence of Ac2O/AcONa, the corresponding chromen-2-one derivatives **4**, **8** and **10** were obtained. Compounds **4**,**8** and **10** were also synthesized through the hydrolysis of 2-imino derivatives using EtOH/HCl. A number of chromeno[3,4-c]pyridine derivatives **11**,**13**,**14** and **16** were prepared from the reaction of 2-iminochromenes with activated nitriles. Most of the synthesized compounds were screened in vitro for their antibacterial and antifungal activities,by measuring the minimal inhibitory concentration values (MICs), against strains ofS. aureus, S. epidermidis , B. subtilis, P. vulgaris, K. pneumonia, S.flexneri, A. fumigates, A.clavatus and Candida albicans using two fold serial dilution method method. Ampicillin, gentamycin and amphotricin B were used as reference standards for antibacterial and antifungal activities respectively. Some of the synthesized compounds have been found to exhibit considerable antibacterial and antifungal activities, reaching, in certain cases, the same level of antimicrobial activity as the standard antibacterial agent Ampicilline and antifungal agent Flucanazole, especially compound 3b that showed promising broad spectrum antibacterial and antifungal properties against all the tested strains. Generation of 3D pharmacophore model was combined to explore the structural requirements controlling the observed antibacterial properties estimated by the effect of the synthesized compounds.*

Keywords: Chromenone, Benzochromene, Chromenocoumarin, Chromeno-pyridine Micheal addition, Antimicrobial and 3D Pharmacophore.

INTRODUCTION

Chromene derivatives have attracted considerable interest due to their diverse biological properties and therapeutical applications, namely as anti-HIV, anti-tuberculosis, anti-inflammatory and anti-fungal agents [1,2]. Naturally occurring chromenes were found in plants [3] and have been used as valuable leads for the design and synthesis of

new pharmacophores for medicinal chemistry and drug development. 2-Imino-2H-chromene-3-carboxamide **I** was reported as an efficient inhibitor of tyrosine kinases of potentially use as oncolytic agent [4]. Compound **II** was described as an anti-inflammatory agent [5].



I **II**

Chromenopyrimidine derivative possesses potent activity against tumer cell lines[6]. Moreover, coumarin derivatives are well known to act as anticoagulant and antibacterial agents [7-10].A recent approach started with the reaction of substituted salicylaldehydes with diethylmalonate to generate the ethyl 2-oxo2*H*-chromene-3-carboxylate. Hydrolysis of the ester function and activation of the carboxylic acid as an acyl chloride, allowed the preparation of the amide substitution upon reaction with primary amines[11]. Encourage by the above finding and due to wide applications of Chromene derivatives, that make these interesting compounds attractive for further backbone derivatization and screening as novel therapeutic agents, it was planned to synthesize new series of chromene derivatives by usingone-step synthesis to be screened in vitro for their antibacterial and antifungal activities.

MATERIALS AND METHODS

All chemicals and solvents were commercially available and used without purification. Melting points were determined by open glass capillary method on a Cintex melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer spectrometer in KBr pellets.^{1}H NMR spectra were obtained on a Varian Gemini 300 MHz spectrometer using TMS as internal standard; chemical shifts are reported as (ppm). Mass spectra were obtained on GCMS\QP 1000 Ex mass spectrometer at 70 ev. Elemental analyses were performed on a LECO-932 analyzer at the Department of Chemistry, Faculty of Science, Cairo University, Egypt. Microbiology screening was carried out in the Regional Center for Microbiology and Biotechnology (RCMB), Antimicrobial unit test organisms, Al-azhr University, Cairo, Egypt.

Preparation of: 3a,b, 7a,b, 9b

A mixture of equimolar amounts of **1a-c** (0.01 mol), salicyaldehyde (0.01 mol), or 2-hydroxy naphthaldehyde (0.01 mol) or 7-hydroxy-5-methoxy-coumarin-6-carboxaldehyde (0.01 mol) and amm. acetate (0.015 mol) was refluxed in ethanol (30 mL) for 3 h. The solid product formed was collected by filtration and recrystallized from a suitable solvent to give **3**, **7** and **9**.

2-Imino-N-(p-tollyl)-2H-chromene-3-carboxamide (3a)

Yield 80% (Dioxan); mp 160-161ºC; IR cm^{-1} (KBr) 3403, 3316 (2NH), 2923 (CH-aliph), 1681 (C=O);^{1}HNMR (300 MHz, DMSO-d_6) δ = 2.2 (s, 3H, CH_3), 6.2-7.8 (m, 4H, Ar-H), 8.5 (s, 1H, H-4), 9.1, 12.6(2s, 2H, 2NH; cancelled with D_2O);MS: m/z 280 (22%), M+1(3.2%), 278 (100%); Anal. Calcd for$C_{17}H_{14}N_2O_2$. Calcd: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.51; H, 5.10; N, 10.05.

N-(p-Chlorophenyl)-2-imino-2H-Chromene-3-carboxamide (3b)

Yield 82% (Dioxan); mp 245-7ºC;IR cm^{-1} (KBr) 3336, 3274 (2NH), 2976 (CH-aliph), 1683 (C=O^{1}HNMR (300 MHz, DMSO-d_6) δ = 7.2-7.8 (m, 4H, Ar-H), 8.5 (s, 1H, chromene-H4), 9.1, 12.9(2s, 2H, 2NH; cancelled with D_2O);MS: m/z 298 (3%), M+2 (5.3%), 263 (M-35; 100); Anal. Calcd for $C_{16}H_{11}ClN_2O_2$. Calcd: C, 64.33; H, 3.71; N, 9.38. Found: C, 64.40; H, 3.65; N, 9.50.

3-Imino-N-(4-tolyl)-3H-benzo[f]chromene-3-carboxamide(7a)

Yield 60% (Dioxan); mp 235-7ºC;IR cm^{-1} (KBr) 3324, 3298 (2NH), 2925 (CH-aliph), 1684 (C=O);^{1}HNMR (300 MHz, DMSO-d_6) δ = 2.3 (s, 3H, CH_3),7.1-8.2 (m, 6H, Ar-H), 8.4 (s, 1H, chromene-4H), 9.2, 12.4 (2s, 2H, 2NH; cancelled with D_2O); MS: m/z 328 (15 %), 195 (100%); Anal. Calcd for $C_{21}H_{16}N_2O_2$. Calcd: C, 76.81; H, 4.91; N, 8.53. Found: C, 76.75; H, 5.05; N, 8.40.

N-(4-chlorophenyl)-3-imino-3H-benzo[f]chromene-2-carboxamide (7b)
Yield 65% (Dioxan); mp 217-8ºC; IR cm^{-1} (KBr) 3242 (NH), 2924 (CH-aliph), 1671 (C=O);^{1}HNMR (300 MHz, DMSO-d_6) δ = 7.4-8.4 (m, 6H, Ar-H), 8.5 (s, 1H, chromene-4H), 9.1, 12.9(2s, 2H, 2NH; cancelled with D_2O); MS: m/z 348.5 (4%), 313 [(M-35;-Cl); 100 %]; Anal. Calcd for $C_{20}H_{13}ClN_2O_2$. Calcd: C, 68.87; H, 3.76; N, 8.03. Found: C, 68.90; H, 3.70; N, 7.95.

N-(p-chlorophenyl)-2-imino-5-methoxy-8-oxo-2,8-dihydropyrano[3,2-g]-chromenecarboxamide (9):
Yield 60% (DMF); mp 310-1ºC;IR cm^{-1} (KBr) 3325 (NH), 2970 (CH-aliph), 1735, 1689 (2C=O);^{1}HNMR (300 MHz, DMSO-d_6) δ = 4.2 (s, 3H, OCH_3), 6.2-(d, 1H, H-6), 7.05-7.52 (AB-system, 4H, ArH), 8.1(d, 1H, H-7), 8.5(s, 1H,H-4), 9.5, 12.8(2s, 2H, 2NH; cancelled with D_2O); MS: m/z 396.5 [M^+ (27.15%), M^{+1} (10.53%)], 286 (100%); Anal. Calcd for $C_{20}H_{13}ClN_2O_5$. Calcd: C, 60.53; H, 3.28; N, 7.06. Found: C, 60.45; H, 3.35; N, 7.00.

Preparation of: 4a,b, 8a,b and 10
Method A: To a solution of **1** (0.01mol) in acetic anhydride (20mL), o-hydroxyaldehydes (0.01 mol) and sod.acetate (0.5g) were added. The mixture was refluxed for 1hr, the solid product after cooling was collected by filtration and recrystallized from the proper solvent to give **4**, **8** and **10**.

Method B: A solution of the corresponding iminochromene derivatives **3**,**7** and **9** (0.01 mol) in ethanol (30 mL), hydrochloric acid or acetic acid (5mL) was refluxed for 1h. the solid product after cooling was collected by filtration and recrystallized from the proper solvent.

2-Oxo-N-(4-tolyl)-2H-chromene-3-carboxamide (4a)
Yield 73% (DMF); mp 235-6ºC;IR cm^{-1} (KBr) 3271 (NH), 2925 (CH-aliph), 1705, 1661 (2C=O); ^{1}HNMR (300 MHz, DMSO-d_6) δ = 6.2-7.8 (m, 4H, Ar-H), 8.3 (s, 1H, chromenone-H4), 9.1 (s, H, 2NH; cancelled with D_2O); MS: m/z 279 (14%), 174 (100%); Anal. Calcd for $C_{17}H_{13}NO_3$. Calcd: C, 73.11; H, 4.69; N, 5.02. Found: C, 73.15; H, 4.60; N, 5.05.

N-(4-Chlorophenyl)-2-Oxo-2H-chromene-3-carboxamide (4b)
Yield 65% (DMF); mp 230-1ºC; IR cm^{-1} (KBr) 3264 (NH), 2925 (CH-aliph), 1700, 1663 (2C=O);^{1}HNMR (300 MHz, DMSO-d_6) δ = 7.4-8.0 (m, 4H, Ar-H), 8.8 (s, 1H, chromenone-H), 10.6, (s, 1H, NH; cancelled with D_2O), MS: m/z 299 (25%), 173 [M-ClC_6H_4-N; 100%]; Anal. Calcd for $C_{16}H_{10}ClNO_3$. Calcd: C, 64.12; H, 3.36; N, 4.67. Found: C, 64.20; H, 3.25; N, 4.50.

3-Oxo-N-(p-tolyl)-3H-benzo[f]chromene-2-carboxamide (8a)
Yield 55% (Methanol); mp 260-1ºC;IR cm^{-1} (KBr) 3208 (NH), 2923 (CH-aliph), 1712, 1654 (2C=O); ^{1}HNMR (300 MHz, DMSO-d_6) δ = 2.4 (s, 3H, CH_3), 7.1-8.2 (m, 6H, Ar-H), 8.2 (s, 1H, chromenone-H4), 9.2, (s, H, NH; cancelled with D_2O); MS: m/z329(25.5%), 315[M-CH_3;100%]; Anal. Calcd for $C_{21}H_{15}NO_3$. Calcd: C, 76.58; H, 4.59; N, 4.25. Found: C, 76.45; H, 4.59; N, 4.10.

N-(p-Chlorophenyl)-3-Oxo-3H-benzo[f]chromene-2-carboxamide (8b)
Yield 57% (Methanol); mp 230-2ºC;IR cm^{-1} (KBr) 3242 (NH), 2924 (CH-aliph), 1671 (C=O); ^{1}HNMR (300 MHz, DMSO-d_6) δ = 6.6-8.0 (m, 6H, Ar-H), 8.4 (s, 1H, chromenone-H4), 8.9 (s, H, NH; cancelled with D_2O); MS: m/z349.5 (17%), 220 (100%); Anal. Calcd for $C_{20}H_{12}ClNO_3$. Calcd: C, 68.68; H, 3.46; N, 4.00. Found: C, 68.70; H, 3.40; N, 4.00.

N-(4-chlorophenyl)-5-methoxy-2,8-dioxo-2H,8H-pyrano[3,2-g]chromene-3-carboxa-mide (10):
Yield 55% (Acetic); mp 246-7ºC;IR cm^{-1} (KBr) 3425 (NH), 2923 (CH-aliph), 1728, 1662 (2C=O);MS: m/z 396 (19.1%), 398 [M+2, 6.8 %], 361 (M-Cl, 100%); Anal. Calcd for $C_{20}H_{12}Cl\ NO_6$. Calcd: C, 60.38; H, 3.03, N, 3.52. Found: C, 60.30; H, 3.10, N, 3.62.

Preparation of: 12a,b, 13, 14 and 16
A mixture of 2-iminochromenes **(3b** or **7b** or **15)** (0.01 mol), malononitrile and / or ethyl cyanoacetate (0.01 mol) and amm.acetate (0.015) in ethanol (30 mL) was refluxed for 3h. The solid product was collected by filtration and recrystallized to furnish (**12a,b**, **13**,**14** and **16**).

2-Amino-5-imino-4-oxo-3-(p-tolyl)-3,5-dihydro-4H-chromeno[3,4-c]py-ridine-1-carbo-nitrile (12a)
Yield 40% (Acetic); mp 311-2ºC;IR cm^{-1} (KBr) 3439, 3348 (NH_2, NH), 22075 (C≡N), 1650 (C=O-amide);^{1}HNMR (300 MHz, DMSO-d_6) δ = 2.3(s, 3H, CH_3), 7.1-8.2 (m, 10H, Ar-H + NH_2; cancelled with D_2O), 9.3, (s, H, NH; cancelled with D_2O); MS: m/z 342 (3.2%), 300 (100%); Anal. Calcd for $C_{20}H_{14}N_4O_2$. Calcd: C, 70.17; H, 4.12; N, 16.37. Found: C, 70.10; H, 4.15; N, 16.0.

3-(p-Chlorophenyl)-2-hydroxy-5-imino-4-oxo-3,5-dihydro-4H-chromen-o[3,4-c]pyri-dine-1-carbonitrile (12b)
Yield 41% (Acetic); mp 302-4ºC;IR cm^{-1} (KBr) 3349, 3176 (OH, NH), 2196 (C≡N), 1715 (C=O);^{1}HNMR (300 MHz, DMSO-d_6) δ = 7.3-7.6 (m, 8H, Ar-H), 8.8 (s, 1H, NH; cancelled with D_2O), 9.2 (s, 1H, OH; cancelled with D_2O); Anal. Calcd for $C_{19}H_{10}ClN_3O_3$. Calcd: C, 62.74; H, 2.77; N, 11.55. Found: C, 62.80; H, 2.10; N, 11.45.

2-Amino-3-(p-chlorophenyl)-5-imino-4-oxo-3,4a,5,10b-tetrahydro-4H-chr-omeno[3,4-c]pyridine-1-carbonitrile (13)
Yield 51% (Acetic); mp 302-4ºC;IR cm^{-1} (KBr) 3444, 3348 (NH_2, NH), 2208 (C≡N), 1662 (C=O); δ^{1}HNMR (300 MHz, DMSO-d_6) δ = 4.71, 4.9 (2d, 2H, CH-3,4), 7.3-7.6 (m, 8H, Ar-H), 7.7 (s, 2H, NH_2; cancelled with D_2O), 8.9 (s, 1H, NH; cancelled with D_2O); MS: m/z 364.5 (20.1%), 366 [M+2, 5.4 %], 220 (100%); Anal. Calcd for$C_{19}H_{13}ClN_4O_2$**.** Calcd: C, 62.56; H, 3.59; N, 15.36. Found: C, 62.40; H, 3.60; N, 15.20.

2-Amino-3-(p-chlorophenyl)-5-imino-4-oxo-3,5-dihydro-4H-benzo[5,6]-chromeno[3,4-c]pyridine-1-carbonitrile (14):
Yield 51% (Dioxan); mp 302-4ºC;IR cm^{-1} (KBr) 3330, 3214 (NH_2, NH), 2216 (C≡N), 1715, 1682 (2C=O);^{1}HNMR (300 MHz, DMSO-d_6) δ = 7.6-8.6 (m, 10H, Ar-H), 8.9, 9.3 (2s, 3H, NH_2, NH; cancelled with D_2O);MS: m/z 412.5 (10.5%), 414 [M+2, 6 %], 315 (100%); Anal. Calcd for $C_{23}H_{13}ClN_4O_2$. Calcd. C, 66.92; H, 3.17; N, 13.57. Found: C, 66.80; H, 3.20; N, 13.40.

2-Amino-N-(4-chlorophenyl)-5-imino-4-oxo-3,5-dihydro-4H-chromeno-[3,4-c]pyridine-1-carboxamide (16):
Yield 53% (Dioxan); mp 305-6ºC;IR cm^{-1} (KBr) 3268, 3133 (NH_2, NH), 22075 (C≡N), 2960(CH-aliph), 1667 (C=O);^{1}HNMR (300 MHz, DMSO-d_6) δ = 7.4-7.8 (m, 8H, Ar-H), 9.0, 9.2, 10.3 (3s, 3H, 3NH; cancelled with D_2O), 10.5 (br, 2H, NH_2; cancelled with D_2O); MS: m/z 380 (9.8%), m/z=253 [(M-Cl.$C_6H_4NH_2$) 100; Anal. Calcd for $C_{19}H_{13}ClN_4O_3$. Calcd: C, 59.93; H, 3.44; N, 14.71. found: C, 59.85; H, 3.51; N, 14.61.

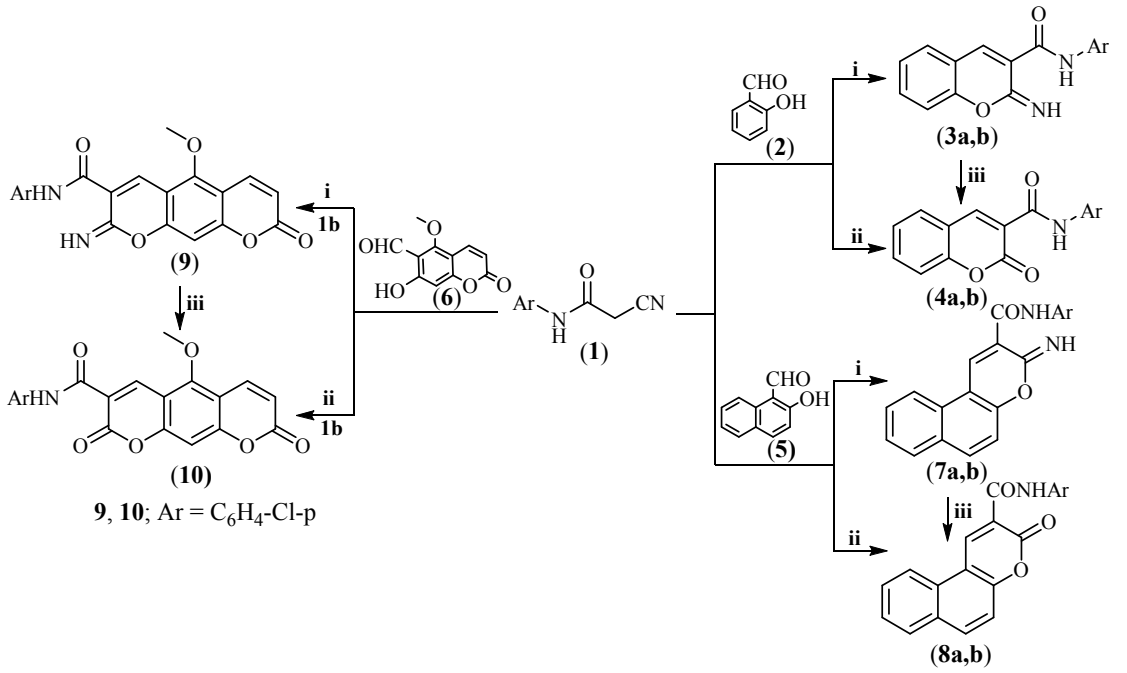
Antimicrobial screening
The disks of Whatman filter paper were prepared with standard size (6.0 mm diameter) and kept into 1.0 Oz screw capped wide mouthed containers for sterilization. These bottles are kept into hot air oven at a temperature of 150 ºC. Then, the standard sterilized filter paper disks impregnated with a solution of the test compound in DMF (100 µL, 5 mg/mL) were placed on nutrient agar plate seeded with the appropriate test organism in triplicates. Standard concentrations of 10^6 CFU/mL (Colony Forming Units/mL) and 10^4 CFU/mL were used for antibacterial and antifungal assay, respectively. Pyrex glass Petri dishes (9 cm in diameter) were used and two disks of filter paper were inoculated in each plate. The utilized test organisms were *S.aureus* (RCMB 010027), *S. epidermidis* (RCMB 010024) and *B. subtilis* (RCMB 010063) as examples of Gram-positive bacteria and *P. vulgaris* (RCMB010085), *K. pneumonia* (RCMB 010093) and *S. flexneri* (RCMB 0100542) as examples of Gram-negative bacteria. They were also evaluated for their in vitro antifungal potential against *A. fumigates* (RCMB 02564), *A. clavatus* (RCMB 02593) and *C.albicans* (RCMB 05035) fungal strain. Ampicillin and gentamycin were used as standard antibacterial agents; while amphotericin B was used as standard antifungal agent. DMF alone was used as control at the same above-mentioned concentration and due this there was no visible change in bacterial growth. The plates were incubated at 37 ºC for 24 h for bacteria and for 48 h at 25 ºC for fungi. The mean zone of inhibition measured in mm ± standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms. Compounds that showed significant growth inhibition zones using the twofold serial dilution technique were further evaluated for their minimal inhibitory concentrations (MICs).

Minimal inhibitory concentration (MIC) measurement
The microdilution susceptibility test in Müller-Hinton Broth (Oxoid) and Subouraud Liquid Medium (Oxoid) was used for the determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds, ampicillin, gentamycin, amphotericin B and sulfisoxazole were prepared in DMF at concentrations 1000 µg/mL. Each stock solution was diluted with standard method broth (Difco) to prepare serial twofold dilutions in the range of 500-0.007 µg/mL. 10 mL of the broth containing about 10^6 CFU/mL of test bacteria or 10^4 CFU/mL

of the test fungus was added to each well of 96-well microtiter plate. The sealed microplates were incubated at 37 ºC for 24 h for antibacterial activity and at 25 ºC for 48 h for antifungal activity in a humid chamber. At the end of the incubation period, the minimal inhibitory concentrations (MIC) values were recorded as the lowest concentrations of the substance that had no visible turbidity. Control experiments with DMF and uninoculated media were run parallel to the test compounds under the same conditions.

RESULTS AND DISCUSSION

An important research area explored by our research group is the synthesis new chromene derivatives [12-14]. As part of this work, we now report a simple synthesis of 2-imino and 2-oxo-2*H*-chromene-3-charboxamide from salicylaldehydes and cyanoacetamides. These chromene and coumarin carb-oxamide derivatives are useful as synthetic intermediates for a variety of further transformations and as promising sources of bioactive molecules.In view of the above-mentioned findings, and as a continuation of our effort to identify new candidates [15-19]. A wide variety of cyanoacetamides **1a,b**were prepared by direct combination of ethyl cyanoacetate and primary amines, inspired by a procedure previously described by Gazit et al [20]. cyclocondensation of cyanoacetanilides **1a,b**with salicylaldehyde(**2**) in ethanolic ammonium acetate afforded 2-imino-3-(N-substituted-phenyl-car-boxamido)-2H-chromenes **3a,b** as colored solid in high yield(scheme1).The structures of compounds **3a,b** were established by elemental analysis and spectral data. IR spectrum of compound **3a** showed absorption bands at 3403, 3316 (NH) and 1681cm^{-1} (C=O). ^{1}H NMR (DMSO-d_6) spectrum of compound **3a** was characterized by the presence of singlet signals at 2.2 and 8.5, 9.1 ppm corresponding to CH_3, chromene-H4 with two singlet signals at 9.1, 12.6 ppm (2s, 2H, 2NH; cancelled with D_2O). The mass spectra of **3a,b** displayed the correct molecular ions expected for the proposed molecular formula. The fragmentation patterns were in accord with the assigned structure. The primary cleavage of the molecular ion occurred at the amide bond giving rise to the acylium ion m/z 172 (chart 1).On the other hand, interaction of **1a,b** with salicylaldehyde in the presence of Ac_2O/AcONa, 3-(*N*-substituted-phenylcarboxamido)chromen-2-ones **4a,b** were obtained in reasonably good yield. IR spectrum of compound **4a**showed absorption bands at 3271 (NH) and 1705 cm^{-1} (C=O; lactone).^{1}HNMR spectrum (DMSO-d_6) of compound**4b**revealed tow singlet at 8.8 and at 10.6 ppm assigned for chromene-H and NH protons, respectively. Mass spectrum of compound **4b** reflected the right a molecular ion at 299 (25%) with a base peak at m/z = 173 (M-Cl.C_6H_4NH). The structure of compound **4** was further confirmed through their synthesis upon hydrolysis of **3** with EtOH/HCl or acetic acid. Similarly, compounds **1a or 1b** were allowed to react with 2-hydroxy-naphthaldehyde **(5)** and 7-hydroxy-5-methoxycoumar-in-6-carbox-aldehyde **(6)** in presence of EtOH/$AcONH_4$, giving 2-imino-benzo[f]chromenes **7a,b** and 2-iminopyrano[2,3-*g*]coumar-ins **9** with high yield. Benzocoumarins**8a,b** and pyrano[2,3-*g*]coumarin **10** were synthesized via interaction of compound **1** with compound **5** and/or**6** in the presence of acetic anhydride and sodium acetate. Compounds**8** and **10** were also obtained upon the treatment of compounds **7** and **9** with ethanol containing hydrochloric acid or acetic acid (scheme1).The suggested structures**7**, **8**, **9** and **10** were established on the basis of both elemental and spectral data.IR spectrum of the compound **7a**, showed absorption bands at 3324, 3298 and 1684 cm^{-1} assignable for 2NH and carboxamide groups,respectively.^{1}HNMR spectrum of **9** in DMSO-$d_6$revealed signals at δ 4.2 (s, 3H, OCH_3), 6.42 (d, 1H, H-6), 7.05-7.52 (m,5H, Ar-H), 8.1(d,1H,H7), 8.5(s,1H,H-4) and 9.5, 12.8 (2s, 2H, 2NH). Furthermore, the mass spectrum of **9**showed a molecular ion peak at m/z = 396 (27.15%) together base peak at m/z = 286.

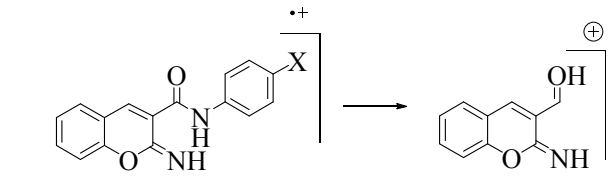


9, **10**; Ar = C_6H_4-Cl-p

3, 4, 7, 8 a; Ar = $C_6H_4CH_4$-p
b; Ar = C_6H_4Cl-p

i = EtOH/$AcONH_4$, ii = Ac_2 O/AcONa , iii = EtOH/HCl or AcOH

Scheme 1

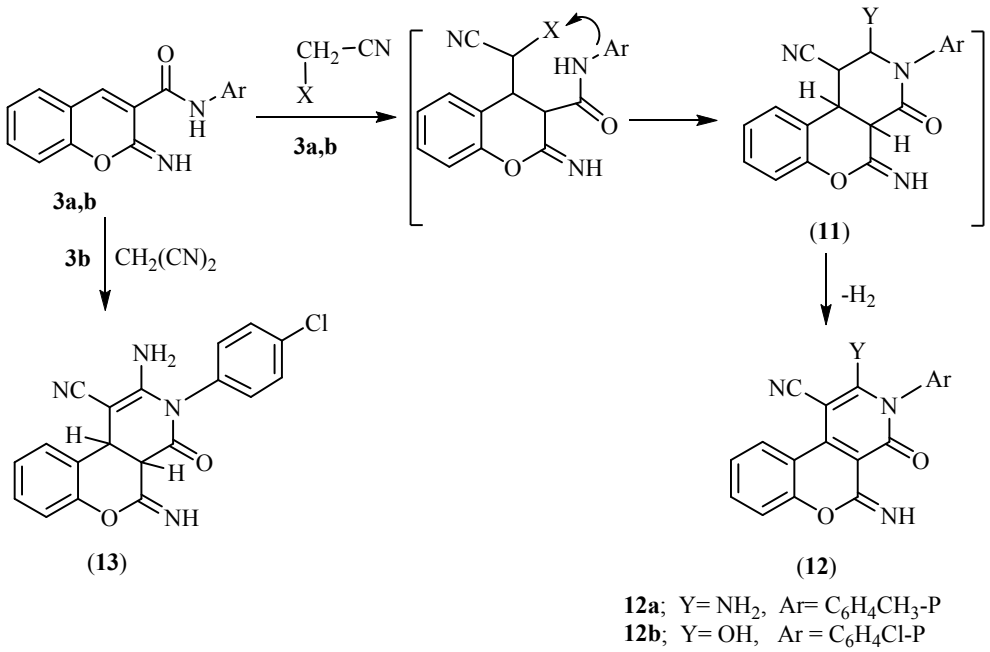


3a; m/z = 278 (100%) ⟶ **3a**; m/z =172 (20%)

3b; m/z = 298 (3%) ⟶ **3b**; m/z =172 (30%)

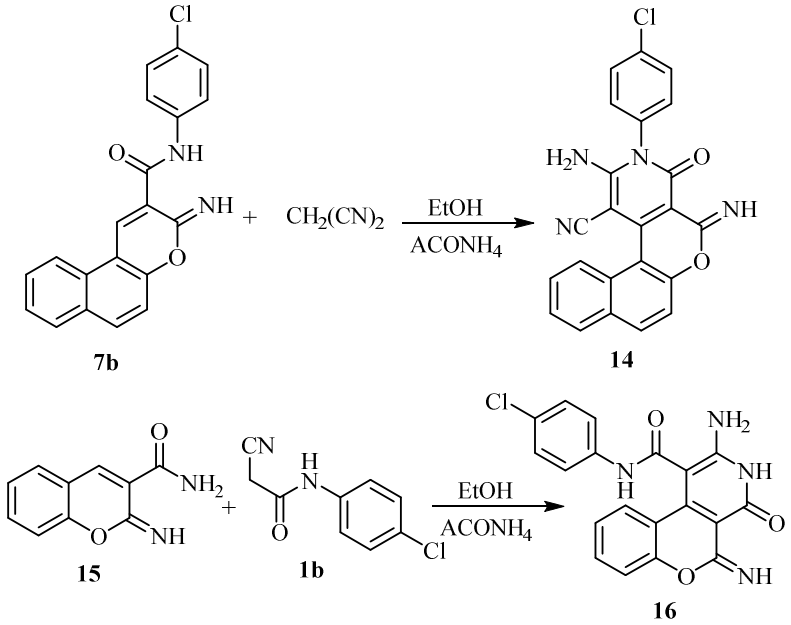
Chart 1

Moreover, the resulting chromene derivatives have latent functional substituents which have the potential for further chemical transformations as new routes for condensed chromene with possible biological activity.Reaction of 2-iminochromene derivatives **3a,b**with malononitrile and/orethyl cyanoacetate in refluxing ethanol containing catalytic amount of amm. acetate afforded in each case a product with analytical and spectral data in good agreement with chromeno[3,4-*c*]pyridine derivatives **12a,b** (scheme 2).The structuresof**12** have been confirmed on the basis of elemental analysis and spectral data. IR spectrum of **12a** showed absorption bands at 3439, 3348 (NH_2 NH), 2207 (CN) and 1650 cm^{-1} (C=O;amide).Mass spectrum of**12a** showed a molecular ion peak at m/z 342 (3.2%) with a base peak at m/z 300 [M-(CH_3 + HCN)]. ^{1}HNMR spectrum of **12b** in DMSO-$d_6$exhibited signals at δ 7.3-7.6 (m, 8H, Ar-H), 8.8 (s, 1H, NH) and9.2 (s, 1H, OH) ppm.The formation of **12a,b** was assumed to proceed via the addition of an active methylene group to the activated double bond of chromene giving Michael adduct, which underwent cyclized to the respective dihydrochromeno-pyridine **11** as an intermediate for 5-iminochromenopyridine derivatives**12a,b**.On the other hand, the dihydrochromenopyridine derivative **13** was obtained upon treatment of **3b** with malononitrile in boiling EtOH/$AcONH_4$.^{1}HNMR spectrum of compound**13** in DMSO-$d_6$was characteristic by the presence of two doublets at δ 4.71, 4.9corresponding to pyridine-H4and H-3, in addition to two singlet at δ 7.7 and 8.9 ppm for NH_2 and NH, respectively.



Scheme 2

In addition, Benzochromenopyridine derivative **14** was obtained via interaction of **7b** with malononitrile under the same condition of EtOH/$AcONH_4$.The structure of **14**could be established for the reaction product based on elemental analysis and spectral data.^{1}HNMR spectrum of **14** in DMSO-d_6 showed the following signals: 7.6-8.6 (m, 10H, Ar-H), 8.9, 9.0 (2s, 3H, NH_2 + NH; canaelled with D_2O). Finally, cyanoacetanilide**1b** was used as an active methylene compound which reacted with 2-iminochromene-3-carbboxamide **(15)**[21]in 1: 1 molar ratios in the presence of ammonium acetate to afford the chromenopyridine derivative **16**(scheme 3). Its ^{1}HNMR spectrum in DMSO-d_6 exhibited signals at δ 9.0, 9.2, 10.3 and 10.5 corresponding to three NH and NH_2protons.



Scheme 3

Biological activity:

Antibacterial activity

The antimicrobial screening and minimal inhibitory concentrations of the tested compounds were carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University, and Cairo, Egypt. The synthesized compounds **3a**,**b**, **4a**,**b**,**7a**,**b**,**8a**,**b**,**9**, **10**, **12a**,**b**,**13**,**14** and **16** were tested in vitro for their antibacterial activity against six strains of bacteria three Gram positive bacteria; *Staphylococcus aureus* (RCMB 010027), *Staphylococcus epidermidis* (RCMB 010024),*Bacillis subtilis* (RCMB 010063)andthree gram negative bacteria; *Proteous vulgaris* (RCMB010085), *Klebsiella pneumonia* (RCMB 010093) and *Shigella flexneri* (RCMB 0100542).Based on the results of disc-diffusion method on Gram +ve, Gram –ve bacteria and fungus species, the compounds that showed potential antibacterial activity (**3a**,**b**, **4a**,**b**, **7a**,**b**, **8a**,**b**, **9**,**10**, **12a**,**b** and **13**) were subjected to MIC test. The results are summarized in table1.Where the minimal inhibitory concentrations (MICs) for compounds that showed significant growth inhibition zones (>10 mm) were determined using twofold serial dilution method [22,23].

Some of the tested compounds showed promising antibacterial reaching, in certain cases, the same level of antimicrobial activity as the standard antibacterial agent Ampicilline and Gentamycin. In general compounds **3a**,**b**, **4a**,**b**, **7b** and **8b** were the most effective in inhibiting the growth of Gram-positive and Gram-negative strains of bacteria, with an MIC value ranging from (0.06-7.81 mg/mL) especially compound **3b,** that showed the highest rate of inhibition against all the tested bacterial strains, with an MIC value ranging from (0.06-3.9mg/mL) for antibacterial activity. It showed two fold of activity of ampicillin against *S.epidermis*, equipotent of activity to gentamycin against *K.pneumonia* and *S.flexneri*, three fourths of activity of gentamycin against *P.vulgaris*, about 0.1 of activity of ampicillin against *B.subtilis*, and almost half of the activity of ampicillin against *S.aureus*. Compounds **3a**, **4a**,**4b** and **7b** showed considerable antibacterial activity against all the tested bacterial strains withMIC value ranging from (0.24-7.81 mg/mL).With the exception of *S.epidermis* compound **8b** showed moderate activity against all the tested bacterial strains with MIC value ranging from (0.48-3.9 mg/mL). Compound **7a**showed the same moderate antibacterial activity against two of Gram -ve bacteria **(***K.pneumonia* and *S.flexneri* MIC 3.9 mg/mL), while compounds**9** showed moderate activity against only one strain of Gram -ve bacteria(*P.vulgaris*MIC 7.81 mg/mL). Finally; with the exception of *P.vulgaris*, compounds **9**,**10**,**12a**,**b**,**13**showed poor activity toward all the tested bacterial strains.

Table 1 Antimicrobial minimal inhibitory concentrations (MIC, mg/mL) of some new synthesized compounds

Compounds	Inhibitory %inhibitory %					
	Gram-positive bacteria			Gram-negative bacteria		
	S. aureus	***S. epidermidis***	***B. subtilis***	***P. vulgaris***	***K. pneumonia***	***S. flexneri***
3a	0.97	1.95	0.24	7.81	1.95	1.95
3b	0.24	0.24	0.06	3.9	0.24	0.48
4a	3.9	0.24	1.95	15.6	1.95	3.9
4b	0.97	0.97	0.24	3.9	0.48	1.95
7a	62.5	31.25	31.5	31.25	3.9	3.9
7b	0.97	0.24	0.24	7.81	1.95	1.95
8a	62.5	31.25	62.5	15.6	3.9	7.81
8b	3.9	31.25	1.95	3.9	0.48	0.48
9	31.25	62.5	15.63	7.81	15.63	31.25
10	31.25	31.25	15.63	62.5	31.25	31.25
12a	125	62.5	62.5	62.5	62.5	125
12b	15.6	15.63	15.63	125	31.25	31.25
13	125	125	62.5	125	-	62.5
AMPI	0.06	0.48	0.007	NT	NT	NT
GEN	NT	NT	NT	1.95	0.24	0.48

AMPI, Ampicillin; GEN, Gentamycin.
NT., Not tested.
(-), No activity.

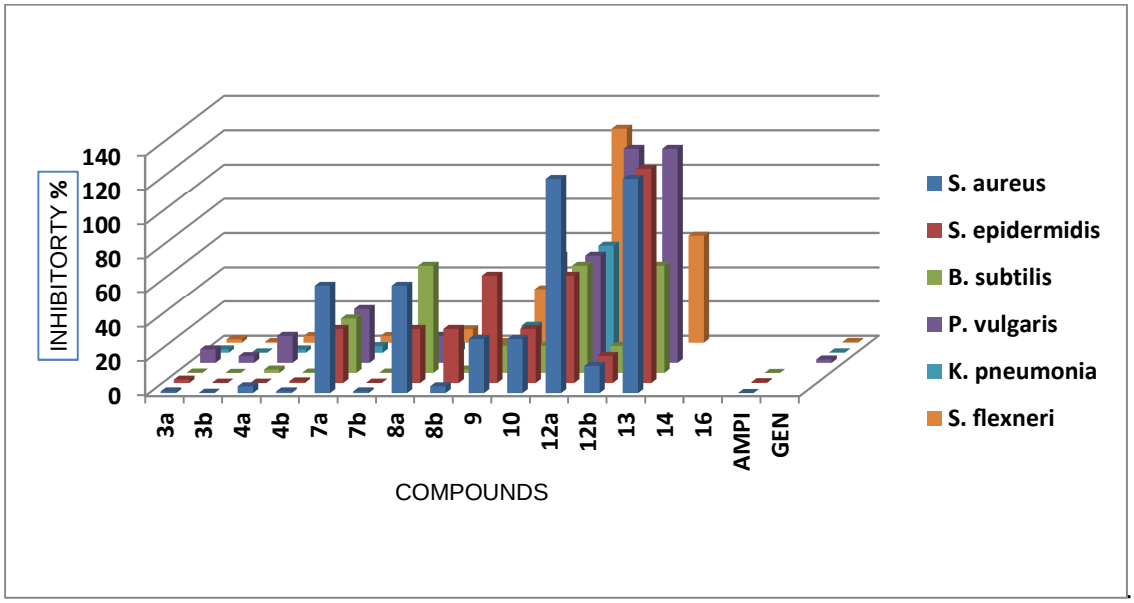


Figure 1: Graphical representation of in vitro antibacterial activity of the newly synthesized compounds

Antifungal activity

The synthesized compounds **3a**,**b**, **4a**,**b**, **7a**,**b**, **8a**,**b**, **9**,**10**, **12a**,**b**, **13** and **14** also evaluated for their potential antifungal activities against the following fungal strains; *Aspergillus fumigates* (RCMB 02564), *Aspergillus clavatus* (RCMB 02593) and *Candida albicans* (RCMB 05035)by the agar well diffusion method; Amphotricin was used as standards for comparison of antifungal activity.

For antifungal activity compounds **3a**,**b**, **4a**,**b**, **7a**,**b** and **8a**,**b**, were most effective in inhibiting the growth of all the tested fungal strains with MIC (0.24-7.81). Especially compound 3b which exerted half of activity of ampicillin against *C.albicans*, three fourths of activity of ampicillin against *A.clavulate* and one of activity of ampicillin against A. fumigates. With the exception of Compound **12a**,**b**. *C.albicans*, were more sensitive to almost all the tested compounds with MIC value (0.24-7.81 mg/mL) than the other tested fungal strains.

Table 2 Antifungal minimal inhibitory concentrations (MIC, mg/mL) of some new synthesized compounds:

Compounds	Antifugal activity (Inhibitory%)		
	A. fumigates	*A. clavatus*	*C. albicans*
3a	3.9	7.81	0.48
3b	1.95	3.9	0.24
4a	7.81	15.63	0.97
4b	3.9	7.81	0.97
7a	3.9	15.63	1.95
7b	1.95	7.81	0.97
8a	7.81	15.63	0.97
8b	3.9	7.81	1.95
9	15.6	15.6	7.81
10	31.25	15.6	1.95
12a	125	62.5	-
12b	62.5	62.5	250
13	125	125	-
Amph.	0.97	1.95	0.24

AMPH, Amphotricin B;
(-), No activity.

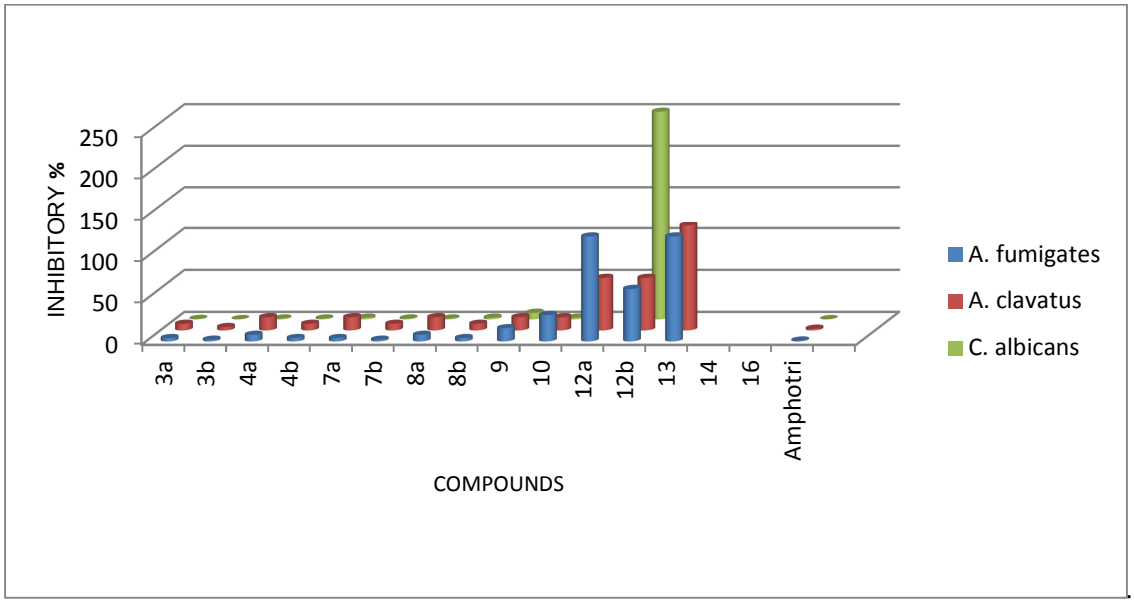


Figure 2: Graphical representation of in vitro antifungal activity of the newly synthesized compounds

Molecular modeling study (Pharmacophore modeling)

In this study, pharmacophore generation was performed using Discovery Studio 2.5 software. The 3D QSAR Pharmacophore Generation protocol (Catalyst HypoGen algorithm)[24] was used to derive structure activity relationship hypothesis models (3D QSAR pharmacophore models) from a set of ligands with known activity values on a given biological target. The training set is composed of thirteen synthesized compounds from the present study with measured MIC for the synthesized compounds against each strain of the tested bacteria for the generation of pharmacophore models considered as the certainty value. The uncertainty value was set as 1.5 instead of the default value 3.0. The pharmacophore features used are hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), hydrophobic (HYP), ring aromatic (RA) and negative ionizable features. Fisher validation was applied and set to 95% significance. Pharmacophores were then generated in HypoGen module and the top ten scoring hypotheses were exported. HypoGen identifies features common to the active compounds and excludes features common to the inactive compounds within conformational allowable regions of space. It further estimates the activity of our newly synthesized and tested compounds using regression parameters. The parameters were computed by regression analysis using the relationship of geometric fit value versus the activity. The better the geometric fit the greater the activity prediction of the compound.

Pharmacophore study results;

Ten predictive pharmacophore models (hypothesis) were generated via aligning different conformations of the represented training set ligands to bind with the generated pharmacophore models. All of the generated pharmacophore models for top pharmacophore model generated, Hypothesis 10, 1, 8, 5, 5, 5 for the Gram-positive strains (*S. aureus*, *S. epidermidis*, *B. subtilis*) and for the Gram-negative strains (*P. vulgaris*, *K. pneumonia*, *S. flexneri*) respectively, except P. vulgaris, that contained three chemical features; two HBA and one HYP. It worth to be mentioned that, the results of antibacterial activity were consistent with pharmacophore fit score values where it was observed that the most potent antibacterial compounds showed the best fit values. From the results, it is important to note that inthe best mapping conformations of the ligand compound**3b**,the imino group(NH) of chromene is mostly fits with one of feature of hydrogen bond (HBA) or (HBD) while the C=Ogroup of carboxamide fits with the other hydrogen bond (HBA) or (HBD), either benzene or pyran moiety of benzopyran fits with (HYP-1), and the terminal phenyl ring of triclosan fits with (HYP_2) or (RA), **Figure 3, 4.**

The previous analysis enabled us to point out several structural requirements as mentioned in the above discussion for the observed antibacterial activity, which can be summarized in figure5.

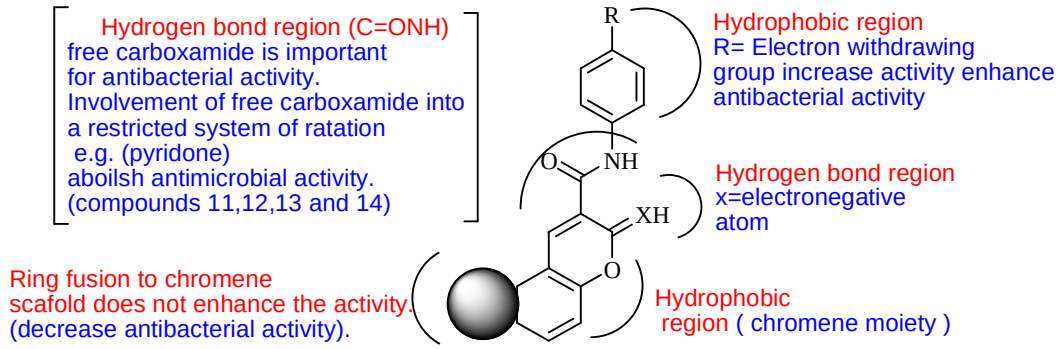


Figure 5: Structural requirements for the newly synthesized compounds as anti-bacterial agents

Pharmacophore validation

The pharmacophore models generated should be statistically significant, able to predict the activities of new chemical compounds and retrieve active compounds from the database. The selection of the generated pharmacophore models 10, 1, 8, 5, 5, 5 for *S. aureus*, *S. epidermidis*, *B. subtilis*, *P. vulgaris*, *K. pneumonia and S. flexneri* respectively was be based on its validation using four methods; cost analysis, Activity prediction, and Fisher validation test. HypoGen selects the best hypothesis by applying a cost analysis. The quality of the generated pharmacophore hypothesis was evaluated by considering the cost functions calculation by HypoGen module during hypothesis generation. In detail, the null cost and fixed cost of the ten pharmacophore hypothesis were equal to (222.358, 42.518), (203.603, 44.1958), (263.33, 46.908), (91.707, 43.061), (138.597, 41.062) and (263.334, 46.90) respectively for Hypothesis (10, 1, 8, 5, 5, 5) also they were chosen as the best generated pharmacophore hypothesis as they are characterized by the lowest total cost 110.072, 114.270, 142.441, 94.272, 92.378 and 78.710 the best correlation coefficient (0.818, 0.7522, 0.7732, 0.826, 0.891 and 0.846) which indicates the capability of the pharmacophore model to predict the activity of the training set compounds. The pharmacophore models (hypothesis 10, 1, 8, 5, 5, 5) were also validated through activity prediction of the synthesized structures as training set. It should be noted that the predicated MIC by the 3D Pharmacophore QSAR model were very close to those experimentally observed, indicating that these models can be safely applied for prediction of anti-microbial activity of newly synthesized compounds. Fischer validation is another approach for pharmacophore model validation. This validation method checks the correlation between the chemical structures and biological activity. This method generates pharmacophore hypothesis using the same parameters as those used to develop the original pharmacophore hypothesis by randomizing the activity data of the training set compounds.

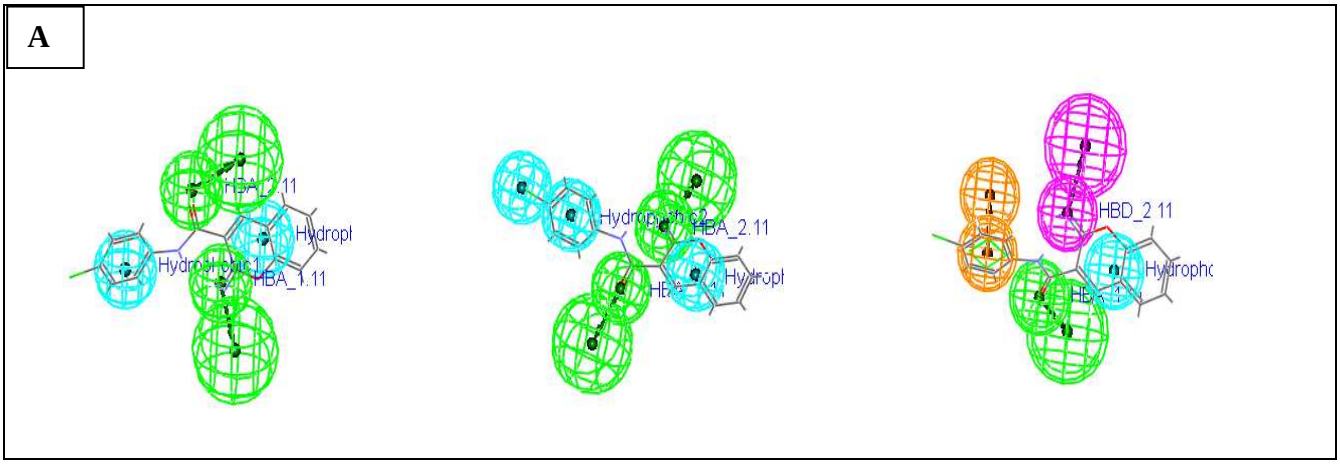


Figure 3: Best generated pharmacophore model for Gram-positive strains, (1A) for *S. aureus* showed synthesized structure compound (3b) fitted in the pharmacophore with fit value 9.79, (2A) showed The best generated pharmacophore model for *S. epidermidis* where synthesized structure compound (3b) fitted in the pharmacophore with fit value 7.25. (3A) The best generated pharmacophore model for *B. subtilis* that showed the synthesized structure compound (3b) fitted in the pharmacophore with fit value 8.08, with the features considered hydrogen bond acceptor (HBA) colored in green, hydrogen bond donor (HBD) colored in violet, ring aromatic (RA) colored in orange, hydrophobic (HYP) colored in cyan

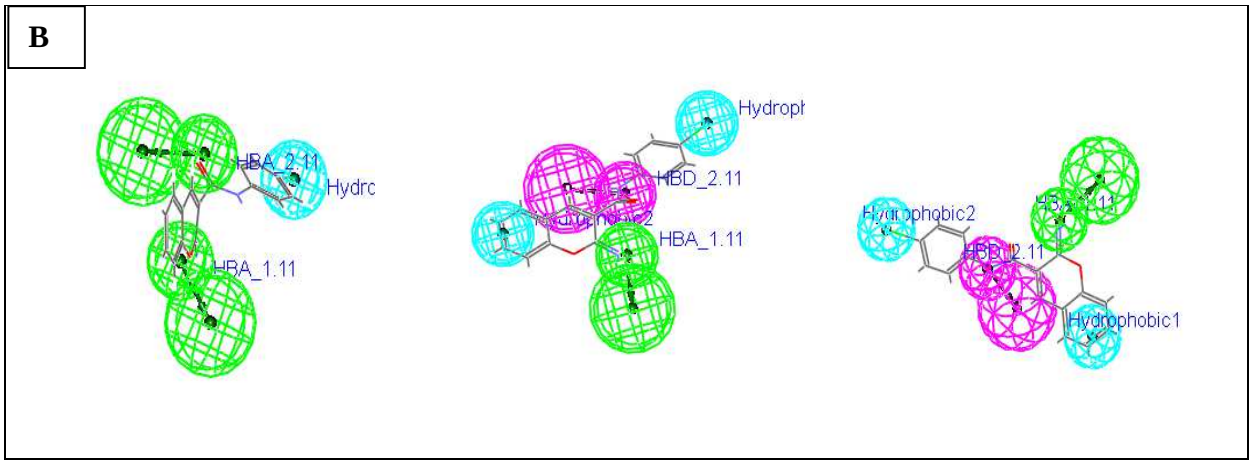


Figure 4: Best generated pharmacophore model for Gram-negative strains, (1B) for *P. vulgaris* showed synthesized structure compound (3b) fitted in the pharmacophore with fit value 6.12, (2B) showed The best generated pharmacophore model for *K. pneumonia* where synthesized structure compound (3b) fitted in the pharmacophore with fit value 8.84. (3B) the best generated pharmacophore model for *S. flexneri* that showed the synthesized structure compound (3b) fitted in the pharmacophore with fit value 7.80, with the features considered hydrogen bond acceptor (HBA) colored in green, hydrogen bond donor (HBD) colored in violet, and hydrophobic (HYP) colored in cyan

Table 3: Fit values and predicted activities the synthesized compounds mapped with the generated 3D-pharmacophore models

Compound	*S. aureus*			*S. epidermidis*			*B. subtilis*		
	Predicted activity	Exp. Activity MIC (mg / ml)	Fit value	Predicted activity	Exp. Activity MIC (mg / ml)	Fit value	Predicted activity	Exp. Activity MIC (mg / ml)	Fit value
3a	0.97	**0.97**	9.43	2.88	**1.95**	6.96	1.58	**0.24**	6.13
3b	0.43	**0.24**	9.79	0.18	**0.24**	7.25	0.07	**0.06**	8.08
4a	1.56	**0.39**	9.67	0.74	**1.95**	6.46	2.79	**1.95**	5.98
4b	2.57	**0.97**	9.45	0.37	**0.97**	8.02	2.41	**0.24**	6.06
7a	47.01	**62.5**	8.19	4.21	**31.25**	7.23	2.13	**31.5**	6.13
7b	0.86	**0.97**	9.93	1.61	**0.24**	7.51	0.43	**0.24**	6.15
8a	23.05	**62.5**	9.61	10.45	**31.25**	6.58	2.93	**62.5**	5.99
8b	3.72	**3.9**	9.29	11.42	**31.25**	6.63	3.28	**1.95**	6.02
9	28.98	**31.25**	8.40	18.92	**62.5**	6.15	5.54	**15.63**	6.02
10	7.418	**31.25**	8.99	22.12	**31.25**	6.23	2.40	**15.63**	6.08
12a	120.69	**125**	7.78	64.94	**62.5**	5.74	32.78	**62.5**	5.59
12b	15.39	**15.6**	8.67	52.71	**15.63**	6.17	28.26	**15.63**	5.75
13	156.72	**125**	7.67	62.96	**125**	5.53	28.17	**62.5**	5.59

Table 3 cont.: Fit values and predicted activities the synthesized compounds mapped with the generated 3D-pharmacophore models

Compound	*P. vulgaris*			*K. pneumonia*			*S. flexneri*		
	Predicted activity	Exp. Activity MIC (mg / ml)	Fit value	Predicted activity	Exp. Activity MIC (mg / ml)	Fit value	Predicted activity	Exp. Activity MIC (mg / ml)	Fit value
3a	12.51	**7.81**	5.92	2.71	**1.95**	8.09	2.96	**1.95**	7.38
3b	7.92	**3.9**	6.12	0.48	**0.24**	8.84	0.51	**0.48**	7.80
4a	16.57	**15.6**	5.80	4.33	**1.95**	7.89	6.80	**3.9**	7.74
4b	2.51	**3.9**	6.61	0.44	**0.48**	8.88	2.86	**1.95**	7.02
7a	10.87	**31.25**	5.98	2.36	**3.9**	8.15	2.69	**3.9**	6.92
7b	6.74	**7.81**	6.19	1.21	**1.95**	8.44	1.80	**1.95**	6.66
8a	13.99	**15.6**	5.87	3.87	**3.9**	7.94	6.77	**7.81**	7.38
8b	8.52	**3.9**	6.08	0.39	**0.48**	8.93	0.42	**0.48**	6.14
9	15.56	**7.81**	5.82	12.06	**15.63**	7.44	15.56	**31.25**	7.75
10	31.62	**62.5**	5.52	19.47	**31.25**	7.24	19.54	**31.25**	7.78
12a	68.54	**62.5**	5.18	41.47	**62.5**	6.91	117.34	**125**	6.58
12b	106.37	**125**	4.99	40.18	**31.25**	6.92	34.96	**31.25**	7.95
13	90.49	**125**	5.06	2.71	**1.95**	6.09	68.63	**62.5**	6.50

CONCLUSION

In conclusion, a series of some novel chromene derivatives were synthesized and established on the basis of spectral as well as analytical results. The in-vitro antimicrobial activities of the newly synthesized compounds were evaluated for their antibacterial and antifungal activities**.**Some of the synthesized compounds revealed promising antibacterial and antifungal activities. Close survey of the MIC values indicate that compound**3b** showed the maximum potent activities against all tested organisms with an MIC value ranging from (0.06-3.9 mg/mL), while compounds **3a**, **4a**,**b**, **7b** and **8b** showed considerable activities against the most Gram -ve and Gram +ve tested strains of bacteria. Compounds **3a**,**b**, **4a**,**b**, **7a**,**b** and**8a**,**b** were most effective in inhibiting the growth of all the tested fungal strains with MIC (0.24-7.81). 3D QSAR pharmacophore model created to explore the observed antibacterial activity against the tested strains of bacteria; interestingly the results of antibacterial activity were consistent with pharmacophore fit score values where it was observed that the most potent antibacterial compounds showed the best fit values.Thus, these compounds could be used as lead structure for designing more potent antibacterial agents.

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