بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

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MONA MAGHRABY

Faculty of Women
For Arts, Science and Education
Chemistry Department

Study on Some Heterocyclic Compounds of Expected Biological Activity

A Thesis Submitted for the Master's Degree
In

Organic Chemistry

Presented By

Hebat Allah Mahmode Said Mahmode Radwan
B.Sc. (2013)

To

Chemistry Department
Faculty of Women for Arts, Science and Education
Ain Shams University

Supervised By

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(2021)

Faculty of Women
For Arts, Science and Education
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Study on Some Heterocyclic Compounds of Expected Biological Activity

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Approval of Faculty Council **Approval of University Council**

/ / 2021 **/ / 2021**

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1. Synthesis, Characterization, Biological Activity of Novel 1*H*-benzo[*f*]chromene and 12*H*-benzo[*f*]chromeno[2,3-*d*]-pyrimidine Derivatives

Heba K. Abd El-Mawgoud, **Hebat Allah M. Radwan**, Fatma El-Mariah and Ahmed M. El-Agrody; *Letters in Drug Design & Discovery*, **15** (8), 857 – 865 (2018).

2. Single-Crystal Structure and Antimicrobial Activity of Ethyl 3-Amino-1-(4-chlorophenyl)-9-hydroxy-1*H*-benzo[*f*]-chromene-2-carboxylate Combined with Ethyl *α*-Cyano-4-chlorocinnamate

Heba A. M. Radwan, H. K. A. El-Mawgoud, F. El-Mariah, A. M. El-Agrody, A. E. Amr, M. A. Al-Omar and H. A. Ghabbour; *Russian Journal of General Chemistry*, **90** (2), 299-304 (2020).

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Letters in Drug Design & Discovery, 2018, *15*, 857-865

RESEARCH ARTICLE

Synthesis, Characterization, Biological Activity of Novel 1*H*-benzo[*f*]-chromene and 12*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidine Derivatives

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Abstract: ***Background*:** Chromene, benzochromene and their derivatives have been considered as an important class of oxygen-containing heterocycles. There has been increasing interest in the study of chromenes and benzochromenes due to their biological and pharmacological activities.

***Methods*:** 3-Amino-1-(4-chlorophenyl)-9-hydroxy-1*H*-benzo[*f*]chromene-2-carbonitrile (**3**) was used as precursor for the synthesis of novel 1*H*-benzo[*f*]chromene (**4,8-11**) and 12*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidine (**5-7,12-14**) derivatives via reaction of compounds **3** with appropriate chemical reagents. The structures of the synthesized compounds were confirmed on the basis of spectral data, IR, ^{1}H NMR, ^{13}C NMR and MS data. The targeted compounds were tested *in-vitro* for their antimicrobial activity and showed congruent results against the most tested microorganisms compared to the standard drugs Gentamycin and Ketoconazol. The Structure Activity Relationship (SAR) study for the target compounds agreed with the *in-vitro* essays and confirmed higher potent antimicrobial activity against some of the tested microorganisms.

***Results*:** In this study, the antimicrobial activity of the synthesized compounds **3–14** was examined and showed congruent results against the most tested microorganisms compared to the standard drugs Gentamycin and Ketoconazol.

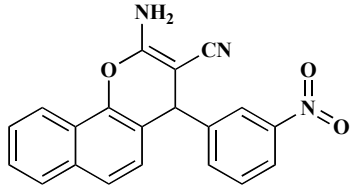
***Conclusion*:** Several 1*H*-benzo[*f*]chromene (**4,8-11**) and 12*H*-benzo[*f*]chromeno[2,3-*d*]-pyrimidine (**5-7,12-14**) derivatives were synthesized in good yields, starting from *β*-enaminonitrile **3** and elucidated on the basis of spectral data. An antimicrobial study has been performed and some compounds showed congruent results against the most tested microorganisms compared to the standard drugs Gentamycin and Ketoconazol.

Keywords: 1*H*-benzo[*f*]chromene, 12*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidine, Antimicrobial, SAR, gentamycin, ketoconazol.

1. INTRODUCTION

An aminochromene and aminobenzochromene moieties represent a significant class of heterocyclic framework containing oxygen atom. They are considered an interesting medicinal scaffold in drug systems due to their valuable biological and pharmacological activities. Numerous reports on aminochromene and aminobenzochromene derivatives explained such activities, for example antimicrobial [1-5], antioxidant [6], TNF-α inhibitor [7], antitubercular [8], anticoagulant, antispasmolytic, estrogenic [9], anticancer [10], hypertensive [11], anti-HIV [12], anti-inflammatory [13], herbicidal, analgesic and anticonvulsant [14] effects and activities.

A key feature is that the lipophilic nature of the benzochromene derivatives helps in crossing the cell membrane easily [15]. For example, 2-amino-4-(3-nitrophenyl)-4*H*-benzo[*h*]chromene-3-carbonitrile (LY290181) is a potent antiproliferative agent for a variety of cell types and inhibition of mitosis and microtubule [16, 17] as shown in Fig. (**1**).



LY290181
A potent antiproliferative agent

Fig. (1). Structure of LY290181.

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In addition, 2-amino-4-(4-chloro/2-nitro/4-nitrophenyl)-4*H*-benzo[*h*]chromene-3-carbonitrile and 3-amino-1-(4-chloro/4-bromophenyl)-1*H*-benzo[*f*]chromene-3-carbonitrile have good cytotoxic and apoptotic effects on human cancer cell lines namely, MCF-7, MDA-MB-231, T-47D, SK-N-MC, KB, HepG-2, and PC3 [18] as shown in Fig. (**2**).

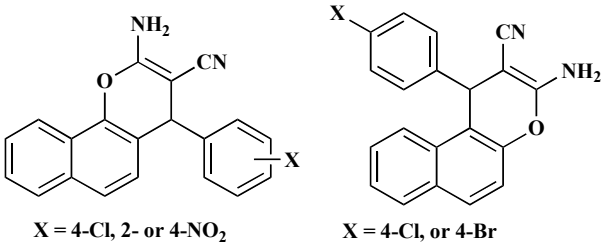


Fig. (2). 4*H*-Benzo[*h*]chromene and 1*H*-benzo[*f*]chromene derivatives with cytotoxic and apoptotic effects.

Furthermore, several reports have demonstrated that 7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine and 12*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidine moieties are promising and attractive scaffolds for the development of potent antimicrobial agents [19-21] as shown in Fig. (**3**).

In the light of the previous observations and benefits, and in continuation of our previous work in developing synthetic strategies for synthesis of heterocyclic compounds containing benzochromene moieties, we have synthesized some novel 1*H*-benzo[*f*]chromene and 12*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidine derivatives. Structures of the target compounds were examined for their antimicrobial activities in comparison to the standard drugs Gentamycin and Ketoconazol. The Structure Activity Relationship (SAR) was discussed in this research.

2. EXPERIMENTAL

2.1. Methods and Materials

Commercial-grade solvents and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. Melting points were measured with a Stuart Scientific (UK) apparatus and were uncorrected. IR spectra were determined as KBr pellets on a Jasco FT/IR 460 plus spectrophotometer (Jasco, Japan). ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Varian Gemini-300BB at 300 MHz spectrometer (Varian, USA). The MS were measured using a Shimadzu GC/MS-QP5050A spectrometer (Shimadzu, Japan). Elemental analyses were carried out at the Regional Centre for Mycology & Biotechnology (RCMP), Al-Azhar University, Cairo, Egypt and the results were between ± 0.3%. Analytical thin layer chromatography (TLC) was performed on silica gel precoated F_{254} Merck plates and to check the purity of the compounds.

2.2. Synthesis of the Compounds

2.2.1. Synthesis of 3-amino-1-(4-chlorophenyl)-9-hydroxy-1H-benzo[f]chromene-2-carbonitrile (3)

Compound **3** was prepared according to literature [22].

2.3. General Procedure for the Preparation of Compounds 4 and 5

A solution of (**3**) (0.01 mol) in acetic anhydride (20 mL) was refluxed for ½ or 3 h. The solvent was removed under reduced pressure and the solid obtained was collected and washed with cold methanol, filtered, dried and recrystallized from ethanol or benzene to afford **4** and **5** respectively. The physical and spectral data of compounds **4** and **5** are as follows:

2.3.1. 9-Acetoxy-3-acetylamino-1-(4-chlorophenyl)-1H-benzo[f]chromene-2-carbonitrile (4)

Pale yellow needles; M.p. 242-243 ^{o}C; yield 83 %; IR (FTIR/KBr) υ (cm $^{-1}$): 3341 (NH), 3079, 3061, 3032, 2916, 2871 (CH), 2188 (CN), 1748 (C=O, acetoxy), 1655 (C=O, acetylamino); ^{1}H-NMR (DMSO-d$_6$) δ: 2.27 (s, 3H, NHCOCH$_3$), 2.28 (s, 3H, OCOCH$_3$), 5.29 (s, 1H, H-1), 7.02-7.99 (m, 10H, Ar-H and NH); MS (EI, 70 eV) *m/z* (rel. intensity): 434 (M$^+$+2, 3.96), 432 (M$^+$, 11.73) with a base peak at 237 (100); Anal. Calcd for $C_{24}H_{17}ClN_2O_4$ (432.86): C, 66.59; H, 3.96; N, 6.47. Found: C, 66.41; H, 3.79; N 6.31 %.

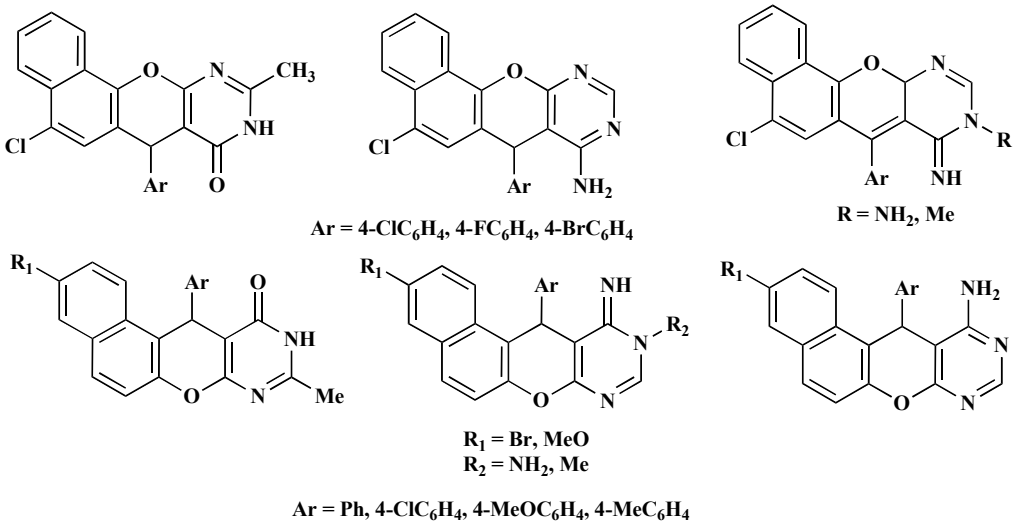


Fig. (3). 7*H*-Benzo[*h*]chromeno[2,3-*d*]pyrimidine and 12*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidine with antimicrobial agents.

2.3.2. 2-Acetoxy-9-methyl-10,11-dihydro-12-(4-chlorophenyl)-11H-benzo[f]chromeno[2,3-d]pyrimidin-11-one (5)

Yellow needles; M.p. 310-311 ^{o}C; yield 81 %; IR (FTIR/KBr) υ (cm $^{-1}$): 3340 (NH), 3061, 3013, 2927, 2860 (CH), 1760 (C=O, acetoxy), 1643 (C=O, cyclic); ^{1}H-NMR (DMSO-d_6) δ: 2.28 (s, 3H, CH_3), 2.30 (s, 3H, $OCOCH_3$), 5.69 (s, 1H, H-1), 7.32-8.02 (m, 9H, Ar-H), 12.53 (bs, 1H, NH); MS (EI, 70 eV) *m/z* (rel. intensity): 434 (M^++2, 0.9), 432 (M^+, 2.55) with a base peak at 279 (100); Anal. Calcd for $C_{24}H_{17}ClN_2O_4$ (432.86): C, 66.59; H, 3.96; N, 6.47. Found: C, 66.42; H, 3.83; N 6.14 %.

2.3.3. 11-Amino-2-hydroxy-12-(4-chlorophenyl)-12H-benzo[f]chromeno[2,3-d]pyrimidine (6)

Method (a): A mixture of **3** (0.01 mol) and formamide (0.02 mol) was stirred at reflux for 6 h. The solvent was removed under vacuum. The solid obtained was recrystallized from benzene to give **6** as colourless crystals; M.p. 229-230 ^{o}C; yield 71 %; IR (FTIR/KBr) υ (cm $^{-1}$): 3481, 3327, 3120 (OH & NH_2), 3019 2927 (CH), 1632 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 5.89 (s, 1H, H-12), 7.03-8.02 (m, 9H, Ar-H), 7.21 (bs, 2H, NH_2, exchangeable with D_2O), 8.11 (s, 1H, H-9), 9.93 (bs, 1H, OH, exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6) δ: 162.91, 162.47, 156.97, 156.87, 148.72, 143.10, 132.88, 131.77, 130.71, 129.97, 129.71, 128.83, 125.71, 117.50, 116.07, 114.52, 106.07, 97.26, 34.31; MS (EI, 70 eV) *m/z* (rel. intensity): 377 (M^++2, 9.19), 375 (M^+, 27.89) with a base peak at 264 (100); Anal. Calcd for $C_{21}H_{14}ClN_3O_2$ (375.81): C, 67.12; H, 3.75; N, 11.18. Found: C, 67.31; H, 3.89; N, 11.33 %.

Method (b): A mixture of the imadates **8** or **9** (0.01 mol) in absolute methanol (20 mL) and NH_3 gas bubbled in methanol under stirring at room temperature for 2 h, and then the mixture was left overnight. The solid product was collected and crystallized from benzene to give **6** (m.p., mixed m.p., identical IR and MS spectra).

2.3.4. 2-Benzoxy-9-phenyl-10,11-dihydro-12-(4-chlorophenyl)-12H-benzo[f]chromeno[2,3-d]pyrimidin-11-one (7)

A solution of **3** (0.01 mol) with benzoyl chloride (20 mL) was heated for 6 h. The excess of benzoyl chloride was removed under reduced pressure and the solid obtained was collected and washed with cold methanol, filtered, dried and recrystallized from benzene to afford **7** as colourless crystals; M.p. 290-291 ^{o}C; yield 70 %; IR (FTIR/KBr) υ (cm $^{-1}$): 3338 (NH), 3067, 2925, 2857 (CH), 1732 (C=O, benzoxy), 1646 (C=O, cyclic) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 5.85 (s, 1H, H-1), 7.25-8.18 (m, 19H, Ar-H), 12.93 (bs, 1H, NH); MS (EI, 70 eV) *m/z* (rel. intensity): 558 (M^++2, 1.19), 556 (M^+, 3.89) with a base peak at 77 (100); Anal. Calcd for $C_{34}H_{21}ClN_2O_4$ (556.99): C, 73.32; H, 3.80; N, 5.03. Found: C, 73.51; H, 3.99; N 5.21 %.

2.4. General Procedure for the Synthesis of the Imidates 8, 9 and Imidine 10

A mixture of **3** (0.01 mol), triethyl orthoformate (0.01 mol) and acetic anhydride (20 mL) or without acetic anhydride was refluxed for 2 h. The solvent was removed under reduced pressure and the resulting solid was washed with methanol and recrystallized from benzene to afford **8** and **9** respectively. The physical and spectral data of compounds **8** and **9** were as follows:

2.4.1. 9-Acetoxy-3-ethoxymethyleneamino-1-(4-chlorophenyl)-1H-benzo[f]chromene-2-carbonitrile (8)

Colourless crystals; M.p. 185-186 ^{o}C; yield 86 %; IR (FTIR/KBr) υ (cm $^{-1}$): 3066, 3025, 2982, 2934, 2891 (CH), 2204 (CN), 1766 (C=O, acetoxy), 1653 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 1.32 (t, J = 7.2 Hz, 3H, CH_3), 4.33 (q, J = 7.2 Hz, 2H, CH_2), 5.58 (s, 1H, H-1), 7.25-8.03 (m, 9H, Ar-H), 8.72 (s, 1H, N=CH); ^{13}C-NMR (DMSO-d_6) δ: 169.62, 162.53, 157.52, 149.91, 148.12, 142.86, 132.42, 131.25, 130.57, 130.36, 129.83, 129.59, 129.41, 121.44, 118.16, 117.44, 115.40, 114.01, 81.18, 64.49, 38.87, 21.36, 14.31; MS (EI, 70 eV) *m/z* (rel. intensity): 448 (M^++2, 6.01), 446 (M^+, 17.13) with a base peak at 293 (100); Anal. Calcd for $C_{25}H_{19}ClN_2O_4$ (446.88): C, 67.19; H, 4.29; N, 6.27. Found: C, 67.29; H, 4.38; N, 6.39 %.

2.4.2. 1-(4-Chlorophenyl)-3-ethoxymethyleneamino-9-hydroxy-1H-benzo[f]chromene-2-carbonitrile (9)

Colourless crystals; M.p. 162-163 ^{o}C; yield 87 %; IR (FTIR/KBr) υ (cm $^{-1}$): 3382 (OH), 3065, 3026, 2978, 2932, 2896 (CH), 2206 (CN), 1650 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 1.31 (t, J = 7.2 Hz, 3H, CH_3), 4.33 (q, J = 7.2 Hz, 2H, CH_2), 5.37 (s, 1H, H-1), 6.94-7.85 (m, 9H, Ar-H), 8.69 (s, 1H, N=CH), 9.87 (s, 1H, OH); ^{13}C-NMR (DMSO-d_6) δ: 162.43, 157.42, 156.98, 147.97, 143.03, 132.38, 130.73, 130.27, 129.91, 129.26, 129.17, 128.79, 126.05, 118.34, 117.83, 113.97, 112.07, 81.11, 64.42, 38.37, 14.33; MS (EI, 70 eV) *m/z* (rel. intensity): 406 (M^++2, 10.39), 404 (M^+, 31.43) with a base peak at 368 (100); Anal. Calcd for $C_{23}H_{17}ClN_2O_3$ (404.85): C, 68.23; H, 4.23; N, 6.92. Found: C, 68.40; H, 4.39; N. 7.11 %.

2.4.3. 1-(4-chlorophenyl)-3-dimethylaminomethyleneamino-9-hydroxy-1H-benzo[f]chromene-2-carbonitrile (10)

Method (a): A mixture of **3** (0.01 mol) and *N,N*-dimethylformamide dineopentyl acetal (DMF-DPA) (0.01 mol) in benzene (30 mL) was refluxed for 3 h. The solvent was removed under reduced pressure and the resulting solid was washed with methanol and recrystallized from ethanol /benzene to give imidine **10** as colourless crystals; M.p. 171-172^{o}C; yield 89 %; IR (FTIR/KBr) υ (cm $^{-1}$): 3221 (OH), 2924, 2853 (CH), 2207 (CN), 1622 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 3.16, 3.00 (s, 6H, $2CH_3$), 5.20 (s, 1H, H-1), 6.96-8.64 (m, 9H, Ar-H), 8.46 (s, 1H, N=CH), 9.80 (s, 1H, OH); ^{13}C-NMR (DMSO-d_6) δ: 159.69, 156.87, 155.53, 148.38, 144.19, 132.52, 131.81, 130.64, 129.93, 129.57, 129.18, 125.77, 120.56, 117.52, 114.08, 112.90, 106.14, 73.58, 40.92, 34.67; MS (EI, 70 eV) *m/z* (rel. intensity): 405 (M^++2, 3.39), 403 (M^+, 13.23) with a base peak at 57 (100); Anal. Calcd for $C_{23}H_{18}ClN_3O_2$ (403.86): C, 68.40; H, 4.49; N, 10.40. Found: C, 68.61; H, 4.69; N, 10.59 %.

Method (b): A mixture of imadates **8** or **9** (0.01 mol) and dimethylamine in methanol (30 mL), was stirred for 1 h. and the mixture was left overnight. The solid product was

collected by filtration, washed with methanol and recrystallized from ethanol/benzene to afford **10** (m.p., mixed m.p., identical IR and MS spectra).

2.5. General Procedure for the Synthesis of Compounds 11-13

A mixture of imadates **8** or **9** (0.01 mol) and diethylamine, methylamine, or hydrazine hydrate (0.01 mol) in absolute methanol (20 mL), was stirred for 1 h at room temperature and the mixture was left overnight. The solid product was collected by filtration, washed with cold methanol and recrystallized from ethanol/benzene to afforded compounds **11-13**. The physical and spectral data of compounds **11-13** were as follows:

2.5.1. 1-(4-Chlorophenyl)-3-diethylaminomethyleneamino-9-hydroxy-1H-benzo[f]chromene-2-carbonitrile (11)

Colourless crystals; M.p. 260-261 ^{o}C; yield 84 or 82%; IR (FTIR/KBr) υ (cm $^{-1}$): 3391 (OH), 3021, 2970, 2924, 2856 (CH), 2184 (CN), 1647 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 1.31 (t, J = 6.9 Hz, 3H, CH_3), 4.34 (q, J = 6.9 Hz, 2H, CH_2), 5.06 (s, 1H, H-1), 6.94-7.82 (m, 9H, Ar-H), 8.46 (s, 1H, N=CH), 9.82 (s, 1H, OH); MS (EI, 70 eV) *m/z* (rel. intensity): 433 (M^++2, 10.39), 431 (M^+, 31.43) with a base peak at 368 (100); Anal. Calcd for $C_{25}H_{22}ClN_3O_2$ (431.91): C, 69.52; H, 5.13; N, 9.73. Found: C, 69.34; H, 4.98; N 9.55 %.

2.5.2. 12-(4-Chlorophenyl)-2-hydroxy-11-imino-10-methyl-10,11-dihydro-12H-benzo[f]chromeno[2,3-d]pyrimidine (12)

Colourless needles; M.p. 260-261^{o}C; yield 82 or 80%; IR (FTIR/KBr) υ (cm $^{-1}$): 3375 (OH), 3048, 2955, 2918, 2850 (CH), 1644 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 3.30 (s, 3H, CH_3), 5.73 (s, 1H, H-1), 7.00-7.77 (m, 10H, Ar-H, NH), 8.09 (1H, s, H-9), 9.87 (s, 1H, OH); ^{13}C-NMR (DMSO-d_6) δ: 156.97, 156.52, 151.70, 148.18, 143.28, 132.80, 131.68, 130.62, 130.42, 129.62, 128.69, 125.79, 117.46, 115.09, 114.25, 106.25, 99.54, 35.86, 35.46; MS (EI, 70 eV) *m/z* (rel. intensity): 391 (M^++2, 10.39), 389 (M^+, 31.43) with a base peak at 368 (100); Anal. Calcd for $C_{22}H_{16}ClN_3O_2$ (431.91): C, 67.78; H, 4.14; N, 10.78. Found: C, 67.93; H, 4.29; N. 10.94 %.

2.5.3. 10-Amino-12-(4-chlorophenyl)-2-hydroxy-11-imino-10,11-dihydro-12H-benzo[f]chromeno-[2,3-d]pyrimidine (13)

Colourless crystals; M.p. 254-255 ^{o}C; yield 88 or 85 %; IR (FTIR/KBr) υ (cm $^{-1}$): 3434, 3365, 3324, 3297 (OH, NH & NH_2), 3065, 3028, 2924, 2864 (CH), 1646 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 5.68 (s, 1H, H-12), 6.98-7.79 (m, 12H, Ar-H, NH, NH_2), 8.04 (1H, s, H-9), 9.85 (s, 1H, OH); ^{13}C-NMR (DMSO-d_6) δ: 157.00, 155.74, 154.84, 151.12, 148.46, 143.28, 132.74, 131.58, 130.67, 130.26, 129.62, 128.53, 125.80, 117.49, 114.81, 114.26, 105.96, 99.85, 36.10; MS (EI, 70 eV) *m/z* (rel. intensity): 392 (M^++2, 6.09), 390 (M^+, 18.26) with a base peak at 364 (100); Anal. Calcd for $C_{21}H_{15}ClN_4O_2$ (390.82): C, 64.54; H, 3.87; N, 14.34. Found: C, 64.71; H, 3.99; N. 14.45 %.

2.6. Synthesis of Schiff Base 14

2.6.1. 10-Benzylideneamino-12-(4-chlorophenyl)-2-hydroxy-11-imino-10,11-dihydro-12H-benzo[f]chromeno[2,3-d] pyrimidine (14)

A mixture of the aminoimino **13** (0.01 mol) and benzaldehyde (0.01 mol) in ethanolic piperidine solution (20 mL) was refluxed for 2 h. The solid product was collected by filtration, washed with cold methanol and recrystallized from benzene to afford compound **14**. The physical and spectral data of compound **14** as yellow crystals; M.p. 314-315 ^{o}C; yield 80 %; IR (FTIR/KBr) υ (cm $^{-1}$): 3431, 3362 (OH & NH), 3081, 3060, 3032, 2985, 2926 (CH), 1631 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 6.62 (s, 1H, H-12), 7.08-8.37 (m, 14H, Ar-H), 8.39 (1H, s, H-9), 10.01 (s, 1H, N=CH), 11.25 (bs, 1H, NH); MS (EI, 70 eV) *m/z* (rel. intensity): 480 (M^++2, 1.27), 478 (M^+, 3.79) with a base peak at 374 (100); Anal. calcd for $C_{28}H_{19}ClN_4O_2$ (478.93): C, 64.54; H, 3.87; N, 14.34. Found: C, 64.38; H, 3.72; N, 14.20 %.

2.7. Antibacterial Evaluation

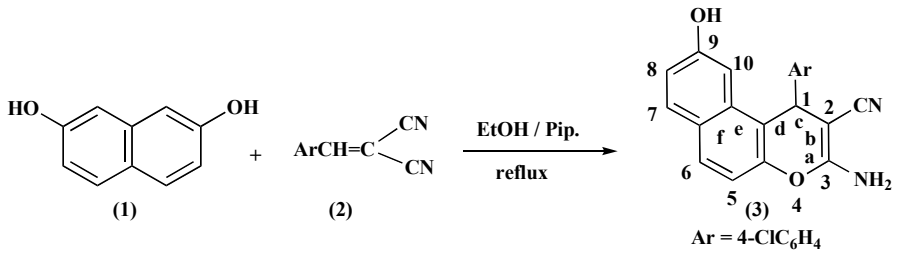
The targeted compounds **3-14** were tested *in-vitro* for their antimicrobial activities by the agar diffusion method using Mueller-Hinton agar medium for bacteria and Sabouraud's agar medium for fungi [23, 24]. The tested microorganisms were obtained from the Regional Center for Mycology & Biotechnology (RCMP), Al-Azhar University. The assayed collection included three Gram-positive species (*Staphylococcus aureus, Bacillus subtilis* and *Staphylococcus epidermtitis*), three Gram-negative bacteria (*Enterococcus cloaca*, *Escherichia coli* and *Salmonella typhimurium*) using the standard antibiotic Gentamycin (5 mg/mL) as reference drugs and two fungi (*Aspergillus fumigates* and *Aspergillus flavus*) using the standard antibiotic Ketoconazol (5 mg/mL). The mean zone of inhibition in mm ± standard deviation beyond the well diameter (6 mm) was determined using a 5 µg/mL concentration of the tested compounds. The inhibitory effects of the synthetic compounds against these organisms are listed in Tables **1** & **2**.

3. RESULTS AND DISCUSSION

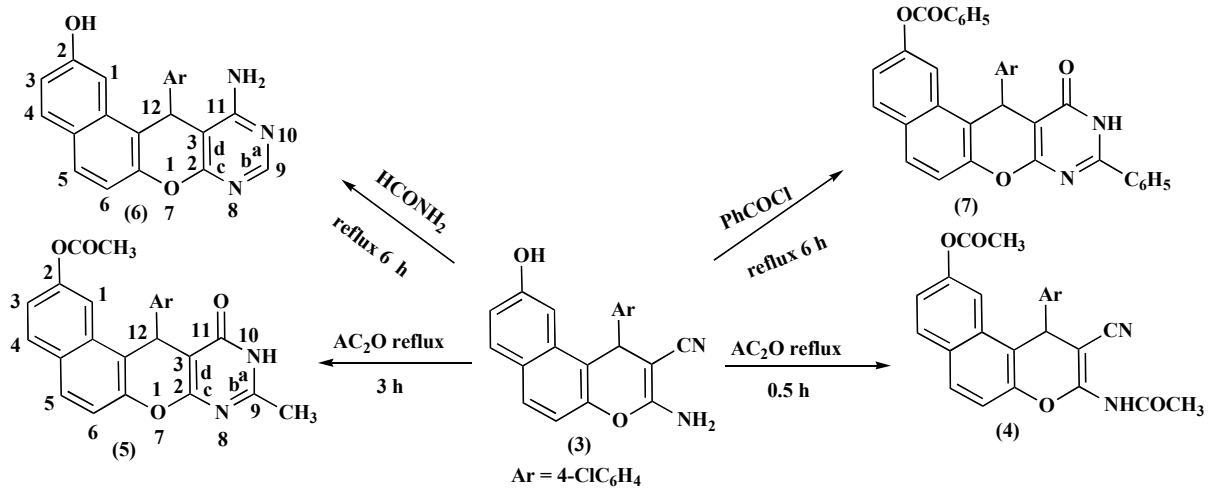
3.1. Chemistry

Treatment of naphthalene-2,7-diol (**1**) with *α*-cyano-*p*-chlorocinnamonitrile (**2**) in ethanolic piperidine solution under reflux afforded the corresponding 1:1 adduct 3-amino-1-(4-chlorophenyl)-9-hydroxy-1*H*-benzo[*f*]chromene-2-carbonitrile (**3**) as illustrated in Scheme **1**.

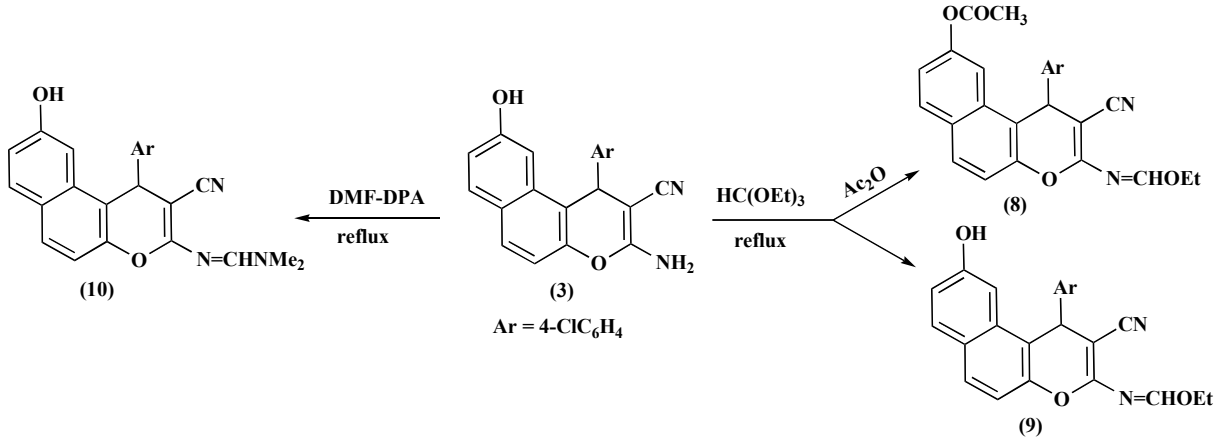
Interaction of 3-amino-1-(4-chlorophenyl)-9-hydroxy-1*H*-benzo[*f*]chromene-2-carbonitrile (**3**) with acetic anhydride for 0.5 h afforded the open chain product 9-acetoxy-3-acetylamino-1-(4-chlorophenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile (**4**), while heating of **3** with acetic anhydride for 3 h gave the cycloaddition product 2-acetoxy-9-methyl-10,11-dihydro-12-(4-chlorophenyl)-12*H*-benzo[*f*]chromeno-[2,3-*d*]pyrimidin-11-one (**5**) respectively, with acylation of the hydroxyl group at 9-position into the acetoxy group (Scheme **2**). Besides, condensation of **3** with formamide under reflux for 6 h gave the cycloaddition product 11-amino-



Scheme 1. Synthetic routes to compound **3**.



Scheme 2. Synthetic protocol for compounds **4-7**.

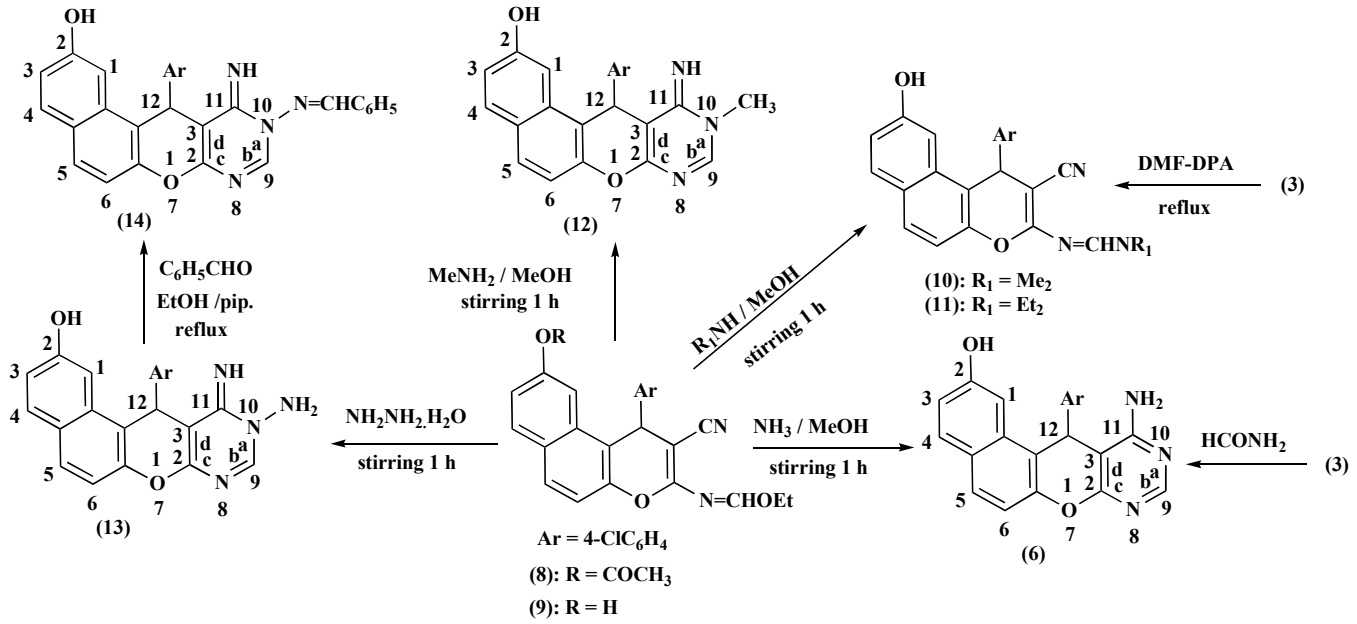


Scheme 3. Synthesis of 4*H*-chromene derivatives (**8-10**).

2-hydroxy-12-(4-chlorophenyl)-12*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidine (**6**), while benzoylation of **3** with benzoyl chloride under reflux gave the cycloaddition product 2-benzoxy-9-phenyl-10,11-dihydro-12-(4-chlorophenyl)-12*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidin-11-one (**7**) with benzoylation of the hydroxyl group at 9-position into the benzoxy group (Scheme **2**). The 1-position of compound **3, 4**, 12-postion of compound **5-7** is chiral center and all the reactions were controlled using TLC technique.

Interaction of **3** with triethyl orthoformate in acetic anhydride under reflux gave 9-acetoxy-3-ethoxymethyleneamino-1-(4-chlorophenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile (**8**) with acylation of the hydroxyl group at 9-position, while reaction of **3** with the neat triethyl orthoformate afforded 1-(4-chlorophenyl)-3-ethoxymethyleneamino-9-hydroxy-1*H*-benzo[*f*]chromene-2-carbonitrile (**9**). Condensation of **3** with dimethylformamide-dineopentylacetal (DMF-DPA) in benzene under reflux afforded 1-(4-chlorophenyl)-3-dimethylaminomethyleneamino-9-hydroxy-1*H*-benzo[*f*]chromene-2-carbonitrile (**10**) as shown in Scheme **3**. The 1-position of compounds **8-10** are chiral center and all the reactions were controlled using TLC technique.

Ammonolysis of imidates **8** and **9** with NH_3 gas bubbled in absolute methanol at room temperature under stirring for 1 h giving the cycloaddition product 11-amino-2-hydroxy-12-(4-chlorophenyl)-12*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidine



Scheme 4. Synthesis of novel 1*H*-benzo[*f*]chromenes and 12*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidines (**6,10-14**).

(**6**) (m.p., mixed m.p., identical IR and MS spectra) with deacylation of the acetoxy group at 2-position into hydroxyl group in the case of imidate **8**. The aminopyrimidine derivatives (**6**) can be obtained as previously described from the reaction of **3** with formamide (Scheme **2**) (m.p., mixed m.p., identical IR and MS spectra). Treatment of imidates **8** and **9** with dimethylamine or diethylamine in absolute methanol at room temperature under stirring for 1 h gave the open chain product 1-(4-chlorophenyl)-3-dimethylaminomethyleneamino-9-hydroxy-1*H*-benzo[*f*]chromene-2-carbonitrile (**10**) and 1-(4-chlorophenyl)-3-diethylaminomethyleneamino-9-hydroxy-1*H*-benzo[*f*]chromene-2-carbonitrile (**11**) (m.p., mixed m.p., identical IR and MS spectra) respectively, with deacylation of the acetoxy group at 2-position into hydroxyl group in the case of imidate **8**. Imidine **10** can be obtained as previously described from the reaction of compound **3** and DMF-DPA (Scheme **3**) (m.p., mixed m.p., identical IR and MS spectra). Moreover, reaction of imidates **8** and **9** with methylamine or hydrazine hydrate under the same conditions afforded the cycloaddition products 12-(4-chlorophenyl)-2-hydroxy-11-imino-10-methyl-10,11-dihydro-12*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidine (**12**) and 10-amino-12-(4-chlorophenyl)-2-hydroxy-11-imino-10,11-dihydro-12*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidine (**13**) (m.p., mixed m.p., identical IR and MS spectra) with deacylation of the acetoxy group at 2-position into hydroxyl group in the case of imidate **8**. Finally, reaction of the aminoimino compound (**13**) with benzaldehyde afforded the open chain Schiff base product 10-benzylideneamino-12-(4-chlorophenyl)-2-hydroxy-11-imino-10,11-dihydro-12*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidine (**14**). These results are depicted in Scheme **4**.

3.2. Antimicrobial Activity

The targeted synthesized compounds **3-14** were tested for their *in-vitro* antimicrobial activity against different microorganisms representing Gram-positive (*Staphylococcus aureus, Bacillus subtilis* and *Staphylococcus epidermtitis*), Gram-negative bacteria (*Enterococcus cloaca*, *Escherichia coli* and *Salmonella typhimurium*) using the standard antibiotic Gentamycin (5 mg/mL) as reference drug and fungi (*Aspergillus fumigates, Aspergillus flavus* and *Candida Albicans*) using the standard antibiotic Ketoconazol (5 mg/mL). The compounds were tested for their activities at concentration of 5 mg/mL using inhibition zone diameter in mm as criterion for the antimicrobial activity [23, 24] and the results are shown in Tables **1** & **2**.

3.3. SAR Studies

The Structure Activity Relationship (SAR) studies of compounds **3-14** revealed that compounds **13, 11, 6** and **3** with inhibitory effects of 21±0.8, 21±0.3, 20±0.7 and 20±0.5 mg/mL showed activity against *Staphylococcus aureus* close to standard antibiotic Gentamycin (24±1.2 mg/mL), implying that the 12*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidine moiety with hydrophilic or hydrophobic groups (-OH-2, -NH_2-10, =NH-11) was preferred than 1*H*-benzo[*f*]chromene moiety with hydrophilic or hydrophobic groups (-OH-9, -N=CHNEt_2-3), while compounds **3, 13, 11** and **6** showed almost equipotent or near activity against *Bacillus subtilis* with inhibitory effects ranging 25–20 mg/mL as compared to the standard antibiotic Gentamycin (26±0.8 mg/mL) and compound **6** (20±1.1 mg/mL) exhibited moderate activity against *Staphylococcus epidermtitis* as compared to the standard antibiotic Gentamycin (28±1.3 mg/mL), suggesting that a 1*H*-benzo[*f*]chromene moiety with hydrophilic or hydrophobic groups (-OH-9, -NH_2-3) was more effective than a 12*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidine moiety with hydrophilic or hydrophobic groups (-OH-2, -NH_2-10, =NH-11). Moreover, compounds **11, 6, 13** and **3** showed good activity against *Escherichia coli* with inhibitory effects ranging 27–23 mg/mL as compared to the standard antibiotic Gentamycin (30±1.5 mg/mL), while compounds **13, 6** and **3** with inhibitory effects of 24±1.3, 22±1.3 and 22±0.2 were 1.4, 1.3, 1.3 times more active than the standard antibiotic Gentamycin (17±0.4 mg/mL) against *Salmonella typhimurium* and

Table 1. Antibacterial screening for compounds 3-14 (5 mg/mL).

Compounds	Gram Positive Bacteria			Gram Negative Bacteria		
	S. aureus RCMB 010010	*B. subtilis* RCMB 015	*S. epidermtitis* RCMB 009	*E. cloaca* RCMB 001	*E. coli* RCMB 010052	*S. typhimurium* RCMB 006
3	20±0.5	25±0.8	17±0.4	15±0.4	23±0.3	22±0.2
4	NA	12±0.9	12±0.3	NA	NA	12±0.6
5	9±0.7	12±0.5	NA	13±0.7	12±0.6	13±1.2
6	20±0.7	20±1.4	20±1.1	14 ±1.3	26±1.2	22±1.3
7	16±1.1	13±1.3	NA	13±0.7	16±0.8	13±0. 5
8	11±1.1	15±0.5	9±0. 3	14±0. 7	12±0.8	17±2.1
9	10±1.2	14±1.8	12±1.4	13±1.9	11±2.1	16±1.1
10	10±1.4	9±1.3	8±1.2	NA	7±1.1	9±1.3
11	21±0.3	21±0.7	17±0.6	12±0.9	27±0.4	23±0.8
12	17±1.1	20±0.3	18±0.5	NA	17±1.3	16±1.1
13	21±0.8	23±1.1	18±0.9	10±1.3	25±1.4	24±1.3
14	12±1.4	13±1.3	13±0.8	NA	14±1.0	15±0.8
Gentamycin	24±1.2	26±0.8	28±1.3	27±0.6	30±1.5	17±0.4

NA = not active; Diameter of the hole = 6 mm; Data are expressed in the form of mean ± SD.

Table 2. Antifungal screening for compounds 3-14 (5 mg/mL).

Compounds	Fungi		
	A. fumigates RCMB 002008	*A. flavus* RCMB 002002	*C. Albicans* RCMB 005003
3	14±1.1	NA	13±1.8
4	NA	NA	16±0.4
5	NA	NA	15±0.2
6	NA	13±0.1	16±0.3
7	NA	11±0.2	NA
8	NA	13±0.7	14±0.9
9	NA	NA	15±0.5
10	NA	NA	NA
11	NA	NA	14±0.1
12	15±1.3	16±1.7	12±0.4
13	15±0.1	14±0.6	15±0.5
14	NA	NA	18±0.8
Ketoconazol	17±1.2	16±1.1	20±0.2

NA = not active; Diameter of the hole = 6 mm; Data are expressed in the form of mean ± SD.

compound **8** (17±2.1) was equipotent as Gentamycin, suggesting that a 12*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidine moiety was more effective than a 1*H*-benzo[*f*]chromene moiety. In addition, compounds **12, 13** and **3** showed almost equipotent or near activity against *Aspergillus fumigates* with inhibitory effects of (15±1.3, 15±0.1 and 14±1.1 mg/mL) as compared to the standard antibiotic Ketoconazol (17±1.2 mg/mL), while compound **12** with inhibitory effect