International Journal of Pharmacy and Pharmaceutical Sciences

Print ISSN: 2656-0097 | Online ISSN: 0975-1491 Vol 11, Issue 9, 2019

Original Article

SYNTHESIS AND UTILITY OF NEW POLYCYCLIC COMPOUNDS AS POTENTIAL ANTIMICROBIALS BASED ON CHROMENE MOIETY

MAHMOUD N. ABDELAZIZ[1], EMAN S. ZARIE[2,3*], ALAADIN E. SARHAN[3]

[1]Faculty of Science (Girls), Chemistry Department, Al-Azhar University, Nasr City, Cairo, Egypt, [2]Nanochemistry and Nanoengineering, School of Chemical Engineering, Department of Chemistry and Materials Science, Aalto University, Kemistintie 1, 00076 Aalto, Finland, [3]Department of Therapeutical Chemistry, Pharmaceutical Division, National Research Centre, Dokki 12311, Giza, Egypt
Email: eman.zarie@aalto.fi

Received: 28 May 2019 Revised and Accepted: 30 Jul 2019

ABSTRACT

Objective: The present research aims to synthesize some new polycyclic compounds including chromene moiety and study their antimicrobial activity.

Methods: Several new polycyclic systems including chromene scaffold incorporated with pyridine, pyrimidine, imidazopyrimidine, and imidazodiazocine were achieved *via* condensation reaction of chromene derivative under the proper condition with various reagents namely; cyanothioacetamide, phenyl isothiocyanate, malononitrile, carbon disulfide, benzaldehyde, triethylorthoformate, and 1,4-dichlorobutane. Moreover, a chlorodiazenyl chromene derivative was reacted with some substances possessing active–CH_2-bridge such as ethyl cyanoacetate and malononitrile to end up with hydrazono compounds. Such compounds were eventually cyclized with hydrazine hydrate to form pyrazole and oxopyrazole derivatives. Moreover, compound 1 was treated with benzoyl acetone, and then followed by cyclization with malononitrile to provide the corresponding 2-amino14-(4-methoxyphenyl)-4-methy-5-phenyl-14H-benzo[5,6] chromeno [2,3H][1,6]naphthyridine-3-carbonitrile (20).

Results: The results of the antimicrobial screening *in vitro* revealed that the inhibition zone (mm) of the synthesized compounds 1-3, 5 and 8 implied their optimum antibacterial activity, while the compounds 4, 6 and 9-13, 15 showed a moderate to weak antibacterial activity against multiple species of *B. subtilis, S. aureus, E. coli and P. aeruginosa*. In contrast, the compounds 1, 6, 11, 15 showed high antifungal activities against different species of *A. flavinand C. albicans*, while the other compounds exhibit a moderate to poor antifungal activity.

Conclusion: It is remarkable that a series of chromene derivatives synthesized by a simple and available method leads to a molecule of promising antimicrobial activity. Further research is recommended to approve the importance of polycyclic systems for various applications.

Keywords: Chromene-3-carbonitrile, Chromenes, Polycyclic compounds, Pyrimidines, Fused ring, Antimicrobial activity

DOI: http://dx.doi.org/10.22159/ijpps.2019v11i9.34338

INTRODUCTION

The resistance of bacteria to antimicrobial drugs is a well-known phenomenon [1-2]. As a result, new drugs based on less toxic materials are needed and should be designed and developed. The chromene nuclei have drawn wide attention for many applications in the field of biology, chemotherapy, pharmacology, therapeutical chemistry, and materials science. Chromenes are consisting of benzene and pyrane fused ring called benzopyrans which used as starting materials for the synthesis of bioactive structures [3-25]. Since ten years ago, the chromene derivatives have been extensively used as active compounds to treat cancer [26, 27] inflammation [28], cardiovascular diseases (CVD) [29]. Moreover, they have been used as antimicrobial [30-32], antioxidant [33] antiviral [34], anthelminthic [35], anti-HIV [36], TNF-α-inhibitor [37], estrogenic [38, 39] antitubercular [40-41] herbicidal [42], anticonvulsant, antiparkinsonian [43, 44], antidepressant [45] and analgesic [46, 47] activity. Moreover, some of chromene scaffold are well known as antimicrobial drugs and their analogues such as novobiocin, chlorobiocin and coumermycin Al [48-50]. In the present research, we aimed to synthesize some new polycyclic compounds including chromene moiety and study their antimicrobial activity.

MATERIALS AND METHODS

Melting points were determined on the digital melting point apparatus (Electrothermal 9100, Electrothermal Engineering Ltd, serial No. 8694, Rochford, United Kingdom) and were uncorrected. IR data were measured on a Perkin-Elmer 1600 FTIR (Perkin-Elmer, USA) in KBr discs. ^{1}H and ^{13}C–NMR spectra were recorded with a Bruker Avance spectrometer (300 and 75MHz) (Bruker, Germany) in DMSO-d_6 and chemical shifts were determined in δppm to TMS as the standard internal solvent. Mass spectra (EI) were measured on GCMS-QP 1000Ex70ev, Schimadz spectrometer, Japan (EI) at Micro Analytical Center, Cairo University, Egypt. Elemental analysis was measured by using a Perkin-Elmer microanalysis at 24°C. The new derivatives were tested *in vitro* with respect to their antimicrobial activity at the Micro Analytical Center Faculty of Science, Cairo University. All reagents and solvents were of commercial grade.

Synthesis of 11-amino-12-(4-methoxyphenyl)-9-thioxo-9,12-dihydro-8*H*-benzo [5,6]chromeno[2,3-*b*]pyridin-10-carbonitrile (2)

Cyanothioacetamide (0.01 mol) was added to a mixture of compound **1** (0.01 mol) in ethanol (30 ml) including a few drops of piperidine. The reaction mixture was allowed to reflux for 8 h then cooled down, after that transferred onto ice-cold water, and neutralized with HCl (10 %). the filtration process was used to separate the resultant solid, which was cleaned and recrystallized from ethanol.

Synthesis of 11-imino-12-(4-methoxyphenyl)–10–phenyl, 10, 11, dihydro-8H-benzo [5,6] chromeno[2,3-*b*]pyrimidin-9-(12*H*) thione (3)

Phenyl isothiocyanate (0.01 mol) was added to the solution of compound **1** (0.01 mol) in dry dioxane (20 ml) including 1 ml pyridine for 10 h. the reaction mixture was heated under reflux. The separated solid was formed after cooling at 25 °C and neutralized with HCl (10 %). The precipitated product was filtered, washed with H_2O, dried and recrystallized from ethanol.

Synthesis of 9,11-diamino-12–(4-methoxyphenyl)-12*H*–benzo [5,6]chromeno[2,3-*b*]pyridin-10-carbonitrile (4)

A solution of compound **1** (0.01 mol) and malononitrile (0.01 mol) in absolute ethanol (30 ml) containing a few drops of triethylamine

was heated under reflux for 9 h. The obtained solid was filtered and recrystallized from ethanol.

Synthesis of 12-(4-methoxyphenyl)-8,12-dihydro-9H-benzo [5,6]chromeno[2,3-d]pyrimidin-9,11(10*H*)-dithione (5)

A mixture of compound **1** (0.01 mol) and an aqueous solution of 20 % KOH in dimethyl sulfoxide (20 ml) was stirred, and then carbon disulfide (0.03 mol) was added in several portions during 30 min, after 1 h at 25 °C. The precipitated solid was separated, washed many times with H_2O, and recrystallized from dimethylformamide.

Synthesis of 2-(4,5-dihydro-1*H*-imidazol-2-yl)-1-(4-methoxy-phenyl)-1*H*-benzo[*f*]-chromen-3-amine (6)

To a suspension of compound **1** (0.01 mol) in ethylenediamine (3 ml), carbon disulfide CS_2 (1 ml) was added dropwise and the reaction mixture was refluxed underwater bath control for 8 h. After cooling, the deposited solid was filtered off, dried, and recrystallized from benzene.

Synthesis of 14-(4-methoxyphenyl)-5-phenyl-3,5,6,14-tetra-hydro-2*H*-benzo[5,6]-chromeno[3,2-*e*]imidazo[1,2-c] pyrimidine (7)

A solution of compound **6** (0.01 mol) and benzaldehyde (0.01 mol) in absolute ethanol (50 ml) containing 0.1 ml of conc. HCl was refluxed for 14 h. After cooling, the separated solid was collected and recrystallized from ethanol.

Synthesis of 14-(4-methoxyphenyl)–2,3,6,14-tetrahydro-5-*H*-benzo[5,6]chromeno [3,2-e]imidazo [1,2-*c*]pyrimidin-5-thione (8)

A mixture of compound **6** (0.01 mol) and carbon disulfide CS_2 (10 ml) in dry pyridine (25 ml) was refluxed under water-bath for 22 h. After cooling, the reaction mixture was poured onto cold water. The formed solid was filtered off, dried and recrystallized from ethanol.

Synthesis of 14-(4-methoyphenyl)-3,14-dihydro-2*H*-benzo [5,6] chromeno[3,2-*e*] imidazo[1,2-*c*]pyrimidine (9)

A solution of compound **6** (0.01 mol), triethyl orthoformate (30 ml) and acetic anhydride (15 ml) were refluxed for 8h. The precipitate that obtained after cooling was filtered, dried and recrystallized from benzene.

Synthesis of 14-(4-methoxyphenyl)-2,3,6,14-tetrahydro-benzo [5,6]chromeno[3,2-*e*]imidazo[1,2-*c*]pyrimidin-5-one (10)

A solution of **6** (0.011 mol) with ethylchroformate (0.011 mol) in dry pyridine (30 ml) was refluxed for 1 h. The separated solid whichformed was filtered, dried and recrystallized from dioxane.

Synthesis of 14-(4-methoxyphenyl)-*N*-phyenyl-2,3-dihydro-14*H*-benzo[5,6]-chromeno [3,2-*e*]imidazo[1,2-*c*] pyrimidin-5-amine (11)

A solution of **6** (0.011 mol) and phenyl isothiocyanate (0.011 ml) in dry pyridine (10 ml) was heated under reflux for 7-8 h. After cooling, the precipitate was formed by pouring the mixture into cold water which filtered off, dried and recrystallized from DMF/ethanol.

Synthesis of 5-(chloromethyl)-14–(4–methoxyphenyl)–3,14-dihydro-2H-benzo [5,6]-chromeno[3,2-*e*]imidazo[1,2-*c*] pyrimidine (12)

A solution of compound **6** (0.011 mol) and chloroacetyl chloride (0.011 mol) in glacial acetic acid (30 ml) was heated under reflux overwater bath for 15 min. The precipitate that obtained by adding of aqueous sodium acetate was filtered off, dried and recrystallized from methanol.

Synthesis of 17-(4-methoxyphenyl)-2,3,5,6,7,8,16-octahydro-benzo[5,6]chromeno [3,2-e]imidazo[1,2-a][1,5]diazocine (13)

A solution of compound **6** (0.01 mol) potassium carbonate anhydrous (3.03 g), and1, 4-dichlorobutane (0.01 mol) in dimethylformamide (20 ml). The reaction mixture was stirred at 60 °C for 9 h. The reaction mixture was cooled down by adding cold water (50 ml), and then titrated with 10 % hydrochloric acid to reach pH7. The separated solid was collected by filtration, washed with distilled water and recrystallized from ethanol.

Diazotization of compound 1:Synthesis of 3–(chlorodiazenyl)–1–(4-methoxyphenyl)–1*H*-benzo[*f*]chromen-2-carbonitrile (14)

To a suspended solution of compound 1 (0.01 mol) in conc. HCl (3 ml), a solution of sodium nitrite (0.01 mol) in cold water (2 ml) was added over 15 min at 0-5 °C. The diazonium salt was used freshly in the next step.

General procedure for the synthesis of compounds 15, 17

The diazonium salt solution **14** (0.005 mol) was added slowly to a stirred solution of malononitrile and/or ethyl cyanoacetate (0.005 mol) in acetone (20 ml) at 0-5 °C. The reaction was left overnight under stirring at room temperature. The separated product was then collected by filtration and washed with ethanol.

(*E*)–3-(3,5-diamino-1*H*-pyrazol–4–y)diazenyl)-1-(4-methoxy-phenyl)–1*H*-benzo[*f*]chromeno-2-carbonitrile (16)

A solution of compound **15** (0.01 mol) and NH_2NH_2 99 % (5 ml) in ethanol (20 ml) was refluxed for 5 h. The excess solvent was evaporated under reduced pressure in vacuum. The separated solid was collected by filtration, air-dried and recrystallized from ethanol.

3–(2,(3-Amino-5-oxo-1,5-dihydro-4*H*–pyrazol–4–ylidene)-hydrazinyl)–1*H*-benzo[*f*]chromene–2–carbonitrile (18)

A mixture of compound **17** (0.01 mol) and NH_2NH_2 99 % (3 ml) in absolute ethanol (20 ml) was refluxed for 5 h. The precipitate that formed after cooling was filtered off, dried and recrystallized from absolute ethanol.

Synthesis of 1-(11-amino-12-(4-methoxyphenyl)-9-pyhenyl-12*H*-benzo [5,6]-chromeno[2,3-*b*]pyridin-10-yl)ethan-1-one (19)

The reaction mixture of an equimolar amount of compound **1** (0.012 mol) and benzoyl acetone (0.012 mol) in ethanol (20 ml) in the presence of sodium ethoxide was heated under reflux for 10 h. The mixture was cooled down and then poured onto cold H_2O. The formed solid was collected by filtration and recrystallized from ethanol.

Synthesis of 2-amino14-(4-methoxyphenyl)-4-methy-5-phenyl-14*H*-benzo [5,6] chromeno [2,3H][1,6]naphthyridine-3-carbonitrile (20).

A mixture of compound 19 (0.012 mol) and malononitrile (0.012 mol) was allowed to heat under reflux in ethanol (25 ml) including a few drops of piperidine as a catalyst for 16 h. The separated solid that obtained after cooling was filtered off, washed with cold ethanol and recrystallized from DMF/ethanol.

Antimicrobial evaluation

Antibacterial and antifungal activity of the newly synthesized compounds was determined *in vitro* by the disc diffusion method [51] using a nutrient agar medium against the following microorganisms, *Staphylococcus aureus* (RCMB 29213), *Bacillus subtilis* (RCMB 010010) (Gram-positive bacteria), *Escherichia coli* (RCMB 27853), *Pseudomonas aeuroginosa* (RCMB 25922) (Gram-negative bacteria) and fungal species like *Candida albicans* (RCMB 05036), *Asperigullusflavin* (RCMB 02568). All strains were provided from the culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. Ampicillin was the standard drug for antibacterial screening and amphotericin B was used as a standard for antifungal screening.

Method of testing

The sterilized media was poured onto the sterilized Petri dishes (20 ml, each Petri dish) and allowed to solidify. Wells of 6 mm diameter were made in the solidified media (Nutrient and MacConky agar media for bacteria and on Sabouraud dextrose agar for fungus.) with the help of sterile borer. A sterile swab was used to evenly distribute microbial suspension over the surface of solidified media and solutions of the tested samples (5 mg/ml) were added to each well with the help of micropipette. The inhibition zones (IZ) of the test compounds were

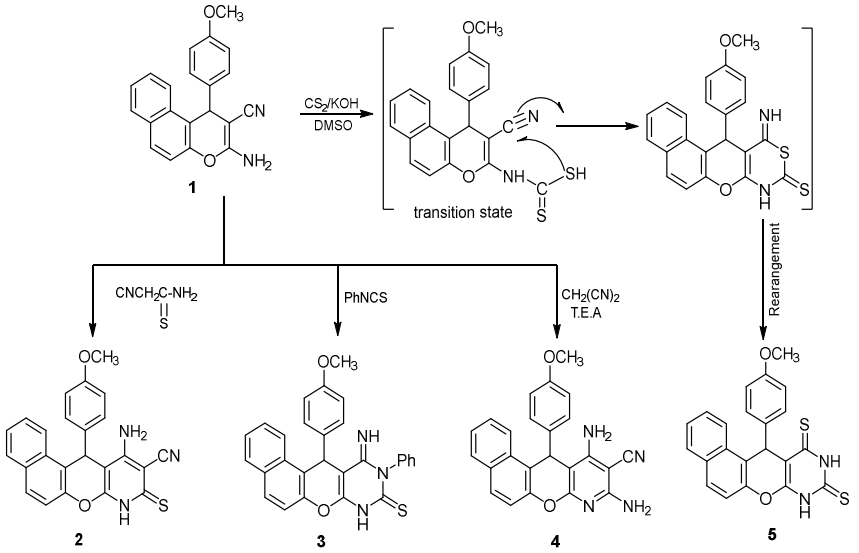
measured after 24-48 h incubation at 37 °C for bacteria and after 5 d incubation at 28 °C for fungi. The experiment was performed in triplicate and the average zone of inhibition was calculated.

RESULTS AND DISCUSSION

Chemistry

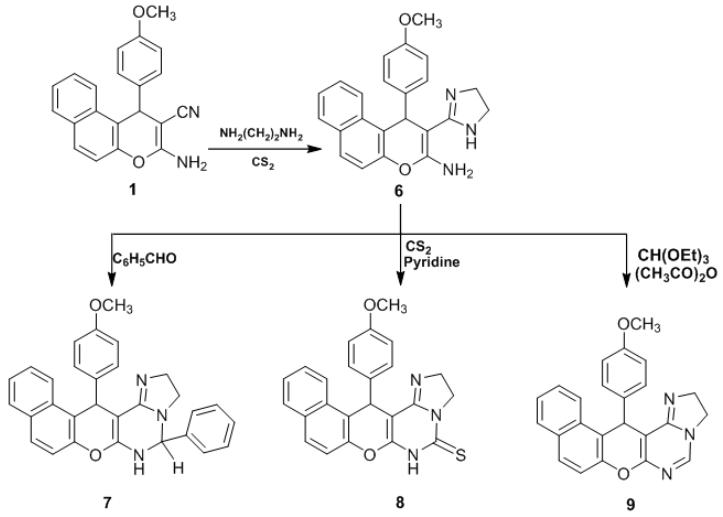
The pathway for the preparation of the target compounds is outlined under schemes 1-4. 2-Amino-1-(4-methoxyphenyl)-1*H*-benzo[*f*]chromene-3-carbonitrile (1) is the key compound in our study for the production of its functionalized derivatives [52]. Compound 1 was condensed with cyanothioacetamideunder basic condition to give11-amino-12-(4-methoxyphenyl)-9-thioxo-9,12-dihydro-8*H*-benzo[5, 6] chromeno[2,3-*b*]pyridin-10-carbonitrile (2) (Scheme 1). The NMR spectra revealed two D_2O replaceable main peaks at 6.9 and 8.3 ppm related to the presence of NH_2 and NH protons. Additionally, compound 1 was allowed to react with phenyl isothiocyanatein order to form 11-imino-12-(4-methoxyphenyl)-10-phenyl,10,11-dihydro-8*H*-benzo[5, 6]chromeno[2,3-*d*] pyrimidin-9-(12*H*) thione (3). The NMR spectra of compound 3 showed two D_2O changeable singles at 8.3 and 8.4 ppm corresponding to NH and =NH, respectively. Basic catalyzed reaction of 1 with malononitrile afforded 9,11-diamino-12-(4-methoxyphenyl)-14,12*H*-benzo [5,6] chromeno[2,3-*b*]pyridin-10-carbonitrile (4). Interaction of compound 1 with carbon disulfide in dimethyl sulfoxide gave 9-mercapto [2,3-*d*]pyrimidin-11-thione derivative (5) (Scheme 1). The IR spectrum of compound 5 implied characteristic peak at 3100 cm^{-1}which correlated to the NH group.

Furthermore, the reaction of 1 with ethylenediamine and a few drops of carbon disulfide ended up with 2-imidazolyl benzo [*f*]chromene derivative (6) (Scheme 2). The ^1H NMR spectrum of compound 6 indicated two D_2O replaceable peaks at δ 8.2 and 8.3 ppm corresponding to NH_2 and NH protons, respectively.



Scheme 1: Synthesis of benzo [5,6] chromeno [2,3-*b*]pyridine derivatives

Heating of compound **6** with benzaldehyde resulted in the corresponding 5-phenyl-benzochromenoimidazopyrimidine (**7)** as shown in Scheme 2. Moreover, benzochromeno-imidazopyridine-5-thione (**8)** was achieved by reaction of compound **6** with carbon disulfide as described in Scheme 2. The ^1H NMR spectrum of compound **8** showed signals at δ 6.8 ppm and δ 8.3 ppm attributed to pyridine-C_5 proton and NH proton. Polycyclic systems were achieved through the reaction of compound **6** with triethyl orthoformate in acetic anhydride that led to the formation of benzochromeno-imidazopyridine (9).

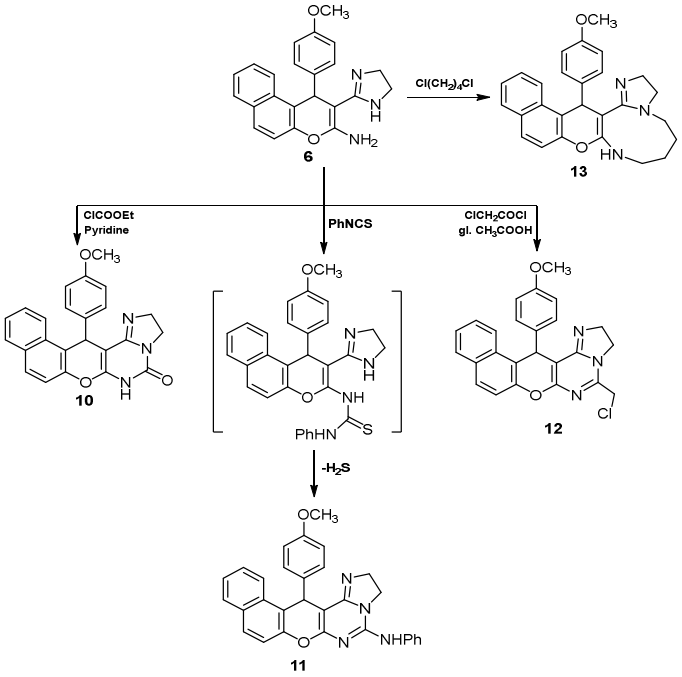


Scheme 2: Synthesis of benzo[5,6]-chromeno[3,2-*e*]imidazo[1,2-c]pyrimidine derivatives

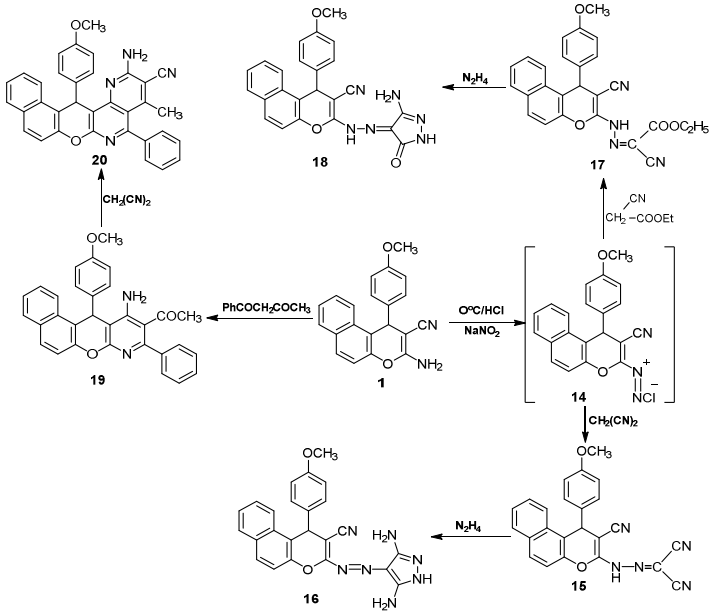
On the other hand, compound 6 was treated with ethyl chloroformate in pyridine to produce the imidazo[1,2-*c*]pyrimidin-5-one derivative (10). Imidazo [1,2-*c*]pyrimidin-5-amine derivative (11) was obtained *via* intermolecular cyclization of the compound 6 with phenyl isothiocyanate. Compound 6 was heated with chloroacetyl chloride in glacial acetic acid at 60 °C to provide 5-(chloromethyl)-14-(4-methoxyphenyl)-3,14-dihydro-2*H*-benzo [5,6] chromeno[3,2-*e*]imidazo[1,2-*c*]pyrimidine (12) (Scheme 3). IR data of compound 12 indicated a peak at υ 744.5 cm^{-1} for C-Cl and disappearance of NH_2 and NH groups that could be related to their involvement in the cyclization process. The mass spectrum of compound 12 showed a molecular ion peak at *m/z* 472 (M^+, 2%), 406 (M^++2, 8%), which indicate the presence of Cl atom. The cyclo condensation of compound 6 with 1,4-dichlorobutane in DMF in the presence of anhydrous potassium carbonate led to the formation of imidazo[1,2-a][1,5]diazocine derivative (13).

Benzochromene 2-diazonium chloride 14 was achieved by diazotization of compound 1 through its treatment with cold HCl and $NaNO_2$ aqueous solution. Coupling of compound 14 with active methylene compounds namely; malononitrile and ethylcyanoacetate led to the production of the hydrazono derivatives 15 and 17, respectively. Pyrazole derivatives 16 and 18 were created through the reaction of compounds 15 and/or 17 with hydrazine hydrate.

Treatment of compound 1 with benzoyl acetone in the presence of C_2H_5ONa led to the formation of 19, which on turn reacted with malononitrile under the basic condition to produce benzo [5,6] chromeno [3,2,-*h*] [1,6]naphtharidin-3-carbnitrile derivative (20) (Scheme 4).



Scheme 3: Synthesis of benzo[5,6]chromeno[3,2-*e*]imidazo[1,2-*c*] pyrimidin-5-amine (10, 11, 12) and 2,3,5,6,7,8,16-octahydro-benzo[5,6]chromeno[3,2-e]imidazo[1,2-a][1,5]diazocine (13)



Scheme 4: Synthesis of benzo[5,6]chromene (16, 18), benzo[5,6]chromeno[2,3-*b*] pyridine (19) and benzo[5,6]chromeno[2,3H][1,6]naphthyridine (20)

Spectral characterization of synthesized compounds

11-Amino-12-(4-methoxyphenyl)-9-thioxo-9,12-dihydro-8*H*-benzo[5,6]chromeno[2,3-*b*]pyridin-10-carbonitrile (2)

Black crystals; yield 83 %; mp 182 °C; Anal Calcd (%) for $C_{24}H_{17}N_3O_2S$ (411.48): C, 70.05; H, 4.16; N, 10.21; found (%) C, 70.1; H, 4.2; N; 10.1; IR (KBr, cm^{-1}): 3379, 3321 (NH, NH_2); 3001 for all (CH–aromatic); 2194 (C≡N); 1562 (C=C), 1273 (C=S); ^{1}H NMR (DMSO–d_6, δ ppm): 3.8 (s, 3H, OCH_3), 6.3 (s, 1H, pyran–H), 6.9 (s, 2H, NH_2), 7.1-7.9 (m, 10H, Ar–H), 8.3 (s, 1H, NH); [13]C–NMR δ 26.3, 53.9, 63.1, 105.9, 117.9, 118.1, 118.8, 121.01, 123.6, 127.1, 128.2, 128.5, 128.7, 129.7, 129.8, 129.9, 131.1, 136.6, 143.8, 150.1, 156.8, 161.8.

11-Imino-12-(4-methoxyphenyl)–10–phenyl,10,11,dihydro-8H-benzo[5,6]chromeno[2,3-*b*]pyrimidin-9-(12*H*)thione (3)

Buff crystals; yield 62 %; mp 132 °C; Anal Calcd (%) for $C_{28}H_{21}N_3O_2S$ (463.14): C, 72.55; H, 4.57; N, 9.06; found (%) C, 72.1; H, 4.6; N, 9.1.; IR (KBr, cm^{-1}): 3336, 3309 (NH), 3001 (CH–aromatic), 1689 (C=N), 1527 (C=C), 1277 (C=S); ^{1}H NMR (DMSO-d_6, δ ppm): 3.8 (s, 3H, OCH_3), 6.3 (s, H, pyran–H), 7.0-7.9 (m, 15H, Ar–H), 8.3 (s, 1H, NH, D_2O exchangeable), 8.5 (s, 1H, NH, D_2O exchangeable).

9,11-Diamino-12–(4-methoxyphenyl)-12*H*–benzo[5,6] chromeno[2,3-*b*]pyridin-10-carbonitrile (4)

White powder; yield 83 %; mp 142 °C; Anal Calcd (%) for $C_{24}H_{18}N_4O_2$ (394.14): C, 73.68; H, 4.60; N, 14.20; found (%) C, 73.1; H, 4.5; N, 14.1; IR (KBr, cm^{-1}): 3390; 3288 (NH_2), 3011 (CH–aromatic), 2200 (CN), 1610 (C=N), 1530 (C=C); ^{1}H NMR (DMSO–d_6, δ ppm): 3.8 (s, 3H, OCH_3), 6.3 (s, 1H, pyran–H), 6.8 (s, 2H, NH_2, D_2O exchangeable), 6.9 (s, 2H, NH_2. D_2O exchangeable), 7.1-7.9 (m, 10H, Ar–H).

12-(4-Methoxyphenyl)-8,12-dihydro-9H-benzo[5,6]chromeno [2,3-d]pyrimidin-9,11(10*H*)-dithione (5)

White powder; yield 55 %; mp>300 °C; Anal Calcd (%) for $C_{22}H_{16}N_2O_2S_2$ (404.07): C, 65.32; H, 3.99; N, 6.93; found (%) C, 65.1; H, 3.5; N, 6.7; IR (KBr, cm^{-1}): 3100 (NH), 3012 (CH–aromatic), 1609 (C=N), 1531 (C=C); ^{1}H NMR (DMSO-d_6, δppm): 3.8 (s, 3H, OCH_3), 6.3 (s, 1H, pyran–H), 7.01-7.8 (m, 10H, Ar–H), 8.1(s,1H, NH, D_2O, exchangeable), 8.3 (s, 1H, NH, D_2O exchangeable).

2-(4,5-Dihydro-1*H*-imidazol-2-yl)-1-(4-methoxyphenyl)-1*H*-benzo[*f*]-chromen-3-amine (6)

Black brown powder; yield 65 %; mp 152 °C; Anal Calcd (%) for $C_{23}H_{21}N_3O_2$ (371.16): C, 74.37; H, 5.70; N, 11.31; found (%): C, 74.1; H, 5.6; N, 11.1; IR (KBr, cm^{-1}): 3360, 33.48 (NH, NH_2), 3002 (CH-aromatic), 1604 (C=N), 1573 (C=C); (^{1}H NMR-d_6, δ ppm): 3.8 (s, 3H, OCH_3) 6.3 (s, 1H, pyran-H) 6.55-6.57 (m, 2H, imidazolidine-C_4–H), 6.80-7.00 (m, 2H, imidazolidine–C_5–H), 7.1-7.9 (m, 10H, Ar–H), 8.2 (s, 2H, NH_2, D_2O exchangeable); 8.3 (s, 1H, NH, D_2O exchangeable);[13]C, NMR (DMSO-d_6, δ ppm):, 21.9, 55.6, 99.5, 113.2, 118.9, 119.2, 120.6, 121.9, 122.1, 122.5, 123.2, 123.7, 125.4, 126.3, 127.9, 128.3, 143.5, 158.6, 159.1, 160.1, 160.2.

14-(4-Methoxyphenyl)-5-phenyl-3,5,6,14-tetrahydro-2*H*-benzo[5,6]chromeno[3,2-*e*]imidazo[1,2-c]pyrimidine (7)

White crystal; yield 82 %; mp>300 °C; Anal Calcd (%) for $C_{30}H_{25}N_3O_2$ (459.54): C, 78.41; H, 5.48; N, 9.14; found (%): C, 78.1; H, 5.3; N, 9.2. IR (KBr, cm^{-1}): 3320 (NH); 3005, (CH-aromatic); 1561 (C=N); 1510 (C=C);^{1}H NMR (DMSO-d_6, δ ppm); 3.1 (s, 3H, OCH_3), 3.6 (m, 2H, imidazo-C_2-H), 3.8 (m, 2H, imidazo–C_3-H), 6.3 (s, 1H, pyran–H),, 6.8 (s, H, pyrimidine-H), 7.04–7.5 (m, 15H, Ar–H), 8.5 (s, H, NH, D_2O exchangeable).

14-(4-Methoxyphenyl)–2,3,6,14-tetrahydro-5-*H*-benzo [5,6]chromeno[3,2-e]imidazo [1,2-*c*]pyrimidin-5-thione (8)

Brown powder; yield 70 %; mp 222 °C; Anal Calcd (%) for $C_{24}H_{19}N_3O_2S$ (413): C, 68.7; M, 4.6; N, 10.1; found (%): C, 69.6; H, 4.5; N, 10.2.; IR (KBr, cm^{-1}): 3241 (NH); 3000 (CH–aromatic); 1650 (C=N); (C=C), 1447 (C=S);^{1}H NMR (DMSO-d_6, δ ppm): 3.1 (s, 3H, OCH_3), 3.6 (m, 2H, imidazo–C_2-H), 3.8 (m, 2H, imidazo-C_3-H), 6.3 (s, 1H, pyran–H), 7.05-7.9 (m, 10H, Ar–H), 8.4 (s, 1H, NH, D_2O exchangeable).

14-(4-Methoyphenyl)-3,14-dihydro-2*H*-benzo[5,6]chromeno [3,2-*e*] imidazo[1,2-*c*] pyrimidine (9)

Brown powder; yield 75 %: mp 252 °C; Anal Calcd (%) for $C_{24}H_{19}N_3O_2$ (381.15): C, 75.57; H, 5.02; N, 11.02; found (%): C, 75.1; H, 5.1; N, 11.1; IR (KBr, cm^{-1}): 3008 (CH-aromatic); 1601 (C=N), 1573 (C=C); (^{1}H NMR–d_6, δ ppm): 3.1 (s, 3H, OCH_3), 3.6 (m, 2H, imidazo-C_2H), 3.8 (m, 2H, imidazo-C_3–H), 6.1 (s, 1H, pyran-H), 6.9 (s, H, pyrimidine–H), 7.11-7.58 (m, 10H, Ar–H);[13]C NMR: δ 35.1, 46.1, 55.1, 57.2, 109.7, 117.2, 117.3, 119.8, 120.8, 120.9, 121.6, 121.8, 128.7, 129.4, 129.8, 129.9, 130.1, 132.2, 135.1, 135.7, 137.7, 138.8, 140.1, 140.2.

14-(4-Methoxyphenyl)-2,3,6,14-tetrahydro-benzo[5,6] chromeno[3,2-*e*]imidazo[1,2-*c*]pyrimidin-5-one (10)

White crystals; yield 65 %; mp>300 °C; Anal Calcd (%): for $C_{24}H_{19}N_3O_3$ (397.14): C, 72.53; H, 4.82; N, 10.57; found (%): C, 72.4; H, 4.7; N, 10.4; IR (KBr, cm^{-1}): 3007 (CH-aromatic), 1602 (C=O), 1553 (C=C); ^{1}H NMR (DMSO-d_6, δ ppm): 3.8 (s, 3H, OCH_3), 6.1 (s, H, pyran–H), 5.4 (m, 2H, imidazo–C_2-H), 6.1 (m, 2H, imidazo-C_3-H), 7.01-7.6 (m, 10H, Ar–H) 8.3 (s, H, NH, D_2O exchangeable).

14-(4-Methoxyphenyl)-*N*-phyenyl-2,3-dihydro-14*H*-benzo [5,6]chromeno[3,2-*e*]imidazo[1,2-*c*] pyrimidin-5-amine (11)

Brown crystals; yield 65 %; mp 287 °C; Anal Calcd (%) for $C_{30}H_{24}N_4O_2$ (472.19): C, 76.25; H, 5.12; N, 11.80; found (%). C, 76.1; H, 5.1; N, 11.7; IR (KBr, cm^{-1}): 3320 (NH), 3007 (CH–aromatic), 1602 (C=N), 1510 (C=C);^{1}H NMR (DMSO-d_6, δ ppm): 3.8 (s, 3H, OCH_3), 6.1 (s, 1H, pyran–H), 5.3 (m, 2H, imidazo-C_2–H), 6.5 (m, 2H, imidazo C_3–H), 7.1–7.9 (m, 15H, Ar–H), 8.3 (s, H, NH, D_2O exchangeable);[13]C (DMSO-d_6, δ ppm): 36.2, 56.0, 57.6, 109.8, 117.1, 117.2, 119.7, 120.9, 121.0, 123.6, 123.6, 125.3, 127.5, 128.8, 129.4,129.5, 129.6, 129.8, 129.9, 130.1, 132.2, 142.7, 142.8, 147.1, 160.1, 162.1

5-(Chloromethyl)-14–(4–methoxyphenyl)–3,14-dihydro-2H-benzo[5,6]chromeno[3,2-*e*]imidazo[1,2-*c*]pyrimidine (12)

Brown crystal; yield 60 %; mp>300 °C; Anal Calcd (%) for $C_{25}H_{20}ClN_3O_2$ (429.90) C; 69.85; H, 4.69; N, 9.77; found (%): C; 69.7; H, 4.5; N, 9.6; IR (KBr, cm^{-1}): 3001 (CH–aromatic), 1601 (C=N), 1510 (C=C), 745 (C–Cl);^{1}H NMR (DMSO–d_6, δ ppm): 3.1 (s, 3H, OCH_3, 3.6 (m, 2H, imidazo-C_2–H), 3.8 (m, 2H, imidazole–C_3–H), 6.3 (s, 1H, pyran–H), 5.1 (s, 2H, CH_2–Cl), 7.1-7.9 (m, 10H, Ar–H)

17-(4-methoxyphenyl)-2,3,5,6,7,8,16-octahydro-benzo [5,6]chromeno[3,2-e]imidazo[1,2-a][1,5]diazocine (13)

Brown crystals; yield 83 %; mp 177 °C; Anal Calcd (%) for $C_{27}H_{27}N_3O_2$ (425.21): C, 76.19; H, 6.3; N, 9.8; found (%): C, 76.1; H, 9.2; N, 9.1; IR (KBr, cm^{-1}) 3332 (NH); 3005 (CH–aromatic); 1651 (C=N), 1521(C=C); ^{1}H NMR (DMSO–d_6, δppm): 3.1 (m 2H, C-$_6$–H), 3.3 (m, 2H, C_7–H), 3.8 (s, 3H, OCH_3), 4.6 (m, 2H, C_5–H), 5.1 (s, H, pyran–H), 5.3 (m, 2H, imidazo–C_2–H), 6.5 (m, 2H, imidazo-C_3–H) 7.17-7.91 (m, 10H, Ar–H), 8.1 (s, H, NH, D_2O exchangeable).

***N*-(2-Cyano-1-(4-methoxyphenyl)-1*H*-benzo[*f*]chromen-3-yl) carbonohydrazonoyldicyanide (15)**

Brown crystals; yield 75 %; mp 132 °C; Anal Calcd (%) for $C_{24}H_{15}N_5O_2$ (405.12): C, 71.10; H, 3.73; N, 17.27; found (%): C, 71.2; H, 3.5; N, 17.1; IR (KBr, cm^{-1}) 3332 (NH), 3001 (CH–aromatic), 2198 (C≡N); 1643 (C=N) 1504 (C=C);^{1}H NMR (DMSO-$d_6$$\delta$ppm): 3.8 (s, 3H, OCH_3), 4.3(s, 1H, pyran–H), 7.1–7.9 (m, 10H, Ar–H), 8.3 (s, H, NH, D_2O exchangeable).

(*E*)–3-(3,5-Diamino-1*H*-pyrazol–4–y)diazenyl)-1-(4-methoxyphenyl)–1*H*-benzo[*f*]chromeno-2-carbonitrile (16)

Brown powder; yield 57 %; mp 212 °C; Anal Calcd (%) for $C_{24}H_{19}N_7O_2$ (437.16): C, 65.89; H, 4.38; N, 22.41; found (%): C, 65.7; H, 4.1; N, 22.3; IR (KBr, cm^{-1}): 3387, 3336, 3208 (NH, NH_2) 2187 (CN), 1651 (C=N), 1562 (C=C), 1408 (N=N);^{1}H NMR (DMSO-d_6, δ ppm): 3.8 (s, 3H, OCH_3), 6.1 (s, 1H, pyran–H), 6.9 (s, 1H, NH, pyrazole-NH, D_2O exchangeable), 7.1-7.8 (m, 10H, Ar–H), 8.4 (s, 4H, $2NH_2$, pyrazole, D_2O exchangeable).

(*Z*) Ethyl-2-cyano-2-(2-(2-cyano-1-(4-methoxyphenyl)-1*H*-benzo [*f*] chromene-3-yl) hydrazono) acetate (17)

Brown crystals; yield 69 %; mp 122°C; Anal Calcd (%)| for $C_{26}H_{20}N_4O_4$ (452.46): C, 69; H, 4.4; N, 12.3; found (%): C, 68.1; H, 4.3; N, 12.3; IR (KBr, cm^{-1}): 3357 (NH): 3008 (CH-aromatic), 2194 (CN); 1698 (C=O), 1612 (C=N), 1573 (C=C);^{1}H NMR (DMSO–d_6, δ ppm): 1.2 (t, 3H, CH_3, J = 7.2 Hz), 4.6 (q, 2H, CH_2, J = 7.2 Hz), 3.8 (s, 3H, OCH_3), 6.1 (s, 1H, pyran–H), 7.05-7.8 (m, 10H, Ar–H), 8.6 (s, H, NH, D_2O exchangeable).

3–(2,(3-Amino-5-oxo-1,5-dihydro-4*H*–pyrazol–4–ylidene)-hydrazinyl)–1*H*-benzo[*f*]chromene–2–carbonitrile (18)

Brown crystal; yield 65 %; mp 232 °C; Anal Calcd (%) for $C_{24}H_{18}N_6O_3$(438.14): C; 65.75; H, 4.14; N, 19.17; found (%): C; 65.6; H, 4; N, 19.2; IR (KBr, cm^{-1}): 3325, 3209 (NH, NH_2), 2197 (CN), 1680 (C=O), 1602 (C=N), 1581 (C=C); ^{1}H NMR (DMSO–d_6, δ ppm): 3.8 (s, 3H, OCH_3), 6.01 (s, 1H, pyran–H) 7.01-7.6 (m, 10H, Ar-H), 8.1 (s, 2H, pyrazole–C_3-NH_2, D_2O exchangeable); 8.2 (s, 1H, pyrazole–NH, D_2Oexchangeable), 8.3 (s, 1H, C_3–NH, D_2O exchangeable).

1-(11-Amino-12-(4-methoxyphenyl)-9-pyhenyl-12*H*-benzo[5,6]chromeno[2,3-*b*]pyridin-10-yl)ethan-1-one (19)

Pale bag powder; yield 45 %; mp 122 °C; Anal Calcd (%) for $C_{31}H_{24}N_2O_3$ (472.18): C, 78.78; H, 5.08; N, 5.92; found (%): C, 78.3; H, 5.4; N, 5.8; IR (KBr, cm^{-1}): 3325 (NH_2), 3001 (CH–aromatic), 1712 (C=O), 1666 (C=N), 1563 (C=C);^{1}H NMR (DMSO–d_6, δ ppm): 3.3 (s, 3H, $COCH_3$), 3.8 (s, 3H, OCH_3), 6.1 (s, 1H, pyran–H), 7.1-7.8 (m, 15H, Ar–H), 8.6 (s, 2H, NH_2, D_2O exchangeable).

2-Amino14-(4-methoxyphenyl)-4-methy-5-phenyl-14*H*-benzo [5,6]chromeno-[2,3H][1,6]naphthyridine-3-carbonitrile (20)

Brown powder; yield 82 %; mp>300°C; Anal Calcd (%) for $C_{34}H_{24}N_4O_2$ (520.19): C, 78.44; H, 4.65; N, 10.76; found (%): C, 78.3; H, 4.5; N, 10.6; IR (KBr, cm^{-1}): 3320 (NH_2), 3002 (CH-aromatic), 2212 (CN), 1656 (C=N), 1560 (C=C);^{1}H NMR(DMSO–d_6, δ ppm) 3.02 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 6.3 (s, 1H, pyran–H), 7.1-7.9 (m, 15H, Ar–H), 8.3(s, 2H, NH_2);[13]C NMR: δ 23.1, 55.1, 63.2, 117.1, 118.1, 119.1, 123.2, 125.3, 128.1, 128.8, 28.9, 130.1,130.2,130.6, 130.7, 130.9, 140.1, 140.2, 140.5, 140.6, 140.7, 140.8, 140.9, 141.2, 147.1, 150.3, 151.1, 152.1, 153.1, 160.1.

Antimicrobial activity

The screening results are tabulated in table 1. Among all the tested compounds, 1-3, 5 and 8 implied their optimum antibacterial activity while the compounds 4, 6 and 9-13, 15 showed a moderate to weak antibacterial activity against *B. subtilis*, *S. aureus, E. coli* and *P. aeruginesa*. In contrast, the compounds 1, 6, 11, 15 are highly active against *A. flavin* and *C. albicans* while the other compounds exhibit a moderate to weak activity.

Table 1: Antimicrobial activity of most active compounds

Antimicrobial activity of most active compounds						
Inhibition zone (mm)±Standard deviation[a]						
Compd. No.	***B. subtilis*(RCMB 010010)**	***S. aureus* (RCMB 29213)**	***E. coli* (RCMB 27853)**	***P. aeruginosa* (RCMB 25922)**	***C. albicans* (RCMB 05036)**	***A. fumigatus* (RCMB 02568)**
	Test compounds 5 mg/ml					
1	19.0±0.00	20.1±0.12	23.0±0.17	18.5±0.07	13.2±0.25	15.7±1.4
2	12.1±0.17	14.1±.05	20.1±0.04	18.1±0.25	11.0±0.77	14.7±0.25
3	24.6±0.05	20.5±0.05	19.1±0.1	21.7±0.44	NA	NA
4	11.5±1.19	10.1±0.17	10.0±0.11	12.4±0.77	11.1±0.32	10.1±0.97
5	18.0±0.07	19.0±0.24	12.2±0.17	12.7±0.25	13.3±0.74	11.6±0.54
6	9.09±0.17	10.1±0.1	10.2±0.25	0.0	14.1±0.14	16.0±0.21
7	9.9±0.11	9.1±0.51	13.4±0.14	NA	13.5±1.4	12.1±0.13
8	16.4±0.19	14.4±0.12	17.4±0.18	20.8±0.77	NA	NA
9	9.8±0.15	10.2±0.00	11.1±0.90	9.2±0.84	10.4±1.5	NA
10	10.0±0.05	9.7±0.10	12.7±0.25	NA	11.7±0.97	10.7±1.5
11	8.9±0.01	NA	10.1±0.05	12.0±0.57	16.6±1.5	12.5±0.01
12	15.0±0.06	11.1±0.11	NA	13.7±0.55	11.0±0.52	NA
13	9.5±0.01	11.5±0.04	9.5±0.14	12.7±0.91	8.5±0.97	NA
15	15.1±.03	12.2±0.51	11.6±0.26	13.1±0.25	11.2±0.97	13.4±1.2
Ampicillin	26.1±0.01	21.4±0.91	25.9±0.70	26.0±0.81	NA	NA
Amphotericin B	NA	NA	NA	NA	17.4±0.58	21.7±1.2

a: each value is the mean of three values, NA: no activity, RCMB: The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt

Structure-activity relationship (SAR)

Based on previously obtained antimicrobial activity of chromene derivatives, we performed the SAR studies on their activity against B. subtilis, S. aureus, E. coli and P. aeruginosa, A. flavin and C. albicans. By inspection of the experimental results of the antimicrobial activity of the synthesized chromene derivatives, the following structural activity relationship assumptions are suggested.

1-The pyrimidine moiety with incorporated with a sulfur atom in compounds 3, 5 and 8 is necessary to have higher antibacterial activities towards *B. subtilis, S. aureus, E. coli* and *P. aeruginesa.*

2-It is interesting to point out that imidazole moiety in compounds 6, 11 and 15 is necessary to have higher antifungal activity against *A. flavin* and *C. albicans*. The studies also emphasize the important role of cyano group in compound 1 in antimicrobial activity, which led to design new potentially active chromene, antimicrobial agents. In general, the above-mentioned results suggest that the new chromene derivatives may provide valuable leads for the synthesis and development of novel antifungal agents

CONCLUSION

In conclusion, various chromene derivatives were synthesized in efficient and easy process. Our study focused on the synthesis of new heterocyclic compounds as more powerful antimicrobial activities. In this research, we have shown the preparation procedure of new synthesized heterocyclic compounds containing a benzochromene moiety and evaluated the antimicrobial activities of all the innovative synthesized derivatives. Antimicrobial results revealed that compounds 4, 6 and 9-13, 15 showed a moderate to weak antibacterial activity against *B. subtilis*, *S. aureus, E. coli* and *P. aeruginesa*. In contrast, the compounds 1, 6, 11, 15 have higher activity towards *A. flavin* and *C. albicans* while the other compounds exhibit a moderate to weak activity. The chemical configurations of the newly synthesized compounds were clarified by spectroscopic techniques such as IR, NMR, and mass spectral data and elemental analysis.

ACKNOWLEDGMENT

Faculty of Science, Cairo University, microanalytically center for carrying out the biological screening.

AUTHORS CONTRIBUTIONS

EMAN S. ZARIE conceived the idea, supervised the experiments, analyzed the results and drafted the manuscript. MAHMOUD N. ABDELAZIZ performed the experiments. ALAADIN E. SARHAN contributed to the idea development.

CONFLICTS OF INTERESTS

Authors declare no conflicts of interest

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