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Synthesis and Antimicrobial Activities of 2*H*-Chromene-3-Carboxamide Derivatives: Experimental and Computational Studies

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ABSTRACT

Coupling reaction between Baylis–Hillman-derived 2*H*-chromene-3-carboxylic acid derivatives and some amines afforded the corresponding 2*H*-chromene-3-carboxamides. The synthesized carboxamides and their acid precursors were screened for their in vitro antifungal and antibacterial activities using nystatin and streptomycin, respectively, as standard drugs. Among the tested compounds, it has been found that compounds **3a**, **3c**, and **4c** (minimum inhibitory concentration = 0.062 mg/mL) exhibited better activities than the reference drug, streptomycin (minimum inhibitory concentration = 0.125 mg/mL) against *Bacillus cereus*. Compound **4a** showed the best inhibitory profile against gram-negative bacterial strains, while compound **4b** appeared to be the most active against fungal strains *Candida albicans* and *Aspergillus niger*. Molecular quantum chemical calculations suggested that the activities of the compounds against gram-negative bacterial strains could have some correlations with the electron-donating abilities of the molecules, while their activities against gram-positive bacterial strains showed some correlations with the electron-accepting abilities of the molecules.

Keywords: 2*H*-chromene-3-carboxylic acid, 2*H*-chromene-3-carboxamide, dicyclohexylcarbodiimide, streptomycin, nystatin

INTRODUCTION

Multidrug resistance remains a major problem in the treatment of bacterial infections and necessitates the need for the development of new therapeutic agents with improved activity against microbial agents. Benzopyrans occur ubiquitously in plants, and members of this family have displayed interesting biological activities.[1,2] Though less common than other benzopyran compounds, 2*H*-chromene (2*H*-1-benzopyran) possesses

an array of pharmacological effects.[3] Both natural and synthetic chromene derivatives have been reported to exhibit antitumor, antivascular,[4] antimicrobial, antifungal,[5,6,7] and antioxidant activities.[8] 2*H*-chromene-3-carboxylic acid derivatives have been reported as analgesic, inflammatory agents and endothelin A-receptor antagonists.[9,10] Various synthetic methods have led to the preparation of these multifunctional compounds such as ring-closing metathesis,[11,12,13] alkaline hydrolysis of 3-cyano-2*H*-chromenes,[14] Baylis–Hillman reactions of salicylaldehydes and acrylonitrile, and recently by chemo-selective base-mediated cyclization of *tert*-butyl acrylate-derived Baylis–Hillman adducts.[15]

In continuation of our interest in the development of new antimicrobial agents[16] and investigation of their mode of action, the present work reports the synthesis of some 2*H*-chromene-3-carboxamides with observed antimicrobial activities. The 2*H*-chromene-3-carboxamides were synthesized *via* the Baylis–Hillman methodology, characterized using infrared (IR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopic techniques. Antimicrobial activities of the synthesized compounds were evaluated using the agar-well diffusion method, while molecular-based explanations to the biological activities of the compounds were sought with theoretical density functional theory calculations.

MATERIALS AND METHODS

Melting points were measured on a Gallenkamp electrothermal melting point apparatus. IR spectra were recorded on a PerkinElmer spectrum 100 Fourier Transform Infrared (FTIR) spectrometer. NMR spectra were recorded on Bruker AMX 400 and BioSpin 600 spectrometers at 303 K in DMSO-d_6 or $CDCl_3$ and calibrated using solvent signals δ_H: 7.26 for residual $CDCl_3$, δ_H: 2.50 for residual DMSO-d_6, δ_C: 77.0 ($CDCl_3$), and 39.5 ppm (DMSO-d_6). Column chromatography was carried out using Merck silica gel 60-200 mesh size and a mixture of *n*-hexane:ethyl acetate as eluent. Thin-layer chromatography was carried out using precoated Merck silica gel 60 F_{254} plates and viewed under UV light at 254/365-nm wavelength.

General Procedure for the Synthesis of *Tert*-Butyl-3(2-Hydroxyphenyl)-Methylenepropanoate Esters 2a-c

Baylis–Hillman adducts **2a-c** were synthesized by reacting salicylaldehyde (**1a-c**) derivatives with *tert*-butyl acrylate using diazabicyclo[2.2.2]octane (DABCO) according to literature procedure.[17] A mixture of salicylaldehyde (3.5 mL, 33 mmol), *tert*-butyl acrylate (4.8 mL, 33 mmol), and 0.5 g (4.5 mmol) of DABCO catalyst in 21-mL $CHCl_3$ was sealed in a round-bottomed flask and left to stir for 3 weeks at room temperature. The mixture of products obtained were separated using column chromatography with a mixture of hexane-EtOAc: 9:1 as eluent to give *tert*-butyl-3-hydroxy-3-(2-hydroxyphenyl)-2-methylenepropanoate **2a** as a white solid (2.3 g, 28%), m.p. 109°C–111°C (Lit. 108°C–110°C).[17]

Compound **2b** was obtained as a white solid (4.27 g, 47%), m.p. 178°C–181°C (Lit. 185°C–187°C).[17]

Compound **2c** was obtained as a white solid (4.3 g, 44%), m.p. 182°C–185°C (Lit. 186°C–188°C).[17]

The ^1H and ^{13}C NMR data of compounds **2a-c** agreed with the reported literature data.[17]

General Procedure for the Synthesis of 2*H*-Chromene-3-Carboxylic Acid Analogues 3a-c

2*H*-chromene-3-carboxylic acid analogues **3a-c** were prepared using the method of

Faridoon et al. (2015) via base-mediated cyclization of Baylis–Hillman adducts **2a-c** (Scheme 1).[15] Thus, the synthesis of **3a** has been achieved by boiling a mixture of *tert*-butyl-3-hydroxy-3-(2-hydroxyphenyl)-2-methylenepropanoate **2a** (1.0 g, 4 mmol) and KOH (0.896 g, 16 mmol) in 16 mL of water under reflux for 46 h. After 46 h, the solution was left to cool at room temperature, and then, the reaction mixture was acidified with 8 mL of 2-M HCl. This was left to precipitate, filtered, washed with water, and dried to afford 2*H*-chromene-3-carboxylic acid **3a** as a yellow solid (0.26 g, 37%), m.p. 189°C–192°C (Lit. 192°C–194°C).[11]

Compound **3b** was obtained as a yellow solid (0.46 g, 62.4%), m.p. 234°C–236°C (Lit. 240°C–241°C).[14]

Compound **3c** was obtained as a brown solid (0.43g, 56%), m.p. 238°C–240°C (Lit. 247°C–248°C).[15]

The ^{1}H and ^{13}C NMR data of compounds **3a-c** agreed with the reported literature data.[11,14,15]

Synthesis of *N*-Benzyl-2*H*-Chromene-3-Carboxamide 4a

2*H*-Chromene-3-carboxylic acid **3a** (200 mg, 1.14 mmol) and dicyclohexylcarbodiimide (DCC; 260 mg, 1.25 mmol) were dissolved in 5 mL of dichloromethane and allowed to cool in an ice bath for 30 minutes; then, benzylamine (122 mg, 1.14 mmol) was added, and the mixture was stirred continuously for 16 h. The mixture was then filtered and washed with dichloromethane, and the filtrate was concentrated to obtain a brown solid **4a** (170 mg, 56.5%), m.p. 153°C–156°C. IR spectrum (neat, ν_{max} cm^{-1}) 3300 cm^{-1} due to secondary N-H stretching frequency, 1631 cm^{-1} due to C=O stretching of a conjugated amide, olefinic C=C stretch at 1573 cm^{-1}, bands at 1242 and 1064 cm^{-1} are due to C-N stretching of amide.

Synthesis of *N*-Benzyl-6-Chloro-2*H*-Chromene-3-Carboxamide 4b

6-Chloro-2*H*-chromene-3-carboxylic acid **3b** (200 mg, 0.95 mmol) and DCC (215 mg, 1.05 mmol) were dissolved in 5 mL of dichloromethane and allowed to cool in an ice bath for 30 minutes; then, benzylamine (102 mg, 0.95 mmol) was added, and the mixture was stirred continuously for 16 h. The mixture was then filtered and washed with dichloromethane, and the filtrate was concentrated to obtain a yellow solid (93 mg, 32.7%), m.p. 159°C–162°C. IR spectrum (neat, ν_{max} cm^{-1}) 3323 cm^{-1} due to secondary N-H stretching frequency, 1627 cm^{-1} due to C=O stretching of a conjugated amide, olefinic C=C stretch at 1573 cm^{-1}, bands at 1228 and 1153 cm^{-1} are due to C-N stretching of amide.

Synthesis of *N*-Benzyl-6-Bromo-2*H*-Chromene-3-Carboxamide 4c

6-Bromo-2*H*-chromene-3-carboxylic acid **3c** (200 mg, 0.78 mmol) and DCC (178 mg, 0.86 mmol) were dissolved in 5 mL of dichloromethane and allowed to cool in an ice bath for 30 minutes; then , benzylamine (84 mg, 0.78 mmol) was added, and the mixture was stirred continuously for 16 h. The mixture was then filtered and washed with dichloromethane, and the filtrate was concentrated to obtain a yellow solid (81 mg, 30.3%), m.p. 164°C–166°C. IR spectrum (neat, ν_{max} cm^{-1}) 3325 cm^{-1} due to secondary N-H stretching frequency, 1624 cm^{-1} due to C=O stretching of a conjugated amide, olefinic C=C stretch at 1573 cm^{-1}, bands at 1205 and 1128 cm^{-1} are due to C-N stretching of amide.

Synthesis of 2*H*-Chromene-3-Carboxamide 4d

2*H*-Chromene-3-carboxylic acid **3a** (0.5 g, 2.85 mmol) and DCC (0.65 g, 3.125 mmol) were dissolved in 7 mL of dichloromethane

and allowed to cool in an ice bath for 30 minutes; then, ammonia (48.5 mg, 2.85 mmol) was added, and the mixture was stirred continuously for 16 h. The mixture was then filtered and washed with dichloromethane, and the filtrate was concentrated to obtain a brownish-red solid (187 mg, 37.6%), m.p. 137°C–141°C. IR spectrum (neat, ν_{max} cm^{-1}) 3435 and 3385 cm^{-1} due to primary N-H stretching, 1660 cm^{-1} due to C=O stretching of a conjugated amide, olefinic C=C stretch at 1610 cm^{-1}, bands at 1242 and 1172 cm^{-1} are due to C-N stretching of amide.

Antimicrobial Evaluation

1. Sensitivity Testing

The synthesized compounds were evaluated for antimicrobial activity using the agar-well diffusion method as described in literature.[18,19] Most of the test organisms were collected from the National Collection for Industrial Bacteria (NCIB) while few were locally isolated organisms (LIO). The gram-positive bacteria utilized were *Bacillus cereus* (NCIB 6349), *Clostridium sporogenes* (NCIB 532), *Pseudomonas aeruginosa* (NCIB 950), *Bacillus stearothermophilus* (NCIB 8222), *Streptococcus faecalis* (NCIB 775), and *Bacillus anthracis* (LIO), and the gram-negative ones used were *Escherichia coli* (NCIB 86), *Klebsiella pneumoniae* (NCIB 418), *Proteus vulgaris* (LIO), and *Shigella spp* (LIO). The compounds were also evaluated in vitro for their antifungal activity against *Aspergillus niger* (LIO), *Aspergillus fumigatus* (LIO), and *Candida albicans* (LIO) fungal strains. A standardized sterile disc (6-mm diameter) impregnated with a solution of the test compound in dimethyl sulphoxide (DMSO) (1 mg/mL) was placed on an agar plate seeded with the appropriate test organism in triplicates. The bacterial isolates were first grown in nutrient broth for 18 h before use, while the fungal isolates were allowed to grow on potato dextrose agar medium (PDA) at 25°C until they sporulate. The bacterial isolates were thereafter incubated at 37°C for 24 h after which they were observed for zones of inhibition. Plates containing fungal isolates were incubated at 25°C for 96 h and later observed for zones of inhibition. Streptomycin and nystatin were used as standard antibacterial and antifungal agents, respectively.

2. Minimum Inhibitory Concentration (MIC) Measurement

The MIC test was carried out using the method of Akinpelu and Kolawole.[20] Twofold dilutions of the compounds were prepared, and 2-mL aliquots of different concentrations of the solutions were added to 18 mL of presterilized molten nutrient agar at 40°C to give final concentrations between 10 and 0.079 mg/mL. The medium was poured into sterile petri dishes and allowed to set. The surfaces of the media were allowed to dry under a laminar flow before streaking with 18-h-old bacterial cultures. The different compound concentrations were dispensed into each well and properly labeled. The preparation was left to diffuse before incubating at 37°C for 24 h for the bacterial strains and 25°C for 96 h for the fungal strains. The lowest concentration of antimicrobial agent that completely prevented the growth of the microorganisms was taken as the MIC of the compound.

Computational Details

Full geometry optimization of the molecules was accomplished in the gas phase at the RB3LYP/6-31+(d,p) level of theory.[21,22,23,24,25] The optimized geometry of each molecule was identified to represent the ground state energy

minimum as the force constant calculations gave no negative frequency. The Gaussian 09 software[26] was utilized for the calculations. The energies of the frontier molecular orbitals, that is, energy of the highest occupied molecular orbital energy (E_{HOMO}) and energy of the lowest unoccupied molecular orbital energy (E_{LUMO}), were recorded and utilized to derive further reactivity indices according to the equations:

$$\Delta E = E_{LUMO} - E_{HOMO} \quad (1)$$

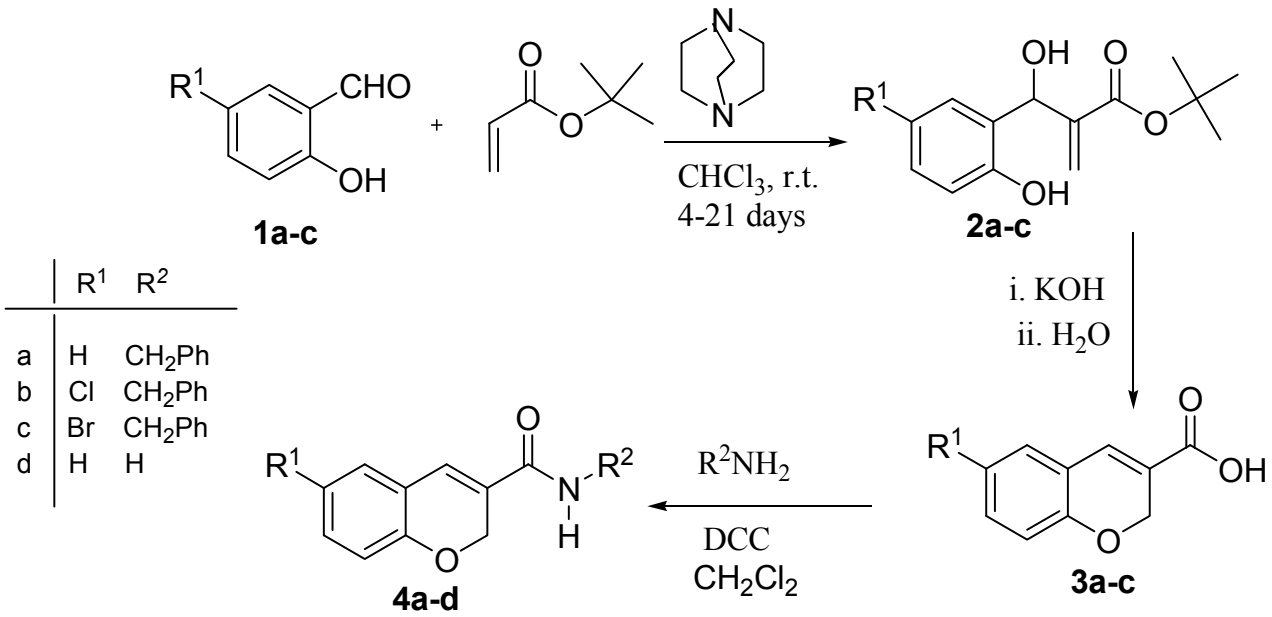
$$\chi = \frac{1}{2}\left(E_{LUMO} + E_{HOMO}\right) \quad (2)$$

Additional reactivity parameters such as octanol–water partition coefficient (log *P*), polarizability, number of hydrogen bond donor (HBD), and number of hydrogen bond acceptor (HBA) were derived with the quantitative structure activity relationship (QSAR) tool encoded in Spartan 10 (V1.0.1) software.

RESULTS

The NMR and IR spectroscopic data obtained for Baylis–Hillman adducts **2a-c** and 2*H*-chromene-3-carboxylic acid derivatives **3a-c** were in accordance with literature.[15,17] Scheme 1 shows the route to the chromene-3-carboxamides synthesized.

The synthesized compounds were screened against pathogenic microorganisms, which include five gram–positive and five gram–negative bacteria and three fungi. The results of zones of inhibition for both bacterial and fungal strains are reported in Tables 1 and 2, respectively, while the results for MIC of the bacterial and fungal strains are presented in Tables 3 and 4, respectively.



	R1	R2
a	H	CH_2Ph
b	Cl	CH_2Ph
c	Br	CH_2Ph
d	H	H

Scheme 1. Synthesis of chromene-3-carboxamides.

Table 1. Sensitivity Pattern of Zones of Inhibition Exhibited by Test Compounds on Bacterial Isolates Zones of Inhibition (mm)

Bacterial Isolates	3a	4a	3b	4b	3c	4c	4d	Streptomycin
E. coli (-)	15	15	12	14	14	15	16	20
P. aeruginosa (-)	14	17	11	14	12	16	14	00
P. vulgaris (-)	10	18	00	14	00	15	12	19
K. pneumoniae (-)	15	20	14	15	14	17	16	20
Shigella spp (-)	13	18	00	12	00	00	12	21
B. stearothermophilus (+)	12	13	11	14	11	11	00	23
C. sporogenes (+)	11	10	14	00	13	00	16	25
S. faecalis (+)	18	12	00	00	14	11	12	00
B. anthracis (+)	13	11	00	00	00	10	17	00
B. cereus (+)	17	13	11	12	14	12	16	23

(-): gram-negative bacteria.
(+): gram-positive bacteria.

Table 2. Sensitivity Pattern of Zones of Inhibition Exhibited by Test Compounds on Fungal Isolates Zone of Inhibition (mm)

Fungal Isolates	3a	4a	3b	4b	3c	4c	4d	Nystatin
Aspergillus niger	00	18	14	21	13	20	17	20
Aspergillus fumigatus	10	16	11	18	11	15	11	22
Candida albicans	10	00	14	20	12	19	16	18

Table 3. MIC of Test Compounds on Bacterial Isolates

Bacterial Isolates	3a	4a	3b	4b	3c	4c	4d	Streptomycin
E. coli (-)	0.250	0.062	0.500	0.031	0.250	0.500	0.125	0.062
P. aeruginosa (-)	0.250	0.031	0.500	0.250	0.125	0.062	0.250	ND
P. vulgaris (-)	0.500	0.062	ND	0.062	ND	0.125	0.016	0.031
K. pneumoniae (-)	0.500	0.016	0.125	0.125	0.062	0.062	0.125	0.062
Shigella spp (-)	0.062	0.016	ND	0.250	ND	ND	0.250	0.031
B. stearothermophilus (+)	0.125	0.016	0.500	0.062	0.250	0.125	ND	0.062
C. sporogenes (+)	0.031	0.062	0.062	ND	0.062	ND	0.031	0.031
S. faecalis (+)	0.062	0.031	ND	ND	0.125	0.125	0.25	ND
B. anthracis (+)	0.031	0.500	ND	ND	ND	0.500	0.016	ND
B. cereus (+)	0.062	0.125	0.250	0.125	0.062	0.062	0.0625	0.125

ND: not determined.

(-): gram-negative bacteria.
(+): gram-positive bacteria.

Table 4. MIC of Test Compounds on Fungal Strains

Fungal Isolates	3a	4a	3b	4b	3c	4c	4d	Nystatin
Aspergillus niger	ND	0.031	0.062	0.016	0.062	0.031	0.125	0.031
Aspergillus fumigatus	0.500	0.500	0.500	0.016	0.25	0.125	0.25	0.016
Candida albicans	0.125	ND	0.250	0.031	0.125	0.062	0.062	0.125

ND: not determined.

Table 5. Quantum Chemical Parameters for the Studied Compounds

Compound	E_{HOMO}	E_{LUMO}	ΔE_{L-H}	χ	Dipole Moment	log *P*	HBD	HBA	Polarizability
3a	−6.285	−2.289	3.996	4.287	1.649	1.370	1	2	54.560
3b	−6.396	−2.517	3.879	4.457	2.184	1.930	1	2	55.700
3c	−6.375	−2.520	3.855	4.448	2.148	2.200	1	2	56.060
4a	−6.158	−1.843	4.315	4.000	2.908	2.690	0	3	63.230
4b	−6.277	−2.063	4.214	4.170	4.239	3.240	0	3	64.360
4c	−6.254	−2.067	4.187	4.160	4.219	3.510	0	3	64.720
4d	−6.181	−1.999	4.182	4.090	3.201	0.720	0	3	54.770

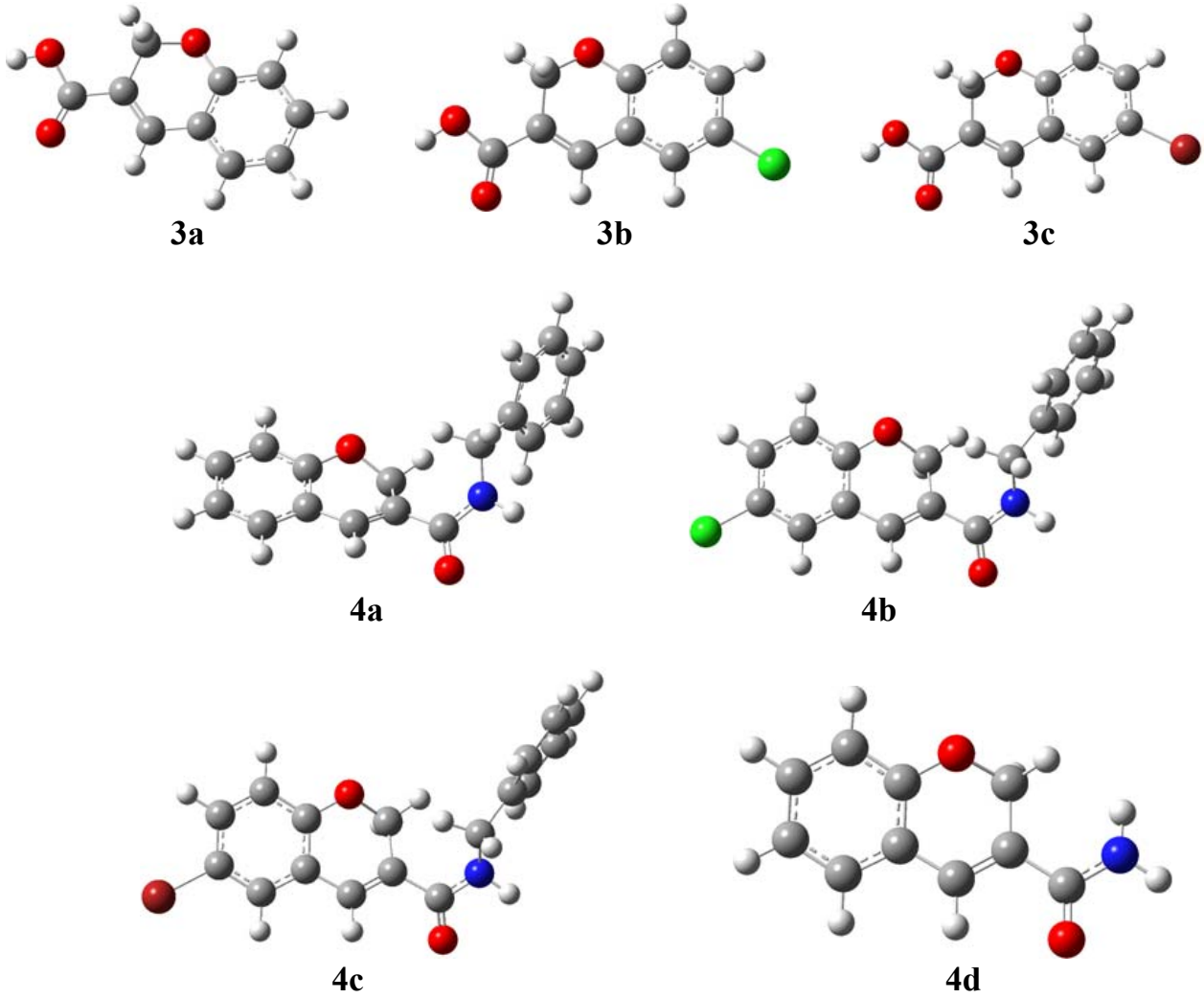


Figure 1. Optimized molecular structures of the studied compounds obtained with B3LYP/6-31+G(d,p) model.

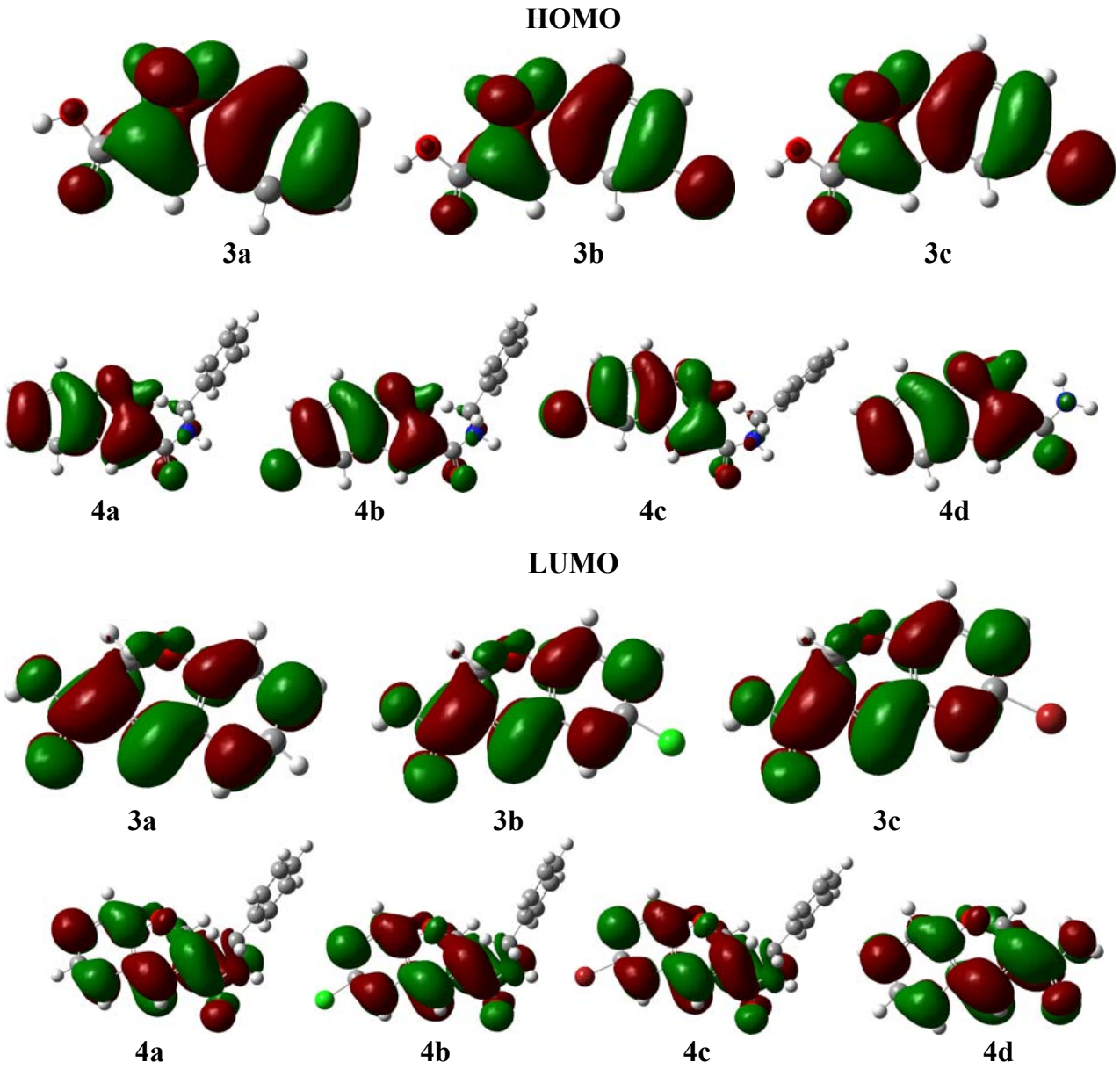


Figure 2. HOMO and LUMO canonical surfaces of the studied compounds obtained with B3LYP/6-31+G(d,p) model and displayed at 0.02 isovalue.

DISCUSSION

Synthesis

The adducts **2a-c** were prepared from the Baylis–Hillman reaction of 2-hydroxybenzaldehydes and *tert*-butyl acrylate using DABCO as catalyst[17] and subsequently cyclized regioselectively to 2*H*-chromene-3-carboxylic acid derivatives (**3a-c**) *via* base-mediated catalysis using an earlier reported procedure.[15] Coupling reaction was then employed in the synthesis of the 2*H*-chromene-3-carboxamide derivatives because the reaction between carboxylic acids and amines do not occur readily as the acid

rather protonates the amine to form unreactive carboxylate, which prevents further reaction. Hence, DCC was used as a coupling agent in the reaction of the 2*H*-chromene-3-carboxylic acid derivatives with the amines used. The 2*H*-chromene-3-carboxylic acid derivatives were added to benzylamine with DCC as catalyst, the mixture was allowed to stir for 16 h at room temperature and filtered, and the filtrate was evaporated to afford 2*H*-chromene-3-carboxamides in moderate yield up to 57%. The IR spectrum of *N*-benzyl-2*H*-chromene-3-carboxamide **4a**, for example, showed the presence of a secondary N-H stretching frequency at 3300 cm^{-1}, C=O stretching frequency at 1631 cm^{-1}, N-H bending frequency at 1573 cm^{-1}, C-N stretching frequency at 1242 cm^{-1}, and 1064 cm^{-1} of the amide. The disappearance of the broad OH stretch in the IR spectrum and the negative response to the sodium bicarbonate solution test confirmed the absence of a carboxylic acid group in the final products.

Antimicrobial Activity

The antimicrobial sensitivity of the 2*H*-chromene-3-carboxylic acid derivatives **3a-c** and 2*H*-chromene-3-carboxamides **4a-d** was assayed using agar diffusion technique against selected gram-positive bacteria, gram-negative bacteria, and fungal strains using streptomycin and nystatin as standard drugs, respectively. At low concentrations, streptomycin inhibits the growth of bacteria through the induction of prokaryotic ribosomes to misread mRNA.[27] It also prevents initiation of protein synthesis and eventually leads to microbial cell death. In humans, the ribosomes are structurally different from that of bacteria, thereby allowing the selectivity of streptomycin for antibacterial activity.[28] On the other hand, nystatin is a polyene antifungal antibiotic that is both fungicidal and fungistatic against a wide variety of yeasts.[29]

The results of the antimicrobial sensitivity testing were recorded for each test compound as the average diameter of zones of inhibition of bacterial and fungal growth around the disc in millimeters as shown in Tables 1 and 2. From the antibacterial screening, compound **4a** showed similar zones of inhibition as streptomycin on *Klebsiella pneumoniae* (20 mm). Compounds **3a**, **4a**, **4d,** and **4c** were sensitive to *B. anthracis*, *S. faecalis*, and *P. aeruginosa* with the zones of inhibition ranging from 10 to 18 mm, whereas these organisms developed resistance to streptomycin and no inhibition zone was observed.

Most of the tested compounds showed good sensitivity with zones of inhibition up to 21 mm in diameter against the fungal strains.

The MIC test was carried out on the nine clinical isolates earlier mentioned.[18] The MIC results for the selected bacterial strain are shown in Table 3. The result showed that compounds **3a-c** and **4a-d** exhibited weak to moderate growth inhibitory activity against the tested bacterial strains as revealed by MIC values between 0.016 and 0.5 mg/mL. Among the tested compounds, compounds **3a**, **3c,** and **4c** (MIC = 0.062 mg/mL) showed a better activity higher than the reference drug streptomycin (MIC = 0.125 mg/mL) against *Bacillus cereus*. Compounds **3a** and **4d** also showed equipotent activity with streptomycin (MIC = 0.031 mg/mL) against *Clostridium sporogenes*. Compound **4a** showed a better activity than streptomycin against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Shigella spp*, and *Bacillus stearothermophilus* and also a similar activity against *Escherichia coli* compared to the reference drug. Compound **4c** showed a similar activity as the reference drug against *Klebsiella pneumoniae*, and compound **4d** showed a better activity than the reference drug (streptomycin) against *Proteus vulgaris* and *Bacillus cereus*.

Compounds **4a** and **4c** showed a similar activity with the reference drug (nystatin) against Aspergillus *niger* (MIC = 0.031 mg/mL). As presented in Table 4, compounds **4b**, **4c**, and **4d** showed a better activity than the reference drug (nystatin) against *Candida albicans*, and only compound **4b** showed a better activity (MIC = 0.016 mg/mL) than the reference drug (MIC = 0.031 mg/mL) against *Aspergillus niger* and equipotent activity (MIC = 0.016 mg/mL) with the reference drug against *Aspergillus fumigatus* fungal strains. However, none of compounds **3a–c** showed a better activity than that of the reference drug (nystatin) against all the fungal strains.

Computational Study: Molecular Reactivity and Antibacterial Activity

There have been reports on the correlation between chemical reactivity and in vitro activities of organic compounds.[30,31,32] The reactivity of each compound at and/or around the receptor site and hence its antibacterial activity depend largely on its molecular and electronic structure. The molecular geometry of a chemotherapeutic agent and its ability to fit into the receptor environment for adequate ligand–receptor interaction are other important factors. The degree of hydrophobicity of the organic molecule and the extent of polarity of the receptor are known to influence the ease and extent of approach of the molecule to the attack site.[30,31,32] Therefore, it is not unexpected that antibacterial agents with dissimilar molecular structures, electronic structures, functional groups, and chemical reactivity exhibit different bactericidal efficiency. Their activities against gram-positive and gram-negative bacteria might also differ.

In view of this, relative activities of the investigated compounds against gram-negative and gram-positive bacteria can therefore be correlated with their chemical reactivities, which are inferred from the quantum chemically derived parameters. The correlations between global chemical reactivities of the carboxylic acid derivatives (**3a**, **3b**, and **3c**) and their activities against gram-positive and gram-negative bacteria are compared with those of their respective *N*-benzylcarboxamide derivatives (**4a, 4b, 4c,** and **4d**).

The quantum chemical descriptors used to correlate the experimental zone of inhibition data are listed in Table 5. The increasing trend of the highest occupied molecular orbital energy (E_{HOMO}) and electronegativity (χ) for the carboxylic acid derivatives is **3b < 3c < 3a**, which suggests that **3a** has the highest tendency of electron donation to a receptor site with propensity for electron acceptance. This trend is similar to the order of antibacterial activity of the molecules against gram-negative bacteria. Similarly, the *N*-benzylcarboxamide derivatives exhibited E_{HOMO} and χ values in the order **4b < 4c < 4d < 4a**, and they have slightly higher E_{HOMO} and lower χ values than their respective carboxylic acid derivatives. The trend of activities of the compounds against gram-negative bacteria suggests that the molecules combat the bacteria by donating charges and complexing with the receptor. This further suggests that the receptor site of the gram-negative bacteria might be electrophilic and the mechanism of bactericidal activity of the molecules (against gram-negative bacteria) is ligand–receptor complexation. More so, since each of the *N*-benzyl-3-carboxamide derivatives has higher E_{HOMO} and lower χ values than its corresponding carboxylic acid derivative, it can be assumed that the higher anti-gram-negative bacteria activities of an *N*-benzyl-3-carboxamide derivative compared to its correspondingly similar carboxylic acid derivative is due to the better electron donating ability of the *N*-benzyl-3-carboxamide derivative.

The results in Table 5 further show that each *N*-benzyl-3-carboxamide derivative has a

higher dipole moment than the corresponding carboxylic acid derivative. This suggests that the *N*-benzyl-3-carboxamide-based molecules have higher dipole–dipole interactions with the receptor sites than the carboxylic acid derivative, which enhance their anti-gram-negative bacteria activities. All the *N*-benzyl-3-carboxamide derivatives with the exception of **4d** showed higher log *P* (octanol/water partition function) than the carboxylic acid derivatives. *N*-benzyl-3-carboxamide derivatives also possess three HBAs and higher polarizability than the carboxylic acid derivatives. All these data support better electron donating ability of the carboxamide derivatives.

On the other hand, the carboxylic acid derivatives (**3a, 3b,** and **3c**) seem to exhibit higher activity against gram-positive bacteria than their corresponding *N*-benzyl-3-carboxamide derivatives (**4a, 4b, 4c,** and **4d**). In finding the link between the sensitivity patterns of the molecules against gram-positive bacteria and their quantum chemical parameters, the results in Table 5 suggest that the higher anti-gram-positive bacteria activity of a carboxylic acid derivative compared to its corresponding *N*-benzyl-3-carboxamide derivative might be related to the lower E_{LUMO} and higher χ values. Therefore, each carboxylic acid derivative has the tendency to interact with the receptor site of gram-positive bacteria better than the respective *N*-benzyl-3-carboxamide, and the activity might be enhanced by the charge-accepting ability of the carboxylic acid derivative.

The optimized molecular structures (Fig. 1) and frontier molecular orbitals distributions (Fig. 2) of the compounds do not provide direct quantitative correlation with the antibacterial activities. However, they present additional data that explain the differences in molecular and electronic structures of the molecules. As shown in Figure 1, the carboxylic acid functional group is nearly planar to the pyran ring, while the N- and O-atoms of the carboxamide twist by nearly the same angle upward and downward the plane of the pyran ring. The highest occupied molecular orbital (HOMO) of the carboxylic acid derivatives is distributed over the entire benzopyran ring and extended to the sp^2 O-atom of the –COOH group. The Cl- and Br- atoms in **3b** and **3c**, respectively, are also involved in the HOMO distribution. The lowest unoccupied molecular orbital (LUMO) of the carboxylic acid derivatives is distributed over the entire benzopyran ring and the –COOH group but excluding the halogen atoms in **3b** and **3c**. The HOMO and LUMO of the *N*-benzyl-3-carboxamide derivatives are delocalized over the benzopyran ring and extended to the carboxamide group. The benzyl moiety of the *N*-benzyl-3-carboxamide derivatives is not involved in the HOMO and LUMO distributions.

CONCLUSION

The reaction between 2*H*-chromene-3-carboxylic acid derivatives with various amines gave the corresponding 2*H*-chromene-3-carboxamide analogues in yields up to 57% using DCC as the coupling agent in CH_2Cl_2. The results obtained from the in vitro antimicrobial experiments showed that among the tested compounds, compound **4a** emerged as the most active, with activity higher than streptomycin, especially against the gram-negative bacterial strains. Compound **4b** appeared to be the most active against fungal strains of *Candida albicans* and *Aspergillus niger*, with activity higher than the reference drug (nystatin). The 2*H*-chromene-3-carboxamide derivatives had better activity than their 2*H*-chromene-3-carboxylic acid counterparts. This improved activity could be due to amide functional group on the 2*H*-chromene-3-carboxamide derivatives. Molecular quantum chemical

parameters provided further explanations to the observed biological activities of the molecules. Thus, this work will be very useful for further studies in terms of toxicity effects to improve their biological and pharmacological activities.

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