European Journal of Medicinal Chemistry 45 (2010) 1552–1559

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and study of some new 1,3-isoindoledione derivatives as potential antibacterial agents

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ARTICLE INFO

Article history:
Received 9 June 2009
Received in revised form
12 December 2009
Accepted 23 December 2009
Available online 13 January 2010

Keywords:
Dibenzobarallene
Isoindole
Benzimidazole
Benzothiazole
Bacillus thuringiensis
Escherichia coli

ABSTRACT

In an effort to establish new candidates with improved antibacterial activities, we reported here the synthesis and *in vitro* antibacterial evaluation of various series of 2-substituted-3a,4,9,9a-tetrahydro-4,9-benzeno-benz[f]isoindole-1,3-diones: 4-acetyl-phenyl **2**, 2,2-dibromoacetylphenyl **3**, benzimidazole **4**, acetylbenzimidazole **5**, aminophenyl **6**, acetamide **7**, naphthalene **8**, disulfide **9**, mercaptophenyl **10**, hydroxyphenyl **11**, phenyl ester **12**, triazole **13**, benzothiophene **14**, benzothiazole **15** phenylazo **16a**, **b** and aminomethane **17** derivatives. The newly synthesized compounds were characterized by (IR, ^{1}H NMR, ^{13}C NMR and mass spectrum studies). Representative compounds of the synthesized products were established and evaluated as antibacterial agents.

1. Introduction

In the past decades, the synthesis of heterocyclic compounds has been a subject of great interest due to their wide applicability. Heterocyclic compounds occur very widely in natural and are essential to life. Among a large variety of heterocyclic compounds, heterocycles containing isoindole moiety are of interest because the show some pharmacological and biological activities [1–4] (Fig. 1).

Isoindole derivatives were reported to possess anti-neoplastic and antiviral drugs [5], antimalarial [6] and anti Mycobacterium tuberculosis activity [7], antitumor [8] and antimicrobial activities [9]. In view of the above mentioned findings and as continuation of our effort [10–12], to identify new candidates that may be of value in designing new, potent, selective and less toxic antimicrobial agent, we report herein the synthesis of some new heterocycles incorporating isoindole moiety starting from dibenzobarallene [13] (Fig. 1).

2. Results and discussion

2.1. Chemistry

The synthetic procedures adopted to obtain the target compounds are depicted in Schemes 1–3. Dibenzobarallene was prepared according to the previous reported method [13]. The reaction of **1** with 4-aminoacetophenone in DMF yielded 4-acetyl-phenyl derivatives **2**. The structure of **2** was established on the basis of both analytical and spectral data. The ^{1}H NMR spectrum of **2** revealed the presence of singlet signal at δ 2.5 characteristic for $COCH_3$ protons. Moreover, the ^{13}C NMR spectrum displayed signals at δ 196.7 and 26.5 due to acetyl moiety. Bromination of **2** in acetic acid afforded the *geminal* dibromo derivative **3** which was established on the basis of analytical and spectral data. The ^{1}H NMR spectrum of compound **3** displayed singlet signal at δ 6.5 due to $CHBr_2$ proton. The ^{13}C NMR spectrum was characterized by downfield signals at δ 39.1 and 206.4 which were assigned to O═$CHBr_2$ moiety.

Cyclization of **3** by refluxing with *o*-phenylenediamine was studied in the aim of formation of new benzoimidazole derivative with potential biological activities [14,15]. Thus, it reacted with *o*-phenylenediamine in DMF containing a catalytic amount of TEA to give benzimidazole derivative **4** which was established on the basis of analytical and spectral data. The ^{1}H NMR spectrum displayed clearly the presence of singlet signal at δ 9.4 due to one proton of NH group (Scheme 1).

The present work was also extended to include the reaction of **1** with *o*-phenylenediamine, *o*-aminothiophenol and *o*-aminophenol by adapting the previously published procedure for the analogues reaction [16]. Refluxing of equimolar amounts of **1** and *o*-phenylenediamine in acetic acid afforded *N*-acetylbenzimidazole

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doi:10.1016/j.ejmech.2009.12.064

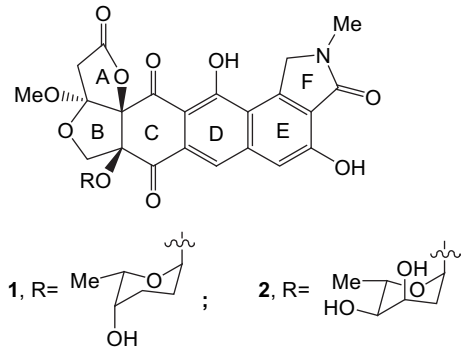


Fig. 1. Structures of lactonamycin (1) and *Z*-lactonamycin (**2**).

derivative **5**, while the isoindole derivative **6** was formed when the reaction was carried out in DMF or dioxane/pyridine instead of acetic acid (Scheme 2).

The formation of both **5** and **6** was explained *via* the intermediate [A⇋A′]. (Scheme 2) Acylation of compound **6** using acetyl chloride afforded phenyl acetamide derivative **7**. The structures **5**–**7** were confirmed on the basis of analytical and spectral data, which are in good agreement with its formulations. The mass spectrum of **5** exhibited beside the molecular ion peak at *m*/*z* 408 which is adopted with its molecular formula $C_{26}H_{20}N_2O_3$, two fragment ion peaks at *m*/*z* 348 and 178 (base peak) corresponding to [M^+ − (CH_2═C═O, H_2O)] and anthracene, respectively. The mass spectrum of **7** exhibited beside the molecular ion peak at *m*/*z* 408 which is in agreement with its molecular formula $C_{26}H_{20}N_2O_3$, three fragment ion peaks at *m*/*z* 366, 349 and 178 (base peak) corresponding to [M^+ − (CH_2═C═O)], [M^+ − (CH_2═C═O, NH_3)] and anthracene, respectively. It is note worthily that, the molecular ion peaks of both **5** and **7** are the same at *m*/*z* 408, but the fragment ion patterns were differ.

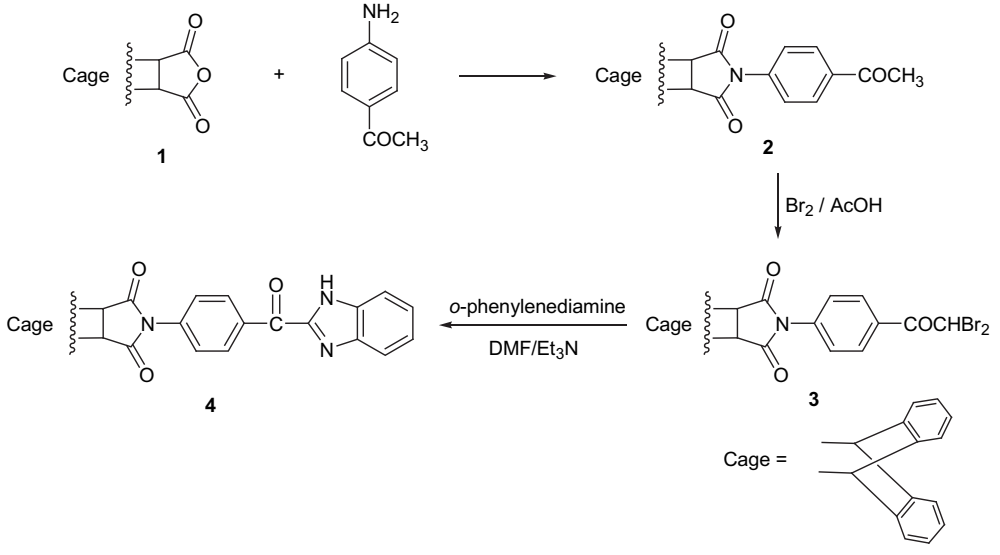
Moreover, compound **6** was reacted with 1,2-naphthoquinone-4-sulphonic acid sodium salt in water/DMF to give the unexpected product **8** according to the plausible mechanism in Scheme 3. The structure of **8** was established on basis of elemental analysis and spectral data. The mass spectrum exhibited peaks at *m*/*z* 870, 692 and 514 corresponding to [M^+], [M^+ − anthracene] and [M^+ − 2 anthracene]. The ^{1}H NMR spectrum displayed among other signals at δ 8.1 and 8.4 due to NH and C_3–H of quinonoid system protons. ^{13}C NMR spectrum of **8** exhibited signal at 181.0 due to C═O of 1,2-quinonoid system (Scheme 3).

The reaction of **1** with *o*-aminothiophenol in acetic acid, gave the disulfide **9**, whereas a mixture of **9** and **10** was obtained when the reaction carried out in DMF or dioxane/pyridine instead of acetic acid. The formation of **10** may be interpreted through the auto-oxidation of **9**. The structures of **9** and **10** were assigned on the basis of their mass spectra. The mass spectrum of **9** exhibited the molecular ion peak at *m*/*z* 764, which is in agreement with its molecular formula $C_{48}H_{32}N_4O_4S_2$. The base peak at *m*/*z* 178 corresponding to anthracene and the other fragment ion peaks at *m*/*z* 732 and 383 due to [$M^+ - S$] and [(1/2 M^+) + 1], whereas, the mass spectrum of **10** exhibited the molecular ion peak at *m*/*z* 383.

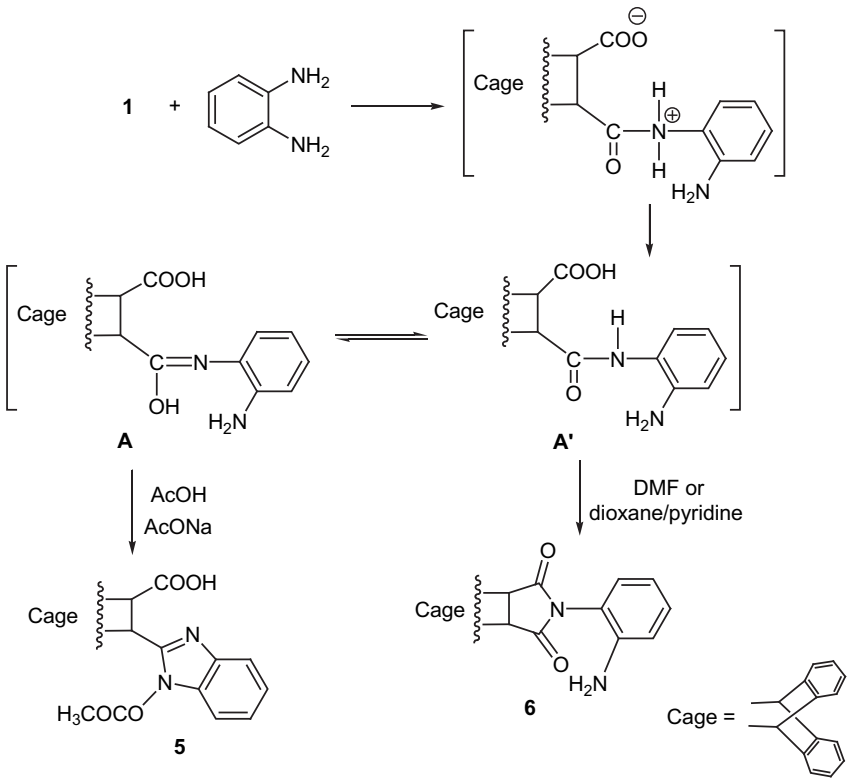
On the other hand, 2-hydroxyphenyl derivative **11** was obtained by refluxing of **1** with *o*-aminophenol in acetic acid, DMF or dioxane/pyridine. The structure **11** was established on the basis of IR, ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectrum displayed singlet signal at δ 9.9 due to OH proton, beside all signals in the expected regions. Moreover, its ^{13}C NMR spectrum revealed signals at δ 142.1, 153.8 and (162.7, 176.4) due to carbons of C–N, C–OH and 2C═O, respectively.

Treatment of compound **11** with *p*-toluenesulphonyl chloride in DMF and in the presence of a catalytic amount of TEA gave the corresponding ester derivative **12**. (Scheme 4) The structure **12** was confirmed on the basis of analytical and spectral data. The ^{1}H NMR spectrum clearly displayed singlet signal at δ 2.4 due to CH_3 proton. Also, its ^{13}C NMR spectrum revealed signals at 21.6 due to CH_3 carbon (Scheme 4).

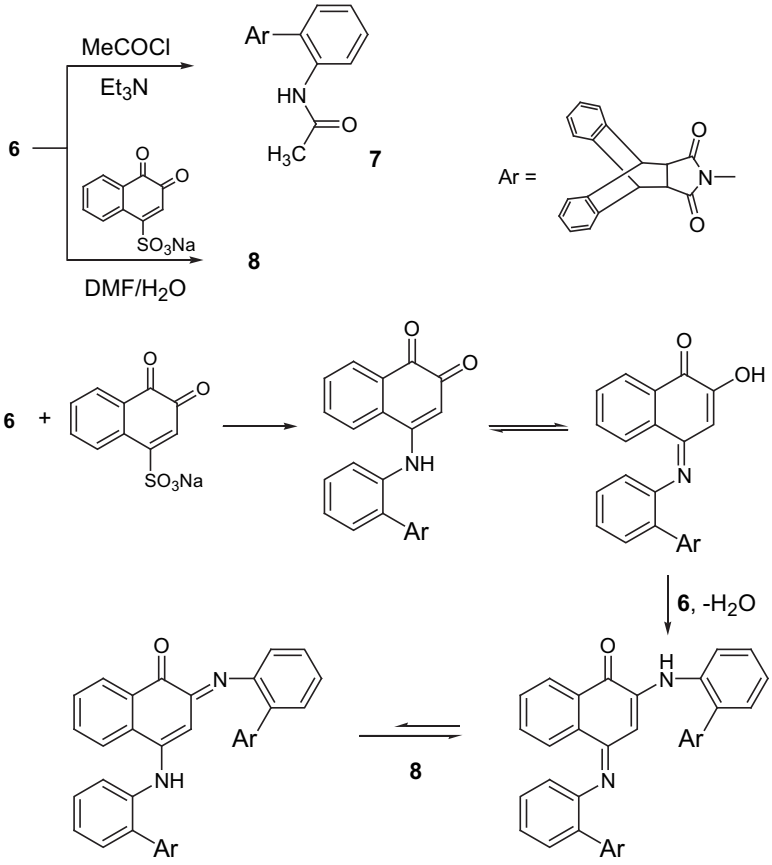
Moreover, the present work was also extended to include the reaction of **1** with some selective amino compounds, namely 3-amino[1,2,4]-triazole, ethyl-2-amino-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate [17], 2-amino-6-methylbenzothiazole [18], 4-aminoarylazobenzene [19] and tris-(hydroxymethyl)amino methane. Thus, refluxing of **1** with 3-amino-[1,2,4]-triazole in acetic acid/sodium acetate or in DMF afforded the corresponding isoindole derivative **13**. The structure **13** was established on the basis of both analytical and spectral data. The ^{1}H NMR spectrum displayed a clearly singlet signal at δ 8.4 due to one proton of C_5–H of triazole moiety and a singlet signal at δ 7.9 due to NH proton. Also, the ^{13}C NMR spectrum showed signals at δ 150.3, 144.2 characteristic for C-3 and C-5 of triazole ring.



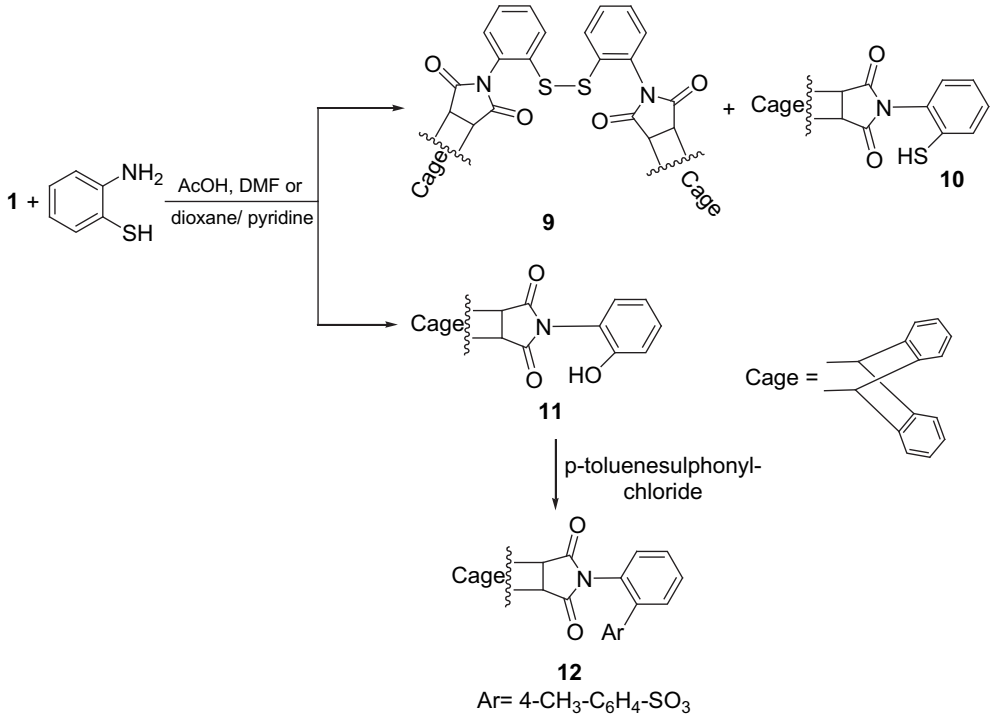
Scheme 1.



Scheme 2.



Scheme 3.



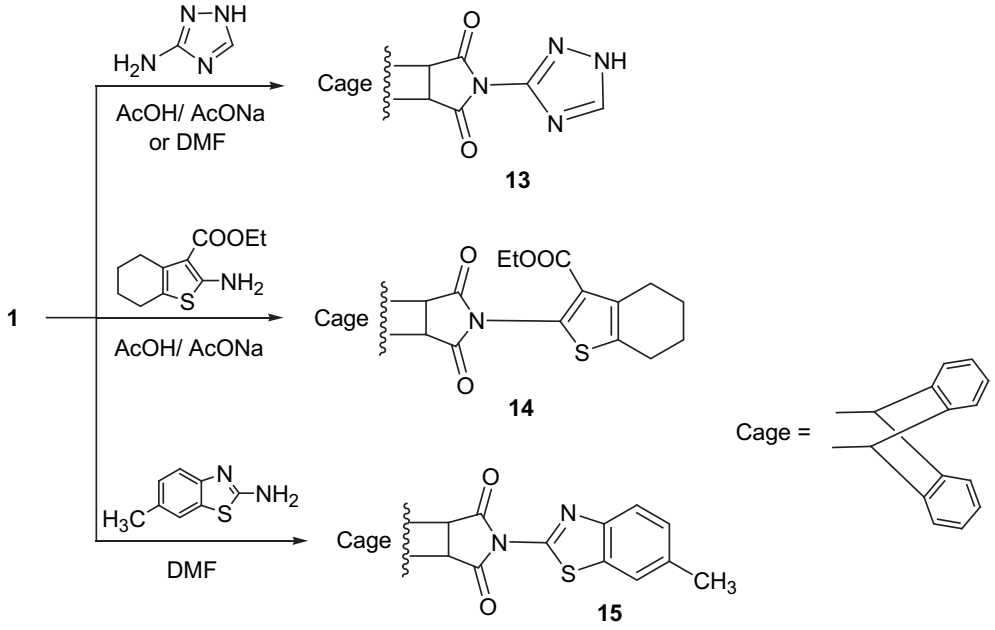
Scheme 4.

Ethyl-2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate was reacted with **1** in acetic acid/sodium acetate to yield the corresponding derivative **14** which was established on the basis of both elemental analysis and spectral data. The ^{1}H NMR spectrum displayed signals of ethyl protons at δ 1.2, 4.2 and multiplet signals at 1.8–2.8 due to eight protons of $4CH_2$ groups. ^{13}C NMR spectrum showed signals at δ 14.0, 21.7, 22.2, 22.5, 25.0 and 26.1 characteristic for CH_3, $5CH_2$ carbons, respectively.

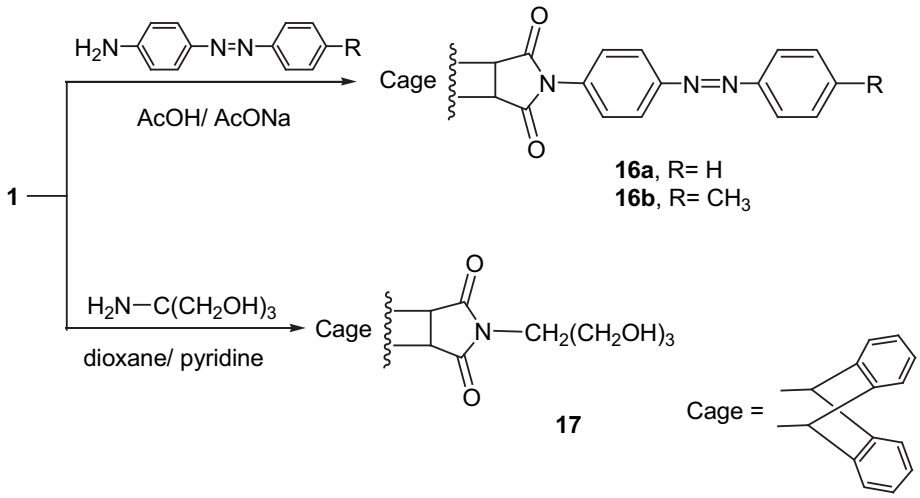
Isoindoldione derivatives **15** was obtained from the reaction of the adduct **1** with 2-amino-6-methylbenzothiazole in DMF. The spectral data of this compound supported the assigned structure **15** (Scheme 5).

It was found that, compounds containing arylazomethylene and *N*-cyclic imide bearing arylazo moiety exerts antimicrobial properties [20,21]. Thus, *p*-aminoarylazobenzene derivatives were reacted with **1** in DMF or in glacial acetic acid/anhydrous sodium acetate to afford 2-arylazobenzene isoindole derivatives **16a**, **b**. The structures of **16a**, **b** were ascertained through their analytical and spectral data. IR spectra of **16a**, **b** showed bands at 1503–1500 cm^{-1} attributed to azo groups.

The pharmacological importance [22,23], of tris-(hydroxymethyl)-aminomethane prompted us to investigate the synthesis of some new derivatives of expected biological activity. Thus, the adduct **1** was reacted with tris-(hydroxy methyl)-aminomethane



Scheme 5.



Scheme 6.

in dioxane/pyridine to yield the derivative **17** in good yield. The structure of **17** was established on the basis of its elemental analysis and spectral data. The ^{1}H NMR spectrum of **17** displayed signals at δ 3.6 and 3.9 due to $3CH_2$ and 3OH protons groups, respectively. Also, the ^{13}C NMR spectrum revealed signals at δ 67.6, 57.8 and 38.3 due to $(CH_2OH)_3$ and N–C carbons, respectively (Scheme 6).

3. Pharmacology

Seventeen compounds were screened *in vitro* for their antimicrobial activities against two strains of bacteria *Bacillus thuringiensis* and *Escherichia coli* by the agar diffusion technique [24]. The bacteria were maintained on nutrient agar. DMSO showed no inhibition zones. The agar media were incubated with different microorganism culture tested. After 24 h of incubation at 30 °C, the diameter of inhibition zone (mm) was measured (Table 1). *Ampicillin* and *chloramphenicol* were purchased from Egyptian market and used in a concentration 2 mg mL^{-1} as references.

The results depicted in Table 1 revealed that compounds **4** and **15** exhibited interesting high antibacterial activities against the reference drugs. It is worth mentioning that incorporation of isoindole to the benzimidazole or benzotriazole produce a high antibacterial activities.

In conclusion, we reported herein a simple and convenient route for the synthesis of some new heterocycles based on isoindole moiety for antibacterial evaluation.

4. Experimental

4.1. General

All melting points are in degree centigrade, are uncorrected and were measured on Gallenkamp electric melting point apparatus. TLC analysis was carried out on silica gel 60 F_{254} precoated aluminum sheets. IR spectra were recorded on Waffer technique on a MATSON 5000 FTIR spectrometer, Faculty of Science, Mansoura University. ^{1}H NMR spectra were determined on a Varian XL 200 MHz, Faculty of Science, Cairo University, a Brucker WP 300 Georg–August University Goettingen, Germany and a Brucker AC 300 Eberhard–Karls University, Tuebingen, Germany, in $CDCl_3$ or DMSO solvent using TMS as internal standard. ^{13}C NMR spectra were determined on Brucker AC 300 Eberhard–Karls University, Tuebingen, Germany, in $CDCl_3$ or DMSO solvent using TMS as internal standard. Mass spectrum recorded on Finnegan MAT 212 instrument and elemental analyses (C, H, and N) were carried out in The Microanalytical Center of Cairo Univ., Egypt.

4.1.1. Synthesis of 2-[4-acetyl-phenyl]-3a,4,9,9a-tetrahydro-4,9-benzeno-benz[f]isoindole-1,3-dione (**2**)

A mixture of **1** (6.76 g; 0.05 mol) and *p*-aminoacetophenone (13.8 g; 0.05 mol) in DMF (30 mL) was heated under reflux for 3 h. The reaction mixture was then poured into a beaker contain cooled water. The separated product was crystallized from ethanol–benzene to give **2**. Yield 70%, 13.7 g; mp 278 °C; IR (KBr): ν/cm^{-1} = 2846 (aliphatic C–H), 1770, 1702, 1654 (3CO); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 2.5 (s, 3H, $COCH_3$), 3.3 (s, 2H, C_{11}–H, C_{12}–H), 4.8 (s, 2H, C_9–H, C_{10}–H), 6.8 (d, 2H, Ar–H), 7.1–7.6 (m, 8H, Ar–H) and 7.9 (d, 2H, Ar–H); ^{13}C NMR ($CDCl_3$): δ_{ppm} = 196.7, 175.5, 167.4, 141.1, 138.6, 136.8, 135.4, 129.0, 128.2, 127.2, 126.9, 126.4, 125.1, 124.3, 111.7, 47.0, 45.8, and 26.5; EIMS (m/z) (%) = 393 (M^+, 0.6), 231 (0.2), 200 (12.2), 178 (100.0), 116 (3.0), and 90 (3.8). Anal. for $C_{26}H_{19}NO_3$ (393.43): calcd.: C 79.37, H 4.87, N 3.56%; found: C 79.49, H 4.96, N 3.69%.

Table 1
Inhibition zone (mean diameter of inhibition in mm) as a criterion of antibacterial activities of the newly synthesized compounds.

Compound No.	Inhibition zone in mm	
	Gram-positive bacteria "*Bacillus thuringiensis*"	Gram-negative bacteria "*Escherichia coli*"
2	15	12
3	17	14
4	20	23
5	17	13
6	17	12
7	18	16
8	17	17
9	17	17
10	18	16
11	17	16
12	15	18
13	17	15
14	15	14
15	26	29
16a	13	14
16b	11	14
17	15	14
Reference drugs		
Ampicillin	18	19
Chloramphenicol	23	20

*4.1.2. Synthesis of 2-[4-(2,2-dibromo-acetyl)-phenyl]-3a,4,9,9a-tetrahydro-4,9-benzeno-benz[f]isoindole-1,3-dione (**3**)*

A mixture of bromine (1.6 g; 0.01 mol) in glacial acetic acid (15 mL) was added drop wise over a period of 30 min to a hot solution of **1** (3.9 g; 0.01 mol) in acetic acid (75 mL) and acetic anhydride (0.5 mL). The reaction mixture was heated on water bath at 80–90 °C for 3 h. The separated product was then crystallized from ethanol–benzene afforded dibromo derivative **3**. Yield 45%, 2.5 g; mp 250 °C; IR (KBr): $\nu/cm^{-1} = 2881$ (aliphatic C–H) and 1774, 1707, 1681 cm^{-1} (3CO); ^1H NMR ($CDCl_3$): $\delta_{ppm} = 3.4$ (s, 2H, C_{11}–H, C_{12}–H), 4.8 (s, 2H, C_9–H, C_{10}–H), 6.5 (s, 1H, $CHBr_3$), 6.8 (d, 2H, Ar–H), 7.1–7.5 (m, 8H, Ar–H) and 8.1 (d, 2H, Ar–H); ^{13}C NMR ($CDCl_3$): $\delta_{ppm} = 184.8$, 175.4, 141.0, 138.6, 136.5, 130.5, 127.2, 126.9, 126.6, 125.1, 124.3, 47.1, 45.9 and 39.1; EIMS (*m*/*z*) (%) = 552 ($M^+ + 2$, 0.1), 551 ($M^+ + 1$, 24), 550 (M^+, 0.2), 378 (0.9), 344 (0.1), 264 (5.1), 178 (100.0), 176 (9.5) and 54 (3.3). Anal. for $C_{26}H_{17}Br_2NO_3$ (551.23): calcd.: C 56.65, H 3.11, N 2.54%; found: C 56.72, H 3.14, N 2.60%.

*4.1.3. Synthesis of 2-[4-(1H-benzoimidazole-2-carbonyl)-phenyl]-3a,4,9,9a-tetrahydro-4,9-benzeno-benz-[f]isoindole-1,3-dione (**4**)*

A solution of **3** (0.55 g; 0.001 mol), *o*-phenylenediamine (0.11 g; 0.001 mol) and TEA (0.5 mL) in DMF (20 mL) was warmed on water bath at 95 °C for 5 h. The separated product was crystallized from DMF-ethanol to give **4**. Yield 90%, 0.45 g; mp 272 °C; IR (KBr): $\nu/cm^{-1} = 3315$ (NH), 2928 (aliphatic C–H), and 1774, 1707, 1681 cm^{-1} (3CO); ^1H NMR ($CDCl_3$): $\delta_{ppm} = 3.1$ (s, 2H, C_{11}–H, C_{12}–H), 4.8 (s, 2H, C_9–H, C_{10}–H), 6.8 (d, 2H, Ar–H), 7.1–7.5 (m, 12H, Ar–H), 8.0 (d, 2H, Ar–H) and 9.4 (s, H, NH); Anal. for $C_{32}H_{21}N_3O_3$ (495.53): calcd.: C 77.56, H 4.27, N 8.48%; found: C 77.59, H 4.33, N 8.55%.

*4.1.4. Synthesis of 12-[1-acetyl-1H-benzimidazol-2-yl]-9,10-dihydro-9,10-ethanoanthracene-11-carboxylic acid (**5**)*

A mixture of **1** (1.38 g; 0.005 mol), *o*-phenylenediamine (0.54 g; 0.005 mol) and fused sodium acetate (0.41 g; 0.005 mol) in glacial acetic acid (20 mL) was refluxed for 4 h. The solvent was concentrated under reducing pressure. The separated product washed with water and crystallized from methanol–benzene afforded compound **5**. Yield 65%, 1.32 g; mp 263 °C; IR (KBr): $\nu/cm^{-1} = 2934$ (OH) and 1757, 1714 (2CO); EIMS (*m*/*z*) (%) = 408 (M^+, 0.5), 348 (1.0), 290 (0.5), 227 (0.7), 202 (1.0), 178 (100.0), 152 (7.1), 114 (1.5) and 63 (1.0). Anal. for $C_{26}H_{20}N_2O_4$ (424.45): calcd.: C 73.57, H 4.75, N 6.60%; found: C 73.60, H 4.79, N 6.64%.

*4.1.5. Synthesis of 2-[2-amino-phenyl]-3a,4,9,9a-tetrahydro-4,9-benzeno-benz[f]isoindole-1,3-dione (**6**)*

A mixture of **1** (1.38 g; 0.005 mol), *o*-phenylenediamine (0.54 g; 0.005 mol) in dioxane/pyridine (20 mL; 3.1 V) or DMF (20 mL) was heated under reflux for 5 h. The reaction mixture poured in ice water. The separated product was crystallized from ethanol–benzene to give isoindole-1,3-dione **6**. Yield 92.8%, 1.7 g; mp 277 °C; IR (KBr): $\nu/cm^{-1} = 3458$, 3369 (NH_2) and 1775, 1707 cm^{-1} (2CO); ^1H NMR (DMSO): $\delta_{ppm} = 3.3$ (s, 2H, C_{11}–H, C_{12}–H), 4.7 (s, 2H, C_9–H, C_{10}–H), 5.2 (s, 2H, NH_2) and 7.1–7.6 (m, 12H, Ar–H); EIMS (*m*/*z*) (%) = 366 (M^+, 30.0), 266 (2.6), 203 (3.6), 188 (8.8), 178 (100), 119 (8.8) and 79 (3.5). Anal. for $C_{24}H_{18}N_2O_2$ (366.41): calcd.: C 78.67, H 4.95, N 7.65%; found: C 78.72, H 5.01, N 7.71%.

*4.1.6. Synthesis of N-[2-(1,3-dioxo-1,3,3a,4,9,9a-hexahydro-4,9-benzeno-benz[f]isoindol-2-yl)-phenyl]-acetamide (**7**)*

A mixture of **6** (0.5 g; 0.0013 mol), acetyl chloride (5 mL) and TEA (0.5 mL) was heated on water bath at 70 °C for 15 min. The reaction mixture was allowed to cool. The separated product was crystallized from benzene–ethanol to afford acetamide derivative **7**. Yield 94%, 0.5 g; mp 264 °C; IR (KBr): $\nu/cm^{-1} = 3372$, (NH) and 1775, 1706, 1629 (3CO); EIMS (*m*/*z*) (%) = 408 (M^+, 6.2), 366 (2.2), 349 (0.5), 277 (0.1), 203 (1.8), 202 (2.6), 178 (100), 152 (3.5) and 89 (1.8). Anal. for $C_{26}H_{20}N_2O_3$ (408.45): calcd.: C 76.45, H 4.94, N 6.86%; found: C 76.57, H 4.50, N 6.94%.

*4.1.7. Synthesis of 2-[2-{4-(2-[1,3-dioxo-1,3,3a,4,9,9a-hexahydro-4,9-bezeno-benz[f]isoindol-2-yl]-phenylimino)-1-oxo-naphthalen-2-yl-amino}-phenyl]-3a,4,9,9a-tetrahydro-4,9-benzeno-benz[f]isoindole-1,3-dione (**8**)*

A mixture of **6** (1.83 g; 0.005 mol) and 1,2-naphthoquinone-4-sulphonic acid sodium salt (1.56 g; 0.006 mol) in water/DMF (20 mL; 1:1 V) was refluxed for 1 h. The separated product was crystallized from benzene–ethanol to afford isoindole derivative **8**. Red crystals; yield 70%, 1.53 g; mp 256 °C; IR (KBr): $\nu/cm^{-1} = 3256$ (NH) and 1766, 1715, 1659 (3CO); ^1H NMR ($CDCl_3$): $\delta_{ppm} = 3.2$–3.6 (m, 4H, C_{11}–H, C_{12}–H), 4.7–4.9 (m, 4H, C_9–H, C_{10}–H), 6.9–7.6 (m, 20H, Ar–H), 8.1 (d, 1H, NH) and 8.4 (s, 1H, C_3 of quinonoid system); ^{13}C NMR ($CDCl_3$): $\delta_{ppm} =$ 181, 175.9, 175.3, 156.2, 141.2, 141.0, 138.8, 134.7, 133.6, 130.8, 129.9, 129.4, 128.1, 127.1, 127.0, 126.8, 126.6, 126.4, 125.1, 124.3, 124.1, 122.9, 121.2, 98.0, 47.3, 47.0, 45.8, 45.7 and 45.5; EIMS (*m*/*z*) (%) = 870 (M^+, 8.8), 692 (M^+ − anthracene, 98.0), 514 (M^+ − 2 anthracene, 23.0), 418 (3.5), 178 (100) and 89 (6.2). Anal. for $C_{58}H_{38}N_4O_5$ (870.95): calcd.: C 79.98, H 4.40, N 6.43%; found: C 80.08, H 4.52, N 6.53%.

*4.1.8. Synthesis of 2,2′-bis[1,3-dioxo-1,3,3a-,4,9,9a-hexahydro-4,9-benzeno-benz[f]isoindol-2-yl]diphenyl-disulphide (**9**) and 2-[2-(mercapto-phenyl)]-3a,4,9,9a-tetrahydro-4,9-benzeno-benz[f]-isoindole-1,3-dione (**10**)*

A mixture of **1** (1.38 g; 0.005 mol), *o*-aminothiophenol (0.63 g; 1.005 mol) and fused sodium acetate (0.5 g; 0.006 mol) in glacial acetic acid (20 mL) was refluxed for 5 h. The separated solid washed with water and crystallized from DMF to give **9**, 91% yield. Also, the previous reaction was carried out in dioxane/pyridine (20 mL; 3.1 V) or DMF (20 mL) instead of acetic acid/sodium acetate; in this case the reaction mixture was refluxed for 3 h, left to cool. The precipitated solid product filtered off, crystallized from DMF to give **9**, 40% yield and 45%, respectively. The filtrate was poured into a beaker contain ice and the separated solid product crystallized from benzene–ethanol to give **10**, 19%, 21% yield, respectively.

Compound **9**: mp >320 °C; IR (KBr): $\nu/cm^{-1} = 1775$, 1710 (2CO) and 560 (–S–S–); EIMS (*m*/*z*) (%) = 764 (M^+, 48.6), 732 (0.1), 586 (2.6), 502 (0.1), 408 (0.5), 383 (6.2), 381 (0.8), 231 (0.4), 204 (9.7), 178 (100), 89 (0.4) and 44 (1.7). Anal. for $C_{48}H_{32}N_2O_4S_2$ (764.91): calcd.: C 75.37, H 4.22, N 3.66%; found: C 75.32, H 4.19, N 3.61%.

Compound **10**: White powder; mp 315 °C; IR (KBr): $\nu/cm^{-1} =$ 2552, (SH) and 1770, 1712 (2CO); EIMS (*m*/*z*) (%) = 383 (M^+, 16.0), 351 (0.8), 276 (0.1), 231 (0.6), 202 (7.0), 178 (100), 176 (5.3), 152 (3.5) and 96 (0.9). Anal. for $C_{24}H_{17}NO_2S$ (383.46): calcd.: C 75.17, H 4.47, N 3.65%; found: C 75.23, H 4.53, N 3.75%.

*4.1.9. Synthesis of 2-[2-hydroxy-phenyl]-3a,4,9,9a-tetrahydro-4,9-benzeno-benz[f]isoindole-1,3-dione (**11**)*

A mixture of **1** (1.38 g; 0.005 mol), *o*-aminophenol (0.55 g; 0.005 mol) and fused sodium acetate (0.7 g; 0.007 mol) in glacial acetic acid (20 mL) was heated under reflux for 4 h. The separated product washed with water and crystallized from methanol–benzene to give **11**, 95% yield. Compound **11** was also obtained in 98% yield if the reaction was carried out in dioxane/pyridine (20 mL; 3.1 V) or DMF (20 mL) instead of acetic acid/sodium acetate. Yield 95%, 98%, 1.75 g; mp 266 °C; IR (KBr): $\nu/cm^{-1} = 3282$–3034 (OH) and 1775, 1707 (2CO); ^1H NMR (DMSO): $\delta_{ppm} = 3.3$ (s, 2H, C_{11}–H, C_{12}–H), 4.9 (s, 2H, C_9–H, C_{10}–H), 7–7.9 (m, 12H, Ar–H) and 9.9 (s, 1H, OH); ^{13}C NMR (DMSO): $\delta_{ppm} =$ 176.4, 162.7, 153.8, 142.1, 139.9, 130.7, 128.8, 127.0, 126.8, 125.3, 124.8, 119.3, 117.0, 47.1 and 45.3; Anal. for $C_{24}H_{17}NO_3$ (367.40): calcd.: C 78.46, H 4.66, N 3.81%; found: C 78.50, H 4.76, N 3.98%.

*4.1.10. Synthesis of toluene-4-sulphonic acid-2-[1,3-dioxo-1,3,3a,4,9,9a-hexahydro-4,9-benzeno-benz[f]isoindol-2-yl]-phenyl ester (**12**)*

A solution of **11** (0.725 g; 0.002 mol), *p*-toluene sulphonyl chloride (0.38 g; 0.002 mol) and few drops of TEA in DMF (10 mL) was heated on water bath at 90 °C for 5 h. The separated product was crystallized from ethanol–benzene to afford phenyl ester derivative **12**. Yield 67%, 0.7 g; mp 234 °C; IR (KBr): $\nu/cm^{-1} = 2958$ (aliphatic C–H), 1769, 1713 (2CO) and 1410 (SO_3); ^1H NMR ($CDCl_3$): $\delta_{ppm} = 2.4$ (s, 3H, CH_3), 3.2 (s, 2H, C_{11}–H, C_{12}–H), 4.8 (d, 2H, C_9–H, C_{10}–H) and 7.1–7.8 (m, 16H, Ar–H); ^{13}C NMR ($CDCl_3$): $\delta_{ppm} = 174.7, 145.6, 145.0, 141.2, 138.9, 132.3, 130.2, 129.8, 129.0, 128.2, 127.4, 126.9, 126.8, 125.1, 124.3, 123.1, 47.1, 45.6$ and 21.6; Anal. for $C_{31}H_{23}NO_5S$ (521.58): calcd.: C 71.38, H 4.44, N 2.69%; found: C 71.46, H 4.53, N 2.79%.

*4.1.11. Synthesis of 2-(1H-[1,2,4]triazol-3-yl)-3a,4,9,9a-tetrahydro-4,9-benzeno-benz[f]isoindole-1,3-dione (**13**)*

A mixture of **1** (1.38 g; 0.005 mol), 3-amino-1,2,4-triazole (0.5 g; 0.006 mol) and fused sodium acetate (0.6 g; 0.007 mol) in glacial acetic acid (15 mL) was heated under reflux for 3 h. The separated product was washed with water and crystallized from ethanol–benzene to give **13**, 88% yield. Also, the reaction afforded compound **13**, 65% yield when carried out in DMF instead of acetic acid. Yield 88%, 1.5 g; 65%, 1.1 g; mp 330 °C; IR (KBr): $\nu/cm^{-1} = 3110$ (NH), 2922 (aliphatic C–H) and 1770, 1678 (2CO); ^1H NMR ($CDCl_3$): $\delta_{ppm} = 3.4$ (s, 2H, C_{11}–H, C_{12}–H), 4.8 (s, 2H, C_9–H, C_{10}–H), 7.2–7.4 (m, 8H, Ar–H), 7.9 (s, 1H, NH) and 8.4 (s, 1H, C_5–H (triazole)); ^{13}C NMR ($CDCl_3$): $\delta_{ppm} = 167.2, 150.3, 144.2, 139.8, 138.2, 125.3, 125.0, 123.3, 122.7, 45.5$ and 43.6; EIMS (*m*/*z*) (%) = 342 (M^+, 2.6), 260 (0.3), 204 (1.3), 202 (3.5), 178 (100), 152 (2.1), 84 (1.1) and 66 (3.5). Anal. for $C_{20}H_{14}N_4O_2$ (342.35): calcd.: C 70.17, H 4.12, N 16.37%; found: C 70.28, H 4.27, N 16.43%.

*4.1.12. Synthesis of 2-[1,3-dioxo-1,3,3a,4,9,9a-hexahydro-4,9-benzeno-benz-[f]isoindol-2-yl]-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethyl ester (**14**)*

A mixture of **1** (1.38 g; 0.005 mol), 2-amino-3-ethoxycarbonyl-4,5,6,7-tetrahydrobenzo[b]thiophene (1.13 g; 0.005 mol) and fused sodium acetate (0.41 g; 0.005 mol) in glacial acetic acid (20 mL) was heated under reflux for 3 h. The reaction mixture was poured in to a beaker contain cooled water. The separated product was crystallized from ethanol to give **14**, 80% yield and 92% yield if the reaction was carried out in DMF instead of acetic acid. Yield 80%, 1.93 g; 92%, 2.2 g; mp 203 °C; IR (KBr): $\nu/cm^{-1} = 2955$ (aliphatic C–H) and 1772, 1724, 1713 (3CO); ^1H NMR ($CDCl_3$): $\delta_{ppm} = 1.2$ (t, 3H, CH_3), 1.8 (m, 4H, H–$C_{5'}$–H, C_6–H), 2.6 (m, 2H, H–C_7–H), 2.8 (m, 2H, H–C_4–H), 3.4 (s, 2H, C_{11}–H, C_{12}–H), 4.2 (t, 2H, O–CH_2), 4.8 (s, 2H, C_9–H, C_{10}–H) and 7.1–7.4 (m, 8H, Ar–H); ^{13}C NMR ($CDCl_3$): $\delta_{ppm} = 175.1, 161.5, 141.1, 138.5, 136.4, 135.4, 128.0, 127.2, 126.8, 125.0, 124.2, 60.2, 47.2, 45.8, 26.1, 25.0, 22.5, 22.2, 21.7$ and 14.0; EIMS (*m*/*z*) (%) = 383 (M^+, 1.3), 437 (0.9), 409 (0.2), 305 (0.5), 259 (8.8), 231 (2.6), 178 (100), 138 (1.7), 110 (4.4), 84 (15.0) and 54 (7.0). Anal. for $C_{29}H_{25}NO_4S$ (483.58): calcd.: C 72.03, H 5.21, N 2.90%; found: C 72.14, H 5.27, N 2.96%.

*4.1.13. Synthesis of 2-[benzothiazol-2-yl]-3a,4,9,9a-tetrahydro-4,9-benzeno-benz[f]isoindole-1,3-dione (**15**)*

A solution of **1** (2.76 g; 0.001 mol), 2-amino-6-methyl-benzothiazole (1.64 g; 0.001 mol) in DMF (20 mL) was heated under reflux for 4 h. The separated product was washed with hot DMF to give **15**. Yield 85%, 3.58 g; mp 320 °C; IR (KBr): $\nu/cm^{-1} = 2952$ (aliphatic C–H) and 1772, 1707 (2CO); EIMS (*m*/*z*) (%) = 422 (M^+, 15.3), 325 (14.6), 256 (14.5), 202 (17.3), 178 (100) and 74 (20.6). Anal. for $C_{26}H_{18}N_2O_2S$ (422.50): calcd.: C 73.91, H 4.29, N 6.63%; found: C 73.88, H 4.25, N 6.59%.

*4.1.14. Synthesis of 2-[4-(phenylazo/tolylazo)-phenyl]-3a,4,9,9a-tetrahydro-4,9-benzeno-benz[f]isoindole-1,3-dione (**16a**, **b**)*

General procedure: A mixture of **1** (2.76 g; 0.01 mol), 4-amino-phenylazobenzene or 4-aminotolylazobenzene (0. 01 mol) and fused sodium acetate (1 g; 0.012 mol) in glacial acetic acid (75 mL) was heated under reflux for 3–4 h. The separated product was washed with water and crystallized from methanol–benzene to give **16a** and **16b**, respectively.

Compound **16a**: Red crystals; yield 80%, 3.64 g; mp 260 °C; IR (KBr): $\nu/cm^{-1} = 2952$ (aliphatic C–H), 1770, 1703 (2CO) and 1503 (N=N); ^1H NMR (DMSO): $\delta_{ppm} = 3.5$ (s, 2H, C_{11}–H, C_{12}–H), 4.8 (s, 2H, C_9–H, C_{10}–H) and 7.1–7.9 (m, 17H, Ar–H); EIMS (*m*/*z*) (%) = 455 (M^+, 18.7), 352 (0.3), 350 (10.6), 332 (0.6), 277 (2.8), 203 (2.5), 202 (4.6), 178 (100), 152 (5.5), 105 (5.5) and 77 (20.0). Anal. for $C_{30}H_{21}N_3O_2$ (455.51): calcd.: C 79.10, H 4.65, N 9.22%; found: C 79.19, H 4.73, N 9.30%.

Compound **16b**: Red crystals; yield 77%, 3.61 g; mp 244 °C; IR (KBr): $\nu/cm^{-1} = 2968, 2922$ (aliphatic C–H), 1773, 1705 (2CO) and 1500 (N=N); EIMS (*m*/*z*) (%) = 469 (M^+, 4.8), 455 (2.4), 350 (3.1), 291 (1.4), 204 (2.2), 202 (5.7), 178 (100), 152 (10.9) and 91 (24.5). Anal. for $C_{31}H_{23}N_3O_2$ (469.53): calcd.: C 79.30, H 4.94, N 8.95%; found: C 79.38, H 5.01, N 9.02%.

*4.1.15. Synthesis of 2-[2-hydroxy-1,1-bis-hydroxymethyl-ethyl]-3a,4,9,9a-tetrahydro-4,9-benzeno-benz[f]isoindole-1,3-dione (**17**)*

A mixture of **1** (2.76 g; 0.01 mol) and 2-amino-2-hydroxymethyl-1,3-propandiol (0.73 g; 0.01 mol) in DMF (20 mL) and was heated under reflux for 3 h. The reaction mixture was poured into a beaker contain ice water. The separated product was crystallized from ethanol–benzene to give **17**. Yield 94%, 3.1 g; mp 220 °C; IR (KBr): $\nu/cm^{-1} = 3410$ (OH), 2910 (aliphatic C–H) and 1732, 1703 (2CO); ^1H NMR ($CDCl_3$/DMSO): $\delta_{ppm} = 3.1$ (s, 2H, C_{11}–H and C_{12}–H), 3.6 (s, 6H, $3CH_2$), 3.9 (s, 3H, 3OH), 4.8 (s, 2H, C_9–H and C_{10}–H) and 7.1–7.6 (m, 8H, Ar–H); ^{13}C NMR ($CDCl_3$/DMSO): $\delta_{ppm} = 177.5, 139.9, 137.4, 124.8, 124.7, 123.1, 122.4, 67.6, 57.8, 44.5, 43.7$ and 38.3; EIMS (*m*/*z*) (%) = 379 (M^+, 0.9), 343 (1.1), 332 (2.4), 331 (10.0), 276 (57.0), 202 (10.6), 178 (100), 152 (11.3), 140 (4.1), 104 (19.4), 82 (2.6) and 68 (2.6). Anal. for $C_{22}H_{23}NO_5$ (381.42): calcd.: C 69.28, H 6.08, N 3.67%; found: C 69.37, H 6.14, N 3.74%.

4.2. In vitro antimicrobial activity

The tested compounds were evaluated by the agar diffusion technique [24], using a 2 mg mL^{-1} solution in DMSO. The test organisms were *B. thuringiensis* as gram-positive bacteria and *E. coli* as gram-negative bacteria. A control using DMSO without the test compound was included for each organism. *Ampicillin* and *chloramphenicol* in DMSO were used as reference drugs.

Acknowledgements

To Dr. S. Bondock and Dr. E. Abd El-Latif, Chemistry Department, Faculty of Science, Mansoura University, for carrying out spectral measurements, also, to Dr. A. Mohamadin and Dr. A. El-Morsey, Botany Department, Faculty of Science, Mansoura University, for microbiological screening, they are greatly acknowledged.

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