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Synthesis and Antimicrobial Screening of Novel Benzoxazinophenothiazine Derivatives

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The acid catalyzed synthesis of benzoxazinophenothiazine derivatives is reported. The intermediates 6-chloro-5*H*-benzo[a]phenothiazin-5-one (**1**), 11-amino-6-chloro-9-thio-8,10-diazabenzo[a]phenoxazin-5-one (**2**) and 6,8-dichlorobenzo[a]phenoxazine-5-one (**3**) were prepared by the condensation of 2,3-dichloro-1,4-naphthoquinone with 2-aminothiophenol, 4,5-diamino-6-hydroxypyrimidin-2-thiol and 2-amino-6-chlorophenol respectively in an alkaline medium. Further alkaline condensation of the intermediates, **1** and **2** each with 4-aminothiophenol yielded 14-nitrobenzo[a][1,4]benzoxazino[3,2-c]phenothiazine and 15-amino-13-thio-12,14-diazabenzo[a][1,4]-benzoxazino[3,2-c]phenothiazine correspondingly. Another complex ring system, 12-chloro-9-methoxy-8-azabenzo[a][1,4]benzoxazino-[3,2-c]phenothiaizne was accomplished in a good yield by the condensation of **3** with 3-amino-6-methoxypyridin-2-thiol. The structural confirmation was done using UV-visible spectroscopy, FT-IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. The synthesized compounds were screened against some micro-organisms and the results showed that the complex derivatives were significantly active against the microorganisms.

Keywords: Antimicrobial screening, Acid catalysis, Benzoxazinophenothiazine, Diazabenzo derivatives, Phenoxazin-5-ones.

INTRODUCTION

The chemistry of nitrogen/sulphur hetero atom containing aromatic compounds is becoming more popular as an area of research[1]. Phenothiazine derivatives have been shown to possess a broad spectrum of pharmacological activity depending on their particular structure like antiparkinsonian[2,3], tranquilizer[4], anti-inflamatory[5-7], antimalarial[8,9], antipsychotic[10-12], antimicrobial[13-15], anti-tubercular[16-18], antitumor[19,20], antihistaminic[21,22], analgesic[23], prion disease drug[24]. In textile, paint and plastic industries, they are used as dyes and pigments[25,26] and in agricultural industries as insecticides[27]. In petroleum industries, they are found useful as antioxidants in lubricants and fuels[28]. It has been observed that some phenothiazines inhibit intracellular replication of viruses including human immunodeficiency viruses (HIV)[29]. On the other hand, some have been reported to exhibit significant anticancer activity[30,31]. Owing to the wide range of applications of phenothiazine, intensive research has been in progress for more derivatives with highly improved pharmacological and biological activities. Hence, several articles describing the successful synthesis of these derivatives had been reported especially on the angular derivatives including the non-aza and the congeneric aza-analogues, but there are still limited literatures on the complex derivatives of this phenothiazine ring system. The past work done was based on their dye and pigment properties, not much is known of their antimicrobial properties. Therefore, the authors describe in this present work the synthesis of complex aza derivatives of benzoxazinophenothiazine and their antimicrobial screening.

EXPERIMENTAL

All chemicals used were of laboratory grade (Sigma Aldrich). The melting points were determined with a Fischer John's apparatus and were uncorrected. UV/visible spectra were recorded on UV-2500PC series spectrophotometer using matched 1 cm quartz cells. The IR spectra were recorded on 8400S FT-IR spectrometer using KBr discs (NARICT, ZARIA). The ^{1}H NMR and ^{13}C NMR were scanned at University of Strathclyde, Scotland on a JEOL FX-90Q spectrometer using TMS as internal standard (chemical shift in δ). Elemental analysis was carried on CHN rapid analyzer and the antimicrobial screening was done at the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

6-Chloro-10-nitro-5*H*-benzo[a]phenoxazin-5-one (1): To a mixture of 2-amino-4-nitrophenol (2.0 g, 13 mmol) and sodium acetate (2.0 g, 24 mmol) in a 250 mL two-necked round bottom flask fitted with magnetic stirrer, thermometer and a reflux condenser, was added benzene (100 mL) and DMF

(10 mL). The mixture was boiled for 1 h and 2,3-dichloro-I,4-naphthoquinone (3 g, 13 mmol) was added and refluxed with constant stirring for 6 h at 70-75 °C. At the end of reaction, benzene was distilled off and the slurry poured into 150 mL of water and stirred to dissolve the inorganic salt. It was chilled overnight, filtered and recrystallized from methanol-acetone to yield 6-chloro-10-nitro-5*H*-benzo[a]phenoxazin-5-one (**1**) as red powder. Yield 2.3 g, (77 %), m.p. 268-270 °C. UV/visible (MeOH) λ_{max}: 304 (log ε 2.569), 344 (log ε 2.593), 711 (log ε 1.736), 759 (log ε 1.750). IR (KBr, ν_{max}, cm^{-1}): 3097 (C-H str), 1653 (C=O str), 1581 (NO_2 str), 1533, 1458 (Ar C=C str), 1329, 1250 (C-N str), 1130 (C-O), 762, 719 cm^{-1} (C-Cl str). ^{1}H NMR (400 MHz, DMSO): δ 8.10 (m, 3H), 7.92 (m, 4H); ^{13}C NMR (400 MHz, DMSO): δ 117.4, 119.1, 121.2, 122.0, 124.7, 126.4, 130.8, 131.1, 131.5, 134.5, 137.3, 140.5, 143.4, 159.2, 163.4 and 175.3. Analysis calculated for $C_{16}H_7N_2O_4Cl$: C, 58.82, H, 2.16, Cl, 10.35, N, 8.57; Found: C, 58.95, H, 2.21, Cl, 10.98, N, 8.70.

11-Amino-6-chloro-9-thio-8,10-diazabenzo[a]phenoxazin-5-one (2): The same procedure as 6-chloro-10-nitro-5*H*-benzo[a]phenoxazin-5-one (**1**) using 4,5-diamino-6-hydroxypyrimidin-2-thiol (3 g, 18 mmol), sodium acetate (1.7 g, 20 mmol) and 2,3-chloro-1,4-naphthoquinone (4.3 g, 18 mmol) to afford **2** as reddish brown solid after recrystallization from methanol-acetone. Yield 1.5 g (70 %), m.p. 295-296 °C. UV-visible (MeOH) λ_{max}: 327 (log ε 1.08), 418 (log ε 1.36), 507 (log ε 1.00). IR (KBr, ν_{max}, cm^{-1}): 3404, 3308 (NH_2), 3075 (C-H str), 1645 (C=O), 1573 (C=N str), 1450 (Ar C=C str), 1037 (C-O), 899 (C-H out-of-plane bend), 748 (C-Cl str). ^{1}H NMR (400 MHz, DMSO): δ 7.34 (4H, m), 6.2 (2H, s). ^{13}C NMR (400 MHz, DMSO): δ 118.1, 124.7, 125.9, 126.4, 130.8, 131.1, 131.7, 134.6, 141.3, 147.1, 150.4, 154.3, 168.0, 178.0. Analysis calculated for $C_{14}H_7N_4O_2SCl$: C, 50.79, H, 2.12, Cl, 10.73, N, 16.93, S, 9.68; Found: C, 51.02, H, 2.01, Cl, 10.79, N, 16.70, S 9.68.

6,8-Dichlolorobenzo[a]phenoxazin-5-one (3): Same as described earlier using 2-amino-6-chlorophenol (3.0 g, 21 mmol), sodium acetate (1.7 g, 20 mmol) and 2,3-dichloro-1,4-naphthoquinone (4.8 g, 21 mmol) to afford 6,8-chlorobenzo[a]-phenoxazin-5-one as orange powder after recrystallization from methanol-acetone. Yield = 2.83 g (80 %); m.p. 265 °C; UV-visible (MeOH) ν_{max}: 283 (log ε 2.00), 352 (log ε 2.57). IR (KBr, ν_{max}, cm^{-1}): 3090 (C-H str), 1663 (C=O str), 1572 (C=N str), 1477 (ArC=C str), 1269, 1130 (C-O str), 824 (C-Cl str). ^{1}H NMR (400 MHz, DMSO): δ 6.92 (4H, m), 6.4 (3H, m). ^{13}C NMR (400 MHz, DMSO): δ 116.1, 120.7, 123.9, 126.4, 129.8, 130.1, 132.7, 133.5, 140.3, 147.5, 152.2, 155.3, 167.0, 170.0. Analysis calculated for $C_{16}H_7NO_2Cl$: C, 60.76, H, 2.22, Cl, 22.47, N, 4.43; Found: C, 59.92, H, 2.31, Cl, 22.51, N, 4.40.

14-Nitrobenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (4): 2-Aminothiophenol (1.0 g, 8 mmol) and anhydrous sodium carbonate (1.0 g, 9 mmol) were weighed into a 250 mL reaction flask equipped with reflux condenser and thermometer. Benzene (100 mL) and DMF (10 mL) were added and the mixture warmed in water bath with stirring using magnetic stirrer for 1 h for complete dissolution. 6-Chloro-10-nitrobenzo[a]phenoxazin-5-one (2.42 g, 8 mmol) was later added and the mixture was refluxed with constant stirring for 10 h. At the end of reaction period, benzene was distilled off and 100 mL of water was poured in the reaction flask, agitated and transferred into a beaker. It was heated to near boiling and stirred to dissolve inorganic salt, chilled and filtered to obtain dark residue which was recrystallized from methanol/acetone to give 2 as dark red powder. Yield 1.4 g, (90 %); m.p. 310-311 °C; UV-visible (MeOH) λ_{max}: 343 (log ε 1.887), 670 (log ε 1.371), 758 (log ε 1.329). IR (KBr, ν_{max}, cm^{-1}): 3091(C-H str), 1640 (C=N str), 1587 (NO_2) 1519, 1465 (ArC=C str), 1321, 1244 (C-O str), 1140, 1076 (C-S), 949 (aromatic C-H in-plane bend), 685 cm^{-1} (C-S str). ^{1}H NMR (400 MHz, DMSO): δ 7.51 (4H, m), 6.9 (3H, m), 6.2 (4H, m). ^{13}C NMR (400 MHz, DMSO): δ 109.8, 110.6, 115.3, 117.0, 118.1, 124.7, 125.9, 126.4, 127.0, 130.8, 131.1, 131.7, 134.6, 136.0, 138.1, 139.9, 141.3, 147.1, 150.4, 154.3, 162.3. Analysis Calculated for $C_{22}H_{11}N_3O_3S$: C, 66.49, H, 2.79, N, 10.57, S, 8.07; Found: C, 66.70, H, 2.82, N, 10.63, S, 8.13.

15-Amino-13-thio-12,14-diazabenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (5): The same procedure as 14-nitrobenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (**4**) was employed using 2-aminothiophenol (1.0 g, 8 mmol) and anhydrous sodium carbonate (1.0 g, 9 mmol) but 11-Amino-6-chloro-9-thiol-8,10-diazabenzo[a]phenoxazin-5-one (2.2 g, 8 mmol) was employed in the second stage instead of 6-chloro-10-nitrobenzo[a]phenoxazin-5-one (2.42 g, 8 mmol) to give 15-Amino-13-thio-12,14-diazabenzo[a][1,4]-benzoxazino[3,2-c]phenothiazine as purple red powder after recrystallization from methanol/acetone. Yield = 1.0 g (8 %); m.p. 330-331 °C; UV-visible (MeoH) λ_{max}: 314 (log ε 2.668), 373 (log ε 2.472), 379 (log ε 2.472), 482 (log ε 2.452). IR (KBr, ν_{max}, cm^{-1}): 3398, 3320 (-NH_2), 3061 (C-H str), 1635 (C=N str), 1506, 1443 (Ar C=C str), 1304, 1250 (C-O str), 759, 681, 546 cm^{-1} (C-S str). ^{1}H NMR (400 MHz, DMSO): δ 6.98 (4H, m), 6.45 (4H, m), 6.2 (2H, s). ^{13}C NMR (400 MHz, DMSO): δ 111.0, 114.4, 116.2, 118.1, 124.7, 125.6, 126.9, 127.0, 130.0, 131.2, 131.7, 134.6, 136.1, 138.9, 139.9, 150.4, 154.3, 159.0, 160.9, 162.3. Analysis calculated for $C_{20}H_{11}N_5OS_2$: C, 59.83, H, 2.76, N, 17.44, S, 15.97; Found: C, 59.96, H, 2.98, N, 17.56, S, 15.60.

12-Chloro-9-methoxy-8-azabenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (6): Using the same procedure as for compound **4** and employing 3-amino-6-methoxypyridin-2-thiol (1.5 g, 10 mmol), anhydrous sodium carbonate (1.0 g, 9 mmol) and 6,8-dichlorobenzo[a]phenoxazin-5-one (2.8 g, 10 mmol), compound **6** was obtain as orange yellow powder. m.p. 350 °C. UV-visible (MeOH) λ_{max}: 284 (log ε 1.46), 336 (log ε 2.15), 467 (log ε 2.15), 467 (log ε 2.27). IR (KBr, ν_{max}, cm^{-1}): 3080 (C-H str), 1641, 1586 (C=N str), 1279 (C-O str), 1080, 1017 (arom. C-H in-plane bend), 469 cm^{-1} (C-S str). ^{1}H NMR (400 MHz, DMSO): δ 6.86 (4H, m), 6.51 (2H, *J* 7.96 Hz, d), 6.12 (3H, m). ^{13}C NMR (400 MHz, DMSO): δ 45.5, 99.9, 113.4, 115.3, 117.9, 122.2, 123.6, 125.3, 127.0, 127.8, 130.9, 131.8, 132.1, 134.6, 136.2, 138.9, 139.9, 150.4, 159.0, 160.9, 162.3. Analysis calculated for $C_{22}H_{12}N_3O_2ClS$: C, 63.23, H, 2.89, Cl, 8.48, N, 10.06, S, 7.67; Found: C, 63.46, H, 2.93, Cl, 8.64, N, 10.12, S, 7.60.

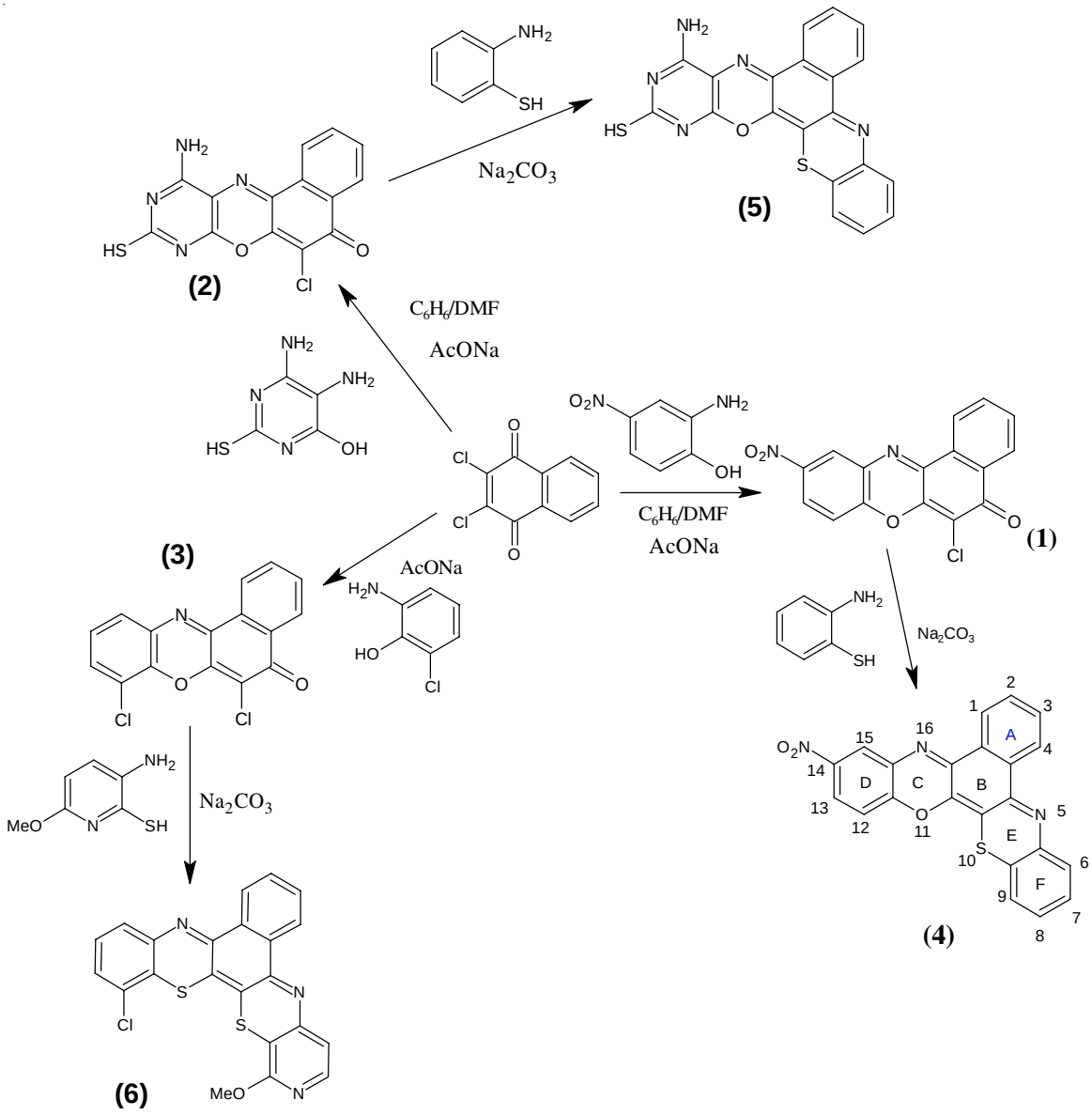
RESULTS AND DISCUSSION

2,3-Dichloro-1,4-naphthoquinone was refluxed with 2-amino-4-nitro-thiophenol to yield 6-chloro-10-nitro-5*H*-

benzo[a]phenothiazin-5-one (**1**). Compound **1** on treatment with 2-amino-thiophenol in the presence of anhydrous Na_2CO_3 under reflux yielded 14-ntirobenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (**4**). The second derivative, 15-amino-13-thio-12,14-diazabenzoxazino[3,2-c]phenothaizine (**5**) was synthesized as purple red powder by condensation of 2-amino-thiophenol with a mole of 11-amino-6-chloro-9-thio-8,10-diazabenzo[a]-phenoxazin-5one 2 which was obtained by the reaction of the 2,4-dichloro-1,4-naphthoquinone with in a basic medium (**Scheme-I**).

However, the condensation of 1 mol of 6-chloro-2-amino-thiophenol with 1 mol of 2,4-dichloro-1,4-naphthoquinone in a basic medium afforded **3** which on further reaction with 6-methoxy-3-amino-pyridine-2-thiol gave 12-chloro-9-methoxy-8-azabenzo[a][1,4]benzoxazino[3,2-c]phenothiazine **6** as orange yellow powder (**Scheme-I**).

The IR spectra of compounds **1** and **3** showed NH stretching vibration as weak bands in the region of 3489-3090 cm^{-1} and the C=O as strong band at 1663 cm^{-1} and 1653 cm^{-1} respectively due to its conjugation with double bonds. In compound **3**, the two peaks between 3403 and 3306 cm^{-1} is assigned to $-NH_2$. The strong bands at 1572, 1574 and 1582 cm^{-1} were assigned to C=N in compounds **1, 2** and **3**, respectively. The absorption band of C-Cl appeared between 825 and 719 cm^{-1}; likewise the appearance of NO_2 stretch at 1533 cm^{-1} in compound **1**. The disappearance of the bands due to C=O and C-Cl and the appearance of new bands due to C=N and C-S at 1582-1572 cm^{-1} and 773-754 cm^{-1} respectively coupled with shifts in absorption maxima in UV-visible spectra because of double bond extensions indicated the formation of **4, 5** and **6** from **1, 2** and **3** respectively. Furthermore, the bands at 3381 cm^{-1} (NH), 3092 cm^{-1} (C-H str aromatic), 1620 cm^{-1} (C=N str), 1519-1456 cm^{-1} (ArC=C), 1587 cm^{-1} (NO_2) and 685 cm^{-1} (C-S-C in the thiazine ring) were in agreement with the assigned structure of compound **4** likewise those at 3399-3320 cm^{-1} (NH_2), 3119 (aromatic C-H str), 3061 cm^{-1} (aromatic C-H str), 1636-1600 cm^{-1} (C=N), 1443 cm^{-1} (aromatic C=C), 1304-1250 cm^{-1} (C-O-C str.) and 760-546 cm^{-1} (C-S-C in the thiazine ring) were consistent with



Scheme-I

the structure of compound **5**. In compound **6**, the appearance of the bands at 3080 cm^{-1} (aromatic C-H str), 1641-1585 cm^{-1} (C=N), 1279 cm^{-1} (C-O str) and 773-687 cm^{-1} (C-Cl str) were in agreement with structure of **6**.

In the ^{1}H NMR spectra of compounds **4-6**, the multiplet due to aromatic protons appeared in the region δ 8.66-7.00. The proton of the -SH and $-NH_2$ groups in compound **5** appeared at δ 12.15 and 8.53 respectively. The peaks due to the two protons in pyridine ring of compound **6** occurred between δ 7.66-7.01. The $-OCH_3$ protons in compound **6** showed a singlet in the region δ 2.40-4.28. The structural confirmation of the synthesized compounds was achieved with elemental analysis. With the exception of compounds **4** and **5** which showed few peaks due to the insolubility, the ^{13}C NMR of others led credence to the establishment of their structures.

Antimicrobial activity: All the synthesized compounds were screened for their antimicrobial activity at concentration 20 mg/disc in agar media following the method of Bauer *et al.*[32]. Using ciprofloxacin, an antibacterial and ketoconazole, an antifungal as reference drugs, the compounds were screened against eight (8) micro-organisms, *viz*: *Bacillus subtitis, Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Candida albicans* and *Aspergillus niger.* This was carried under sensitivity test and minimium inhibitory concentration (MIC).

Sensitivity test: This assay was conducted by applying agar-well diffusion method[33] using a concentration of 20 mg/mL of each compound. From the result (Table-1), most of the compounds showed significant activity against the test organisms except compound **4** that was resistant to all of them. Compounds **1**, **2**, **5** are sensitive to *Candida albicans* while **3** was only active against gram-negative bacteria. Compounds **3** and **6** were sensitive to both *Candida albicans* and *A. niger.*

Minimum inhibitory concentration (MIC) determination: This was carried out using agar dilution following the procedure outlined by Chemical Laboratory Standards Institute (CLSI)[34] using the following serial dilutions 1, 0.5, 0.25, 0.125 and 0.0625 mg/mL. Almost all the synthesized phenothiazine derivatives were active against the microorganisms even at very low concentrations following from the fact that the lower the MIC values, the higher the activity. Compound **2** has the lowest MIC values against bacteria (*B. cereus, B. subtitis, S. aureus* and *E. coli*) which ranged from 0.0457-0.1040 mg/mL (Table-2). There was no MIC for compound **4**. Compound **3** was inactive for both bacteria and fungi likewise **1** was inactive against *A. niger*. Compound **5** was inactive against *E. pneumonia* and *A. niger*. All the MIC values of the synthesized compounds were well above the reference drugs except compound **5** where its value is below for *P. aeruginosa* (Table-2).

Conclusion

The acid catalyzed synthesis of benzoxazinophenothiazine derivatives was successfully carried out. The structures were supported by spectral and elemental analysis. The synthesized compounds showed fascinating antibacterial and antifungal activity.

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TABLE-1
RESULTS OF ANTIMICROBIAL SENSITIVITY TEST OF THE SYNTHESIZED COMPOUNDS

S/N	Gram-negative bacteria			Gram-positive bacteria			Fungi organism	
	B. cereus	*B. subtilis*	*S. aureaus*	*E. coli*	*P. aeruginosa*	*K. pneumonia*	*C. albicans*	*A. niger*
1	++	+	+	++	+	+	++	–
2	+++	+	+	+	+	+	+++	–
3	–	–	–	++	+	+++	+	+
4	–	–	–	–	–	–	–	–
5	+	+	++	+++	++	–	+	–
6	+	++	–	–	+	+	+	+
Rf1	+	++	+	+++	++	+++	–	–
Rf2	–	–	–	–	–	–	+++	++

+++ = Highly sensitive (18-22); ++ = Moderately sensitive (13-17); + = Sensitive (8-12); Rf1 = (Ciprofloxacin, antibacterial); – = Resistance (< 8) Rf2 = (Ketoconazole, antifungal).

TABLE-2
MINIMUM INHIBITORY CONCENTRATION OF THE COMPOUNDS (mg/mL)

S/N	Gram-negative bacteria			Gram-positive bacteria			Fungi organism	
	B. cereus	*B. subtilis*	*S. aureaus*	*E. coli*	*P. aeruginosa*	*K. pneumonia*	*C. albicans*	*A. niger*
1	0.0661	0.1380	0.1318	0.144	0.1738	0.1585	0.151	–
2	0.0457	0.0758	0.0794	0.104	0.1258	0.1850	0.085	0.13
3	–	–	–	0.132	0.6996	0.1850	0.085	0.13
4	–	–	–	–	–	–	–	–
5	0.0832	0.1905	0.0501	0.125	0.0661	–	0.158	–
6	0.1585	0.1445	–	–	0.1819	0.1514	0.120	0.1
Rf1	0.0335	0.0567	0.0212	0.021	0.1677	0.0389	–	–
Rf2	–	–	–	–	–	–	0.073	0.2

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