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## DESIGN AND SYNTHESIS OF NEW HYBRID TRIAZINE-INDOLE DERIVATIVES AS POTENTIAL ANTIMICROBIAL AGENTS AGAINST HOSPITAL RESISTANT STRAINS

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**Abstract** – A new series of antimicrobial [2-(5,6-diaryl-1,2,4-triazin-3-yl)-1*H*-indole] have been synthesized *via* microwave technology. The synthesized compounds were found to be potent against various clinical isolates of Gram-positive and Gram-negative bacteria as well as hospital resistant strains, MRSA and MSSA when compared to the drugs Cephtriaxone, Gentamycin and Levofloxacin.

The rise of multidrug resistance has prompted renewed interest in the development of novel antimicrobial agents. It is not surprising that pathogenic species have adopted survival mechanisms to the existing antimicrobial drugs. The increase incidence of bacterial resistance to a large number of antibacterial agents such as glycopeptides, sulfonamides,  $\beta$ -lactams, nitroimidazole, quinolones, tetracyclins, chloroamphenicol, macrolides is becoming a major concern.<sup>1,2</sup> The emergence of multidrug resistance Gram-positive and Gram-negative bacteria considered to be the current drive to produce new agents targeting novel sites that may circumvent resistance is critical to the long-term control of bacterial infection.<sup>3</sup>

Sepsis caused by methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of severe diseases and mortality worldwide. Many organs are vulnerable to MRSA infections, including skin, kidney, and other organs, resulting in serious life-threatening pathogenesis.<sup>4</sup> Community- and nosocomial-acquired

MRSA are common pathogens with resistance towards various classes of antibiotics, including methicillin, penicillin, and amoxicillin.<sup>5</sup>

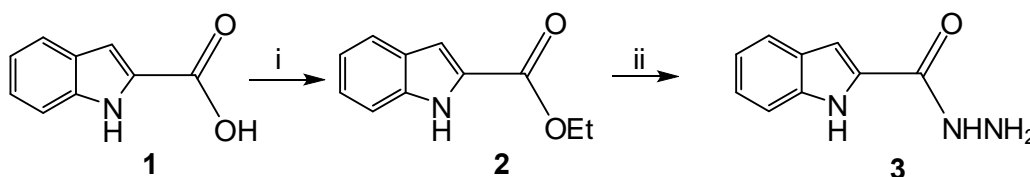
Therefore, in 2010 the Infectious Disease Society of America (IDSA) has issued a challenge, which seeks a global commitment to create antibiotic research and development enterprise powerful enough to produce 10 new systemic antibiotics by the year 2020.<sup>6</sup>

Indole nucleus and its derivatives are considered as privileged scaffold in medicinal chemistry,<sup>7-10</sup> that constitutes an important class of therapeutic agents, including anticancer,<sup>11</sup> antioxidant,<sup>12</sup> antirheumatoid,<sup>13</sup> and anti-HIV,<sup>14</sup> and also play a vital role in immune system drugs.<sup>15</sup> Indole moiety was reported as a main component of potent antimicrobial agents.<sup>16</sup> Another important heterocyclic compound of particular importance is the triazine moiety. Triazine and their derivatives possess a variety of biological activities such as hypertension and inhibition of platelets,<sup>17</sup> antileukemic,<sup>18</sup> antiinflammatory,<sup>19</sup> and potent neuroprotective agents.<sup>20</sup> The triazine moiety also is a main structural element in antimalarial,<sup>21</sup> anticancer,<sup>22</sup> antifungal,<sup>23</sup> anticonvulsant,<sup>24</sup> antibacterial,<sup>25</sup> and antiviral,<sup>26</sup> compounds. Certain compounds containing a 1,2,4-triazine nucleus have been reported to possess pesticidal,<sup>27</sup> neuropharmacological,<sup>28</sup> analgesic and antidepressant properties.<sup>29</sup>

In continuation of a program directed toward drug design and discovery (references from our work below), we report herein the synthesis of new hybrid scaffolds combining indole and substituted triazine to achieve maximum *in vitro* antimicrobial activity against Gram-negative and Gram-positive bacteria, including hospital resistant strains.<sup>30-33</sup>

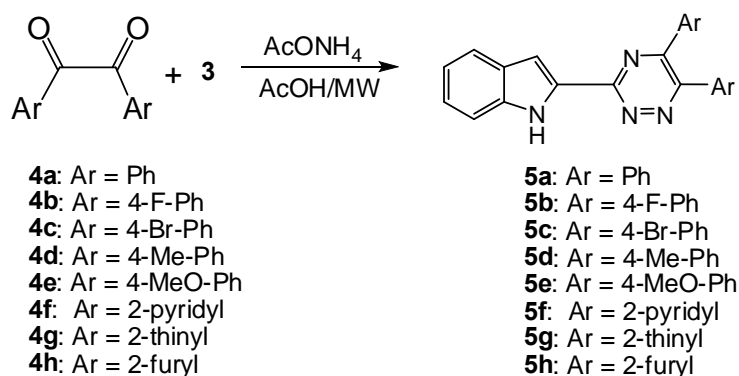
### Chemistry

The newly prepared compounds were synthesized starting from the commercially available indole 2-carboxylic acid **1**, which was converted to the ethyl ester **2** *via* conventional Fischer esterification (Scheme 1). Hydrazone derivative **3** was produced under the microwave condition *via* reaction of the ester in presence of excess hydrazine hydrate to deliver hydrazone **3**. With the latter in hand, it was anticipated to construct the heterocycles triazines applying Biotage Initiator 2.5 under microwave assisted organic synthesis as described in Scheme 2. Thus, hydrazone **3** was mixed with symmetrical benzil compounds **4a-h** in acetic acid and ammonium acetate to produce after work up highly pure compounds in fairly good yields.



**Scheme 1.** Synthesis of the hydrazone compound: i) EtOH/H<sub>2</sub>SO<sub>4</sub>, MW. ii) NH<sub>2</sub>NH<sub>2</sub>, EtOH, MW

All the synthesized compounds were identified through physical analysis. HRMS-ESI gave exact molecular ions for all the compounds. The structures of the new derivatives were unambiguously determined from their corresponding  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra. Chemical shifts of aromatic protons on triazine core appeared as expected in the range of  $\delta$  7.00–9.50 ppm, with multiplicities and coupling constants reflecting the substitution pattern in each compound.  $^1\text{H}$  NMR spectra of the synthesized derivatives showed multiplet signals in the aromatic region typical for non-symmetrically substituted triazine. The NH protons of the indole moiety appeared at 9.50–9.60 ppm for all the synthesized compounds.  $^{13}\text{C}$  NMR spectra of the new compounds **5a-h** were in full agreement with the proposed structures. DEPT experiments were used equivocally to confirm the proposed structures.



**Scheme 2.** Synthesis of the triazine compounds **5a-h**

### Antibacterial studies

The title compounds were evaluated *in vitro* against three Gram-positive *Staphylococcus epidermidis*, methicillin-sensitive *Staphylococcus aureus* ATCC 25923 (MSSA), methicillin-resistant *Staphylococcus aureus* ATCC33591 (MRSA), two standard Gram negative bacteria: *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27583. In addition, clinical (hospital acquired) isolates for *E. coli*, *Klebsiella pneumoniae* (*K.p*), *Pseudomonas aeruginosa* (*P.a*) and *Proteus vulgaris* (*P.v*) were also used for comparison. Broth micro-dilution method, using Mueller-Hinton broth medium (Himedia), was used to determine the antimicrobial activity.<sup>34,35</sup> Gentamicin, levofloxacin and cephtriaxone were used as the positive control. The obtained results, depicted in Table 1 as MIC values, revealed that many of the new scaffolds could effectively, to some extent, inhibit the growth of all tested strains *in vitro*.

As indicated in Table 1, MIC values in the range of 1.85–62.50  $\mu\text{g/mL}$  against both Gram-positive and Gram-negative strains were found. Interestingly, almost all the tested derivatives indicated appreciable activities against MRSA and MSSA species. Compounds **5e**, **5f** and **5h** were found to be two folds more potent against MRSA than the positive controls used in this study. On the other hand, compounds **5b**, **5e** and **5f** were found to be four times more potent against MSSA compared to cephtriaxone and gentamycine

controls. However, compound **5h** possessed twice the activity against MSSA compared to cephtriaxone and gentamycine.

Compounds **5b**, **5c** and **5e** gave the best inhibitory activity against most of the Gram-negative strains tested in this study. Compounds **5b** and **5e** possess stronger antimicrobial activities compared to the reference antimicrobial agents cephtriaxone, gentamycin and levofloxacin.

**Table 1.** In vitro antibacterial activity (MIC  $\mu\text{g/mL}$ ) of compounds **5a-h**

Compound	Gram-negative						Gram-positive		
	<i>E.Coli</i> <sup>a</sup>	<i>E. Coli</i> <sup>*</sup>	<i>K.p</i> <sup>*</sup>	<i>P.v</i> <sup>*</sup>	<i>P.a</i> <sup>b</sup>	<i>P.a</i> <sup>*</sup>	<i>MRSA</i> <sup>c</sup>	<i>MSSA</i> <sup>d</sup>	<i>S. Epidermidis</i>
<b>5a</b>	R	31.25	15.75	7.5	31.25	62.5	15.75	62.5	62.5
<b>5b</b>	1.85	7.5	7.5	3.75	7.5	1.85	1.85	7.5	1.85
<b>5c</b>	7.5	7.5	31.25	15.75	15.75	1.85	15.75	7.5	7.5
<b>5d</b>	15.75	15.75	31.25	31.25	31.25	62.5	15.75	62.5	62.5
<b>5e</b>	1.85	3.75	7.5	3.75	15.75	1.85	7.5	3.75	7.5
<b>5f</b>	7.5	7.5	15.75	15.75	62.5	3.75	7.5	3.75	3.75
<b>5g</b>	31.25	154.75	15.75	31.25	31.25	62.5	R	62.5	31.25
<b>5h</b>	15.75	7.5	3.85	7.5	15.75	7.5	7.5	7.5	1.85
Cephtriaxone	1.85	15.5	7.85	R	R	3.75	15.5	15.5	1.85
Gentamycin	15.5	R	7.85	15.5	1.85	7.5	15.5	15.5	15.5
Levofloxacin	7.85	62.5	7.85	7.5	7.5	1.85	15.5	7.85	3.75

<sup>a</sup> (*ATCC25922*); <sup>b</sup> (*ATCC 27583*); <sup>c</sup> (*ATCC 33591*); <sup>d</sup> (*ATCC 25923*); \* These bacteria species were clinical isolates, supplied by the Clinical Hospital of Rashid-Dubai, United Arab Emirates

At this point in time, structural-activity relationships could be drawn around triazine aryl moieties of the designed compounds. It is evident that the bromine and methoxy substituted aryl moieties were the most active compounds. Besides, the fluorophenyl armed triazine was found to be the most potent among others.

In conclusion, a series of new indole-triazine hybrids were successfully synthesized from commercially available starting materials applying microwave assisted synthesis. The in vitro antibacterial evaluation of these motifs showed that the synthesized compounds could effectively inhibit the growth of all tested bacteria including methicillin-resistant and multidrug resistant *S. aureus* and some of these lead scaffolds displayed two to four fold stronger antimicrobial activities in comparison with the positive controls used in this study.

These findings form the foundation for redirected efforts aimed at developing novel analogs as preclinical trials candidates as antibacterial. Furthermore, the mechanism of action of these compounds is under investigations in our laboratories.

## EXPERIMENTAL

<sup>1</sup>H NMR spectra were recorded on a Bruker Avance III-500 MHz spectrometer with TMS as an internal standard. <sup>13</sup>C NMR spectra were recorded at 125 MHz using a Bruker Avance III-500 MHz spectrometer with TMS as an internal standard. Chemical shifts were reported as  $\delta$ -values in ppm. The multiplicities of carbon atoms were determined from DEPT experiments. High resolution Mass spectra (HRMS) were recorded in positive ion mode by Electrospray Ionization using a Bruker Daltonics Apex IV, 7.0 T Ultra Shield Plus. The samples were dissolved in chloroform, diluted in spray solution (MeOH/water 1:1 v/v + 0.1% formic acid) and infused using a syringe pump with a flow rate of 2  $\mu$ L/min. External calibration was conducted using arginine cluster in a mass range  $m/z$  175-871. For all HRMS data, mass error: 0.00–0.50 ppm. Melting points (mp) were determined on an Electrothermal Melting point Apparatus and were uncorrected in  $^{\circ}$ C. Solvents used in this study were obtained from Scharlau, Fluka and Aldrich. All reactions were monitored by thin layer chromatography (TLC) using Merck aluminum plates pre-coated with silica gel PF254; 20  $\times$  20  $\times$  0.25 mm, and detected by visualization of the plate under UV lamp ( $\lambda$  = 254 or 365 nm). Spots were also detected by spraying with anisaldehyde- sulphuric acid in EtOH, followed by heating to 140  $^{\circ}$ C. All microwave reactions were performed using Biotage Initiator 2.5, the closed Teflon vessel was irradiated with variable power to keep a constant temperature over the reaction time.

### Ethyl 1*H*-indole-2-carboxylate (2)

A mixture of indole-2-carboxylic acid (**1**) (0.161 mg, 1 mmol), EtOH (4-5 mL), and concentrated sulfuric acid (0.5 mL) was placed in 5 mL sealed vial and subjected to coherent microwave radiation for 20 min at 140  $^{\circ}$ C. The reaction mixture was poured on crushed ice and then neutralized with sodium bicarbonate solution, the precipitated product was recrystallized from ethanol to yield 180 mg of a colorless powder, 95% yield.

### 1*H*-Indole-2-carbohydrazide (3)

A mixture of ethyl 1*H*-indole-2-carboxylate (**2**) (189 mg, 1 mmol), EtOH (3-4 mL), and hydrazine hydrate (10 mmol) was placed in 5 mL sealed vial and subjected to coherent microwave radiation for 15 min at 140  $^{\circ}$ C. The reaction mixture was poured on crushed ice and the precipitated product was recrystallized from ethanol to produce a quantitative product.

**2-(5,6-Diaryl/heteroaryl-1,2,4-triazin-3-yl)-1H-indoles 5a-h****General procedure for the synthesis of 5a-h**

A mixture of 1H-indole-2-carbohydrazide (**3**) (249 mg, 1 mmol), acetic acid (4 mL), ammonium acetate (10 eq), and benzil compound (1.2 mmol) was placed in 5 mL sealed vial and subjected to two successive coherent microwave radiation for 20 min at 140 °C. The reaction mixture was poured on a mixture of ammonia and crushed ice to produce a yellow precipitate which was further separated using TLC to produce pure compounds (40-75% yield).

**2-(5,6-Diphenyl-1,2,4-triazin-3-yl)-1H-indole (5a)**

This derivative was synthesized according to the general procedure above. Yield: 75%; Yellow solid; mp 163-164 °C. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 7.71 (bd, *J* = 8 Hz, 1H), 7.66 (bd, *J* = 8 Hz, 1H), 7.65 (bs, 1H), 7.41-7.60 (m, 11H), 7.26 (bt, *J* = 7 Hz, 1H), 7.10 (bt, *J* = 7 Hz, 1H). <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>): 157.8, 156.3, 155.6, 138.8, 136.1, 136.0, 133.6, 131.1, 130.3, 129.8, 128.9, 128.8, 128.5, 124.4, 124.3, 122.0, 120.5, 113.0, 106.1. ESI-HRMS: Calc. for C<sub>23</sub>H<sub>17</sub>N<sub>4</sub><sup>+</sup> [M+H]<sup>+</sup>: 349.14477. Found: 349.14479.

**2-(5,6-Bis(4-fluorophenyl)-1,2,4-triazin-3-yl)-1H-indole (5b)**

This derivative was synthesized according to the general procedure above. Yield: 70%; Yellow solid; mp 170-172 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 9.56 (bs, 1H), 7.77 (bd, *J* = 7 Hz, 1H), 7.69 (m, 3H), 7.62 (d, *J* = 5.3 Hz, 1H), 7.61 (d, *J* = 5.3 Hz, 1H), 7.52 (bdd, *J* = 0.8, 1 Hz, 1H), 7.35 (m, 1H), 7.20 (m, 1H); 7.11-7.15 (m, 4H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 165.4, 164.7, 163.4, 162.7, 156.9, 154.88, 154.1, 132.6, 132.1, 132.0, 131.5, 131.4, 131.4, 131.3, 128.7, 125.0, 122.2, 120.8, 116.1, 116.0, 115.9, 115.8. ESI-HRMS: Calc. for C<sub>23</sub>H<sub>15</sub>F<sub>2</sub>N<sub>4</sub><sup>+</sup> [M+H]<sup>+</sup>: 385.12593. Found: 385.12593.

**2-(5,6-Bis(4-bromophenyl)-1,2,4-triazin-3-yl)-1H-indole (5c)**

This derivative was synthesized according to the general procedure above. Yield 65%; Yellow solid; mp 235-236 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 9.53 (bs, 1H), 7.77 (bd, *J* = 8 Hz, 1H), 7.69 (m, 1H), 7.48-7.60 (m, 9H); 7.34-7.37 (m, 1H), 7.19-7.22 (m, 1H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 57.0, 154.9, 153.9, 137.7, 134.2, 134.1, 132.5, 132.4, 132.1, 132.0, 131.2, 128.9, 126.0, 125.1, 124.52 122.3, 120.9, 111.8, 107.4. ESI-HRMS: Calc. for C<sub>23</sub>H<sub>15</sub>Br<sub>2</sub>N<sub>4</sub><sup>+</sup> [M+H]<sup>+</sup>: 504.96580. Found: 504.96588.

**2-(5,6-Di(*p*-tolyl)-1,2,4-triazin-3-yl)-1H-indole (5d)**

This derivative was synthesized according to the general procedure above. Yield 65%; Yellow solid; mp 215-216 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 9.60 (bs, 1H), 7.77 (d, *J* = 8 Hz, 1H), 7.68 (bd, *J* = 1.5 Hz, 1H), 7.60 (d, *J* = 8 Hz, 2H), 7.52 (bd, *J* = 8 Hz, 2H), 7.51 (d, *J* = 9 Hz, 1H), 7.33 (t, *J* = 7 Hz, 1H), 7.18-7.23 (m,

5 H); 2.43 (s, 6H).  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  156.7, 155.9, 155.1, 141.3, 139.7, 137.5, 133.1, 132.9, 132.8, 129.8, 129.3, 129.3, 129.3, 128.8, 124.8, 124.7, 122.13, 120.6, 111.7, 106.6, 21.5, 21.4. ESI-HRMS: Calc. for  $\text{C}_{25}\text{H}_{21}\text{N}_4^+ [\text{M}+\text{H}]^+$ : 377.17607. Found: 377.17613.

### 2-(5,6-Bis(4-methoxyphenyl)-1,2,4-triazin-3-yl)-1H-indole (5e)

This derivative was synthesized according to the general procedure above. Yield 70%; Yellow solid; mp 209-211 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.54 (s, 1H), 7.97 (bd,  $J = 9$  Hz, 2H), 7.77 (bd,  $J = 8$  Hz, 1H); 7.70 (d,  $J = 9$  Hz, 2H), 7.66 (bs, 1H), 7.60 (d,  $J = 9$  Hz, 2H); 7.51 (bd,  $J = 8$  Hz, 2H), 7.34 (bt,  $J = 8$  Hz, 1H), 7.19 (bt,  $J = 8$  Hz, 1H), 7.09 (bd,  $J = 12$  Hz, 1H), 3.91 (s, 3H), 3.88 (s, 3H). ESI-HRMS: Calc. for  $\text{C}_{25}\text{H}_{21}\text{N}_4\text{O}_2^+ [\text{M}+\text{H}]^+$ : 409.16590. Found: 409.16589.

### 2-(5,6-Di(pyridin-2-yl)-1,2,4-triazin-3-yl)-1H-indole (5f)

This derivative was synthesized according to the general procedure above. Yield 45%; Yellow solid; mp 158-159 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.66 (bs, 1H), 8.41 (d,  $J = 5$  Hz, 1H), 8.37 (d,  $J = 5$  Hz, 1H), 8.23 (d,  $J = 8$  Hz, 1H), 7.90-7.94 (m, 2H), 7.73-7.78 (m, 2H), 7.51 (bd,  $J = 8$  Hz, 1H), 7.28-7.37 (m, 4H), 7.27 (bt,  $J = 8$  Hz, 1H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  157.3, 155.6, 154.8, 154.7, 154.6, 148.8, 148.6, 137.7, 137.0, 136.9, 132.6, 128.8, 125.0, 124.6, 124.5, 123.9, 122.3, 120.8, 111.8, 107.6. ESI-HRMS: Calc. for  $\text{C}_{21}\text{H}_{15}\text{N}_6^+ [\text{M}+\text{H}]^+$ : 351.13527. Found: 351.13497.

### 2-(5,6-Di(thiophen-2-yl)-1,2,4-triazin-3-yl)-1H-indole (5g):

This derivative was synthesized according to the general procedure above. Yield 40%; Yellow solid; mp 178-179 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.51 (bs, 1H), 7.65-7.66 (m, 2H), 7.61 (dd,  $J = 5, 1$  Hz, 1H), 7.58 (dd,  $J = 1, 4$  Hz, 2H), 7.50-7.52 (m, 2H), 7.35 (bt,  $J = 8$  Hz, 1H), 7.16-7.21 (m, 2H), 7.10 (dd,  $J = 4, 5$  Hz, 1H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  156.2, 149.3, 148.2, 139.2, 137.6, 137.2, 132.5, 132.4, 131.8, 131.3, 129.3, 129.2, 128.7, 124.9, 122.2, 120.8, 111.8, 107.1. ESI-HRMS: Calc. for  $\text{C}_{19}\text{H}_{13}\text{N}_4\text{S}_2^+ [\text{M}+\text{H}]^+$ : 361.05761. Found: 361.05802.

### 2-(5,6-Di(furan-2-yl)-1,2,4-triazin-3-yl)-1H-indole (5h)

This derivative was synthesized according to the general procedure above. Yield 40%; Yellow solid; mp 162-164 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.61 (bs, 1H), 7.77 (d,  $J = 8$  Hz, 1H), 7.72 (d,  $J = 1.5$  Hz, 1H), 7.69 (d,  $J = 1.5$  Hz, 1H), 7.66 (d,  $J = 1.5$  Hz, 1H), 7.52 (d,  $J = 8$  Hz, 1H), 7.34 (bt,  $J = 8$  Hz, 1H), 7.20 (bt,  $J = 8$  Hz, 1H), 7.13 (d,  $J = 3.4$  Hz, 1H), 7.03 (d,  $J = 3.5$  Hz, 1H), 6.68 (dd,  $J = 2, 3$  Hz, 2H); 6.64 (dd,  $J = 2, 3.5$  Hz, 2H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  156.4, 148.6, 146.7, 144.5, 144.3, 143.9, 137.6, 132.6, 128.7,

124.7, 122.2, 120.7, 117.9, 113.1, 112.8, 112.2, 111.7, 107.2. ESI-HRMS: Calc. for  $C_{19}H_{13}N_4O_2^+ [M+H]^+$ : 329.10330. Found: 329.10313.

### **In vitro antimicrobial procedure**

Gram-positive strains namely; *Staphylococcus epidermidis*, methicillin-sensitive *Staphylococcus aureus* ATCC 25923 (MSSA), methicillin-resistant *Staphylococcus aureus* ATCC33591 (MRSA), two standard Gram-negative bacterial strains namely; *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27583 along with the clinical (hospital acquired) isolates for *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris* were used to evaluate the minimum inhibitory concentration of designed compounds.<sup>34,35</sup> Thus, the bacteria were inoculated into Luria broth medium containing 1% tryptone, 0.5% yeast extract, 0.5% sodium chloride. The pH of the medium was adjusted to 7.2 with sterile phosphate buffered saline and incubated at 37 °C for 24 h. The optical density of the bacteria from mid-log phase of growth was measured at 540 nm and diluted in fresh medium to obtain an optical density of 0.004 (corresponding to  $5 \times 10^5$  colony forming units/mL). To each well of the ELISA plate, 200  $\mu$ L of diluted bacterial suspension was added and graded concentrations (0.2-500  $\mu$ g/50  $\mu$ L) of the synthesized compounds and standard antibiotics (Cephtriaxone, Gentamycin and Levofloxacin) in 20% H<sub>2</sub>O/DMSO were added and incubated at 37 °C for 24 h. At the end of incubation the effect of the drugs on the growth of organisms was monitored by measuring the optical density at 540 nm using an ELISA reader. The MIC was defined as the lowest concentration of the antibiotic or test sample allowing no visible growth.

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