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Synthesis of 2-substituted indoles and evaluation of their antibacterial activity and inhibitory effects on the efflux pump of methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

A series of 2-arylindoles were synthesized and characterized through spectral analysis. Their antibacterial activity and synergistic effect against multidrug-resistant *Staphylococcus aureus* were pharmacologically studied. 2-(4-Aminophenyl)-1*H*-indole (**4k**) showed a potent inhibitory activity at 15.6 µg/ml against *Bacillus subtilis* ATCC 6633 and *Salmonella typhi* ATCC 19430. 2-(3-Nitrophenyl)-1*H*-indole (**4j**) at 15.6 µg/ml displayed a potent antibacterial activity only against *S. typhi* ATCC 19430. All the synthesized compounds elicited a synergistic effect against methicillin-resistant *S. aureus* Sp6, drug resistance, and efflux pump genes strain. 2-(2-Hydroxyphenyl)-1*H*-indoles **4g**, **4j**, and **4k** were combined with tetracyclines, thereby decreasing the minimum inhibitory concentration of tetracyclines. Molecular docking showed that interaction with key amino acids occurred at the active site of the NorA efflux pump. Prediction of the toxicity using the ADMET study was also reported.

INTRODUCTION

The development of new pharmaceutical agents is one of the greatest challenges in overcoming widely spread epidermics and drug resistance. Furthermore, the ineffectiveness of medications such as antimicrobial drugs cause an increase in mortality, morbidity, and public health problems. The major components of resistance to many classes of antimicrobials are efflux mechanisms (Reygaert, 2018). In these mechanisms, efflux pump inhibitors are used to stop bacteria from pumping antibiotics out of cells, and it has been reported that 5-nitro-2-phenylindole '…..(INF55) (**1**) (Fig. 1) participates in the NorA efflux pump of Gram-positive bacteria and increases the susceptibility of *Staphylococcus aureus* to antibiotics (Markham *et al*., 1999). Substituents on the phenyl ring in INF55 (**1**) are also important for biological activities; that is, the 2,5-dimethoxyl group on the aryl ring (**2**) increases the inhibitory activity of NorA (Samosorn *et al*., 2006). This indicated that the substituents on the phenyl ring of the 2-arylindole nucleus affect biological activity. Similarly, the substitutions of the benzene ring of the indole nucleus diverted the biological activity, which were reported in the literature (Lal and Snape, 2012; Naik *et al*., 2014; Williams *et al*., 2013). Among these, 5-methoxy-2-phenylindole (**3**) was reported to be a significant antibacterial activity against Gram-positive *Bacillus cereus* minimum inhibitory concentrations (MIC) = 3.9 µg/ml. Furthermore, 5-amidine-2-arylindole bearing a 3,5-disubstituted aryl ring (**4**) remarkably affects the inhibition of acid-sensing ion channels (Kuduk *et al*., 2009). In addition, the 2-aryl-substituted indole derivative (**5**) is a bacterial histidine kinase inhibitor (Deschenes *et al*., 1999).

With the wide-ranging biological activities, the 2-arylindole scaffold is among the privileged frameworks to study the antibacterial activity and its inhibited NorA efflux pump-resistant mechanism. Thus, the goal of this study is to investigate the 2-arylindoles with various substituents on the aryl ring with

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their antibacterial activities and the ability to potentiate the activity of commercially available drugs and overcome drug resistance.

Indole is synthesized using various synthesis methods, including Fischer indole synthesis (Cho *et al*., 2009), Bischler synthesis (Cossío *et al*., 2008), Madelung cyclization (Imanishi *et al*., 1996), and transition metal catalyzation (Heck and Terpko, 1979; Larock and Yum, 1991). However, in terms of synthesis efficiency, these methods are limited by their requirement of several steps to be completed (Dai *et al*., 2001; Ezquerra *et al*., 1996; Rudisill and Stille, 1989; Taylor *et al*., 1985). Furthermore, only a few methods are available to substitute the indole ring at the C2 position because the C3 position is more reactive than the C2 position for electrophilic aromatic substitution. Nevertheless, an *N*-protected indole can undergo organometallic substitution at the C2 position.

In this study, Fischer indole synthesis was applied as a simple and effective method to prepare 2-substituted indoles. The antibacterial activities of the synthesized compounds and their synergistic antibacterial potential with tetracyclines against the clinical isolate of methicillin-resistant *S. aureus* (MRSA) Sp6 were evaluated and described. Their synergistic mechanism was also investigated through molecular docking.

MATERIALS AND METHODS

General information

Melting points (m.p.) were determined using a Stuart Scientific SMP 2 m.p. apparatus and uncorrected. ^{1}H- and ^{13}C-NMR spectra were obtained with (D) d_6-DMSO solutions at 300 MHz for ^{1}H and 75 MHz for ^{13}C with a Bruker AVANCE 300 spectrometer. Tetramethylsilane was used as an internal standard. Mass spectra were recorded with a Polaris Q or Hewlett Packard 5973 mass spectrometer. Reagents and solvents were supplied by Acros, Aldrich, Fluka, and TCI.

General procedure for the synthesis of 2-substituted indoles (4a–m)

Phenylhydrazine (**1**) (1.5 eq.) was mixed with ketone (**2a–m**) (1.0 eq.) in 50% acetic acid (20 ml). The mixture was added to a beaker containing polyphosphoric acid (1.0 eq.), stirred while being heated in an oil bath for 30 minutes. The reaction mixture was cooled to room temperature and added to iced water (50 ml) to yield a black precipitate. The precipitate was crystallized in ethanol to obtain 2-substituted indoles (**4a–m**).

*2-Methylindole (**4a**)*

The title compound was synthesized from **1** (0.50 ml, 5.1 mmol) and acetone (**2a**) (0.5 ml, 6.8 mmol) to afford **4a** (0.44 g, 66% yield) as a pale yellow substance with m.p. of 55.3°C–56.5°C [(Nakazaki, 1976); 58°C–60°C]; ^{1}H NMR (300 MHz, $CDCl_3$); δ: 7.83 (br, N), 7.50 (d, J = 7.4 Hz, 1H), 7.28 (d, J = 7.4 Hz, 1H), 7.11 (td, J = 1.4, 7.1 Hz, 1H), 7.06 (td, J = 1.4, 7.1 Hz, 1H), 6.21 (s, 1H), 2.45 (s, 3H) ppm.

*2-tert-Butyl indole (**4b**)*

The title compound was synthesized from **1** (0.60 ml, 6.1 mmol) and 1,1,1-trimethylacetone (**2b**) (0.90 ml, 6.1 mmol) to afford **4b** (0.49 g, 46% yield) as dark solids; m.p. 71.3°C–72.8°C [(Fuher *et al*., 2019); 75°C–76°C]; ^{1}H NMR (300 MHz, $CDCl_3$); δ: 7.94 (br, NH), 7.54 (d, J = 7.6 Hz, 1H), 7.32 (d, J = 7.6 Hz, 1H), 7.12 (t, J = 7.6 Hz, 1H), 7.06 (t, J = 7.6 Hz, 1H), 6.26 (s, 1H), 1.40 (s, 9H) ppm.

*2-Phenyl-1H-indole (**4c**)*

The title compound was synthesized from **1** (0.60 ml, 60 mmol) and acetophenone (**2c**) (0.50 ml, 40 mmol) to afford **4c** (6.49 g, 84% yield) as yellow crystals; m.p. 187.9°C–188.3°C [(Bremner *et al*., 2008); 190.5°C–191.0°C]; ^{1}H NMR (300 MHz, DMSO-d_6); δ: 6.90 (1H, s), 6.99 (1H, t, J = 7.5 Hz), 7.10 (1H, t, J = 7.5 Hz), 7.3 (1H, t, J = 7.8 Hz), 7.41 (1H, d, J = 7.5 Hz), 7.46 (2H, t, J = 7.8 Hz), 7.53 (1H, d, J = 7.8 Hz), 7.86 (2H, d, J = 7.8 Hz), 11.53 (1H, s); ^{13}C-NMR (DMSO-d_6); δ: 100.0, 110.9, 120.3, 120.7, 112.4, 125.2, 127.7, 129.9, 129.3, 132.4, 136.8, 137.9; HRES-MS m/z calcd for $[M]^+$ $C_{14}H_{11}N$: 194.0970, found: 194.0966.

*2-(4-Methylphenyl)-1H-indole (**4d**)*

The title compound was synthesized from **1** (6.0 ml, 60 mmol) and 4-methylacetonephenone (**2d**) (6.56 g, 40 mmol) to afford **4d** (6.88 g, 83% yield) as yellow crystals; m.p. 218.4°C–219.7°C; ^{1}H NMR (300 MHz, DMSO-d_6); δ: 2.34 (3H, s), 6.83 (1H, s), 6.98 (1H, t, J = 7.8 Hz), 7.08 (1H, t, J = 7.8 Hz), 7.28 (2H, d, J = 8.1 Hz), 7.39 (1H, d, J = 7.8 Hz), 7.56 (1H, d, J = 7.8 Hz), 7.75 (2H, d, J = 8.1 Hz), 11.47 (1H, s);); ^{13}C-NMR (DMSO-d_6); δ: 20.9, 98.2, 111.3, 119.5, 120.0, 121.5, 125.0, 128.8, 129.5, 129.6, 136.9, 137.1, 137.9 [(Shi *et al*., 2008); ^{1}H NMR (300 MHz, acetone-d_6); δ: 2.29 (3H, s), 6.80 (1H, s), 7.00–6.95 (1H, m), 7.08–7.03 (2H, m), 7.23 (1H, d, J = 7.8), 7.38–7.34 (1H, m), 7.52 (1H, d, J = 9.0 Hz), 7.71 (2H, t, J = 8.4 Hz), 10.56 (1H, s). ^{13}C NMR (75 MHz, acetone-d_6); δ: (21.0, 99.2, 111.8, 120.2, 120.8, 122.3, 125.7, 130.2, 130.8, 137.1, 138.1)]; HRES-MS m/z calcd for $[M]^+$ $C_{15}H_{14}N$: 208.1126, found: 208.1131.

*2-(3-Methoxyphenyl)-1H-indole (**4e**)*

The title compound was synthesized from **1** (6.0 ml, 60 mmol) and 3-methoxyacetophenone (**2e**) (6.56 g, 40 mmol) to afford **4e** (7.41 g, 83% yield) as dark yellow crystals; m.p. 128.9°C–130.0°C; ^{1}H NMR (300 MHz, DMSO-d_6); δ: 3.84 (3H, s), 6.88 (1H, d, J = 8.1 Hz), 6.92 (1H, s), 7.00 (1H, t, J = 7.8 Hz), 7.10 (1H, t, J = 8.1 Hz), 7.36 (1H, t, J = 8.1 Hz), 7.40 (1H, d, J = 8.1 Hz), 7.44 (1H, s), 7.45 (1H, d, J = 7.8 Hz), 7.53 (1H, d, J = 7.8 Hz), 11.53 (1H, s); ^{13}C-NMR (DMSO-d_6); δ: 55.6, 99.3, 110.5, 111.6, 113.4, 117.8, 119.8, 120.4, 122.0, 128.5, 130.4, 133.8, 137.3, 137.7, 160.0 [(Shi *et al*., 2008); ^{1}H NMR (300 MHz, $CDCl_3$); δ: 3.85 (3H, s), 6.74 (1H, s), 6.84 (1H, d, J = 8.4 Hz), 7.08 (1H, s), 7.43–7.24 (4H, m), 7.61 (2H, d, J = 7.2 Hz), 8.23 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$); δ: 55.8, 99.8, 102.2, 111.6, 112.6, 125.0, 127.5, 128.9, 129.7, 138.6, 152.0, 154.4]; HRES-MS m/z calcd for $[M]^+$ $C_{15}H_{14}NO$: 224.1075, found: 224.1060.

*2-(4-Methoxyphenyl)-1H-indole (**4f**)*

The title compound was synthesized from **1** (6.0 ml, 60 mmol) and 4-methoxyacetophenone (**2f**) (6.56 g, 40 mmol) to afford **4f** (7.14 g, 80% yield) as dark yellow crystals; m.p. 221.0°C–222.5°C; ^{1}H NMR (300 MHz, DMSO-d_6); δ: 3.83 (3H, s), 6.75 (1H, s), 6.97 (1H, t, J = 6.9 Hz), 7.03 (2H, d, J = 6.9 Hz), 7.06 (1H, t, J = 9.0 Hz), 7.37 (1H, d, J = 6.9 Hz), 7.49 (1H, d,

J = 6.9 Hz), 7.79 (2H, d, *J* = 9.0 Hz), 11.42 (1H, s);); ^{13}C-NMR (DMSO-d_6); *δ*: 55.4, 97.5, 111.2, 114.5, 119.4, 119.8, 121.2, 125.0, 126.5,1 29.0, 137.1, 138.0, 159.0 [(Shi *et al*., 2008); ^{1}H NMR (300 MHz, acetone-d_6); *δ*: 3.86 (3H, s), 6.76 (1H, s), 7.01–6.97 (4H, m), 7.38 (1H, d, *J* = 9.0), 7.53 (1H, d, *J* = 7.5 Hz), 7.85–7.77 (2H, m), 10.55 (1H, s). ^{13}C NMR (75 MHz, acetone-d_6); *δ*: 60.4, 103.5, 116.6, 120.0, 125.1, 125.5, 126.9, 131.1, 132.1, 135.2, 143.1, 143.8, 165.1]; HRES-MS m/z calcd for $[M]^+$ $C_{15}H_{14}NO$: 224.1075, found: 224.1088.

*2-(2-Hydroxyphenyl)-1H-indole (**4g**)*

The title compound was synthesized from **1** (6.0 ml, 60 mmol) and 2-hydroxyacetophenone (**2g**) (4.80 ml, 40 mmol) to afford **4g** (5.01 g, 60% yield) as dark green crystals; m.p. 221.0°C–223.0°C; ^{1}H NMR (300 MHz, DMSO-d_6); *δ*: 6.82–6.91 (3H, m), 7.04 (1H, d, *J* = 8.7 Hz), 7.06 (1H, d, *J* = 8.7 Hz), 7.21 (1H, t, *J* = 8.0 Hz), 7.28 (1H, t, *J* = 8.7 Hz), 7.29 (1H, t, *J* = 8.7 Hz), 7.54 (1H, d, *J* = 8.0 Hz), 9.43 (1H, s), 12.80 (1H, s); ^{13}C-NMR (DMSO-d_6); *δ*: 112.6, 116.7, 117.7, 119.0, 119.4, 120.4, 120.6, 127.5, 129.5, 131.6, 136.5, 145.0, 147.9, 157.4 [(Snape *et al*., 2013); ^{1}H NMR (300 MHz; CDCl3); *δ*: 6.0–5.0 (1H, br s), 6.87 (1H, m, Ar), 6.91 (1H, dd, *J* = 0.9 and 8.1 Hz, Ar), 7.04 (1H, td, *J* = 1.1 and 7.6 Hz, Ar), 7.26–7.11 (3H, m, Ar), 7.42 (1H, d, *J* = 8.1 Hz, Ar), 7.66 (1H, d, *J* = 7.8 Hz, Ar), 7.70 (1H, dd, *J* = 1.6 and 7.8 Hz, Ar), 9.22 (1H, br s); ^{13}C-NMR (75 MHz; CDCl3); *δ*: 100.2, 116.6, 119.1, 120.1, 120.4, 121.5, 122.2, 128.4, 128.9, 134.8, 136.4, 152.0]; HRES-MS m/z calcd for $[M]^+$ $C_{14}H_{12}NO$: 210.0919, found: 210.0908.

*2-(3-Hydroxyphenyl)-1H-indole (**4h**)*

The title compound was synthesized from **1** (6.0 ml, 60 mmol) and 3-hydroxyacetophenone (**2h**) (5.44 g, 40 mmol) to afford **4h** (5.02 g, 60% yield) as violet crystals; m.p. 220.0°C–222.0°C; ^{1}H NMR (300 MHz, DMSO-d_6); *δ*: 6.84 (1H, s), 6.97 (1H, t, *J* = 7.8 Hz), 7.07 (1H, t, *J* = 7.8 Hz), 7.13 (1H, d, *J* = 7.8 Hz), 7.35 (1H, t, *J* = 7.8 Hz), 7.38 (1H, t, *J* = 7.8 Hz), 7.51 (1H, d, *J* = 7.8 Hz), 7.55 (1H, d, *J* = 7.8 Hz), 7.67 (1H, s), 11.67 (1H, s); ^{13}C-NMR (DMSO-d_6); *δ*: 99.0, 111.6, 120.1, 114.7, 116.8, 119.6, 120.2, 121.7, 128.7, 129.8, 133.5, 137.3, 137.6, 157.9 [(Kim *et al*., 2016); ^{1}H NMR (400 MHz, DMSO-d_6); *δ*: 6.73 (1H, d, *J* = 7.6 Hz), 6.79 (1H, s), 7.38 (1H, dd, *J* = 8.0, 2.4 Hz), 6.98 (1H, td, *J* = 7.6, 2.6 Hz), 7.08 (1H, td, *J* = 7.5 Hz, 2.3 Hz), 7.31–7.20 (3H, m), 7.51 (1H, dd, *J* = 7.5, 2.0 Hz), 9.55 (1H, s), 11.44 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6); *δ*: 98.5, 111.3, 111.9, 114.5, 116.0, 119.3, 120.0, 121.4, 128.6, 129.9, 133.5, 137.0, 127.8, 157.8]; HRES-MS m/z calcd for $[M]^+$ $C_{14}H_{12}NO$: 210.0919, found: 210.0934.

*2-(4-Hydroxyphenyl)-1H-indole (**4i**)*

The title compound was synthesized from **1** (6.0 ml, 60 mmol) and 4-hydroxyacetophenone (**2i**) (5.44 g, 40 mmol) to afford **4i** (5.10 g, 61% yield) as yellow crystals; m.p. 240.0°C–241.5°C; ^{1}H NMR (300 MHz, DMSO-d_6); *δ*: 6.68. (1H, s), 6.86 (1H, d, *J* = 8.7 Hz), 6.96 (1H, t, *J* = 7.2 Hz), 7.04 (1H, t, *J* = 7.2 Hz), 7.21 (1H, s), 7.37 (1H, d, *J* = 7.2 Hz), 7.47 (1H, d, *J* = 7.2 Hz), 7.68 (1H, d, *J* = 8.7 Hz), 11.31 (1H, s) [(Tsuchimoto *et al*., 2005); ^{1}H NMR (300 MHz, acetone-d_6); *δ*: d 6.71 (1H, dd, *J* = 2.1, 0.9 Hz,), 6.91 (2H, dt, *J* = 9.0, 2.4 Hz), 6.97 (1H, ddd, *J* = 7.8, 6.8, 1.2 Hz), 6.97 (1H ddd, *J* = 8.0, 7.0, 1.2 Hz), 7.33–7.38 (1H, m), 7.48–7.53 (1H, m), 7.69 (2H, dt, *J* = 8.7, 2.5 Hz), 8.52 (1H, s), 10.47 (1H, bs)]; ^{13}C-NMR (DMSO-d_6); *δ*: 97.0, 114.2, 115.3, 119.7, 121.0, 123.6, 126.6, 126.7, 128.8, 136.0, 137.0, 157.2; HRES-MS m/z calcd for $[M]^+$ $C_{14}H_{12}NO$: 210.0919, found: 210.0903.

*2-(3-Nitrophenyl)-1H-indole (**4j**)*

The title compound was synthesized from **1** (6.0 ml, 60 mmol) and 3-nitroacetophenone (**2j**) (6.56 g, 40 mmol) to afford **4j** (7.05 g, 74% yield) as dark yellow crystals; m.p. 169.2°C–171.3°C; ^{1}H NMR (300 MHz, DMSO-d_6); δ: 7.06 (1H, t, *J* = 7.8 Hz), 7.13 (1H, s), 7.16 (1H, t, *J* = 7.8 Hz), 7.40 (1H, d, *J* = 8.1 Hz), 7.57 (1H, d, *J* = 7.8 Hz), 7.73 (1H, d, *J* = 7.8 Hz), 8.13 (1H, d, *J* = 8.1 Hz), 8.83 (1H, d, *J* = 8.1 Hz), 8.70 (1H, s), 11.42 (1H, s); ^{13}C-NMR (DMSO-d_6); *δ*: 87.1, 111.7, 117.1, 118.5, 120.8, 123.3, 125.1, 130.2, 132.8, 138.7, 141.7, 146.5, 148.5, 161.0 [(Shi *et al*., 2008); ^{1}H NMR (300 MHz, acetone-d_6); *δ*: 6.65 (1H, s), 7.38–7.16 (4H, m), 7.66–7.58 (2H, m), 7.81 (1H, d, *J* = 9.0), 8.21 (1H, d, *J* = 9.0), 8.35 (1H, s), 10.53 (1H, s); ^{13}C-NMR (75 MHz, acetone-d_6); *δ*: 103.2, 109.8, 120.3, 120.8, 122.6, 123.6, 127.6, 129.5, 134.4, 134.9, 138.7, 148.3]; HRES-MS m/z calcd for $[M]^+$ $C_{14}H_{11}N_2O_2$: 239.0821, found: 239.0843.

*2-(4-Nitrophenyl)-1H-indole (**4k**)*

The title compound was synthesized from **1** (6.0 ml, 60 mmol) and 4-nitroacetophenone (**2k**) (6.56 g, 40 mmol) to afford **4k** (7.60 g, 80% yield) as dark yellow crystals; m.p. 173.2°C–175.3°C [(Westwell *et al*., 2013); 168°C–172°C]; ^{1}H NMR (300 MHz, DMSO-d_6); *δ*: 6.87 (1H, s), 6.99 (1H, t, *J* = 7.5 Hz), 7.09 (1H, t, *J* = 7.5 Hz), 7.31 (2H, d, *J* = 9.0 Hz), 7.38 (1H, d, *J* = 7.5 Hz), 7.53 (1H, d, *J* = 7.5 Hz), 7.90 (2H, d, *J* = 9.0 Hz), 11.42 (1H, s); ^{13}C-NMR (DMSO-d_6); *δ*: 102.5, 111.7, 119.9, 120.8, 123.1, 124.36, 125.5, 128.4, 135.2, 137.9, 138.6, 145.8; HRES-MS m/z calcd for $[M]^+$ $C_{14}H_{11}N_2O_2$: 239.0821, found: 239.0803.

*2-(4-Fluorophenyl)-1H-indole (**4l**)*

The title compound was synthesized from **1** (6.0 ml, 60 mmol) and 4-fluoroacetophenone (**2l**) (0.50 ml, 40 mmol) to afford **4l** (6.80 g, 80% yield) as dark green crystals; m.p. 172.9°C–173.5°C; ^{1}H NMR (300 MHz, DMSO-d_6); *δ*: 6.87 (1H, s), 6.99 (1H, t, *J* = 7.5 Hz), 7.09 (1H, t, *J* = 7.5 Hz), 7.31 (2H, dd, *J* = 9.0, 9.0 Hz), 7.38 (1H, d, *J* = 7.5 Hz), 7.53 (1H, d, *J* = 7.5 Hz), 7.90 (2H, dd, *J* = 9.0, 5.4 Hz), 11.42 (1H, s); ^{13}C-NMR (DMSO-d_6); *δ*: 99.2, 111.8, 116.2, 116.5,120.0, 120.6, 122.2, 127.4, 127.5, 129.1, 137.2, 137.69 [(Shi *et al*., 2008); ^{1}H NMR (300 MHz, acetone-d_6); *δ*: 6.84 (1H, s), 7.24–7.01 (3H, m), 7.42 (1H, d, *J* = 10.2 Hz), 7.56 (1H, d, *J* = 8.1 Hz), 7.89–7.84 (1H, m), 10.58 (1H, s); ^{13}C NMR (75 MHz, acetone-d_6); *δ*: 99.6, 111.7, 116.1, 116.3, 120.2, 122.4, 127.5, 127.6, 129.9, 138.1, 164.3]; HRES-MS m/z calcd for $[M]^+$ $C_{14}H_{11}FN$: 212.0876, found: 212.0853.

*2-(4-Aminophenyl)-1H-indole (**4m**)*

The title compound was synthesized from **1** (6.0 ml, 60 mmol) and 4-aminoacetophenone (**2m**) (5.40 g, 40 mmol) to afford **4m** (6.99 g, 84% yield) as dark green crystals; m.p. 261.4°C–262.8°C [(Westwell *et al*., 2013); 168°C–172°C]; ^{1}H NMR (300 MHz, DMSO-d_6); *δ*: 6.71 (1H, s), 6.67–7.00 (2H, br), 6.96 (1H, t, *J* = 7.8 Hz), 6.97 (2H, d, *J* = 7.8 Hz), 7.04 (1H, t, *J* = 7.8 Hz), 7.36 (1H, d, *J* = 7.8 Hz), 7.46 (1H, d, *J* = 7.8 Hz), 7.77

(2H, d, J = 7.8 Hz), 11.39 (1H, s) [(Westwell *et al*., 2013); ^{1}H NMR (500 MHz, DMSO-d_6); δ: 5.27 (2H, s), 6.57 (1H, s), 6.64 (2H, d, J = 8.4 Hz), 6.93 (1H, d, J = 8.5 Hz), 7.00 (1H, m), 7.32 (1H, d, J = 8.2 Hz), 7.43 (1H, d, J = 8.8 Hz), 7.52 (2H, d, J = 8.4 Hz), 11.16 (1H, s)]; ^{13}C-NMR (DMSO-d_6); δ: 95.9, 111.1, 114.6, 119.3, 119.5, 120.6, 120.7, 126.3, 129.3, 136.9, 139.3, 148.0; HRES-MS m/z calcd for $[M]^+$ $C_{14}H_{13}N_2$: 209.1079, found: 209.1090

General procedure for the amidation of 4m with acid bromide derivatives (6a–f)

Acid bromide (**5**) (1.7 eq.) was added to a solution of 2-(4-aminophenyl)-1*H*-indole (**4m**) (1.0 eq.) and Et_3N (0.8 eq.) in dry THF (2.5 ml) at 0°C. The mixture was stirred for 2 hours and water (25 ml) was added. The mixture was extracted with CH_2Cl_2 (3 × 20 ml). The organic phase was washed with sat. $NaHCO_3$ and dried with anh. Na_2SO_4. Then, the solvent was evaporated under vacuum and purified via column chromatography (silica gel, 1:2 hexane:EtOAc) to give the amide derives (**6a–f**).

*2-(4-Acetamidophenyl)-1H-indole (**6a**)*

The title compound was synthesized from **4m** (75 mg, 0.36 mmol), Et_3N (0.05 ml, 0.36 mmol), and acetyl bromide (0.05 ml, 0.6 mmol) to obtain 2-(4-acetamidophenyl)-1*H*-indole (**6a**) (47.8 mg, 53% yield) as a brown solid; m.p. 286.5°C–288.2°C [(Ackermann *et al*., 2009); 283.9°C–286.8°C]; ^{1}H NMR (300 MHz, DMSO-d_6); δ: 11.42 (br, NH), 10.04 (br, NH), 7.75 (d, J = 8.7 Hz, 2H), 7.65 (d, J = 8.7 Hz, 2H), 7.49 (d, J = 7.7 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.07 (t, J = 8.0 Hz, 1H), 6.97 (t, J = 7.9 Hz, 1H), 6.79 (s, 1H), 2.07 (s, 3H) ppm; ^{13}C-NMR (DMSO-d_6); δ: 21.4 (CH_3), 96.5, 111.9, 114.5 (×2), 118.6, 119.3, 120.7, 121.7, 127.7 (×2), 128.9, 136.0, 139.5, 147.8, 172.5 [(Ackermann *et al*., 2009); ^{1}H-NMR (300 MHz, DMSO-d_6); δ: 2.10 (3H, s), 6.80 (1H, m), 7.02–6.96 (1H, m), 7.11–7.06 (1H, m), 7.42–7.40 (1H, m), 7.52 (1H, d, J = 7.7 Hz), 7.71 (2H, d, J = 8.6 Hz), 7.81 (2H, d, J = 8.6 Hz), 10.04 (1H, s), 11.42 (1H, s). ^{13}C-NMR (126 MHz, DMSO-d_6); δ: 24.0, 97.8, 111.1, 119.2, 119.2, 119.7, 121.2, 125.4, 127.0, 128.8, 137.0, 137.7, 138.6, 168.3]; HRES-MS m/z calcd for $[M]^+$ $C_{16}H_{14}N_2O$: 250.1106, found 250.1079.

*2-(4-Propioamidophenyl)-1H-indole (**6b**)*

The title compound was synthesized from **4m** (64 mg, 0.31 mmol), Et_3N (0.05 ml, 0.4 mmol), and propanoyl chloride (0.05 ml, 0.6 mmol) to obtain 2-(4-propioamidophenyl)-1H-indole (**6b**) (50.0 mg, 62% yield) as a pale orange solid; m.p. 256.1°C–257.2°C; ^{1}H NMR (300 MHz, DMSO-d_6); δ: 1.11 (3H, t, J = 7.5 Hz), 2.35 (2H, q, J = 7.5 Hz), 6.81 (1H, s), 6.98 (1H, t, J = 7.4 Hz), 7.07 (1H, t, J = 8.0 Hz), 7.38 (1H, d, J = 8.0 Hz), 7.50 (1H, d, J = 8.0 Hz), 7.68 (2H, d, J = 8.4 Hz), 7.79 (2H, d, J = 8.4 Hz), 9.97 (1H, br, NH), 11.45 (1H, br, NH); ^{13}C-NMR (DMSO-d_6); δ: 9.5, 26.6, 96.8, 111.1, 114.8 (×2), 119.4, 119.8, 120.7, 121.5, 127.6 (×2), 129.4, 135.6, 138.5, 148.2, 173.2; HRES-MS m/z calcd for $[M]^+$ $C_{17}H_{16}N_2O$: 264.1263, found 264.1222.

*2-(4-Octanamidophenyl)-1H-indole (**6c**)*

The title compound was synthesized from **4m** (70 mg, 0.34 mmol), Et_3N (0.05 ml, 0.36 mmol), and octanoyl chloride (0.10 ml, 0.59 mmol) to obtain 2-(4-octanamidophenyl)-1H-indole (**6c**) (50 mg, 44% yield) as a white solid; m.p. 239.2°C–240.2°C; ^{1}H NMR (300 MHz, DMSO-d_6); δ: 0.88 (3H, t, J = 6.9 Hz), 1.35–1.20 (8H, m), 1.70–1.55 (2H, m), 2.33 (2H, t, J = 7.4 Hz, 2H), 6.80 (1H, s), 6.98 (1H, t, J = 7.0 Hz), 7.07 (1H, t, J = 7.0 Hz), 7.38 (1H, d, J = 8.0 Hz), 7.50 (1H, d, J = 7.8 Hz), 7.68 (2H, d, J = 8.7 Hz), 7.78 (2H, d, J = 8.7 Hz), 9.97 (1H, br, NH), 11.43 (1H, br, NH); ^{13}C-NMR (DMSO-d_6); δ: 14.1, 25.5, 29.2, 29.5 (×2), 31.7, 33.7, 40.5, 111.1, 114.8 (×2), 119.4, 119.8, 120.7, 121.5, 127.6 (×2), 129.4, 135.6, 138.5, 148.2, 173.2; HRES-MS m/z calcd for $[M]^+$ $C_{21}H_{24}N_2O$: 320.1889, found 320.1875.

*2-(4-Benzamidophenyl)-1H-indole (**6d**)*

The title compound was synthesized from **4m** (92.3 mg, 0.44 mmol), Et_3N (0.1 ml, 0.72 mmol), and benzoyl chloride (0.10 ml, 0.86 mmol) to obtain 2-(4-benzamidophenyl)-1H-indole (**6d**) (0.12 g, 90% yield) as a brown solid; m.p. 281.7°C–282.8°C; ^{1}H NMR (300 MHz, DMSO-d_6); δ: 6.01 (1H, s), 6.14 (1H, t, J = 7.5 Hz), 6.24 (1H, t, J = 7.3 Hz), 6.55 (1H, d, J = 9.0 Hz), 6.81–6.64 (4H, m), 7.07–6.98 (4H, m), 7.14 (2H, d, J = 7.2 Hz), 9.51 (1H, br, NH), 10.63 (1H, br, NH); ^{13}C-NMR (DMSO-d_6); δ: 96.8, 111.1, 114.7 (×2), 119.6, 119.8, 120.7, 121.7, 126.9 (×2), 128.6 (×2), 130.0, 132.1, 134.2, 136.0, 136.3, 137.6, 137.9, 148.5, 171.6; HRES-MS m/z calcd for $[M]^+$ $C_{21}H_{16}N_2O$: 312.1263, found 312.1249.

*2-(2-Nitrobenzamidophenyl)-1H-indole (**6e**)*

The title compound was synthesized from **4m** (75.0 mg, 0.36 mmol), Et_3N (0.05 ml, 0.36 mmol, 0.8 eq.), and 4-nitrobenzyl chloride (0.11 g, 0.63 mmol) to obtain 2-(2-nitrobenzamidophenyl)-1H-indole (**6e**) (0.10 g, 88% yield) as a yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6); δ: 6.91 (1H, s), 7.03 (1H, t, J = 7.5 Hz), 7.12 (1H, t, J = 7.7 Hz), 7.43 (1H, d, J = 9.0 Hz), 7.56 (1H, d, J = 9.0 Hz), 7.92 (4H, s), 8.26 (2H, d, J = 9.0 Hz), 8.43 (2H, d, J = 9.0 Hz), 10.74 (1H, br, NH), 11.55 (1H, br, NH); ^{13}C-NMR (DMSO-d_6); δ: 107.9, 111.1, 113.2, 113.8, 120.2, 120.5, 121.0, 122.2, 126.1, 129.0, 136.92, 136.97, 147.2, 152.0, 171.9; HRES-MS m/z calcd for $[M]^+$ $C_{21}H_{15}N_3O_3$: 357.1113, found 357.1100.

*2-(4-Nitrobenzamidophenyl)-1H-indole (**6f**)*

The title compound was synthesized from **4m** (0.11 g, 0.52 mmol), Et_3N (0.05 ml, 0.36 mmol), and 4-nitrobenzyl chloride (0.05 g, 1 mmol) to obtain 2-(4-nitrobenzamidophenyl)-1H-indole (6**f**) (88.0 mg, 47% yield) as a yellow solid; m.p. 263.4°C–264.4°C; ^{1}H NMR (300 MHz, DMSO-d_6); δ: 6.86 (1H, s), 7.00 (1H, t, J = 7.4 Hz), 7.10 (1H, t, J = 7.5 Hz), 7.40 (1H, d, J = 8.1 Hz), 7.53 (1H, d, J = 7.8 Hz), 7.84–7.71 (4H, m, 4H), 7.95–7.84 (3H, m), 8.18 (1H, d, J = 7.5 Hz), 10.79 (1H, br, NH), 11.48 (1H, br, NH); ^{13}C-NMR (DMSO-d_6); δ: 107.8, 110.9, 113.1, 113.8, 120.0, 120.4, 121.0, 122.1, 126.1, 128.9, 136.8, 136.9, 147.1, 152.0, 171.8; HRES-MS m/z calcd for $[M]^+$ $C_{21}H_{15}N_3O_3$: 357.1113, found 357.1105.

Determination of antibacterial activity

The MICs of **4a–4 m** and **6a–6f** were tested against *S. aureus* ATCC 25932, *B. cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 10536, and *Salmonella typhi* ATCC 19430 and determined with National Committee for Clinical Laboratory Standards (NCCLS) microbroth dilution methods (National Committee for Clinical Laboratory

Standards, 2000). Kanamycin and chloramphenicol were used as references for antibacterial activity. The test samples were dissolved in DMSO with a range of 512–0.98 µg/ml in nutrient broth supplemented with 10% glucose and 0.05% phenol red (NBGP). Then, 100 µl of each concentration was added to each well (96-well microplate) containing 95 µl of NBGP and 5 µl of inoculum (standardized at 1.5×10^6 cfu/ml by adjusting the optical density to 0.1 at 600 nm. The final concentration of DMSO in the well was less than 1% preliminary analyses with 1% (v/v) DMSO/NBGP affected neither the growth of the test organisms nor the change in color because of this growth). The negative control well contained 195 µl of NBGP and 5 µl of the standard inoculum. The plates were covered with a sterile plate sealer, agitated using a plate shaker to mix the content of the wells, and incubated at 37°C for 24 hours. The assay was repeated twice, and microbial growth was determined by observing the change in the color of the content of the wells (i.e., red = without growth, yellow with growth). The lowest concentration showing no color change was considered the MIC.

Synergistic antibacterial assay

MRSA Sp6 was isolated from pus samples of patients in Nakhon Pathom Hospital (Taechowisan *et al*., 2018). The drug resistance of this strain was detected via a cefoxitin paper method of the American Association of Clinical and Laboratory Standards Institute (CLSI, 2014). Its drug resistance and efflux pump genes were also detected through PCR amplification. The primers of the species-specific *S. aureus* (*femA*) gene, the penicillin-binding protein 2 (*mecA*) gene, and the active efflux pump of the plasmid-located tetracycline (*tetK*) gene were utilized for PCR assay as previously described (Taechowisan *et al*., 2018).

The MICs of methicillin and tetracycline were determined using a twofold dilution method in accordance with the guidelines of the National Committee for Clinical Laboratory Standards (1997). In this procedure, 100 ml of MRSA Sp6 (10^5 cfu/ml) was mixed with different concentrations of methicillin and tetracycline (16, 32, 64, and 128 mg/ml) in a 96-well plate and incubated at 37°C for 24 hours. A control group was prepared by culturing MRSA Sp6 with 0 mg/ml methicillin and tetracycline. Various concentrations (16, 32, 64, and 128 mg/ml) were applied to 100 ml of MRSA Sp6 (10^5 cfu/ml) cells alone or in combination with either methicillin or tetracycline (16, 32, 64, and 128 mg/ml) in 96-well plates to elucidate the effect of methicillin and tetracycline activities in the presence of 2-arylindole derivatives. The plates were incubated at 37°C for 24 hours. A blank control was also prepared by culturing MRSA Sp6 cells in media only without methicillin and tetracycline or 2-arylindole derivatives.

Molecular modeling

The homology model of NorA from *S. aureus* was constructed using a Swiss-Model server and the protein EmrD efflux pump from *E. coli* (PDB ID: 2GFP) because the crystal structure of NorA from *S. aureus* remained unavailable (Zárate *et al*., 2019). Molecular docking was performed with iGEMDOCK v2.1 (Hsu *et al*., 2011) to explore the protein-substrate interactions and binding position of **4g–k** and **4 m** in the active site of NorA from *S. aureus*. Ciprofloxacin, which is known as a good inhibitor of NorA efflux pumps, was also docked into the NorA efflux pump, and its binding energy was compared with those of our compounds.

Prediction of ADMET by computational analysis

The computational prediction of the compounds was performed through the online software SwissADME (http://swissadme.ch) in order to evaluate the pharmacokinetics of the synthesized molecules. The interaction of the synthesized molecules with CYP was used to predict the toxicity properties. Passive human gastrointestinal absorption (HIA) and blood–brain barrier (BBB) were used to predict the pharmacokinetic behaviors of these molecules. Caco-2-permeability was considered as drug-likeness property.

RESULTS AND DISCUSSION

Chemistry

2-Arylindoles were prepared in a manner similar to previously reported Fischer indole synthesis (Hughes, 1993)

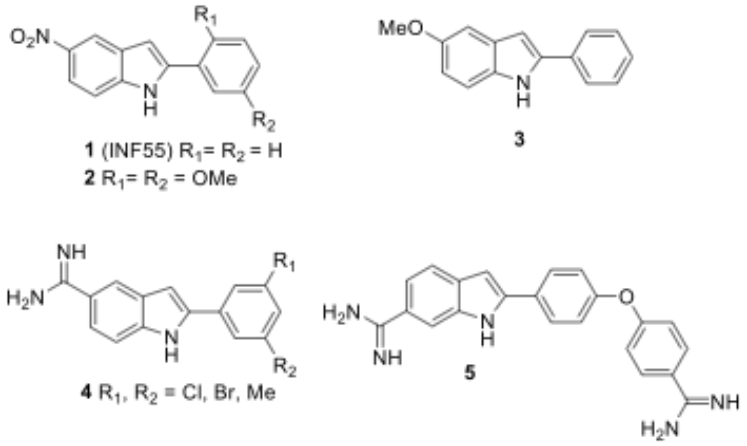


Figure 1. Bioactive 2-arylindole.

Table 1. Synthesis of 2-substituted phenyl-1*H* indole derivatives.

Entry	Ketone	R_1	Time (minute)	Temp (°C)	Product (%)
1	**2a**	CH_3	15	80	**4a** (66)
2	**2b**	$C(CH_3)_3$	15	80	**4b** (46)
3	**2c**	Ph	15	80	**4c** (88)
4	**2d**	*p*-CH_3Ph	30	100	**4d** (83)
5	**2e**	*m*-OCH_3Ph	120	100	**4e** (73)
6	**2f**	*p*-OCH_3Ph	240	100	**4f** (70)
7	**2g**	*o*-phenol	240	100	**4g** (60)
8	**2h**	*m*-phenol	120	100	**4h** (60)
9	**2i**	*p*-phenol	240	100	**4i** (61)
10	**2j**	*m*-NO_2Ph	10	75	**4j** (74)
11	**2k**	*p*-NO_2Ph	5	70	**4k** (80)
12	**2l**	*p*-FPh	30	100	**4l** (80)
13	**2m**	*p*-NH_2Ph	60	100	**4m** (84)

(Scheme 1). After the reaction parameters were screened, the reaction of **1** (1.5 eq.) and methyl ketone derivatives (2; 1.0 eq.) in 50% acetic acid (20 ml) produced moderate to high yields of indole derivatives (**4**) via hydrazone derivatives (**3**). The reaction time and temperature of each product under the optimized conditions are shown in Table 1. The heating duration of most of the aromatic derivatives of **2** at high temperatures should be prolonged. Unidentified products except nitrobenzene substitution (entries 10–11) were obtained at high temperatures (Scheme 2). The structures of all known compounds were confirmed by m.p. and spectroscopic analysis.

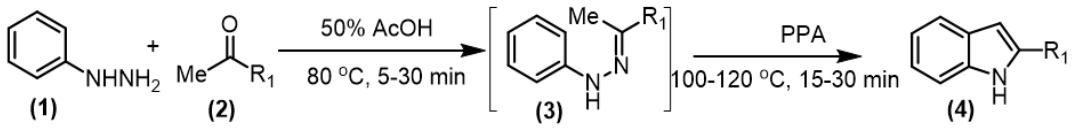
The substitutions of amino groups on the aromatic ring were investigated to study the structure-activity relationship (Scheme 2). Amidation using acid halide (**5**) produced moderated yields of amides (**6a–f**). Then, the antibacterial activities and synergistic effects of the synthesized 2-arylindole derivatives were further evaluated.

Antibacterial activity

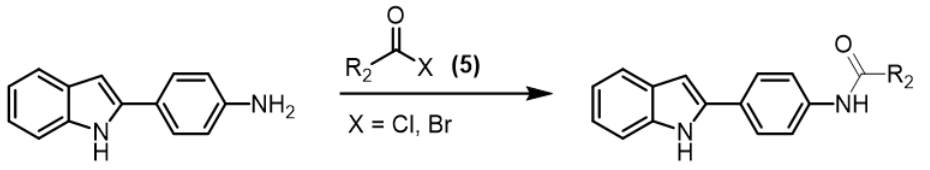
All the synthesized compounds **4a–m** and **6a–f** were tested against *S. aureus* ATCC 25932, *B. cereus* ATCC 7064, *B. subtilis* ATCC 6633, *E. coli* ATCC 10536, and *S. typhi* ATCC 19430 by NCCLS microbroth dilution methods (National Committee for Clinical Laboratory Standards, 1997). Kanamycin and chloramphenicol were used as positive controls, and a solvent was used as a negative control. The results are shown in Table 2 as MIC (µg/ml) unit. Among the compounds tested, **4m** was the most active with an MIC of 15.6 µg/ml against *B. subtilis* and *S. typhi*. Furthermore, **4c**, **4d**, and **4f** selectively inhibited *B. subtilis* and had MICs of 31, 125, and 62 µg/ml, respectively. In addition, **4j** showed antibacterial activities against *S. typhi* at 15.6 µg/ml. Thus, the phenyl ring, substituents, and their positions on the phenyl ring are essential for potency. Compounds **4e** and **4h** with phenyl-bearing electron-donating groups on the *meta*-position have weak or no activity, while compound **4k** bearing electron-withdrawing group on the *meta*-position has selective activity against *S. typhi*. On the other hand, the phenyl-bearing hydrophilic substituents at *para*-position seem to be important for the activity. The protected amino derivatives (**6a–f**) had weak or no antibacterial activity against all the tested bacteria. This may assume that the amide linkage of **4m** affected the hydrophilicity and might obstruct the interaction of these molecules and the bacterial active site.

Synergistic effect

The synthesized compounds were assayed to examine their antibacterial activities against MRSA Sp6. The results revealed that none of them exhibited an inhibitory activity at 128 mg/ml. By comparison, the MIC of tetracycline to MRSA Sp6



Scheme 1. Synthesis of 2-substituted phenyl-1*H* indole derivatives.



6a; R_2= CH_3 (53%)
6b; R_2= CH_2CH_3 (62%)
6c; R_2= $(CH_2)_6CH_3$ (44%)
6d; R_2= Ph (90%)
6e; R_2= 2-NO_2Ph (88%)
6f; R_2= 4-NO_2Ph (47%)

Scheme 2. Preparation of N-substituted aminobenzene derivatives (**6a–f**).

Table 2. *In vitro* antimicrobial activities of compounds against the tested bacteria.

Compound	MIC (µg/ml)				
	Gram-positive			Gram-negative	
	S. aureus	*B. subtilis*	*B. cereus*	*S. typhi*	*E. coli*
4a	na	na	na	nt	na
4b	250	125	62.5	nt	500
4c	>500	31	>500	>500	>500
4d	>500	125	>500	>500	>500
4e	250	>500	>500	>500	250
4f	>500	62	>500	>500	>500
4g	31	250	>500	>500	>500
4h	>500	>500	>500	>500	>500
4i	31	62	>500	500	250
4j	125	>500	>500	15.6	>500
4k	62	125	125	250	125
4l	>500	250	500	>500	>500
4m	>500	15.6	>500	15.6	>500
6a	>500	250	>500	>500	>500
6b	>500	>500	>500	>500	>500
6c	>500	>500	>500	>500	>500
6d	>500	>500	>500	>500	>500
6e	>500	>500	>500	>500	>500
6f	>500	>500	>500	>500	>500
Kanamycin	7.8	£1.9	7.8	7.8	7.8
Chloramphenicol	31.25	£1.9	3.9	£1.9	£1.9

was 256 mg/ml. Nevertheless, the MIC of tetracycline decreased notably when the 2-arylindole derivatives were added. This result demonstrated that the sensitivity of MRSA Sp6 to tetracycline improved. Each experiment was repeated three times (Table 3). **4g**, **4j**, and **4k** elicited significant synergistic effects with tetracycline against MRSA Sp6. Thus, the MIC of tetracycline could be reduced from 256 to 32 mg/ml when it was combined with **4g**, **4j**, and **4k** at a concentration of 16 mg/ml.

Molecular docking

Molecular docking was conducted to explore the protein-substrate interactions and binding positions of the active synergistic compounds, namely, **4g–k** and **4m**, in the active site of NorA from *S. aureus*. These compounds were docked into the active site of the *S. aureus* NorA model prepared with the EmrD efflux pump as a template. Their molecular docking

Table 3. Synergistic effect of 2-arylindoles with tetracyclin against MRSA[a].

Substrates	Tetracyclin (mg/ml)				
	0	16	32	64	128
4a	>128	>128	>128	64	32
4c	>128	64	64	32	16
4e	>128	>128	>128	128	64
4f	>128	128	64	32	16
4g	>128	32	16	16	16
4h	>128	64	32	16	16
4i	>128	64	32	16	16
4j	>128	32	16	16	16
4k	>128	32	16	16	16
4l	>128	32	32	16	16
4 m	>128	64	64	64	32
6a	>128	>128	>128	>128	>128
6b	>128	>128	>128	>128	>128
6c	>128	>128	>128	>128	>128
6d	>128	>128	>128	128	64
6e	>128	>128	128	64	64
6f	>128	>128	>128	128	64

[a]MRSA, Methicillin-resistant *S. aureus* (clinical isolate);: genotype: *mecA*-positive, and *tetK*-positive.

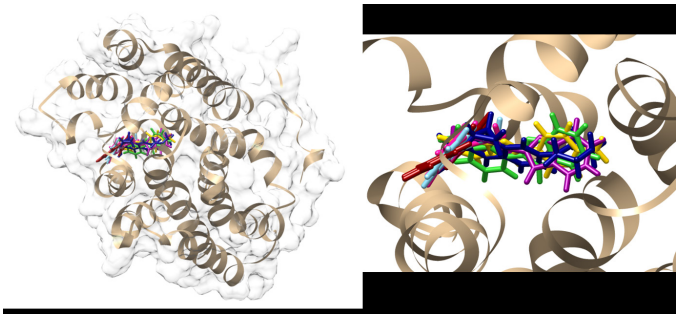


Figure 2. Comparison of the binding positions of **4g** (pink), **4h** (light blue), **4i** (purple), **4j** (green), **4k** (red), **4m** (yellow), and ciprofloxacin (deep blue) in the cavity of the *S. aureus* NorA model prepared using the EmrD efflux pump (PDB ID: 2GFP) as a template.

results were compared with those of the NorA substrate ciprofloxacin. The findings revealed that **4g–k** and **4 m** bound to the cavity of the NorA efflux pump in a position similar to

Table 4. Binding energy (kcal/mol) and residues involved in the interaction of **4g–k**, **4m**, and ciprofloxacin to the NorA efflux pump model.

Compounds	Binding energy (kcal/mol)	Residue implicated in the interaction	
		Hydrogen bond	Hydrophobic
4g	−90.37	Glu222, Ile240	Ile240, Ala243, Ala289
4h	−91.95	Glu222, Trp293	Ile240, Ala243, Ala289
4i	−85.07	Asn290, Asp291	Ile23, Val44, Ile240
4j	−91.73	Asn290, Asp291, Trp293	Val44, Phe47, Ile240, Ala243, Ile244, Tyr292,
4k	−92.00	Ser226	Ile240, Ala243, Ala289
4m	−91.46	Phe47	Val44, Phe47, Ile240, Ala243, Tyr292
Ciprofloxacin	−99.64	Asn290, Asp291, Trp293	Tyr292, Ile240

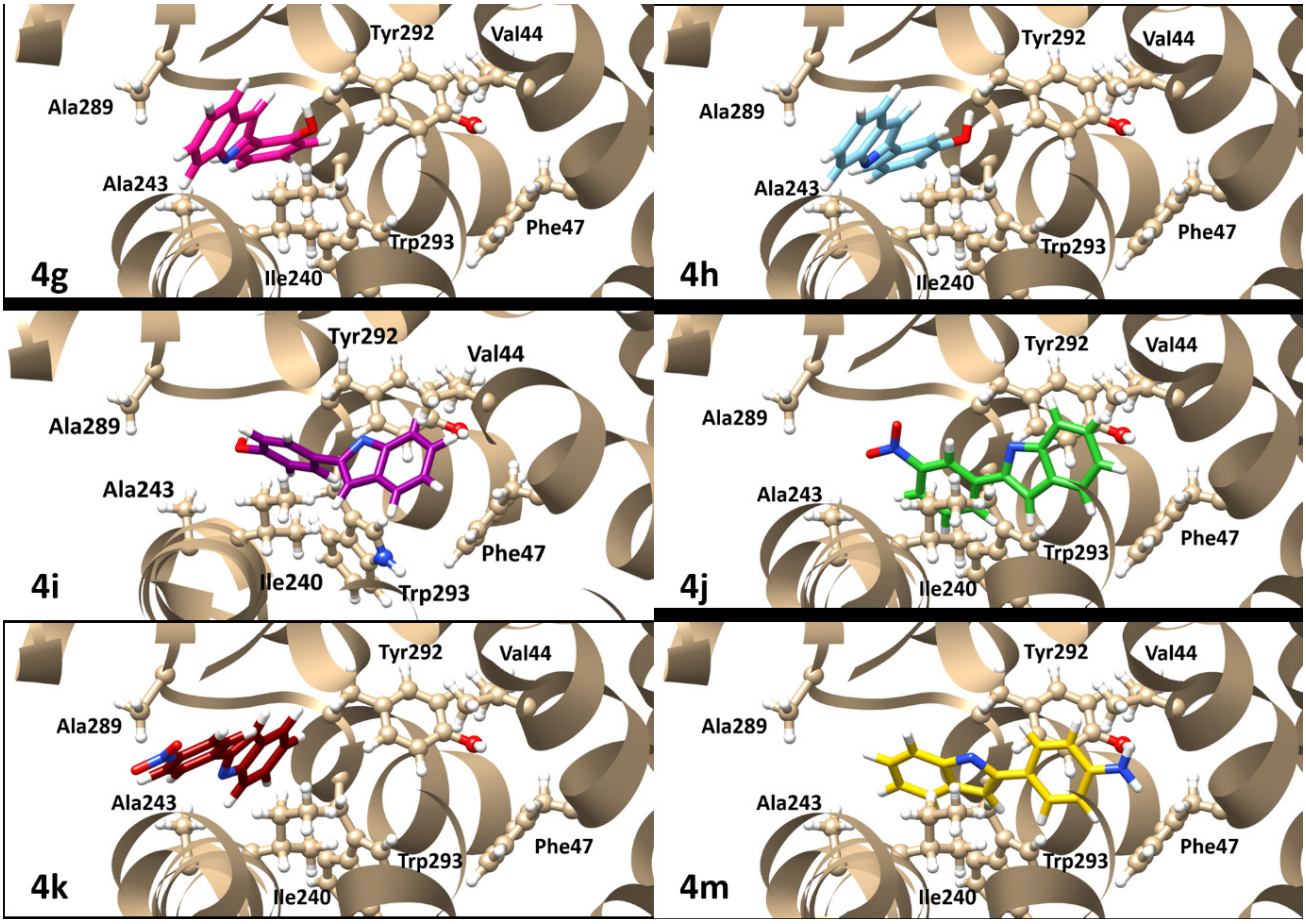


Figure 3. Binding positions of **4g–k** and **4m** in the cavity of the NorA efflux pump model.

Table 5. ADMET properties predicted for compounds **4a–m** and **6a–f**.

	BBB	HIA	CPM	CYP_2C19	CYP_2C9	CYP_2D6		CYP_3A4	
				Inhibition	Inhibition	Inhibition	Substrate	Inhibition	Substrate
4a	1.50	100.00	22.28	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non
4c	13.68	100.00	22.37	Inhibitor	Inhibitor	Non	Weakly	Inhibitor	Non
4e	10.90	95.09	31.32	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non
4f	11.58	95.09	31.32	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non
4g	8.65	91.94	38.13	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non
4h	8.65	91.94	3.53	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non
4i	8.65	91.94	3.53	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non
4j	3.56	91.70	3.61	Inhibitor	Inhibitor	Non	Non	Inhibitor	Weakly
4k	3.29	91.70	3.61	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non
4l	14.23	100.00	29.24	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non
4m	5.95	91.90	6.53	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non
6a	4.25	91.82	39.54	Non	Non	Non	Non	Non	Non
6b	6.43	92.04	44.68	Non	Non	Non	Non	Non	Non
6c	14.55	93.09	54.98	Non	Non	Inhibitor	Non	Non	Non
6d	9.30	93.35	48.59	Non	Non	Non	Non	Non	Non
6e	0.70	92.05	18.47	Non	Non	Non	Non	Non	Weakly
6f	1.59	92.05	19.58	Non	Non	Non	Non	Non	Weakly

BBB, Blood–brain barrier; HIA, Human intestinal absorption; CPM, Caco-2-cells permeability.

that of ciprofloxacin (Fig. 2). The binding energies of **4g–k** and **4m** were −92.00 to −85.07 kcal/mol (Table 4), which were slightly higher than that of ciprofloxacin (−99.64 kcal/mol). Moreover, in the active site of the NorA efflux pump, **4g**, **4h**, and **4k** interacted with key amino acid residues, namely, Ile240, Ala243, and Ala289, respectively, while **4i**, **4j**, and **4m** interacted with Val44, Phe47, and Ile240, respectively (Fig. 3). Therefore, the aromatic moieties of **4g–k** and **4m** played important roles in the binding of these compounds to the hydrophobic core of the NorA efflux pump.

Prediction of ADMET by computational analysis

The ADMET properties of the synthesized compounds were presented in Table 5. This result suggested that most of the compounds were through to cross BBB permeation. For *in vitro* Caco-2 cell permeability, most of the compounds except **4h–k** and **4m** have high permeability and are easy to absorb. All of the compounds showed % HIA in the range of 91.70%–100.00% which were considered to be very well absorbed in the gastrointestinal tract. The toxicity predictions were considered of the cytochrome P450s (CYP), an important enzyme system for drug metabolism that influences clearance rates, toxicity, and interactions with coadministered drugs. All compounds were predicted to be not substrates and not inhibitors for four major CYP isoforms, except for compounds **4a–m**, which were predicted to be CYP2C19, CYP2C9, and CYP3A4 inhibitors. This suggested that these compounds may be metabolized in the liver which may have hepatotoxicity.

CONCLUSION

2-Arylindole derivatives were synthesized, and their antibacterial activity and synergistic effects on multidrug-resistant *S. aureus* were evaluated. The results showed that **4j** combined with tetracycline showed a potent antibacterial activity against *S. typhi* and exhibited a synergistic effect against MRSA Sp6 strain. Furthermore, **4 m** displayed the most potent antibacterial activity against *B. subtilis*, whereas **4j** was the most potent against *S. typhi*. All the synthesized compounds improved the sensitivity of MRSA Sp6 to tetracycline. Molecular docking studies on **4g**, **4h**, and **4k** revealed that interaction with key amino acids occurred at the active site of the NorA efflux pump. However, the toxicity study by ADMET was predicted as hepatotoxicity. Therefore, the 2-arylindole derivatives could be considered as an interesting scaffold and further development of the 2-arylindole derivative with no toxicity might be the leading compounds that could potentiate the activity of commercially available drugs against multidrug-resistant bacteria.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit the paper to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors requirements/guidelines.

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The authors report no financial or any other conflicts of interest in this work.

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