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Synthesis of mono and bis-[3,3-di(indolyl)indolin-2-ones] and evaluation of their antimicrobial activity

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Abstract Magnetite sulfuric acid (Fe_3O_4–SO_3H) magnetic nanoparticles have been used as a heterogeneous, recyclable, and efficient catalyst for synthesis of mono and bis-[3,3-di(indolyl)indolin-2-ones] in good yields at room temperature under ultrasonic irradiation. Some of these compounds were assayed for in-vitro antimicrobial activity.

Keywords Magnetic nanoparticles · Magnetite sulfuric acid · Ultrasonic irradiation · 3,3-Di(indolyl)indolin-2-ones · Antimicrobial activity

Introduction

Indole, a very important heterocyclic compound in medical chemistry, is known as a "privileged scaffold". Indole derivatives have important biological activity, for example antimicrobial [1, 2], anti-HIV [3], antifungal [4], anti-inflammatory [5, 6], anticancer [7], antitumor [8], anti-influenza [9], antibacterial [10], and antimalaria [11]. Isatin is an orange red crystalline compound found in plants, for example *Isatis tinctoria*, *Calanthe discolor*, and *Couroupita guianes* [1, 2]. Isatin derivatives are very useful for development of molecules of biological or pharmaceutical interest [12], including some with anticancer [13], anti-inflammatory [14], antiviral [15], anti-HIV [16], and antibacterial [17] activity.

It has been shown that condensation of indole with isatin to form 3,3-di(indolyl)indolin-2-ones can substantially enhance biological activity [18]. The

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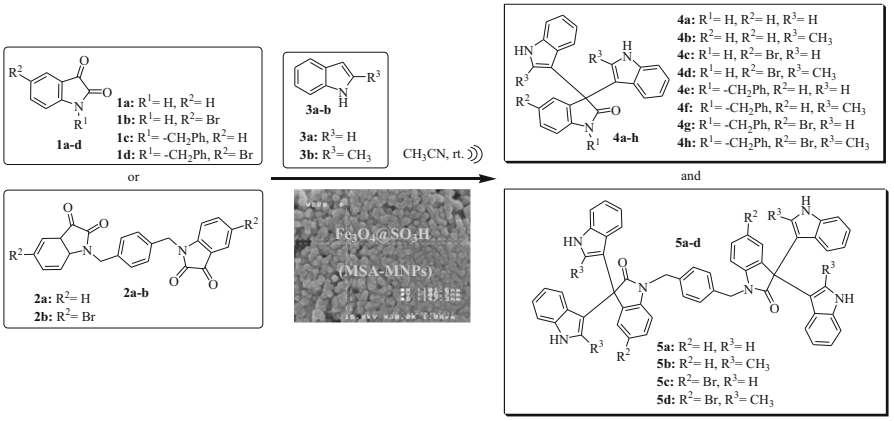
compounds obtained have antibacterial, antiprotozoal, and anti-inflammatory activity and one is a PR (progesterone receptor) agonist [19].

Synthesis of 3,3-di(indolyl)indolin-2-ones has been catalyzed by variety of reagents, for example $KAl(SO_4)_2$ [20, 21], ceric ammonium nitrate (CAN) [22], I_2 [23], *N,N,N,N*-tetramethylguanidinium trifluoroacetate (TMGT) [24], $FeCl_3$ [25], $Bi(OTf)_3$ [26], TfOH [27], and silica sulfuric acid [28], among others. Although 3,3-di(indolyl)indolin-2-ones can be produced by use of these methods, many of the catalysts cannot be reused, and in many instances, long reaction times, drastic reaction conditions, and, sometimes, depending on the nature of the catalyst, tedious workup are needed. The development of new methodology with more efficient reagents, simpler procedures, milder reaction conditions, and higher yields of the products is important.

One of the main objectives of research in recent years has been the design of new catalysts with such specifications as high activity, continuous use, recycling, and environmental safety [29–33]. The development of magnetic nanoparticles has provided opportunities to design and develop a new generation of catalysts with high activity, high selectivity for valuable products, and a long lifetime, and which also make efficient use of energy and are readily separated by use of a simple magnet [34–41].

Application of ultrasonic irradiation in reactions using heterogeneous catalysts is a promising technique. The advantages of such methods, for example reduced reaction times, improved yields, and suppression of side products are well documented [42, 43].

We have recently prepared new magnetite sulfuric acid (Fe_3O_4–SO_3H) magnetic nanoparticles (MSA-MNP) and investigated their catalytic activity in the synthesis of mono, bis, and tris-aryl-14*H*-dibenzo[*a*,*j*]xanthenes [44]. In continuation of our research on the preparation of acid-functionalized magnetic nanoparticles [41] and the use of ultrasonic irradiation for synthesis of organic compounds [41], and because of the biological activity of indole and oxindole derivatives, we report



Scheme 1 Synthesis of mono and bis-[3,3-di(indolyl)indolin-2-ones] (**4a–h** and **5a–d**)

herein a new, green strategy for preparation of mono and bis-[3,3-di(indolyl)indolin-2-one] derivatives **4a–h** and **5a–d** at room temperature by using MSA-MNP as catalyst under the action of ultrasound irradiation. The results showed this method can be use to obtain the products in excellent yields without the need for such purification techniques as column chromatography. Reaction times are short; another advantage of the method is use of an inexpensive and reusable catalyst (Scheme 1).

Compounds **4c–h** and **5d** were screened for antimicrobial activity against *Escherichia coli* PTCC 1330, *Staphylococcus aureus* PTCC, *Pseudomonas aeruginosa* PTCC 1074, and *Candida albicans* PTCC 5027.

Experimental

General

MSA-MNP were prepared as reported elsewhere [44]. Bis-isatins **1c–d** and **2a–b** were prepared by use of a literature method [45]. Products **4c–d** are known compounds; their structures were confirmed by comparison of their physical and spectroscopic data (IR and ^{1}H NMR) with reported data. Melting points were recorded on an Electrothermal digital melting point apparatus and are uncorrected. New products were characterized on the basis of their elemental analysis and their IR, ^{1}H NMR, and ^{13}C NMR spectra. Fourier-transform IR spectra were recorded by use of a Unicom Galaxy Series FTIR 5000 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer at 300 and 75 MHz, respectively. Chemical shifts (δ) are reported relative to TMS as internal standard. Mass spectra were recorded on a Shimadzu QP 1100 EX mass spectrometer operated in EI mode. Elemental analysis was performed by use of a Vario EL III elemental analyzer. Sonication was performed in a Struers Metason 200 HT ultrasonic cleaner with a frequency of 50–60 Hz and an output power of 140 W. The microbial strains *E. coli* PTCC 1330, *S. aureus* PTCC 1337, *P. aeruginosa* PTCC 1074, and *C. albicans* PTCC 5027, used for testing purposes, were purchased from the Persian Type Culture Collection (PTCC). Mueller–Hinton agar was from Merck.

General procedure for the synthesis of mono and bis-[3,3-di(indolyl)indolin-2-ones]

A mixture of isatin **1a–d** or bis-isatin **2a–b** (1 mmol), indole **3a**, **b** (2.1 mmol for **1a–d** or 4.2 mmol for **2a–b**), and MSA-MNP (0.1 g) in CH_3CN (5 mL) was exposed to US irradiation at room temperature for the appropriate time (Table 2). On completion of the reaction, as monitored by TLC, the MSA-MNP were removed by use of an external magnetic field. The solvent was evaporated under reduced pressure and water and ethanol were then added in a 5:1 ratio. The solid products obtained were collected by filtration, then washed with CH_3CN and C_2H_5OH to remove excess indole.

Evaluation of antimicrobial activity

The anti-bacterial assay was performed in accordance with the USP 29-NF25 cylinder plate assay. Briefly, a suspension of microorganisms containing 10^8 cfu/mL (0.5 McFarland) was prepared, and final dilutions of 1.5×10^5 cfu/mL were prepared in sterile normal saline. Mueller–Hinton agar medium was prepared and sterilized by autoclaving at 121 °C and 15 psi for 15 min. The medium was poured into sterile Petri dishes under aseptic conditions in a laminar air-flow chamber. After solidification of the medium, holes 6 mm in diameter were cut into the agar (8 mm thick). These were inoculated by flooding and filled with 100 μL of the different compounds. In general, four cylinders were used per plate. The cylinders were placed on inoculated plates at equal distances. The plates were incubated at 37 °C for 24 and 48 h. Antimicrobial activity was evaluated by measuring the inhibition zone (IZ) around each hole. All the experiments were performed in triplicate.

Minimum inhibitory concentration (MIC) is the lowest concentration of an agent that inhibits visible growth of a microorganism. Inoculated microorganisms were cultured for 12 h in broth; the broth was then diluted to reach the 0.5 McFarland standard. Ninety-six-well plates were prepared by adding 95 μL Mueller–Hinton broth and 5 μL microorganism inoculum to each well. The compounds being tested (100 μg/mL, 100 μL) were added to the first well for each microorganism. Then, 100 μL containing four different concentrations (10, 5, 2.5, and 1.25 μg/mL) were transferred to the other four wells. The last well in each row consisted of 95 μL Mueller–Hinton broth, 100 μL 50 % DMSO, and 5 μL microorganism inoculum, as negative control. The positive control was used as the standard control. The contents of each well were mixed on a shaker for 60 s at 100 rpm and incubated at 37 °C for 24 h (bacteria) and at 25 °C for 72 h (fungi). The turbidities of the microorganism cultures were measured as the optical density at 600 nm by use of a spectrophotometer.

Mono-[di(indolyl)indolin-2-one] (4a) White solid; mp: 241–243 °C; FTIR (KBr): 603, 742, 1014, 1101, 1176, 1222, 1338, 1415, 1471, 1616, 1707, 3055, 3271, and 3400 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 6.78 (2H, t, $J = 7.7$ Hz, H_{arom}), 6.84 (2H, s, H_{arom}), 6.92 (1H, t, $J = 7.5$ Hz, H_{arom}), 6.69–7.03 (1H, m), 7.22–7.24 (4H, m), 7.37 (2H, d, $J = 8.1$ Hz, H_{arom}), 10.58 (1H, s, NH), 10.96 (2H, s, NH) ppm. MS (EI): $m/z = 363$ [M^+]. Anal. calc. for $C_{24}H_{17}N_3O$: C, 79.32; H, 4.72; N, 11.56 %. Found: C, 79.44; H, 4.77; N, 11.51 %.

Mono-[di(indolyl)indolin-2-one] (4b) White solid; mp: 220–224 °C; FTIR (KBr): 605, 744, 1018, 11180, 1300, 1421, 1458, 1616, 1662, 1707, 2920, 3055, 3298, and 3396 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 2.49 (6H, s, CH_3), 6.44 (1H, d, $J = 7.54$ Hz, H_{arom}), 6.60–6.70 (3H, m, H_{arom}), 6.85–6.95 (4H, m, H_{arom}), 7.14 (2H, d, $J = 7.11$ Hz, H_{arom}), 7.20 (2H, d, $J = 7.21$ Hz, H_{arom}), 10.53 (1H, s, NH), 10.85 (s, NH, 2H). MS (EI): $m/z = 391$ [M^+]. Anal. calc. for $C_{26}H_{21}N_3O$: C, 79.77; H, 5.41; N, 10.73 %. Found: C, 79.86; H, 5.48; N, 10.60 %.

Mono-[di(indolyl)indolin-2-one] (4c) White solid; mp: 299–301 °C; FTIR (KBr): 547, 736, 1010, 1105, 1170, 1244, 1336, 1417, 1473, 1616, 1703, 3053,

3360, and 3445 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 6.82 (2H, t, $J = 5.7$ Hz, H_{arom}), 6.88 (2H, d, $J = 1.8$ Hz, H_{arom}), 6.97 (1H, d, $J = 6$ Hz, H_{arom}), 7.03 (2H, t, $J = 5.7$ Hz, H_{arom}), 7.19 (2H, d, $J = 6$ Hz, H_{arom}), 7.29 (1H, d, $J = 1.2$ Hz, H_{arom}), 7.37 (2H, d, $J = 6.3$ Hz, H_{arom}), 7.42 (1H, dd, $J = 6.3$ Hz, H_{arom}), 10.76 (1H, s, NH), 11.02 (2H, s, NH). MS (EI): $m/z = 441$ [M^+]. Anal. calc. for $C_{24}H_{16}BrN_3O$: C, 65.17; H, 3.65; N, 9.50 %. Found: C, 65.23; H, 3.67; N, 9.43 %.

Mono-[di(indolyl)indolin-2-one] (4d) White solid; mp: 300–303 °C; FTIR (KBr): 742, 814, 949, 1020, 1114, 1176, 1244, 1298, 1429, 1473, 1614, 1710, 2916, 3186, 2338, and 3393 cm^{-1}. MS (EI): $m/z = 469$ [M^+]. Anal. calc. for $C_{26}H_{20}BrN_3O$: C, 66.39; H, 4.29; N, 8.93 %. Found: C, 66.44; H, 4.33; N, 8.86 %.

Mono-[di(indolyl)indolin-2-one] (4e) White solid; mp: 243–246 °C; FTIR (KBr): 694, 6740, 916, 1010, 1099, 1168, 1350, 1462, 1606, 1703, 2912, 2970, 3050, and 3257 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 5.02 (s, 2H, CH_2), 6.74 (t, $J = 7.2$ Hz, 2H), 6.87 (s, 2H), 6.98–7.03 (m, 3H), 7.10–7.13 (m, 3H), 7.24–7.33 (m, 5H), 7.35–7.37 (m, 4H), 11.01 (s, 2H, NH) ppm. MS (EI): $m/z = 453$ [M^+]. Anal. calc. for $C_{31}H_{23}N_3O$: C, 82.10; H, 5.11; N, 9.27 %. Found: C, 82.20; H, 5.15; N, 9.21 %.

Mono-[di(indolyl)indolin-2-one] (4f) White solid; mp: 173–176 °C; FTIR (KBr): 698, 740, 1014, 1080, 1172, 1242, 1375, 1456, 1487, 1608, 1668, 1699, 2918, 3036, 3328, and 3398 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 2.50 (6H, s, CH_3), 5.00 (2H, dd, CH_2), 6.36 (1H, d, $J = 8.16$ Hz, H_{arom}), 6.57–6.63 (2H m, H_{arom}), 6.85–6.95 (4H, m, H_{arom}), 7.06 (2H, d, $J = 7.70$ Hz, H_{arom}),7.20–7.25 (8H, m, H_{arom}), 10.88 (s, NH, 1H), 10.92 (s, NH, 1H). MS (EI): $m/z = 481$ [M^+]. Anal. calc. for $C_{33}H_{27}N_3O$: C, 82.30; H, 5.65; N, 8.73 %. Found: C, 82.41; H, 5.58; N, 8.69 %.

Mono-[di(indolyl)indolin-2-one] (4g) White solid; mp: 268–270 °C; FTIR (KBr): 688, 742, 1101, 1167, 1244, 1338, 1419, 1602, 1662, 1701, 2903, 3053, 3329, and 3391 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 5.02 (2H, s, CH_2), 6.76 (2H, t, $J = 6$ Hz, H_{arom}), 6.90 (1H, d, $J = 2.1$ Hz, H_{arom}), 7.03 (2H, t, $J = 5.4$ Hz, H_{arom}), 7.07–7.13 (3H, m, H_{arom}), 7.30–7.39 (8H, m, H_{arom}), 7.47 (2H, dd, $J = 6.3$ Hz, H_{arom}), 11.09 (s, 2H, NH). MS (EI): $m/z = 531$ [M^+]. Anal. calc. for $C_{31}H_{22}BrN_3O$: C, 69.93; H, 4.16; N, 7.89 % Found: C, 70.02; H, 4.20; N, 7.94 %.

Mono-[di(indolyl)indolin-2-one] (4h) White solid; mp: 171–174 °C; FTIR (KBr): 538, 740, 1016, 1170, 1330, 1419, 1456, 1602, 1664, 1703, 2916, 3053, 3337, and 3398 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 2.50 (6H, s, CH_3), 4.99–5.08 (2H, dd, CH_2, H_{arom}), 6.35 (1H, d, $J = 6$ Hz, H_{arom}), 6.60–6.65 (3H, m, H_{arom}), 6.90–6.94 (2H, m, H_{arom}), 7.08 (1H, d, $J = 6.3$ Hz, H_{arom}), 7.22–7.26 (8H, d, $J = 6.9$ Hz, H_{arom}), 7.47–7.50 (1H, dd, $J = 6.3$ Hz, H_{arom}), 10.92 (s, NH, 1H), 11.02 (s, NH, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 12.93, 13.10, 42.93, 52.24, 106.61, 108.22, 109.29, 110.58, 111.42, 113.80, 118.11, 118.58, 118.98, 119.82, 120.03, 126.65, 127.28, 127.33, 127.43, 127.57, 128.44, 128.55, 128.65, 129.41, 130.65, 1320.12, 134.19, 134.88, 134.97, 135.87, 136.92, 141.06, 176.88.

MS (EI): $m/z = 559$ [M^+]. Anal. calc. for $C_{33}H_{26}BrN_3O$: C, 70.72; H, 4.68; N, 7.50; % Found: C, 70.78; H, 4.73; N, 7.43 %.

Bis-[di(indolyl)indolin-2-one] (5a) White solid; mp: 241–244 °C; FTIR (KBr): 738, 914, 1003, 1099, 1242, 1292, 1350, 1496, 1612, 674, 1730, 3134, and 3344 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 4.99 (4H, s, CH_2), 6.64–6.68 (4H, t, $J = 6.0$ Hz, H_{arom}), 6.85 (2H, d, $J = 1.8$ Hz, H_{arom}), 6.95–7.01 (6H, m), 7.08–7.13 (6H, t, $J = 7.5$ Hz, H_{arom}), 7.23–7.27 (4H, t, $J = 5.7$ Hz, H_{arom}), 7.30–7.35 (6H, t, $J = 7.8$ Hz, H_{arom}), 7.45 (2H, s, H_{arom}), 7.54–7.57 (2H, t, $J = 4.2$ Hz, H_{arom}), 10.98 (s, NH, 4H). MS (EI): $m/z = 828$ [M^+]. Anal. calc. for $C_{56}H_{40}N_6O_2$: C, 81.14; H, 4.86; N, 10.14 %. Found: C, 81.24; H, 4.90; N, 10.08 %.

Bis-[di(indolyl)indolin-2-one] (5b) White solid; mp: 264–267 °C; FTIR (KBr): 592, 692, 742, 920, 1020, 1174, 1302, 1361, 1421, 1458, 1606, 1697, 3051 and 3302 cm^{-1}. MS (EI): $m/z = 885$ [M^+]. Anal. calc. for $C_{60}H_{48}N_6O_2$: C, 81.42; H, 5.47; N, 9.50 %. Found: C, 81.53; H, 5.52; N, 9.38 %.

Bis-[di(indolyl)indolin-2-one] (5c) White solid; 248–250 °C; FTIR (KBr): 236, 742, 1012, 1101, 1165, 1244, 1336, 1479, 1604, 1662, 1705, 2864, 2918, 3055, 3294 and 3408 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 5.00 (4H, s, CH_2, H_{arom}), 6.64–6.68 (4H, t, $J = 6.89$ Hz, H_{arom}), 6.91 (4H, s, H_{arom}), 6.96–7.01 (5H, t, $J = 7.03$ Hz, H_{arom}), 7.07 (4H, d, $J = 7.89$ Hz, H_{arom}), 7.14–7.16 (3H, d, $J = 8.08$ Hz, H_{arom}), 7.33–7.38 (8H, t, $J = 7.69$ Hz, H_{arom}), 7.45–7.48 (2H, d, $J = 8.10$ Hz, H_{arom}), 11.06 (s, NH, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 42.69, 52.38, 111.45, 111.51, 111.54, 111.73, 113.09, 113.12, 114.14, 118.38, 120.46, 121.03, 121.08, 124.31, 121.38, 124.48, 125.20, 127.25, 127.95, 128.00, 130.74, 130.91, 135.53, 136.02, 136.72, 136.89, 140.95, 176.53. Anal. calc. for $C_{56}H_{38}Br_2N_6O_2$ C, 68.16; H, 3.88; N, 8.52; %. Found: C, 68.10; H, 3.91; N, 8.47 %.

Bis-[di(indolyl)indolin-2-one] (5d) White solid; mp: 270–272 °C; FTIR (KBr): 522, 744, 814, 1016, 102, 1172, 1242, 1367, 15620, 1593, 1707, 2986, 3059 and 3205 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 2.50 (12H, s, CH_3,), 4.94 (4H, s, CH_2), 6.64–6.68 (4H, t, $J = 5.7$ Hz, H_{arom}), 6.89–6.90 (4H, d, $J = 1.5$ Hz, H_{arom}), 6.97–7.00 (4H, t, $J = 5.7$ Hz, H_{arom}), 7.05–7.07 (4H, d, $J = 6$ Hz, H_{arom}), 7.14–7.16 (4H, d, $J = 6.3$ Hz, H_{arom}), 7.32 (1H, s, H_{arom}), 7.35–7.37 (3H, d, $J = 6.3$ Hz, H_{arom}), 7.45–7.48 (2H, dd, $J = 6.3$ Hz, H_{arom}), 10.83 (4H, s, NH, H_{arom}). Anal. calc. for $C_{60}H_{46}Br_2N_6O_2$: C, 69.10; H, 4.45; N, 8.06 %. Found: C, 69.17; H, 4.39; N, 8.02 %.

Results and discussion

Magnetite sulfuric acid (Fe_3O_4–SO_3H) magnetic nanoparticles (MSA-MNP) were obtained in a simple one-step procedure by direct reaction of chlorosulfonic acid with Fe_3O_4 [29]. Mono-[3,3-di(indolyl)indolin-2-one] derivatives **4a**–**h** were obtained by the reaction of 1 mmol isatin **1a**–**d** with 2.1 mmol indole **3a**, **b** in the presence of 0.1 g MSA-MNP as heterogeneous catalyst. The reaction was

performed in CH_3CN at room temperature under ultrasonic conditions to give products **4a**–**h** in high yields. The same procedure was used for preparation of mono-[3,3-di(indolyl)indolin-2-one] derivatives **5a**–**d** by reaction of bis-isatin **2a**, **b** (1 mmol) with indole **3a**, **b** (4.2 mmol).

To optimize the reaction conditions, reaction of isatin **1a** (1 mmol) and indole **3a** (2.1 mmol) was investigated under different conditions (Table 1). Initially the effect of catalyst loading on the yield of the reaction in CH_3CN was evaluated by varying the amount of catalyst from 0 to 0.15 g under ultrasonic irradiation at room temperature. After 60 min, with 0, 0.05, 0.1, or 0.15 g of MSA-MNP, yields of trace, 70, 93 and 91 %, respectively, were obtained (Table 1, entries 1–4). The solvent, also, was important in this reaction. Methanol, ethanol, tetrahydrofuran (THF), chloroform, and water were also investigated (Table 1, entries 5–9), but CH_3CN was selected as the reaction solvent. The experimental results showed that use of ultrasound radiation and acetonitrile, especially, increased the yield of the reaction (Table 1, entry 3 and 10). Hence, the best reaction conditions were use of 0.1 g MSA-MNP in CH_3CN at room temperature under ultrasonic irradiation (Table 1, entry 3).

After optimization of the reaction conditions, the scope and generality of these conditions with other reactants were examined by using a variety of isatins and indoles (Table 2). The reactions were clean and the products were obtained without formation of by-products. The advantage of use of this catalyst was that it could be easily removed and recycled in a subsequent reaction, without significant reduction of its activity, by simple filtration and extraction. The catalyst could be recycled three times without substantial loss of activity (93 % 1st run; 92 % 2nd run; 89 % 3rd run).

Compounds **4c**, **4g**, **4h** and **5d** at 100 μg/mL were assayed for antibacterial and antifungal activity against *E. coli*, *S. aureus*, *P. aeruginosa*, and *C. albicans*. Disks saturated with DMSO were used as controls. The diameters of inhibition zones (mm) were measured and recorded. As summarized in Table 3, **4g** was the most active against *S. aureus* and **4c** had the lowest activity. We found that compounds **4c**, **4g**, **4h**, and **5d** had no activity against *E. coli* and *P. aeruginosa*. All the compounds were inactive against the fungal species *C. albicans* (Table 3).

Table 1 Optimization of the reaction conditions for synthesis of **4a** under ultrasonic irradiation at room temperature

Entry[a]	Solvent	MSA-MNP	Time (min)	Yield (%)[a]
1	CH_3CN	–	60	10
2	CH_3CN	0.05 g	60	70
3	CH_3CN	0.1 g	60	92
4	CH_3CN	0.15 g	60	93
5	CH_3OH	0.1 g	180	70
6	EtOH	0.1 g	90	84
7	THF	0.1 g	150	60
8	CH_3Cl	0.1 g	200	65
9	H_2O	0.1 g	120	55
10	CH_3CN	0.1 g	60	45[b]

[a] Isolated yields

[b] Without US

Table 2 MSA-MNP-catalyzed synthesis of mono and bis-[3,3-di(indolyl)indolin-2-ones] (**4a**–**5d**) at room temperature under ultrasonic irradiation

Entry	Product	R^1	R^2	R^3	Time (min)	Yield	Mp/Mp (°C) [Lit].
1	**4a**	H	H	H	60	93	241–243/>244–245 [26]
2	**4b**	H	H	CH_3	60	90	220–224
3	**4c**	H	Br	H	60	94	299–301/300 [22]
4	**4d**	H	Br	CH_3	60	96	300–303
5	**4e**	$-CH_2Ph$	H	H	60	87	243–246
6	**4f**	$-CH_2Ph$	H	CH_3	60	90	173–176
7	**4g**	$-CH_2Ph$	Br	H	60	92	268–270
8	**4h**	$-CH_2Ph$	Br	CH_3	60	95	171–174
9	**5a**	–	H	H	120	91	241–244
10	**5b**	–	H	CH_3	120	88	264–267
11	**5c**	–	Br	H	120	82	248–250
12	**5d**	–	Br	CH_3	120	85	270–272

Table 3 In-vitro antimicrobial activity of compounds **4c**, **4g**, **4h**, and **5d**

Entry[a]	Compound	Zone of inhibition (mm)[a]			
		S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*
1	**4c**	12	–	–	–
2	**4g**	23	–	–	–
3	**4h**	19	–	–	–
4	**5d**	17			

[a] Inhibition zone, mm

Table 4 Antibacterial activity of the compounds **4c**, **4g**, **4h**, and **5d** (MIC)

Entry	Compound	MIC[a]			
		S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*
1	**4c**	10 μg/mL	–	–	–
2	**4g**	1.25 μg/mL	–	–	–
3	**4h**	2.5 μg/mL	–	–	–
4	**5d**	10 μg/mL			

[a] Minimum inhibitory concentration

According to Table 3, the maximum zone of inhibition of growth at 100 μg/mL concentration for compound **4g** against *S. aureus* was 23 mm. Minimum inhibitory concentrations (MIC) are given in Table 4. According to the table, the MIC of **4g** was 1.25 μg/mL; this was the highest antimicrobial activity.

Conclusions

In summary, we have demonstrated that MSA-MNP is an efficient heterogeneous catalyst for synthesis of mono and bis-[3,3-di(indolyl)indolin-2-ones] under ultrasound irradiation at room temperature. The simplicity of the reaction procedure, and the inexpensive and reusable catalyst makes this method one of the most efficient for synthesis of this class of compound. The method is simple, practical to perform, and no column chromatography is required for purification. Study of antibacterial activity revealed that four compounds (**4c**, **4g**, **4h**, and **5d**) were active against *S. aureus*. Study of antifungal activity revealed that none of the compounds **4c**, **4g**, **4h**, and **5d** were active against *C. albicans*.

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