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Synthesis and Antibacterial Activity of Novel 4-Bromo-1*H*-Indazole Derivatives as FtsZ Inhibitors

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A series of novel 4-bromo-1*H*-indazole derivatives as filamentous temperature-sensitive protein Z (FtsZ) inhibitors were designed, synthesized, and assayed for their *in vitro* antibacterial activity against various phenotypes of Gram-positive and Gram-negative bacteria and their cell division inhibitory activity. The results indicated that this series showed better antibacterial activity against *Staphylococcus epidermidis* and penicillin-susceptible *Streptococcus pyogenes* than the other tested strains. Among them, compounds **12** and **18** exhibited 256-fold and 256-fold more potent activity than 3-methoxybenzamide (3-MBA) against penicillin-resistant *Staphylococcus aureus*, and compound **18** showed 64-fold better activity than 3-MBA but 4-fold weaker activity than ciprofloxacin in the inhibition of *S. aureus* ATCC29213. Particularly, compound **9** presented the best activity (4 μg/mL) against *S. pyogenes* PS, being 32-fold, 32-fold, and 2-fold more active than 3-MBA, curcumin, and ciprofloxacin, respectively, but it was four times less active than oxacillin sodium. In addition, some synthesized compounds displayed moderate inhibition of cell division against *S. aureus* ATCC25923, *Escherichia coli* ATCC25922, and *Pseudomonas aeruginosa* ATCC27853, sharing a minimum cell division concentration of 128 μg/mL.

Keywords: Antibacterial evaluation / FtsZ inhibitors / Indazole derivatives / Resistant bacteria / Synthesis

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Introduction

Nowadays, the extensive use and misuse of antibiotics have resulted in the emergence and prevalence of bacterial resistance, which has seriously threatened human health. In particular, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *enterococci* (VRE), and penicillin-resistant *Streptococcus pneumoniae* contribute to the enormous difficulties in fighting against bacterial infections [1–3]. Many initial clinical effective antibiotics have been either weak or not active against the resistant bacteria. For purpose of preventing serious medical problem, there is an urgent need for development of new types of antibacterial agents or the expansion of bioactivity of the previous drugs, especially with novel mechanisms of action [4].

Bacterial cell division protein FtsZ (filamentous temperature-sensitive protein Z) is conserved in almost all bacterial pathogens and in several genetic studies has been shown to be essential for bacterial viability [5–8]. Cell division in bacteria occurs at the site of formation of a cytokinetic Z-ring polymeric structure, which comprises FtsZ subunits [9]. During bacterial cell division, FtsZ offers an essential skeleton for the formation of Z-ring in the presence of guanosine triphosphate (GTP) [10–12]. Thus, if FtsZ assembly is restrained or removed, the bacterial cell division will eventually fail, thereby resulting in bacterial apoptosis. The vital role of FtsZ in bacterial cell division renders FtsZ as a promising therapeutic target to develop antibacterial agents with

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selective toxicity to bacterial pathogens. At present, a certain amount of FtsZ-targeting antibacterial agents have been screened from natural sources such as plants, microbial fermentations metabolites, or small molecules designed and synthesized, such as curcumin, 3-MBA, sanguinarine, and chelerythrine (Fig. 1). FtsZ inhibitors can show their destructive effects on the Z-ring by either enhancing or inhibiting FtsZ self-polymerization [13–20].

Sanguinarine with a benzo[c]phenanthridine structure is an antibacterial plant alkaloid, which has been identified as a small molecule altering the Z-ring formation [14]. Chelerythrine, an analog of sanguinarine, has also similar effects on *S. aureus* and *Bacillus subtilis* to sanguinarine. In addition, the presence of hydrophobic functionality at the 1-position of the benzo[c]phenanthridines such as that of compound **1** (Figure 1), has been shown to significantly enhance antibacterial activity relative to either sanguinarine or chelerythrine [21, 22]. More importantly, 5-substituted 1-phenylnaphthalene has been found to be the pharmacophore of these benzo[c]phenanthridines [23]. For example, several 5-substituted 1-phenylnaphthalene derivatives have exerted noteworthy antibacterial activity against methicillin-resistant *S. aureus* with minimum inhibitory concentration (MIC) values from 2.0 to 4.0 μg/mL. Moreover, FtsZ polymerization test results have suggested that the antibacterial activity of the phenylnaphthalenes is associated with their stimulatory impact on the dynamics of FtsZ polymerization.

Indazole molecule has a wide range of biological properties, such as tuberculostatic activity, analgesic and anti-inflammatory [24], hepatoprotective [25], antibacterial [26], antiangiogenic [27], and cytostatic [28]. Therefore, we designed and synthesized a novel series of 4-bromo-1*H*-indazole derivatives (Fig. 1) by replacing the naphthalene and phenyl group of 5-substituted 1-phenylnaphthalene with the indazole and bromine atom, respectively, on the basis of the principle of bioisosterism. Our objective was to explore FtsZ-targeting antibacterial agents with potent antibacterial activity against a wide range of bacteria.

Results and discussion

Chemistry

In the present work, the methods used for preparation of the 4-bromo-1*H*-indazole derivatives **9**–**16** and **17**–**19** are outlined in Scheme 1. Commercially available starting material **2** was converted to 1-bromo-2-methyl-3-nitrobenzene **3** in the presence of ammonium sulfide in ethanol by reflux followed by Sandmeyer reaction. **3** was reduced with ferrum and ammonium chloride in refluxing ethanol and water to give

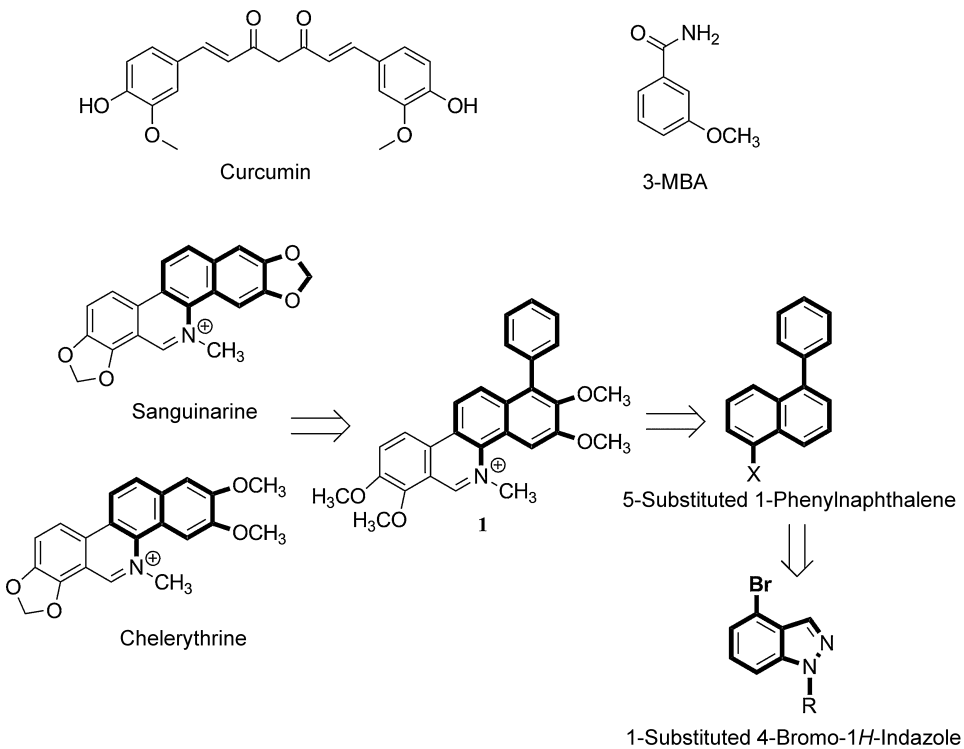

Figure 1. Structures of curcumin, 3-MBA, sanguinarine, chelerythrine, and 1-phenyl-5-methyl-2,3,7,8-tetramethoxybenzo[c]-phenanthridine 1, and the design of target compounds.

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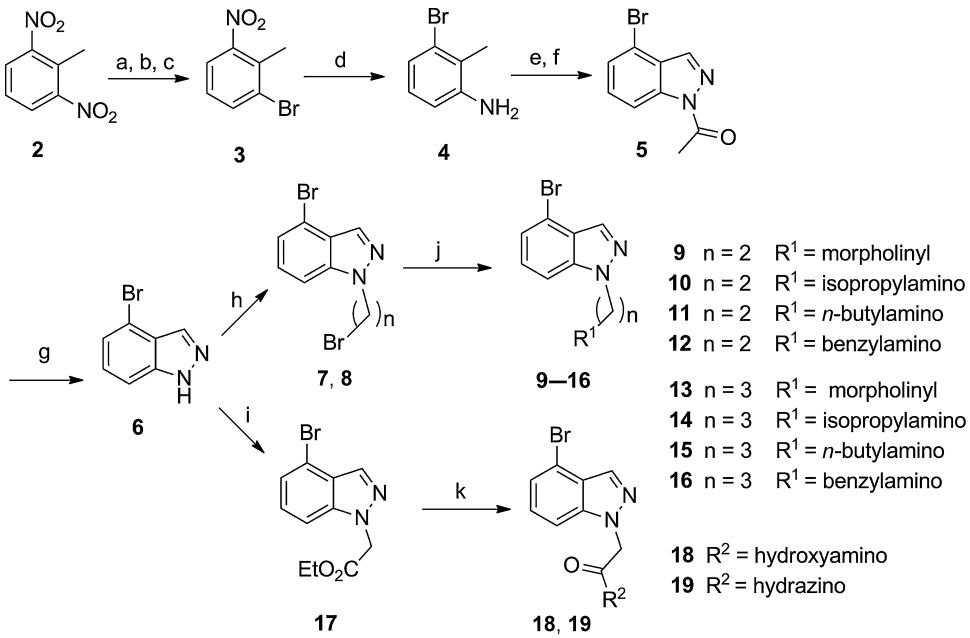

Scheme 1. Reagents and conditions: (a) $(NH_4)_2S$, EtOH, reflux, 79%; (b) HBr/H_2O, $NaNO_2/H_2O$, 0°C, 84%; (c) CuBr/HBr, 25–35°C; (d) Fe, NH_4Cl, N_2, EtOH, reflux; (e) AcOK, $(AcO)_2$, toluene, 0°C, 35°C; (f) *iso*-amyl nitrite, 80°C; (g) 6 N HCl, MeOH; (h) $Br(CH_2)_nBr$, K_2CO_3, DMF, 29–42%; (i) $BrCH_2CO_2Et$, K_2CO_3, DMF, rt., 71.5%; (j) amines, K_2CO_3, DMF, 80°C, or TEA, CH_3CN, rt.; (k) NH_2R^2, MeOH, reflux, 35–51%.

3-bromo-2-methylaniline **4** in 81% yield. **4** reacted with acetic anhydride in the presence of potassium acetate and then condensed with *iso*-amyl nitrite to produce the *N*-acetyl-4-bromo-1*H*-indazole **5**. **5** was hydrolized in mixture solution of 6 N hydrochloric acid in methanol to yield 4-bromo-1*H*-indazole **6** as the key intermediate. **6** reacted with alkyl bromides or bromo esters in the presence of potassium carbonate to give intermediates **7**–**8** in 29–42% yields and **17** in 71.5% yield. **7** or **8** was heated with morpholine at 80°C in the presence of potassium carbonate and catalytic sodium iodide to afford compounds **9** and **13**. At room temperataure, **7** or **8** was converted to compounds **10**–**12** and **14**–**16** by reacting with amines in acetonitrile. **17** was subjected to subsequent nucleophilic substitution with hydroxyamine or hydrazine hydrate to provide compounds **18**–**19**. The structures of the newly synthesized compounds were characterized by MS, ^{1}H NMR, and all the spectral data were in agreement with the proposed structures.

Antibacterial evaluation

The 4-bromo-1*H*-indazole derivatives (**6**, **9**–**16**, and **17**–**19**) were determined for their *in vitro* antibacterial activity and cell division inhibitory activity, respectively. The *in vitro* antibacterial activity was tested using tube dilution method recommended by NCCLS [29] and the cell division inhibitory activity was measured by assessing cell morphology using phase-contrast light microscopy [30]. The tested strains included *S. aureus* ATCC25923, *S. aureus* ATCC29213 (MRSA), *S. aureus* ATCC31007, *S. epidermidis*, *S. pyogenes* PS, *S. pyogenes* PR, *Escherichia coli* ATCC25922, *B. subtilis* ATCC9372, and *Pseudomonas aeruginosa* ATCC27853. *S. aureus* ATCC25923, *B. subtilis* ATCC9372, *E. coli* ATCC25922, and *P. aeruginosa* ATCC27853 were used to determine the cell division inhibitory activity of the synthesized compounds. The results in the unit of μg/mL are shown in Tables 1 and 2.

The synthesized compounds universally exhibited better antibacterial activity against *S. epidermidis* and *S. pyogenes* PS than the other tested strains. Among them, many compounds showed superior or comparable activity against *S. epidermidis* to ciprofloxacin. Especially, compound **9** bearing the morpholine group in this series displayed the best antibacterial activity with an MIC value of 4 μg/mL against *S. pyogenes* PS, showing over 32-fold, 32-fold, and 2-fold better activity than 3-MBA, curcumin, and ciprofloxacin, respectively, but it was four times less active than oxacillin sodium. In the inhibition of *S. aureus* PR, the most active compounds **12** and **18** with the benzylaminoethyl and hydroxycarbamoylmethyl groups at 1-position of the indazole, respectively, exhibited the same MIC value of 16 μg/mL, being 256-fold, 8-fold, 8-fold, and 8-fold better than 3-MBA, curcumin, oxacillin sodium, and ciprofloxacin, respectively. Furthermore, compound **18** also showed the strongest activity with an MIC value of 32 μg/mL against *S. aureus* ATCC29213 in all of the tested compounds, being 64-fold, 4-fold, and 4-fold more potent than 3-MBA, curcumin, and oxacillin sodium, but 4-fold weaker than ciprofloxacin. Besides, all of the compounds showed same activity as oxacillin

Table 1. The *in vitro* antibacterial activity of the 4-bromo-1*H*-indazole derivatives (μg/mL).

Compounds	*S. aureus* ATCC25923[a]	*S. aureus* ATCC29213[b]	*S. aureus* PR[c]	*B. subtilis* ATCC9372[d]	*E. coli* ATCC25922[e]	*P. aeruginosa* AT CC27853[f]	*S. epidermidis*[g]	*S. pyogenes* PS[h]	*S. pyogenes* PR[i]
6	>128	128	>128	128	128	128	64	64	>128
9	128	128	128	128	128	>128	32	4	>128
10	>128	>128	64	128	128	128	>128	64	>128
11	>128	>128	>128	128	128	128	128	128	>128
12	128	128	16	128	128	128	64	64	>128
13	128	128	128	128	128	>128	128	64	>128
14	>128	>128	>128	128	128	>128	64	32	>128
15	128	>128	32	128	128	>128	32	64	64
16	128	128	>128	128	128	>128	128	128	>128
17	>128	>128	>128	128	>128	128	>128	>128	128
18	128	32	16	128	128	128	64	128	>128
19	>128	>128	>128	128	128	128	>128	>128	>128
3-MBA	2048	2048	4096	4096	ND	ND	ND	>128	>128
Curcumin	>128	>128	>128	32	>128	>128	ND	>128	ND
Oxacillin sodium	8	128	>128	128	128	128	8	1	1
Ciprofloxacin	8	8	>128	8	8	4	128	8	128

ND, not determined.
[a] *S. aureus* ATCC25923: penicillin-susceptible strain.
[b] *S. aureus* ATCC29213: methicillin-resistant strain.
[c] *S. aureus* PR: penicillin-resistant strain isolated clinically, not characterized.
[d] *B. subtilis* ATCC9372: penicillin-susceptible strain.
[e] *E.coli* ATCC25922: penicillin-susceptible strain.
[f] *P. aeruginosa* ATCC27853: penicillin-susceptible strain.
[g] *S. epidermidis*: penicillin-resistant strain isolated clinically, not characterized.
[h] *S. pyogenes* PS: penicillin-susceptible strain.
[i] *S. pyogenes* PR: penicillin-resistant strain.

Table 2. The cell division inhibitory activity of the 4-bromo-1*H*-indazole derivatives (μg/mL).

Compounds	*S. aureus* ATCC25923[a)]	*B. subtilis* ATCC 9372[b)]	*E. coli* ATCC25922[c)]	*P. aeruginosa* ATCC 27853[d)]
6	>128	256	128	>128
9	>128	>128	>128	>128
10	>128	>128	128	>128
11	>128	>128	128	128
12	128	>128	>128	>128
13	>128	>128	>128	>128
14	>128	>128	128	>128
15	>128	>128	>128	>128
16	>128	>128	>128	>128
17	>128	>128	128	>128
18	>128	>128	128	>128
19	>128	>128	128	>128
3-MBA	WT	512	ND	ND
Curcumin	>128	16	256	ND
Oxacillin sodium	>128	>128	>128	>128
Ciprofloxacin	>128	>128	>128	>128

WT, no effect on morphology at 2048–4096 μg/mL; ND, not determined.
[a)] *S. aureus* ATCC25923: penicillin-susceptible strain.
[b)] *B. subtilis* ATCC9372: penicillin-susceptible strain.
[c)] *E. coli* ATCC25922: penicillin-susceptible strain.
[d)] *P. aeruginosa* ATCC27853: penicillin-susceptible strain.

sodium, but weaker activity than curcumin and ciprofloxacin in the activity against *B. subtilis* ATCC9372, and most of all the compounds displayed comparable potency to oxacillin sodium and much better activity than curcumin, but weaker activity than ciprofloxacin against *E. coli* ATCC25922, *P. aeruginosa* ATCC27853.

Moreover, although precursor **6** exerted moderate or weak activity against all the tested bacterial strains, many derivatives of **6** displayed greatly improved antibacterial activity, in which compounds **9** exhibited 16-fold improved activity against *S. pyogenes* PS while compound **12** showed 8-fold improved activity against *S. aureus* ATCC29213 in comparison with **6**. The subseries of **13**–**16** with a linkage of the three carbon atoms connected between the indazole and alkylamino group displayed a similar trend in antibacterial activity to the subseries of **9**–**12** with a linkage of the two carbon atoms connected between them. All the above results indicated that the alkylamino side chains at the 1-position of the indazole were beneficial to the antibacterial activity, and the length of the linkage between the indazole and alkylamino group had almost no effect on the antibacterial activity. In another subseries of **18**–**19**, compound **18** bearing the amide side chain at the 1-position of the indazole showed significantly increased activity 4-fold and 8-fold better than **6** against *S. aureus* ATCC29213 and *S. aureus* PR.

As seen from Table 2, some synthesized compounds displayed moderate inhibition of cell division against *E. coli* ATCC25922 sharing a minimum cell division concentration of 128 μg/mL, which basically fitted the *in vitro* antibacterial activity against *E. coli* ATCC25922. Among them, compound **12** showed improved on-target activity against *S. aureus* ATCC25923 in comparison to 3-MBA. In the inhibition of *B. subtilis* ATCC9372, few compounds displayed improved on-target activity. In contrast, compounds **6, 10, 11, 14,** and **17**–**19** showed slightly improved on-target activity against *E. coli* ATCC25922 compared with curcumin. In all synthesized compounds, only compound **11** against *P. aeruginosa* ATCC27853 shared a minimum cell division concentration of 128 μg/mL. Therefore, the above results indicated that they were promising FtsZ inhibitors with on-target activity. However, the cell inhibitory activity of some compounds was not accordant to their *in vitro* antibacterial activity, which implied that they could have other mechanism against the bacteria.

Conclusion

In conclusion, a series of 4-bromo-1*H*-indazole derivatives were designed, synthesized, and evaluated for *in vitro* antibacterial activity and cell inhibitory activity against various Gram-positive and Gram-negative bacteria. Compounds bearing the alkylamino side chains **9**–**16** generally displayed better *in vitro* activity aganist *S. epidermidis* and *S. pyogenes* PR than the other tested strains. In particular, compound **9** showed the best activity with an MIC value of 4 μg/mL against *S. pyogenes* PS in the tested compounds, displaying 32-fold, 32-fold, and 2-fold more potent activity than 3-MBA, curcumin, and ciprofloxacin, respectively, but it

was four times less active than oxacillin sodium. In the inhibition of *S. aureus* PR, the most active compounds were **12** and **18** with an MIC value of 16 μg/mL, being 256-fold, 8-fold, 8-fold, and 8-fold better than 3-MBA, curcumin, oxacillin sodium, and ciprofloxacin, respectively. Compound **18** showed 256-fold better activity than 3-MBA but 4-fold weaker activity than ciprofloxacin against *S. aureus* ATCC29213. In cell division inhibitory activity, some synthesized compounds displayed moderate inhibition of cell division against *S. aureus* ATCC25923, *E. coli* ATCC25922, and *P. aeruginosa* ATCC27853 sharing a minimum cell division concentration of 128 μg/mL.

Experimental

Chemistry

Thin-layer chromatography (TLC) was done using 0.25 mm precoated silica gel plates (Qingdong Yumingyuan silica gel reagent factory, Shandong, China, Yuyuan). The ^{1}H NMR spectra were recorded on Bruker Avance DRX 400 spectrometer (Bruker, Switzerlands) at ambient temperature using TMS as internal reference standard (chemical shift in δ ppm); coupling constants (*J*) were given in Hertz. Mass spectra were recorded on API 4000 instrument (Applied Biosystems, CT). All melting points were determined by open capillary methods and are uncorrected. Column chromatography was performed on a neutral silica column (2.5 × 45 cm) using appropriate eluent.

*1-Bromo-2-methyl-3-nitrobenzene (**3**)*

To a solution of 2,6-dinitrotoluene **2** (9 g, 49.45 mmol) in ethanol at 79°C was slowly added $(NH_4)_2S$ (60 mL). The reaction was refluxed till complete conversion took place, which was monitored by TLC. It took about 2 h. The reaction mixture was then cooled to room temperature and filtered to remove solid. The combined solutions were evaporated in vacuum and residue thus obtained was recrystallized from ethanol and water to yield 2-methyl-3-nitroaniline as a yellow crystalline solid. Yield: 79.0%, mp 87–91°C.

To a suspension of 2-methyl-3-nitroaniline (1.5 g, 9.87 mmol) in water (4 mL) was added concentrated HBr (10 mL) at room temperature. The contents were cooled to 0–5°C and a solution of sodium nitrite (0.72 g, 10.35 mmol) in water (3 mL) was added dropwise at 0–5°C over a period of 3–5 min. After stirring at 0–5°C for another 20–30 min, the reaction mixture was then added to a cuprous bromide (1.4 g, 9.87 mmol) in concentrated HBr (14 mL) with stirring. The resulting reaction mixture was stirred at 30–35°C for another 30 min. Finally, precipitated solid was filtered, washed with water, saturated $NaHCO_3$, water and dried under vacuum to yield 1.79 g of 1-bromo-2-methyl-3-nitrobenzene **3** as milky white solid. Yield: 84.0%, mp 38–40°C.

*3-Bromo-2-methylaniline (**4**)*

To a solution of **3** (1.79 g, 8.29 mmol) in a mixture of ethanol (21 mL) and water (7 mL) was added Fe (2.32 g, 41.45 mmol) and NH_4Cl (0.24 g, 4.40 mmol) at room temperature. The reaction was refluxed till complete conversion took place, which was monitored by TLC. It took about 2 h. The reaction mixture was then cooled to room temperature and filtered to remove solid. The combined solutions were evaporated under reduced pressure and the mixture was diluted with H_2O and extracted with ethyl acetate. The organic layer was washed consecutively with water and saturated brine, and dried over anhydrous Na_2SO_4. The dried organic layer was filtered and evaporated under pressure to dryness and residue was purified on a column of silica gel eluting with 10% ethyl acetate: petroleum ether. 3-Bromo-2-methylaniline **4** was obtained as a light yellow liquid. Yield: 81%.

*N-Acetyl-4-bromo-1H-indazole (**5**)*

To **4** (1.43 g, 7.69 mmol) was added toluene (23 mL) and potassium acetate (0.91 g, 9.23 mmol). This mixture was cooled to 0°C with stirring, and then acetic anhydride (2.35 g, 23.06 mmol) was added dropwise over 2 min. The reaction mixture was allowed to gradually warm up to room temperature and stirred for 1 h (monitored by TLC). The solution was heated to 80°C and then isoamyl nitrite (2.2 g, 18.46 mmol) was added, which was stirred for 7 h at 80°C until the reaction was completed (TLC, petroleum ether/ethyl acetate 2:1). Cooled to room temperature, the reaction mixture was washed with 5% $NaHCO_3$ to pH 7 and then washed with water again. The organic layer was dried over anhydrous Na_2SO_4 and then evaporated *in vacuo* to give N-acetyl-4-bromo-1*H*-indazole **5** as orange crystal. Yield: 89.4%.

*4-Bromo-1H-indazole (**6**)*

To **5** (1.6 g, 6.69 mmol) was added methanol (25.9 mL) and then 6 N HCl (15.6 mL). The resulting solution was stirred at room temperature until the reaction was completed after 7 h (monitored by TLC, petroleum ether/ethyl acetate 2:1). After evaporation of methanol *in vacuo*, the residue was extracted with ethyl acetate (50 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude was purified by flash chromatography (petroleum ether/ethyl acetate, 5:1) to yield 4-bromo-1*H*-indazole (0.8 g) as milky white solid, mp 164–166°C, yield 67.1%. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 10.25 (s, 1H, NH), 8.11 (s, 1H, CH=N), 7.45 (d, *J* = 8.0 Hz, 1H, Ar–H), 7.34 (d, *J* = 8.0 Hz, 1H, Ar–H), 7.26 (t, *J* = 8.0 Hz, 1H, Ar–H). ESI–MS *m/z* calcd for $C_7H_5BrN_2$, 195.96; found: $[M+H^+]$ 197.2.

*General methods for 1-N-bromoalkyl-4-bromo-1H-indazoles (**7** and **8**)*

In a round-bottomed flask with mechanical stirrer was added **6** (0.23 g, 1.17 mmol) and DMF and then K_2CO_3. The mixture was stirred for 10 min and then 1,2-dibromoethane or 1,3-dibromopropane (5.85 mmol) was added dropwise. The reaction mixture was stirred for 12 h at room temperature and diluted with water. The mixture solution was extracted

with ethyl acetate (25 mL × 3) and washed with brine. The organic was combined, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 30:1) to yield compounds **7** and **8** in yields of 51.6% and 40.1%, respectively.

*General methods for 1-N-aminoalkyl-4-bromo-1H-indazoles (**9**–**16**)*

A solution of **7** or **8** (0.63 mmol) in DMF was heated with morpholine (1.2 mmol) at 80°C in the presence of K_2CO_3 (0.98 mmol) and NaI (0.06 mmol). The mixture solution was extracted with ethyl acetate (15 mL × 3) and washed with brine. The organic was combined, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* and the crude was purified by flash chromatography (petroleum ether/ethyl acetate, 1:1) to afford compounds **9** and **13**. To a solution of **7** or **8** (0.63 mmol) in CH_3CN was added triethylamine (0.17 mL). The solution was stirred for 5–10 min and added amines (1.2 mmol) gradually. The resulting mixture was stirred for 24 h at room temperature and then evaporated under reduced pressure. The residue was dissolved in ethyl acetate and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure to dryness. The crude was purified by flash chromatography (petroleum ether/ethyl acetate, 1:1) to yield compounds **10**–**12**, **14**–**16** in yields ranging from 22.3% to 61.5%.

*1-N-[2-(4-Morpholinyl)ethyl]-4-bromo-1H-indazole (**9**)*

Pale yellow crystalline solid, mp 80–82°C, yield 61.5%, $R_f = 0.48$ (petroleum ether/ethyl acetate, 1:3). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.01 (s, 1H, CH=N), 7.38 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.30 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.23 (t, $J = 8.0$ Hz, 1H, Ar–H), 4.50 (t, $J = 6.8$ Hz, 2H, CH_2), 3.65 (t, $J = 4.8$ Hz, 4H, $2CH_2$), 2.87 (t, $J = 6.8$ Hz, 2H, CH_2), 2.49 (t, $J = 4.8$ Hz, 4H, $2CH_2$). ESI–MS *m/z* calcd for $C_{13}H_{16}BrN_3O$, 309.05; found: $[M+H^+]$ 310.4.

*1-N-(2-Isopropylaminoethyl)-4-bromo-1H-indazole (**10**)*

White solid, mp 155–159°C, yield 46.1%, $R_f = 0.51$ (dichloromethane/methanol, 8:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.01 (s, 1H, CH=N), 7.56 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.28 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.22 (t, $J = 8.0$ Hz, 1H, Ar–H), 4.94 (t, $J = 6.8$ Hz, 2H, CH_2), 3.52 (t, $J = 6.8$ Hz, 2H, CH_2), 3.29 (q, $J = 6.4$ Hz, 1H, CH), 1.45 (d, $J = 6.4$ Hz, 6H, $2CH_3$). ESI–MS *m/z* calcd for $C_{12}H_{16}BrN_3$, 281.05; found: $[M+H^+]$ 282.4.

*1-N-(2-n-Butylaminoethyl)-4-bromo-1H-indazole (**11**)*

White solid, mp 178–180°C, yield 34.3%, $R_f = 0.48$ (dichloromethane/methanol, 8:1). ^{1}H NMR (400 MHz, DMSO-D_6): δ (ppm) = 8.10 (s, 1H, CH=N), 7.82 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.41–7.34 (m, 2H, Ar–H), 4.71 (t, $J = 6.4$ Hz, 2H, CH_2), 3.26 (t, $J = 6.4$ Hz, 2H, CH_2), 2.76 (t, $J = 7.6$ Hz, 2H, CH_2), 1.52–1.44 (m, 2H, CH_2), 1.31–1.23 (m, 3H, CH_2, and 1H of NH), 0.85 (t, $J = 7.2$ Hz, 3H, CH_3). ESI–MS *m/z* calcd for $C_{13}H_{18}BrN_3$, 295.07; found: $[M+H^+]$ 296.4.

*1-N-(2-Benzylaminoethyl)-4-bromo-1H-indazole (**12**)*

Light yellow solid, mp 104–107°C, yield 54.5%, $R_f = 0.48$ (petroleum ether/ethyl acetate, 1:2). ^{1}H NMR (400 MHz, DMSO-D_6): δ (ppm) = 8.03 (s, 1H, CH=N), 7.74 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.37–7.31 (m, 2H, Ar–H), 7.29–7.19 (m, 5H, Ar–H), 4.50 (t, $J = 6.4$ Hz, 2H, CH_2), 3.66 (s, 2H, CH_2), 2.94 (t, $J = 6.4$ Hz, 2H, CH_2), 2.32 (s, 1H, NH). ESI–MS *m/z* calcd for $C_{16}H_{16}BrN_3$, 329.05; found: $[M+H^+]$ 330.4.

*1-N-[3-(4-Morpholinyl)propyl]-4-bromo-1H-indazole (**13**)*

Yellow liquid, yield 57%, $R_f = 0.33$ (petroleum ether/ethyl acetate, 2:1). ^{1}H NMR (400 MHz, MeOD): δ (ppm) = 7.99 (s, 1H, CH=N), 7.60 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.25–7.31 (m, 2H, Ar–H), 4.47 (t, $J = 6.4$ Hz, 2H, CH_2), 3.62 (t, $J = 6.4$ Hz, 4H, $2CH_2$), 2.33 (brs, 4H, $2CH_2$), 2.25 (t, $J = 7.2$ Hz, 2H, CH_2), 2.12–2.06 (m, 2H, CH_2). ESI–MS *m/z* calcd for $C_{14}H_{18}BrN_3O$, 323.06; found: $[M+H^+]$ 324.4.

*1-N-(3-Isopropylaminopropyl)-4-bromo-1H-indazole (**14**)*

White solid, mp 201–203°C, yield 35.7%, $R_f = 0.66$ (dichloromethane/methanol, 8:1). ^{1}H NMR (400 MHz, DMSO-D_6): δ (ppm) = 8.90 (s, 1H, NH), 8.08 (s, 1H, CH=N), 7.83 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.41–7.34 (m, 2H, Ar–H), 4.58 (t, $J = 6.8$ Hz, 2H, CH_2), 3.23 (q, $J = 6.4$ Hz, 1H, CH), 2.90 (t, $J = 7.2$ Hz, 2H, CH_2), 2.24–2.17 (m, 2H, CH_2), 1.20 (d, $J = 6.4$ Hz, 6H, $2CH_3$). ESI–MS *m/z* calcd for $C_{13}H_{18}BrN_3$, 295.07; found: $[M+H^+]$ 296.4.

*1-N-(3-n-Butylaminopropyl)-4-bromo-1H-indazole (**15**)*

White solid, mp 153–157°C, yield 22.3%, $R_f = 0.58$ (dichloromethane/methanol, 8:1). ^{1}H NMR (400 MHz, DMSO-D_6): δ (ppm) 8.99 (s, 1H, NH), 8.08 (s, 1H, CH=N), 7.82 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.40–7.34 (m, 2H, Ar–H), 4.57 (t, $J = 6.8$ Hz, 2H, CH_2), 2.90–2.80 (m, 4H, $2CH_2$), 2.21 (t, $J = 7.6$ Hz, 2H, CH_2), 1.58–1.54 (m, 2H, CH_2), 1.33–1.27 (m, 2H, CH_2), 0.91 (t, $J = 7.2$ Hz, 3H, CH_3). ESI–MS *m/z* calcd for $C_{14}H_{20}BrN_3$, 309.08; found: $[M+H^+]$ 310.5.

*1-N-(3-Benzylaminopropyl)-4-bromo-1H-indazole (**16**)*

Colourless liquid, yield 31.8%, $R_f = 0.45$ (dichloromethane/methanol, 8:1). ^{1}H NMR (400 MHz, MeOD): δ (ppm) = 7.85 (s, 1H, CH=N), 7.45 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.21–7.10 (m, 7H, Ar–H), 4.35 (t, $J = 6.8$ Hz, 2H, CH_2), 3.55 (s, 2H, CH_2), 2.42 (t, $J = 7.2$ Hz, 2H, CH_2), 2.02–1.95 (m, 2H, CH_2). ESI–MS *m/z* calcd for $C_{17}H_{18}BrN_3$, 343.07; found: $[M+H^+]$ 344.4.

*1-N-Ethyl ethanoate-4-bromo-1H-indazole (**17**)*

To a solution of 4-bromo-1*H*-indazole **6** (0.4 g, 2.03 mmol) in DMF was added K_2CO_3 (0.42 g, 3.05 mmol). The mixture was stirred for 5–10 min, and then ethyl bromoacetate (0.51 g, 3.05 mmol) was added. The resulting mixture was stirred 16 hours at room temperature and then diluted with water. The mixture solution was extracted with ethyl acetate (30 mL × 3) and washed with brine. The organic was combined, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 30:1) to yield compound **17**.

Light yellow solid, yield 71.5%, mp 58–60°C, $R_f = 0.65$ (petroleum ether/ethyl acetate, 1:2). ^{1}H NMR (400 MHz, DMSO-D_6): δ (ppm) = 8.09 (s, 1H, CH=N), 7.71 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.41 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.34 (d, $J = 8.0$ Hz, 1H, Ar–H), 5.43 (s, 2H, CH_2), 4.15 (q, $J = 7.2$ Hz, 2H, CH_2), 1.19 (t, $J = 7.2$ Hz, 3H, CH_3). ESI–MS *m/z* calcd for $C_{11}H_{11}BrN_2O_2$, 282.00; found: [M+H$^+$] 283.3.

*1-N-Hydroxycarbamoylmethyl-4-bromo-1H-indazole (**18**)*
A solution of hydroxylamine was obtained by dissolving $H_2NOH \cdot HCl$ (0.37 g, 5.3 mmol) in 3 mL of methanol and stirring it with a solution of KOH (0.3 g, 5.3 mmol) in 2 mL of methanol for 20 min. After the KCl precipitated out was filtered, the hydroxylamine solution was added dropwise to the ice cooled solution of **17** (0.3 g, 1.06 mmol). The mixture was refluxed until the reaction was completed. The solid that precipitated out was filtered and was recrystallized from ethanol to furnish compound **18**. White crystalline solid, yield 37%, mp 188–201°C, $R_f = 0.51$ (dichloromethane/methanol, 8:1). ^{1}H NMR (400 MHz, DMSO-D_6): δ (ppm) = 10.94 (s, 1H, CONH), 9.08 (s, 1H, OH), 8.05 (s, 1H, CH=N), 7.68 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.40–7.32 (m, 2H, Ar–H), 5.03 (s, 2H, CH_2). ESI–MS *m/z* calcd for $C_9H_8BrN_3O_2$, 268.98; found: [M+H$^+$] 270.4.

*1-N-aminocarbamoylmethyl-4-bromo-1H-indazole (**19**)*
17 (0.31 g, 1.10 mmol) and hydrazine hydrate (0.071 g, 1.21mmol) in methanol was refluxed for about 2 h. The reaction mixture was then cooled to room temperature and filtered. The residue was recrystallized from ethanol to furnish compound **19**. White crystalline solid, yield 51%, mp 210–212°C, $R_f = 0.65$ (dichloromethane/methanol, 8:1). ^{1}H NMR (400 MHz, DMSO-D_6): δ (ppm) = 9.45 (s, 1H, CONH), 8.04 (s, 1H, CH=N), 7.67 (d, $J = 8.0$ Hz, 1H, ArH), 7.39–7.31 (m, 2H, CH_2), 5.07 (s, 2H, CH_2), 4.32 (d, $J = 2.8$ Hz, 2H, NH_2). ESI–MS *m/z* calcd for $C_9H_9BrN_4O$, 268.00; found: [M+H$^+$] 269.4.

Antibacterial evaluation

In vitro antibacterial assay
The MIC values were determined using tube dilution method recommended by NCCLS [29]. Bacterial strains were maintained on Mueller Hinton Agar (MHA) medium for 24 h at 37°C. The bacteria were prepared by suspension in 10 mL of sterile water for colonies from culture on MHA medium. Mueller Hinton Broth (MHB) was used for bacteria in this test. The cell density of each inoculum was adjusted in sterile water of a 0.5 McFarland standard. In this method, various concentrations of the 4-bromo-1*H*-indazole derivatives were prepared from 128 to 0.25 μg/mL in sterile tubes No. 1–10. A hundred microliters of sterile MHB was poured in each sterile tube followed by adding 200 μL of the test compound in tube 1. Twofold serial dilutions were carried out from tube 1 to tube 10 and excess broth (100 μL) was discarded from the last tube no. 10. To each tube, 100 μL of standard inoculum (1.5×10^8 cfu/mL) was added. 3-MBA, curcumin, oxacillin sodium, and ciprofloxacin were used as controls. Turbidity was observed after incubating the inoculated tubes at 37°C for 24 h. The last tube with no growth of microorganism was recorded to represent the MIC value expressed in μg/mL.

Cell division inhibitory assay
Cell division inhibitory activity of the tested compounds was performed as described previously [30]. Overnight cultures were grown in starvation medium supplemented with 1% hydrolyzed casein and then diluted in starvation medium supplemented with 3% hydrolyzed casein (*B. subtilis*) or in Mueller Hinton medium (*S. aureus*, *E. coli*, and *P. aeruginosa*) and grown at 37°C. The culture was diluted to A600 of −0.06, and 10 μL of aliquots were added to transparent 96-well microtiter plates containing dilutions of the 4-bromo-1*H*-indazoles and ciprofloxacin as controls in 100 μL volumes of medium. After incubation for approximately 5 h (4–5 generations) at 37°C, 20 μL of culture samples were transferred to poly-L-lysine-coated slides for microscopy. Cell morphology was assessed by phase-contrast light microscopy. Lowest concentration at which filamentation of *B. subtilis*, *E. coli*, and *P. aeruginosa* or ballooning of *S. aureus* was recorded represented the cell division inhibitory activity indicating on-target activity.

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