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5-Bromo-1-(4-chlorobenzyl)-1*H*-indole-2-carboxamides as new potent antibacterial agents

https://doi.org/10.1515/hc-2018-0107
Received June 23, 2018; accepted October 19, 2018; previously published online November 13, 2018

Abstract: Ten 5-bromoindole-2-carboxamides were synthesized, characterized and evaluated for antibacterial activity against pathogenic Gram-negative bacteria *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella* Typhi using gentamicin and ciprofloxacin as internal standards. Compounds **7a–c**, **7g** and **7h** exhibit high antibacterial activity with a minimum inhibitory concentration (MIC) of 0.35–1.25 μg/mL. Compounds **7a–c** exhibit antibacterial activities that are higher than those of the standards against *E. coli* and *P. aeruginosa*.

Keywords: antibacterial activity; indole; indole-2-carboxamide.

Introduction

The treatment of bacterial infections is a challenging therapeutic problem because of the increasing resistance of disease-causing bacteria against existing antibacterial agents [1, 2]. Infections with Gram-negative bacteria are especially worrisome [3]. Considering the magnitude of ever-growing antibacterial resistance, it is necessary to discover novel chemical entities with improved pharmacological profiles. Natural and synthetic heterocycles containing an indole moiety [4] are known for their significant biological activities [5–22]. Compounds with an indole-2-carboxamide scaffold (Figure 1) are of special interest (Figure 1). In this paper, we report the synthesis and antibacterial activities of indole-2-carboxamides **7a–j** (Scheme 1).

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Results and discussion

As can be seen from Scheme 1, the intermediate product **6** was prepared starting with commercially available 4-bromoaniline (**1**). Diazotization of **1** followed by reduction afforded 4-bromophenylhydrazine (**2**) [23]. Condensation of **2** with ethyl pyruvate followed by cyclization of the resultant product **3** furnished ethyl 5-bromo-1*H*-indole-2-carboxylate (**4**) [23]. Benzylation of **4** with 4-chlorobenzyl chloride gave *N*-benzylated ester **5**, the hydrolysis of which furnished the desired carboxylic acid **6**. Coupling of the carboxylic acid **6** with appropriate amines using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC.HCl), hydroxybenzotriazole (HOBt) and N,N-diisopropylethylamine (DIPEA)/triethylamine (TEA) in dimethylformamide (DMF) afforded the target carboxamides **7a–j** in good to excellent yields.

Compounds **7a–j** were evaluated for their *in vitro* antibacterial activity against pathogenic Gram-negative bacteria *Klebsiella pneumoniae* (ATCC 27736), *Escherichia coli* (ATCC 9637), *Pseudomonas aeruginosa* (ATCC BAA427) and *Salmonella enterica* serovar Typhi (ATCC 19430) [24]. The experiments were conducted using the broth microdilution technique described by Clinical and Laboratory Standards Institute (CLSI), 2012 (formerly NCCLS) [24]. The activities were compared with those of antibacterial drugs gentamicin and ciprofloxacin. The results are reported in Table 1.

Among the tested compounds, carboxamides **7a–c**, **7g** and **7h** show good antibacterial activity against *E. coli* and *P. aeruginosa* with a minimum inhibitory concentration (MIC) of 0.15–3.25 μg/mL. When compared with the commercial references (gentamicin and ciprofloxacin) as positive controls, the activity of **7c** is identical with that of ciprofloxacin and, more significantly, better than the activity of gentamicin against *E. coli*. Compounds **7a** and **7b** show a 2.5- to 3-fold greater effect than both standards against *E. coli*. Compound **7c** exhibits excellent activity against *Salmonella* Typhi and is 1.6 and 3-fold more active than gentamicin and ciprofloxacin, respectively.

Conclusions

New 1-benzyl-5-bromoindole-2-carboxamides **7a–j** were synthesized and screened *in vitro* for antibacterial activity.

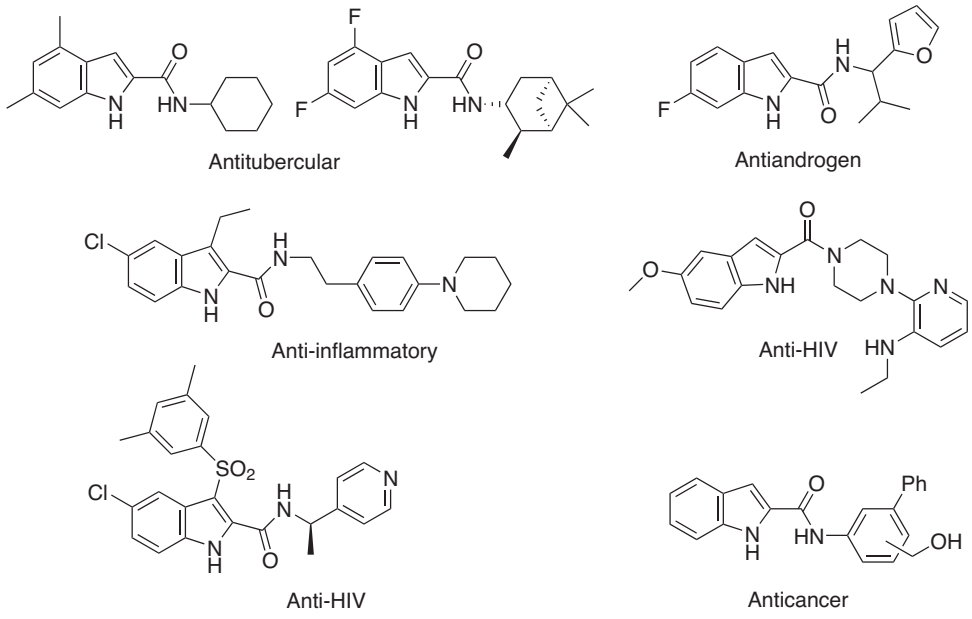
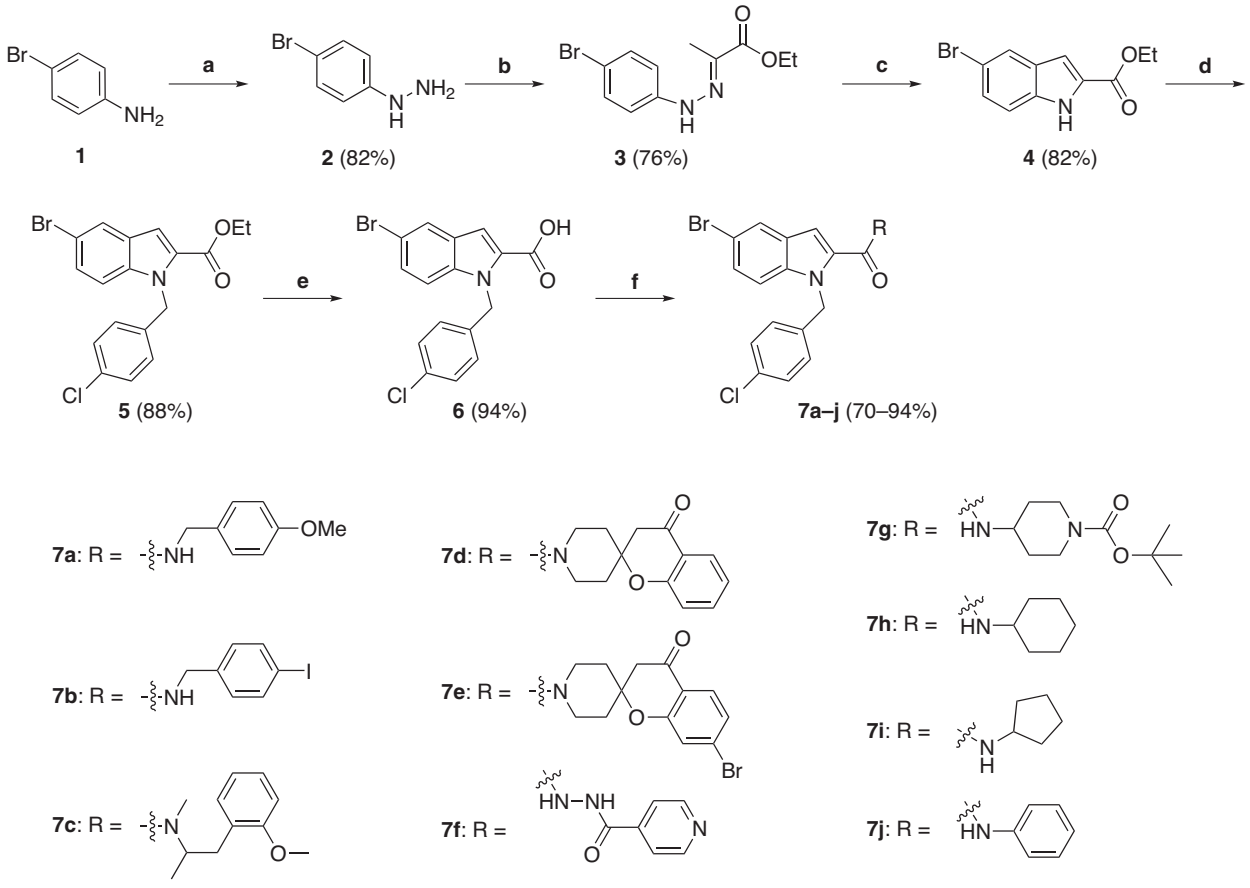


Figure 1 Reported bioactive indole-2-carboxamides.



Scheme 1 Synthesis of indole-2-carboxamides: (a) (i) $NaNO_2$, HCl, 0°C, 15 min; (ii) $SnCl_2$, HCl, 0°C, 4 h; (b) ethyl pyruvate, EtOH, argon, reflux 5.5 h; (c) polyphosphoric acid (PPA), 120°C, 20–30 min; (d) 4-chlorobenzyl chloride, Cs_2CO_3, DMF, 60°C, 16 h; (e) LiOH, THF, H_2O, EtOH, stirring, room temperature (rt), 3–4 h; (f) amine, EDC · HCl, HOBt, DIPEA, DMF, 0°C to rt, 20–30 h.

Table 1 *In vitro* antibacterial activity of compounds **7a–j** against pathogenic bacteria.

Compound	MIC (μg/mL)			
	K. pneumonia ATCC 27736	*E. coli* ATCC 9637	*P. aeruginosa* ATCC BAA427	*S.* Typhi ATCC 19430
7a	12.25	0.35	3.25	>50
7b	12.5	0.39	6.25	>50
7c	1.25	1.05	0.15	1.05
7d	12.5	12.25	12.25	>50
7e	12.25	12.5	6.35	>50
7f	>50	>50	>50	>50
7g	25.0	12.25	0.15	>50
7h	12.25	6.25	0.65	>50
7i	>50	7.39	6.25	>50
7j	30.6	8.42	20.7	>50
Gentamicin	0.25	1.25	3.02	1.65
Ciprofloxacin	0.5	1.05	1.25	3.01

Many compounds show moderate to excellent antibacterial activity compared to the reference drugs. Compounds **7a**,**b** show higher antibacterial activity than standard drugs against *E. coli* while compounds **7c**, **7g** and **7h** are more active against *P. aeruginosa* than the standard drugs.

Experimental

Reagents and solvents were purchased from commercial sources and used without further purification. Melting points were determined in open capillary tubes and are uncorrected. Synthesis of compounds was monitored by thin-layer chromatography (TLC) on silica gel-G plates of 0.5-mm thickness and spots were visualized by treatment with iodine and UV light. All compounds were purified by crystallization. Mass spectra were recorded on the Shimadzu GC-MS-QP-2010 model using the direct inlet probe technique. The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in dimethyl sulfoxide-d_6 (DMSO-d_6) solutions on a Bruker spectrometer.

4-Bromophenylhydrazine (2)

This compound was obtained by using a previously published procedure [23]; yield 82% of orange crystals; ^{1}H NMR: δ 7.2 (d, J = 8.8 Hz, 2H), 6.9 (s, 1H), 6.7 (d, J = 8.8 Hz, 2H), 4.4 (s, 2H); ^{13}C NMR: δ 113.4, 117.2, 133.6, 134.3.

Ethyl pyruvate 4-bromophenylhydrazone (3)

A mixture of compound **2** (5.0 g, 26.7 mmol) and ethyl pyruvate (3.7 g, 32.1 mmol) in EtOH (30 mL) was heated under reflux under argon for 5 h. After cooling, the resultant precipitate was filtered and washed with water. The crude product was triturated with cyclohexane and filtered to give a yellow solid **3** [23]; yield 76% of yellow crystals; ^{1}H NMR: δ 9.9 (s, 1H), 7.4 (d, J = 8.8 Hz, 2H), 7.2 (d, J = 8.8 Hz, 2H), 4.2 (q, J = 7.2 Hz, 2H), 2.1 (s, 3H), 1.3 (t, J = 7.2 Hz, 3H); ^{13}C NMR: δ 12.4, 14.7, 60.8, 112.6, 116.1, 132.2, 133.3, 144.2, 165.3.

Ethyl 5-bromo-1*H*-indole-2-carboxylate (4)

A mixture of ethyl pyruvate 4-bromophenylhydrazone **3** (5.0 g, 17.5 mmol) and polyphosphoric acid (44 g) was heated to 120°C for 0.5 h. The mixture was then cooled, poured into ice-cold water and neutralized with saturated aqueous sodium bicarbonate. The product was extracted with EtOAc and the extract was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give **4** [23]; yield 82% of pale-yellow crystals; ^{1}H NMR: δ 12.0 (s, 1H), 7.8 (d, J = 0.8 Hz, 1H), 7.4 (dd, J = 8.8 Hz and 0.8 Hz, 1H), 7.3 (dd, J = 8.8 Hz and 1.2 Hz, 1H), 7.1 (d, J = 1.2 Hz, 1H), 4.3 (q, J = 7.2 Hz, 2H), 1.3 (t, J = 7.2 Hz, 3H); ^{13}C NMR: δ 13.8, 61.2, 109.1, 113.8, 114.1, 121.8, 124.9, 126.1, 133.4, 135.6, 162.3.

Ethyl 1-(4-chlorobenzyl)-5-bromo-1*H*-indole-2-carboxylate (5)

4-Chlorobenzyl chloride (2.1 mL, 13.0 mmol) was added to a suspension of cesium carbonate (4.25 g, 13.0 mmol) and ethyl 5-bromo-1*H*-indole-2-carboxylate (**4**, 3.5 g. 13.0 mmol) in DMF (35 mL). The mixture was stirred at 60°C for 6 h (monitored by TLC), poured into water (25 mL) and extracted with EtOAc (3 × 20 mL). The extract was washed with a saturated solution of $NaHCO_3$ (20 mL), water (10 mL), brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was subjected to silica gel chromatography eluting with hexanes/EtOAc (90:10) to afford 4.5 g (88%) of compound **5** as a white solid; yield 88%; ^{1}H NMR: δ 7.8 (d, J = 1.5 Hz, 1H), 7.6 (d, J = 8.1 Hz, 2H), 7.5 (dd, J = 8.5 Hz and 1.5 Hz, 1H), 7.4 (d, J = 8.5 Hz, 1H), 7.3 (d, J = 8.1 Hz, 2H), 7.1 (s, 1H), 5.8 (s, 2H), 4.3 (q, 2H), 1.3 (t, 3H); ^{13}C NMR: δ 14.5, 47.2, 61.2, 110.5, 113.8, 125.1, 127.7, 128.2, 128.5, 128.7, 128.9, 132.2, 137.6, 138.0, 161.3.

1-(4-Chlorobenzyl)-5-bromo-1*H*-indole-2-carboxylic acid (6)

A solution of ethyl 1-(4-chlorobenzyl)-5-bromo-1*H*-indole-2-carboxylate (**5**, 3.0 g, 7.6 mmol) in tetrahydrofuran (THF) (24 mL) was treated dropwise with a solution of lithium hydroxide monohydrate (0.63 g, 11.5 mmol) in water (6 mL). The mixture was stirred at room temperature for 16 h and then acidified to pH 2–3 with 1N HCl. The mixture was extracted with EtOAc (3 × 30 mL). The extract was dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The product **6** was isolated by silica gel chromatography eluting with hexanes/EtOAc (50:50); yield 2.61 g (94%) of a white solid; ^{1}H NMR: δ 11.2 (s, 1H), 7.9 (d, J = 1.5 Hz, 1H), 7.6 (d, J = 8.1 Hz, 2H), 7.5 (dd, J = 8.5 Hz and 1.5 Hz, 1H), 7.4 (d, J = 8.5 Hz, 1H), 7.3 (d, J = 8.1 Hz, 2H), 7.1 (s, 1H), 5.8 (s, 2H); ^{13}C NMR: δ 46.9, 110.3, 113.6, 113.7, 125.0, 127.7, 127.9, 128.5, 128.9, 129.7, 132.1, 137.7, 137.9, 162.9.

General procedure for synthesis of 1-(4-chlorobenzyl)-5-bromo-1*H*-indole-2-carboxamides 7a–j

A solution of 1-(4-chlorobenzyl)-5-bromo-1*H*-indole-2-carboxylic acid (**6**, 250 mg, 0.68 mmol) in DMF/CH_2Cl_2 (10 mL) was cooled to 0°C and treated with EDC · HCl (261 mg, 1.36 mmol), HOBt (110 mg, 0.82 mmol) and DIPEA/TEA (12 µL, 1.36 mmol). The mixture was stirred for 30 min, treated with an amine (0.82 mmol) and stirred for an additional 20–30 h at room temperature. After completion of the reaction (monitored by TLC), the mixture was diluted with dichloromethane (20 mL) and washed with saturated solution of NH_4Cl (20 mL), water (20 mL) and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The product **7a–j** was isolated by flash chromatography on silica gel eluting with hexanes/EtOAc (95:5).

5-Bromo-1-(4-chlorobenzyl)-*N*-(4-methoxybenzyl)-1*H*-indole-2-carboxamide (7a) Yield 82% of white crystals; mp 270–272°C; ^{1}H NMR: δ 9.1 (t, J = 4.0 Hz, 1H), 7.9 (s, 1H), 7.5 (d, J = 8.0 Hz, 1H), 7.4 (d, J = 8.0, 1H), 7.3 (d, J = 8.1 Hz, 2H), 7.2 (s, 1H), 7.1 (d, J = 8.1 Hz, 2H), 7.0 (d, J = 8.0 Hz, 2H), 6.8 (d, J = 8.0 Hz, 2H), 5.8 (s, 2H), 4.3 (d, J = 4.0 Hz, 2H), 3.7 (s, 3H); ^{13}C NMR: δ 41.6, 46.4, 55.1, 104.8, 113.0, 113.7, 124.0, 126.4, 127.7, 128.5, 131.3, 131.7, 132.9, 136.7, 137.5, 158.2, 161.3; MS (ESI): *m/e* 482 $(M)^+$, 484 $(M+2)^+$, 486 $(M+4)^+$. Anal. Calcd for $C_{24}H_{20}BrClN_2O_2$: C, 59.58; H, 4.17; N, 5.79. Found: C, 59.51; H, 4.09; N, 5.73.

5-Bromo-1-(4-chlorobenzyl)-*N*-(4-iodobenzyl)-1*H*-indole-2-carboxamide (7b) Yield 78% of white crystals; mp 284–286°C; ^{1}H NMR: δ 9.1 (t, J = 5.8 Hz, 1H), 7.7 (s, 1H), 7.5 (d, 1H), 7.4 (d, 1H), 7.3 (d, 2H), 7.1 (m, 3H), 6.9 (d, J = 8.2 Hz, 2H), 6.7 (d, J = 8.2 Hz, 2H), 5.7 (s, 2H), 4.3 (d, J = 5.8 Hz, 2H); ^{13}C NMR: δ 45.4, 50.0, 92.0, 108.6, 116.7, 117.4, 127.7, 130.1, 131.4, 132.2, 135.0, 135.4, 136.7, 140.4, 141.3, 161.9; MS (ESI): *m/e* 578 $(M)^+$, 580 $(M+2)^+$, 582 $(M+4)^+$. Anal. Calcd for $C_{23}H_{17}BrClIN_2O$: C, 47.66; H, 2.96; N, 4.83. Found: C, 47.61; H, 2.89; N, 4.77.

5-Bromo-1-(4-chlorobenzyl)-*N*-(1-(2-methoxyphenyl)propan-2-yl)-*N*-methyl-1*H*-indole-2-carboxamide (7c) Yield 76% of white crystals; mp 291–293°C; ^{1}H NMR: δ 8.0 (d, J = 1.4 Hz, 1H), 7.6 (d, J = 8.1 Hz, 1H), 7.5 (dd, J = 8.1 Hz and 1.4 Hz, 1H), 7.4 (d, J = 8.5 Hz, 2H), 7.3 (s, 1H), 7.2 (d, J = 8.5 Hz, 2H), 6.9–7.1 (m, 4H), 5.8 (s, 2H), 4.2 (m, 1H), 3.8 (s, 3H), 3.4 (s, 3H), 2.9 (dd, 1H), 2.6 (dd, 1H), 1.2 (d, 3H); ^{13}C NMR: δ 18.4, 32.7, 33.4, 49.5, 55.70, 56.7, 104.5, 112.3, 113.4, 123.9, 126.5, 127.1, 127.8, 127.9, 128.7, 130.7, 131.5, 132.8, 135.7, 137.6, 161.0; MS (ESI): *m/e* 524 $(M)^+$, 526 $(M+2)^+$, 528 $(M+4)^+$. Anal. Calcd for $C_{27}H_{26}BrClN_2O_2$: C, 61.67; H, 4.98; N, 5.33. Found: C, 61.59; H, 4.94; N, 5.28.

1′-(5-Bromo-1-(4-chlorobenzyl)-1*H*-indole-2-carbonyl) spiro[chroman-2,4′-piperidin]-4-one (7d) Yield 73% of white crystals; mp >300°C; ^{1}H NMR: δ 8.1 (d, J = 1.5 Hz, 1H), 7.7 (d, J = 8.1 Hz, 1H), 7.6 (dd, J = 8.1 Hz and 1.5 Hz, 1H), 7.5 (m, 1H), 7.4 (dd, J = 8.4 Hz and 1.3 Hz, 1H), 7.3 (d, J = 8.5 Hz, 2H), 7.2 (s, 1H), 7.1 (d, J = 8.5 Hz, 2H), 7.0 (m, 2H), 5.8 (s, 2H), 3.3–3.4 (m, 4H), 2.7 (s, 2H), 1.7–1.9 (m, 4H); ^{13}C NMR: δ 33.7, 37.8, 43.6, 49.5, 68.0, 104.8, 113.7, 114.3, 119.1, 120.7, 123.7, 126.4, 126.9, 127.8, 128.5, 130.9, 132.3, 135.0, 137.8, 159.4, 164.7, 189.8; MS (ESI): *m/e* 562 $(M)^+$, 564 $(M+2)^+$, 566 $(M+4)^+$. Anal. Calcd for $C_{29}H_{24}BrClN_2O_3$: C, 61.77; H, 4.29; N, 4.97. Found: C, 61.73, H, 4.23, N, 4.94.

7-Bromo-1′-(5-bromo-1-(4-chlorobenzyl)-1*H*-indole-2-carbonyl) spiro[chroman-2,4′-piperidin]-4-one (7e) Yield 79% of white crystals; mp >300°C; ^{1}H NMR: δ 8.2 (d, J = 1.5 Hz, 1H), 7.8 (d, J = 8.1 Hz, 1H), 7.7 (dd, J = 8.1 Hz and 1.5 Hz, 1H), 7.6 (d, J = 8.2 Hz, 1H), 7.4 (d, J = 1.3 Hz, 1H), 7.3 (d, J = 8.5 Hz, 2H), 7.2 (dd, J = 8.2 Hz and 1.3 Hz, 1H), 7.1 (s, 1H), 7.0 (d, J = 8.5 Hz, 2H), 5.7 (s, 2H), 3.3–3.4 (m, 4H), 2.7 (s, 2H), 1.7–1.9 (m, 4H); ^{13}C NMR: δ 33.8, 37.9, 43.7, 49.6, 57.7, 104.9, 113.6, 116.3, 120.0, 122.9, 123.7, 126.7, 127.0, 128.0, 128.7, 130.3, 130.5, 132.6, 135.3, 138.0, 159.6, 165.2, 190.1; MS (ESI): *m/e* 640 $(M)^+$, 642 $(M+2)^+$, 644 $(M+4)^+$, 646 $(M+6)^+$. Anal. Calcd for $C_{29}H_{23}Br_2ClN_2O_3$: C, 54.19; H, 3.61; N, 4.36. Found: C, 54.12; H, 3.58; N, 4.34.

5-Bromo-1-(4-chlorobenzyl)-*N*′-isonicotinoyl-1*H*-indole-2-carbohydrazide (7f) Yield 79% of white crystals; mp 268–270°C; ^{1}H NMR: δ 9.0 (d, J = 8.4 Hz, 2H), 8.5 (s, 2H), 8.0 (d, J = 1.5 Hz, 1H), 7.9 (d, J = 8.4 Hz, 2H), 7.8 (d, J = 8.1 Hz, 1H), 7.7 (dd, J = 8.1 Hz and 1.5 Hz, 1H), 7.4 (d, J = 8.5 Hz, 2H), 7.3 (s, 1H), 7.2 (d, J = 8.5 Hz, 2H), 5.8 (s, 2H); ^{13}C NMR: δ 49.8, 68.7, 104.6, 113.5, 121.0, 123.8, 126.8, 127.9, 128.7, 131.5, 132.6, 135.3, 138.1, 140.7, 149.9, 161.3, 164.9; MS (ESI): *m/e* 482 $(M)^+$, 484 $(M+2)^+$, 486 $(M+4)^+$. Anal. Calcd for $C_{22}H_{16}BrClN_4O_2$: C, 54.62; H, 3.33; N, 11.58. Found: C, 54.57; H, 3.29; N, 11.56.

tert-Butyl-4-(5-bromo-1-(4-chlorobenzyl)-1*H*-indole-2-carboxamido)piperidine-1-carboxylate (7g) Yield 87% of white crystals; mp 290–292°C; ^{1}H NMR: δ 8.5 (d, 1H), 8.1 (d, J = 1.5 Hz, 1H), 7.7 (d, J = 8.1 Hz, 1H), 7.6 (dd, J = 8.1 Hz and 1.5 Hz, 1H), 7.4 (d, J = 8.5 Hz, 2H), 7.3 (s, 1H), 7.2 (d, J = 8.5 Hz, 2H), 5.8 (s, 2H), 3.5 (m, 1H), 3.2–3.4 (m, 4H), 1.6–1.8 (m, 4H), 1.4 (s, 9H); ^{13}C NMR: δ 27.9, 30.1, 43.1, 47.5, 49.6, 79.5, 104.7, 113.5, 123.7, 126.8, 127.9, 128.6, 131.5, 132.7, 138.2, 135.3, 159.1, 160.7. HRMS. Calcd for $(M+H)^+$: *m/z* 546.1154. Found: *m/z* 546.1148.

5-Bromo-1-(4-chlorobenzyl)-*N*-cyclohexyl-1*H*-indole-2-carboxamide (7h) Yield 94% of white crystals; mp 232–234°C; ^{1}H NMR: δ 8.3 (d, 1H), 8.2 (d, J = 1.5 Hz, 1H), 7.8 (d, J = 8.2 Hz, 1H), 7.7 (dd, J = 8.2 Hz and 1.5 Hz, 1H), 7.4 (d, J = 8.4 Hz, 2H), 7.3 (s, 1H), 7.2 (d, J = 8.4 Hz, 2H), 5.8 (s, 2H), 3.6 (m, 1H), 1.5–1.7 (m, 5H), 1.2–1.3 (m, 5H); ^{13}C NMR: δ 24.9, 25.5, 32.2, 49.4, 51.3, 1047, 113.6, 123.8, 126.8, 128.0, 128.4, 131.4, 132.8, 135.5, 138.3, 160.5. HRMS. Calcd for $(M+H)^+$: *m/z* 445.0677. Found: *m/z* 445.0677.

5-Bromo-1-(4-chlorobenzyl)-*N*-cyclopentyl-1*H*-indole-2-carboxamide (7i) Yield 92% of white crystals; mp 230–232°C; ^{1}H NMR: δ 8.4 (d, 1H), 8.1 (d, J = 1.4 Hz, 1H), 7.7 (d, J = 8.3 Hz, 1H), 7.6 (dd, J = 8.3 Hz and 1.4 Hz, 1H), 7.4 (d, J = 8.4 Hz, 2H), 7.3 (s, 1H), 7.2 (d, J = 8.4 Hz, 2H), 5.8 (s, 2H), 3.6 (s, 1H), 1.6–1.8 (m, 4H), 1.3–1.5 (m, 4H); ^{13}C NMR: δ 23.3, 32.7, 49.3, 56.3, 104.5, 113.6, 123.8, 126.7, 127.9, 128.2, 131.3, 132.8, 135.5, 138.4, 160.4; MS (ESI): *m/e* 430 $(M)^+$, 432 $(M+2)^+$, 434 $(M+4)^+$. Anal. Calcd for $C_{21}H_{20}BrClN_2O$: C, 58.42; H, 4.67; N, 6.49. Found: C, 58.37; H, 4.60; N, 6.43.

5-Bromo-1-(4-chlorobenzyl)-*N*-phenyl-1*H*-indole-2-carboxamide (7j) Yield 90% of white crystals; mp 238–240°C; ^{1}H NMR: δ 8.9 (b, 1H), 8.1 (s, 1H), 7.8 (dd, 1H), 7.7 (dd, 1H), 7.6 (d, 2H), 7.5 (d, 2H), 7.4 (s, 1H), 7.3 (d, 2H), 7.1 (d, 2H), 5.8 (s, 2H); ^{13}C NMR: δ 49.2, 110.6, 113.7, 114.9, 120.6, 121.4, 123.5, 127.3, 128.2, 130.4, 133.1, 135.4, 137.2, 142.2, 162.4; MS (ESI): *m/e* 438 $(M)^+$, 440 $(M+2)^+$, 442 $(M+4)^+$. Anal. Calcd for $C_{22}H_{16}BrClN_2O$: C, 60.09; H, 3.67; N, 6.37. Found: C, 60.03; H, 3.64; N, 6.31.

In vitro *antibacterial activity*

Compounds **7a–j** were evaluated for their *in vitro* antibacterial activity against pathogenic Gram-negative bacteria *K. pneumoniae* (ATCC 27736), *E. coli* (ATCC 9637), *P. aeruginosa* (ATCC BAA427) and

S. enterica subsp. *enterica* serovar Typhi (ATCC 19430) by using the broth microdilution technique described by the CLSI, 2012 [24]. Gentamicin and ciprofloxacin were used as standard drugs for comparison of antibacterial activity. DMSO was used as a solvent or negative control. To clarify any effect of DMSO on the antibacterial activity, separate studies were carried out with solutions of only DMSO and these studies showed no activity against any microbial strains. The MIC of tested compounds was determined using the 2-fold serial dilution technique by assaying at 51.2, 25.6, 12.8, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, 0.1 and 0.05 μg/mL concentrations along with standards at the same concentrations.

Acknowledgments: The authors are grateful to the Department of Postgraduate Studies and Research in Chemistry, Dnyanopasak College, Parbhani and Shri Chhatrapati Shivaji College, Omerga, for providing laboratory facilities and to the Indian Institute of Chemical Technology, Hyderabad, India, for spectral data.

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