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Potent antimicrobial activity of 3-(4,5-diaryl-1*H*-imidazol-2-yl)-1*H*-indole derivatives against methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

A new series of antimicrobial derivatives [3-(4,5-diaryl-1*H*-imidazol-2-yl)-1*H*-indole)] have been synthesized with potent activity against strains of *Staphylococcus aureus*, including methicillin-resistant strains (MRSA). Compound **17** [3-(4,5-bis(4-fluorophenyl)-1*H*-imidazol-2-yl)-5-bromo-1*H*-indole], the most active derivative was shown to inhibit the growth of all Gram-positive strains tested, including vancomycin resistant *Enterococcus faecalis* and *Enterococcus faecium* with no activity against Gram-negative bacteria.

Infections caused by multi-drug resistant bacteria are of major health concern worldwide. Particularly important are infections caused by the Gram-positive bacteria *Staphylococcus aureus* and species of the genus Enterococcus, due to increasing incidence of infections caused by these microorganisms in hospitals and communities, and their ability of developing antibiotic resistance to multiple antibiotics.[1] New antibacterial agents to treat infections caused by these Gram-positive bacteria have recently been introduced, including the semi-synthetic streptogramins quinupristin/dalfopristin, daptomycin, the synthetic oxazolidinone linezolid and tigecycline.[2] These new treatment options are welcome additions to the armamentarium against infections caused by Gram-positive bacteria. However, the identification of intrinsically resistant strains, reports of emergence of resistance and serious side effects for some of these new agents, make the development of a diversified pipeline of antimicrobials a necessity.[1]

Previously, we reported the synthesis and preliminary biological characterization of new 2,4,5 tri-substituted imidazoles, including 3-(4,5-diaryl-1*H*-imidazol-2-yl)-1*H*-indoles, some of which exhibited antibacterial, antifungal or anticancer activities.[3] We report herein the synthesis and structure-antibacterial activity relationship of a series of thirty 3-(4,5-diaryl-1*H*-imidazol-2-yl)-1*H*-indole derivatives against bacterial strains of *S. aureus*, including methicillin-resistant strains (MRSA). We also determined the spectrum of activity of one of the most active compounds identified through these studies, compound **17** (3-(4,5-bis (4-fluorophenyl)-1*H*-imidazol-2-yl)-5-bromo-1*H*-indole), against bacterial strains that belong to several Gram-positive and Gram-negative bacterial species. This series of derivatives was obtained via classical condensation of an aldehyde with a dicarbonyl compound to produce an imidazole ring system,[4] with 1*H*-indole-3-carboxyldehyde as our choice of aldehyde, along with symmetrical and unsymmetrical benzil derivatives.[5]

Scheme 1 shows the general synthetic strategy used to synthesize these compounds, and Table 1 shows their structural features.[6] The antimicrobial activity was evaluated by determining the minimal inhibitory concentration (MIC) using the microdilution susceptibility test.[7,8] MIC values were defined as the lowest concentration at which no visible growth is observed. The results showed a wide range of antimicrobial activities among the different derivatives tested with MIC values ranging from >64 μg/mL to 1 μg/mL (Table 2). The most active compounds belonged to compounds having the 5-bromoindole moiety. Derivatives **17** and **22** exhibited activities against MRSA comparable to that of vancomycin (MIC 1–2 μg/mL) and higher activity than oxacillin (MIC 16 to >64 μg/mL) and linezolid (2–4 μg/mL).

Table 2, shows the superiority of compounds having bromine on the indole moiety at position 5 over compounds with a methyl group at position 2 in the same moiety, with the exception of compounds **19** and **23**. For example, MICs of compounds **16**, **17**, **20**, **21**, **22**, **24**, **27**, and **30** are lower than those of the corresponding compounds with the methyl group at position 2 namely **1, 2**, **5**, **6**, **7**, **9**,

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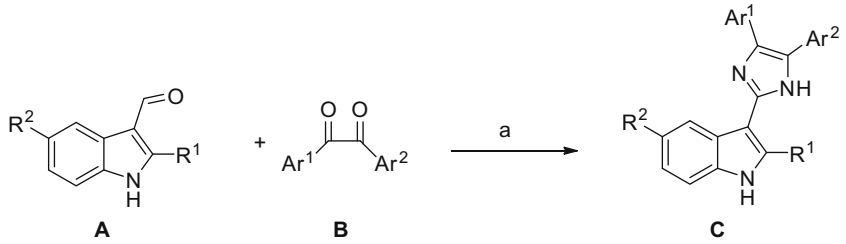
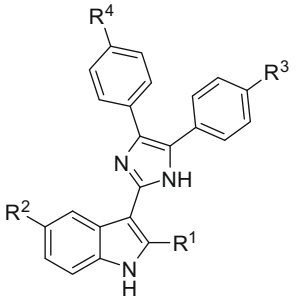


Scheme 1. Synthesis of the target compounds of general formula **C** from indole derivatives **A** and benzil derivatives **B**. Reagents: (a) NH_4Ac, AcOH, reflux 4–8 h.

Table 1
Structures of synthetic 3-(4,5-diaryl-1*H*-imidazol-2-yl)-1*H*-indole compounds



Compound	R^1	R^2	R^3	R^4	Compound	R^1	R^2	R^3	R^4
1	CH_3	H	H	H	**16**	H	Br	H	H
2	CH_3	H	F	F	**17**	H	Br	F	F
3	CH_3	H	$N(CH_3)_2$	$N(CH_3)_2$	**18**	H	Br	$N(CH_3)_2$	$N(CH_3)_2$
4	CH_3	H	Br	Br	**19**	H	Br	Br	Br
5	CH_3	H	Cl	Cl	**20**	H	Br	Cl	Cl
6	CH_3	H	CH_3	CH_3	**21**	H	Br	CH_3	CH_3
7	CH_3	H	H	Cl	**22**	H	Br	H	Cl
8	CH_3	H	H	NO_2	**23**	H	Br	H	NO_2
9	CH_3	H	H	F	**24**	H	Br	H	F
10	CH_3	H	O-Ph	O-Ph	**25**	H	Br	O-Ph	O-Ph
11	CH_3	H	Ph	Ph	**26**	H	Br	Ph	Ph
12	CH_3	H	H	Br	**27**	H	Br	H	Br
13	CH_3	H	H	Ph	**28**	H	Br	H	Ph
14	CH_3	H	NO_2	NO_2	**29**	H	Br	NO_2	NO_2
15	CH_3	H	H	CH_3	**30**	H	Br	H	CH_3

Table 2
In vitro antimicrobial activity (MIC μg/mL) of synthetic compounds tested against strains of *Staphylococcus aureus*[7,8]

Compound	MRSA	MSSA	Compound	MRSA	MSSA
1	4–8	8	**16**	4	4
2	4–32	4	**17**	1	1
3	2–4	2	**18**	2–4	2
4	2–4	2–4	**19**	>64	>64
5	4	4	**20**	2–4	2–4
6	8	8	**21**	4	2–4
7	8	8	**22**	1	1
8	8	8	**23**	>64	>64
9	4–8	4	**24**	2	2
10	>64	>64	**25**	>64	>64
11	>64	>64	**26**	>64	>64
12	4	4	**27**	2	2
13	2–4	4	**28**	4	4
14	>64	>64	**29**	>64	>64
15	4–8	4	**30**	2	2
L	2–4	1–2	V	1–2	1
Ox	16–>64	0.5–4			

L = linezolid; V = vancomycin; Ox = oxacillin.

Table 3
Spectrum of in vitro activity (MIC; μg/mL) of compound **17** against several bacterial species

Strain	ATCC #	**17**	V
B. subtillis	14579	2	1
B. subtillis	6633	2	0.25
E. faecalis	29212	2	4
E. faecalis	51299	2	16
E. faecium	51559	2	>64
M luteus	10240	1	0.25
S. epidermidis	13518	2	0.5
S epidermidis	12228	2	2
S. epidermidis	35983	2	4
S .saprophyticus	15305	2	2
S. pneumoniae	49150	2	4
S. dysgalactiae	12388	2	0.5
S. dysgalactiae	12394	2	1
S. pyogenes	19615	2	1
S. sanguinis	10556	1	1
S. agalactiae	13813	2	1
S. agalactiae	12386	2	2
E. coli	12435	>64	>64
E. coli	35333	>64	>64
P. aeruginosa	19142	>64	>64
P. aeruginosa	39324	>64	>64
S. typhimurium	14028	>64	>64
S. typhimurium	13311	>64	>64

V = vancomycin.

12, and **13.** Substitutions in the phenyl ring at positions 4 and 5 of the imidazole moiety with either nitro, phenoxy or phenyl groups abrogated the activity.

Compounds with halogenated phenyl groups in the imidazole moiety exhibited higher activity than compounds with other substituents at the same positions. Compound **17** was the most active from this series of derivatives. The spectrum of activity of compound **17** against different microorganisms, including Gram-positive and Gram-negative bacteria, was investigated. In addition, its activity was compared with that of vancomycin.

Compound **17** exhibited consistent activity toward all Gram-positive strains tested (Table 3), with MIC values ranging from 1 to 2 μg/mL, including *Enterococcus faecium* (ATCC # 51559) and *Enterococcus faecalis* (ATCC # 51299), both of which are resistant to vancomycin (MIC >64 μg/mL and 16 μg/mL, respectively). Compound **17,** however, showed no activity against Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. These results suggest the selectivity of this new synthetic compound for Gram-positive bacteria.[9]

In conclusion, the structure-antibacterial activity relationship of a new series of 3-(4,5-diaryl-1*H*-imidazol-2-yl)-1*H*-indole derivatives has been described, with some derivatives exhibiting potent in vitro antimicrobial activity against various strains of MRSA and other Gram-positive bacteria. Several synthesized compounds exhibited comparable activity to that of vancomycin and higher activity than oxacillin and linezolid.

The characterization of the antimicrobial spectrum of compound **17** indicates a selective activity of this derivative for Gram-positive bacteria. Studies are currently underway to characterize the mode of action of this series of compounds, including the investigation of possible common mechanisms involved in the growth inhibition of Gram-positive bacteria and cancer cells, since several derivatives were also capable of inhibiting human colon carcinoma cell proliferation (data not shown).[3b] Additional studies are also underway to determine the *in vivo* efficacy of selected compounds in several animal models of infection.

References and notes

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5. In this study two indole-3-carboxaldehydes were used, both commercially available. The benzyl compounds either symmetrical or unsymmetrical were synthesized based on the following literature: (a) Ogata, Y.; Takagi, K.; Fujii, Y. *J. Org. Chem.* **1972**, *37*, 4026; (b) Adams, R.; Marvel, C. S. In *Organic Synthesis*; Whiley J. and Sons: New York, 1941; Coll Vol. 1. p 94; (c) Fisher, A.; Grigor, B. A.; Packer, J.; Vaughan, J. *J. Am. Chem. Soc.* **1961**, *83*, 4208; (d) Chi, K.-W.; Yusubov, M. S.; Filimonov, V. D. *Synth. Commun.* **1994**, *24*, 2119; (e) Armesto, D.; Horspool, W. M.; Ortiz, M. J.; Perez-Ossorio, R. *Synthesis* **1988**, *10*, 799; f Baach, H. C. U.S. Patent 3,551385, 1970.; (g) Lau, K.; Arnold, F. *Org. Prep. Proced. Int.* **1980**, *12*, 327.
6. All the synthetic compounds were fully characterized and show satisfactory physicochemical properties. In a typical experiment 1 mmol (1 equiv) of the indole-3-carboxaldehyde was combined with 1.05 equiv of the benzyl derivative and 20 equiv of ammonium acetate in 5 ml acetic acid. The reaction mixture was refluxed for 3–4 h and the reaction monitored by TLC. The work up of the reaction was done by adding the reaction to a stirred ice-water in which a precipitate formed. The precipitated product was air dried and recrystallized from ethanol. All the compounds were produced in a quantitative yield. ^{1}H NMR for selected compounds: **4**, mp = 240–245 °C: ^{1}H NMR ($CDCl_3$): δ = 7.47 (d, 4H), 7.30–7.34 (m, 1H, 7.14–7.19 (m, 3H), 2.68 (s, 3H). Compound **5**: mp = 165–167 °C; ^{1}H NMR (DMSO-d_6): δ = 12.13 (s, 1H), 11.33 (s, 1H), 7.94 (d, 2H), 7.57 (d, 2H), 7.39 (br d, 2H), 7.35 (d(1H), 7.05–7.12 (m, 3H), 2.5 (s, 3H). Compound **9**: mp = 247–250 °C; ^{1}H NMR ($CDCl_3$): δ = 7.78 (br s, 1H), 7.59 (d, 2H), 7.54 (d, 2H), 7.35–7.39 (m, 2H), 7.28–7.34 (m, 2H), 7.13–7.18 (2H), 7.01–7.05 (m, 2H), 2.72 (br s, 3H). Compound **26**: mp = 155.158 °C; ^{1}H NMR ($CDCl_3$): δ = 8.08 (d, 4H), 8.07 (br s, 1H), 7.75 (d, 4H), 7.28–7.50 (m, 10H), 7.12 (br d, 2H), 6.97 (br s, 1H).
7. National Council of Clinical Laboratory Services (NCCLS). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically M7-A5, National Committee on Clinical Laboratory Standards, 2000; Vol. 20, pp 2. Briefly, 10 μL stock dilutions of each derivative carried out in 50% DMSO were added to microculture wells containing 90 μL volumes of the corresponding bacterial cultures in Mueller–Hinton broth medium, to final concentrations from 64 μg/mL to 0.06 μg/mL.
8. A collection of two methicillin susceptible strains (MSSA); ATCC-6538 and ATCC-29213, and six methicillin-resistant strains (MRSA); 1A-218, 1A-318, 1B-374, 1B-315, 1B-185 and 1B-387 were included in the study representing two different epidemic isolates (CMRSA-1A and CMRSA-1B). They were identified by molecular typing by sentinel hospitals as part of the Canadian Nosocomial Infection Surveillance program: Simor, A. E.; Ofner-Agostini, M.; Bryce, E.; Green, K.; McGeer, A.; Mulvey, M.; Paton, S. *C.M.A.J.* **2001**, *165*, 21.
9. The selectivity of this series of compound for Gram-positive bacteria might be related to an antimicrobial mechanism specific for Gram-positive bacteria, or to their inability to penetrate the outer membrane present in Gram-negative bacteria. The characterization of the mechanism of action of these novel compounds is underway.