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Unprecedented C-2 Arylation of Indole with Diazonium Salts: Syntheses of 2,3-Disubstituted Indoles and their Antimicrobial Activity

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Abstract

A novel reaction of indole with aryldiazonium salts leading to the formation of 2-aryl-3-(arylazo)indoles was discovered. The products were found to possess potent anti-MRSA and anti-LLVRE activities. The SAR studies indicate that the potentially metabolically labile azo functionality can be replaced with ether oxygen and thioether sulphur atoms without any loss of activity.

Keywords

MRSA; low-level VRE; indole; arylation

Nosocomial infections caused by multi-drug resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) or vancomycin-resistant *Enterococcus faecalis* (VRE) present a considerable challenge in the clinic.[1,2] Therefore, the search for new antibacterial agents capable of combating these microorganisms continues unabated.[3–5] Because a number of azo-containing compounds have been reported to exhibit promising antimicrobial properties,[6–10] we initiated a project aimed at the synthesis of diverse azo-containing heterocycles and their evaluation for antibacterial and antifungal activities with the emphasis on hospital-acquired infections. These efforts led to the identification of a compound active against MRSA, whose structure was originally assigned as 2,3-di(*p*-chlorophenylazo)indole (Compound **A**, Figure 1).

This compound was originally synthesized by the reaction of indole with 2.5 equiv of *p*-$ClPhN_2^+Cl^-$, which gave 87% of the C-3 monoazo product (**1a** in Scheme 1) and only 3% of compound **A**. The subsequent optimization of this procedure resulted in the use of 4 equiv of *p*-$ClPhN_2^+BF_4^-$ and afforded an improved 56% yield of this desired product. Although the NMR spectra of compound **A** were consistent with its originally assigned structure as

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2,3-di(*p*-chlorophenylazo)indole, the mass spectral analysis suggested that only one azo group was present. While either C-2 or C-3 positioning of the azo functionality was theoretically possible, the mechanistic considerations led us to propose structure **2a** (Figure 1, Scheme 1) for this indole derivative.

To confirm the structure of **2a** unequivocally, this compound was synthesized by an independent route, involving the Fisher synthesis of indole **3** and its subsequent reaction with *p*-$ClPhN_2^+BF_4^-$ (Scheme 2a). The spectral data for the obtained compound were indistinguishable with those for **2a**. The reaction in Scheme 1 also allowed for the synthesis of analogues **2b–d**, while the unsymmetrical derivative **2e** was obtained via a stepwise introduction of the substituents (Scheme 2b).

It could be surmised that mechanistically this new process involves an *ipso*-attack at C-3 of the indole ring[11] with the formation of the geminal diazo compound **B** ("path a" in Scheme 3). The homolytic bond breakage and elimination of nitrogen can then lead to radical recombination at C-3 (**C**) or more sterically accessible C-2 (**D**). The 1,5 hydrogen shift results in the observed product **2**. However, this mechanism is inconsistent with the formation of **2e** (in Scheme 2b), which proceeds without scrambling of the halogens. Therefore, a more likely mechanism is based on the Meerwein-type arylation shown as "path b." It involves an aromatic radical formation from the diazonium salt and addition of this radical at C-2 position of the indole to form azo-stabilized radical **F**, which results in product **2**. The aryl radical formation from the diazonium salt can be promoted by either acetate or dioxane (shown is Scheme 3) as has been reported previously for the metal-free Meerwein arylations.[12,13] Although the classical Meerwein arylations of olefins are promoted with copper or palladium salts,[14] the use of $Cu(OAc)_2$ or $Pd(OAc)_2$ did not result in higher yields in our case.

The evaluation of compounds **2a–e** for antibacterial activity showed promising results, specifically against gram-positive organisms (see Table 1). However, we had concerns about the stability of the azo functionality under physiological conditions. Therefore, to explore the SAR within this series of compounds we first addressed the issue of whether this moiety is absolutely required for activity. Thus, we synthesized the "carba" analogues **4** and **5**, utilizing Fujiwara/Heck chemistry and varying the number of equivalents of the olefin (Scheme 4).[15] Distyrylindole **5** was then converted to carbazole **6** upon reflux in xylenes in the presence of Pd/C. We believe this approach provides a useful synthetic access to the carbazole skeleton.[16]

We also explored a bioisosteric substitution of the azo group with sulfur and oxygen atoms. Such compounds were prepared using the Fisher indole synthesis starting with the requisite arylthia- and aryloxa-acetophenones (Scheme 5). To our knowledge, these syntheses constitute the first examples of the preparation of 2-aryl-3-aryloxa- and 2-aryl-3-arylthia-indoles (**7a–c** and **8a–g**) using the Fisher reaction.

Finally, compound **9**, containing the sulfone isostere of the azo moiety, was prepared by oxidation of **7a** with MCPBA (Scheme 6a),[17] while compound **10**, the carbono analogue, was obtained by acylation of **3** with *p*-Cl-PhCOCl (Scheme 6b).[18,19]

The data in Table 1 indicate that all our compounds that show antibacterial activity inhibit the growth of gram-positive microorganisms only. Furthermore, there is no discrimination between the bacterial strains on the basis of their resistance status. Thus, all 3-azo-indole derivatives synthesized (**1a,b** and **2a–e**) are equally potent toward the susceptible *S. aureus* strain (*S. aureus* 29213), its resistant counterpart MRSA (*S. aureus* BAA-44) or low-level vancomycin-resistant *Enterococcus* (LLVRE, *E. faecalis* 51299). Although the activity is lost when the 3-azo functionality is replaced by "carba" bridges (**4–6**), or sulfono (**9**) and

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carbono (**10**) groups, even more potent compounds result from substituting the 3-azo moiety with the thioether sulphur (**7a–c**) and ether oxygen (**8a–g**) atoms. For example, aryloxyindole **8b** is submicromolar toward MRSA. These results compare favorably with the clinically used antibiotics vancomycin and penicillin G, which are less potent in this minipanel of gram-positive bacteria and show organism-dependent growth inhibitory properties.

The synthesized indole derivatives were also tested against a nosocomial fungus *C. albicans* (Table 1). Only the 3-monoazo compounds **1a** and **1b** exhibited modest levels of activity, which is consistent with an earlier report of antifungal activities associated with 3-phenylazoindole.[25] Importantly, we found that the antibacterial properties of our promising analogues **7a–c** and **8a–g** are paralleled with an antiproliferative effect against cultured human cancer cells, such as HeLa (Table 1). These findings warrant future rodent studies, in which the toxicity of these compounds can be properly evaluated.

In conclusion, an unprecedented reaction of indole with aryldiazonium salts affords 2-aryl-3-(arylazo)indoles, which display promising anti-MRSA and anti-LLVRE activities. The successful bioisosteric substitution of the labile azo group with ether oxygen and thioether sulphur atoms indicates that the azo functionality is not required for activity and, thus, the potential metabolic instability of these indole-based antibacterial agents is not of concern. Experiments aimed at elucidating the mode of action of these compounds in gram-positive bacteria are underway and will be reported in due course.

Acknowledgments

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19. *Selected synthetic procedure* ***(2a–d)***: To a solution of indole (0.001 mol) in dioxane (2 mL) were added a solution of sodium acetate (0.004 mol) in water (0.5 mL) and an appropriate diazonium tetrafluoroborate (0.004 mol) at room temperature. The reaction mixture was stirred at room temperature for six hours and then water was added (10 mL). After the extraction with AcOEt, the organic layer was dried (Na_2SO_4) and the solvent was removed under reduced pressure. The product was purified by flash chromatography on silica gel (hexane-ethyl acetate, 15:1). Selected characterization data (**2a**): 56%; ^{1}H NMR ($CDCl_3$) δ 8.60–8.59 (m, 1H), 7.90-7.87 (d, *J*=8.52 Hz, 2H), 7.80-7.77 (d, *J*=8.52 Hz, 2H), 7.50-7.47 (d, *J*=8.52 Hz, 2H), 7.46-7.43 (d, *J*=8.79 Hz, 2H), 7.36-7.31 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 135.7, 134.8, 132.7, 130.5, 129.3, 129.2, 125.1, 123.8, 123.3, 119.8, 111.2; HRMS *m/z* (ESI) calcd for $C_{20}H_{14}Cl_2N_3$ ($M+H^+$) 366.0565, found 366.0557.

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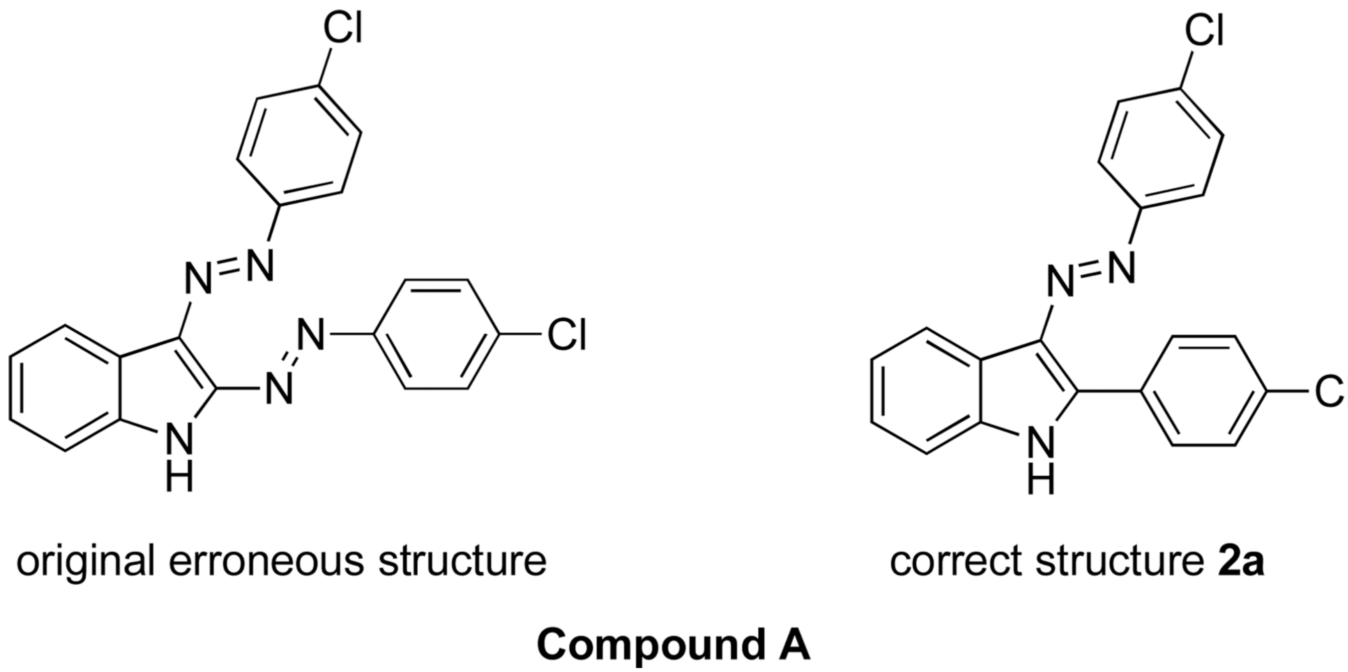

Figure 1.
The originally assigned erroneous and corrected structures for compound **A**.

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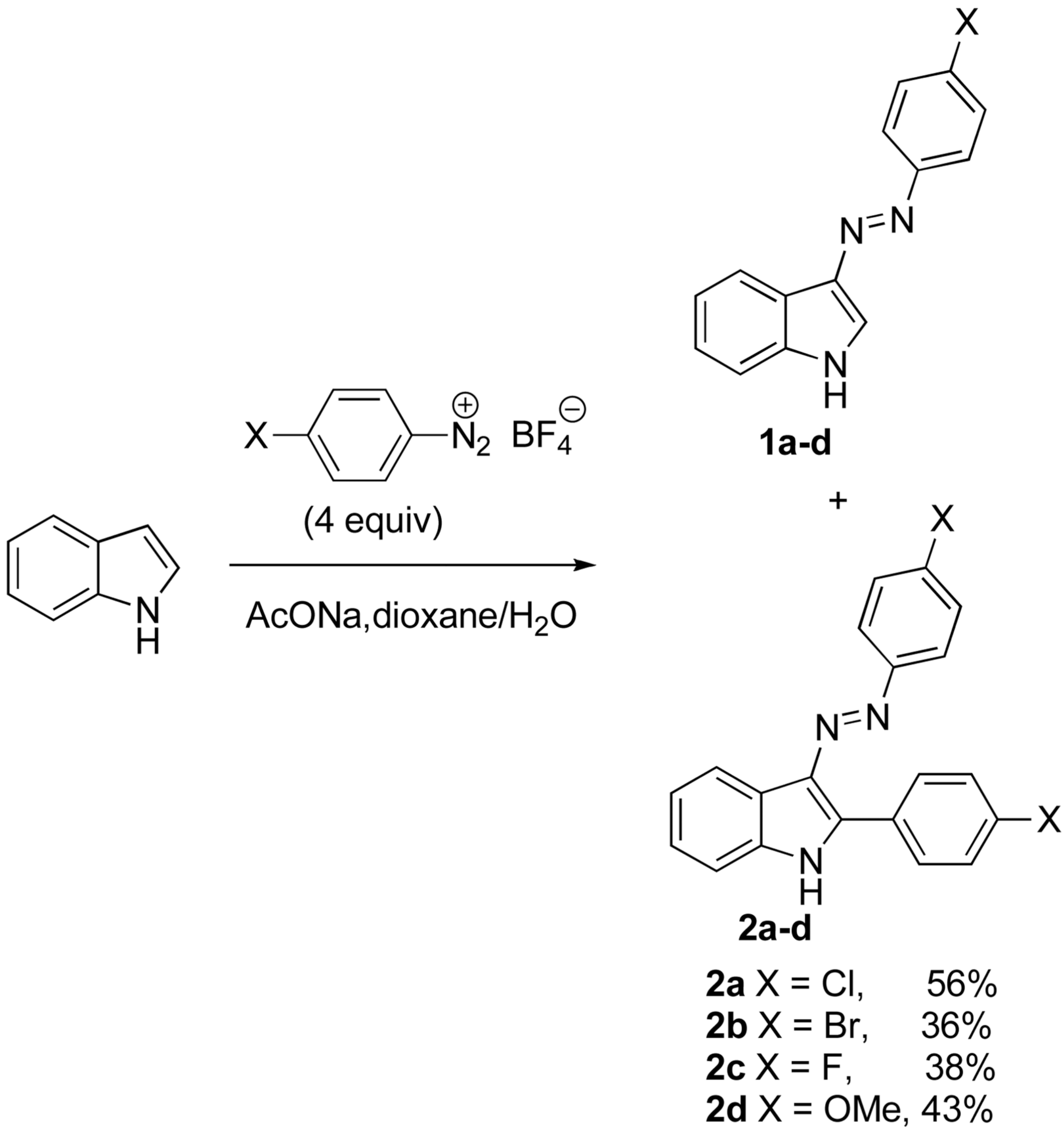
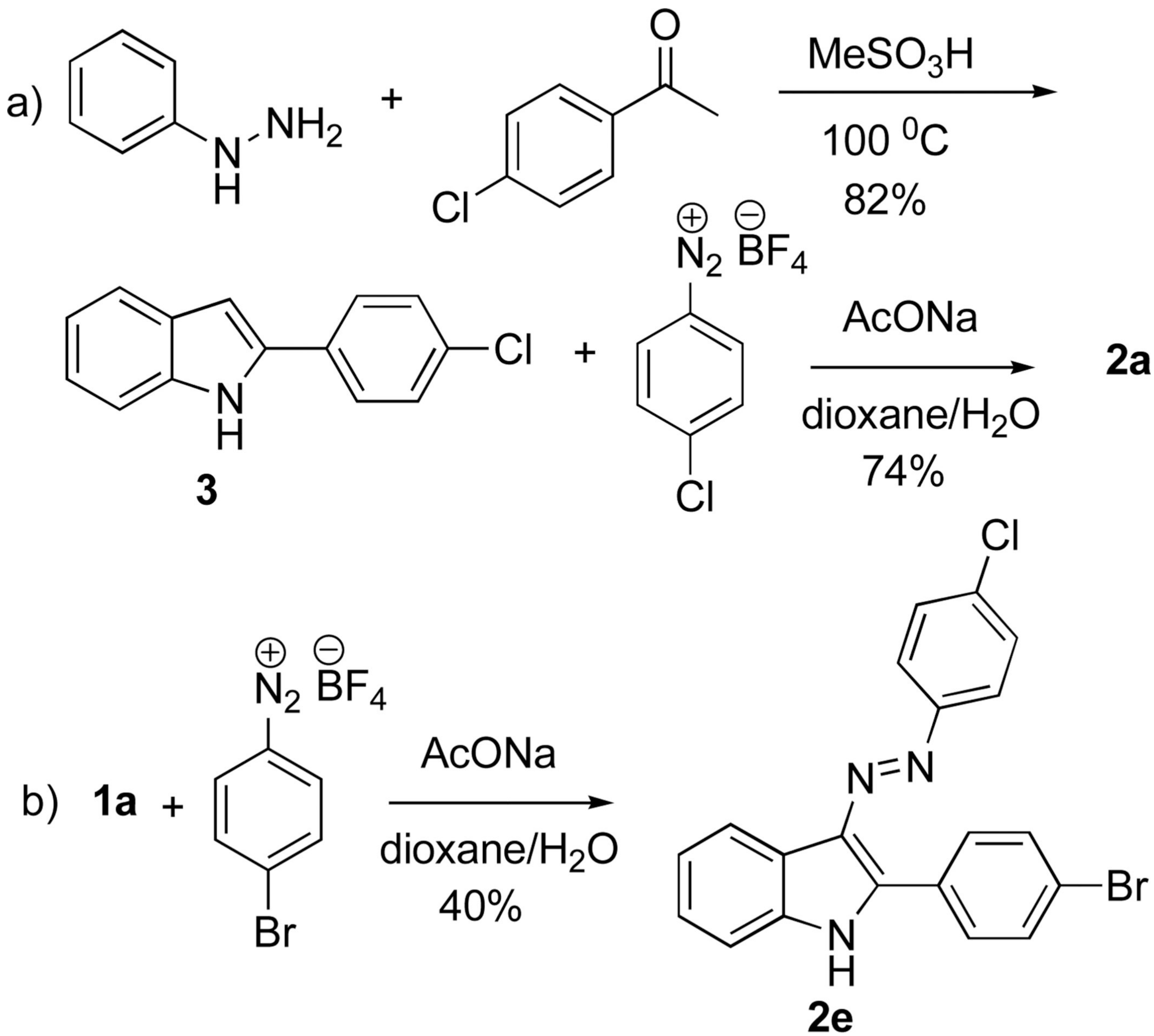
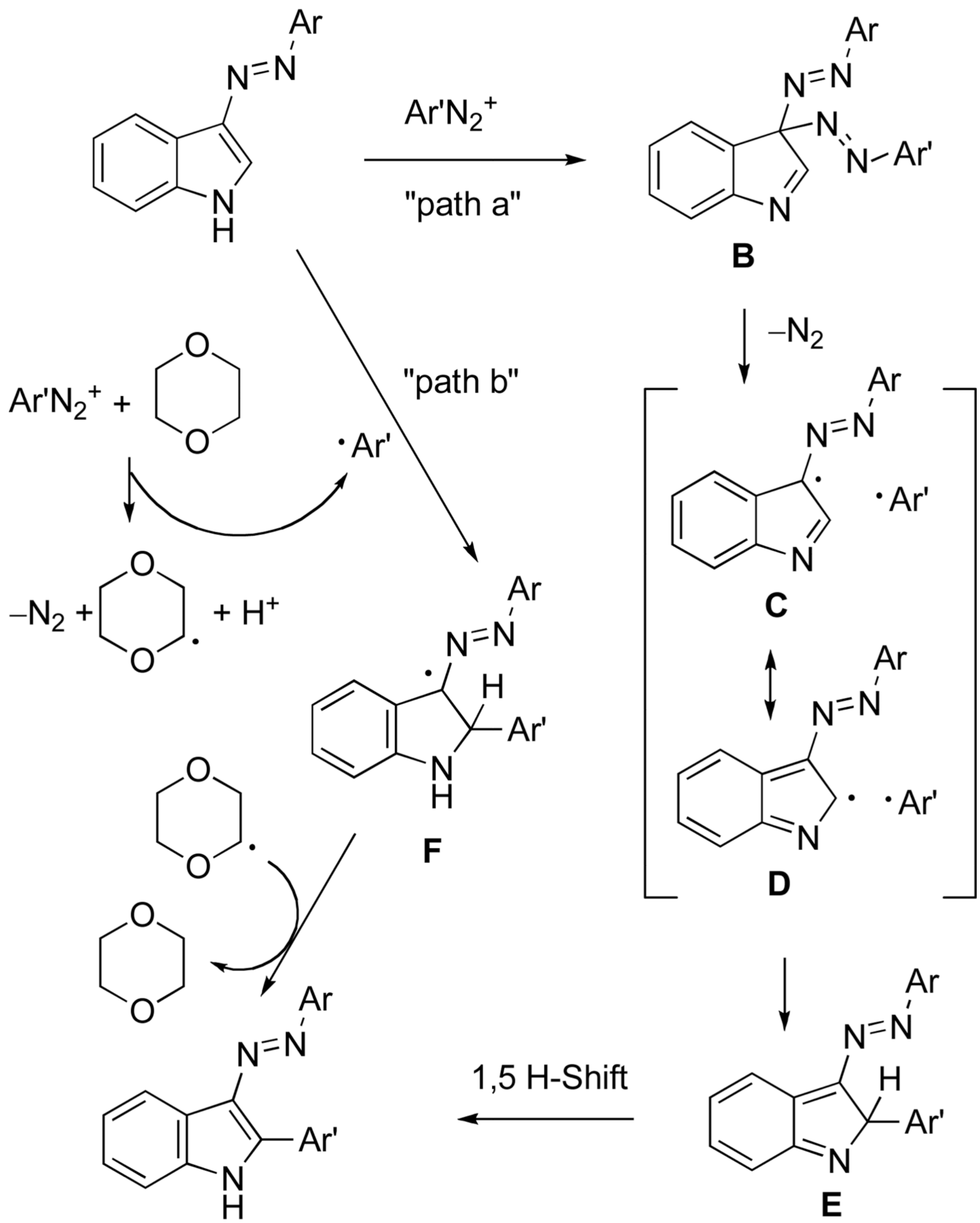


Scheme 1.


a)
MeSO3H
100 0C
82%
3
AcONa
dioxane/H2O
74%
2a
b) 1a
AcONa
dioxane/H2O
40%
2e

Scheme 2.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N
H
Ar'N2+
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Ar
N=N
N
N
Ar'
N
B
−N2
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Ar'N2+ +
O
O
·Ar'
−N2 +
O
O
+ H+
Ar
N=N
·
·Ar'
N
C
Ar
N=N
H
Ar'
N
H
F
Ar
N=N
·
·Ar'
N
D
O
O
·
O
O
Ar
N=N
Ar'
N
H
2
1,5 H-Shift
Ar
N=N
H
Ar'
N
E

Scheme 3.

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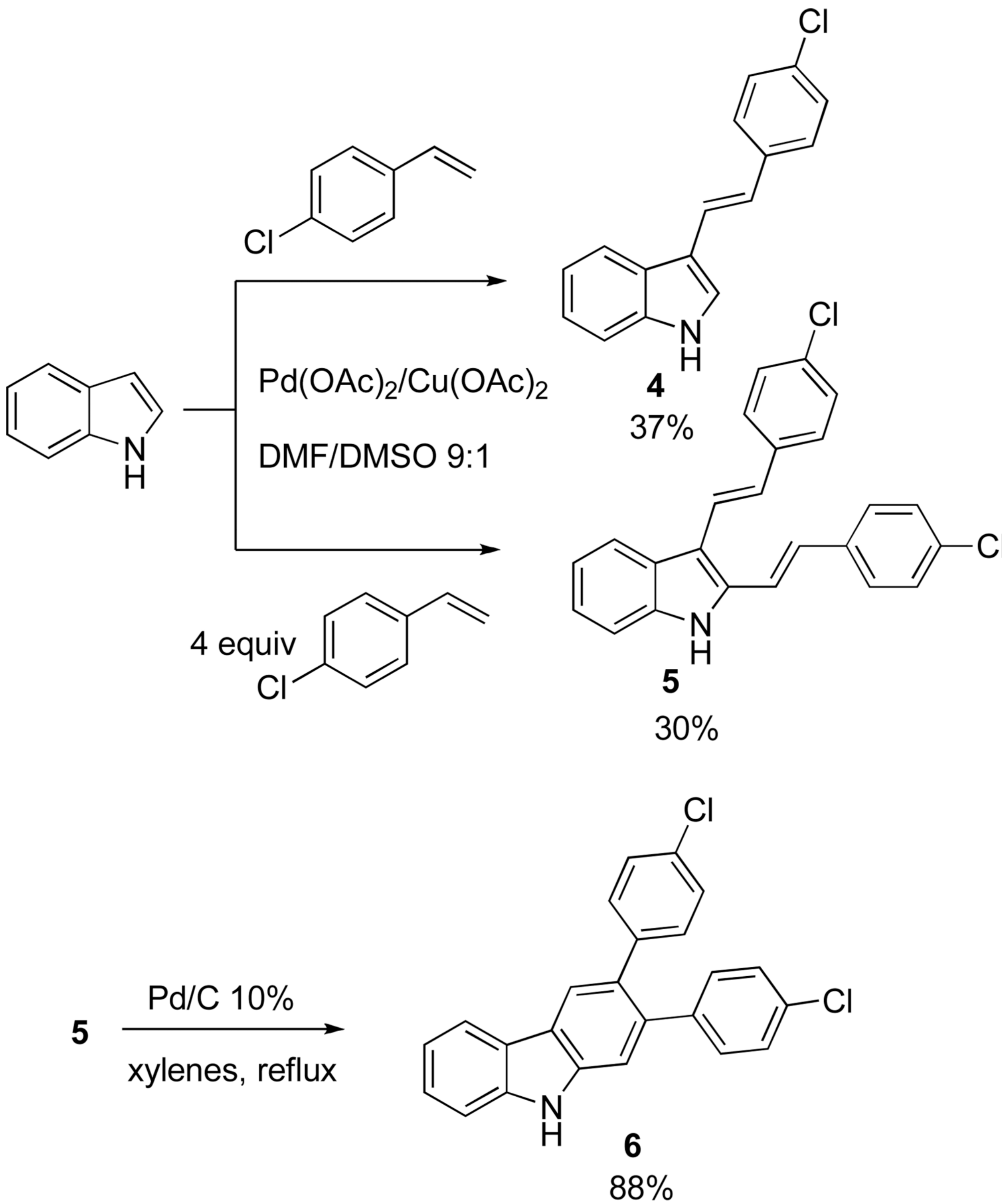

Scheme 4.

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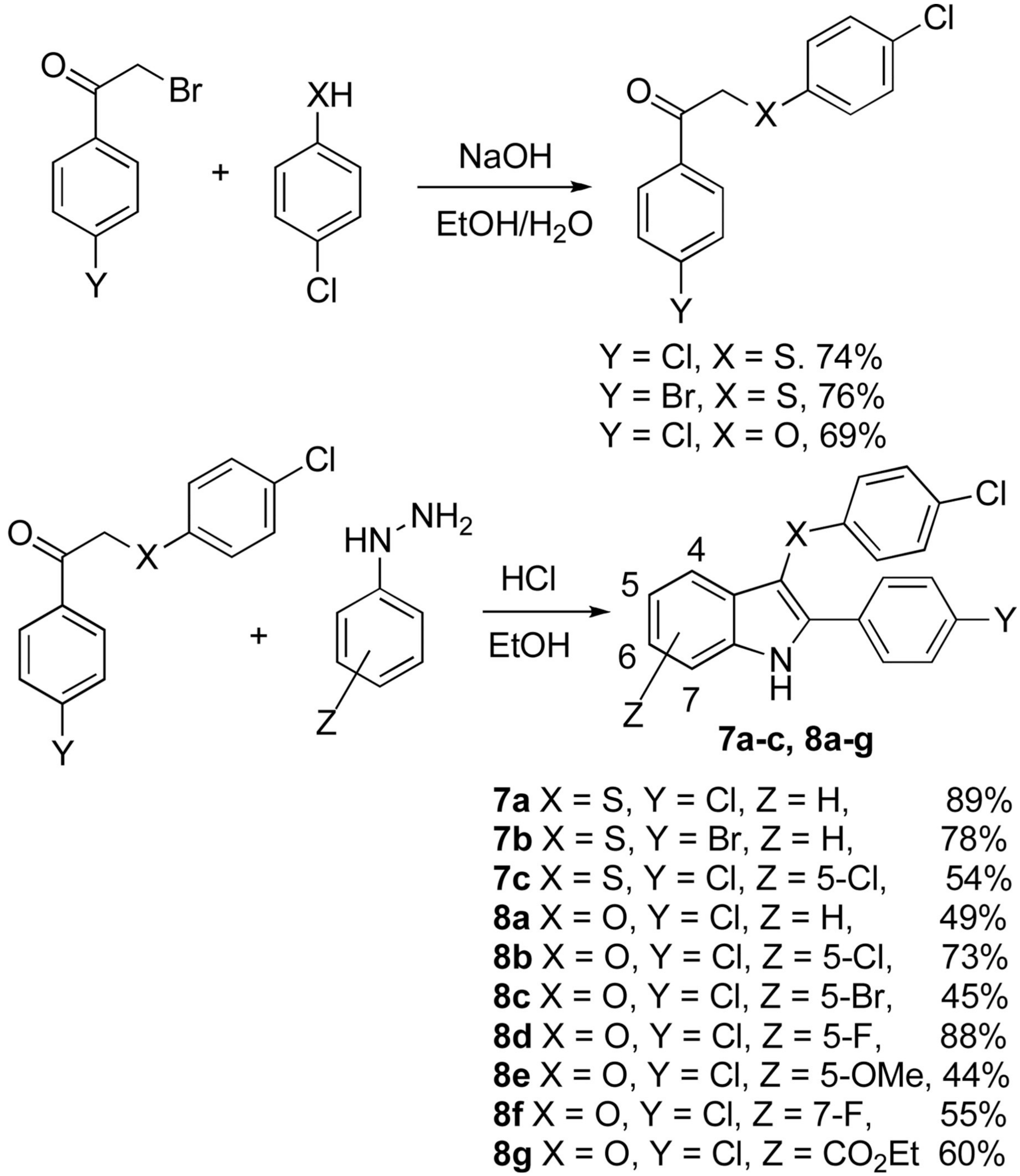
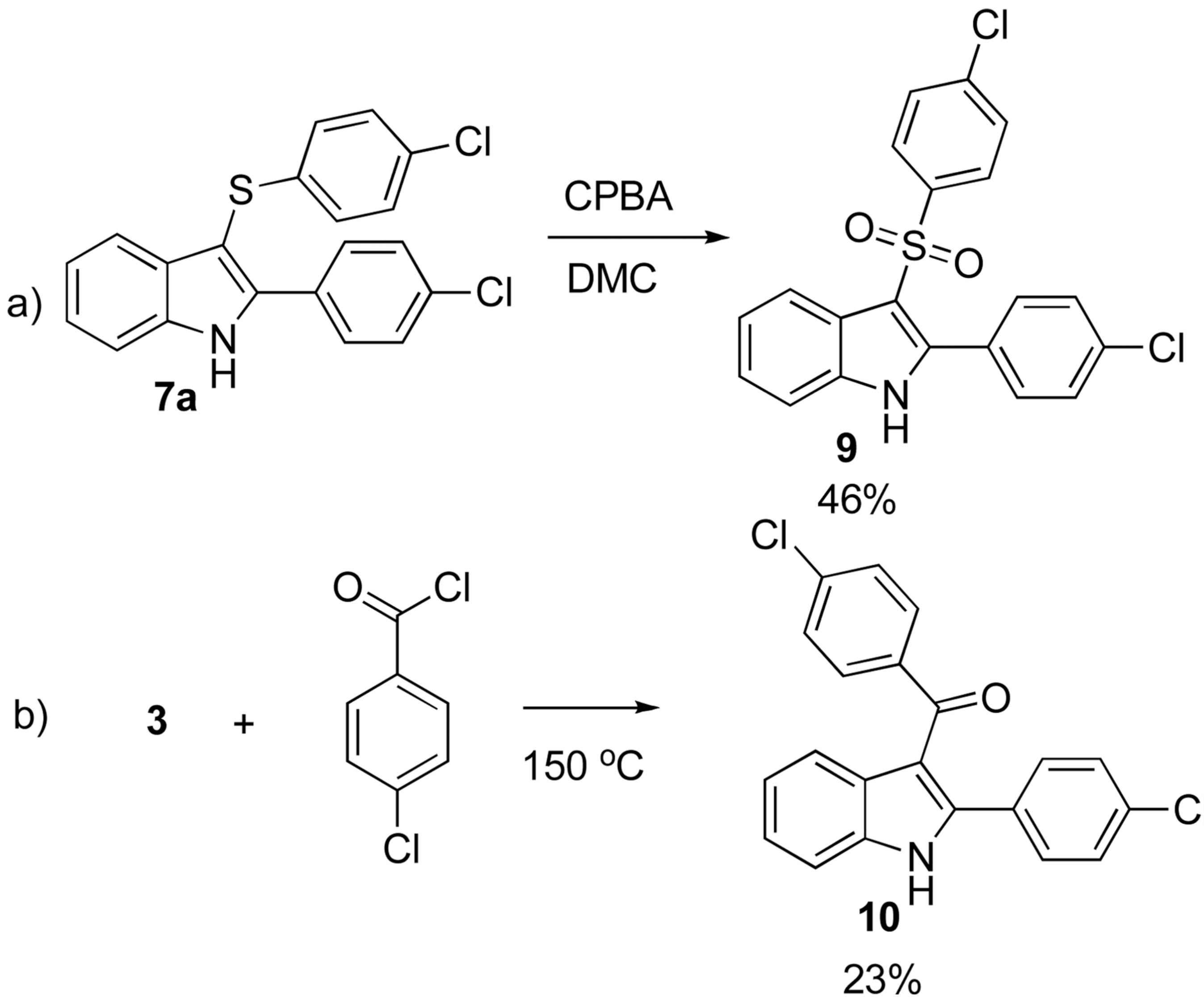


Scheme 5.


a)
S
Cl
Cl
N
H
7a
CPBA
DMC
Cl
O
S
O
Cl
N
H
9
46%
b)
3
+
O
Cl
Cl
150 °C
Cl
O
Cl
N
H
10
23%

Scheme 6.

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Table 1

Antimicrobial (MICs, μM) and Cancer Cell Growth Inhibitory (GI_{50}, μM) Activities of the Synthesized Indole Derivatives[a]

Compound	Gram-(+) bacterial strains			Gram-(−) bacterial strains		Fungus	Cancer cell line
	S. aureus[b] BAA-44	*S. aureus*[c] 29213	*E. faecalis*[d] 51299	*P. aeruginosa* 27853	*A. baumannii* 15151	*C. albicans* 26555	HeLa
1a	12.5	12.5	25	>100	>100	12.5	23 ± 3[e]
1b	6.3	6.3	12.5	>100	>100	25	49 ± 5
2a	1.6	0.6	8	>100	>100	>100	26 ± 0
2b	12.5	6.3	6.3	>100	>100	>100	9 ± 1
2c	1.6	1.3	3.1	>100	>100	>100	12 ± 1
2d	50	3.1	> 100	>100	>100	>100	23 ± 1
2e	6.3	6.3	6.3	>100	>100	>100	13 ± 0
3	>100	>100	>100	>100	>100	>100	>100
4	>100	25	>100	>100	>100	>100	64 ± 5
5	>100	>100	> 100	> 100	> 100	> 100	69 ± 5
6	>100	>100	>100	> 100	> 100	> 100	16 ± 1
7a	3.1	1.6	3.1	>100	>100	>100	6 ± 1
7b	3.1	1.6	3.1	>100	>100	>100	12 ± 2
7c	1.3	1.6	6.3	>100	>100	>100	8 ± 1
8a	3.1	1.6	12.5	>100	>100	>100	9 ± 0
8b	0.6	1.6	12.5	>100	>100	>100	8 ± 2
8c	3.1	3.1	12.5	>100	>100	>100	3 ± 0
8d	1.6	1.6	6.3	>100	>100	>100	13 ± 3
8e	6.3	12.5	25	>100	>100	>100	24 ± 2
8f	1.3	1.6	3.1	>100	>100	>100	13 ± 4
8g	6.3	12.5	50	>100	>100	>100	19 ± 1
9	>100	>100	>100	>100	>100	>100	14 ± 1
10	>100	>100	>100	>100	>100	>100	19 ± 1
Vancomycin	2.8	2.8	86.3	ND[f]	ND	ND	ND
Penicillin G	>100	23.9	11.9	ND	ND	ND	ND

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[a]Bacterial suspensions were adjusted to ~5×10^5 CFU/mL.[20,21] The suspensions were treated with compounds, serially diluted 2-fold and were incubated according to ATCC recommendations for 18 hours. Fungal testing was accomplished according to a previously published method.[22] Bacterial and fungal viability was determined using the MTT method due to coloring of compounds.[23] HeLa testing was also accomplished according to a previously published method.[24]

[b]Methicillin-resistant *S. aureus*

[c]Susceptible *S. aureus* strain

[d]Low-level vancomycin-resistant *Enterococcus*

[e]The data are presented as GI_{50} ± SD from four experiments.

[f]ND = not determined