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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Iodine (III)-mediated synthesis of some 2-aryl/hetarylbenzoxazoles as antibacterial/antifungal agents

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Abstract Ten 2-aryl/hetarylbenzoxazoles (**5a**, **5b**, and **6a**–**h**) were synthesized via oxidative cyclization of Schiff bases (**3a**, **3b**, and **4a**–**h**) with 1.1 equivalent of iodobenzene diacetate (IBD) in methanol. All of these 2-aryl/hetarylbenzoxazoles (**5a**, **5b**, and **6a**–**h**) were tested in vitro for their antibacterial and antifungal activities against *Bacillus subtilis, Bacillus stearothermophilus, Escherichia coli, and Pseudomonas putida.* These compounds also were screened for their antifungal activity against *Aspergillus flavus* and *Aspergillus niger*. Biological activity of these compounds was compared with those of commercially available antibiotics, chloramphenicol and antifungal agent cycloheximide. Most of these compounds, **5a**, **5b**, **6a**, **6b**, **6d**, **6e**, **6g**, **6h**, were equipotent or more potent than these commercial drugs at concentration 100 μg/ml.

Keywords Iodobenzene diacetate · Schiff bases · 2-hetarylbenzoxazoles · Antibacterial activity · Antifungal activity

Introduction

Organohypervalent iodine (III) reagents find wide utility in organic synthesis (Varvoglis, 1981; Merkushev, 1987; Moriarty *et al.*, 1990; Moriarty and Prakash, 1998, 2001; Prakash, 1995; Stang and Zhdankin, 1996; Koser, 2002; Zhdankin and Stang, 2002; Prakash *et al.*, 1994a, b) Many of these reactions lead to heterocyclic compounds, such as benzoxazoles, thiazoles, and pyrazoles, which are associated

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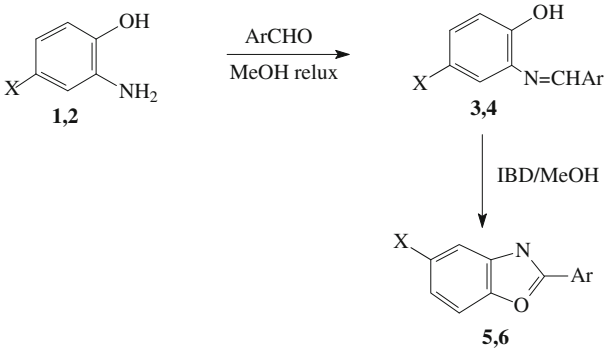
with significant biological properties (Grimmet *et al.*, 1979; Deluca and Kerwin, 1997). Organoiodine (III)-mediated oxidative approach is preferred to other existing oxidative methods because of the low toxicity, simple experimentation, and easy handling of these reagents compared with other oxidants, such as DDQ (Chang *et al.*, 2002), PCC (Praveen *et al.*, 2008). In view of the various biological properties, such as cholesteryl ester transfer protein inhibitors (Harikrishnan *et al.*, 2008), transthyretin amyloloidogenesis inhibitors (Johnson *et al.*, 2008) are associated with 2-substituted benzoxazoles and encouraged by promising results on the iodine (III)-mediated oxidative cyclization of Schiff's bases (Prakash *et al.*, 1997, 2003, 2006). We synthesized some 2-aryl/hetarylbenzoxazoles (**5a–5b** and **6a–h**) from Schiff bases (**3a–3b** and **4a–h**) to evaluate their antifungal and antibacterial activities.

Results and discussion

Chemistry

2-Aryl/hetaryl benzoxazoles were prepared from oxidation of Schiff's bases with IBD according to our recently disclosed procedure (Prakash *et al.*, 1997, 2003, 2006). It has been found that the reaction between Schiff's bases and 1.1 equivalent of IBD in methanol occurs smoothly to afford the corresponding 2-substituted benzoxazoles (**5a**, **5b**, and **6a–h**) (Scheme 1 and Table 1). The structures of Schiff bases (**3a**, **3b** and **4a–h**) and 2-aryl/hetarylbenzoxazoles (**5a–5b** and **6a–h**) were elaborated by their spectral data and elemental analysis.



1,3,5	X	Ar
a	H	2-thienyl
b	H	5-methyl-2-thienyl

2,4,6	X	Ar
a	Cl	2-thienyl
b	Cl	3-methyl-2-thienyl
c	Cl	5-methyl-2-thienyl
d	Cl	3-pyridyl
e	Cl	C_6H_5
f	Cl	4-MeC_6H_4
g	Cl	4-$NO_2C_6H_4$
h	Cl	3-OMe-4-OHC_6H_3

Scheme 1

Table 1 In vitro antifungal activity

Compound	Concentration (μg/ml)	% Age inhibition	
		A flavus	*A niger*
5a	10 μg	21.05	41.71
	50 μg	63.15	64.70
	100 μg	88.47	82.35
5b	10 μg	24.31	04.89
	50 μg	51.61	05.23
	100 μg	87.99	16.64
6a	10 μg	21.05	04.89
	50 μg	47.36	5.88
	100 μg	84.24	11.76
6b	10 μg	21.05	17.47
	50 μg	36.84	47.05
	100 μg	84.21	76.49
6c	10 μg	13.74	12.44
	50 μg	24.92	63.87
	100 μg	37.47	71.88
6d	10 μg	15.78	17.64
	50 μg	26.31	41.71
	100 μg	78.94	76.47
6e	10 μg	29.04	08.93
	50 μg	51.42	05.88
	100 μg	86.23	21.86
6f	10 μg	20.11	04.19
	50 μg	43.23	07.47
	100 μg	66.91	21.38
6g	10 μg	19.94	43.19
	50 μg	49.61	66.93
	100 μg	78.54	73.62
6h	10 μg	29.04	08.93
	50 μg	51.42	05.88
	100 μg	86.23	21.86
Cycloheximide	10 μg	15.78	11.76
	50 μg	63.15	23.52
	100 μg	78.94	35.29

Experimental

Chemical synthesis

Melting points were determined in open capillaries in electrical melting point apparatus and are uncorrected. The IR (KBr) and ^{1}HNMR spectra were recorded on Buck Scientific IR M-500 and Bruker (300 MHz) spectrophotometers, respectively.

The compounds **5b**, **6a–d** gave satisfactory analytical results (within ± 0.4 of the theoretical values).

*Schiff Bases **3a**, **3b**, and **4a–h***

General procedure Schiff bases were prepared by refluxing 10 mmol of *o*-amino/*p*-chloro-*o*-aminophenol and 15 mmol of appropriate aldehyde in methanol. The Schiff bases precipitated on cooling the reaction mixture and were recrystallized from methanol.

*Characterization data of Schiff bases **3a**, **3b**, and **4a–d** is given* **3b**: m.p. 60–61°C; yield 50%; IR (KBr) cm^{-1} 1589, 2930, 3343 (O–H) str. ^1H NMR ($CDCl_3$); δ 2.50 (s, 3H, $–CH_3$), 6.79–6.81 (m, 1H), 6.84–6.90 (m, 1H), 6.969–7.00 (dd, 1H, $J_1 = 1.5$ Hz, $J_2 = 8.1$ Hz), 7.12–7.15 (m, 1H), 7.15–7.18 (m, 1H), 7.30–7.31 (m, 1H), 8.72 (s, 1H, = C–H).

4a: m.p. 90–91°C; yield 84%; IR (KBr) cm^{-1} 1589, 2924, 3360 (O–H) str. ^1H NMR ($CDCl_3$); δ 6.93–6.96 (m, 1H), 7.13–7.27 (m, 4H), 7.55–7.58 (m, 2H), 8.82 (s, 1H, = C–H).

4b: m.p. 130–131°C; yield 89%; IR (KRr) cm^{-1} 1589, 2930, 3327 (O–H) str. ^1H NMR ($CDCl_3$); δ 2.51 (s, 3H, $–CH_3$), 6.92–6.97 (m, 1H), 7.12–7.16 (dd, 1H), 7.26–7.28 (m, 2H), 7.45–7.49 (m, 1H), 8.71 (s, 1H, = C–H).

4c: m.p. 96–98°C; yield 65%; IR (KBr) cm^{-1} 1590, 2925, 3357 (O–H) str. ^1H NMR ($CDCl_3$); δ 2.53 (s, 3H, $–CH_3$), 6.83–6.94 (m, 2H), 7.10–7.13 (dd, 1H), 7.24–7.36 (m, 2H), 8.63 (s, 1H, = C–H).

4d: m.p. 135–137°C; yield 87%; IR (KBr) cm^{-1} 1571, 2900, 3333 (O–H) str. ^1H NMR ($CDCl_3$); δ 6.97–7.00 (d, 1H, J = 8.7 Hz), 7.19–7.22 (dd, 1H, J1 = 2.1 Hz, J2 = 8.7 Hz), 7.45–7.49 (m, 1H), 8.28–8.29 (m, 1H), 8.72 (s, 1H, = C–H), 8.75–8.76 (m, 1H), 9.07–9.09 (m, 1H).

4e: m.p. 81–82°C; lit m.p. 81–82°C (Prakash *et al.*, 1997, 2006); yield 70%.

4f: m.p. 93–94°C; lit m.p. 92°C (Prakash *et al.*, 1997, 2006); yield 76%.

4g: m.p. 184–185°C; lit m.p. 186°C (Prakash *et al.*, 1997, 2006); yield 60%.

4h: m.p. 130–131°C; lit m.p. 133°C (Prakash *et al.*, 1997, 2006); yield 63%.

*2-aryl/hetarylbenzoxazoles (**5a**, **5b**, and **6a–h**)*

General procedure To a solution of Schiff' base (1 mmol) in 10 ml, methanol was added IBD (1.1 mmol) and the mixture was stirred for 15–20 minutes. Solvent was evaporated in *vacuo* to bring the volume to one-third. Crystalline solids, separated out on keeping the reaction mixture at room temperature. Solids, thus obtained, were filtered and washed with methanol followed by petroleum ether to get *2-aryl/hetarylbenzoxazoles* (**5a**, **5b**, and **6a–h**).

*Characterization data of 2-aryl/hetarylbenzoxazoles (**5a**, **5b**, and **6a–h**) are given:* **5b**: ^1H NMR ($CDCl_3$); δ 2.51 (s, 3H, $–CH_3$), 7.19–7.23 (m, 2H), 7.34–7.37 (m, 2H), 7.55–7.59 (m,1H), 7.74–7.77 (m, 1H), 7.93–7.95 (m, 1H); ^{13}C NMR

($CDCl_3$); δ 15.63, 110.28, 119.60, 124.58, 126.74, 130.28, 142.23, 145.78, 152.24; $C_{12}H_9NOS$; Found (C, 66.83; H, 4.14; N, 6.48%); Requires (C, 66.97; H, 4.18; N, 6.51%). m/z M^+ 215.

6a: ^1H NMR ($CDCl_3$); δ 7.21–7.24 (dd, 1H, $J_1 = 6.9$ Hz, $J_2 = 1.2$ Hz), 7.31–7.34 (dd, 1H, $J_1 = 6.9$ Hz, $J_2 = 1.2$ Hz), 7.43–7.50 (m, 1H), 7.60–7.62 (m, 1H), 7.93–7.95 (m, 1H); ^{13}C NMR ($CDCl_3$); δ 111.10, 119.74, 125.30, 128.36, 129.10, 130.49, 143.20, 149.04, 160.32; $C_{11}H_6NOSCl$ Found (C, 56.02; H, 2.44; N, 5.88%); requires (C, 56.05; H, 2.54; N, 5.94%). m/z M^+ 235, 237.

6b: ^1H NMR ($CDCl_3$); δ 2.51(s, 3H, $-CH_3$), 7.02–7.03 (d, 1H, J = 5.1 Hz), 7.28–7.38 (m, 1H), 7.46–7.49 (m, 3H), 7.73–7.74 (m, 1H); ^{13}C NMR ($CDCl_3$); δ 15.68, 110.42, 119.82, 124.72, 125.07, 129.68, 130.23, 142.02, 150.45; $C_{12}H_8NOSCl$ Found (C, 57.62; H, 3.16; N, 5.58%); Requires (C, 57.71; H, .20; N, 5.61%). m/z M^+ 249, 251.

6c: ^1H NMR ($CDCl_3$); δ 2.52(s, 3H, $-CH_3$), 6.85–6.86 (m, 1H), 7.26–7.29 (m, 2H), 7.42–7.45 (m, 1H), 7.71–7.73 (m, 1H); ^{13}C NMR ($CDCl_3$); δ 15.70, 110.96, 119.52, 124.96, 126.90, 130.88, 143.23, 146.28, 150.24; $C_{12}H_8NOSCl$ Found (C, 57.62; H, 3.16; N, 5.58%); Requires (C, 57.71; H, 3.20; N, 5.61; O, 6.41%). m/z M^+ 249, 251.

6d: ^1H NMR ($CDCl_3$); δ 7.22–7.34 (m, 2H), 7.46–7.49 (m, 2H), 7.60–7.94 (m, 2H), 8.89–8.99 (m, 1H); ^{13}C NMR ($CDCl_3$); δ 111.10, 119.74, 125.30, 128.37, 130.86, 149.04; $C_{12}H_7N_2OCl$ Found (C, 62.58; H, 2.99; N, 12.14%); Requires (C, 62.60; H, 3.04; N, 12.17%). m/z M^+ 230, 232.

Biological investigation

In vitro antifungal assay

Potato dextrose media (PDA media) was prepared in the flask and sterilized. Stock solutions of 500 μg/ml of each compound were prepared in dimethylsulfoxide (DMSO) and were appropriately diluted to get final concentration of 10, 50, and 100 μg/ml. A volume of 100 μl of each sample was poured in the well in PDA media containing sterilized Petri plates. Mycelial discs taken from the standard cultures (*Aspergillus flavus* and *Aspergillus niger*) of fungi were grown on PDA medium for 5–7 days. These cultures were used for the purpose of inoculation in the center sterilized Petri dish, aseptically. Standard cultures inoculated at 28 ± 1°C were used as the control. The efficacy of each sample was determined by measuring radial mycelial growth. The radial growth of the colony was measured in two directions at right angle to each other, and the average of two replicates was recorded in each case. Data were expressed as percent inhibition over control from the size of colonies. The percentage inhibition given in the table was calculated using the formula:

$$\% \text{ inhibition } = (C - T) \times 100/C$$

where C = diameter of fungus colony in the control plate after 96-hour incubation, T = diameter of fungus colony in the tested plate after 96-hour incubation.

In vitro antibacterial assay

Primary screening

The antibacterial activities of the newly synthesized compounds were evaluated by Agar Well Diffusion Assay technique (NCCLS, 2000; McFarland, 1907; Greenwood *et al.*, 1997) against two gram-positive bacteria, i.e., *Bacillus subtilis* (MTCC 121) and *Bacillus stearothermophilus* (MTCC 8508), and two gram-negative bacteria, i.e., *E. coli* (MTCC 51) and *Pseudomonas putida*. The bacterial cultures were maintained on the nutrient agar media by subculturing them on the fresh slants after every 4–6 weeks and incubating them at the appropriate temperature for 24 hours. All stock cultures were stored at 4°C. For the evaluation of antimicrobial activity of the synthetic compounds, suspension of each test microorganism was prepared. Turbidity of each suspension was adjusted to 0.5 McFarland units by suspending the cultures in sterile, distilled water. The size of final a volume of 20 ml of agar media was poured into each Petri plate and plates were swabbed with broth cultures of the respective microorganisms and kept for 15 minutes for adsorption to take place. Using a punch, ≈8-mm diameter well was bored in the seeded agar plates and a 100-µl volume of each test compound reconstituted in DMSO was added into the wells. DMSO was used as control for all the test compounds. After holding the plates at room temperature for 2 hours to allow diffusion of the compounds in to the agar, the palates were incubated at 37°C for 24 hours. Antibacterial activity was determined by measuring the inhibition zone diameter. The entire tests were made in triplicates and mean of the diameter of inhibition was calculated. The antimicrobial activities of the compounds were compared against the standard drugs. Inoculum was adjusted to $5x10^7$ CFU/ml.

A volume of 20 ml of agar media was poured into each Petri plate and plates were swabbed with broth cultures of the respective microorganisms and kept for 15 minutes for adsorption to take place. Using a punch, ≈8-mm diameter well was bored in the seeded agar plates and a 100-µl volume of each test compound reconstituted in DMSO was added into the wells. DMSO was used as control for all the test compounds. After holding the plates at room temperature for 2 hours to allow diffusion of the compounds in to the agar, the palates were incubated at 37°C for 24 hours. Antibacterial activity was determined by measuring the inhibition zone diameter. The entire tests were made in triplicates and mean of the diameter of inhibition was calculated. The antimicrobial activities of the compounds were compared against the standard drugs.

Conclusions

All ten chemically synthesized compounds, **5a**, **b**, **6a–h**, were tested in vitro for their antifungal activities against two fungi, *A. flavus* and *A. niger*, and antibacterial activity against two gram-positive bacteria (*B. subtilis, B. stearothermophilus*) and two gram-negative bacteria (*E. coli, P. putida*).

Most of these compounds possessed excellent in vitro antifungal activity against *A. flavus*. Of these synthesized compounds, **5a**, **6b**, **6c**, **6d**, and **6g** were active against both fungi: *A. flavus* and *A. niger*. The activities of the compounds **5a–b**, **6a–h** also were compared with commercially available antibiotic, cycloheximide, (Table 2; Fig. 1). It was found that **5a**, **5b**, **6a**, **6b**, **6e**, and **6h** were more potent than cycloheximide against *A. flavus*, whereas **5a**, **6b**, **6c**, **6d**, and **6g** were more active than cycloheximide against *A. niger*. It also was found that compounds having

Table 2 Physical data of 2-aryl/hetarylbenzoxazoles (**5a**, **5b**, **6a–h**)

Compounds	M.pt (°C)	Lit. M.pt (°C)	Yield (%)
5a	105–106	105–106 (Chang, *et al.*, 2002)	70
5b	107–108	–	65
6a	120–121	–	75
6b	134–135	–	76
6c	99–100	–	60
6d	194–195	–	63
6e	101–102	101–102 (Prakash *et al.*, 1997, 2006)	62
6f	135–136	138 (Prakash *et al.*, 1997, 2006)	60
6g	234–238	240 (Prakash *et al.*, 1997, 2006)	65
6h	185–186	186 (Prakash *et al.*, 1997, 2006)	55

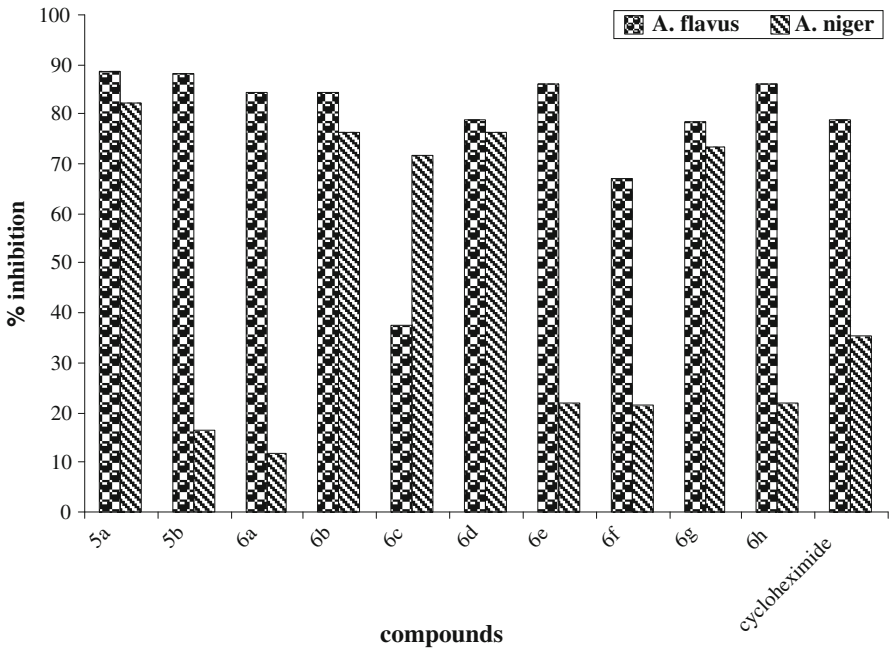


Fig. 1 In vitro antifungal assay of test compounds

Table 3 In vitro antibacterial activity of newly synthesized compounds by using well diffusion method

Compound	Concentration (μg/ml)	Diameter of zone of growth inhibition[a] (%)			
		B·S	*B.St*	*E.C*	*P.put*
5a	10	12.66	10.33	11.66	09.66
	50	21.26	20.43	26.28	24.34
	100	31.72	31.33	36.66	33.33
5b	10	–	–	12.45	13.47
	50	11.33	12.66	21.74	22.33
	100	17.33	19.66	34.29	35.45
6a	10	19.66	21.33	17.74	19.66
	50	30.53	32.66	31.77	33.66
	100	41.66	39.66	43.33	44.77
6b	10	10.34	12.66	10.33	12.12
	50	21.67	23.44	24.56	25.67
	100	29.33	31.84	33.17	35.06
6c	10	–	–	–	–
	50	10.33	12.66	17.44	18.33
	100	16.66	18.47	31.74	32.66
6d	10	13.33	11.33	12.66	09.66
	50	22.16	21.43	27.33	25.33
	100	33.33	32.33	36.74	35.33
6e	10	10.21	10.66	19.92	15.21
	50	26.47	27.21	33.87	57.66
	100	36.74	36.44	45.33	76.33
6f	10	17.22	17.92	18.62	14.22
	50	31.37	30.87	32.77	54.56
	100	42.13	43.33	43.33	73.47
6g	10	–	–	–	–
	50	10.13	12.26	17.14	18.13
	100	17.22	19.37	30.24	31.16
6h	10	09.33	10.66	09.33	12.66
	50	20.43	21.33	21.43	26.33
	100	31.33	31.74	31.33	37.33
Chloramphenicol	10	26.33	25.33	20.33	19.33
	50	47.20	53.20	50.40	43.40
	100	64.20	77.20	65.40	71.24

B.S = Bacillus subtilis; B.St = Bacillus stearothermophilus; E.C = Escherichia coli; P.put = Pseudomonas putida

[a] Mean of three replicates—no activity

thienyl or substituted thienyl (**5a**, **6a**–**6c**) or nitro group **6g** were found to possess more antifungal activity than other benzoxazoles synthesized.

These benzoxazoles possessed good antibacterial activity against both gram-positive (*B. subtilis, B. stearothermophilus*) and gram-negative bacteria (*E. coli, P. putida*). Their activities were compared with commercial antibiotics (chloramphenicol). The compounds **6e**, **6f** were found to be more potent than chloramphenicol

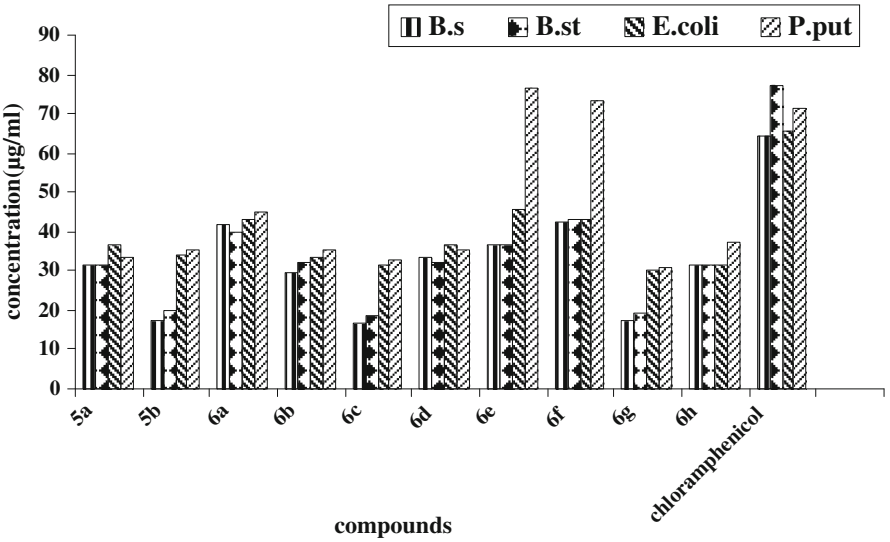


Fig. 2 In vitro antibacterial assay of test compounds

against *P. putida* (Table 3; Fig. 2). The five chlorobenzoxazoles showed activity comparable with five unsubstituted derivatives.

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