

Inorganic anion induced fluorescence quenching of a few benzimidazoles and their monocations

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Fluorescence quenching of seven benzimidazoles and their monocations by inorganic anions in 1-2% methanol/H₂O mixtures, has been studied. The quenching efficiency can be correlated linearly with the oxidation potentials of the anions. Results have indicated that quenching cannot be explained purely by the electron transfer mechanism. The non-fluorescent collision complex formed between the quencher and the excited state fluorophore may decay either to the triplet state or to the ground state depending on the values of the respective rate constants. Higher values of k_q for the fluorescence quenching of cations are due to the presence of coulombic interactions. The static quenching component can be explained with the help of a sphere of action model and the radius of the sphere of action varies from molecule to molecule.

Fluorescence quenching of neutral molecules or their monocations by inorganic anions finds a lot of applications in determining the distribution of fluorophores and their binding sites in the micelles and lipid bilayers¹⁻⁴. Thus, it is necessary to examine the fluorescence quenching of these fluorophores by the inorganic anions before these molecules can be used as probe molecules in ionic micelles or lipid biomolecules. Although the results can be explained either by heavy atom effect or by electron transfer mechanism, single mechanism is not enough to explain the fluorescence quenching by inorganic ions. Shizuka *et al*⁵⁻⁷ have pointed out that the electron or charge transfer is the key step in the anion-induced fluorescence quenching of aromatic molecules. This leads to the formation of either exciplex or a collision complex which decays to ground state by radiationless deactivation.

The present study involves the fluorescence quenching of seven benzimidazoles and their monocations (benzimidazole, BI; 2-methylbenzimidazole, MBI; 1-ethyl-2-methylbenzimidazole, EMBI; 5,6-dimethylbenzimidazole, DMBI; 2-phenylbenzimidazole, PBI and 1-ethyl-2-phenylbenzimidazole, EPBI) by four inorganic anions (Cl⁻, Br⁻, SCN⁻ and I⁻), using steady state and nanosecond time resolved fluorescence spectroscopy. We have also varied the ionic strength of

the solution to see the effect of these parameters on the second order fluorescence quenching rate constant (k_q), as well as, to explain the spectral characteristics of these molecules in ionic micelles¹⁻⁴.

Materials and Methods

All the benzimidazoles (Aldrich, U.K.) were further purified as reported earlier⁸. 2-PBI (99% gold label from Aldrich) and AnalR grade H₂SO₄, K₂SO₄, KCl, KBr, KI and KSCN were used as such. Acetonitrile and methanol (AR, E. Merck) were further purified by literature procedures. The pH of the solutions were adjusted using phosphate buffers.

Procedures used to prepare solutions, to carry out spectral measurements, to do the calculations and the apparatus used were exactly similar as described in our recent papers^{3,9}. The concentrations of the fluorophores were in the range of $1-2 \times 10^{-5}$ M in 1-2% ethanol-aqueous solution (v/v), whereas the concentrations of the quenchers were in the range of 0.01 to 0.01 M. The wavelengths used for exciting the fluorophores were 280, 290 and 290 nm for BI and PBI respectively. Since the results obtained from the solutions, with and without passing nitrogen were the same within experimental error, nitrogen was not used.

Results and Discussion

Quenching at low concentrations of inorganic anions

No changes were observed in the spectral characteristics of BI's ($pH=9$) and their monocations ($pH=3$, adjusted with the addition of H_2SO_4) in the presence of various inorganic anions having concentrations varying from 0 to 1 M , indicating clearly that no ground state complex is formed between these species. Observation of similar absorption spectra, recorded after carrying out fluorescence spectra, further indicate that no chemical reaction is occurring.

Decrease in the fluorescence intensity of each BI's without the appearance of any new band, in the presence of quencher concentration upto 0.1 M , with the exception of Cl^- and F^- ions (for Cl^- ions 4 M NaCl is used) indicates that no direct exciplex is formed between the quencher and excited BI's and their monocations. Use of sodium salts resulted in the fluorescence quenching to the same extent as that in the presence of potassium salts, thus showing that the inorganic cations play no role in the fluorescence quenching.

Decrease in fluorescence intensity in the case of Br^- , I^- and SCN^- can be interpreted by the Stern-Volmer relation, i.e.

$$\left(\frac{\tau_0}{\tau}\right) = \frac{I_0}{I} = 1 + K_{sv}[Q] = 1 + k_q \tau_0 [Q] \quad \dots (1)$$

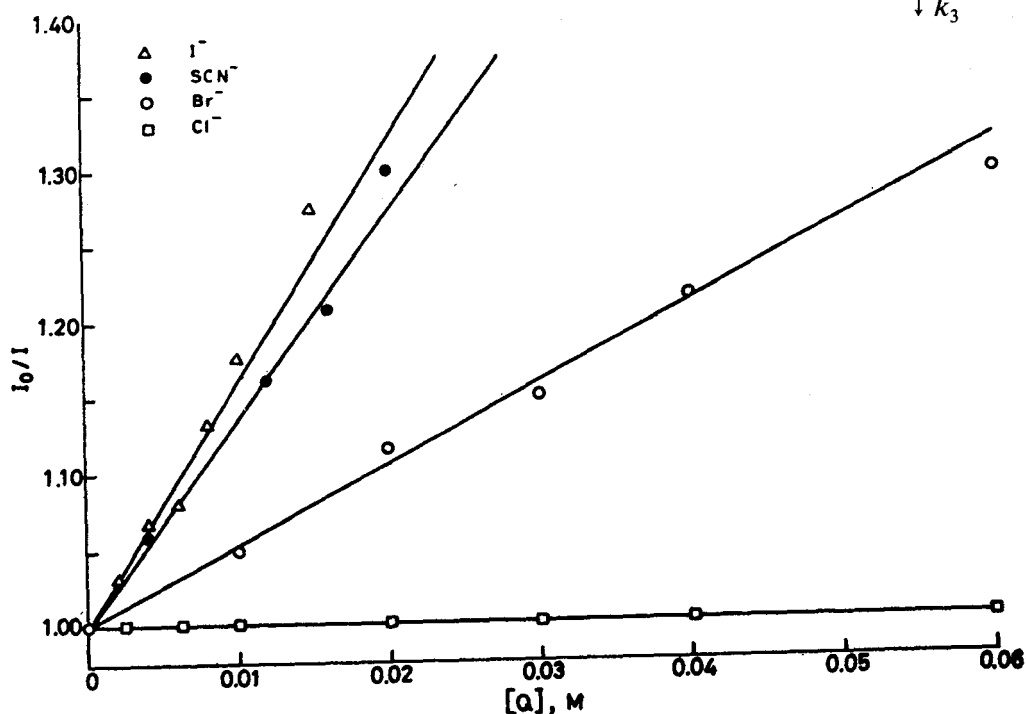


Fig. 1—Plot of I_0/I versus $[Q]$ concentration, $[PBI] = 2 \times 10^{-5} M$, containing 1% methanol, $\mu = 1.0 M (K_2SO_4)$

where I and I_0 are the fluorescence intensities, τ and τ_0 are the singlet state lifetimes of the fluorophores with and without the presence of quenchers. K_{sv} is the Stern-Volmer constant ($k_q \tau_0$), k_q is the second order quenching rate constant. Figure 1 represents the Stern-Volmer plot and the values of k_q calculated from the slopes, (using K_{sv} and τ_0) are compiled in Table 1. Singlet state energies of the fluorophores (E_A), oxidation potential [$E(X^-/X)$] and the transition energy for the charge transfer to solvent (E_{CTTS}) of the respective quencher are also compiled in Table 1. It is quite evident from the data of Table 1 that the values of k_q decrease with increase in the oxidation potential and E_{CTTS} of the quencher species and increase with increase in E_A of the fluorophore, with the exception of PBI and EPBI. In the case of PBI and EPBI although the singlet state energy is much less than that of BI, the values of k_q are only half of that of BI for SCN^- and I^- ions but twice as large as that for Br^-/BI combination. Further k_q values for $I^- - BI$ and $I^- - MBI$ combinations approach diffusion controlled limit ($1.0 \times 10^{10} M^{-1} s^{-1}$), indicating that every collision is of unit efficiency in quenching the fluorescence, whereas for other BI's - X^- combination it varies between 0.01 to 1.

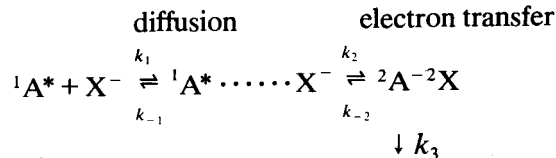


Table 1—Quenching rate constants (k_q), lifetime (τ_0), singlet state energy (E_{A^*}), oxidation potential ($E(X^-/X)$), ΔG_{RW} (Rehm-Weller) parameters for anions at 1M ionic strength (K_2SO_4)

Compound	τ_0 (ns)	E_{A^*} (eV)	$k_q \times 10^{-9} (M^{-1} s^{-1})$			$-\Delta G'_{RW} (eV)$		
			Br ⁻	SCN ⁻	I ⁻	Br ⁻	SCN ⁻	I ⁻
BI	5.5	4.36	1.01	8.3	10.1	2.36	2.86	2.96
MBI	4.1	4.33	0.34	7.8	11.9	2.33	2.83	2.93
EMBI	5.5	4.28	0.13	5.7	7.0	2.28	2.78	2.88
DMBI	10.8	4.21	0.14	4.6	7.0	2.21	2.71	2.81
TMBI	4.5	4.18	0.094	1.0	2.0	2.18	2.68	2.78
PBI	2.9	3.91	1.72	4.8	5.9	1.91	2.41	2.51
EPBI	3.5	3.86	1.6	3.5	5.5	1.86	2.31	2.41
$E(X^-/X)$			2.0	1.5	1.4			
E_{CTTS}			6.24	5.59	5.46			

In the above mechanism, $^1A^* \cdots X^-$ is simply an encounter complex, formed by the diffusion of excited fluorophore ($^1A^*$) and the quencher inorganic anion (X^-), $^2A^- \cdots X^-$ is the charge transfer complex formed by the transfer of an electron or charge from halide ion to the fluorophore and k_3 is the decay of the radical pair.

A similar scheme has been given for the quenching of a neutral donor-acceptor system by Rehm-Weller¹⁰. Equation (2) can be derived using the steady state approximation.

$$k_q = \frac{k_1}{1 + \frac{k_{-1}}{k_2} \left(1 + \frac{k_{-2}}{k_3}\right)} \quad \dots (2)$$

The free energy change, ΔG (in eV) in the electron transfer process can be calculated from Eqs (3) and (4) (given by Rehm and Weller¹⁰ and Trenin and Hayon¹¹ respectively)

$$\Delta G_{RN} = E(X^-/X) - E_{1/2} - E_{A^*} \quad \dots (3)$$

$$\Delta G_{TH} = E_{CTTS} - E_{1/2} - E_{A^*} - 4.7 \quad \dots (4)$$

$E_{1/2}$ is the half wave reduction potential of 1A .

We have not been able to determine $E_{1/2}$ values for these molecules because these are greater than those of the solvents. The free energy changes ($\Delta G'_{RW}$ and $\Delta G'_{TH}$) can be expressed as

$$\Delta G'_{RW} = \Delta G_{RW} + E_{1/2} = E(X^-/X) - E_{A^*} \quad \dots (5)$$

$$\Delta G'_{TH} = \Delta G_{TH} + E_{1/2} = E_{CTTS} - E_{A^*} - 4.7 \quad \dots (6)$$

It is thus expected that the activation energy to electron transfer will become larger and the rate constant smaller with $\Delta G'$ becoming more positive for any given anion. The values of $\Delta G'_{RW}$ so calculated are compiled in Table 1, along with k_q values for different quencher-fluorophore combi-

nation. Similar values can be calculated for $\Delta G'_{TH}$ parameters.

Qualitatively it is clear from the data in Table 1 that the results obtained for the quenching follow an electron transfer mechanism i.e. k_q increases if we go from Cl^- ion ($7.9 \times 10^7 M^{-1} s^{-1}$ only for BI has been determined and similar behaviour is expected by other BI's also) to I^- , k_q decreases if the number of electron-donating methyl groups increase on benzimidazole and k_q increases if an electron-withdrawing phenyl group is attached to BI moiety. Similar behaviour has been observed in number of cases which involve the inorganic anion-induced fluorescence quenching^{5,6}. Quantitatively, the results seem to be more complex and cannot be explained by a simple electron transfer mechanism, k_1 and k_{-1} are the diffusion controlled rate constants and thus will be independent of oxidation and reduction potentials. If electron transfer is the rate determining step, ΔG (and so $\Delta G'$) will be acting as thermodynamic barrier for electron transfer. Assuming k_3 to be much faster than k_{-2} , Eq. (2) will reduce to

$$k_q = \frac{k_1}{k_{-1}} k_2 \quad \dots (7)$$

and will lead to Eq. (9) after combining with Eq. (8), where A_2 is the frequency factor

$$k_2 = A_2 e^{-\Delta G/RT} \quad \dots (8)$$

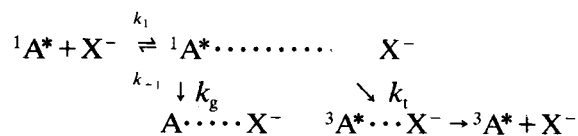
$$k_q = \frac{k_1}{k_{-1}} A_2 e^{-\Delta G/RT} \quad \dots (9)$$

In the absence of exact values of ΔG , we can compare our results taking approximate values. I^- -BI and I^- -MBI form donor-acceptor couples where k_q are close to diffusion controlled rate

constants in water ($9.8 \times 10^9 M^{-1} s^{-1}$ at 298 K) and thus will have $\Delta G \approx 0$. This will give rise to a minimum value of $E_{1/2}$ (2.41 eV) for MPBI and a maximum value of $E_{1/2}$ (3.00 eV) for BI. The lower value of $E_{1/2}$ for MPBI as compared to BI is expected but $E_{1/2}$ for TMBI should be greater than that of BI. This is because the electron-withdrawing group decreases and the electron donating-group increases the reduction potential of the parent molecule, BI. Even assuming $E_{1/2}$ for TMBI same as that of BI (3.0 eV), ΔG_{RW} for TMBI is nearly 0.20 eV less than that of BI. This will predict k_q for I^- -TMBI combination to be three order of magnitude less than that for I^- -BI combination. Similarly, for any Br^- -BI's combination, k_q will be nearly 10^{-6} times less than that for I^- -BI's combination. Thus from our results, it seems that an electron transfer mechanism is unable to explain the quenching kinetics.

The other possible mechanism to explain the fluorescence quenching rate constant can be heavy atom effect, which depends upon the spin-orbit coupling constant of the quencher. Our results, as compiled in Table 1, do not show any regular dependence on the atomic number of the heaviest atom of the quencher. For example, all the BI's are more efficiently quenched by SCN^- ion than by Cl^- and Br^- ions. Anomalous behaviour as shown by SCN^- ion as quencher has been observed by many workers^{7,12}.

In the absence of a clear cut mechanism for fluorescence quenching of these systems, it may be proposed that a quantum mechanical coupling of higher lying electron transfer states, which can induce radiationless transitions from first singlet state of BI's to other electronic states, may be predominant in these cases, also as earlier proposed and confirmed by Watkin¹³ and Shizuka *et al.*⁵⁻⁷ in some other cases. As stated by them^{5,6,13}, this mechanism involves the coming together of fluorophore and the quencher to form a loose complex, whose binding energy is much less than $k_b T$, and the loose complex formed is non-fluorescent. In the absence of any other evidence, it may be proposed that this loose complex may produce the molecules in the triplet state by intersystem crossing or may decay to ground state by internal conversion. The reaction can be represented by Scheme 1,



Scheme 1

where k_g and k_t are the rate constants for internal conversion and intersystem crossing respectively. From our data it is very difficult to predict whether ${}^1A^* \cdots \cdots X^-$ dissociate to S_0 state or to T_1 state, i.e. whether $k_g \gg k_t$ or $k_t \ll k_g$, although the existence of latter is preferred.

Fluorescence quenching of monocations

Table 2 gives the quenching rate constants, singlet state energy and lifetimes of monocations, oxidation potential and E_{CTS} energies of the inorganic anions. The monocations of alkyl substituted benzimidazoles possesses two fluorescence bands⁸, k_q values for both the bands have been determined, with the exception of BI^{+*} and $TMBI^{+*}$. In the former case, the $\pi \rightarrow \pi^*$ emission is not observed, whereas in latter case, CT emission is so weak that its excited state lifetime could not be determined. The trend observed in k_q values is the same as noticed in neutral molecules, i.e. $k_q(Cl^-) < k_q(Br^-) < k_q(SCN^-) < k_q(I^-)$, except that values of k_q observed in case of protonated species are greater than those observed for the neutral ones. Further the values of k_q observed for $\pi \rightarrow \pi^*$ emission is more than that observed for the CT band. The other important feature of the results shown in Table 2 is that k_q for PBI^{+*} and $MPBI^{+*}$ is less than that for BI^{+*} . This behaviour is different from that observed for neutral BI's.

Although $\Delta G'_{RW}$ and $\Delta G'_{TH}$ values have not been listed here, data follow exactly the same trend as noticed for neutral molecules (Table 1) and thus the results can be explained on the same lines. Data in Table 2 indicate that the value of k_q of BI^{+*} by Cl^- ion is nearly two order of magnitude greater than that for neutral molecule ($7.9 \times 10^7 M^{-1} s^{-1}$). Although we have not calculated k_q for other neutral BI- Cl^- combinations (because one need $[Cl^-] \gg 1 M$) similar trend will hold. The larger value of k_q can be explained as follows: Fluorophore (BI^{+*}) and the quencher (X^-) are oppositely charged particles and will thus involve attractive Coulombic interactions which are much stronger than those present between the neutral BI^* and the halide ions. The latter kind of interactions are of contact type. This attractive Coulomb interaction will increase the probability of electron being transferred from halide ion to BI^{+*} in the counter complex and consequently deactivate the complex to BI^+ and halide ion. The lower value of k_q for PBI^{+*} in comparison to methyl substituted BI^{+*} , is due to the fact that positive charge in case of PBI^{+*} is completely delocalised over the complete mole-

Table 2—Lifetimes (τ_0) and singlet state energy (E_{A^*}) of the monocations of BI's, k_q for different anions, $E(X^-/X)$ and E_{CTTS} of inorganic anions at 1 M ionic strength (K_2SO_4)

Compd	τ_0 (ns)		E_{A^*} (*) eV	$k_q \times 10^{-9} (M^{-1} s^{-1})$							
				Cl ⁻		Br ⁻		SCN ⁻		I ⁻	
	$\pi \rightarrow \pi^*$	CT		$\pi \rightarrow \pi^*$	CT	$\pi \rightarrow \pi^*$	CT	$\pi \rightarrow \pi^*$	CT	$\pi \rightarrow \pi^*$	CT
BI	—	10.3	3.99 ^a	—	9.9	—	11.04	—	10.7	—	12.2
MBI	9.9	12.7	4.45	4.6	5.4	7.1	4.9	13.6	11.3	15.1	11.2
EMBI	4.4	9.1	4.42	11.8	5.5	18.9	9.4	22.3	11.0	25.0	11.9
DMBI	7.8	12.0	4.31	9.0	6.5	10.6	7.1	15.5	11.5	19.2	11.1
TMBI	6.5	—	4.28	2.4	—	7.9	—	10.2	—	10.8	—
PBI	3.1	—	3.87	3.4	—	5.4	—	9.5	—	11.1	—
EPBI	4.7	—	3.91	4.4	—	6.4	—	9.2	—	11.0	—
$E(X^-/X)$ (eV)				2.55	2.0					1.5	1.4
E_{CTTS} (eV)				7.07	6.24					5.59	5.46

a = CT state

cule^s rather than on tertiary nitrogen atom of methyl substituted BI⁺*. Because of this the charge density at any particular atom of PBI⁺* will be less than that in case of methyl substituted BI⁺*s. This will decrease the Coulombic interaction and thus the value of k_q .

Fluorescence quenching at higher concentration of quencher

At high concentration of I⁻ (0.1 M) the simple Stern-Volmer plot departs from linearity, with upward curvature. The limit of linearity is higher in the case of neutral BI's as compared to their monocations. In the absence of any chemical reactions of BI*^s, it is clear that fluorescence quenching at high [I⁻] involves static quenching. Out of the two static quenching mechanisms, the complex formation in the ground state can be rejected as no change is observed in the absorption spectra. The second mechanism, sphere of action model¹⁴, seems to be consistent with our results i.e. a linear Stern-Volmer plot observed at low concentration indicates the presence of dynamic quenching whereas departure from linearity at high concentration suggests that sphere of action model is predominant. Under first conditions, the excited state lifetime should decrease, whereas in the latter stages lifetime should not change. To test this, we have measured the lifetimes of PBI in the presence of 0.02 and 0.04 M I⁻ concentration and these are 2.19 and 1.81 ns respectively.

The lifetimes at further higher [I⁻] could not be determined because the lifetimes at higher concentration of quenchers are too small to be measured with our instrument. But the decrease in lifetime does tell that up to this concentration of I⁻ the fluorescence quenching is dynamic. Under these conditions, the modified Stern-Volmer equation is given by

$$\frac{I_0}{I} = (1 + k_q \tau_0 [Q]) \exp \left(\frac{V N_{av} [Q]}{1000} \right) \quad \dots (10)$$

where N_{av} is the Avogadro's number and V is volume in cm³; equal to $4/3 \pi R^3$, where R is the radius of the sphere of action. The radii of the sphere of action determined for I⁻-BI's combination are compiled in Table 3. Although the values of radii of the sphere of action so obtained are not as accurate as those of k_q (because of approximations used in these calculations), it is sufficient for qualitative discussion. Except for DMBI and TMBI, the radii of the sphere of action lies between 8 to 9 Å. This is larger than the collision diameter of 5 Å, between BI and I⁻ anion. The values of the radii so obtained are consistent with the efficiency of quenching by I⁻ ions toward BI's. In the case of Br⁻ ion, the active volume element is much smaller and leads to radii which are close to collision diameters. This is attributable to the weakness in the quenching ability of Br⁻ in comparison to I⁻, because the contact of

Table 3— K_{SV} (M^{-1}), k_q ($10^9 M^{-1} s^{-1}$) by I^- ions and radii of sphere of action for BI's and BI^+ at $\mu = 1.0 M$ (K_2SO_4)

Compd	Neutral			Monocation		
	K_{SV}	k_q	R Å	K_{SV}	k_q	R Å
BI	54	10.1	8.4	103	10.0	11.2
MBI	48.3	11.9	7.3	95	9.6	10.6
EMBI	37.5	6.9	8.3	100	22.7	9.3
DMBI	36.0	6.9	5.6	114	14.6	9.3
TMBI	22.0	2.03	5.8	60	9.2	8.4
PBI	17.3	5.9	7.6	32	10.5	10.3
MPBI	19.3	5.5	8.4	41	8.8	11.2

the quencher with the fluorophore does not always lead to subsequent fluorescence quenching.

The radii of sphere of action calculated for I^- and BI^{+*} 's combination (compiled in Table 3) are larger than the values for the neutral molecules. This seems consistent, because the interactions between the neutral BI's and I^- ion are of the contact type, whereas those between the monocations and I^- are of the Coulombic type. The former are effective over smaller distances and the latter ones extend over longer distances.

Effect of ionic strength on quenching constants

The values of k_q for both neutral and monocations of BI and PBI by iodide ions determined at 0.25, 0.5, 0.75 and 1.0 M ionic strengths, maintained by the addition of Na_2SO_4 are compiled in Table 4. As expected, the values of k_q remain nearly unchanged for the neutral species but decrease for monocations. Simple and extended Debye-Huckel theories are not valid at such high ionic strength. But qualitatively, the results observed are consistent with the fact that the rate constant of any process involving interaction of oppositely charged species decreases with increase in ionic strength.

In conclusion it may be mentioned that inorganic ion-induced fluorescence quenching is a complex phenomenon. The first step involves the formation of charge transfer exciplex and this

Table 4— K_{SV} (M^{-1}) and k_q ($10^9 M^{-1} s^{-1}$) by I^- ions for BI^+ and PBI^+ at different ionic strengths (K_2SO_4)

Ionic strength	BI^+		PBI^+	
	K_{SV}	k_q	K_{SV}	k_q
0.25	156	15.2	48	15.7
0.50	165	16.0	44	14.4
0.75	130	12.6	37.5	12.3
1.00	103	10.0	32.0	10.5

complex may come to ground state either via internal conversion or through the formation of triplet state involving intersystem crossing mechanism. Increase in quenching rate constant for cations are due to the presence of Coulombic attractive interactions. The sphere of action model plays major role at higher quencher concentration.

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