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Polyamine receptors containing anthracene as fluorescent probes for ketoprofen in H2O/EtOH solution / Romano, G.M., Mummolo, L., Savastano, M., Paoli, P., Rossi, P., Prodi, L., Bencini, A.. - In: CHEMICAL COMMUNICATIONS. - ISSN 1359-7345. - STAMPA. - 58:(2022), pp. 7022-7025. [10.1039/D2CC01107G]

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DOI: 10.1039/D2CC01107G

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09 October 2026

COMMUNICATION

Polyamine receptors containing anthracene and their Zn(II) complexes as fluorescent probes for ketoprofen in aqueous media

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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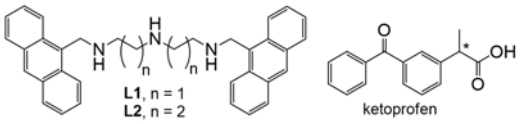
Triamine receptors containing anthracene units are able to bind and sense ketoprofen *via* fluorescence enhancement in aqueous media exploiting their protonation and Zn(II) binding features, which result to be tuned by the interaction with the analyte.

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most worldwide used pharmaceuticals. Their chemical stability and widespread, often uncontrolled, human consumption and ubiquitous veterinary use, accompanied by lack of efficient methodologies for removal, storage, and disposal of waste, is leading to their increasing presence in aquatic environment.[1] So far, they have been classified as emerging pollutants, due to their recently proved ecotoxicity and suspected long-term adverse effects on human health and biota in general.[2,3] On the other hand, their release in the environment is still often poorly regulated and monitored.[2] In this panorama, there is an effective challenge for economically viable, highly sensitive and rapid response sensors to be used for protection of human/animal health and ecosystems. Fluorescent sensors have shown to match these requirements.[4]

In this context, several nanostructured assemblies have been used for optical signal of NSAIDs, including polyethyleneimine-passivated Cd/S quantum dots (QDs)[5] or CdSe/ZnS QDs capped with N-acetyl-L-cysteine methyl ester,[6] carbon dots-containing imprinted polymers,[7] chitosan-stabilized Ag nanoparticles (NPs),[8] hybrid organic-Ag NPs assemblies,[9,10] arrays of monolayered Au NPs,[11] poly(*p*-aryleneethynylene polymers,[12] or hydrogel-embedded chemosensors.[13] Less attention has been paid to fluorescent small molecules able to detect NSAIDs. On the other hand, NSAIDs feature some common structural features. In fact, they are normally composed by a carboxylic group linked to an aromatic portion. Binding and sensing of carboxylic acid are normally achieved by using receptors containing hydrogen bonding site[14] and may result a hard task, especially in aqueous media, where solvation efficiently competes with the binding process.[14] Examples are limited to cinchona alkaloids[15] or calixpyrroles-based[16] chemosensors and a BINOL-containing macrocycle,[17] able to optically signal NSAIDs in non-aqueous solvents.

Receptors **L1** and **L2**, obtained by using a well-known procedure for terminal alkylation of linear polyamines,[18] are composed by a diethylen- or a dipropylen-triamine unit, two 'classic' ligands used in coordination chemistry, linked at their extremities *via* methylene bridges, to the 9-position of an anthracene unit, probably the most exploited fluorophore (Scheme 1). To test these simple receptors as NSAIDs probes in aqueous media, we chose ketoprofen (KP), one of the most common NSAIDs, which does not feature fluorescence emission.



Scheme 1

L1 and **L2** can protonate in aqueous media even at neutral pH to give polyammonium cations able to interact with the carboxylic group of NSAIDs, including KP, normally in its anionic form at pH 7, *via* charge-charge and H-bonding interactions. The anthracene moieties can give hydrophobic and/or π-stacking interactions with the aromatic section of KP. Furthermore, both triamines are known for their ability to bind metals, including Zn(II),[19] which can also be used as binding site for carboxylate groups.

Indeed, potentiometric titrations, performed in water/EtOH 50:50 (Vol:Vol) mixture (the ligands are not sufficiently soluble in pure water in the conditions of potentiometric measurements) pointed out that the receptors can bind up to three or two protons in the case of **L1** and **L2**, respectively. At neutral pH, **L1** is mainly in its [H**L1**]$^+$ form, while in the case of **L2** the not-protonated amine is present in solution together with

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

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[H**L2**]$^+$, in relevant percentages. Finally, the presence of di- and/or three-protonated species features the solution behaviour of these receptors at acidic pH values. However, the polyammonium cations of both receptors form 1:1 adducts with KP in its anionic form. The stability constants of the adducts, depending on the charge of the receptor, range from 3.36 to 3.76 in the case of **L1** and from 2.47 to 2.98 log units for **L2** (Table S1, ESI).

To investigate the ability of the receptor to optically signal KP, we performed a titration by adding to solutions of **L1** and **L2**, buffered at pH 7, increasing amount of this NSAID, which is in its deprotonated anionic form at pH 7 (the pK_a of KP is 5.31 in our experimental conditions). In these conditions, both **L1** and **L2** show the typical structured band of anthracene derivatives both in their absorption (figure S11 and S12, respectively) and fluorescence spectra. Two excited state lifetimes can be obtained for both **L1** (1.0 and 6.5 ns; Φ = 0,083) and **L2** (1.2 and 6.3 ns; Φ = 0,061); in both cases being the shorter component the most abundant one (ca 10:1). These data are compatible with the occurrence of a photoinduced electron transfer (PET) process between the non-protonated amines (as discussed below) and the anthracene units.[21,22] A deeper investigation shows also that both ligands have a tail in the fluorescence spectrum around the 450-600 nm range, that is much more pronounced in **L1**, that can be attributed to the formation of excimers between the two anthracene moieties.

As shown in Fig. 1a for **L1**, addition of KP induces up to a 3-fold increase of the fluorescence emission of the anthracene band. Interestingly enough, emission at 414 nm increases almost linearly up to the addition of 1 equiv. of the substrate (inset of Fig. 1; a linear increase is also observed upon addition of more than 1 eq. of KP, but with a higher slope, up to 2:1 KP:**L1** molar ratio, to achieve an almost constant value for molar ratios greater than 3, suggesting the successive formation of complexes with 1:1 and 1:2 stoichiometry between **L1** and KP. At the same time, the shorter excited state lifetime undergoes

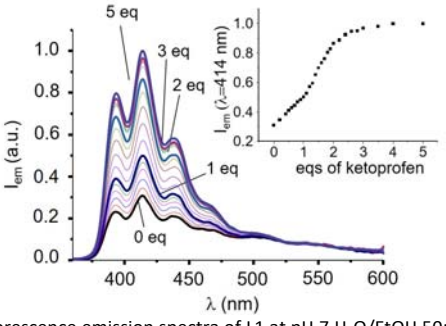

Fig. 1. Fluorescence emission spectra of L1 at pH 7 H_2O/EtOH 50:50 (Vol:Vol) and (inset) emission intensity at 414 nm in the presence of increasing amounts of KP.

a two-fold elongation up to 2.1 ns, while the other experience a more limited change to 7.1 ns. Moreover, the tail in the 450-600 nm becomes less prominent (Figure S12). A similar behaviour is also observed in the case of **L2** (Fig. S12), although in this case the emission enhancement is less marked. This behaviour appears in apparent contrast with the potentiometric study of these systems, which did not show the formation of 1:2 complexes. On the other hand, the formation constants of the 1:1 adducts are rather low (see above) and the constants for the addition of a second guest species to the host are normally lower. In this context, potentiometric titrations present poorer sensitivity than fluorescence measurements and complexed species with formation constants lower than 2 log. units are usually not detected. Noteworthy, emission enhancement is also observed in pure water at pH 7, although, in this case, the response of the probe is less intense than in ethanol/water mixture (Figure S17).

However, the observed emission enhancement in the presence of KP may depend on the host-guest binding mode, which, in principle, can be influenced by interactions between the aromatic units of both receptors and substrate and/or by charge-charge and hydrogen bonding contacts between the protonated polyamine chain and the carboxylate group of KP. To elucidate this point, we performed ^{1}H NMR titrations in the presence of KP. Interestingly, addition of increasing amounts of KP to a solution of **L1** buffered at pH 7 gives rise to a progressive downfield shift of the resonance of CH_2 (2_{AL}) and to a simultaneous upfield shift of the CH_2 (3_{AL}) signal (Fig. 2a). These results strongly suggest that host-guest binding takes place *via* charge-charge and hydrogen bonding interactions that would occur between the carboxylate group of KP and the triamine group, monoprotonated at this pH value, of the receptor. In the [H**L1**]$^+$ species, the proton is localized on the central amine group of the polyamine chain, more basic than the 'benzylic' amines, adjacent to the aromatic units,[20,21] as also demonstrated by the analysis of the pH dependence of the ^{1}H

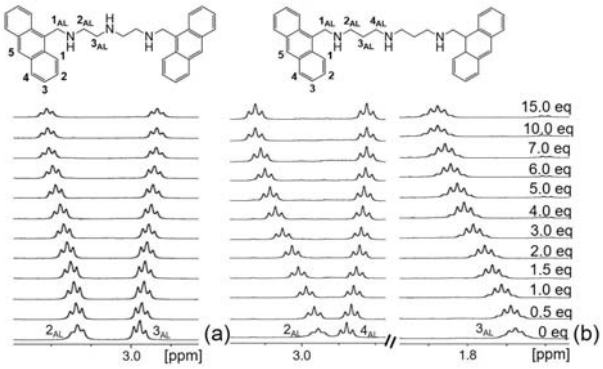

Fig. 2. Aliphatic signals of the ^{1}H NMR spectra of L1 (a) and L2 (b) at pH 7 in the presence of increasing amounts of KP. The signal of 1_{AL} is superimposed to the HOD resonance and cannot be confidently monitored.

NMR signals of **L1** and **L2** (Fig. S22).‡ Considering that, in aliphatic amines, protonation is normally accompanied by a downfield shift of the signal of the adjacent methylene unit,[20] the shifts in opposite directions of the resonances 2_{AL} and 3_{AL} in **L1** can be reasonably ascribed to a proton transfer process of the acidic proton from the central nitrogen to the benzylic amine group, induced by KP binding. A similar behaviour is also observed for the signal of the CH_2 groups of **L2** adjacent to central (4_{AL}) and the benzylic amine groups (2_{AL}), respectively (Fig. 2b). This proton transfer from the farther central nitrogen to the closer benzylic amine groups of **L1** and **L2** upon KP binding

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is expected to make the PET process less efficient, thus justifying the observed enhancement of anthracene emission. An additional element that can lead to the fluorescence increase is the observed decrease – caused by the complexation process – of the efficiency of the deactivation channel leading to the formation of the excimer.

The anthracene signals are not affected by the presence of KP (Fig. S21), indicating that the aromatic units are likely not directly involved in substrate binding *via* π-stacking. On the other hand, no interaction is detected by potentiometry with carboxylate anions with smaller hydrophobic units, including acetate, benzoate and ibuprofen. The latter also do not induce significant changes in the **L1** and **L2** emission (Fig. S18). This would suggest that the overall hydrophobic character of **L1** and **L2**, due to the presence two large anthracene units, contribute to stabilize the adducts with KP, containing a diphenylmethane moiety, in aqueous media, with respect to less hydrophobic carboxylates.

Binding *via* charge-charge contacts and H-bond interactions is also supported by the crystal structure of the $[H_2\mathbf{L2}](KP)_2{\cdot}0.75EtOH{\cdot}2.75H_2O$ complex. Fig. 3 shows the asymmetric unit, containing the KP_A and KP_B anions, together with carboxylate groups of the symmetry related KP_A' and KP_B' KP units. The non-symmetry related KP_A and KP_B anions are bound to the ligand *via* 2 salt-bridges each (KP_A: N2···O1A, N3···O2A'; KP_B: N2···O1B, N3···O1B'), while a further stabilizing H-bond (N1···O3B) involves the not-protonated amino group and KP_B carbonyl function. This structure also supports the 1:2 host-guest stoichiometry found in solution, although at the solid-state it is realized via a larger 2:4 adduct (Fig. S25). It is also interesting to notice how KP_B accepts to form a bifurcated salt-bridge, generally less stable than standard ones (average salt-bridge length: KP_A, 2.69±0.04 Å, vs KP_B, 2.73 ± 0.08 Å), in order to maintain its carbonyl-amine H-bond, indicating that this second stabilizing contribution (and some cooperativity effects) overcome the energy loss.[23] This structure also demonstrates that anion coordination can effectively influence the localization of the acidic protons within the polyamine backbone. In the $[H_2\mathbf{L2}](KP)_2$ complex the two acidic protons are localized on one benzylic group and on the central nitrogen of the aliphatic chain. This is an unexpected positioning considering that in polyprotonated polyamine chains acidic protons distribution is normally regulated by the minimization of the electrostatic repulsion between ammonium groups, and in the $[H_2L]^{2+}$ cation (L = **L1** or **L2**) the two acidic protons are both localized on the benzylic nitrogen.[‡] In the present case, interaction of one KP unit with central ammonium group allows for the formation of a further stabilizing hydrogen bond between the carbonyl oxygen of KP and the not-protonated amine group, which, in turn, would be lost in the case of interaction of KP with two ammonium groups of **L2**.

As expected considering the ability of the triamine chains of **L1** and **L2** in Zn(II) binding,[19] both **L1** and **L2** form stable 1:1 complexes with Zn(II), which can be isolated as solid complexes from H_2O/EtOH 1:1 solutions. As normally observed for Zn(II) complexes with polyamine-based fluorescent ligands,[22] both complexes are emissive (Φ = 0.20 and 0.059, respectively). The large difference in the fluorescence quantum yields of these two complexes can be attributed to the known lower binding ability of the dipropylen-triamine unit with respect to the diethylen- triamine one,[24] leading to the presence of a weakly bound nitrogen atom, thus able to partially quench the fluorescence of anthracene. More interestingly, addition of KP to the Zn(II) complexes of **L2** induces a *ca* 90% enhancement of the anthracene emission (see Fig. 4), while a *ca* 20% intensity decrease has been observed in the case of **L1**, most probably because of the different starting quantum yields of the two complexes. As in the case of receptors in the absence of the metal, the observed changes account for the formation of 1:2 adducts between the Zn(II) complexes and KP, in good agreement with the ability of Zn(II), which is likely coordinated by the three nitrogens of the receptors in the $[ZnL]^{2+}$ (L = **L1** or **L2**) complexes, to expand its coordination sphere, easily achieving an overall pentacoordination.

These results demonstrate that simple molecular receptors, obtained by straightforward coupling of a linear polyamine with common fluorogenic units, can be used for binding and sensing – with the advantageous feature of some selectivity in the fluorescence response – of elusive analytes in aqueous media, such as NSAIDs, exploiting the proton and metal binding features of this class of receptors. Of note, the sensing properties appear to be tuned by distribution of the ammonium groups within the polyamine chain, which is, in turn, controlled by anion coordination.

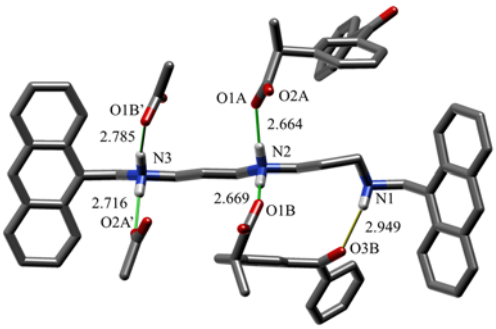
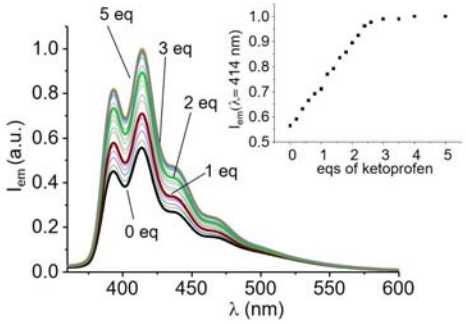


Fig. 3. A depiction of main interactions as found in the crystal structure of the complex. Asymmetric unit, comprising one ligand molecule and 2 KP anions, KP_A and KP_B, is completely shown. Carboxylate groups of symmetry-related KP_A and KP_B have been added to show complete H-bond environment of the $[H_2L2]^{2+}$ cation. Salt-bridges depicted in green, H-bond in yellow.



Fig, 4. Fluorescence emission spectra of the Zn(II) complex with **L2** at pH 7 H_2O/EtOH 50:50 (Vol:Vol) and (inset) emission intensity at 414 nm in the presence of increasing amounts of KP.

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There are no conflicts to declare. Financial support from MUR (Italian Ministry for University and Research) within the PRIN 2017 project 2017EKCS35 is gratefully acknowledged.

Notes and references

‡ Analysis of the ^{1}H NMR spectra of **L1** (Fig. S2, ESI) recorded at different pH values shows that the resonances of the ligands are not affected by pH decreasing from 10 to 8, where the not protonated ligand is basically the only species present in solution. Protonation of **L1** to give [H**L1**]$^+$ species occurs below pH 8 and is accompanied by a marked downfield shift of the signal of the methylene group 3_{AL}, adjacent to the central nitrogen and to a minor shift for methylene 2_{AL}, suggesting that the first protonation step occurs on the central amine of the aliphatic chain. Further pH decrease below pH 6 and consequent formation of the [H_2**L1**]$^{2+}$ species induces a remarkable downfield shift for the signal of the CH_2 2_{AL} and negligible shift for the CH_2 3_{AL}, indicating that in [H_2**L1**]$^{2+}$ the two ammonium groups are localized on the two lateral benzylic amine groups, thus achieving a minimization of the repulsion between positive charge. Interestingly enough, the emission intensity of **L1** is strongly pH dependent and remarkably increases with the formation of [H_2L1]$^{2+}$, accounting for inhibition of the PET process from the benzylic amines to anthracene. An almost equal behavior is found for **L2**, in which the pH dependence of the ^{1}H NMR signals of the CH_2 groups 2_{AL} and 4_{AL} accounts for protonation of the central nitrogen in the [H**L2**]$^+$ species and of both benzylic amines in [H_2**L2**]$^+$.

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