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Fluorescent Probes from Aromatic Polycyclic Nitrile Oxides: Isoxazoles versus Dihydro-1λ³,3,2λ⁴-Oxazaborinines

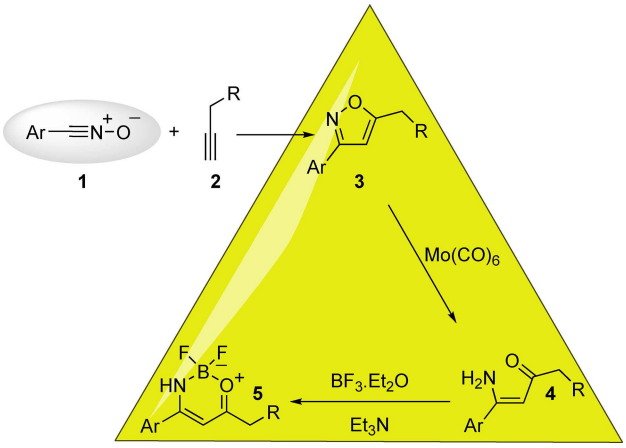
Mattia Moiola,[a] Antonio Bova,[a] Stefano Crespi,[a] Misal G. Memeo,[a] Mariella Mella,[a] Herman S. Overkleeft,[b] Bogdan I. Florea,[b] and Paolo Quadrelli*[a]

Dedicated to the memory of Prof. Pierluigi Caramella

Anthracenenitrile oxide undergoes 1,3-dipolar cycloaddition reaction with propargyl bromide affording the expected isoxazole as single regioisomer, suitably synthetically elaborated and functionalized with a protected triple bond. The introduction of a bromine atom at the position C10 of the anthracene moiety allows for inserting a variety of aromatic and heterocyclic substituents through Suzuki coupling. A two-way synthetic route can lead to simple isoxazole derivatives or, after N–O bond reductive cleavage and BF_3 complexation, enamino ketone boron complexes. The photophysical properties of both the substituted isoxazoles and the corresponding boron complexes were investigated to show the potentialities for the employment as fluorescent tags in imaging techniques. The quite good quantum yield values confirm the suitability of these compounds in the cellular environment. Scope and limitations of the methodology are discussed.

1. Introduction

The chemistry of isoxazoles dates from 1888,[1] when Claisen proposed the correct structure for a compound isolated some years before from the reaction of hydroxylamine with benzoylacetone. In 1891, Claisen published a paper in which the fundamental outline of the isoxazole chemistry was reported; after that fundamental work, a few other authors explored the isoxazole chemistry but the reemergence of interest in these heterocycles[2] must be ascribed to Quilico and co-workers as a consequence of their research on the reaction of nitric acid with C≡C triple bond-bearing compounds initiated in the thirties of last century.[3] 1,3-Dipolar cycloadditions of nitrile oxides **1** to alkynes of type **2** can be undoubtedly considered a pillar of the synthesis of isoxazoles **3** (Scheme 1). The uses of isoxazoles themselves and their elaborated compounds in organic synthesis have been already accounted in several books and papers, being a mature subject of organic chemistry.[2,4] Among these methods, it is worth mentioning the readily cleavage at the N–O bond by several reducing agents,[2] and metal carbonyl [$Mo(CO)_6$] in particular, to afford the β-enamino ketones of type **4**.[5] The importance of these structures lies into the presence of two functional groups, the amino and the carbonyl groups that, among others, can serve as ligands in coordinative compounds. A typical case is that of boron complexation leading to the 2,2-difluoro-dihydro-1λ³,3,2λ⁴-oxazaborinine of type **5**. The versatility of this strategy was recently applied to synthesize structures designed for imaging. Albeit the number of works devoted to this topic is increasing, the applications of the probes constructed via this pathway remain quite low so far.[6]



Scheme 1. From nitrile oxides to 3,5-disubstituted isoxazoles, N–O bond reductive cleavage and boron complexation: the synthetic route to fluorescent probes. Ar = Anthryl.

In a recent conceptual work we successfully employed the 1,3-dipolar cycloaddition of stable aromatic nitrile oxides to afford novel fluorescent compounds. The reported strategy is the cornerstone to furnish boron-substituted complexes suitable for biochemical applications; the nitrile oxide-based protocol is cleaner and selective with dipolarophiles and can be a useful alternative to the use of azides. Indeed, once properly derivatized can found proper use in the activity-based protein

[a] Dr. M. Moiola, Dr. A. Bova, Dr. S. Crespi, Dr. M. G. Memeo, Prof. M. Mella, Prof. P. Quadrelli
Department of Chemistry
University of Pavia
Viale Taramelli 12, 27100 – Pavia (Italy)
E-mail: paolo.quadrelli@unipv.it
[b] Prof. H. S. Overkleeft, Dr. B. I. Florea
Institute of Chemistry
University of Leiden
Einsteinweg 55, 2333 CC Leiden (The Netherlands)

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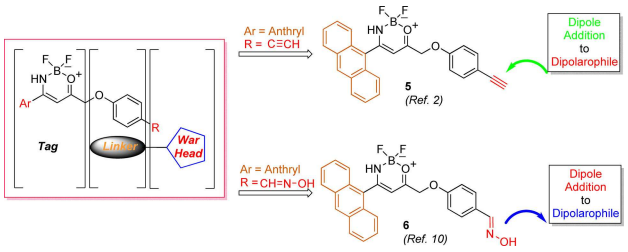
profiling (ABPP).[7] ABPP has emerged as one of the most powerful tools to gain insights into complex biological systems,[8] and the aim resides in the visualization of the active forms of the enzymes using chemical probes directed to the active site of a target protein, resulting in the selective labeling of the sole catalytically active form of the enzyme.[9]

From a structural point of view, chemical probes consist of three different parts: a tag for recognition, a linker of variable length and a warhead (ligation handle) containing the functional groups to link the probe with the target substrate with highly specific interactions that make the probe selective for a well-defined biological structure (see Scheme 2). In our previous work we detailed the synthetic strategy and the fluorescence study of compound **5** that structurally follows the aforementioned paradigms. The compound is synthesized starting from a 1,3-dipolar cycloaddition reaction between a 4-(prop-2-yn-1-yloxy)benzene of type **2** (R = –C≡C–TMS), affording the 5-substituted isoxazole **3** in very good yields as a single regioisomer. The outcome of the transformation nicely follows the predictions based on the frontier orbital theory.[7] Reductive cleavage of the N–O bond and complexation with BF_3 furnishes the corresponding fluorescent boron complex **5** (Scheme 1). The molecule bears an anthryl substituent (Ar = 9-anthryl) on the tag and a triple C≡C bond on the warhead end terminus (R = –C≡CH) of the probe, suitable for click-chemistry applications and late-stage functionalization. Furthermore, we also presented a strategy to prepare chemical probes that maintain the same tag structure, while bearing warheads prone to an orthogonal functionalization compared to **5**. Indeed, in compound **5** the triple bond requires the use of a substrate bearing a dipole (typically an azido-derivative) to connect the two sides of the chemical reporter (Scheme 2). The newly designed compound **6** bears an oxime moiety on the warhead that is suitable to be oxidized to nitrile oxide. Hence, the probe is another 1,3-dipole that can be attached to a double (or triple) bond located on a target substrate. Being a nitrile oxide, probe **6** can enter the ligation process without the help of metal ions that normally are needed in azide-based protocols, affording a metal-free methodology to be easily applied in a cellular environment.

The two chemical probes **5** and **6** have been compared from the photophysical point of view and tested by coupling them with differently functionalized epoxomicin derivatives. Ultimately, competitive ABPP assays will be performed with the aim to verify the maintenance of proteasome inhibitor properties and possible differences in terms of selectivity. To detect the fluorescence of the probes adducts to epoxomicin while they bind the proteasome, several different light sources were tried to excite the fluorophores, but unluckily they did not match the excitation wavelength of our probes and no fluorescence were registered. Some possible explanations were considered. We cannot state if these probes maintained their fluorescence during the interaction with the proteasome; it was also possible that the fluorophores maintained their optical properties but the excitation was not efficient. Alternatively, we cannot exclude a potential quenching or degradation (instability of the boron complex) during the interaction with the proteasome.[10]



Scheme 2. Probe structures: dipolarophile and dipole precursor ligation handles structures.

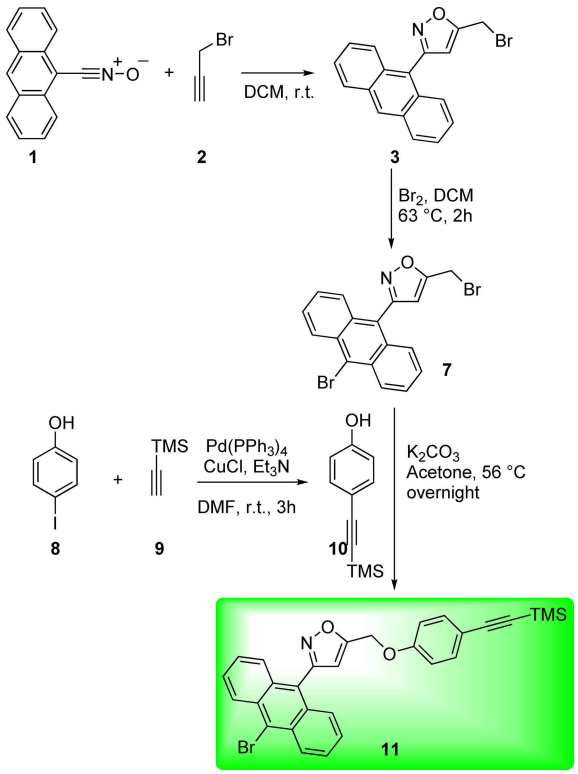
For these reasons, we started revising and enlarging the library of compounds prepared on the base of isoxazole chemistry in order to synchronize better synthesis and application, fluorescent activity and stability of products. Suitably designed isoxazoles can show intense fluorescent properties, too, even stronger than the corresponding boron complexes. That is why in this study, we present the synthetic approach to fluorescent brand-new compounds, comparing the photochemical behavior of the *uncomplexed* and *complexed* probe couples with the aim to enhance the fluorescence quantum yields and stability of compounds in order to become competitive and possibly more convenient than commercial ABPP traditional probes.

2. Results and Discussion

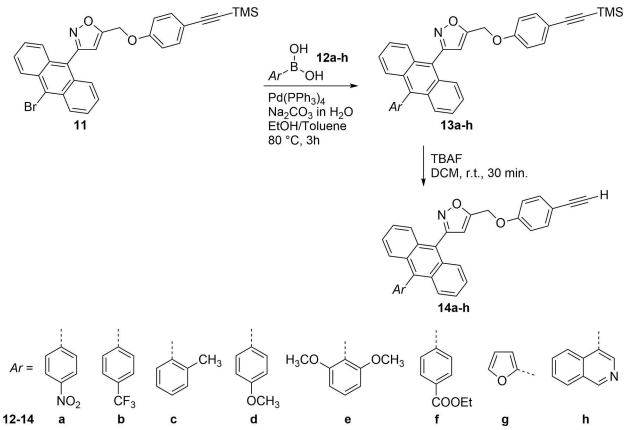
Anthracenenitrile oxide **1** was prepared in very good yields (81%) from the commercially available corresponding oxime under standard NCS treatment in chloroform solution at 0 °C for 3 h, by adapting literature reported procedures.[11,12] The solid stable aromatic nitrile oxide **1** can be stored for months at low temperature and was used, without any further purification or particular adaptations of experimental procedures, in the 1,3-dipolar cycloaddition with propargyl bromide (**2**) (Scheme 3).[7] The 3-(anthracen-9-yl)-5-(bromomethyl)isoxazole (**3**) was obtained as single regioisomer in 75% yield as a yellow solid (m.p. 81–85 °C from ethanol) and was submitted to bromination reaction under mild conditions (Br_2, DCM at 63 °C for 2 h) to insert a bromine atom in the position 10 of the anthracene moiety, to afford the 3-(10-bromoanthracen-9-yl)-5-(bromomethyl)isoxazole (**7**) in 80% yield as a straw coloured high melting solid (m.p. 129–132 °C from ethanol).[13]

The commercially available 4-iodophenol (**8**) was derivatized with ethynyltrimethylsilane (**9**) under typical Pd(0)-catalyzed conditions to afford the 4-((trimethylsilyl)ethynyl)phenol (**10**) in 80% yield. The ethynyl derivative **10** was coupled with the isoxazole derivative **7** by treatment with mild basic conditions, to afford compound **11** (80% yield, m.p. 126–130 °C from ethanol) that represent the starting point for a series of derivatizations at the carbon C10 of the anthracene moiety.

From the structural point of view all the compounds were fully characterized. In particular compound **3** shows in the ^1H NMR spectrum ($CDCl_3$) the diagnostic signal of the H4 isoxazole



Scheme 3. Synthetic pathway to the 3-(10-bromoanthracen-9-yl)-5-((4-((trimethylsilyl)ethynyl) phenoxy)methyl)isoxazole (**11**).



Scheme 4. Probe synthesis (*I*): from isoxazole derivative **11** to compounds **14a–h**.

Table 1. Yields, physical chemical data of compounds **13a–h** and **14a–h**.

Compd.	Yield (%)	Mp (°C) (from Cy/AcOEt)	^{1}H NMR (δ, $CDCl_3$)
13			
a	80	211–215	–
b	72	178–182	–
c	63	124–128	1.49 (s, 3H, CH_3)
d	66	Sticky oil	3.99 (s, 3H, OCH_3)
e	30	Sticky oil	3.59 (s, 6H, OCH_3)
f	74	Semi solid/oil	1.50 (t, 3H, CH_3); 4.51 (q, 2H, OCH_2)
g	–	–	–
h	44	211–214	–
14			
a	70	206–208	–
b	81	176–178	–
c	59	198–200	1.91 (s, 3H, CH_3)
d	66	181–183	3.99 (s, 3H, OCH_3)
e	4	175–180	3.59 (s, 6H, OCH_3)
f	32	65–68	1.51 (t, 3H, CH_3); 4.51 (q, 2H, OCH_2)
g	84	104–105	–
h	81	185–190	–

proton at δ 6.59 as a singlet, also found in the bromo-derivative **7** at δ 6.57. In the ^{1}H NMR spectrum ($CDCl_3$) of compound **11** the signal of the TMS protecting group can be found at δ 0.27 along with the H4 isoxazole proton singlet at δ 6.61.

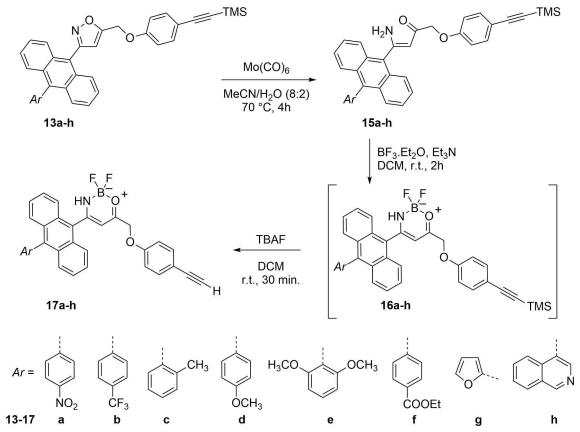
The isoxazole derivative **11** was then coupled with a series of boronic acids **12a–j** according to a typical Pd(0)-catalyzed Suzuki procedure[14] to afford the 10-substituted anthracen-9-yl-isoxazoles **13a–h** in good yields whose triple bond was deported under standard TBAF conditions[7] leading to the desired fluorescent isoxazole derivatives **14a–h** in very good yields (Scheme 4).

Table 1 collects the chemical yields and the relevant physical-chemical data of both compounds **13** and **14**. As a general comment, the yields are pretty good in all the cases, except for **13e** and **14e** that were found quite unstable, and the synthetic procedures were standardized for all the substituents introduced and were found robust and reliable.

Just in one case the intermediate **13g** could not be isolated because TMS deprotection occurred under the experimental conditions affording the final compound **14g**. Most of the products are solids with a few exceptions.

The structures were confirmed on the basis of the corresponding analytical and spectroscopic data. In particular, in the ^{1}H NMR spectra ($CDCl_3$) the most relevant changes in compounds **13**, compared with **11**, is the thicken of the aromatic region between δ 7.0–8.5 ppm and the presence of new signals in the cases of products **13c–f** due to the phenyl ring substitution.

A second synthetic route was followed to prepare the boron complexes relative to the isoxazoles compounds **14a–h** and the experimental steps are shown in Scheme 5. The N–O bond cleavage[15] in the isoxazole moieties was performed using Mo $(CO)_6$ in a mixed solvent of CH_3CN–H_2O (8:2, 70 °C, 4 h) to give the (*Z*)-4-amino-4-(10-aryl-substituted-anthracen-9-yl)-1-(4-((trimethylsilyl)ethynyl)phenoxy) but-3-en-2-ones **15a–h** as a yellow/yellowish solids in 50–80% yields. As previously reported,[7,10] the signals shown in the ^{1}H NMR spectrum denote the existence of an intramolecular hydrogen bond between the amino group and the carbonyl functionality obtained from the N–O bond cleavage. As a consequence, one of the NH_2 protons is found in the range δ 5.63–5.90 ppm while the other one is strongly deshielded in the range δ 10.44–10.53 ppm. These latters are the direct evidence of the six-membered cyclic array kept together by the hydrogen bonding. Likewise, the newly



Scheme 5. Probe synthesis (*II*): from isoxazole derivatives **13a–h** to compounds **17a–h**.

formed carbonyl groups can be found in the ^{13}C NMR spectra around δ 194 ppm.

The enaminoketones **15a–h** were used as ligand for boron complexation,[16] achieved by addition of 10 equivalents of $BF_3 \cdot Et_2O$ in an anhydrous DCM solution, in the presence of excess Et_3N. Protected boron complexes **16a–h** were not isolated since the reaction conditions promoted partial deprotection from TMS group in all the cases. As a consequence, the synthetic protocol was properly adapted in order to skip immediately on the final products **17a–h** by treating the resulting reaction mixtures under standard TBAF conditions leading to the desired boron complexes **17a–h**. The yields are in the range 30–60%. With the exception of compound **17e** that could not be synthetized because of the very low yields of **13e** available, the structures of all the other boron complexes were confirmed by full characterization by analytical and spectroscopic methods and, in particular, in the ^1H NMR spectra ($CDCl_3$) the presence of the ≡CH proton signals at δ 3.03 ppm for all the compounds clearly indicated the successful deprotection. With reference to the synthetic route shown in Scheme 5, we precise that the entire described methodology is valid for all the compounds with the single exception of **17g** that was obtained directly from **14g**, since the TMS protection could not be saved during the synthesis. The experimental section reports the characterization of the enamino ketone **15g′** having the structure shown in Scheme 5 without the TMS group.

We performed some UV-Vis and fluorescence studies on both the isoxazole derivatives **14a–h** and the corresponding boron complexes **17a–h**, comparing the results with those previously obtained for compounds **5** and **6** (see Scheme 2).[7,10] These studies will be propaedeutic to evaluate the potential use of the new compounds as chemical probes in ABPP investigations.

The oxime derivative **6** showed an absorption band at 384 nm ($\varepsilon = 5.00 \cdot 10^3\ mol^{-1}$) with a shoulder at 238 nm and vibrational peaks. Analogously, the BF_2 complex **5** shows an absorption band at 387 nm ($\varepsilon = 8.20 \cdot 10^3\ mol^{-1}$) along with several vibrational peaks.[7,10] Table 2 resumes the main photophysical characteristics of compounds **14a–h** and **17a–h**. The absorption and emission spectra of compounds **14c** and **17c** in DCM are depicted in Figure 1, here reported as paradigmatic examples. All the remaining spectra in all the solvents are collected in the Supporting Information. Both the isoxazoles and the BF_2 complexes possess an absorption around 400 nm, characterized by a vibrational structure, attributable to the anthryl moiety. Interestingly, the change in functional groups on the phenyl ring does not drastically shift the absorption peak in the near UV.

The fluorescence emission spectra of **14a–h** and **17a–h** are not influenced in their relative shapes and λ_{em} (emission wavelength) by the change of solvents. The fluorescence of

Table 2. Quantum Yields (Φ_F) values for compounds **14a–h** and **17a–h** in the listed solvents.[a]

	DCM					MeOH					DMSO/H_2O				
	λ_{ads}	λ_{em}	Stk[b]	ε^{χ}	Φ_F	λ_{ads}	λ_{em}	Stk[b]	ε^{χ}	Φ_F	λ_{ads}	λ_{em}	Stk[b]	ε^{χ}	Φ_F
14															
a	394	418	24	$8.37.10^3$	0.00	391	419	28	$8.09.10^3$	0.00	391	421	30	$6.89.10^3$	0.00
b	393	436	43	$8.71.10^3$	0.90	390	427	37	$7.65.10^3$	0.92	394	433	39	$6.07.10^3$	0.17
c	394	434	40	$6.76.10^3$	0.99	391	425	34	$6.48.10^3$	>0.99	396	432	36	$6.62.10^3$	0.91
d	395	441	46	$1.17.10^4$	0.14	392	443	51	$9.20.10^3$	0.11	397	452	55	$7.45.10^3$	0.05
e	394	432	38	$6.99.10^3$	0.94	391	425	34	$6.41.10^3$	0.96	396	437	41	$2.18.10^3$	0.65
f	394	438	44	$7.02.10^3$	0.73	391	434	43	$6.16.10^3$	0.97	398	442	44	$7.48.10^3$	0.71
g	395	430	35	$7.44.10^3$	0.13	392	423	31	$6.76.10^3$	0.10	393	435	42	$8.37.10^3$	0.22
h	395	429	34	$9.70.10^3$	0.81	392	424	32	$7.84.10^3$	0.74	396	434	38	$9.40.10^3$	0.89
17															
a	396	437	41	$3.48.10^5$	0.02	392	430	38	$9.97.10^3$	0.00	396	434	38	$8.91.10^3$	0.00
b	395	491	96	$7.88.10^3$	0.10	391	495	104	$7.89.10^3$	0.02	396	491	95	$8.01.10^3$	0.03
c	395	496	101	$6.91.10^3$	0.07	392	496	104	$6.56.10^3$	0.02	396	497	101	$6.68.10^3$	0.02
d	397	511	114	$8.38.10^3$	0.08	393	493	100	$9.10.10^3$	0.01	397	463	66	$7.17.10^3$	0.05
e	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
f	396	492	96	$1.19.10^4$	0.09	392	490	98	$1.27.10^4$	0.04	396	482	86	$7.51.10^3$	0.04
g	396	483	87	$7.82.10^3$	0.01	392	460	68	$7.77.10^3$	0.01	396	455	59	$7.30.10^3$	0.01
h	396	488	92	$3.90.10^3$	0.12	392	486	94	$7.25.10^3$	0.03	402	460	58	$1.27.10^3$	0.08

a. Concentration range $3 \cdot 10^{-7}$–$5 \cdot 10^{-5}$ M in all the solvents. b. Stk, Stokes shifts. – Dielectric constants: DCM, 8.9; MeOH, 33; DMSO/H_2O, 55. – Viscosities (cP at 20 °C):[18] DCM, 0.44; MeOH, 0.55; DMSO/H_2O, 3.30. c. Molar absorptivity, ε (L.mol^{-1}.cm^{-1}).

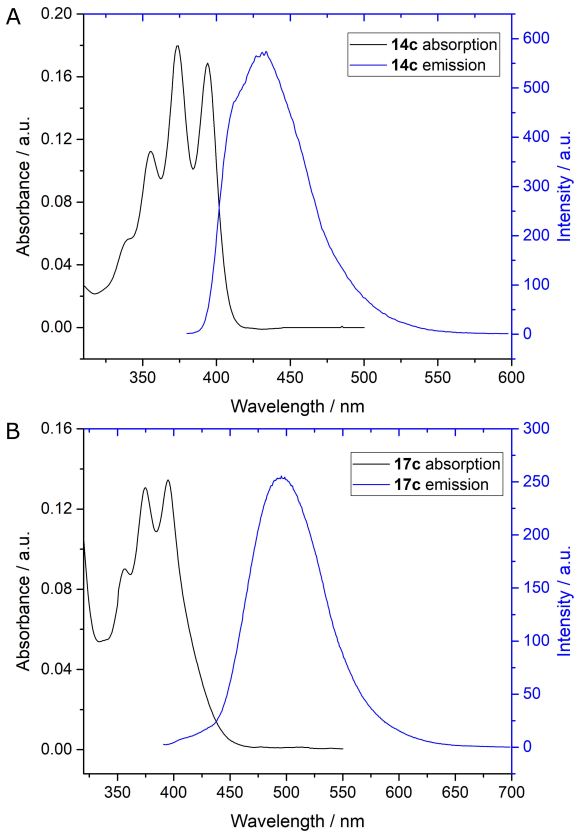


Figure 1. UV-vis (black) and fluorescence (blue) spectra of compounds **14c** (A) and **17c** (B) in DCM ($2\cdot10^{-5}$ M solutions).

14a–h is characterized by a λ_{em} shifted around ca. 40 nm compared to the absorption wavelength (λ_{abs}). Compounds **17a–h** are characterized by higher Stokes shifts that reach the 100 nm. These findings suggest that the dipole moments of the molecule in the ground and excited states are almost the same.[17] These results show the same trend observed for compound **5** and **6**.[2] The quantum yields of fluorescence emission (Φ_F) are considerably higher in the isoxazole derivatives. Compound **14c** possess almost quantitative emission in the solvents examined, with MeOH the one in which the better performances are obtained ($\Phi_F > 0.99$). The nature of the substituents has a role in the observed Φ_F in compounds **14a–h**. Both the electronic and steric nature of the moieties modify the emission properties of the final compound. Indeed, the most fluorescent compounds possess ortho substituents in the aryl moiety attached to the anthryl one (**14c** and **14e**). It is possible that the increased steric hindrance diminishes the electronic interactions between the anthryl moiety and its substituents, or shields the chromophore from the interaction with solvent molecules that could deactivate the excited state.

In this case, the electronic nature of the substituent in ortho is not relevant for the Φ_F. We can postulate that the ortho substituents block the anthryl and aryl rings in perpendicular fashion, more than the para ones. In the latter case the electronic effects are more pronounced. Hence, the electron attracting groups (**14b** and **14f** and electron poor heteroaromatics **14h**) give better results if compared with electron rich rings (**14d** and **14g**). In compounds **5** and **6**,[7,10] the change in viscosity and polarity of the solvent mixture – hence moving from DCM, to MeOH and DMSO–H_2O 1:1 – is accompanied with a decrease in the Φ_F. Such an effect is not pronounced in the series of the isoxazoles. Indeed compounds **14c**, **e** and **14f** are promising for further testing their applicability as molecular probes.

All the BF_2 derivatives are, on the other hand, weakly fluorescent suggesting a potential limitation in biological environment. All the nitro derivatives are outliers in both the two series of compounds analyzed. The compounds are non-fluorescent, except for **17a** in DCM ($\Phi_F = 0.02$) and possess the lower Stokes shift. The facile Intersystem Crossing to the triplet state due to the presence of the NO_2 chromophore could explain the absence of the fluorescence in the nitro substituted molecules.[19]

The difference between the two series of compounds analyzed is mirrored by the nature of the excited state populated after irradiation, calculated at the TD-CAM-B3LYP/def2-TZVP//B3LYP/6-31G(d) level of theory.[20] In the case of compounds **14**, the excitation is confined to the anthryl moiety (exemplified in Figure 2 by compound **14b**). On the other hand, compounds **17** show also a partial charge transfer to the boracycle (**17b** in Figure 2).

3. Conclusions

In conclusion, this work has contributed to show the possible and reliable application of the nitrile oxide chemistry in the biomedical field, especially as starting material for the synthesis of some fluorescent probes. The chemistry of isoxazoles in the field of imaging probes seems to be promising and a valuable alternative to azides, avoiding the use of copper (metals are detrimental in a biological environment) and the chemistry is cleaner and safer; the construction of the boron complexes is simple, reliable and robust.[13] The methodology can be applied to a variety of structures having the aldehydes as starting compounds. In this light, we have demonstrated the possibility to expand the application of 1,3-dipolar cycloaddition reactions in order to obtain a group of easy-derivatizable fluorescent probes. Starting from a known stable nitrile oxide and adapting a simple chemical approach, we were able to obtain two families of fluorescent compounds of type **14** and **17**. One of the key points of the present project was the enhancement of the fluorescence quantum yields; this goal was achieved by preparing derivatized isoxazole compounds.

The optical properties of these compounds suggest the potential for the employment as fluorescent tags in imaging techniques. In spite of the drops of quantum yields values for compound **6** with respect to **5**, the quite good quantum yields values maintained by some of these compounds in the DMSO/H_2O mixture confirm the suitability of these compounds in the cellular environment. The ABPP assays performed did not give us an ultimate answer about the behavior of compounds **5** and **6** in SDS-page analyses. The coupling tests performed showed a

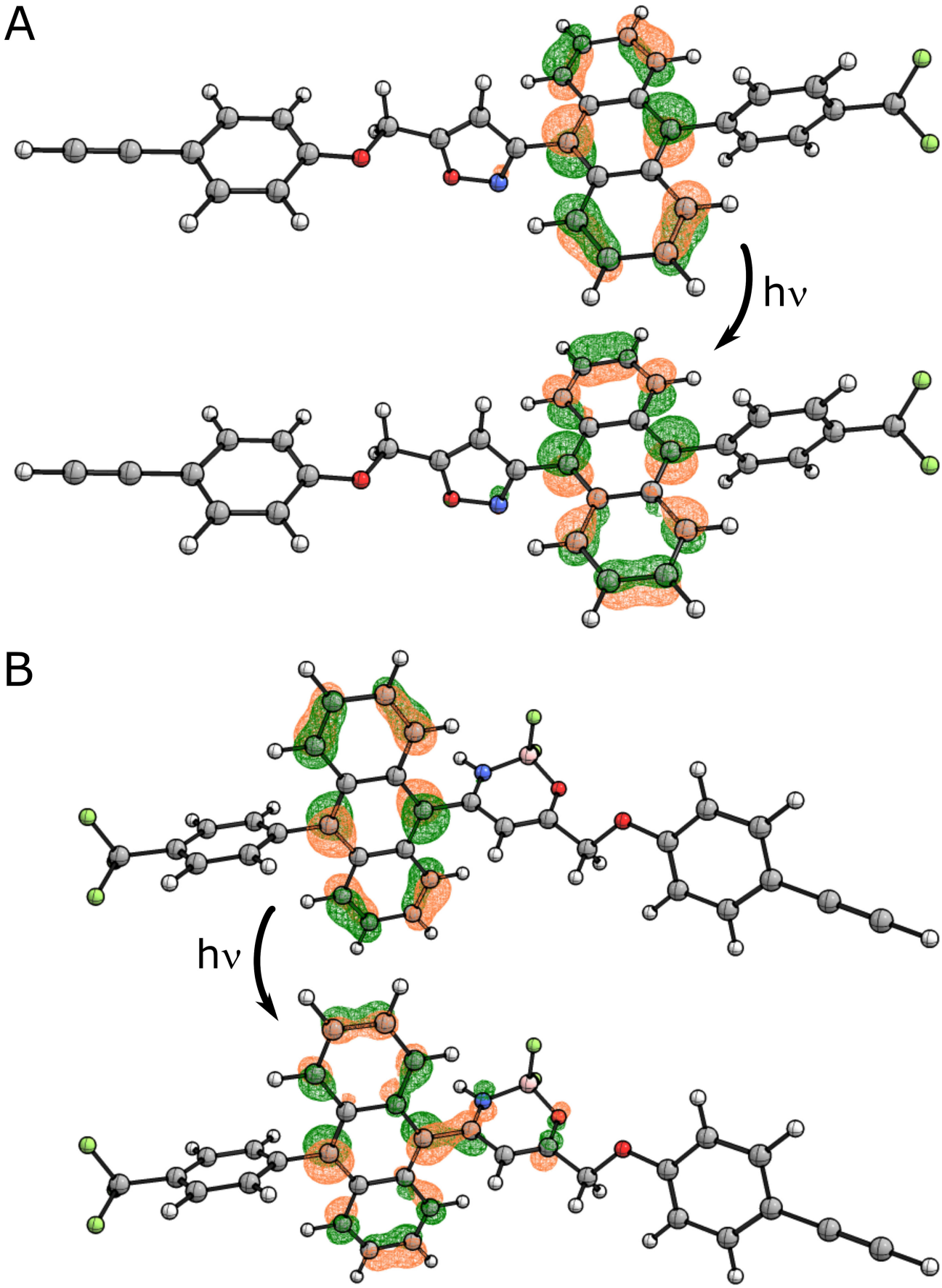


Figure 2. Electronic orbitals involved in the lower allowed electronic transition from (A) HOMO (top) to LUMO (bottom) of **14b**; (B) HOMO (top) to LUMO (bottom) of **17b**. The TD-CAM-B3LYP/def2-TZVP//B3LYP/6-31G(d) level of theory was applied.

good reactivity for the probes **5** and **6** even with short peptide chains such as the Epoxomicin. Improvements on the fluorescence properties of enamino ketone-based boron complexes were needed but the results demonstrated the better performances of the isoxazoles. These results reasonably indicate the isoxazole as candidate for imaging studies. The tuning of the optical properties of the probes is a crucial point for the application in imaging techniques: at the moment the shift towards higher wavelengths remains a target to be achieved with the proposed chemical approach. Future developments of the synthetic strategy are currently under investigation; specifically we have designed the synthetic approach to fully conjugated isoxazole structures of type **18** and the corresponding boron complexes of type **19** with the specific target to have further insights on the relative bathochromic shifts in the presence of the reported substituents on the aromatic moieties (Figure 3).

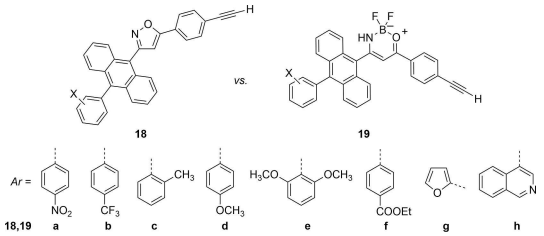


Figure 3. Future perspectives in the synthesis of fluorescent compounds.

Having some of the synthetized isoxazoles unexpected intense fluorescent properties, even stronger than the corresponding boron complexes, the planned SDS-page analyses on coupled epoxomicin compounds will direct future developments in the application of nitrile oxide chemistry to the synthesis of fluorescent tags.

Experimental Section

General. Melting points (mp) are uncorrected. Elemental analyses were performed on a FlashSmart™ elemental analyzer. IR spectra were registered using a FT-IR Perkin-Elmer RX-1 spectrometer [Nujol mulls or dissolving the analyzed products in DCM (film)]. ^{1}H-NMR and ^{13}C-NMR were registered on Bruker AVANCE 300 spectrometers in deuterated solvent solutions as specified. The chemical shifts are expressed in δ (ppm), using tetramethylsilane as internal reference. Coupling constants (*J*) are expressed in Hertz (Hz).

Chromatographic columns were performed using Kieselgel 60, 70–230 (Merck), with a BIOTAGE MPLC or BIOTAGE Isolera One with KP-SIL columns; the elution solvents were cyclohexane/ethyl acetate from 9:1 to pure ethyl acetate.

UV-Vis spectra were registered with a Jasco V-550 UV/VIS Spectrophotometer; the fluorescence spectra were registered with a Perkin-Elmer LS 55 Luminescence Spectrometer.

Materials. Anthracenenitrile oxide **1** was prepared from the corresponding commercially available oxime according to known procedure.[12] Solvents and other reagents were purchased and used without any further purification.

*Synthesis of the 3-(anthracen-9-yl)-5-(bromomethyl)isoxazole **3**.* In a 250 mL flask 4.45 g (20.3 mmol) of anthracenenitrile oxide (**1**), dissolved in 50 mL anhydrous DCM, were added dropwise to 80 mL anhydrous DCM solution of 2.65 g (2 mL, 22.3 mmol) of propargyl bromide (**2**). The reaction was kept in the dark and under stirring at room temperature for 48 hours. Then the mixture was diluted with DCM (30 mL) and the solution was washed with Brine (3×50 mL) and dried over anhydrous Na_2SO_4.

The solvent was evaporated and the crude residue was purified by column chromatography, giving a yellow solid of **3** in 75% yield (5.20 g).

Mp: 81–85 °C from cyclohexane/ethyl acetate. FT-IR: $\nu_{C=N}$ 1598 cm^{-1}; $\nu_{C=C}$ 1626 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 4.68 (s, 2H, CH_2); 6.59 (s, 1H, C≡C–H); 7.51 (m, 4H, anthr.); 7.87 (m, 2H, anthr.); 8.08 (m, 2H, anthr.); 8.60 (s, 1H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): 18.6; 107.2; 122.4; 125.0; 125.3; 125.4; 126.6; 128.5; 129.1; 130.5; 131.0; 161.2; 167.8. Elemental Analysis: Calculated for $C_{18}H_{12}BrNO$ (MW = 338.20): C, 63.93; H, 3.58; N, 4.14. Found: C, 63.94; H, 3.58; N, 4.13.

*Synthesis of the 3-(10-bromoanthracen-9-yl)-5-(bromomethyl)isoxazole **7**.* In a 500 mL round-bottom flask 2.60 g (0.833 mL, 16.3 mmol) of Br_2, diluted in 40 mL anhydrous DCM, were added dropwise to 250 mL anhydrous DCM solution of 5.00 g (14.8 mmol) of cycloadduct **3**. The reaction was kept in the dark heating at reflux and under stirring for 2 hours. Then the mixture was diluted with DCM (20 mL) and the solution was washed with 5% $Na_2S_2O_4$ solution (3×35 mL) and water (3×50 mL) and finally dried over anhydrous Na_2SO_4.

The solvent was evaporated and the crude residue was purified by column chromatography, giving a yellow solid of **7** in 90% yield (5.56 g).

Mp: 128–132 °C from cyclohexane/ethyl acetate. FT-IR: $\nu_{C=N}$ 1598 cm^{-1}; $\nu_{C=C}$ 1636 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 4.68 (s, 2H, CH_2); 6.57 (s, 1H, C≡C–H); 7.51 (m, 2H, anthr.); 7.63 (m, 2H, anthr.); 7.48 (d, 2H, J = 9 Hz, anthr.); 8.63 (d, 2H, J = 9 Hz, anthr.). ^{13}C-NMR (δ, $CDCl_3$): 18.1; 106.8; 122.2; 122.8; 125.4; 126.4; 127.7; 129.7; 130.6; 160.6; 167.7. Elemental Analysis: Calculated for $C_{18}H_{11}Br_2NO$ (MW = 417.10): C, 51.83; H, 2.66; N, 3.36. Found: C, 51.84; H, 2.67; N, 3.35.

*Synthesis of the 3-(10-bromoanthracen-9-yl)-5-((4-((trimethylsilyl)ethynyl)phenoxy)methyl)isoxazole **11**.* In a 250 mL round-bottom flask 7.70 g (55.6 mmol) of K_2CO_3 were added to 120 mL acetone solution of 2.90 g (6.95 mmol) of compound **7** and the mixture heated at 56 °C. An acetone solution (100 mL) of 1.71 g (9.04 mmol) of 4-((trimethylsilyl)ethynyl)phenol (**10**) was then added dropwise and the mixture was allowed to react at reflux and under stirring overnight. The mixture was then filtered and the solvent evaporated at reduced pressure. The brown residue was taken up with DCM (100 mL) and the organic phase washed with brine (3×40 mL) and finally dried over anhydrous Na_2SO_4.

Evaporation of the solvent left the crude compound **11** that was purified by column chromatography, giving a yellow fluorescent solid in 82% yield (3.00 g).

Mp: 126–130 °C from cyclohexane/ethyl acetate. FT-IR: $\nu_{C=N}$ 1598 cm^{-1}; $\nu_{C=C}$ 1676 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 0.27 (s, 9H, CH_3); 5.40 (s, 2H, CH_2); 6.61 (s, 1H, C≡C–H); 7.00 (AA′BB′, 2H, arom.); 7.51 (m, 4H, anthr.); 7.66 (m, 2H, anthr.); 7.81 (AA′BB′, 2H, arom.); 8.64 (m, 2H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): −0.1; 61.5; 77.1; 107.0; 114.7; 125.9; 126.7; 127.1; 128.1; 130.1; 131.1; 133.6; 157.7; 160.7; 168.1. Elemental Analysis: Calculated for $C_{29}H_{24}BrNO_2Si$ (MW = 526.51): C, 66.16; H, 4.59; N, 2.66. Found: C, 66.15; H, 4.58; N, 2.65.

General procedure for the synthesis of the 3-(10-aryl-subnstituted-anthracen-9-yl)-5-((4-((trimethylsilyl)ethynyl)phenoxy)methyl)isoxazole ***13a–h.*** In a 100 mL round-bottom flask 0.50 g (0.95 mmol) of compound **11** were dissolved in 15 mL toluene. An ethanol solution (10 mL) of 1.90 mmol of the boronic acids **12a–h** was mixed with the previously prepared solution of **11**. A water solution (5 mL) of 700 mg (6.60 mmol) of Na_2CO_3 was then added and the mixtures were allowed to react at room temperature under stirring and nitrogen atmosphere for 30 minutes. $Pd(PPh_3)_4$ (17 mg, 0.014 mmol) were then added to the reaction mixtures and the resulting solution was heated at 80 °C for 3 h. The mixtures were then cooled down, 50 mL water were added and the organic phases separated. The water phases were furtherly extracted with DCM (3×30 mL) and finally dried over anhydrous Na_2SO_4.

Evaporation of the solvent left the crude compounds **13a–h** that were purified by column chromatography, giving a yellow fluorescent solids in very good yield.

13a: Yield: 80%. Mp: 211–215 °C from ethyl acetate. FT-IR: $\nu_{C=N}$ 1560 cm^{-1}; $\nu_{C=C}$ 1603 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 0.27 (s, 9H, CH_3); 5.43 (s, 2H, CH_2); 6.68 (s, 1H, C≡C–H); 7.02 (AA′BB′, 2H, arom.); 7.50 (m, 4H, anthr.); 7.68 (m, 2H, anthr.); 7.89 (AA′BB′, 2H, arom.); 8.51 (m, 2H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): −0.1; 61.5; 77.1; 106.9; 114.7; 123.7; 125.8; 126.0; 126.1; 126.4; 129.2; 130.1; 132.1; 133.6; 145.8; 147.6; 157.7; 160.9; 168.1. Elemental Analysis: Calculated for $C_{35}H_{28}N_2O_4Si$ (MW = 568.70): C, 73.92; H, 4.96; N, 4.93. Found: C, 73.92; H, 4.97; N, 4.94.

13b: Yield: 72%. Mp: 178–182 °C from ethyl acetate. FT-IR: $\nu_{C=N}$ 1604 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 0.28 (s, 9H, CH_3); 5.42 (s, 2H, CH_2); 6.67 (s, 1H, C≡C–H); 7.03 (AA′BB′, 2H, arom.); 7.44 (m, 4H, anthr.); 7.62 (m, 4H, arom. and anthr.); 7.90 (m, 2H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): −0.1; 61.5; 77.1; 107.0; 114.7; 125.3; 125.4; 125.6; 125.7; 126.3; 126.5; 129.5; 130.1; 131.4; 133.6; 142.5; 157.7; 161.0; 168.0. Elemental Analysis: Calculated for $C_{36}H_{28}F_3NO_2Si$ (MW = 591.61): C, 73.08; H, 4.77; N, 2.37. Found: C, 73.06; H, 4.78; N, 2.36.

13c: Yield: 63%. Mp: 124–128 °C from ethyl acetate. FT-IR: $\nu_{C=N}$ 1604 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 0.34 (s, 9H, CH_3); 1.49 (s, 3H, CH_3); 5.38 (s, 2H, CH_2); 6.69 (s, 1H, C≡C–H); 7.02 (d, 2H, J = 9 Hz, arom.); 7.44 (m, 8H, anthr.); 7.93 (d, 2H, J = 9 Hz, arom.). ^{13}C-NMR (δ, $CDCl_3$): −0.0; 26.9; 61.4; 107.2; 114.8; 125.5; 125.7; 125.9; 126.3; 126.8; 128.1; 129.5; 130.1; 130.4; 130.9; 133.6; 137.6; 157.8; 161.2; 167.9. Elemental Analysis: Calculated for $C_{36}H_{31}NO_2Si$ (MW = 537.73): C, 80.41; H, 5.81; N, 2.60. Found: C, 80.40; H, 5.82; N, 2.59.

13d: Yield: 66%. Sticky oil. FT-IR: $\nu_{C=N}$ 1602 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 0.28 (s, 9H, CH_3); 3.99 (s, 3H, OCH_3); 5.41 (s, 2H, CH_2); 6.66 (s, 1H, C≡C–H); 7.02 (AA′BB′, 2H, arom.); 7.17 (d, 2H, anthr.); 7.41 (m, 4H, anthr.); 7.77 (AA′BB′, 2H, arom.); 7.85 (m, 2H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): −0.0; 55.4; 61.6; 107.2; 113.9; 114.8; 125.2; 125.6; 126.3; 127.4; 130.2; 130.4; 132.2; 133.7; 157.9; 159.3; 161.4; 167.9. Elemental Analysis: Calculated for $C_{36}H_{31}NO_3Si$ (MW = 553.73): C, 78.09; H, 5.64; N, 2.53. Found: C, 78.09; H, 5.66; N, 2.53.

13e: Yield: 30%. Sticky oil. FT-IR: $\nu_{C=N}$ 1603 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 0.28 (s, 9H, CH_3); 3.59 (s, 6H, OCH_3); 5.39 (s, 2H, CH_2); 6.68 (s, 1H, C≡C–H); 6.84 (AA′BB′, 2H, arom.); 7.01 (d, 2H, anthr.); 7.41 (m, 4H, anthr.); 7.66 (AA′BB′, 2H, arom.); 7.85 (m, 2H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): −0.0; 55.8; 61.5; 104.4; 114.7; 124.9; 125.6; 126.0; 126.8; 129.8; 130.1; 130.5; 133.6; 157.8; 158.8; 161.5; 167.5. Elemental Analysis: Calculated for $C_{37}H_{33}NO_4Si$ (MW = 583.76): C, 76.13; H, 5.70; N, 2.40. Found: C, 76.15; H, 5.71; N, 2.41.

13f: Yield: 74%. Sticky oil (semi-solid compound). FT-IR: $\nu_{C=N}$ 1609, $\nu_{C=O}$ 1716 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 0.27 (s, 9H, CH_3); 1.50 (t, 3H, J = 7 Hz, CH_3); 4.51 (q, 2H, J = 7 Hz, OCH_2); 5.42 (s, 2H, CH_2); 6.66 (s, 1H, C≡C–H); 7.02 (AA′BB′, 2H, arom.); 7.51 (m, 6H, anthr.); 7.86 (AA′BB′, 2H, arom.); 8.32 (m, 2H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): −0.0; 14.4; 61.2; 61.7; 107.1; 114.9; 125.6; 125.7; 126.4; 126.8; 129.5; 129.7; 130.3; 131.3; 133.7; 157.9; 161.2; 166.5; 168.1. Elemental Analysis: Calculated for $C_{38}H_{33}NO_4Si$ (MW = 595.77): C, 76.61; H, 5.58; N, 2.35. Found: C, 76.60; H, 5.55; N, 2.34.

13g: this compound could not be isolated because, under the reaction conditions, deprotection occurred to afford **14g**.

13h: Yield: 44%. Mp: 211–214 °C from ethyl acetate. FT-IR: $\nu_{C=N}$ 1610 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 0.27 (s, 9H, CH_3); 5.45 (s, 2H, CH_2); 6.73 (s, 1H, C≡C–H); 7.02 (d, 2H, arom.); 7.51 (m, 7H, anthr.); 7.93 (d, 2H, arom.); 8.22 (d, 1H, arom.); 8.62 (s, 1H, arom.); 9.54 (s, 1H, arom.). ^{13}C-NMR (δ, $CDCl_3$): −0.0; 61.5; 107.1; 114.7; 125.3; 125.8; 125.9; 126.4; 126.5; 127.8; 128.0; 128.2; 130.0; 130.2; 130.7; 133.0; 152.2; 157.7; 161.0; 168.0. Elemental Analysis: Calculated for $C_{44}H_{34}N_2O_2Si$ (MW = 650.85): C, 81.20; H, 5.27; N, 4.30. Found: C, 81.20; H, 5.26; N, 4.31.

General procedure for the synthesis of the 3-(10-aryl-substituted-anthracen-9-yl)-5-((4-ethynylphenoxy)methyl)isoxazole ***14a–h.*** In a 100 mL flask a DCM solution of compounds **13a–h** (0.50 g) was treated with 1 mL of TBAF solution (1 M in THF) for 30 minutes. The organic phases were then washed with water (3×40 mL) and dried over anhydrous Na_2SO_4.

Evaporation of the solvent left the crude compounds **14a–h** that were purified by column chromatography, giving a yellow fluorescent solids in very good yield.

14a: Yield: 70% Mp: 206–208 °C from ethyl acetate. FT-IR: $\nu_{C=N}$ 1604; $\nu_{C\equiv C}$ 2306; $\nu_{\equiv C-H}$ 3296 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 5.44 (s, 2H, CH_2); 6.68 (s, 1H, C≡C–H); 7.05 (AA′BB′, 2H, arom.); 7.53 (m, 4H, anthr.); 7.68 (m, 2H, anthr.); 7.89 (AA′BB′, 2H, arom.); 8.51 (m, 2H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): 61.5; 107.0; 114.8; 123.7; 125.8; 126.0; 126.1; 126.4; 129.2; 130.1; 132.1; 133.7; 145.8; 147.6; 157.9; 160.9; 168.0. Elemental Analysis: Calculated for $C_{32}H_{20}N_2O_4$ (MW = 496.52): C, 77.41; H, 4.06; N, 5.64. Found: C, 77.40; H, 4.07; N, 5.65.

14b: Yield: 81%. Mp: 176–178 °C from ethyl acetate. FT-IR: $\nu_{C=N}$ 1604; $\nu_{C\equiv C}$ 2306; $\nu_{\equiv C-H}$ 3296 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 3.06 (s, 1H, ≡CH); 5.43 (s, 2H, CH_2); 6.68 (s, 1H, C≡C–H); 7.03 (AA′BB′, 2H, arom.); 7.44 (m, 4H, anthr.); 7.62 (m, 4H, arom. and anthr.); 7.90 (m, 2H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): 61.5; 107.0; 114.8; 125.3; 125.4; 125.6; 125.7; 126.3; 126.5; 129.5; 130.1; 131.4; 133.7; 137.7; 157.9; 161.0; 167.9. Elemental Analysis: Calculated for $C_{33}H_{20}F_3NO_2$ (MW = 519.52): C, 76.29; H, 3.88; N, 2.70. Found: C, 76.28; H, 3.88; N, 2.72.

14c: Yield: 59%. Mp: 198–200 °C from ethyl acetate. FT-IR: $\nu_{C=N}$ 1606; $\nu_{C\equiv C}$ 2306; $\nu_{\equiv C-H}$ 3297 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 1.91 (s, 3H, CH_3); 3.07 (s, 1H, ≡CH); 5.43 (s, 2H, CH_2); 6.70 (s, 1H, C≡C–H); 7.05 (d, 2H, J = 9 Hz, arom.); 7.48 (m, 8H, anthr.); 7.87 (d, 2H, J = 9 Hz, arom.). ^{13}C-NMR (δ, $CDCl_3$): 19.3; 61.1; 106.7; 114.4; 125.0; 125.2; 125.4; 125.8; 126.4; 127.6; 129.0; 129.6; 129.9; 130.5; 133.3; 157.6; 160.9; 167.3. Elemental Analysis: Calculated for $C_{33}H_{23}NO_2$ (MW = 465.55): C, 85.14; H, 4.98; N, 3.01. Found: C, 85.14; H, 4.99; N, 3.00.

14d: Yield: 66%. Mp: 181–183 °C from ethyl acetate. FT-IR: $\nu_{C=N}$ 1601; $\nu_{C\equiv C}$ 2306; $\nu_{\equiv C-H}$ 3232 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 3.06 (s, 1H, ≡CH); 3.99 (s, 3H, OCH_3); 5.42 (s, 2H, CH_2); 6.66 (s, 1H, C≡C–H); 7.04 (AA′BB′, 2H, arom.); 7.17 (d, 2H, anthr.); 7.44 (m, 4H, anthr.); 7.78 (AA′BB′, 2H, arom.); 7.86 (m, 2H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): 55.3; 61.5; 107.1; 113.8; 114.9; 125.1; 125.4; 126.1; 127.2; 130.1; 130.3; 132.1; 133.7; 158.0; 159.1; 161.3; 167.7. Elemental Analysis: Calculated for $C_{33}H_{23}NO_3$ (MW = 481.55): C, 82.31; H, 4.81; N, 2.91. Found: C, 82.30; H, 4.82; N, 2.92.

14e: Yield: 4%. Mp: 175–180 °C from ethyl acetate. FT-IR: $\nu_{C=N}$ 1605; $\nu_{C\equiv C}$ 2107; $\nu_{\equiv C-H}$ 3289 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 3.06 (s, 1H, ≡CH); 3.59 (s, 6H, OCH_3); 5.39 (s, 2H, CH_2); 6.68 (s, 1H, C≡C–H); 6.83

(AA′BB′, 2H, arom.); 7.03 (d, 2H, anthr.); 7.44 (m, 4H, anthr.); 7.67 (AA′BB′, 2H, arom.); 7.85 (m, 2H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): 55.4; 61.1; 103.8; 114.4; 124.5; 125.2; 125.6; 126.4; 129.4; 129.7; 130.1; 133.3; 157.6; 158.4; 161.1; 167.1. Elemental Analysis: Calculated for $C_{34}H_{25}NO_4$ (MW = 511.58): C, 79.83; H, 5.93; N, 2.74. Found: C, 79.84; H, 5.92; N, 2.73.

14f: Yield: 32%. Mp: 65–68 °C from ethyl acetate. FT-IR: $\nu_{C=N}$ 1606; $\nu_{C=O}$ 1715; $\nu_{C\equiv C}$ 2107; $\nu_{\equiv C-H}$ 3292 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 1.51 (t, 3H, J = 7 Hz, CH_3); 3.06 (s, 1H, ≡CH); 4.51 (q, 2H, J = 7 Hz, OCH_2); 5.42 (s, 2H, CH_2); 6.68 (s, 1H, C=C–H); 7.04 (AA′BB′, 2H, arom.); 7.51 (m, 6H, anthr.); 7.88 (AA′BB′, 2H, arom.); 8.32 (m, 2H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): 14.3; 61.1; 61.5; 107.1; 114.8; 125.6; 126.3; 126.7; 128.0; 129.4; 129.6; 130.6; 131.1; 133.7; 158.0; 161.1; 166.4; 168.3. Elemental Analysis: Calculated for $C_{34}H_{25}NO_4$ (MW = 523.18): C, 80.29; H, 4.81; N, 2.68. Found: C, 80.30; H, 4.86; N, 2.62.

14g: Yield: 84%. Mp: 104–105 °C from ethyl acetate. FT-IR: $\nu_{C=N}$ 1606; $\nu_{C\equiv C}$ 2306; $\nu_{\equiv C-H}$ 3297 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 3.06 (s, 1H, ≡CH); 5.41 (s, 2H, CH_2); 6.65 (s, 1H, C=C–H); 6.77 (m, 1H, fur.); 7.05 (m, 2H, anthr.); 7.49 (m, 5H, anthr.); 7.85 (m, 2H, fur.); 7.97 (m, 1H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): 61.5; 107.0; 114.8; 125.6; 125.9; 126.0; 126.3; 126.5; 126.7; 127.2; 127.9; 128.2; 130.2; 131.1; 143.1; 158.0; 161.0; 167.9. Elemental Analysis: Calculated for $C_{36}H_{23}NO_3$ (MW = 517.58): C, 83.54; H, 4.48; N, 2.71. Found: C, 83.53; H, 4.45; N, 2.75.

14h: Yield: 81%. Mp: 185–190 °C from ethyl acetate. FT-IR: $\nu_{C=N}$ 1605; $\nu_{C\equiv C}$ 2305; $\nu_{\equiv C-H}$ 3295 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 3.07 (s, 1H, ≡CH); 5.46 (s, 2H, CH_2); 6.74 (s, 1H, C=C–H); 7.06 (AA′BB′, 2H, arom.); 7.16 (d, 1H, J = 9 Hz, isoq.); 7.32–7.56 (m, 9H, anthr.); 7.70 (t, 1H, J = 7 Hz, isoq.); 7.93 (AA′BB, 2H, arom.); 8.21 (d, 1H, J = 8 Hz, isoq.); 8.63 (s, 1H, anthr.); 9.54 (s, 1H, isoq.). ^{13}C-NMR (δ, $CDCl_3$): 61.1; 75.9; 82.7; 106.7; 114.4; 115.2; 123.6; 124.9; 125.4; 125.5; 126.0; 126.2; 127.4; 127.6; 127.8; 129.8; 130.3; 130.8; 132.7; 133.3; 135.8; 143.7; 151.9; 157.6; 160.7; 167.6. Elemental Analysis: Calculated for $C_{35}H_{22}N_2O_2$ (MW = 502.57): C, 83.65; H, 4.41; N, 5.57. Found: C, 83.64; H, 4.41; N, 5.56.

General procedure for the synthesis of the 4-amino-4-(10-aryl-substitutedanthracen-9-yl)-1-(4-((trimethylsilyl)ethynyl)phenoxy)but-3-en-2-one ***15a–h***. In a 100 mL round-bottom flask 0.47 mmol of compounds **13a–h** were dissolved in 50 mL MeCN/H_2O 8:2 solution. The solutions were kept under stirring under nitrogen atmosphere for 15 minutes. After this period of time 0.47 mmol of $Mo(CO)_6$ were added and the mixtures were allowed to react heating at 70 °C for 4 h. The mixtures were then cooled down, 80 mL of a mixture of DCM/$CHCl_3$ 1:1 were added and the organic phases separated. The organic phases were washed with 3 × 40 mL brine. The collected organic phases were then dried over anhydrous Na_2SO_4.

Evaporation of the solvent left the crude compounds **15a–h** that were purified by column chromatography, giving a yellow fluorescent solids in very good yield.

15a: Yield: 79%. Mp: 169–173 °C from ethyl acetate. FT-IR: $\nu_{C=O}$ 1590; $\nu_{C\equiv C}$ 2305; ν_{NH2} 3296 and 3476 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 0.25 (s, 9H, CH_3); 4.69 (s, 2H, CH_2); 5.64 (bs, 1H, NH); 5.82 (s, 1H, C=C–H); 6.85 (AA′BB′, 2H, arom.); 7.53 (m, 6H, anthr.); 8.15 (d, 2H, anthr.); 8.49 (AA′BB′, 2H, arom.); 10.49 (bs, 1H, NH). ^{13}C-NMR (δ, $CDCl_3$): −0.1; 29.6; 71.8; 77.3; 95.8; 114.5; 123.7; 125.5; 126.0; 126.2; 126.6; 127.5; 129.1; 132.0; 132.1; 133.4; 136.1; 145.6; 158.2; 161.5; 194.6. Elemental Analysis: Calculated for $C_{35}H_{30}N_2O_4Si$ (MW = 570.72): C, 73.66; H, 5.30; N, 4.91. Found: C, 73.65; H, 5.31; N, 4.91.

15b: Yield: 71%. Mp: 116–119 °C from ethyl acetate. FT-IR: $\nu_{C=O}$ 1586; $\nu_{C\equiv C}$ 2154; ν_{NH2} 3472 and 3943 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 0.26 (s, 9H, CH_3); 4.68 (s, 2H, CH_2); 5.63 (bs, 1H, NH); 5.83 (s, 1H, C=C–H); 6.84 (d, 2H, anthr.); 7.52 (m, 7H, arom. and anthr.); 7.89 (d, 2H, anthr.); 8.13 (d, 2H, arom.); 10.49 (bs, 1H, NH). ^{13}C-NMR (δ, $CDCl_3$): −0.1; 26.8; 71.8; 92.6; 95.8; 104.9; 114.5; 115.8; 125.4; 125.8; 126.4; 126.5; 128.0; 129.3; 131.3; 131.4; 133.4; 142.2; 158.3; 161.8; 194.5. Elemental Analysis: Calculated for $C_{36}H_{30}NO_2Si$ (MW = 593.72): C, 72.83; H, 5.09; N, 2.36. Found: C, 72.82; H, 5.07; N, 2.35.

15c: Yield: 50%. Mp: 212–214 °C from ethyl acetate. FT-IR: $\nu_{C=O}$ 1587; $\nu_{C\equiv C}$ 2155; ν_{NH2} 3472 and 3944 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 0.27 (s, 9H, CH_3); 1.89 (s, 3H, CH_3); 4.68 (s, 2H, CH_2); 5.69 (bs, 1H, NH); 5.85 (s, 1H, C=C–H); 6.86 (d, 2H, anthr.); 7.42 (m, 8H, arom. and anthr.); 8.13 (d, 2H, anthr.); 10.53 (bs, 1H, NH). ^{13}C-NMR (δ, $CDCl_3$): −0.1; 19.7; 71.8; 95.8; 105.0; 114.5; 125.4; 125.5; 126.4; 126.7; 128.0; 129.3; 130.5; 130.8; 130.9; 133.4; 137.6; 158.3; 162.3; 194.2. Elemental Analysis: Calculated for $C_{36}H_{33}NO_2Si$ (MW = 539.75): C, 80.11; H, 6.16; N, 2.60. Found: C, 80.12; H, 6.16; N, 2.61.

15d: Yield: 70%. Mp: 126–129 °C from ethyl acetate. FT-IR: $\nu_{C=O}$ 1604; $\nu_{C\equiv C}$ 2155; ν_{NH2} 3469 and 3943 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 0.26 (s, 9H, CH_3); 3.98 (s, 3H, OCH_3); 4.69 (s, 2H, CH_2); 5.70 (bs, 1H, NH); 5.83 (s, 1H, C=C–H); 6.86 (AA′BB′, 2H, arom.); 7.16 (d, 2H, anthr.); 7.41 (m, 6H, anthr); 7.73 (AA′BB′, 2H, arom.); 8.13 (d, 2H, anthr.); 10.49 (bs, 1H, NH). ^{13}C-NMR (δ, $CDCl_3$): −0.1; 26.8; 55.3; 71.8; 95.8; 105.0; 113.9; 114.7; 125.2; 125.3; 126.3; 126.8; 129.9; 130.2; 130.5; 130.8; 130.9; 133.4; 137.6; 158.3; 162.5; 194.2. Elemental Analysis: Calculated for $C_{36}H_{33}NO_3Si$ (MW = 555.75): C, 77.80; H, 5.99; N, 2.52. Found: C, 77.81; H, 5.98; N, 2.53.

15e: This compound was not prepared because of the extremely low amounts of the precursor.

15f: Yield: 72%. Mp: 114–117 °C from ethyl acetate. FT-IR: $\nu_{C=O}$ 1715; $\nu_{C=O}$ 1602; $\nu_{C\equiv C}$ 2155; ν_{NH2} 3392 and 3944 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 0.27 (s, 9H, CH_3); 1.48 (t, 3H, J = 7 Hz, CH_3); 4.46 (q, 2H, J = 7 Hz, OCH_2); 4.62 (s, 2H, CH_2); 5.81 (s, 1H, C=C–H); 5.90 (bs, 1H, NH); 6.81 (AA′BB′, 2H, arom.); 7.35 (d, 2H, anthr.); 7.48 (m, 2H, anthr); 7.59 (d, 2H, anthr); 8.13 (AA′BB′, 2H, arom.); 8.26 (m, 2H, anthr.); 10.46 (bs, 1H, NH). ^{13}C-NMR (δ, $CDCl_3$): −0.1; 14.3; 26.8; 61.1; 71.6; 95.7; 105.0; 114.5; 115.7; 125.4; 125.7; 126.4; 126.5; 127.9; 129.2; 129.6; 129.9; 131.0; 131.1; 133.4; 137.8; 143.2; 158.2; 162.2; 166.3; 194.3. Elemental Analysis: Calculated for $C_{38}H_{35}NO_4Si$ (MW = 597.79): C, 76.35; H, 5.90; N, 2.34. Found: C, 76.36; H, 5.90; N, 2.34.

15g′: Yield: 58%. Mp: 166–168 °C from ethyl acetate. FT-IR: $\nu_{C=O}$ 1735; $\nu_{C=O}$ 1654; $\nu_{C\equiv C}$ 2305; ν_{NH2} 3296 and 3470 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 3.03 (s, 1H, ≡CH); 4.68 (s, 2H, CH_2); 5.81 (s, 1H, C=C–H); 5.90 (bs, 1H, NH); 6.81 (AA′BB′, 2H, arom.); 7.35 (d, 2H, anthr.); 7.48 (m, 2H, anthr); 7.59 (d, 2H, anthr); 8.13 (AA′BB′, 2H, arom.); 8.26 (m, 2H, anthr.); 10.44 (bs, 1H, NH). ^{13}C-NMR (δ, $CDCl_3$): 14.1; 60.3; 71.7; 75.8; 110.9; 112.5; 114.6; 125.3; 125.5; 125.6; 126.2; 126.4; 126.9; 127.3; 128.0; 128.3; 129.0; 130.1; 133.5; 143.1; 149.7; 158.4; 166.3; 194.3. Elemental Analysis: Calculated for $C_{38}H_{35}NO_4Si$ (MW = 597.79): C, 76.35; H, 5.90; N, 2.34. Found: C, 76.36; H, 5.90; N, 2.34.

15h: Yield: 56%. Mp: > 240 °C from ethyl acetate. FT-IR: $\nu_{C=N}$ 1603; $\nu_{C\equiv C}$ 2157 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 0.27 (s, 9H, CH_3); 4.70 (s, 2H, CH_2); 5.70 (bs, 1H, NH); 5.89 (s, 1H, C=C–H); 6.88 (m, 2H, isoq.); 7.41 (m, 11H, isoq. And anthr.); 8.17 (m, 3H, anthr); 8.47 (s, 1H, isoq.); 9.54 (s, 1H, anthr.); 10.55 (bs, 1H, NH). ^{13}C-NMR (δ, $CDCl_3$): −0.1; 71.7; 77.1; 92.6; 95.8; 104.9; 114.5; 115.8; 125.1; 125.4; 125.6; 126.2; 126.3; 126.4; 126.6; 128.0; 128.1; 128.2; 130.5; 131.6; 132.0; 132.1; 133.4; 142.7; 151.7; 158.3; 161.7. Elemental Analysis: Calculated for $C_{38}H_{32}N_2O_2Si$ (MW = 576.77): C, 79.13; H, 5.59; N, 4.88. Found: C, 79.13; H, 5.60; N, 4.47.

General procedure for the synthesis of the 4-(10-aryl-substituted-anthracen-9-yl)-6-((4-ethynylphenoxy)methyl)-2,2-difluoro-2,3-dihydro-1λ^3,3,2λ^4-oxazaborinine ***17a–h***. In a 100 mL flask 0.30 mmol of compounds **15a–h** were dissolved in 30 mL anhydrous DCM and 7.9 mmol distilled Et_3N were added. The solutions were kept under

stirring under nitrogen atmosphere for 10 minutes. After this period of time excess $BF_3 \cdot Et_2O$ (1 mL) was added and the mixtures were allowed to react at room temperature for 2 h. The mixtures were then diluted with 40 mL DCM and the organic phases washed with 3×40 mL brine. The collected organic phases were then dried over anhydrous Na_2SO_4.

In a 100 mL flask a DCM solution of compounds **16a–h** was treated with 1 mL of TBAF solution (1 M in THF) for 30 minutes. The organic phases were then washed with water (3×40 mL) and dried over anhydrous Na_2SO_4.

Evaporation of the solvent left the crude compounds **17a–h** that were purified by column chromatography, giving a yellow fluorescent solids in very good yield. Compound **17e** was not synthetized because of the extremely low yields of compound **13e** available for all the synthetic steps.

17a: Yield: 41%. Mp: 190–198 °C from ethyl acetate. FT-IR: $\nu_{C=C}$ 1606 cm^{-1}; $\nu_{C\equiv C}$ 2306; $\nu_{C\equiv H}$ 3340; ν_{NH} 3361 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 3.04 (s, 1H, ≡C–H); 4.98 (s, 2H, CH_2); 6.30 (s, 1H, C=C–H); 6.87 (AA′BB′, 2H, arom.); 7.47 (m, 3H, anthr.); 7.61 (m, 5H, anthr.); 7.74 (bs, 1H, NH); 7.89 (d, 2H, anthr.); 8.53 (AA′BB′, 2H, arom.). ^{13}C-NMR (δ, $CDCl_3$): 26.8; 97.8; 114.5; 123.8; 124.2; 126.5; 126.6; 127.3; 127.8; 129.0; 131.9; 133.7; 144.9; 157.4; 177.7. Elemental Analysis: Calculated for $C_{32}H_{21}BF_2N_2O_4$ (MW = 546.34): C, 70.35; H, 3.87; N, 5.13. Found: C, 70.35; H, 3.88; N, 5.14.

17b: Yield: 52%. Mp: 240–244 °C from ethyl acetate. FT-IR: $\nu_{C=C}$ 1618 cm^{-1}; $\nu_{C\equiv C}$ 2305; $\nu_{C\equiv H}$ 3341; ν_{NH} 3361 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 3.03 (s, 1H, ≡C–H); 4.97 (s, 2H, CH_2); 6.30 (s, 1H, C=C–H); 6.87 (d, 2H, arom.); 7.46 (m, 3H, anthr.); 7.59 (m, 5H, anthr.); 7.79 (bs, 1H, NH); 7.90 (m, 2H, arom. and anthr.). ^{13}C-NMR (δ, $CDCl_3$): 76.3; 97.9; 114.5; 124.1; 125.5; 125.6; 126.2; 126.9; 127.4; 127.6; 129.2; 131.2; 133.7; 157.5; 177.5. Elemental Analysis: Calculated for $C_{33}H_{21}BF_5NO_2$ (MW = 569.34): C, 69.62; H, 3.72; N, 2.46. Found: C, 69.61; H, 3.71; N, 2.45.

17c: Yield: 58%. Mp: 139–144 °C from ethyl acetate. FT-IR: $\nu_{C=C}$ 1619 cm^{-1}; $\nu_{C\equiv C}$ 2305; $\nu_{C\equiv H}$ 3297; ν_{NH} 3360 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 1.89 (s, 3H, CH_3); 3.03 (s, 1H, ≡C–H); 4.97 (s, 2H, CH_2); 6.30 (s, 1H, C=C–H); 6.89 (d, 2H, arom.); 7.49 (m, 8H, arom. and anthr.); 7.77 (b, 1H, NH); 7.88 (m, 2H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): 26.8; 77.1; 98.0; 114.5; 124.0; 125.9; 127.2; 127.6; 128.3; 129.2; 130.1; 130.6; 133.7; 157.5; 177.1. Elemental Analysis: Calculated for $C_{33}H_{24}BF_2NO_2$ (MW = 515.37): C, 76.91; H, 4.69; N, 2.72. Found: C, 76.92; H, 4.70; N, 2.73.

17d: Yield: 39%. Mp: 210–215 °C from ethyl acetate. FT-IR: $\nu_{C=C}$ 1607 cm^{-1}; $\nu_{C\equiv C}$ 2305; $\nu_{C\equiv H}$ 3298; ν_{NH} 3361 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 3.03 (s, 1H, ≡C–H); 3.98 (s, 3H, OCH_3); 4.96 (s, 2H, CH_2); 6.30 (s, 1H, C=C–H); 6.86 (AA′BB′, 2H, arom.); 7.15 (d, 2H, anthr.); 7.42 (m, 2H + 1H, anthr. and NH); 7.54 (m, 2H, anthr.); 7.77 (AA′BB′, 2H, arom.); 7.85 (d, 2H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): 55.3; 67.4; 76.3; 83.0; 98.0; 113.9; 114.5; 123.8; 125.6; 127.4; 127.5; 127.6; 129.6; 129.8; 131.8; 133.7; 157.5; 159.3; 174.2; 177.1. Elemental Analysis: Calculated for $C_{33}H_{24}BF_2NO_3$ (MW = 531.37): C, 74.59; H, 4.55; N, 2.64. Found: C, 74.60; H, 4.52; N, 2.64.

17f: Yield: 28%. Mp: > 225 °C (dec.) from ethyl acetate. FT-IR: $\nu_{C=C}$ 1624; $\nu_{C=O}$ 1717 cm^{-1}; $\nu_{C\equiv C}$ 2305; $\nu_{C\equiv H}$ 3299; ν_{NH} 3360 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 1.48 (t, 3H, J = 7 Hz, CH_3); 3.03 (s, 1H, ≡C–H); 3.98 (s, 3H, OCH_3); 4.49 (q, 2H, OCH_2); 4.97 (s, 2H, CH_2); 6.30 (s, 1H, C=C–H); 6.86 (AA′BB′, 2H, arom.); 7.53 (m, 8H + 1H, anthr. and NH); 7.87 (AA′BB′, 2H, arom.); 8.29 (d, 2H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): 14.3; 61.2; 76.4; 97.9; 114.5; 124.0; 126.1; 127.4; 127.5; 127.6; 129.6; 129.8; 131.8; 133.7; 157.5; 166.2; 177.3. Elemental Analysis: Calculated for $C_{35}H_{26}BF_2NO_4$ (MW = 573.40): C, 73.31; H, 4.57; N, 2.44. Found: C, 73.32; H, 4.58; N, 2.44.

17g: Yield: 40%. Mp: 175–179 °C from ethyl acetate. FT-IR: $\nu_{C=C}$ 1618; $\nu_{C\equiv C}$ 2305; $\nu_{C\equiv H}$ 3296; ν_{NH} 3360 cm^{-1}. ^{1}H-NMR (δ, $CDCl_3$): 3.03 (s, 1H, ≡C–H); 4.96 (s, 2H, CH_2); 6.27 (s, 1H, C=C–H); 6.77 (m, 1H, fur.); 6.86 (m, 2H, anthr.); 7.42–7.85 (m, 7H, fur., arom. and NH); 7.98 (d, 2H, anthr.). ^{13}C-NMR (δ, $CDCl_3$): 67.4; 77.1; 83.0; 97.8; 111.0; 112.9; 114.5; 123.9; 126.5; 126.9; 127.4; 130.7; 133.7; 149.1; 157.4; 173.9; 177.5. Elemental Analysis: Calculated for $C_{30}H_{20}BF_2NO_3$ (MW = 491.30): C, 73.34; H, 4.10; N, 2.85. Found: C, 73.36; H, 4.11; N, 2.85.

17h: Yield: 12%. Mp: > 240 °C from ethyl acetate. FT-IR: $\nu_{C=C}$ 1637; $\nu_{C\equiv C}$ 2157 cm^{-1}, $\nu_{C\equiv H}$ 3296; ν_{NH} 3360 cm^{-1}. ^{1}H-NMR (δ, DMSO): 5.10 (s, 2H, CH_2); 6.20 (s, 1H, NH); 6.27 (s, 1H, C=C–H); 7.07 (m, 2H, isoq.); 7.41 (m, 12H, isoq. And anthr.); 7.83 (m, 2H, anthr); 8.38 (s, 1H, isoq.); 8.59 (s, 1H, anthr.); 11.30 (bs, 1H, NH). ^{13}C-NMR (δ, DMSO): 67.1; 79.6; 83.2; 97.7; 115.2; 124.4; 124.5; 126.3; 126.4; 126.8; 127.0; 127.5; 128.3; 128.4; 129.7; 131.5; 132.9; 133.6; 135.1; 135.2; 144.0; 144.3; 153.2; 157.7; 172.2; 175.5. Elemental Analysis: Calculated for $C_{35}H_{23}BF_2N_2O_2$ (MW = 552.39): C, 76.10; H, 4.20; N, 5.07. Found: C, 76.11; H, 4.21; N, 5.06.

UV-Vis and Fluorescence Spectroscopic Analysis. In order to assess the optical properties of the synthetized fluorescent probes **14a–h** and **17a–h** and in order to evaluate their potential use in ABPP, we have performed some UV-Vis and fluorescence spectroscopic analysis. These analyses were performed in 3 different solvents with different polarity (DCM, MeOH), considering also the possible applications in the cellular environment (DMSO/H_2O 1:1).

UV-Vis spectra of the compounds **14a–h** and **17a–h** were registered in some different solvents (DCM, MeOH and DMSO/H_2O 1:1) with a Jasco V-550 UV/VIS Spectrophotometer. The maxima of absorption were acquired and all the obtained spectra are reported in graphics in the SI.

Fluorescence spectra of compounds **14a–h** and **17a–h** were registered in different solvents (DCM, MeOH and DMSO/H_2O 1:1) with a Perkin-Elmer LS 55 Luminescence Spectrometer. The maxima of excitation and emission were acquired and the fluorescence spectra are reported in the graphics in the SI.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: isoxazoles · enamino-ketones · fluorescent probes · boron complexes · photophysical studies

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