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Synthesis of fluorescent tetracyclic lactams by a "one pot" three steps palladium-catalyzed borylation, Suzuki coupling (BSC) and lactamization DNA and polynucleotides binding studies

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Abstract

Tetracyclic lactams (benzothieno[2,3-*c*]quinolones) were prepared by "one pot" three steps palladium-catalyzed borylation, Suzuki coupling (BSC) and lactamization, starting from *ortho*-haloanilines and alkyl 3-bromobenzo[*b*]thiophene-2-carboxylates. The former were used as the components to be borylated with pinacolborane, and the latter as the brominated component in the Suzuki coupling. The amidation occurred with loss of the alkyl alcohol, presumably in the Suzuki coupling product, giving the corresponding tetracyclic lactam. This constitutes a novel application of the BSC reaction using sterically hindered substrates. In this work studies of absorption and fluorescence in several solvents and in presence of salmon sperm DNA or synthetic double-stranded (ds) heteropolynucleotides, poly(dA–dT)·(dA–dT) and poly(dG–dC)·(dG–dC), were performed. The binding constant values ($K_i = 2.6 \times 10^5$ to $4.5 \times 10^5\ M^{-1}$) point to a high affinity of the lactams to DNA. It was shown that the intercalation is the preferred mode of binding and that the substituted new lactams (with F or OMe) exhibit a higher affinity for A–T regions.

Quenching experiments with iodide show that the methoxylated lactam is the more intercalative in DNA. The same type of experiments using this compound bound to heteropolynucleotides show a very low accessibility ($f_a = 0.07$) of the lactam in poly(dG–dC)·(dG–dC) to the quencher showing a large majority of intercalative binding while the high affinity for A–T regions together with a higher accessibility ($f_a = 0.25$) point to the possibility of both intercalative (75%) and groove modes of binding.

Keywords: Palladium-catalyzed BSC reaction; Lactamization; Benzothieno[2,3-*c*]quinolones; Fluorescence; DNA and polynucleotides binding

1. Introduction

Two main methods were used in the synthesis of benzothieno[2,3-*c*]quinolin-6(5*H*)-ones **I** (Fig. 1): photocyclization of benzo[*b*]thiophene-2-carboxanilides [1] or of several 3-chlorobenzo[*b*]thiophene-2-carboxanilides [2–5]. Some of the benzothieno[2,3-*c*]quinolinones prepared by these methods have shown antitumoral activity against several types of malignant cell lines (carcinomas and melanoma) [4,5] and their intercalative DNA binding properties were proven [5].

Recently another method appeared in the literature for the synthesis of this type of compounds (*N*-methylated) by a "one pot' consecutive palladium-catalyzed regioselective aryl–aryl and *N*-aryl coupling of *o*-iodotoluene with 3-bromobenzo[*b*]thiophene-2-carboxylic acid methylamide using $Pd(OAc)_2$, tri-2-furylphosphine as ligand, norbornene, K_2CO_3 as base, in DMF at 50 °C. Insertion and deinsertion of norbornene during the catalytic cycle, originates a product with a C–C bond in the *ortho*-position relative to the position of the C–N cyclization [6].

We have already described successfully the application of the "one pot" two steps palladium-catalyzed, borylation and Suzuki coupling (BSC reaction) using Baudoin's conditions [7] to the synthesis of 2-methyl-2′-nitrodiaryl compounds in the benzo[*b*]thiophene series [8]. These compounds were prepared

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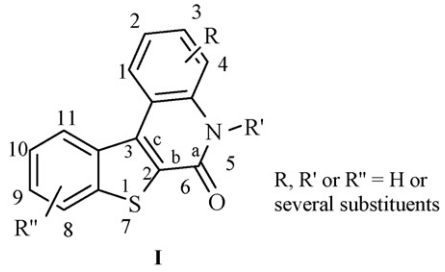

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Fig. 1. General structure of benzothieno[2,3-*c*]quinolin-6(5*H*)-ones.

as precursors of thienocarbazoles, analogues of the natural antitumoral pyridocarbazoles (ellipticine and olivacine), by reductive cyclization [8].

Here we describe the use of the palladium-catalyzed BSC methodology, including also an *in situ* amidation reaction, to synthesize new substituted benzothieno[2,3-*c*]quinolin-6(5*H*)-ones. Like many planar polycyclic aromatic molecules, they may intercalate between DNA base pairs, which seems to be an essential (but not sufficient) step for antitumoral activity. The understanding of the influence of different substituents on the binding to nucleic acids is also crucial to new drug design [9,10].

The interaction of the new substituted lactams obtained with natural double-stranded salmon sperm DNA and with synthetic ds-polyheteronucleotides was studied by absorption and fluorescence spectroscopies. DNA binding studies of the already known non-substituted lactam [3] were also performed, for comparison.

2. Experimental

2.1. Synthesis

2.1.1. General remarks

Melting points were determined on a Gallenkamp apparatus and are uncorrected. The ^{1}H NMR spectra were measured on a Varian Unity Plus at 300 MHz. Spin–spin decoupling techniques were used to assign the signals. The ^{13}C NMR spectra were measured in the same instrument at 75.4 MHz (using DEPT θ 45°). Mass spectra (EI) and HRMS were made by the mass spectrometry service of University of Vigo-Spain.

Column chromatography was performed on Macherey-Nagel silica gel 230–400 mesh using solvent gradients. Petroleum ether refers to the boiling range 40–60 °C.

2.1.2. General procedure for "one pot" palladium-catalyzed borylation and Suzuki coupling (BSC) and amidation (Scheme 1)

A dried Schlenk tube was charged under Ar with dry dioxane (4 mL), 2-bromoaniline, 2-bromo-4-fluoroaniline or 2-chloro-5-methoxyaniline hydrochloride (0.500 mmol), $Pd(OAc)_2$ (5 mol%), 2-(dicyclohexylphosphino)biphenyl (20 mol%), pinacolborane (3 equiv., unless stated) and NEt_3 (4 equiv.) was added. The mixture was left stirring for 2 h at 100 °C. After cooling the methyl or ethyl 3-bromobenzo[*b*]thiophene-2-carboxylate (1 equiv.) and $Ba(OH)_2{\cdot}8H_2O$ (3 equiv.) were added, and the mixture was heated again at 100 °C for two more hours.

2.1.3. 6-Oxo-5,6-dihydro[1]benzo[b]thieno [2,3-c]quinoline (**1**)

The procedure above was followed, using 2-bromoaniline (0.0860 g, 0.500 mmol) and ethyl 3-bromobenzo[*b*]thiophene-2-carboxylate with addition also of H_2O (0.5 mL) in the second step. A precipitate came out which was filtered after cooling the reaction mixture. The solid was washed with H_2O (10 mL) and it was poured in ethyl acetate (20 mL) stirring for 2 h at 40 °C. The remaining solid was removed by filtration and removal of solvent of the filtrate gave lactam **1**, as a bege solid (0.0503 g, 40%), mp > 300 °C (lit. [3] >280 °C). ^{1}H NMR (DMSO-d_6): 7.36–7.44 (m, 1H, ArH), 7.54–7.62 (m, 2H, ArH), 7.64–7.72 (m, 2H, ArH), 8.24–8.30 (m, 1H, ArH), 8.77 (d, J = 7.8 Hz, 1H, ArH), 8.90–8.95 (m, 1H, ArH), 12.20 (br s, 1H, NH).

2.1.4. 2-Fluoro-6-oxo-5,6-dihydro[1]benzo[b] thieno[2,3-c]quinoline (**2**)

The procedure described above was followed, using 2-bromo-4-fluoroaniline (0.0950 g, 0.500 mmol) and methyl 3-bromobenzo[*b*]thiophene-2-carboxylate. After cooling water and ethyl acetate were added and a precipitate came out and it was filtered. The solid was then poured into ethyl acetate (20 mL) and heated with stirring for 2 h at 40 °C. The remaining solid was filtered and solvent removal of the filtrate gave lactam **2** as a colourless solid (0.0710 g, 50%) mp > 300 °C. ^{1}H NMR (DMSO-d_6): 7.45–7.52 (m, 1H, 3-H), 7.56 (dd, J = 9.2 and 5.4 Hz, 1H, 4-H), 7.64–7.72 (m, 2H, ArH), 8.24–8.28 (m, 1H, ArH), 8.46 (dd, J = 10.6 and 2.7 Hz, 1H, 1-H), 8.87–8.90 (m, 1H, ArH), 12.31 (broad s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): 109.05 (108.89, 109.21, d, J = 24.45 Hz, 1-CH), 116.70 (116.54, 116.86, d, J = 23.9 Hz, 3-CH), 117.86 (117.81, 117.92, d, J = 8.9 Hz, C), 118.70 (118.63, 118.75, d, J = 8.6 Hz, 4-CH), 124.12 (CH), 125.68 (CH), 126.02 (CH), 127.53 (CH), 133.48 (C), 134.78 (C), 135.03 (134.78, 135.01, d, J = 3.17 Hz, C), 135.24 (C), 141.11 (C), 157.59 (156.012, 159.170, d, J = 238.2 Hz, CF), 157.91 (C=O); MS (EI) m/z (%) 271 (M^+ + 2, 6), 270 (M^+ + 1, 17.4), 269 (M^+, 100); HRMS: $C_{15}H_8FNOS$ requires M^+ 269.0311. Found 269.0303.

2.1.5. 3-Methoxy-6-oxo-5,6-dihydro[1]benzo[b]thieno [2,3-c]quinoline (**3**)

The procedure described above was followed, using 2-chloro-5-methoxyaniline hydrochloride (100 mg, 0.500 mmol), pinacolborane (5 equiv.) and ethyl 3-bromobenzo[*b*]thiophene-2-carboxylate. After cooling ethyl acetate was added and the mixture was filtered. To the filtrate H_2O was added and the phases were separated. The organic phase was dried and filtered, and the solvent removal gave an oil. This was submitted to column chromatography using as eluents from 50% ethyl acetate/petroleum ether till 100% ethyl acetate. Lactam **3** was

isolated as a colourless solid (0.060 g, 40%), mp > 300 °C. ^{1}H NMR (DMSO-d_6): 3.86 (s, 3H, OMe), 7.00 (dd, J = 9.0 and 2.7 Hz, 1H, 2-H), 7.07 (d, J = 2.7 Hz, 1H, 4-H), 7.64–7.70 (m, 2H, ArH), 8.21–8.24 (m, 1H, ArH), 8.67 (d, J = 9.0 Hz, 1H, 1-H), 8.85–8.88 (m, 1H, ArH), 12.18 (broad s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): 55.37 (OCH_3), 99.85 (4-CH), 110.92 (2-CH), 111.52 (C), 124.16 (CH), 125.03 (1-CH), 125.70 (CH), 125.80 (CH), 127.49 (CH), 129.33 (C), 135.36 (C), 136.26 (C), 139.54 (C), 141.40 (C), 158.21 (C), 159.53 (C) ppm; MS (EI) m/z (%) 283 ($M^+ + 2$, 7), 282 ($M^+ + 1$, 19), 281 (M^+, 100), 238 ($M^+ - 43$, 48); HRMS: $C_{16}H_{11}NO_2S$ requires M^+ 281.0511. Found 281.0516.

2.2. Spectroscopic measurements

Absorption spectra were recorded in a Shimadzu UV-3101PC UV–vis–NIR spectrophotometer. Fluorescence measurements were performed using a Fluorolog 3 spectrofluorimeter. For fluorescence quantum yield determination (λ_{exc} = 335 nm), the solutions were previously bubbled for 20 min with ultrapure nitrogen. Fluorescence spectra were corrected for the instrumental response of the system. The fluorescence quantum yields (Φ_s) were determined using the standard method (Eq. (1)) [11]. 9,10-Diphenylanthracene in ethanol was used as reference, Φ_r = 0.95 [12].

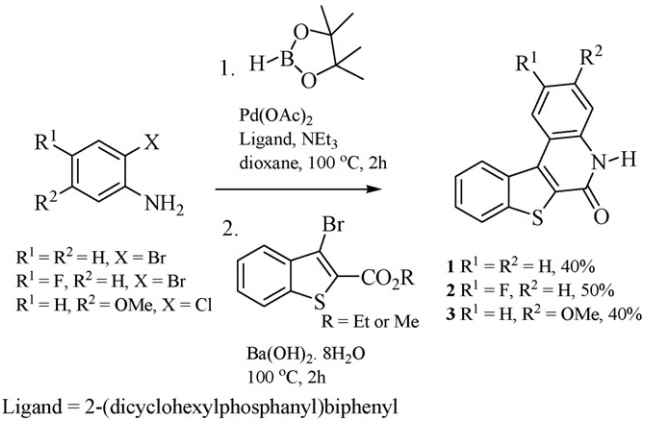
$$\Phi_s = \left(\frac{A_r F_s n_s^2}{A_s F_r n_r^2}\right) \times \Phi_r \quad (1)$$

A is the absorbance at the excitation wavelength, F the integrated emission area and n is the refraction index of the solvents used. Subscripts refer to the reference (r) or sample (s) compound.

All solutions were prepared using spectroscopic grade solvents and Milli-Q grade water.

Natural double-stranded salmon sperm DNA was obtained from Invitrogen, while synthetic double-stranded heteropolynucleotides, poly(dA–dT)·(dA–dT) and poly(dG–dC)·(dG–dC), were obtained from Sigma–Aldrich. Salmon sperm DNA, heteropolynucleotides and lactams stock solutions were prepared in 10^{-2} M Tris–HCl buffer (pH 7.2), with 10^{-3} M EDTA and 0.1 M sodium chloride. The DNA and polynucleotide concentrations in number of bases (or phosphate groups) were determined from the molar extinction coefficients [10], ε = 6600 M^{-1} cm^{-1} at 260 nm for DNA, ε = 8400 M^{-1} cm^{-1} at 254 nm for poly(dG–dC)·(dG–dC) and ε = 6600 M^{-1} cm^{-1} at 260 nm for poly(dA–dT)·(dA–dT).

For DNA or synthetic polynucleotides interaction studies, the absorption and fluorescence titrations were performed by keeping the concentration of lactams constant (4×10^{-6} M) while varying the nucleic acid concentration, in order to obtain increasing [nucleic acid]/[compound] ratios. This was done by mixing the required volume of lactam and DNA (or polynucleotide) stock solutions, while the total volume of the mixture was kept constant. All solutions were left to stabilize. The absorbance at excitation wavelengths was always less than 0.1, in order to avoid inner filter effects.



Scheme 1. Synthesis of lactams **1–3** by "one pot" three steps palladium-catalyzed BSC and lactamization.

3. Results and discussion

3.1. Synthesis

In this work we present the synthesis of benzothieno[2,3-*c*]quinolin-6(5*H*)-ones **1–3** (Scheme 1) by a "one pot" three steps palladium-catalyzed borylation and Suzuki coupling (BSC reaction) and amidation, from *ortho*-haloanilines, as the component to be borylated with pinacolborane (forming a C–B bond) and alkyl 3-bromobenzo[*b*]thiophene-2-carboxylates, earlier prepared by us [13], as the brominated component in the Suzuki coupling (forming a C–C bond). The amidation reaction occurred presumably in the Suzuki coupling intermediate product, which was not possible to isolate, through a nucleophilic attack of the nitrogen atom of the amine on the carbonyl of the ester group with loss of methanol or ethanol. The conditions used, namely the electron-rich sterically hindered ligand, 2-(dicyclohexylphosphanyl)biphenyl and the barium hydroxide as base are indicated for sterically hindered substrates and, as postulated by Baudoin et al. [7] the borylation should be performed on a component bearing an *ortho*-EDG (electron-donating group) with the other Suzuki coupling component having an *ortho*-EWG (electron-withdrawing group).

This constitutes a novel method for the synthesis of these types of tetracyclic lactams and compounds **2** and **3**, as far as our knowledge, are new ones and were fully characterized.

This method allows the use of two bromo components conveniently substituted as starting materials. The palladium-catalyzed borylation *in situ* avoids the preparation and isolation of boronic acids or esters and occurs with atom economy because in general a halogen–lithium exchange preceeds the boron-transmetalation. The borylation occurred either using *ortho*-bromo or *ortho*-chloroanilines which enhances the scope of the reaction.

3.2. Fluorescence studies in several solvents

Fluorescence properties of lactams **1–3** were studied in several solvents. As an example, the absorption and emission spectra of compounds **1–3** in dichloromethane are displayed in

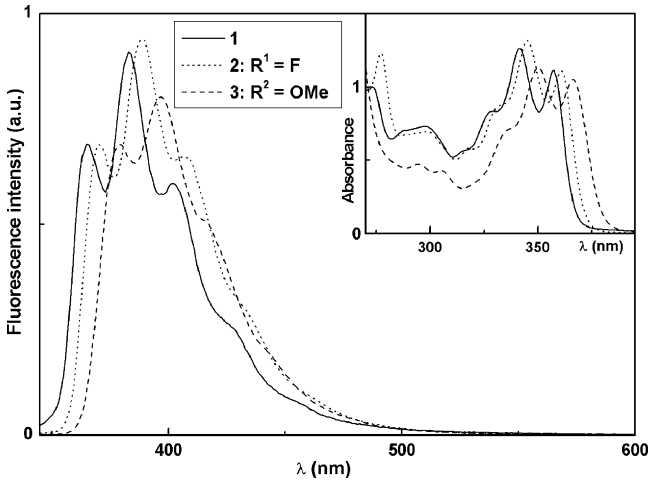


Fig. 2. Normalized fluorescence (at peak of maximum energy) spectra of 4×10^{-6} M solutions of compounds **1–3** in dichloromethane ($\lambda_{exc} = 335$ nm). Inset: Absorption spectra of 10^{-4} M solutions of the same compounds in dichloromethane.

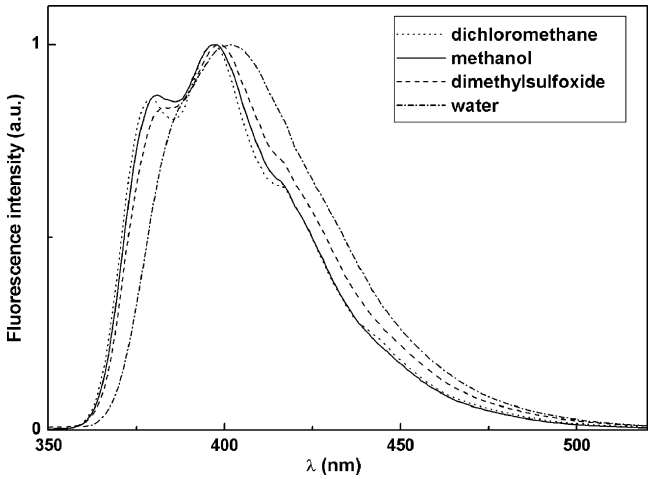


Fig. 3. Normalized fluorescence spectra (at maximum emission) of 4×10^{-6} M solutions of lactam **3** in several solvents.

Fig. 2. It can be observed that the substitution with an OMe group (EDG) in compound **3** produces a significant red shift of absorption and emission spectra, changing also the vibrational peaks ratio, when compared with the spectra of the non-substituted compound **1**. Compound **2** exhibits a smaller red shift (Fig. 2), may be due to the dual character of the F atom, which is an EDG by mesomeric effect (+M) and an EWG by inductive effect (−I).

The fluorescence quantum yields were determined in several solvents (Table 1), showing that the compound with an OMe group exhibits higher Φ_F values in all solvents. For all compounds, the quantum yields in protic solvents decrease with increasing solvent hydrogen bonding capability (Φ_F in ethanol > Φ_F in methanol > Φ_F in water), may be due to an increase of S → T intersystem crossing efficiency through H-bond formation between lactams and solvent. The lactam group can establish H-bonds through the carbonyl (acceptor) and the N–H (donor).

Compounds **1** and **2** exhibit small solvent effects in both absorption and emission maxima wavelengths, λ_{abs} and λ_{em}. For lactam **3**, a significant red shift and loss in vibrational structure of the fluorescence spectra can be observed in polar solvents, with a structureless emission in water (Fig. 3). This behaviour may be due to the electron-donating properties of the OMe group, which can increase the charge transfer character (ICT) of the excited state.

3.3. Spectroscopic studies of interaction with salmon sperm DNA and with synthetic double-stranded polynucleotides

The interaction of the new lactams **2** and **3** with natural double-stranded salmon sperm DNA was studied by absorption and fluorescence. DNA binding studies of the non-substituted lactam **1** were also performed for comparison.

Fig. 4 shows the emission spectra of the unsubstituted lactam **1** with increasing [DNA]/[compound] ratio, where [DNA] is expressed in number of bases or phosphates, using the molar extinction coefficient at 260 nm, $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ [10]. An enhancement in emission intensity is observed with increasing DNA concentration. No detectable changes are observed for [DNA]/[lactam] < 2. In absorption spectra (inset of Fig. 4) the differences are negligible. No more variations in emission are observed for [DNA]/[compound] above 18, indicating total compound binding at this [DNA]/[compound] ratio (spectra corresponding to ratio 18 and 25 are overlapped).

A similar behaviour in absorption and emission is displayed by lactam **2** (substituted with a F atom) with increasing DNA content (Fig. 5).

Table 1
Fluorescence quantum yields (Φ_F) and absorption and emission maxima wavelengths (λ_{abs} and λ_{em}) for lactams **1–3**

Solvent	Compound **1**			Compound **2**			Compound **3**		
	λ_{abs} (nm)	λ_{em} (nm)	Φ_F[a]	λ_{abs} (nm)	λ_{em} (nm)	Φ_F[a]	λ_{abs} (nm)	λ_{em} (nm)	Φ_F[a]
Cyclohexane	339	380	0.06	346	386	0.08	348	389	0.10
Dichloromethane	342	383	0.11	345	388	0.13	350	397	0.39
DMF	343	388	0.11	347	394	0.16	350	397	0.30
DMSO	343	388	0.07	347	395	0.12	351	399	0.20
Ethanol	342	382	0.13	345	387	0.19	350	397	0.29
Methanol	342	382	0.10	345	387	0.16	350	398	0.27
Water	342	383	0.05	346	387	0.07	351	403	0.08

[a] Relative to 9,10-diphenylanthracene in ethanol ($\Phi_r = 0.95$) [12].

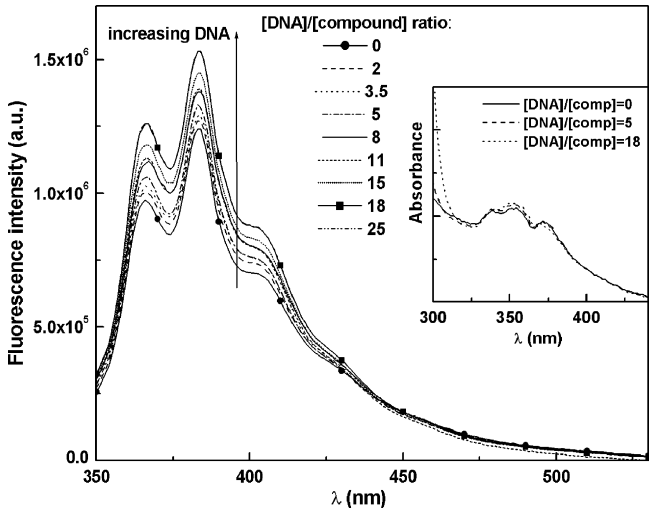


Fig. 4. Fluorescence spectra of lactam **1** (4×10^{-6} M) in 10^{-2} M Tris–HCl buffer (pH 7.2), with increasing DNA content. Inset: Absorption spectra for different [DNA]/[compound] ratios.

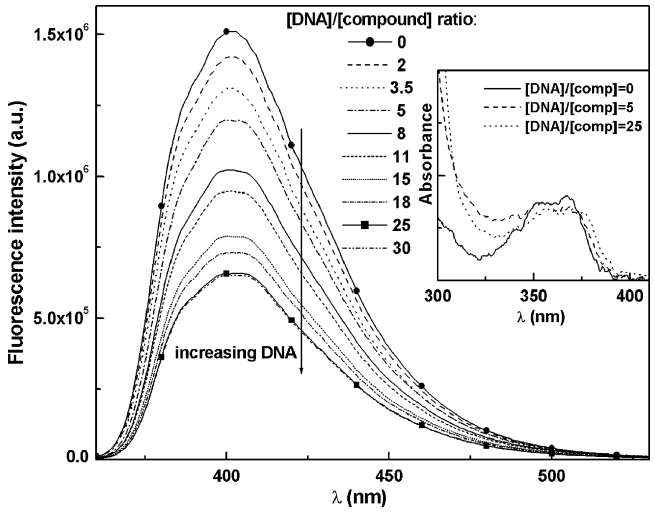


Fig. 6. Fluorescence spectra of lactam **3** (4×10^{-6} M) in 10^{-2} M Tris–HCl buffer (pH 7.2), with increasing DNA content. Inset: Absorption spectra for different [DNA]/[compound] ratios.

However, for lactam **3** (with a OMe group) a pronounced decrease in fluorescence emission is observed (Fig. 6). This may indicate a different type of interaction of lactam **3** with DNA bases, namely by charge transfer processes induced by the presence of the OMe group. For both substituted lactams **2** and **3**, total binding (inferred from the invariance of emission with increasing [DNA]) is attained at [DNA]/[lactam] = 25. For lactam **3**, changes in absorption spectrum in the long wavelength region are observed, especially for high [DNA]/[lactam] ratio (inset of Fig. 6). This result may suggest that compound **3** may present a higher degree of intercalation in double-stranded DNA than compounds **1** and **2** [14].

For the new lactams **2** and **3**, the base sequence binding preference was also investigated, using synthetic ds-heteropolynucleotides, poly(dA–dT)·(dA–dT) and poly(dG–dC)·(dG–dC). Normalized emission spectra for lactams **2** and **3**, in the absence and in presence of nucleic acids (DNA and synthetic polynucleotides) are presented in Figs. 7 and 8. At insets are shown the absorption spectra. Figs. 9 and 10 display the ratio of maximum emission intensities in the presence (I) and absence (I_0) of nucleic acids for several [nucleic acid]/[compound] ratios, for lactam **2** and **3**, respectively.

Comparing I/I_0 curves for lactams **2** and **3** in the presence of DNA and heteropolynucleotides (Figs. 9 and 10), it seems that the behaviour of both lactams bound to DNA is more similar to that observed in poly(dA–dT)·(dA–dT) than in poly(dG–dC)·(dG–dC), specially for lactam **2**.

The binding constants and binding site sizes were determined by the modified Scatchard equation, given by McGhee and von Hippel [15,16]:

$$\frac{r}{c_f} = K_i(1 - nr)\left[\frac{1 - nr}{1 - (n-1)r}\right]^{n-1} \quad (2)$$

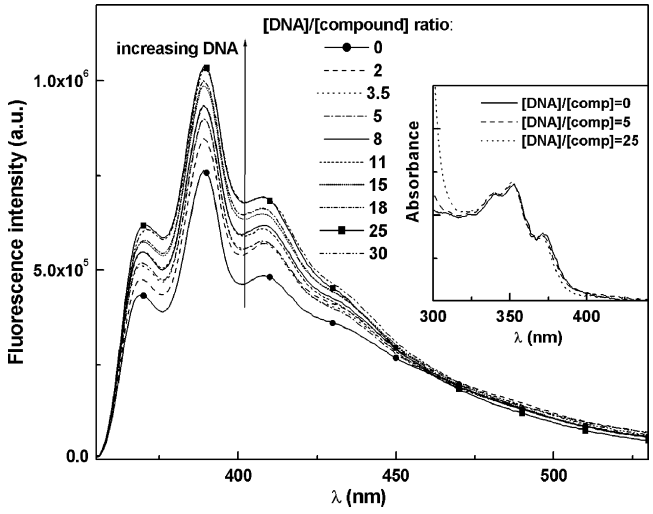


Fig. 5. Fluorescence spectra of lactam **2** (4×10^{-6} M) in 10^{-2} M Tris–HCl buffer (pH 7.2), with increasing DNA content. Inset: Absorption spectra for different [DNA]/[compound] ratios.

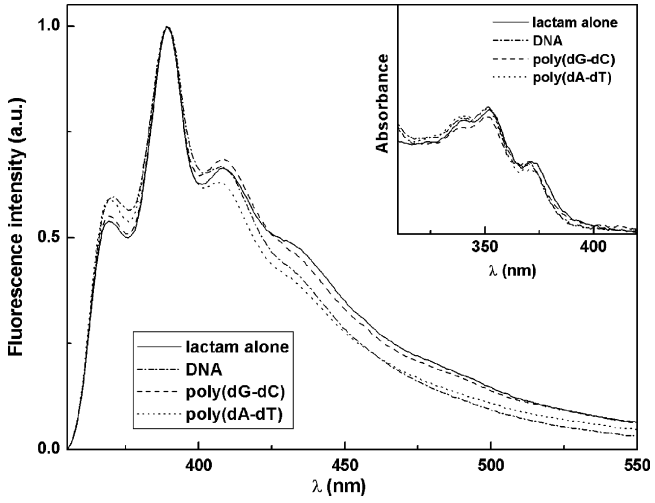


Fig. 7. Normalized fluorescence spectra of lactam **2** (4×10^{-6} M) solutions in Tris–HCl buffer (pH 7.2), for [nucleic acid]/[compound] = 0 and 25. Inset: Corresponding absorption spectra.

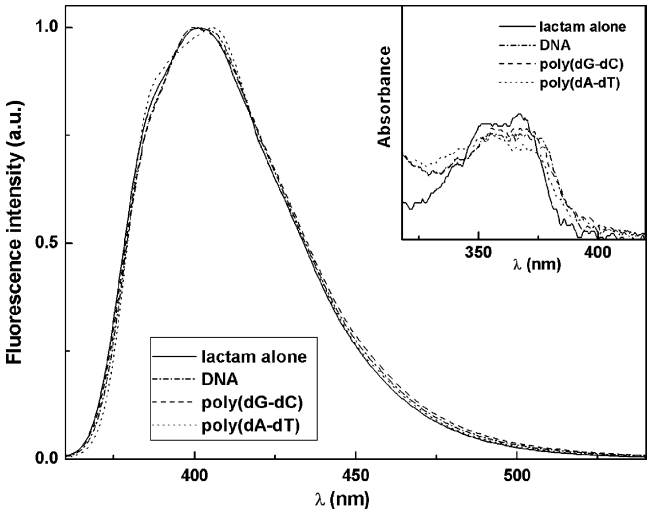


Fig. 8. Normalized fluorescence spectra of lactam **3** (4×10^{-6} M) solutions in Tris–HCl buffer (pH 7.2), for [nucleic acid]/[compound] = 0 and 25. Inset: Corresponding absorption spectra.

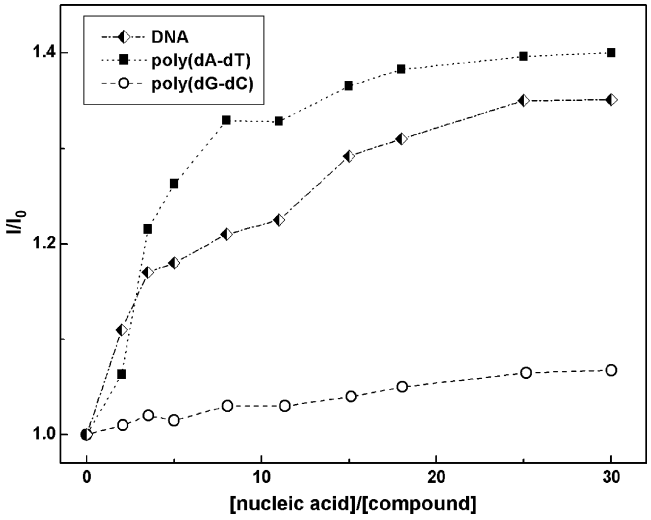


Fig. 9. Ratio of maximum emission intensities in the presence (I) and absence (I_0) of nucleic acids for lactam **2** at several [nucleic acid]/[compound] ratios.

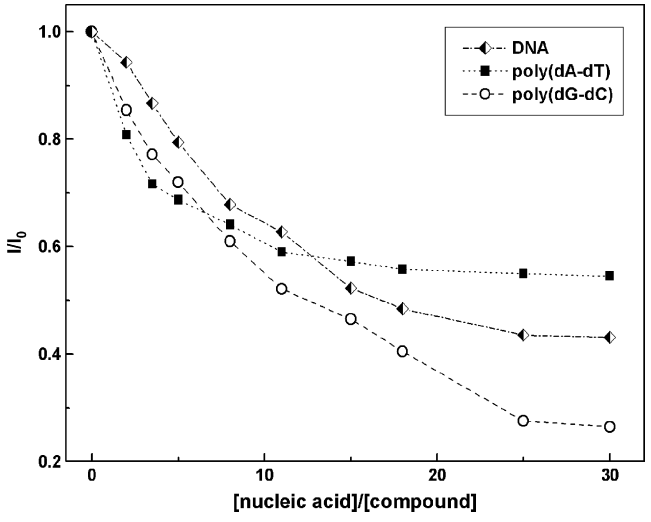


Fig. 10. Ratio of maximum emission intensities in the presence (I) and absence (I_0) of nucleic acids for lactam **3** at several [nucleic acid]/[compound] ratios.

Table 2
Values of binding constants (K_i) and binding site sizes (n) for lactam interaction with DNA and synthetic heteropolynucleotides

Compound	Nucleic acid	K_i (M^{-1})	n
1 ($R^1 = R^2 = H$)	Salmon sperm DNA	$(4.5 \pm 0.5) \times 10^5$	9.1 ± 2.7
2 ($R^1 = F$)	Salmon sperm DNA	$(2.6 \pm 0.3) \times 10^5$	7.3 ± 2.2
	Poly(dA–dT)·(dA–dT)	$(6.6 \pm 0.6) \times 10^5$	6.4 ± 1.9
	Poly(dG–dC)·(dG–dC)	$(8.6 \pm 0.8) \times 10^4$	8.3 ± 2.5
3 ($R^2 = OMe$)	Salmon sperm DNA	$(4.1 \pm 0.4) \times 10^5$	9.2 ± 2.8
	Poly(dA–dT)·(dA–dT)	$(7.8 \pm 0.7) \times 10^5$	6.1 ± 1.8
	Poly(dG–dC)·(dG–dC)	$(2.4 \pm 0.2) \times 10^5$	9.1 ± 2.7

where K_i is the intrinsic binding constant, n the binding site size in base pairs, r the ratio c_b/[nucleic acid], and c_b and c_f are the concentrations of bound and free compound, respectively.

The fluorescence measurements data were fitted by least squares methods to obtain the values of the binding constants (K_i) and the number of base pairs between consecutive intercalated compound molecules (n). The results are presented in Table 2.

The binding constant values are slightly smaller than those obtained for similar compounds (using concentrations of 6×10^{-5} M) with a methyl ester and an isopropylamidino groups in each benzene ring, with the amidine group predominantly protonated [5]. Our results can be attributed to the fact that lactams **1–3** are neutral molecules. In our case we need also a higher [nucleic acid]/[compound] ratio to achieve complete binding in spectral terms which can be due to the low lactam concentration used in our assays (4×10^{-6} M). This compound concentration influence was also observed in binding studies of a pyrene derivative with polynucleotides [17].

Lactam **3** presents a similar DNA binding constant to lactam **1** and higher binding constants than lactam **2** in all the nucleic acids used. The new substituted lactams **2** and **3** exhibit higher affinity for A–T binding sites, as the binding constants are larger for poly(dA–dT)·(dA–dT) than for poly(dG–dC)·(dG–dC). The binding site sizes are also smaller for A–T sequences than for G–C sequences (Table 2), showing that more G–C base pairs are excluded between consecutive intercalated lactam molecules. It has been reported that many cationic drug intercalators exhibit slight to pronounced preferences for binding at dG–dC sites, while polycyclic aromatic molecules without ionic sites or groups prefer intercalation at alternating dA–dT sequences [18], which is in agreement with our results. Despite this, a higher affinity for poly(dG–dC)·(dG–dC) of the methoxylated lactam **3** is observed than for the fluorinated lactam **2** (Table 2).

Fluorescence quenching experiments with iodide ion were also performed for compounds **1–3** in the presence of DNA. The quenching data were first plotted according to the Stern–Volmer relation (Eq. (3)) [19]:

$$\frac{I_0}{I} = 1 + K_{SV}[Q] \tag{3}$$

where I_0 and I are, respectively, the fluorescence intensities in the absence and in the presence of quencher (I^-), K_{SV} the Stern–Volmer constant and [Q] is the quencher concentration.

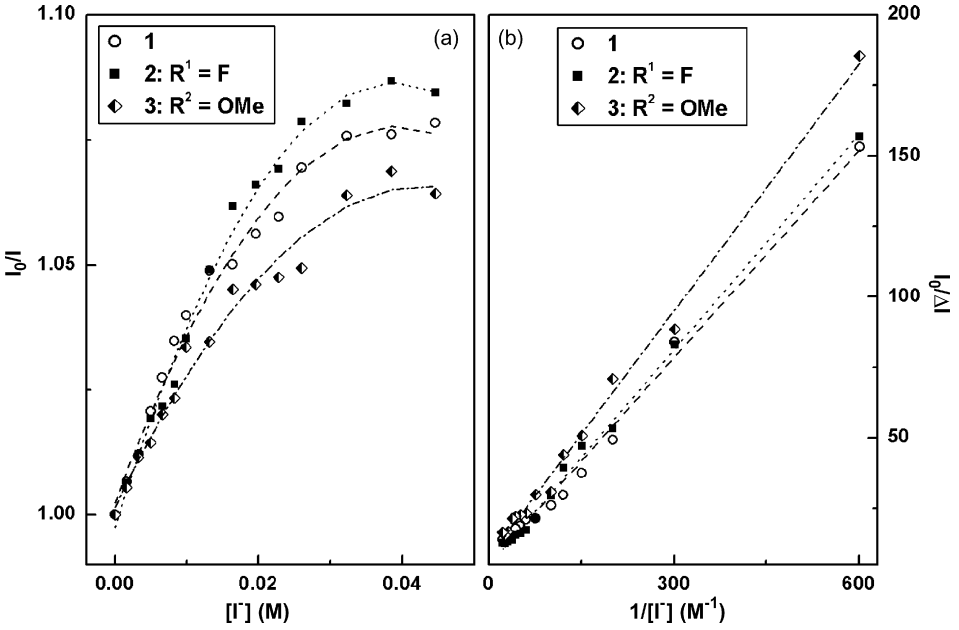


Fig. 11. (a) Stern–Volmer plots for quenching with iodide ion (I^-) of lactams **1**–**3** with salmon sperm DNA; (b) corresponding modified Stern–Volmer plots.

Stern–Volmer plots are non-linear (Fig. 11a), with a downward curvature. This means that not all fluorescent molecules are accessible to the quencher. In this case, the system contains heterogeneously emitting sites (some compound molecules accessible to the quencher and other not accessible) and the Stern–Volmer equation must be modified [20] as follows:

$$\frac{I_0}{\Delta I} = \frac{1}{f_a} + \frac{1}{f_a K_{SV}[Q]} \quad (4)$$

where $\Delta I = I_0 - I$ and f_a is the accessibility to quencher. From the plots of $I_0/\Delta I$ versus $1/[Q]$, it is possible to obtain the accessibilities to quencher (Fig. 11b). The results are summarized in Table 3.

Anionic quenchers can be used to distinguish between DNA binding modes [17,21,22]. Intercalated chromophores are less accessible to quenching by iodide due to electrostatic repulsion between the negatively charged DNA and iodide ion [22]. Compounds which are bound at the DNA surface (groove binding or electrostatic binding) are more accessible and therefore emission from these molecules can be quenched more efficiently. As lactams are neutral molecules, electrostatic binding to nucleic acids is not expected to occur. Therefore, the fraction of lac-

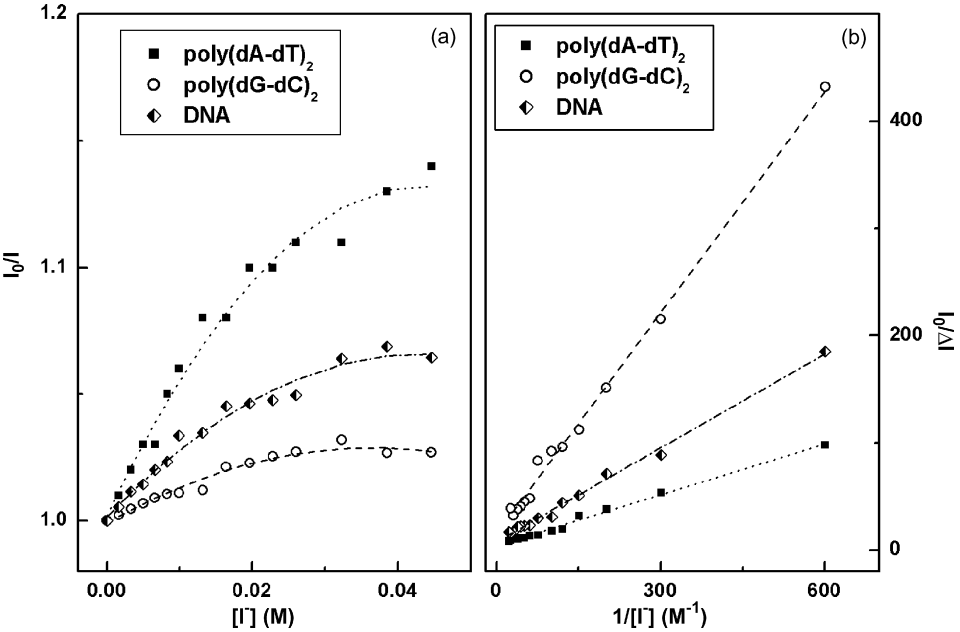


Fig. 12. (a) Stern–Volmer plots for quenching with iodide ion of lactam **3** with DNA, poly(dA–dT)·(dA–dT) and poly(dG–dC)·(dG–dC); (b) corresponding modified Stern–Volmer plots.

Table 3
Values of the accessibilities (f_a) to the quencher (I^-) and Stern–Volmer constants for lactams **1**–**3** bound to DNA and heteropolynucleotides

Compound	Nucleic acid	K_{SV} (M^{-1})	f_a
1 ($R^1 = R^2 = H$)	Salmon sperm DNA	20.2	0.20
2 ($R^1 = F$)	Salmon sperm DNA	18.7	0.21
3 ($R^2 = OMe$)	Salmon sperm DNA	25.8	0.13
	Poly(dA–dT)·(dA–dT)	25.1	0.25
	Poly(dG–dC)·(dG–dC)	21.4	0.07

tam molecules accessible to the external quencher (f_a) should correspond to bound molecules at the grooves.

The calculated accessibilities show that for lactam **3**, 87% of the emitting sites are located inside the double helix, while for compounds **1** and **2** this value is around 80%. This shows that the intercalative binding is the preferred form of interaction of the three lactams with DNA, intercalation being more effective for lactam **3**. This compound is also the one that exhibits more significant spectral changes in absorption by interaction with nucleic acids (*vd*. Fig. 8).

Quenching experiments with I^- were also performed for the more intercalative lactam **3** bound to heteropolynucleotides, poly(dA–dT)·(dA–dT) and poly(dG–dC)·(dG–dC). As observed for DNA, Stern–Volmer plots exhibit a downward curvature (Fig. 12a). Modified Stern–Volmer plots (Eq. (3)) are displayed in Fig. 12b and K_{SV} and f_a values are presented in Table 3.

The results show that, for poly(dA–dT)·(dA–dT), 75% of the emitting sites are located inside the double helix, while this value attains 93% for poly(dG–dC)·(dG–dC). Despite the lower binding constant (Table 2) for poly(dG–dC)·(dG–dC), lactam **3** shows a pronounced intercalation between G–C base pairs. For poly(dA–dT)·(dA–dT), the accessibility to the quencher I^- is higher, together with a large value for the binding constant (Table 2). This is possibly due to the occurrence also of groove binding in A–T regions, together with intercalation in large majority of lactam **3** in A–T base pairs.

4. Conclusions

We have applied a novel method to the synthesis of bioactive benzothieno[2,3-*c*]quinolin-6(5*H*)-ones (tetracyclic lactams), consisting of "one pot" three steps, palladium-catalyzed borylation and Suzuki coupling (BSC), and lactamization. Two new substituted (F or OMe) compounds were prepared and completely characterized.

Salmon sperm DNA and synthetic heteropolynucleotides, poly(dA–dT)·(dA–dT) and poly(dG–dC)·(dG–dC) binding studies were performed for the new lactams. These exhibit high binding constants to DNA, the intercalation being the preferred mode of binding. Studies with heteropolynucleotides show a higher affinity of the new lactams for A–T base sequences. The methoxylated lactam is the more intercalative compound in DNA as demonstrated by quenching experiments using iodide ion, with determination of the quencher accessibilities to bound compounds. Iodide ion quenching measurements using the latter compound bound to heteropolynucleotides show a large majority (93%) of intercalative binding in poly(dG–dC)·(dG–dC), while in A–T regions intercalative (75%) and also groove binding may occur for this compound.

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