Synthesis and Characterization of Some Novel 2-(Trifluoromethyl)pyrimido-[1,2-*a*]benzimidazoles and Pyrimido[1,2-*a*]benzimidazol-2*H*)-ones of Biological Interest

Nilo Zanatta,*[a] Simone S. Amaral,[a] Andressa Esteves-Souza,[b] Aurea Echevarria,*[b] Patrícia B. Brondani,[a] Darlene C. Flores,[a] Helio G. Bonacorso,[a] Alex F. C. Flores,[a] Marcos A. P. Martins[a]

[a] Núcleo de Química de Heterociclos (NUQUIMHE), Departamento de Química, Universidade Federal de Santa Maria, 97.105-900, Santa Maria, RS, Brazil
Fax +55(55)2208031; E-mail: zanatta@base.ufsm.br

[b] Departamento de Química, Universidade Federal Rural do Rio de Janeiro, 23851-970, Seropédica, RJ, Brazil
E-mail: echevarr@ufrrj.br

Received 24 February 2006

Abstract: The synthesis of some potentially active 2-(trifluoromethyl)pyrimido[1,2-*a*]benzimidazoles and pyrimido[1,2-*a*]benzimidazol-2(1*H*)-ones by the cyclization of 4-alkoxyvinyl trifluoro(chloro)methyl ketones with 2-aminobenzimidazole is described. The structure of the products was assigned based on ^{1}H and ^{13}C NMR as well as 2D-NMR experiments. Some of the obtained products exhibited significant DNA-topoisomerase I inhibitory activity.

Key words: 2-aminobenzimidazole, pyrimidobenzimidazole, enones, halogenated heterocycles, DNA-topoisomerase I inhibitory activity

Imidazopyrimidines and their analogues have been found to be of pharmacological interest for a long time. More specifically, imidazo[1,2-*a*]pyrimidine derivatives have already been described as benzodiazepine receptor agonists,[1] as well as antiviral,[2] antitumor,[3] and antimicrobial agents,[4] and calcium-channel blockers.[5]

Trifluoromethyl-substituted pyrimidines and their condensed cyclic derivatives are well known to possess great importance in the medicinal and agricultural fields.[6] Pyrimidobenzimidazoles bearing a trifluoromethyl group have been synthesized from the cyclization reaction of 2-aminoazole with trifluoromethylated β-dicarbonyl compounds,[7] 3-trifluoroacetyl lactams,[8] 4-trifluoroacetyl-2,3-dihydropyrroles[9] or β-trifluoroacetyl vinyl sulfones.[6]

Pyrimido[1,2-*a*]benzimidazol-2-ones have been synthesized by the reaction of propiolic esters[10] and α,β-unsaturated esters[10a,11] with 2-aminobenzimidazole. Other synthetic methods to obtain pyrimidobenzimidazoles with other type of substituents, not directly related to this work, have also been reported.[12]

Despite the variety of methods available for the synthesis of pyrimidobenzimidazoles,[10–12] the synthetic potential of 4-alkoxy-1,1,1-trihaloalk-3-ene-2-ones **1a–f** and **5a–e** as precursors of this class of heterocycles has not been tested yet. Enones **1** and **5** have the following advantage over the traditional methods to prepare pyrimidobenzimidazoles: (i) they are easily prepared by acylation of enol ethers[13] and acetals,[14] (ii) the reactions are more regioselective than 1,3-dicarbonyl compounds, and (iii) they allow one to introduce a wider range of substituents in the final product, compared with propiolic esters and diethyl ethoxymethylenemalonates. In addition, considering that two possible structures can be formed in the cyclization step, a concise characterization of the regioisomers, based on modern NMR spectral data such as 2D HMBC and NOESY, is limited in the literature.[15]

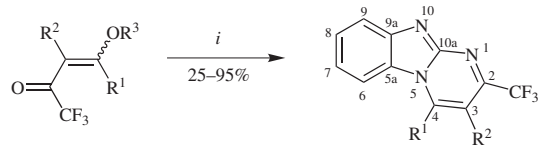
The DNA-topoisomerases are ubiquitous nuclear enzymes that play crucial roles in DNA metabolism events such as replication, transcription, recombination, repair, chromatin assembly and chromosome segregation.[16,17] DNA-topoisomerase type I enzymes are monomeric and catalyze an ATP-independent relaxation of DNA supercoils by transiently breaking and relegating single-stranded DNA. Topoisomerase inhibitors have gained wide clinical significance due to their efficacy as the principal intracellular targets of important antimicrobial and antitumor agents.[18]

Based on these facts, and as an extension of the research developed in our laboratory,[19] we wish to report the synthesis and structure assignment of some novel 2-(trifluoromethyl)pyrimido[1,2-*a*]benzimidazoles and pyrimido-[1,2-*a*]benzimidazol-2(1*H*)-ones from the cyclocondensation reactions of 2-aminobenzimidazole hydrobromide (**2**) and 2-aminobenzimidazole (**3**) with 4-alkoxyvinyl trihalomethyl ketones **1a–f** and **5a–e**. The obtained products were tested for DNA-topoisomerase inhibitory activity.

Scheme 1 outlines the synthesis of novel 2-(trifluoromethyl)pyrimido[1,2-*a*]benzimidazoles **4–f** from the cyclization reaction of 4-alkoxyvinyl trifluoromethyl ketones **1a–f** with 2-aminobenzimidazoles **2** and **3**. The best conditions to obtain the trifluoromethylpyrimidines **4a–f** were achieved using 2-aminobenzimidazole hydrobromide (**2**) with triethylamine in toluene (acetonitrile for **4e**). This procedure promoted a significant reduction in reaction times (see Table 1), if compared with the cyclization using the free base, 2-aminobenzimidazole (**3**). We believe that the triethylamine salt formed in situ when the

SYNTHESIS 2006, No. 14, pp 2305–2312
Advanced online publication: 28.06.2006
DOI: 10.1055/s-2006-942444; Art ID: M00906SS

benzimidazole hydrobromide **2** was used increases the medium polarity, allowing a more efficient dehydration that occurs on the last step of the formation of products **4** and, as a consequence, the reaction proceeded faster than when the free base benzimidazole (**3**) was used. In both cyclizations, the reaction was regiospecific, showing the formation of only the 2-trifluoromethylpyrimido[1,2-*a*]benzimidazole derivatives, except for the compound **4c** that furnished the 4-trifluoromethylpyrimido isomer in smaller amount (70:30%, respectively). The reaction mechanism suggests a Michael addition of an imidazo nitrogen on the β-carbon of the 4-alkoxyvinyl trifluoromethyl ketones **1a–f**, followed by the attack of the exocyclic amino group of the 2-aminobenzimidazole on the carbonyl of the ketones **1a–f**, as the general five- and six-membered cyclization mechanism proposed by the reaction of 4-alkoxyvinyl trifluoromethyl ketones with dinucleophiles of the N–N and N–C–N type, respectively.[20]



1a–f **4a–f**

Compound	R^1	R^2	R^3
a	H	H	Et
b	H	Me	Et
c	Me	H	Me
d	Ph	H	Me
e	H	-$(CH_2)_2$-	
f	H	-$(CH_2)_3$-	

4e: $R^2 = (CH_2)_2OH$
4f: $R^2 = (CH_2)_3OH$

Scheme 1 *Reagents and conditions*: *i*) 2-aminobenzimidazole hydrobromide (**2**) or 2-aminobenzimidazole (**3**), Et_3N, toluene or MeCN, reflux, 1–72 h.

Scheme 2 shows the reaction of 4-alkoxyvinyl trichloromethyl ketones **5a–f** with 2-aminobenzimidazoles **2** and **3**. The reactions were carried out using the same conditions for the trifluorinated analogues **1a–f**. The cyclization was achieved through the elimination of the trichloromethyl group, giving pyrimido[1,2-*a*]benzimidazol-2(1*H*)-ones **6** and **7** or the dihydropyrido analogues **8** as the reaction products. The elimination of the trichloromethyl group in the cyclization reaction of 4-alkoxyvinyl trichloromethyl ketones with other amidine derivatives has been reported previously.[21] Although the synthesis of pyrimido[1,2-*a*]benzimidazol-2(1*H*)-ones has been extensively explored,[10–12] 4-alkoxyvinyl trichloromethyl ketones **5a–e** had never been used as precursors of these compounds

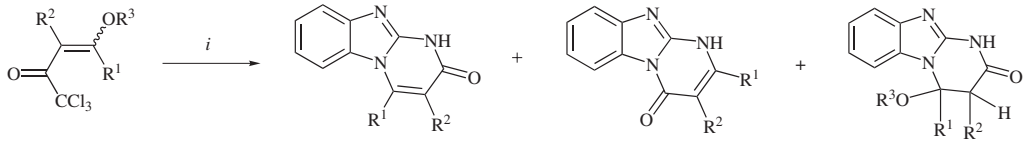
Table 1 Preparation of 2-(Trifluoromethyl)pyrimido [1,2-*a*]benzimidazoles **4a–f**

Compound	2-Aminobenzimidazole	Time (h)[a]	Yield (%)	Product
1a	**2**	1	95	**4a**
1a	**3**	2	95	**4a**
1b	**2**	6	80	**4b**
1b	**3**	72	–[b]	**4b**
1c	**2**	10	70	**4c**[c]
1c	**3**	72	–[b]	**4c**[c]
1d	**2**	24	65	**4d**
1e	**2**	72	25	**4e**
1f	**2**	60	65	**4f**

[a] Reaction condition: toluene (MeCN for **4e**), Et_3N, reflux.
[b] Incomplete reaction.
[c] Regioisomer mixture: **4c/4c′** (70:30%, respectively).

before. Thus, this work additionally aims to explore the synthetic potential of the enones **5a–e** for the synthesis of pyrimido[1,2-*a*]benzimidazol-2(1*H*)-ones. The cyclization reaction of **5a–e** with 2-aminobenzimidazoles **2** and **3** furnished different compounds and regioisomers depending on the substituents of the enones **5a–e** (e.g. R^1 and R^2) and the reaction conditions. Table 2 shows the composition products obtained as a function of different enone structures and reaction conditions.

The results show that, in general, the cyclization of **5** with the 2-aminobenzimidazole free base **3** gave better yields than its hydrobromide form **2** but the reaction times when using either **2** or **3** were basically the same. One can also observe that substituents in both R^1 and R^2 positions, such as in **5b–d**, increased the reaction times and raised the possibility of regioisomer formation. An interesting result was given by the reaction of the enone **5a** with 2-aminobenzimidazole (**3**). When this reaction was carried out in toluene it gave only the pyrimido-2-one derivative **6a** but, when carried out in acetonitrile, only the dihydropyrimido-2-one product **8a** was obtained; both products **6a** and **8a**, in high yields. For the reaction of the enones **5b–d** with **2** or **3**, a mixture of isomers **6** and **7** was usually obtained, with the reaction favoring the formation of compounds **6** (Table 2). In our hands, the mixture of products **6c/7c** and **6d/7d** could not be isolated. For these compounds only the NMR data (taken from the mixture of isomers) of the major compounds **6c** and **6d** were reported. The composition product of the reaction of the enone **5e**



5a–e **6** **7** **8**

Scheme 2 *Reagents and conditions*: *i*) 2-aminobenzimidazole hydrobromide (**2**) or 2-aminobenzimidazole (**3**), Et_3N, reflux, 0.25–72 h.

Table 2 Preparation of Pyrimido[1,2-*a*]benzimidazol-2(1*H*)-ones **6**–**8**

Compound	R^1	R^2	R^3	Benzimidazole	Reaction conditions[a]	Products[b]			Yield (%)[c]
						6	**7**	**8**	
5a	H	H	Et	**2**	toluene, reflux, 0.5 h	100	–	–	90
5a	H	H	Et	**3**	toluene, reflux, 2 h	100	–	–	90
5a	H	H	Et	**2**	MeCN, r.t., 0.25 h	–	–	100	80
5a	H	H	Et	**3**	MeCN, r.t., 0.25 h	–	–	100	90
5b	H	Me	Et	**2**	toluene, reflux, 24 h	70	30	–	25
5b	H	Me	Et	**3**	toluene, reflux, 16 h	70	30	–	65
5b	H	Me	Et	**2**	MeCN, reflux, 24 h	100	–	–	6
5b	H	Me	Et	**3**	MeCN, reflux, 24 h	100	–	–	20
5c	Me	H	Et	**2**	toluene, reflux, 48 h	70	30		25
5c	Me	H	Me	**3**	toluene, reflux, 48h	70	30	–	60
5d	Ph	H	Me	**2**	toluene, reflux, 24h	70	30		14
5d	Ph	H	Me	**3**	toluene, reflux, 24 h	70	30	–	50
5e	H	-$(CH_2)_3$-		**2**	toluene, reflux, 72 h	–	–	100	25
5e	H	-$(CH_2)_3$-		**3**	toluene, reflux, 72 h	100	–	–	–[d]
5e	H	-$(CH_2)_3$		**2**	MeCN, reflux, 8 h	–	–	100	12
5e	H	-$(CH_2)_3$-		**3**	MeCN, reflux, 1 h	100	–	–	60

[a] Molar proportion between reagents **5a**–**e**/Et_3N/2-aminobenzimidazole) = 1:1:1.
[b] Isolated isomer proportion (%).
[c] Isolated yield.
[d] Incomplete reaction.

with **2** and **3** seems to be more sensitive to the 2-aminobenzimidazoles **2** and **3** used. When the free base 2-aminobenzimidazole (**3**) was used, only product **6e** was isolated and, when the 2-aminobenzimidazole hydrobromide (**2**) was used, only product **8e** was obtained.

In order to determine the pyrimido[1,2-*a*]benzimidazole structure, ^1H and ^{13}C NMR data of **4a**,**b** and **6a**,**b** were compared with the NMR data of representative compounds, such as 2-amino-4-trifluoromethyl pyrimidine (standard I)[22] and 5-*H*-thiazolo[3,2-*a*] pyrimidin-5-one (standard II),[20a] to be used as the model compounds for the H-4 and C-4 nuclei of **4a**,**b** and **6a**,**b** (Table 3). The 2-amino-*N*-methylbenzimidazole (standard III)[23] was taken as the model of comparison of the same molecular residue of the pyrimido[1,2-*a*]benzimidazoles (Table 4). Table 3 shows that the H-6 of standard I and the H-7 of standard II are, on average, 1.3 ppm more shielded than the corresponding H-4 of compounds **4a**,**b** and **6a**,**b**. Table 3 also shows that the C-6 of standard I and the C-7 of standard II are, on average, 24.4 ppm more deshielded than the corresponding C-4 of the compounds **4a**,**b** and **6a**,**b**. Standard III (Table 4) shows that the H-7 and C-7 of 2-aminobenzimidazole have a trend similar to that shown by the pyrimido moiety. Here, H-7 is also more shielded and C-7 is more deshielded than the corresponding H-6 and C-6 of **4a**,**b** and **6a**,**b**. Deshielded hydrogens attached to shielded carbons in both the 4 and 6 positions of compounds **4** and **6** suggest a steric hindrance effect between H-4 and H-6.[24]

Table 3 ^1H and ^{13}C NMR Data for Compounds **4a**,**b**, **6a**,**b** and Standard **I** and **II**

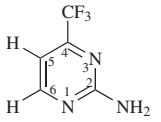
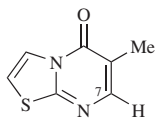
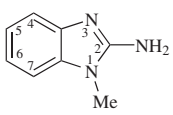
Compound	δ	
	H-n	C-n
	8.5 (H-6)	161.4 (C-6)
Standard **I**[22]		
4a	9.91 (H-4)	139.4 (C-4)
4b	9.74 (H-4)	137.8 (C-4)
	7.93 (H-7)	160.6 (C-7)
Standard **II**[20a]		
6a	9.79 (H-4)	138.8 (C-4)
6b	8.73 (H-4)	130.5 (C-4)

Table 4 ^{1}H and ^{13}C NMR Data for Compounds **4a**,**b**, **6a**,**b** and Standard **III**

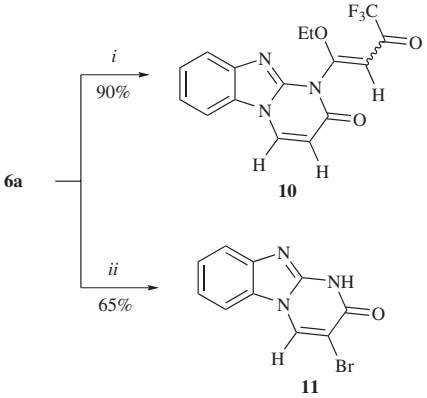
Compound	δ	
	H-n	C-n
	7.70 (H-7)	115.4 (C-7)
Standard **III**[23]		
4a	8.48 (H-6)	113.4 (C-6)
4b	8.35 (H-6)	113.1 (C-6)
6a	8.42 (H-6)	113.7 (C-6)
6b	7.85 (H-6)	109.9 (C-6)

Furthermore, these two hydrogens should also experience deshielding by a ring current effect; H-4 from the benzimidazole portion and H-6 from the pyrimido residue.[24] This trend was considered to be the key information in assigning the correct structure of the title compounds as 2-(trifluoromethyl)pyrimido[1,2-*a*]benzimidazoles and pyrimido[1,2-*a*]benzimidazol-2(1*H*)-ones.

In order to confirm our hypothesis, a NOESY experiment of compound **4b** was carried out. In the NOESY spectrum, a distinct cross-peak between H-4 and H-6 was observed, which confirmed the spatial proximity of these two nuclei. In addition, compound **4a** had already been synthesized by Nenajdenko et al.[6] and their NOE results are in agreement with our proposed structure.

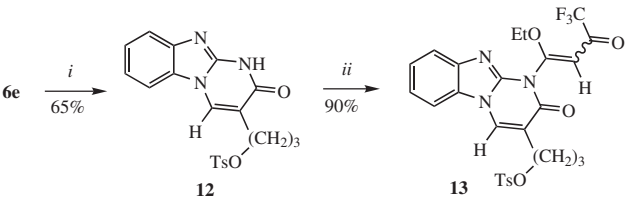
The hexahydropyranopyrimido ring closure of **8e** seems to be accomplished with *cis*-configuration. This indication is obtained from the coupling constants between H-3 and H-4 of 3.8 Hz, which suggests an *axial-equatorial* relationship between these nuclei. The *trans* configuration for related hexahydropyranopyrimidines reports coupling constants of 9.1 Hz.[21a]

Compounds **6a** and **6e**, which were isolated in higher yields and purities, were derivatized in an attempt to improve their solubility in organic solvents and to increase their scope for biological assays. Compound **10** was synthesized from the N-alkylation of **6a** with 4,4-diethoxy-1,1,1-trifluorobut-3-en-2-one (**9**) using potassium carbonate in acetone. Compound **6a** was brominated using NBS in chloroform to give compound **11** (Scheme 3).



Scheme 3 *Reagents and conditions*: *i*) 4,4-diethoxy-1,1,1-trifluorobut-3-en-2-one (**9**), K_2CO_3, acetone, reflux, 4 h; *ii*) NBS, $CHCl_3$, r.t., 24 h.

The alkylation reaction of **6e** furnished a mixture of N-alkylated and O-alkylated products. In order to obtain only the N-alkylated product of **6e**, the OH group was first protected using *p*-toluenesulfonyl chloride and pyridine in acetonitrile to give compound **12**. Without further purification, compound **13** was synthesized through the N-alkylation reaction of **12** with 4,4-diethoxy-1,1,1-trifluorobut-3-en-2-one (**9**) using potassium carbonate in acetone (Scheme 4).



Scheme 4 *Reagents and conditions*: *i*) TsCl, MeCN, Py, reflux, 16 h; *ii*) 4,4-diethoxy-1,1,1-trifluorobut-3-en-2-one (**9**), K_2CO_3, acetone, reflux, 4 h.

Unfortunately, compounds **10** and **13** decomposed when submitted to purification and could not be tested in the biological assays.

DNA-Topoisomerase Inhibitory Activity

The conversion of supercoiled plasmid DNA to relaxed DNA by topoisomerase I was examined in the presence of **4a**, **4b**, **4d**, **4f**, **6a**, **6e**, **8a**, **8e**, **11** and **12** derivatives. Campothecin, a well-known DNA-topoisomerase I enzyme inhibitor, was used as a positive control.[25,26]

The results were observed by the alteration of the electrophoretic mobility of *p*BR322 plasmid DNA by the combined action of the enzyme and the drugs. The results were analyzed after development with ethidium bromide in UV light, and the record was photographed with a digital camera. The preliminary assays were performed at 200 μM as the higher drug concentration, and the derivatives with inhibitory activity were screened at 20 μM and 2 μM. The results indicate the dose-dependent inhibition of DNA-topoisomerase I catalytic activity and the importance of the steric effect of the β-carbonyl moiety. For the trifluoromethylated compounds **4a**, **4b**, **4d** and **4f**, the results indicated significant inhibitory activity for all derivatives at 200 μM. However, only compound **4a** (unsubstituted derivative) did not show catalytic effect against DNA-topoisomerase I at 2 μM indicating the importance of the hydrophobic effect.

In the pyrimidinones series **6a**, **6e**, **8a**, **8e**, **11** and **12**, only compound **8a** did not show significant catalytic effect against the enzyme at 200 μM. When screened for 20 μM and 2 μM, all compounds showed a catalytic effect against

DNA-topoisomerase I at 2 μM, except compound **12**. The results indicate the dose-dependent inhibition of DNA-topoisomerase I catalytic activity. Thus, the pyrimido[1,2-*a*]benzimidazol-2(1*H*)-one derivatives showed a DNA-topoisomerase I inhibitory effect, indicating a significant performance for this class. The potential in vitro and in vivo cytotoxic effects are under investigation.

In conclusion, a series of 2-(trifluoromethyl)pyrimido[1,2-*a*]benzimidazoles **4a**–**f** and pyrimido[1,2*a*]benzimidazol-2(1*H*)-ones **6a**–**e**, **8a**, and **8e** were synthesized from the 4-alkoxyvinyl trifluoro(chloro)methyl ketones **1a**–**f** and **5a**–**e** via cyclocondensation reactions with 2-aminobenzimidazoles **2** and **3**. Several conditions were tested in order to improve the reaction times, compound purities, yields, and selectivity. The synthesis of different compounds was possible by maintaining the same reagents and making simple variation on the reaction conditions. The conversion of supercoiled plasmid DNA to relaxed DNA by topoisomerase I was examined in the presence of **4a**, **4b**, **4d**, **4f**, **6a**, **6e**, **8a**, **8e**, **11** and **12** derivatives in 200 μM, 20 μM and 2 μM concentrations. The assays results showed a significant inhibitory effect for both series of compounds, indicating a significant performance for this class. All compounds were active in 2 μM concentrations, except for compound **4a** in the trifluoromethylated series and for compounds **8a** (>200 μM) and **12** (20 μM) in the pyrimidinone series.

The syntheses of compounds **1a**–**f**, **5a**–**e**[13,14] and **2**[27] were reported in the literature. All melting points were determined on a MQAPF-301 apparatus and are uncorrected. The CHN microanalyses were performed on a PerkinElmer 2400 elemental analyzer from the Department of Chemistry of the São Paulo University (USP), São Paulo, SP, Brazil. Mass spectra were recorded on a HP 5973 MSD connected to a HP 6890 GC. The GC was equipped with a split-splitless injector, auto-sampler, cross-linked HP-5 capillary column (30 m, 0.32 mm of internal diameter), and He was used as the carrier gas. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX200 or DPX400 spectrometer in DMSO-d_6 with TMS as the internal reference. ^{19}F and 2D-NMR spectra such as COSY, HMQC, HMBC, and NOESY were recorded on a Bruker DPX400 spectrometer. Fluorobenzene was used as the internal reference for the ^{19}F NMR spectra.

2-(Trifluoromethyl)pyrimido[1,2-*a*]benzimidazole (4a); Typical Procedure

Et_3N (0.2 mL, 1.4 mmol) was added to a solution of **2** (0.3 g, 1.4 mmol) in toluene (10 mL). The mixture was stirred for 15 min at r.t. After that, the ketone **1a** (0.24 g, 1.4 mmol) was added and the mixture was stirred under reflux until the reaction was complete (see Table 1). The solvent was evaporated under reduced pressure. A mixture of $CHCl_3$–H_2O (10:30 mL) was added to the residual solid to dissolve the Et_3N salt and the product was filtered and dried in a desiccator under vacuum; mp 307–308 °C ($CHCl_3$–MeOH) (Lit.[6] mp 290–292 °C).

^{1}H NMR (DMSO-d_6/TMS): δ = 9.91 (d, 1 H, *J* = 7.0 Hz, H-4), 8.48 (d, 1 H, *J* = 8.0 Hz, H-6), 7.99 (d, 1 H, *J* = 8.0 Hz, H-9), 7.69 (t, 1 H, *J* = 8.0 Hz, H-8), 7.64 (d, 1 H, *J* = 7.0 Hz, H-3), 7.57 (t, 1 H, *J* = 8.0 Hz, H-7).

^{13}C NMR (DMSO-d_6/TMS): δ = 150.8 (q, $^2J_{C,F}$ = 35.7 Hz, C-2), 148.0 (C-10a), 144.5 (C-9a), 139.4 (C-4), 127.2 (C-8), 126.9 (C-5a), 122.6 (C-7), 121.8 (C-9), 120.5 (q, $^1J_{C,F}$ = 274.1 Hz, CF_3), 113.4 (C-6), 102.0 (C-3).

^{19}F NMR (DMSO-d_6/fluorobenzene): δ = –66.93 (CF_3).

Anal. Calcd for $C_{11}H_6F_3N_3$ (237.18): C, 55.70; H, 2.55; N, 17.72. Found: C, 55.78; H, 2.77; N, 17.53.

3-Methyl-2-(trifluoromethyl)pyrimido[1,2-*a*]benzimidazole (4b)

Mp 299–301 °C ($CHCl_3$–MeOH).

^{1}H NMR (DMSO-d_6/TMS): δ = 9.74 (s, 1 H, H-4), 8.35 (d, 1 H, *J* = 8.4 Hz, H-6), 7.95 (d, 1 H, *J* = 8.4 Hz, H-9), 7.64 (t, 1 H, *J* = 8.4 Hz, H-8), 7.53 (t, 1 H, *J* = 8.4 Hz, H-7), 2.49 (s, 3 H, CH_3).

^{13}C NMR (DMSO-d_6/TMS): δ = 149.8 (q, $^2J_{C,F}$ = 33.8 Hz, C-2), 147.3 (C-10a), 144.5 (C-9a), 137.8 (C-4), 126.9 (C-8), 126.5 (C-5a), 122.5 (C-7), 120.91 (q, $^1J_{C,F}$ = 275.4 Hz, CF_3), 119.7 (C-9), 113.1 (C-6), 112.1 (C-3), 14.2 (CH_3).

^{19}F NMR (DMSO-d_6/fluorobenzene): δ = –65.58 (CF_3).

Anal. Calcd for $C_{12}H_8F_3N_3$ (251.21): C, 57.37; H, 3.21; N, 16.73. Found: C, 57.48; H, 3.36; N, 16.70.

4-Methyl-2-(trifluoromethyl)pyrimido[1,2-*a*]benzimidazole (4c) (Major Isomer)

Isomer proportion: 70:30.

^{1}H NMR (DMSO-d_6/TMS): δ = 8.37 (d, 1 H, *J* = 8.3 Hz, H-6), 7.99 (d, 1 H, *J* = 8.3 Hz, H-9), 7.68 (t, 1H, *J* = 8.3 Hz, H-8), 7.53–7.49 (m, 2 H, H-3, H-7), 3.21 (s, 3 H, CH_3).

^{13}C NMR (DMSO-d_6/TMS): δ = 154.1 (C-10a), 150.2 (q, $^2J_{C,F}$ = 35.0 Hz, C-2), 149.2 (C-9a), 144.9 (C-4), 127.6 (C-5a), 126.8 (C-8), 122.5 (C-7), 120.5 (q, $^1J_{C,F}$ = 275.0 Hz, CF_3), 119.6 (C-9), 116.7 (C-6), 102.6 (C-3), 20.7 (CH_3).

^{19}F NMR (DMSO-d_6/fluorobenzene): δ = –66.26 (CF_3).

4-Phenyl-2-(trifluoromethyl)pyrimido[1,2-*a*]benzimidazole (4d)

Mp 167–169 °C ($CHCl_3$–MeOH) (Lit.[28] mp 188 °C).

^{1}H NMR (DMSO-d_6/TMS): δ = 8.44–8.39 (m, 2 H, H-6, H-9), 8.22 (s, 1 H, H-3), 8.04–7.95 (m, 2 H, H-7, H-8), 7.67–7.48 (m, 5 H, C_6H_5).

^{13}C NMR (DMSO-d_6/TMS): δ = 159.8 (C-10a), 150.5 (C-9a), 144.8 (C-4), 135.3 (C_6H_5), 133.8 (q, $^2J_{C,F}$ = 37.1 Hz, C-2), 131.9, 129.0, 127.8 (C_6H_5), 126.4 (C-8), 125.5 (C-5a), 123.1 (C-7), 120.0 (C-9), 119.8 (q, $^1J_{C,F}$ = 272.4 Hz, CF_3), 113.9 (C-6), 104.2 (C-3).

GC/MS (EI, 70 eV): *m*/*z* (%) = 313 (M^+, 100), 244 (21), 90 (33).

3-(1-Hydroxyethyl)-2-(trifluoromethyl)pyrimido[1,2-*a*]benzimidazole (4e)

The reaction was carried out in MeCN instead of toluene; mp 147–151 °C ($CHCl_3$–MeOH).

^{1}H NMR (DMSO-d_6/TMS): δ = 9.72 (s, 1 H, H-4), 8.45 (d, 1 H, *J* = 8.3 Hz, H-6), 7.97 (d, 1 H, *J* = 8.3 Hz, H-9), 7.66 (t, 1 H, *J* = 8.3 Hz, H-8), 7.50 (t, 1 H, *J* = 8.3 Hz, H-7), 3.74 (t, 2 H, *J* = 5.4 Hz, H-1′), 2.98 (t, 2 H, *J* = 5.4 Hz, H-2′).

^{13}C NMR (DMSO-d_6/TMS): δ = 149.6 (q, $^2J_{C,F}$ = 33.8 Hz, C-2), 147.1 (C-10a), 144.6 (C-9a), 138.7 (C-4), 127.0 (C-8), 126.4 (C-5a), 122.4 (C-7), 120.9 (q, $^1J_{C,F}$ = 275.8 Hz, CF_3), 119.6 (C-9), 113.7 (C-3), 113.2 (C-6), 60.4 (C-1′), 31.2 (C-2′).

GC/MS (EI, 70 eV): *m*/*z* (%) = 281 (M^+, 40), 250 (100), 102 (14), 76 (4.5).

3-(1-Hydroxypropyl)-2-(trifluoromethyl)pyrimido[1,2-*a*]benzimidazole (4f)

Mp 83–85 °C ($CHCl_3$–MeOH).

^{1}H NMR (DMSO-d_6/TMS): δ = 9.68 (s, 1 H, H-4), 8.42 (d, 1 H, *J* = 8.1 Hz, H-6), 7.92 (d, 1 H, *J* = 8.1 Hz, H-9), 7.61 (t, 1 H, *J* = 8.1 Hz, H-8), 7.50 (t, 1 H, *J* = 8.1 Hz, H-7), 4.65 (s, 1 H, OH), 3.55 (t, 2 H, *J* = 5.4 Hz, H-1′), 2.87 (t, 2 H, *J* = 5.4 Hz, H-3′), 1.87 (m, 2 H, H-2′).

^{13}C NMR (DMSO-d_6/TMS): δ = 149.5 (q, $^2J_{C,F}$ = 33.4 Hz, C-2), 147.1 (C-10a), 144.6 (C-9a), 137.8 (C-4), 127.0 (C-8), 126.6 (C-5a), 122.4 (C-7), 120.98 (q, $^1J_{C,F}$ = 275.6 Hz, CF_3), 119.6 (C-9), 116.8 (C-3), 113.4 (C-6), 59.9 (C-1′), 33.2 (C-3′), 24.5 (C-2′).

^{19}F NMR (DMSO-d_6/fluorobenzene): δ = –63.96 (CF_3).

GC/MS (EI, 70 eV): *m*/*z* (%) = 295 (M^+, 88), 250 (100), 182 (16).

Pyrimido[1,2-*a*]benzimidazol-2(1*H*)-one (6a); Typical Procedure A

Et_3N (0.2 mL, 1.4 mmol) was added to a solution of **2** (0.3 g, 1.4 mmol) in toluene (10 mL). The mixture was stirred for 15 min at r.t. After that, the ketone **5a** (0.31 g, 1.4 mmol) was added and the mixture was stirred under reflux until the reaction time was complete (see Table 2). The solvent was evaporated under reduced pressure. A mixture of $CHCl_3$–H_2O (10:30 mL) was added to the residual solid to dissolve the Et_3N salt and the product was filtered and dried in a desiccator under vacuum.

Pyrimido[1,2-*a*]benzimidazol-2(1*H*)-one (6a); Typical Procedure B

Et_3N (0.2 mL, 1.4 mmol) was added to a solution of **3** (0.19 g, 1.4 mmol) in toluene (10 mL). The mixture was stirred for 15 min at r.t. After that, the ketone **5a** (0.31 g, 1.4 mmol) was added and the mixture was stirred under reflux until the reaction time was complete (see Table 2). The solvent was evaporated under reduced pressure. A mixture of $CHCl_3$–H_2O (10:30 mL) was added to the residue to dissolve the Et_3N and the product was filtered and dried in a desiccator under vacuum; mp 254–257 °C ($CHCl_3$–MeOH, dec.) {Lit.[10b] mp 336–339 °C (probably, this is the pyrimido[1,2-*a*]benzimidazol-4(1*H*)-one isomer!)}.

^{1}H NMR (DMSO-d_6/TMS): δ = 12.26 (br s, 1 H, NH), 9.79 (d, 1 H, *J* = 7.3 Hz, H-4), 8.42 (d, 1 H, *J* = 8.1 Hz, H-6), 7.94 (d, 1 H, *J* = 8.1 Hz, H-9), 7.77 (d, 1 H, *J* = 7.3 Hz, H-3), 7.63 (t, 1 H, *J* = 8.1 Hz, H-8), 7.53 (t, 1 H, *J* = 8.1 Hz, H-7).

^{13}C NMR (DMSO-d_6/TMS): δ = 161.1 (C-2), 147.2 (C-10a), 143.4 (C-9a), 138.8 (C-4), 127.1 (C-8), 126.8 (C-5a), 123.2 (C-7), 119.1 (C-9), 113.7 (C-6), 102.9 (C-3).

4-Ethoxy-3,4-dihydropyrimido[1,2-*a*]benzimidazol-2(1*H*)-one (8a); Typical Procedure C

Et_3N (0.2 mL, 1.4 mmol) was added to a solution of **3** (0.19 g, 1.4 mmol) in MeCN (10 mL). The mixture was stirred for 15 min at r.t. After that, **5a** (0.31 g, 1.4 mmol) was added and the mixture was stirred at r.t. (under reflux for **5b** and **5e**) until the reaction time was complete (see Table 2). The solvent was evaporated under reduced pressure. A mixture of $CHCl_3$–H_2O (10:30 mL) was added to the residue to dissolve the Et_3N and the product was filtered and dried in a desiccator under vacuum; mp 339–342 °C ($CHCl_3$–MeOH).

^{1}H NMR (DMSO-d_6/TMS): δ = 11.63 (br s, 1 H, NH), 7.58 (d, 1 H, *J* = 8.2 Hz, H-6), 7.43 (d, 1 H, *J* = 8.2 Hz, H-9), 7.16–7.13 (m, 2 H, H-7), 6.00–5.8 (m, 1 H, H-4), 3.71–3.67 (m, 1 H, OCH_2), 3.41–3.38 (m, 1 H, OCH_2), 3.32–3.27 (m, 1 H, H-3), 2.82–2.50 (m, 1 H, H-3), 1.02 (t, 3 H, *J* = 7.0 Hz, CH_3).

^{13}C NMR (DMSO-d_6/TMS): δ = 167.2 (C-2), 147.3 (C-10a), 141.6 (C-9a), 132.9 (C-5a), 122.1 (C-8), 121.4 (C-7), 117.6 (C-9), 109.4 (C-6), 77.6 (C-4), 64.1 (OCH_2), 37.7(C-3), 14.9 (CH_3).

3-Methylpyrimido[1,2-*a*]benzimidazol-2(1*H*)-one (6b); Typical Procedure C

Mp 284–285 °C ($CHCl_3$–MeOH, dec.).

^{1}H NMR (DMSO-d_6/TMS): δ = 12.57 (br s, 1 H, NH), 8.73 (s, 1 H, H-4), 7.85 (d, 1 H, *J* = 7.1 Hz, H-6), 7.50 (d, 1 H, *J* = 7.1 Hz, H-9), 7.33–7.19 (m, 2 H, H-7, H-8), 2.00 (s, 3 H, CH_3).

^{13}C NMR (DMSO-d_6/TMS): δ = 162.9 (C-2), 147.1 (C-10a), 140.4 (C-9a), 130.5 (C-4), 128.3 (C-5a), 123.8 (C-8), 120.8 (C-7), 116.8 (C-9), 113.9 (C-3), 109.9 (C-6), 13.2 (CH_3).

4-Methylpyrimido[1,2-*a*]benzimidazol-2(1*H*)-one (6c); Typical Procedure B

^{1}H NMR (DMSO-d_6/TMS): δ = 12.70 (br s, 1 H, NH), 8.38 (d, 1 H, *J* = 7.9 Hz, H-6), 7.53 (d, 1 H, *J* = 7.9 Hz, H-9), 7.43 (t, 1 H, *J* = 7.9 Hz, H-7), 7.29 (t, 1 H, *J* = 7.9 Hz, H-8), 5.84 (s, 1 H, H-3), 2.31 (s, 3 H, CH_3).

^{13}C NMR (DMSO-d_6/TMS): δ = 159.2 (C-2), 148.2 (C-9a, C-10a), 126.7 (C-4, C-5a), 125.4 (C-8), 121.2 (C-7), 115.0 (C-9), 113.5 (C-6), 98.4 (C-3), 21.6 (CH_3).

3-Phenylpyrimido[1,2-*a*]benzimidazol-2(1*H*)-one (6d);[11b] Typical Procedure B

^{1}H NMR (DMSO-d_6/TMS): δ = 12.97 (br s, 1 H, NH), 8.49 (d, 1 H, *J* = 8.0 Hz, H-6), 8.15–8.10 (m, 2 H, H-8, H-9), 7.49–7.52 (m, 6 H, H-7, C_6H_5), 6.63 (s, 1 H, H-3).

^{13}C NMR (DMSO-d_6/TMS): δ = 159.8 (C-2), 149.5 (C-10a), 137.0 (C-9a), 130.2 (C-4), 129.0, 128.6, 128.4, 127.0 (C_6H_5), 126.2 (C-8), 125.7 (C-5a), 121.8 (C-7), 115.7 (C-9), 111.0 (C-6), 97.1 (C-3).

3-(3-Hydroxypropyl)pyrimido[1,2-*a*]benzimidazol-2(1*H*)-one (6e); Typical Procedure C

Mp 269–270 °C ($CHCl_3$–MeOH).

^{1}H NMR (DMSO-d_6/TMS): δ = 11.70 (br s, 1 H, NH), 7.80 (s, 1 H, H-4), 7.43–7.39 (m, 2 H, H-6, H-9), 7.09–7.04 (m, 2 H, H-7, H-8), 4.04 (t, 2 H, *J* = 5.0 Hz, H-1′), 2.31 (t, 2 H, *J* = 5.0 Hz, H-3′), 1.84 (quintet, 2 H, *J* = 5.0 Hz, H-2′).

^{13}C NMR (DMSO-d_6/TMS): δ = 167.2 (C-2), 153.3 (C-4), 147.8 (C-9a, C-10a), 120.9 (C-5a, C-7, C-8, C-9), 113.7 (C-6), 108.6 (C-3), 66.1 (C-1′), 20.7 (C-3′), 18.9 (C-2′).

GC/MS (EI, 70 eV): *m*/*z* (%) = 243 (M^+, 18), 213 (28), 111 (100), 83 (28).

Anal. Calcd for $C_{13}H_{13}N_3O_2$ (243.26): C, 64.19; H, 5.39; N, 17.27. Found: C, 64.19; H, 5.10; N, 17.58.

2a,6a,3,4,5,6-Hexahydropyran[2′,3′:6,5]pyrimido[1,2-*a*]benzimidazol-2-one (8e); Procedure A

Mp 260–262 °C ($CHCl_3$–MeOH).

^{1}H NMR (DMSO-d_6/TMS): δ = 11.70 (br s, 1 H, NH), 7.49 (dd, 1H, *J* = 3.4 Hz, 2.2 Hz, H-6), 7.43 (dd, 1 H, *J* = 3.4 Hz, 2.3 Hz, H-9), 7.15–7.13 (m, 2 H, H-8, H-7), 5.92 (d, 1 H, *J* = 3.6 Hz, H-4), 3.89–3.87 (m, 1 H, H-1′), 3.75–3.70 (m, 1 H, H-1′), 3.23–3.20 (m, 1 H, H-3), 2.49–2.45 (m, 1 H, H-3′), 1.83–1.77 (m, 1 H, H-3′), 1.47–1.43 (m, 2 H, H-2′).

^{13}C NMR (DMSO-d_6/TMS): δ = 168.5 (C-2), 147.4 (C-10a), 141.8 (C-9a), 131.8 (C-5a), 122.1 (C-8), 121.2 (C-7), 117.4 (C-9), 109.1 (C-6), 77.6 (C-4), 66.5 (C-1′), 39.2 (C-3), 21.6 (C-3′), 21.4 (C-2′).

CG/MS (EI, 70 eV): *m*/*z* (%) = 243 (M^+, 34), 213 (18), 111 (100), 83 (18).

Anal Calcd for $C_{13}H_{13}N_3O_2$ (243.26): C, 64.19; H, 5.39; N, 17.27. Found: C, 64.62; H, 5.44; N, 16.91.

1-(1-Ethoxy-4,4,4,-trifluoro-3-oxobuten-1-yl)pyrimido[1,2-*a*]benzimidazol-2(1*H*)-one (10)

K_2CO_3 (1.38 g, 1.0 mmol) was added to a solution of **6a** (0.185 g, 1.0 mmol) in acetone (15 mL). The mixture was stirred for 15 min at r.t. Ketone **9** (0.212 g, 1.0 mmol) was added and the mixture was

stirred under reflux for 4 h. The solvent was partially evaporated under reduced pressure. The product was collected by filtration and dried in a desiccator under vacuum. From the recrystallization of **10** from $CHCl_3$–MeOH, only **6a** was recovered.

^{1}H NMR (DMSO-d_6/TMS): δ = 9.84 (d, 1 H, *J* = 7.1 Hz, H-4), 8.44 (d, 1 H, *J* = 7.9 Hz, H-6), 7.95 (d, 1 H, *J* = 7.9 Hz, H-9), 7.75 (d, 1 H, *J* = 7.1 Hz, H-3), 7.64 (t, 1 H, *J* = 7.9 Hz, H-8), 7.52 (t, 1 H, *J* = 7.9 Hz, H-7), 4.57 (s, 1 H, H-2 enone), 3.91 (q, 2 H, *J* = 7.1 Hz, OCH_2), 1.13 (t, 3 H, *J* = 7.1 Hz, CH_3).

^{13}C NMR (DMSO-d_6/TMS): δ = 167.9 (C-2), 166.5 (q, $^2J_{C,F}$ = 27.2 Hz, C-3 enone), 160.5 (C-1 enone), 147.4 (C-10a), 144.5 (C-9a), 138.4 (C-4), 126.9 (C-8), 126.7 (C-5a), 122.5 (C-7), 119.7 (qua, $^1J_{C,F}$ = 291.8 Hz, CF_3), 119.5 (C-9), 113.1 (C-6), 102.2 (C-3), 76.2 (C-2 enone), 56.1 (OCH_2), 14.7 (CH_3).

3-Bromopyrimido[1,2-*a*]benzimidazol-2(1*H*)-one (11)

NBS (0.178 g, 1.0 mmol) was added to a solution of **6a** (0.185 g, 1.0 mmol) in $CHCl_3$ (20 mL). The mixture was stirred at r.t. for 24 h. The mixture was extracted with H_2O (3 × 10 mL) in order to eliminate the residual NBS. The product was collected by filtration as a reddish solid and dried in a desiccator under vacuum; mp 270–274 °C ($CHCl_3$–MeOH).

^{1}H NMR (DMSO-d_6/TMS): δ = 11.60 (br s, 1 H, NH), 10.21 (s, 1 H, H-4), 8.46 (d, 1 H, *J* = 8.0 Hz, H-6), 7.96 (d, 1 H, *J* = 8.0 Hz, H-9), 7.67 (t, 1 H, *J* = 8.0 Hz, H-8), 7.56 (t, 1 H, *J* = 8.0 Hz, H-7).

^{13}C NMR (DMSO-d_6/TMS): δ = 161.5 (C-2), 147.9 (C-10a), 144.5 (C-9a), 134.1 (C-4), 128.5 (C-8), 126.4 (C-5a), 124.0 (C-7), 122.1 (C-9), 113.6 (C-6), 111.1 (C-3).

3-(1-Tosylpropyl)pyrimido[1,2-*a*]benzimidazol-2(1*H*)-one (12)

p-Toluenesulfonyl chloride (0.190 g, 1.0 mmol) and pyridine (0.1 mL, 1.0 mmol) were added to a solution of **6e** (0.243 g, 1.0 mmol) in MeCN (15 mL). The mixture was stirred under reflux for 16 h. The solvent was evaporated under reduced pressure. A mixture of $CHCl_3$–H_2O (10:30 mL) was added to the residual solid to dissolve the Et_3N salt and the product was filtered and dried in a desiccator under vacuum; mp 174–176 °C ($CHCl_3$–MeOH).

^{1}H NMR (DMSO-d_6/TMS): δ = 12.73 (br s, 1 H, NH), 7.96 (s, 1 H, H-4), 7.69 (m, 2 H, H-6, H-8), 7.55 (d, 2 H, *J* = 8.0 Hz, Ar), 7.40 (m, 2 H, H-9, H-7), 7.14 (d, 2 H, *J* = 7.8 Hz, Ar), 4.14 (t, 2 H, *J* = 5.6 Hz, H-1′), 2.34 (t, 2 H, *J* = 5.6 Hz, H-3′), 2.29 (s, 3 H, CH_3), 1.89 (quin, 2 H, *J* = 5.6 Hz, H-2′).

^{13}C NMR (DMSO-d_6/TMS): δ = 165.7 (C-2), 156.1 (C-4), 144.7 (C-10a), 143.8 (C-9a), 138.1 (Ar), 128.5 (C-5a, Ar), 128.1 (C-8, C-7), 125.4 (C-9, C-6), 124.5, 113.2 (Ar), 107.6 (C-3), 66.7 (C-1′), 20.6 (CH_3), 20.3 (C-2′), 18.5 (C-3′).

1-(1-Ethoxy-4,4,4-trifluoro-3-oxobuten-1-yl)-3-(1-tosylpropyl)pyrimido[1,2-*a*]benzimidazol-2(1*H*)-one (13)

K_2CO_3 (0.138 g, 1.0 mmol) was added to a solution of **12** (0.398 g, 1.0 mmol) in acetone (15 mL). The mixture was stirred for 15 min at r.t. The enone **9** (0.212 g, 1.0 mmol) was added and the mixture was stirred under reflux for 4 h. The solvent was partially evaporated under reduced pressure. The product was collected by filtration and dried in a desiccator under vacuum. From the recrystallization of **13** from $CHCl_3$–MeOH, only **12** was recovered.

^{1}H NMR (DMSO-d_6/TMS): δ = 7.81 (s, 1 H, H-4), 7.50–7.40 (m, 2 H, Ar), 7.12–7.04 (m, 2 H, Ar), 4.53 (s, 1 H, H-2 enone), 4.04 (t, 2 H, *J* = 5.0 Hz, H-1′), 3.88 (q, 2 H, *J* = 7.0 Hz, OCH_2), 2.27–2.30 (m, 5 H, H-3′, CH_3), 1.84 (quin, 2 H, *J* = 5.0 Hz, H-2′), 1.11 (t, 3 H, *J* = 7.0 Hz, $OCCH_3$).

^{13}C NMR (DMSO-d_6/TMS): δ = 168.1 (C-2), 167.0 (q, $^2J_{C,F}$ = 30.9 Hz, C-3 enone), 153.1 (C-4), 147.7 (C-10a), 145.5 (9a, C-1 enone), 137.5 (Ar), 127.9 (C-8, C-7, C-5a, Ar), 125.4 (C-6, C-9, Ar), 120.7 (Ar), 119.5 (q, $^1J_{C,F}$ = 280.0 Hz, CF_3) 108.5 (C-3), 76.4 (C-2 enone), 66.0 (C-1′), 56.2 (OCH_2), 20.6 (C-2′, CH_3), 18.7 (C-3′), 14.6 ($OCCH_3$).

Biological Assays; Materials for DNA-relaxation

The DNA-topoisomerase I drug screening kit from TopoGEN contained supercoiled (form I) plasmid substrate DNA (25 μg in 10 mL of TE buffer). TE buffer (10 mM tris-HCl at pH 7.5 and 1 mM EDTA), and the assay buffers 10 mM tris-HCl pH 7.9, 1 mM EDTA, 0.15 M NaCl, 0.1% BSA, 0.1 mM spermidine, 5% glycerol were used. The DNA utilized was supercoiled *p*BR322 plasmid purchased from Sigma. The loading buffer contained 25% bromophenol blue, 50% glycerol and 10% SDS. The agarose and the substances utilized in these assays were purchased from the Sigma.

Topo I Assay

The topo I inhibition was determined by relaxation assay and was carried out as described in the TopoGEN screening kit. For topo I, one unit of the enzyme was utilized to relax 0.25 μg of the supercoiled *p*BR322 plasmid DNA. The reaction mixture (10 μL) contained the drug, DNA, assay buffer, 1 U of topo I and H_2O. The mixture was incubated at 37 °C for 30 min, and the reaction was finalized by the addition of 1 μL of dye solution containing 25% bromophenol blue, 50% glycerol and 10% SDS.

Reaction products were loaded onto a 1% agarose gel containing ethidium bromide. Electrophoresis was carried out in tris-acetate-EDTA pH 8.5 at 15 V for 3.5 h and then photographed with a digital camera by illumination.

Acknowledgment

The authors thank the financial support from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (Universal grant No. 477682/01-4) and fellowship from CAPES (S.S.A.) and CNPq (P.B.B., D.C.F., A.E.S, and A.E.).

References

(1) Trapani, G.; Franco, M.; Latrofa, A.; Genchi, G.; Iacobazzi, V.; Ghiani, C. A.; Maciocco, E.; Liso, G. *Eur. J. Med. Chem.* **1997**, *32*, 83.
(2) Gueiffieri, A.; Blache, Y.; Chapat, J. P.; Elhakmaoui, E. M. E.; Andrei, G.; Snoeck, R.; De Clercq, E.; Chavignon, O.; Teulade, J. C.; Fauvelle, F. *Nucleosides Nucleotides* **1995**, *14*, 551.
(3) Dalla Via, L.; Gia, O.; Magno, S. M.; Da settimo, A.; Marini, A. M.; Primofiore, G.; Da Settimo, F.; Salaerno, S. *Il Farmaco* **2001**, *65*, 159.
(4) (a) Rival, Y.; Grassy, G.; Michel, G. *Chem. Pharm. Bull.* **1992**, *40*, 1170. (b) Rival, Y.; Grassy, G.; Taudou, A.; Ecalle, R. *Eur. J. Med. Chem.* **1991**, *26*, 13.
(5) Almirante, L.; Polo, L.; Mugnani, A.; Provincialli, E.; Rugarli, P.; Gambá, A.; Olivi, A.; Murmann, W. *J. Med. Chem.* **1965**, *9*, 29.
(6) Krasovsky, A. L.; Hartulyari, A. S.; Nenajdenko, V. G.; Balenkova, E. S. *Synthesis* **2002**, 133.
(7) Kreutzberger, A.; Leger, M. *J. Heterocycl. Chem.* **1981**, *18*, 1587.
(8) Kawase, M.; Hirabayashi, M.; Saito, S.; Yamamoto, K. *Tetrahedron Lett.* **1999**, *40*, 2541.
(9) Bouillon, J.; Janousek, Z.; Viehe, H. G.; Tinant, B.; De Clercq, J. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2907.
(10) (a) Dunwell, D. W.; Evans, D. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1588. (b) Chow, A. W.; Jakas, D. R.; Trotter, B. P.; Hall, N. M.; Hoover, J. R. E. *J. Heterocycl. Chem.* **1973**, *10*, 71. (c) Reimlinger, H.; Peiren, M. A.; Merenyi, R. *Chem.*

Ber. **1972**, *105*, 794. (d) Wade, J. J.; Hegel, R. F.; Toso, C. B. *J. Org. Chem.* **1979**, *44*, 1811. (e) Troxler, F.; Weber, H. P. *Helv. Chim. Acta* **1974**, *57*, 2356.
(11) (a) Ogura, H.; Kawano, M.; Itoh, T. *Sch. Pharm. Sci.* **1973**, *21*, 2019; *Chem. Abstr.* **1973**, *79*, 137078. (b) Al-Jallo, H. N.; Muniem, M. A. *J. Heterocycl. Chem.* **1978**, *15*, 849. (c) Da Settimo, A.; Primofiore, G.; Da Settimo, F.; Marini, A. M.; Taliani, S.; Salermo, S.; Dalla Via, L. *J. Heterocycl. Chem.* **2003**, *40*, 1091.
(12) (a) Nawrocka, W.; Sztuba, B.; Kowalska, M. W.; Liszkiewicz, H.; Wietrzyk, J.; Nasulewicz, A.; Pelczynska, M.; Opolski, A. *Il Farmaco* **2004**, *59*, 83. (b) Antaki, H.; Petrow, V. *J. Chem. Soc.* **1951**, 901. (c) Elnagdi, M. H.; Wamhoff, H. *Chem. Lett.* **1981**, 419. (d) Da Settimo, A.; Primofiore, G.; Da Settimo, F.; Pardi, G.; Simorini, F.; Marini, A. M. *J. Heterocycl. Chem.* **2002**, *39*, 1007.
(13) Colla, A.; Martins, M. A. P.; Clar, G.; Krimmer, S.; Fisher, P. *Synthesis* **1991**, 483.
(14) (a) Martins, M. A. P.; Siqueira, G. M.; Flores, A. F. C.; Clar, G.; Zanatta, N. *Quím. Nova* **1994**, *17*, 24; *Chem. Abstr.* **1994**, *122*, 187063a. (b) Martins, M. A. P.; Bastos, G. P.; Bonacorso, H. G.; Zanatta, N.; Flores, A. F. C.; Siqueira, G. M. *Tetrahedron Lett.* **1999**, *40*, 4309.
(15) Asobo, P. F.; Wahe, H.; Mbafor, J. T.; Nkengfack, A. E.; Fomum, Z. T.; Sopbue, E. F.; Döpp, D. *J. Chem. Soc., Perkin Trans. 1* **2001**, 457.
(16) Champoux, J. J. *Annu. Rev. Biochem.* **2001**, *70*, 369.
(17) Wang, J. C. *Annu. Rev. Biochem.* **1996**, *65*, 635.
(18) Singh, S. K.; Ruchebman, A. L.; Li, T. K.; Liu, A.; Liu, A. F.; Lavoie, E. J. *J. Med. Chem.* **2003**, *46*, 2254.
(19) (a) Flores, A. C. F.; Brondani, S.; Pizzuti, L.; Martins, M. A. P.; Zanatta, N.; Bonacorso, H. G.; Flores, D. C. *Synthesis* **2005**, 2744. (b) Zanatta, N.; Flores, D. C.; Amaral, S. S.; Bonacorso, H. G.; Martins, M. A. P.; Flores, A. C. F. *Synlett* **2005**, 3079. (c) Zanatta, N.; Flores, D. C.; Madruga, C. C.; Flores, A. C. F.; Bonacorso, H. G.; Martins, M. A. P. *Tetrahedron Lett.* **2006**, *47*, 573.
(20) (a) Bonacorso, H. G.; Lourega, R. V.; Wastowsky, A. D.; Flores, A. F. C.; Zanatta, N.; Martins, M. A. P. *Tetrahedron Lett.* **2002**, *43*, 9315. (b) Martins, M. A. P.; Sinhorin, A. P.; Rosa, A.; Flores, A. F. C.; Wastowsky, A. D.; Pereira, C. M. P.; Flores, D. C.; Beck, P.; Freitag, R. A.; Brondani, S.; Cunico, W.; Bonacorso, H. G.; Zanatta, N. *Synthesis* **2002**, 2353. (c) Bonacorso, H. G.; Martins, M. A. P.; Bittencourt, S. R. T.; Lourega, R. V.; Zanatta, N.; Flores, A. F. C. *J. Fluorine Chem.* **1999**, *99*, 177.
(21) (a) Zanatta, N.; Lopes, E. C. S.; Fantinel, L.; Bonacorso, H. G.; Martins, M. A. P. *J. Heterocycl. Chem.* **2002**, *39*, 943. (b) Zanatta, N.; Madruga, C. C.; Marisco, P. C.; Flores, D. C.; Bonacorso, H. G.; Martins, M. A. P. *J. Heterocycl. Chem.* **2000**, *37*, 1213. (c) Zanatta, N.; Barichello, R.; Pauletto, M. M.; Bonacorso, H. G.; Martins, M. A. P. *Tetrahedron Lett.* **2003**, *44*, 961. (d) Flores, A. F. C.; Zanatta, N.; Rosa, A.; Brondani, S.; Martins, M. A. P. *Tetrahedron Lett.* **2002**, *43*, 5005.
(22) Zanatta, N.; Cortelini, M. F. M.; Carpes, M. J. S.; Bonacorso, H. G.; Martins, M. A. P. *J. Heterocycl. Chem.* **1997**, *34*, 509.
(23) Carotti, P.; Ceccotti, C.; Da Settimo, A.; Palla, F.; Primofiore, G. *J. Heterocycl. Chem.* **1986**, *23*, 1833.
(24) (a) Levy, G. C.; Lichter, L. L.; Nelson, G. L. *Carbon-13 Nuclear Magnetic Resonance Spectroscopy*, 2nd ed.; Wiley: New York, **1980**. (b) Abraham, R. J.; Loftus, P. *Proton and Carbon-13 NMR Spectroscopy*; Heyden: London, **1980**.
(25) (a) Carpenter, A. J.; Porter, A. C. G. *Mol. Biol. Cell.* **2004**, *15*, 5700. (b) Ackerman, P.; Glover, C. V.; Osheroff, N. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3164.
(26) Larsen, A. K.; Escargueil, A. E.; Skladanwski, A. *Pharmacol. Therap.* **2003**, *99*, 167.
(27) Rastogi, R.; Sharma, S. *Synthesis* **1983**, 861.
(28) Kreutzberger, A.; Leger, M. *J. Fluorine Chem.* **1982**, *20*, 777.