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

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Abstract: The synthesis of some potentially active 2-(trifluoromethyl)pyrimido[1,2-a]benzimidazoles and pyrimido[1,2-a]benzimidazol-2(1H)-ones by the cyclization of 4-alkoxyvinyl trifluorochloromethyl ketones with 2-aminobenzimidazole is described. The structure of the products was assigned based on 1H and 13C 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,2 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-aminooazole with trifluoromethylated β-dicarbonyl compounds,7 3-trifluoroacetyl lactams,8 4-trifluoroacetyl-2,3-dihydropyroles or β-trifluoroacetyl vinyl sulfones.6

Pyrimido[1,2-a]benzimidazol-2-ones have been synthesized by the reaction of propionic esters9 and α,β-unsaturated esters10a,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 ethers13 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 propionic esters and diethyl ethoxymethylenemalonates. In addition, considering that two possible structures can be formed in the cyclization step, a concise characterization of the regiosomers, 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(1H)-ones from the cyclodecondensation 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 (acetanilide 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...
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-α]benzimidazole derivatives, except for the compound 4c that furnished the 4-trifluoromethylpyrimido iso in smaller amount (70:30%, respectively). The reaction mechanism suggests a Michael addition of an imidazolium 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 di- nuclophiles of the N–N and N–C–N type, respectively.20

Scheme 1 Reagents and conditions: i) 2-aminobenzimidazole hydrobromide (2) or 2-aminobenzimidazole (3), Et3N, toluene or MeCN, reflux, 1–72 h.

Scheme 2 shows the reaction of 4-alkoxyvinyl trifluoromethyl 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 trifluoromethyl group, giving pyrimido[1,2-α]benzimidazol-2(1H)-ones 6 and 7 or the dihydropyrido analogues 8 as the reaction products. The elimination of the trifluoromethyl group in the cyclization reaction of 4-alkoxyvinyl trifluoromethyl ketones with other amidine derivatives has been reported previously.21 Although the synthesis of pyrimido[1,2-α]benzimidazol-2(1H)-ones has been extensively explored,10–12 4-alkoxyvinyl trifluoromethyl ketones 5a–e had never been used as precursors of these compounds before. Thus, this work additionally aims to explore the synthetic potential of the enones 5a–e for the synthesis of pyrimido[1,2-α]benzimidazol-2(1H)-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. R1 and R2) 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 R1 and R2 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 iso-mers) of the major compounds 6c and 6d were reported. The composition product of the reaction of the enone 5e

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>2-Aminobenzimidazole Time (h)</th>
<th>Yield (%)</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>18</td>
<td>95</td>
<td>4a</td>
</tr>
<tr>
<td>4a</td>
<td>25</td>
<td>95</td>
<td>4a</td>
</tr>
<tr>
<td>4b</td>
<td>25</td>
<td>95</td>
<td>4b</td>
</tr>
<tr>
<td>4c</td>
<td>25</td>
<td>95</td>
<td>4c</td>
</tr>
<tr>
<td>4d</td>
<td>25</td>
<td>95</td>
<td>4d</td>
</tr>
<tr>
<td>4e</td>
<td>25</td>
<td>95</td>
<td>4e</td>
</tr>
<tr>
<td>4f</td>
<td>25</td>
<td>95</td>
<td>4f</td>
</tr>
</tbody>
</table>

Table 2: Table 1 Prepared by Dr. John Doe

Scheme 2 Reagents and conditions: i) 2-aminobenzimidazole hydrobromide (2) or 2-aminobenzimidazole (3), Et3N, reflux, 0.25–72 h.
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 13C 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-aminobenzimidazole-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 2
Preparation of Pyrimido[1,2-a]benzimidazol-2(1H)-ones 6-8

<table>
<thead>
<tr>
<th>Compound</th>
<th>R&lt;sup&gt;1&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
<th>R&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Benzimidazole</th>
<th>Reaction conditions&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Products&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Yield (%)&lt;sup&gt;c&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>5a</td>
<td>H</td>
<td>H</td>
<td>Et</td>
<td>2</td>
<td>toluene, reflux, 0.5 h</td>
<td>100 – –</td>
<td>90</td>
</tr>
<tr>
<td>5a</td>
<td>H</td>
<td>H</td>
<td>Et</td>
<td>3</td>
<td>toluene, reflux, 2 h</td>
<td>100 – –</td>
<td>90</td>
</tr>
<tr>
<td>5a</td>
<td>H</td>
<td>H</td>
<td>Et</td>
<td>2</td>
<td>MeCN, r.t., 0.25 h</td>
<td>– – 100</td>
<td>80</td>
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<tr>
<td>5a</td>
<td>H</td>
<td>H</td>
<td>Et</td>
<td>3</td>
<td>MeCN, r.t., 0.25 h</td>
<td>– – 100</td>
<td>90</td>
</tr>
<tr>
<td>5b</td>
<td>H</td>
<td>Me</td>
<td>Et</td>
<td>2</td>
<td>toluene, reflux, 24 h</td>
<td>70 30 –</td>
<td>25</td>
</tr>
<tr>
<td>5b</td>
<td>H</td>
<td>Me</td>
<td>Et</td>
<td>3</td>
<td>toluene, reflux, 16 h</td>
<td>70 30 –</td>
<td>65</td>
</tr>
<tr>
<td>5b</td>
<td>H</td>
<td>Me</td>
<td>Et</td>
<td>2</td>
<td>MeCN, reflux, 24 h</td>
<td>100 – –</td>
<td>6</td>
</tr>
<tr>
<td>5b</td>
<td>H</td>
<td>Me</td>
<td>Et</td>
<td>3</td>
<td>MeCN, reflux, 24 h</td>
<td>100 – –</td>
<td>20</td>
</tr>
<tr>
<td>5c</td>
<td>Me</td>
<td>H</td>
<td>Et</td>
<td>2</td>
<td>toluene, reflux, 48 h</td>
<td>70 30 –</td>
<td>25</td>
</tr>
<tr>
<td>5c</td>
<td>Me</td>
<td>H</td>
<td>Me</td>
<td>3</td>
<td>toluene, reflux, 48 h</td>
<td>70 30 –</td>
<td>60</td>
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<tr>
<td>5d</td>
<td>Ph</td>
<td>H</td>
<td>Me</td>
<td>2</td>
<td>toluene, reflux, 24 h</td>
<td>70 30 –</td>
<td>14</td>
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<tr>
<td>5d</td>
<td>Ph</td>
<td>H</td>
<td>Me</td>
<td>3</td>
<td>toluene, reflux, 24 h</td>
<td>70 30 –</td>
<td>50</td>
</tr>
<tr>
<td>5e</td>
<td>H</td>
<td>-(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;-</td>
<td>2</td>
<td>toluene, reflux, 72 h</td>
<td>– – 100</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>5e</td>
<td>H</td>
<td>-(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;-</td>
<td>3</td>
<td>toluene, reflux, 72 h</td>
<td>100 – –</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>5e</td>
<td>H</td>
<td>-(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;-</td>
<td>2</td>
<td>MeCN, reflux, 8 h</td>
<td>– – 100</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>5e</td>
<td>H</td>
<td>-(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;-</td>
<td>3</td>
<td>MeCN, reflux, 1 h</td>
<td>100 – –</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Molar proportion between reagents 5a-e/Et<sub>3</sub>N/2-aminobenzimidazole = 1:1:1.
<sup>b</sup> Isolated isomer proportion (%).
<sup>c</sup> Isolated yield.
<sup>d</sup> Incomplete reaction.

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>δ&lt;sup&gt;h&lt;/sup&gt;</th>
<th>H-n</th>
<th>C-n</th>
</tr>
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<tbody>
<tr>
<td>4a</td>
<td>8.5 (H-6)</td>
<td>161.4 (C-6)</td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>9.91 (H-4)</td>
<td>139.4 (C-4)</td>
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<tr>
<td>4b</td>
<td>9.74 (H-4)</td>
<td>137.8 (C-4)</td>
<td></td>
</tr>
<tr>
<td>6a</td>
<td>7.93 (H-7)</td>
<td>160.6 (C-7)</td>
<td></td>
</tr>
<tr>
<td>6b</td>
<td>8.73 (H-4)</td>
<td>130.5 (C-4)</td>
<td></td>
</tr>
</tbody>
</table>
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. 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(1H)-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. 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.

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).

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).

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. Camptothecin, a well-known DNA-topoisomerase I enzyme inhibitor, was used as a positive control.

The results were observed by the alteration of the electrophoretic mobility of ρBR322 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(1H)-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(1H)-ones 6a–e, 8a, and 8e were synthesized from the 4-alkoxyvinyl trifluoro(chloro)methyl ketones 1a–f and 5a–e via cyclodepsimation reactions with 2-amino-benzimidazoles 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 for compounds 6a–e via cyclodepsimation reactions with 2-amino-benzimidazoles 2 and 3.

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, and 2 were reported in the literature. All melting points were determined on a PerkinElmer 2400 elemental analyzer from the Department of Chemistry of the São Paulo University (USP), São Paulo, SP, Brazil. Mass spectra were reported on an 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. The solvent was evaporated under reduced pressure. A 1H NMR (DMSO-d6) and NOESY were recorded on a Bruker DPX400 spectrometer. Fluorobenzene was used as the internal reference for the 19F NMR spectra. The reactions were carried out in MeCN instead of toluene; mp 147–151 °C (CHCl3–MeOH).

The reaction was carried out in MeCN instead of toluene; mp 147–151 °C (CHCl3–MeOH).
1H NMR (DMSO-d6/TMS): δ = 9.68 (s, 1 H, H-4), 8.42 (d, 1 H, J = 8.1 Hz, H-5), 7.92 (d, 1 H, J = 8.1 Hz, H-6), 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').

13C NMR (DMSO-d6/TMS): δ = 149.5 (q, 1J_C = 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 = 275.6 Hz, CF3), 119.6 (C-9), 116.8 (C-6), 113.4 (C-3), 59.9 (C-1'), 33.2 (C-3'), 24.5 (C-2').

19F NMR (DMSO-d6/fluorobenzene): δ = -63.96 (CF3).

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

Pyrimido[1,2-a]benzimidazol-2(1H)-one (6a); Typical Procedure A
Et3N (0.2 mL, 1.4 mmol) was added to a solution of 2 (0.3 g, 1.4 mmol) in toluene (10 mL). After 15 min at r.t., the mixture was filtered and dried in a desiccator under vacuum.

Pyrimido[1,2-a]benzimidazol-2(1H)-one (6a); Typical Procedure B
After that, the mixture was stirred under reflux until the reaction was complete (see Table 2). The solvent was evaporated under reduced pressure. A mixture of CHCl3–H2O (10:30 mL) was added to the residue to dissolve the Et3N salt and the product was filtered and dried in a desiccator under vacuum.

4-Ethoxy-3,4-dihydropyrimido[1,2-a]benzimidazol-2(1H)-one (8a); Typical Procedure C
Et3N (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 mixture was stirred under reflux until the reaction was complete (see Table 2). The solvent was evaporated under reduced pressure. A mixture of CHCl3–H2O (10:30 mL) was added to the residue to dissolve the Et3N and the product was filtered and dried in a desiccator under vacuum; mp 245–257 °C (CHCl3–MeOH, dec.) [Lit.16 mp 336–339 °C (probably, this is the pyrimido[1,2-a]benzimidazol-4(1H)-one isomer)].

4-Hydroxy-3,4-dihydropyrimido[1,2-a]benzimidazol-2(1H)-one (8a); Typical Procedure C
After that, the mixture was stirred until the reaction time was complete (see Table 2). The solvent was evaporated under reduced pressure. A mixture of CHCl3–H2O (10:30 mL) was added to the residue to dissolve the Et3N and the product was filtered and dried in a desiccator under vacuum; mp 339–342 °C (CHCl3–MeOH).

4-Hydroxy-3,4-dihydropyrimido[1,2-a]benzimidazol-2(1H)-one (8a); Typical Procedure C
After that, the mixture was stirred under reflux for 2h and 5e until the reaction time was complete (see Table 2). The solvent was evaporated under reduced pressure. A mixture of CHCl3–H2O (10:30 mL) was added to the residue to dissolve the Et3N and the product was filtered and dried in a desiccator under vacuum; mp 339–342 °C (CHCl3–MeOH).

4-Methylpyrimido[1,2-a]benzimidazol-2(1H)-one (6c); Typical Procedure B
After that, 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 CHCl3–H2O (10:30 mL) was added to the residue to dissolve the Et3N salt and the product was filtered and dried in a desiccator under vacuum.

4-Methylpyrimido[1,2-a]benzimidazol-2(1H)-one (6c); Typical Procedure B
H NMR (DMSO-d6/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, H-8, CH3), 6.63 (s, 1 H, H-3).

3-(3-Hydroxypropyl)pyrimido[1,2-a]benzimidazol-2(1H)-one (6e); Typical Procedure C
H NMR (DMSO-d6/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').

19F NMR (DMSO-d6/TMS): δ = 167.2 (C-2), 153.3 (C-4), 147.8 (C-9a), 120.9 (C-5a), 121.8 (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).


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(1H)-one (8e); Procedure A
H NMR (DMSO-d6/TMS): δ = 1.170 (br s, 1 H, NH), 7.49 (dd, 1 H, J = 3.4 Hz, 2.2 Hz, H-6), 7.43 (ddd, 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').

1C NMR (DMSO-d6/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').

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 CHCl₃–MeOH, only 6a was recovered.

1H NMR (DMSO-d₆/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 = 6.5 Hz, H-1), 1.89 (quin, 2 H, J = 5.6 Hz, H-3 enone), 1.37 (t, 3 H, J = 7.1 Hz, CH₃), 1.13 (t, 3 H, J = 7.1 Hz, CH₃), 1.11 (t, 3 H, J = 7.1 Hz, CH₃), 0.89 (t, 3 H, J = 6.5 Hz, CH₃).

13C NMR (DMSO-d₆/TMS): δ = 167.9 (C-2), 166.5 (q, JCF = 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, JCF = 291.8 Hz, CF₃), 119.5 (C-9), 113.1 (C-6), 102.2 (C-3), 76.2 (C-2 enone), 56.1 (OCH₃), 14.7 (CH₃).

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 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 pBR322 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 pBR322 plasmid DNA. The reaction mixture (10 µL) contained the drug, DNA, assay buffer, 1 U of topo I and H₂O. 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.

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