Full Paper

Synthesis and *In Vitro* Protozoocidal Evaluation of Novel Diazabicyclic Tropolone Derivatives

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The synthesis and *in vitro* antiparasitic activity of twenty-seven novel diazabicycles based on tropolone ethers is presented. The compounds can be readily prepared by means of a high-yielding hetero Diels–Alder reaction using simple and readily available starting materials. Several of the new diazabicycles have *in vitro* activities against *Trypanosoma cruzi*, *Leishmania donovani*, *Trypanosoma brucei rhodesiense,* and chloroquine-resistant *Plasmodium falciparum* that are comparable or superior to those of currently employed protozoocidal agents.

Keywords: Hydrazynes / Parasitoses / Protozoocidal activity / Synthesis / Tropolone derivatives

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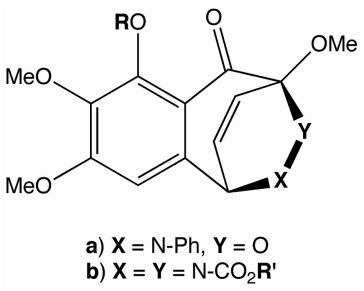
Introduction

Protozoan parasitoses such as malaria, sleeping sickness, kala-azar, and chagas disease represent some of the most important health concerns in tropical and sub-tropical regions of Africa, South America, and Southeast Asia [1]. Despite recent efforts, the adaptability of the causative microorganisms has hampered the development of usable vaccines, and the success of vector control initiatives has been limited [1, 2]. Consequently, chemotherapy is still at the forefront in the battle against these ailments. Unfortunately, most of the drugs employed in the treatment of parasitic infections are non-specific, considerably toxic and have adverse side-effects. In addition, their widespread use and misuse has induced the selection of drug-resistant strains of protozoa which are becoming increasingly difficult to combat [3–5]. As a result, the demand for novel and easily accessible compounds with antiparasitic potential and improved pharmacological properties will continue to grow at a steady pace [1, 6].



a) **X** = N-Ph, **Y** = O
b) **X** = **Y** = N-CO_2**R'**

Figure 1. General structure of benzotropolone-based protozoocidal oxazine (**a**) and hydrazine (**b**) bicycles [7–9]. **R** can be H-, alkyl-, Ar-CH_2-, alkylCO-, or Ar-CO-, and **R′** is CH_3-, CH_3CH_2-, or $(CH_3)_2CH$-.

We have recently described the design, synthesis, and *in vitro* protozoocidal evaluation of a series of oxazino and hydrazo bicycles (Fig. 1) [7–9]. The activities of these compounds, which were easily obtained through a high-yielding hetero Diels–Alder reaction between benzotropolone derivatives and suitable dienophiles, ranged from limited against *Plasmodium falciparum* and *Trypanosoma brucei rohdesiense*, to good against *Leishmania donovani* and *Typanosoma cruzi*. Indeed, the activities of several of the novel diazabicycles against the latter two parasites rivaled or surpassed those of currently employed drugs.

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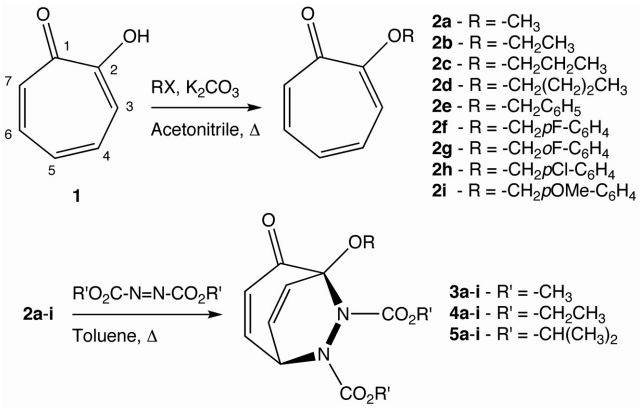
As stated in our previous reports, the strategy behind the design of these compounds involves the incorporation of –N–O– and –N–N– bonds into readily accessible molecular frameworks [7–9]. Their relatively low bond dissociation energies (BDEs), ranging from 35 to 38 kcal/mol [10], make these moieties prone to facile homolytic cleavage. In theory, radicals generated through such a process have the potential to induce oxidative stress in the protozoa, a mechanism of action that has been established for several other antiparasitic agents [11–16]. While further studies are required to elucidate their mode of action, the fact that oxazines structurally unrelated to those depicted in Fig. 1 have shown protozoocidal activity supports this hypothesis [17].

Although the benzotropolones provided a convenient scaffold for the preparation of bicyclic oxazines and hydrazines, their structure leads to final products with non-ideal pharmacological properties. For example, the molecular weight of several of these compounds, in excess of 500 g/mol, could adversely affect their bioavailability and makes them unattractive as potential drug leads. As cited above, Gamenara and co-workers recently reported a series of antiparasitic bicyclic oxazines based on smaller monoterpene scaffolds [17]. Their results prompted us to turn our attention to similarly sized molecular frameworks bearing activated dienes. Among the vast number of candidate compounds, tropolones and tropanes are particularly well suited for our purposes. Not only are their structures compatible with previously developed synthetic strategies [7–9], but the chemistry and multi-gram scale preparation of these compounds has been documented at length [18, 19]. In this manuscript, we describe the synthesis and antiparasitic evaluation of an exploratory series of tropolone-based endocyclic hydrazines. As detailed in the following sections, several of these compounds displayed activities against *T. cruzi* and *L. donovani* that are superior to those of chemotherapies currently employed against these trypanosomatids.



Scheme 1. Numbering scheme used in the text and synthetic route to diazabicycles **3a–i**, **4a–i**, and **5a–i**.

Results and discussion

Chemistry

The first step toward the preparation of the new series of diazabicycles involved the generation of a library of tropolone ethers with structures corresponding to those of earlier series of benzotropolone-based oxazino and hydrazo bicycles (compounds **2a–2i**, Scheme 1). As noted in our previous reports, the precursors needed to prepare these derivatives are readily available, and they provide a small but representative series of alkyl and aryl substitutions at the C2 position of the tropolone [7–9]. Although it has been reported that the synthesis of this type of derivatives requires the use of crown ethers and phase-transfer catalysts [20], we found that standard Williamson etherification conditions were quite effective. As a matter of fact, treatment of tropolone **1** with three to five molar equivalents of the appropriate alkyl or benzyl halide and solid K_2CO_3 in refluxing acetonitrile led to the desired 2-alkoxy and 2-benzyloxytropanes in 52% to quantitative yields.

Following the preparation of ethers **2a–2i**, the final products were obtained using hetero Diels–Alder reaction conditions described by us in earlier reports [8, 9]. Thus, treatment of the tropanes with a two-fold molar excess of the electron-deficient diazo-containing dienophiles dimethyl, diethyl, and diisopropylazodicarboxylate (DMAD, DEAD, and DIAD, respectively) in refluxing toluene led cleanly to the desired diazabicyles. Once again, the dienophiles employed in the cycloadditions were selected so as to yield compounds with substitution patterns analogous to those of the already studied benzotropolone-based series [8, 9]. As we have observed in the past, the success of the reactions could be easily established by ^{1}H-NMR [7–9]. In particular, the signals for the C3 and C6 protons shift upfield, respectively, from 6.8 and 7.2 ppm in the starting materials to 5.8 and 5.3 ppm in the final products. This evidences the conversion of the flat pseudo-aromatic tropane ring into a bicyclic non-planar system. Finally, it is worth mentioning that with a few exceptions, the yields of the hetero Diels–Alder reactions ranged from 50% to nearly quantitative, corroborating the robustness of the synthetic methodology.

Table 1. *In vitro* protozoocidal activity of diazabicycles **3a**–**3i**, **4a**–**4i**, and **5a**–**5i**.

	IC_{50} (μM)[a]											
	T. cruzi			*L. donovani*[b]			*T. brucei rhodesiense*			*P. falciparum K1*		
Compound	**3**	**4**	**5**	**3**	**4**	**5**	**3**	**4**	**5**	**3**	**4**	**5**
a	11.17	7.58	4.74	–	–	1.87	3.80	3.49	1.14	15.00	5.90	5.45
b	6.16	6.31	3.41	–	–	5.09	2.90	1.87	1.16	15.80	4.90	4.04
c	6.01	2.24	2.63	2.25	4.77	–	1.98	1.28	4.40	26.50	4.30	3.19
d	8.56	2.15	2.60	–	1.15	2.63	1.60	1.13	0.70	11.40	3.30	1.91
e	5.32	< 1.10	1.74	–	1.15	1.19	1.19	0.66	0.90	14.40	4.70	1.25
f	3.84	< 1.10	1.26	–	3.66	5.93	1.60	0.80	0.80	12.80	3.30	2.29
g	2.69	1.69	1.41	–	1.45	–	1.12	0.90	0.44	2.90	3.50	1.52
h	1.13	< 1.10	2.55	–	1.16	1.20	1.02	0.73	0.49	5.10	3.50	1.95
i	2.31	1.90	2.59	–	3.83	1.52	1.29	1.00	0.52	10.5	3.22	2.30

[a] Benznidazole (IC_{50} 4.00, μM, *T. cruzi*), pentostam (IC_{50} 84.42, μM, 20.43 μgSb^V/mL, *L. donovani*), pentamidine (2.50×10^{-3} μM, *T. brucei rhodesiense*), and chloroquine (0.84 μM, *P. falciparum* K1) were used as controls. Compound concentrations of 30.0, 10.0, 3.0, 1.0, 0.3, and 0.1, μg/mL were used in the IC_{50} determinations. All reported values are averages of three independent repeats.

[b] Dashes indicate that the compound was toxic to macrophages.

Biology

The antiparasitic activity of the tropolone-based diazabicycles was evaluated against *T. cruzi*, *L. donovani*, *T. brucei rhodesiense*, and chloroquine-resistant *P falciparum* (strain K1), and results are presented in Table 1. Inspection of the data reveals that the former two parasites are particularly susceptible to these compounds. In the case of *T. cruzi*, activities ranged from three-fold lower to nearly fourfold higher than benznidazole, which had an IC_{50} of 4.00 μM in the same assay. Of the 27 compounds, 19 had trypanocidal activities higher than the control, with hydrazines **4e**, **4f**, and **4h** displaying IC_{50}s below 1.1 μM. Results for *L. donovani* were even more encouraging, revealing that all hydrazines non-toxic to macrophages in the assay were considerably more active than sodium stibogluconate (pentostam). Activities against this parasite ranged from more than one order of magnitude higher than the control for compound **5f**, to nearly two orders of magnitude higher in the case of diazabicycles **4d** and **4e**. As it was observed for our previous series of benzotropolone-based hydrazines [9], the data presented in Table 1 also show that the activity of the compounds correlates with the lipophilicity of their ether and carbamate groups. This finding further emphasizes the importance of membrane permeability in the activity of this class of compounds [7–9].

While none of the Diels–Alder adducts in this new series had activities comparable to pentamidine in the *T. Brucei-rhodesiense* assay, some of them, including **5e** and **5g**, were only half as active as the control against chloroquine-resistant *P falciparum* K1. As was the case for trypanosomatids discussed above, there is a clear correlation between the hydrophobicity of the compounds and their activity against these protozoa.

Table 2. *In vitro* toxicity of diazabicycles **3a**–**3i**, **4a**–**4i**, and **5a**–**5i**.

	LD_{50} (μM)[a]		
Compound	**3**	**4**	**5**
a	1.06	1.15	0.82
b	1.27	2.32	0.59
c	0.77	0.42	0.60
d	0.82	0.79	0.80
e	2.05	0.46	1.61
f	0.67	0.47	0.79
g	0.40	0.76	0.51
h	0.62	1.72	0.89
i	0.92	0.81	0.47

[a] Cytotoxicities where assayed against KB cells using podophyllotoxin as standard (LD_{50} 0.50×10^{-3} μM). Compound concentrations of 300.0, 30.0, 3.0, and 0.3 μg/mL were used in the ED_{50} determinations. All reported values represent the average of three independent repeats.

The cytotoxicity of the tropolone-based hydrazines against KB cells is summarized in Table 2. Although they are all considerably less toxic than the control employed in the assay, the cytotoxicity of all of the compounds is quite high. Despite this, and even considering that some of the compounds were also toxic to macrophages (Table 1), it is worth noting that, in contrast to their protozoocidal activity, the cytotoxicity of the compounds is not related to their hydrophobicity. This could indicate that compound toxicity is linked more closely to the structure of the molecular framework than to the struc-

ture of the substituents, and could in principle be exploited to increase the therapeutic index of future series of diazabicycles.

Conclusions

To summarize, we described the preparation of 27 new bicyclic hydrazines and their evaluation as potential protozoocidals. The synthetic route to these compounds is based on simple and readily accessible tropolone ethers and relies on well-established and high-yielding chemical transformations. Despite most of the diazabicycles in this series displayed significant cytotoxicity, many of them were considerably more active than currently employed chemotherapies against *T. cruzi* and *L. donovani*. The results for these two parasites agree with earlier findings for similar compounds [7–9, 17], and are consistent with the fact that these trypanosomatids are particularly susceptible to oxidative stress [11–16].

In light of the results presented here and taking into account those from earlier reports, we are evaluating additional modifications to the tropane scaffold that could improve the selectivity of the compounds against protozoa. Of special interest is the reduction or derivatization of unsaturations in the seven-membered ring. In addition, we will study the mechanism of action of these compounds in more detail in an attempt to better guide the design of future series of compounds. Findings from these ongoing investigations will be reported shortly.

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Experimental

Chemistry

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected (A. H. Thomas Co., Philadelphia, PA, USA). Flash column chromatography was carried out using 230–400 mesh silica gel (Sigma-Aldrich, St. Louis, MO, USA). IR spectra were obtained on Perkin-Elmer Spectrum 1000 and Thermo-Electron Nicolet Avatar 370 DTGS FT-IR spectrophotometers (Perkin-Elmer Inc., Waltham, MA, USA; Thermo Fisher Scientific Inc., Waltham, MA, USA). NMR experiments were performed on a Bruker AVANCE 400 spectrometer operating at ^{1}H and ^{13}C frequencies of 400.13 and 100.61 MHz, respectively (Bruker BioSpin GmbH, Rheinstetten, Germany). Spectra of ethers **2a**–**2i** were recorded in $CDCl_3$ at room temperature, while those of all the Diels–Alder adducts were obtained in DMSO-d_6 at 90°C. Chemical shifts (δ) are in ppm and relative to tetramethylsilane, and coupling constants (*J*) are reported in Hz. ESI-MS spectra were recorded on a Varian 1200L triple quadrupole mass spectrometer (Varian Inc., Palo Alto, CA, USA). Tropolone **1** was prepared following the procedure described by Minns [19].

*General procedure for the preparation of tropolone ethers **2a–2i***

The appropriate alkyl or benzyl halide (12.3–24.6 mmol) was added gradually to a heterogeneous mixture of tropolone (**1**, 8.2 mmol) and K_2CO_3 (32.8 mmol) in acetonitrile (20 mL) under a nitrogen atmosphere. The resulting suspension was allowed to reflux until no starting materials were detectable by TLC (24 to 72 hours). The CH_3CN was then evaporated *in vacuo* to yield a solid that was resuspended in water (20 mL), and the resulting aqueous solution extracted with CH_2Cl_2 (2×20 mL). The combined organic extracts were washed with water (20 mL), dried with anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. Solid crude products were recrystallized from ether-hexanes, while oils were purified by column chromatography using hexanes-EtOAc (3 : 1 to 1 : 3) as eluting solvent.

*2-Methoxytropone **2a***

Obtained as an oil from **1** and iodomethane in 65% yield. ^{1}H-NMR: δ 3.93 (3H, s), 6.73 (1H, dd, *J* = 0.6, *J* = 10.1), 6.85 (1H, dddd, *J* = 0.6, *J* = 3.0, *J* = 6.3, *J* = 10.4), 7.07 (1H, ddd, *J* = 0.9, *J* = 10.1, *J* = 10.4), 7.21 (2H, AB). ^{13}C-NMR: δ 56.8, 112.9, 128.4, 133.2, 137.1, 137.4, 165.9, 180.9.

*2-Ethoxytropone **2b***

Obtained as an oil from **1** and bromoethane in 53% yield. ^{1}H-NMR: δ 1.54 (3H, t, *J* = 6.6), 4.15 (2H, q, *J* = 6.6), 6.75 (1H, dd, *J* = 0.7, *J* = 9.9), 6.86 (1H, dddd, J = 0.7, *J* = 3.3, *J* = 5.7, *J* = 10.5), 7.07 (1H, ddd, *J* = 1.0, *J* = 9.9, *J* = 10.5), 7.23 (2H, AB). ^{13}C-NMR: δ 14.7, 65.4, 113.7, 128.2, 133.3, 136.9, 137.3, 165.4, 181.0.

*2-Propoxytropone **2c***

Obtained as an oil from **1** and 1-bromopropane in 52% yield. ^{1}H-NMR: δ 1.07 (3H, t, *J* = 7.2), 1.95 (2H, sex, *J* = 7.2), 4.02 (2H, t, *J* = 7.2), 6.75 (1H, dd, *J* = 0.6, *J* = 10.0), 6.85 (1H, dddd, *J* = 0.6, *J* = 3.3, *J* = 5.7, *J* = 10.5), 7.06 (1H, ddd, *J* = 1.0, *J* = 10.0, *J* = 10.5), 7.22 (2H, AB). ^{13}C-NMR: δ 10.8, 22.4, 71.3, 113.8, 128.2, 133.3, 136.9, 137.4, 165.6, 181.0.

*2-Butoxytropone **2d***

Obtained as an oil from **1** and 1bromobutane in 62% yield. ^{1}H-NMR: δ 0.97 (3H, t, *J* = 7.5), 1.51 (2H, sex, *J* = 7.3), 1.89 (2H, quin, *J* = 7.1), 4.05 (2H, t, *J* = 6.9), 6.75 (1H, dd, *J* = 0.7, *J* = 10.0), 6.84 (1H, dddd, *J* = 0.7, *J* = 3.3, *J* = 5.9, *J* = 10.2), 7.05 (1H, ddd, *J* = 1.0, *J* = 10.0, *J* = 10.2) 7.21 (2H, AB). ^{13}C-NMR: δ 14.2, 19.6, 31.0, 69.6, 113.7, 128.1, 133.3, 136.8, 137.3, 165.6, 181.0.

*2-Benzyloxytropone **2e***

Obtained from **1** and benzyl bromide in quantitative yield. Mp. 81–82°C. ^{1}H-NMR: δ 5.21 (2H, s), 6.80 (1H, dd, *J* = 0.7, *J* = 9.9), 6.85 (1H, dddd, *J* = 0.7, *J* = 1.8, *J* = 7.4, *J* = 10.4), 6.99 (1H, ddd, *J* = 1.0, *J* = 9.9, *J* = 10.4), 7.24 (2H, AB), 7.38 (5H, m). ^{13}C-NMR: δ 71.2, 115.2, 127.5, 128.6, 128.7, 129.1, 133.1, 135.7, 136.9, 137.7, 164.8, 181.0.

2-(p-Fluorobenzyloxy)tropone ***2f***

Obtained from **1** and *p*-fluorobenzyl bromide in 88% yield. Mp. 90 – 91°C. ^{1}H-NMR: δ 5.20 (2H, s), 6.79 (1H, bd, *J* = 9.7), 6.86 (1H, bddd, *J* = 3.0, *J* = 6.2, *J* = 10.1), 7.00 (1H, bdd, *J* = 9.7, *J* = 10.1), 7.06 (2H, dd, *J* = 8.5, $^3J_{HF}$ = 8.7), 7.23 (2H, AB), 7.42 (2H, dd, $^4J_{HF}$ = 5.4, *J* = 8.5). ^{13}C-NMR: δ 70.7, 115.3, 116.1 ($^2J_{CF}$ = 21.6), 128.9, 129.5 ($^3J_{CF}$ = 8.0), 131.6 ($^4J_{CF}$ = 3.3), 132.9, 136.8, 137.9, 163.0 ($^1J_{CF}$ = 247.7), 164.7, 181.0.

2-(o-Fluorobenzyloxy)tropone ***2g***

Obtained from **1** and *o*-fluorobenzyl chloride in 78% yield. Mp. 87 – 88°C. ^{1}H-NMR: δ 5.31 (2H, s), 6.85 (1H, dd, *J* = 0.6, *J* = 10.0), 6.88 (1H, dddd, *J* = 0.6, *J* = 2.6, *J* = 6.8, *J* = 10.6), 7.03 (1H, ddd, *J* = 1.0, *J* = 10.0, *J* = 10.6), 7.10 (1H, m), 7.18 (1H, m), 7.25 (2H, AB), 7.32 (1H, m), 7.57 (1H, m). ^{13}C-NMR: δ 64.9 ($^3J_{CF}$ = 4.6), 115.1, 115.7 ($^2J_{CF}$ = 20.9), 123.0 ($^2J_{CF}$ = 13.4), 125.0 ($^4J_{CF}$ = 3.7), 128.9, 130.0 ($^3J_{CF}$ = 3.7), 130.4 ($^3J_{CF}$ = 8.2), 132.9, 136.8, 137.9, 160.5 ($^1J_{CF}$ = 245.5), 164.6, 181.0.

2-(p-Chlorobenzyloxy)tropone ***2h***

Obtained from **1** and *p*-chlorobenzyl bromide in 84% yield. Mp. 106 – 107°C. ^{1}H-NMR: δ 5.19 (2H, s), 6.75 (1H, dd, *J* = 0.6, *J* = 9.9), 6.84 (1H, dddd, *J* = 0.6, *J* = 2.9, *J* = 6.1, *J* = 10.6), 6.97 (1H, ddd, *J* = 1.0, *J* = 9.9, *J* = 10.6), 7.21 (2H, AB), 7.35 (4H, AA′BB′). ^{13}C-NMR: δ 70.5, 115.4, 128.916, 129.0, 129.4, 132.9, 134.3, 134.5, 136.9, 137.9, 164.6, 181.0.

2-(p-Methoxybenzyloxy)tropone ***2i***

Obtained from **1** and *p*-methoxybenzyl chloride in quantitative yield. Mp. 115 – 117°C. ^{1}H-NMR: δ 3.82 (3H, s), 5.21 (2H, s), 6.82 (1H, dd, *J* = 0.6, *J* = 9.9), 6.85 (1H, dddd, *J* = 0.6, *J* = 2.4, *J* = 6.9, *J* = 10.6), 6.92 (2H, AA′XX′), 6.99 (1H, ddd, *J* = 1.0, *J* = 9.9, *J* = 10.6), 7.23 (2H, AB), 7.37 (2H, AA′XX′). ^{13}C-NMR: δ 55.7, 71.2, 114.6, 115.2, 127.8, 128.5, 129.3, 132.9, 136.7, 137.8, 160.0, 165.0, 181.2.

General procedure for the preparation of the azodicarboxylate Diels–Alder adducts ***3a–3i, 4a–4i,*** *and* ***5a–5i***

The appropriate azodicarboxylate (8.2-24.7 mmol) was added to a solution of the desired tropolone ether (**2a** – **2i**, 1.1-6.9 mmol) in toluene (25 mL), and the resulting mixture heated to reflux. Once TLC indicated that the starting material was consumed (18 to 72 hours), the solvent was removed under reduced pressure. The crude product was then triturated with hexanes-ether to eliminate unreacted dienophile, and, if necessary, further purified by column chromatography using hexanes-EtOAc (1 : 3 to 3 : 1) as eluting solvent.

Dimethyl-1-methoxy-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***3a***

Obtained from **2a** in 98% yield. Mp. 165 – 166°C. IR (KBr disc, cm^{-1}): 1106 (m, C-O-C, ether), 1630 (m, C=C), 1722 (s, C=O, ketone), 1748 (s, C=O, carbamate), 2958 (m, C-C-H), 3067 (w, C=C – H). ^{1}H-NMR: δ 3.35 (3H, s), 3.65 (3H, s), 3.75 (3H, s), 5.32 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.81 (1H, d, *J* = 11.4), 6.03 (1H, dd, *J* = 0.8, *J* = 9.0), 6.89 (1H, dd, *J* = 8.0, *J* = 11.4), 7.29 (1H, dd, *J* = 5.8, *J* = 9.0). ^{13}C-NMR: δ 51.8, 54.2, 54.6, 55.0, 94.6, 127.0, 131.2, 140.4, 146.6, 157.9, 158.1, 189.1. ESI-MS: 305.0 [M+Na].

Dimethyl-1-ethoxy-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***3b***

Obtained from **2b** in 89% yield. Mp. 201 – 202°C. IR (KBr disc, cm^{-1}): 1103 (m, C-O-C, ether), 1631 (m, C=C), 1724 (s, C=O, ketone), 1747 (s, C=O, carbamate), 2951 (m, C-C-H), 3062 (w, C=C – H). ^{1}H-NMR: δ 1.20 (3H, t, *J* = 7.1), 3.65 (3H, s), 3.74 (3H, s), 3.63 (2H, ABX_3), 5.30 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.80 (1H, d, *J* = 11.4), 6.02 (1H, dd, *J* = 0.8, *J* = 9.0), 6.88 (1H, dd, *J* = 8.0, *J* = 11.4), 7.26 (1H, dd, *J* = 5.8, *J* = 9.0). ^{13}C-NMR: δ 15.9, 54.2, 54.6, 55.0, 60.3, 94.2, 127.7, 131.2, 140.3, 146.2, 157.9, 158.1, 189.2. ESI-MS: 319.0 [M+Na].

Dimethyl-1-propoxy-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***3c***

Obtained from **2c** in 50% yield. Mp. 110-111°C. IR (KBr disc, cm^{-1}): 1112 (m, C-O-C, ether), 1634 (m, C=C), 1716 (s, C=O, ketone/carbamate), 2959 (m, C-C-H), 3074 (w, C=C – H). ^{1}H-NMR: δ 0.92 (3H, t, *J* = 7.3), 1.60 (2H, sex, *J* = 7.3), 3.53 (2H, ABX_2), 3.64 (3H, s), 3.74 (3H, s), 5.31 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.80 (1H, d, *J* = 11.4), 6.01 (1H, dd, *J* = 0.8, *J* = 9.0), 6.89 (1H, dd, *J* = 8.0, *J* = 11.4), 7.26 (1H, dd, *J* = 5.8, *J* = 9.0). ^{13}C-NMR: δ 11.1, 23.2, 54.1, 54.6, 55.0, 66.3, 94.2, 127.6, 131.3, 140.2, 146.2, 157.9, 158.1, 189.2. ESI-MS: 333.1 [M+Na].

Dimethyl-1-butoxy-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***3d***

Obtained from **2d** in 61% yield. Mp. 100 – 101°C. IR (KBr disc, cm^{-1}): 1105 (m, C-O-C, ether), 1629 (m, C=C), 1709 (s, C=O, ketone), 1725 and 1752 (s, C=O, carbamate), 2958 (m, C-C-H), 3063 (w, C=C – H). ^{1}H-NMR: δ 0.91 (3H, t, *J* = 7.3), 1.39 (2H, sex, *J* = 7.3), 1.58 (2H, m), 3.58 (2H, ABX_2), 3.64 (3H, s), 3.74 (4H, s), 5.31 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.80 (1H, d, *J* = 11.4), 6.01 (1H, dd, *J* = 0.8, *J* = 9.0), 6.88 (1H, dd, *J* = 11.4, *J* = 8.0), 7.26 (1H, dd, *J* = 5.8, *J* = 9.0). ^{13}C-NMR: δ 14.2, 19.5, 32.0, 54.1, 54.6, 55.0, 64.3, 94.2, 127.5, 131.3, 140.2, 146.2, 157.9, 158.1, 189.2. ESI-MS: 347.1 [M+Na].

Dimethyl-1-benzyloxy-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***3e***

Obtained from **2e** in 88% yield. Mp. 149-150°C. IR (KBr disc, cm^{-1}): 1108 (m, C-O-C, ether), 1634 (m, C=C), 1726 (s, C=O, ketone), 1742 (s, C=O, carbamate), 2956 (m, C-C-H), 3063 (w, C=C – H). ^{1}H-NMR: δ 3.66 (3H, s), 3.77 (3H, s), 4.67 (2H, AB), 5.36 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.85 (1H, d, *J* = 11.4), 6.16 (1H, dd, *J* = 0.8, *J* = 9.0), 6.92 (1H, dd, *J* = 8.0, *J* = 11.4), 7.32 (1H, dd, *J* = 5.8, *J* = 9.0), 7.35 (5H, m). ^{13}C-NMR: δ 54.3, 54.8, 55.1, 66.6, 94.2, 127.1, 128.4, 128.7, 129.0, 131.1, 138.3, 140.6, 146.5, 158.0, 158.2, 189.2. ESI-MS: 381.1 [M+Na].

Dimethyl-1-(p-fluorobenzyloxy)-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***3f***

Obtained from **2f** in quantitative yield. Mp. 166 – 168°C. IR (KBr disc, cm^{-1}): 1109 (m, C-O-C, ether), 1725 (s, C=O, ketone), 1742 (s, C=O, carbamate), 2958 (m, C-C-H). ^{1}H-NMR: δ 3.66 (3H, s), 3.77 (3H, s), 4.65 (2H, AB), 5.36 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.1), 5.85 (1H, d, *J* = 11.4), 6.16 (1H, dd, *J* = 0.8, *J* = 9.0), 6.92 (1H, dd, *J* = 8.1, *J* = 11.4), 7.17 (2H, dd, *J* = 8.6, $^3J_{HF}$ = 8.8), 7.32 (1H, dd, *J* = 5.8, *J* = 9.0), 7.43 (2H, dd, $^4J_{HF}$ = 5.7, *J* = 8.6). ^{13}C-NMR: δ 54.3, 54.8, 55.2, 65.8, 94.2, 115.8 ($^2J_{CF}$ = 21.4), 127.2, 130.7 ($^3J_{CF}$ = 8.2), 131.2, 134.7 ($^4J_{CF}$ =

2.9), 140.5, 146.5, 158.0, 158.1, 162.6 ($^1J_{CF}$ = 243.3), 188.9. ESI-MS: 399.0 [M+Na].

Dimethyl-1-(o-fluorobenzyloxy)-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***3g***

Obtained from **2g** in quantitative yield. Mp. 116 – 118°C. IR (KBr disc, cm^{-1}): 1108 (m, C-O-C, ether), 1633 (m, C=C), 1723 (s, C=O, ketone/carbamate), 2959 (m, C-C-H). ^{1}H-NMR: δ 3.66 (3H, s), 3.77 (3H, s), 4.73 (2H, AB), 5.37 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.85 (1H, d, *J* = 11.4), 6.16 (1H, dd, *J* = 0.8, *J* = 9.0), 6.93 (1H, dd, *J* = 11.4, *J* = 8.0), 7.18 (1H, m), 7.23 (1H, m), 7.34 (1H, dd, *J* = 5.8, *J* = 9.0), 7.39 (1H, m), 7.55 (1H, m). ^{13}C-NMR: δ 54.3, 54.7, 55.2, 60.4 ($^3J_{CF}$ = 4.4), 94.1, 115.9 ($^2J_{CF}$ = 21.2), 125.1 ($^4J_{CF}$ = 3.5), 125.4 ($^2J_{CF}$ = 14.3), 127.0, 130.7 ($^3J_{CF}$ = 8.2), 131.2, 131.4 ($^3J_{CF}$ = 4.2), 140.5, 146.8, 158.0, 158.1, 161.0 ($^1J_{CF}$ = 246.1), 188.8. ESI-MS: 399.1 [M+Na].

Dimethyl-1-(p-chlorobenzyloxy)-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***3h***

Obtained from **2h** in 75% yield. Mp. 149 – 151°C. IR (KBr disc, cm^{-1}): 1109 (m, C-O-C, ether), 1633 (m, C=C), 1724 (s, C=O, ketone/carbamate), 2956 (m, C-C-H). ^{1}H-NMR: δ 3.66 (3H, s), 3.77 (3H, s), 4.66 (2H, AB), 5.36 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.85 (1H, d, *J* = 11.4), 6.16 (1H, dd, *J* = 0.8, *J* = 9.0), 6.93 (1H, dd, *J* = 8.0, *J* = 11.4), 7.33 (1H, dd, *J* = 5.8, *J* = 9.0), 7.42 (4H, s). ^{13}C-NMR: δ 54.3, 54.8, 55.2, 65.7, 94.2, 127.1, 129.0, 130.4, 131.2, 133.2, 137.5, 140.5, 146.6, 158.0, 158.1, 188.8. ESI-MS: 415.0 [M+Na].

Dimethyl-1-(p-methoxybenzyloxy)-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***3i***

Obtained from **2i** in 43% yield. Mp. 96 – 98°C. IR (KBr disc, cm^{-1}): 1104 (m, C-O-C, ether), 1633 (m, C=C), 1717 (s, C=O, ketone/carbamate), 2959 (m, C-C-H), 3064 (w, C=C-H). ^{1}H-NMR: δ 3.67 (3H, s), 3.77 (3H, s), 3.79 (3H, s), 4.59 (2H, AB), 5.35 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.84 (1H, d, *J* = 11.4), 6.15 (1H, dd, *J* = 0.8, *J* = 9.0), 6.92 (1H, dd, *J* = 8.0, *J* = 11.4), 6.94 (2H, AA′XX′), 7.30 (2H, AA′XX′), 7.31 (1H, dd, *J* = 5.8, *J* = 9.0). ^{13}C-NMR: δ 54.3, 54.7, 55.1, 56.1, 66.3, 94.2, 114.7, 127.4, 130.3, 130.5, 131.2, 140.4, 146.4, 158.0, 158.1 60.0, 189.0. ESI-MS: 411.0 [M+Na].

Diethyl-1-methoxy-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***4a***

Obtained from **2a** in 69% yield. Mp. 104-106°C. IR (KBr disc, cm^{-1}): 1114 (m, C-O-C, ether), 1633 (m, C=C), 1719 (s, C=O, ketone), 1747 (s, C=O, carbamate), 2986 (m, C-C-H), 3066 (w, C=C-H). ^{1}H-NMR: δ 1.15 (3H, t, *J* = 7.0), 1.22 (3H, t, *J* = 7.0), 3.36 (3H, s), 4.11 (2H, ABX_3), 4.18 (2H, q, *J* = 7.0), 5.32 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.81 (1H, d, *J* = 11.4), 6.02 (1H, dd, *J* = 0.8, *J* = 9.0), 6.90 (1H, dd, *J* = 8.0, *J* = 11.4), 7.29 (1H, dd, *J* = 5.8, *J* = 9.0). ^{13}C-NMR: δ 14.9, 15.0, 51.8, 54.8, 63.1, 63.5, 94.5, 127.2, 131.3, 140.4, 146.6, 157.4, 157.5, 189.2. ESI-MS: 333.1 [M+Na].

Diethyl-1-ethoxy-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***4b***

Obtained from **2b** in 87% yield. Mp. 155 – 156°C. IR (KBr disc, cm^{-1}): 1112 (m, C-O-C, ether), 1629 (m, C=C), 1720 (s, C=O, ketone), 1746 (s, C=O, carbamate), 2986 (m, C-C-H), 3060 (w, C=C-H). ^{1}H-NMR: δ 1.15 (3H, t, *J* = 7.0), 1.20 (3H, t, *J* = 7.0), 1.22 (3H, t, *J* = 7.0), 3.64 (2H, ABX_3), 4.11 (2H, q, *J* = 7.0), 4.17 (2H, qd, *J* = 1.8, *J* = 7.1), 5.31 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.80 (1H, d, *J* = 11.4), 6.01 (1H, dd, *J* = 0.8, *J* = 9.0), 6.88 (1H, dd, *J* = 8.0, *J* = 11.4), 7.26 (1H, dd, *J* = 5.8, *J* = 9.0). ^{13}C-NMR: δ 15.0 (2), 15.9, 54.8, 60.2, 63.0, 63.5, 94.2, 127.8, 131.3, 140.4, 146.3, 157.5 (2), 189.3. ESI-MS: 347.1 [M+Na].

Diethyl-1-propoxy-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***4c***

Obtained from **2c** in 38% yield. Mp. 119 – 120°C. IR (KBr disc, cm^{-1}): 1110 (m, C-O-C, ether), 1633 (m, C=C), 1712 (s, C=O, ketone/carbamate), 2964 (m, C-C-H). ^{1}H-NMR: δ 0.92 (3H, t, *J* = 7.3), 1.15 (3H, t, *J* = 7.0), 1.22 (3H, t, *J* = 7.0), 1.60 (2H, q, *J* = 7.3), 3.55 (2H, ABX_2), 4.10 (2H, ABX_3), 4.17 (2H, ABX_3), 5.31 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.80 (1H, d, *J* = 11.4), 6.00 (1H, dd, *J* = 0.8, *J* = 9.0), 6.88 (1H, dd, *J* = 8.0, *J* = 11.4), 7.26 (1H, dd, *J* = 5.8, *J* = 9.0). ^{13}C-NMR: δ 11.2, 14.9, 15.0, 23.3, 54.8, 63.0, 63.5, 66.3, 94.2, 127.7, 131.3, 140.3, 146.3, 157.4, 157.5, 189.3. ESI-MS: 361.1 [M+Na].

Diethyl-1-butoxy-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***4d***

Obtained from **2d** in 50% yield. Mp. 63 – 64°C. IR (KBr disc, cm^{-1}): 1110 (m, C-O-C, ether), 1720 (s, C=O, ketone), 1753 (s, C=O, carbamate), 2959 (m, C-C-H), 3061 (w, C=C-H). ^{1}H-NMR: δ 0.91 (3H, t, *J* = 7.3), 1.15 (3H, t, *J* = 7.0), 1.22 (3H, t, *J* = 7.0), 1.39 (2H, sex, *J* = 7.3), 1.58 (2H, m), 3.60 (2H, ABX_2), 4.10 (2H, ABX_3), 4.17 (2H, ABX_3), 5.31 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.80 (1H, d, *J* = 11.4), 6.01 (1H, dd, *J* = 0.8, *J* = 9.0,), 6.88 (1H, dd, *J* = 11.4, *J* = 8.0), 7.26 (1H, dd, *J* = 5.8, *J* = 9.0). ^{13}C-NMR: δ 14.2, 14.9, 15.0, 19.5, 32.1, 54.8, 63.0, 63.4, 64.2, 94.2, 127.7, 131.3, 140.3, 146.3, 157.4, 157.5, 189.3. ESI-MS: 352.1 [M+Na].

Diethyl-1-benzyloxy-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***4e***

Obtained from **2e** in 98% yield. Mp. 121 – 123°C. IR (KBr disc, cm^{-1}): 1104 (m, C-O-C, ether), 1633 (m, C=C), 1716 (s, C=O, ketone/carbamate), 2970 (m, C-C-H), 3066 (w, C=C-H). ^{1}H-NMR: δ 1.15 (3H, t, *J* = 7.0), 1.22 (3H, t, *J* = 7.0), 4.13 (2H, ABX_3), 4.21 (2H, ABX_3), 4.68 (2H, AB), 5.36 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.85 (1H, d, *J* = 11.4), 6.16 (1H, dd, *J* = 0.8, *J* = 9.0), 6.93 (1H, dd, *J* = 8.0, *J* = 11.4), 7.33 (1H, dd, *J* = 5.8, *J* = 9.0), 7.35 (5H, m). ^{13}C-NMR: δ 14.9, 15.0, 55.0, 63.2, 63.6, 66.5, 94.2, 127.4, 128.3, 128.6, 129.0, 131.3, 138.5, 140.5, 146.6, 157.4, 157.5, 189.0. ESI-MS: 409.1 [M+Na].

Diethyl-1-(p-fluorobenzyloxy)-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***4f***

Obtained from **2f** in 81% yield. Mp. 92 – 94°C. IR (KBr disc, cm^{-1}): 1100 (m, C-O-C, ether), 1634 (m, C=C), 1712 (s, C=O, ketone), 1721 and 1743 (s, C=O, carbamate), 2986 (m, C-C-H), 3080 (w, C=C-H). ^{1}H-NMR: δ 1.15 (3H, t, *J* = 7.0), 1.22 (3H, t, *J* = 7.0), 4.12 (2H, ABX_3), 4.21 (2H, ABX_3), 4.67 (2H, AB), 5.36 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.85 (1H, d, *J* = 11.4), 6.16 (1H, dd, *J* = 0.8, *J* = 9.0), 6.93 (1H, dd, *J* = 8.0, *J* = 11.4), 7.17 (2H, dd, *J* = 8.6, $^3J_{HF}$ = 8.8), 7.33 (1H, dd, *J* = 5.8, *J* = 9.0), 7.43 (2H, dd, $^4J_{HF}$ = 5.7, *J* = 8.6). ^{13}C-NMR: δ 14.9, 15.0, 55.0, 63.2, 63.6, 65.8, 94.2, 115.8 ($^2J_{CF}$ = 21.4), 127.3, 130.7 ($^3J_{CF}$ = 8.2), 131.3, 134.7 ($^4J_{CF}$ = 3.1), 140.6, 146.6, 157.4, 157.5, 162.7 ($^1J_{CF}$ = 243.5), 188.9. ESI-MS: 427.1 [M+Na].

Diethyl-1-(o-fluorobenzyloxy)-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***4g***

Obtained from **2g** in 71% yield. Mp. 121 – 122°C. IR (KBr disc, cm^{-1}): 1102 (m, C-O-C, ether), 1633 (m, C=C), 1720 (s, C=O, ketone/

carbamate), 2985 (m, C-C-H), 3063 (w, C=C-H). ^{1}H-NMR: δ 1.14 (3H, t, *J* = 7.0), 1.23 (3H, t, *J* = 7.0), 4.11 (2H, ABX_3), 4.21 (2H, ABX_3), 4.75 (2H, AB), 5.37 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.85 (1H, d, *J* = 11.4), 6.16 (1H, dd, *J* = 0.8, *J* = 9.0), 6.94 (1H, dd, *J* = 8.0, *J* = 11.4), 7.17 (1H, m), 7.23 (1H, m), 7.34 (1H, dd, *J* = 5.8, *J* = 9.0), 7.38 (1H, m), 7.56 (1H, m). ^{13}C-NMR: δ 14.9 (2), 55.0, 60.3 ($^3J_{CF}$ = 4.6), 63.2, 63.7, 94.1, 115.9 ($^2J_{CF}$ = 21.0), 125.1 ($^4J_{CF}$ = 3.5), 125.4 ($^2J_{CF}$ = 14.5), 127.1, 130.6 ($^3J_{CF}$ = 8.2), 131.3 ($^3J_{CF}$ = 4.2), 131.3, 140.6, 146.8, 157.3, 157.5, 161.0 ($^1J_{CF}$ = 245.8), 188.9. ESI-MS: 427.1 [M+Na].

Diethyl-1-(p-chlorobenzyloxy)-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***4h***

Obtained from **2h** in 95% yield. Mp. 86 – 89°C IR (KBr disc, cm^{-1}): 1093 (m, C-O-C, ether), 1633 (m, C=C), 1722 (s, C=O, ketone/carbamate), 2985 (m, C-C-H). ^{1}H-NMR: δ 1.14 (3H, t, *J* = 7.0), 1.22 (3H, t, *J* = 7.0), 4.12 (2H, ABX_3), 4.20 (2H, ABX_3), 4.68 (2H, AB), 5.36 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.85 (1H, d, *J* = 11.4), 6.16 (1H, dd, *J* = 0.8, *J* = 9.0), 6.93 (1H, dd, *J* = 8.0, *J* = 11.4), 7.33 (1H, dd, *J* = 5.8, *J* = 9.0), 7.41 (4H, s). ^{13}C-NMR: δ 14.9, 15.0, 55.0, 63.3, 63.7, 65.7, 94.2, 127.3, 129.0, 130.3, 131.3, 133.2, 137.5, 140.6, 146.7, 157.4, 157.5, 188.9. ESI-MS: 443.1 [M+Na].

Diethyl-1-(p-methoxybenzyloxy)-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***4i***

Obtained from **2i** in 71% yield. Mp. 97 – 98°C. IR (KBr disc, cm^{-1}): 1100 (m, C-O-C, ether), 1615 (m, C=C), 1708 (s, C=O, ketone/carbamate), 2988 (m, C-C-H), 3060 (w, C=C-H). ^{1}H-NMR: δ 1.15 (3H, t, *J* = 7.0), 1.22 (3H, t, *J* = 7.0), 3.78 (3H, s), 4.12 (2H, ABX_3), 4.21 (2H, ABX_3), 4.60 (2H, AB), 5.35 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.84 (1H, d, *J* = 11.4), 6.14 (1H, dd, *J* = 0.8, *J* = 9.0), 6.92 (1H, dd, *J* = 8.0, *J* = 11.4), 6.93 (2H, AA′XX′), 7.30 (2H, AA′XX′), 7.31 (1H, dd, *J* = 5.8, *J* = 9.0). ^{13}C-NMR: δ 15.0 (2), 55.0, 56.1, 63.2, 63.6, 66.3, 94.2, 114.7, 127.5, 130.3, 130.5, 131.3, 140.5, 146.4, 157.4, 157.5, 160.0, 189.0. ESI-MS: 439.2 [M+Na].

Diisopropyl-1-methoxy-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***5a***

Obtained from **2a** in 53% yield. Mp. 144 – 146°C. IR (KBr disc, cm^{-1}): 1114 (m, C-O-C, ether), 1634 (m, C=C), 1715 (s, C=O, ketone/carbamate), 2984 (m, C-C-H), 3062 (w, C=C-H). ^{1}H-NMR: δ 1.16 (3H, d, *J* = 6.2), 1.17 (3H, d, *J* = 6.2), 1.22 (3H, d, *J* = 6.2), 1.23 (3H, d, *J* = 6.2), 3.35 (3H, s), 4.83 (1H, sep, *J* = 6.2), 4.89 (1H, sep, *J* = 6.2), 5.30 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.79 (1H, d, *J* = 11.4), 5.99 (1H, dd, *J* = 0.8, *J* = 9.0), 6.88 (1H, dd, *J* = 8.0, *J* = 11.4), 7.27 (1H, dd, *J* = 5.8, *J* = 9.0). ^{13}C-NMR: δ 22.2, 22.3, 22.4, 22.5, 51.7, 54.6, 70.9, 71.2, 94.3, 127.3, 131.3, 140.3, 146.6, 156.9, 157.0, 189. ESI-MS: 339.2 [M+H].

Diisopropyl-1-ethoxy-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***5b***

Obtained from **2b** in 70% yield. Mp. 128 – 130°C. IR (KBr disc, cm^{-1}): 1112 (m, C-O-C, ether), 1636 (m, C=C), 1715 (s, C=O, ketone/carbamate), 2983 (m, C-C-H). ^{1}H-NMR: δ 1.16 (3H, d, *J* = 6.2), 1.17 (3H, d, *J* = 6.2), 1.20 (3H, t, *J* = 7.1), 1.22 (3H, d, *J* = 6.2), 1.23 (3H, d, *J* = 6.2), 3.64 (2H, ABX_3), 4.83 (1H, sep, *J* = 6.2), 4.88 (1H, sep, *J* = 6.2), 5.29 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.78 (1H, d, *J* = 11.4), 5.98 (1H, dd, *J* = 0.8, *J* = 9.0), 6.87 (1H, dd, *J* = 8.0, *J* = 11.4), 7.24 (1H, dd, *J* = 5.8, *J* = 9.0). ^{13}C-NMR: δ 15.9, 22.2, 22.3, 22.4, 22.5, 54.6, 60.2, 70.9, 71.2, 94.1, 128.0, 131.3, 140.3, 146.2, 156.9 (2), 189.4. ESI-MS: 353.2 [M+H].

Diisopropyl-1-propoxy-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***5c***

Obtained from **2c** in 42% yield. Mp. 98 – 99°C. IR (KBr disc, cm^{-1}): 1110 (m, C-O-C, ether), 1633 (m, C=C), 1715 (s, C=O, ketone), 1738 (s, C=O, carbamate), 2984 (m, C-C-H). ^{1}H-NMR: δ 0.92 (3H, t, *J* = 7.3), 1.16 (3H, d, *J* = 6.2), 1.17 (3H, d, *J* = 6.2), 1.22 (3H, d, *J* = 6.2), 1.23 (3H, d, *J* = 6.2), 1.60 (2H, sex, *J* = 7.3), 3.54 (2H, ABX_2), 4.83 (1H, sep, *J* = 6.2), 4.88 (1H, sep, *J* = 6.2), 5.29 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.79 (1H, d, *J* = 11.4), 5.98 (1H, dd, *J* = 0.8, *J* = 9.0), 6.87 (1H, dd, *J* = 8.0, *J* = 11.4), 7.25 (1H, dd, *J* = 5.8, *J* = 9.0). ^{13}C-NMR: δ 11.2, 22.2, 22.4 (2), 22.5, 23.3, 54.6, 66.3, 70.9, 71.2, 94.0, 127.7, 131.3, 140.3, 146.2, 156.9, 157.0, 189.4. ESI-MS: 367.2 [M+H].

Diisopropyl-1-butoxy-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***5d***

Obtained from **2d** in 56% yield. Mp. 71 – 72°C. IR (KBr disc, cm^{-1}): 1110 (m, C-O-C, ether), 1635 (m, C=C), 1715 (s, C=O, ketone), 1727 and 1743 (s, C=O, carbamate), 2983 (m, C-C-H), 3068 (w, C=C-H). ^{1}H-NMR: δ 0.91 (3H, t, *J* = 7.3), 1.16 (3H, d, *J* = 6.2), 1.17 (3H, d, *J* = 6.2), 1.21 (3H, d, *J* = 6.2), 1.22 (3H, d, *J* = 6.2), 1.39 (2H, sex, *J* = 7.3), 1.57 (2H, m), 3.58 (2H, ABX_2), 4.82 (1H, sep, *J* = 6.2), 4.88 (1H, sep, *J* = 6.2), 5.29 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.1), 5.79 (1H, d, *J* = 11.4), 5.98 (1H, dd, *J* = 0.8, *J* = 9.0), 6.87 (1H, dd, *J* = 8.1, *J* = 11.4), 7.25 (1H, dd, *J* = 5.8, *J* = 9.0). ^{13}C-NMR: δ 14.3, 19.5, 22.2, 22.3, 22.4, 22.5, 32.1, 54.6, 64.1, 70.9, 71.2, 94.0, 127.7, 131.4, 140.3, 146.3, 156.9, 157.0, 189.5. ESI-MS: 381.2 [M+H].

Diisopropyl-1-benzyloxy-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***5e***

Obtained from **2e** in 91% yield. Mp. 99 – 100°C. IR (KBr disc, cm^{-1}): 1104 (m, C-O-C, ether), 1634 (m, C=C), 1710 (s, C=O, ketone), 1742 (s, C=O, carbamate), 2984 (m, C-C-H). ^{1}H-NMR: δ 1.15 (3H, d, *J* = 6.2), 1.18 (3H, d, *J* = 6.2), 1.22 (3H, d, *J* = 6.2), 1.24 (3H, d, *J* = 6.2), 4.68 (2H, AB), 4.86 (1H, sep, *J* = 6.2), 4.92 (1H, sep, *J* = 6.2), 5.35 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.1), 5.84 (1H, d, *J* = 11.4), 6.16 (1H, dd, *J* = 0.8, *J* = 9.0), 6.93 (1H, dd, *J* = 8.1, *J* = 11.4), 7.32 (1H, dd, *J* = 5.8, *J* = 9.0), 7.35 (5H, m). ^{13}C-NMR: δ 22.1, 22.2, 22.4, 22.5, 54.8, 66.5, 71.1, 71.4, 94.1, 127.5, 128.3, 128.6, 129.0, 131.4, 138.5, 140.5, 146.6, 158.0 (2), 189.1. ESI-MS: 437.1 [M+Na].

Diisopropyl-1-(p-fluorobenzyloxy)-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***5f***

Obtained from **2f** in 80% yield. Mp. 120 – 121°C. IR (KBr disc, cm^{-1}): 1107 (m, C-O-C, ether), 1634 (m, C=C), 1719 (s, C=O, ketone/carbamate), 2980 (m, C-C-H), 3061 (w, C=C-H). ^{1}H-NMR: δ 1.15 (3H, d, *J* = 6.2), 1.17 (3H, d, *J* = 6.2), 1.22 (3H, d, *J* = 6.2), 1.23 (3H, d, *J* = 6.2), 4.66 (2H, AB), 4.85 (1H, sep, *J* = 6.2), 4.91 (1H, sep, *J* = 6.2), 5.35 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.84 (1H, d, *J* = 11.4), 6.16 (1H, dd, *J* = 0.8, *J* = 9.0), 6.93 (1H, dd, *J* = 8.0, 11.4), 7.17 (2H, dd, *J* = 8.6, $^3J_{HF}$ = 8.8), 7.32 (1H, dd, *J* = 5.8, *J* = 9.0), 7.43 (2H, dd, $^4J_{HF}$ = 5.7, *J* = 8.6). ^{13}C-NMR: δ 22.2, 22.3, 22.4, 22.5, 54.8, 65.8, 71.2, 71.4, 94.1, 115.8 ($^2J_{CF}$ = 21.2), 127.4, 130.7 ($^3J_{CF}$ = 8.1), 131.3, 134.7 ($^4J_{CF}$ = 2.9), 140.6, 146.6, 156.9, 157.0, 162.6 ($^1J_{CF}$ = 244.1), 189.0. ESI-MS: 455.1 [M+Na].

Diisopropyl-1-(o-fluorobenzyloxy)-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***5g***

Obtained from **2g** in 82% yield. Mp. 118 – 119°C. IR (KBr disc, cm^{-1}): 1099 (m, C-O-C, ether), 1633 (m, C=C), 1705 (s, C=O,

ketone), 1739 (s, C=O, carbamate), 2988 (m, C-C-H), 3060 (w, C=C-H). ^{1}H-NMR: δ 1.14 (3H, d, *J* = 6.2), 1.16 (3H, d, *J* = 6.2), 1.22 (3H, d, *J* = 6.2), 1.24 (3H, d, *J* = 6.2), 4.74 (2H, AB), 4.84 (1H, sep, *J* = 6.2), 4.92 (1H, sep, *J* = 6.2), 5.36 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.85 (1H, d, *J* = 11.4), 6.16 (1H, dd, *J* = 0.8, *J* = 9.0), 6.93 (1H, dd, *J* = 8.0, *J* = 11.4), 7.18 (1H, m), 7.23 (1H, m), 7.34 (1H, dd, *J* = 5.8, *J* = 9.0), 7.38 (1H, m), 7.60 (1H, m). ^{13}C-NMR: δ 22.2, 22.3 (2), 22.4, 54.8, 60.2 ($^3J_{CF}$ = 4.4), 71.2, 71.4, 94.0, 115.8 ($^2J_{CF}$ = 21.2), 125.1 ($^4J_{CF}$ = 3.3), 125.4 ($^2J_{CF}$ = 14.3), 127.2, 130.6 ($^3J_{CF}$ = 8.1), 131.2 ($^3J_{CF}$ = 4.4), 131.3, 140.6, 146.9, 156.9, 157.0, 161.0 ($^1J_{CF}$ = 242.6), 188.9. ESI-MS: 455.1 [M+Na].

Diisopropyl-1-(p-chlorobenzyloxy)-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***5h***

Obtained from **2h** in 71% yield. Mp. 120 – 121°C. IR (KBr disc, cm^{-1}): 1111 (m, C-O-C, ether), 1633 (m, C=C), 1713 (s, C=O, ketone/carbamate), 2982 (m, C-C-H), 3063 (w, C=C-H). ^{1}H-NMR: δ 1.15 (3H, d, *J* = 6.2), 1.17 (3H, d, *J* = 6.2), 1.22 (3H, d, *J* = 6.2), 1.23 (3H, d, *J* = 6.2), 4.68 (2H, AB), 4.85 (1H, sep, *J* = 6.2), 4.91 (1H, sep, *J* = 6.2), 5.35 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.85 (1H, d, *J* = 11.4), 6.16 (1H, dd, *J* = 0.8, *J* = 9.0), 6.93 (1H, dd, *J* = 8.0, *J* = 11.4), 7.32 (1H, dd, *J* = 5.8, *J* = 9.0), 7.42 (4H, s). ^{13}C-NMR: δ 22.2, 22.3, 22.4, 22.5, 54.8, 65.7, 71.2, 71.4, 94.1, 127.3, 129.0, 130.3, 131.3, 133.2, 137.5, 140.6, 146.7, 156.9, 157.0, 189.0. ESI-MS: 471.1 [M+Na].

Diisopropyl-1-(p-methoxybenzyloxy)-2-oxo-6,7-diazabicyclo[3.2.2]nona-3,8-diene-6,7-dicarboxylate ***5i***

Obtained from **2i** in 44% yield. Mp. 108 – 109°C. IR (KBr disc, cm^{-1}): 1099 (m, C-O-C, ether), 1715 (s, C=O, ketone), 1729 and 1743 (s, C=O, carbamate), 2983 (m, C-C-H), 3069 (w, C=C-H). ^{1}H-NMR: δ 1.17 (3H, d, *J* = 6.2), 1.19 (3H, d, *J* = 6.2), 1.23 (3H, d, *J* = 6.2), 1.25 (3H, d, *J* = 6.2), 3.79 (3H, s), 4.62 (2H, AB), 4.88 (1H, sep, *J* = 6.2), 4.93 (1H, sep, *J* = 6.2), 5.35 (1H, ddd, *J* = 0.8, *J* = 5.8, *J* = 8.0), 5.84 (1H, d, *J* = 11.4), 6.15 (1H, dd, *J* = 0.8, *J* = 9.0), 6.92 (1H, dd, *J* = 8.0, *J* = 11.4), 6.94 (2H, AA'XX'), 7.30 (2H, AA'XX'), 7.31 (1H, dd, *J* = 5.8, *J* = 9.0). ^{13}C-NMR: δ 22.3, 22.4, 22.4, 22.5, 54.7, 56.1, 66.2, 71.1, 71.3, 94.1, 114.7, 127.6, 130.3 (2), 130.5, 131.4, 140.5, 146.5, 157.0, 160.0, 189.2. ESI-MS: 467.1 [M+Na].

Protozoocidal and cytotoxicity assays

The *in vitro* activity of compounds **3a-3i**, **4a-4i**, and **5a-5i** against *L. donovani*, *T. cruzi*, *P. falciparum*, and *T. brucei rhodesiense*, as well as their cytotoxicity against KB cells, was evaluated following protocols detailed in earlier reports [8].

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