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Synthesis and antimicrobial activity of new (4,4,4-trihalo-3-oxo-but-1-enyl)-carbamic acid ethyl esters, (4,4,4-trihalo-3-hydroxy-butyl)-carbamic acid ethyl esters, and 2-oxo-6-trihalomethyl-[1,3]oxazinane-3-carboxylic acid ethyl esters

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Abstract—This work presents a three-step synthesis of a new series of 4-substituted 2-oxo-6-trihalomethyl-[1,3]oxazinane-3-carboxylic acid ethyl esters, from β-alkoxyvinyl trihalomethyl ketones of general formula X_3C–C(O)–CH=C(R)–OR^1, where R = H, Me, Ph, and 4-Me-Ph; R^1 = Me and Et; and X = F and Cl. The Michael addition–substitution of the ethyl carbamate on β-alkoxyvinyl trihalomethyl ketones furnished the corresponding (4,4,4-trihalo-3-oxo-but-1-enyl)-carbamic acid ethyl esters. These compounds underwent reduction with $NaBH_4$ leading to the respective (4,4,4-trihalo-3-hydroxy-butyl)-carbamic acid ethyl esters. The 3-hydroxy-butyl carbamates were submitted to cyclization reaction with triphosgene to give a series of 4-substituted 2-oxo-6-trihalomethyl-[1,3]oxazinane-3-carboxylic acid ethyl esters. The in vitro antimicrobial activity, of some of the three new series of the title compounds, was assessed against a panel of microorganisms including yeast like fungi, bacteria, and algae, and their minimal inhibitory concentration and minimal fungicidal, bactericidal, and algacidal concentrations were determined. Some of the analyzed carbamates exhibited significant in vitro antimicrobial activity.

1. Introduction

A higher incidence of microbial infections caused by fungi, yeast, bacteria, and algae has been documented since the 1980s with the parallel emergence of either new pathogens or the resistance phenomenon. Fungi deserve attention because they are emerging as important nosocomial pathogens causing severe morbidity and mortality in immunocompromised patients. Modern therapies and management such as bone marrow or solid-organ transplants, new and more aggressive chemotherapy have resulted in a rapidly expanding number of immunosuppressed patients. These patients now survive longer and become highly susceptible to life-threatening fungal infections.[1] Concomitant with the increased incidence of fungal infections there has been a dramatic increase in the use of antifungals for the treatment of both systemic and localized fungal infections. In this context, the expanded use of antifungal agents has accelerated the development of antifungal drug resistance followed by frequent therapeutic failures and increasing mortality rate.[2] The most important and dreadful microorganisms include fungi such as *Candida albicans*, *Candida glabrata*, *Candida dubliniensis*, and *Cryptococcus neoformans*, and bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. *Prototheca zopfii*, an alga of medical and veterinary concern may, be added to this list.[3] The antimycotic drugs available for the treatment of systemic fungal infections are limited (11 active compounds)

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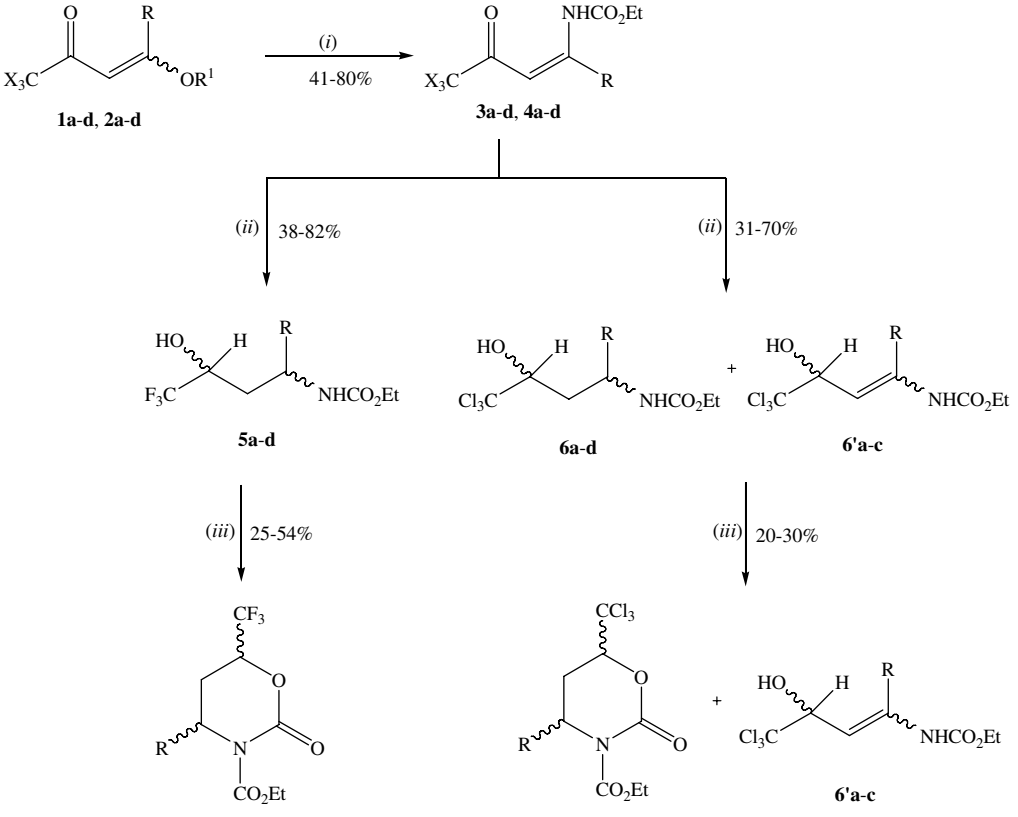
which can be divided into four classes as follows: (i) polyene macrolides; (ii) azole derivatives; (iii) DNA and RNA inhibitors; and (iv) 1,3-β-glucan synthase inhibitors (echinocandins).[4] Hence, there is a great demand for novel agents with a widened spectrum of activity and reduced toxicity.

Biological evaluation of carbamate function containing compounds was reported recently.[5,6] The introduction of a carbamate group in organic molecules has shown promising antimicrobial activity.[7] Furthermore, carbamate groups are frequently used as prodrug linkers or as versatile synthetic building blocks that allow one to combine structure novelty with the potential for streamline synthesis.[5,8] Carbamates are attractive due to the easy preparation and chemical stability.[9,10] Also, cyclic carbamates are described in the literature as biologically active compounds showing, for example, antitubercular activity.[11] On the other hand, it has been reported that the introduction of a trifluoromethyl group in heterocycles frequently results in much more potent activity than that of the parent compounds, a fact that is probably related to the high lipophilicity of the trifluoromethyl group.[12] Recently, it has been shown that, in some cases, trichloromethyl containing heterocycles are more active than the corresponding trifluoromethyl substituted analogues[13] as, for example, NTPDase inhibitory effect in synaptosomes from rat cerebral cortex,[13a] antinociceptive effect in mice,[13b] and hypothermic and antipyretic effects.[13c]

In the course of our research concerning the application of β-alkoxyvinyl trihalomethyl ketones to obtain trihalomethylated compounds and considering the pharmacological and synthetic importance of carbamates,[9,10] 1,3-aminoalcohols,[14] 1,3-oxazinan-2-ones,[15] and trihalogenated compounds,[12,13] we are reporting the synthesis of 4-substituted 2-oxo-6-trihalomethyl-[1,3]oxazinane-3-carboxylic acid ethyl esters **7a–d** and **8a–d**, as well as their intermediate compounds (4,4,4-trihalo-3-oxo-but-1-enyl)-carbamic acid ethyl esters **3a–d** and **4a–d** and the respective (4,4,4-trihalo-3-hydroxy-butyl)-carbamic acid ethyl esters **5a–d** and **6a–d**, which are all new compounds (Scheme 1). The obtained trihalomethylated carbamates were evaluated for their in vitro antimicrobial activity against 18 microorganisms such as yeast like fungi, bacteria, and alga considered important human pathogens.



Compound	R	R^1
a	H	Et
b	Me	Me
c	Ph	Me
d	4-Me-Ph	Me

X	Compounds
F	**1**, **3**, **5**, **7**
Cl	**2**, **4**, **6**, **8**

Scheme 1. Reagents and conditions: (i) NH_2COOEt, $CH_2Cl_2/CHCl_3$, *p*-TsOH, 50/70 °C, 24–48 h; (ii) $NaBH_4$, EtOH, rt/70 °C, 5–24 h; (iii) $(CCl_3O)_2CO$, $(CH_2)_2Cl_2$, Et_3N, 85 °C, 5–22 h.

2. Chemistry

Scheme 1 outlines the synthetic strategy developed to obtain the [1,3]oxazinan-2-ones **7a–d** and **8a–d**. This strategy starts with the reaction of the readily available 4-alkoxy-1,1,1-trifluoro[chloro]-alk-3-en-2-ones (**1** and **2**)[16] with ethyl carbamate to give a series of (4,4,4-trihalo-3-oxo-but-1-enyl)-carbamic acid ethyl esters **3a–d** and **4a–d**.

Although the Michael addition reaction of amines to 4-alkoxy-1,1,1-trihalo-alk-3-en-2-ones (enones), followed by the elimination of the 4-alkoxy group, leading to the corresponding enamino ketones has been widely reported,[17] the reaction of weak nitrogen nucleophiles such as amides and carbamates with enones is not very well developed.[18] In this work, the reaction of enones **1** and **2** with ethyl carbamate was carried out in dichloromethane or chloroform in the presence of *p*-toluenesulfonic acid under reflux for 24–48 h. It was observed that the substitution on the β-carbon makes the attack of the ethyl carbamate on the β-alkoxyvinyl ketones more difficult. For the 1-substituted 4,4,4-trihalo-3-oxo-but-1-enyl carbamates (e.g., **3b–d** and **4b–d**) it was necessary to use an excess of ethyl carbamate and longer reflux time in order to obtain the desired products. Compounds **3** and **4** are in the *Z* form, which is evidenced by the deshielding nature of the N–H chemical shift in the range of 10.5–11.5 ppm.[17]

The reduction of compounds **3** and **4** with sodium borohydride in ethanol at room temperature furnished the corresponding (4,4,4-trihalo-3-hydroxy-butyl)-carbamic acid ethyl esters **5** and **6**, in moderate to good yields. The 4,4,4-trihalo-3-oxo-but-1-enyl carbamates **3a–d** and **4d** underwent reduction of both the carbon–carbon double bond and the carbonyl giving only the corresponding trihalomethylated 3-hydroxy-butyl carbamates **5a–d** and **6d**, while the 4,4,4-trichloro-3-oxo-but-1-enyl carbamates **4a–c** furnished a mixture of the desired **6a–c** and the partially reduced 4,4,4-trichloro-3-hydroxy-alk-1-enyl carbamates **6′a–c** in approximately 1:1 ratio. In our hands, the separation of compounds **6a–c** from **6′a–c** was not possible by column chromatography, so the cyclization step was carried out with the mixture of both **6** and **6′**. It is interesting to note that the reduced compounds, such as **5b–d** and **6b–d**, which have more than one stereogenic center, presented only a single set of signals in both ^{1}H and ^{13}C NMR spectra. In addition, a single peak was observed in the total ion current, registered on a GC–MS equipped with an achiral capillary column. This is an indication that only a pair of enantiomers was formed and that the reduction reaction was stereoselective.

The structures of all synthesized compound were analyzed by GC–MS, ^{1}H, and ^{13}C NMR spectroscopy and the data are reported in Section 6. Figure 1 shows the atom numbering used for the NMR assignment of compounds **3–8**.

The major evidence for the reduction of the 4,4,4-trihalo-3-oxo-but-1-enyl carbamates **3a–d** and **4a–d** to the respective 3-hydroxy-butyl carbamates **5a–d** and **6a–d** comes from the chemical shift changes observed for the C-2, C-3, and C-4 (see Fig. 1). For example, the chemical shift of the C-2 (C=O) shifted from the range of 179–182 ppm, for the 3-oxo-but-1-enyl carbamates **3a–d** and **4a–d**, to 66–84 ppm for the 3-hydroxy-butyl carbamates **5a–d** and **6a–d**. The chemical shift of the carbons of the carbon–carbon double bond (C-3 and C-4) shifted from the range of 94–99 and 146–165 ppm, respectively, for the 3-oxo-but-1-enyl carbamates, to 29–38 and 36–53 ppm, respectively, for the 3-hydroxy-butyl carbamates. Also the reduction can be confirmed from the ^{1}H NMR spectra by observing the disappearance of the doublet and the doublet of doublets of the vinylic hydrogens H-3 and H-4 in the range of 5.7–6.5 and 7.6–8.0 ppm, respectively, for the 4,4,4-trihalo-3-oxo-but-1-enyl carbamate to appear as complex multiplets in the range of 1.6–2.6 and 3.5–5.3 ppm, respectively, for the 3-hydroxy-butyl carbamates. The 3-hydroxy-butyl carbamates also showed a multiplet at about 3.3–4.3 ppm relative to the H-2, absent in the enaminones.

The [1,3]oxazinan-2-ones **7** and **8** were obtained by the cyclization of 3-hydroxy-butyl carbamates **5** and **6** with triphosgene in dichloroethane in the presence of triethylamine, under argon atmosphere, for 5 or 22 h at reflux. The literature reports that the cyclization of 1,3-amino alcohols to construct 1,3-oxazin-2-ones has been done using phosgene[14,19] or by an intramolecular cyclization of a 1,3-hydroxy carbamate in the presence of a strong base.[20,21] In this work, the inconvenience of handling the highly toxic phosgene gas was avoided by using a crystalline stable solid triphosgene[22] to successfully perform the cyclocarbonylation of the 3-hydroxy-butyl carbamates **5** and **6** to the respective [1,3]oxazinan-2-ones **7** and **8**. We also tried to perform the intramolecular cycli-

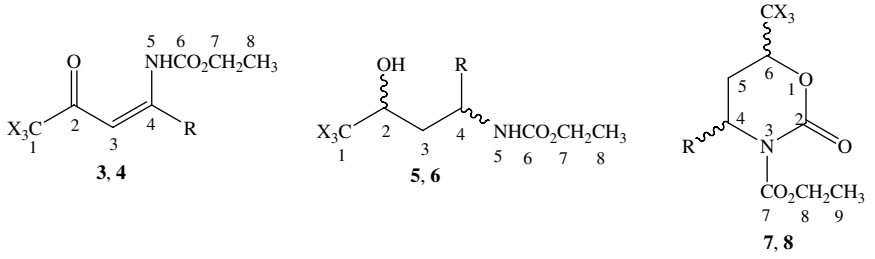


Figure 1. Atom numbering used for the NMR assignment of compounds **3–8**.

zation of **5** and **6** in the presence of strong bases, according to the literature,[20,21] but these procedures did not furnish the corresponding [1,3]oxazinan-2-ones **7** and **8**.

The cyclization of the mixtures of **6a–c** and **6′a–c** carried out with triphosgene underwent cyclization only the double reduced compounds **6a–c** furnishing the respective [1,3]oxazinan-2-ones **8a–c**. Thus, the product mixture of **6′a–c** and **8a–c** was purified by column chromatography obtaining **8a–c** as pure compounds and **6′a–c** were not recovered. Table 1 reports the yields, melting points, and elemental analysis of all compounds synthesized in this work.

The [1,3]oxazinan-2-ones **7a–d** and **8b–d** display similar ^{1}H and ^{13}C NMR spectra as the corresponding amino alcohol precursors. The main evidence of the formation of compounds **7** and **8** is the appearance of a second carbonyl carbamate in the range of 146–148 ppm. Furthermore, it was observed that the resonance of H-6 of **7** and **8** moved downfield, in average, 0.40 ppm in relation to the respective (H-2) of the 3-hydroxy-butyl carbamate **3** and **4** (see Fig. 1). In addition, the molecular ion in the mass spectra showed that the oxazinanes **7** and **8** have 26 mass units more than the corresponding 3-hydroxy-butyl carbamates **5** and **6**.

The oxazinanes **7b–d** and **8b–d** have more than one stereogenic center but as for the 3-hydroxy-butyl carbamates precursors **5b–d** and **6b–d** they present only one set of signals in both the ^{1}H and the ^{13}C NMR spectra. In addition, a single peak was observed in the mass chromatogram registered on a GC–MS equipped with an achiral capillary column. This indicates that only a pair of enantiomers has been obtained for the compounds **7b–d** and **8b–d**. To confirm this hypothesis, ^{1}H and ^{13}C NMR spectra were registered in the presence of tris-[3-trifluoromethyl-hydroxymethylen-*d*-camphorate]-europium, a chiral lanthanide shift reagent, in a molar ratio of 1:0.3 of the **7d** and the lanthanide, respectively. The NMR spectra registered under this condition show that some signals in both the ^{1}H and the ^{13}C spectra were duplicated and exhibited the same intensity, suggesting that the [1,3]oxazinan-2-ones **7b–d** and **8b–d** were obtained as a racemic mixture rather than mixtures of diastereoisomers.

The 4-substituted 6-trichloromethyl-[1,3]oxazinan-2-ones are good probes to obtain information about the three-dimensional structure of these compounds by analyzing the coupling constants between the hydrogens of the backbone structure (H-4, H-5/H-5′, and H-6). The trichloromethylated oxazinanes **8b–d** are better probes than the trifluoromethylated analogues **7b–d** because the splitting pattern of the H-6 signal is more simple to analyze due to the lack of further coupling with the fluorine nuclei. For example, compound **8c** shows coupling constants between the H-6 and H-5 axial and

Table 1. Melting points, yields, and elemental analysis of compounds **3–8**

Compound	Mp (°C)	Yield[a] (%)	Molecular formula	Elemental analysis					
				Calculated			Found		
				C	H	N	C	H	N
3a	69–72	80	$C_7H_8NO_3F_3$	39.82	3.82	6.63	39.81	3.81	6.44
3b	Oil	70	$C_8H_{10}NO_3F_3$	42.67	4.48	6.22	42.90	4.49	6.09
3c	Oil	64	$C_{13}H_{12}NO_3F_3$	54.36	4.21	4.88	54.63	4.23	4.61
3d	Oil	47	$C_{14}H_{14}NO_3F_3$	55.82	4.68	4.65	57.13	4.93	4.71
4a	118–123	80	$C_7H_8NO_3Cl_3$	32.27	3.10	5.38	32.54	2.95	5.27
4b	Oil	48	$C_8H_{10}NO_3Cl_3$	35.00	3.67	5.10	35.13	3.91	4.86
4c	Oil	68	$C_{13}H_{12}NO_3Cl_3$	46.39	3.59	4.16	47.09	3.29	3.80
4d	Oil	41	$C_{14}H_{14}NO_3Cl_3$	47.96	4.02	3.99	48.31	3.97	3.64
5a	Oil	78	$C_7H_{12}NO_3F_3$	39.07	5.62	6.51	38.88	5.56	6.95
5b	Oil	58	$C_8H_{14}NO_3F_3$	41.92	6.16	6.11	41.85	5.95	5.97
5c	Oil	38	$C_{13}H_{16}F_3NO_3$	53.61	5.54	4.81		[b]	
5d	108–110	82	$C_{14}H_{18}NO_3F_3$	55.08	5.94	4.59	55.51	5.88	4.61
6a	Oil	40	$C_7H_{12}Cl_3NO_3$	31.78	4.57	5.29		[b]	
6b	Oil	40	$C_8H_{14}Cl_3NO_3$	34.49	5.07	5.03		[b]	
6c	Oil	36	$C_{13}H_6Cl_3NO_3$	45.84	4.73	4.11		[b]	
6d	Oil	31	$C_{14}H_{18}NO_3Cl_3$	47.41	5.12	3.95	47.27	5.51	4.06
6′a	Oil	35	$C_7H_{10}Cl_3NO_3$	32.03	3.84	5.34		[b]	
6′b	Oil	30	$C_8H_{12}Cl_3NO_3$	34.75	4.37	5.06		[b]	
6′c	Oil	30	$C_{13}H_{14}Cl_3NO_3$	46.11	4.17	4.14		[b]	
7a	80–85	51	$C_8H_{10}NO_4F_3$	39.84	4.18	5.81	39.77	4.16	5.65
7b	Oil	25	$C_9H_{12}NO_4F_3$	42.36	4.74	5.49	42.69	4.74	5.57
7c	95–100	54	$C_{14}H_{14}NO_4F_3$	53.00	4.45	4.41	52.98	4.45	4.48
7d	60–70	28	$C_{15}H_{15}NO_4F_3$	54.38	4.87	4.23	54.64	4.86	3.98
8a			$C_8H_{10}Cl_3NO_4$	33.07	3.47	4.82		[b]	
8b	79–83	22	$C_9H_{12}Cl_3NO_4$	35.49	3.97	4.60	35.97	3.95	4.47
8c	153–156	20	$C_{14}H_{14}Cl_3NO_4$	45.86	3.85	3.82	46.08	3.91	3.54
8d	Oil	20	$C_{15}H_{16}Cl_3NO_4$	47.33	4.24	3.68		[b]	

[a] Yields after purification.
[b] Not registered.

H-5 equatorial of 11.2 and 1.8 Hz, respectively. Also, the observed coupling constant between H-4 and H-5 axial and H-5 equatorial is 10.9 and 7.6 Hz, respectively. These values of coupling constants are consistent with a chair conformation and that the CCl_3 occupies an equatorial position and the 4-substituent group is in a pseudo-equatorial position. All the other synthesized 4-substituted 6-trihalomethyl-[1,3]oxazinan-2-ones showed similar coupling constants and, therefore, they should have the same three-dimensional structure. This observation is in agreement with the conformational assignment study of some 3-acyloxy-1,3-oxazinanes done by Ali and co-workers.[23] According to these observations the compounds **7b–d** and **8b–d**, which have two stereogenic centers, but presented only a single set of signals in both the ^{1}H and the ^{13}C NMR spectra, should be composed by a pair of enantiomers with configuration 4-(*R*)/6-(*R*) and 4-(*S*)/6-(*S*).

3. Biological activity

The in vitro antimicrobial activity of the carbamate compounds was assessed against a panel of microorganisms including yeast like fungi such as *C. albicans* ATCC 44373, *C. dubliniensis* CBS 7987, *C. glabrata* ATCC 10231, *C. neoformans* var. gattii (sorotype D) ATCC 28952, and *Saccharomyces cerevisiae* ATCC 2601, bacteria such as *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27850, *S. aureus* ATCC 25923 and *Micrococcus luteus* ATCC 9341, *Staphylococcus haemolyticus* (clinically isolated), *Staphylococcus warneri* (clinically isolated), *Staphylococcus saprophyticus* (clinically isolated), *Enterococcus faecalis* ATCC 29212, and the alga *P. zopfii* (clinically isolated). All microorganisms were compared to the control drugs: fluconazole, imipenem, and amphotericin B for fungi, bacteria, and alga, respectively. The minimal inhibitory concentration (MIC) and minimal fungicidal, bactericidal, and algacidal concentrations were determined by broth microdilution methods according to NCCLS standards.[24] Compounds were dissolved in DMSO (1 mL) and the solutions were diluted with medium. By further progressive dilutions with test medium the required concentrations (64, 32, 16, 8, 4, 2, 1, 0.5 and 0.25 μg/mL) were obtained. The antimicrobial activities were evaluated based on minimal inhibitory concentration (MIC) according to the procedures NCCLS M27-A2 for fungi and algae and, NCCLS M7-A4 for bacteria that grow aerobically. Bacteria were inoculated into Mueller–Hinton agar and after overnight growth, four or five colonies were directly suspended in saline solution so that the turbidity matches the turbidity of the McFarland standard ($\approx 10^8$ cfu/mL). The suspension was diluted 1:100 in saline followed by new dilution 1:20 in Mueller–Hinton broth, resulting in a final inoculum concentration of 5×10^4 cfu for well.

Fungi and *P. zopfii* were inoculated into potato dextrose agar and the procedures of inoculum standardization were similar among them; however, the medium test was RPMI 1640 broth. Each well of the microdilution tray was filled with 100 μL of compound diluted in 100 μL of the inoculum. The plates were incubated at 35 °C for 24 h for bacteria and *Candida species*; 72 h for *S. cerevisae*, *C. neoformans*, and *P. zopfii*. Growth or a lack thereof in the antimicrobial agent containing wells was determined by comparison with the growth control, indicated by turbidity. All tests were carried out in duplicate and the lowest concentration that completely inhibited visible growth of the organism was recorded as the MIC.

The minimal fungicidal, bactericidal, and algacidal concentrations were determined by subculture of 20 μL of the content of each well that remained clear. The media employed were Sabouraud's dextrose agar for fungi and *P. zopfii*, and Mueller–Hinton agar for bacteria. The plates were incubated at 35 °C for the same duration as for the MIC determinations and the lowest concentration required to demonstrate complete absence of growth was named cidal.

The interpretation of the results was based on fluconazole breakpoints for the fungi, amphotericin B for the *Prototheca*, and based on imipenem for bacterial pathogens, according to M27-A2[24] and M7-A4[25] techniques, respectively.

4. Results and discussion

Thirteen of the new synthesized compounds **3a**,**d**, **4a–d**, **5a**,**b**,**d**, **7a**,**c**,**d**, and **8c** were evaluated for their in vitro antimicrobial activity against a panel of microorganisms including yeast like fungi, bacteria, and alga by determining their minimal inhibitory concentration (MIC) and minimal fungicidal, bactericidal, and algacidal concentrations by broth microdilution methods according to NCCLS standards.[24] Some of the evaluated compounds exhibited significant in vitro activity against the tested microorganism strains with a MIC range of 0.5–8 μg/mL; a moderate antifungal activity was also found with the MIC range varying from 16 to 32 μg/mL (Tables 2 and 3). When we consider the susceptibility of the microorganisms, another point deserves attention. In general, *C. albicans* was similar or more resistant than *C. dubliniensis* which is in agreement with the literature for antifungal agents and compounds like NaCl, alcohols, and others.[26,27] In general *C. glabrata*, *C. tropicalis*, and *C. krusei* showed susceptibility patterns similar to *C. albicans*. Based on breakpoints established for fluconazole (M27-A2), the three varieties of *C. neoformans* studied were sensitive to three compounds such as **4b**,**c** and **7d**.

When we compared the MICs with the MCM (minimal cidal concentrations) in 160 (68.3%) of the cases, the MICs were similar to MCMs and in 74 (31.7%) of the cases the MCMs were one or more dilution more elevated. When the MICs or MCMs were $\geqslant 64$ μg/mL, the comparison was not established. The compounds with a trichloromethyl group showed higher activity in the three types of tested microorganisms. Compounds **4c** and **b**, where R = Ph and CH_3, respectively, exhibited higher activity. Considering the compounds bearing

Table 2. Antibacterial activity in vitro of carbamates (MIC/MBC, μg/mL)

Compound	MIC[a]/MBC[b]							
	Bacteria Gram-positive						Bacteria Gram-negative	
	S. a.[c]	S. h.[d]	S. w.[e]	S. s.[f]	M. l.[g]	E. f.[h]	E. c.[i]	P. a.[j]
3a	16/64	16/32	16/64	32/64	16/64	16/64	16/64	32/64
3d	64/64	32/64	64/64	64/>64	64/64	64/64	64/64	64/64
4a	32/64	32/64	32/64	32/64	>64/>64	32/64	64/64	64/64
4b	1/1	1/2	1/1	2/2	1/1	1/1	16/32	64/64
4c	2/2	1/2	2/2	2/2	2/2	2/2	4/4	2/2
4d	32/>64	32/>64	32/64	32/>64	8/16	32/>64	32/32	32/32
5a	16/16	16/32	16/16	16/16	8/64	16/64	64/>64	32/>64
5b	64/64	64/>64	64/64	64/64	64/>64	64/64	64/64	64/64
5d	64/>64	64/>64	64/64	64/64	64/64	64/>64	64/>64	32/>64
7a	32/64	32/64	32/32	32/64	64/64	32/64	64/>64	64/64
7c	>64	>64	64/>64	>64	>64	>64	64/>64	64/>64
7d	64/64	64/>64	64/64	64/64	64/>64	64/64	64/>64	32/>64
8c	>64	>64	64/>64	>64	>64	>64	64/>64	64/>64
I[k]	0.06	0.06	0.06	0.06	0.03	0.06	0.06	2.0

[a] Minimal inhibitory concentration.
[b] Minimal bactericidal concentration microorganisms.
[c] *Staphylococcus aureus* ATCC 25923.
[d] *Staphylococcus haemolyticus* (clinical isolate).
[e] *Staphylococcus warneri* (clinical isolate).
[f] *Staphylococcus saprophyticus* (clinical isolate).
[g] *Micrococcus luteus* ATCC 9341.
[h] *Enterococcus faecalis* ATCC 29212.
[i] *Escherichia coli* ATCC 25922.
[j] *Pseudomonas aeruginosa* ATCC 27850.
[k] Control: imipenem.

Table 3. Antifungal activity in vitro of carbamates (MIC/MFC, μg/mL)

Compound	MIC[a]/MFC[b]									
	Fungi									Alga
	C. a.[c]	C. d.[d]	C. g.[e]	C. t.[f]	C. k.[g]	C. n.[h]	C. n.[i]	C. n.[j]	S. c.[k]	P. z.[l]
3a	64/64	64/64	64/>64	64/64	64/64	16/32	16/64	32/32	64/64	64/64
3d	16/64	8/32	16/32	16/32	16/64	>64	>64	>64	>64	>64
4a	32/32	64/64	32/32	32/32	32/32	32/32	32/64	32/32	16/16	64/64
4b	16/64	8/16	16/32	16/64	16/64	2/2	2/4	2/2	4/8	4/4
4c	32/64	16/16	32/32	32/64	32/64	4/4	4/4	2/4	4/4	0.5/0.5
4d	32/64	64/64	64/>64	32/64	32/64	32/32	32/64	32/32	32/64	8/8
5a	32/64	64/64	64/64	32/32	32/64	16/64	16/32	32/64	64/64	64/64
5b	64/64	64/64	64/64	64/64	64/64	64/64	64/>64	64/64	32/64	32/32
5d	64/64	64/64	64/64	64/64	64/64	16/16	16/16	16/32	32/64	64/64
7a	64/64	64/64	64/>64	64/64	64/64	16/32	16/64	16/32	64/64	64/64
7c	64/64	64/>64	64/>64	64/64	64/>64	32/32	32/32	32/64	64/>64	64/64
7d	32/32	32/32	64/64	32/64	32/32	8/32	8/32	8/16	32/64	64/64
8c	64/64	64/64	64/64	64/64	64/64	32/64	32/32	32/32	64/64	64/64
F[m]	4.0	2.0	8.0	4.0	64	2.0	2.0	2.0	1.0	
A[n]										0.5

[a] Minimal inhibitory concentration.
[b] Minimal fungicidal concentration microorganisms.
[c] *Candida albicans* ATCC 44373.
[d] *Candida dubliniensis* CBS 7987.
[e] *Candida glabrata* ATCC 10231.
[f] *Candida tropicalis* ATCC 750.
[g] *Candida krusei* ATCC 6258.
[h] Cryptococcus *neoformans* var. gattii (sorotype B) ATCC 56990.
[i] Cryptococcus *neoformans* var. gattii (sorotype C) ATCC 24066.
[j] Cryptococcus *neoformans* var. grubii (sorotype A) ATCC 90012.
[k] *Saccharomyces cerevisiae* ATCC 2601.
[l] *Prototheca zopfii* (clinical isolate).
[m] Control: fluconazole.
[n] Control: amphotericin B.

the trichloromethyl group, compound **4b** (R = CH_3) showed the best bacteriostatic activity from the series of compounds tested to Gram-positive cocci (*S. aureus*, *S. haemolyticus*, *S. warneri*, *S. saprophyticus*, *E. faecalis*, and *M. luteus*). Compound **4b** also showed better bacteriostatic activity against other microorganisms if compared with compound **4a** (R = H). The **4a** showed moderate antifungal activity against *S. cerevisiae*. Compound **4c** (R = Ph) was the most active compound against the alga *P. zopfii* with MIC similar to that obtained for amphotericin B. The activity of **4c** was also significant against *P. aeruginosa* as well as for Gram-negative rods and Gram-positive cocci. The antifungal activity of **4c** was more significant against *C. neoformans* and *S. cerevisiae*, and showed moderate activity against *Candida* species. In general, **4c** showed a large spectrum of antimicrobial activity.

One interesting result was demonstrated for the compound **4d** (R = 4-Me-Ph). The antimicrobial activity of **4d** was restricted to *P. zopfii* and *M. luteus*, both with moderate susceptibility to this compound. Although, **4d** has only a methyl group attached to the phenyl residue more than **4c**, the former exhibited much lower activity against all studied microorganisms if compared with **4c**. These results show that small changes in the R group induced significant differences in antimicrobial activity. In addition, the cyclic compound **8c** also exhibited much lower activity than its precursor **4c**.

Compound **3a** showed a weak fungistatic activity against *C. neoformans* which was absent in *Candida* species. Bacteriostatic activity to Gram-positive cocci and Gram-negative rod as *E. coli* was also observed. In contrast, the **3d** exhibited a specific anti-Candida activity and the activity against *C. dubliniensis* must be emphasized. The 3-hydroxy-butyl carbamates **5a** and **d** showed very similar results to their precursors **3a** and **d**. The 1,3-oxazinan-2-one **7a** exhibited activity similar to its precursor **5a** against *C. neoformans*, but it was inactive against other micoorganisms. Compound **7d** was similar to its precursor **5d** but its anticryptococcal activity was more pronounced.

5. Conclusion

In conclusion, this work presented a convenient three-step synthesis of a new series of 4-substituted 6-trihalomethyl-1,3-oxazinan-2-ones **7a**–**d** and **8a**–**d** from the readily available enones **1a**–**d** and **2a**–**d**. An important step of this strategy was the preparation of the new series of trihalomethylated 3-oxo-but-1-enyl carbamates **3a**–**d** and **4a**–**d**, by an unprecedented reaction of a very weak carbamate nucleophile with β-alkoxyvinyl trihalomethyl ketones **1** and **2**. Another important reaction was the double reduction of compounds **3** and **4** that was achieved in a single reaction step to furnish a new series of trihalomethylated 3-hydroxy carbamates **5a**–**d** and **6a**–**d**. Some compounds of the three studied series exhibited significant antimicrobial activity against pathogenic microorganisms including fungi, bacteria, and alga. Some compounds deserve additional studies due to their anti-Cryptococcus and anti-Candida specificity. It was observed that the antimicrobial activity is very sensitive to the R-substituent and that the trichloromethylated compounds, in general, showed higher activity than the trifluoromethylated analogues.

6. Experimental

The solvents were purified and dried before used and the 4-alkoxy-1,1,1-trifluoro[chloro]alk-3-en-2-ones were prepared according to the literature procedures.[16] The CHN elemental analysis was performed on a Perkin-Elmer 2400 CHN elemental analyzer (University of São Paulo, SP, Brazil). Melting points were determined with a Reichert Thermovar apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were acquired on a Bruker DPX 200 spectrometer (^1H at 200.13 MHz and ^{13}C at 50.32 MHz) or on a Bruker DPX 400 (^1H at 400.13 MHz and ^{13}C at 100.62 MHz) in $CDCl_3$ or DMSO-d_6 and using TMS as the internal reference. The mass spectrums were registered in a HP 5973 MSD connected to a HP 6890 GC and interfaced by a HP Pentium PC. The GC was equipped with a split–splitless injector, autosampler, cross-linked HP 5 capillary column (30 m, 0.32 mm, of internal diameter), and helium was used as the carrier gas.

6.1. General procedure for the synthesis of (4,4,4-trihalo-3-oxo-but-1-enyl)-carbamic acid ethyl esters (3a–d and 4a–d)

To a solution of ethyl carbamate (1.33 g, 15 mmol for **1a** and **2a** and 2.00 g, 22.5 mmol for **1b**–**d** and **2b**–**d**) in chloroform or dichloromethane (10 mL), a solution of **1** or **2** (15 mmol) in chloroform or dichloromethane (10 mL) was slowly added under stirring at room temperature. 4-Toluenesulfonic acid (catalytic amount) was added to the reaction vessel and the stirring was continued for 24 h at 50 °C (for **3a** and **4a**), 24 h at 70 °C (for **3c**,**d** and **4c**,**d**), and 48 h at 70 °C (for **3b** and **4b**). The isolation of compounds was carried out by neutralization of the reaction mixture with sodium carbonate solution (1 M) and water (10 mL) followed by extraction with chloroform or dichloromethane (3× 10 mL). The organic layers were combined, dried with anhydrous magnesium sulfate, and the solvent was removed with rotatory evaporator. Compounds **3b**,**c** and **4b**,**c** were purified by florisil column chromatography (Aldrich 100–200 mesh) using hexane as the eluant. The compound **3d** and **4d** were purified by silica gel column chromatography (Aldrich 60A, 230–400 Mesh) using hexane/dichloromethane, 2:1 as the eluant. Compounds **3a** and **4a** were purified by recrystallization from hexane.

6.1.1. (4,4,4-Trifluoro-3-oxo-1-but-1-enyl)-carbamic acid ethyl ester (3a). MS EI (70 ev): *m*/*z* (%) = 211 (M^+, 52), 166 (31), 142 (100), 114 (62), 70 (96); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.30 (t, 3H, J_{H8-H7} = 7.0 Hz, H-8), 4.32 (q, 2H, J_{H7-H8} = 7.0 Hz, H-7), 5.77 (d, 1H, J_{H3-H4} = 8.4 Hz, H-3), 7.67 (dd, 1H, J_{H4-H3} = 8.4 Hz, J_{H4-H5} = 12.0 Hz, H-4), 10.5 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.6 (C-8), 62.7 (C-7), 94.0 (C-3), 115.5

(q, J_{C-F} = 287.4 Hz, CF_3), 146.7 (C-4), 152.3 (C-6), 181.2 (q, J_{C-F} = 35.3 Hz, C-2).

6.1.2. (4,4,4-Trifluoro-1-methyl-3-oxo-but-1-enyl)-carbamic acid ethyl ester (3b). MS EI (70 ev): *m/z* (%) = 225 (M^+, 60), 196 (5), 180 (38), 156 (85), 128 (63), 110 (87), 68 (36); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.21 (t, 3H, J_{H8-H7} = 7.0 Hz, H-8), 2.42 (s, 3H, CH_3), 4.16 (q, 2H, J_{H7-H8} = 7.2 Hz, H-7), 5.55 (s, 1H, H-3), 11.47 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.9 (C-8), 21.7 (CH_3), 62.5 (C-7), 96.4 (C-3), 116.3 (q, J_{C-F} = 287.2 Hz, CF_3), 152.1 (C-6), 165.0 (C-4), 179.6 (q, J_{C-F} = 34.3 Hz, C-2).

6.1.3. (4,4,4-Trifluoro-3-oxo-1-phenyl-1-but-1-enyl)-carbamic acid ethyl ester (3c). MS EI (70 ev): *m/z* (%) = 287 (M^+, 31), 242 (10), 218 (100), 190 (60), 77 (55); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.24 (t, 3H, J_{H8-H7} = 7.2 Hz, H-8), 4.14 (q, 2H, J_{H7-H8} = 7.2 Hz, H-7), 5.81 (s, 1H, H-3), 7.43 (m, 5H, Ph), 11.18 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.9 (C-8), 62.7 (C-7), 98.6 (C-3), 116.3 (q, J_{C-F} = 287.8 Hz, CF_3), 127.5, 128.1, 130.9, 134.3 (Ph), 151.7 (C-6), 163.4 (C-4), 180.1 (q, J_{C-F} = 35.3 Hz, C-2).

6.1.4. [4,4,4-Trifluoro-1-(4-methyl-phenyl)-3-oxo-but-1-enyl]-carbamic acid ethyl ester (3d). MS EI (70 ev): *m/z* (%) = 301 (M^+, 42), 272 (4), 256 (10), 232 (92), 204 (50), 91 (51); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.26 (t, 3H, J_{H8-H7} = 7.0 Hz, H-8), 2.40 (s, 3H, CH_3), 4.15 (q, 2H, J_{H7-H8} = 7.0 Hz, H-7), 5.81 (s, 1H, H-3), 7.23 (d, 2H, $J_{H10-H11}$ = 8.0 Hz, Ph), 7.34 (d, 2H, $J_{H11-H10}$ = 8.0 Hz, Ph), 11.17 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0 (C-8), 21.4 (CH_3), 62.6 (C-7), 98.4 (C-3), 116.3 (q, J_{C-F} = 287.8 Hz, CF_3), 127.7, 128.9, 131.3, 141.7 (Ph), 151.9 (C-6), 163.6 (C-4), 180.0 (q, J_{C-F} = 34.6 Hz, C-2).

6.1.5. (4,4,4-Trichloro-3-oxo-but-1-enyl)-carbamic acid ethyl ester (4a). MS EI (70 ev): *m/z* (%) = 259 (M^+, 2), 142 (83), 114 (100); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.26 (t, 3H, J_{H8-H7} = 7.0 Hz, H-8), 4.22 (q, 2H, J_{H7-H8} = 7.0 Hz, H-7), 6.38 (d, 1H, J_{H3-H4} = 13.5 Hz, H-3), 7.99 (dd, 1H, J_{H4-H3} = 13.3 Hz, J_{H4-H5} = 1.8 Hz, H-4), 10.50 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0 (C-8), 62.2 (C-7), 96.1 (C-3), 96.5 (CCl_3), 146.6 (C-4), 152.9 (C-6), 180.4 (C-2).

6.1.6. (4,4,4-Trichloro-1-methyl-3-oxo-but-1-enyl)-carbamic acid ethyl ester (4b). MS EI (70 ev): *m/z* (%) = 273 (M^+, 4), 228 (4), 156 (59), 128 (100); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.31 (t, 3H, J_{H8-H7} = 7.2 Hz, H-8), 2.52 (s, 3H, CH_3), 4.22 (q, 2H, J_{H7-H8} = 7.0 Hz, H-7), 5.91 (s, 1H, H-3), 11.22 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.2 (C-8), 22.2 (CH_3), 62.3 (C-7), 95.1 (C-3), 96.3 (CCl_3), 152.3 (C-6), 163.3 (C-4), 182.2 (C-2).

6.1.7. (4,4,4-Trichloro-3-oxo-1-phenyl-but-1-enyl)-carbamic acid ethyl ester (4c). MS EI (70 ev): *m/z* (%) = 335 (M^+, 2), 218 (82), 190 (65), 172 (100), 146 (13), 77 (41); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.17 (t, 3H, J_{H8-H7} = 7.0 Hz, H-8), 4.06 (q, 2H, J_{H7-H8} = 7.0 Hz, H-7), 6.05 (s, 1H, H-3), 7.39 (m, 5H, Ph), 10.73 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.0 (C-8), 62.5 (C-7), 96.2 (CCl_3), 98.0 (C-3), 127.6, 128.1, 130.6, 134.9 (Ph), 151.9 (C-6), 161.9 (C-4), 182.2 (C-2).

6.1.8. [4,4,4-Trichloro-1-(4-methyl-phenyl)-3-oxo-but-1-enyl]-carbamic acid ethyl ester (4d). MS EI (70 ev): *m/z* (%) = 349 (M^+, 3), 304 (3), 232 (100), 204 (78), 118 (72), 91 (40); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.25 (t, 3H, J_{H8-H7} = 7.2 Hz, H-8), 2.40 (s, 3H, CH_3), 4.14 (q, 2H, J_{H7-H8} = 7.2 Hz, H-7), 6.13 (s, 1H, H-3), 7.23 (d, 2H, $J_{H10-H11}$ = 8.0 Hz, Ph), 7.35 (d, 2H, $J_{H11-H10}$ = 8.0 Hz, Ph), 10.80 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$,100 MHz) δ 14.1 (C-8), 21.5 (C-13), 62.5 (C-7), 96.3 (CCl_3), 97.8 (C-3), 127.7, 128.9, 132.0, 141.3 (Ph), 152.1 (C-6), 162.2 (C-4), 182.2 (C-2).

6.2. General procedure for the synthesis of (4,4,4-trihalo-3-hydroxy-butyl)-carbamic acid ethyl esters (5a–d and 6a-d)

To a solution of 3-oxo-but-1-enyl carbamates **3** and **4** (10 mmol) in anhydrous ethanol (10 mL) was slowly added sodium borohydride (0.45 g, 12 mmol) under vigorous stirring at room temperature. The stirring was continued for 5 h at room temperature (for **3a**–**d**) or 24 h at 70 °C (for **4a**–**d**). The solvent was removed by rotatory evaporator, dichloromethane (15 mL) and water (15 mL) were added, and the mixture was stirred at room temperature for 15 min. The organic layer was separated and the water phase was extracted with dichloromethane (2× 15 mL). The organic layers were combined, dried under magnesium sulfate, and the solvent was removed by rotatory evaporator. Compounds **5a** and **b** were purified by florisil column chromatography (Aldrich 100–200 mesh) with hexane as the eluant. Compound **5c** was purified by silica gel column chromatography (Aldrich 60A, 230–400 Mesh), with dichloromethane as the eluant. Compound **5d** was purified by recrystallization from hexane. The compound **6d** was purified by dissolution in a mixture of 5% of dichloromethane in hexane. In this condition, a solid (impurity) precipitated and was filtered off. The product **6d** was obtained as oil and was purified by column chromatography as the compound **5c**. Our effort to separate the mixture of 3-hydroxy-butyl carbamates **6a**–**c** from 3-hydroxyvinyl carbamates **6′a**–**c** (partially reduced compounds) was unsuccessful. The mixture of **6** and **6′** was obtained in a ratio of approximately 1:1.

6.2.1. (4,4,4-Trifluoro-3-hydroxy-butyl)-carbamic acid ethyl ester (5a). MS EI (70 ev): *m/z* (%) = 215 (M^+, 3), 186 (14), 170 (18), 142 (12), 102 (100); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.27 (t, 3H, J_{H8-H7} = 7.2 Hz, H-8), 1.66-1.94 (m, 2H, H-3), 3.27–3.40 (m, 1H, H-4), 3.53 (1H, OH), 3.69–3.73 (m, 1H, H-4), 4.11 (q, 2H, J_{H7-H8} = 7.0 Hz, H-7), 4.87–4.90 (m, 1H, H-2), 5.23–5.25 (m, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.2 (C-8), 29.7 (C-4), 36.5 (C-3), 61.1 (C-7), 67.6 (q, J_{C-F} = 31.1 Hz, C-2), 125.0 (q, J_{C-F} = 279.9 Hz, CF_3), 157.8 (C-6).

6.2.2. (4,4,4-Trifluoro-3-hydroxy-1-methyl-butyl)-carbamic acid ethyl ether (5b). MS EI (70 ev): *m/z* (%) = 229 (M^+, 3), 214 (39), 200 (9), 184 (16), 160 (3), 116 (100), 88 (24); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.16 (t, 3H, J_{H8-H7} = 6.8 Hz, H-8), 1.16 (d, 3H, J_{H9-H4} = 6.4 Hz,

CH_3), 1.66–1.79 (m, 2H, H-3), 3.49 (m, 1H, OH), 3.80–4.00 (m, 2H, H-4, H-2), 4.03 (q, 2H, J_{H7-H8} = 7.2 Hz, H-7), 4.83 (1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.4 (C-8), 21.4 (CH_3), 36.9 (C-4), 44.6 (C-3), 61.1 (C-7), 68.7 (q, J_{C-F} = 31.0 Hz, C-2), 125.0 (q, J_{C-F} = 280.2 Hz, CF_3), 156.6 (C-6).

6.2.3. (4,4,4-Trifluoro-3-hydroxy-1-phenyl-butyl)-carbamic acid ethyl ester (5c). MS EI (70 ev): *m/z* (%) = 291 (M^+, 3), 218 (4), 178 (100); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.18 (t, 3H, J_{H8-H7} = 7.0 Hz, H-8), 2.22–2.30 (m, 2H, H-3), 3.61–3.77 (m, 2H, H-4, OH), 4.06 (q, 2H, J_{H7-H8} = 7.2 Hz, H-7), 4.86–4.89 (m, 1H, H-2), 5.32 (1H, NH), 7.32–7.35 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.4 (C-8), 36.4 (C-3), 52.9 (C-4), 61.3 (C-7), 69.0 (q, J_{C-F} = 31.5 Hz, C-2), 124.9 (q, J_{C-F} = 280.2 Hz, CF_3), 126.6, 128.1, 129.0, 140.4 (Ph), 156.2 (C-6).

6.2.4. (4,4,4-Trifluoro-3-hydroxy-1-(4-methyl-phenyl)-butyl)-carbamic acid ethyl ester (5d). MS EI (70 ev): *m/z* (%) = 305 (M^+, 7), 232 (43), 214 (7), 192 (100), 91 (52); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.12 (t, 3H, J_{H8-H7} = 7.2 Hz, H-8), 2.00–2.09 (m, 2H, H-3), 2.26 (s, 3H, CH_3), 3.62–3.69 (m, 2H, H-4, OH), 3.99 (q, 2H, J_{H7-H8} = 7.2 Hz, H-7), 4.74–4.77 (m, 1H, H-2), 5.14 (s, 1H, NH), 7.10–7.20 (m, 4H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.3 (C-8), 20.9 (CH_3), 36.4 (C-3), 52.5 (C-4), 61.2 (C-7), 68.2 (q, J_{C-F} = 31.15 Hz, C-2), 125.0 (q, J_{C-F} = 280.2 Hz, CF_3), 126.5, 129.6, 137.5, 137.7 (Ph), 156.3 (C-6).

6.2.5. (4,4,4-Trichloro-3-hydroxy-butyl)-carbamic acid ethyl ester (6a). MS EI (70 ev): *m/z* (%) = 263 (M^+, 1), 234 (3), 190 (4), 117 (32), 102 (100); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.22–1.29 (m, 3H, H-8), 1.77 (m, 1H, H-3), 2.25 (s, 1H, OH), 2.57–2.61 (m, 1H, H-3), 3.40–3.71 (m, 2H, H-4), 4.07-4.20 (m, 2H, H-7), 4.27 (m, 1H, H-2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.4 (C-8), 32.0 (C-3), 37.7 (C-4), 61.1 (C-7), 80.6 (C-2), 103.2 (CCl_3), 156.4 (C-6).

6.2.6. (4,4,4-Trichloro-3-hydroxy-1-but-1-enyl)-carbamic acid ethyl ester (6′a). MS EI (70 ev): *m/z* (%) = 217 (M^+−45, 2), 147 (3), 91 (100), 73 (90); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.22–1.29 (m, 3H, H-8), 2.25 (s, 1H, OH), 4.07–4.20 (m, 2H, H-7), 4.53 (m, 1H, H-2), 5.10 (m, 1H, H-3), 6.91 (m, 2H, H-4, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.9 (C-8), 61.6 (C-7), 79.3 (C-2), 103.5 (CCl_3), 128.3 (C-3), 156.2 (C-6), 157.3 (C-4).

6.2.7. (4,4,4-Trichloro-3-hydroxy-1-methyl-butyl)-carbamic acid ethyl ester (6b). MS EI (70 ev): *m/z* (%) = 262 (M^+–15, 4), 190 (4), 116 (100); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.17 (t, 3H, J_{H8-H7} = 7.2 Hz, H-8), 1.26 (d, 3H, J_{CH_3-H4} = 6.0 Hz, CH_3), 1.68–1.90 (m, 2H, H-3, OH), 2.04–2.25 (m, 1H, H-3), 3.56–3.67 (m, 1H, H-4), 3.76–3.90 (m, 1H, H-2), 4.04 (q, 2H, J_{H7-H8} = 7.2 Hz, H-7), 4.93 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.3 (C-8), 21.0 (CH_3), 31.1 (C-3), 44.9 (C-4), 60.7 (C-7), 84.3 (C-2), 97.7 (CCl_3), 157.6 (C-6).

6.2.8. (4,4,4-Trichloro-3-hydroxy-1-methyl-but-1-enyl)-carbamic acid ethyl ester (6′b). MS EI (70 ev): *m/z* (%) = 230 (M^+−45, 4), 216 (67), 136 (86), 114 (86), 71 (100); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.17 (t, 3H, J_{H8-H7} = 7.2 Hz, H-8), 1.87 (s, 3H, CH_3), 1.68–1.90 (s, 1H, OH), 4.04 (q, 2H, J_{H7-H8} = 7.2 Hz, H-7), 4.62–4.69 (dd, 1H, $_{H2-H3}$ = 11.4 Hz, J_{H2-OH} = 2.6 Hz, H-2), 4.74–4.91 (m, 1H, H-3), 6.42 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.3 (C-8), 20.4 (CH_3), 60.6 (C-7), 80.6 (C-2), 95.0 (CCl_3), 103.9 (C-3), 152.7 (C-4), 156.2 (C-6).

6.2.9. (4,4,4-Trichloro-3-hydroxy-1-phenyl-butyl)-carbamic acid ethyl ester (6c). MS EI (70 ev): *m/z* (%) = 339 (M^+, 1), 266 (16), 178 (100), 77 (33); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.08–1.27 (m, 3H, H-8,), 2.13–2.25 (m, 2H, H-3, OH), 2.38–2.42 (m, 1H, H-3), 3.99 (q, 2H, J_{H7-H8} = 7.0 Hz, H-7), 4.12–4.23 (m, 1H, H-4), 4.82–4.88 (m, 1H, H-2), 5.21 (br s, 1H, NH), 7.27–7.30 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.4 (C-8), 38.3 (C-3), 53.4 (C-4), 61.1 (C-7), 79.5 (C-2), 97.5 (CCl_3), 126.1, 127.2, 128.9, 133.3, (Ph), 156.0 (C-6).

6.2.10. (4,4,4-Trichloro-3-hydroxy-1-phenyl-but-1-enyl)-carbamic acid ethyl ester (6′c). MS EI (70 ev): *m/z* (%) = 292 (M^+−45, 11), 176 (9), 104 (100), 77 (72); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.08–1.27 (m, 3H, H-8), 2.13–2.25 (s, 1H, OH), 3.74–3.79 (m, 1H, H-2), 3.99 (q, 2H, J_{H7-H8} = 7.0 Hz, H-7), 4.84–4.88 (m, 1H, H-3), 5.21 (br s, 1H, NH), 7.27–7.30 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1 (C-8), 60.1 (C-7), 72.4 (C-2), 99.5 (CCl_3), 125.5 (C-4), 126.6, 127.5 (Ph), 128.4 (C-3), 129.1, 139.1 (Ph), 154.8 (C-6).

6.2.11. [4,4,4-Trichloro-3-hydroxy-1-(4-methyl-phenyl)-butyl]-carbamic acid ethyl ester (6d). MS EI (70 ev): *m/z* (%) = 353 (M^+, 2), 280 (11), 192 (100), 148 (8), 118 (40), 91 (42); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.20 (t, 3H, J_{H8-H7} = 7.20 Hz, H-8), 2.26 (s, 3H, CH_3), 2.29 (m, 2H, H-3), 3.72–3.76 (m, 1H, H-4), 4.00 (q, 2H, J_{H7-H8} = 7.20 Hz, H-7), 4.77–4.81 (m, 1H, H-2), 5.11 (s, 1H, NH), 7.07–7.19 (m, 4H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.5 (C-8), 21.1 (CH_3), 38.4 (C-3), 53.3 (C-4), 61.1 (C-7), 80.8 (q, J_{C-F} = 31.2 Hz, C-2), 103.6 (CCl_3), 126.1, 126.6, 129.6, 137.7 (Ph), 156.0 (C-6).

6.3. General procedure for the synthesis of 2-oxo-6-trihalomethyl-[1,3]oxazinane-3-carboxylic acid ethyl esters (7a–d and 8a–d)

To a solution of 2-hydroxy carbamates **5** and **6** (5 mmol) in 1,2-dichloroethane (10 mL), triethylamine (0.70 mL, 5 mmol) and triphosgene (1.483 g, 5 mmol) were added under stirring at room temperature. The stirring was continued for 5 h (for **5a–d**) or 22 h (for **6a–d**) at 80 °C. The mixture was allowed to cool and the triethylammonium salt was filtered off. The solution was washed with water (4× 15 mL). The aqueous layer was extracted with dichloromethane (3× 10 mL). The organic layers were combined, dried under magnesium sulfate, and the solvent was removed by rotatory evaporator. Compounds **7d** and **8b–d** were purified by silica gel column chromatography (Aldrich 60A, 230–400 Mesh), with hexane/dichloromethane, 1:1 as the eluant. Compound **7b** was purified by silica gel column chromatography (Aldrich 60A, 230–400 Mesh), with hexane/dichloromethane, 2:1 as the eluant. Compounds **7a**

and **c** were purified by recrystallization from a mixture of 5% of chloroform in hexane. Compound **8a** could not be purified and it was detected by GC–MS.

6.3.1. 2-Oxo-6-trifluoromethyl-[1,3]oxazinane-3-carboxylic acid ethyl ester (7a). MS EI (70 ev): *m/z* (%) = 241 (M^+, 5), 214 (81), 196 (5), 169 (92), 152 (12), 69 (33); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.36 (t, 3H, J_{H9-H8} = 7.0 Hz, H-9), 2.05–2.42 (m, 2H, H-5), 3.62–3.77 (m, 1H, H-4), 4.04–4.15 (m, 1H, H-4), 4.34 (q, 2H, J_{H8-H9} = 7.0 Hz, H-8), 4.64–4.77 (m, 1H, H-6); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0 (C-9), 21.1 (C-5), 42.0 (C-4), 64.0 (C-8), 74.0 (q, J_{C-F} = 34.5 Hz, C-6), 122.2 (q, J_{C-F} = 278.2 Hz, CF_3), 146.4 (C-2), 152.8 (C-7).

6.3.2. 4-Methyl-2-oxo-6-trifluoromethyl-[1,3]oxazinane-3-caboxylic acid ethyl ester (7b). MS EI (70 ev): *m/z* (%) = 255 (M^+, 5), 228 (6), 168 (100), 124 (22), 70 (77); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.35 (t, 3H, J_{H9-H8} = 7.2 Hz, H-9), 1.42 (d, 3H, J_{CH3-H4} = 6.2 Hz, CH_3), 1.91 (ddd, 1H, $J_{H5(ax)-H5(eq)}$ = 13.7 Hz, $J_{H5(ax)-H6}$ = 11.6 Hz, $J_{H5(ax)-H4}$ = 8.8 Hz, H-5), 2.57 (ddd, 1H, $J_{H5(eq)-H5(ax)}$ = 13.7 Hz, $J_{H5(eq)-H4}$ = 8.7 Hz, $J_{H5(eq)-H6}$ = 3.1 Hz, H-5), 4.33 (q, 2H, J_{H8-H9} = 7.0 Hz, H-8), 4.41–4.59 (m, 2H, H-6, H-4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0 (C-9), 22.6 (CH_3), 29.9 (C-5), 49.5 (C-4), 63.8 (C-8), 72.3 (q, J_{C-F} = 34.6 Hz, C-6), 122.0 (q, J_{C-F} = 277.3 Hz, CF_3), 148.2 (C-2), 152.8 (C-7).

6.3.3. 2-Oxo-4-phenyl-6-trifluoromethyl-[1,3]oxazinane-3-carboxylic acid ethyl ester (7c). MS EI (70 ev): *m/z* (%) = 317 (M^+, 15), 272 (2), 244 (100), 132 (14), 77 (71); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.12 (t, 3H, J_{H9-H8} = 7.0 Hz, H-9), 2.18 (ddd, 1H, $J_{H5(ax)-H5(eq)}$ = 14.2 Hz, $J_{H5(ax)-H6}$ = 11.0 Hz, $J_{H5(ax)-H4}$ = 10.8 Hz, H-5), 2.70 (ddd, 1H, $J_{H5(eq)-H5(ax)}$ = 14.2 Hz, $J_{H5(eq)-H4}$ = 7.8 Hz, $J_{H5(eq)-H6}$ = 2.0 Hz, H-5), 4.13 (q, 2H, J_{H8-H9} = 7.2 Hz, H-8), 4.71 (m, 1H, $J_{H6-H5(ax)}$ = 11.0 Hz, $J_{H6-H5(eq)}$ = 2.0 Hz, H-6), 5.25 (dd, 1H, $J_{H4-H5(ax)}$ = 10.8 Hz, $J_{H4-H5(eq)}$ = 7.8 Hz, H-4), 7.23–7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.7 (C-9), 31.8 (C-5), 58.0 (C-4), 63.8 (C-8), 72.3 (q, J_{C-F} = 35.0 Hz, C-6), 121.9 (q, J_{C-F} = 277.30 Hz, CF_3), 125.2, 128.3, 129.1, 140.9 (6C, Ph), 147.9 (C-2), 152.3 (C-7).

6.3.4. 2-Oxo-4-(4-methyl-phenyl)-6-trifluoromethyl-[1,3]oxazinane-3-carboxylic acid ethyl ester (7d). MS EI (70 ev): *m/z* (%) = 331 (M^+, 22), 258 (100), 91 (51), 118 (68); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.15 (t, 3H, J_{H9-H8} = 7.1 Hz, H-9), 2.18 (ddd, 1H, $J_{H5(ax)-H5(eq)}$ = 14.4 Hz, $J_{H5(ax)-H6}$ = 11.4 Hz, $J_{H5(ax)-H4}$ = 10.5 Hz, H-5), 2.33 (s, 3H, CH_3), 2.67 (ddd, 1H, $J_{H5(eq)-H5(ax)}$ = 14.4 Hz, $J_{H5(eq)-H4}$ = 7.8 Hz, $J_{H5(eq)-H6}$ = 1.6 Hz, H-5), 4.15 (q, 2H, J_{H8-H9} = 7.0 Hz, H-8), 4.59–4.74 (dd, 1H, $J_{H6-H5(ax)}$ = 11.4 Hz, $J_{H6-H5(eq)}$ = 1.6 Hz, H-6), 5.21 (dd, 1H, $J_{H4-H5(ax)}$ = 10.5 Hz, $J_{H4-H5(eq)}$ = 7.8 Hz, H-4), 7.16 (m, 4H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.6 (C-9), 20.9 (CH_3), 31.8 (C-5), 57.7 (C-4), 63.6 (C-8), 72.2 (q, J_{C-F} = 34.3 Hz, C-6), 121.9 (q, J_{C-F} = 277.50 Hz, CF_3), 125.2, 129.6, 137.9, 138.0 (Ph), 147.9 (C-2), 152.2 (C-7).

6.3.5. 2-Oxo-6-trichloromethyl-[1,3]oxazinane-3-carboxylic acid ethyl ester (8a). This compound could not be purified. It was only detected by GC–MS. MS EI (70 ev): *m/z* (%) = 289 (M, 3), 262 (29), 217 (17), 157 (12), 119 (35), 100 (100).

6.3.6. 4-Methyl-2-oxo-6-trichloromethyl-[1,3]oxazinane-3-carboxylic acid ethyl ester (8b). MS EI (70 ev): *m/z* (%) = 303 (M^+, 1), 186 (12), 116 (96), 72 (100); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.37 (t, 3H, J_{H9-H8} = 7.2 Hz, H-9), 1.44 (d, 3H, J_{CH_3-H4} = 6.4 Hz, CH_3), 2.01 (ddd, 1H, $J_{H5(ax)-H5(eq)}$ = 13.8 Hz, $J_{H5(ax)-H6}$ = 11.2 Hz, $J_{H5(ax)-H4}$ = 8.7 Hz, H-5), 2.86 (ddd, 1H, $J_{H5(eq)-H5(ax)}$ = 13.8 Hz, $J_{H5(eq)-H4}$ = 8.6 Hz, $J_{H5(eq)-H6}$ = 2.8 Hz, H-5), 4.34 (q, 2H, J_{H8-H9} = 7.2 Hz, H-8), 4.42–4.50 (m, 1H, H-4), 4.64 (dd, 1H, $J_{H6-H5(ax)}$ = 11.2 Hz, $J_{H6-H5(eq)}$ = 2.8 Hz, H-6); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1 (C-9), 22.7 (CH_3), 32.9 (C-5), 49.7 (C-4), 63.8 (C-8), 83.1 (C-6), 96.4 (CCl_3), 148.4 (C-2), 152.9 (C-7).

6.3.7. 2-Oxo-4-phenyl-6-trichloromethyl-[1,3]oxazinane-3-carboxylic acid ethyl ester (8c). MS EI (70 ev): *m/z* (%) = 365 (M^+, 9), 292 (53), 250 (13), 177 (41), 104 (100), 77 (95); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.07 (t, 3H, J_{H9-H8} = 7.0 Hz, H-9), 2.21 (ddd, 1H, $J_{H5(ax)-H5(eq)}$ = 14.0 Hz, $J_{H5(ax)-H6}$ = 11.2 Hz, $J_{H5(ax)-H4}$ = 10.9 Hz, H-5), 2.93 (ddd, 1H, $J_{H5(eq)-H5(ax)}$ = 14.0 Hz, $J_{H5(eq)-H4}$ = 7.6 Hz, $J_{H5(eq)-H6}$ = 1.8 Hz, H-5), 4.08 (q, 2H, J_{H8-H9} = 7.2 Hz, H-8), 4.71 (dd, 1H, $J_{H6-H5(ax)}$ = 11.2 Hz, $J_{H6-H5(eq)}$ = 1.8 Hz H-6), 5.18 (dd, 1H, $J_{H4-H5(ax)}$ = 10.9 Hz, $J_{H4-H5(eq)}$ = 7.6 Hz, H-4), 7.19–7.31 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.8 (C-9), 34.8 (C-5), 58.1 (C-4), 63.8 (C-8), 83.0 (C-6), 96.3 (CCl_3), 125.3, 128.3, 129.1, 141.2 (6C, Ph), 148.1 (C-2), 152.3 (C-7).

6.3.8. 2-Oxo-4-(4-methyl-phenyl)-6-trichloromethyl-[1,3]oxazinane-3-carboxylic acid ethyl ester (8d). MS EI (70 ev): *m/z* (%) = 379 (M^+, 11), 191 (46), 118 (100), 91 (98); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.16 (t, 3H, J_{H9-H8} = 7.0 Hz, H-9), 2.18-2.30 (ddd, 1H, $J_{H5(ax)-H5(eq)}$ = 14.2 Hz, $J_{H5(ax)-H6}$ = 11.4 Hz, $J_{H5(ax)-H4}$ = 10.8 Hz, H-5), 2.34 (s, 3H, CH_3), 2.97 (ddd, 1H, $J_{H5(eq)-H5(ax)}$ = 14.2 Hz, $J_{H5(eq)-H4}$ = 7.8 Hz, $J_{H5(eq)-H6}$ = 1.8 Hz, H-5), 4.16 (q, 2H, J_{H8-H9} = 7.0 Hz, H-8), 4.78 (dd, 1H, $J_{H6-H5(ax)}$ = 11.4 Hz, $J_{H6-H5(eq)}$ = 1.6 Hz, H-6), 5.21 (dd, 1H, $J_{H4-H5(ax)}$ = 10.8 Hz, $J_{H4-H5(eq)}$ = 7.8 Hz, H-4), 7.17 (m, 4H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.9 (C-9), 21.1 (CH_3), 34.8 (C-5), 57.9 (C-4), 63.8 (C-8), 83.0 (C-6), 96.4 (CCl_3), 125.3, 129.8, 138.2 (Ph), 148.2 (C-2), 152.4 (C-7).

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