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The carbonate analogues of 5′-halogenated resiniferatoxin as TRPV1 ligands

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ABSTRACT

A series of carbonate analogues of 5′-halogenated RTX have been investigated in order to examine the effect of the carbonate group as a linker and the role of halogens in the reversal of activity from agonism to antagonism for rat and human TRPV1 heterologously expressed in Chinese hamster ovary cells. The carbonate analogues showed similar activities to the corresponding RTX derivatives in rat TRPV1 but lower potency in human TRPV1. 5-Halogenation converted the agonists to partial agonists or full antagonists and the extent of antagonism reflected the order of I > Br > Cl > F, with a somewhat greater extent of antagonism for the derivatives of the 4-amino RTX surrogates compared to the corresponding derivatives of RTX itself. The carbonate analogues of I-RTX (**60**) and 5-bromo-4-amino-RTX (**66**) were potent and full antagonists with $K_{i(ant)} = 2.23$ and 2.46 nM, respectively, for rat TRPV1, which were ca. 5-fold more potent than I-RTX (**2**) under our conditions. The conformational analysis of the I-RTX-carbonate (**60**) indicated that its bent conformation was similar to that of I-RTX, consistent with compound **60** and I-RTX showing comparable potent antagonism.

1. Introduction

Resiniferatoxin (RTX, **1**) [1,2], isolated from *Euphorbia resinifera*, is an extremely potent irritant tricyclic diterpene in which the phorbol-related diterpene is connected to homovanillic acid at C-20 [3,4]. RTX has proven to function pharmacologically as an ultrapotent agonist for the transient receptor potential vanilloid 1 (TRPV1) channel, displaying 10^3- to 10^4-fold greater potency than the prototypic agonist capsaicin [5].

RTX was found to evoke large inward currents in dorsal root ganglion (DRG) neurons and TRPV1 transfected cell lines [6]. The actions of RTX are mediated by binding, with picomolar affinity, directly to the capsaicin-binding site on the TRPV1 receptor [7]. Whereas capsaicin under normal conditions produces only short term desensitization of TRPV1 mediated responses, the apparent desensitization to RTX can be of very long duration, lasting for weeks [8,9]. RTX is being developed as a potent desensitizing agent for neurons in the treatment of urinary urge incontinence and the pain associated with diabetic neuropathy, as well as for cancer pain [10–13].

Due to synthetic inaccessibility, the investigation of structure–activity relationships (SAR) for RTX derivatives was limited to partial modifications starting from RTX or ROPA (resiniferonol 9,13,14-orthophenylacetate) based on the three structural regions including the A-region (4-hydroxy-3-methoxyphenyl), B-region (C_{20} ester), and C-region (diterpene) in Fig. 1 [14–24]. In the SAR of RTX, it should be noted that a divergence in potencies between ligand binding and agonism for TRPV1 ligands is frequently observed [17] and may reflect the distinction between the properties of the small fraction of the receptor at the cell surface and the predominant proportion in internal membranes [25].

Previous SAR in the A-region of RTX indicated that substitution of a phenol isosteric amine or methanesulfonamide on RTX provided metabolically stable surrogates, **2** and **3**, with comparable receptor potency. For example, whereas RTX-amine **2** (EC_{50} = 0.46 nM) showed ca. 2-fold less potency than

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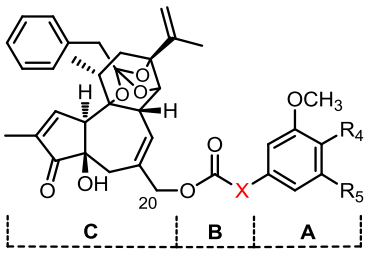


	X	R_4	R_5
1	CH_2	OH	H
2	CH_2	NH_2	H
3	CH_2	$NHSO_2CH_3$	H
4	CH_2	OH	I
5	O	OH	H
6	O	OH	I

Fig. 1. Resiniferatoxin and its carbonate analogs.

RTX (EC_{50} = 0.27 nM) [24], RTX-methanesulfonamide **3** (EC_{50} = 0.106 nM) was found to be 2.5-fold more potent than RTX for rat TRPV1 heterologously expressed in CHO cells [22].

A series of 5′-halogenated RTX analogues have been investigated in order to examine the effect of halogenation in the A-region of RTX and RTX-amine on their binding and their functional pattern of agonism/antagonism for rat TRPV1 [24]. Halogenation at the 5-position in the A-region of RTX and of RTX-amine shifted the agonism of the parent compounds toward antagonism. The extent of antagonism was greater as the size of the halogen increased while the binding affinities were similar. Consequently, 5-bromo-4-amino RTX showed very potent and full antagonism with $K_{i(ant)}$ = 2.81 nM, which was thus 4.5-fold more potent than 5′-iodo RTX (I-RTX) (**4**) [18,19], previously reported as a potent TRPV1 antagonist. Molecular modelling analyses revealed that 5′-halogenation rendered the B-region conformation of RTX to be bent, permitting the 3-methoxy to form an internal hydrogen bond with the C4–OH of the diterpene rather than to interact with the receptor [24].

Appendino et al. recently characterized the carbonate surrogates of RTX and I-RTX in a structure activity analysis of the benzylic methylene side chain using hTRPV1 expressed in HEK-293 cells. Surprisingly, whereas the carbonate analogue of RTX (**5**) showed weak agonism with 720-fold less potency than RTX, the carbonate analogue of the potent antagonist I-RTX (**6**) displayed agonism rather than antagonism and showed extreme, picomolar potency, which was ca. 4-fold more potent than RTX for hTRPV1 expressed in HEK cells [23].

This striking result prompted us to investigate the SAR in the carbonate surrogates of 5′-halogenated analogues of RTX and 4′-amino RTX as part of our continuing efforts to characterize the influence of halogenation and isosteric substitution of the phenolic group in the A-region, which appear to be key modifications for the functional activity and potency toward TRPV1.

In this paper, we describe the syntheses, receptor activities for rat and human TRPV1 heterologously expressed in CHO cells, and the molecular modelling of the carbonate analogues of RTX surrogates and their 5'-halogenated derivatives.

2. Result and discussion

2.1. Chemistry

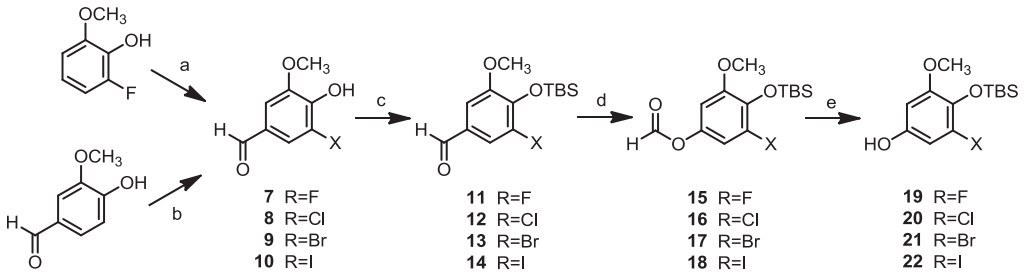
For the syntheses of the 5-halogenated 4-phenolic A-region of RTX-carbonates, the *O*-TBS protected 5-halo-4-hydroxy-3-methoxyphenols (**19**–**22**) were synthesized from the corresponding aldehydes (**7**–**10**) in 3 steps as shown in Scheme 1.

The 5-fluoro vanillin **7** was prepared from commercially available 2-fluoro-6-methoxyphenol by Mannich-type formylation and 5-chloro vanillin **8** was synthesized from vanillin by basic α-chlorination. 5-Bromo and 5-iodo vanillins (**9**, **10**) were obtained from commercial sources. TBS-protection followed by Baeyer–Villiger oxidation of **7**–**10** provided the formates **15**–**18**, respectively, which were hydrolyzed to the 5-halo phenols **19**–**22**.

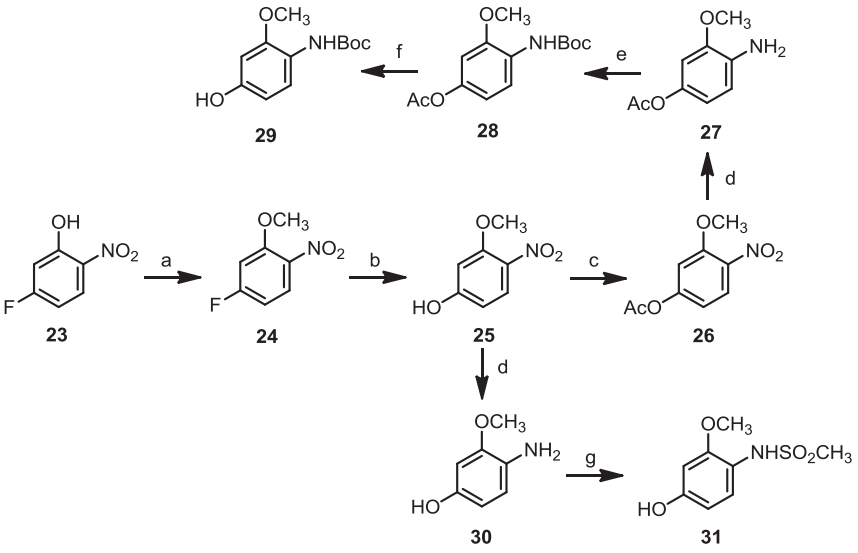
Next, for the syntheses of the 4-amino and 4-methane sulfonylamino A-region of RTX-carbonates, the 4-amino or 4-mesylamino-3-methoxy phenol (**29**, **31**) were synthesized starting from 5-fluoro-2-nitrophenol **23** in multi-steps as depicted in Scheme 2. *O*-Methylation followed by hydroxide substitution of **23** afforded the key intermediate **25**, which was converted to the phenol **29** or **31** in 4 or 2 steps, respectively, by conventional methods.

Finally, for the syntheses of the 5-halogenated 4-amino A-region of RTX-carbonates, the 5-halo-4-amino-3-methoxyphenols (**37**, **48**–**50**) were synthesized starting from 3,5-difluorophenol **32** as described in Scheme 3. Phenol **32** was nitrated and then protected by methoxymethyl (MOM) to give the key intermediate **34**. Methoxy substitution and nitro reduction followed by acidic hydrolysis of **34** provided the 5-fluoro intermediate **37**. On the other hand, the treatment of **34** with ammonia produced the 5-amino intermediate **38** whose amino group was converted to the corresponding halogens by the Sandmeyer reaction to provide **39**–**41**, respectively. Methoxy substitution, MOM deprotection and subsequent nitro reduction of **39**–**41** provided 5-halo-4-amino phenols **48**–**50**, respectively.

The final RTX carbonate analogues were generally synthesized from the 4-nitrophenyl carbonate of RTX (**52**), prepared from



Scheme 1. Reactions and conditions: (a) i) 37% formaldehyde, 40% NMe_2, reflux, 2 h, ii) MeI, $CHCl_3$, rt, 18 h, iii) AcOH/H_2O, HMTA, HCl, 120 °C, 2 h; (b) X = Cl: NCS, NaH, THF, 0 °C to rt, overnight; (c) TBS-Cl, NEt_3, DMAP, CH_2Cl_2, 0 °C, 2 h; (d) *m*-CPBA, CH_2Cl_2, rt, overnight; (e) K_2CO_3, MeOH, rt, overnight.



Scheme 2. Reactions and conditions: (a) MeI, K_2CO_3, acetone, reflux, 3 h; (b) NaOH, H_2O, DMSO, rt, overnight; (c) Ac_2O, pyridine, THF, rt, 5 h; (d) 10% Pd/C, H_2, MeOH/THF, rt, 1 h; (e) Boc_2O, $NaHCO_3$, H_2O/THF, 0 °C-rt, overnight; (f) $NaHCO_3$, H_2O, MeOH, rt, 48 h; (g) MsCl, pyridine.

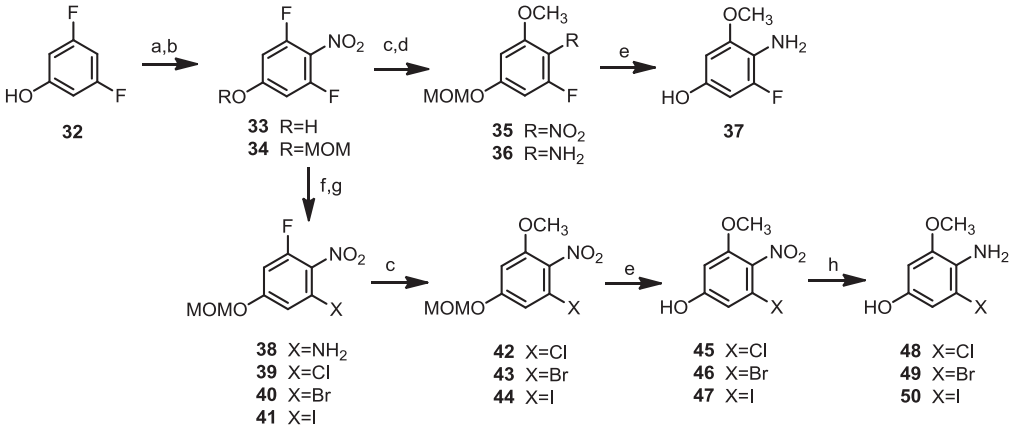
commercially available ROPA (resiniferonol-9,13,14-orthophenyl acetate) by the substitution reaction with the appropriate phenols of the A-region (**19**–**22**, **29**, **31**, **37**, **48**–**50**) followed by deprotection if necessary, as shown in Scheme 4.

2.2. Receptor activity

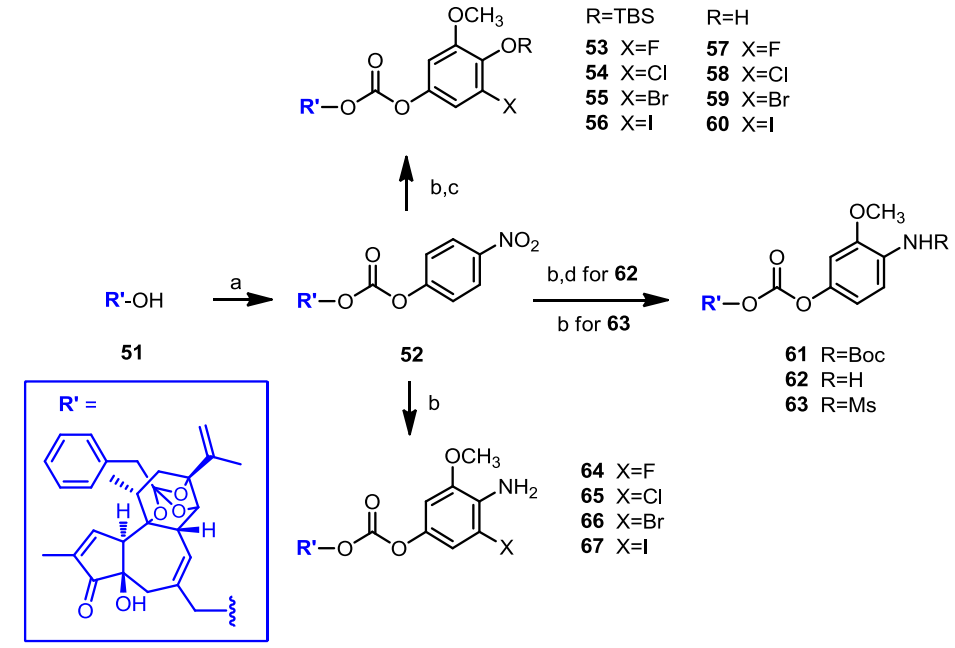
The synthesized resiniferatoxin carbonate analogues were evaluated for their binding affinities (expressed as K_i values) using a competitive binding assay with [^{3}H]RTX and for their agonism/antagonism using a functional $^{45}Ca^{2+}$ uptake assay (expressed as EC_{50} and $K_{i(ant)}$ values) for rat and human TRPV1 heterologously expressed in Chinese hamster ovary (CHO) cells [26]. In this system, resiniferatoxin (**1**) displayed $K_i = 0.043$ nM and $EC_{50} = 0.27$ nM for rTRPV1[22] and 5′-Iodo resiniferatoxin (**4**) exhibited $K_i = 0.61$ nM and $K_{i(ant)} = 12.2$ nM for rTRPV1 [24]. The receptor binding and functional activities of the synthesized RTX-carbonate analogues are listed in Table 1 for rat TRPV1 and Table 2 for human TRPV1.

As previously found in a series of 5′-halogenated RTX analogues [24], 5-halogenation on the A-region in the RTX-carbonates progressively shifted the activity toward antagonism as the size of the halogen increased (I > Br > Cl > F). 5-Fluorination yielded the full agonist **57** with $EC_{50} = 4.97$ nM, which was ca. 5-fold less potent than RTX for its functional activity for rTRPV1. 5-Chlorination caused a partial shift in function, providing the partial antagonist **59** with $K_{i(ant)} = 26.2$ nM and 13% residual agonism. 5-Bromination and iodination afforded the full and potent antagonists **59** and **60** with K_i (ant) = 5.20 and 2.23 nM, which were 2-fold and 5-fold more potent than I-RTX. The 5-halogenated RTX-carbonate analogues (**57**–**60**) bound to TRPV1 with lower affinity than did RTX (**1**) but showed little difference as the size of the halogen increased, with a range of $K_i = 1.17$–1.66 nM.

The previous report had described that compound **6** (synthesized as compound **60** here), the carbonate surrogate of I-RTX **4**, was an ultrapotent agonist for hTRPV1 in HEK-293 cells with $EC_{50} = 4.7$ pM and with ca. 4-fold better potency than RTX ($EC_{50} = 19$ pM) [23]. This finding could not be confirmed in our experiments in which compound **6** (or compound **60**) was found to be a potent antagonist with 5-fold better potency than I-RTX, indicating that the replacement of the benzylic methylene with



Scheme 3. Reactions and conditions: (a) HNO_3, CH_2Cl_2, 0 °C to rt, overnight; (b) MOM-Cl, DIPEA, CH_2Cl_2, 0 °C to rt, overnight; (c) NaOMe, MeOH, rt, 8 h; (d) Pd/C, H_2, THF–EtOH, overnight; (e) CF_3CO_2H, CH_2Cl_2, rt, 3 h; (f) NH_3 in MeOH, rt, 2 days; (g) X = Cl: *t*-BuONO, $Cu(II)Cl_2$, CH_3CN, 0 °C to rt, 1 h, X = Br: *t*-BuONO, Cu(I)Br, CH_3CN, rt, 1 h, 65 °C, 3 h, X = I: *t*-BuONO, I_2, CH_3CN, 60 °C, 2 h, rt, overnight; (h) hydrazine hydrate, $FeCl_3$–$6H_2O$, carbon, MeOH, 65 °C, reflux, 1–10 h.



Scheme 4. Reactions and conditions: (a) 4-nitrophenyl chloroformate, NEt_3, CH_2Cl_2, 0 °C to rt, overnight; (b) R–OH, CH_2Cl_2, rt, overnight; (c) TBAF, THF, rt; (d) CF_3CO_2H, CH_2Cl_2, 0 °C.

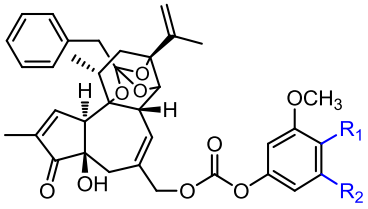
oxygen in I-RTX did not change the functional antagonism of I-RTX but further increased its antagonism for rat TRPV1 (and for human TRPV1, see below).

Next, the carbonate analogues of 4-amino (**62**) and 4-methylsulfonylamino RTX (**63**) were explored. Compounds **62** and **63** showed full agonism but exhibited 4.5- and 5.5-fold less potency in binding affinity and 4- and 40-fold less potency for agonism compared to the parent compounds **2** and **3**, respectively. The result was similar to that of RTX previously reported [23].

Finally, we investigated the SAR of the carbonate analogues of 5-halogenated 4-amino RTX (**64**–**67**). Similar to the previous findings, 5-halogenation on the A-region in 4-amino RTX-carbonate (**62**) progressively shifted the agonism toward antagonism as the size of the halogen increased and the extent of antagonism was further favoured compared to the corresponding RTX-carbonates. 5-Fluorination produced partial agonist **64** with 83% residual agonism. However, 5-chlorination, bromination and iodination yielded the full antagonists **65**–**67**. The binding affinities and potencies for antagonism of **65**–**67** had similar values with ranges of K_i = 1.83–3.01 nM and $K_{i(ant)}$ = 2.46–4.06 nM. These values were also similar to that of compound **60**.

We further evaluated the synthesized RTX-carbonates for their binding affinities and functional activities with human TRPV1 (Table 2). The SAR analysis showed a similar pattern to that in

Table 1
Receptor activities of RTX-carbonates in rat TRPV1.



	R_1	R_2	RTX binding (K_i = nM)	Agonism (EC_{50} = nM)	Antagonism (K_i = nM)
1[c] (RTX)			0.043	0.27 (±0.13)	NE
4[c] (I-RTX)			0.61 (±0.08)	NE	12.2 (±4.0)
57	OH	F	1.17 (±0.30)	4.97 (±0.98)	NE
58	OH	Cl	1.64 (±0.49)	ND[a]	21.3 (±6.5)[b]
59	OH	Br	1.64 (±0.33)	NE	5.20 (±0.39)
60	OH	I	1.66 (±0.36)	NE	2.23 (±0.22)
2			0.13 (±0.03)	0.46 (±0.61)	NE
3			0.228 (±0.051)	0.106 (±0.031)	NE
62	NH_2	H	0.58 (±0.15)	1.84 (±0.42)	NE
63	NHMs	H	1.28 (±0.48)	4.62 (±0.61)[a]	NE
64	NH_2	F	2.21 (±0.36)	3.17 (±0.78)[a]	NE
65	NH_2	Cl	2.50 (±0.11)	NE	4.06 (±0.36)
66	NH_2	Br	1.83 (±0.26)	NE	2.46 (±0.76)
67	NH_2	I	3.01 (±0.53)	NE	2.68 (±0.66)

NE: not effective.
Values represent the mean ± SEM of three or more independent experiments of K_i or EC_{50}.
[a] The mean % of agonism relative to capsaicin: 13% for **58**, 89% for **63**, 83% for **64**.
[b] The mean % of antagonism of the capsaicin stimulated $^{45}Ca^{2+}$ uptake response: 75% for **58**.
[c] Values from ref 24.

Table 2
Receptor activities of RTX-carbonates in human TRPV1.

	R_1	R_2	RTX binding (K_i = nM)	Agonism (EC_{50} = nM)	Antagonism (K_i = nM)
1 (RTX)			1.23 (±0.22)	0.92 (±0.19)	
4 (I-RTX)			11.8 (±3.1)	NE	5.87 (±1.3)
57	OH	F	138 (±39)	16.3 (±1.9)	NE
58	OH	Cl	50 (±11)	183 (±42)[a]	451 (±120)[b]
59	OH	Br	33.3 (±3.9)	NE	512 (±72)
60	OH	I	49 (±10)	NE	189 (±42)
62	NH_2	H	140 (±39)	6.28 (±1.33)	NE
63	NHMs	H	82 (±17)	16.5 (±4.2)	NE
64	NH_2	F	830 (±330)	17.9 (±4.5)[a]	NE
65	NH_2	Cl	330 (±170)	NE	1400 (±140)[b]
66	NH_2	Br	212 (±73)	NE	666 (±95)
67	NH_2	I	990 (±200)	NE	1800 (±190)

Values represent the mean ± SEM of three or more independent experiments of K_i or EC_{50}.
[a] The mean % of agonism relative to capsaicin: 52% for **58**, 90% for **64**.
[b] The mean % of antagonism of the capsaicin stimulated $^{45}Ca^{2+}$ uptake response: 28% for **58**, 85% for **64**.

rTRPV1 in which 5-halogenation on the A-region in RTX-carbonate shifted the activity toward antagonism progressively as the size of halogen increased.

The differences between the hTRPV1 and the rTRPV1 were the following: 1) The activities of I-RTX (**4**) and its carbonate analogue (**60**) indicated that the substitution of the benzylic methylene with oxygen did not affect activity for rat TRPV1 but reduced the potency for human TRPV1. 2) The 4-amino RTX-carbonates showed reduced affinities for hTRPV1 compared to the corresponding RTX-carbonates. 3) The partial agonists/antagonists, **58**, **63** and **64**, showed a little more agonism for the hTRPV1 than for the rTRPV1.

2.3. Molecular modelling

In order to investigate the structural basis for the antagonist activity of I-RTX-carbonate (**60**), we performed conformational analysis using the SYBYL Grid Search method. The resulting lowest energy conformer was selected and the distance for this conformer between the A-region methoxy group and the diterpene hydroxyl group was compared with that for the lowest energy conformers of RTX and I-RTX.

As shown in Fig. 2, the conformation of the I-RTX-carbonate (**60**) was similar to that of I-RTX (**4**) but different from that of RTX [24]. The I-RTX-carbonate demonstrated a bent conformation, and the methoxy group appeared to form internal H-bonding with the hydroxyl group of the diterpene core as observed in I-RTX, which also showed antagonist activity. RTX, which is an agonist, did not show this internal H-bonding and that could allow its A-region to interact with residues in the binding site, driving agonism. Our modelling is thus consistent with our biological findings that the I-RTX-carbonate functions as an antagonist, not as an agonist, and provides a potential structural basis for its biological behaviour.

3. Conclusion

We have investigated the carbonate analogues of 5′-halogenated RTX and its 4-amino surrogate in order to explore the effect of oxygen substitution for the benzylic methylene on the A-region and the role of halogens in the reversal of activity from agonism to antagonism. The carbonate analogues showed similar activities to the corresponding RTX ones in rat TRPV1 but lower potency in human TRPV1. 5-Halogenation converted the agonists to partial or full antagonists, and the extent of antagonism reflected the order of I > Br > Cl > F. Antagonism was further favoured in derivatives of the 4-amino RTX surrogate compared to derivatives of RTX itself. The carbonate analogues of I-RTX (**60**) and 5-bromo-4-amino-RTX (**65**) proved to be potent and full antagonists with $K_{i(ant)} = 2.23$ and 2.46 nM, respectively, which were ca. 5-fold more potent than I-RTX (**2**) under our conditions. Of particular note, the results with compound **60** (same as compound **6**) are strikingly different from the previous report [23] in which the compound was described as an ultrapotent agonist with ca. 4-fold better potency than RTX for hTRPV1 in HEK-293. The conformational analysis of the I-RTX-carbonate (**60**) indicated that its bent conformation was similar to that of I-RTX rather than that of RTX, consistent with compound **60** showing potent antagonism as does I-RTX.

4. Experimental

4.1. General

All chemical reagents were commercially available. Melting points were determined on a Büchi Melting Point B-540 apparatus and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230–400 mesh, Merck. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a JEOL JNM-LA 300 and Bruker Avance 400 MHz FT-NMR spectrometer. Chemical shifts are reported in ppm units with Me_4Si as a reference standard. Mass spectra were recorded on a VG Trio-2 GC–MS and 6460 Triple Quad LC/MS.

4.1.1. 3-Fluoro-4-hydroxy-5-methoxy-benzaldehyde (**7**)

Commercially available 2-fluoro-6-methoxyphenol (1.00 g, 6.8 mmol) was added to a solution of 40% dimethylamine (1.6 mL, 12.4 mmol) and 37% formaldehyde (0.92 mL, 12.4 mmol) in ethanol (20 mL). The mixture was refluxed for 2 h, cooled to room temperature, and concentrated *in vacuo* to give a solid. The solid was triturated with ether and the solvent was removed under vacuum. The product was dissolved in chloroform (20 mL) and iodomethane (4.0 mL, 64.2 mmol) was added. The reaction mixture was stirred for 18 h at room temperature and filtered. Without purification the filtrate was dissolved in acetic acid (20 mL) and H_2O (20 mL) and then HMTA (1.4 g, 10.3 mmol) was added. The reaction mixture was heated to 120 °C. After 2 h, conc. HCl (0.8 mL) was added and it was heated for a further 5 min and cooled. The reaction mixture was extracted with EtOAc and water. The organic layer was dried with Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography to afford **7** as a white solid (0.29 g, 25%).

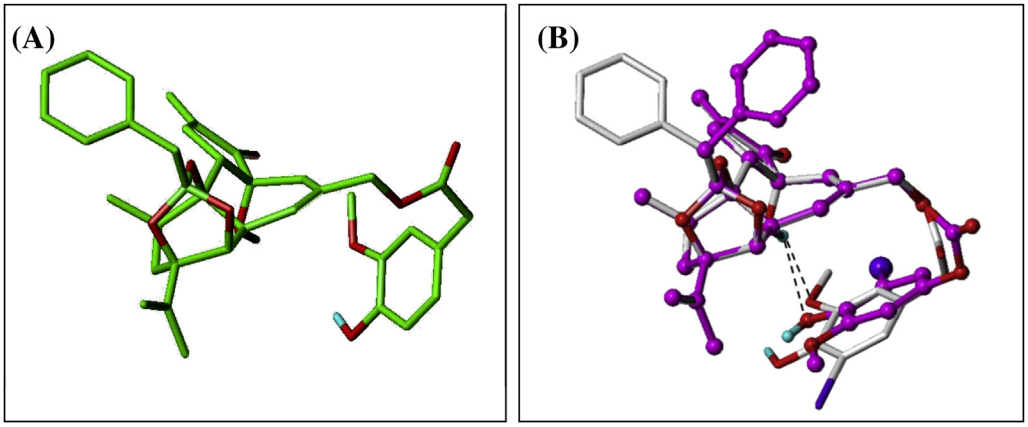


Fig. 2. The lowest energy conformers of (A) RTX and (B) I-RTX superimposed with I-RTX-carbonate. Carbon atoms are shown in green for RTX, white for I-RTX, and magenta for I-RTX-carbonate. RTX and I-RTX are depicted as capped stick. I-RTX-carbonate is in ball-and-stick. Hydrogen bonds are displayed in black dashed lines, and non-polar hydrogens are undisplayed for clarity.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.80 (d, 1H, J = 1.08 Hz, CHO), 7.28–7.31 (m, 1H, Ar), 5.98 (bs, 1H, OH), 4.00 (s, 3H, OCH_3).

*4.1.2. 3-Chloro-4-hydroxy-5-methoxy-benzaldehyde (**8**)*

A solution of vanillin (0.50 g, 3.3 mmol) in THF (10 mL) was cooled to 0 °C and NaH (0.25 g, 6.6 mmol) and *N*-chlorosuccinimide (0.53 g, 4.0 mmol) was added. The reaction mixture was stirred for 16 h at room temperature and diluted with EtOAc. The mixture was acidified with 1 N HCl and extracted with EtOAc several times. The combined organic layers were washed with water and brine and dried over Na_2SO_4. The layer was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by recrystallization to afford **8** as a white solid (0.4 g, 64%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.80 (s, 1H, CHO), 7.50 (d, 1H, J = 1.68 Hz, Ar), 7.34 (d, 1H, J = 1.65 Hz, Ar), 6.41 (s, 1H, OH), 4.00 (s, 3H, OCH_3).

*4.1.3. 3-Bromo-4-hydroxy-5-methoxy-benzaldehyde (**9**)*

Commercially available.

*4.1.4. 3-Iodo-4-hydroxy-5-methoxy-benzaldehyde (**10**)*

Commercially available.

*4.1.5. 3-Fluoro-4-(tert-butyl-dimethyl-silanyloxy)-5-methoxy-benzaldehyde (**11**)*

To a cooled solution of **7** (0.46 g, 2.7 mmol) in CH_2Cl_2 (10 mL) at 0 °C were added DMAP (0.13 g, 1.1 mmol), triethylamine (0.46 mL, 3.3 mmol), and *t*-butyldimethylsilyl chloride (0.45 g, 3.0 mmol). The reaction mixture was stirred for 5 h at room temperature and diluted with CH_2Cl_2. The organic layer was washed with water and brine and dried with Na_2SO_4. The suspension was filtered and concentrated *in vacuo*. The residue was purified by column chromatography to afford **11** as a white solid (0.69 g, 89%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.80 (d, 1H, J = 1.08 Hz, CHO), 7.22 (m, 2H, Ar), 3.89 (s, 3H, OCH_3), 1.02 (s, 9H, *t*-Bu), 0.20 (d, 6H, J = 0.9 Hz, Si–CH_3).

*4.1.6. 3-Chloro-4-(tert-butyl-dimethyl-silanyloxy)-5-methoxy-benzaldehyde (**12**)*

The titled compound was prepared from **8** by following the procedure for the synthesis of **11** as a white solid in 66% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.80 (s, 1H, CHO), 7.49 (d, 1H, J = 1.83 Hz, Ar), 7.30 (d, 1H, J = 1.83 Hz, Ar), 3.88 (s, 3H, OCH_3), 1.04 (s, 9H, *t*-Bu), 0.23 (s, 6H, Si–CH_3).

*4.1.7. 3-Bromo-4-(tert-butyl-dimethyl-silanyloxy)-5-methoxy-benzal dehyde (**13**)*

The titled compound was prepared from **9** by following the procedure for the synthesis of **11** as a white solid in 54% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.77 (s, 1H, CHO), 7.62 (d, 1H, J = 1.83 Hz, Ar), 7.31 (d, 1H, J = 1.83 Hz, Ar), 3.85 (s, 3H, OCH_3), 1.02 (s, 9H, *t*-Bu), 0.23 (s, 6H, Si–CH_3).

*4.1.8. 3-Iodo-4-(tert-butyl-dimethyl-silanyloxy)-5-methoxy-benzal dehyde (**14**)*

The titled compound was prepared from **10** by following the procedure for the synthesis of **11** as a white solid in 80% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.75 (s, 1H, CHO), 7.83 (d, 1H, J = 1.28 Hz, Ar), 7.33 (d, 1H, J = 1.00 Hz, Ar), 3.84 (s, 3H, OCH_3), 1.04 (s, 9H, *t*-Bu), 0.26 (s, 6H, Si–CH_3).

*4.1.9. Formic acid 4-(tert-butyl-dimethyl-silanyloxy)-3-fluoro-5-methoxy-phenyl ester (**15**)*

A mixture of **11** (0.10 g, 0.35 mmol) and m-CPBA (0.12 g, 0.68 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature for 16 h. The reaction mixture was diluted with EtOAc, washed with 10% $NaHCO_3$ two times, and dried with Na_2SO_4. The suspension was filtered and the filtrate was concentrated *in vacuo* to afforded **15** as a white solid (0.07 g, 63%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (s, 1H, COOH), 6.56 (dd, 1H, J = 2.7 Hz, 10.2 Hz, Ar), 6.47 (t, 1H, J = 2.4 Hz, Ar), 3.80 (s, 3H, OCH_3), 1.01 (s, 9H, *t*-Bu), 0.16 (s, 6H, Si–CH_3).

*4.1.10. Formic acid 4-(tert-butyl-dimethyl-silanyloxy)-3-chloro-5-methoxy-phenyl ester (**16**)*

The titled compound was prepared from **12** by following the procedure for the synthesis of **15** as a white solid in 63% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (s, 1H, COOH), 6.78 (d, 1H, J = 2.76 Hz, Ar), 6.57 (d, 1H, J = 2.73 Hz, Ar), 3,79 (s, 3H, OCH_3), 1.03 (s, 9H, *t*-Bu), 0.20 (s, 6H, Si–CH_3).

*4.1.11. Formic acid 4-(tert-butyl-dimethyl-silanyloxy)-3-bromo-5-methoxy-phenyl ester (**17**)*

The titled compound was prepared from **13** by following the procedure for the synthesis of **15** as a white solid in 58% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (s, 1H, COOH), 6.93 (d, 1H, J = 2.76 Hz, Ar), 6.61 (d, 1H, J = 2.73 Hz, Ar), 3.79 (s, 3H, OCH_3), 1.04 (s, 9H, *t*-Bu), 0.21 (s, 6H, Si–CH_3).

*4.1.12. Formic acid 4-(tert-butyl-dimethyl-silanyloxy)-3-iodo-5-methoxy-phenyl ester (**18**)*

The titled compound was prepared from **14** by following the procedure for the synthesis of **15** as a white solid in 60% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.77 (s, 1H, COOH), 7.85 (d, 1H, J = 1.83 Hz, Ar), 7.35 (d, 1H, J = 1.83 Hz, Ar), 3.86 (s, 3H, OCH_3), 1.06 (s, 9H, *t*-Bu), 0.28 (s, 6H, Si–CH_3).

*4.1.13. 4-(tert-Butyl-dimethyl-silanyloxy)-3-fluoro-5-methoxy-phenol (**19**)*

To a solution of **15** in MeOH (10 mL) was added 10% K_2CO_3 solution (0.59 mL). The reaction mixture was stirred for 12 h at room temperature, diluted with water, neutralized with 10% HCl and extracted with EtOAc several times. The combined extracts were dried with Na_2SO_4 and evaporated *in vacuo*. The residue was purified by column chromatography to afford **19** as a white solid (0.05 g, 74%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.18–6.22 (m, 2H, Ar), 4.51 (s, 1H, OH), 3.77 (s, 3H, OCH_3), 0.99 (s, 9H, *t*-Bu), 0.13 (s, 1H, Si–CH_3); LRMS *m/z* (FAB) 273 (M + H).

*4.1.14. 4-(tert-Butyl-dimethyl-silanyloxy)-3-chloro-5-methoxy-phenol (**20**)*

The titled compound was prepared from **16** by following the procedure for the synthesis of **19** as a white solid in 98% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.42 (d, 1H, J = 2.94 Hz, Ar), 6.32 (d, 1H, J = 2.94 Hz, Ar), 4.66 (s, 1H, OH), 3.76 (s, 3H, OCH_3), 1.02 (s, 9H, *t*-Bu), 0.16 (s, 1H, Si–CH_3); LRMS *m/z* (FAB) 289 (M + H).

*4.1.15. 4-(tert-Butyl-dimethyl-silanyloxy)-3-bromo-5-methoxy-phenol (**21**)*

The titled compound was prepared from **17** by following the procedure for the synthesis of **19** as a white solid in 96% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.58 (d, 1H, J = 2.91 Hz, Ar), 6.36 (d, 1H, J = 2.94 Hz, Ar), 4.66 (s, 1H, OH), 3.76 (s, 3H, OCH_3), 1.03 (s, 9H, *t*-Bu), 0.18 (s, 1H, Si–CH_3); LRMS *m/z* (FAB) 333 (M + H).

*4.1.16. 4-(tert-Butyl-dimethyl-silanyloxy)-3-iodo-5-methoxy-phenol (**22**)*

The titled compound was prepared from **18** by following the procedure for the synthesis of **19** as a white solid in 24% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.80 (d, 1H, $J = 2.94$ Hz, Ar), 6.40 (d, 1H, $J = 2.76$ Hz, Ar), 3.75 (s, 3H, OCH_3), 1.04 (s, 9H, *t*-Bu), 0.23 (s, 1H, Si–CH_3).

*4.1.17. 4-Fluoro-2-methoxy-1-nitro-benzene (**24**)*

To a stirred solution of **23** (0.60 g, 3.82 mmol) in acetone (20 mL) was added K_2CO_3 (1.1 g, 7.6 mmol) and iodomethane (0.5 mL, 7.6 mmol). The reaction mixture was refluxed for 3 h and concentrated *in vacuo*. The residue was diluted with EtOAc, washed with water and brine and dried over Na_2SO_4. The suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography to afford **24** as a white solid (0.63 g, 96%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (m, 1H, Ar), 6.76 (dd, 1H, $J = 8.20$, 1.84 Hz, Ar), 6.71 (m, 1H, Ar), 3.94 (s, 3H, OCH_3).

*4.1.18. 3-Methoxy-4-nitro-phenol (**25**)*

To a stirred solution of **24** (0.63 g, 3.7 mmol) in DMSO (5.3 mL) was added 30% NaOH (2.5 mL, 18.0 mmol) solution. The mixture was stirred for 21 h at room temperature, cooled to 0 °C and acidified with 6 N HCl. The mixture was pour into 5% HCl and extracted with ether several times. The combined organic layers were washed with 5% HCl three times, then with brine, and dried over Na_2SO_4. The suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography to afford **25** as a white solid (0.37 g, 60%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, 1H, $J = 8.79$ Hz, Ar), 6.54 (d, 1H, $J = 2.19$ Hz, Ar), 6.44 (dd, 1H, $J = 8.97$, 2.37 Hz, Ar), 5.73 (s, 1H, OH) 3.95 (s, 3H, OCH_3); LRMS *m/z* (FAB) 170 (M + H).

*4.1.19. Acetic acid 3-methoxy-4-nitro-phenyl ester (**26**)*

To a stirred solution of **25** (0.30 g, 1.77 mmol) in THF (10 mL) was added acetic anhydride (0.33 mL, 3.6 mmol) and pyridine (0.29 mL, 3.6 mmol). The reaction mixture was stirred for 5 h at room temperature and concentrated *in vacuo*. The residue was diluted with EtOAc, washed with 1 N HCl and with brine and dried over Na_2SO_4. The suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography to afford **26** as a white solid (0.37 g, 99%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, 1H, $J = 8.79$ Hz, Ar), 6.87 (d, 1H, $J = 2.22$ Hz, Ar), 6.80 (dd, 1H, $J = 8.97$, 2.19 Hz, Ar), 3.95 (s, 3H, OCH_3), 2.34 (s, 3H, Ac).

*4.1.20. Acetic acid 4-amino-3-methoxy-phenyl ester (**27**)*

A suspension of **45** (0.37 g, 1.77 mmol) and 10% Pd/C (0.04 g) in MeOH (10 mL) was hydrogenated at room temperature for 2 h under a balloon of hydrogen gas. The mixture was filtered through a bed of Celite and the filtrate was concentrated *in vacuo* to afford **27** as a white solid (0.37 g, >99%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.67 (d, 1H, $J = 8.25$ Hz, Ar), 6.50–6.55 (m, 2H, Ar), 3.83 (s, 3H, OCH_3), 3.73 (s, 2H, NH_2), 2.27 (s, 3H, Ac); LRMS *m/z* (FAB) 181 (M + H).

*4.1.21. Acetic acid 4-tert-butoxycarbonylamino-3-methoxy-phenyl ester (**28**)*

To a cooled solution of **27** (0.10 g, 0.05 mmol) in THF and H_2O (1:1, 10 mL) at 0 °C was added $NaHCO_3$ (0.07 g, 0.8 mmol) and Boc_2O (0.07 g, 0.3 mmol). The reaction mixture was stirred for 24 h at room temperature and diluted with EtOAc. The organic layer was washed with water and brine and dried over Na_2SO_4. The suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography to afford **28** as a white solid (0.04 g, 50%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, 1H, $J = 8.36$ Hz, NH), 6.96 (s, 1H, Ar), 6.63–6.66 (dd, 1H, $J = 8.76$, 2.36 Hz, Ar), 6.60 (d, 1H, $J = 2.36$ Hz), 3.83 (s, 3H, OCH_3), 2.26 (s, 3H, Ac), 1.50 (s, 9H, Boc).

*4.1.22. (4-Hydroxy-2-methoxy-phenyl)-carbamic acid tert-butyl ester (**29**)*

A mixture of **28** (0.04 g, 0.15 mmol) and saturated $NaHCO_3$ solution (1 mL) in MeOH and H_2O (2:1, 3 mL) was stirred for 32 h at room temperature, cooled to 0 °C and acidified with 10% HCl. The mixture was diluted with EtOAc and washed with water and brine and dried over Na_2SO_4. The suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography to afford **29** (0.03 g, 93%) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.83 (s, 1H, NH), 6.81 (s, 1H, Ar), 6.43 (s, 1H, Ar), 6.43–6.36 (dd, 1H, $J = 8.58$, 2.76 Hz, Ar), 4.62 (s, 1H, OH), 3.83 (s, 3H, OCH_3), 1.51 (s, 9H, Boc); LRMS *m/z* (FAB) 239 (M + H).

*4.1.23. 4-Amino-3-methoxy-phenol (**30**)*

The titled compound was prepared from **25** by following the procedure for the synthesis of **27** as a white solid in 99% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.62 (d, 1H, $J = 8.40$ Hz, Ar), 6.38 (d, 1H, $J = 2.58$ Hz, Ar), 6.22 (dd, 1H, $J = 8.25$, 2.55 Hz, Ar), 3.80 (s, 3H, OCH_3).

*4.1.24. N-(4-Hydroxy-2-methoxy-phenyl)-methanesulfonamide (**31**)*

To a cooled solution of **30** (0.40 g, 2.65 mmol) in pyridine (18 mL) at 0 °C was added methane sulfonyl chloride (0.21 mL, 2.6 mmol). The reaction mixture was stirred at room temperature overnight and diluted with EtOAc. The organic layer was washed with 1 N HCl, water and brine and dried over Na_2SO_4. The suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography to afford **31** as a white solid (0.4 g, 71%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 (d, 1H, $J = 8.55$ Hz, Ar), 6.48 (d, 1H, $J = 2.15$ Hz, Ar), 6.34 (dd, 1H, $J = 8.30$, 2.40 Hz, Ar), 3.83 (s, 3H, OCH_3), 2.83 (s, 3H, CH_3SO_2); LRMS *m/z* (FAB) 218 (M + H).

*4.1.25. 3,5-Difluoro-4-nitro-phenol (**33**)*

To a cooled solution of 3,5-difluorophenol **32** (0.50 g, 3.8 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added conc. HNO_3 (0.55 mL, 3.7 mmol) dropwise over 10 min. The reaction mixture was stirred at 0 °C for 1 h, quenched with cold water, stirred and poured into a separatory funnel. The aqueous layer was extracted with EtOAc several times and the combined organic layers were dried over Na_2SO_4. The suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography to afford **33** as a white solid (0.23 g, 34%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.52–6.58 (m, 2H).

*4.1.26. 1,3-Difluoro-5-methoxymethoxy-2-nitro-benzene (**34**)*

To a stirred solution of **33** (0.10 g, 0.57 mmol) in CH_2Cl_2 (8 mL) was added chloromethyl methyl ether (0.05 mL, 0.63 mmol) and DIPEA (0.12 mL, 0.68 mmol). After stirring for 3 h at room temperature, the reaction mixture was evaporated *in vacuo*. The residue was purified by column chromatography to afford **34** as a white solid (0.11 g, 88%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.71–6.77 (m, 2H), 5.21 (s, 2H), 3.49 (s, 3H).

*4.1.27. 1-Fluoro-3-methoxy-5-methoxymethoxy-2-nitro-benzene (**35**)*

To a stirred solution of **34** (0.11 g, 0.50 mmol) in MeOH (8 mL) was added NaOMe (25 wt.% in MeOH, 0.34 mL, 1.5 mmol).

After stirring for 8 h at room temperature, the reaction mixture was evaporated *in vacuo*. The residue was purified by column chromatography to afford **35** as a white solid (0.11 g, 95%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.45–6.54 (m, 2H), 5.19 (s, 2H), 3.90 (s, 3H), 3.48 (s, 3H).

*4.1.28. 2-Fluoro-6-methoxy-4-methoxymethoxy-phenylamine (**36**)*

A suspension of **8** (0.10 g, 0.43 mmol) and 10% Pd/C (0.010 g) in THF (5 mL) and ethanol (5 mL) was hydrogenated for 20 h under a balloon of hydrogen gas. The reaction mixture was filtered by washing with EtOAc and the combined filtrates were concentrated *in vacuo*. The residue was purified by column chromatography to afford **36** as a white solid (0.080 g, 99%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.45 (dd, 1H, J = 2.4, 11.7 Hz), 6.36–6.38 (m, 1H), 5.07 (s, 2H), 3.84 (s, 3H), 3.47 (s, 3H).

*4.1.29. 4-Amino-3-fluoro-5-methoxy-phenol (**37**)*

To a cooled solution of **36** (0.05 g, 0.25 mmol) in CH_2Cl_2 (0.8 mL) at 0 °C was added trifluoroacetic acid (0.2 mL). After stirring for 3 h at room temperature, the reaction mixture was evaporated *in vacuo*. The residue was dissolved with EtOAc and then washed with saturated $NaHCO_3$ solution, water, and brine and dried over Na_2SO_4. The suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography to afford **37** as a white solid (0.026 g, 66%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.21–6.22 (m, 1H), 6.13 (dd, 1H, J = 2.4, 11.7 Hz), 3.81 (s, 3H).

*4.1.30. 3-Fluoro-5-methoxymethoxy-2-nitro-phenylamine (**38**)*

To a stirred solution of **34** (0.10 g, 0.46 mmol) in MeOH (3 mL) was added ammonia (2.0 M solution in MeOH, 7.0 mL). After stirring for 2 days at room temperature, the reaction mixture was evaporated *in vacuo*. The residue was purified by column chromatography to afford **38** as a white solid (0.026 g, 25%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.17–6.22 (m, 2H), 5.16 (s, 2H), 3.47 (s, 3H).

*4.1.31. 1-Chloro-3-fluoro-5-methoxymethoxy-2-nitro-benzene (**39**)*

To a partially dissolved solution of copper (II) chloride (0.038 g, 0.28 mmol) and *t*-BuONO (0.029 g, 0.28 mmol) in acetonitrile (4 mL) was added a suspension of **38** (0.040 g, 0.18 mmol) in acetonitrile (4 mL) at 0 °C. After all the aniline had been added, the reaction mixture was stirred for 1 h at room temperature, poured into 0.5 N HCl and extracted with EtOAc several times. The combined organic layers were washed with brine and dried over Na_2SO_4. The suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography to afford **39** as a white solid (0.033 g, 78%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.97–6.98 (m, 2H), 6.86 (dd, 1H, J = 2.5, 11.2 Hz), 5.20 (s, 2H), 3.48 (s, 3H).

*4.1.32. 1-Bromo-3-fluoro-5-methoxymethoxy-2-nitro-benzene (**40**)*

To a solution of **38** (0.050 g, 0.23 mmol) and Cu(I)Br (0.050 g, 0.35 mmol) in acetonitrile (5 mL) was added a solution of *t*-BuONO (0.036 g, 0.35 mmol) in acetonitrile (5 mL) in a dropwise manner at 0 °C. The mixture was extracted with EtOAc several times. The combined organic layers were washed with 2 N HCl and $NaHCO_3$ solution, then with brine, and finally dried over Na_2SO_4. The suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography to afford **40** as a white solid (0.022 g, 34%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.14–7.15 (m, 2H), 6.90 (dd, 1H, J = 2.4, 11.0 Hz), 5.20 (s, 2H), 3.48 (s, 3H).

*4.1.33. 1-Fluoro-3-iodo-5-methoxymethoxy-2-nitro-benzene (**41**)*

To a heated solution of iodine (0.45 g, 1.8 mmol) and *t*-BuONO (0.13 g, 1.3 mmol) in acetonitrile (5 mL) at 60 °C was added **11** (0.040 g, 0.18 mmol) in acetonitrile (5 mL). The reaction mixture was stirred for an additional 2 h at 60 °C and then stirred at room temperature overnight. The mixture was quenched with aqueous Na_2SO_4 solution and extracted with EtOAc several times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography to afford **41** as a white solid (0.043 g, 83%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.37 (m, 2H), 6.91 (dd, 1H, J = 2.4, 11.0 Hz), 5.19 (s, 2H), 3.48 (s, 3H).

*4.1.34. 1-Chloro-3-methoxy-5-methoxymethoxy-2-nitro-benzene (**42**)*

To a stirred solution of **39** (0.033 g, 0.14 mmol) in MeOH (5 mL) was added NaOMe (25 wt.% in MeOH, 0.1 mL). After stirring for 3 h at 60 °C, the reaction mixture was evaporated *in vacuo*. The residue was purified by column chromatography to afford **42** as a white solid (0.035 g, 99%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.74 (d, 1H, J = 2.4 Hz), 6.59 (d, 1H, J = 2.4 Hz), 5.18 (s, 2H), 3.87 (s, 3H), 3.48 (s, 3H).

*4.1.35. 1-Bromo-3-methoxy-5-methoxymethoxy-2-nitro-benzene (**43**)*

The titled compound was prepared from **40** by following the procedure for the synthesis of **42** as a white solid in 99% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.89 (d, 1H, J = 2.2 Hz), 6.63 (d, 1H, J = 2.2 Hz), 5.18 (s, 2H), 3.87 (s, 3H), 3.48 (s, 3H).

*4.1.36. 1-Iodo-3-methoxy-5-methoxymethoxy-2-nitro-benzene (**44**)*

The titled compound was prepared from **41** by following the procedure for the synthesis of **42** as a white solid in 67% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 (d, 1H, J = 2.4 Hz), 6.65 (d, 1H, J = 2.2 Hz), 5.17 (s, 2H), 3.85 (s, 3H), 3.48 (s, 3H).

*4.1.37. 4-Amino-3-chloro-5-methoxy-phenol (**45**)*

The titled compound was prepared from **42** by following the procedure for the synthesis of **37** as a white solid in 62% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.47 (d, 1H, J = 2.2 Hz), 6.39 (d, 1H, J = 2.4 Hz), 3.85 (s, 3H).

*4.1.38. 3-Bromo-5-methoxy-4-nitro-phenol (**46**)*

The titled compound was prepared from **43** by following the procedure for the synthesis of **37** as a white solid in 65% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.63 (d, 1H, J = 2.2 Hz), 6.43 (d, 1H, J = 2.2 Hz), 3.84 (s, 3H).

*4.1.39. 3-Iodo-5-methoxy-4-nitro-phenol (**47**)*

The titled compound was prepared from **44** by following the procedure for the synthesis of **37** as a white solid in 78% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.87 (d, 1H, J = 2.2 Hz), 6.49 (d, 1H, J = 2.4 Hz), 3.84 (s, 3H).

*4.1.40. 4-Amino-3-chloro-5-methoxy-phenol (**48**)*

To a stirred solution of **45** (0.045 g, 0.22 mmol), ferric chloride hexahydrate (2 mg, 0.01 mmol) and activated carbon (22 mg) was added hydrazine monohydrate (0.22 mL, 2.9 mmol). After stirring for 24 h at 60 °C, the reaction mixture was filtered through Celite. The filtrate was evaporated *in vacuo*. The residue was purified by column chromatography to afford **48** as a white solid (8 mg, 21%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.41 (d, 1H, J = 2.5 Hz), 6.49 (d, 1H, J = 2.5 Hz), 3.84 (s, 3H).

*4.1.41. 4-Amino-3-bromo-5-methoxy-phenol (**49**)*

The titled compound was prepared from **46** by following the procedure for the synthesis of **48** as a white solid in 28% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.56 (d, 1H, J = 2.5 Hz), 6.36 (d, 1H, J = 2.5 Hz), 3.81 (s, 3H).

*4.1.42. 4-Amino-3-iodo-5-methoxy-phenol (**50**)*

The titled compound was prepared from **47** by following the procedure for the synthesis of **48** as a white solid in 20% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.76 (d, 1H, J = 2.4 Hz), 6.40 (d, 1H, J = 2.5 Hz), 3.80 (s, 3H).

*4.1.43. Compound **52***

To a stirred solution of ROPA **51** (50 mg, 0.11 mmol) in CH_2Cl_2 (8 mL) was added 4-nitrophenyl chloroformate (86 mg, 0.43 mmol) followed by triethylamine (0.02 mL, 0.16 mmol) at 0 °C. After stirring overnight at room temperature, the reaction mixture was diluted with EtOAc. The organic layer was washed with saturated $NaHCO_3$, water and brine, and dried over Na_2SO_4. The suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography to afford **52** (60 mg, 87%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, 2H, J = 9.2 Hz), 7.48 (s, 1H, Ar), 7.33–7.35 (m, 4H), 7.19–7.27 (m, 3H), 6.05 (s, 1H), 4.62–4.72 (m, 4H), 4.27 (d, 1H, J = 2.6 Hz), 3.21 (s, 2H), 3.16 (bs, 2H), 2.54–2.66 (m, 2H), 2.12–2.30 (m, 3H), 1.83 (m, 3H), 1.51 (s, 3H), 0.96 (d, 3H, J = 7.1 Hz); LRMS *m/z* (FAB) 630 (M + H).

4.1.44. General procedure for RTX carbonates

To a stirred solution of **52** (20 mg, 0.032 mmol) in CH_2Cl_2 (5 mL) was added phenol (0.064 mmol, 2 eq.) and DMAP (4 mg, 0.035 mmol). After stirring overnight at room temperature, the reaction mixture was diluted with EtOAc. The organic layer was washed with saturated $NaHCO_3$, water and brine, and dried over Na_2SO_4. The suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography to afford the final carbonate.

*4.1.45. Compound **53***

The titled compound was prepared from **19** by following the general procedure for RTX carbonates as a white solid in 92% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (s, 1H, Ar), 7.35–7.38 (m, 2H), 7.20–7.31 (m, 3H), 6.60 (dd, 1H, J = 2.8, 10.4 Hz), 6.52 (m, 1H), 6.02 (s, 1H), 4.64–4.76 (m, 4H), 4.27 (d, 1H, J = 2.6 Hz), 3.79 (s, 3H, OCH_3), 3.21 (s, 2H), 3.16 (bs, 2H), 2.55–2.65 (m, 2H), 2.02–2.30 (m, 3H), 1.83 (m, 3H), 1.52 (s, 3H), 0.95–1.00 (m, 12H), 0.13 (s, 6H); LRMS *m/z* (FAB) 763 (M + H).

*4.1.46. Compound **54***

The titled compound was prepared from **20** by following the general procedure for RTX carbonates as a white solid in 71% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (s, 1H, Ar), 7.35–7.38 (m, 2H), 7.20–7.31 (m, 3H), 6.84 (d, 1H, J = 2.8 Hz), 6.63 (d, 1H, J = 2.8 Hz), 6.02 (s, 1H), 4.64–4.76 (m, 4H), 4.27 (d, 1H, J = 2.6 Hz), 3.79 (s, 3H, OCH_3), 3.21 (s, 2H), 3.16 (bs, 2H), 2.55–2.65 (m, 2H), 2.02–2.30 (m, 3H), 1.83 (m, 3H), 1.52 (s, 3H), 0.95–1.00 (m, 12H), 0.20 (s, 6H); LRMS *m/z* (FAB) 779 (M + H).

*4.1.47. Compound **55***

The titled compound was prepared from **21** by following the general procedure for RTX carbonates as a white solid in 66% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (s, 1H, Ar), 7.35–7.38 (m, 2H), 7.20–7.31 (m, 3H), 6.99 (d, 1H, J = 2.8 Hz), 6.67 (d, 1H, J = 2.8 Hz), 6.02 (s, 1H), 4.64–4.76 (m, 4H), 4.27 (d, 1H, J = 2.6 Hz), 3.79 (s, 3H, OCH_3), 3.21 (s, 2H), 3.16 (bs, 2H), 2.55–2.65 (m, 2H), 2.02–2.30 (m, 3H), 1.83 (m, 3H), 1.52 (s, 3H), 0.95–1.00 (m, 12H), 0.20 (s, 6H); LRMS *m/z* (FAB) 823 (M + H).

*4.1.48. Compound **56***

The titled compound was prepared from **22** by following the general procedure for RTX carbonates as a white solid in 93% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (s, 1H, Ar), 7.35–7.38 (m, 2H), 7.20–7.31 (m, 3H), 7.19 (d, 1H, J = 2.8 Hz), 6.70 (d, 1H, J = 2.8 Hz), 6.02 (s, 1H), 4.63–4.75 (m, 4H), 4.27 (d, 1H, J = 2.6 Hz), 3.77 (s, 3H, OCH_3), 3.21 (s, 2H), 3.16 (bs, 2H), 2.55–2.65 (m, 2H), 2.02–2.30 (m, 3H), 1.83 (m, 3H), 1.52 (s, 3H), 0.95–1.00 (m, 12H), 0.20 (s, 6H); LRMS *m/z* (FAB) 871 (M + H).

*4.1.49. Compound **57***

To a cooled solution of **53** (14 mg, 0.018 mmol) in THF (1 mL) at 0 °C was added tetrabutylammonium fluoride (0.027 mL, 0.027 mmol) slowly. After stirring 2 h at room temperature, the reaction mixture was quenched with saturated $NaHCO_3$ solution. The organic layer was washed with water and brine and dried over Na_2SO_4. The suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography to afford **57** as a white solid (8 mg, 68%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (s, 1H, Ar), 7.33–7.35 (m, 2H), 7.19–7.27 (m, 3H), 6.65 (dd, 1H, J = 2.5, 10.6 Hz), 6.55 (m, 1H), 6.00 (s, 1H), 5.25 (b, 1H), 4.63–4.72 (m, 4H), 4.25 (d, 1H, J = 2.6 Hz), 3.88 (s, 3H, OCH_3), 3.19 (s, 2H), 3.13 (bs, 2H), 2.53–2.60 (m, 2H), 2.02–2.27 (m, 3H), 1.81 (s, 3H), 1.51 (s, 3H), 0.95 (d, 3H, J = 7.1 Hz); LRMS *m/z* (FAB) 649 (M + H), HRMS *m/z* (FAB) calculated 649.2449 (M + H), found 649.2427.

*4.1.50. Compound **58***

The titled compound was prepared from **54** by following the procedure for the synthesis of **57** as a white solid in 63% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.33–7.35 (m, 2H), 7.19–7.27 (m, 3H), 6.82 (d, 1H, J = 2.6 Hz), 6.65 (d, 1H, J = 2.6 Hz), 6.00 (s, 1H), 5.70 (b, 1H), 4.63–4.73 (m, 4H), 4.24 (d, 1H, J = 2.6 Hz), 3.88 (s, 3H, OCH_3), 3.19 (s, 2H), 3.13 (bs, 2H), 2.54–2.61 (m, 2H), 2.00–2.27 (m, 4H), 1.81 (s, 3H), 1.50 (s, 3H), 0.95 (d, 3H, J = 7.1 Hz); LRMS *m/z* (FAB) 665 (M + H), HRMS *m/z* (FAB) calculated 665.2154 (M + H), found 665.2149.

*4.1.51. Compound **59***

The titled compound was prepared from **55** by following the procedure for the synthesis of **57** as a white solid in 67% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.33–7.35 (m, 2H), 7.19–7.27 (m, 3H), 6.97 (d, 1H, J = 2.6 Hz), 6.69 (d, 1H, J = 2.6 Hz), 6.00 (s, 1H), 5.79 (b, 1H), 4.63–4.73 (m, 4H), 4.25 (d, 1H, J = 2.6 Hz), 3.88 (s, 3H, OCH_3), 3.19 (s, 2H), 3.13 (bs, 2H), 2.54–2.61 (m, 2H), 2.00–2.27 (m, 4H), 1.81 (s, 3H), 1.50 (s, 3H), 0.95 (d, 3H, J = 7.1 Hz); LRMS *m/z* (FAB) 709 (M + H), HRMS *m/z* (FAB) calculated 711.1636, found 709.1639.

*4.1.52. Compound **60***

The titled compound was prepared from **56** by following the procedure for the synthesis of **57** as a white solid in 49% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.33–7.35 (m, 2H), 7.19–7.27 (m, 3H), 7.14 (d, 1H, J = 2.6 Hz), 6.71 (d, 1H, J = 2.6 Hz), 6.00 (s, 1H), 5.97 (b, 1H), 4.63–4.73 (m, 4H), 4.25 (d, 1H, J = 2.6 Hz), 3.86 (s, 3H, OCH_3), 3.19 (s, 2H), 3.13 (bs, 2H), 2.54–2.61 (m, 2H), 2.00–2.27 (m, 4H), 1.81 (s, 3H), 1.50 (s, 3H), 0.95 (d, 3H, J = 7.1 Hz); LRMS *m/z* (FAB) 757 (M + H), HRMS *m/z* (FAB) calculated 757.1510, found 757.1495.

*4.1.53. Compound **61***

The titled compound was prepared from **31** by following the general procedure for RTX carbonates as a white solid in 67% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (d, 1H, J = 8.5 Hz), 7.44 (s, 1H), 7.33–7.35 (m, 2H), 7.19–7.27 (m, 3H), 6.78–6.81 (m, 2H), 6.67 (b, 1H), 6.01 (s, 1H), 4.63–4.73 (m, 4H), 4.25 (d, 1H, J = 2.6 Hz), 3.85 (s, 3H, OCH_3), 3.19 (s, 2H), 3.13 (bs, 2H), 2.93 (s, 3H), 2.55–2.62 (m, 2H), 2.11–2.27 (m, 3H), 1.81 (s, 3H), 1.50 (s, 3H), 0.95 (d, 3H, J = 7.1 Hz); LRMS *m/z* (FAB) 708 (M + H), HRMS *m/z* (FAB) calculated 708.2479, found 708.2485.

4.1.54. Compound **62**

The titled compound was prepared from **29** by following the general procedure for RTX carbonates as a white solid in 50% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04–8.07 (m, 1H), 7.46 (s, 1H), 7.35–7.38 (m, 2H), 7.20–7.31 (m, 3H), 7.00 (b, 1H), 6.70–6.78 (m, 2H), 6.02 (s, 1H), 4.64–4.75 (m, 4H), 4.26 (d, 1H, J = 2.7 Hz), 3.86 (s, 3H, OCH_3), 3.21 (s, 2H), 3.15 (bs, 2H), 2.56–2.65 (m, 2H), 2.11–2.31 (m, 3H), 1.83 (m, 3H), 1.50 (s, 12H), 0.98 (d, 3H, J = 7.1 Hz); LRMS *m/z* (FAB) 730 (M + H).

4.1.55. Compound **63**

To a cooled solution of **62** (14 mg, 0.019 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added trifluoroacetic acid (0.01 mL). After stirring 40 min at 0 °C, the reaction mixture was diluted with CH_2Cl_2, washed with $NaHCO_3$ and brine, and dried over $MgSO_4$. The suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography to afford **63** as a white solid (9 mg, 75%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.33–7.35 (m, 2H), 7.19–7.27 (m, 3H), 6.59–6.65 (m, 3H), 6.00 (s, 1H), 4.62–4.71 (m, 4H), 4.24 (d, 1H, J = 2.7 Hz), 3.82 (s, 3H, OCH_3), 3.19 (s, 2H), 3.13 (bs, 2H), 2.53–2.61 (m, 2H), 2.02–2.33 (m, 4H), 1.81 (m, 3H), 1.50 (s, 12H), 0.95 (d, 3H, J = 7.1 Hz); LRMS *m/z* (FAB) 630 (M + H), HRMS *m/z* (FAB) calculated 630.2703, found 630.2707.

4.1.56. Compound **64**

The titled compound was prepared from **37** by following the general procedure for RTX carbonates as a white solid in 58% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (s, 1H, Ar), 7.33–7.35 (m, 2H), 7.19–7.27 (m, 3H), 6.57 (dd, 1H, J = 2.3, 10.6 Hz), 6.47 (m, 1H), 6.00 (s, 1H), 4.62–4.72 (m, 4H), 4.24 (d, 1H, J = 2.6 Hz), 3.83 (s, 3H, OCH_3), 3.66 (b, 2H), 3.19 (s, 2H), 3.13 (bs, 2H), 2.53–2.60 (m, 2H), 2.02–2.27 (m, 3H), 1.81 (s, 3H), 1.51 (s, 3H), 0.96 (d, 3H, J = 7.1 Hz); LRMS *m/z* (FAB) 648 (M + H), HRMS *m/z* (FAB) calculated 648.2609, found 648.2610.

4.1.57. Compound **65**

The titled compound was prepared from **48** by following the general procedure for RTX carbonates as a white solid in 69% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 1H, Ar), 7.33–7.35 (m, 2H), 7.19–7.27 (m, 3H), 6.77 (d, 1H, J = 2.2 Hz), 6.56 (d, 1H, J = 2.2 Hz), 6.00 (s, 1H), 5.27 (s, 1H), 4.62–4.72 (m, 4H), 4.24 (d, 1H, J = 2.6 Hz), 4.07 (b, 2H), 3.83 (s, 3H, OCH_3), 3.19 (s, 2H), 3.13 (bs, 2H), 2.53–2.60 (m, 2H), 2.02–2.27 (m, 3H), 1.81 (s, 3H), 1.51 (s, 3H), 0.96 (d, 3H, J = 7.1 Hz); LRMS *m/z* (FAB) 664 (M + H), HRMS *m/z* (FAB) calculated 664.2306, found 664.2309.

4.1.58. Compound **66**

The titled compound was prepared from **49** by following the general procedure for RTX carbonates as a white solid in 35% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 1H, Ar), 7.33–7.35 (m, 2H), 7.19–7.27 (m, 3H), 6.91 (d, 1H, J = 2.2 Hz), 6.59 (d, 1H, J = 2.2 Hz), 6.00 (s, 1H), 4.62–4.72 (m, 4H), 4.24 (d, 1H, J = 2.6 Hz), 4.11 (b, 2H), 3.83 (s, 3H, OCH_3), 3.19 (s, 2H), 3.13 (bs, 2H), 2.53–2.60 (m, 2H), 2.02–2.27 (m, 3H), 1.81 (s, 3H), 1.51 (s, 3H), 0.96 (d, 3H, J = 7.1 Hz); LRMS *m/z* (FAB) 708 (M + H), HRMS *m/z* (FAB) calculated 708.1803, found 708.1812.

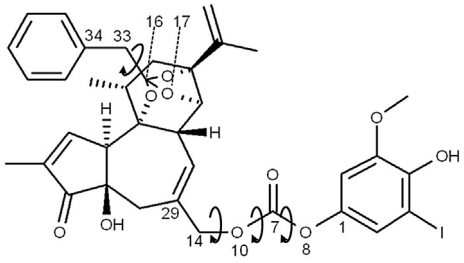
4.1.59. Compound **67**

The titled compound was prepared from **50** by following the general procedure for RTX carbonates as a white solid in 51% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.33–7.35 (m, 2H), 7.19–7.27 (m, 3H), 7.09 (d, 1H, J = 2.3 Hz), 6.62 (d, 1H, J = 2.3 Hz), 5.99 (s, 1H), 4.62–4.72 (m, 4H), 4.24 (d, 1H, J = 2.6 Hz), 3.81 (s, 3H, OCH_3), 3.19 (s, 2H), 3.13 (bs, 2H), 2.53–2.60 (m, 2H), 2.08–2.27 (m, 3H), 1.81 (s, 3H), 1.50 (s, 3H), 0.95 (d, 3H, J = 7.1 Hz); LRMS *m/z* (FAB) 756 (M + H), HRMS *m/z* (FAB) calculated 756.1670 (M + H), found 756.1686.

4.2. Molecular modelling

The 3D structure of the I-RTX-carbonate was generated with Concord and energy minimized using the MMFF94s force field and MMFF94 charge until the rms of the Powell gradient was 0.05 kcal mol^{-1} A^{-1} in SYBYL 8.1.1 (Tripos Int., St. Louis, MO, USA). The conformational analysis was carried out using the SYBYL Grid Search method (force field: MMFF94s; charges: MMFF94; minimization method: powell; termination: gradient 0.05 kcal mol^{-1} A^{-1}; and max iterations: 10,000). The torsion angles defined for the Grid Search were C29–C14–O10–C7, C14–O10–C7–C8, O10–C7–C8–C1 and O17–C16–C33–C34, and they were rotated in 30° increments. 20,736 unique conformers were generated, and 678 conformers were selected with the distance of 2.5–3.5 Å between the methoxy oxygen and hydroxyl hydrogen in the diterpene. The lowest energy conformer was selected and superimposed with those of RTX and I-RTX using the Fit Atoms tool in SYBYL. All computation calculations were performed on an Intel® XeonTM Quad-core workstation with Linux Cent OS release 4.6.



I-RTX-carbonate

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