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Synthesis and biological evaluation of novel imidazole-containing macrocycles

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ABSTRACT

A new family of compounds made of a 5-aryl-1*H*-imidazole motif included in a macrocycle has been designed and synthesized. The synthesis of the imidazole core makes use of our previously developed method for the regioselective preparation of 1,2,5-trisubstituted imidazoles while the construction of the macrocycle is based on a three steps sequence: S_NAr, Suzuki coupling, and RCM reaction. Biological evaluation of synthesized imidazole-containing macrocycles revealed that they display actual binding activity toward A_3 adenosine (*h*) receptor, dopamine D_1 (*h*) receptor, chloride channel (GABA-gated), and choline transporter (*h*) CHT1.

1. Introduction

4(5)-Aryl-1*H*-substituted imidazoles are central substructures of many compounds exhibiting biological and pharmacological properties.[1,2] Hence, this motif is found in numerous drugs, such as *anti*-inflammatory agents, kinase inhibitors, antagonists of CB_1 cannabinoid, and glucagon receptors, antibacterial agents. For these reasons, the synthesis of new structures containing this framework has attracted much attention from both academia and industry.

Recently, we reported the synthesis of novel large ring 1,3-bridged 2-azetidinones related to the carbapenem family.[3] This combined experimental and theoretical study showed that the inclusion of the azetidinone motif in a macrocycle allows acting on its conformational flexibility and hence investigating the importance of geometrical factors on biological properties.[4]

This prompted us to consider the synthesis and the biological evaluation of novel 5-aryl-1*H*-imidazole-containing macrocycles. The envisioned structures (**1**) consist in large rings (≥15 atoms) incorporating a 5-aryl-1*H*-imidazole motif by connection at C2 and *ortho*-aryl positions (Fig. 1).

Our planned strategy for the synthesis of these macrocycles (**1**) is based on the ring closing metathesis (RCM) reaction[5] of

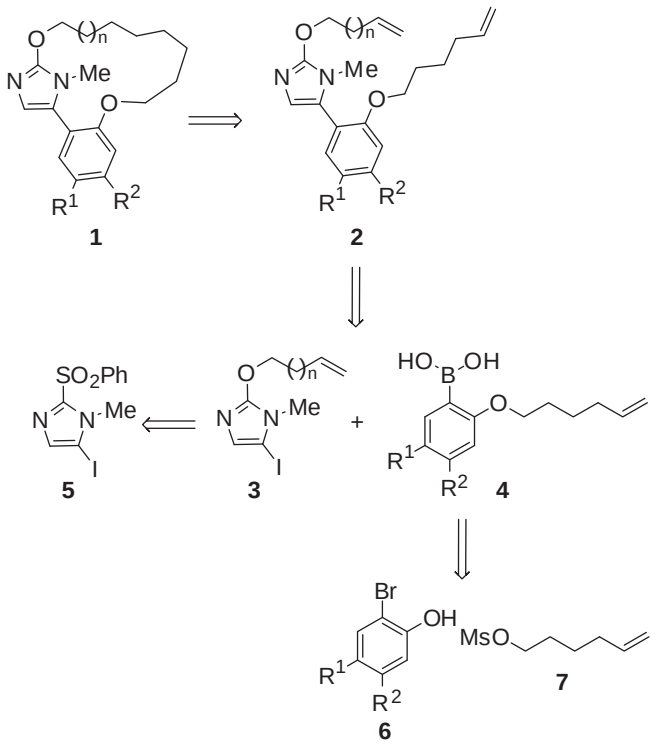

Figure 1. Designed strategy for the synthesis of imidazole-containing macrocycles **1**.

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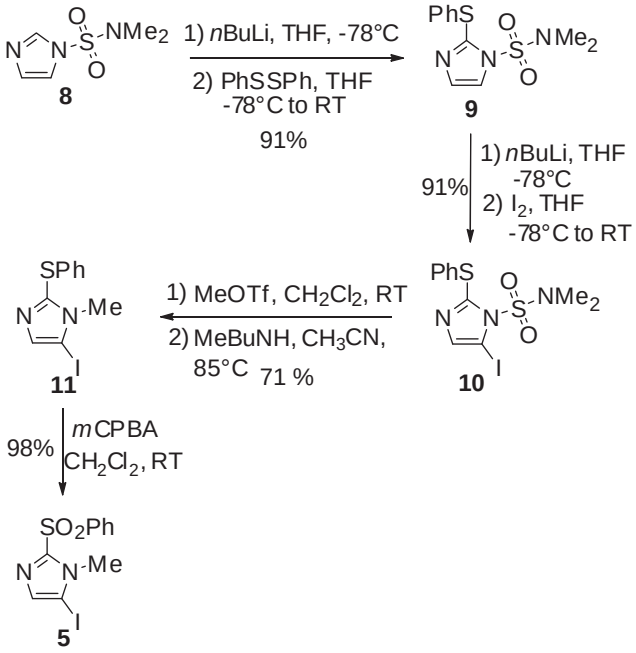


Scheme 1. Synthesis of key intermediate **5**.

1,2,5-trisubstituted imidazoles **2**. These intermediates could be obtained by successive functionalization of imidazole **5**: aromatic nucleophilic substitution (S_NAr) will allow placing the first alkene chain in position 2 while substitution at carbon 5 by Suzuki coupling will provide diene substrate **2**. In order to explore the influence of aryl substitution on biological activities, different aryl boronic acids have been envisaged (variations on R^1 and R^2). The size of the macrocycle has also been varied (n=1 or 3).

2. Results and discussion

2.1. Synthesis

The first step of our synthesis is the preparation of intermediate **5**. We have recently reported a divergent and regioselective method for the synthesis of 1,2,4- and 1,2,5-trisubstituted imidazoles.[6] Application of this methodology to imidazole **5** allows its preparation, on gram-scale, in four steps with a good overall yield (58%) (Scheme 1). This intermediate in hand (it can be stored for months), we subjected it to further functionalization in positions 2 and 5.

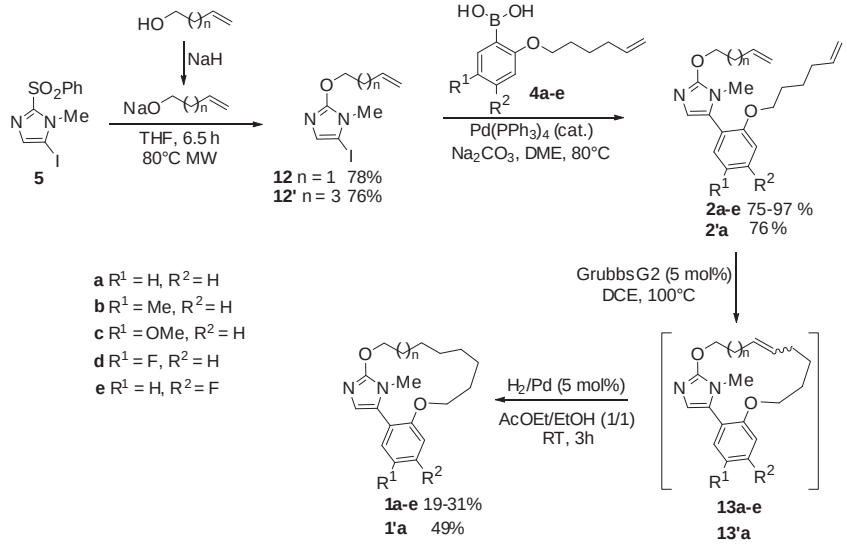
Placing of the first alkene chain at position 2 of the imidazole could be done by aromatic nucleophilic substitution of the phenyl sulfone group by an alkenoxide (Scheme 2). Under classical heating bath conditions the reaction necessitated extended reaction times (>24 h) and led to low yields (<50%) whatever the nature of the counterion (Na^+, K^+, Li^+). However, we found that microwave heating allows high conversion after only 6.5 h.[7] In order to investigate the influence of the size of the macrocycle, the reaction was performed with 5-buten-1-ol and 7-hexen-1-ol, leading to **12** and **12′** in 78% and 76% yield, respectively. The next step in our strategy is the substitution of the iodo atom by an aryl group using a Suzuki coupling.

In order to explore substituent effects on the biological activities, a variety of boronic acids were prepared (variations on R^1 and R^2). These latter were easily obtained from corresponding (commercially available) substituted bromophenols according to a two steps procedure (Scheme 3). In the first step, alkylation of phenols allows introducing the alkene chain (**6**→**14**). Then, the obtained ethers are subjected to a halogen–metal exchange and added to triisopropylborate to yield, after hydrolysis, the corresponding boronic acids (**4a**–**e**).[8]

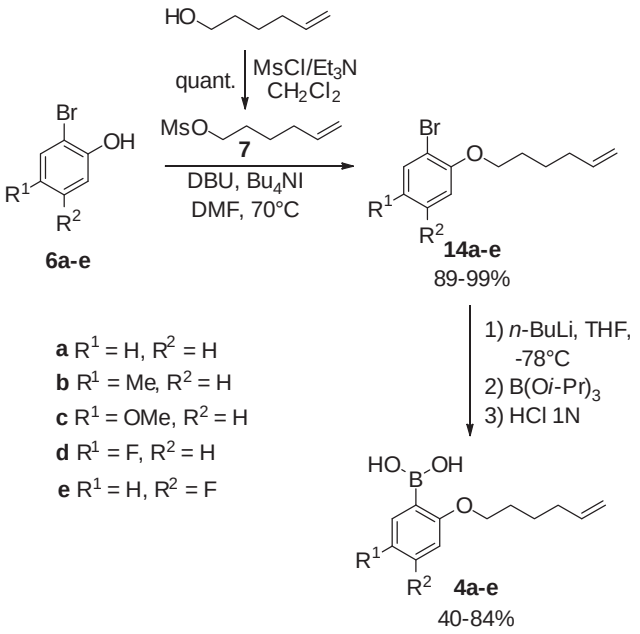
The Suzuki coupling between the synthesized boronic acids (**4a**–**e**) and 5-iodo-1*H*-imidazoles **12** and **12′**, under microwave heating, leads to the desired 1,2,5-trisubstituted imidazoles **2a**–**e** and **2′a** in good yields (see Scheme 2).[9]

These dienes (**2a**–**e** and **2′a**) were then subjected to the last key step of our synthesis, the RCM reaction. Under heating in the presence of second generation Grubbs catalyst, the dienes furnish macrocycles **13a**–**e** and **13′a** as ca. 50/50 mixtures of *E* and *Z* isomers (determined by ^{1}H NMR) (see Scheme 2).[10] Alkenes were not isolated but directly hydrogenated to provide macrocycles **1a**–**e**. The moderate yields (19–49% over the two steps) are mainly due to low conversion in the RCM reaction. All our attempts to improve this step by using other catalysts (Grubbs first generation, Hoveyda, etc.) or additives, such as Ti(O*i*-Pr)$_4$, were unsuccessful.

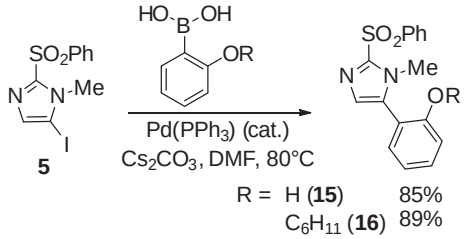
Thus, the designed aryl imidazole-containing macrocycles (**1a**–**e** and **1′a**) were synthesized in eight steps (longest linear sequence) in 8–16% overall yield. To investigate the importance of the C2 and *ortho* aryl substitution on the biological activity, we have also synthesized compounds **15** and **16** (Scheme 4).



Scheme 2. Synthesis of macrocycles **1a**–**e**.



Scheme 3. Synthesis of boronic acids **4a**–**e**.



Scheme 4. Synthesis of **14** and **15**.

2.2. Biological evaluation

In order to optimally assess the 'biological diversity' of these early discovery compounds, we then submitted compound **1a** to the CEREP's Diversity Profile (CEREP, Paris, France). The diversity profile of compound **1a** has been evaluated by performing test binding against a panel of 71 receptors and 16 enzymes (see Experimental section). The obtained data showed that four targets were hinted by this compound: adenosine A_3 adenosine (*h*) receptor (A(3)AR), dopamine D_1 (*h*) receptor (DA D1), Cl^- channel (GABA-gated), and choline transporter (*h*) CHT1. These results suggest a possible activity and application of this compound in the central nervous system (CNS) and for inflammatory affection, as briefly summarized below.

Adenosine receptors (ARs) are major targets of caffeine and theophylline. Adenosine has potent effects on both the cardiovascular and immune systems. Thus, A(3)AR agonists may be a new family of drugs to be developed as potent inhibitors of autoimmune-inflammatory diseases.[11]

Dopamine is thought to play a role in such a diverse array of processes as motor control, neuroendocrine and cardiovascular regulation as well as in regulation of cognition, emotion and reward.[12–15] Although the first DA D1 receptor selective ligand was introduced more than two decades ago, clinically useful D1 receptor selective ligands are rare.

$GABA_A$ receptors are chloride ion channels that mediate fast synaptic transmission. $GABA_A$ receptors are the major inhibitory neurotransmitter receptors in the brain and the site of action of many clinically important drugs.[16] Drugs that enhance synaptic gamma-aminobutyric acid (GABA)ergic neurotransmission are widely utilized in the clinical setting.

Choline plays an important role in the synthesis of the membrane phospholipid components of the cell membranes. Abnormal choline transport and metabolism have been implicated in a number of neurodegenerative disorders such as Alzheimer's and Parkinson's disease.[17,18]

In summary, these four hinted targets present sustained pharmacological interest and therefore new compounds from this series could generate novel lead compounds by means of pursuing of the methodological approach we have initiated here.

Thus, all the synthesized macrocycles, as well as several key intermediates in their synthesis, were submitted to specific binding analysis on these four targets (Table 1). Interestingly, these assays show that most of our compounds conserved the highly binding activity toward these receptors. Results are given in percentages of inhibition of control specific binding.

Table 1
Specific binding tests (concentration=10 μM)

Compound	% Inhibition of control specific binding			
	Adenosine A_3 (*h*) receptor	Dopamine receptor D_1 (*h*)	Cl-channel (GABA-gated)	Choline transporter (*h*) (CHT1)
5	27	−11	2	0
15	52	23	3	−6
16	96	55	80	28
2a	93	84	86	16
2b	96	93	63	75
2c	96	54	79	63
2d	94	51	58	65
2e	96	88	72	73
2′a	96	87	74	81
13a	65	70	32	38
1a	84	69	52	52
1b	94	97	67	87
1c	73	56	50	46
1d	95	90	49	47
1e	88	80	54	43
1′a	99	99	73	98

The panel of compounds tested enabled us to draw some very preliminary hypotheses. First, the binding affinity patterns of **5**, **14**, and **15** (as compared to **1a** and **2a**) suggests the importance of the 5-aryl and, to a lower extent, 2-alkoxy substituents in the biological activity.

A careful analysis of results for **1a**–**e** reveals the rather low influence of aryl substitution (R^1, R^2) on the binding properties of the macrocycles toward A_3 (*h*) receptor and Cl^- channel (GABA-gated). For the two other targets, dopamine D_1 (*h*) receptor and choline transporter (*h*) CHT1, significant variations are observed with the methyl substituted macrocycle (**1b**) displaying the best activities (better than its non-substituted analog **1a**).

The influence of macrocyclization can also be investigated by comparing binding properties of **1a**–**e** and **2a**–**e**. This analysis shows that formation of the 15-membered macrocycle (n=1) has no, or a negative, effect on the binding activity toward A(3)AR, DA D1, and Cl^- channel (GABA-gated).[19] This slight decrease in binding activities can probably be accounted for by the lower flexibility of macrocyclic compounds. Indeed, an increase in binding activity is observed by reduction of the double bond (**13a** to **1a**). Interestingly, for choline transporter target, the opposite trend is observed in the case of non-substituted (**1a**/**2a**) and 4-methylated (**1b**/**2b**) derivatives: in these cases, binding activity is significantly increased by macrocyclization. For the three other substrates (**1c**–**e**/**2c**–**e**), the activity toward choline transporter target decreases with cyclization.

The importance of flexibility is further illustrated by the increase in biological activities observed with the enlargement of the size of the macrocycle (n=1 (**1a**) to n=3 (**1′a**)). For 17-membered macrocycle, cyclization is found to be beneficial for binding affinity toward the four targets; macrocycle **1′a** displaying the higher binding activity toward the four identified targets among all synthesized compounds.

Given the high binding affinity displayed by the macrocycles and the pharmacological interest of the four hinted targets, we went further and determined the IC_{50} values for the more potent compound (**1′a**) as well as for the corresponding non-cyclized product (**2′a**) (Table 2). The IC_{50} values for these compounds confirm their high binding activities and highlight the interest presented by this novel family of compounds. A comparison of the IC_{50} values obtained for **1′a** and **2′a** corroborates also the beneficial character of the macrocyclisation on the binding affinity.

Table 2
IC_{50} values for **2′a** and **1′a**

Compound	IC_{50} (μM)[a]			
	Adenosine A_3 (h) receptor	Dopamine receptor D_1 (h)	Cl-channel (GABA-gated)	Choline transporter (h) (CHT1)
2′a	1.2	6.5	7.3	3.3
1′a	0.85	1.1	7.2	3.2

[a] Each experiment was run in duplicate and the values shown are the average of the two (see Experimental section for full details).

3. Conclusion

In conclusion, we have designed and synthesized a series of novel 5-aryl imidazole-containing macrocycles. The first part of our synthesis makes use of the method we recently developed for the regioselective synthesis of 1,2,5-trisubstituted imidazoles, which allows preparing **5** on large scale. Our strategy for the construction of the macrocycle is based on a three steps sequence: S_NAr, Suzuki coupling, and RCM reaction. This method allowed preparing a variety of novel macrocyclic substrates in eight steps in good overall yields.

Biological evaluation of all synthesized macrocycles, as well as of key intermediates in their synthesis, has been performed. These assays revealed that synthesized 5-aryl imidazole-containing macrocycles display significant binding activity toward A_3 adenosine (h) receptor, dopamine D_1 (h) receptor, Cl^- channel (GABA-gated), and choline transporter (h) CHT1. These receptors are important actors in the CNS and inflammation and their ligands could play major roles in the treatments of diverse neurological and inflammatory disorders.

4. Experimental section

4.1. Synthesis

General procedures and key spectroscopic data are reported below for the non-substituted (R^1, R^2=H) macrocycle (**1a**) with full data, for all other derivatives, in the Supplementary data. Purity of all tested compounds has been determined by HPLC. Purity is >98%, unless mentioned otherwise.

Synthesis and characterization of **5** and **12** were previously reported.[6]

*4.1.1. General procedure for S_NAr (**5**→**12**).* Alcohol (5 equiv) was added to a solution of sodium hydride (383 mg of a 60 wt% suspension in mineral oil, 9.58 mmol, 4.6 equiv) in dry THF (7 mL), under argon. The solution was stirred until no more gas evolution was observed and then added to a solution of 1-methyl-5-iodo-2-phenylsulfonylimidazole (**5**, 725 mg, 2.08 mmol, 1 equiv) in dry THF (35 mL). The reaction mixture was transferred into a sealed tube and heated under microwave for 6.5 h at 80 °C. Ethyl acetate (50 mL) and 10% sodium hydroxide solution (30 mL) were successively added. The organic phase was further washed twice with a 10% solution of sodium hydroxide (30 mL). Combined aqueous phases were extracted with ethyl acetate (20 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by flash chromatography over silica.

*4.1.2. General procedure for alkylation of bromophenols (**6**→**14**).* To a solution of 2-bromophenol (0.75 mL, 1.12 g, 6.47 mmol) in anhydrous DMF (10 mL) were successively added, under argon, 1,8-diazabicyclo[5.4.0]undec-7-ene (1.48 mL, 1.51 g, 9.70 mmol, 1.5 equiv), hex-5-en-1-yl methanesulfonate[20] (**7**, 1.5 g, 8.42 mmol, 1.3 equiv), and tetra-*n*-butylammonium iodide (73 mg, 0.20 mmol, 0.03 equiv). The mixture was heated at 70 °C for 7 h and then stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue was taken up in 100 mL of ether. Water (15 mL) and 5 mL of HCl 1 M were successively added. The phases were separated and the organic phase was washed with water (2×20 ml). The combined aqueous phases were extracted with ether (2×30 ml). The organic phases were combined, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by flash chromatography using cyclohexane/ethyl acetate 95/5 as eluent.

*4.1.3. Characterization of 2-bromo-1-(hex-5-en-1-yloxy)benzene (**11a**).* Colorless oil; yield: 1.47 g (91%); ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.53 (dd, J=1.6, 7.9 Hz, 1H), 7.24 (ddd, J=1.6, 7.4, 8.3 Hz, 1H), 6.88 (dd, J=1.3, 8.3 Hz, 1H), 6.82 (dt, J=1.4, 7.6 Hz, 1H), 5.89–5.78 (m, 1H), 5.01–4.99 (m, 2H), 4.03 (t, J=6.4 Hz, 2H), 2.20–2.10 (m, 2H), 1.90–1.80 (m, 2H), 1.70–1.60 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=155.6, 138.7, 133.5, 128.5, 121.8, 114.9, 113.3, 112.4, 69.1, 33.5, 28.7, 25.4 ppm; IR: ν=3074, 2941, 2858, 1535, 1500, 1491, 1369, 1244, 1142, 912 cm^{-1}; MS (CI) m/z: 254; HRMS calcd for $C_{12}H_{15}O^{79}Br$: 254.0301, found: 254.0314.

*4.1.4. General procedure for the synthesis of boronic acids (**14**→**4**).* In a flame-dried two-neck round-bottomed flask was introduced, under argon, 2-(hex-5-enyloxy)bromobenzene (**14a**, 1.3 g, 5.1 mmol, 1 equiv) and 20 mL of anhydrous THF. The solution was then cooled to −78 °C and ^{n}BuLi 2.5 M in hexanes (2.65 mL, 6.63 mmol, 1.3 equiv) was added dropwise. The resulting solution was stirred for 1 h at −78 °C and triisopropylborate (2.4 mL, 1.96 g, 10.02 mmol, 2.0 equiv) was then added dropwise at −78 °C. The mixture was allowed to warm up slowly to room temperature without removing the cooling bath and stirred overnight. Ethyl acetate (10 mL) was added to the suspension and hydrogen chloride 1 N was added until pH=1 (about 7 mL). The two phases were separated and the aqueous phase was extracted with ethyl acetate (3×20 mL). The organic phases were combined and washed with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The pale yellow oil was purified by flash chromatography with cyclohexane/ethyl acetate 85/15 as eluent.

*4.1.5. Characterization of 2-(hex-5-en-1-yloxy)phenylboronic acid (**4a**).* Colorless oil; yield: 945 mg (84%); ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.87 (dd, J=7.3, 1.7 Hz, 1H), 7.45–7.40 (m, 1H), 7.03 (t, J=7.3 Hz, 1H), 6.90 (d, J=8.3 Hz, 1H), 6.04 (s, 2H), 5.89–5.75 (m, 1H), 5.08–4.98 (m, 2H), 4.09 (t, J=6.6 Hz, 2H), 2.19–211 (m, 2H), 1.90–1.80 (m, 2H), 1.65–1.55 ppm (m, 2H); ^{13}C NMR (300 MHz, $CDCl_3$): δ=164.1, 138.2, 137.0, 132.9, 121.3, 115.3, 110.9, 68.3, 33.4, 28.7, 25.4 ppm; IR: ν=3409, 3074, 2926, 2854, 1641, 1599, 1576,

1487, 1448, 1340, 1225, 910 cm^{-1}; MS (CI) *m/z*: 220, 219; HRMS calcd for $C_{12}H_{17}BO_3$: 220.1265, found: 220.1265.

*4.1.6. General procedure for Suzuki coupling (**12**→**2**).* Tetrakis(triphenylphosphino)palladium (24.9 mg, 0.03 equiv) and imidazole **12** (200 mg, 0.719 mmol, 1 equiv) in dimethoxyethane (2 mL) were introduced in a microwave tube. Boronic acid (1.1 equiv) in dimethoxyethane (2 mL), water (2 mL), and an aqueous solution of sodium carbonate (20%, 1.2 mL, 3 equiv) were successively added. The resulting mixture was then degassed for 30 min by means of a flow of argon and placed in a microwaves oven at 105 °C and 200 W for 1 h. Ethyl acetate (50 mL) and water (25 mL) were added. The phases were separated and the aqueous phases were extracted with ethyl acetate (25 mL). The organic phases were combined, washed with a saturated aqueous solution of NaCl (25 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by flash chromatography with cyclohexane/ethyl acetate 85/15 as eluent.

*4.1.7. Characterization of 2-(but-3-en-yloxy)-5-[2-(hex-5-en-1-yloxy)phenyl]-1-methyl-1H-imidazole (**2a**).* Yellow oil; yield: 176 mg (75%); Purity=91%; ^1H NMR (300 MHz, $CDCl_3$): δ=7.33 (td, *J*=8.2, 1.5 Hz, 1H), 7.25 (dd, *J*=7.5, 1.5 Hz, 1H), 6.98 (dt, *J*=7.4, 1.5 Hz, 1H), 6.94 (d, *J*=8.2 Hz, 1H), 6.62 (s, 1H), 5.97–5.71 (m, 2H); 5.20–4.94 (m, 4H); 4.46 (t, *J*=6.7 Hz, 2H), 3.97 (t, *J*=6.6 Hz, 2H), 3.24 (s, 3H), 2.61–2.58 (m, 2H), 2.10–2.03 (m, 2H), 1.79–1.70 (m, 2H), 1.52–1.42 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=156.9, 153.3, 138.5, 134.4, 132.1, 129.6, 126.6, 121.7, 120.8, 120.0, 117.2, 114.9, 112.2, 68.45, 68.41, 33.7, 33.4, 29.5, 28.7, 25.4 ppm; IR: ν=2924, 2860, 1535, 1500, 1370, 1248, 1030, 735 cm^{-1}; MS (APCI) *m/z*: 327; HRMS calcd for $C_{20}H_{27}N_2O_2$: 327.2073, found: 327.2064.

*4.1.8. General procedure for RCM reaction (**2**→**13**).* Diene **2** (225 mg, 1 equiv) and 1,2-dichloroeethane (140 ml) were introduced in a flask under argon. The mixture was heated at reflux and Grubbs catalyst (second generation) (26.8 mg, 0.315 mmol, 0.05 equiv) was added. After 24 h, a second fraction of Grubbs catalyst (13.4 mg, 0.158 mmol, 0.025 equiv) was added. After 24 h, the mixture was cooled down to room temperature and potassium isocyanoacetate[20] (39 mg) was added. After 1 h of stirring, the solvent was evaporated. The crude product was purified by flash chromatography over silica with cyclohexane/ethyl acetate 75/25 as eluent.

*4.1.9. General procedure for hydrogenation (**13**→**1**).* Pd/C 10% catalyst (24 mg, 0.0222 mmol, 0.05 equiv) was added to a solution of imidazole **13** (145 mg, 0.442 mmol, 1 equiv) in 20 mL of a 1:1 mixture of ethanol and ethyl acetate. The resulting suspension was degassed by means of a flow of argon for 30 min and then stirred under hydrogen atmosphere for 3 h at room temperature. The suspension was filtered through a Celite pad, washed with ethyl acetate and evaporated under reduced pressure. The crude product was purified by flash chromatography using cyclohexane/ethyl acetate 70/30 as eluent.

*4.1.10. Characterization of 21-methyl-8,17-dioxa-19,21-diazatricyclo[16.2.1.0^{2,7}]henicosa-1(20),2,4,6,18-pentaene (**1a**).* Colorless oil; yield: 23.2 mg were obtained from 40 mg of **13a** (19% over the two steps); Purity=84%; ^1H NMR (300 MHz, $CDCl_3$): δ=7.33 (td, *J*=8.2, 1.5 Hz, 1H), 7.25 (dd, *J*=7.5, 1.5 Hz, 1H), 6.98 (dt, *J*=7.4, 1.5 Hz, 1H), 6.94 (d, *J*=8.2 Hz, 1H), 6.62 (s, 1H), 5.97–5.71 (m, 2H); 5.20–4.94 (m, 4H); 4.46 (t, *J*=6.7 Hz, 2H), 3.97 (t, *J*=6.6 Hz, 2H), 3.24 (s, 3H), 2.61–2.58 (m, 2H), 2.10–2.03 (m, 2H), 1.79–1.70 (m, 2H), 1.52–1.42 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=156.9, 153.3, 138.5, 134.4, 132.1, 129.6, 126.6, 121.7, 120.8, 120.0, 117.2, 114.9, 112.2, 68.45, 68.41, 33.7, 33.4, 29.5, 28.7, 25.4 ppm; IR (on fumaric salt): ν=2927, 2854, 1715, 1531, 1452, 1269, 1248, 1165, 1072, 754 cm^{-1}; MS (ESI) *m/z*: 301; HRMS calcd for $C_{18}H_{25}N_2O_2$: 301.1916, found: 301.1919.

4.1.11. General procedure for formation of fumaric salts. The imidazole compound (1 equiv) was dissolved in ethanol (0.1 M). A solution of fumaric acid (1 equiv) in ethanol (0.05 M) was added. The mixture was stirred overnight and then concentrated under reduced pressure. The correct 1:1 ratio was checked by ^1H NMR.

4.2. Biological assays

Assays have been performed on fumaric salts (see above). Purity of all tested compounds has been determined by HPLC. Purity is >98%, unless mentioned otherwise. The Diversity Profile was performed with 10 μM of compound 1a by CEREP (France). The Diversity profile is composed of 71 receptor binding and 16 enzyme assays. The 87 assay panel is broadly defined with roughly an equal number of selective, central and peripheral therapeutically relevant targets. The lists of all targets and their reference ligands used as controls, as well as experimental conditions, could be obtained at CEREP's web site.[21] Following the analysis of the data obtained in the first set of results, all synthesized compounds were analyzed (by CEREP, France) for their binding activity toward four targets hinted by **1a**. All compounds (test and reference) were analyzed in duplicate.

The specific ligand binding to the receptors is defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabeled ligand. The results are expressed as a percent of control specific binding ((measured specific binding/control specific binding)×100) and as a percent inhibition of control specific binding (100−((measured specific binding/control specific binding)×100)) obtained in the presence of tested compound.

The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) and Hill coefficients (n_H) were determined by non-linear regression analysis of the competition curves generated with mean replicate values using Hill equation curve fitting $Y=D+[(A-D)/(1+(C/C_{50})^{nH})]$, where Y=specific binding, D=minimum specific binding, A=maximum specific binding, C=compound concentration, $C_{50}=IC_{50}$, and n_H=slope factor. This analysis was performed using a software developed at CEREP (Hill software) and validated by comparison with data generated by the commercial software SigmaPlot® 4.0 for Windows® (© 1997 by SPSS Inc.).

The inhibition constants (K_i) were calculated using the Cheng Prusoff equation($K_i=IC_{50}/(1+(L/K_D))$, where *L*=concentration of radioligand in the assay, and K_D=affinity of the radioligand for the receptor).

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Supplementary data

Full spectroscopic data, for all derivatives, are included as Supplementary data. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.04.070.

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