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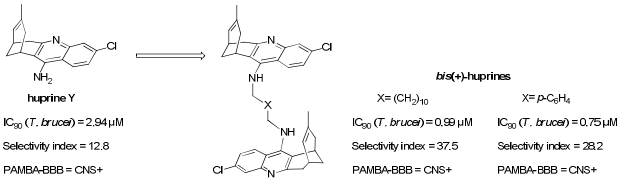

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Synthesis and antiprotozoal activity of oligomethylene- and *p*-phenylene-*bis*(methylene)-linked *bis*(+)-huprines

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ABSTRACT

We have synthesized a series of dimers of (+)-(7*R*,11*R*)-huprine Y and evaluated their activity against *Trypanosoma brucei*, *Plasmodium falciparum*, rat myoblast L6 cells and human acetylcholinesterase (hAChE), and their brain permeability. Most dimers have more potent and selective trypanocidal activity than huprine Y and are brain permeable, but they are devoid of antimalarial activity and remain active against hAChE. Lead optimization will focus on identifying compounds with a more favourable trypanocidal/anticholinesterase activity ratio.

In the last few years, implementation of prevention and control measures has significantly reduced the burden of tropical protozoan diseases such as human African trypanosomiasis (HAT or sleeping sickness) and malaria. However, approximately 70 million people remain at risk of HAT, and in the case of malaria, half of the world's population live in countries where the disease is endemic. Thus, these parasitic infections continue to pose a serious health threat, especially in developing regions.[1,2]

The causative agents of HAT and malaria are the single-celled parasites *Trypanosoma brucei gambiense* or *T. brucei rhodesiense*, and several species of the genus *Plasmodium*, amongst which, *P. falciparum* is the most common and deadly. The parasites are transmitted through the bite of infected insects, namely *Glossina* flies (tsetse flies) for HAT and *Anopheles* mosquitoes for malaria.

In HAT, following an initial hemolymphatic phase, parasites can cross the blood–brain barrier (BBB) and infect the central nervous system (CNS), leading to severe neurological symptoms. Without treatment, death is inevitable when the disease has reached this late stage. In malaria, the parasites multiply initially ~~in the liver~~, and then in the bloodstream. In severe cases, they can become sequestered within brain capillaries, particularly in children, causing the so-called cerebral malaria, frequently with fatal consequences.

Current options to reduce the burden of HAT and malaria are far from ideal.[3-5] There is no licensed vaccine for either infection, with vector control and public health measures being the main means of prevention. Currently registered drugs are problematic, with toxicity and resistance being major problems. For example, although five drugs have been approved for the treatment of HAT (pentamidine, suramin, melarsoprol, nifurtimox and eflornithine), their activity can be stage and/or species specific, they display a range of toxic side effects, and require strict and complicated parenteral administration regimens.[6] This type of specialized infrastructure is often unavailable in the poor rural settings where HAT is endemic. Drug resistance continues to emerge and undermine clinical effectiveness. Increased resistance has been observed for the trypanocidal agent melarsoprol. In the case of malaria, chloroquine is no longer widely effective and rising resistance against the current front line drug artemisinin is a potential threat to global health. Overall, there is an acute need to

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develop novel drugs for HAT and malaria that can circumvent the limitations of existing therapies.

Several approaches have been proposed to speed up the antiprotozoal drug pipeline. These include high-throughput screening of large compound libraries, new strategies to functionally validate novel druggable targets involved in key steps of the parasite life-cycle,[7-10] or the simultaneous inhibition of two or more key biological targets with combination therapies or multitarget-directed ligands.[11-13] Increasingly, the search for novel antiprotozoal agents also involves the repositioning of existing drugs registered for other applications[14] or the synthesis of new chemical entities endowed with antiprotozoal activity.[15-17]

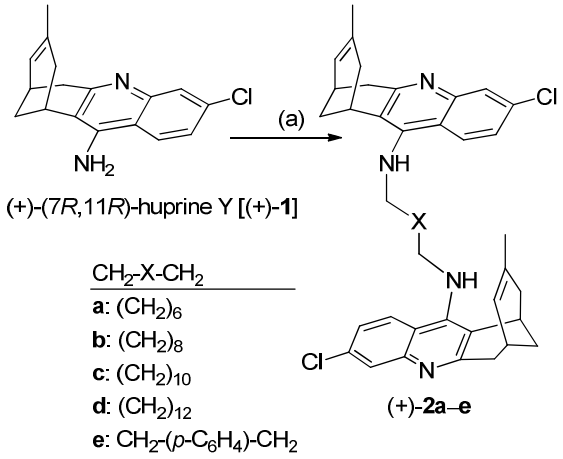
In recent years, new compounds bearing the 4-amino-7-chloroquinoline core of chloroquine, or other aminoquinoline moieties, have been assessed as novel trypanocidal or antimalarial agents, or as dual agents endowed with both activities.[18-21] The development of single molecules that have potency against different protozoan diseases (such as HAT and malaria) has been regarded as an feasible approach, with potential economic savings.[22] We recently reported that the aminoquinoline derivatives huprines, a structural class initially developed as inhibitors of the enzyme acetylcholinesterase, are moderately effective and selective trypanocidal agents, with some also being active against a chloroquine-resistant strain of *P. falciparum*.[23,24] In particular, the 4-amino-7-chloroquinoline derivative **1** (huprine Y, Scheme 1) exhibited the lowest IC_{50} value of the series against *T. brucei* (IC_{50} = 0.61 μM; IC_{90} = 2.94 μM), with one of the best selectivity indices over rat myoblast L6 cells (SI = 13) among the entire set of tested huprines.[23]

Molecular dimerization of compounds with known antiprotozoal activity constitutes a strategy that can be used to overcome drug resistance.[25] This approach has proven successful for dimers of 4-aminoquinolines, in which the two constituting units were connected through linkers of different length or containing different functional groups.[26-29]

Here, we report the synthesis of dimers of huprine Y, in which the two huprine moieties have been connected through oligomethylene linkers of different length, or with a *p*-phenylene-*bis*(methylene) tether. To this end, enantiopure (+)-(7*R*,11*R*)-huprine Y [(7*R*,11*R*)-**1**, Scheme 1], the least active enantiomer in terms of acetylcholinesterase (AChE) inhibition activity,[30-32] has been used. The dimeric *bis*(+)-huprines have been tested against cultured bloodstream forms of *T. brucei* and *P. falciparum*, and their cytotoxicity against mammalian cells, inhibitory activity against human AChE, and brain permeability have been assessed.

The synthesis of hexa-, octa-, deca-, and dodeca-methylene linked *bis*-huprines (+)-**2a**–**d** and the *p*-phenylene-*bis*(methylene)-linked *bis*-huprine (+)-**2e** was carried out by reaction of 2 equivalents of (+)-(7*R*,11*R*)-huprine Y with 1 equivalent of the corresponding α,ω-dihaloalkane, using KOH as the base in DMSO at room temperature for three days (Scheme 1). After silica gel column chromatography purification, *bis*(+)-huprines (+)-**2a**–**d** were obtained in moderate yields (21–50% yields, whereas (+)-**2e** was obtained in a lower yield (11%) along with the byproduct resulting from the monoalkylation of (+)-**1** (12% yield).

Bis-huprines (+)-**2a**–**e** were converted into the corresponding dihydrochlorides for their chemical characterization (specific rotation, melting point, IR, ^{1}H and ^{13}C NMR, HRMS, and elemental analysis) and biological profiling.[33]



Scheme 1. Reagents and conditions: (a) (+)-**1** (2 equiv.), KOH, DMSO, 2 h; then, α,ω-dihaloalkane (1 equiv.) in DMSO, rt, 3 days.

Bis-huprines (+)-**2a**–**e** were first tested *in vitro* against cultured bloodstream forms of *T. brucei*. All of the *bis*-huprines exhibited IC_{50} values in the range 0.50–0.89 μM. Their IC_{90} values of around 1 μM (0.73–1.09 μM) (Table 1), were significantly lower than the parent huprine Y (IC_{90} = 2.94 μM). Given the narrow range of potencies of the different *bis*-huprines, the length of the linker or the presence of a benzene ring within the linker do not seem to have a strong influence on the trypanocidal activity of *bis*(+)-huprines. Thus, the increased trypanocidal potency of *bis*-huprines, relative to huprine Y, might be ascribed to the dimerization strategy, even though the mechanisms responsible for inhibition of trypanosome growth or for the enhanced activity are not known.

Bis-huprines (+)-**2a**–**e** were also evaluated against the chloroquine-resistant K1 strain of *P. falciparum*. Even though some huprines have been reported to exhibit moderately potent antiplasmodial properties,[23] huprine Y did not exhibit significant activity (IC_{50} > 10 μM). Huprine Y bears the 4-amino-7-chloroquinoline moiety, thought to be an antimalarial pharmacophore responsible for inhibition of heme dimerization.[20,34] Since dimerization of other 4-aminoquinoline compounds increased antiplasmodial potency and/or overcame the chloroquine resistance mechanism,[26-29] we hypothesized that dimerization of huprine Y to *bis*-huprines (+)-**2a**–**e** might also enhance activity. However, no noticeably increased antiplasmodial potency was observed for the dimeric compounds, which exhibited IC_{50} values > 5 μg/mL (i.e. > 6–7 μM), much higher than that of artemisinin (IC_{50} = 91 nM) used in this assay as a positive control. The improvement of potency against chloroquine resistant strains of *P. falciparum* of other *bis*(4-aminoquinoline) derivatives relative to the corresponding monomeric compounds has been ascribed mainly to the doubling of the number of protonatable nitrogen atoms in the dimers, which might lead to more efficient trapping in the acidic digestive vacuole of the parasite and prevention of heme polymerization.[25,29] The failure of *bis*-huprines to show antiplasmodial activity might be indicative of the fact that these compounds cannot hit the biological target of chloroquine and other 4-aminoquinoline derivatives despite their structural similarity. Indeed, we have recently found that the parent huprine Y, unlike chloroquine, shows no inhibition of β-haematin formation, whereas several huprine analogues that possess antiplasmodial activity are effective inhibitors of β-haematin formation (unpublished results).

Table 1
Trypanocidal, cytotoxic and hAChE inhibitory activity of *bis*-huprines (+)-**2a**–**e**[a]

Compd	*T. brucei* IC_{50} µM	*T. brucei* IC_{90} µM	L6 cells IC_{50} µM	SI[b]	hAChE IC_{50} nM
(+)-**2a**	0.89 ± 0.02	1.09 ± 0.01	1.61 ± 0.07	1.8	192 ± 18
(+)-**2b**	0.52 ± 0.01	0.74 ± 0.02	4.92 ± 0.15	9.5	72.5 ± 8.3
(+)-**2c**	0.50 ± 0.01	0.73 ± 0.01	7.71 ± 0.70	15.4	17.5 ± 3.8
(+)-**2d**	0.76 ± 0.03	0.99 ± 0.05	28.5 ± 2.9	37.5	431 ± 22
(+)-**2e**	0.57 ± 0.02	0.75 ± 0.02	16.1 ± 0.3	28.2	nd[c]
1	0.61 ± 0.03[d]	2.94 ± 0.20[d]	7.80 ± 0.47[d]	12.8	0.61 ± 0.03[e]

[a] *In vitro* activity against bloodstream form of *T. brucei* (pH 7.4) and rat myoblast L6 cells expressed at the concentration that inhibited growth by 50% (IC_{50}) and 90% (IC_{90}, for trypanocidal activity). Data are the mean of triplicate experiments ± SEM.

[b] SI: Selectivity index is the ratio of cytotoxic to trypanocidal IC_{50} values.

[c] Not determined.

[d] Taken from ref. 23.

[e] Taken from ref 35.

The cytotoxicity of *bis*-huprines was assessed *in vitro* using rat skeletal myoblast L6 cells. These compounds displayed a modest toxicity against the mammalian cells, exhibiting IC_{50} values in the range 1.6–28.5 µM (Table 1). A clear structure-cytotoxicity trend was found, with cytotoxicity decreasing with increased tether length and with the presence of a benzene ring within the linker. Thus, the dodecamethylene-linked *bis*-huprine (+)-**2d** was found to be 18-, 6-, and 4-fold less cytotoxic than the hexa-, octa-, and deca-methylene counterparts (+)-**2a**, (+)-**2b**, and (+)-**2c**, respectively, whereas the *p*-phenylene-*bis*(methylene)-linked *bis*-huprine (+)-**2e** was 10-fold less cytotoxic than the oligomethylene-linked *bis*-huprine with a similar tether length, (+)-**2a.** Thus, the longest homologue (+)-**2d** and the *p*-phenylene-*bis*(methylene)-linked *bis*-huprine (+)-**2e** displayed the highest selectivity indices for trypanocidal over cytotoxic activity (SI = 37.5 and 28.2, respectively).

Bis-huprines possess two units of (+)-huprine Y, which is the distomer for AChE inhibition. Even though the dextrorotatory enantiomers of huprine Y and some hybrid derivatives are less potent AChE inhibitors than the racemic mixtures and the levorotatory counterparts, they typically exhibit activities in the nanomolar to low micromolar range.[32,35] To assess potential toxicity issues arising from AChE inhibition by *bis*-huprines, their inhibitory activity against human recombinant AChE (hAChE) was evaluated by the method of Ellman.[36] As expected, *bis*-huprines (+)-**2a**-**d** are 30-700-fold less potent hAChE inhibitors than racemic huprine Y, but they exhibit more potent anticholinesterase than trypanocidal activity (2-29-fold), with IC_{50} values for hAChE inhibition in the two-three digit nanomolar range (Table 1). The least active compound against hAChE is (+)-**2d**, which is the dimer with the best selectivity index for trypanocidal over cytotoxic activity. However, even in this case, its 2-fold higher anticholinesterase activity could still be a concern in terms of further development.

Permeability across the BBB is a necessary condition for drug candidates against HAT that are to be effective against late-stage disease, when the CNS is invaded by the parasite.

Table 2
BBB predicted permeabilities of *bis*-(+)-huprines (+)-**2a**–**e** and parent huprine Y

Compd	P_e (10^{-6} cm s^{-1})[a]	Prediction
(+)-**2a**	11.1 ± 0.3	CNS+
(+)-**2b**	13.9 ± 1.0	CNS+
(+)-**2c**	8.7 ± 1.5	CNS+
(+)-**2d**	17.4 ± 0.7	CNS+
(+)-**2e**	8.3 ± 0.6	CNS+
huprine Y, **1**	23.8 ± 2.7[b]	CNS+

[a] Values are expressed as mean ± SD of three independent experiments.

[b] Taken from ref. 32.

The BBB permeability of *bis*-huprines (+)-**2a**–**e** was assessed *in vitro* through the widely used parallel artificial membrane permeability assay (PAMPA-BBB), using a lipid extract of porcine brain as the artificial membrane.[37] Assay validation was performed by comparing the experimentally observed permeabilities [P_e (exp)] of fourteen marketed drugs with the permeabilities reported in the literature [P_e (lit)], which provided a good linear correlation: P_e (exp) = 1.4974 P_e (lit) – 0.8434 (R^2 = 0.9428). Taking into account this equation and the limits established by Di *et al.*,[35] three ranges of BBB permeation were established: high BBB permeation (CNS+) for those compounds with P_e (10^{-6} cm s^{-1}) > 5.1; low BBB permeation (CNS–) for those compounds with P_e (10^{-6} cm s^{-1}) < 2.1; and uncertain BBB permeation (CNS±) for those compounds with 5.1 > P_e (10^{-6} cm s^{-1}) > 2.1. All the *bis*-huprines exhibited permeabilities clearly above the threshold established for high BBB permeation (Table 2), even though they seem to be less permeable than monomeric huprine Y. This may arise because they are dibasic compounds and will mostly be in the diprotonated form, whereas monomeric huprine, with only one basic nitrogen atom, will be monoprotonated under the assay conditions.

In summary, we report the synthesis of a series of dimeric *bis*(4-aminoquinoline) derivatives, which are composed of two units of (+)-(7*R*,11*R*)-huprine Y connected through oligomethylene linkers of different length or a *p*-phenylene-*bis*(methylene) linker. We also describe the assessment of the different *bis*(+)-huprines on the growth of bloodstream forms of *T. brucei* and *P. falciparum*, and of rat skeletal myoblast L6 cells, as well as their hAChE inhibitory activity and BBB permeability. All of the *bis*(+)-huprines exhibited potent trypanocidal activity, with IC_{50} and IC_{90} values in the submicromolar range. However, they did not exhibit significant antiplasmodial activity, and, conversely, they were found to potently inhibit hAChE. As trypanocidal agents, *bis*(+)-huprines are more potent than monomeric huprine Y and some of them, particularly the dodecamethylene- and *p*-phenylene-*bis*(methylene)-linked dimers (+)-**2d** and (+)-**2e**, are less cytotoxic and, hence, more selective for *T. brucei* over rat L6 cells growth inhibition than huprine Y. All the *bis*(+)-huprines have been predicted to have the ability to cross the BBB, thereby being potentially useful for the treatment of late-stage HAT. Overall, *bis*(+)-huprines (+)-**2d** and (+)-**2e** emerge as interesting lead compounds for further trypanocidal drug development. Future research should focus not only on increasing trypanocidal potency, but decreasing cytotoxicity and AChE inhibitory activity.

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Supplementary data

Supplementary data (synthetic procedures and chemical characterization of *bis*(+)-huprines and biological methods) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/...

References and notes

1. Simarro, P. P.; Cecchi, G.; Franco, J. R.; Paone, M.; Diarra, A.; Ruiz-Postigo, J. A.; Fevre, E. M.; Mattioli, R. C.; Jannin, J. G. *PLoS Negl. Trop. Dis.* **2012**, *6*, e1859.
2. World Health Organization. World Malaria Report 2013.
3. Espuelas, S.; Plano, D.; Nguewa, P.; Font, M.; Palop, J. A.; Irache, J. M.; Sanmartín, C. *Curr. Med. Chem.* **2012**, *19*, 4259.
4. Hu, L.; Patel, A.; Bondada, L.; Yang, S.; Wang, M. Z.; Munde, M.; Wilson, W. D.; Wenzler, T.; Brun, R.; Boykin, D. W. *Bioorg. Med. Chem.* **2013**, *21*, 6732.
5. Faist, J.; Seebacher, W.; Kaiser, M.; Brun, R.; Saf, R.; Weis, R. *Bioorg. Med. Chem.* **2013**, *21*, 4988.
6. Phillips, M. A. *Mol. Microbiol.* **2012**, *86*, 10.
7. Fairlamb, A. H. *Nature* **2012**, *482*, 167.
8. Alsford, S.; Eckert, S.; Baker, N.; Glover, L.; Sanchez-Flores, A.; Leung, K. F.; Turner, D. J.; Field, M. C.; Berriman, M.; Horn, D. *Nature* **2012**, *482*, 232.
9. Gilbert, I. H. *J. Med. Chem.* **2013**, *56*, 7719.
10. Spinks, D.; Shanks, E. J.; Cleghorn, L. A. T.; McElroy, S.; Jones, D.; James, D.; Fairlamb, A. H.; Frearson, J. A.; Wyatt, P. G.; Gilbert, I. H. *ChemMedChem* **2009**, *4*, 2060.
11. Belluti, F.; Uliassi, E.; Veronesi, G.; Bergamini, C.; Kaiser, M.; Brun, R.; Viola, A.; Fato, R.; Michels, P. A. M.; Krauth-Siegel, R. L.; Cavalli, A.; Bolognesi, M. L. *ChemMedChem* **2014**, *9*, 371.
12. Njogu, P. M.; Chibale, K. *Curr. Med. Chem.* **2013**, *20*, 1715.
13. Prati, F.; Uliassi, E.; Bolognesi, M. L. *MedChemComm* **2014**, *5*, 853.
14. Njoroge, M.; Njuguna, N. M.; Mutai, P.; Ongarora, D. S. B.; Smith, P. W.; Chibale, K. *Chem. Rev.* **2014**, in press, DOI: 10.1021/cr500098f.
15. Persch, E.; Bryson, S.; Todoroff, N. K.; Eberle, C.; Thelemann, J.; Dirdjaja, N.; Kaiser, M.; Weber, M.; Derbani, H.; Brun, R.; Schneider, G.; Pai, E. F.; Krauth-Siegel, R. L.; Diederich, F. *ChemMedChem* **2014**, *9*, 1880.
16. Stich, A.; Ponte-Sucre, A.; Holzgrabe, U. *Lancet Infect. Dis.* **2013**, *13*, 733.
17. Ferrins, L.; Rahmani, R.; Baell, J. B. *Future Med. Chem.* **2013**, *5*, 1801.
18. Upadhayaya, R. S.; Dixit, S. S.; Földesi, A.; Chattopadhyaya, J. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2750.
19. Rashad, A. A.; Jones, A. J.; Avery, V. M.; Baell, J.; Keller, P. A. *ACS Med. Chem. Lett.* **2014**, *5*, 496.
20. Musonda, C. C.; Yardley, V.; Carvalho de Souza, R. C.; Ncokazi, K.; Egan, T. J.; Chibale, K. *Org. Biomol. Chem.* **2008**, *6*, 4446.
21. Musonda, C. C.; Gut, J.; Rosenthal, P. J.; Yardley, V.; Carvalho de Souza, R. C.; Chibale, K. *Bioorg. Med. Chem.* **2006**, *14*, 5605.
22. Chibale, K. *Pure Appl. Chem.* **2005**, *77*, 1957.
23. Defaux, J.; Sala, M.; Formosa, X.; Galdeano, C.; Taylor, M. C.; Alobaid, W. A. A.; Kelly, J. M.; Wright, C. W.; Camps, P.; Muñoz-Torrero, D. *Bioorg. Med. Chem.* **2011**, *19*, 1702.
24. Oluwafemi, A. J.; Okanla, E. O.; Camps, P.; Muñoz-Torrero, D.; Mackey, Z. B.; Chiang, P. K.; Seville, S.; Wright, C. W. *Nat. Prod. Commun.* **2009**, *4*, 193.
25. Kaur, K.; Jain, M.; Reddy, R. P.; Jain, R. *Eur. J. Med. Chem.* **2010**, *45*, 3245.
26. Girault, S.; Grellier, P.; Berecibar, A.; Maes, L.; Lemière, P.; Muray, E.; Davioud-Charvet, E.; Sergheraert, C. *J. Med. Chem.* **2001**, *44*, 1658.
27. Ayad, F.; Tilley, L.; Deady, L. W. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2075.
28. Cowman, A. F.; Deady, L. W.; Deharo, E.; Desneves, J.; Tilley, L. *Aust. J. Chem.* **1997**, *50*, 1091.
29. Girault, S.; Grellier, P.; Berecibar, A.; Maes, L.; Mouray, E.; Lemière, P.; Debreu, M.-A.; Davioud-Charvet, E.; Sergheraert, C. *J. Med. Chem.* **2000**, *43*, 2646.
30. Camps, P.; Contreras, J.; Font-Bardia, M.; Morral, J.; Muñoz-Torrero, D.; Solans, X. *Tetrahedron: Asymmetry* **1998**, *9*, 835.
31. Camps, P.; El Achab, R.; Morral, J.; Muñoz-Torrero, D.; Badia, A.; Baños, J. E.; Vivas, N. M.; Barril, X.; Orozco, M.; Luque, F. J. *J. Med. Chem.* **2000**, *43*, 4657.
32. Viayna, E.; Sola, I.; Bartolini, M.; De Simone, A.; Tapia-Rojas, C.; Serrano, F. G.; Sabaté, R.; Juárez-Jiménez, J.; Pérez, B.; Luque, F. J.; Andrisano, V.; Clos, M. V.; Inestrosa, N. C.; Muñoz-Torrero, D. *J. Med. Chem.* **2014**, *57*, 2549.
33. The synthetic procedure for the preparation of *bis*(+)-huprines is exemplified by the synthesis and chemical characterization of (+)-**2d**: (+)-*N,N'-bis*[(7*R*,11*R*)-3-chloro-6,7,10,11-tetrahydro-9-methyl-7,11-methanocicloocta[*b*]quinolin-12-yl]-1,12-dodecanediamine. A suspension of (+)-huprine Y, (+)-**1** (500 mg, 1.76 mmol, 2 equiv) and finely powdered KOH (85% purity, 347 mg, 5.26 mmol, 6 equiv), and 4 Å molecular sieves (approx. 1 g) in anhydrous DMSO (10 mL) was stirred, heating every 10 min approximately with a heat gun for 1 h and at rt one additional hour, and then treated with a solution of 1,12-dibromododecane (289 mg, 0.88 mmol, 1 equiv) in DMSO (4 mL) dropwise for 30 min. The reaction mixture was stirred at room temperature for 3 days, diluted with 5N NaOH (100 mL) and H_2O (50 mL), and extracted with EtOAc (2×200 mL). The combined organic extracts were washed with H_2O (2×50 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a yellow oil (614 mg), which was purified through column chromatography (40–60 µm silica gel, CH_2Cl_2/50% aq. NH_4OH 100:0.2), to afford *bis*-huprine (+)-**2d** (323 mg, 50% yield); R_f 0.75 (CH_2Cl_2/MeOH/50% aq. NH_4OH 9:1:0.05). A solution of (+)-**2d** (223 mg, 0.30 mmol) in CH_2Cl_2 (4 mL) was filtered through a 0.2 µm PTFE filter, treated with methanolic HCl (0.43 N, 4.2 mL) and evaporated under reduced pressure. The resulting solid was washed with pentane (3×2 mL) to give, after drying at 65 ºC/2 Torr for 48 h, (+)-**2d**·2HCl (199 mg) as a yellowish solid: $[\alpha]_D^{20}$ = + 239 (MeOH, *c* = 0.6); mp 179–181 °C; IR (KBr) ν 3500–2500 (max at 3208, 3050, 3002, 2924, 2849, 2733, N-H, N^+-H and C-H st), 1631, 1582, 1567, 1514 (ar-C-C and ar-C-N st) cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 1.22–1.48 [complex signal, 16H, 3(10)-H_2, 4(9)-H_2, 5(8)-H_2, 6(7)-H_2], 1.58 [s, 6H, 9'(9")-CH_3], 1.80–1.94 [complex signal, 6H, 2(11)-H_2, 13'(13")-H_{syn}], 1.93 [d, *J* = 17.6 Hz, 2H, 10'(10")-H_{endo}], 2.08 [br d, *J* = 12.4 Hz, 2H, 13'(13")-H_{anti}], 2.55 [dd, *J* = 17.6 Hz, *J'* = 4.4 Hz, 2H, 10'(10")-H_{exo}], 2.77 [m, 2H, 7'(7")-H], 2.87 [d, *J* = 17.6 Hz, 2H, 6'(6")-H_{endo}], 3.21 [dd, *J* = 17.6 Hz, *J'* = 5.2 Hz, 2H, 6'(6")-H_{exo}], 3.45 [m, 2H, 11'(11")-H], 3.98 [t, *J* = 6.8 Hz, 4H, 1(12)-H_2], 4.85 (s, NH, NH^+), 5.58 [br d, *J* = 4.0 Hz, 2H, 8'(8")-H], 7.54 [dd, *J* = 9.2 Hz, *J'* = 1.2 Hz, 2H, 2'(2")-H], 7.77 [br s, 2H, 4'(4")-H], 8.39 [d, *J* = 9.2 Hz, 2H, 1'(1")-H]; ^{13}C NMR (100.6 MHz, CD_3OD) δ 23.5 [CH_3, 9'(9")-CH_3], 27.3 [CH, C11'(11")], 27.83 [CH_2, C3(10)], 27.85 [CH, C7'(7")], 29.3 [CH_2, C13'(13")], 30.3 (CH_2) and 30.6 ($2CH_2$) [C4(9), C5(8), C6(7)], 31.2 [CH_2, C2(11)], 36.0 [CH_2, C10'(10")], 36.1 [CH_2, C6'(6")], 49.7 [CH_2, C1(12)], 115.6 (C) and 117.6 (C) [C11a'(11a"), C12a'(12a")], 119.1 [CH, C4'(4")], 125.2 [CH, C8'(8")], 126.6 [CH, C2'(2")], 129.5 [CH, C1'(1")], 134.5 [C, C9'(9")], 140.2 [C, C3'(3")], 141.0 [C, C4a'(4a")], 151.2 [C, C5a'(5a")], 156.9 [C, C12'(12")]. HRMS (ESI) calcd for ($C_{46}H_{56}{}^{35}Cl_2N_4$ + H^+): 735.3955, found 735.3960. Anal. calcd for ($C_{46}H_{56}Cl_2N_4$·2HCl·1.25H_2O): C 66.46%, H 7.34%, N 6.74%; found: C 66.63%, H 7.66%, N 6.23%.
34. Egan, T. J. *Drug Des. Rev.* **2004**, *1*, 93.
35. Viayna, E.; Gómez, T.; Galdeano, C.; Ramírez, L.; Ratia, M.; Badia, A.; Clos, M. V.; Verdaguer, E.; Junyent, F.; Camins, A.; Pallàs, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M. P.; Rodríguez-Franco, M. I.; Bidon-Chanal, A.; Luque, F. J.; Camps, P.; Muñoz-Torrero, D. *ChemMedChem* **2010**, *5*, 1855.
36. Ellman, G. L.; Courtney, K. D.; Andres, V. Jr.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.
37. Di, L.; Kerns, E. H.; Fan, K.; McConnell, O. J.; Carter, G. T. *Eur. J. Med. Chem.* **2003**, *38*, 223.