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Design and synthesis of 1,2-annulated adamantane piperidines with anti-influenza virus activity

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ABSTRACT

1–2 Annulated adamantane piperidines **4**, **6**, **16**, **17**, **19**, **23** and **25** were synthesized and evaluated for anti-influenza A virus activity. The stereoelectronic requirements for optimal antiviral potency were investigated. Piperidine **23** proved to be the most active of the compounds tested against influenza A virus, being 3.5-fold more active than amantadine, equipotent to rimantadine and 15-fold more potent than ribavirin. It is noteworthy that piperidine **23** displayed one of the highest selectivity indexes (SI > 732) among aminoadamantanes or other cage structure amines tested till now.

1. Introduction

In the face of the persistent threat of human influenza A (H3N2, H1N1) and B infections, the outbreaks of avian influenza (H5N1) in Southeast Asia, and the potential of a new human or avian influenza A variant to unleash a pandemic, there is much concern about the shortage in both the number and supply of effective anti-influenza virus agents.[1–4] The current avian H5N1 originated in 1997 in Hong-Kong and has spread (through birds) to Southeast Asia and other countries, with occasional transmission to humans (almost 400 human cases, more than half of which were fatal). Whether the current avian H5N1 will evolve further to cause a pandemic through either mutation of the current H5N1 virus ('antigenic drift') or reassortment of this avian influenza virus with a human (or other non-avian) influenza virus ('antigenic shift')[5] is unpredictable at present.

The adamantane derivatives amantadine and rimantadine (α-methyl adamantanemethanamine) are specifically active against influenza A. They interfere with the viral uncoating process through a direct interaction with the viral matrix (M2) protein, which functions as a proton channel.[6] These drugs have been postulated to block the transmembrane channel formed by the tetrameric M2 helix.[7] Over the past twelve years our group has synthesized many potent aminoadamantane derivatives, mainly heterocycles, the most potent of which usually bear a six-membered ring (I–VI) and are shown in Figure 1.[8–10] It is noteworthy that these compounds, bearing the pharmacophore group of rimantadine and a six-membered ring carbon skeleton in the vicinity of the adamantane moiety, have increased anti-influenza A virus activity and excellent selectivity. Parent molecules **4**, **16**, and **23** contain the 1-aminoethyl pharmacophore group of drug Rimantadine extended into a piperidine. The rigid carbon frame work in the piperidines fits better, than a free rotating group, into a lipophilic pocket in the M2 receptor. Thus, we sought mainly to compare the anti-influenza A virus potency of these structurally modified rimantadines with the activity of Rimantadine and Amantadine. The effect of *N*-substitution was also investigated since it appeared to be important for activity against influenza A virus.

We now describe the synthesis and biological evaluation of adamantanopiperidines **4**, **6**, **16**, **17**, **19**, **23** and **25** (Fig. 2), and show that they contain structural features necessary for antiviral activity.

2. Results and discussion

2.1. Chemistry

For the synthesis of the piperidine **4** (Scheme 1), 2-oxo-1-adamantanepropionic acid **1**[11] was used as starting material. The acid **1** was converted to the oxime **2** upon treatment with hydroxylamine hydrochloride in the presence of sodium acetate. Catalytic hydrogenation of **2** over Raney nickel, under high temperature, gave lactam **3**. Reduction of lactam **3** with $LiAlH_4$ in THF afforded

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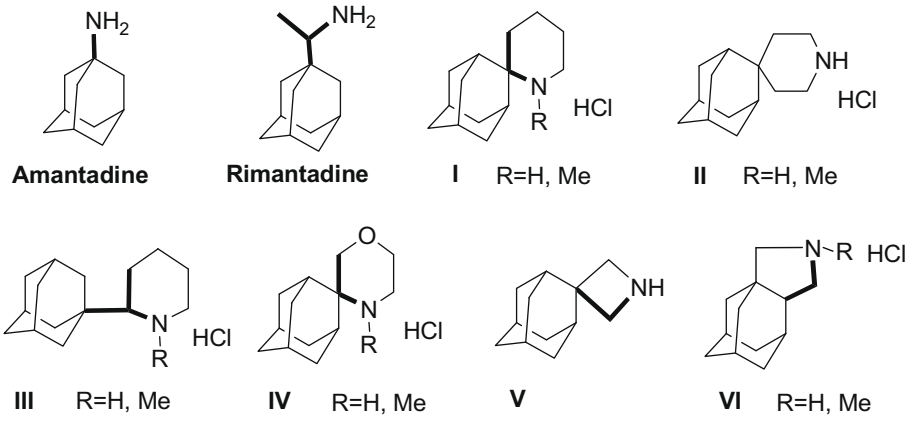


Figure 1.

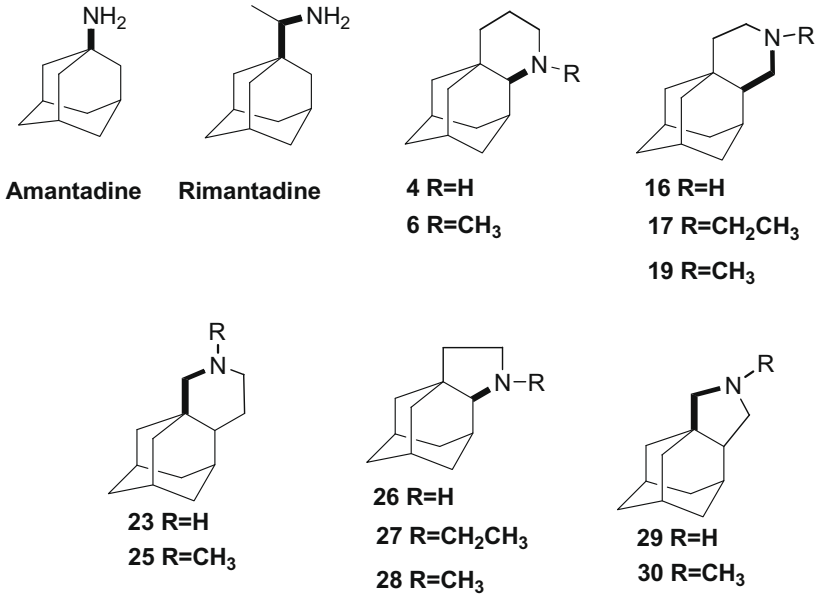
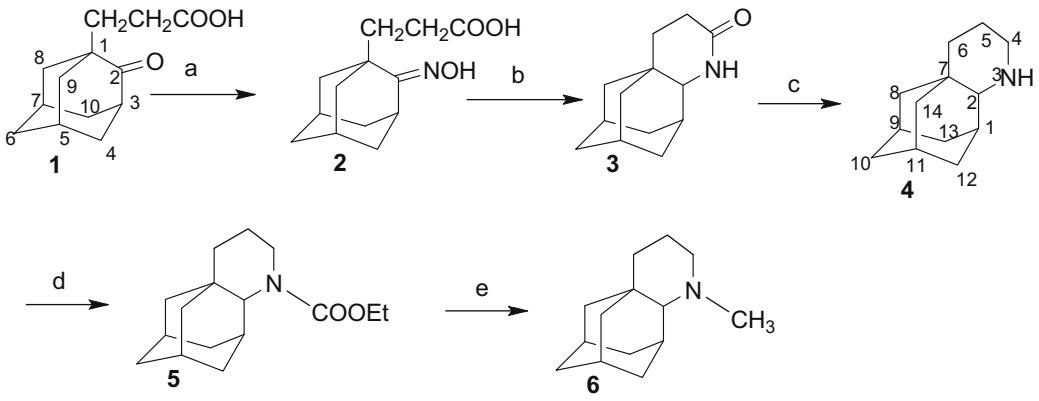


Figure 2.



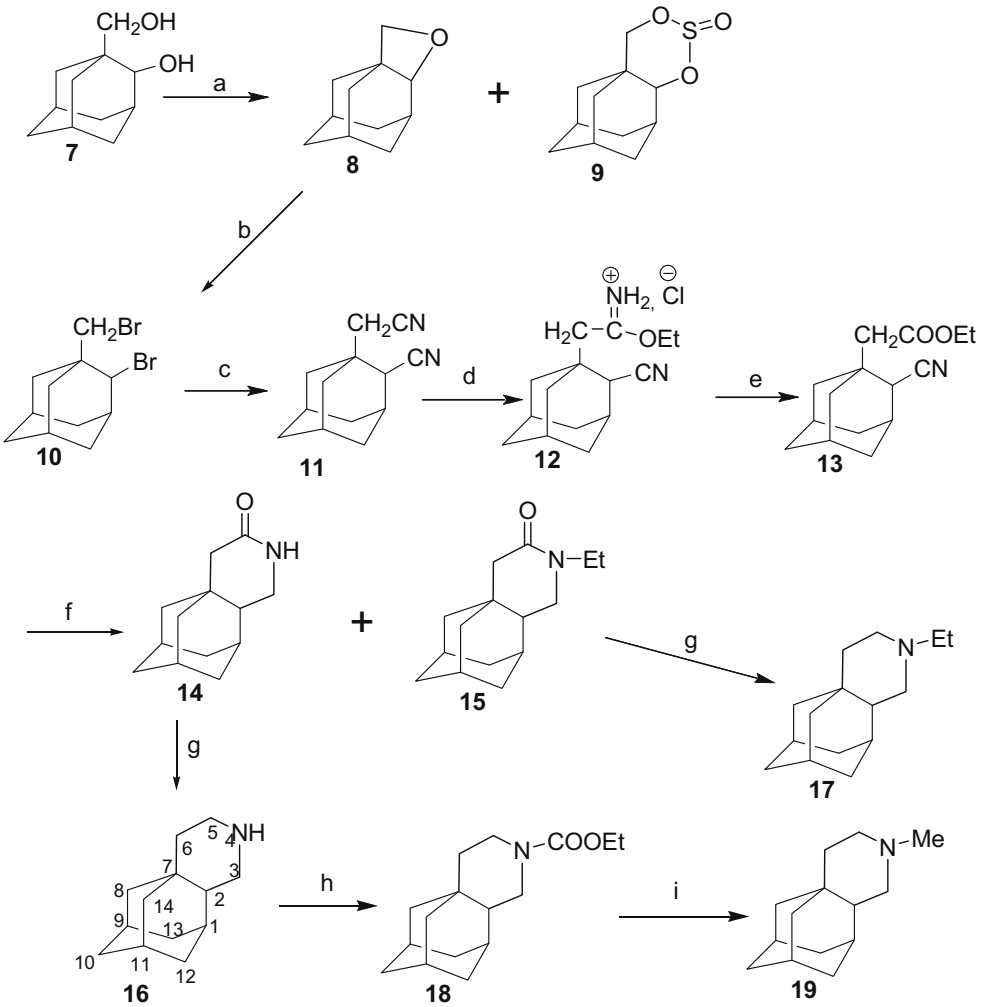
Scheme 1. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, $CH_3COONa{\cdot}3H_2O$, CH_3CH_2OH/H_2O (9:1), reflux, 5 h (94%); (b) H_2/Ni-Raney, EtOH, 50 p.s.i., 200 °C, 4 h (94%); (c) $LiAlH_4$, THF, 20 h, reflux (98%); (d) Et_3 N, $ClCOOC_2H_5$, ether, 24 h, 25 °C (quant.); (e) $LiAlH_4$, THF, 20 h, 50 °C (96%).

the piperidine **4**. N-Acylation of the piperidine **4** followed by reduction of the intermediate carbamate **5** with $LiAlH_4$ gave the *N*-methyl derivative **6**.

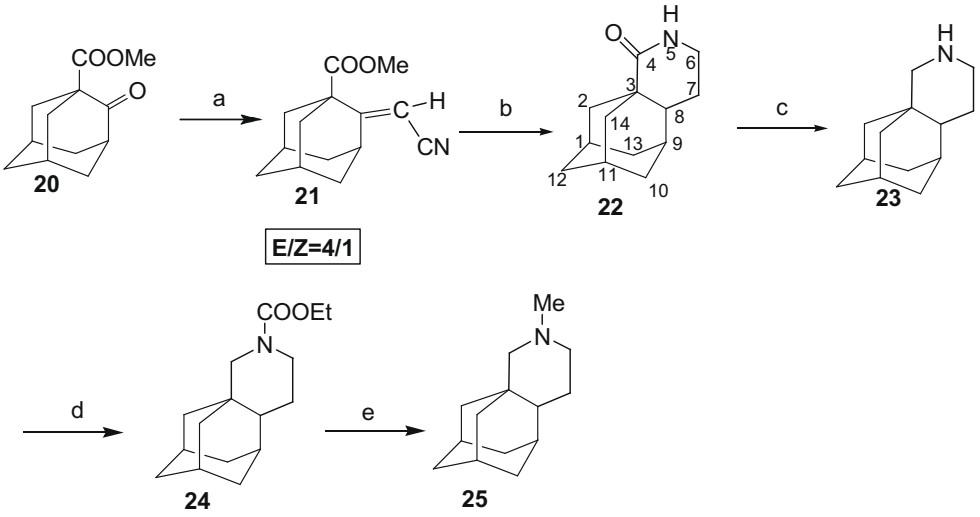
The synthetic route to the piperidine **16** is shown in Scheme 2 and involved diol **7**[12] as starting material. Reaction of the diol **7** with thionyl chloride[13] in ether resulted in the formation of a mixture consisting of two different products: the oxetane **8** and cyclic sulfite **9**. Temperature seems to be of critical importance in the conversion of diol **3** into the respective oxetane. It was found that the best yield for oxetane **8** (98%) was obtained at 43 °C, since negligible cyclic sulfite **9** formation (2%) was observed at this temperature. Reaction of oxetane **8** with bromine, triphenylphosphine in benzonitrile[14] provided the dibromo derivative **10** in 38% yield, which on heating for 4 h with NaCN/DMSO at 170 °C gave dinitrile **11** in an excellent yield. When dinitrile **11** was left in a saturated ethanolic solution of gaseous HCl at room temperature for 10 days, it was converted to the imino ether hydrochloride **12** in a very high yield, which was then hydrolyzed, under mild conditions, to the cyanoester **13**. Hydrogenation of cyanoester **13** over Raney nickel catalyst in ethanol gave a mixture of lactams **14** and **15**, which were reduced with $LiAlH_4$ in THF to the piperidines **16** and **17**, respectively. N-Acylation of the piperidine **16** followed by reduction of the intermediate carbamate **18** with $LiAlH_4$ gave the *N*-methyl derivative **19**.

N-Alkylation of heterocyclic secondary amines during hydrogenation has rarely been reported in the literature and only in cases where the reaction is run at high temperatures.[15] A plausible explanation for the observed N-alkylation of lactam **14** might be the anticipated stability to disproportionation of the solvent (primary alcohols: ethanol, methanol), which, under the specific reaction conditions is rendered sufficiently electrophilic. Nucleophilic attack of the latter by the primary aminoesters, formed during the hydrogenation, gives the secondary aminoesters which, in turn, cyclized to the corresponding N-alkylated lactams.

The annulated piperidine **23** was prepared by the route shown in Scheme 3, starting from ketoester **20**[9f] which was homologated with diethylcyanomethylphosphonate using a Horner–Wadsworth–Emmons reaction[16] to afford nitrile **21** in excellent (95%) yield. The nitrile **21** was found to be a mixture of *E* and *Z* stereoisomers. The stereochemistry around the C=C bond was investigated using NOE spectroscopy and it was found to be in favor of the *E*-stereoisomer (*E*/*Z*, 4/1 and *E*/*Z*, 9/1 when ethyl ester was used instead of methyl ester). Hydrogenation of the mixture of nitriles **21** over Raney nickel catalyst afforded the piperidinone **22**. Subsequent reduction of δ-lactam **22** with $LiAlH_4$ in THF gave the parent piperidine **23** which was then converted into the carbamate **24** through N-acylation. Finally, reduction of the carbamate **24** with $LiAlH_4$ led to the corresponding *N*-methyl piperidine **25**.



Scheme 2. Reagents and conditions: (a) (I) $SOCl_2$, Et_2O, 43 °C, 2.5 h (98%); (b) Br_2, C_6H_5CN, Ph_3P, 122 °C, 4 h (38%); (c) NaCN, DMSO, 170 °C, 4 h (88%); (d) EtOH–HCl, 10 days, 30 °C (82%); (e) H_2O, HCl, ether, 24 h, 30 °C (86%); (f) H_2/Ni-Raney, EtOH, 55 p.s.i., 140 °C, 7 h (**14**:21%, **15**:26%); (g) $LiAlH_4$, THF, 20 h, reflux (98%); (h) Et_3N, $ClCOOC_2H_5$, ether, 24 h, 25 °C (quant.); (i) $LiAlH_4$, THF, 20 h, 50 °C (96%).



Scheme 3. Reagents and conditions: (a) $(EtO)_2POCH_2CN$, C_6H_6, NaH, 1 h at 20 °C and then 15 min at 65 °C (95%); (b) H_2/Ni-Raney, EtOH, 50 p.s.i., 150 °C, 10 h (40%); (c) $LiAlH_4$, THF, 10 h, reflux (91%); (d) Et_3 N, $ClCOOC_2H_5$, ether, 24 h, 25 °C (quant.); (e) $LiAlH_4$, THF, 20 h, 50 °C (96%).

3. Biological activity

The antiviral efficacy of the new aminoadamantane heterocycles **4**, **6**, **16**, **17**, **19**, **23** and **25** was examined in vitro against influenza A (H3N2 subtype) and was compared to the activity of pyrrolidines **26**, **27**, **28**, **29** and **30**, the syntheses of which have already been described elsewhere,[9f] amantadine, rimantadine and ribavirin (Table 1). The antiviral assay used was identical to that previously reported.[17] There was a good correlation between the antiviral EC_{50} values obtained by CPE and MTS assay, and, hence, only the latter values are shown in Table 1.

The data presented in Table 1 indicate that compounds **23** and **29** elicit potent anti-influenza A virus activity, with a selectivity index (SI) of 732 and 200, respectively. Pyrrolidine **29** was endowed with the most potent anti-influenza A virus activity; it proved to be at least fourfold more potent than amantadine, equipotent to rimantadine and 19-fold more active than ribavirin. Piperidine **4** exhibited a twofold higher potency than ribavirine and had slightly lower potency than amantadine. It is noteworthy that piperidine **23** was more active than amantadine, while this compound was comparable to rimantadine in both antiviral activity and cytotoxicity, resulting in a similarly high selectivity index for **23** and rimantadine. The piperidine compound **4**, and the *N*-alkyl compounds **6**, **27**, **28** and **30**, had intermediate activity (EC_{50} ranging from 2 to 8 μM), with **4** and **27** having promising selectivity (SI above 100). Compounds **16**, **17**, **19**, **25**, were devoid of anti-influenza virus activity. Conformational studies have revealed that the most populated conformer (~95% at ambient temperature) of the protonated *N*-methyl derivative of piperidine **16** has adamantyl group in equatorial position and N–Me group in axial position. This preference leaves the N^+–H pharmacophore group in equatorial orientation and any hydrogen bonding interaction is distorted by the voluminous adamantyl group.

Table 1
Anti-influenza virus A (H3N2) activity and cytotoxicity of 1,2-annulated adamantane five,six-membered heterocyclic analogues[a] in MDCK cells[b]

Compound	EC_{50}[c,e] (μM)	MCC[d] (μM)	SI (ratio MCC/EC_{50})
4	4.1 ± 3.6 (4)	439	106
6	4.4 ± 2.6 (3)	83	19
16	N/A	—	—
17	N/A	—	—
19	N/A	—	—
23	0.6 ± 0.4 (4)	439	732
25	N/A	—	—
26	2.2 ± 1.1 (4)	468	217
27	3.4 ± 2.7 (4)	439	128
28	7.7 ± 2.9 (3)	83	11
29	0.5 ± 0.3 (4)	94	200
30	2.4 ± 1.6 (3)	83	35
Amantadine	2.0	>500	>250
Rimantadine	0.36	>500	1389
Ribavirin	8.7	20	2

N/A: not active at subtoxic concentrations or the highest concentration tested (~500 μM).

[a] All the compounds were tested as hydrochlorides.

[b] MDCK, Madin-Darby canine kidney cells; virus strain: influenza A/Hong Kong/7/87 (H3N2).

[c] Concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

[d] Minimal cytotoxic concentration, or concentration causing microscopically detectable changes in cell morphology.

[e] Data are shown as mean ± SD (in brackets: number of independent determinations).

All compounds were inactive against influenza B virus, which is in accordance with their putative mode of action, namely interaction with the influenza A virus M2 protein, which is different in influenza B virus.

Finally, all the new compounds were also tested for their trypanocidal activity, but none had significant activity against *Trypanosoma brucei*.

4. Conclusion

The aim of this study was to examine the anti-influenza A virus activity of 1,2-annulated adamantane analogues **4**, **6**, **16**, **17**, **19**, **23** and **25** and to correlate their potency to the ring size and the distance of the amine nitrogen atom from the adamantane skeleton. Three interesting points arise from this analysis: (i) extension from a five- to a six-membered ring retains the potency against influenza A virus. The present SAR study indicates that large and extended lipophilic moieties in the vicinity of adamantane skeleton are compatible with biological activity and suggests that there is a complementary acceptor group/site within the lumen of the M2 channel pore. (ii) Moving the amine nitrogen atom from the 2-adamantyl carbon enhances activity (*compound* **4** *vs* **23** *and* **26**

vs ***29***, Table 1) and (iii) non-substituted compounds are more active, with N-alkylation causing a clear reduction in potency and/or selectivity (*compound* **23** *vs* **25**, **26** *vs* **27** and **28** and **29** *vs* **30**). The N-substitution of the parent amines with other alkyl substituents was not attempted, as it has already been reported that the small size of the *N*-alkyl group enhances the activity of the respective compounds against influenza A/H3N2 strains.[9e]

5. Experimental

Melting points were determined using a Büchi capillary apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 833 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker MSL 400 spectrometer, respectively, using $CDCl_3$ as solvent and TMS as internal standard. Carbon multiplicities were established by DEPT experiments. The 2D NMR experiments (HMQC, COSY and NOESY) were performed for the elucidation of the structures of the new compounds.

Microanalyses were carried out by the Service Central de Microanalyse (CNRS) France, and the results obtained had a maximum deviation of ±0.4% from the theoretical value.

5.1. 3-[-2-(hydroxyimino)tricyclo[3.3.1.1^{3,7}]dec-1-yl]propanoic acid (2)

To a solution of ketoacid **1** (1.00 g, 4.5 mmol) in ethanol (22 mL) was added hydroxylamine hydrochloride (674 mg, 9.6 mmol) and $CH_3COONa{\cdot}3H_2O$ (3.00 g, 22.0 mmol). The mixture was refluxed for 5 h and was then evaporated to dryness under reduced pressure. Water was added and the mixture was acidified with concd HCl under cooling (0 °C). The white solid oxime acid **2** formed was filtered off, washed with H_2O and dried (1.02 g, 94%); mp 182 °C (dec.; CH_3OH–Et_2O); IR (mull) ν 3302 cm^{-1} (OH), 1702 cm^{-1} (C=O), 1656 cm^{-1} (C=N); ^{1}H NMR(400 MHz, $CDCl_3$), δ: 1.71–1.90 (complex m, 12H, 4, 6, 8, 9, 10-H, –C*H*$_2$CH$_2$COOH), 2.03 (br s, 2H, 5, 7-H), 2.44 (t, 2H, J = 8.2 Hz, –CH$_2$C*H*$_2$COOH), 3.62 (br s, 1H, 3-H), 4.93 (br s, 1H, NO*H*) ppm. ^{13}C NMR (100 MHz, $CDCl_3$), δ: 30.3 (5-C), 30.4 (3, 7-C, –*C*H$_2$COOH), 35.1 (6-C), 37.9 (4, 10-C), 39.2 (8, 9-C), 41.9 (1-C), 45.9 (–*C*H$_2$CH$_2$COOH), 167.8 (C=N), 179.5 (–*C*OOH) ppm. Anal. Calcd for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07. Found: C, 65.70; H, 7.93.

5.2. 3-Azatetracyclo[7.3.1.1^{7,11}.0^{2,7}]tetradecan-4-one (3)

A solution of the oxime acid **2** (1.48 g, 6.2 mmol) in dry ethanol (20 mL) was hydrogenated in the presence of Raney nickel catalyst under a pressure of 50 psi, at 200 °C, for 4 h. The solution was filtered to remove catalyst, and the filtrate was evaporated to dryness to afford an oily product, which was treated with a mixture of Et_2O/petroleum ether 1/2 and the precipitate formed was filtered and washed with cold petroleum ether (−15 °C) to afford lactam **3** (1.20 g, 94%) as a white solid; mp 207 °C (acetone); IR (mull) ν 3171 cm^{-1} (NH), 1653 cm^{-1} (C=O); ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.17 (d, 1H, J = 12.4 Hz, 14e-H), 1.39–1.95 (comlex m, 14H, 1, 6, 8, 9, 10, 11, 12, 13 14a-H), 2.30–2.43 (m, 2H, 5-H), 3.26 (s, 1H, 2-H), 6.20 (br s, 1H, NH) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 27.4 (11-C), 28.0 (5-C), 28.4 (9-C), 29.6 (12-C), 31.2 (7-C), 32.6 (1-C), 33.8 (13-C), 34.0 (14-C), 36.0 (8-C), 37.1 (10-C), 43.0 (6-C), 60.6 (2-C), 172.7 (C=O) ppm. Anal. Calcd for $C_{13}H_{19}NO$: C, 76.06; H, 9.33. Found: C, 76.35; H, 9.26.

5.3. General procedures for the synthesis of piperidines 4, 16, 17 and 23

To a stirred suspension of $LiAlH_4$ (4 equiv) in dry THF (50 mL) was added a solution of the requisite lactam (1 equiv) in dry THF (40 mL). The reaction mixture was refluxed for 12 h and was then hydrolyzed with water and a 5% NaOH solution under ice cooling. The inorganic precipitate was filtered off and washed with Et_2O, and the filtrate was evaporated under vacuum to afford a viscous oily product (yield almost quantitative). The residue was dissolved in ether and the resulting dry Et_2O solution of the free amine was treated with a saturated ethanolic solution of gaseous HCl under ice cooling. The precipitate was filtered off, washed with cold Et_2O and dried to afford the hydrochloride salt as a white solid.

5.4. 3-Azatetracyclo[7.3.1.1^{7,11}.0^{2,7}]tetradecane (4)

Yield 98%; Hydrochloride, white solid: mp >250 °C (EtOH–Et_2O). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 0.95–1.05 (m, 2H, 12e, 14e-H), 1.19 (br d, 1H, 12a-H), 1.25–1.28 (m, 2H, 5a, 6a-H), 1.38 (br q, 3H, 6e, 10-H), 1.53–1.70 (comlex m, 6H, 5e, 8, 12, 13-H), 1.80 (br d, 3H, 9, 11-H, NH), 2.24 (dd, 1H, J = 12.8, 2.0 Hz, 14a-H), 2.43 (br s, 1H, 2-H), 2.53–2.59 (m, 1H, 4a-H), 3.02–3.06 (m, 1H, 4e-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm) 22.4 (5-C), 28.4 (11-C), 28.9 (9-C), 31.0 (10-C), 32.5 (7-C), 34.1 (1-C), 35.2 (14-C), 37.8 (13-C), 38.0 (8-C), 38.6 (12-C), 45.3 (6-C), 47.8 (4-C), 65.3 (2-C) ppm. Hydrochloride: Anal. Calcd for $C_{13}H_{22}NCl$: C, 68.55; H, 9.73. Found: C, 68.31; H, 9.58.

5.5. General procedures for the synthesis of *N*-methyl piperidines 6, 19, and 25

To a solution of the requisite piperidine (1 equiv) and triethylamine (2 equiv) in dry ether (20 mL) was added dropwise and under ice cooling a solution of ethyl chloroformate (1.5 equiv) in dry ether (10 mL). The mixture was stirred for 24 h at 25 °C, poured into an ice-water mixture and extracted with ether. The organic phase was washed with water, cold HCl (3%), water, dried (Na_2SO_4) and evaporated in vacuo. The liquid carbamate ester (quantitative yield) was used without further purification.

A solution of the carbamate (1 equiv) in dry THF (20 mL) was added dropwise under ice cooling to a suspension of $LiAlH_4$ (5 equiv) in dry THF (15 mL). The mixture was gently refluxed for 20 h, hydrolyzed with water and NaOH (5%), dried (Na_2CO_3), filtered off and concentrated in vacuo. The residue was dissolved in ether and extracted with a 10% HCl solution. The aqueous phase was made alkaline with solid Na_2CO_3, and the oil which separated was extracted with ether, dried (Na_2CO_3), and evaporated to dryness under vacuum to give (quantitative yield) the viscous oily, free corresponding amine, which was converted to its hydrochloride salt (white solid).

5.6. 3-Methyl-3-azatetracyclo[7.3.1.1^{7,11}.0^{2,7}]tetradecane (6)

Yield 96%; hydrochloride, white crystals: mp >250 °C. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 0.96–1.01 (m, 2H, 12e, 14e-H), 1.15 (br d, 1H, 12a-H), 1.25–1.42 (m, 4H, 1, 5a, 6a, 6e-H), 1.58–1.62 (m, 4H, 2, 8a, 13-H), 1.78–2.08 (comlex m, 10H, 4a, 5e, 8e, 9, 10, 11-H, C*H*$_3$), 2.36 (br d, 1H, 14a-H), 2.83 (br d, 1H, 4e-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 21.6 (5-C), 28.1 (11-C), 28.5 (1-C), 29.3 (9-C), 30.3 (10-C), 32.9 (7-C), 36.8 (14-C), 37.8 (13-C), 37.9 (8-C), 39.0 (12-C), 42.4 (*C*H$_3$), 45.5 (6-C), 58.7 (4-C), 73.2 (2-C) ppm. Anal. Calcd for $C_{14}H_{24}NCl$: C, 69.54; H, 10.00. Found: C, 69.19; H, 9.93.

5.7. 3-Oxatricyclo[5.3.1.1.^{5,9}0^{2,5}]dodecane (8) and 3,5,4-dioxathiatetracyclo[7.3.1.1.^{7,11}0^{2,7}]tetradecan-4-oxide (9)

Freshly distilled thionyl chloride (3.47 g, 29.2 mmol) in dry ether (20 mL) was added dropwise (~1 h) to a solution of the diol **7** (2.50 g, 13.3 mmol) in dry ether (30 mL) under ice cooling. After stirring at 43 °C for 2.5 h, the reaction mixture was poured into an ice/water mixture (30 mL). The organic phase was washed with

H_20, a solution of $NaHCO_3$ (foaming) and H_2O again, dried (Na_2SO_4) and evaporated under reduced pressure. The oily residue was purified by flash column chromatography on silica gel eluting with *n*-pentane-ether (1.6:1) to give first the liquid, in rt, oxetane **8** (2.14 g, 98%) and then the dioxathiane **9** (62 mg) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) oxetane **8**, δ: 1.27–1.33 (m, 2H, 6a, 12e-H), 1.43 (m, 1H, 6e-H), 1.52 (d d, 1H, J = 12.6, 1.6 Hz, 10e-H), 1.60–1.67 (m, 1H, 11a-H), 1.70–1.77 (m, 2H, 8a, 11e-H), 1.79–1.87 (m, 2H, 1, 8e-H), 1.89–1.93 (m, 2H, 7, 10a-H), 1.98 (br s, 1H, 9-C), 2.53 (d d, 1H, J = 12.8, 2.0 Hz, 12a-H), 3.19 (d, 1H, J_{AB} = 11.2 Hz, 4A-H), 4.49 (d, 1H, J_{AB} = 11.0 Hz, 4B), 4.94 (br s, 1H, 2-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 27.0 (9-C), 27.5 (7-C), 30.0 (10-C), 31.3 (1-C), 34.1 (12-C), 34.3 (5-C), 36.4 (6-C), 36.9 (11-C), 38.0 (6-C), 67.3 (4-C), 73.6 (2-C) ppm. HRMS (ESI, m/z) calcd for $C_{11}H_{16}O$ [M + H] 164.2468, found 164.2464.

Dioxathiane **9** mp 83 °C (ether–*n*-pentane); ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.26–1.31 (m, 2H, 13a, 14e-H), 1.40–1.51 (m, 2H, 12e, 13e-H), 1.59–1.72 (m, 3H, 10a, 8-H), 1.82–1.86 (m, 1H, 10e-H), 1.94–2.04 (m, 4H, 1, 9, 11, 12a-H), 2.58 (d d, 1H, J = 13.0, 2.0 Hz, 14a-H), 3.74 (d, 1H, J_{AB} = 12.0 Hz, 6A-H), 4.04 (d, 1H, J_{AB} = 12.0 Hz, 6B-H), 4.36 (d, 1H, J = 1.6 Hz, 2-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 27.0 (11-C), 27.3 (9-C), 29.6 (12-C), 32.3 (1-C), 33.0 (7-C), 34.0 (14-C), 35.9 (10-C), 36.8 (8-C), 36.9 (13-C), 76.3 (6-C), 82.7 (2-C) ppm. HRMS (ESI, m/z) calcd for $C_{11}H_{16}O_3S$ [M + H] 228.3116, found 228.3113.

5.8. 2-Bromo-1-tricyclo[3.3.1.1^{3,7}]decanemethyl bromide (10)

A solution of triphenyldibromophosphorane was prepared by the dropwise addition of Br_2 (2.78 g, 17.4 mmol) in benzonitrile (12 mL) to a solution of triphenylphosphine (4.57 g, 17.4 mmol) in benzonitrile (15 mL) and the resulting solution was stirred at 122 °C under argon atmosphere. To this solution was added in one portion the oxetane derivative **8** (2.26 g, 13.7 mmol) and the mixture was heated at 122 °C for 4 h. The mixture was cooled to room temperature, *n*-pentane was added and the precipitate formed was removed by filtration and washed with *n*-pentane. The washings were combined and the upper layer was removed and evaporated under vacuum to give a viscous oil. The remaining benzonitrile was removed by fractional distillation in vacuo ($Eb_{0.01\ mmHg}$ 50–60 °C) and the residue was purified by flash column chromatography on silica gel eluting with cyclohexane to give the dibromide **10** (1.60 g, 38%) as a white solid; mp 62 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.35 (dd, 1H, J = 10.0, 2.0 Hz, 9e-H), 1.53–1.88 (complex m, 6H, 4e, 6a, 8, 10-H), 1.94–2.00 (m, 4H, 5, 6e, 7, 9a-H), 2.24 (m, 2H, 3, 4a-H), 3.10 (d, 1H, J = 10.1 Hz, CH_A–H), 3.46 (d, 1H, J = 10.1 Hz, CH_B–H), 4.55 (s, 1H, 2-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 26.9 (5-C), 27.7 (7-C), 31.3 (4-C), 35.6 (9-C), 37.0 (3, 8-C), 37.9 (1, 10-C), 40.8 (6-C), 46.2 (CH_2), 65.7 (2-C) ppm. Anal. Calcd for $C_{11}H_{16}Br_2$: C, 42.89; H, 5.23. Found: C, 42.77; H, 5.01.

5.9. 2-Cyano-1-tricyclo[3.3.1.1^{3,7}]decaneacetonitrile (11)

A mixture of dibromide **10** (1.55 g, 5.0 mmol) and NaCN (3.20 g, 65.0 mmol) in DMSO (40 mL) was stirred at 170 °C for 4 h. The mixture was cooled to room temperature, poured into 40 mL of water and extracted with Et_2O. The organic extracts were washed with water and brine and dried over anhydrous Na_2SO_4. After removal of the solvent in vacuo, the residue was purified by column chromatography on silica gel, using cyclohexane, cyclohexane- Et_2O (1:1) and Et_2O as eluents, to give the solid nitrile **11** (0.88 g, 88%); mp 68 °C. IR (mull) ν 2235 cm^{-1} (CN). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.60–1.78 (complex m, 7H, 4e, 6a, 8, 9, 10a-H), 1.83–1.89 (m, 2H, 6e, 10e-H), 2.00–2.07 (m, 3H, 4a, 5, 7-H), 2.32 (d, 2H, J = 16.8 Hz, 3, CH_A–H), 2.42 (d, 1H, J = 16.8 Hz, CH_B–H), 2.82 (br s, 1H, 2-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 27.0 (5-C), 27.2 (7-C), 30.2 (CH_2), 31.0 (3-C), 32.0 (4-C), 33.9 (1-C), 35.4 (10-C), 35.5 (6-C), 37.9 (9-C), 40.3 (8-C), 40.8 (2-C), 116.3 (CH_2CN), 119.4 (CN) ppm. Anal. Calcd for $C_{13}H_{16}N_2$: C, 76.56; H, 8.57. Found: C, 76.31; H, 8.35.

5.10. Ethyl (2-cyanotricyclo[3.3.1.1^{3,7}]dec-1-yl)acetate (13)

Dinitrile **11** (790 mg, 3.9 mmol) was dissolved in a chilled absolute ethanol solution (20 mL) which was saturated with gaseous HCl. The flask was stoppered and the solution left to stand at room temperature for 10 days. The solvent was then evaporated under reduced pressure, dry ether was added and the precipitated iminoether hydrochloride **12** was filtered off and washed with dry ether: yield 0.9 g (82%).

The hydrochloride salt **12** (0.88 g, 3.1 mmol) was dissolved in a mixture of water (20 mL), ether (80 mL) and HCl 36% (1 mL). The resulting mixture was stirred for 24 h at 30 °C, the organic phase separated and the aqueous layer was extracted with ether. The combined ether extracts were washed with water and dried (Na_2SO_4). The solvent was removed in vacuo to give 650 mg (86%) of the cyanoester **13** as an oil: IR (Nujol) ν 2235 cm^{-1} (CN), 1732 cm^{-1} (C=O). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.24 (t, 3H, J = 7.1 Hz, CH_3), 1.53 (br d, 1H, 4e-H), 1.61–1.73 (m, 6H, 6a, 8, 9e, 10-H), 1.78 (m, 1H, 9a-H), 1.88 (m, 1H, 4a-H), 1.97 (br q, 2H, 5, 7-H), 2.03 (br d, 1H, 6e-H), 2.19 (d, 1H, J_{AB} = 14.7 Hz, CH_A–H), 2.23 (br d, 1H, 3-H), 2.41 (d, 1H, J_{AB} = 14.7 Hz, CH_B–H), 3.11 (br s, 1H, 2-H), 4.10 (q, 2H, J = 7.1 Hz, –OCH_2CH$_3$) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 14.0 (CH_3), 26.3 (7-C), 26.5 (5-C), 30.7 (8, 10-C), 33.4 (1, 3-C), 34.8 (4, 9-C), 37.6 (6-C), 39.4 (CH_2COOCH$_2$CH$_3$), 42.1 (2-C), 60.7 (COOCH_2CH$_3$) 122.9 (CN), 169.3 (C=O) ppm.

5.11. 4-Azatetracyclo[7.3.1.1^{7,11}.0^{2,7}]tetradecan-5-one (14) and 4-ethyl-4-azatetracyclo [7.3.1.1^{7,11}.0^{2,7}]tetradecan-5-one (15)

Cyanoester **13** (0.69 g, 2.8 mmol) in dry ethanol (20 mL) was hydrogenated in the presence of Raney-Ni under a pressure of 55 psi, at 140 °C, for 7 h. The solution was filtered to remove catalyst, and the filtrate was evaporated to dryness. The solid formed was chromatographed on a silica gel column (Et_2O–MeOH 3/2) to afford first pure *N*-ethyl piperidone **15** (170 mg, 26%) and then piperidone **14** (120 mg, 21%) as white solids.

Piperidone **14** mp 186 °C; IR (mull) ν 3163 cm^{-1} (NH), 1657 cm^{-1}(C=O). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.28 (br d, 1H, 14e-H), 1.40–2.00 (complex m, 15H, 1, 2, 6, 8, 9, 10, 11, 12, 13, 14a-H), 3.15 (m, 1H, 3a-H), 3.46 (t, 1H, J = 12.1 Hz, 3e-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 28.1 (11-C), 28.3 (9-C), 29.8 (1-C), 31,1 (7-C), 31.2 (12-C), 36.6 (14-C), 37.1 (13-C), 38.2 (10-C), 41.0 (2-C), 43.1 (3-C), 45.0 (8-C), 45.4 (6-C), 172.1 (C=O) ppm. Anal. Calcd for $C_{13}H_{19}NO$: C, 76.06; H, 9.33. Found: C, 75.79; H, 9.10.

N-Ethyl piperidone **15** mp 85 °C; IR (mull) ν 1632 cm^{-1} (C=O). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.09 (t, 3H, J = 7.2 Hz, CH_3), 1.24 (br d, 1H, 14e-H), 1.36–1.40 (m, 1H, 8a-H), 1.50 (dd, 1H, J = 13.0, 2.2 Hz, 12e-H), 1.54–1.94 (complex m, 11H, 1, 2, 8e, 9, 10, 11, 12a, 13, 14a-H), 1.99 (s, 2H, 6-H), 3.06 (q, 1H, J = 6.5 Hz, 3a-H), 3.33–3.45 (m, 3H, 3e-H, CH_2CH$_3$) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 12.1 (CH_3), 28.1 (11-C), 28.4 (9-C), 29.8 (1-C), 31,2 (12-C), 31.5 (7-C), 36.5 (14-C), 37.2 (13-C), 38.2 (10-C), 41.6 (CH_2CH$_3$), 41.8 (2-C), 45.2 (8-C), 45.9 (6-C), 48.1 (3-C), 168.8 (C=O) ppm. Anal. Calcd for $C_{15}H_{23}NO$: C, 77.21; H, 9.93. Found: C, 77.40; H, 10.05.

5.12. 4-Azatetracyclo[7.3.1.1^{7,11}.0^{2,7}]tetradecane (16)

Yield 52%; hydrochloride, white crystals: mp >250 °C (EtOH–Et_2O). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 1.02–1.19 (m, 3H, 6, 14e-H), 1.34–1.43 (m, 3H, 1, 13-H), 1.56–1.70 (complex m, 8H, 8, 10, 12, 13-H), 1.88 (br d, 2H, 9, 11-H), 2.19 (br d, 1H, 14a-H), 2.56

(dd, 1H, J = 12.4, 4.0 Hz, 3a-H), 2.75–2.87 (m, 3H, 3e, 5a, 5e-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm) 28.6 (11-C), 29.2 (9-C), 31.4 (1-C), 31.6 (12-C), 35.1 (14-C), 38.2 (10-C), 39.1 (8-C), 39.4 (7-C), 40.2 (13-C), 41.8 (6-C), 46.6 (5-C), 47.0 (3-C), 47.7 (2-C) ppm. Anal. Calcd for $C_{13}H_{22}NCl$: C, 68.55; H, 9.73. Found: C, 68.30; H, 9.61.

5.13. 4-Ethyl-4-azatetracyclo[7.3.1.1^{7,11}.0^{2,7}]tetradecane (17)

Yield 98%; hydrochloride, white crystals: mp >250 °C (EtOH–Et_2O). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.04 (m, 4H, 14e-H, CH_3), 1.15 (br d, 1H, 6a-H), 1.35–1.53 (complex m, 4H, 6e, 8, 12e-H), 1.62–1.72 (m, 7H, 1, 2, 10, 12a, 13-H), 1.90 (br d, 2H, 9, 11-H), 2.07–2.19 (m, 3H, 5a, 3a, 14a-H), 2.38 (q, 2H, J = 7.2 Hz, CH_2), 2.50 (dd, J = 11.1, 3.8 Hz, 1H, 3e-H), 2.66 (br d, 1H, 5e-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 12.2 (*C*H_3), 28.8 (11-C), 29.4 (9-C), 31.1 (7-C), 31.8 (1-C), 31.9 (12-C), 35.0 (14-C), 38.3 (10-C), 39.1 (6-C), 39.2 (13-C), 45.9 (2-C), 46.6 (8-C), 49.0 (5-C), 52.8 (***C***H$_2$), 54.1 (3-C) ppm. Anal. Calcd for $C_{15}H_{26}NCl$: C, 70.42; H, 10.24. Found: C, 70.64; H, 10.06.

5.14. 4-Methyl-4-azatetracyclo[7.3.1.1^{7,11}.0^{2,7}]tetradecane (19)

Yield 96%; hydrochloride, white solid: mp >250 °C (EtOH–Et_2O). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.05 (br d, 1H, 14e-H), 1.17 (br d, 1H, 6a-H), 1.35–1.58 (complex m, 4H, 6e, 8, 12e-H), 1.61–1.80 (m, 7H, 1, 2, 10, 12a, 13-H), 1.80 (br d, 2H, 9, 11-H), 2.12–2.24 (m, 3H, 5a, 3a, 14a-H), 2.29 (s, 3H, CH_3), 2.43 (br d, 1H, 3e-H), 2.59 (br d, 1H, 5e-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 28.7 (11-C), 29.3 (9-C), 31.6 (1-C), 31.8 (7, 12-C), 34.9 (14-C), 38.2 (10-C), 39.1 (6-C), 39.3 (13-C), 46.0 (2-C), 46.5 (8-C, ***C***H$_3$), 51.3 (5-C), 56.5 (3-C) ppm. Anal. Calcd for $C_{14}H_{24}NCl$: C, 69.54; H, 10.00. Found: C, 69.43; H, 9.93.

5.15. Methyl (2-cyanomethylidene)tricyclo[3.3.1.1^{3,7}]decane-1-carboxylate (21)

A dry, three-necked flask equipped with stirrer, thermometer, condenser, and dropping funnel was purged with dry argon and charged with 0.23 g. (5.8 mmol) of a 60% dispersion of sodium hydride in mineral oil and dry benzene (10 mL). To this stirred mixture was added dropwise diethylcyanomethyl-phosphonate (1.02 g, 5.8 mmol). During the addition period the temperature is maintained at 30–35°, and cooling was employed if necessary. Evolution of hydrogen was noted. After addition of diethylcyanomethylphosphonate was completed, the mixture was stirred for 1 h at rt to ensure complete reaction. To this nearly clear solution was added dropwise ketoester **20** (1.3 g, 5.8 mmol). The mixture was then heated at 63–65 °C for 15 min. The resulting product was cooled to 15–20 °C, and the mother liquor was decanted from the precipitate. This gummy precipitate was washed well by mixing it at 60 °C with several portions of benzene (10 mL). Benzene was removed in vacuo to give a viscous oil which was filtered through a short-column of silica gel (SiO_2), using Et_2O as eluent, to afford 1.35 g of the corresponding nitrile **21** in a mixture of *E* and *Z* stereoisomers (yield 95%). IR (mull) ν 2217 cm^{-1} (CN), 1731 cm^{-1} (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) **E/Z 4/1**, δ: 1.75–2.00 (complex m, 8H, 4, 6, 8a, 9, 10a-H), 2.12 (br s, 2H, 5, 7-H), 2.30 (dd, 2H, J = 13.4, 2.0 Hz, 8e, 10e-H), 2.58 (br s, 1H, 3-H), 3.81/3.74 (s, 3H, CH_3), 4.84/5.22 (s, 1H, C*H*-CN) **E/Z 4/1** ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 27.5 (5, 7-C), 35.2 (6-C), 38.5 (4-C), 39.0 (9-C), 41.6 (8, 10-C), 42.0 (3-C), 49.1 (1-C), 52.0 (CH_3), 88.9 (C=C*H*), 115.4 (CN), 169.5 (2-C), 173.7 (C=O) ppm. Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41. Found: C, 73.01; H, 7.68.

5.16. 5-Azatetracyclo[7.3.1.1^{3,11}.0^{3,8}]tetradecan-4-one (22)

Cyanoester **21** (1.00 g, 4.3 mmol) in dry ethanol (20 mL) was hydrogenated in the presence of Raney-Ni under a pressure of 50 psi, at 150 °C, for 10 h. The solution was filtered to remove catalyst, and the filtrate was evaporated to dryness. The solid formed was treated with *n*-pentane and chilled at −15 °C. The precipitate was filtered and washed with cold *n*-pentane to afford piperidinone **23** (330 mg, 40%) as a white solid; mp 165 °C (ether); IR (mull) ν (NH) 3424 cm^{-1}, (C=O) 1653 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.46 (dd, 1H, J = 12.8, 2.4 Hz, 7e-H), 1.52–1.60 (m, 2H, 10e, 14e-H), 1.66–1.82 (complex m, 7H, 9, 10a, 12, 2a, 13-H), 1.90–2.02 (m, 4H, 1, 2e, 8, 11-H), 2.07–2.11 (m, 1H, 7a-H), 2.26 (dd, 1H, J = 12.6, 2.0 Hz, 14a-H), 3.27 (m, 2H, 6-H), 6.17 (br s, 1H, NH) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm) 23.8 (7-C), 27.9 (11-C), 28.4 (1-C), 30.9 (10-C), 32.5 (9-C), 35.7 (2-C), 37.2 (12-C), 38.3 (13-C), 38.8 (14-C), 40.1 (3-C), 41.9 (6-C), 43.2 (8-C), 178.0 (C=O) ppm. Anal. Calcd for $C_{13}H_{19}NO$: C, 76.06; H, 9.33. Found: C, 75.95; H, 9.19.

5.17. 5-Azatetracyclo[7.3.1.1^{3,11}.0^{3,8}]tetradecane (23)

Yield 91%; hydrochloride, white solid; mp >250 °C (EtOH–Et_2O). ^{1}H NMR (400 MHz, $CDCl_3$), δ 1.08 (d, 1H, J = 12.4 Hz, 14e-H), 1.15 (br d, 1H, 7a-H), 1.34 (m, 2H, 12-H), 1.46 (m, 1H, 10e-H), 1,51 (s, 1H, 8-H), 1.56 (s, 1H, 9-H), 1.61–1.80 (complex m, 7H, 2, 7e, 10a, 13-H, NH), 1.94 (br t, 2H, 1, 11-H), 2.21–2.26 (m, 2H, 4a, 14a-H), 2.42 (d, 1H, J = 12.5 Hz, 4e-H), 2.57 (td, J = 12.4, 12.3 Hz 1H, 6a-H), 3.08 (dd, 1H, J = 12.4, 4.1 Hz, 6e-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm) 27.8 (7-C), 28.7 (11-C), 29.0 (1-C), 31.1 (10-C), 32.7 (3-C), 33.3 (9-C), 34.8 (14-C), 38.5 (13-C), 39.1 (2-C), 43.7 (12-C), 46.3 (8-C), 47.7 (6-C), 59.4 (4-C) ppm. Anal. Calcd for $C_{13}H_{22}NCl$: C, 68.55; H, 9.73. Found: C, 68.70; H, 9.65.

5.18. 5-Methyl-5-azatetracyclo[7.3.1.1^{3,11}.0^{3,8}]tetradecane (25)

Yield 94%; hydrochloride, white solid: mp >250 °C (EtOH–Et_2O). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.19–1.26 (m, 2H, 7a, 14e-H), 1.29–1.35 (m, 2H, 8, 12a-H), 1.40–1.52 (m, 3H, 4a, 10e, 12e-H), 1,62–1.69 (m, 2H, 9, 13a-H), 1.78–1.85 (m, 2H, 2a, 13e-H), 1.75–1.86 (complex m, 3H, 2e, 6a, 10a-H), 1.89–1.98 (complex m, 3H, 1, 7e, 11-H), 2.16 (s, 3H, CH_3), 2.28 (m, 2H, 4e, 14a-H), 2.86–2.90 (m, 1H, 6e-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm) 27.2 (7-C), 28.7 (11-C), 28.8 (1-C), 31.1 (10-C), 32.8 (9-C), 32.9 (3-C), 36.3 (14-C), 38.5 (13-C), 39.3 (2-C), 43.9 (12-C), 45.4 (8-C), 47.0 (*C*H_3), 57.3 (6-C), 69.4 (4-C) ppm. Anal. Calcd for $C_{14}H_{24}NCl$: C, 69.54; H, 10.00. Found: C, 69.23; H, 9.89.

5.19. Anti-influenza virus assay

Human influenza A/H3N2 virus (strain A/Hong Kong/7/87) was added to semi-confluent Madin-Darby canine kidney (MDCK) cells (cultured in 96-well plates) at a multiplicity of infection of 50 CCID50 per well. Serial dilutions of the test compounds were added at the time of virus infection, and the plates were incubated for 72 h at 35 °C. Then, the antiviral activity was estimated from the inhibitory effect on virus-induced cytopathic effect, as determined by microscopical examination and the formazan-based MTS cell viability test. Cytotoxicity of the test compounds was expressed as the compound concentration causing minimal changes in cell morphology (MCC).

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