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8-Methylureido-10-amino-10-methyl-imidazo[1,2-*a*]indeno[1,2-*e*] pyrazine-4-ones: Highly In Vivo Potent and Selective AMPA Receptor Antagonists

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Abstract—Water soluble 8-methylureido-10-amino-10-methyl-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one **4** represents a novel class of highly potent and selective AMPA receptors antagonists with in vivo activity. The dextrorotatory isomer **(+)-4** was found to display the highest affinity with an IC_{50} of 10 nM. It also exhibited very good anticonvulsant effects after ip, sc and iv administration in mice subjected to electrical convulsions (MES) and ip in audiogenic seizure-e in DBA/2 mice ($ED_{50's} \leq 10$ mg/kg).

Introduction

The principal output pathways to the mammalian brain use L-glutamic acid as a neurotransmitter, as do numerous excitatatory local circuits in the cortex hippocampus and cerebellum. Glutamate is released from glutamatergic nerve terminals in response to depolarization. It then crosses the synaptic cleft and acts on post-synaptic receptors. This physiological process is part of the normal function of rapid excitatory synaptic transmission. Glutamate activates three major types of ionotropic receptors: *N*-methyl-D-aspartate (NMDA), α-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanoic acid (AMPA) and kainate as well as metabotropic receptors.[1] Overstimulation of these receptors by excessive endogenous glutamate can initiate neuronal cell death. Thus, antagonists at these receptors are considered as attractive therapeutic targets in many neurological disorders, such as pain, amyotrophic lateral sclerosis, anxiety, epilepsy, Parkinson's disease and cerebral ischemia.[2]

AMPA ionotropic receptors are of particular interest, and several compounds belonging to various chemical families have been reported to be effective AMPA antagonists. Representative examples are **YM90K**,[3] **MPQX**,[4] **(-)LY293558**[5] and **LY300164**.[6] To date, this later derivative seems to be one of the most clinically advanced drugs. In addition, the *N*-phosphonoalkyl-5-aminomethylquinoxaline-2,3-dione derivative **1**[7] and the spiro-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one derivative **(+)-2**[8] have affinities for both the AMPA and the glycine/NMDA receptors. Recently, we described the 8-methylureido-10-substituted-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one such as **3**[9] which also demonstrates interesting anticonvulsant properties (Figure 1, Table 1).

As part of our efforts towards the discovery of new AMPA antagonists, we now report the synthesis of 10-amino-10-methyl-8-methylureido-4,5-dihydro-4-oxo-10*H*-imidazo[1,2-*a*] indeno[1,2-*e*]pyrazines **4** and its enantiomers **(+)-4** and **(−)-4**.[10] The best enantiomer **(+)-4** displayed high affinity for the AMPA receptors and potent anticonvulsant effects in vivo in both maximal electroshock convulsions (MES) and audiogenic convulsions in mice (Scheme 1, Table 1).

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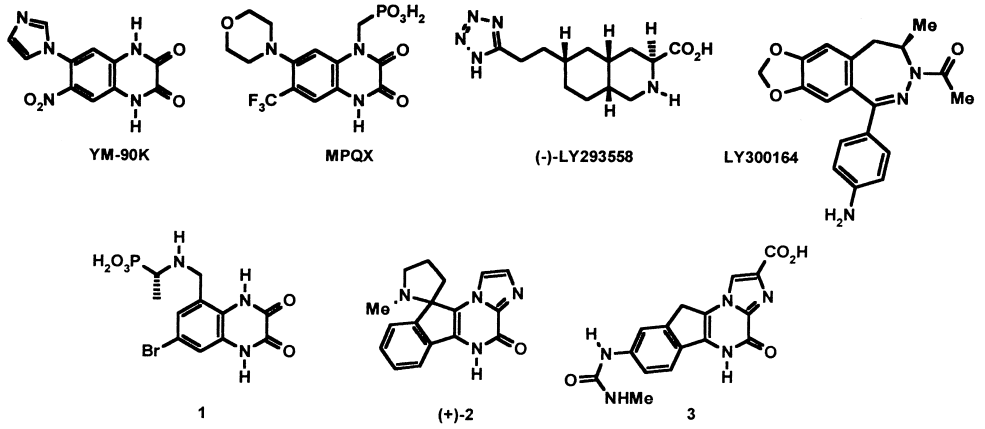


Figure 1. Chemical structure of the most promizing AMPA antagonist.

Table 1. In vitro and in vivo activities of **4, (−)-4, (+)-4, 5, 11, 12, YM-90K** and **(-)-LY-293558**

Compound	Receptor affinity		Anticonvulsant activity		Antagonist activity[e]
	AMPA[a]	Glycine/NMDA[a]	MES[b,c]	DBA/2[b,d]	
4	51	14,000	5.7 ip	4.7 ip	25
(−)-4	42	> 1,000,00	80 ip		30
(+)-4	10	32,000	10 ip	1.8 ip	29.5
			2.5 iv		see Figure 3
			4.6 sc		
5	760	3000	62 ip		1800
11	220	38,000	> 80 ip	80 ip	90
12	2500	1400	30 ip		6126
YM-90K	350	10,000	12 ip	15 ip	260
			12 iv		
(-)-LY-293558	600	> 10,000	4 ip		230
			3.4 iv		

[a]IC_{50} values (nM) are mean of at least 3 determinations, each in triplicate.
[b]pretreatment time: ip and ip: 30 min., iv: 5 min; vehicle for ip and sc: 1% Tween-80 in water, vehicle for iv: saline.
[c]ED_{50} values (mg/kg) are defined as the dose which protected 50% of the animals from a tonic convulsion (6 male CD1 mice/dose of compound, with at least 3 doses plus 1 group receiving vehicle alone).
[d]ED_{50} values (mg/kg) are defined as the dose which protected 50% of the animals from an audiogenically induced tonic convulsion (6 DBA/2 mice/dose with 3 doses plus vehicle treated group).
[e]IC_{50} values (nM, except for **(+)-4** Kb value in nM, and **5** Kb value in nM from ref 11) for inhibition of currents generated by 50 μM kainate in *Xenopus* oocytes injected with rat brain mRNA.

Results and Discussion

Chemistry

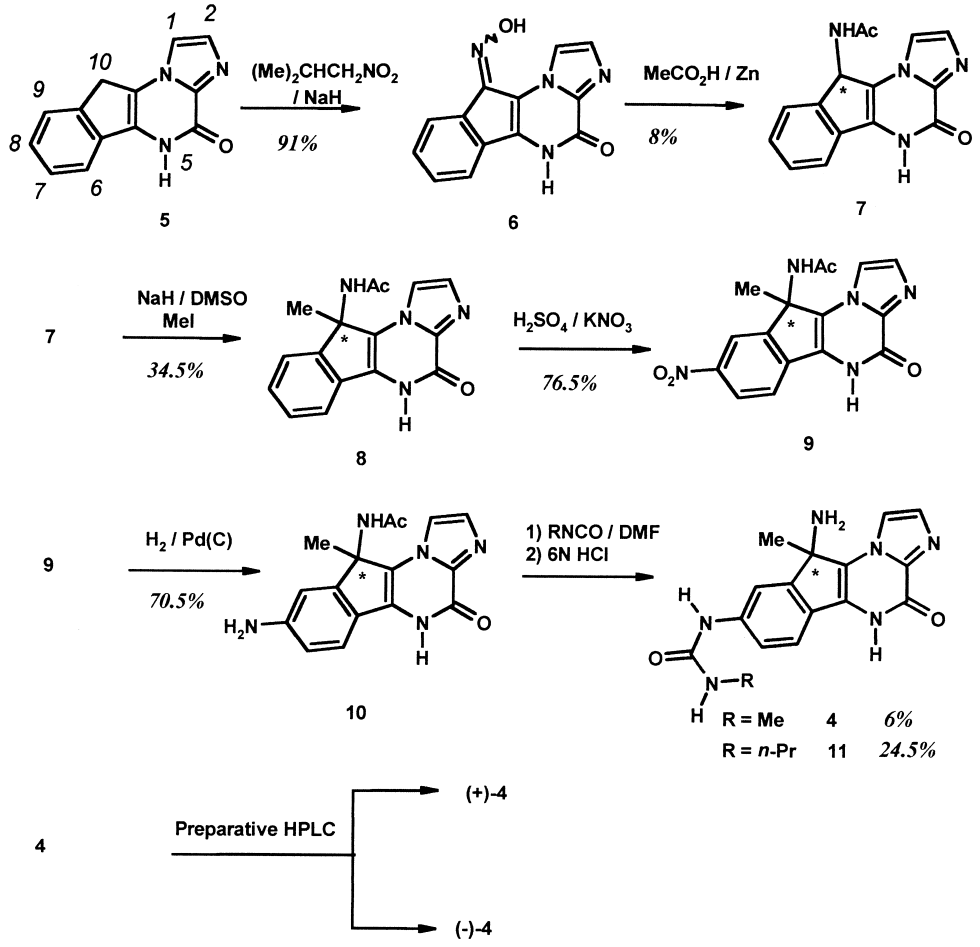
The synthesis and isolation of the targeted 10-amino-10-methyl-8-methylureido-imidazo[1,2-*a*] indeno[1,2-*e*]pyrazine-4-one derivatives **4, (+)-4, (−)-4** and **11** are outlined in Scheme 1. This route involves the reaction of **5**[12] with isoamyl nitrite in the presence of NaH, followed by the action of Zn in acetic acid giving **7** with a low yield. Then, the regioselective methylation of **7** in position 10 — using methyl iodide as the electrophile and NaH as base — gave **8** with 34.5% yield. The regioselective nitration of **8** using KNO_3 in concentrated sulfuric acid produced **9**. It was followed by the hydrogenation of the nitro group in the presence of a catalytic amount of Pd/C (10%) affording **10** with 54% overall yield. Finally, condensation of methylisocyanate or *n*-propylisocyanate in DMF, followed by *N*-acetyl deprotection with 6N HCl, gave, as expected, compounds **4** and **11** with 6% and 24.5% overall yield respectively.

The encouraging AMPA affinity of **4** (Table 1) prompted us to examine each of the enantiomers **(+)-4** and **(−)-4**. They were separated in an optically pure form from the racemic compound **4** by preparative chiral HPLC.

Biological activity

Binding studies. The affinities for AMPA receptors and the glycine modulatory site pertaining to the NMDA receptor were evaluated in in vitro binding assays using [^{3}H]-AMPA and [^{3}H]-5,7-dichlorokynurenate ([^{3}H]-DCKA) as selective ligands on rat cortical membrane preparations. Biological results for compounds **4**, **(+)-4, (−)-4, 5**, **11**, **12**, **YM90K** and **(-)-LY293558** are given in Table 1.

Introduction of a methylurea group in position **8** of the imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one cycle of **12** (which displayed only moderate combined AMPA and glycine/NMDA affinities with $IC_{50's}$ of 2.5 and 1.4 μM respectively;[8] chemical structure, see Figure 2) increased the AMPA binding potency 50-fold while the discrimination



Scheme 1. Synthesis of **4**, **(−)-4**, **(+)-4**, and **11**.

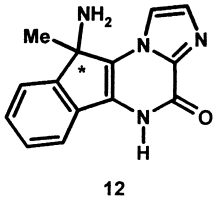


Figure 2. Chemical structure of **12**.[8]

for the AMPA receptor versus the glycine-binding site gained at least 250-fold (**4** versus **12**). On the other hand, substitution of the *N*-methyl group of **4** by a *n*-propyl reduced the AMPA binding potency 4-fold (**11** versus **4**), while still retaining high selectivity versus the glycine-binding site (170-fold). The **(+)-4** isomer displayed a 4-fold greater potency at the AMPA receptors ($IC_{50} = 10$ nM) than did the levorotatory molecule **(−)-4** ($IC_{50} = 42$ nM). Selectivity versus the glycine/NMDA receptors was in excess of 3200-fold for the dextrorotatory isomer.

In comparison with **5**, **YM90K** or **(−)-LY-293558**, our most potent isomer, **(+)-4b** exhibited a higher potency (between 35- to 76-fold) at the AMPA receptors and retained the selectivity (> 300-fold) against the glycine site of the NMDA receptors.

Functional studies. The functional activity of **4**, **(-)-4**, **(+)-4**, **5**, **11**, **12**, **YM90K** and **(−)-LY-293558** at AMPA receptors was determined using kainate-evoked currents in *Xenopus* oocytes injected with rat brain mRNAs 11. The potency of these ligands at AMPA receptors was examined using current response analysis. All compounds exhibited antagonist intrinsic activity against responses elicited by the non-desensitizing AMPA agonist kainate. There was an overall good correlation between the IC_{50} in this functional model and the binding affinities (Table 1). The mechanism of the antagonist activity was studied in some details for the most interesting compound **(+)-4** showing a Kb value of 29.5 nM (Fig. 3).

In vivo studies. The anticonvulsant effects were evaluated both in normal male CD1 swiss white mice submitted to an electric shock (Maximal Electroshock, MES) following ip, sc and iv administrations, and through audiogenic convulsions in DBA/2 mice following ip administration 30 min before challenges. Compounds **4**, **(+)–4** and **(−)-4** demonstrated moderate to potent in vivo activity against both types of convulsion (Table 1). Compounds **(+/−)-4** and **(+)-4** were found to have greater activity in in vivo models ($ED_{50} = 4.7$–10 mg/kg) following ip administration, unlike the levorotatory isomer **(−)-4** which was between 8- and 14-fold less potent in the MES test. Due to a good solubility in physiological saline

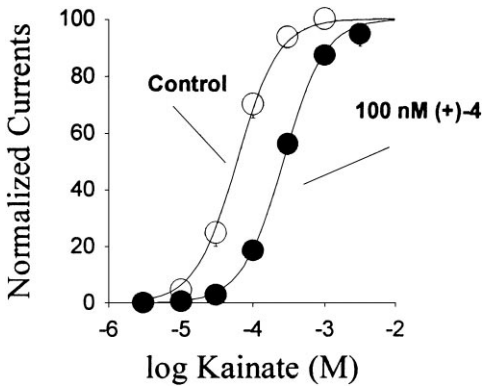


Figure 3. Antagonist activity of compound **(+)-4** against functional responses mediated by AMPA receptors in *Xenopus* oocytes injected with rat brain mRNA. In the presence of 100 nM of compound **(+)-4**, a parallel rightward shift of the kainate concentration–response curve was seen, indicating competitive antagonism at AMPA receptors. The equilibrium constant K_b calculated from the concentration-ratio was 29.5 nM (Hill 1.5).

Table 2. Duration of action of **(+)-4**, and YM-90K in the MES test following iv administration

Pretreatment time (min.)	5	15	30	60	180
ED_{50}[a](mg/kg)					
(+)-4	2.5	2.5	5	10	> 20
YM-90K	6.2	nd	24	40	> 40

[a]ED_{50} values (mg/kg) are defined as the dose which protected 50% of the animals from a tonic convulsion (6 male CD1 mice/dose of compound, with at least 3 doses plus 1 group receiving vehicle alone).

solution (~2 g/L), we evaluated the most potent derivative **(+)-4** by iv route in the MES test. This compound exhibited markedly potent activity with an ED_{50} of 2.5 mg/kg (5 and 15 min. before challenge) showing a long duration of action as reported in Table 2 (ED_{50} ≤10 mg/kg when administered 60 min before challenge). Compound **(+)–4** also showed a good anticonvulsant activity by sc route with an ED_{50} of 4.6 mg/kg (administered 30 min before challenge). In addition, **(+)-4** exhibited potent anticonvulsant effect in DBA/2 mouse model with an ED_{50} of 1.8 mg/kg ip

Starting from **5**,[12] the introduction of a gem methyl and amino groups in position 10 increased 2-fold the in vivo potency (ip administration) in the MES test (**12** versus **5**). The addition of a methylurea group in position 8 of **12** led to the title compound **4** showing a 5-fold higher potency. Isomer **(+)-4** displayed a similar activity to **YM90K** in the MES (ip administration). On the other hand it exhibited a higher level of biological activity following iv administration and a rather long duration of action suitable for activity close to what was observed with **(-)-LY-293558**. The 8-methylureido derivative **4** was found at least 15-fold more potent in vivo than the corresponding 8-*n*-propylureido analogue **11**.

Conclusion

This study reports a novel series of highly water-soluble 8-methylureido-10-amino-10-methyl-imidazo[1,2-*a*]indeno [1,2-*e*]pyrazine-4-one derivative **(+)-4** possessing one of the highest selective affinities reported to date for the AMPA receptor (IC_{50} = 10 nM). Functionally, it was able to inhibit kainate-induced currents in the nanomolar range (Kb: 29.5 nM). This compound also exhibited potent anticonvulsant effects following i.p., s.c. and i.v. administrations (ED_{50} ≤10 mg/kg). In addition, **(+)-4** has shown a long duration of action in the MES test following iv administration (ED_{50} ≤10 mg/kg when administered 60 min before challenge).

Experimental

In vitro assay

[^{3}H] AMPA binding. Radiolabelled AMPA binding assays in rat cerebral cortex membranes were performed as previously described[13] with the following modifications. Briefly, rats were decapitated and their cerebral cortices were removed on ice and immediately frozen at −80 °C for at least 1 h. The tissue was rapidly thawed, homogenized with a Polytron® in 20 vol of cold (4 °C) sucrose (0.32 M) and centrifuged at 1000×*g* for 20 min. The supernatant was re-centrifuged at 17,500×*g* for 20 min at 4 °C. The resulting pellet was suspended in 50 vol of ice-cold distilled water, incubated at 37 °C for 30 min and centrifuged at 32,000×*g* for 20 min at 4 °C. This procedure was repeated in order to remove any endogenous glutamate. The biological material thus obtained, was suspended in 50 vol of HEPES buffer (10 mM pH 7.5) and centrifuged at 32,000×*g* for 20 min at 4 °C. The pellet was finally re-suspended in 30 vol of ice-cold HEPES buffer and was frozen at −80 °C until use. On the day of the binding assays, the membranes were thawed and centrifuged at 32,000×*g* for 20 min at 4 °C. This procedure was repeated and the final pellet was suspended in a pH 7.5-buffer containing 10 mM KH_2PO_4 and 100 mM KSCN at a concentration of 0.2 mg protein/ml. Membranes were then incubated for 30 min at 4 °C with [^{3}H]AMPA (10 nM), the compound under study or 1 mM L-glutamate for determination of the non-specific binding. The binding interaction was terminated by filtration through glass fiber filters (Printed filtermat A) for Betaplate TM scintillation counter experiment using a Skatron micro cell harvester. The filters were immediately rinsed with 5 mL of cold buffer. The radioactivity remaining on the filters was measured by liquid scintillometry. Protein levels were measured by the method of Bradford (Bio-Rad Protein Assay).

[^{3}H] 5,7-Dichlorokynurenic acid binding. Affinity for the glycine site on the NMDA receptor was determined using radiolabelled 5,7-dichlorokynurenic acid in membranes from rat cerebral cortex according to previously published methods.[14,15] Briefly, rats were decapitated and their cerebral cortices removed on ice and frozen at −80 °C for at least 1 h. The tissue was rapidly thawed, homogenized with a Polytron® in 10 vol of cold (4 °C) sucrose (0.32 M) and centrifuged at 1000×*g* for 10 min. The supernatant was re-centrifuged at 20,000×*g* for 20 min. The resulting pellet was suspended in 20

volumes of ice-cold distilled water and centrifuged at 8000×g for 20 min. The supernatant and buff layer were collected and centrifuged at 48,000×*g* for 20 min. The pellet was suspended in 20 vol of ice-cold distilled water and centrifuged at 48,000×*g* for 20 min. The final pellet was frozen at −20°C until use. On the day of the binding assays, the membranes were thawed and suspended in 20 vol of HEPES-KOH buffer (50 mM, pH 7.5), incubated at 37 °C for 20 min and then centrifuged at 48,000×*g* for 10 min. This procedure was repeated and the final pellet was re-suspended in the appropriate buffer at a concentration of 0.1 mg protein/mL. Incubation was then implemented for 10 min at 4 °C with [^{3}H]5,7-dichlorokynurenic acid (20 nM), the compound under study or 1 mM glycine for determination of the non-specific binding. The binding interaction was terminated by filtration through Whatman GF/B glass fiber filters, and filters were immediately rinsed three times with 4 mL of cold HEPES-KOH buffer (pH 7.5, containing 10 mM magnesium sulfate). The radioactivity remaining on the filters was measured by liquid scintillometry. Protein levels were measured by the method of Bradford (Bio-Rad Protein Assay).

In vivo assay

Anticonvulsant activity was evaluated in groups of male mice (CD1 Charles River) against tonic convulsions induced by supramaximal electroshock, according to previously described methods[16] or against audiogenic seizures in 21 day old DBA/2 mice.[17] For supramaximal electroshock, tested compounds were administered to groups of 6 male mice (bw 18–22 g) at at least 3 sequential doses by ip and sc routes in 1% tween in distilled water and by iv route in physiological saline, with a control group of 6 mice receiving vehicle alone. Animals then remained in their home cage. Thirty min later a 50 mA, 50 Hz electric shock was administered via ocular electrodes for 0.2 s. The ED_{50} was defined as the dose of tested compounds which protected 50% of animals from tonic convulsions. For audiogenic seizures, groups of 6 DBA/2 mice were treated with the compound ip at at least 3 sequential doses 30 min before being exposed to a 126 db sound at 12 KHz. The occurrence of wild running behavior, clonic and tonic convulsions was observed. The ED_{50} was defined as the dose of tested compound which protected 50% of animals from tonic convulsions.

Functional assay

The antagonist activity of **4, (−)-4, (+)-4, 5, 11, 12, YM90K** and **(-)-LY-293558** at AMPA receptors was determined using kainate-evoked currents in *Xenopus* oocytes injected with rat brain mRNAs as previously described.[11] In brief, oocytes were removed from ovarian lobes of anaesthetized *Xenopus laevis* (150–300 g) and defolliculated for 1 h with collagenase (2 mg/mL) in Barth's medium containing: NaCl (88 mM), KCl (1 mM), $MgSO_4$ (0.82 mM), $NaHCO_3$ (2.4 mM), $Ca(NO_3)_2$ (0.33 mM), $CaCl_2$ (0.41 mM) and HEPES (10 mM). Oocytes were then injected with 50 ml poly (A)+mRNAs extracted from 10 to 12-day-old rat cerebral cortex. Injected oocytes were maintained for at least 48 h at 19 °C in Barth's medium containing streptomycin (10 mg/mL) and penicillin (100 units/ml) before being used for voltage-clamp experiments. On the day of study, oocytes were mounted individually in a small recording chamber (300 μL) and superfused with a Ringer medium containing: NaCl (90 mM), KCl (1 mM), $MgCl_2$ (1 mM), $CaCl_2$ (1 mM), HEPES (5 mM) at a flow rate of 6 mL/min. Current responses were recorded with 3 M KCl (0.8–2.5 MΩ)-filled glass microelectrode at a holding potential of −60 mV with a two-electrodes voltage-clamp amplifier. Tested compounds were prepared as a 10^{-3} to 10^{-2} M stock solution in DMSO, except for **LY-293558** directly prepared in the recording Ringer medium. Appropriate final bath concentrations were prepared by dilution in this medium for all other compounds. For IC_{50} studies, kainate at a supra-threshold and sub-maximal concentration (50 μM) was used as the agonist. Concentration–response curves were constructed in the absence and in the presence of increasing concentrations of test compounds. For pK_b determination, concentration-response curves to kainate were constructed in the absence and in the presence of 100 nM of **(+)-4**. The amplitudes of the evoked responses were expressed as a percentage of the maximal pre-drug baseline control responses. EC50 values were determined by a non-linear least square regression procedure according to a sigmoidal equation (Graphpad Prism 2.01).

Chemistry

General

Commercially available reagents were used as received from suppliers. Solvents (THF, diethyl ether) were dried over 4Å molecular sieves. The progress of the reactions was monitored by TLC on silica gel (Merck Kieselgel $60F_{254}$). Melting points were determined using a Reicher–Kofler apparatus and are uncorrected. All ^{1}H NMR spectra were recorded at 200, 250 or 300 MHz using Bruker spectrometers in DMSO-d_6 solutions. Chemical shifts are reported in δ ppm with DMSO as internal standard (δ = 2.5 ppm). Coupling constants are given in Hz. ^{1}H are identified according to numbers provided in Scheme 1. IR spectra (KBr dispersions) were recorded between 4000 and 400 cm^{-1} using a 60SXR Nicolet FT–IR spectrophotometer. Mass spectra were obtained on a VG Autospec (LSIMS, Cs gun at 35 kV; in glycerol/thioglycerol as a matrix) or on a Nermag R10-10B (CIMS, NH_3). Elemental analyses were determined using Fisons 1108 analysers while rotations were obtained with a Perkin Elmer 341 polarimeter. Karl Fischer as well as classical titrimetric methods were also used for % water or halogen atom determinations.

10-Hydroxyimino-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one (6). To a suspension of **5** (22.3 g, 0.1 mol) in dry DMSO (300 mL) was added portion-wise 80% NaH (7.2 g, 0.24 mol) at room temperature under nitrogen atmosphere. The resulting mixture was stirred for 1 h. Then, isoamyl nitrite (12.9 g, 15 mL, 0.11 mol) in dry

DMSO (40 mL) was added dropwise and the reaction mixture was stirred for an additional 1h. Water (50 mL) was added and the reaction mixture was poured onto an ice/water mixture (~550g/1.1 L) and acidified to pH = 4 with acetic acid (20 mL). The precipitate thus obtained was washed with water (2×200 mL) and then dried in vacuo to give 23 g of title compound (91%). It was recrystallized in hot acetone to give **6** as an orange solid: mp > 260 °C; ^{1}H NMR (200 MHz):13 (1H, brs, H_5), 12.7 (1H, brs, OH), 8.2 (1H, dd, J = 8 Hz, 1.5 Hz, H_6), 8 (1H, brs, H_1), 7.8 (1H, dd, J = 8 Hz, 1.5 Hz, H_9), 7.6 (1H, brs, H_2), 7.5 to 7.35 (2H, m, H_7, H_8). Attempts to determine the orientation of the oxime group by nOe failed due to the fast exchange rates of mobile protons (H_5, OH and residual H_2O); IR: 3250, 1670, 1005 (oxime); CIMS m/z 253 (MH^+). Anal. $C_{13}H_8N_4O_2$ requires C, 61.90%; H, 3.20%; N, 22.21%; O, 12.69%; found C, 62.04%; H, 3.23%; N, 22.34%; O, 12.86%.

10-Acetamido-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one (7). To a stirred suspension of **6** (5.25 g, 20 mmol) in acetic acid (100 mL) was added portion wise Zn (2.9 g, 44 mAtg) at room temperature. The reaction was complete after heating the solution for 3 h at 80–90 °C. Then, the reaction mixture was cooled to room temperature and acetic acid (100 mL) was added. The resulting solid was collected and filtered through Celite. The filtrate was evaporated in vacuo to give a residue which was triturated with water (100 mL) to afforded a brown solid. This solid was washed with water (10 mL) and acetone (10 mL) and then recrystallized in hot DMF to give 0.43 g of **7** (8%) as a pale yellow solid: mp > 260 °C; ^{1}H NMR (200 MHz): 12.5 (1H, brs, H_5), 8.60 (1H, d, J = 8.5 Hz, NH), 7.8 (1H, brd, J = 8 Hz, H_6), 7.65 (1H, brs, H_1), 7.6 (1H, brs, H_2), 7.55–7.35 (3H, m, H_7, H_8, H_9), 6.15 (1H, d, J = 8.5 Hz, H_{10}), 2 (3H, s, Me); CIMS m/z 281 (MH^+). Anal. $C_{15}H_{12}N_4O_2$ requires C, 64.28%; H, 4.32%; N, 19.99%; O, 11.42%; found C, 64.12%; H, 4.56%; N, 20.06%; O, 11.78%.

10-Acetamido-10-methyl-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one (8). To a suspension of **7** (3.54 g, 126 mmol) in dry DMSO (80 mL) was added portion wise 80% NaH (0.86 g, 36 mmol) at room temperature under nitrogen atmosphere. The resulting mixture was stirred for 20 min., and then methyl iodide (1.7 g, 12 mmol) in dry DMSO (8 mL) was added drop-wise .The reaction mixture was stirred at room temperature for an additional 1h. Water (40 mL) was added and then the mixture was poured onto ice:water (~300 g:760 mL). The resulting solution was acidified until pH = 4 with acetic acid (4 mL). The organic layer was then separated and poured into water (200 mL). The precipitate was washed with water (2×80 mL) and treated with hot ethanol (20 mL) to give **8** as a solid (1.28 g, 34.5%): mp > 260 °C; R_f = 0.25 (CH_2Cl_2:MeOH 80:20); ^{1}H NMR (200 MHz): 12.4 (1H, brs, H_5), 8.60 (1H, brs, NH), 7.9 (1H, brs, H_1), 7.8 (1H, dd, J = 8 Hz, 1.5 Hz, H_6), 7.65 (1H, brs, H_2), 7.55–7.35 (3H, m, H_7, H_8, H_9), 1.8 (3H, s, Me), 1.7 (3H, s, Me); LSIMS m/z 295 (MH^+). Anal. $C_{16}H_{14}N_4O_2$ requires C, 65.30%; H, 4.79%; N, 19.04%; O,10.87%; found C, 65.62%; H, 4.44%; N, 18.85%; O,10.82%.

8-Nitro-10-acetamido-10-methyl-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one (9). To a solution of concentrated sulfuric acid (150 mL) at 5 °C was added **8** (13.6 g, 46 mmol) portion-wise, and the reaction mixture was stirred for 30 min at 5 °C. Then, potassium nitrate (4.67 g, 46 mmol) was added, and the reaction mixture was stirred for an additional 1.5 h at the same temperature. Then, the reaction mixture was poured onto ice water (1 L) to yield a suspension. The solid was collected by filtration, washed with water, and dried in vacuo to give **9** as a yellow solid (12 g, 76.5%): mp > 260 °C; ^{1}H NMR (200 MHz): 12.8 (1H, brs, H_5), 8.8 (1H, brs, NH), 8.3 (2H, m, H_7, H_9), 8.1 (1H, brs, H_1), 8 (1H, d, J = 8 Hz, H_6), 7.85 (1H, brs, H_2), 1.85 (3H, s, Me), 1.7 (3H, s, Me); IR: 1530, 1345 (NO_2); CIMS m/z 340 (MH^+).

8-Amino-10-acetamido-10-methyl-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one (10). A solution of **9** (12 g, 35 mmol) in DMF (200 mL) was hydrogenated at atmospheric pressure using Pd/C (10%) (1.5 g) as catalyst at room temperature. The suspension was filtered through Celite and the filtrate was evaporated to give a residue which was recrystallized from an ethanol:water mixture (75:25) to give **10** as a red solid (7.7 g, 70.5%): mp > 260 °C; R_f = 0.25 (CH_2Cl_2:MeOH 85:15); ^{1}H NMR (300 MHz): 12.4 (1H, brs, H_5), 8.65 (1H, brs, NH), 7.9 (1H, brs, H_1), 7.65 (1H, brs, H_2), 7.5 (1H, d, J = 8 Hz, H_6), 6.85 (1H, d, J = 1.5 Hz, H_9), 6.65 (1H, dd, J = 8 Hz, 1.5Hz, H_7), 1.8 (3H, s, Me), 1.65 (3H, s, Me); IR: 3430, 3350, 1625 (NH_2); CIMS m/z 310 (MH^+).

8-Methylureido-10-amino-10-methyl-imidazo[1,2-*a*]indeno [1,2-*e*]pyrazine-4-one. 2HCl.H_2O (4). To a stirred solution of **10** (7 g, 22 mmol) in dry DMF (130 mL) was added drop wise methylisocyanate (4.7 mL, 80 mmol) at room temperature. The reaction mixture was allowed to stir for 15 h and concentrated in vacuo. The crude residue was filtered, washed successively with water and ethyl acetate and finally refluxed with 6N HCl (20 mL) for 2 h and slowly cooled to room temperature. The resulting precipitate was filtered and washed with acetone to afford **4** as a pale yellow solid (0.6 g, 6%): mp > 260 °C; ^{1}H NMR (200 MHz): 13.1 (1H, brs, H5), 9.55 (3H, brs, NH_3^+), 9.35 (1H, brs, NH), 9 (1H, brs, H_1), 8.15 (1H, brs, H_9), 7.95 (1H, brs, H_2), 7.8 (1H, d, J = 8 Hz, H_6), 7.60 (1H, brd, J = 8 Hz, H_7), 6.5 (1H, brs, NH), 2.7 (3H, s, NMe), 1.9 (3H, s, Me); LSIMS m/z 325 (MH^+). Anal. $C_{16}H_{16}N_6O_2.2HCl\ .H_2O$, requires C, 46.28%; H, 4.85%; Cl, 17.07; N, 20.24%; O, 11.56%; found C, 46.36%; H, 4.72%; N, 20.03%; O, 11.81%.

8-Methylureido-10-acetamido-10-methyl-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one derivatives (+)-4 and (−)-4. The two enantiomers **(+)-4** and **(−)-4** were prepared in optically pure form from the racemic compound **4** by preparative HPLC using a column packed with a chiral stationary phase (Chiracel OD) and eluted with a 20:80 mixture of heptane:ethanol and 0.05% of TEA. Fifteen runs were necessary for the separation, starting from 6 g of **4** (flow-rate: 35 mL/min, detection: UV (265 nm), column diameter: 60 mm, column length:

23 cm) giving **(+)-4** as a pale yellow solid [1.47 g, 49%, mp > 200 °C, $\alpha_D^{20} = +\ 81.0 \pm 1.0$ (CH_3CO_2H, c = 2)] and **(−)-4** as a white solid (1.79 g, 59.6%, mp > 200 °C, $\alpha_D^{20} = -90.3 \pm 1.2$ (CH_3CO_2H, c = 2)). Enantiomeric homogeneity of both enantiomers **(+)-4** (> 90%) and **(−)-4** (> 98%) was evaluated by analytical HPLC using the same chiral phase.

(+)-4. ^{1}H NMR (300 MHz): 12.25 (1H, brs, H_5), 8.7 (1H, s, NH), 8.2 (1H, d, $J = 0.5$ Hz, H_1), 7.8 (1H, d, $J = 1.5$ Hz, H_9), 7.65 (1H, d, $J = 8$ Hz, H_6), 7.6 (1H, d, $J = 0.5$ Hz, H_2), 7.35 (1H, dd, $J = 8$ Hz, 1.5 Hz, H_6), 6.1 (1H, brq, NH), 2.7 (3H, d, $J = 4.5$ Hz, NMe), 1.55 (3H, s, Me).^{1}H attributions were revealed with nOe enhancements observed between the gem methyl in position 10 and H_1, H_9 on one hand, and the ureido NH proton with H_7, H_9 on the other. IR: 3330, 1675, 1565 (urea); LSIMS *m/z* 325 (MH^+). Anal. $C_{16}H_{16}N_6O_2 .C_2H_6O$, requires C, 58.36%; H, 5.99%; N, 22.69%; O, 12.96%; found C, 58.45%; H, 6.04%; N, 22.78%; O, 13.12%.

(−)-4. Anal. $C_{16}H_{16}N_6O_2 .C_2H_6O$, requires C, 58.36; H, 5.99%; N, 22.69%; O, 12.96%; found C, 58.61%; H, 6.03%; N, 23.06%; O, 12.86%. Other analytical data—except rotation—are identical to **(+)–4**.

8-*n*-Propylureido-10-amino-10-methyl-imidazo[1,2-*a*]indeno [1,2-*e*]pyrazine-4-one. HCl.3H$_2$O (11). To a stirred solution of **10** (2 g, 6 mmol) in dry DMF (15 mL) was added drop wise *n*-propylisocyanate (1.8 mL, 19 mmol) at room temperature. The reaction mixture was allowed to stir for 12 h. The resulting precipitate was collected and washed with DMF and then acetone. The crude residue was finally refluxed with 6N HCl (20 mL) for 2 h and slowly cooled to room temperature. The resulting precipitate was filtered and washed with acetone to give **11** as a pale yellow solid (0.65 g, 24.5%): mp 220 °C; ^{1}H NMR (250 MHz): 12.2 (1H, brs, H_5), 9.5 (3H, bs, NH_3^+), 9.2 (1H, brs, NH), 8.85 (1H, s, H1), 8.2 (1H, bs, H_9), 7.85 (2H, m, H_2, H_6), 7.55 (1H, dd, $J = 8$ Hz, 1.5Hz, H_7), 6.7 (1H, brs, NH), 3.1 (2H, brt, $J = 7$Hz, NCH_2), 1.9 (3H, s, Me), 1.5 (2H, m, CH_2), 0.9 (3H, t, $J = 7$ Hz, Me); IR: 3250, 1695, 1545 (urea); LSIMS *m/z* 353 (MH^+). Anal. $C_{18}H_{20}N_6O_2 .HCl.3H_2O$ requires C, 48.83%; H, 6.14%; Cl, 8.00%; N, 18.97%; O, 18.06%; found C, 48.63%; H, 6.18%; Cl, 8.05%; N, 19.24%.

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