Synthesis of Some New Linear and Chiral Macrocyclic Pyridine Carbazides as Analgesic and Anticonvulsant Agents

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A series of 2,6-disubstituted pyridine derivatives were prepared from 2,6-diacetylpyridine or 2,6-dicarbonyl pyridine dichloride as starting materials. Reaction of 2,6-diacetylpyridine **1** with hydroxylamine hydrochloride or different aromatic aldehydes afforded the corresponding 2,6-diacetylpyridine dioxime and 2,6-*bis*-[β-(2-thienyl)acryloyl]pyridine derivatives **2** and **3**, respectively. Additionally, $N^2,N^{2'}$-(pyridine-2,6-dicarbonyl)-L-amino acid hydrazides **5** were prepared starting from 2,6-dicarbonyl pyridine dichloride *via* the corresponding esters **4**. Compound **3** was reacted with hydroxylamine hydrochloride to afford the 2,6-*bis*-[β-(2-thienyl)acryloyl-oxime]-pyridine derivative **6**. Treatment of compounds **2** or **6** with phenyl isocyanate or phenyl isothiocyanate in refluxing dioxane gave the corresponding semicarbazide or thiosemicarbazide derivatives **7** and **8**, respectively. Their treatment with toluene-3,5-diisocyanate afforded the macrocyclic semicarbzides **9** and **10**, respectively. The chiral thiosemicarbazides **11a,b** were however, prepared by treating compounds **5a,b** with phenyl isothiocyanate followed by cyclization with sodium hydroxide (2N) yielding the triazoles **12a,b**. Finally, the hydrazides **5a,b** were treated with toluene-3,5-diisocyanate to afford the chiral macrocyclic tetrapeptide semicarbazides **13a,b** in reasonable yields, while the expected cyclic dipeptide **14** was not formed. The structure assignments of the new compounds were based on chemical and spectroscopic evidence.

The pharmacological screening showed that many of these compounds have good analgesic and anticonvulsant activities comparable to Voltarine® and Carbamazapine® used as reference drugs.

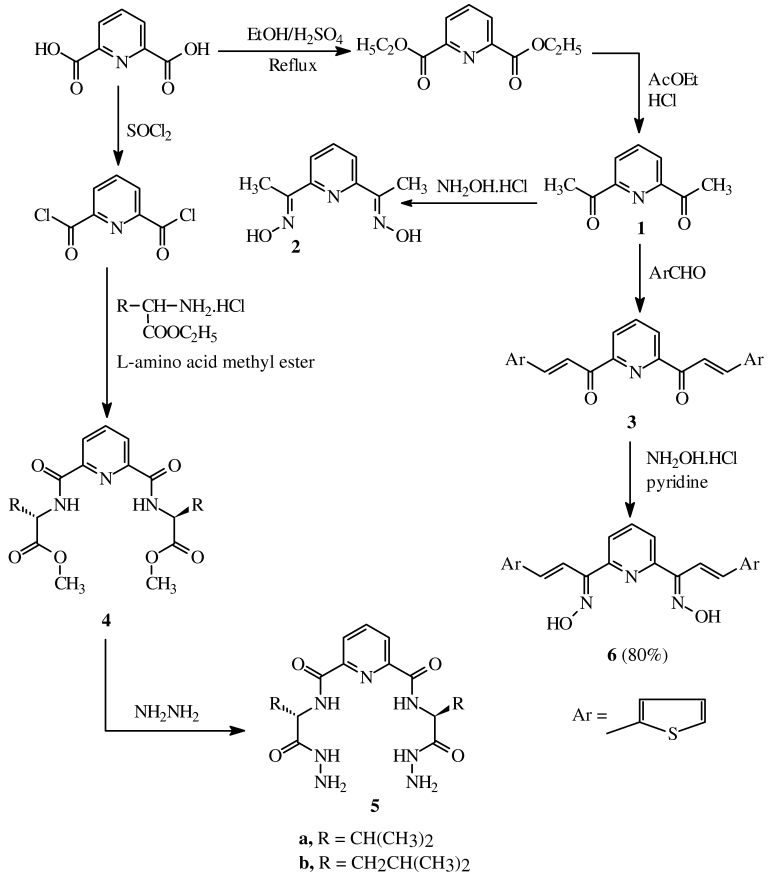
Key words: 2,6-Disubstituted Pyridine, Semicarbazides, Thiosemicarbazides, Chiral Macrocyclics, Anticonvulsants, Analgesics

Introduction

In previous work we reported that certain substituted pyridines and their chiral macrocyclic derivatives have antimicrobial and antiinflammatory activities [1 – 6], and antitumor properties [7 – 9]. We also demonstrated that some peptidopyridine derivatives exhibit a general ionophoric potency for divalent cations [10] and are useful for assembling novel thiocyanate-selective membrane sensors [11]. Recently, some new 2,6-disubstituted pyridine derivatives were synthesized which exhibit analgetic, antipa rkinson and androgenic anabolic activities [12, 13]. On the other hand, semicarbazide, thiosemicarbazide and macrocyclic pyridine derivatives show promising biological activities [14 – 16]. In view of these observations and as continuation of our previous work on pyridine chemistry, we have synthesized some new macrocyclic compounds containing pyridine moieties and tested their selected biological activities.

Results and Discussion

In our previous work we reported the synthesis and a preliminary biological activity screening of several chiral macrocyclic derivatives based on $N^2,N^{2'}$-(pyridine-2,6-dicarbonyl)-L-amino acid hydrazides (**5a,b**) [5] which were obtained from the corresponding ester **4** according to the published procedures [1, 2]. Similarly, 2,6-diacetylpyridine dioxime **2** and 2,6-*bis*-[β-(2-thienyl)acryloyl]pyridine **3** were prepared as starting materials according to literature reports [4, 17]. Condensation of compound **3** with hydroxylamine hydrochloride in pyridine afforded the corresponding 2,6-*bis*[β-(thienyl)acryloyl]pyridine dioxime **6** (Scheme 1). The IR spectrum of compound **6** showed bands corresponding $\tilde{\nu}$ (OH) and $\tilde{\nu}$ (C=N) and while devoid of bands corresponding $\tilde{\nu}$ (C=O). The structure of starting materials **2**, **3** and **5** were confirmed by elemental analysis as well as spectroscopic data in comparison with authentic samples [3 – 5, 17].

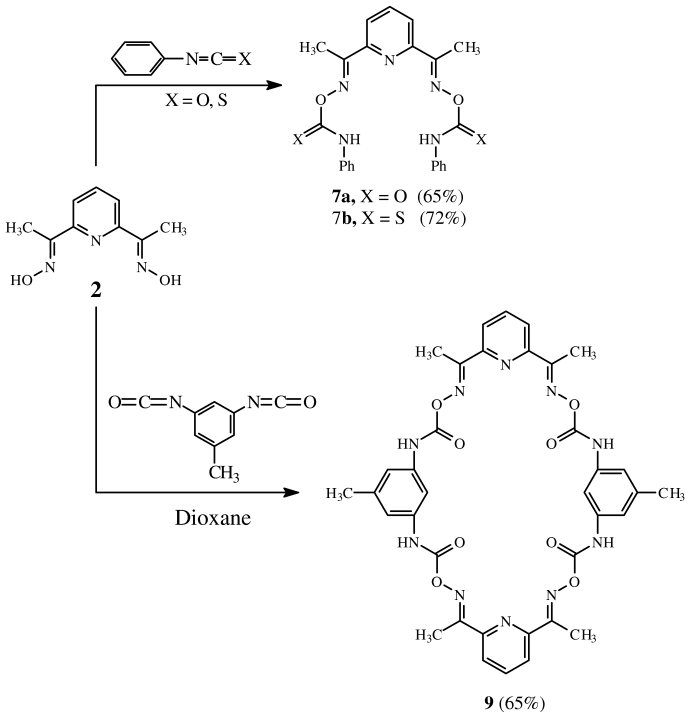


Scheme 1.

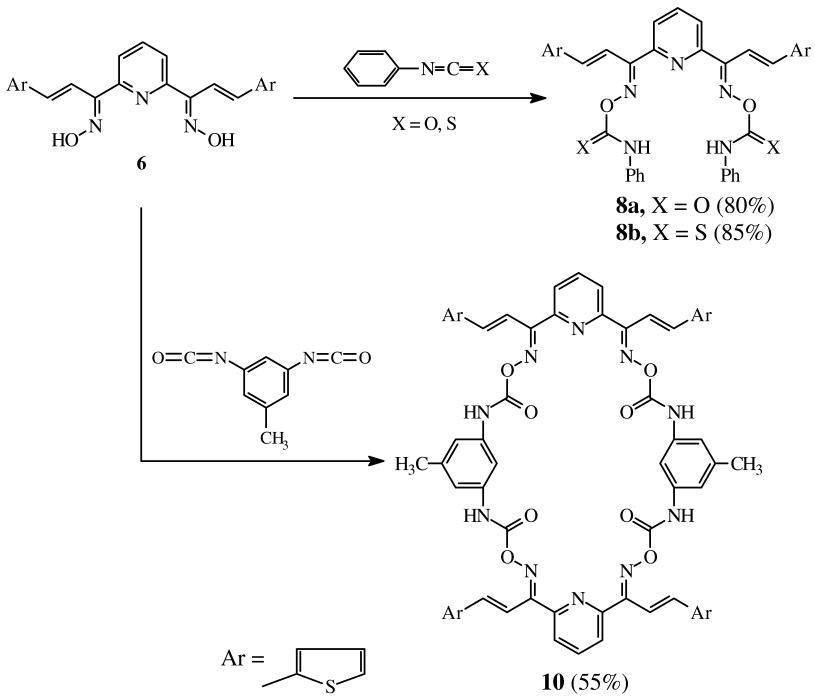
Treatment of 2,6-diacetylpyridine dioxime (**2**) with phenyl isocyanate or phenyl isothiocyanate in refluxing dioxane afforded the corresponding semicarbazides and thiosemicarbazides **7a,b**, respectively. When compound **2** was treated with toluene-3,5-diisocyanate in refluxing dioxane, the corresponding macrocyclic semicarbazide **9** was obtained (Scheme 2). The IR spectra of compounds **7** and **9** showed bands corresponding to $\tilde{\nu}$ (NH), $\tilde{\nu}$ (C=O) and $\tilde{\nu}$ (C=S), while devoid of bands corresponding to OH present in compound **2**.

2,6-*Bis*-[β-(2-thienyl)acryloyl]pyridine dioxime derivative **6** was reacted with phenyl isocyanate or phenyl isothiocyanate in refluxing dioxane to afford the corresponding 2,6-bis-semi- and thiosemicarbazides **8a,b**, respectively. While, reaction of **6** with toluene-3,5-diisocyanate yielded the macrocyclic semicarbazide derivatives **10** (Scheme 3).

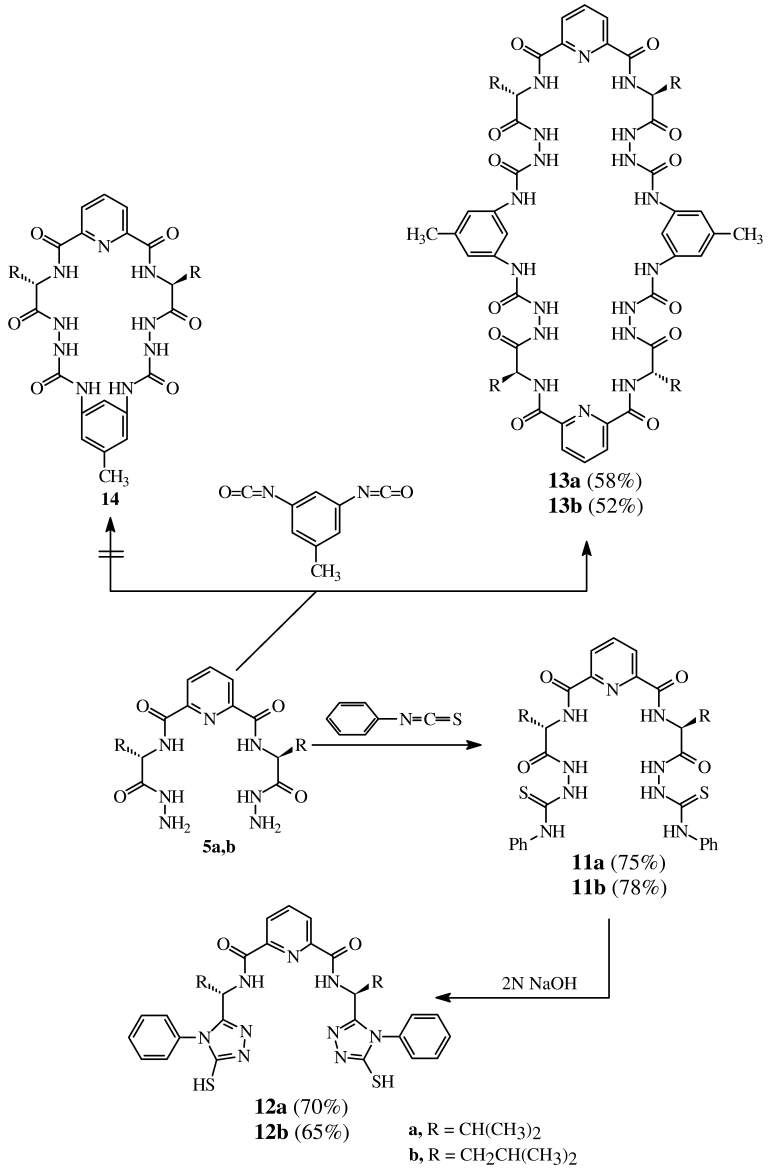
In addition, treatment of $N^2,N^{2'}$-(pyridine-2,6-dicarbonyl)-L-amino acid hydrazides (**5a,b**) with phenyl isothiocyanate in refluxing dioxane afforded the corresponding chiral thiosemicarbazide derivatives **11a,b**, respectively. The IR spectra of compounds **11** included bands corresponding to NH of amide groups while devoid of bands corresponding to NH_2 present in the parent compounds **5**. Compounds **11a, b** could be cyclized by the action of sodium hydroxide (2N) affording the corresponding 2,6-*bis*-triazole derivatives **12a,b**, respectively. The IR spectra of compounds **12** showed week bands at 2250 cm^{-1} corresponding to $\tilde{\nu}$ (SH) and devoid of bands corresponding to $\tilde{\nu}$ (C=S). When compounds **5a,b** were reacted with toluene-3,5-diisocyanate under the same conditions, the chiral macrocyclic tetrapeptide semicarbazide pyridine derivatives **13a,b** were obtained after purification by column chromatography using chloroform/methanol as eluent (Scheme 4). As expected, the mass spectral data confirmed that the bridged cyclic dipeptides **14a,b** were not formed [2].



Scheme 2.



Scheme 3.



Scheme 4.

Pharmacological Screening

Seven representative compounds were studied with respect to their analgesic and anticonvulsant activities.

Analgesic activity

All tested compounds exhibited analgesic activities (Table 1). The most potent was **13b** which showed the same activity as Voltarine® after 45 min. and had even higher activity after 60, 90 and 120 min., respectively. Similarly, the analgesic activities of **7a** and **10** approached those of Voltarine®.

Anticonvulsant activity

While compounds **8b** and **9** were devoid of any anticonvulsant activity, and provided no protection against yohinobine-induced clonic seizures, compounds **7a**, **10** and **13a** showed interesting anticonvulsant activities. Their relative potencies to Carbamazebene® were 0.60, 0.96 and 0.72. Compounds **13a** and **13b** were

Table 1. Analgesic activity (mice) of the new compounds compared to Voltarene®.

Comp. No.	Analgesic activity after 10 min	20 min	30 min	45 min	60 min	90 min	120 min
Voltarine®	1	1	1	1	1	1	1
7a	0.56	0.58	0.70	0.75	0.75	0.80	0.78
8b	0.80	0.84	0.88	0.89	0.89	0.89	0.89
9	0.52	0.54	0.56	0.50	0.50	0.50	0.45
10	0.80	0.90	0.89	0.92	0.92	0.92	0.93
12b	0.60	0.62	0.70	0.73	0.73	0.73	0.75
13a	0.30	0.38	0.38	0.42	0.43	0.41	0.50
13b	0.96	0.97	0.96	1.00	1.10	1.18	1.38

Table 2. Anticonvulsant activity of some new compounds as compared to Carbamazepine® in mice.

Comp. No.	ED_{50} Value (mg/kg)	Relative potency of Carbamazepine®
Control	0	0
Carbamazepine®	29	1
7a	50	0.60
8b	no protection	–
9	no protection	–
10	31	0.96
12b	35	1.72
13a	15	1.94
13b	13	2.20

even more potent than Carbamazebene® (1.94 and 2.20, relative potency) (Table 2).

Experimental Section

Melting points are uncorrected and were taken on Electrothermal IA 9000 Digital Melting Point Apparatus. Analytical data were obtained from the Microanalytical Unit, Cairo University, Egypt. The IR spectra (KBr) were recorded on a Pe Unicam SP-1000 Spectrometer. The NMR spectra were measured with Varian Gemini 270 MHz in DMSO-d_6 and the chemical shifts were recorded in δ-scale ppm relative to TMS as an internal standard. The mass spectra were taken at 70 eV with a Finning SSQ 7000 GC/MS Spectrometer using Electron Ionization Technique (EI). All reactions were followed by TLC (Silica gel, aluminum sheets $60F_{254}$, Merck). The starting materials **2**, **3** and **5** were prepared according to the reported procedures [1, 2, 4, 5, 17].

Synthesis of 2,6-bis-[β-(2-thienyl)acryloyl]pyridine dioxime (6)

A mixture of 2,6-*bis*-[β-(2-thienyl)acrylopyl]pyridine (**3**) (0.35 g, 1 mmol) and hydroxylamine hydrochloride (0.192 g, 2 mmol) in dry pyridine (30 ml) was refluxed for 6 h. The reaction mixture was cooled, poured into ice-water and neutralized with 1N hydrochloric acid. The product was extracted with ethyl acetate, dried over anhydrous sodium sulphate, evaporated under reduced pressure. The obtained residue was triturated with ether, and solidified by washing with *n*-hexane/petroleum ether (40 – 60 °C). Finally the obtained solid was collected by filtration, dried and crystallized from methanol to give compound (**6**).

M. p. 160 – 2 °C (MeOH). – IR (film): $\tilde{\nu} = 3525 - 3460$ (OH), 1670 (C=N), 1605 – 1595 cm^{-1} (C=C). – ^{1}H NMR (270 MHz, DMSO-d_6). $\delta = 2.30$ (s, 2H, $2 \times OH$, exchangeable with D_2O), 6.60 (d, $J = 14.60$ Hz, 2H, $2 \times CH$-thiophene), 7.00 (d, $J = 14.65$ Hz, 2H, $2 \times CH$-C=N), 7.30 – 7.60 (m, 6H, $2 \times$ thiophene-*H*), 8.10 – 8.30 (m, 3H, pyr-*H*). – $^{13}C\{^1H\}$ NMR (270 MHz, DMSO-d_6). $\delta = 120.10$, 121.20, 128.45, 132.20 (all thiophene-*C*), 134.80, 137.50 (4*C*H), 158.60 (2*C*=N), 124.10, 139.3, 148.45 (pyr-*C*). – MS (EI, 70 eV): *m/z* (%) = 381 (5) [M^+], 347 (100) [M^+-2OH], 215 (80) [M^+-$2C_4H_3S$]. – $C_{19}H_{15}N_3O_2S_2$ (381.46): calcd. C 59.82, H 3.96, N 11.01; found C 59.75, H 3.88, N 10.98.

Synthesis of 2,6-bis-(phenylcarbazide)pyridine derivatives (7a,b)

A mixture of 2,6-diacetylpyridine dioxime (**2**) (0.2 g, 1 mmol) and phenyl isocyanate or phenyl isothiocyanate (2 mmol) in 50 ml dry dioxane containing 2 ml of triethylamine was heated under reflux for 7 h. The solvent was evaporated under reduced pressure and the residue was solidified with petroleum ether (40 – 60 °C). The obtained solid was filtered off and crystallized from the proper solvents to give the corresponding semi- and thiosemicarbazide derivatives **7a,b**, respectively.

*2,6-Bis-(phenylsemicarbazide)pyridine derivative (***7a***)*

M. p. 158-160 °C (dioxane). – IR (film): $\tilde{\nu} = 3340 - 3280$ (NH), 1710 – 1690 (C=O), 1665 – 1650 cm^{-1} (C=N). – ^{1}H NMR (270 MHz, DMSO-d_6). $\delta = 1.80$ (s, 6H, 2CH_3), 6.90 – 7.10 (m, 10H, 2Ph-*H*), 7.90 – 8.25 (m, 3H, pyr-*H*), 8.50 (s, 2H, 2N*H*, exchangeable with D_2O). – $^{13}C\{^1H\}$ NMR (270 MHz, DMSO-d_6). $\delta = 16.90$, 17.10 (2CH_3), 119.20, 127.80, 128.25, 134.50 (all Ph-*C*), 124.15, 139.0, 147.90 (all pyr-*C*), 150.10 (2*C*=N), 156.65 (2*C*=O). – MS (EI, 70 eV): *m/z* (%) = 431 (15) [M^+], 277 (65) [M^+-$2C_6H_5$], 191 (80) [M^+-2PhNHC≡O], 120 (100) [PhNHC≡O]. – $C_{23}H_{21}N_5O_4$ (431.45): calcd. C 64.02, H 4.90, N 16.23; found C 64.00, H 4.86, N 16.18.

*2,6-Bis-(phenylthiosemicarbazide)pyridine derivative (***7b***)*

M.p. 130 – 2 °C (dioxane). – IR (film): $\tilde{\nu} = 3330 - 3300$ (NH), 1670 – 1660 (C=N), 1225 – 1210 cm^{-1} (C=S). – ^{1}H NMR (270 MHz, DMSO-d_6). $\delta = 1.90$ (s, 6H, 2CH_3), 4.20 – 4.60 (bs, 2H, 2N*H*, exchangeable with D_2O), 6.85 – 7.00 (m, 10H, 2Ph-*H*), 8.10 – 8.30 (m, 3H, pyr-*H*). – $^{13}C\{^1H\}$ NMR (270 MHz, DMSO-d_6). $\delta = 17.90$, 18.05 (2CH_3), 120.05, 127.95, 131.0, 134.40 (all Ph-*C*), 124.20,

138.15, 148.10 (all pyr-*C*), 150.60 (2*C*=N), 178.60 (2*C*=S). – MS (EI, 70 eV): *m/z* (%) = 463 (10) [M^+], 309 (25) [M^+-$2C_6H_5$], 191 (65) [M^+-2PhNHC≡S], 153 (100) [PhNHC-SOH]. -$C_{23}H_{21}N_5O_2S_2$ (463.57): calcd. C 59.59, H 4.56, N 15.10, S 13.83; found C 59.52, H 4.48, N 15.05, S 13.78.

Synthesis of 2,6-bis-[β-(2-thienyl)acryloylcarbazide]pyridine derivatives (8a, b)

A mixture of 2,6-*bis*-[β-(2-thienyl)acryloyl]pyridine dioxime (**6**) (0.38 g, 1 mmol) and phenyl isocyanate or phenyl isothiocyanate (2 mmol) in 50 ml dry dioxane containing 2 ml of triethylamine was heated under reflux for 10 h. After cooling, the obtained solid was filtered off, washed with diethyl ether, dried and crystallized from acetic acid/water to give the corresponding semi- and thiosemicarbazide derivatives (**8a,b**), respectively.

*2,6-Bis-[β-(2-thienyl)acryloylphenylsemicarbazide]pyridine derivative (***8a***)*

M. p. 190 – 5 °C (AcOH/H_2O). – IR (film): $\tilde{\nu}$ = 3400 – 3390 (NH), 1710 – 1690 (C=O), 1610 cm^{-1} (C=C). – ^{1}H NMR (270 MHz, DMSO-d_6). δ = 6.45 (d, J = 14.55 Hz, 2H, 2C*H*-thiophene), 6.80 (d, J = 14.60 Hz, 2H, 2C*H*-C=N), 7.10 – 7.60 (m, 16H, Ar-*H*), 8.10 – 8.25 (m, 3H, pyr-*H*), 8.60 (s, 2H, 2N*H*, exchangeable with D_2O). – ^{13}C{^{1}H} NMR (270 MHz, DMSO-d_6). δ = 121.25, 127.30, 127.95, 134.10 (all Ph-*C*), 134.35, 136.20 (4*C*H), 124.60, 138.45, 147.65 (all pyr-*C*), 157.30 (2*C*=O), 160.10, 160.40 (2*C*=N), 119.90, 120.65, 128.10, 148.10 (all thiophene-*C*). – MS (EI, 70 eV): *m/z* (%) = 619 (6) [M^+], 453 (15) [M^+-$2C_4H_3S$], 435 (100) [M^+-2PhNH]. – $C_{33}H_{25}N_5O_4S_2$ (619.71): calcd. C 63.95, H 4.06, N 11.30, S 10.34; found C 63.88, H 4.00, N 11.20, S 10.28.

*2,6-Bis-[β-(2-thienyl)acryloylphenylthiosemicarbazide]pyridine derivative (***8b***)*

M. p. 210 – 2 °C (AcOH/H_2O). – IR (film): $\tilde{\nu}$ = 3390 – 3360 (NH), 1675 (C=N), 1230 (C=S), 1600 cm^{-1} (C=C). – ^{1}H NMR (270 MHz, DMSO-d_6). δ = 4.50 – 4.60 (bs, 2H, 2N*H*, exchangeable with D_2O), 6.30 (d, J = 14.60 Hz, 2H, 2 C*H*-thiophene), 6.55 (d, J = 14.65 Hz, 2H, 2C*H*-C=N), 6.95 – 7.40 (m, 16H, Ar-*H*), 8.00 – 8.15 (m, 3H, pyr-*H*). – ^{13}C{^{1}H} NMR (270 MHz, DMSO-d_6). δ = 122.0, 127.20, 128.15, 136.45 (all Ph-*C*), 133.95, 136.10 (4*C*H), 123.85, 138.30, 146.90 (all pyr-*C*), 120.0, 121.05, 127.95, 133.25 (all thiophene-*C*), 159.80, 159.85 (2*C*=N), 178.45 (2*C*=S). – MS (EI, 70 eV): *m/z* (%) = 651 (8) [M^+], 485 (10) [M^+-$2C_4H_3S$], 467 (100) [M^+-2PhNH], 213 (85) [485-PhNHC≡S]. – $C_{33}H_{25}N_5O_2S_4$ (651.83): calcd. C 60.80, H 3.86, N 10.74, S 19.67; found C 60.76, H 3.78, N 10.68, S 19.59.

Synthesis of macrocyclic semicarbazide pyridine (9)

A mixture of 2,6-diacetylpyridine dioxime (**2**) (0.193 g, 1 mmol) and toluene-3,5-diisocyanate (0.174 g, 1 mmol) in 50 ml dry dioxane containing 2 ml of triethylamine was refluxed for 12 h. The solvent was evaporated under reduced pressure and the oily product was triturated with *n*-hexane, petroleum ether (40 – 60 °C). The obtained solid was filtered off, dried and purified by column chromatography with chloroform/ethanol (9:1, *v/v*) ratio as eluent to give the corresponding macrocyclic semicarbazide pyridine derivative (**9**).

M. p. 142 – 4 °C (MeOH). – IR (film): $\tilde{\nu}$ = 3400 – 3340 (NH), 1705 – 1695 (C=O), cm^{-1} (C=N). – ^{1}H NMR (270 MHz, DMSO-d_6). δ = 0.95 (s, 6H, 2Ph-CH_3), 1.85 (s, 12H, 4 × CH_3), 6.95 (s, 4H, 2Ph-*H*-2,6), 7.35 (s, 2H, 2Ph-*H*-4), 8.15 – 8.35 (m, 6H, pyr-*H*), 8.45 (s, 4H, 4N*H*, exchangeable with D_2O). – ^{13}C{^{1}H} NMR (270 MHz, DMSO-d_6). δ = 15.60, 20.2 (4CH_3), 104.30, 118.20, 133.80, 135.90 (all Ph-*C*), 124.10, 138.15, 147.05 (all pyr-*C*), 148.30, 148.45 (2*C*=N), 156.90, 157.0 (2*C*=O). – MS (EI, 70 eV): *m/z* (%) = 734 (12) [M^+], 674 (35) [M^+-4CH_3], 543 (65) [M^+-$C_9H_9N_3O_2$], 176 (100) [$C_9H_8N_2O_2$]. -$C_{36}H_{34}N_{10}O_8$ (734.72): calcd. C 58.85, H 4.66, N 19.06; found C 58.80, H 4.60, N 18.98.

Synthesis of macrocyclic 2,6-bis-[β-(2-thienyl)acryloylphenylsemicarbazide]-pyridine derivative (10)

A mixture of 2,6-bis-[β-(2-thienyl)acryloyloxime]pyridine (**6**) (0.76 g, 2 mmol) and toluene-3,5-diisocyanate (0.438 g, 2 mmol) in 50 ml dry dioxane containing 2 ml of triethylamine was refluxed for 12 h. The solvent was evaporated under reduced pressure and the oily product purified by column chromatography with chloroform: ethanol (9.5:0.5, *v/v* ratio) as eluent to give the macrocyclic compound (**10**).

M. p. 136 – 8 °C (MeOH). – IR (film): $\tilde{\nu}$ = 3460 – 3410 (NH), 1710 – 1695 (C=O), 1680 – 1670 (C=N), 1600 cm^{-1} (C=C). – ^{1}H NMR (270 MHz, DMSO-d_6). δ = 0.85 (s, 6H, 2Ph-CH_3), 6.3 (d, J = 14.45 Hz, 4H, 4 C*H*-thiophene), 6.70 (d, J = 14.50 Hz, 4H, 4C*H*-C=N), 7.10 – 7.40 (m, 12H, 4 × thiophene-*H*), 6.85 (s, 4H, 2Ph-*H*-2,6), 7.50 (s, 2H, 2Ph-*H*-4), 8.10 – 8.20 (m, 6H, pyr-*H*), 8.60 (s, 4H, 4 × *NH*, exchangeable with D_2O). – ^{13}C{^{1}H} NMR (270 MHz, DMSO-d_6). δ = 19.50 (2CH_3), 104.80, 117.20, 134.65, 136.75 (all Ph-*C*), 135.0, 137.10 (all *C*H), 124.10, 138.25, 147.10 (all pyr-*C*), 121.0, 122.05, 128.95, 132.25 (all thiophene-*C*), 156.80, 156.95 (all *C*=O), 158.60, 158.70 (all *C*=N). - MS (EI, 70 eV): *m/z* (%) = 1111 (18) [M^+], 776 (28) [M^+-4C_4H_3S], 731 (24) [M^+-$C_{19}H_{13}N_3O_2S_2$], 565 (100) [731-2C_4H_3S], 379 (85) [$C_{19}H_{13}N_3O_2S_2$]. – $C_{56}H_{42}N_{10}O_8S_4$ (1111.25): calcd. C 60.52, H 3.80, N 12.60; found C 60.48, H 3.74, N 12.55.

Synthesis of chiral 2,6-bis-(phenylthiosemicarbazide) pyridine derivatives (11a, b)

A mixture of $N^2,N^{2'}$-(pyridine-2,6-dicarbonyl)-L-amino acid hydrazides (**5a,b**) (1 mmol) and phenyl isothiocyanate (0.27 g, 2 mmol) in 50 ml dry dioxane containing 1 ml of triethylamine was heated on a water bath at (80 °C) for 8 h. The obtained solid was filtered off, washed with diethyl ether, dried and crystallized from the proper solvent to give the corresponding chiral thiosemicarbazide derivatives (**11a,b**).

*Chiral isopropyl phenylthiosemicarbazide pyridine derivative (***11a***)*

M. p. 166 – 8 °C (EtOH). $-[\alpha]_D^{30} = +15$ (DMF). – IR (film): $\tilde{\nu} = 3350 - 3310$ (NH), 1695 – 1985 (C=O), 1230 – 1225 cm^{-1} (C=S). – ^{1}H NMR (270 MHz, DMSO-d_6). $\delta = 1.00 - 1.10$ (m, 12H, $4 \times CH_3$), 2.10 – 2.30 (m, 2H, $2 \times CH(CH_3)_2$), 4.15 – 4.30 (s, 2H, *CH*-NH), 4.60 (bs, 4H, 4CS*NH*, exchangeable with D_2O), 6.85 – 7.10 (m, 10H, 2Ph-*H*), 8.00 – 8.30 (m, 3H, pyr-*H*), 8.55 (s, 2H, 2CO*NH*, exchangeable with D_2O), 8.85 (s, 2H, 2CO*NH*, exchangeable with D_2O). – $^{13}C\{^1H\}$ NMR (270 MHz, DMSO-d_6). $\delta = 15.90$, 17.0 (all CH_3), 26.25 [$CH(CH_3)_2$], 57.10 (*C*HNH), 122.15, 123.25, 130.65, 134.60 (all Ph-*C*), 124.15, 139.10, 148.35 (all pyr-*C*), 173.0 (2*C*=S), 163.20, 174.10 (all *C*=O). – MS (EI, 70 eV): *m/z* (%) = 663 (14) [M^+], 577 (25) [M^+-2CH$(CH_3)_2$], 509 (32) [M^+-2Ph], 331 (100) [M^+-2PhNHCSNHNH]. -$C_{31}H_{37}N_9O_4S_2$ (663.81): calcd. C 56.09, H 5.61, N 18.99, S 19.66; found C 55.99, H 5.56, N 18.85, S 19.58.

*Chiral isobutyl phenylthiosemicarbazide pyridine derivative (***11b***)*

M. p. 156 – 8 °C (dioxane). – $[\alpha]_D^{30} = +10$ (DMF). – IR (film): $\tilde{\nu} = 3340 - 3310$ (NH), 1705 – 1695 (C=O), 1225 cm^{-1} (C=S). – ^{1}H NMR (270 MHz, DMSO-d_6). $\delta = 1.65 - 175$ (m, 12H, $4 \times CH_3$), 1.90 – 2.00 (m, 4H, $2CH_2$), 2.10 – 2.30 (m, 2H, $2 \times CH(CH_3)_2$), 4.30 – 4.40 (s, 2H, *CH*NH), 4.65 (bs, 4H, 4CS*NH*, exchangeable with D_2O), 7.10 – 7.35 (m, 10H, 2Ph-*H*), 8.10 – 8.25 (m, 3H, pyr-*H*), 8.90 (s, 2H, 2CO*NH*, exchangeable with D_2O), 8.45 (s, 2H, 2CO*NH*, exchangeable with D_2O). – $^{13}C\{^1H\}$ NMR (270 MHz, DMSO-d_6). $\delta = 19.45$, 20.25 (all CH_3), 22.15 [$CH(CH_3)_2$], 33.65 (CH_2), 56.90 (*C*HNH), 120.25, 127.30, 128.10, 134.45 (all Ph-*C*), 124.40, 139.15, 148.60 (all pyr-*C*), 172.60 (2*C*=S), 162.10, 175.60 (all *C*=O). – MS (EI, 70 eV): *m/z* (%) = 691 (25) [M^+], 577 (45) [M^+-2CH_2CH$(CH_3)_2$], 537 (13) [M^+-2Ph], 359 (100) [M^+–2PhNHCSNHNH]. -$C_{33}H_{41}N_9O_4S_2$ (691.86): calcd. C 57.28, H 5.97, N 18.22, S 9.26; found C 57.16, H 5.88, N 18.17, S 9.21.

Synthesis of chiral bis-(triazolyl)pyridine derivatives (12a, b)

A suspension of thiosemicarbazides **11a,b** (1 mmol) in sodium hydroxide (2N, 5 ml) was refluxed for 2 h. The reaction mixture was cooled then acidified with acetic acid (pH 3). The formed solid was filtered off, washed with water and crystallized from the proper solvent to give the corresponding bis-triazolyl derivatives (**12a,b**).

*Compound (***12a***)*

M. p. 210 – 2 °C (AcOH/H_2O). – $[\alpha]_D^{30} = +15$ (DMF). – IR (film): $\tilde{\nu} = 3350 - 3340$ (NH), 2250 – 2240 (SH), 1695 cm^{-1} (C=O). – ^{1}H NMR (270 MHz, DMSO-d_6). $\delta = 1.0 - 1.15$ (m, 12H, $4 \times CH_3$), 2.10 – 2.40 (m, 2H, $2 \times CH(CH_3)_2$), 4.20 – 4.30 (m, 2H, $2 \times CH$NH), 7.0 – 7.30 (m, 10H, 2Ph-*H*), 8.10 – 8.40 (m, 3H, pyr-*H*), 8.50 (s, 2H, 2CO*NH*, exchangeable with D_2O), 8.95 (bs, 2H, 2S*H*, exchangeable with D_2O). – $^{13}C\{^1H\}$ NMR (270 MHz, DMSO-d_6). $\delta = 16.85$, 17.45 (all CH_3), 22.10 [$CH(CH_3)_2$], 56.90 (*C*HNH), 123.50, 127.10, 128.00, 133.55 (all Ph-*C*), 124.25, 139.15, 148.15 (all pyr-*C*), 162.10, 162.30 (2*C*=O), 140.55, 149.65 (triazole-*C*). – MS (EI, 70 eV): *m/z* (%) = 627 (7) [M^+], 541 (48) [M^+-2CH$(CH_3)_2$], 473 (15) [M^+-2Ph], 163 (100) [$C_7H_5N_3O_2$]. — $C_{31}H_{33}N_9O_2S_2$ (627.78): calcd. C 59.31, H 5.29, N 20.08, S 10.21; found C 59.25, H 5.24, N 20.00, S 10.18.

*Compound (***12b***)*

M. p. 184 – 6 °C (AcOH/H_2O). – $[\alpha]_D^{30} = +10$ (DMF). – IR (film): $\tilde{\nu} = 3375 - 3350$ (NH), 2245 – 2235 (SH), 1695 cm^{-1} (C=O). – ^{1}H NMR (270 MHz, DMSO-d_6). $\delta = 0.95 - 1.10$ (m, 12H, $4 \times CH_3$), 2.0 – 2.25 (m, 2H, $2 \times CH(CH_3)_2$), 2.30 – 2.40 (m, 4H, $2CH_2$), 4.25 – 4.40 (m, 2H, $2 \times CH$NH), 6.90 – 7.15 (m, 10H, 2Ph-*H*), 8.15 – 8.30 (m, 3H, pyr-*H*), 8.65 (s, 2H, 2CO*NH*, exchangeable with D_2O), 8.70 (bs, 2H, 2S*H*, exchangeable with D_2O). – $^{13}C\{^1H\}$ NMR (270 MHz, DMSO-d_6). $\delta = 18.90$, 19.45 (all CH_3), 23.75 [$CH(CH_3)_2$], 34.65 (CH_2), 57.0 (*C*HNH), 123.60, 127.20, 127.90, 134.50 (all Ph-*C*), 123.95, 138.65, 148.25 (all pyr-*C*), 161.90, 162.0 (2*C*=O), 140.85, 149.0 (triazole-*C*). – MS (EI, 70 eV): *m/z* (%) = 655 (12) [M^+], 541 (16) [M^+-2CH_2CH$(CH_3)_2$], 501 (23) [M^+-2Ph], 163 (100) [$C_7H_5N_3O_2$]. – $C_{33}H_{37}N_9O_2S_2$ (655.83): calcd. C 60.43, H 5.68, N 19.22, S 9.77; found C 60.38, H 5.62, N 19.18, S 9.69.

Synthesis of chiral macrocyclic semicarbazide derivatives (13a,b)

A mixture of $N^2,N^{2'}$-(pyridine-2,6-dicarbonyl)-L-amino acid hydrazides (**5a,b**) (1 mmol) and toluene-3,5-diisocyanate (0.174 g, 1 mmol) in 50 ml dry dioxane

containing 2 ml of triethylamine. The reaction mixture was refluxed for 6 h, the solvent was evaporated under reduced pressure and the oily product was triturated with *n*-hexane, petroleum ether (40 – 60 °C). The obtained solid was filtered off, dried and purified by column chromatography with chloroform:ethanol (9:1, *v/v* ratio) as eluent to give the corresponding macrocyclic semicarbazide derivatives (**13a,b**).

*Macrocyclic compound (***13a***)*

M. p. 170 – 2 °C (MeOH). – $[\alpha]_D^{30} = +5$ (DMF). – IR (film): $\tilde{\nu} = 3400 - 3390$ (NH), 1700 – 1690 (C=O), 1670 cm^{-1} (C=N). – ^{1}H NMR (270 MHz, DMSO-d_6). $\delta =$ 0.80 (s, 6H, 2Ph-CH_3), 0.95 – 1.50 (m, 24H, 8 × CH_3), 2.25 – 2.45 (m, 4H, 4 × *CH*$(CH_3)_2$), 4.30 – 4.50 (m, 4H, C*H*NH), 7.00 (s, 4H, 2Ph-*H*-2,6), 7.30 (s, 2H, 2Ph-*H*-4), 8.15 – 8.30 (m, 6H, pyr-*H*), 8.65 (br. s, 8H, 8CO*NH*, exchangeable with D_2O), 8.85 (br. s, 8H, 8CO*NH*, exchangeable with D_2O). – $^{13}C\{^1H\}$ NMR (270 MHz, DMSO-d_6). $\delta = 16.90$, 17.75, 21.35 (all *C*H_3), 22.80, 23.10 [*C*H$(CH_3)_2$], 57.10, 57.30 (*C*HNH), 104.10, 128.10, 128.45, 134.65, 136.50 (all Ph-*C*), 124.25, 138.80, 147.90 (all pyri-*C*), 145.30, 162.20, 174.50 (all *C*=O). – MS (EI, 70 eV): *m/z* (%) = 1135 (5) [M^+], 962 (13) [M^+-4CH$(CH_3)_2$], 803 (22) [M^+-$C_{17}H_{21}N_3O_4$], 331 (100) [$C_{17}H_{21}N_3O_4$]. – $C_{52}H_{66}N_8O_{12}$ (1135.20): calcd. C 55.01, H 5.85, N 22.20; found C 54.96, H 5.78, N 22.01.

*Macrocyclic Compound (***13b***)*

M. p. 120 – 2 °C (EtOH). – $[\alpha]_D^{30} = +15$ (DMF). – IR (film): $\tilde{\nu} = 3380 - 3360$ (NH), 1705 – 1695 (C=O), 1680 cm^{-1} (C=N). – ^{1}H NMR (270 MHz, DMSO-d_6). $\delta =$ 0.90 (s, 6H, 2Ph-CH_3), 1.55 – 1.75 (m, 24H, 8 × *CH*$_3$), 2.0 – 2.15 (m, 8H, 4*CH*$_2$), 2.30 – 2.45 (m, 4H, 4 × *CH*$(CH_3)_2$), 4.40 – 4.55 (m, 4H, 4 × *CH*NH), 6.90 (s, 4H, 2Ph-*H*-2,6), 7.25 (s, 2H, 2Ph-*H*-4), 8.05 – 8.20 (m, 6H, pyr-*H*), 8.70 (br. s, 8H, 8CO*NH*, exchangeable with D_2O), 8.85 (br. s, 8H, 8CO*NH*, exchangeable with D_2O). – $^{13}C\{^1H\}$ NMR (270 MHz, DMSO-d_6). $\delta = 19.10$, 20.0, 20.50 (all *C*H_3), 21.50, 22.10 [*C*H$(CH_3)_2$], 35.60, 36.20 (all *C*H_2), 56.75, 56.90 (*C*HNH), 104.35, 119.50, 134.30, 136.10 (all Ph-*C*), 123.90, 138.85, 148.50 (all pyr-*C*), 145.70, 163.0, 174.15 (all *C*=O). – MS (EI, 70 eV): *m/z* (%) = 1191 (5) [M^+], 962 (58) [M^+-4CH_2CH$(CH_3)_2$], 934 (12) [962-2CH_3], 831 (16) [M^+-$C_{19}H_{25}N_3O_4$], 559 (100) [$C_{19}H_{25}N_3O_4$]. – $C_{56}H_{74}N_{18}O_{12}$ (1191.31): calcd. C 56.46, H 6.26, N 21.16; found C 56.38, H 6.18, N 21.04.

Analgesic activity

Sixty mice of both sexes weighting from 20 – 25 g were divided into 10 groups. A group was kept as control (received saline) and the second received vehicle (Gumacaccia), while the third received Voltarine® as a reference drug, whereas the other groups received **7a**, **8b**, **9**, **10**, **12b**, **13a** and **13b** (SC administration). Mice were dropped gently in a dry glass beaker of one liter capacity maintained at ∼ 55 °C. Normal reaction times in seconds for all animals were determined at time intervals of 10, 20, 30, 45, 60, 90 and 120 min. This is the interval extending from the instant the mouse reaches the hot beaker till the animals licks its feet or jamb out of the beaker (dose 5 mg/kg). Relative potencies to Voltarine® were determined (Table 1).

Anticonvulsant activity

Male Webster mice (20 – 30 g) were individually placed in clear plastic cylinder and the tested compounds were administrated intrapretonial (5 mg/kg), 30 min prior to a dose of 45 mg/kg of yohimbine.HCl. The animals were observed for onset and number of clonic seizures. Evaluation ED_{50} values of compounds with 95% confidence limit were calculated for the antagonism of yohimbine-induced clonic seizures by means of the Lichtfield-Wilcoxon procedure [18, 19] (Table 2).

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