Full Paper

Anti-inflammatory, Analgesic, Anticonvulsant and Antiparkinsonian Activities of Some Pyridine Derivatives Using 2,6-Disubstituted Isonicotinic Acid Hydrazides

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A series of novel thiazolo derivatives **2–15** was synthesized by initial condensation of 2,6-dihydroxyisonicotinohydrazide **1** and 2-chloro-6-hydrazinylisonicotinohydrazide **11** with different organic reagents. The pharmacological screening showed that many of these obtained compounds have good anti-inflammatory, analgesic, anticonvulsant, and antiparkinsonian activities comparable to diclofenac potassium, Voltarene®, Carbamazepine®, and Benzotropene® as reference drugs. Initially the acute toxicity of the compounds was assayed via the determination of their LD_{50}. The structures of newly synthesized compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, MS spectral data and elemental analysis. The detailed synthesis, spectroscopic data, LD_{50} and pharmacological activities of the synthesized compounds were reported.

Keywords: Bis-imides / Isonicotinic acid hydrazides / Pharmacological activities / Triazoles

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Introduction

In a previous work we reported that certain of our newly substituted heterocyclic compounds exhibited antiparkinsonian [1], antitumor [2–4], antimicrobial [5–7], and anti-inflammatory [8, 9] activities. Pyrazoles present an interesting group of compounds many of which possess widespread pharmacological properties such as analgesic, antipyretic, and antirheumatic activities [10, 11]. In addition, the heterocyclic nitrogen derivatives exhibited a general ionophoric potency for divalent cations [12] and used a novel thiocyanate-selective membrane sensor [13]. Recently, the pharmacological and antitumor activities of many compounds containing heterocyclic ring have been reviewed [14–23]. In view of these reports and in continuation of our previous works in heterocyclic chemistry, we have herein synthesized some new derivatives containing thiophene ring fused with cyclohexane ring for the evaluation of their anti-inflammatory, analgesic, anticonvulsant, and antiparkinsonian activities comparable to diclofenac potassium, Voltarene®, Carbamazepine®, and Benzotropene® as reference drugs.

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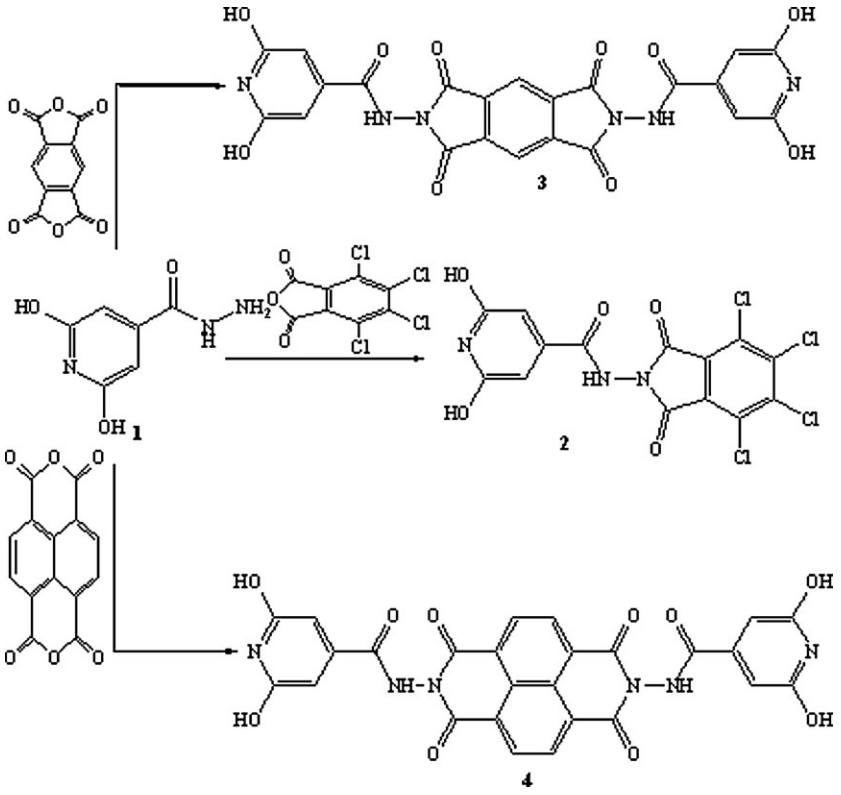
Results and discussion

Chemistry

Condensation of compound **1** with acid anhydrides, namely, 3,4,5,6-tetrachloro-phthalic anhydride, 1,2,4,5-benzenetetracarboxylic acid dianhydride and 1,4,5,8-naphthalenetetracarboxylic acid dianhydride in refluxing glacial acetic acid afforded the corresponding imide derivative **2**, benzene-bis-substituted pyridine derivative **3**, and naphthalene-bis-substituted pyridine derivative **4**, respectively (Scheme 1).

Moreover, fusion of **1** with ammonium thiocyanate gave the corresponding 2-mercaptotriazole derivative **5**, which was condensed with chloroacetic acid in a mixture of acetic acid/acetic anhydride in the presence of anhydrous sodium acetate to yield the corresponding thiazolopyrimidine derivative **6**. Compound **6** contains an active methylene group, as such it condensed with benzaldehyde in the presence of anhydrous sodium acetate and glacial acetic acid/acetic anhydride mixture to yield the arylmethylene **7**. However, the latter was also prepared directly from **5** by the action of chloroacetic acid, benzaldehyde, and anhydrous



Scheme 1. Synthetic routes of compounds **2–4**.

sodium acetate in the presence of acetic acid/acetic anhydride mixture. Treatment of the hydrazide **1** with carbon disulphide in refluxing alcoholic potassium hydroxide afforded the corresponding oxadiazole-2-thione derivative **8**, which was condensed with hydrazine hydrate to afford the corresponding 1-amino-thiazole-2-thione derivative **9**. Condensation of compound **9** with, 3,4,5,6-tetrachlorophthalic anhydride in refluxing glacial acetic acid afforded the corresponding imide derivative **10** (Scheme 2).

The reaction of compound **11** with different arylsulfonyl chloride, namely, benzenesulfonyl chloride or *p*-toluenesulfonyl chloride in the presence of TEA gave the corresponding compounds **12a** and **12b**. Also, cyclization of hydrazide **11** with diethylmalonate, pentane-2,4-dione or ethyl 3-oxobutanoate afforded the corresponding substituted pyrazole derivatives **13–15**, respectively according to the literature procedures (Scheme 3) [24].

Pharmacological screening

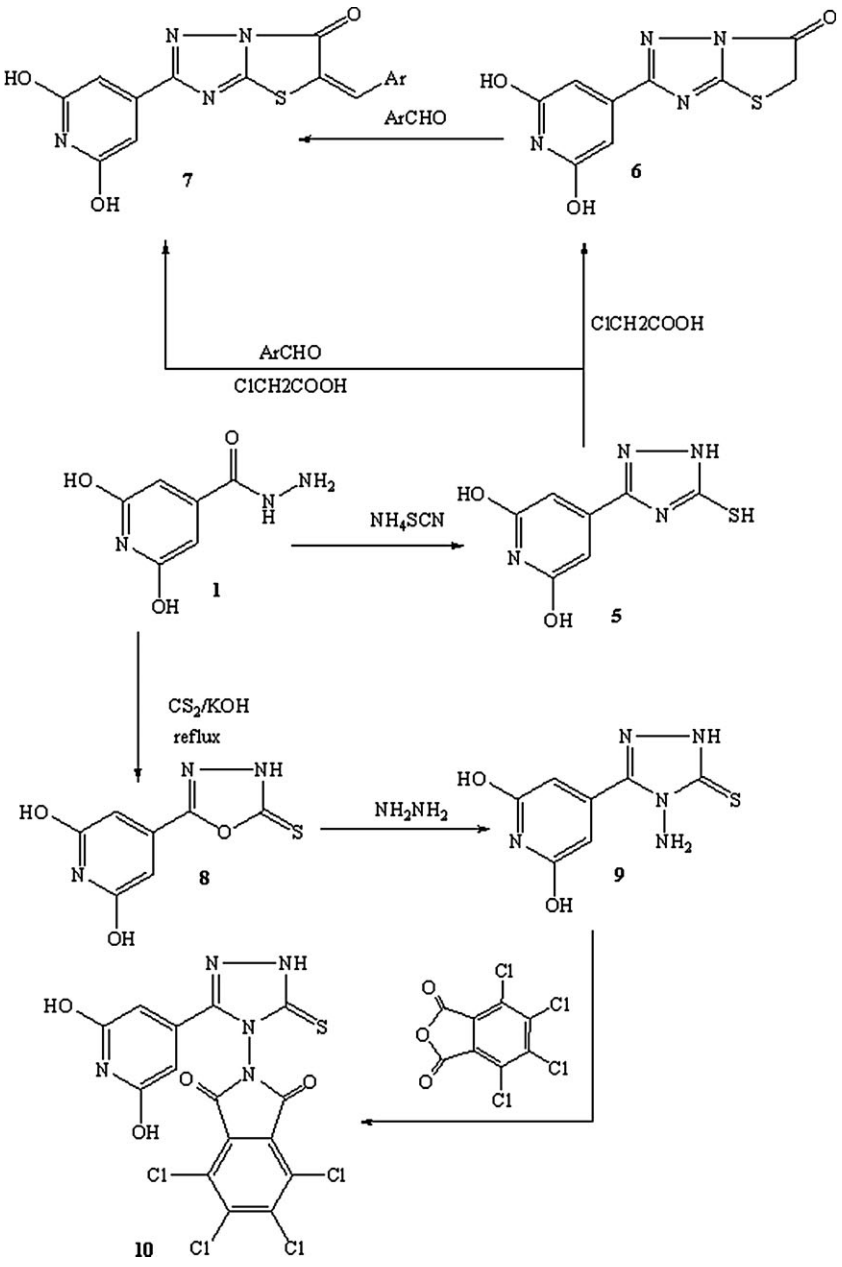
All animals were obtained from the Animal House Colony, Research Institute of Ophthalmology, Giza, Egypt. Initially, the acute toxicity of the compounds was assayed determining their LD_{50}. Interestingly, all the synthesized compounds were less toxic than valdecoxib (Table 1). Then, the newly synthesized compounds were screened pharmacologically for their anti-iniflammatory, analgesic, anticonvulsant, and anti-parkinsonian activities using male albino rats (Tables 2–5). The ethical committee of the National Research Centre, Cairo, Egypt, approved the protocol of this study.

Anti-inflammatory activity

Regarding the protection against carrageenan-induced edema, all tested compounds, were found to be more potent than diclofenac potassium. For these compounds, a similar activity profile was realized for the inhibition of plasma PGE2. Concerning the anti-inflammatory activities, the descending order of activity is **12a** > **3** > **12b** > **6** > **4** > **7** > **10** > **15** > **5** > **9** > **13** > **2**. Compounds **12a**, **4**, **12b**, **6**, and **4** are the most active products (Table 2).

Analgesic activity

All tested compounds exhibited analgesic activity in a hot-plate assay (Table 3). Interestingly, the analgesic activities of all the tested compounds were more potent than valdecoxib as a reference drug (Table 3) and, compared to valdecoxib after 120 min these analgesic activities were increased. Compounds **12a** > **12b** > **3** > **6** > **4** > **7** > **10** > **15** > **5** > **9** > **13** > **2** are arranged in descending order of analgesic potency. Compound **12a** showed more than three times the



Scheme 2. Synthetic routes of compounds **5–10**.

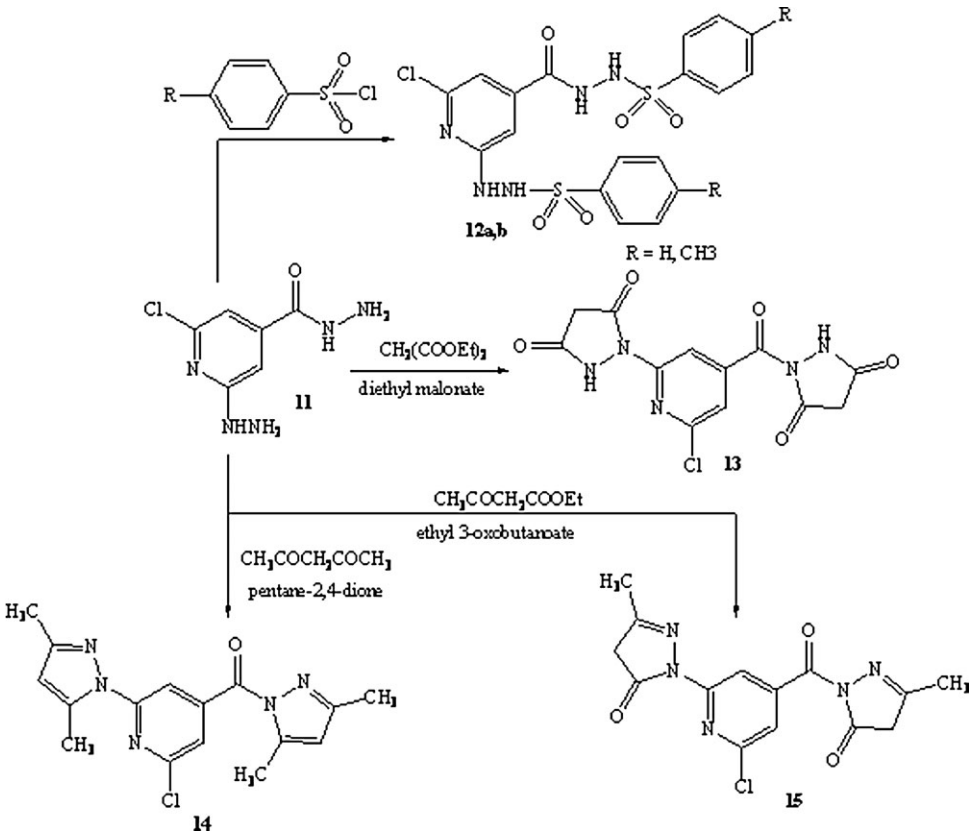
activity of valdecoxib, while compounds **6**, **12b**, **3**, and **4** showed double activity as compared to valdecoxib after two hours.

Anticonvulsant Activity

From Table 4, compounds **9** and **15** are devoid of any anticonvulsant activity where they provide no protection against yohimobine-induced clonic seizures. Compounds **3**, **10**, and **12a** showed interesting anticonvulsant activities, their relative potencies to Carbamazebene® are 0.72, 0.885, and 0.82. Compounds **4** and **12b** are even more potent than Carbamazebene® (1.90 and 2.18, relative potency).

Antiparkinsonian Activity

Compounds **12a**, **10**, and **3** showed moderate activity (relative potencies to Benzotropene® 0.42, 0.44, and 0.62). Compounds **12b** and **4** are the most potent antiparkinsonic agents (0.84 relative potency) (Table 5).



Scheme 3. Synthetic routes of compounds **12–15**.

Table 1. Acute toxicity (LD_{50}) of the synthesized compounds.

Compound	LD_{50} [mg/kg]
2	1618.14 ± 0.23
3	2034.89 ± 0.12
4	2210.87 ± 0.18
5	1250.87 ± 0.12
6	2112.55 ± 0.11
7	2205.78 ± 0.18
9	1620.00 ± 0.14
10	1345.98 ± 0.14
12a	2512.56 ± 0.17
12b	2100.89 ± 0.17
13	1356.09 ± 0.15
15	1272.87 ± 0.17
Valdecoxib	1180.01 ± 0.23
Diclofenac potassium	1750.87 ± 0.12
Carbamazepine	2312.55 ± 0.11
Benzotropene	2405.78 ± 0.18

Experimental

Chemistry

Melting points were determined on open glass capillaries using an Electrothermal IA 9000 digital melting point apparatus and corrected. Elemental analyses were performed on Elementar, Vario EL, Microanalytical Unit, National Research Center, Cairo, Egypt and were found within ±0.2% of the theoretical values. Infrared spectra were recorded on Carl Zeiss Spectrophotometer model ''UR 10'' spectrophotometer using the KBr disc technique. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Varian Gemini 270 MHz spectrometer (DMSO-d_6) and the chemical shifts are given in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard. The mass spectra were measured using a Finnigan SSQ 7000 mass spectrometer. Follow up of the reactions and checking the purity of the compounds was made by TLC on silica gel aluminum sheets (Type 60 F_{254}, Merck, Darmstadt, Germany).

*2,6-Dihydroxy-N-(4,5,6,7-tetrachloro-1,3-dioxoisoindolin-2-yl)isonicotinamide **2***

A mixture of hydrazide **1** (0.17 g, 1 mmol) and 3,4,5,6-tetrachlorophthalic anhydride (0.29 g, 1 mmol) in glacial acetic acid (50 mL)

Table 2. Anti-inflammatory activities of some new synthesized compounds.

Groups	Dose [mg/kg]	% Protection against edema	% Inhibition of plasma PGE2
2	2.5	77.88 ± 0.090	57.57 ± 0.051
	5.0	91.00 ± 0.061	76.00 ± 0.041
3	2.5	89.12 ± 0.076	84.15 ± 0.041
	5.0	98.09 ± 0.065	86.00 ± 0.031
4	2.5	93.44 ± 0.075	75.71 ± 0.041
	5.0	98.56 ± 0.064	80.00 ± 0.061
5	2.5	91.16 ± 0.064	73.44 ± 0.049
	5.0	95.12 ± 0.075	78.56 ± 0.041
6	2.5	97.35 ± 0.055	81.27 ± 0.041
	5.0	98.85 ± 0.045	82.50 ± 0.053
7	2.5	85.95 ± 0.052	59.43 ± 0.051
	5.0	98.16 ± 0.052	81.09 ± 0.055
9	2.5	82.10 ± 0.076	74.56 ± 0.041
	5.0	93.12 ± 0.060	54.00 ± 0.041
10	2.5	85.16 ± 0.056	61.14 ± 0.041
	5.0	96.19 ± 0.067	77.11 ± 0.041
12a	2.5	92.46 ± 0.090	63.16 ± 0.031
	5.0	99.30 ± 0.080	86.16 ± 0.071
12b	2.5	90.26 ± 0.033	62.12 ± 0.051
	5.0	99.00 ± 0.032	83.00 ± 0.049
13	2.5	79.28 ± 0.078	57.90 ± 0.041
	5.0	92.90 ± 0.095	77.11 ± 0.042
15	2.5	81.00 ± 0.072	63.11 ± 0.041
	5.0	95.80 ± 0.065	79.88 ± 0.031
Diclofenac potassium	2.5	70.14 ± 0.061	54.00 ± 0.041
	5.0	75.23 ± 0.083	70.00 ± 0.051

All results were significantly different from the standard and normal control value at $P \leq 0.05$.

was heated under reflux for 6 h. The reaction mixture was concentrated under reduced pressure; the obtained solid was filtered off and crystallized from AcOH/H_2O to yield the imide derivative **2**. Yield (76%), m.p. 256°C; IR (KBr, ν, cm^{-1}): 3422 (OH), 3233 (NH), 1661, 1710 (C=O); ^{1}H-NMR (DMSO-d_6, 270 MHz) δ (ppm): 6.85 (s, 2H, Pyri-H), 11.05, 12.95 (2s, 3H, 2 OH + NH, exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, 67.5 MHz) δ (ppm): 95.10, 127.96, 132.55, 134.12, 142.32, 147.54, 164.23, 164.76; EIMS: *m/z* (%) 437 (M, 12) and at 324 (100, base peak). Elemental analysis for $C_{14}H_5Cl_4N_3O_5$ (437.02): Calcd.: C, 38.48; H, 1.15; Cl, 32.45; N, 9.62. Found: C, 38.40; H, 1015; Cl, 32.38; N, 9.56.

*General procedure for the synthesis of bis-imide derivatives **3** and **4***

A mixture of compound **1** (0.35 g, 2 mmol) and dianhydrides, namely, 1,2,4,5-benzenetetracarboxylic acid dianhydride and 1,4,5,8-naphthalenetetracarboxylic acid dianhydride (1 mmol) in glacial acetic acid (50 mL) was heated under reflux for 6 h.

Table 3. Analgesic activities of some new synthesized compounds.

Compound	Comparative analgesic potency to valdecoxib after time [min]					
	10 min	20 min	30 min	60 min	90 min	120 min
Valdecoxib	1.00	1.00	1.00	1.00	1.00	1.00
2	0.40 ± 0.01	0.40 ± 0.03	0.45 ± 0.04	0.58 ± 0.05	0.89 ± 0.08	1.11 ± 0.07
3	0.64 ± 0.02	0.76 ± 0.07	0.81 ± 0.07	1.15 ± 0.15	1.43 ± 0.12	2.15 ± 0.14
4	0.55 ± 0.01	0.56 ± 0.03	0.85 ± 0.05	0.89 ± 0.08	1.12 ± 0.12	2.22 ± 0.21
5	0.47 ± 0.01	0.47 ± 0.03	0.55 ± 0.05	0.67 ± 0.09	0.87 ± 0.09	1.47 ± 0.14
6	0.61 ± 0.02	0.61 ± 0.05	0.90 ± 0.03	1.00 ± 0.01	1.18 ± 0.10	2.12 ± 0.12
7	0.51 ± 0.01	0.53 ± 0.05	0.81 ± 0.07	0.85 ± 0.08	1.11 ± 0.11	1.89 ± 0.08
9	0.45 ± 0.01	0.45 ± 0.03	0.50 ± 0.04	0.65 ± 0.06	0.80 ± 0.08	1.30 ± 0.08
10	0.45 ± 0.01	0.48 ± 0.04	0.61 ± 0.06	0.76 ± 0.07	0.99 ± 0.09	1.63 ± 0.06
12a	0.72 ± 0.03	0.83 ± 0.07	0.98 ± 0.09	1.22 ± 0.12	1.63 ± 0.16	3.05 ± 0.14
12b	0.65 ± 0.01	0.63 ± 0.06	0.97 ± 0.08	1.00 ± 0.01	1.24 ± 0.12	2.16 ± 0.11
13	0.41 ± 0.01	0.40 ± 0.04	0.50 ± 0.05	0.61 ± 0.06	0.97 ± 0.09	1.28 ± 0.16
15	0.45 ± 0.02	0.47 ± 0.03	0.59 ± 0.05	0.74 ± 0.07	0.99 ± 0.09	1.58 ± 0.04

All results were significantly different from the standard and normal control value at $P \leq 0.05$.

Table 4. Anticonvulsant activity of the new compounds and Carbamazepine® via ED_{50} needed to antagonize yohimbine seizure.

Comp. no.	ED_{50} value (mg/kg)	Relative potency to Carbamazepine®
Control	0	0
Carbamazepine®	29	1
3	50	0.72
4	15	1.90
9	No protection	–
10	31	0.885
12a	35	0.82
12b	13	2.18
15	No protection	–

The obtained solid was filtered off and crystallized to yield the corresponding bis-imide derivatives **2** and **3**, respectively.

N,N′-(1,3,5,7-Tetraoxopyrrolo[3,4-f]isoindole-2,6 (1H,3H,5H,7H)-diyl)bis(2,6-di-hydroxyisonicotinamide) ***3***

Yield (82%), m.p. 232°C (AcOH); IR (KBr, ν, cm^{-1}): 3428 (OH), 3312 (NH), 1668, 1718 (C=O); ^{1}H-NMR (DMSO-d_6, 270 MHz) δ (ppm): 6.78 (s, 4H, Pyri-H), 7.86 (s, 2H, Ar-H), 10.95, 12.76 (2s, 6H, 4 OH + 2 NH, exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, 67.5 MHz) δ (ppm): 94.90, 125.12, 134.18, 142.15, 147.64, 164.32, 164.80; EIMS: *m/z* (%) 520 (M^+, 18) and at 214 (100, base peak). Elemental analysis for $C_{22}H_{12}N_6O_{10}$ (520.36): Calcd.: C, 50.78; H, 2.32; N, 16.15. Found: C, 50.71; H, 2.26; N, 16.05; O, 30.68.

N,N′-(1,3,6,8-Tetraoxobenzo[lmn][3,8]phenanthroline-2,7 (1H,3H,6H,8H)-diyl)bis (2,6-dihydroxyisonicotinamide) ***4***

Yield (84%), m.p. >300°C (AcOH); IR (KBr, ν, cm^{-1}): 3418 (OH), 3318 (NH), 1665, 1722 (C=O); ^{1}H-NMR (DMSO-d_6, 270 MHz) δ (ppm): 6.86 (s, 4H, Pyri-H), 7.46–7.88 (m, 4H, Ar-H), 10.98, 12.68 (2s, 6H, 4 OH + 2 NH, exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, 67.5 MHz) δ (ppm): 94.99, 119.75, 134.28, 138.98, 142.05, 147.72, 158.76, 164.67; EIMS: *m/z* (%) 570 (M^+, 8) and at 138 (100, base peak). Elemental analysis for $C_{26}H_{14}N_6O_{10}$ (570.42): Calcd.: C, 54.74; H, 2.47; N, 14.73. Found: C, 54.68; H, 2.42; N, 14.66.

4-(5-Mercapto-1H-1,2,4-triazol-3-yl)pyridine-2,6-diol ***5***

A mixture of compound **1** (0.17g, 1 mmol) and ammonium thiocyanate (2.0 g, 5 mmol) was fused at 200°C for 1 h. The obtained solid mass was triturated with hot water, cooled and acidified with conc. hydrochloric acid (pH 3). The formed precipitate was filtered off, washed with water, dried and crystallized from methanol to give compound **5**. Yield (76%), m.p. 246°C; IR (KBr, ν, cm^{-1}): 3396 (OH), 3320 (NH), 1600 (C=N); ^{1}H-NMR (DMSO-d_6, 270 MHz) δ (ppm): 6.76 (s, 2H, Pyri-H), 10.88 (s, 2H, 2 OH, exchangeable with D_2O), 12.56 (s, 1H, NH, exchangeable with D_2O), 12.86 (s, 1H, SH, exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, 67.5 MHz) δ (ppm): 92.65, 142.85, 147.98, 148.78, 158.96; EIMS: *m/z* (%) 210 (M^+, 68) and at 100 (100, base peak). Elemental analysis for $C_7H_6N_4O_2S$ (210.21): Calcd.: C, 40.00; H, 2.88; N, 26.65; S, 15.25. Found: C, 39.86; H, 2.82; N, 26.59; S, 15.18.

2-(2,6-Dihydroxypyridin-4-yl)thiazolo[3,2-b][1,2,4]triazol-6(5H)-one ***6***

A mixture of **5** (0.2g, 1 mmol) and chloroacetic acid (0.1g, 1 mmol) was dissolved in 40 mL of a mixture of AcOH/Ac_2O (1:3) in the presence of 3 g anhydrous sodium acetate and was refluxed for 7 h. The reaction mixture was cooled and poured onto cold water with stirring, the solid formed was filtered off and crystallized from ethanol to give compounds **6**. Yield (66%), m.p. 210°C; IR (KBr, ν, cm^{-1}): 3382 (OH), 3298 (NH), 1710 (C=O), 1605 (C=N); ^{1}H-NMR (DMSO-d_6, 270 MHz) δ (ppm): 3.74 (s, 2H, CH_2-thiazole), 6.78 (s, 2H, Pyri-H), 11.05 (s, 2H, 2 OH, exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, 67.5 MHz) δ (ppm): 30.50, 88.90, 143.05, 147.84, 150.78, 159.45, 198.02; EIMS: *m/z* (%) 250 (M^+, and as base peak). Elemental analysis for $C_9H_6N_4O_3S$ (250.23): Calcd.: C, 43.20; H, 2.42; N, 22.39; S, 12.81. Found: C, 43.07; H, 2.36; N, 22.33; S, 12.76.

5-Benzylidene-2-(2,6-dihydroxypyridin-4-yl)thiazolo[3,2-b] [1,2,4]triazol-6(5H)-one ***7***

Method A

A mixture of **5** (0.2 g, 1 mmol), chloroacetic acid (0.1 g, 1 mmol), anhydrous sodium acetate (1.5 g) in 40 mL, a mixture of AcOH/Ac_2O (1:3) and benzaldehyde (0.106 g, 1 mmol) was refluxed for 6 h. The reaction mixture was cooled and poured onto ice-water, the obtained solid was collected by filtration and crystallized from ethanol to give compound 7 in 76% yield. M.p. 252°C; IR

Table 5. Antiparkinsonian activity of the new compounds as compared with Benzotropene®.

Comp. no.	Salivation & lacrimation score	Tremors score	% decrease from Oxotremerine® rectal temp.	Relative potency to Benzotropene®
Control	0	0	0	0
Oxotremerine®	3	3	0	0
Benzotropene®	1	1	25	1
3	2	2	15	0.62
4	1	1	20	0.84
9	3	3	4	0.16
10	2	2	11	0.44
12a	2	2	10	0.42
12b	1	1	20	0.84
15	3	3	3	0.12

(KBr, ν, cm^{-1}): 3366 (OH), 1700 (C=O), 1600 (C=N); ^{1}H-NMR (DMSO-d_6, 270 MHz) δ (ppm): 6.82 (s, 2H, Pyri-H), 7.15–798 (m, 6H, Ar-H + benzylic-H), 11.24 (s, 2H, 2 OH, exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, 67.5 MHz) δ (ppm): 86.90, 126.76, 127.98, 128.34, 132,00, 134.55, 142.86, 145.00, 148.01, 148.65, 159.5, 196.15; EIMS: *m/z* (%) 338 (M^+, 16), and at 130 (100, base peak). Elemental analysis for $C_{16}H_{10}N_4O_3S$ (338.34): Calcd.: C, 56.80; H, 2.98; N, 16.56; S, 9.48. Found: C, 56.75; H, 2.92; N, 16.50; S, 9.42.

Method B

A mixture of **6** (0.25 g, 1 mmol) and benzaldehyde (0.106 g, 1 mmol) in 40 mL, a mixture of AcOH/Ac_2O (1:3) was refluxed for 5 h, allowed to cool, then poured onto water the solid formed was collected by filtration and crystallized to yield compound **7** in 82% yield, as identified by its m.p., mixed m.p. and R_f value on TLC by comparison with authentic sample from method A.

*5-(2,6-Dihydroxypyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione **8***

To a solution of **1** (0.17g, 1 mmol) in hot alcoholic potassium hydroxide (0.15 g, 50 mL), carbon disulphide (30 mL) was added. The reaction mixture was heated in water bath for 6 h, the excess carbon disulphide evaporated under reduced pressure, cooled and treated with acetic acid (5 mL). The resulting solid was collected by filtration, dried and crystallized from acetic acid to give compound **8**. Yield (82%), m.p. 178°C; IR (KBr, ν, cm^{-1}): 3415 (OH), 3345 (NH), 1605 (C=N), 1165 (C=S); ^{1}H-NMR (DMSO-d_6, 270 MHz) δ (ppm): 6.84 (s, 2H, pyri-H), 8.56 (s, 1H, NH, exchangeable with D_2O), 11.25 (s, 2H, 2 OH, exchangeable with D_2O); EIMS: *m/z* (%) 210 (M^+ – 1, 100 and as base peak). Elemental analysis for $C_7H_5N_3O_3S$ (211.20): Calcd.: C, 39.81; H, 2.39; N, 19.90; S, 15.18. Found: C, 39.75; H, 2.34; N, 19.84; S, 15.08.

*4-Amino-3-(2,6-dihydroxypyridin-4-yl)-1H-1,2,4-triazole-5(4H)-thione **9***

A mixture of compound **8** (0.2 g, 1 mmol) and hydrazine hydrate (0.4 g, 8 mmol) in absolute ethanol (30 mL) was heated under reflux for 6 h. The reaction mixture was evaporated under reduced pressure to dryness, washed with *n*-hexane and solidified with diethyl ether. The obtained solid was filtered off, dried and crystallized from ethanol to give *N*-aminotriazole derivative **9**. Yield (68%), m.p. 242°C; IR (KBr, ν, cm^{-1}): 3396 (OH), 3298–2204 (NH_2, NH), 1608 (C=N), 1158 (C=S); ^{1}H-NMR (DMSO-d_6, 270 MHz) δ (ppm): 5.05 (s, 2H, NH_2 exchangeable with D_2O), 6.78 (s, 2H, pyri-H), 11.18 (s, 2H, 2 OH, exchangeable with D_2O), 12.98 (s, 1H, NH, exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, 67.5 MHz) δ (ppm): 94.05, 139.56, 147.25, 159.56, 194.15; EIMS: *m/z* (%) 225 (M^+, 8) and at 115 (100, base peak). Elemental analysis for $C_7H_7N_5O_2S$ (225.23): Calcd.: C, 37.33; H, 3.13; N, 31.09; S, 14.24. Found: C, 37.24; H, 3.04; N, 30.98; S, 14.17.

*4,5,6,7-Tetrachloro-2-(3-(2,6-dihydroxypyridin-4-yl)-5-thioxo-1H-1,2,4-triazol-4(5H)-yl)isoindoline-1,3-dione **10***

Compound **10** was synthesized by using the same procedure of synthesis of derivative **2** but using compound **9** as a starting material. Yield (75%), m.p. 265°C; IR (KBr, ν, cm^{-1}): 3415 (OH), 3312 (NH), 1605 (C=N), 1160 (C=S); ^{1}H-NMR (DMSO-d_6, 270 MHz) δ (ppm): 6.84 (s, 2H, pyri-H), 11.38 (s, 2H, 2 OH, exchangeable with D_2O), 13.05 (s, 1H, NH, exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, 67.5 MHz) δ (ppm): 93.86, 127.76, 132.98, 134.50, 137.56, 147.75, 159.46, 163.89, 196.15; EIMS: *m/z* (%) 493 (M^+, 18) and at 281 (100, base peak). Elemental analysis for $C_{15}H_5Cl_4N5O_4S$ (493.11): Calcd.: C, 36.54; H, 1.02; Cl, 28.76; N, 14.20; S, 6.50. Found: C, 36.47; H, 1.00; Cl, 28.70; N, 14.06; S, 6.44.

*General procedure for the synthesis of sulfonyl compounds **12a** and **12b***

A mixture of hydrazide **11** (0.2 g, 1 mmol) and the aryl halides, namely, benzene sulfonyl chloride or *p*-toluene sulfonyl chloride (2 mmol) in absolute ethanol (20 mL) was refluxed for 8 h. The formed precipitate was filtered off, dried and crystallized from acetic acid/water to give compounds **12a** and **12b**, respectively.

*N′-(6-Chloro-4-(2-(phenylsulfonyl)hydrazinecarbonyl) pyridin-2-yl)benzene-sulfono-hydrazide **12a***

Yield (78%), m.p. 186°C; IR (KBr, ν, cm^{-1}): 3412 (OH), 3335–3228 (NH), 1680 (C=O), 1368, 1160 (SO_2); ^{1}H-NMR (DMSO-d_6, 270 MHz) δ (ppm): 6.94, 7.15 (2s, 2H, pyri-H), 7.35–7.88 (m, 10H, Ar-H), 8.13, 8.25, 9.54, 10.86 (4s, 4H, 4 NH, exchangeable with D_2O), 11.42 (s, 2H, 2 OH, exchangeable with D_2O); EIMS: *m/z* (%) 482 (M^+, 18) and at 310 (100, base peak). Elemental analysis for $C_{18}H_{16}ClN_5O_5S_2$ (481.93): Calcd.: C, 44.86; H, 3.35; Cl, 7.36; N, 14.53; S, 13.31. Found: C, 44.80; H, 3.28; Cl, 7.29; N, 14.48; S, 13.26.

*N′-(6-Chloro-4-(2-tosylhydrazinecarbonyl)pyridin-2-yl)-4-methylbenzene-sulfono-hydrazide **12b***

Yield (72%), m.p. 232°C; IR (KBr, ν, cm^{-1}): 3398 (OH), 3342–3232 (NH), 1685 (C=O), 1370, 1166 (SO_2); ^{1}H-NMR (DMSO-d_6, 270 MHz) δ (ppm): 2.28 (s, 6H, 2 CH_3), 6.90, 7.18 (2s, 2H, pyri-H), 7.42–7.86 (m, 8H, Ar-H), 8.10, 8.22, 9.62, 10.82 (4s, 4H, 4 NH, exchangeable with D_2O), 11.38 (s, 2H, 2 OH, exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, 67.5 MHz) δ (ppm): 21.05, 104.20, 117.82, 127.85, 128.78, 132.68, 136.98, 145.18, 149.65, 159.32, 164.52; EIMS: *m/z* (%) 510 (M^+, 28) and at 155 (100, base peak). Elemental analysis for $C_{20}H_{20}ClN_5O_5S_2$ (509.99): Calcd.: C, 47.10; H, 3.95; Cl, 6.95; N, 13.73; S, 12.57. Found: C, 47.00; H, 3.90; Cl, 6.90; N, 13.67; S, 12.50.

*General procedure for the synthesis of compounds **13–15***

A mixture of compound **11** (0.2 g, 1 mmol) and diethylmalonate, pentane-2,4-dione or ethyl 3-oxobutaneoate (2 mmol) in glacial acetic acid for 6–8 h. After cooling, the formed precipitate was filtered off, dried and crystallized from acetic acid to give the corresponding compounds **13–15**, respectively.

*1-(6-Chloro-4-(3,5-dioxopyrazolidine-1-carbonyl)pyridin-2-yl)pyrazolidine-3,5-dione **13***

Yield (78%), m.p. 256°C; IR (KBr, ν, cm^{-1}): 3410 (OH), 3265 (NH), 1686 (C=O); ^{1}H-NMR (DMSO-d_6, 270 MHz) δ (ppm): 3.25 (s, 4H, cyclic CH_2), 7.34, 7.87 (2s, 2H, pyri-H), 9.86, 9.92 (2s, 2H, 2 NH, exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, 67.5 MHz) δ (ppm): 45.65, 46.48, 112.30, 118.85, 145.75, 149.88, 152.68, 164.84, 167.92, 169.65, 171.85; EIMS: *m/z* (%) 337 (M^+, 8) and at 238

(100, base peak). Elemental analysis for $C_{12}H_8ClN_5O_5$ (337.68): Calcd.: C, 42.68; H, 2.39; Cl, 10.50; N, 20.74. Found: C, 42.60; H, 2.32; Cl, 10.45; N, 20.68.

(2-Chloro-6-(3,5-dimethyl-1H-pyrazol-1-yl)pyridin-4-yl) (3,5-dimethyl-1H-pyrazol-1-yl)methanone ***14***

Yield (58%), m.p. 265°C; IR (KBr, ν, cm^{-1}): 3418 (OH), 1682 (C=O); ^{1}H-NMR (DMSO-d_6, 270 MHz) δ (ppm): 2.34–2.42 (m, 12H, 4 CH_3), 7.12–7.98 (m, 4H, pyri-H + CH-pyrazole); ^{13}C-NMR (DMSO-d_6, 67.5 MHz) δ (ppm): 11.78, 12.70, 12.82, 12.98, 108.88, 109.10, 111.68, 120.38, 139.98, 142.28, 144.45, 149.92, 150.76, 151.48, 152.87, 164.65; EIMS: *m/z* (%) 330 (M^+ + 1, 12) and at 234 (100, base peak). Elemental analysis for $C_{16}H_{16}ClN_5O$ (329.78): Calcd. C, 58.27; H, 4.89; Cl, 10.75; N, 21.24. Found: C, 58.21; H, 4.84; Cl, 10.70; N, 21.20.

1-(6-Chloro-4-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazole-1-carbonyl)pyridin-2-yl)-3-methyl-1H-pyrazol-5(4H)-one ***15***

Yield (70%), m.p. 198°C; IR (KBr, ν, cm^{-1}): 3406 (OH), 1708 (C=O), 1680 (C=O); ^{1}H-NMR (DMSO-d_6, 270 MHz) δ (ppm): 1.92 (s, 6H, 2 CH_3), 2.35 (s, 4H, 2 CH_2), 7.12, 7.98 (2s, 2H, pyri-H); ^{13}C-NMR (DMSO-d_6, 67.5 MHz) δ (ppm): 15.22, 15.54, 41.32, 41.76, 111.98, 118.76, 145.82, 149.00, 152.55, 158.68, 162.18, 169.43, 171.95; EIMS: *m/z* (%) 333 (M^+, 8) and at 236 (100, base peak). Elemental analysis for $C_{14}H_{12}ClN_5O_3$ (333.73): Calcd.: C, 50.39; H, 3.62; Cl, 10.62; N, 20.99. Found: C, 50.32; H, 3.56; Cl, 10.58; N, 20.94.

Pharmacological screening

Determination of acute toxicity (LD_{50})

The LD_{50} was determined by using rats. They were injected with different increasing doses of the synthesized compounds. The dose that killed 50% of the animals was calculated according to Austen *et al.* [25] (Table 1).

Anti-inflammatory activity

Purpose and rational

For the determination of the antiphlogistic potency of the synthesized compounds, two standard tests were realized at a dose level 2.5 and 5 mg/kg body weight of the rats, namely, the protection against carrageenan induced edema according to Winter *et al*. [26] and the inhibition of plasma PGE2. The latter is known as a good confirming indicator for the carrageenan-induced rat paw edema [27].

Carrageenan-induced edema (rats paw test)

Groups of adult male albino rats (150–180 g), each of eight animals were orally dosed with tested compounds at a dose level of 2.5–5 mg/kg one hour before the carrageenan challenge. Foot paw edema was induced by subplantar injection of 0.05 mL of a 1% suspension of carrageenan in saline into the plantar tissue of one hind paw. An equal volume of saline was injected to the other hind paw and served as control. Four hours after drug administration, the animals were decapitated, blood was collected, and the paws were rapidly excised. The average weight of edema was examined for the treated as well as for the control group, and the percentage inhibition of weight of edema was evaluated. Diclofenac potassium (5 mg/kg) was employed as standard reference to which the tested compounds were compared (Table 2).

Estimation of plasma prostaglandin E2 (PGE2)

Heparinized blood samples were collected from rats ($n = 8$), plasma was separated by centrifugation at 12 000 × *g* for 2 min at 40°C, immediately frozen, and stored at 20°C until use. The design correlate EIA prostaglandin E2 (PGE2) kit (Aldrich, Steinheim, Germany) is a competitive immunoassay for the quantitative determination of PGE2 in biological fluids. The kit uses a monoclonal antibody to PGE2 to bind, in a competitive manner, the PGE2 in the sample after a simultaneous incubation at room temperature. The excess reagents were washed away and the substrate was added. After a short incubation time, the enzyme reaction was stopped, and the yellow color generated was read on a microplate reader DYNATech, MR 5000 at 405 nm (Dynatech Industries Inc., McLean, VA, USA). The intensity of the bound yellow color is inversely proportional to the concentration of PGE2 in either standard or samples.

Analgesic activity

Purpose and Rationale

Sixty Webster mice of both sexes weighting 20–25 g were divided into ten groups. One group was kept as control (received saline), the second group received vehicle (gum acacia), and the third one received valdecoxib as a reference drug, whereas the other groups received the test compounds (s.c. administration). Mice were dropped gently in a dry glass beaker of 1 dm^3 capacity maintained at 55–55.5°C. Normal reaction time in seconds for all animals was determined at time intervals of 10, 20, 30, 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 jump out of the beaker (dose 5 mg/kg) [28]. The relative potencies to valdecoxib were determined (Table 3).

Anticonvulsant Activity

Purpose and Rationale

Antagonism against yohimbine-induced seizures in mice is considered to be a predictive model of potential anxiolytic and GABA-mimetic [29].

Procedure

Male Webster mice (20–30 g) were individually placed in clear plastic cylinder and the tested compounds were administered intraperitoneal (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 [30] (Table 4).

Antiparkinsonian Activity

Purpose and Rationale

The muscarinic agonists Tremorine® and Oxotremorine® induce parkinisonian-like signs such as tremor, attaxia, spasticity, salivation, lacrimation, and hypothermia. These signs are antagonized by antiparkinsonian agents.

Procedure

Groups of eight mail mice (18–20 g) were used. They were dosed orally with the tested compounds (5 mg/kg) or the standard (Benzotropene® mesilate, 5 mg/kg) [31] 1 h prior to the administration of 0.5 mg/kg of Oxotremerine® S.C. Rectal temperature was measured before administration of the compounds and one hour after Oxotremerine® dosage. The scores for tremor, attaxia, spasticity, salivation, lacrimation, and hypothermia were recorded as zero (absent), one (slight), two (medium), and three (high) (Table 5).

Statistical Analysis

Analysis of variance (ANOVA) was carried out to determine any significant differences among concentration effects ($p < 0.05$).

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