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Synthesis, lipophilicity and biological evaluation of indole-containing derivatives of 1,3,4-thiadiazole and 1,2,4-triazole

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Abstract

3-[(2-Methyl-1*H*-3-indolyl)methyl]-4-aryl-4,5-dihydro-1*H*-1,2,4-triazole-5-thiones **6a–c** and their respective *N*-{5-[(2-methyl-1*H*-3-indolyl)methyl]-1,3,4-thiadiazol-2-yl}-*N*-arylamines **7a,b** have been prepared. The antidepressant profile of **6a,c** and **7a** was studied on mice with respect to that of the analogous 3-(1*H*-1-indolylmethyl)-4-aryl-4,5-dihydro-1*H*-1,2,4-triazole-5-thiones **1a–c** and the respective *N*-{5-[(2-methyl-1*H*-3-indolyl)methyl]-1,3,4-thiadiazol-2-yl}-*N*-arylamines **2a–c**, the synthesis and antimicrobial potency of which we have recently reported. Behavioral effects, induced by the members of both series, in conjunction with their activity in some specific tests (forced swim, pentetrazole convulsions) on mice, show that these derivatives cross the blood–brain barrier and could develop an antidepressant activity comparable to that of imipramine. Blood–brain barrier penetration is also supported by the lipophilicity data obtained for all analogs.

Keywords: Indole; 1,3,4-Thiadiazole; 1,2,4-Triazole; Lipophilicity; Antidepressant activity

1. Introduction

Derivatives of 1,3,4-thiadiazole and 1,2,4-triazole are known to exhibit anti-inflammatory [1–3], antiviral [4,5], analgesic [6,7], antimicrobial [8–12], anticonvulsant [13–16] and antidepressant activity [17], the latter being usually explored by the forced swim test [18,19]. Among the pharmacological profiles of 1,3,4-thiadiazoles and 1,2,4-triazoles, their antimicrobial, anticonvulsant and antidepressant properties seem to be the best documented. Furthermore, although limited, there are examples in the literature on the antibacterial [20–22] and antidepressant activity of indolic molecules [23].

We have recently reported on the design, synthesis and antibacterial activity of new indolic derivatives of triazoles **1a–c** and thiadiazoles **2a–c** (Table 1), emphasizing in particular the strategy of combining two chemically different but pharmacologically compatible molecules (the indole nucleus and the five-membered heterocycles) in one frame [24]. In view of the pharmacological profiles of these two chemical moieties, as described above, we considered it interesting to further explore the biological properties of compounds **1a–c** and **2a–c**. In particular, we examined their behavioral effects as well as their activity in specific tests (forced swim, pentetrazole convulsions) on mice, in order to ascertain their entry into the brain and to possibly highlight their tranquilizing, antidepressant or anticonvulsant potential. Furthermore, in order to probe the stereoelectronic requirements for optimal effectiveness of the indolic component of these molecules, we made further structural modifications by relocating the 4-aryl-4,5-dihydro-1*H*-1,2,4-triazole-5-thione moiety and its respective thiadiazole from N-1 to C-3 and introducing a methyl group at C-2 of the indole nucleus. Three of the newly synthesized compounds, **6a,c** and **7a** (Table 1), were tested for their antidepressant and anticonvulsant activity by employing the aforementioned tests.

As the desired action of the compounds is manifested in the central nervous system (CNS), their lipophilicity was also investigated. Calculation procedures applied for the estimation of partition coefficients were further supported by experimental determination. Since high log *P* values cannot be measured with accuracy by the classical shake-flask method [25], log k_w values determined by high-performance liquid chromatography (HPLC) were also considered as lipophilicity indices [26,27].

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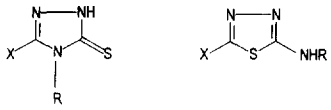
Table 1
Structures of the indole-containing derivatives of 1,3,4-thiadiazole and 1,2,4-triazole



1a-c
6a-c

2a-c
7a,b

Comp.	X	R
1a	1-indolylmethyl	phenyl
1b	1-indolylmethyl	*p*-tolyl
1c	1-indolylmethyl	*α*-naphthyl
2a	1-indolylmethyl	phenyl
2b	1-indolylmethyl	*p*-tolyl
2c	1-indolylmethyl	*α*-naphthyl
6a	2-methyl-3-indolylmethyl	phenyl
6b	2-methyl-3-indolylmethyl	*p*-tolyl
6c	2-methyl-3-indolylmethyl	*α*-naphthyl
7a	2-methyl-3-indolylmethyl	phenyl
7b	2-methyl-3-indolylmethyl	*α*-naphthyl

2. Chemistry

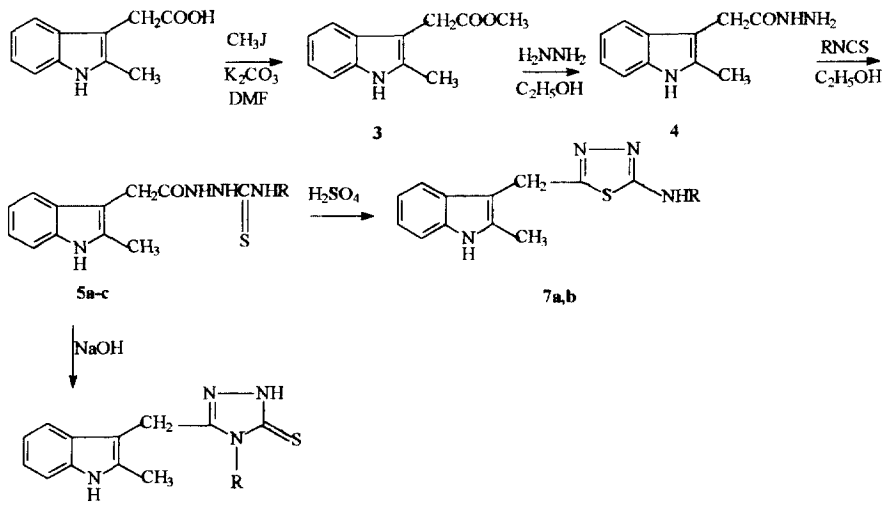
The synthetic pathway followed for the preparation of the target molecules **6a–c** and **7a,b** is depicted in Scheme 1. Commercially available 2-methyl-3-indoleacetic acid was reacted with iodomethane in the presence of potassium carbonate to give ester **3**. The latter was converted to the desired hydrazide **4** in 73% yield upon treatment with hydrazine hydrate in ethanol. The hitherto unknown thiosemicarbazides **5a–c** were obtained upon the reaction of acid hydrazide **4** with aryl isothiocyanates in ethanol. Cyclization of **5a–c** with sodium hydroxide or with sulfuric acid resulted to the formation of 3-[(2-methyl-1*H*-3-indolyl)methyl]-4-aryl-4,5-dihydro-1*H*-1,2,4-triazole-5-thiones **6a–c** and *N*-{5-[(2-methyl-1*H*-3-indolyl)methyl]-1,3,4-thiadiazol-2-yl}-*N*-arylamines **7a,b**, respectively. It is interesting to note that compounds **6a–c** were present in the solid state in the C=S form as indicated by their IR spectra (absence of absorption in the region of 2500–2600 cm^{-1} for S–H stretching and presence of two absorption maxima at 1342 and 1320 cm^{-1}, characteristic of the C=S group in this type of compound [28]). The C=S form is present also in dimethylsulfoxide, as suggested by the respective ^{13}C NMR spectral data.

2.1. Experimental

Melting points were taken in glass capillary tubes on a Büchi 530 apparatus and are uncorrected. Infrared (IR) spectra were run as potassium bromide disks on a Perkin-Elmer 883 spectrophotometer. The nuclear magnetic resonance (^1H and ^{13}C NMR) spectra were recorded on Bruker AC 200 and 300 MHz spectrometers. Chemical shift (δ) values are expressed in ppm relative to tetramethysilane (TMS) as an internal standard and coupling constant (J) values in Hz. Microanalyses were carried out by Service Central de Microanalyses of CNRS in Vernaison, France, and the experimental values were within ±0.4% of theoretical values. For compounds **5a** and **7b**, satisfactory elemental analysis could not be obtained due to rapid decomposition. Spectral (IR, NMR) data were compatible with the assigned structures in all cases.

2.1.1. Methyl 2-(2-methyl-1H-3-indolyl)acetate 3

A mixture of 2-methyl-3-indoleacetic acid (1.53 g, 8.10 mmol), potassium carbonate (1.10 g, 10 mmol), iodomethane (0.61 ml, 10 mmol) and *N*,*N*-dimethylformamide (15 ml) was stirred under nitrogen at room temperature for 10 h. The mixture was diluted with ethyl acetate and washed with water. The organic layer was dried (Na_2SO_4) and the solvent was removed under reduced pressure. The resulting oil was used without any further purification (1.20 g, 73%). ^1H



Scheme 1. Synthetic pathway followed for the preparation of the target molecules **6a–c**, **7a,b**.

NMR[200] ($CDCl_3$, ppm): δ 2.30 (s, 3H, CH_3–indole), 3.64 (s, 3H, OCH_3), 3.69 (s, 2H, CH_2–indole), 7.11 (m, 3H, ArH), 7.99 (m, 1H, ArH), 8.40 (b s, indole–N*H*). ^{13}C NMR[50] ($CDCl_3$, ppm): δ 11.00, 29.40, 51.36, 105.02, 110.23, 117.97, 119.50, 121.18, 129.42, 134.00, 136.01, 164.00.

2.1.2. 2-(2-Methyl-1H-3-indolyl)ethanohydrazide ***4***

A solution of the ester **3** (3.30 g, 16.24 mmol), hydrazine hydrate (3 ml) and ethanol (4ml) was refluxed for 1 h. The solution was evaporated to dryness and the resulting solid was recrystallized from ethanol (2.27 g, 73%), m.p. 160°C. ^1H NMR[300] [$(CD_3)_2SO$, ppm]: δ 2.30 (s, 3H, CH_3–indole), 3.47 (s, 2H, CH_2–indole), 6.77 (b s, 2H, NH_2), 7.03 (m, 2H, ArH), 7.18 (m, 1H, arom), 7.29 (m, 1H, arom), 8.01 (s, 1H, NH).

2.1.3. General procedure for the preparation of the thiosemicarbazides ***5a–c***

A mixture of the acid hydrazide **4** (0.42 g, 4.03 mmol) and the appropriate isothiocyanate (5.97 mmol) in ethanol (20 ml) was refluxed for 0.5 h. The solution was allowed to reach ambient temperature and the resulting solid was collected and recrystallized from ethanol to give the title compounds as white powders.

2.1.3.1. N1-Phenyl-2-[2-(2-(2-methyl-1H-3-indolyl)acetyl-1-hydrazinecarbothioamide ***5a***

78% yield, m.p. 168–169°C; IR (KBr, cm^{-1}): ν 3410 (N–H), 1670 (C=O), 1450 (C=S); ^1H NMR[200] [$(CD_3)_2SO$, ppm]: δ 2.37 (s, 3H, CH_3–indole), 3.48 (s, 2H, CH_2–indole), 6.91 (m, 3H, ArH), 7.31 (m, 6H, ArH), 7.83 (m, 3H, NH), 10.44 (s, 1H, NHCO).

2.1.3.2. N1-p-Tolyl-2-[2-(2-(2-methyl-1H-3-indolyl)acetyl-1-hydrazinecarbothioamide ***5b***

81% yield, m.p. 188°C; IR (KBr, cm^{-1}): ν 3400 (N–H), 1675 (C=O), 1460 (C=S); ^1H NMR[200] [$(CD_3)_2SO$, ppm]: δ 2.10 (s, 3H, PhCH_3), 2.56 (s, 3H, CH_3–indole), 3.61 (s, 2H, CH_2–indole), 7.05 (m, 2H, ArH), 7.18 (m, 2H, ArH), 7.30 (m, 3H, ArH), 7.59 (d, J = 7.57 Hz, 1H, ArH), 9.41 (s, 1H, NH), 10.05 (s, 1H, NH) 10.84 (s, 1H, NH). *Anal.* $C_{19}H_{20}N_4OS$ (C, H).

2.1.3.3. N1-α-Naphthyl-2-[2-(2-(2-methyl-1H-3-indolyl)-acetyl-1-hydrazinecarbothioamide ***5c***

92% yield, m.p. 190°C; IR (KBr, cm^{-1}): ν 3400 (N–H), 1675 (C=O), 1470 (C=S); ^1H NMR[200] [$(CD_3)_2SO$, ppm]: δ 2.35 (s, 3H, CH_3–indole), 3.66 (s, 2H, CH_2–indole), 6.93 (m, 2H, ArH), 7.22 (m, 1H, ArH), 7.49 (m, 5H, ArH), 7.90 (m, 3H, ArH), 9.68 (s, 1H, NH), 9.78 (s, 1H, NH), 10.14 (s, 1H, NH), 10.80 (s, 1H, NH). *Anal.* $C_{22}H_{20}N_4OS$ (C, H).

2.1.4. General procedure for the preparation of the 3-[(2-methyl-1H-3-indolyl)methyl]-4-aryl-4,5-dihydro-1H-1,2,4-triazole-5-thiones ***6a–c***

A solution of the appropriate thiosemicarbazide (0.59 mmol) in sodium hydroxide (5%, 6 ml) was stirred at room temperature for 0.5 h. The resulting solution was poured into ice water and acidified to pH 6 with 5% HCl. The precipitate formed was filtered, washed with water and recrystallized from ethanol.

2.1.4.1. 3-[(2-Methyl-1H-3-indolyl)methyl]-4-phenyl-4,5-dihydro-1H-1,2,4-triazole-5-thione ***6a***

77% yield, m.p. > 200°C; IR (KBr, cm^{-1}): ν 3400 (N–H), 1340, 1322 (C=S); ^1H NMR[200] [$(CD_3)_2SO$, ppm]: δ 1.72 (s, 3H, CH_3–indole), 3.87 (s, 2H, CH_2–indole), 6.88 (m, 2H, ArH), 7.10 (m, 4H, ArH), 7.45 (m, 3H, ArH), 10.68 (s, 1H, indole–N*H*), 13.67 (s, 1H, triazole–N*H*); ^{13}C NMR[50] [$(CD_3)_2SO$, ppm]: δ 10.87, 21.89, 102.95, 109.01, 117.79, 118.83, 120.67, 128.19, 128.68, 131.27, 135.53, 137.72, 139.97, 152.04, 167.00. *Anal.* $C_{18}H_{16}N_4S$ (C, H).

2.1.4.2. 3-[(2-Methyl-1H-3-indolyl)methyl]-4-(4-methyl-phenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione ***6b***

84% yield, m.p. > 200°C; ^1H NMR[200] [$(CD_3)_2SO$, ppm]: δ 1.80 (s, 3H, PhCH_3), 2.35 (s, 3H, CH_3–indole), 3.85 (s, 2H, CH_2–indole), 6.89 (m, 4H, ArH), 7.21 (m, 4H, ArH), 10.71 (s, 1H, indole–N*H*); ^{13}C NMR[50] [$(CD_3)_2SO$, ppm]: δ 10.97, 21.34, 21.89, 103.05, 110.98, 117.88, 118.89, 120.70, 128.29, 128.51, 130.29, 131.74, 133.79, 135.59, 139.49, 152.24, 166.43. *Anal.* $C_{19}H_{18}N_4S \cdot 0.5H_2O$ (C, H).

2.1.4.3. 3-[(2-Methyl-1H-3-indolyl)methyl]-4-(1-naphthyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione ***6c***

88% yield, m.p. > 200°C; ^1H NMR[200] [$(CD_3)_2SO$, ppm]: δ 2.32 (s, 3H, CH_3–indole), 3.76 (s, 2H, CH_2–indole), 6.88 (m, 3H, ArH), 7.08 (m, 1H, ArH), 7.13–7.20 (m, 2H, ArH), 7.29 (m, 1H, ArH), 7.53 (m, 2H, ArH), 8.02 (m, 2H, ArH), 10.39 (s, 1H, NH); ^{13}C NMR[50] [$(CD_3)_2SO$, ppm]: δ 9.37, 20.91, 101.53, 109.76, 116.64, 117.75, 119.59, 121.03, 125.99, 126.17, 126.54, 127.02, 127.55, 128.76, 129.44, 132.43, 132.21, 134.47, 135.68, 151.59, 167.99. *Anal.* $C_{22}H_{18}N_4S \cdot 1.5H_2O$ (C, H).

2.1.5. General procedure for the preparation of N-{5-[(2-methyl-1H-3-indolyl)methyl]-1,3,4-thiadiazol-2-yl}-N-arylamines ***7a,b***

A mixture of the appropriate thiosemicarbazide **5a–c** (1 mmol) in cold concentrated sulfuric acid (3 ml) was stirred for 10 min. The resulting solution was then allowed to reach ambient temperature, left stirring for 15 min and poured cautiously into ice cold water. The reaction mixture was made alkaline to pH 8 with aqueous ammonia and the precipitated product was filtered. The cake was washed with water and recrystallized from ethanol.

2.1.5.1. N-{5-[(2-Methyl-1H-3-indolyl)methyl]-1,3,4-thiadiazol-2-yl}-N-phenylamine **7a**

85% yield, m.p. > 200°C; IR (KBr, cm^{-1}): ν 3400 (N–H), ^1H NMR[300] [$(CD_3)_2SO$, ppm]: δ 2.56 (s, 3H, CH_3–indole), 4.34 (s, 2H, CH_2–indole), 7.05 (m, 3H, ArH), 7.34 (m, 4H, ArH), 7.46 (d, J = 7.68 Hz, 1H, ArH), 7.58 (d, J = 8.08 Hz, 1H, ArH), 10.98 (s, 1H, indole–NH), 11.00 (s, 1H, NHPh); ^{13}C NMR[75] [$(CD_3)_2SO$, ppm]: δ 10.10, 24.90, 107.50, 111.04, 117.64, 117.80, 119.03, 120.86, 122.03, 128.10, 129.48, 133.95, 140.00, 141.15, 161.80. *Anal.* $C_{18}H_{16}N_4S$ (C, H).

2.1.5.2. N-{5-[(2-Methyl-1H-3-indolyl)methyl]-1,3,4-thiadiazol-2-yl}-N-(1-naphthyl)amine **7b**

88% yield, m.p. > 200°C (ethanol/ether); ^1H NMR[300] [$(CD_3)_2SO$, ppm]: δ 2.56 (s, 3H, CH_3–indole), 4.34 (s, 2H, CH_2–indole), 7.04 (m, 3H, ArH), 7.16 (s, 1H, –indole–NH), 7.32 (m, 2H, ArH), 7.54 (m, 3H, ArH), 7.96 (d, J = 8.04 Hz, 1H, ArH), 8.02 (d, J = 8.04 Hz, 1H, ArH), 8.20 (d, J = 9.11 Hz, 1H, ArH), 8.92 (dd, J = 9.52 and 2.43 Hz, 1H, ArH), 10.98 (s, 1H, NH–naphthyl); ^{13}C NMR[75] [$(CD_3)_2SO$, ppm]: δ 11.78, 25.2, 106.99, 111.14, 115.36, 117.91, 119.17, 120.98, 122.21, 125.32, 126.01, 126.35, 125.56, 128.11, 128.68, 130.42, 133.52, 135.72, 137.89, 140.21, 162.19, 166.62.

3. Pharmacology

Compounds **1a–c**, **2a–c**, **6a,c** and **7a** were tested for antidepressant and anticonvulsant activity. The increase of the anti-immobility action of the above compounds is listed in Table 2. None of the compounds tested showed any significant anticonvulsant effects against clonic or tonic activity, induced by pentetrazole, with the exception of some proconvulsant action exhibited by the *N*-substituted analogs **1a–c** and **2a–c**.

3.1. Experimental

Adults (4 months) male Balb-C mice weighing 23.7 ± 0.4 g were obtained from the Hellenic Pasteur Institute. The indole derivatives were dispersed in aqueous solution of Tween-80 (5%) and administered intraperitoneally to mice in a volume of 10 μl/g of body weight. Controls received the same volume of Tween-80 (5%). Imipramine hydrochloride was dissolved in Tween-80 (5%) and pentetrazole in saline solution.

3.1.1. Toxicology

The indole derivatives were administered to mice at doses from 1 to 1000 mg/kg (i.p.). Mice were observed daily, for ten days, in order to detect deaths or undesirable effects (ataxia, convulsions, paralysis, diarrhea, etc.).

3.1.2. Behavioral observations

Mice received the indole derivatives (1–800 mg/kg, i.p.) and, one hour later, they were observed for 15 min in the open field. Their reactivity to external (audio) stimulus was also observed 30 min, 2 and 4 h after the administration.

3.1.3. Potential antidepressant activity

Mice received the indole derivatives (1–800 mg/kg, i.p.) or imipramine (30 mg/kg) and, one hour later, they were forced to swim in a glass cylinder (9 cm diameter) containing water (at 25°C ± 1) of depth 6 cm [18,19]. Mice swam for 6 min, but the observation of the anti-immobility activity (time during which mice tried to escape) operated only in the last 4 min.

Table 2
Pharmacological (antidepressive) evaluation of indole-containing derivatives of 1,3,4-thiadiazole and 1,2,4-triazole

Comp.	Dose (mg/kg, i.p.)	Anti-immobility action [a]: mean ± S.E.M.	Increase of anti-immobility action (%)
1a–c, 2a–c	10–50	49 ± 4.73–90 ± 21.3 [b]	80–220
Control	0	21 ± 4.2–32 ± 5.0	0
Imipramine	30	42 ± 4.7 [b]	100
Control	0	21 ± 4.2	0
6a	600	143 ± 16.0 [c]	320
6b	nt	nt	nt
6c	600	132 ± 16.7 [c]	288
Control	0	34 ± 8.9	0
7a	200	124 ± 15.6 [c]	276
7b	nt	nt	nt
Control	0	33 ± 4.5	0
Imipramine	30	81 ± 15.2 [b]	145

[a] Time (s) during which mice try to escape.
nt = not tested.
[b] $p < 0.02$.
[c] $p < 0.001$.

3.1.4. Anticonvulsant action

One hour after the administration of the indole derivatives (1–800 mg/kg, i.p.), mice received pentetrazole (90 mg/kg, i.p.). In order to evaluate the potential anticonvulsant or proconvulsant action of the above-mentioned compounds on the induction and generalization of the pentetrazole seizures, the latency of the appearance of the first convulsions, the latency of the tonic activity and of the lethality as well as the intensity of the convulsions were measured by employing methods previously described [29–32], and utilizing the scale inspired by Iadarola and Gale [30], i.e.: 0, no convulsions; 1, mild clonic activity; 2, important clonic activity; 3, tonic activity with extension of the legs. The unpaired Student's test was used for determination of statistical significances.

3.2. Lipophilicity

3.2.1. Calculation of partition coefficients

Octanol–water partition coefficients were estimated according to the modified Rekker's fragmental system [33] and modified Ghose–Crippen atomic contribution system [34], implemented in PrologP (version 2.1, CompuDrug) as the CDR and ATOMIC5 option. ClogP for Windows (version 1.0.0, Biobyte) was also used, but due to missing fragment values only the log P of compounds **7a** and **7b** could be estimated. Partial calculation within each homologous subset was also performed by adding the appropriate hydrophobic fragmental constants to experimentally obtained log P values. In that case Rekker's values were used and a correction of one $C_M = 0.219$ was added for the naphthyl derivatives to account for ring condensation [35].

3.2.2. Measurement of partition coefficients

Octanol–water partition coefficients were measured using the shake-flask method [25]. Dilute aqueous solutions of the compounds were agitated with octanol for 2 h. The two phases were mutually saturated before the experiment. The volume ratio of the two phases was chosen so that an adequate amount of the solute remained in the aqueous phase after equilibration. Centrifugation followed for 30 min at 2500 rpm. The aqueous phase was analysed spectrophotometrically before and after equilibration using a Perkin-Elmer Lambda 7 UV/VIS spectrophotometer, or by HPLC applying the conditions described below.

Each determination was performed at least in triplicate and the mean values are reported.

3.2.3. Determination of log k_w

Capacity factors values were determined by HPLC using a Waters instrument equipped with a UV detector. An octadecyl silanized silica (ODS) column (25 cm × 4 mm i.d.) prepacked with LiChrosorb RP-18 (particle size μm) stationary phase was used. Mobile phases were made up volumetrically using bidistilled Millipore water and different proportions of methanol (HPLC grade) in the range 75–45%. All solutions were purified and degassed by filtration using a Millipore Milli-Q system. Dilute solutions of the test compounds ($\approx 10^{-5}$–10^{-6}) were prepared in methanol and each compound was injected at least twice. Retention times t_r were measured at ambient temperature. The flow rate was adjusted to be 1.2 ml/min and the column dead-time t_o was determined using the organic modifier as the non-retained compound. Isocratic capacity factors log k, defined as $\log[(t_r - t_o)/t_o]$, were linearly extrapolated to 0% methanol to yield log k_w, according to the relationship $\log k = -S\varphi + \log k_w$ (φ being the methanol volume fraction and S the corresponding slope)

All lipophilicity data are reported in Table 3. Partial calculated log P values are considered together with the experimental log P data, since calculation concerns only a small additive part of the molecule. Experimental log P and log k_w values are in relatively good agreement, while log P estimates generated by the calculation procedures are significantly higher. Correlation between log k_w and log P leads to the following equation with satisfactory statistics:

$$\log P = -2.157(\pm 0.884) + 1.597(\pm 0.295) \log k_w \quad (1)$$

$$n = 5, \quad r = 0.953, \quad s = 0.254, \quad F = 29.17$$

where n is the number of data points, r the correlation coefficient, and s the standard deviation. Within the homologous subsets, log P values increase regularly from phenyl to tolyl to naphthyl derivatives, as expected. The lipophilicity of the N-substituted compounds and that of the C-3 substituted is of the same magnitude. Significant differentiation in lipophilicity was observed between the triazole and thiadiazole derivatives, the latter being more lipophilic. This observation is consistent within all data sets.

4. Results and discussion

Administration of the triazole and thiadiazole derivatives of both series to mice (10–30 mg/kg, i.p.) did not cause any appreciable change in their behavior. However, a minimal hyper-reactivity to external stimulus was noticed upon administration of the N-substituted derivatives **1a–c** and **2a–c** which was more pronounced in the cases of **2a** and **2c**. All members of both series tested induced some hypomobility and sedation in high doses which was more significant above 400 mg/kg, i.p.

The potential antidepressant activities of the triazoles and thiadiazoles listed in Table 2 were assessed in mice using the forced swim test (FST). As the results indicate, all the compounds tested cause a strong statistically equivalent anti-immobility action which, according to Porsolt et al. [18], could imply antidepressant activity. In detail, the N-substituted analogs enhanced the anti-immobility action by 80–220% ($p < 0.02$) when given at dose levels of 10–50 mg/kg, i.p., the effect appearing to have an inverted U-shape dose–response pattern with a maximum at 30 mg/kg. These anti-

Table 3
Experimental and calculated lipophilicity data

Comp.	log P_{cdr} [a]	Clog P [b]	log P_{ats5} [c]	log k_w [d]	log P [e]	Δ [g]
1a	2.57		3.26	2.65	2.05	0.60
1b	3.09		3.67	3.19	(2.57) [f]	0.62
1c	3.84		4.46	3.39	(3.12) [f]	0.27
2a	4.05		4.30	3.51	3.73	−0.22
2b	4.57		4.70	4.04	(4.24) [f]	−0.20
2c	5.32		5.50	4.57	(4.80) [f]	−0.23
6a	3.37		3.16	2.46	1.87	0.59
6b	3.89		3.56	2.94	2.55	0.39
6c	4.65		4.36	3.08	(3.02) [f]	0.06
7a	4.86	4.91	4.19	3.26	2.71	0.55
7b	6.13	6.08	5.39	4.26	(3.78) [f]	0.48

[a] log P calculated according to modified Rekker's system by PrologP using CDR option.
[b] log P calculated according to Leo–Hansch system by ClogP.
[c] log P calculated according to modified Ghose–Crippen system by PrologP using ATOMIC5 option.
[d] Lipophilicity indexes determined by HPLC.
[e] Octanol–water log P values determined by the shake-flask method.
[f] Octanol–water log P values obtained from the log P values of the phenyl derivative.
[g] Difference between log k_w and octanol–water log P values.

immobility action values are comparable with that of imipramine which at the dose level of 30 mg/kg, i.p., causes an increase of 100–145% ($p<0.02$) [18,19]. It is noteworthy that administration of the *N*-substituted derivatives in high doses (> 100 mg/kg, i.p.) led to disappearance of their anti-immobility effect.

The anti-immobility action exerted by the C-3 substituted analogs **6a,c** and **7a** is even more interesting, as an approximately twofold increase was noticed (300%, $p<0.001$) with respect to imipramine (100–145%) when administered at doses of 100–300 mg/kg, i.p., in the case of **7a** (inverted U-shape effect with a maximum at 200 mg/kg), and above 600 mg/kg, i.p., in the cases of **6a,c**. (It should be noted that for imipramine 30 mg/kg, i.p., constitutes its most performant dose [18,19], since ataxia, tremor and convulsions are observed at higher doses.)

Although a structure–activity correlation within the *N*- and C-3 substituted series is not straightforward, there is a difference in activity between each member of each series and its respective counterpart. Specifically, the C-3 substituted triazoles **6a,c** are more potent than their respective *N*-substituted derivatives **1a,c**. An analogous difference in activity was noticed between the C-3 substituted thiadiazole **7a** and its *N*-substituted counterpart **2a**.

The lipophilicity data obtained for the C-3 substituted molecules **6a–c** and **7a,b** do not appear to be appreciably different from those of their respective *N*-substituted analogs, **1a–c** and **2a–c**. On the other hand, there is a lack of differentiation in activity within each homologous series, despite the increase in lipophilicity. These findings indicate that other factors beyond lipophilicity may be critical for the behavior of the compounds.

The marked differentiation in the doses of administration between the C-3 substituted thiadiazole **7a** and the analogous C-3 substituted triazole derivative **6a** (the former was administered in only one-third of the dose) to produce the same anti-immobility action is probably due to the difference in the positioning of the phenyl group in the two compounds and/or to the discrete biological properties of the thiadiazole and triazole nuclei.

None of the compounds tested showed any significant anticonvulsant effects against clonic or tonic activity, induced by pentetrazole, with the exception of some proconvulsant action exhibited by the *N*-substituted analogs **1a–c** and **2a–c**. At low doses (10–30 mg/kg, i.p.) these molecules produced a decrease of 40–60% ($p<0.05$) in the latency of the first convulsions induced by pentetrazole.

Finally, all the compounds tested exhibited negligible toxicity as they incurred neither deaths nor undesirable side-effects such as ataxia, paralysis, convulsions, diarrhea, etc., even when administered at very high doses (up to 1000 mg/kg, i.p.).

In conclusion, this preliminary investigation showed that all the tested compounds seem to penetrate the blood–brain barrier and develop a central activity free from toxicity. Moreover, in the forced swim test system, which is routinely used to detect potential antidepressants, the *N*-substituted molecules exhibited potency at least equivalent to that of imipramine while the newly synthesized C-3 substituted analogs were significantly more active than the control.

We now have two structurally diverse series of potential 'second generation antidepressants' [3], which should be valuable tools in the elucidation of the mechanism of action of compounds of this type.

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References

[1] P.C. Unangst, G.P. Shrum, D.T. Connor, R.D. Dyer, D.J. Schrier, Novel 1,2,4- oxadiazoles and 1,2,4 thiadiazoles as dual 5-lipoxygenase and cycloxygenase inhibitors, J. Med. Chem. 35 (1992) 3691–3698.

[2] M.D. Mullican, M.W. Wilson, D.T. Connor, C.R. Kostlan, D.J. Schrier, R.D. Dyer, Design of 5-(3,5-di-tert-butyl-4-hydroxyphenyl)-1,3,4-thiadiazoles, 1,3,4-oxadiazoles and 1,2,4-triazoles as orally-active non-ulcerogenic antiinflammatory agents, J. Med. Chem. 36 (1993) 1090–1099.

[3] D.H. Boschelli, D.T. Connor, D.A. Bornemeier, R.D. Dyer, J.A. Kennedy, P.J. Kuipers, G.C. Okonkwo, D.J. Schrier, C.D. Wright, 1,3,4-Oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole analogs of the Fenamates: in vitro inhibition of cycloxygenase and 5-lipoxygenase activities, J. Med. Chem. 36 (1993) 1802–1810.

[4] D.H. Jones, R. Slack, S. Squires, K.R.H. Wooldridge, Antiviral chemotherapy. I. The activity of pyridine and quinoline derivatives against Neurovaccinia in mice, J. Med. Chem. 8 (1965) 676–680.

[5] R.W. Sidwell, R.K. Robins, I.W. Hillyard, Ribavirin: an antiviral agent, Pharmacol. Ther. 6 (1979) 123–128.

[6] J.K. Sughen, T. Yoloye, Medicinal applications of indole derivatives, Pharm. Acta Helv. 58 (1978) 64–68.

[7] V.I. Kelarev, R.A. Karakhanov, S.Sh. Gasanvo, G.V. Morozova, K.P. Kuatbekova, Synthesis of derivatives of 1,3,4 oxa(thia)diazole and 1,2,4-triazole containing 3-indolylmethyl radicals, J. Org. Chem. USSR 29 (1993) 323–329.

[8] Sh.A. Shams El-Dine, A.A.B. Hazzaa, Synthesis of compounds with potential fungicidal activity, Pharmazie 29 (1974) 761–768.

[9] T. Misato, K. Ko, Y. Honma, K. Konno, E. Taniyama, Jpn. Kokai 77 25 028 (1977) [Chem. Abstr. 87 (1977) 14705a].

[10] J.H. Reisdorff, W. Brandes, H. Scheinpflug, B. Homeyer, P. Roessler, Ger. Offen. 2 533 604 (1977) [Chem. Abstr. 88 (1978) 137064b].

[11] G. Van Reet, J. Heeres, L. Wals, US Patent 4 160 838 (1979) [Chem. Abstr. 91 (1979) 175361z].

[12] N.S. Habib, S. Abdel-Hamid, M. El-Hawash, Synthesis of benzimidazole derivatives as potential antimicrobial agents, Farmaco 44 (1989) 1225–1232.

[13] M.R. Stillings, A.P. Welbourn, D.S. Walter, Substituted 1,3,4-thiadiazoles with anticonvulsant activity. 2. Aminoalkylderivatives, J. Med. Chem. 29 (1986) 2280–2284.

[14] J.M. Kane, M.A. Staeger, C.R. Dalton, F.P. Miller, M.W. Dudley, A.M.L. Ogden, J.H. Kehne, H.J. Ketteler, T.C. McCloskey, Y. Senyah, , P.A. Chmielewski, J.A. Miller, 5-Aryl-3-(alkylthio)-4*H*-1,2,4-triazoles as selective antagonists of strychnine-induced convulsions and potential antispastic agents, J. Med. Chem. 37 (1994) 125–132.

[15] C. Ainsworth, N.R. Easton, M. Livezey, D.E. Morrison, W.R. Gibson, The anticonvulsant activity of 1,2,4-triazoles, J.Am. Pharm. Assoc. 5 (1962) 383–389.

[16] C.B. Chapleo, M. Myers, P.L. Myers, J.F. Saville, A.C.B. Smith, M.R. Stillings, I.F. Tulloch, D.S. Walter, A.P. Welbourn, Substituted 1,3,4-thiadiazoles with anticonvulsant activity. 1. Hydrazines, J. Med. Chem. 29 (1986) 2273–2280.

[17] J.M. Kane, M.W. Dudley, S.M. Sorensen, F.P. Miller, 2,4-Dihydro-3*H*-1,2,4-triazole-3-thiones as potential antidepressant agents, J. Med. Chem. 31 (1988) 1253–1258.

[18] R.D. Porsolt, A. Bertin, M. Jalfre, Behavioral despair in mice: a primary screening test for antidepressants, Arch. Int. Pharmacodyn. 229 (1977) 327–336.

[19] A. Vamvakides, Effet de quelques dérivés du GABA de la glycine ou de l'acide glutamique sur le test de la nage forcée chez la souris, Ann. Pharm. Fr. 48 (1990) 154–159.

[20] F. Fujikawa, I. Yamashita, T. Seno, M. Sasaki, M. Naito, S. Tsukuma, Chemotherapeutics for Mycobacterium tuberculosis. Synthesis and antibacterial activity on M. Tuberculosis of indole 3-carboxaldehyde derivatives, Yakugaku Zasshi 86 (a) (1966) 861–864 [Chem. Abstr. 65 (1966) 20086h].

[21] L. Canoira, J. Gonzalo Rodriguez, J.B. Subirats, J.-A. Escario, I. Jimenez, A.R. Martinez-Fernandez, Synthesis, structure and antifungal activity of 3-(2′-nitrovinyl)indoles, Eur. J. Med. Chem. 24 (1989) 39–42.

[22] S. Masanori, O. Toshiharu, T. Hiroko, Y. Yoshitoku, Jpn. Kokai, Tokyo Koho JP 01 31 762 [89 31 762] (1989) [Chem. Abstr. 111 (1989) 153624s].

[23] C. Krishna, K.C. Joshi, P. Chand, Biologically active indole derivatives, Pharmazie 37 (1982) 1–12.

[24] A. Tsotinis, A. Varvaresou, T. Calogeropoulou, Th. Siatra-Papastaikoudi, A. Tyligada, Synthesis and antimicrobial evaluation of indole containing derivatives of 1,3,4-thiadiazole, 1,2,4-triazole and their open-chain counterparts, Arzneim. Forsch. 47 (1) (1997) 307–310.

[25] J. Dearden, G.M. Bresnen, The measurement of partition coefficients, Quant. Struct.–Activity Relat. 7 (1996) 133–144.

[26] Th. Braumann, Determination of hydrophobic parameters by reversed-phase liquid chromatography: theory, experimental techniques and application in studies on quantitative structure–activity relationships, J. Chromatogr. 373 (1986) 191–225.

[27] A. Bechalany, A. Tsantili-Kakoulidou, N. El Tayar, B. Testa, Measurement of lipophilicity indices by reversed-phase high-performance liquid chromatography: comparison of two stationary phases and various eluents, J. Chromatogr. 541 (1991) 221–229.

[28] H. Dziewońska, Infra-red spectra of some 3,4,5-substituted derivatives of 1,2,4-triazole, Spectrochim. Acta A 23 (1967) 1195–1202.

[29] M. Kilian, H.H. Frey, Central monoamines and convulsive thresholds in mice and rats, Neuropharmacology 12 (1973) 681–692.

[30] M.J. Iadarola, K. Gale, Substantia nigra: site of anticonvulsant activity mediated by γ-aminobutyric acid, Science 218 (1982) 1237–1240.

[31] A. Vamvakides, Effet du GABA-linoléamide et du linoléamide de glycine sur les convulsions au pentaméthylènetétrazole, J. Pharmacol. 17 (1986) 323–327.

[32] G.B. Foscolos, N. Colocouris, G. Fytas, P. Maracos, N. Pouli, A. Vamvakides, Synthesis and pharmacological study of some new β-(dialkylaminomethyl)-γ-butyrolactones and their tetrahydrofuran analogues, Farmaco 51 (1996) 19–26.

[33] R.F. Rekker, H.M. de Kort, The hydrophobic fragmental constant: an extension to a 1000 data point set, Eur. J. Med. Chem. 14 (1979) 479–488.

[34] A.K. Ghose, G.M. Crippen, Atomic physicochemical parameters for three-dimensional structure-directed quantitative structure–activity relationships. I. Partition coefficients as a measure of hydrophobicity, J. Comput. Chem. 7 (1986) 565–577.

[35] R.F. Rekker, R. Mannhold, Calculation of Drug Lipophilicity, VCH, Weinheim, 1992, pp. 47–53.