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Original article

Synthesis, hypnotic properties and molecular modeling studies of 1,2,7,9-tetraaza-spiro[4.5]dec-2-ene-6,8,10-triones

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ABSTRACT

1,3-Dipolar cycloaddition reaction of nitrilimines with 5-arylidene-1,3-dimethyl-2,4,6-pyrimidinetriones **1a**–**i** afforded 7,9-dimethyl-1,3,4-triaryl-1,2,7,9-tetraaza-spiro[4.5]dec-2-ene-6,8,10-triones **3a**–**k** in a high regioselective manner. Single crystal X-ray study of **3d** added a conclusive support for the assigned structure. Potentiating effects of the synthesized compounds **3a**–**k** (at a dose of 25 mg/kg body weight) on hypnotic action of sodium thiopental (at a dose of 75 mg/kg body weight i.p.) were investigated in vivo using Albino mice according to the standard method. Most of the tested compounds revealed promising hypnotic potentiating effects especially compounds **3k** and **3e** that could be nominated as short-acting hypnotics. A hypothesis of molecular modeling study, including fitting of the synthesized compounds into 3D-pharmacophore using Discovery Studio 2.5 software and their docking into optimized homology model of $GABA_A$-α_1 showed good results consistent with the observed pharmacological properties.

1. Introduction

Many efforts to develop novel CNS-acting drugs especially, sedative/hypnotic drugs with increased potency and reduced side effects, have been made in order to assure the availability of suitable alternatives for the treatment of sleep-disorders. Problems with sleeping in the general population are associated with a year-to-year increase in the aging rate and mental disorders including depression, neurosis and dementia. The current clinical approach employs different varieties of benzodiazepine derivatives that are used as hypnotic agents for those having difficulties in falling asleep and maintaining sleep. However, these agents are often associated with adverse effects such as amnesia, nightmares and prolonged sleepiness [1,2]. Additionally, surgical procedures require the administration of several intravenous drugs to ensure hypnosis, analgesia, relaxation and control of visceral reflex responses. The use of intravenous drugs adds flexibility and permits the administration of lower doses of inhalational anesthetic agents. General anesthesia most often is initiated by an injection of thiopental, an ultra-short acting barbiturate, to induce sleep prior to administration of the agents that are necessary for maintaining anesthesia during the surgical procedure. Ultra-short acting barbiturates have an important place in the practice of anesthesiology [3]. Most of anxiolytic, hypnotic, muscle-relaxant and anticonvulsant active agents act allosterically to influence central γ-aminobutyric acid (GABA)-mediated neurotransmission [4]. GABA mediates the inhibitory neurotransmission in brain via $GABA_A$ and the $GABA_B$ receptors [5–7]. $GABA_A$ receptor has been implicated in a number of neurological diseases and ligands associated with it have been identified as potential therapeutic agents [8,9]. Hence, the pharmacotherapy of various neurological and psychiatric diseases such as generalized anxiety disorders, sleep disturbances, muscle spasms and seizure disorders involves the modulation of GABA-mediated synaptic transmission in CNS [10–14].

In the present work it is intended to investigate synthesis of tetraaza-spiro[4.5]dec-2-ene-6,8,10-trione analogs utilizing 1,3-dipolar cycloaddition reaction of nitrilimines to 1,3-dimethyl-2,4,6-pyrimidinetriones (barbiturate derivatives) possessing exocyclic olefinic linkage at the 5-position. Needless to say that, the newly synthesized spiro-containing heterocycles are still having the barbiturate nucleus so, still possessing most of the pharmacological properties belong to this ring system especially, the unique

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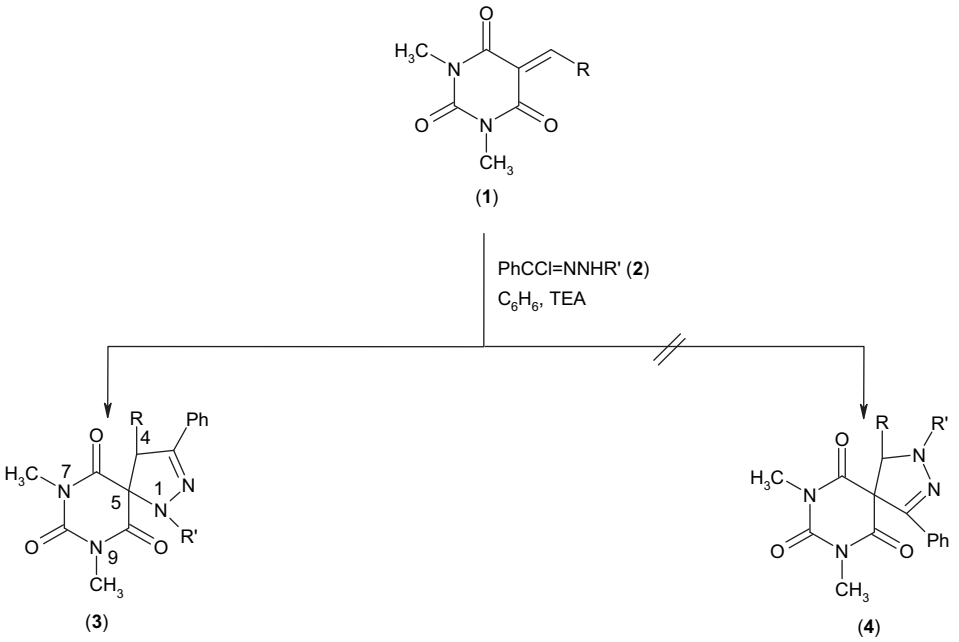
sedative/hypnotic effects. Recent publication describing the anti-convulsant and sedative effects of 5-substituted bromopyrazolinic spirobarbiturates [15] also supported our assumption directed toward developing promising bioactive targets through the present study. A hypothesis of molecular modeling study will be also considered in the present work to explore the intermolecular interactions taking place between the test set of the synthesized compounds and the target protein that may assist in validating the observed pharmacological properties and distinguishing the attained structure–activity relationships.

2. Results and discussion

2.1. Chemistry

Reaction of 5-arylidene-1,3-dimethyl-2,4,6-pyrimidinetriones **1a**–**i** with nitrilimines (generated in situ via triethylamine dehydrohalogenation of the corresponding hydrazonoyl chlorides **2a**, **b**) in refluxing benzene afforded single products as exhibited by TLC. The structures of the isolated products were established to be 7,9-dimethyl-1,3,4-triaryl-1,2,7,9-tetraaza-spiro[4.5]dec-2-ene-6,8,10-triones **3a**–**k** rather than their regio-isomeric forms 2,3,7,9-tetraaza-spiro[4.5]dec-1-ene-6,8,10-triones **4a-k** explaining the high regioselectivity of the conducted reactions under the applied conditions (Scheme 1). The structures of **3a**–**k** were assigned based on spectroscopic (IR, ^{1}H NMR, ^{13}C NMR, MS) and elemental analyses data.

IR spectra of **3a**–**k** reveal a strong carbonyl stretching vibration band at $\nu = 1685$–1697 cm^{-1}. ^{1}H NMR spectra of **3a**–**k** exhibit the pyrazolinyl *H*-4 as a sharp singlet signal at $\delta = 5.23$–5.81. The appearance of this characteristic signal at the mentioned chemical shift value region is consistent with many other similar reported spiro-containing ring systems supporting the assigned regio-isomeric form especially, 2′,4′-dihydro-2′,4′,5′-triaryl-spiro[2*H*-indene-2,3'-[3*H*]pyrazole]-1,3-diones which reveal their pyrazolinyl *H*-4′ signal at $\delta = 5.14$–5.48 [16] and 1,3,4-triaryl-8,8-dimethyl-7,9-dioxa-1,2-diaza-spiro[4.5]dec-2-ene-6,10-diones that exhibit their pyrazolinyl *H*-4 at $\delta = 5.33$–5.68 [17]. Additionally, the methyl groups attached to the pyrimidinyl nitrogens (*N*-7, *N*-9) are observed as singlet signals at $\delta = 2.65$–2.98, 3.45–3.51. ^{13}C NMR spectrum of **3d** (as a representative example) adds a good support for the established structure, revealing the methine (HC-4) and spiro



(1)

(3)

(4)

1a, R = Ph
1b, R = 4-ClC$_6$H$_4$
1c, R = 2,4-Cl$_2$C$_6$H$_3$
1d, R = 4-FC$_6$H$_4$
1e, R = 4-H$_3$CC$_6$H$_4$
1f, R = 4-H$_3$COC$_6$H$_4$
1g, R = 4-(H$_3$C)$_2$NC$_6$H$_4$
1h, R = 2-thienyl
1i, R = 5-methyl-2-furanyl

2a, R' = Ph
2b, R' = 3-ClC$_6$H$_4$

3a, R = R' = Ph
3b, R = Ph, R' = 3-ClC$_6$H$_4$
3c, R = 4-ClC$_6$H$_4$, R' = Ph
3d, R = 4-ClC$_6$H$_4$, R' = 3-ClC$_6$H$_4$
3e, R = 2,4-Cl$_2$C$_6$H$_3$, R' = Ph
3f, R = 4-FC$_6$H$_4$, R' = Ph
3g, R = 4-H$_3$CC$_6$H$_4$, R' = Ph
3h, R = 4-H$_3$COC$_6$H$_4$, R' = Ph
3i, R = 4-(H$_3$C)$_2$NC$_6$H$_4$, R' = Ph
3j, R = 2-thienyl, R' = Ph
3k, R = 5-methyl-2-furanyl, R' = Ph

Scheme 1. Synthetic route of compounds **3a**–**k**.

carbons (*C*-5) at $\delta = 63.7, 77.9$, respectively which are in good accord with many other reported similar structures [16–21]. The carbonyl carbons are observed at $\delta = 150.6, 163.8, 166.1$, while the methyl carbons attached to *N*-7, *N*-9 are appeared at $\delta = 29.1, 30.1$. ^{13}C NMR spectra of the other synthesized analogues revealed similar observations (*c.f.* Section 4). Mass spectral data (EI-MS) of the synthesized compounds **3a**, **c**–**e**, **g**–**k** add good support for the assigned structure revealing the corresponding parent ion peak with considerable relative intensity value (*c.f.* Section 4).

Single crystal X-ray study of **3d** allows confirmation of the established structure (Fig. 1). Theoretical calculations were processed by both AM1 and PM3 methods to compare the observed geometric parameters "geometric parameters obtained experimentally through single crystal X-ray studies and theoretically calculated ones with both AM1 and PM3 methods are presented in Table 1 of supplementary material". The geometries of **3d** were optimized by the molecular mechanics force field (MM+) followed by either semi-empirical AM1 [22] or PM3 [23,24] methods implemented in the HyperChem 8.0 package. The structures were fully optimized without fixing any parameters, thus bringing all geometric variables to their equilibrium values. The energy minimization protocol employed the Polak–Ribiere conjugated gradient algorithm. Convergence to a local minimum was achieved when the energy gradient was $\leq$0.01 kcal mol^{-1}. The RHF method was used in the spin pairing for the two semi-empirical tools [25,26].

2.2. Sodium thiopental-induced hypnosis potentiations of the synthesized compounds

Potentiating effects of the synthesized compounds **3a**–**k** on the hypnotic action of sodium thiopental were investigated in vivo using Albino mice according to the reported standard method [15,27,28]. From the observed results (Table 1), it has been noticed that a few of the synthesized compounds enhanced (shorten) the induction time (time elapsed between injection of sodium thiopental and loss of the righting reflex) relative to the control experiment (induction time = 2.66 min.), which are **3j, 3b, 3f, 3e** and **3d** (induction time, 1.79, 1.85, 2.00, 2.12 and 2.30 min., respectively). However, most of the tested compounds revealed promising hypnotic potentiating effects (i.e. increased the sleeping time relative to the control experiment, which is the time interval between loss and recuperation of the righting reflex), especially compounds **3k**, **3e**, **3f**, **3d** and **3g** (% increase in sleeping time relative to the control experiment, 904.0, 501.2, 358.6, 340.9 and 311.3, respectively).

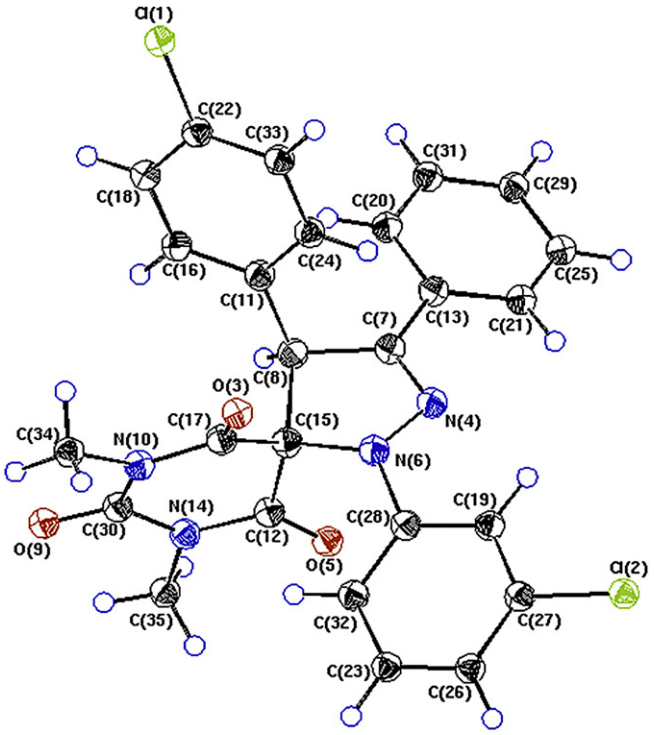


Fig. 1. ORTEP projection of single crystal X-ray diffraction of **3d**.

Structure–activity relationships based on the observed sodium thiopental-induced hypnotic potentiations of the synthesized compounds indicated that, introduction of a *m*-chloro substituent at the phenyl group attached to the 1-position of 1,2,7,9-tetraaza-spiro [4.5]dec-2-ene-6,8,10-triones intensively enhanced the hypnotic potentiating properties as exhibited in pairs **3a**, **3b** (% increase in sleeping time relative to the control experiment, 1.3, 192.0, respectively) and **3c**, **3d** (% increase in sleeping time relative to the control experiment, 20.0, 340.9, respectively). Additionally, the type of substitution of the phenyl group oriented at the 4-position of the constructed heterocycles seems a controlling factor for exhibiting the total observed pharmacological properties. It has been noticed that, attachment of the phenyl group located at the 4-position of the synthesized heterocycles with an electron-donating function, greatly decreases the potentiating hypnotic action. The stronger the electron-donating property of that substituent, the decrease in the observed sleeping period as exhibited in compounds **3h**, **3i** and **3g** (% increase in sleeping time relative to the control experiment, −20.3, 299.4 and 311.3, respectively). Otherwise, attachment of the phenyl group located at the 4-position of the prepared heterocycles with an electron-withdrawing residue enhances greatly the observed potentiating hypnotic effects as exhibited in compounds **3c**, **3e** and **3f** (% increase in sleeping time relative to the control experiment, 20.0, 501.2 and 358.6, respectively). Meanwhile, introduction of a furanyl function at the 4-position of the synthesized compound seems much more promising for constructing a hypnotic active agent compared to the case of adopting a thienyl moiety, as exhibited in pairs **3j** and **3k** (% increase in sleeping time relative to the control experiment, −25.8, 904.0, respectively).

Toxicological studies of the most promising prepared hypnotic active agents (**3d**–**g, i** and **k**) were performed using LD_{50} standard method in mice [29] at a dose of 250, 500 and 750 mg/kg (body weight) "that is, 10–30 fold of the used hypnotic effective dose (25 mg/kg body weight)". It has been noticed that no mortality rates or any toxic symptoms were observed after 24 h post-administrations explaining the safe behavior of the tested compounds at the experimentally applied doses.

A hypothesis of molecular modeling study including fitting the synthesized compounds into a generated 3D-pharmacophore and their docking into optimized homology model of $GABA_A$-α_1 was reported in the supplementary material.

3. Conclusion

From all the above it could be concluded that, 1,3-dipolar cycloaddition reactions of nitrilimines with 5-arylidene-1,3-dimethyl-2,4,6-pyrimidinetriones **1a**–**i** proceed regioselectively affording 7,9-dimethyl-1,3,4-triaryl-1,2,7,9-tetraaza-spiro[4.5]dec-2-ene-6,8,10-triones **3a**–**k** in good yields (71–81% yield). The constructed heterocycles possess promising hypnotic potentiating effects especially, compounds **3k** and **3e** that could be nominated as short-acting hypnotics. A hypothesis of molecular modeling study was undertaken for exploring the intermolecular interactions

Table 1
Sodium thiopental-induced hypnosis potentiations of the synthesized compounds.

Entry	Compound	R	R′	Induction time (min ± SE mean)	Sleeping time (min ± SE mean)	% Increase in sleeping time
1	Control	–	–	2.66 ± 0.23	21.81 ± 1.81	–
2	**3a**	Ph	Ph	3.00 ± 0.39	22.09 ± 6.58	1.3
3	**3b**	Ph	3-ClC_6H_4	1.85 ± 0.20*	63.68 ± 16.02	192.0
4	**3c**	4-ClC_6H_4	Ph	3.47 ± 0.53*	26.17 ± 6.04	20.0
5	**3d**	4-ClC_6H_4	3-ClC_6H_4	2.30 ± 0.12	96.15 ± 12.39*	340.9
6	**3e**	2,4-$Cl_2C_6H_3$	Ph	2.12 ± 0.27	131.13 ± 21.15*	501.2
7	**3f**	4-FC_6H_4	Ph	2.00 ± 0.22	100.02 ± 24.59*	358.6
8	**3g**	4-$H_3CC_6H_4$	Ph	2.92 ± 0.11	89.71 ± 21.73*	311.3
9	**3h**	4-$H_3COC_6H_4$	Ph	5.25 ± 0.37*	17.38 ± 1.65	−20.3
10	**3i**	4-$(H_3C)_2NC_6H_4$	Ph	2.91 ± 0.10	87.10 ± 34.42*	299.4
11	**3j**	2-thienyl	Ph	1.79 ± 0.26*	16.19 ± 1.43	−25.8
12	**3k**	5-methyl-2-furanyl	Ph	2.66 ± 0.27	218.97 ± 30.37*	904.0

*Statistically significant from the control at $p < 0.05$.

taking place between the test set of the synthesized compounds and the target protein which revealed good results consistent with the observed pharmacological properties.

4. Experimental

Melting points were recorded on a Stuart SMP3 digital melting point apparatus. IR spectra (KBr) were recorded on a JASCO 6100 FT-IR spectrophotometer. ^{1}H NMR spectra were recorded on a Varian MERCURY 300 MHz spectrometer. ^{13}C NMR spectra were recorded on JEOL AS 500 (125 MHz) and Varian MERCURY 300 (75 MHz) spectrometers. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX (EI, 70 eV) spectrometer. The starting compounds **1a**–**i** [30–34] and **2a**, **b** [35,36] were prepared according to the previously reported procedures.

*4.1. Reaction of **1a**–**i** with hydrazonoyl chlorides **2a**, **b** (general procedure)*

A mixture of equimolar amounts of **1a**–**i** (5 mmol) and the corresponding **2a**, **b** in dry benzene (25 ml) containing triethylamine (7.5 mmol) was boiled under reflux for the appropriate time. The reaction mixture was filtered while hot to remove triethylamine hydrochloride then, evaporated till dryness under reduced pressure and the remaining residue was triturated with methanol (5 ml) so, the separated solid material was collected and crystallized from a suitable solvent affording the corresponding **3a**–**k**.

*4.1.1. 7,9-Dimethyl-1,3,4-triphenyl-1,2,7,9-tetraaza-spiro[4.5]dec-2-ene-6,8,10-trione (**3a**)*

Reaction time 7 h, colorless crystals from *n*-butanol, mp 214–216 °C, yield 82%. IR: ν_{max}/cm^{-1} 1687 (C=O), 1594, 1496 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 2.66 (s, 3H, CH_3), 3.51 (s, 3H, CH_3), 5.29 (s, 1H, *H*-4), 6.89–7.53 (m, 15H, arom. H). ^{13}C NMR (DMSO-d_6): δ 28.3, 29.4 (CH_3), 64.1 (*HC*-4), 77.5 (*C*-5 "spiro-carbon"), 113.6, 119.6, 120.8, 126.3, 128.4, 128.5, 128.7, 128.8, 128.9, 129.5, 130.4, 133.3, 141.9 (arom. *C*), 145.3 (*C*-3), 150.0, 163.5, 166.1 (C=O). MS: *m*/*z* (%) 438 (M, 73), 333 (51), 332 (41), 276 (9), 275 (17), 248 (13), 247 (9). Anal. Calcd. for $C_{26}H_{22}N_4O_3$ (438.49): C, 71.22; H, 5.06; N, 12.78. Found: C, 71.43; H, 5.20; N, 12.94.

*4.1.2. 1-(3-Chlorophenyl)-7,9-dimethyl-3,4-diphenyl-1,2,7,9-tetraaza-spiro[4.5]dec-2-ene-6,8,10-trione (**3b**)*

Reaction time 9 h, colorless crystals from *n*-butanol, mp 156–158 °C, yield 81%. IR: ν_{max}/cm^{-1} 1690 (C=O), 1590, 1480 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 2.65 (s, 3H, CH_3), 3.51 (s, 3H, CH_3), 5.28 (s, 1H, *H*-4), 6.45–7.53 (m, 14H, arom. H). ^{13}C NMR (DMSO-d_6): δ 28.3, 29.5 (CH_3), 64.3 (*HC*-4), 77.6 (*C*-5 "spiro-carbon"), 112.0, 113.6, 119.4, 120.6, 126.6, 128.48, 128.53, 128.8, 129.2, 129.4, 130.0, 130.4, 133.1, 133.8, 143.4 (arom. *C*), 146.7 (*C*-3), 150.0, 163.3, 165.8 (C=O). Anal. Calcd. for $C_{26}H_{21}ClN_4O_3$ (472.94): C, 66.03; H, 4.48; N, 11.85. Found: C, 66.14; H, 4.57; N, 12.08.

*4.1.3. 4-(4-Chlorophenyl)-7,9-dimethyl-1,3-diphenyl-1,2,7,9-tetraaza-spiro[4.5]dec-2-ene-6,8,10-trione (**3c**)*

Reaction time 7 h, colorless crystals from *n*-butanol, mp 230–232 °C, yield 76%. IR: ν_{max}/cm^{-1} 1695 (C=O), 1595, 1494 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 2.76 (s, 3H, CH_3), 3.50 (s, 3H, CH_3), 5.25 (s, 1H, *H*-4), 6.90–7.50 (m, 14H, arom. H). ^{13}C NMR (DMSO-d_6): δ 28.4, 29.4 (CH_3), 62.9 (*HC*-4), 77.3 (*C*-5 "spiro-carbon"), 113.7, 119.8, 120.5, 120.6, 126.3, 128.5, 128.6, 128.89, 128.95, 130.1, 131.2, 132.3, 133.5, 141.9 (arom. *C*), 145.2 (*C*-3), 150.0, 163.5, 165.8 (C=O). MS: *m*/*z* (%) 472 (M, 45), 474 [(M + 2), 16], 367 (36), 366 (35), 310 (5), 309 (12), 282 (8), 281 (4). Anal. Calcd. for $C_{26}H_{21}ClN_4O_3$ (472.94): C, 66.03; H, 4.48; N, 11.85. Found: C, 65.89; H, 4.37; N, 12.03.

*4.1.4. 1-(3-Chlorophenyl)-4-(4-chlorophenyl)-7,9-dimethyl-3-phenyl-1,2,7,9-tetraaza-spiro[4.5]dec-2-ene-6,8,10-trione (**3d**)*

Reaction time 10 h, colorless crystals from n-butanol, mp 249–251 °C, yield 79%. IR: ν_{max}/cm^{-1} 1693 (C=O), 1589, 1478 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 2.76 (s, 3H, CH_3), 3.50 (s, 3H, CH_3), 5.23 (s, 1H, *H*-4), 6.43–7.50 (m, 13H, arom. H). ^{13}C NMR (DMSO-d_6): δ 29.1, 30.1 (CH_3), 63.7 (*HC*-4), 77.9 (*C*-5 "spiro-carbon"), 112.6, 114.2, 120.1, 127.1, 129.2, 129.3, 130.0, 130.3, 131.1, 131.8, 132.7, 134.2, 134.3, 143.8 (arom. *C*), 147.1 (*C*-3), 150.6, 163.8, 166.1 (C=O). MS: *m*/*z* (%) 506 (M, 59), 508 [(M + 2), 48], 510 [(M + 4), 17], 367 (100), 366 (77), 310 (20), 309 (28), 282 (19), 281 (15). Anal. Calcd. for $C_{26}H_{20}Cl_2N_4O_3$ (507.38): C, 61.55; H, 3.97; N, 11.04. Found: C, 61.49; H, 3.84; N, 11.25.

*4.1.5. 4-(2,4-Dichlorophenyl)-7,9-dimethyl-1,3-diphenyl-1,2,7,9-tetraaza-spiro[4.5]dec-2-ene-6,8,10-trione (**3e**)*

Reaction time 9 h, yellow crystals from ethanol, mp 193–195 °C, yield 71%. IR: ν_{max}/cm^{-1} 1696 (C=O), 1593, 1496 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 2.89 (s, 3H, CH_3), 3.45 (s, 3H, CH_3), 5.81 (s, 1H, *H*-4), 6.91–7.48 (m, 13H, arom. H). ^{13}C NMR (DMSO-d_6): δ 28.5, 29.3 (CH_3), 59.8 (*HC*-4), 76.6 (*C*-5 "spiro-carbon"), 113.8, 120.1, 126.1, 127.9, 128.8, 129.0, 129.2, 129.4, 129.6, 133.3, 133.9, 134.7, 141.7 (arom. *C*), 144.5 (*C*-3), 150.0, 163.3, 165.6 (C=O). MS: *m*/*z* (%) 506 (M, 32), 508 [(M + 2), 25], 510 [(M + 4), 6], 401 (21), 400 (12), 344 (3), 343 (5), 316 (5), 315 (4). Anal. Calcd. for $C_{26}H_{20}Cl_2N_4O_3$ (507.38): C, 61.55; H, 3.97; N, 11.04. Found: C, 61.43; H, 3.93; N, 10.98.

*4.1.6. 4-(4-Fluorophenyl)-7,9-dimethyl-1,3-diphenyl-1,2,7,9-tetraaza-spiro[4.5]dec-2-ene-6,8,10-trione (**3f**)*

Reaction time 7 h, colorless crystals from *n*-butanol, mp 208–210 °C, yield 75%. IR: ν_{max}/cm^{-1} 1686 (C=O), 1597, 1500 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 2.75 (s, 3H, CH_3), 3.50 (s, 3H, CH_3), 5.27 (s,

1H, *H*-4), 6.90–7.51 (m, 14H, arom. H). Anal. Calcd. for $C_{26}H_{21}FN_4O_3$ (456.48): C, 68.41; H, 4.64; N, 12.27. Found: C, 68.19; H, 4.51; N, 12.08.

4.1.7. 7,9-Dimethyl-1,3-diphenyl-4-(4-methylphenyl)-1,2,7,9-tetraaza-spiro[4.5]dec-2-ene-6,8,10-trione (**3g**)

Reaction time 10 h, colorless crystals from n-butanol, mp 175–177 °C, yield 80%. IR: ν_{max}/cm^{-1} 1692 (C=O), 1597, 1496 (C=N, C=C). ^1H NMR ($CDCl_3$): δ 2.32 (s, 3H, $ArCH_3$), 2.68 (s, 3H, NCH_3), 3.50 (s, 3H, NCH_3), 5.26 (s, 1H, *H*-4), 6.85–7.53 (m, 14H, arom. H). ^{13}C NMR (DMSO-d_6): δ 20.6 (Ar–CH_3), 28.3, 29.4 (NCH_3), 64.0 (*HC*-4), 77.5 (*C*-5 "spiro-carbon"), 113.6, 119.6, 120.5, 126.3, 128.5, 128.8, 128.87, 128.97, 129.3, 130.2, 130.4, 138.1, 142.0 (arom. *C*), 145.5 (*C*-3), 150.1, 163.6, 166.1 (C=O). MS: *m*/*z* (%) 452 (M, 81), 347 (62), 346 (63), 290 (9), 289 (19), 262 (12), 261 (7). Anal. Calcd. for $C_{27}H_{24}N_4O_3$ (452.52): C, 71.67; H, 5.35; N, 12.38. Found: C, 71.52; H, 5.15; N, 12.55.

4.1.8. 7,9-Dimethyl-1,3-diphenyl-4-(4-methoxyphenyl)-1,2,7,9-tetraaza-spiro[4.5]dec-2-ene-6,8,10-trione (**3h**)

Reaction time 9 h, colorless crystals from *n*-butanol, mp 229–231 °C, yield 77%. IR: ν_{max}/cm^{-1} 1685 (C=O), 1597, 1496 (C=N, C=C). ^1H NMR ($CDCl_3$): δ 2.73 (s, 3H, NCH_3), 3.50 (s, 3H, NCH_3), 3.78 (s, 3H, OCH_3), 5.25 (s, 1H, *H*-4), 6.81–7.53 (m, 14H, arom. H). ^{13}C NMR (DMSO-d_6): δ 28.4, 29.4 (NCH_3), 55.1 (OCH_3), 63.7 (*HC*-4), 77.5 (*C*-5 "spiro-carbon"), 113.6, 113.8, 119.5, 120.7, 125.0, 126.3, 128.5, 128.75, 128.86, 130.4, 130.7, 142.0, 145.5 (arom. *C*), 150.1 (*C*-3), 159.3, 163.7, 166.2 (C=O). MS: *m*/z (%) 468 (M, 59), 363 (35), 362 (46), 306 (4), 305 (12), 278 (8), 277 (5). Anal. Calcd. for $C_{27}H_{24}N_4O_4$ (468.52): C, 69.22; H, 5.16; N, 11.96. Found: C, 69.27; H, 5.19; N, 12.07.

4.1.9. 7,9-Dimethyl-4-(4-dimethylaminophenyl)-1,3-diphenyl-1,2,7,9-tetraaza-spiro[4.5]dec-2-ene-6,8,10-trione (**3i**)

Reaction time 10 h, colorless crystals from n-butanol, mp 208–210 °C, yield 83%. IR: ν_{max}/cm^{-1} 1697 (C=O), 1604, 1496 (C=N, C=C). ^1H NMR ($CDCl_3$): δ 2.73 (s, 3H, NCH_3), 2.94 [s, 6H, $N(CH_3)_2$], 3.49 (s, 3H, NCH_3), 5.23 (s, 1H, *H*-4), 6.70–7.55 (m, 14H, arom. H). ^{13}C NMR (DMSO-d_6): δ 28.4, 29.3 (NCH_3), 64.5 (*HC*-4), 77.7 (*C*-5 "spiro-carbon"), 111.6, 113.5, 119.4, 119.6, 120.6, 126.4, 128.4, 128.6, 128.9, 130.1, 130.7, 142.1, 145.7 (arom. *C*), 150.1 (*C*-3), 150.2, 163.8, 166.4 (C=O). MS: *m*/*z* (%) 481 (M, 85), 376 (39), 375 (95), 318 (5), 291 (9), 290 (5). Anal. Calcd. for $C_{28}H_{27}N_5O_3$ (481.56): C, 69.84; H, 5.65; N, 14.54. Found: C, 69.97; H, 5.82; N, 14.57.

4.1.10. 7,9-Dimethyl-1,3-diphenyl-4-(2-thienyl)-1,2,7,9-tetraaza-spiro[4.5]dec-2-ene-6,8,10-trione (**3j**)

Reaction time 9 h, colorless crystals from *n*-butanol, mp 191–193 °C, yield 86%. IR: ν_{max}/cm^{-1} 1686 (C=O), 1596, 1497 (C=N, C=C). ^1H NMR ($CDCl_3$): δ 2.85 (s, 3H, CH_3), 3.51 (s, 3H, CH_3), 5.57 (s, 1H, *H*-4), 6.80–7.57 (m, 13H, arom. H). ^{13}C NMR (DMSO-d_6): δ 28.6, 29.4 (CH_3), 58.8 (*HC*-4), 77.4 (*C*-5 "spiro-carbon"), 113.6, 119.8, 120.5, 126.3, 127.0, 128.2, 128.5, 128.9, 129.6, 130.4, 134.7, 141.9 (arom. *C*), 144.9 (*C*-3), 150.1, 163.3, 165.8 (C=O). MS: *m*/*z* (%) 444 (M, 62), 446 [(M + 2), 7], 339 (56), 338 (74), 282 (7), 281 (16), 254 (10), 253 (5). Anal. Calcd. for $C_{24}H_{20}N_4O_3S$ (444.52): C, 64.85; H, 4.54; N, 12.60. Found: C, 65.07; H, 4.60; N, 12.76.

4.1.11. 7,9-Dimethyl-1,3-diphenyl-4-(5-methyl-2-furanyl)-1,2,7,9-tetraaza-spiro[4.5]dec-2-ene-6,8,10-trione (**3k**)

Reaction time 10 h, colorless crystals from *n*-butanol, mp 169–171 °C, yield 81%. IR: ν_{max}/cm^{-1} 1688 (C=O), 1594, 1492 (C=N, C=C). ^1H NMR ($CDCl_3$): δ 2.25 (s, 3H, furanyl CH_3), 2.98 (s, 3H, NCH_3), 3.51 (s, 3H, NCH_3), 5.41 (s, 1H, *H*-4), 5.89–7.59 (m, 12H, arom. H). ^{13}C NMR (DMSO-d_6): δ 13.1 (Ar–CH_3), 28.8, 29.4 (NCH_3), 58.3 (*HC*-4), 76.3 (*C*-5 "spiro-carbon"), 107.1, 112.7, 113.5, 119.7, 120.8, 126.0, 128.5, 128.9, 130.6, 141.9, 142.9, 144.5 (arom. *C*), 150.2 (*C*-3), 153.1, 163.3, 165.8 (C=O). MS: *m*/*z* (%) 442 (M, 52), 337 (58), 336 (58), 280 (3), 279 (9), 252 (4), 251 (2). Anal. Calcd. for $C_{25}H_{22}N_4O_4$ (442.48): C, 67.86; H, 5.01; N, 12.66. Found: C, 67.98; H, 5.24; N, 12.74.

4.2. Single crystal X-ray crystallographic data of **3d**

Full crystallographic details, excluding structure factors have been deposited at Cambridge Crystallographic Data Centre (CCDC) as supplementary publication number CCDC 828750. For X-ray crystallographic studies, compound **3d** was recrystallized as prismatic colorless crystals from *n*-butanol. The crystallographic data were collected at $T = 298$ K on a Kappa CCD Enraf Nonius FR 590 diffractometer using a graphite monochromator with Mo-K_α radiation ($\lambda = 0.71073$ Å). The crystal structures were determined by SIR92 [37] and refined by maXus [38] (Bruker Nonius, Delft and MacScience, Japan). Chemical formula $C_{26}H_{20}Cl_2N_4O_3$, $M_r = 507.377$, orthorhombic, crystallizes in space group $Pna2_1$. Cell lengths "$a = 11.4832(3)$, $b = 16.4905(5)$, $c = 12.4679(3)$ Å". Cell angles "$\alpha = 90.00$, $\beta = 90.00$, $\gamma = 90.00$°", $V = 2360.97(11)$ Å^3, $Z = 4$, $D_c = 1.427$ mg/m^3, θ values 2.910–26.022°, absorption coefficient μ (Mo-K_α) = 0.31 mm^{-1}, $F(000) = 1048$. The unique reflections measured 2621 of which 1627 reflections with threshold expression $I > 3\sigma(I)$ were used in the structural analysis. Convergence for 316 variable parameters by least-squares refinement on F^2 with $w = 1/[\sigma^2(F_0^2) + 0.10000F_0^2]$. The final agreement factors were $R = 0.037$ and $wR = 0.067$ with a goodness-of-fit of 1.491.

4.3. Potentiation of hypnotic effect of sodium thiopental

Potentiating effects of the synthesized compounds **3a**–**k** on hypnotic action of sodium thiopental was conducted according to the reported standard method [15,27,28]. Albino mice (≈20 g) were divided into 12 groups of 6 animals each. Administration of the tested compounds (**3a**–**k**) emulsified in Tween 80/normal saline in a dose of 25 mg/kg body weight were given intraperitoneally. The control group was given vehicle only. One hour later all mice were administered with sodium thiopental (75 mg/kg body weight i.p.) in physiological saline. Induction time (time elapsed between injection of sodium thiopental and loss of the righting reflex) and sleeping time (time interval between loss and recuperation of the righting reflex) were recorded. Data were collected, checked, revised and analyzed. Quantitative variables from normal distribution were expressed as means ± SE "standard error". The significant difference between groups was tested by using one-way ANOVA available in SPSS 11 followed by post-hoc test and the chosen level of significance was $p < 0.05$.

% Increase in sleeping time was expressed as percentage increase in sleeping durations in treated animal groups relative to the control group (Table 1).

$$\%\text{increase in sleeping time} = \frac{T_d - T_c}{T_c} \times 100$$

where, T_c and T_d are the sleeping times for the control and drug-treated animal groups, respectively.

4.4. LD_{50} determination

The toxicological behavior of the most promising prepared hypnotic active agents (**3d**–**g**, **i** and **k**) were studied using the standard known LD_{50} method in mice [29]. Albino mice weighing 20–25 g were divided into 21 groups of 6 animals each. Administrations of the tested compounds (**3d**–**g**, **i** and **k**) emulsified in Tween 80/normal saline at doses of 250, 500 and 750 mg/kg (body

weight) were given intraperitoneally. The control groups were given vehicle only. The toxic symptoms, mortality rates and postmortem findings in each group were recorded 24 h postadministration. LD_{50} of the tested compounds was calculated according to the following formula:

$$LD_{50} = D_m - \frac{\sum (zxd)}{n}$$

where D_m = the largest dose which killed all animals, z = mean of dead animals between two successive groups, d = the constant factor between two successive doses, n = number of animals in each group, and Σ = the sum of $(z \times d)$.

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Appendix. Supplementary information

The supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejmech.2011.07.058. These data include MOL files and InChiKeys of the most important compounds described in this article.

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