

Efficient microwave synthesis and anti-cancer evaluation of new substituted 2,4-diazaspiro[5.5]undecane-1,5,9-trione (or 1,3,5,9-tetraone) derivatives

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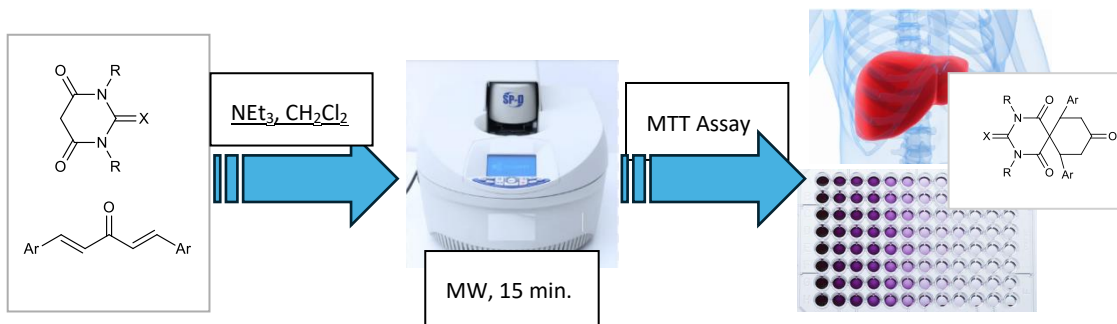
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Abstract

Derivatives of 2,4-diazaspiro[5.5]undecane-1,5,9-trione (or 1,3,5,9-tetraone) (3a-j) were synthesized via the condensation of *N,N*-dimethyl barbituric acid (1a) and 1,3-diethylthiobarbituric acid (1b) with penta-1,4-diene-3-one derivatives (2a-e) using a microwave-assisted method. In this study, we aim to synthesize novel 2,4-diazaspiro[5.5]undecane-1,5,9-trione (or 1,3,5,9-tetraone) derivatives and conduct in vitro anticancer experiments to assess the biological activity of the newly synthesized compounds and previously reported ones from the literature. Optimal reaction conditions were determined for both traditional and microwave methods, showing that the microwave-assisted approach provides short reaction times and high yields (78-92%). The synthesized compounds were characterized using ¹H-NMR, ¹³C-NMR, LC-MS, and elemental analyses, confirming their structures and all these compounds showed anti-cancer effects on liver adenocarcinoma cells at different levels.



Keywords: Diazaspiro, Microwave, Synthesis, Anti-cancer, Divinylketone

Introduction

Azaspiro compounds have gained a lot of attention in the pharmaceutical world.^{1,2} Nitrogen-containing spiro-heterocyclic is an essential synthetic motif found in various naturally occurring chemicals, with a wide range of pharmacological and biological effects.^{3,4} Much of the research focuses on producing novel compounds and studying their biological properties, such as fungicidal, herbicidal, antibacterial, and antimicrobial.^{5–9} Moreover, the biological effects of barbituric acid and thiobarbituric acid derivatives is increasing the attention on synthesizing 2,4-diazaspiro[5.5]undecane derivatives.^{10–12}

Several strategies for the synthesis of these derivatives have been suggested in the literature.^{13,14} For instance, a straightforward and incredibly effective method for synthesizing diazaspiro compounds has been developed, utilizing diarylideneacetone derivatives along with *N,N*-dimethyl barbituric acid.¹⁵ A facile, efficient, and environmentally friendly approach for synthesizing diazaspiro[5.5]undecane-1,5,9-trione derivatives has been devised using *p*-TSA and ultrasound irradiation.¹⁶ A simple synthesis approach is provided for substituting 2,4-diazaspiro[5.5]undecane-1,5,9-trione derivatives using a one-pot reaction using easily available starting materials.¹⁷ In another study conducted in 2015 investigating cytotoxic action of diazaspiro[5.5]undecane-1,3,5,9-tetraone derivatives against PC-3, Hela, MCF-7, and 3T3 cell lines, the derivatives were found to be more powerful α -glucosidase inhibitors than the common medication acarbose.¹⁸ Many studies have been done on the interactions between carbonyl compounds including ketones, aldehydes, esters with barbituric acid and 2-thiobarbituric acid.^{19–21} and α , β -unsaturated carbonyl compounds with barbituric acid and 2-thiobarbituric acid.^{6, 17, 22–28} In the other study, novel pyrimidine-2,4,6-trione derivatives have been synthesized as highly potential biological activity anti-diabetic agent using *N,N*-dimethylbarbituric acid and enone derivatives.²⁹ Spiro heterocycles and their derivatives have been synthesized using dimedone and the produced compounds were tested for their antibacterial activity in vitro against gram-negative bacteria *E. coli* and gram-positive bacteria *S. aureus*.³⁰

A literature review reveals limited research on the reactivity of α,β -unsaturated carbonyl compounds with *N,N*-dimethylbarbituric acid and 1,3-diethylthiobarbituric acid^{25,31,32} and there was limited information for the biological activities of the products of the combination of 1a (or 1b) with the 1,5-diaryl-1,4-pentadien-3-ones derivatives. So, with this purpose, we chose a series of 1,5-diaryl-1,4-pentadien-3-ones for interaction with *N,N*-dimethylbarbituric acid and 1,3-diethylthiobarbituric acid to synthesize medicinally active molecules and made the in-vitro anticancer studies.

Islam M.S.¹⁵ and Aggarwal K. 25 propose a possible mechanism for the formation of diazaspiro compounds. The structure representation for the diazaspiro derivative is given in Figure 1.

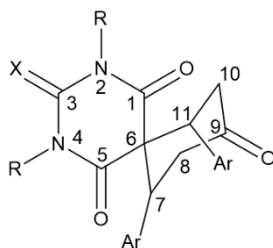


Figure 1. Structure numbering of diazaspiro derivative

Results and Discussion

Microwave irradiation presents a valuable alternative for numerous conventional reactions offering benefits such as increased yields, reduced reaction times³³⁻³⁴, and minimized byproducts.³⁵ This work aimed to develop microwave-assisted synthesis procedures derivatives of 2,4-diazaspiro[5.5]undecane-1,5,9-triones (or 1,3,5,9-tetraone) via a cascade cyclization process involving the [5+1] double Michael addition reaction. In this study, traditional and Microwave-assisted reactions were compared. As a traditional method, the yields of the products were found to be lower compared to those obtained in microwave reactions. When these two reaction methods were compared, the microwave method was selected for all reactions due to the longer reaction time and lower yield of the traditional method. The MW process produces high-quality results and shortens reaction times from 2-3 hours to about 15-20 minutes.

The synthesis of new substituted 2,4-diazaspiro[5.5]undecane-1,5,9-trione (or 1,3,5,9-tetraone) derivatives has garnered considerable attention in recent years due to their potential applications in medicinal chemistry and material science. These compounds exhibit interesting pharmacological activities and have shown promise as building blocks for the design of novel drug candidates. In this study, 6 novel compounds (**3d**, **3e**, **3g**, **3h**, **3i** and **3j**) and 4 compounds that are already in the literature (**3a**, **3b**, **3c** and **3f**) have been synthesized and their anti-cancer effects have been investigated on SK-HEP-1 adenocarcinoma cell line. According to activity results, compound **3a** showed the strongest anticancer activity ($112 \pm 12 \mu\text{M}$) whereas compounds **3b** and **3j** showed a weak cytotoxic effect ($>300 \mu\text{M}$) against adenocarcinoma cell line SK-HEP-1 in Table 1. As a result, all these compounds show anti-cancer effects on liver adenocarcinoma cells.

Table 1. IC₅₀ values of diazaspiro derivatives on SK-HEP-1 cells assessed by MTT test

Compound	IC ₅₀ (μM)
3a	112 ± 12
3b	>300
3c	114.9 ± 30.85
3d	264.8 ± 20.22
3e	239.7 ± 68.7
3f	167.1 ± 11.28
3g	177.2 ± 32.8
3h	128.0 ± 21.33
3i	139.7 ± 26.92
3j	>300

Conclusions

In conclusion, the synthesis of substituted 2,4-diazaspiro[5.5]undecane-1,5,9-trione (or 1,3,5,9-tetraone) derivatives represents a significant advancement in the search for new bioactive compounds particularly in the field of anticancer drug development. Using a microwave assisted approach we successfully condense *N,N*-dimethylbarbituric acid and 1,3-diethylthiobarbituric acid with penta-1,4-diene-3-one derivatives to create novel compounds with promising biological profiles. The in vitro anticancer evaluations underscore the therapeutic potential of these compounds, suggesting that structural modifications within this class may enhance efficacy and selectivity against cancer cells. This work contributes to the current understanding of 2,4-diazaspiro[5.5]undecane-1,5,9-trione (or 1,3,5,9-tetraone) derivatives' synthesis and highlights their emerging role as scaffolds for anticancer agents. By situating our findings within the broader context of existing literature we emphasize the importance of continued research into these derivatives, aiming to foster novel strategies for designing anticancer compounds with improved biological activity.

Experimental Section

Chemicals

N,N-Dimethylbarbituric acid (**1a**), 1,3-diethylthiobarbituric acid (**1b**), benzaldehyde, thiophene-2-carboxaldehyde, 4-fluorobenzaldehyde, 5-methylfurfural, *o*-anisaldehyde, *p*-tolualdehyde, dichloromethane and triethylamine were obtained from Sigma-Aldrich and Merck. Analytical grade chemicals were utilized unless otherwise specified. (1*E*,4*E*)-1,5-diphenylpenta-1,4-dien-3-one (**2a**), (1*E*,4*E*)-1,5-di(thiophen-2-yl)penta-1,4-dien-3-one (**2b**), (1*E*,4*E*)-1,5-bis(4-fluorophenyl)penta-1,4-dien-3-one (**2c**), (1*E*,4*E*)-1,5-bis(5-methylfuran-2-yl)penta-1,4-dien-3-one (**2d**), (1*E*,4*E*)-1,5-bis(2-methoxyphenyl)penta-1,4-dien-3-one (**2e**), (1*E*,4*E*)-1,5-di-*p*-tolylpenta-1,4-dien-3-one (**2f**).

The ^1H and ^{13}C NMR spectra were acquired using CDCl_3 as the solvent and TMS as the internal reference, and the measurements were conducted on a Bruker Avance 400 MHz Spectrometer. The MS spectra were obtained using an Agilent 19091 N-136 GC–MS instrument. For the microwave-irradiated reactions, a CEM-Focused Microwave™ Synthesis System was employed, featuring a continuous microwave power delivery system with an adjustable power output ranging from 0 to 300 W (± 30 W), along with programmable settings and infrared temperature control.

The reaction requires the coupling of *N,N*-dimethylbarbituric acid (**1a**) and 1,3-diethylthiobarbituric acid (**1b**) with derivatives of penta-1,4-diene-3-one (**2a-f**) catalyzed by triethylamine at microwave. The method developed demonstrates exceptional efficiency in producing diazaspiro[5.5]undecane derivatives **3a-j**, achieving yields of up to 98 % from easily accessible symmetric divinylketones **2a-f** with aryl and heteroaryl substituents (Figure 2).

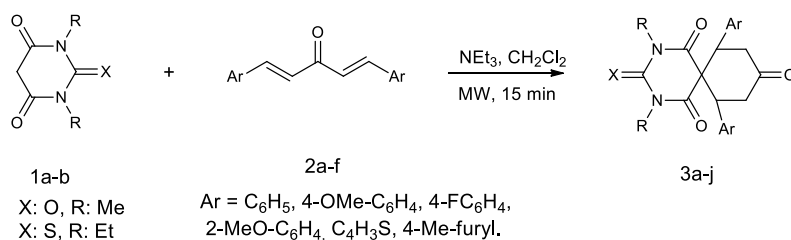
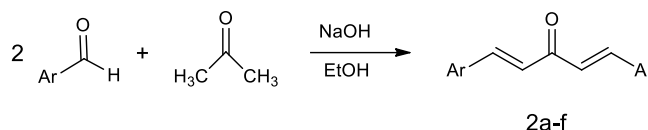


Figure 2. Synthesis reaction of **1a-b** and **2a-2f** compounds.

Synthesis of penta-1,4-diene-3-one derivatives (2a-f): Penta-1,4-dien-3-one derivatives (**2a-f**) were synthesized through an aldol condensation reaction involving substituted benzaldehydes and acetone in a 2:1 ratio, using an ethanolic NaOH solution as the catalyst as described in our previous research.³⁴ These derivatives were produced via a simple Claisen–Schmidt condensation method (Figure 3).

**Figure 3.** The general synthesis reaction of penta-1,4-diene-3-one derivatives

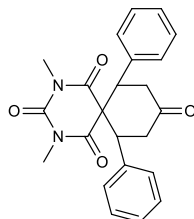
General microwave procedure for the synthesis of 2,4-diazaspiro[5.5]undecane-1,5,9-trione (or 1,3,5,9-tetraone) derivatives (3a-3j). A mixture of penta-1,4-diene-3-one derivatives (**2a-f**) (1 mmol), and *N,N*-dimethyl barbituric acid (**1a**) (1 mmol, 0.156 g) or 1,3-diethylthiobarbituric acid (**1b**) (1 mmol, 0.200 g) were weighed into a Microwave flask and added 5 mL CH₂Cl₂ and triethylamine (1.25 mmol, 0.128 g). The reaction mixture was heated under microwave irradiation (200 W, 40 °C) for 15 minutes. The reaction steps are followed by TLC testing. The reaction mixture was poured into 10 mL of cold water and extracted with chloroform (3x20 mL). The organic extracts were dried with MgSO₄. Column chromatography (gradient, from Hexane: Ethyl Acetate (4/1) was used after solvent evaporation.

General conventional method for the synthesis of 2,4-diazaspiro[5.5]undecane-1,5,9-trione (or 1,3,5,9-tetraone) derivatives (3a-3j). A mixture of penta-1,4-diene-3-one derivatives (**2a-f**) (1 mmol), and *N,N*-dimethyl barbituric acid (**1a**) (1 mmol, 0.156 g) or 1,3-diethylthiobarbituric acid (**1b**) (1 mmol, 0.200 g) were weighed into a flask and added 5 mL CH₂Cl₂ and triethylamine (1.25 mmol, 0.128 g). Experiments were conducted at reaction temperatures ranging from room temperature to 60 °C for 2-3 hours. The reaction steps are followed by TLC testing. The reaction mixture was poured into 10 mL of cold water and extracted with chloroform (3x20 mL). The organic extracts were dried with MgSO₄. Column chromatography (gradient, from Hexane: Ethyl Acetate (4/1) was used after solvent evaporation.

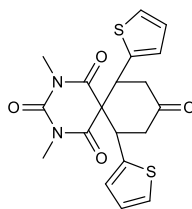
Cell culture and cytotoxicity assay

SK-HEP-1 adenocarcinoma cells were kept in DMEM supplemented with 10% FBS, 100 U/mL penicillin, 2 mM L-glutamine, 100 mg/mL streptomycin, and NEAA at 37 °C in humidified CO₂ (5%). (ESCO CelCulture® CO₂ incubator) SK-HEP-1 cancer cells were subjected to a 48-hour cytotoxicity assay 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to evaluate the anti-cancer activities of synthesized compounds. At a 5x10³ cells/ml density, cells were seeded into 48-well plates and subjected to varying doses of the chemicals dissolved in a maintenance medium. The use of dimethyl sulfoxide (DMSO) as the solvent-control group was implemented. The effect of DMSO at maximum chemical concentrations was determined to be nonsignificant, even though the final concentration of DMSO was consistently kept below 1%. Following the 48-hour treatment, the MTT assay proceeded as previously described.³⁷ MTT assays have been carried out three times independently.

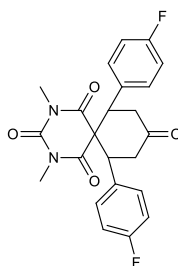
2,4-Dimethyl-7,11-diphenyl-2,4-diazaspiro[5.5] undecane-1,3,5,9-tetraone (3a). ^{15, 18, 25} White solid, (452 mg, 85%). Mp 218-222 °C. ¹H-NMR (400 MHz, CDCl₃) δ 7.24-7.21 (m, 6H, Ar-H); 7.07 – 7.04 (m, 4H, Ar-H); 4.03-3.99 (dd, 2H, *J* 14.0, 4.0 Hz, H₇ and H₁₁); 3.76-3.68 (m, 2H, H_{8a} and H_{10a}); 2.85 (s, 3H, CH₃), 2.83 (s, 3H, CH₃), 2.66-2.58 (m, 2H, H_{8e} and H_{10e}).



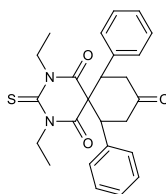
2,4-Dimethyl-7,11-di(thiophen-2-yl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (3b). ^{15, 18} Yellow solid, (512 mg, 87%). Mp 218-222 °C. ¹H-NMR (400 MHz, CDCl₃) δ 7.15 (d, 2H, *J* 5.2 Hz, Ar-H); 6.87-6.85 (m, 2H, Ar-H); 6.77 (d, 2H, *J* 3.2 Hz, Ar-H), 4.31-4.26 (dd, 2H, *J* 14.4, 4.8 Hz, H₇ and H₁₁); 3.62-3.54 (t, 2H, *J* 15.2 Hz, H_{8a} and H_{10a}); 3.06 (s, 3H, CH₃), 3.04 (s, 3H, CH₃), 2.76-2.71 (dd, 2H, *J* 15.2, 4.8 Hz, H_{8e} and H_{10e}).



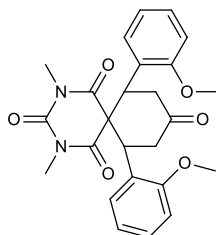
7,11-Bis(4-fluorophenyl)-2,4-dimethyl-2,4-diazaspiro [5.5]undecane-1,3,5,9-tetraone (3c). ^{18, 25, 32} White solid, (380 mg, 88 %). Mp 152-156 °C. ¹H-NMR (400 MHz, CDCl₃) δ 7.06-7.02 (m, 4H, Ar-H); 6.96-6.92 (m, 4H, Ar-H); 4.01-3.97 (dd, 2H, *J* 14.4, 4.8 Hz, H₇ and H₁₁); 3.69-3.62 (t, 2H, *J* 14.8 Hz, H_{8a} and H_{10a}); 3.03 (s, 3H, CH₃), 2.90 (s, 3H, CH₃), 2.61-2.56 (dd, 2H, *J* 15.2, 4.8 Hz, H_{8e} and H_{10e}).



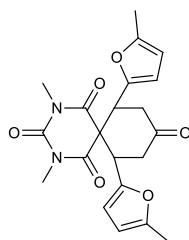
2,4-Dimethyl-7,11-bis(5-methylfuran-2-yl)-2,4-diazaspiro [5.5]undecane-1,3,5,9-tetraone (3d). White solid, (450 mg, 84 %). Mp 178-182 °C. ¹H-NMR (400 MHz, CDCl₃) δ 5.90 – 5.80 (m, 4H, Ar-H); 4.03-3.98 (dd, 2H, *J* 14.4, 4.8 Hz, H₇ and H₁₁); 3.41-3.33 (m, 2H, H_{8a} and H_{10a}); 3.22 (s, 3H, CH₃), 3.12 (s, 3H, CH₃), 2.72-2.61 (dd, 2H, *J* 14.6, 4.8 Hz, H_{8e} and H_{10e}); 2.11 (s, 6H, CH₃). ¹³C-NMR (100 MHz, CDCl₃) δ 207.63, 206.67 (C=O), 171.12, 168.29, 139.55, 126.88, 126.16, 126.09, 125.46, 61.31, 46.01, 44.37, 43.90, 43.48, 11.82. MS: 398.41 (399 [M+1]). Anal. calc. for C₂₁H₂₂N₂O₆ (398.41): C 63.31, H 5.57, N 7.03; found: C 63.28, H 5.59, N 7.04



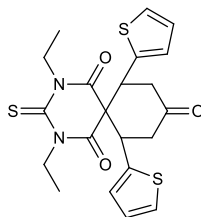
7,11-Bis(2-methoxyphenyl)-2,4-dimethyl-2,4-diazaspiro [5.5]undecane-1,3,5,9-tetraone (3e). White solid, (422 mg, 92%). Mp 242-246 °C. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.20-7.16 (td, 2H, J 8.0, 1.6 Hz, Ar-H), 7.14-7.12 (dd, 1H, J 8.0, 1.2 Hz, Ar-H); 7.01-6.99 (dd, 1H, J 8.0, 1.6 Hz, Ar-H); 6.94-6.90 (td, 1H, J 8.0, 0.8 Hz, Ar-H); 6.83-6.79 (m, 2H, Ar-H); 6.71-6.69 (dd, 1H, J 8.4, 0.8 Hz, Ar-H); 4.65-4.60 (dd, 1H, J 14.6, 5.2 Hz, H_7); 4.51-4.47 (dd, 1H, J 14.4, 3.2 Hz, H_{11}); 3.76 (s, 3H, CH_3), 3.59 (s, 3H, CH_3), 3.53 (t, 1H, H_{8a}), 3.17 (s, 1H, H_{10a}); 2.80 (s, 6H, CH_3); 2.61-2.56 (dd, 2H, J 14.6, 4.8 Hz, H_{8e} and H_{10e}); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ 207.84 (C=O), 170.16, 156.81, 150.14, 129.48, 127.53, 126.65, 126.02, 120.95, 120.68, 111.68, 110.51, 57.79, 55.88, 43.13, 28.41. MS: 450.18 (451 [M+1]). Anal. calc. for $\text{C}_{25}\text{H}_{26}\text{N}_2\text{O}_6$ (450.18): C 66.65, H 5.82, N 6.22; found: C 66.53, H 5.90, N 6.26.



2,4-Diethyl-7,11-diphenyl-3-thioxo-2,4-diazaspiro[5.5] undecane-1,5,9-trione(3f). ²³ Yellow solid, (385 mg, 78%). Mp 130-132 °C. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.21-7.19 (m, 6H, Ar-H); 7.08-7.06 (m, 4H, Ar-H); 4.17 (q, 2H, J 7.2 Hz, CH_2CH_3), 4.07-3.97 (m, 4H, CH_2CH_3 and H_7 and H_{11}); 3.77-3.70 (t, 2H, J 14.4 Hz, H_{8a} and H_{10a}); 2.63 (dd, 2H, J 15.6, 4.8 Hz, H_{8e} and H_{10e}), 1.07 (t, 3H, J 7.2 Hz, CH_3), 0.85 (t, 3H, J 7.2 Hz, CH_3).

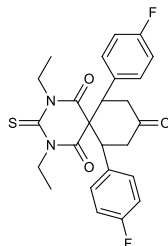


2,4-Diethyl-7,11-di(thiophen-2-yl)-3-thioxo-2,4-diazaspiro[5.5]undecane-1,5,9-trione (3g). White solid, (482 mg, 89%). Mp 220-224 °C. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.15 (dd, 2H, J 5.2, 1.2 Hz, Ar-H); 6.85 (dd, 2H, J 5.2, 3.6 Hz, Ar-H); 6.78 – 6.77 (m, 2H, Ar-H); 4.34-4.30 (dd, 2H, J 14.0, 4.8 Hz, H_7 and H_{11}); 4.23-4.15 (m, 4H, $2\times\text{CH}_2\text{-N}$); 3.59 (t, 2H, J 15.2 Hz, H_{8a} and H_{10a}); 2.74 (dd, 2H, J 15.6, 4.8 Hz, H_{8e} and H_{10e}); 1.08 (t, 3H, J 7.2 Hz, CH_3), 1.01 (t, 3H, J 7.2 Hz, CH_3). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ 205.85 (C=O), 177.51, 169.26, 166.70, 139.55, 126.88, 126.16, 126.09, 125.46, 61.31, 46.01, 44.37, 43.90, 43.48, 11.82. MS: 446.08 (447 [M+1]). Anal. calc. for $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_3\text{S}_3$ (446.08): C 56.48, H 4.97, N 6.27, S 21.54; found: C 56.52, H 4.93, N 6.24, S 21.57.

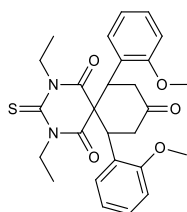


2,4-Diethyl-7,11-bis(4-fluorophenyl)-3-thioxo-2,4-diazaspiro[5.5] undecane-1,5,9-trione (3h). White solid, (405 mg, 88 %). Mp 158-162 °C. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.07-7.02 (m, 4H, Ar-H); 6.94-6.89 (m, 4H, Ar-H);

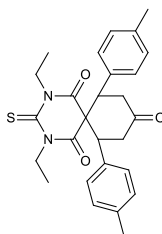
4.17 (q, 2H, J 7.2 Hz, CH_2CH_3), 4.06-3.97 (m, 4H, CH_2CH_3 and H_7 and H_{11}); 3.67 (t, 2H, J 14.4 Hz, H_{8a} and H_{10a}); 2.63 (dd, 2H, J 15.6, 4.8 Hz, H_{8e} and H_{10e}), 1.07 (t, 3H, J 7.2 Hz, CH_3), 0.85 (t, 3H, J 7.2 Hz, CH_3). ^{13}C -NMR (100 MHz, CDCl_3) δ 207.42 (C=O), 176.92, 169.50, 168.84, 166.88, 163.76, 161.29, 132.57, 129.51, 129.43, 115.91, 115.70, 60.61, 50.20, 46.02, 43.63, 43.21, 40.81, 11.91. MS: 470.53 (471 $[\text{M}+1]$). Anal. calc. for $\text{C}_{25}\text{H}_{24}\text{F}_2\text{N}_2\text{O}_3\text{S}$ (470.53): C 63.81, H 5.14, F 8.08, N 5.95, S 6.81; found: C 63.89, H 5.10, F 8.05, N 5.99, S 6.76.



2,4-Diethyl-7,11-bis(2-methoxyphenyl)-3-thioxo-2,4-diazaspiro[5.5]undecane-1,5,9-trione (3i). Yellow solid, (442 mg, 90%). Mp 212-216 °C. ^1H -NMR (400 MHz, CDCl_3) δ 7.20 – 7.15 (m, 2H, Ar-H); 7.04 (dd, 2H, J 7.6, 1.6 Hz, Ar-H); 6.82 – 6.77 (m, 4H, Ar-H); 4.68 (dd, 2H, J 13.6, 4.8 Hz, H_7 and H_{11}); 4.35 (q, 2H, J 7.2 Hz, CH_2CH_3); 3.99 (q, 2H, J 7.2 Hz, CH_2CH_3); 3.76 (s, 6H, O-CH₃), 3.50 (t, 2H, J 14.2, H_{8a} and H_{10a}); 2.63 (dd, 2H, J 16.4, 5.2 Hz, H_{8e} and H_{10e}); 1.22-1.19 (t, 3H, J 7.2 Hz, CH_3), 0.70-0.66 (t, 3H, J 7.2 Hz, CH_3). ^{13}C -NMR (100 MHz, CDCl_3) δ 205.95 (C=O), 174.07, 164.21, 151.94, 125.20, 124.23, 120.59, 116.03, 105.32, 53.16, 50.20, 37.82, 36.98, 35.58. MS: 494.19 (495 $[\text{M}+1]$). Anal. calc. for $\text{C}_{27}\text{H}_{30}\text{N}_2\text{O}_5\text{S}$ (494.19): C 65.57, H 6.11, N 5.66, S 6.48; found: C 65.62, H 6.14, N 5.60, S 6.46.



2,4-Diethyl-3-thioxo-7,11-di-p-tolyl-2,4-diazaspiro[5.5]undecane-1,5,9-trione (3j). White solid, (478 mg, 90%). Mp 178-182 °C. ^1H -NMR (400 MHz, CDCl_3) δ 7.01 – 6.93 (m, 8H, Ar-H); 4.16 (q, 2H, J 7.2 Hz, CH_2CH_3); 4.04-3.96 (m, 4H, CH_2CH_3 , H_7 and H_{11}); 3.69 (t, 2H, J 14.8 Hz, H_{8a} and H_{10a}); 2.59 (dd, 2H, J 15.6, 4.8 Hz, H_{8e} and H_{10e}); 2.25 (s, 6H, CH_3), 1.09 (t, 3H, J 7.2 Hz, CH_3), 0.86 (t, 3H, J 7.2 Hz, CH_3). ^{13}C -NMR (100 MHz, CDCl_3) δ 203.80 (C=O), 172.78, 164.35, 162.43, 133.52, 129.06, 124.68, 124.59, 122.86, 55.99, 46.00, 38.84, 38.53, 38.45, 38.39, 16.29. MS: 462.20 (463 $[\text{M}+1]$). Anal. calc. for $\text{C}_{27}\text{H}_{30}\text{N}_2\text{O}_3\text{S}$ (462.20): C 70.10, H 6.54, N 6.06, S 6.93; found: C 70.15, H 6.51, N 6.01, S 6.96.



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Supplementary Material

Attached.

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