

## Brief Report

DOI: 10.5582/ddt.2011.v5.6.279

# Synthesis and anti-HIV activity of novel 2,4-disubstituted-7-methyl-1,1,3-trioxo-2*H*,4*H*-pyrazolo[4,5-*e*][1,2,4]thiadiazine derivatives

Peng Zhan<sup>1</sup>, Renzhang Yan<sup>1</sup>, Xinyong Liu<sup>1,\*</sup>, Christophe Pannecouque<sup>2</sup>, Myriam Witvrouw<sup>2</sup>, Erik De Clercq<sup>2</sup>, Maria Teresa Molina<sup>3</sup>, Salvador Vega<sup>3</sup>

<sup>1</sup> Institute of Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, Ji'nan, China;

<sup>2</sup> Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium;

<sup>3</sup> Instituto de Quimica Medica (CSIC), c/Juan de la Cierva, Madrid, Spain.

**ABSTRACT:** A series of novel 2,4-disubstituted-7-methyl-1,1,3-trioxo-2*H*,4*H*-pyrazolo[4,5-*e*][1,2,4]thiadiazines (PTDs) was prepared starting from a ring of pyrazolo[4,5-*e*][1,2,4]thiadiazine nuclei with two different alkyl halides obtained by a facile one-pot reaction. The structures of all synthesized compounds were confirmed by <sup>1</sup>H- and <sup>13</sup>C-NMR, infrared spectra (IR), and mass spectra (MS) spectroscopic analysis. Anti-HIV activity was evaluated and none of the compounds were found to inhibit HIV replication in human T-lymphocyte (MT-4) cell culture.

**Keywords:** HIV, pyrazolo[4,5-*e*][1,2,4]thiadiazine, heterocycle, synthesis

## 1. Introduction

Fused heterocyclic thiadiazine derivatives such as pyridothiadiazines, pyrazinothiadiazines, imidazothiadiazines, triazolothiadiazines, and thienothiadiazines have become of particular interest to chemists and biologists because of their broad spectrum of biological activities and potential pharmacological applications in the treatment of cerebro/cardiovascular disease (1,2), cognitive disorders (3), cancers, and viral and bacterial infection (4,5). Among these derivatives, 2,4-disubstituted-1,1,3-trioxo-2*H*,4*H*-thieno [3,4-*e*][1,2,4]thiadiazines (TTDs) have been found to be potent non-nucleoside reverse transcriptase inhibitors (NNRTIs) of human immunodeficiency virus type 1 (HIV-1), which selectively block HIV-1 replication at the reverse transcriptase step (6). The lead compound QM96625 (Figure 1) displayed highly potent activity and selectivity against HIV-1 replication in human T-lymphocyte (MT-4) cells with an EC<sub>50</sub> value of 0.10

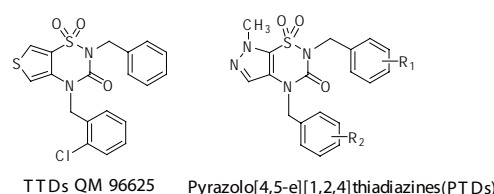
μM, CC<sub>50</sub> value > 119.0 μM, and SI > 1,190 (7,8).

In order to identify new HIV-1 NNRTIs, the current authors recently undertook a study of a series of 2,4-disubstituted-7-methyl-1,1,3-trioxo-2*H*,4*H*-pyrazolo[4,5-*e*][1,2,4]thiadiazines (PTDs) because of the known bioisosterism between TTDs and PTDs (9). This previous work reported a regioselective method for synthesis of *N*<sub>2</sub>- or *N*<sub>4</sub>-monosubstituted PTDs (10) that was used to synthesize *N*<sub>2</sub>,*N*<sub>4</sub>-disubstituted PTDs via a one-pot reaction (11). This work had also evaluated the anti-HIV-1 activity of PTDs and identified some *N*<sub>4</sub>-monosubstituted and *N*<sub>2</sub>,*N*<sub>4</sub>-disubstituted PTDs that exhibited moderate anti-HIV-1 activity. As a continuation of this research on new anti-HIV-1 agents from PTD analogues, a series of novel 2,4-disubstituted-7-methyl-1,1,3-trioxo-2*H*,4*H*-pyrazolo[4,5-*e*][1,2,4]thiadiazines were designed and synthesized based on the one-pot reaction that was previously established. The introduction of substituents at *N*<sub>2</sub> and *N*<sub>4</sub> moieties of the PTD heterocycle resulted in those substituents having the same anti-HIV activity as a series of TTDs. The current research describes the synthesis of novel disubstituted PTDs and screening of their anti-HIV activity based on cell cultures.

## 2. Materials and Methods

### 2.1. General methods

All melting points were determined on a micromelting point apparatus and are uncorrected. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were obtained on a Bruker Avance-600



**Figure 1.** Lead compound in the form of a 2,4-disubstituted thieno[3,4-*e*][1,2,4]thiadiazine (TTD, QM96625) and structures of 2,4-disubstituted-7-methylpyrazolo[4,5-*e*][1,2,4]thiadiazines (PTDs).

\*Address correspondence to:

Dr. Xinyong Liu, Institute of Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, No. 44, Wenhuaxi Road, Ji'nan 250012, China. e-mail: xinyongli@sdu.edu.cn

(600 MHz; Bruker BioSpin, Rheinstetten, Germany) in the indicated solvent. Chemical shifts are expressed in  $\delta$  units with TMS as the internal reference. Infrared spectra (IR) were recorded with a Nexus 470FT-IR spectrometer (Thermo Nicolet, Madison, WI, USA). Mass spectra (MS) were acquired with an LC autosampler device: standard G1313A instrument (Agilent Technologies, Santa Clara, CA, USA) and a AB Sciex API 4000 tandem mass spectrometer (Applied Biosystems, USA) with ESI. All compounds were routinely checked by TLC at 254 nm on pre-coated silica gel G plates with fluorescent indicator, which were prepared in the laboratory. Developed plates were visualized with UV light. Flash column chromatography was performed on a column packed with silica gel 60 (230-400 mesh). Solvents were reagent grade and, when necessary, were purified and dried using standard methods. Concentration of the reaction solutions involved the use of a rotary evaporator at reduced pressure.

## 2.2. General procedure for the preparation of $N_2, N_4$ -disubstituted-7-methyl-1,1,3-trioxo-2H,4H-pyrazolo[4,5-*e*][1,2,4]thiadiazines (**3a-3p**)

Sodium hydride (60% dispersion in mineral oil, 2 equivalent) was incrementally added to a solution of compound (**1**) (1 equivalent) (Scheme 1) in dry DMF in an inert atmosphere ( $N_2$ ) and with the temperature below  $10^\circ\text{C}$ . After 30 min of stirring, the first alkyl halide ( $R_2X$ , 1 equivalent) was added dropwise. The mixture was stirred at room temperature for 20 min and  $30-50^\circ\text{C}$  for 12-20 h (checked by TLC). After the addition of the second alkyl halide ( $R_1X$ , 1 equivalent), the mixture continued to be stirred at  $40-80^\circ\text{C}$  for 12-20 h. After the solvent was evaporated off under reduced pressure, the crude product was purified by recrystallization from ethanol. Specific information on compounds **3a-3p** is indicated in the Appendix.

## 2.3. In vitro anti-HIV assay

The methodology of the anti-HIV assay used here has been previously described (12,13). Stock solutions ( $10\times$  final concentration) of test compounds were added in 25- $\mu\text{L}$  volumes to two sets of triplicate wells so as to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial five-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman Instruments, Fullerton, CA, USA). Untreated control HIV- and mock-infected cell samples were included for each sample.

HIV-1 (IIIB) (14) or HIV-2 (ROD) (15) stock (50  $\mu\text{L}$ ) at 100-300 CCID<sub>50</sub> (cell culture infectious dose) or culture medium was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of the test compound on uninfected cells in order to assess the cytotoxicity of the test compound. Exponentially growing MT-4 cells (16) were

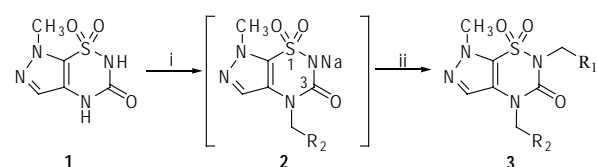
centrifuged for 5 min at 1,000 rpm and the supernatant was discarded. The MT-4 cells were resuspended at  $6 \times 10^5$  cells/mL, and 50- $\mu\text{L}$  volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells was examined spectrophotometrically using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

The MTT assay is based on the reduction of yellow colored MTT (Acros Organics, Geel, Belgium) by mitochondrial dehydrogenase of metabolically active cells to a bluish-purple formazan that can be measured spectrophotometrically. Absorbance was read in an eight-channel computer-controlled photometer (Multiscan Ascent Reader, LabSystems, Helsinki, Finland) at two wavelengths (540 and 690 nm). All data were calculated using the median optical density (OD) of three wells. The 50% cytotoxic concentration ( $CC_{50}$ ) was defined as the concentration of the test compound that reduced the absorbance ( $OD_{540}$ ) of the mock-infected control sample by 50%. The 50% effective concentration ( $EC_{50}$ ) was defined as the compound concentration required to inhibit virus-induced syncytium formation by 50%.

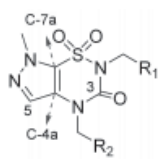
## 3. Results and Discussion

### 3.1. Chemistry

A ring of 7-methyl PTD nuclei (**1**) (Scheme 1) was prepared according to a previously reported procedure (10) and was purified by recrystallization from ethanol to yield a white solid in good yield (84%).  $N_2, N_4$ -disubstituted hetero[1,2,4]thiadiazines are usually prepared by stepwise alkylation. The first step of alkylation at the  $N_2$  position usually produces a mixture of  $N_2$ - and  $O_3$ -monosubstituted products that must be separated by flash column chromatography before the second step of alkylation at the  $N_4$  position. Use of one-pot regioselective alkylation avoids this complicated process (17). In brief, the ring of 7-methyl PTD nuclei (**1**) was dissolved in dry DMF (1 mmol/5 mL), which was cooled to under  $10^\circ\text{C}$ . Two equimolar NaH was added, followed by 1 equimolar  $R_2CH_2X$ , and then 1 equimolar  $R_1CH_2X$  was added when the intermediate of the mono  $N_4$ -substituted product (**2**) was obtained (checked by TLC). The crude product (**3**) was purified by recrystallization from ethanol to yield a white solid in good yield (Scheme 1). A series of  $N_2, N_4$ -disubstituted pyrazolo[4,5-*e*][1,2,4]thiadiazine derivatives (**3a-3p**) were prepared by this method and is listed in Table 1.



**Scheme 1. Reagents:** (i): NaH/ $R_2CH_2X$  (2:1),  $30-80^\circ\text{C}$  (ii):  $R_1CH_2X$ ,  $40-80^\circ\text{C}$ .

**Table 1. Structures and anti-HIV-1<sup>a</sup> and HIV-2<sup>b</sup> of newly synthesized 2,4-disubstituted-7-methyl-1,1,3-trioxo-4,7-dihydropyrazolo [4,3-*e*][1,2,4]thiadiazines (PTDs 3) *in vitro***


Compound	R <sub>1</sub> CH <sub>2</sub>	R <sub>2</sub> CH <sub>2</sub>	EC <sub>50</sub> (μM) <sup>c</sup>		CC <sub>50</sub> (μM) <sup>d</sup>	SI <sup>e</sup>	
			HIV-1 III <sup>B</sup> <sup>a</sup>	HIV-2 ROD <sup>b</sup>		HIV-1 III <sup>B</sup>	HIV-2 ROD
3a	2-Cl-benzyl	2-Cl-benzyl	> 277.2	> 277.2	> 277.2	× 1	× 1
3b	4-Cl-benzyl	2-Cl-benzyl	> 277.2	> 277.2	> 277.2	× 1	× 1
3c	2,4-Cl <sub>2</sub> -benzyl	2-Cl-benzyl	> 253.3	> 198.8	> 251.3	× 1	× 1
3d	2-CN-benzyl	2-Cl-benzyl	> 283.1	> 283.1	≥ 283.1	× 1	× 1
3e	3-CN-benzyl	2-Cl-benzyl	> 283.1	> 283.1	> 283.1	× 1	× 1
3f	4-CN-benzyl	2-Cl-benzyl	> 7.0	> 5.1	7.3	< 1	< 1
3g	4-C <sub>2</sub> H <sub>5</sub> -benzyl	2-Cl-benzyl	> 281.2	> 276.7	≥ 276.7	× 1	× 1
3h	4-(t-Bu)-benzyl	2-Cl-benzyl	> 264.6	> 264.6	> 264.6	× 1	× 1
3i	4-NO <sub>2</sub> -benzyl	2-Cl-benzyl	> 151.7	> 124.8	155.0	< 1	< 1
3j	4-Br-benzyl	2-Cl-benzyl	> 252.3	> 252.3	> 252.3	× 1	× 1
3k	4-NO <sub>2</sub> -benzyl	benzyl	> 252.9	> 238.9	265.8	< 1	< 1
3l	2-Br-benzyl	benzyl	> 271.1	> 271.1	> 271.1	× 1	× 1
3m	4-Br-benzyl	2-Br-benzyl	> 231.5	> 231.5	> 231.5	× 1	× 1
3n	4-Cl-benzyl	2-Br-benzyl	> 252.3	> 252.3	> 252.3	× 1	× 1
3o	2-Br-benzyl	3-Cl-benzyl	> 252.3	> 252.3	> 252.3	× 1	× 1
3p	4-Cl-benzyl	2,4-Cl <sub>2</sub> -benzyl	> 257.5	> 216.3	≥ 216.3	× 1	× 1
Zidovudine					0.0007		35.6
Nevirapine					0.03		683

<sup>a</sup> Anti-HIV-1 activity measured with strain III<sup>B</sup>.<sup>b</sup> Anti-HIV-2 activity measured with strain ROD.<sup>c</sup> Compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1- and HIV-2-induced cytopathogenic effect.<sup>d</sup> Compound concentration that reduces the viability of mock-infected MT-4 cells by 50%.<sup>e</sup> SI: selectivity index (CC<sub>50</sub>/EC<sub>50</sub>).

### 3.2. *In vitro* anti-HIV activity

Compounds **3a-3p** were evaluated for their *in vitro* anti-HIV-1 activity by using the III<sup>B</sup> strain of HIV-1 and the ROD strain of HIV-2. Compounds were monitored for their inhibition of the virus-induced cytopathic effect in MT-4 cells. The results are summarized in Table 1, where the data for zidovudine and nevirapine have been included for comparison purposes. However, none of compounds were found to inhibit HIV replication in MT-4 cell culture. Results revealed that the five-member ring moiety of the heterothiadiazines had a major role in affecting anti-HIV activity. As a result, PTDs had much lower activity than TTDs despite having very similar structures. This might be due to the fact that the pyrazole moiety of pyrazolothiadiazines is more hydrophobic than the thiophene ring of thienothiadiazines, which is ill-suited to the structural requirement for a hydrophobic body as is assumed in the "butterfly" model (17).

### Acknowledgements

The authors gratefully acknowledge financial support from the National Natural Science Foundation of China (NSFC No. 81102320, 30873133, No. 30772629, No. 30371686), Key Project of NSFC for International Cooperation (No. 30910103908), Research Fund for the Doctoral Program

of Higher Education of China (No. 20110131130005, 20110131120037), the Independent Innovation Foundation of Shandong University (IIFSDU, No. 2010GN044), the Shandong Postdoctoral Innovative Science Research Special Program (No. 201002023), and the China Postdoctoral Science Foundation (No. 20100481282).

### References

- Pirotte B, Ouedraogo R, de Tullio P, Khelili S, Somers F, Boverie S, Dupont L, Fontaine J, Damas J, Lebrun P. 3-Alkylamino-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides structurally related to diazoxide and pinacidil as potassium channel openers acting on vascular smooth muscle cells: Design, synthesis, and pharmacological evaluation. *J Med Chem.* 2000; 43:1456-1466.
- Campillo N, Garcia C, Goya P, Alkorta I, Paez JA. Novel bronchodilators: Synthesis, transamination reactions, and pharmacology of a series of pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxides. *J Med Chem.* 2000; 43:4219-4227.
- Pirotte B, Podona T, Diouf O, de Tullio P, Lebrun P, Dupont L, Somers F, Delarge J, Morain P, Lestage P, Lepagnol J, Spedding M. 4*H*-1,2,4-Pyridothiadiazine 1,1-dioxides and 2,3-dihydro-4*H*-1,2,4-pyridothiadiazine 1,1-dioxides chemically related to diazoxide and cyclothiazide as powerful positive allosteric modulators of (*R/S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-

- 4-yl)propionic acid receptors: Design, synthesis, pharmacology, and structure-activity relationships. *J Med Chem.* 1998; 41:2946-2959.
- Martinez A, Esteban AI, Herrero A, Ochoa C, Andrei G, Snoeck R, Balzarini J, De Clercq E. Imidazothiadiazine dioxides: Synthesis and antiviral activity. *Bioorg Med Chem.* 1999; 7:1617-1623.
  - Holla BS, Sarojini BK, Rao BS, Akberali PM, Kumari NS, Shetty V. Synthesis of some halogen-containing 1,2,4-triazolo-1,3,4-thiadiazines and their antibacterial and anticancer screening studies – Part I. *Farmaco.* 2001; 56:565-570.
  - Arranz E, Diaz JA, Ingate ST, Witvrouw M, Pannecouque C, Balzarini J, De Clercq E, Vega S. Novel 1,1,3-trioxo-2*H*,4*H*-thieno [3,4-*e*][1,2,4] thiadiazine derivatives as non-nucleoside reverse transcriptase inhibitors that inhibit human immunodeficiency virus type 1 replication. *J Med Chem.* 1998; 41:4109-4117.
  - Arranz ME, Diaz JA, Ingate ST, Witvrouw M, Pannecouque C, Balzarini J, De Clercq E, Vega S. Synthesis and anti-HIV activity of 1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazines (TTDs): A new family of HIV-1 specific non-nucleoside reverse transcriptase inhibitors. *Bioorg Med Chem.* 1999; 7:2811-2822.
  - Witvrouw M, Arranz ME, Pannecouque C, Declercq R, Jonckheere H, Schmit JC, Vandamme AM, Diaz JA, Ingate ST, Desmyter J, Esnouf R, Van Meervelt L, Vega S, Balzarini J, De Clercq E. 1,1,3-Trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine (TTD) derivatives: A new class of nonnucleoside human immunodeficiency virus type 1 (HIV-1) reverse transcriptase inhibitors with anti-HIV-1 activity. *Antimicrob Agents CH.* 1998; 42:618-623.
  - Patani GA, LaVoie EJ. Bioisosterism: A rational approach in drug design. *Chem Rev.* 1996; 96:3147-3176.
  - Liu XY, Chen NG, Yan RZ, Xu WF, Molina MT, Vega S. Regioselective synthesis of the novel *N<sub>7</sub>*-substituted pyrazolo[4,5-*e*][1,2,4]thiadiazines as potent HIV-1 NNRTIs. *Heterocycles.* 2006; 68:1225-1232.
  - Liu X, Yan R, Chen N, Xu W, Molina MT, Vega S. Regioselective synthesis of novel *N<sub>2</sub>*- and *N<sub>7</sub>*-substituted 7-methylpyrazolo[4,5-*e*][1,2,4]thiadiazines. *Molecules.* 2006; 11:827-836.
  - Pauwels R, Balzarini J, Baba M, Snoeck R, Schols D, Herdewijn P, Desmyter J, De Clercq E. Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. *J Virol Methods.* 1988; 20:309-321.
  - Pannecouque C, Daelemans D, De Clercq E. Tetrazolium-based colorimetric assay for the detection of HIV replication inhibitors: Revisited 20 years later. *Nat Protoc.* 2008; 3:427-434.
  - Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science.* 1984; 224:497-500.
  - Barré-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet C, Axler-Blin C, Vézinet-Brun F, Rouzioux C, Rozenbaum W, Montagnier L. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science.* 1983; 220:868-871.
  - Miyoshi I, Taguchi H, Kubonishi I, Yoshimoto S, Ohtsuki Y, Shiraiishi Y, Akagi T. Type C virus-producing cell lines derived from adult T cell leukemia. *Gann Monogr.* 1982; 28:219-228.
  - Schafer W, Friebe WG, Leinert H, Mertens A, Poll T, von der Saal W, Zilch H, Nuber B, Ziegler ML. Non-nucleoside inhibitors of HIV-1 reverse transcriptase: Molecular modeling and X-ray structure investigations. *J Med Chem.* 1993; 36:726-732.
- (Received September 26, 2011; Revised November 15, 2011; Re-revised November 22, 2011; Accepted November 25, 2011)

## Appendix

### Synthesis of compounds **3a-3p**

2,4-Di(*o*-chlorobenzyl)-7-methyl-1,1,3-trioxo-2*H*,4*H*-pyrazolo[4,5-*e*][1,2,4]thiadiazine (**3a**). Compound (**1**) and 2-chlorobenzyl chloride were allowed to react at 40-50°C for 8 h. After the addition of 2-chlorobenzyl chloride, the mixture was allowed to react at 50-60°C for 20 h. Purification by recrystallization from ethanol yielded compound **3a** as a white solid (80%), mp: 116-118°C. IR (KBr, cm<sup>-1</sup>): 1,699 (C=O), 1,331, 1,195 (SO<sub>2</sub>). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ: 7.80 (s, 1H, PyH), 7.09 (dd, 1H, *J* = 7.58 Hz, *J* = 1.52 Hz, PhH), 7.26-7.51 (m, 7H, PhH), 5.17 (s, 2H, NCH<sub>2</sub>), 5.11 (s, 2H, NCH<sub>2</sub>), 4.08 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 150 MHz) δ: 148.7 (C=O), 132.9, 133.2, 132.1, 131.6, 129.9, 129.5, 129.4, 129.3, 127.8, 127.7, 127.6, 127.5, 126.4 (C-5), 125.9 (C-4a), 122.2 (C-7a), 47.3 (N<sub>2</sub>-CH<sub>2</sub>), 41.7 (N<sub>4</sub>-CH<sub>2</sub>), 39.1 (CH<sub>3</sub>). MS (EI): *m/z* 451.4 [M + H]<sup>+</sup> (calcd for C<sub>19</sub>H<sub>16</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>3</sub>S: 450.03).

2-(*p*-Chlorobenzyl)-4-(*o*-chlorobenzyl)-7-methyl-1,1,3-trioxo-2*H*,4*H*-pyrazolo[4,5-*e*][1,2,4]thiadiazine (**3b**). Compound (**1**) and 2-chlorobenzyl chloride were allowed to react at 40-50°C for 8 h. After the addition of 4-chlorobenzyl chloride, the mixture was allowed to react at 50-60°C for 12 h. Purification by recrystallization from ethanol yielded compound **3b** as a white solid (65%), mp: 128-130°C. IR (KBr, cm<sup>-1</sup>): 1,693 (C=O), 1,331, 1,193 (SO<sub>2</sub>). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ: 7.75 (s, 1H, PyH), 7.50 (dd, 1H, *J* = 7.86 Hz, *J* = 1.23 Hz, PhH), 7.01 (dd, 1H, *J* = 7.63 Hz, *J* = 1.25 Hz, PhH), 7.26-7.41 (m, 6H, PhH), 5.15 (s, 2H, NCH<sub>2</sub>), 5.02 (s, 2H, NCH<sub>2</sub>), 4.08 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 150 MHz) δ: 148.8 (C=O), 133.0, 135.2, 132.1, 132.4, 129.9, 129.8, 129.5, 128.5, 127.7, 127.4, 126.3 (C-5), 125.8 (C-4a), 122.2 (C-7a), 47.2 (N<sub>2</sub>-CH<sub>2</sub>), 43.5 (N<sub>4</sub>-CH<sub>2</sub>), 39.1 (CH<sub>3</sub>). MS (EI): *m/z* 451.4 [M + H]<sup>+</sup> (calcd for C<sub>19</sub>H<sub>16</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>3</sub>S: 450.03).

2-(2,4-Dichlorobenzyl)-4-(*o*-chlorobenzyl)-7-methyl-1,1,3-trioxo-2*H*,4*H*-pyrazolo[4,5-*e*][1,2,4]thiadiazine (**3c**). Compound (**1**) and 2-chlorobenzyl chloride were allowed to react at 40-50°C for 8 h. After the addition of 2,4-dichlorobenzyl chloride, the mixture was allowed to react at 50-60°C for 20 h. Purification

by recrystallization from ethanol yielded compound **3c** as a white solid (55%), mp: 138-140°C. IR (KBr,  $\text{cm}^{-1}$ ): 1,698 (C=O), 1,330, 1,196 ( $\text{SO}_2$ ).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 600 MHz)  $\delta$ : 7.25 (s, 1H, Pyr-H), 7.02 (d, 1H,  $J = 7.26$  Hz, PhH), 7.20-7.43 (m, 6H, PhH), 5.24 (s, 2H,  $\text{NCH}_2$ ), 5.22 (s, 2H,  $\text{NCH}_2$ ), 4.15 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 150 MHz)  $\delta$ : 149.1 (C=O), 133.3, 133.7, 132.5, 131.7, 131.6, 129.7, 129.2, 129.1, 128.6, 128.5, 127.2, 126.0, 125.2, 125.1, 122.6 (C-7a), 46.7 ( $\text{N}_2\text{-CH}_2$ ), 41.4 ( $\text{N}_4\text{-CH}_2$ ), 38.9 ( $\text{CH}_3$ ). MS (EI):  $m/z$  487.3 [ $\text{M} + 3$ ] $^+$  (calcd for  $\text{C}_{19}\text{H}_{15}\text{Cl}_3\text{N}_4\text{O}_3\text{S}$ : 483.99).

*2-(o-Cyanobenzyl)-4-(o-chlorobenzyl)-7-methyl-1,1,3-trioxo-2H,4H-pyrazolo[4,5-e][1,2,4]thiadiazine (3d)*. Compound (**1**) and 2-chlorobenzyl chloride were allowed to react at 40-50°C for 8 h. After the addition of 2-cyanobenzyl chloride, the mixture was allowed to react at 70-80°C for 20 h. Purification by recrystallization from ethanol yielded compound **3d** as a white solid (60%), mp: 130-132°C. IR (KBr,  $\text{cm}^{-1}$ ): 2,226 (CN), 1,695 (C=O), 1,317, 1,192 ( $\text{SO}_2$ ).  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ , 600 MHz)  $\delta$ : 7.77 (s, 1H, PyH), 7.84 (dd, 1H,  $J = 7.73$  Hz,  $J = 1.31$  Hz, PhH), 7.04 (dd, 1H,  $J = 7.57$  Hz,  $J = 1.27$  Hz, PhH), 7.25-7.71 (m, 6H, PhH), 5.22 (s, 2H,  $\text{NCH}_2$ ), 5.15 (s, 2H,  $\text{NCH}_2$ ), 4.09 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C-NMR}$  ( $\text{DMSO-}d_6$ , 150 MHz)  $\delta$ : 48.8 (C=O), 133.7, 139.6, 133.1, 132.8, 132.0, 129.9, 129.5, 128.6, 127.8, 127.7, 127.5, 126.3 (C-5), 125.8 (C-4a), 122.1 (C-7a), 117.2, 110.9 (CN), 47.3 ( $\text{N}_2\text{-CH}_2$ ), 42.4 ( $\text{N}_4\text{-CH}_2$ ), 39.1 ( $\text{CH}_3$ ). MS (EI):  $m/z$  442.4 [ $\text{M} + \text{H}$ ] $^+$  (calcd for  $\text{C}_{20}\text{H}_{16}\text{ClN}_5\text{O}_3\text{S}$ : 441.07).

*2-(m-Cyanobenzyl)-4-(o-chlorobenzyl)-7-methyl-1,1,3-trioxo-2H,4H-pyrazolo[4,5-e][1,2,4]thiadiazine (3e)*. Compound (**1**) and 2-chlorobenzyl chloride were allowed to react at 40-50°C for 8 h. After the addition of 3-cyanobenzyl chloride, the mixture was allowed to react at 70-80°C for 20 h. Purification by recrystallization from ethanol yielded compound **3e** as a white solid (61%), mp: 116-118°C. IR (KBr,  $\text{cm}^{-1}$ ): 2,233 (CN), 1,693 (C=O), 1,329, 1,192 ( $\text{SO}_2$ ).  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ , 600 MHz)  $\delta$ : 7.70 (s, 1H, PyH), 7.79 (s, 1H, PhH), 7.50 (dd, 1H,  $J = 7.86$  Hz,  $J = 1.23$  Hz, PhH), 7.06 (dd, 1H,  $J = 7.63$  Hz,  $J = 1.28$  Hz, PhH), 7.27-7.76 (m, 5H, PhH), 5.15 (s, 2H,  $\text{NCH}_2$ ), 5.10 (s, 2H,  $\text{NCH}_2$ ), 4.08 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C-NMR}$  ( $\text{DMSO-}d_6$ , 150 MHz)  $\delta$ : 148.8 (C=O), 133.0, 138.0, 132.6, 132.1, 131.6, 131.2, 129.9, 129.8, 129.5, 127.6, 127.5, 126.3 (C-5), 125.9 (C-4a), 112.0 (C-7a), 118.8, 111.5 (CN), 47.3 ( $\text{N}_2\text{-CH}_2$ ), 43.2 ( $\text{N}_4\text{-CH}_2$ ), 39.1 ( $\text{CH}_3$ ). MS (EI):  $m/z$  442.4 [ $\text{M} + \text{H}$ ] $^+$  (calcd for  $\text{C}_{20}\text{H}_{16}\text{ClN}_5\text{O}_3\text{S}$ : 441.07).

*2-(p-Cyanobenzyl)-4-(o-chlorobenzyl)-7-methyl-1,1,3-trioxo-2H,4H-pyrazolo[4,5-e][1,2,4]thiadiazine (3f)*. Compound (**1**) and 2-chlorobenzyl chloride were allowed to react at 40-50°C for 8 h. After the addition of 4-cyanobenzyl chloride, the mixture was

allowed to react at 70-80°C for 20 h. Purification by recrystallization from ethanol yielded compound **3f** as a white solid (63%), mp: 164-166°C. IR (KBr,  $\text{cm}^{-1}$ ): 2,227 (CN); 1,690 (C=O); 1,334, 1,193 ( $\text{SO}_2$ ).  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ , 600 MHz)  $\delta$ : 7.78 (s, 1H, PyH), 7.79 (s, 1H, PhH), 7.04 (d, 1H,  $J = 7.61$  Hz, PhH), 7.27-7.85 (m, 7H, PhH), 5.15 (s, 2H,  $\text{NCH}_2$ ), 5.12 (s, 2H,  $\text{NCH}_2$ ), 4.08 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C-NMR}$  ( $\text{DMSO-}d_6$ , 150 MHz)  $\delta$ : 148.9 (C=O), 132.9, 141.9, 132.8, 132.1, 129.9, 129.5, 128.4, 127.9, 127.7, 127.5, 126.3, 126.2 (C-5), 125.9 (C-4a), 122.0 (C-7a), 118.8, 110.5 (CN), 47.3 ( $\text{N}_2\text{-CH}_2$ ), 43.7 ( $\text{N}_4\text{-CH}_2$ ), 39.1 ( $\text{CH}_3$ ). MS (EI):  $m/z$  442.5 [ $\text{M} + \text{H}$ ] $^+$  (calcd for  $\text{C}_{20}\text{H}_{16}\text{ClN}_5\text{O}_3\text{S}$ : 441.07).

*2-(p-Ethylbenzyl)-4-(o-chlorobenzyl)-7-methyl-1,1,3-trioxo-2H,4H-pyrazolo[4,5-e][1,2,4]thiadiazine (3g)*. Compound (**1**) and 2-chlorobenzyl chloride were allowed to react at 40-50°C for 8 h. After the addition of 4-ethylbenzyl chloride, the mixture was allowed to react at 70-80°C for 20 h. Purification by recrystallization from ethanol yielded compound **3g** as a white solid (59%), mp: 112-114°C. IR (KBr,  $\text{cm}^{-1}$ ): 1,699 (C=O), 1,338, 1,192 ( $\text{SO}_2$ ).  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ , 600 MHz)  $\delta$ : 7.74 (s, 1H, PyH), 7.50 (dd, 1H,  $J = 7.75$  Hz,  $J = 0.75$  Hz, PhH), 6.99 (d, 1H,  $J = 7.25$  Hz, PhH), 6.16-7.32 (m, 6H, PhH), 5.16 (s, 2H,  $\text{NCH}_2$ ), 4.99 (s, 2H,  $\text{NCH}_2$ ), 4.08 (s, 3H,  $\text{CH}_3$ ), 2.54-2.58 (q, 2H,  $J = 7.59$  Hz,  $\text{CH}_2$ ), 1.13-1.16 (t, 3H,  $J = 7.59$  Hz,  $\text{CH}_3$ ).  $^{13}\text{C-NMR}$  ( $\text{DMSO-}d_6$ , 150 MHz)  $\delta$ : 148.9 (C=O), 133.0, 133.4, 143.4, 132.1, 129.9, 129.5, 128.0, 127.9, 127.7, 127.4, 126.2 (C-5), 125.8 (C-4a), 122.4 (C-7a), 47.1 ( $\text{N}_2\text{-CH}_2$ ), 44.0 ( $\text{N}_4\text{-CH}_2$ ), 39.1 (Py- $\text{CH}_3$ ), 28.0 (1C,  $\text{CH}_2$ ), 15.8 (1C,  $\text{CH}_3$ ). MS (EI):  $m/z$  445.5 [ $\text{M} + \text{H}$ ] $^+$  (calcd for  $\text{C}_{21}\text{H}_{21}\text{ClN}_4\text{O}_3\text{S}$ : 444.1).

*2-(p-(t-Bu)benzyl)-4-(o-chlorobenzyl)-7-methyl-1,1,3-trioxo-2H,4H-pyrazolo[4,5-e][1,2,4]thiadiazine (3h)*. Compound (**1**) and 2-chlorobenzyl chloride were allowed to react at 40-50°C for 8 h. After the addition of 4-(t-Bu)benzyl chloride, the mixture was allowed to react at 70-80°C for 20 h. Purification by recrystallization from ethanol yielded compound **3h** as a white solid (55%), mp: 140-142°C. IR (KBr,  $\text{cm}^{-1}$ ): 1,700 (C=O), 1,344, 1,189 ( $\text{SO}_2$ ).  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ , 600 MHz)  $\delta$ : 7.75 (s, 1H, PyH), 7.50 (dd, 1H,  $J = 7.90$  Hz,  $J = 1.08$  Hz, PhH), 7.00 (dd, 1H,  $J = 7.52$  Hz,  $J = 1.02$  Hz, PhH), 7.26-7.35 (m, 6H, PhH), 5.16 (s, 2H,  $\text{NCH}_2$ ), 4.99 (s, 2H,  $\text{NCH}_2$ ), 4.08 (s, 3H,  $\text{CH}_3$ ), 1.25 (s, 9H,  $\text{CH}_3$ ).  $^{13}\text{C-NMR}$  ( $\text{DMSO-}d_6$ , 150 MHz)  $\delta$ : 150.2 (C=O), 133.0, 133.1, 148.9, 132.1, 129.9, 129.5, 127.7, 127.6, 127.4, 126.3, 125.8 (C-5), 125.3 (C-4a), 122.4 (C-7a), 47.1 ( $\text{N}_2\text{-CH}_2$ ), 43.9 ( $\text{N}_4\text{-CH}_2$ ), 39.1 ( $\text{CH}_3$ ), 34.4 (C), 31.2 (3C,  $\text{CH}_3$ ). MS (EI):  $m/z$  473.3 [ $\text{M} + \text{H}$ ] $^+$  (calcd for  $\text{C}_{23}\text{H}_{25}\text{ClN}_4\text{O}_3\text{S}$ : 472.13).

*2-(p-Nitrobenzyl)-4-(o-chlorobenzyl)-7-methyl-1,1,3-trioxo-2H,4H-pyrazolo[4,5-e][1,2,4]thiadiazine*

(3i). Compound (1) and 2-chlorobenzyl chloride were allowed to react at 40-50°C for 8 h. After the addition of 4-nitrobenzyl bromide, the mixture was allowed to react at 50-55°C for 12 h. Purification by recrystallization from ethanol yielded compound **3i** as a white solid (70%), mp: 210-212°C. IR (KBr, cm<sup>-1</sup>): 1,689 (C=O), 1,331, 1,193 (SO<sub>2</sub>), 1,520, 1,278 (NO<sub>2</sub>). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ: 7.78 (s, 1H, PyH), 7.49 (dd, 1H, *J* = 7.34 Hz, *J* = 1.28 Hz, PhH), 7.05 (dd, 1H, *J* = 7.68 Hz, *J* = 2.10 Hz, PhH), 7.29-8.21 (m, 6H, PhH), 5.18 (s, 2H, NCH<sub>2</sub>), 5.15 (s, 2H, NCH<sub>2</sub>), 4.09 (s, 3H, CH<sub>3</sub>). MS (EI): m/z 462.4 [M + H]<sup>+</sup> (calcd for C<sub>19</sub>H<sub>16</sub>ClN<sub>5</sub>O<sub>5</sub>S: 461.06).

2-(*p*-Bromobenzyl)-4-(*o*-chlorobenzyl)-7-methyl-1,1,3-trioxo-2H,4H-pyrazolo[4,5-*e*][1,2,4]thiadiazine (3j). Compound (1) and 2-chlorobenzyl chloride were allowed to react at 40-50°C for 8 h. After the addition of 4-bromobenzyl bromide, the mixture was allowed to react at 50-55°C for 12 h. Purification by recrystallization from ethanol gave compound **3j** as a white solid (68%), mp: 148-150°C. IR (KBr, cm<sup>-1</sup>): 1,696 (C=O), 1,330, 1,192 (SO<sub>2</sub>). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ: 7.75 (s, 1H, PyH), 7.53 (d, 2H, *J* = 8.39 Hz, PhH), 7.50 (d, 1H, *J* = 7.76 Hz, PhH), 7.00 (d, 1H, *J* = 7.34 Hz, PhH), 7.28-7.32 (m, 4H, PhH), 5.15 (s, 2H, NCH<sub>2</sub>), 5.00 (s, 2H, NCH<sub>2</sub>), 4.08 (s, 3H, CH<sub>3</sub>). MS (EI): m/z 495.2 [M + H]<sup>+</sup>, 497.2 [M + 3]<sup>+</sup> (calcd for C<sub>19</sub>H<sub>16</sub>BrClN<sub>4</sub>O<sub>3</sub>S: 493.98).

2-(*p*-Nitrobenzyl)-4-benzyl-7-methyl-1,1,3-trioxo-2H,4H-pyrazolo[4,5-*e*][1,2,4]thiadiazine (3k). Compound (1) and bromobenzyl bromide were allowed to react at 30-40°C for 12 h. After the addition of 4-nitrobenzyl bromide, the mixture was allowed to react at 40-50°C for 12 h. Purification by recrystallization from ethanol yielded compound **3k** as a white solid (75%), mp: 202-204°C. IR (KBr, cm<sup>-1</sup>): 1,692 (C=O), 1,327, 1,190 (SO<sub>2</sub>), 1,514, 1,214 (NO<sub>2</sub>). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ: 7.84 (s, 1H, PyH), 8.21 (d, 2H, *J* = 8.72 Hz, PhH), 7.63 (d, 2H, *J* = 8.70 Hz, PhH), 7.26-7.34 (m, 5H, PhH), 5.19 (s, 2H, NCH<sub>2</sub>), 5.10 (s, 2H, NCH<sub>2</sub>), 4.06 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 150 MHz) δ: 149.0 (C=O), 135.8, 144.0, 128.8, 128.7, 127.1, 123.7, 147.1, 127.8, 126.3 (C-5), 125.6 (C-4a), 122.0 (C-7a), 48.8 (N<sub>2</sub>-CH<sub>2</sub>), 43.4 (N<sub>4</sub>-CH<sub>2</sub>), 39.0 (CH<sub>3</sub>). MS (EI): m/z 428.4 [M + H]<sup>+</sup> (calcd for C<sub>19</sub>H<sub>17</sub>N<sub>5</sub>O<sub>5</sub>S: 427.1).

2-(*o*-Bromobenzyl)-4-benzyl-7-methyl-1,1,3-trioxo-2H,4H-pyrazolo[4,5-*e*][1,2,4]thiadiazine (3l). Compound (1) and bromobenzyl bromide were allowed to react at 30-40°C for 12 h. After the addition of 2-bromobenzyl bromide, the mixture was allowed to react at 40-50°C for 12 h. Purification by recrystallization from ethanol yielded compound **3l** as a white solid (74%), mp: 182-184°C. IR (KBr,

cm<sup>-1</sup>): 1,701 (C=O), 1,337, 1,192 (SO<sub>2</sub>). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ: 7.88 (s, 1H, PyH), 7.66 (dd, 1H, *J* = 7.93 Hz, *J* = 1.08 Hz, PhH), 7.19 (dd, 1H, *J* = 7.72 Hz, *J* = 1.10 Hz, PhH), 7.25-7.37 (m, 7H, PhH), 5.12 (s, 2H, NCH<sub>2</sub>), 5.08 (s, 2H, NCH<sub>2</sub>), 4.06 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 150 MHz) δ: 148.9 (C=O), 134.7, 135.9, 132.7, 129.6, 128.9, 128.2, 127.9, 127.6, 127.2, 126.4, 125.7 (C-5), 122.0 (C-4a), 121.7 (C-7a), 48.5 (N<sub>2</sub>-CH<sub>2</sub>), 44.4 (N<sub>4</sub>-CH<sub>2</sub>), 38.9 (CH<sub>3</sub>). MS (EI): m/z 461.4 [M + H]<sup>+</sup>, 463.3 [M + 3]<sup>+</sup> (calcd for C<sub>19</sub>H<sub>17</sub>BrN<sub>4</sub>O<sub>3</sub>S: 460.02).

2-(*p*-Bromobenzyl)-4-(*o*-bromobenzyl)-7-methyl-1,1,3-trioxo-2H,4H-pyrazolo[4,5-*e*][1,2,4]thiadiazine (3m). Compound (1) and 2-bromobenzyl bromide were allowed to react at 30-40°C for 12 h. After the addition of 4-bromobenzyl bromide, the mixture was allowed to react at 40-50°C for 12 h. Purification by recrystallization from ethanol yielded compound **3m** as a white solid (76%), mp: 146-148°C. IR (KBr, cm<sup>-1</sup>): 1,696 (C=O), 1,330, 1,192 (SO<sub>2</sub>). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ: 7.74 (s, 1H, PyH), 7.67 (d, 1H, *J* = 7.81 Hz, PhH), 7.53 (d, 2H, *J* = 8.42 Hz, PhH), 6.93 (d, 1H, *J* = 7.58 Hz, PhH), 7.24-7.32 (m, 4H, PhH), 5.10 (s, 2H, NCH<sub>2</sub>), 5.01 (s, 2H, NCH<sub>2</sub>), 4.08 (s, 3H, CH<sub>3</sub>). MS (EI): m/z 541.2 [M + H]<sup>+</sup> (calcd for C<sub>19</sub>H<sub>16</sub>Br<sub>2</sub>N<sub>4</sub>O<sub>3</sub>S: 537.93).

2-(*p*-Chlorobenzyl)-4-(*o*-bromobenzyl)-7-methyl-1,1,3-trioxo-2H,4H-pyrazolo[4,5-*e*][1,2,4]thiadiazine (3n). Compound (1) and 2-bromobenzyl bromide were allowed to react at 30-40°C for 12 h. After the addition of 4-chlorobenzyl chloride, the mixture was allowed to react at 50-60°C for 20 h. Purification by recrystallization from ethanol yielded compound **3n** as a white solid (70%), mp: 128-130°C. IR (KBr, cm<sup>-1</sup>): 1,695 (C=O), 1,331, 1,192 (SO<sub>2</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 600 MHz) δ: 7.12 (s, 1H, PyH), 7.60 (dd, 1H, *J* = 7.83 Hz, *J* = 1.15 Hz, PhH), 6.88-7.47 (m, 7H, PhH), 5.15 (s, 2H, NCH<sub>2</sub>), 5.08 (s, 2H, NCH<sub>2</sub>), 4.14 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 150 MHz) δ: 149.2 (C=O), 134.1 (C-1'), 137.3 (C-1''), 132.5, 131.6, 129.6, 129.5, 129.0, 128.4, 128.1, 127.2, 126.9, 126.7, 125.2 (C-4a), 125.0 (C-5), 122.6 (C-7a), 46.6 (N<sub>4</sub>-CH<sub>2</sub>), 43.7 (N<sub>2</sub>-CH<sub>2</sub>), 38.9 (CH<sub>3</sub>). MS (EI): m/z 495.2 [M + H]<sup>+</sup>, 497.2 [M + 3]<sup>+</sup> (calcd for C<sub>19</sub>H<sub>16</sub>BrClN<sub>4</sub>O<sub>3</sub>S: 493.98).

2-(*o*-Bromobenzyl)-4-(*m*-chlorobenzyl)-7-methyl-1,1,3-trioxo-2H,4H-pyrazolo[4,5-*e*][1,2,4]thiadiazine (3o). Compound (1) and 3-chlorobenzyl chloride were allowed to react at 40-50°C for 20 h. After the addition of 2-Br benzyl bromide, the mixture was allowed to react at 40-50°C for 12 h. Purification by recrystallization from ethanol yielded compound **3o** as a white solid (70%), mp: 108-110°C. IR (KBr, cm<sup>-1</sup>): 1,680 (C=O), 1,327, 1,192 (SO<sub>2</sub>). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ: 7.91 (s, 1H, PyH), 7.66 (dd, 1H, *J* = 7.40

Hz,  $J = 0.92$  Hz, PhH), 7.20 (d, 1H,  $J = 7.70$  Hz, PhH), 7.23-7.38 (m, 6H, PhH), 5.16 (s, 2H, NCH<sub>2</sub>), 5.07 (s, 2H, NCH<sub>2</sub>), 4.07 (s, 3H, CH<sub>3</sub>). MS (EI):  $m/z$  495.2 [M + H]<sup>+</sup>, 497.2 [M + 3]<sup>+</sup> (calcd for C<sub>19</sub>H<sub>16</sub>BrClN<sub>4</sub>O<sub>3</sub>S: 493.98).

2-(*p*-Chlorobenzyl)-4-(2,4-dichlorobenzyl)-7-methyl-1,1,3-trioxo-2H,4H-pyrazolo[4,5-*e*][1,2,4]thiadiazine (**3p**). Compound (**1**) and 2,4-dichlorobenzyl chloride were allowed to react at 40-50°C for 20 h. After the addition of 4-chlorobenzyl chloride, the mixture was allowed to react at 50-60°C for 20 h. Purification by

recrystallization from ethanol yielded compound **3p** as a white solid (67%), mp: 150-152°C. IR (KBr, cm<sup>-1</sup>): 1,694 (C=O), 1,333, 1,192 (SO<sub>2</sub>). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz)  $\delta$ : 7.78 (s, 1H, PyH), 7.68 (d, 1H,  $J = 2.15$  Hz, PhH), 7.05 (d, 1H,  $J = 8.39$  Hz, PhH), 7.36-7.41 (m, 5H, PhH), 5.12 (s, 2H, NCH<sub>2</sub>), 5.01 (s, 2H, NCH<sub>2</sub>), 4.08 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 150 MHz)  $\delta$ : 148.8 (C=O), 133.2, 135.2, 133.1, 132.4, 132.3, 129.8, 129.4, 129.2, 129.0, 128.9, 128.5, 127.8, 126.3 (C-5), 125.7 (C-4a), 122.2 (C-7a), 47.0 (N<sub>2</sub>-CH<sub>2</sub>), 43.5 (N<sub>4</sub>-CH<sub>2</sub>), 39.1 (CH<sub>3</sub>). MS (EI):  $m/z$  485.3 [M + H]<sup>+</sup> (calcd for C<sub>19</sub>H<sub>15</sub>Cl<sub>3</sub>N<sub>4</sub>O<sub>3</sub>S: 483.99).