

1-1-2007

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SANCAK, KEMAL; ÜNVER, YASEMİN; and ER, MUSTAFA (2007) "Synthesis of 2-Acylamino, 2-Aroylamino and Ethoxycarbonyl Imino-1,3,4-thiadiazoles as Antitumor Agents," *Turkish Journal of Chemistry*. Vol. 31: No. 2, Article 2. Available at: <https://journals.tubitak.gov.tr/chem/vol31/iss2/2>

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# Synthesis of 2-Acylamino, 2-Aroylamino and Ethoxycarbonyl Imino-1,3,4-thiadiazoles as Antitumor Agents

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Received 24.08.2006

2-Amino-1,3,4-thiadiazoles compounds (2a-e) were obtained from the reaction of thiosemi carbazide with 2-cyanomethoxyphenoxyacetonitriles (1). Mono acylamino, mono aroylamino and diethoxycarbonyl imino-1,3,4-thiadiazole compounds (3a-e, 4a-e, 5a-c) were synthesized via the reaction of 2-amino-1,3,4-thiadiazole compounds (2a-e) with acetyl chloride or acetic anhydride, benzoyl chloride or benzoic anhydride and ethyl chloroformate, respectively.

The in vitro antitumor activities of some selected compounds were screened and compounds 2a, 2b, 2c, 3a, 3b, 3c, 5a, 5b and 5c were found to be active.

**Key Words:** 1,3,4-Thiadiazole, acetyl chloride, benzoyl chloride, ethyl chloro formate, antitumor activity.

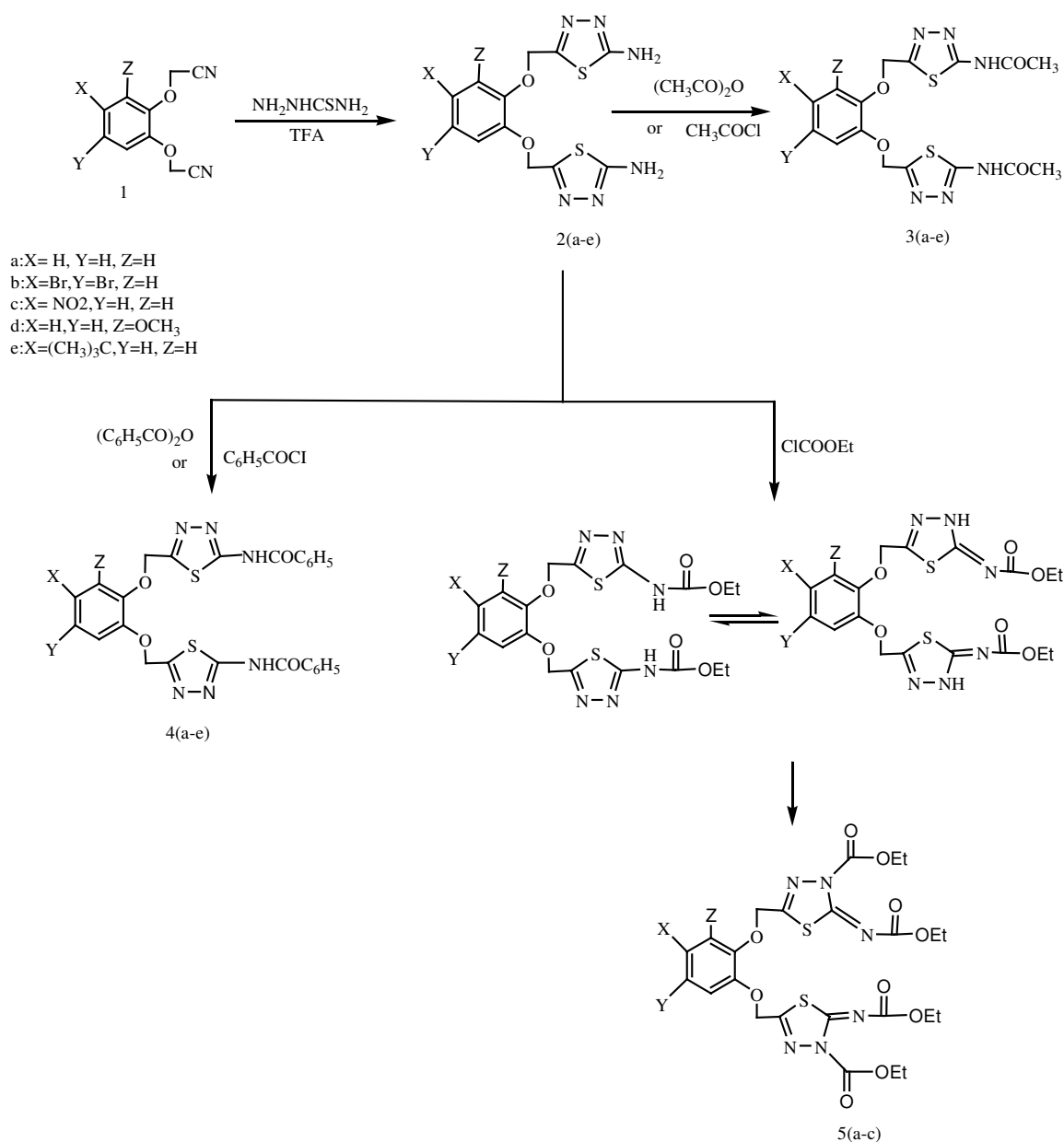
## Introduction

Derivatives of 1,3,4-thiadiazoles and 1,2,4-triazole are known to exhibit anti-inflammatory,<sup>1-3</sup> antiviral,<sup>4,5</sup> analgesic,<sup>6,7</sup> antimicrobial,<sup>8-12</sup> anticonvulsant<sup>13-16</sup> and antidepressant activity,<sup>17</sup> the last being usually explored by the forced swim test.<sup>18,19</sup> 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 of the antibacterial<sup>20-22</sup> and antidepressant activity of indolic molecules.<sup>23</sup> 1,3,4-Thiadiazole and related compounds are of great interest in chemistry owing to their bioactivity of certain plant growth regulating effect as well as antimicrobial activity.<sup>24-25</sup> Antitubercular activities of thiadiazoles linked with aromatic cycles through the methyleneoxy group have also been reported and compounds of this type have shown inhibition on both cyclooxygenase and 5-lipoxygenase activities.<sup>2,26</sup> Lee and coworkers have synthesized some thiadiazoles with antihelminthic activities.<sup>27</sup> More recently, sulfonamide derivatives of 1,3,4-thiadiazoles have been reported to behave as a modulator of anticancer therapies in combination with some cytotoxic compounds.<sup>28-31</sup>

The antitumor activities of 2-amino-1,3,4-thiadiazole (ATDA, NSC-4728) and the related compounds 2-ethylamino-1,3,4-thiadiazole (EATDA) and 2,2'-(methylene-diamino) bis-1,3,4-thiadiazole (NSC-143019)

were found in several experimental tumor systems about 50 years ago.<sup>32,33</sup> 2-Amino-1,3,4-thiadiazole (ATDA), as the most promising compound, was used in phase II clinical trials in patients with different tumors: renal,<sup>34</sup> colon,<sup>35</sup> ovarian,<sup>36</sup> and others.<sup>37</sup> However, due to marked hyperuricemia as well as painful stomatitis, its clinical applicability was limited.<sup>38-41</sup> Nicotinamide was found both to prevent these clinically limiting toxic effects and to suppress antitumor properties.<sup>42</sup> New derivatives with 1,3,4-thiadiazole nucleus as well as Fe(II) / Fe(III) complexes of 2-amino-1,3,4-thiadiazoles have been synthesized and evaluated for their antiproliferative activity against the panel human cancer cell lines.<sup>43</sup>

In view of these facts, the aim of the present study was to obtain 1,3,4-thiadiazoles and 1,3,4-thiadiazole derivatives (Scheme) as antitumor agents.



**Scheme.** Synthetic pathway for the preparation of compounds 2-5.

## Results and Discussion

In the present study, substituted 2-amino-1,3,4-thiadiazoles compounds **2a-e** were obtained from the reaction of thiosemicarbazide with 2-cyanomethoxy phenoxy acetonitriles (**1**) (Scheme). In the IR spectra of compounds **2a-e**, -NH<sub>2</sub> was observed at 3311-3115 cm<sup>-1</sup>. C-O-C stretching frequency was observed at 1124-1140 cm<sup>-1</sup>. In the <sup>1</sup>H-NMR spectra of compounds **2a-e**, the proton signal due to the methylene group (-CH<sub>2</sub>) was recorded at 5.18-5.40 ppm integrating for 2 protons. -NH<sub>2</sub> was observed at 7.30-7.34 ppm integrating for 2 protons (exchangeable with D<sub>2</sub>O). In the <sup>13</sup>C-NMR spectra of compounds **2a-e**, the signals belonging to the thiadiazole ring were observed in the aromatic region while the signal belonging to -CN disappeared. The <sup>13</sup>C signal of the methylene group was observed at 64.41-65.20 ppm in the <sup>13</sup>C-NMR spectra.

The acetylated derivatives (**3a-e**) were afforded when compounds **2a-e** were treated with acetyl chloride or acetic anhydride. In the IR and <sup>1</sup>H-NMR spectra of compounds **3a-e**, no signal derived from amino function was observed. In the IR spectra, -C=O belonging to the acetyl group was observed at 1692-1728 cm<sup>-1</sup>. In the <sup>1</sup>H-NMR spectra of compounds **3a-e**, NH was observed at 12.55-12.58 ppm integrating for one proton (exchangeable with D<sub>2</sub>O). In these compounds, the proton signal of -CH<sub>3</sub> was recorded between 2.02 and 2.49 ppm. The peak belonging to the same group was observed at 21.81-22.08 ppm in the <sup>13</sup>C-NMR spectra of compounds **3a-e**. The peak belonging to the carbonyl function of the acetyl group was observed at 158.58-159.81 ppm in the <sup>13</sup>C-NMR spectra.

Compounds **4a-e** were obtained from the reaction compounds **2a-e** with benzoic anhydride or benzoyl chloride. In the IR and <sup>1</sup>H-NMR spectra of compounds **4a-e**, no signal derived from amino function was observed. In the IR spectra of these compounds, C=O belonging to the benzoyl group was observed at 1670-1676 cm<sup>-1</sup>. In the <sup>1</sup>H-NMR spectra of compounds **4a-e**, NH was observed at 13.07-13.21 ppm integrating for one proton (exchangeable with D<sub>2</sub>O). In these compounds, the peak belonging to the carbonyl function of the benzoyl group was observed at 159.83-160.68 ppm in the <sup>13</sup>C-NMR spectra.

The synthesis of compounds **5a-c** was carried out by the reaction of compounds **2a-e** with ethylchloroformate. Surprisingly, ethoxycarbonylation of compounds **2** resulted in diethoxycarbonylation and gave compounds **5**. It is clearly seen that the basicity of the exocyclic amino nitrogen dramatically decreases after the formation of monoethoxycarbonyl derivatives by the nucleophilic attack of endocyclic nitrogen on the carbonyl carbon of the strong electron-withdrawing ethoxycarbonyl group.<sup>44</sup> In the IR and <sup>1</sup>H-NMR spectra of compounds **5a-c**, no signal derived from -NH was observed. In the <sup>1</sup>H-NMR spectra of these compounds, the proton signals due to ester groups were recorded between 1.30 and 1.32 ppm (-N<sub>endo</sub>COOCH<sub>2</sub>CH<sub>3</sub>) and 1.51-1.56 ppm (-N<sub>exo</sub>COOCH<sub>2</sub>CH<sub>3</sub>) integrating for 3 protons and 4.31-4.36 ppm (-N<sub>endo</sub>COOCH<sub>2</sub>CH<sub>3</sub>) and 4.56-4.58 ppm (-N<sub>exo</sub>COOCH<sub>2</sub>CH<sub>3</sub>) integrating for 2 protons. In the <sup>13</sup>C-NMR spectra of compounds **5a-c**, the signals belonging to the same groups were recorded at 14.2, 14.3, 65.7 and 66.2-66.8 ppm. Although the -C=O function of N<sub>endo</sub>-C=O was observed at 148.1-150.8 ppm, -C=O of N<sub>exo</sub>-C=O was reported at 154.5-155.9 ppm in the <sup>13</sup>C-NMR spectra.

The growth inhibition properties of the selected compounds by the National Cancer Institute, USA, were screened on 3 human tumor cell lines, breast cancer (MCF7), non-small cell lung cancer (NCI-H460) and CNS (8SF-268) as listed in the Table.

According to the results obtained, thiadiazoles and thiadiazole compounds containing acetyl and ethoxy carbonyl groups can be described as a new class of antitumor agents.

**Table.** Antitumor activity data for the selected compounds.

Compound No.	Protocol No.	Growth Percentage of Tumor Cells			Activity
		MCF7	NCI-H460	SF-268	
2a	S723351	0	0	0	Active
2b	S723352	0	0	0	Active
2c	S723353	0	0	0	Active
3a	S723354	0	0	0	Active
3b	S723355	0	0	87	Active
3c	S723356	0	0	0	Active
5a	S723357	0	0	0	Active
5b	S723358	0	0	0	Active
5c	S723359	0	0	0	Active

## Experimental

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected.  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra were recorded on a Varian-Mercury 200 MHz spectrometer. The IR spectra were measured as potassium bromide pellets using a Perkin-Elmer 1600 series FTIR spectrometer. All the chemicals were obtained from Fluka Chemie AG Buchs (Switzerland). Compound 1 was synthesized using the published method.<sup>45</sup>

**General method for synthesis of 5-({2-[(2-amino-1,3,4-thiadiazole-5-yl) methoxy] phenoxy}methyl) 2-amino-1,3,4- thiadiazoles (2)**

A mixture of corresponding compound **1** (0.01 mol) and thiosemicarbazide (0.03 mol) in threefluoroacetic acid (5 mL) at 60-70 °C was stirred for 4 h. The reaction mixture was poured into 200 mL of ice-cold water and neutralized with ammonia. The solid obtained was washed with  $\text{H}_2\text{O}$  and crystallized from appropriate solvent to afford the desired compound.

**5-({2-[(2-amino-1,3,4-thiadiazole-5-yl)methoxy]phenoxy}methyl)2-amino-1,3,4-thiadiazole (2a):** Recrystallized from DMSO-water (1:4) (yield: 89%). mp 183-184 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 3301-3115 ( $\nu_{\text{NH}_2}$ ), 1124 ( $\nu_{\text{C-O-C}}$ );  $^1\text{H-NMR}$  (DMSO- $d_6$ ) $\delta$ : 5.25, (s, 2  $\text{OCH}_2$ ), 6.94-7.16 (m, 4H), 7.30 (s, 2  $\text{NH}_2$ );  $^{13}\text{C-NMR}$  (DMSO- $d_6$ ) $\delta$ : 65.17 ( $\text{OCH}_2$ ), ar C:[147.33 (C), 122.08 (CH), 115.27 (CH)], 169.81, 154.13 (thiadiazole C).

**5-({2-[(2-amino-1,3,4-thiadiazole-5-yl)methoxy]4,5-dibromophenoxy}methyl)2-amino-1,3,4-thiadiazole (2b):** Recrystallized from DMSO-water (1:4) (yield: 83%). mp 231-232 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 3311-3136 ( $\nu_{\text{NH}_2}$ ), 1127 ( $\nu_{\text{C-O-C}}$ );  $^1\text{H-NMR}$  (DMSO- $d_6$ ) $\delta$ : 5.33, (s, 2  $\text{OCH}_2$ ), 7.52-7.68 (m, 2H), 7.34 (s, 2  $\text{NH}_2$ );  $^{13}\text{C-NMR}$  (DMSO- $d_6$ ) $\delta$ : 65.11 ( $\text{OCH}_2$ ), ar C: [147.52 (C), 119.40 (C), 115.48 (CH)], 170.30, 153.47 (thiadiazole C).

**5-({2-[(2-amino-1,3,4-thiadiazole-5-yl)methoxy]4-nitrophenoxy}methyl)2-amino-1,3,4-thiadiazole (2c):** Recrystallized from DMSO-water (1:2) (yield: 75%). mp 244-245 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 3288-3180 ( $\nu_{\text{NH}_2}$ ), 1140 ( $\nu_{\text{C-O-C}}$ );  $^1\text{H-NMR}$  (DMSO- $d_6$ ) $\delta$ : 5.42 (s, 2  $\text{OCH}_2$ ), 7.37-7.48 (m, 2H), 7.96-8.05 (m, 1H), 7.34 (s, 2  $\text{NH}_2$ );  $^{13}\text{C-NMR}$  (DMSO- $d_6$ ) $\delta$ : 64.41 ( $\text{OCH}_2$ ), ar C: [150.07 (C), 146.08 (C), 142.03(C), 117.54 (CH), 112.43 (CH), 108.46 (CH)], 169.81, 152.49 (thiadiazole C).

**5-({2-[(2-amino-1,3,4-thiadiazole-5-yl)methoxy]3-methoxyphenoxy}methyl)2-amino-1,3,**

**4-thiadiazole (2d):** Recrystallized from DMF-water (1:2) (yield: 80%). mp 236-237 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 3274-3126 ( $\nu_{\text{NH}_2}$ ), 1104 ( $\nu_{\text{C-O-C}}$ );  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 5.04 (s,  $\text{OCH}_2$ ), 5.30 (s,  $\text{OCH}_2$ ), 6.71-7.08 (m, 3H), 7.32 (s,  $\text{NH}_2$ ), 7.27 (s,  $\text{NH}_2$ );  $^{13}\text{C-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 55.76 ( $\text{OCH}_3$ ), 65.07 ( $\text{OCH}_2$ ), 68.45 ( $\text{OCH}_2$ ), ar C: [153.97 (C), 153.14 (C), 150.99 (C), 135.90 (CH), 124.22 (CH), 107.09 (CH)], 169.99, 154.46 (thiadiazole C).

**5-({2-[(2-amino-1,3,4-thiadiazole-5-yl)methoxy]4-tertbutylphenoxy}methyl)2-amino-1,3,4-thiadiazole (2e):** Recrystallized from DMF-water (1:2) (yield: 80%). mp 246-247 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 3325-3126 ( $\nu_{\text{NH}_2}$ ), 1019 ( $\nu_{\text{C-O-C}}$ );  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 5.24 (s,  $\text{OCH}_2$ ), 5.29 (s,  $\text{OCH}_2$ ), 6.93-7.14 (m, 3H), 7.30 (s,  $2\text{NH}_2$ );  $^{13}\text{C-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 34.04 (tert butyl C), 31.07 (tert butyl  $\text{CH}_3$ ), 65.20 ( $\text{OCH}_2$ ), ar C:[146.72 (C), 145.04 (C), 144.72 (C), 118.23 (CH), 114.79 (CH), 113.14 (CH)], 169.70, 154.35 (thiadiazole C).

**General method for synthesis of N-[5-({2-[(5-{acetylamino}-1,3,4-thiadiazole-2-yl)methoxy]phenoxy}methyl)-1,3,4-thiadiazole-2-yl] acetamides (3)**

A solution of corresponding compound **2** (0.01 mol) in 50 mL of anhydrous pyridine was treated with a solution of acetyl chloride or acetic anhydride (0.02 mol) in 30 mL of anhydrous pyridine. After the mixture was refluxed for 4 h, it was cooled to room temperature. The solid obtained was recrystallized from appropriate solvent to afford the desired compound.

**N-[5-({2-[(5-{acetylamino}-1,3,4-thiadiazole-2-yl)methoxy]phenoxy}methyl)-1,3,4-thiadiazole-2-yl]acetamide (3a):** Recrystallized from DMSO-water (1:4) (yield: 80%). mp 326-327 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 3185 ( $\nu_{\text{NH}}$ ), 1720 ( $\nu_{\text{C=O}}$ ), 1137 ( $\nu_{\text{C-O-C}}$ );  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 2.20 (s,  $2\text{CH}_3$ ) 5.48 (s,  $2\text{OCH}_2$ ), 6.85-7.21 (m, 4H), 12.55 (s,  $2\text{NH}$ );  $^{13}\text{C-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$  22.03 ( $\text{CH}_3$ ), 64.46 ( $\text{OCH}_2$ ), 158.71 ( $\text{C=O}$ ), ar C:[147.30 (C), 121.93 (CH), 115.43 (CH)], 168.16, 159.94 (thiadiazole C).

**N-[5-({2-[(5-{acetylamino}-1,3,4-thiadiazole-2-yl)methoxy]4,5-dibromophenoxy}methyl)-1,3,4-thiadiazole-2-yl] acetamide (3b):** Recrystallized from DMSO-water (1:2) (yield: 93%). mp 306-307 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 3182 ( $\nu_{\text{NH}}$ ), 1728 ( $\nu_{\text{C=O}}$ ), 1145 ( $\nu_{\text{C-O-C}}$ );  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 2.02 (s,  $2\text{CH}_3$ ), 5.58 (s,  $2\text{OCH}_2$ ), 7.61-7.87 (m, 2H), 12.58 (s,  $2\text{NH}$ );  $^{13}\text{C-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 22.08 ( $\text{CH}_3$ ), 64.46 ( $\text{OCH}_2$ ), 158.58 ( $\text{C=O}$ ), ar C:[146.92 (C), 118.90 (C), 114.95 (CH)], 168.21, 159.33 (thiadiazole C).

**N-[5-({2-[(5-{acetylamino}-1,3,4-thiadiazole-2-yl)methoxy]4-nitro phenoxy}methyl)-1,3,4-thiadiazole-2-yl] acetamide (3c):** Recrystallized from DMSO-water (1:2) (yield: 79%). mp 311-312 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 3168 ( $\nu_{\text{NH}}$ ), 1712 ( $\nu_{\text{C=O}}$ ), 1143 ( $\nu_{\text{C-O-C}}$ );  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 2.18 (s,  $2\text{CH}_3$ ), 5.64 (s,  $2\text{OCH}_2$ ), 7.21-7.38 (m, 2H), 7.98-8.11 (m, 1H), 12.56 (s,  $2\text{NH}$ );  $^{13}\text{C-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 21.81 ( $\text{CH}_3$ ), 64.70 ( $\text{OCH}_2$ ), 158.74 ( $\text{C=O}$ ), ar C:[152.43 (C), 146.41 (C), 140.80 (C), 118.25 (CH), 113.18 (CH), 109.83 (CH)], 168.34, 159.59 (thiadiazole C).

**N-[5-({2-[(5-{acetylamino}-1,3,4-thiadiazole-2-yl)methoxy]3-methoxyphenoxy}methyl)-1,3,4-thiadiazole-2-yl]acetamide (3d):** Recrystallized from DMF-water (1:2) (yield: 68%). mp 295-296 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 3161 ( $\nu_{\text{NH}}$ ), 1692 ( $\nu_{\text{C=O}}$ ), 1101 ( $\nu_{\text{C-O-C}}$ );  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 2.49 (s,  $2\text{CH}_3$ ), 5.45 (s,  $\text{OCH}_2$ ), 5.22 (s,  $\text{OCH}_2$ ), 6.74-7.07 (m, 3H), 12.53 (s,  $2\text{NH}$ );  $^{13}\text{C-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 22.35 ( $\text{CH}_3$ ), 55.79 ( $\text{OCH}_3$ ), 68.24 ( $\text{OCH}_2$ ), 64.76 ( $\text{OCH}_2$ ), 159.81 ( $\text{C=O}$ ), ar C:[153.13 (C), 151.02 (C), 135.87 (C), 124.49 (CH), 106.92 (CH), 106.33 (CH)], 168.54, 160.33 (thiadiazole C).

**N-[5-({2-[(5-{acetylamino}-1,3,4-thiadiazole-2-yl)methoxy]4-tertbutylphenoxy} methyl)-1,3,4-thiadiazole-2-yl]acetamide (3e):** Recrystallized from DMF-water (1:2) (yield: 65%). mp 271-272 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 3168 ( $\nu_{NH}$ ), 1705 ( $\nu_{C=O}$ ), 1145 ( $\nu_{C-O-C}$ );  $^1\text{H-NMR}$  (DMSO- $d_6$ ) $\delta$ : 1.24 (tertbutyl  $\text{CH}_3$ ), 2.18 (s,  $\text{CH}_3$ ), 5.51 (s,  $\text{OCH}_2$ ), 5.45 (s,  $\text{OCH}_2$ ), 6.96-7.18 (m, 3H), 12.56 (s, 2 NH);  $^{13}\text{C-NMR}$  (DMSO- $d_6$ ) $\delta$ : 22.27 ( $\text{CH}_3$ ), 31.02 (tertbutyl  $\text{CH}_3$ ), 34.05 (tertbutyl C), 65.06 ( $\text{OCH}_2$ ), 159.81 ( $\text{C=O}$ ), ar C: [159.51 (C), 146.69 (C), 144.92 (C), 118.44 (CH), 114.85 (CH), 113.26 (CH)], 168.52, 160.09 (thiadiazole C).

**General method for synthesis of N-[5-({2-[(5-{benzoylamino}-1,3,4-thiadiazole-2-yl)methoxy]phenoxy} methyl)-1,3,4-thiadiazole-2-yl]benzamides (4)**

A solution of corresponding compound **2** (0.01 mol) in 50 mL of anhydrous pyridine was treated with a solution of benzoyl chloride or benzoic anhydride (0.02 mol) in 30 mL of anhydrous pyridine. After the mixture was refluxed for 4 h, it was cooled to room temperature. The solid obtained was recrystallized from appropriate solvent to afford the desired compound.

**N-[5-({2-[(5-{benzoylamino}-1,3,4-thiadiazole-2-yl)methoxy]phenoxy} methyl)-1,3,4-thiadiazole-2-yl]benzamide (4a):** Recrystallized from DMSO-water (1:4) (yield: 85%). mp 302-303 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 3166 ( $\nu_{NH}$ ), 1670 ( $\nu_{C=O}$ ), 1140 ( $\nu_{C-O-C}$ );  $^1\text{H-NMR}$  (DMSO- $d_6$ ) $\delta$ : 5.45 (s, 2  $\text{OCH}_2$ ), 6.80-7.22 (m, 4H), 7.35-7.96 (m, 10H), 13.10 (s, 2 NH);  $^{13}\text{C-NMR}$  (DMSO- $d_6$ ) $\delta$ : 64.32 ( $\text{OCH}_2$ ), 159.83 ( $\text{C=O}$ ), ar C: [147.61 (C), 133.11 (C), 131.64 (CH), 128.79 (CH), 128.30 (CH), 122.56 (CH), 115.42 (CH)], 165.31, 160.92 (thiadiazole C).

**N-[5-({2-[(5-{benzoylamino}-1,3,4-thiadiazole-2-yl)methoxy]4,5-dibromophenoxy} methyl)-1,3,4-thiadiazole-2-yl]benzamide (4b):** Recrystallized from DMF-water (1:2) (yield: 86%). mp 306-307 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 3182 ( $\nu_{NH}$ ), 1676 ( $\nu_{C=O}$ ), 1145 ( $\nu_{C-O-C}$ );  $^1\text{H-NMR}$  (DMSO- $d_6$ ) $\delta$ : 5.48 (s, 2  $\text{OCH}_2$ ), 7.30-7.60 (m, 8H), 7.80-8.10 (m, 4H), 13.13 (s, 2 NH);  $^{13}\text{C-NMR}$  (DMSO- $d_6$ ) $\delta$ : 65.14 ( $\text{OCH}_2$ ), 159.55 ( $\text{C=O}$ ), ar C: [146.98 (C), 133.13 (C), 131.19 (CH), 128.78 (CH), 128.40 (CH), 119.14 (CH), 115.28 (CH)], 165.24, 161.21 (thiadiazole C).

**N-[5-({2-[(5-{benzoylamino}-1,3,4-thiadiazole-2-yl)methoxy]4-nitrophenoxy} methyl)-1,3,4-thiadiazole-2-yl] benzamide (4c):** Recrystallized from DMF-water (1:2) (yield: 69%). mp 288-289 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 3135 ( $\nu_{NH}$ ), 1662 ( $\nu_{C=O}$ ), 1144 ( $\nu_{C-O-C}$ ),  $^1\text{H-NMR}$  (DMSO- $d_6$ ) $\delta$  5.86 (s, 2  $\text{OCH}_2$ ), 7.48-7.87 (m, 7H), 8.13-8.31 (m, 6H), 13.21 (s, 2 NH);  $^{13}\text{C-NMR}$  (DMSO- $d_6$ ) $\delta$ : 55.83 ( $\text{OCH}_2$ ), 64.91 ( $\text{OCH}_2$ ), 68.34 ( $\text{OCH}_2$ ), ar C: [160.19 (C), 153.17 (C), 151.06 (C), 135.95 (C), 131.26 (CH), 128.43 (CH), 128.24 (CH), 124.55 (CH), 106.93 (CH), 106.36 (CH)], 165.00, 160.80 (thiadiazole C).

**N-[5-({2-[(5-{benzoylamino}-1,3,4-thiadiazole-2-yl)methoxy] 3-methoxy phenoxy} methyl)-1,3,4-thiadiazole-2-yl]benzamide (4d):** Recrystallized from DMF-water (1:2) (yield: 75%). mp 279- 280 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 3175 ( $\nu_{NH}$ ), 1672 ( $\nu_{C=O}$ ), 1107 ( $\nu_{C-O-C}$ ).  $^1\text{H-NMR}$  (DMSO- $d_6$ ) $\delta$ : 3.82 (s,  $\text{OCH}_3$ ), 5.32 (s,  $\text{OCH}_2$ ), 5.55 (s,  $\text{OCH}_2$ ), 6.92-8.06 (m, 13H), 13.21 (s, 2 NH);  $^{13}\text{C-NMR}$  (DMSO- $d_6$ ) $\delta$ : 55.83 ( $\text{OCH}_3$ ), 64.91 ( $\text{OCH}_2$ ), 68.34 ( $\text{OCH}_2$ ), 160.19 ( $\text{C=O}$ ), ar C: [153.09 (C), 150.08 (C), 142.10 (C), 132.83 (C), 131.26 (CH), 128.90 (CH), 127.50 (CH), 119.30 (CH), 107.60 (CH), 101.40 (CH)], 165.39, 161.67 (thiadiazole C).

**N-[5-({2-[(5-{benzoylamino}-1,3,4-thiadiazole-2-yl)methoxy] 4-tertbutylphenoxy} methyl)-1,3,4-thiadiazole-2-yl] benzamide (4e):** Recrystallized from DMF-water (1:3) (yield: 78%). mp

242-243 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 3174 ( $\nu_{\text{NH}}$ ), 1673 ( $\nu_{\text{C=O}}$  C=O), 1017 ( $\nu_{\text{C-O-C}}$ ).  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 1.26 (tertbutyl  $\text{CH}_3$ ), 5.58 (s,  $\text{OCH}_2$ ), 5.52 (s,  $\text{OCH}_2$ ), 6.99-8.08 (m, 13H), 13.07 (s, 2 NH);  $^{13}\text{C-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 31.05 (tertbutyl  $\text{CH}_3$ ), 34.09 (tertbutyl C), 65.19 ( $\text{OCH}_2$ ), 160.68 (C=O), ar C: [146.78 (C), 145.15 (C), 144.98 (C), 132.83 (C), 131.22 (CH), 128.45 (CH), 128.24 (CH), 118.48 (CH), 114.82 (CH), 113.24 (CH)], 164.90, 160.47 (thiadiazole C).

**General method for synthesis of Ethyl-5-({2-[(4-{ethoxycarbonyl-5-({ethoxy carbonyl} imino)-4,5-dihydro-1,3,4-thiadiazole-2-yl) methoxy] phenoxy} methyl)-2 [(ethoxy carbonyl) imino]-2,3-dihydro-1,3,4-thiadiazole-3-carboxylates (5)**

A solution of corresponding compound **3** (0.005 mol) in 50 mL of anhydrous benzene was treated with a solution of ethyl chloroformate (0.02 mol) in 30 mL of anhydrous benzene. The mixture was refluxed for 4 h. Then the mixture was evaporated and a solid obtained. This was recrystallized from appropriate solvent to afford the desired compound.

**Ethyl-5-({2-[(4-{ethoxycarbonyl-5-({ethoxycarbonyl} imino)-4,5-dihydro-1,3,4-thia diazole-2-yl) methoxy] phenoxy} methyl)-2-[(ethoxycarbonyl) imino]-2,3-dihydro-1,3,4-thiadiazole-3-carboxylate (5a)**: Recrystallized from ethylacetate-petroleum ether (1:4). (yield: 97%). mp 134-135 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 1781 ( $\nu_{\text{N-C=O}}$ ), 1762 ( $\nu_{\text{N-C=O}}$ ), 1196 ( $\nu_{\text{C-O-C}}$ );  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$  1.32 (6H, t,  $\text{N}_{\text{endo}}\text{-COOCH}_2\text{CH}_3$ ), 1.51 (6H, t,  $\text{N}_{\text{exo}}\text{-COOCH}_2\text{CH}_3$ ), 4.31 (4H, q,  $\text{N}_{\text{endo}}\text{-COOCH}_2\text{CH}_3$ ), 4.58 (4H, q,  $\text{N}_{\text{exo}}\text{-COOCH}_2\text{CH}_3$ ), 5.25 (s, 2  $\text{OCH}_2$ ), 7.20-7.35 (m, 4H),  $^{13}\text{C-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 13.08 ( $\text{N}_{\text{endo}}\text{-COOCH}_2\text{CH}_3$ ), 13.08 ( $\text{N}_{\text{exo}}\text{-COOCH}_2\text{CH}_3$ ), 66.7 ( $\text{N}_{\text{endo}}\text{-COOCH}_2\text{CH}_3$ ), 66.9 ( $\text{N}_{\text{exo}}\text{-COOCH}_2\text{CH}_3$ ), 63.12 ( $\text{OCH}_2$ ), 148.22 ( $\text{N}_{\text{endo}}\text{-C=O}$ ) 155.93 ( $\text{N}_{\text{exo}}\text{-C=O}$ ), ar C: [147.63 (C), 123.55 (CH), 115.95 (CH)], 167.98, 162.80 (thiadiazole C).

**Ethyl-5-({2-[(4-{ethoxycarbonyl-5-({ethoxycarbonyl} imino)-4,5-dihydro-1,3,4-thia diazole-2-yl) methoxy] 4,5-dibromophenoxy} methyl)-2-[(ethoxycarbonyl) imino]-2,3-dihydro-1,3,4-thiadiazole-3-carboxylate (5b)**: Recrystallized from ethylacetate-petroleum ether (1:2). (yield: 94%). mp 154-155 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 1780 ( $\nu_{\text{N-C=O}}$ ), 1767 ( $\nu_{\text{N-C=O}}$ ), 1198 ( $\nu_{\text{C-O-C}}$ );  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ )  $\delta$ : 1.32 (6H, t,  $\text{N}_{\text{endo}}\text{-COOCH}_2\text{CH}_3$ ), 1.50 (6H, t,  $\text{N}_{\text{exo}}\text{-COOCH}_2\text{CH}_3$ ), 4.32 (4H, q,  $\text{N}_{\text{endo}}\text{-COOCH}_2\text{CH}_3$ ), 4.58 (4H, q,  $\text{N}_{\text{exo}}\text{-COOCH}_2\text{CH}_3$ ), 5.23 (s, 2  $\text{OCH}_2$ ), 7.25-7.43 (m, 2H);  $^{13}\text{C-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 14.30 ( $\text{N}_{\text{endo}}\text{-COOCH}_2\text{CH}_3$ ), 14.30 ( $\text{N}_{\text{exo}}\text{-COOCH}_2\text{CH}_3$ ), 65.72 ( $\text{N}_{\text{endo}}\text{-COOCH}_2\text{CH}_3$ ), 66.85 ( $\text{N}_{\text{exo}}\text{-COOCH}_2\text{CH}_3$ ), 63.1 ( $\text{OCH}_2$ ), 148.12 ( $\text{N}_{\text{endo}}\text{-C=O}$ ), 154.55 ( $\text{N}_{\text{exo}}\text{-C=O}$ ), ar C: [147.25 (C), 120.47 (CH), 117.70 (CH)], 167.80, 162.82 (thiadiazole C).

**Ethyl-5-({2-[(4-{ethoxycarbonyl-5-({ethoxycarbonyl} imino)-4,5-dihydro-1,3,4-thia diazole-2-yl) methoxy 4-nitrophenoxy} methyl)-2-[(ethoxycarbonyl) imino]-2,3-dihydro-1,3,4-thiadiazole-3-carboxylate (5c)**: Recrystallized from ethylacetate-petroleum ether (1:3). (yield: 91%). mp 164 -165 °C. IR (KBr) ( $\nu$ ,  $\text{cm}^{-1}$ ): 1773 ( $\nu_{\text{N-C=O}}$ ), 1728 ( $\nu_{\text{N-C=O}}$ ), 1194 ( $\nu_{\text{C-O-C}}$ );  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 1.36 (6H, t,  $\text{N}_{\text{endo}}\text{-COOCH}_2\text{CH}_3$ ), 1.54 (6H, t,  $\text{N}_{\text{exo}}\text{-COOCH}_2\text{CH}_3$ ), 4.29 (4H, q,  $\text{N}_{\text{endo}}\text{-COOCH}_2\text{CH}_3$ ), 4.60 (4H, q,  $\text{N}_{\text{exo}}\text{-COOCH}_2\text{CH}_3$ ), 5.38 (s, 2 $\text{OCH}_2$ ), 7.18-7.88 (m, 3H);  $^{13}\text{C-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$ : 14.35 ( $\text{N}_{\text{exo}}\text{-COOCH}_2\text{CH}_3$ ), 14.35 ( $\text{N}_{\text{exo}}\text{-COOCH}_2\text{CH}_3$ ), 65.60 ( $\text{N}_{\text{endo}}\text{-COOCH}_2\text{CH}_3$ ), 66.25 ( $\text{N}_{\text{exo}}\text{-COOCH}_2\text{CH}_3$ ), 63.76 ( $\text{OCH}_2$ ), 150.88 ( $\text{N}_{\text{endo}}\text{-C=O}$ ), 155.20 ( $\text{N}_{\text{exo}}\text{-C=O}$ ), ar C: [153.12 (C), 147.65 (C), 143.12 (C), 120.15 (CH), 113.84 (CH), 118.88 (CH)], 167.90, 160.36 (thiadiazole C).



## Pharmacology

The screening experiments were performed by the Development Therapeutic Program of the National Cancer Institute (NCI), Bethesda MD, USA. Compounds **2a**, **2b**, **2c**, **3a**, **3b**, **3c**, **5a**, **5b** and **5c** were selected by the NCI for screening towards 3 human cell lines, breast cancer (MCF7), non-small cell lung cancer (NCI-H460) and CNS (SF-268). Each cell line was inoculated and preincubated on a microtiter plate. Test agents were then added at a single concentration and culture incubated for 48 h. End-point determinations were performed using alamar blue.<sup>46</sup>

## Acknowledgments

This work was supported by the Research Fund of Karadeniz Technical University. The authors thank the National Cancer Institute, USA, for the antitumor screening results.

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Synthesis of 2-Acylamino, 2-Aroylamino and Ethoxycarbonyl..., *K. SANCAK, et al.*,

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