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Design, synthesis, and biological evaluation of new series of 2-amido-1,3,4-thiadiazole derivatives as cytotoxic agents

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Abstract: A series of novel 1,3,4-thiadiazole derivatives bearing an amide moiety were designed, synthesized, and evaluated for their *in vitro* antitumor activities against HL-60, SKOV-3 and MOLT-4 human tumor cell lines by MTT assay. Ethyl 2-((5-(4-methoxybenzamido)-1,3,4-thiadiazol-2-yl)thio)acetate (**5f**) showed the best inhibitory effect against SKOV-3 cells, with an IC_{50} value of 19.5 μM. In addition, the acridine orange/ethidium bromide staining assay in SKOV-3 cells suggested that the cytotoxic activity of **5f** occurs *via* apoptosis.

Keywords: antitumor agent; cancer; cytotoxic activity; fluorescence; thiadiazole.

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1 Introduction

Cancer has been proposed as the most serious health problem all over the world and is the leading cause of death in humans [1]. During the last few decades, more than 70 % of all cancer deaths occurred in developing and underdeveloped countries [2]. Chemotherapy, one of the main treatment methods, is accompanied by complicated systemic toxicity, serious side effects, high mortality rate, high costs, and development of resistant strains [3]. Therefore, the development of novel chemical structures with high efficacy, low toxicity, a minimum of undesirable side effects, and acceptable resistance profiles is eagerly being pursued [4].

The 1,3,4-thiadiazole ring system is characterized as an important heterocyclic core playing a significant role in medicinal chemistry, especially in the field of antimicrobial agents and chemotherapy [5]. 1,3,4-Thiadiazole derivatives exhibit a broad spectrum of interesting pharmacological properties like antileishmanial, antioxidant, anti-*Helicobacter pylori*, fungicidal, antitubercular, anticancer, anti-inflammatory, antiviral, and anticonvulsant [6–23].

High-throughput screening at Bristol–Myers Squibb revealed (2-acetamido-thiazolyl) thioacetic ester **1** as an inhibitor of cyclin-dependent kinase 2 (CDK2) (Fig. 1) [24]. Moreover, 2-amino-1,3,4-thiadiazole derivatives were also introduced as potential anticancer agents; as shown in Fig. 1, compounds **2** and **3**, with this effective core structure, exhibited good inhibitory activities against Akt/protein kinase B (PKB) [25, 26].

Recently, Zhang et al. reported the antiproliferative properties of novel hybrid molecules containing 1,3,4-oxadiazole and 1,3,4-thiadiazole moieties exemplified by compound **4** in Fig. 1 [27]. According to the above-mentioned findings and in continuation of our interests in exploration of novel heterocyclic scaffolds with anticancer activities [28–31], herein we describe a new series of 1,3,4-thiadiazole derivatives **5a**–**n**, which were designed by replacing the thiazole with a thiadiazole ring and the

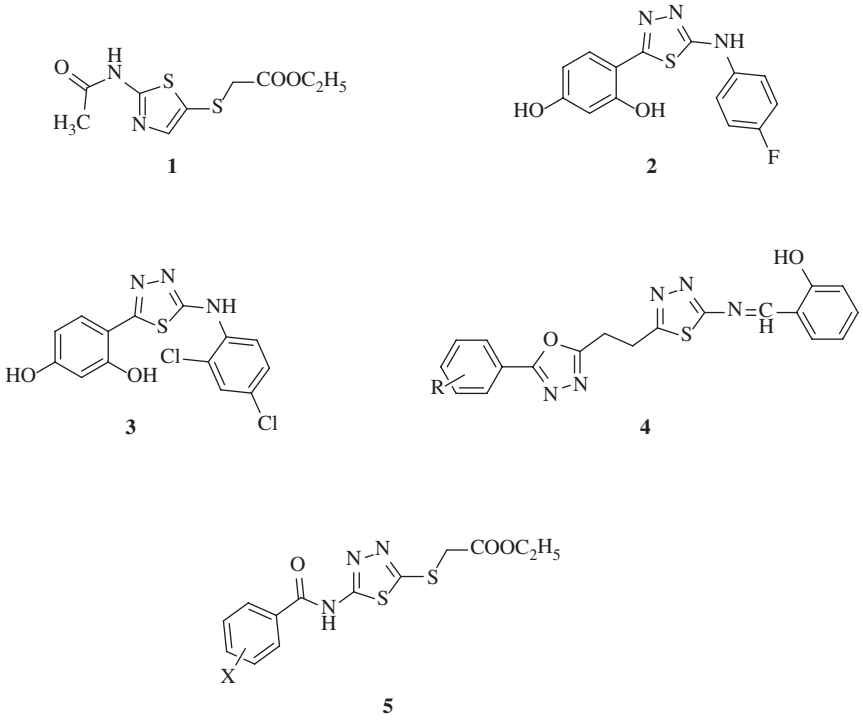


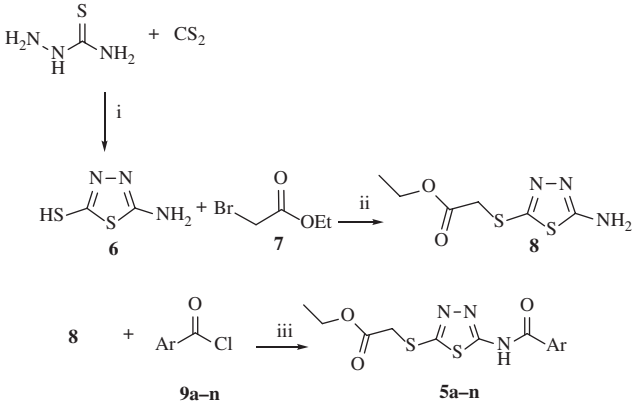
Fig. 1: Compounds **1–4** with 2,5-disubstituted 1,3,4-thiadiazole and thiazole structures were reported as cytotoxic agents. Compounds **5a–n** (see Scheme 1 and Table 1) were designed as new potential cytotoxic agents.

methyl group with substituted phenyl moieties in compound **1** to achieve better cytotoxic effects.

2 Results and discussion

2.1 Chemistry

The target compounds **5a–n** were prepared *via* the synthetic route illustrated in Scheme 1. First, 5-amino-1,3,4-thiadiazole-2-thiol (**6**) was prepared *via* the reaction of thiosemicarbazide and CS_2 in EtOH at reflux temperature [32]. Then, **6** was converted to ethyl 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)acetate (**8**) by the action of ethyl 2-bromoacetate in the presence of potassium hydroxide [33]. In the next step, **8** was reacted with the corresponding aroyl chlorides in the presence of Et_3N at ambient temperature to afford target compounds **5a–n**.



Scheme 1: Synthesis of compounds **5a–n** (for substituent Ar, see Table 1). Reagents and conditions: (i) Na_2CO_3, EtOH, reflux; (ii) KOH, EtOH, r.t.; (iii) Et_3N, THF, reflux. For the syntheses of **6** and **8**, see references [32] and [33], respectively.

2.2 Pharmacology

2.2.1 Cytotoxic assay

The *in vitro* effect of synthesized compounds **5a–n** on cancer cell viability was assessed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay against HL-60 (human promyelocytic leukemia), SKOV-3 (human ovarian carcinoma), and MOLT-4 (human acute lymphoblastic leukemia) cell lines [28].

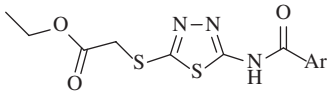
The results of cytotoxic data indicated that all synthesized (2-amido-thiadiazolyl)acetic ester derivatives **5a–n** possess lower cytotoxic potential than doxorubicin and cisplatin in all three cell lines. Moreover, these compounds

were inactive against the MOLT-4 cell line, which could be attributed to rapid hydrolysis of the compounds.

Compound **5f** was the most potent compound against SKOV-3 and HL-60 cell lines with IC_{50} values of 19.5 and 30.1 μM, respectively. In case of the SKOV-3 cell line, compounds **5c** and **5h** exhibited the highest growth inhibitory activities at concentrations of 26.3 and 22.2 μM, respectively. Compounds **5a**, **5c**, and **5h** showed inhibitory effects on viability of HL-60 cells at concentrations <35 μM. As can be seen in Table 1, **5n** showed no growth-inhibiting potential on cancer cell lines within the tested concentrations. According to the obtained data (Table 1), the following structure-activity relationship could be constructed:

1. The presence of an electron-withdrawing group diminishes the cytotoxic activity of the synthesized compounds.
2. Introduction of different substituents such as chlorine, methoxy, and methyl groups into the *ortho* position of the phenyl moiety decreases the cytotoxic potential of the thiadiazole derivatives.
3. Substitution of different groups such as chlorine, methoxy, and methyl at the *meta* and *para* positions of the phenyl moiety potentiates the anticancer activity of the studied compounds.
4. Replacement of the phenyl moiety with other five-membered heterocyclic rings, such as furan and thiophene, leads to a reduced cytotoxic activity.

Table 1: Cell growth inhibitory activity of synthetic compounds **5a**–**n** assessed by the MTT reduction assay.



Compound	Ar	IC_{50} (μM)[a]		
		HL-60 cells	SKOV-3 cells	MOLT-4 cells
5a	C_6H_5	32.4 ± 4.2	27.2 ± 3.0	>100
5b	2-MeC_6H_4	>100	59.8 ± 19.9	>100
5c	3-MeC_6H_4	30.8 ± 6.4	26.3 ± 5.0	>100
5d	4-MeC_6H_4	68.8 ± 14.9	45.4 ± 13.2	>100
5e	2-$OMeC_6H_4$	38.5 ± 4.7	42.3 ± 11.9	>100
5f	4-$OMeC_6H_4$	30.1 ± 2.7	19.5 ± 2.1	>100
5g	2-ClC_6H_4	>100	79.2 ± 11.8	>100
5h	2-ClC_6H_4	33.8 ± 4.4	22.2 ± 0.7	>100
5i	4-ClC_6H_4	88.6 ±10.8	45.0 ± 5.1	>100
5j	2,4-$Cl_2C_6H_4$	59.7 ± 3.3	37.0 ± 6.2	>100
5k	3-FC_6H_4	45.3 ± 9.2	34.5 ± 4.4	>100
5l	3-$NO_2C_6H_4$	>100	72.7 ± 10.3	>100
5m	2-Thienyl	>100	75.1 ± 6.3	>100
5n	2-Furyl	>100	>100	>100
Doxorubicin	–	0.013 ± 0.002	0.018 ± 0.016	0.047 ± 0.015
Cisplatin	–	2.1 ± 0.3	8.5 ± 4.8	3.1 ± 0.1

[a]Values represent mean ± SEM. When compounds were inactive, IC_{50} was reported as more than the maximum tested concentrations.

The acridine orange (AO)/ethidium bromide (EB) double staining technique was used to evaluate the occurrence of apoptosis in SKOV-3 cells, which had been treated with **5a**. AO, a nucleic acid fluorescent cationic dye, permeates all cells and makes the nuclei appear green. EB is only taken up by cells when cytoplasmic membrane integrity is lost, and then stains the nucleus red. Analysis of the AO/EB staining revealed that compound **5f** induced apoptosis in the SKOV-3 cell line. As shown in Fig. 2, the non-apoptotic control cells were stained green, and the late apoptotic cells incorporate EB, thus staining orange, and show condensed and fragmented nuclei.

3 Conclusion

A novel series of ethyl 2-((5-(benzamido)-1,3,4-thiadiazol-2-yl)thio)acetates **5a**–**n** were synthesized with an objective to study their antitumor activities. The designed compounds showed good antiproliferative activities against different cell lines. Some tested compounds including **5c**, **5f**, and **5h** exhibited cytotoxic activities against SKOV-3 and HL-60. Meanwhile, all compounds were inactive against the MOLT-4 cell line, probably due to the rapid hydrolysis of these compounds. The results of an AO/EB staining assay of compound **5f** suggested that the cytotoxic activity of this compound against SKOV-3 cell line occurs *via* apoptosis. These results are therefore conclusive in showing that different benzamide moieties attached to the 1,3,4-thiadiazole core make some of them lead molecules for further optimization in the development of a novel anticancer agent.

4 Experimental section

4.1 Procedure for the synthesis of compound 5a

To a solution of compound **8** [33] (1 mmol, 0.22 g) in anhydrous THF (10 mL) containing triethyl amine (1.5 mmol, 0.15 g), benzoyl chloride (1 mmol, 0.14 g) was added. The reaction mixture was refluxed and monitored by TLC. Upon completion, the reaction mixture was cooled, and the precipitated solid was filtered off and recrystallized from ethanol to give compound **5a**.

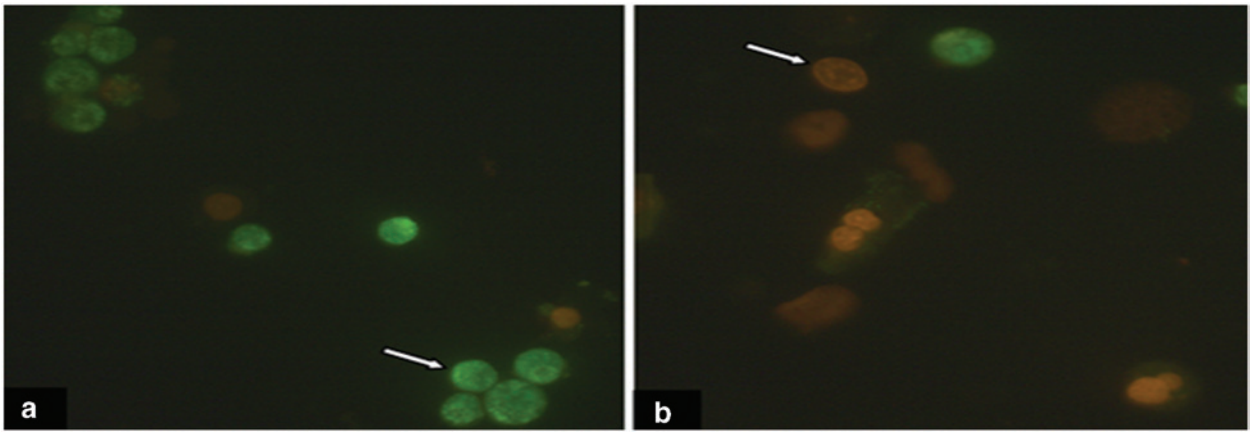


Fig. 2: AO/EB double staining of SKOV-3 cells with characteristic symptoms of apoptosis: (a) DMSO 2 % as control and (b) cells treated with IC_{50} concentration of compound **5f**, respectively, for 24 h. The images of cells were taken with a fluorescence microscope at 400×.

4.1.1 Ethyl 2-((5-benzamido-1,3,4-thiadiazol-2-yl)thio) acetate (5a)

Yield: 81 % (0.26 g). M.p. 174–176 °C. – IR (KBr): ν = 1726, 1659 (C=O) cm^{-1}. – ^1H NMR (400.0 MHz, $CDCl_3$, 25 °C, TMS): δ ppm = 1.26 (t, J = 7.2 Hz, 3H), 4.07 (s, 2H), 4.21 (q, J = 7.2 Hz, 2H), 7.54–7.60 (m, 2H), 7.65 (dt, J = 7.2, 1.2 Hz, 1H), 8.23–8.26 (m, 2H), 12.73 (s, NH). – MS: m/z = 323 $[M]^+$. – $C_{13}H_{13}N_3O_3S_2$: anal. calcd. C 48.28, H 4.05, N 12.99; found C 48.55, H 4.21, N 12.66.

4.1.2 Ethyl 2-((5-(2-methylbenzamido)-1,3,4-thiadiazol-2-yl)thio)acetate (5b)

Yield: 80 % (0.27 g). M.p. 150–152 °C. – IR (KBr): ν = 1727, 1655 (C=O) cm^{-1}. – ^1H NMR (400.0 MHz, $CDCl_3$, 25 °C, TMS): δ ppm = 1.31 (t, J = 7.2 Hz, 3H), 2.55 (s, 3H), 4.00 (s, 2H), 4.25 (q, J = 7.2 Hz, 2H), 7.31–7.38 (m, 2H), 7.47 (tt, J = 7.6, 1.2 H, 1H), 7.80 (dd, J = 7.7, 1.2 Hz, 1H), 12.48 (s, 1H). – ^{13}C NMR (100.0 MHz, $CDCl_3$, 25 °C, TMS): δ ppm = 14.1 (CH_3), 21.4 (SCH_2), 35.1 (CH_3), 62.1 (OCH_2), 126.1 (CH_2Ar), 128.5 (CH_2Ar), 131.6 (CH_2Ar), 131.7 (CH_2Ar), 132.0 (CHAr), 138.3 (CHAr), 158.2 (CAr), 160.9 (CHAr), 167.2 (C=O), 168.0 (C=O). – MS: m/z = 337 $[M]^+$. – $C_{14}H_{15}N_3O_3S_2$: anal. calcd. C 49.83, H 4.48, N 12.45; found C 49.56, H 4.37, N 12.74.

4.1.3 Ethyl 2-((5-(3-methylbenzamido)-1,3,4-thiadiazol-2-yl)thio)acetate (5c)

Yield: 76 % (0.25 g). M.p. 175–177 °C. – IR (KBr): ν = 1727, 1655 (C=O) cm^{-1}. – ^1H NMR (400.0 MHz, $CDCl_3$, 25 °C, TMS): δ ppm = 1.26 (t, J = 7.2 Hz, 3H), 2.46 (s, 3H), 4.02 (s, 2H), 4.20 (q, J = 7.2 Hz, 2H), 7.44–7.46 (m, 2H), 7.98 (brs, 1H), 8.20–8.60 (m, 1H), 12.68 (s, NH). – ^{13}C NMR (100.0 MHz, $CDCl_3$, 25 °C, TMS): δ ppm =14.1 (CH_3), 21.3 (SCH_2), 35.6 (CH_3), 62.1 (OCH_2), 125.7 (CHAr), 128.7 (CHAr), 129.2 (CH_2Ar), 130.8 (CHAr), 134.2 (CH_2Ar), 138.8 (CH_2Ar), 158.5 (CH_2Ar), 161.82 (CAr), 165.7 (C=O), 168.0 (C=O). – MS: m/z = 337 $[M]^+$. – $C_{14}H_{15}N_3O_3S_2$: anal. calcd. C 49.83, H 4.48, N 12.45; found C 49.57, H 4.17, N 12.72.

4.1.4 Ethyl 2-((5-(4-methylbenzamido)-1,3,4-thiadiazol-2-yl)thio)acetate (5d)

Yield: 72 % (0.24 g). M.p. 151–153 °C. – IR (KBr): ν = 1727, 1654 (C=O) cm^{-1}. – ^1H NMR (400.0 MHz, $CDCl_3$, 25 °C, TMS): δ ppm = 1.27 (t, J = 6.8 Hz, 3H), 2.49 (s, 3H), 4.09 (s, 2H), 4.22 (q, J = 6.8 Hz, 2H), 7.36 (d, J = 7.8 Hz, 2H), 8.12 (d, J = 7.8 Hz, 2H), 12.42 (s, NH). – ^{13}C NMR (100.0 MHz, $CDCl_3$): δ ppm = 14.1 (CH_3), 21.7 (SCH_2), 35.4 (CH_3), 62.1 (OCH_2), 128.1 (CHAr), 128.6 (CH_2Ar), 129.5 (CH_2Ar), 144.2 (CHAr), 158.4 (CAr), 161.6 (CAr), 165.8 (C=O), 168.0 (C=O). – MS: m/z = 337 $[M]^+$. – $C_{14}H_{15}N_3O_3S_2$: anal. calcd. C 49.83, H 4.48, N 12.45; found C 49.57, H 4.29, N 12.77.

4.1.5 Ethyl 2-((5-(2-methoxybenzamido)-1,3,4-thiadiazol-2-yl)thio)acetate (5e)

Yield: 84 % (0.29 g). M.p. 184–186 °C. – IR (KBr): ν = 1726, 1654 (C=O) cm^{-1}. – ^1H NMR (400.0 MHz, $CDCl_3$, 25 °C, TMS): δ ppm = 1.26 (t, J = 7.2 Hz, 3H), 4.03 (s, 3H), 4.06 (s, 2H), 4.20 (q, J = 7.2 Hz, 2H), 7.04 (d, J = 8.4 Hz, 1H), 7.11 (dt, J = 7.8, 1.2 Hz, 1H), 7.55 (dt, J = 7.8, 1.2 Hz, 1H), 8.20 (dd, J = 7.8, 1.6 Hz, 1H), 11.20 (s, NH). – MS: m/z = 353 $[M]^+$. – $C_{14}H_{15}N_3O_4S_2$: anal. calcd. C 47.58, H 4.28, N 11.89; found C 47.25, H 4.19, N 11.97.

4.1.6 Ethyl 2-((5-(4-methoxybenzamido)-1,3,4-thiadiazol-2-yl)thio)acetate (5f)

Yield: 68 % (0.24 g). M.p. 144–146 °C. – IR (KBr): ν = 1729, 1664 (C=O) cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ ppm = 1.25 (t, J = 6.8 Hz, 3H), 3.92 (s, 3H), 4.08 (s, 2H), 4.20 (q, J = 6.8 Hz, 2H), 7.04 (d, J = 8.2 Hz, 2H), 8.23 (d, J = 8.2 Hz, 2H), 12.61 (s, NH). – ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ ppm = 14.0 (CH_3), 33.5 (SCH_2), 55.5 (OCH_3), 62.1 (OCH_2), 114.1 (2CAr), 123.0 (CAr), 130.8 (2CAr), 158.3 ($COCH_3$), 162.0 (CAr), 163.8 (CAr), 164.8 (C=O), 168.0 (C=O). – MS: m/z = 353 [M]$^+$. – $C_{14}H_{15}N_3O_4S_2$: anal. calcd. C 47.58, H 4.28, N 11.89; found C 47.81, H 4.46, N 11.60.

4.1.7 Ethyl 2-((5-(2-chlorobenzamido)-1,3,4-thiadiazol-2-yl)thio)acetate (5g)

Yield: 81 % (0.29 g). M.p. 157–159 °C. – IR (KBr): ν = 1729, 1660 (C=O) cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ ppm 1.31 (t, J = 7.2 Hz, 3H), 3.94 (s, 2H), 4.24 (q, J = 7.2 Hz, 2H), 7.41–7.47 (m, 1H), 7.51–7.53 (m, 2H), 7.77 (d, J = 7.6 Hz, 1H), 12.95 (s, NH). – ^{13}C NMR (100.0 MHz, $CDCl_3$, 25 °C, TMS): δ 14.1 (CH_3), 35.1 (SCH_2), 62.2 (OCH_2), 127.1 (CH_2Ar), 130.4 (CH_2Ar), 130.6 (CH_2Ar), 132.2 (CHAr), 132.4 (CHAr), 132.5 (CH_2Ar), 158.6 (CAr), 160.5 (CAr), 164.7 (C=O), 167.8 (C=O). – MS: m/z = 357 [M]$^+$. – $C_{13}H_{12}ClN_3O_3S_2$: anal. calcd. C 43.63, H 3.38, N 11.74; found C 43.50, H 3.59, N 11.51.

4.1.8 Ethyl 2-((5-(3-chlorobenzamido)-1,3,4-thiadiazol-2-yl)thio)acetate (5h)

Yield: 73 % (0.26 g). M.p. 168–170 °C. – IR (KBr): ν = 1728, 1661 (C=O) cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ ppm = 1.26 (t, J = 7.2 Hz, 3H), 4.12 (s, 2H), 4.22 (q, J = 7.2 Hz, 2H), 7.25–7.28 (m, 1H), 7.29 (s, 1H), 7.73 (dd, J = 6.8, 1.2 Hz, 1H), 8.48 (dd, J = 6.8, 1.2 Hz, 1H), 12.67 (s, 1H). – MS: m/z = 357 [M]$^+$. – $C_{13}H_{12}ClN_3O_3S_2$: anal. calcd. C 43.63, H 3.38, N 11.74; found C 43.39, H 3.16, N 11.91.

4.1.9 Ethyl 2-((5-(4-chlorobenzamido)-1,3,4-thiadiazol-2-yl)thio)acetate (5i)

Yield: 80 % (0.28 g). M.p. 181–183 °C. – IR (KBr): ν = 1729, 1660 (C=O) cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ ppm = 1.29 (t, J = 7.2 Hz, 3H), 4.09 (s, 2H), 4.25 (q, J = 7.2 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 8.07 (d, J = 8.4 Hz, 2H), 11.24 (s, NH). – MS: m/z = 357 [M]$^+$. – $C_{13}H_{12}ClN_3O_3S_2$: anal. calcd. C 43.63, H 3.38, N 11.74; found C 43.31, H 3.19, N 12.08.

4.1.10 Ethyl 2-((5-(2,4-dichlorobenzamido)-1,3,4-thiadiazol-2-yl)thio)acetate (5j)

Yield: 76 % (0.30 g). M.p. 164–166 °C. – IR (KBr): ν = 1727, 1662 (C=O) cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ ppm = 1.31 (t, J = 7.2 Hz, 3H), 3.98 (s, 2H), 4.25 (q, J = 7.2 Hz, 2H), 7.65 (d, J = 8.4 Hz, 1H), 7.56 (brs, 1H), 7.75 (d, J = 8.4 Hz, 1H), 12.89 (s, NH). – ^{13}C NMR (100.0 MHz, $CDCl_3$, 25 °C, TMS): δ ppm = 14.1 (CH_3), 34.9 (SCH_2), 62.2 (OCH_2), 127.6 (CHAr), 130.63 (CHAr), 130.66 (CH_2Ar), 130.7 (CHAr), 131.4 (CH_2Ar), 133.2 (CH_2Ar), 138.3 (CH_2Ar), 158.9 (CAr), 160.37 (CAr), 163.75 (C=O), 167.82 (C=O). – MS: m/z = 391 [M]$^+$. – $C_{13}H_{11}Cl_2N_3O_3S_2$: anal. calcd. C 39.80, H 2.83, N 10.71; found C 39.51, H 2.92, N 11.03.

4.1.11 Ethyl 2-((5-(3-fluorobenzamido)-1,3,4-thiadiazol-2-yl)thio)acetate (5k)

Yield: 87 % (0.33 g). M.p. 189–191 °C. – IR (KBr): ν = 1730, 1668 (C=O) cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ ppm = 1.25 (t, J = 7.2 Hz, 3H), 4.09 (s, 2H), 4.19 (q, J = 7.2 Hz, 2H), 7.35 (t, J = 8.0 Hz, 1H), 7.55 (q, J = 7.6 Hz, 1H), 7.98 (d, J = 9.2 Hz, 1H), 8.06 (d, J = 7.6 Hz, 1H), 13.07 (s, NH). – ^{13}C NMR (100.0 MHz, $CDCl_3$, 25 °C, TMS): δ ppm = 14.1 (CH_3), 33.1 (SCH_2), 62.1 (OCH_2), 115.9 (d, CH_2CAr, J = 92.8 Hz), 120.4 (d, CH_2Ar, J = 84.4 Hz), 124.6 (d, CH_2Ar, J = 11.6 Hz), 130.5 (d, CH_2Ar, J = 31.2 Hz), 133.1 (d, CH_2Ar, J = 29.2 Hz), 159.0 (CAr), 161.7 (CAr), 163.95 (C=O), 164.42 (C-FAr, J = 10 Hz), 167.95 (C=O). – MS: m/z = 341 [M]$^+$. – $C_{13}H_{12}FN_3O_3S_2$: anal. calcd. C 45.74, H 3.54, N 12.31; found C 45.38, H 3.25, N 12.13.

4.1.12 Ethyl 2-((5-(3-nitrobenzamido)-1,3,4-thiadiazol-2-yl)thio)acetate (5l)

Yield: 89 % (0.33 g). M.p. 180–182 °C. – IR (KBr): ν = 1730, 1669 (C=O) cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ ppm = 1.25 (t, J = 7.2 Hz, 3H), 4.12 (s, 2H), 4.20 (q, J = 7.2 Hz, 2H), 7.28 (t, J = 4.6 Hz, 1H), 7.73 (d, J = 4.6 Hz, 1H), 8.49 (d, J = 4.6 Hz, 1H), 12.69 (s, NH). – MS: m/z = 368 [M]$^+$. – $C_{13}H_{12}N_4O_5S_2$: anal. calcd. C 42.38, H 3.28, N 15.21; found C 42.61, H 3.09, N 15.54.

4.1.13 Ethyl 2-((5-(thiophene-2-carboxamido)-1,3,4-thiadiazol-2-yl)thio)acetate (5m)

Yield: 82 % (0.27 g). M.p. 175–177 °C. – IR (KBr): ν = 1728, 1663 (C=O) cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ 1.25 (t, J = 7.2 Hz, 3H), 4.12 (s, 2H), 4.20 (q, J = 7.2 Hz,

2H), 7.28 (t, J = 4.6 Hz, 1H), 7.73 (d, J = 4.6 Hz, 1H), 8.49 (d, J = 4.6 Hz, 1H), 12.69 (s, NH). – MS: m/z = 329 $[M]^+$. – $C_{11}H_{11}N_3O_3S_3$: anal. calcd. C 40.11, H 3.37, N 12.76; found C 40.36, H 3.09, N 12.90.

4.1.14 Ethyl 2-((5-(furan-2-carboxamido)-1,3,4-thiadiazol-2-yl)thio)acetate (5n)

Yield: 79 % (0.25 g). M.p. 171–173 °C. – IR (KBr): ν = 1729, 1660 (C=O) cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ ppm = 1.28 (t, J = 7.2 Hz, 3H), 4.08 (s, SCH_2), 4.24 (q, J = 7.2 Hz, 2H), 6.66 (dd, J = 3.6, 1.6 Hz, 1H), 7.58 (dd, J = 3.6, 1.6 Hz, 1H), 7.69 (dd, J = 3.6, 1.6 Hz, 1H), 11.28 (s, 1H, NH). – MS: m/z = 313 $[M]^+$. – $C_{11}H_{11}N_3O_4S_2$: anal. calcd. C 42.16, H 3.54, N 13.41; found C 42.51, H 3.75, N 13.59.

5 Supporting Information

Experimental details of the biological studies and pictures of the ^{1}H NMR and ^{13}C NMR spectra of **5a**–**n** are available online (DOI: 10.1515/znb-2015-0138).

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