Research Article

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Synthesis and antiproliferative evaluation of novel triazolothiadiazine compounds

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Abstract: A novel series of triazolothiadiazines has been successfully synthesized with high efficiency through Knoevenagel condensation, utilizing thiocarbamoyl dihydrazine as the starting material. The structures of these compounds were characterized through ^{1}H-NMR, ^{13}C-NMR, high-resolution mass spectrometry. The MTT assay was utilized to evaluate the *in vitro* cytotoxicity of the synthesized compounds against four human cancer cell lines including SW620, A549, Hela, and MCF-7. Among the tested compounds, (Z)-3-methyl-6-phenyl-7-(3-(trifluoromethyl)benzylidene)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine exhibited notably potent antiproliferative activity against all four human cancer cell lines.

Keywords: 1,2,4-triazole, thiadiazine, anti-proliferative activity, Knoevenagel condensation

1 Introduction

Heterocyclic compounds are a class of organic compounds characterized by the presence of a heterocyclic ring system within their molecular structure, which includes at least one heteroatom in addition to the carbon atoms. These heterocycles are ubiquitous in both natural and synthetic sources and their significant pharmacological properties are renowned. Considerable interest has been garnered in medicinal chemistry by thiadiazines and triazoles, which are part of the nitrogen-containing heterocyclic class.

Thiadiazines are a class of heterocyclic compounds known for their diverse pharmacological activities, which encompass antibacterial [1], antiparasitic [2], and antidepressant effects [3]. These compounds are frequently integrated into the molecular frameworks of various pharmaceuticals as pharmacodynamic moieties, as illustrated by the examples of chlorothiazide, cyclopentothiazide, and bendroflumethiazide. Two isomeric forms based on the positional arrangement of the nitrogen atom within the ring are exhibited by triazoles, which feature a five-membered heterocyclic ring containing nitrogen: 1,2,3-triazole and 1,2,4-triazole. The 1,2,4-triazole isomer has been associated with a broad spectrum of pharmacological activities, including antimicrobial, anticancer, and antiviral effects, as documented in the literature [4–7]. In oncology, significant potential has been shown by this isomer as an inhibitor of aromatase [8,9], protein kinase [10,11], and carbonic anhydrase [12,13]. A number of pharmacologically relevant drugs, such as trimethoprim, anastrozole, and letrozole, featuring the aforementioned heterocyclic structural motif, have been approved for clinical use.

It has been demonstrated that a range of biological activities, including anti-inflammatory [14,15], antitumour [16–20], antimicrobial [21–24], cholinesterase-inhibitory [25], antioxidant [26], antiviral [27], and anticonvulsant properties, are exhibited by the fusion of the 1,2,4-triazole ring with the thiadiazine ring. On the basis of the above, our interest was aroused by the synthesis of novel triazolothiadiazine analogues.

2 Results and discussion

2.1 Synthesis

Novel triazolothiadiazine compounds **7a–7t** were synthesized in accordance with the established methodology, with the synthetic route illustrated in Scheme 1 [28,29]. The intermediates **3a** and **b** were initially obtained through the cyclization of thiocarbamoyl dihydrazide with aliphatic acids. The resulting products were then subjected to the

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subsequent step without further purification. Subsequently, intermediate **3** was condensed with α-bromoacetophenone/4-hydroxybromoacetophenone in order to obtain the key intermediates **5a–c**. Caution must be exercised regarding the amount of solvent used, as either a suboptimal or an excessive quantity can lead to increased by-product formation, thereby impacting the reaction yield. Ultimately, intermediate **5** was condensed with aldehydes possessing diverse substituents through a Knoevenagel condensation reaction, affording the target compounds in a yield range of 55–89%. The structure of the desired compounds was confirmed by ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry.

2.2 Bioactivity studies

The *in vitro* cytotoxic effects of the synthesized compounds were evaluated using the MTT assay on four different human cancer cell lines: SW620, A549, HeLa, and MCF-7. The corresponding IC_{50} values, expressed in μM, are summarized in Table 1.

Encouragingly, when compared to the standard drug cisplatin, the majority of our tested compounds exhibited inhibitory effects on SW620, A549, Hela, and MCF-7 cancer cells. Notably, among the tested compounds, several demonstrated potent antiproliferative activity against SW620 cells, with the following IC_{50} values: **7d**, bearing a trifluoromethyl group at R_3, showed an IC_{50} of 29 μM; **7j**, with a cyano substitution, had an IC_{50} of 26.6 μM; **7i**, featuring a monomethyl group, exhibited an IC_{50} of 24.8 μM; and **7l**, with a trimethoxyl substitution, displayed an IC_{50} of 24.6 μM. For A549 cells, compounds with trifluoromethyl (**7d**), furanyl (**7n**), hydroxyl (**7t**), and bromochlorine substitutions (**7k**) showed enhanced inhibitory activity. The potency order was as follows: **7d** (16.9 μM) was the most potent, followed by **7t** (19.3 μM), **7k** (26.5 μM), and **7n** (29.1 μM). Additionally, against Hela cells, **7c**, which contains a methyl group, **7d** with a trifluoromethyl substitution, **7p** featuring a dimethylamino group, and **7t** substituted with a hydroxyl group, all exhibited significant activity. Notably, **7c** and **7p** demonstrated the strongest inhibitory effects (the structure of the compound is shown in Figure 1), with IC_{50} values of 18.2 and 18.9 μM, respectively. Concurrently, our findings indicated that compounds adorned with ethoxy, naphthylidene, and pyrrolyl groups were essentially ineffective against MCF-7 cells. However, **7d**, which incorporates a trifluoromethyl group, and **7k**, which contains chlorine and bromine atoms, demonstrated enhanced activity.

Preliminary structure–activity relationship analysis revealed that the antiproliferative potency of the target compounds was significantly influenced by the substituents on the triazolothiadiazine ring, with the nature of the R_3 substituent exerting a pronounced effect on activity. Moreover, the incorporation of halogenated groups was found to be particularly beneficial in augmenting the biological potency of these compounds. This enhancement was hypothesized to involve two key mechanistic aspects. First, the electronic effects of halogen atoms were identified as critical modulators of activity. Fluorine atoms, owing to their high electronegativity, were shown to exhibit the most pronounced enhancement, while synergistic electronic effects derived from polyhalogen

Table 1: Antiproliferative activity against SW620, A549, Hela, and MCF-7 cells IC_{50} (μM)

Compd	A549	Hela	MCF-7	SW620
Cisplatin	7.3	6.6	20.3	18.5
7a	28.6	>50	>50	30.5
7b	34.5	>50	>50	42.8
7c	32.6	18.2	33.6	>50
7d	16.9	21.6	31.7	29
7e	>50	40.3	>50	>50
7f	>50	>50	>50	43.6
7g	35.6	42.1	46.6	>50
7h	>50	>50	>50	>50
7i	>50	30.5	>50	24.8
7j	38.6	>50	>50	26.6
7k	26.5	39.5	30.5	>50
7l	38.6	>50	>50	24.6
7m	>50	42.6	>50	40.8
7n	29.1	>50	>50	>50
7o	>50	>50	>50	>50
7p	>50	18.9	>50	>50
7q	>50	>50	>50	>50
7r	39.9	>50	>50	48.1
7s	38.6	48.1	40.5	>50
7t	19.3	28.3	>50	>50

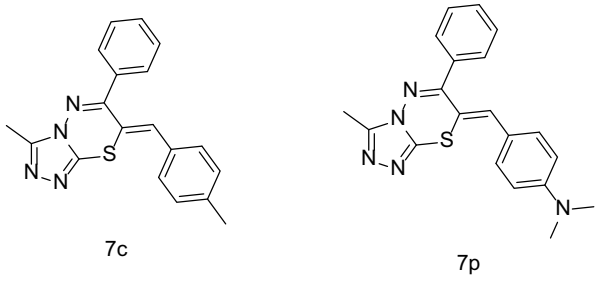
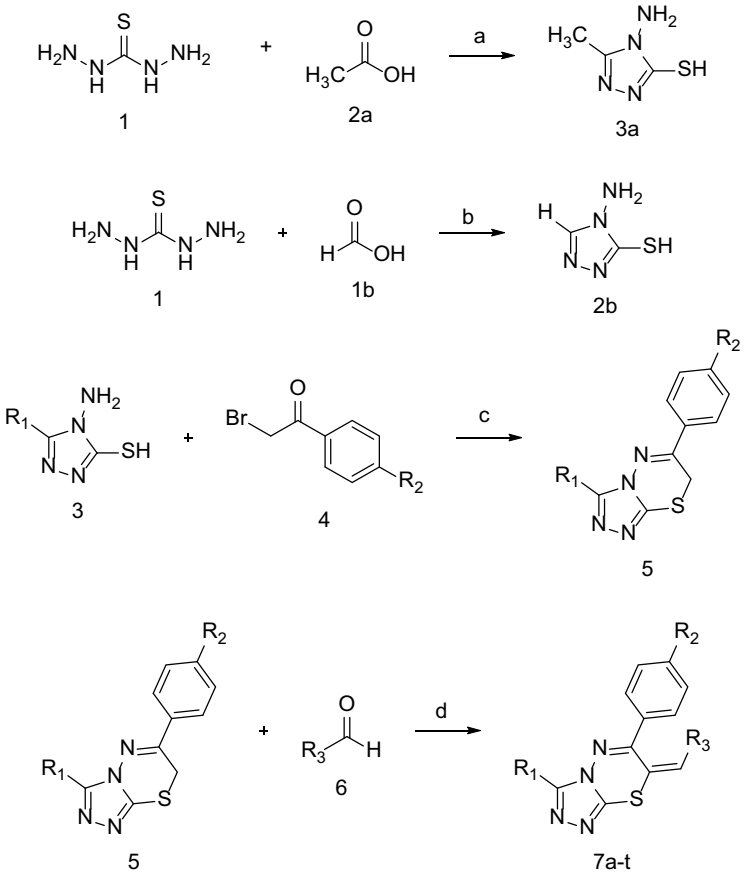


Figure 1: Compounds 7c and 7p.



a: reflux; b: H_2O, reflux; c: EtOH, reflux; d: KOH, CH_3OH, reflux.

7a: R_1=Me; R_2=H; R_3=Ph

7b: R_1=H; R_2=H; R_3=Ph

7c: R_1=Me; R_2=H; R_3=4-MePh

7d: R_1=Me; R_2=H; R_3=3-(CF_3)Ph

7e: R_1=Me; R_2=H; R_3=2-ClPh

7f: R_1=Me; R_2=H; R_3=2-MePh

7g: R_1=Me; R_2=H; R_3=2-FPh

7h: R_1=Me; R_2=H; R_3=2-OC_2H_5Ph

7i: R_1=Me; R_2=H; R_3=2-OMePh

7j: R_1=Me; R_2=H; R_3=2-CNPh

7k: R_1=Me; R_2=H; R_3=5-Br-2-ClPh

7l: R_1=Me; R_2=H; R_3=2,3,4-$(OMe)_3$Ph

7m: R_1=Me; R_2=H; R_3=3-OMePh

7n: R_1=Me; R_2=H; R_3=2-furan

7o: R_1=Me; R_2=H; R_3=naphthaldehyde

7p: R_1=Me; R_2=H; R_3=N,N-dimethylaniline

7q: R_1=Me; R_2=H; R_3=1H-pyrrole

7r: R_1=Me; R_2=H; R_3=4-ClPh

7s: R_1=Me; R_2=H; R_3=2-BrPh

7t: R_1=Me; R_2=OH; R_3=Ph

Scheme 1: Synthesis of triazolothiadiazine derivatives.

substitutions (e.g., trifluoromethyl groups) were associated with significantly higher activity compared to monosubstituted analogues. Second, halogen atoms were linked to reduced steric hindrance relative to bulky substituents (e.g., naphthyl or ethoxy groups), which was proposed to facilitate ligand–receptor binding. Notably, dihalogen-substituted compounds (e.g., Br/Cl) were observed to demonstrate superior activity over monosubstituted counterparts, further supporting the hypothesis that both the number and type of halogen substituents could synergistically modulate biological activities.

3 Conclusion

A series of triazolothiadiazine derivatives have been synthesized with different substituents at triazolyl ring and thiadiazine. Triazolothiadiazine derivatives were synthesized under mild conditions, which yielded well. The spectroscopic analysis was used to characterize the new compounds. The newly synthesized compounds were tested for their anti-cancer properties against MCF-7 cells, Hela cells, A549 cells, and SW620 cells. IC_{50} values of 18.2 and 18.9 μM were exhibited by **7c** and **7p**, respectively, for the strongest inhibition of Hela cells, whereas IC_{50} values of 16.9 and 19.3 μM were shown by **7d** and **7t** as the most potent inhibitors against A549 cells. The most active compounds against the SW620 cell line were found to be **7l** and **7i**, while the most promising ones against the MCF-7 cell line were determined to be **7k** and **7d**. In addition, certain inhibitory activity was shown by the compound **7d** on all four cells. In summary, a leading compound of antitumor drugs has the potential to be developed from the new triazothiodiazine derivative.

4 Experimental

4.1 Experimental details

The melting points were determined using an X-6 microscopic melting point tester. The ^{1}H NMR (400 MHz) and ^{13}C NMR spectra were acquired on a Bruker Avance spectrometer at 298.0 K using $CDCl_3$/DMSO-d_6 as solvent with 16 scans. The mass spectral information of the compounds was obtained using ESI+ mode.

The experimental materials were purchased from Shanghai Xian Ding Biotechnology Co. Ltd and were used directly without any purification.

4.2 Synthesis of 4-amino-5-methyl-4*H*-1,2,4-triazole-3-thiol (3a)

Thiocarbohydrazide (94.21 mmol) was added to acetic acid (659.46 mmol) and the mixture was stirred for 4 h at reflux. The progress of the reaction was monitored using thin-layer chromatography (TLC). Upon completion of the reaction, the mixture was allowed to cool to room temperature, and the crude product was isolated by filtration. The pure product was subsequently obtained by recrystallization from distilled water. Yield: 78%, white solid.

4.3 Synthesis of 4-amino-4*H*-1,2,4-triazole-3-thiol (3b)

Thiocarbazide (94.21 mmol) is added to a mixture of formic acid (197.84 mmol) and water (30.6 mL) and stirred for 4 h at reflux temperature. The progress of the reaction was monitored by TLC. After the reaction was complete, the mixture was cooled to room temperature and filtered through a Büchner funnel to obtain the crude product. The pure product was subsequently obtained by recrystallization from distilled water. Yield: 55%, purple solid.

4.4 Synthesis of 3-methyl-6-phenyl-7*H*-[1,2,4]triazolo [3,4-b][1,3,4] thiadiazine (5a)

In a three-necked round-bottom flask, **3a** (76.82 mmol) and α-bromoacetophenone (76.82 mmol) were combined with anhydrous ethanol (180 mL) and the mixture was refluxed for 5 h. The reaction progress was monitored by TLC. After the reaction was complete, the flask was allowed to cool to room temperature, and the pH of the mixture was carefully adjusted to 8 using a 1 M aqueous ammonia solution with continuous stirring, which led to the precipitation of a solid. The solid was isolated by vacuum filtration using a Büchner funnel, and then dried under controlled conditions to afford a red solid product without the need for further purification. Yield: 90%.

4.5 General method for synthesis of target triazole thiadiazine compounds 7a–t

A mixture of triazolothiadiazine **5a–c** (8.68 mmol), aldehyde **6** (11.29 mmol) in 25 mL of methanol was at reflux

temperature for a period of 8–34 h and monitored by TLC. Once the reaction was complete, the mixture was cooled and diluted with cold water. The crude solid was then purified by column chromatography using a mixture of ethyl acetate and petroleum ether as the eluent.

(Z)-7-benzylidene-3-methyl-6-phenyl-7H-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazine (**7a**). Yield: 78%, yellow solid, m.p.: 237–238°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.88–7.81 (m, 2H), 7.65–7.59 (m, 2H), 7.55–7.52 (m, 3H), 7.52–7.48 (m, 3H), 7.32 (s, 1H), 2.50 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.13, 150.58, 141.17, 137.68, 135.00, 133.19, 131.38, 130.16, 129.85, 129.11, 128.79, 116.33, 9.75. HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{18}H_{14}N_4S$: 319.1012, found: 319.1014.

(Z)-7-benzylidene-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazine (**7b**). Yield: 74%, yellow solid, m.p.: 184–185°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H), 7.85–7.79 (d, 2H), 7.67–7.55 (m, 3H), 7.54–7.47 (m, 5H), 7.31 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.29, 143.49, 141.58, 138.19, 135.07, 133.38, 131.70, 130.04, 129.38, 129.10, 117.05. HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{17}H_{12}N_4S$: 305.0856, found: 305.0860.

(Z)-3-methyl-7-(4-methylbenzylidene)-6-phenyl-7H-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazine (**7c**). Yield: 70%, yellow solid, m.p.: 156–157°C. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.90–7.82 (m, 2H), 7.67–7.55 (m, 7H), 7.26 (s, 1H), 2.71 (s, 3H), 2.53 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-d) δ 156.51, 151.10, 140.70, 140.41, 138.61, 135.42, 131.13, 130.55, 130.08, 129.70, 129.54, 129.00, 116.05, 21.65, 10.13. HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{19}H_{16}N_4S$: 333.1169, found: 333.1172.

(Z)-3-methyl-6-phenyl-7-(3-(trifluoromethyl)benzylidene)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**7d**). Yield: 89%, yellow solid, m.p.: 177–178°C. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.77–7.74 (m, 2H), 7.70–7.61 (m, 3H), 7.61–7.56 (m, 2H), 7.54–7.51 (m, 2H), 7.19 (s, 1H), 2.59 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-d) δ 156.00, 151.51, 138.61, 135.17, 134.30, 132.90, 131.71, 129.93, 129.72, 129.49, 127.01, 126.84, 125.39, 122.68, 119.77, 10.42. HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{19}H_{13}F_3N_4S$: 387.0886, found: 387.0885.

(Z)-7-(2-chlorobenzylidene)-3-methyl-6-phenyl-7H-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazine (**7e**). Yield: 88%, yellow solid, m.p.: 158–159°C. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.81–7.75 (m, 2H), 7.67–7.49 (m, 5H), 7.37–7.34 (m, 2H), 7.30 (s, 1H), 2.60 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-d) δ 155.92, 151.46, 138.83, 137.72, 135.03, 134.68, 132.14, 131.69, 131.43, 130.42, 130.17, 129.94, 129.38, 127.26, 120.36, 10.49. HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{18}H_{13}ClN_4S$: 353.0623, found: 353.0627.

(Z)-3-methyl-7-(2-methylbenzylidene)-6-phenyl-7H-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazine (**7f**). Yield: 84%, yellow solid, m.p.: 185–186°C. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.76–7.75 (m, 2H), 7.59–7.49 (m, 3H), 7.43–7.39 (m, 1H), 7.32–7.21 (m, 4H), 2.59 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.63, 150.61, 140.10, 137.88, 136.96, 134.79, 132.50, 131.38, 130.59, 130.04, 129.74, 129.10, 128.87, 126.00, 118.03, 19.51, 9.78. HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{19}H_{16}N_4S$: 333.1169, found: 333.1171.

(Z)-7-(2-fluorobenzylidene)-3-methyl-6-phenyl-7H-[1,2,4] triazolo[3,4-b][1,3,4] thiadiazine (**7g**). Yield: 85%, yellow solid, m.p.: 167–168°C. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.78–7.75(m, 2H), 7.64–7.48 (m, 4H), 7.45–7.42 (m, 1H), 7.31–7.22 (m, 2H), 7.12–7.10 (m, 1H), 2.60 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.06, 158.58, 155.58, 150.98, 137.76, 134.00, 133.46, 132.78, 131.75, 131.00, 130.07 129.43, 128.80, 125.12, 121.37, 119.83, 116.36, 10.02. HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{18}H_{13}FN_4S$: 337.0918, found: 337.0922.

(Z)-7-(2-methoxybenzylidene)-3-methyl-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**7h**). Yield: 78%, yellow solid, m.p.: 182–183°C. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.80–7.75 (m, 2H), 7.55–7.47 (m, 4H), 7.38–7.35 (m, 2H), 7.04–6.98 (m, 2H), 6.91 (d, J = 1.0 Hz, 1H), 4.03 (d, J = 6.9 Hz, 2H), 2.59 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.87, 156.38, 150.82, 138.57, 137.31, 135.30, 132.28, 131.60, 130.04, 129.35, 122.10, 120.62, 117.12, 112.72, 64.24, 14.90, 10.03. HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{20}H_{18}N_4OS$: 363.1275, found: 363.1277.

(Z)-7-(2-methoxybenzylidene)-3-methyl-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**7i**). Yield: 80%, yellow solid, m.p.: 172–173°C. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.83–7.75 (m, 2H), 7.59–7.45 (m, 3H), 7.38–7.37 (m, 2H), 7.34 (s, 1H), 7.04–7.02 (m, 1H), 6.92–6.91 (m, 1H), 3.79 (s, 3H), 2.58 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.37, 156.33, 150.79, 138.68, 137.65, 135.27, 132.38, 131.74, 130.58, 130.10, 129.38, 121.91, 120.62, 117.36, 111.83, 56.06, 10.03. HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{19}H_{16}N_4OS$: 349.1118, found: 349.1120.

(Z)-2-((3-methyl-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazin-7-ylidene)methyl)benzonitrile (**7j**). Yield: 88%, yellow solid, m.p.: 247–258°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (d, J = 7.6 Hz, 1H), 7.86 (d, J = 7.4 Hz, 1H), 7.77–7.71 (m 1H), 7.69–7.67 (m, 1H), 7.55–7.49 (m, 2H), 7.45–7.38 (m, 3H), 6.64 (s, 1H), 1.82 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.81, 168.85, 163.21, 144.35, 143.31, 137.43, 135.65, 133.56, 131.78, 130.71, 129.32, 128.92, 127.61, 123.82, 122.58, 99.90, 11.50. HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{19}H_{13}N_5S$: 344.0965, found: 344.0971.

(Z)-7-(4-bromo-2-chlorobenzylidene)-3-methyl-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**7k**). Yield: 85%, yellow solid, m.p.: 207–208°C. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.78–7.74 (m, 2H), 7.71–7.70 (m, 1H), 7.58–7.51 (m, 3H), 7.47–7.46 (m, 1H), 7.32–7.30 (m, 1H), 7.19 (s, 1H), 2.61 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.02, 151.08, 137.66, 136.35, 134.48, 134.39, 133.99, 133.28, 132.39, 132.03, 131.89, 130.08, 129.46, 121.47, 120.39, 10.05. HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{18}H_{12}BrClN_4S$: 430.9728, found: 430.9730.

(Z)-3-methyl-6-phenyl-7-(2,3,4-trimethoxybenzylidene)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**7l**). Yield: 82%,

yellow solid, m.p.: 168–169°C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.75–7.73 (m, 2H), 7.57–7.51 (m, 3H), 7.37–7.26 (m, 2H), 6.77–6.75 (m, 1H), 3.92 (s, 3H), 3.84 (s, 3H), 3.74 (s, 3H), 2.58 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.54, 155.91, 152.38, 150.80, 142.09, 138.23, 136.17, 135.50, 131.53, 130.03, 129.32, 125.35, 119.95, 115.71, 108.24, 61.91, 61.03, 56.58, 10.01. HRMS (ESI) *m*/*z* [M + H]$^+$ calcd for $C_{21}H_{20}N_4O_3S$: 409.1329, found: 409.1335.

(Z)-7-(3-methoxybenzylidene)-3-methyl-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**7m**). Yield: 72%, yellow solid, m.p.: 157–158°C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.75–7.73 (m, 2H), 7.61–7.47 (m, 3H), 7.37–7.35 (m, 1H), 7.14 (s, 1H), 7.05–6.92 (m, 3H), 3.83 (s, 3H), 2.58 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.59, 156.31, 150.83, 141.37, 137.96, 135.28, 134.78, 131.64, 130.14, 129.37, 122.60, 116.95, 116.45, 115.38, 55.77, 10.01. HRMS (ESI) *m*/*z* [M + H]$^+$ calcd for $C_{19}H_{16}N_4OS$: 349.1118, found: 349.1119.

(Z)-7-(furan-2-ylmethylene)-3-methyl-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**7n**). Yield: 81%, yellow solid, m.p.: 230–231°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (d, *J* = 1.8 Hz, 1H), 7.73–7.66 (m, 2H), 7.66–7.52 (m, 3H), 7.09 (d, *J* = 3.6 Hz, 1H), 6.87 (s, 1H), 6.77–6.76 (m 1H), 2.46 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.26, 150.93, 149.63, 147.32, 136.97, 135.30, 131.15, 129.90, 129.36, 124.30, 118.49, 113.93, 112.75, 9.99. HRMS (ESI) *m*/*z* [M + H]$^+$ calcd for $C_{16}H_{12}N_4OS$: 309.0805, found: 309.0808.

(Z)-3-methyl-7-(naphthalen-2-ylmethylene)-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**7o**). Yield: 85%, yellow solid, m.p.: 222–223°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (s, 1H), 8.10–8.04 (m, 1H), 7.99–7.97 (m, 2H), 7.88–7.87 (m, 2H), 7.62–7.58 (m, 6H), 7.46 (s, 1H), 2.51 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.40, 150.88, 141.11, 137.86, 135.36, 133.48, 132.80, 131.63, 131.07, 130.71, 130.14, 129.41, 129.10, 128.60, 128.19, 128.12, 127.45, 127.15, 116.92, 10.03. HRMS (ESI) *m*/*z* [M + H]$^+$ calcd for $C_{22}H_{16}N_4S$: 369.1169, found: 369.1173.

(Z)-N,N-dimethyl-4-((3-methyl-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-7-ylidene)methyl)aniline (**7p**). Yield: 65%, yellow solid, m.p.: 198–199°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79–7.73 (m, 2H), 7.57–7.53 (m, 3H), 7.43 (d, *J* = 8.5 Hz, 2H), 7.07 (s, 1H), 6.79 (d, *J* = 8.7 Hz, 2H), 3.00 (s, 6H), 2.47 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.57, 151.61, 150.65, 141.45, 138.13, 135.96, 132.71, 131.37, 130.08, 129.28, 120.64, 111.89, 109.27, 40.22, 10.02. HRMS (ESI) *m*/*z* [M + H]$^+$ calcd for $C_{20}H_{19}N_5S$: 362.1434, found: 362.1435.

(Z)-7-((1H-pyrrol-2-yl)methylene)-3-methyl-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**7q**). Yield: 79%, yellow solid, m.p.: 183–184°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.54 (s, 1H), 7.76–7.67 (m, 2H), 7.66–7.53 (m, 3H), 7.15–7.14 (m, 1H), 7.10 (s, 1H), 6.95–6.89 (m, 1H), 6.40–6.39 (m, 1H), 2.46 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.67, 150.79, 137.38, 135.80, 131.06, 130.04, 129.30, 128.84, 127.09, 124.36, 115.40, 112.16, 107.37, 10.04. HRMS (ESI) *m*/*z* [M + H]$^+$ calcd for $C_{16}H_{13}N_5S$: 308.0965, found: 308.0969.

(Z)-7-(4-chlorobenzylidene)-3-methyl-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**7r**). Yield: 64%, yellow solid, m.p.: 215–216°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91–7.81 (m, 2H), 7.69–7.48 (m, 7H), 7.32 (s, 1H), 2.51 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.24, 150.89, 140.22, 137.87, 135.13, 134.97, 132.36, 132.22, 131.71, 130.14, 129.39, 129.09, 117.33, 10.02. HRMS (ESI) *m*/*z* [M + H]$^+$ calcd for $C_{18}H_{13}ClN_4S$: 353.0623, found: 353.0627.

(Z)-7-(2-bromobenzylidene)-3-methyl-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**7s**). Yield: 78 %, yellow solid, m.p.: 162–163°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92–7.85 (m, 2H), 7.77 (d, *J* = 7.9 Hz, 1H), 7.68 (d, *J* = 7.2 Hz, 1H), 7.60–7.56 (m, 4H), 7.46–7.38 (m, 1H), 7.21 (s, 1H), 2.51 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.23, 151.05, 139.53, 137.55, 134.70, 133.54, 133.39, 132.12, 131.77, 131.01, 130.05, 129.43, 128.34, 123.70, 119.84, 10.06. HRMS (ESI) *m*/*z* [M + H]$^+$ calcd for $C_{18}H_{13}BrN_4S$: 397.0118, found: 397.0126.

(Z)-4-(7-benzylidene-3-methyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-yl)phenol (**7t**). Yield: 55%, yellow solid, m.p.: 201–202°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.96–7.94 (m, 1H), 7.73–7.71 (m, 2H), 7.54–7.48 (m, 5H), 7.45 (s, 1H), 6.93 (d, *J* = 8.6 Hz, 2H), 2.49 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.98, 156.71, 150.61, 142.02, 138.52, 133.54, 131.98, 130.54, 130.35, 128.99, 125.32, 116.49, 116.29, 10.04. HRMS (ESI) *m*/*z* [M + H]$^+$ calcd for $C_{18}H_{14}N_4OS$: 335.0962, found: 335.0965.

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