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1,5-Benzodiazepine derivatives as potential antimicrobial agents: design, synthesis, biological evaluation, and structure–activity relationships†

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36 Novel 1,5-benzodiazepine derivatives were rationally designed and synthesized according to the principle of superposition of bioactive substructures by the combination of 1,5-benzodiazepines, thiophene or thiazole and ester group. The structures of the target compounds have been characterized by IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. The structure of **1v** was further determined using X-ray single crystal diffraction. All synthesized 1,5-benzodiazepine derivatives were evaluated for their *in vitro* antimicrobial activity against *C. neoformans*, *C. neoformans* clinical isolates, *C. albicans*, *E. coli* and *S. aureus*. The bioactive assay results revealed that most of the 1,5-benzodiazepine derivatives exhibited considerable potency against all of the tested strains. In particular, compounds **1v** and **1w** (MIC: 2–6 μg mL^{-1}, MFC: 10–14 μg mL^{-1}) exhibited excellent antifungal activity and were found to be 32–64 and 9–12.8 times more potent than the reference drugs against *C. neoformans*, respectively. Moreover, compound **1v** (MIC: 40 μg mL^{-1}) displayed equipotent antibacterial activity against *E. coli* and *S. aureus* compared to the reference drugs. The most potent of the synthesized compounds **1v** and **1w** were further studied by evaluating their cytotoxicities, and the results showed that they had relatively low level cytotoxicity for BV2 cell. A preliminary study of the structure–activity relationship revealed that substituents in the phenyl ring and the thiophene ring had a great effect on the antimicrobial activity of these compounds. In addition, the thiazole ring at C_2 may be a pharmacophore of these compounds and $COOC_2H_5$ group at C_3 is the best substituent for the maintenance of antimicrobial activities at low concentrations (1.5625 μg per disc).

Introduction

1,5-Benzodiazepines is a class of outstanding organic molecules with a wide array of biological activities and therapeutic functions. They are commonly used as anxiolytic and anticonvulsive drugs.[1] The use of 1,5-benzodiazepines for therapeutic purposes is not confined to the treatment of anxiety and stress conditions because minor changes in their structures can produce various biological activities, and novel applications of these compounds are continuously emerging.[2] Recently, 1,5-benzodiazepines and their derivatives have been also reported to exhibit excellent antibacterial and antifungal properties.[3,4] In addition, previous studies on 1,5-benzodiazepines have indicated that the free ester group present at different positions in the nuclei of the molecules can enhance the pharmacological properties of the compounds, and this effect is attributed to their high hydrophobicity.[5]

Moreover five-membered heterocycle compounds, such as thiophene or thiazole were reported to be significant structural units of potent antimicrobial agents, and they were also vital pharmacodynamic heterocyclic nuclei.[6,7] 1,5-Benzodiazepines composed of five-membered heterocycle compounds show superior biological activity.[8]

In view of the above-mentioned findings, and as a continuation of our effort to identify new candidates that may be valuable in designing new, potent, selective, and less toxic antimicrobial agents, we have reported herein the synthesis of 36 novel 1,5-benzodiazepine derivatives by incorporating a five-membered S-heterocyclic group and an ester group. All the synthesized compounds were screened for their *in vitro* antimicrobial activities against fungi (*C. neoformans* ATCC 32264 and *C. neoformans* clinical isolates, *C. albicans* ATCC 10231) and a representative Gram-negative bacterium (*E. coli* ATCC 44752) and Gram-positive bacteria (*S. aureus* ATCC 25923). This combination was suggested to investigate the influence of such hybridization and structure variation on the anticipated biological activities, hoping to add some synergistic biological sig-

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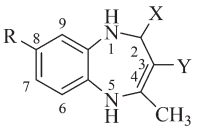


1a-x: **Y=$COOCH_2CH_3$**
1a-c: **X=** thiophen-3-yl **1a:** R=H **1b:** R=CH_3 **1c:** R=Br
1d-f: **X=**thiophen-2-yl **1d:** R=H **1e:** R=CH_3 **1f:** R=Br
1g-i: **X=**3-methylthiophen-2-yl **1g:** R=H **1h:** R=CH_3 **1i:** R=Br
1j-l: **X=**5-methylthiophen-2-yl **1j:** R=H **1k:** R=CH_3 **1l:** R=Br
1m-o: **X=** 3-bromothiophen-2-yl **1m:** R=H **1n:** R=CH_3 **1o:** R=Br
1p-r: **X=** 4-bromothiophen-2-yl **1p:** R=H **1q:** R=CH_3 **1r:** R=Br
1s-u: **X=**5-bromothiophen-2-yl **1s:** R=H **1t:** R=CH_3 **1u:** R=Br
1v-x: **X=** thiazol-2-yl **1v:** R=H **1w:** R=CH_3 **1x:** R=Br **1y:** R = F

2a-g: **Y=$COOCH_3$**
2a-b: **X=** thiophen-3-yl **2a:** R=H **2b:** R=CH_3
2c-d: **X=** thiophen-2-yl **2c:** R=H **2d:** R=CH_3
2e-g: **X=**thiazol-2-yl **2e:** R=H **2f:** R=CH_3 **2g:** R=Br
3a-b: **Y=$COOCH_2CH_2CH_3$**
X= thiazol-2-yl **3a:** R=H **3b:** R=CH_3
4a-b: **Y=$COOCH(CH_3)_2$**
X=thiazol-2-yl **4a:** R=H **4b:** R=CH_3

Fig. 1 Structures of compounds **1–4**.

nificance to target compounds. The structures of the compounds **1–4** are shown in Fig. 1.

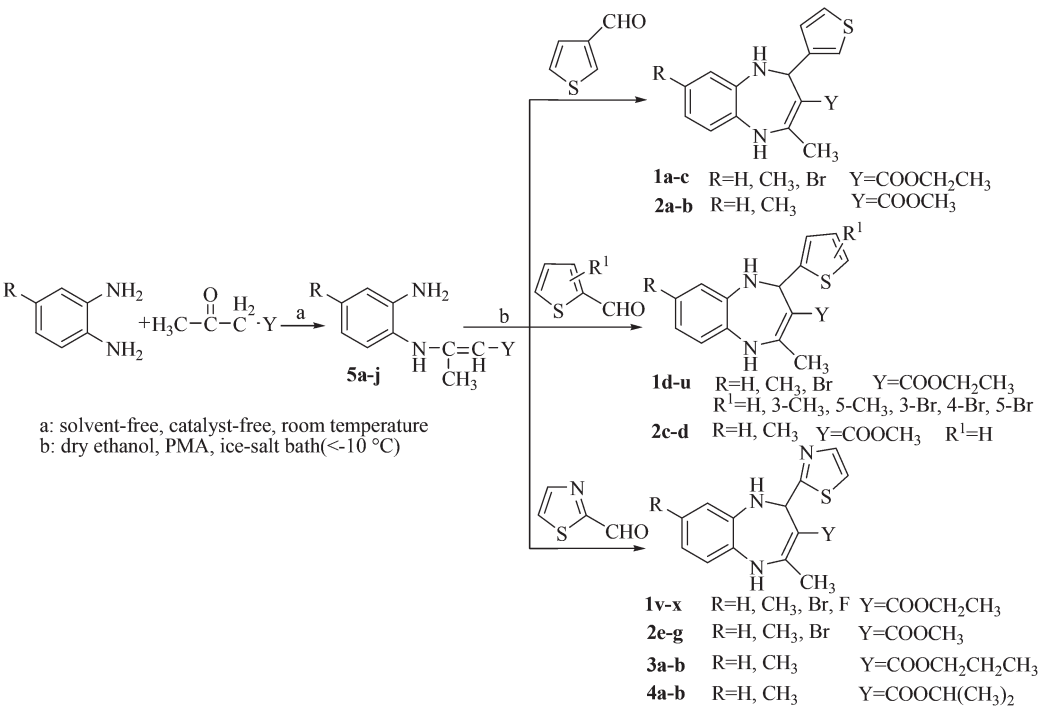
Results and discussion

Synthesis of 1,5-benzodiazepine derivatives 1–4

The novel 1,5-benzodiazepine derivatives **1–4** described in the present study were synthesized as shown in Scheme 1. The nucleophilic addition of substituted *o*-PDA with ethyl acetoacetate under solvent-free, catalyst-free and room-temperature conditions yielded approximately 95% *N-o*-aminoaryl-β-enamino esters **5**. Compound **5** was smoothly converted to the 1,5-benzodiazepine derivatives **1** *via* reaction with substituted thiophene aldehyde or thiazol aldehyde in ethanol at 0 °C in the presence of a catalytic amount of phosphomolybdic acid with a yield of 80–90%. Compounds **2**, **3** and **4** were obtained *via* a similar synthetic route in which ethyl acetoacetate was replaced by the corresponding ester (methyl acetoacetate, acetyl propyl acetate and acetyl isopropyl acetate). All of the compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. To correctly define the structure of **1v**, X-ray analysis was performed. Fig. 2 shows the ORTEP of **1v**.

Biological activity

The 36 target compounds **1–4** were evaluated for their *in vitro* antibacterial activities using the disk-diffusion method at a concentration of 200 μg per disc.[9] The diameter of the inhi-



Scheme 1 Synthesis of benzodiazepines **1–4**.

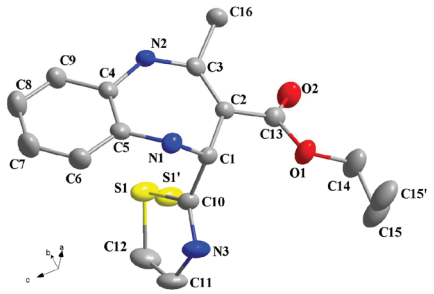


Fig. 2 Crystal structure of **1v**.

bition zone was measured as an indicator of each compound's activity. For ease of visualisation, the zone values obtained from these assays indicate the average diameter (from three trials) of the growth inhibition zones. The margin of error of these measurements was ±1 mm. The antibacterial activity was classified as highly active (>14 mm), moderately active (10 mm–14 mm), slightly active (6 mm–10 mm) and inactive (<6 mm). The zones of the 36 compounds are reported in Table 1.

Table 1 reveals 19 compounds with good antimicrobial activities. Among compounds **1a–1c** with 3-thienyl group at the C_2-position, compounds **1a** and **1b** showed moderate to high activity against all tested fungi and slight broad-spectrum activity against all tested bacteria, whereas compound **1c** was found inactive against the tested microorganisms. In general, **1a** and **1b** exhibited better antimicrobial activity against fungi than against bacteria. Of the compounds **1d–1f** with a 2-thienyl group at the C_2-position, **1d** exhibited high activity against the *C. neoformans* clinical strain, slight activity against *S. aureus*, and no activity against *E. coli*. Compounds **1h–1l** presented a methyl substituent at positions 3 or 5 on the thiophene ring. These compounds showed obvious inhibition potency and specificity to *C. albicans* alone except **1j**.

Compounds **1v–1y**, which were composed of 2-thiazolyl at the C_2-position, showed good to excellent antimicrobial activity against all of the tested microorganisms. Compounds **1v–1y** presented the largest zone activity against *C. neoformans* clinical isolates compared with their activity against the remaining four microorganisms. Compound **1v** in particular exhibited a zone of inhibition of 28.63 mm against *C. neoformans* clinical isolates. In general, the activities of **1v–1y** against bacteria or fungi were in the order **1v** > **1w** > **1y** > **1x**. Compounds **1v–1y** exhibited better antimicrobial activities against fungi compared with their corresponding activities against bacteria and better activities against Gram-positive bacteria than Gram-negative bacteria.

Compounds **1a–1c** and **1v–1y** exhibited reduced inhibition activities, indicating that their activity decreased when R = Br, F. These data suggest that the introduction of Br or F is detrimental to the antimicrobial activity of these compounds. Compounds **1h–1i** and **1k–1l** (X = 3-methyl-2-thienyl or 5-methyl-2-thienyl) show unique antifungal activity against *C. albicans* among the five tested microorganisms.

In order to further explore the influence of substituent **Y** at the C_3-position on the antimicrobial activity of this series of 1,5-benzothiazepines, compounds **2–4** were synthesized by modifying the ester group that caused compounds **1** to exhibit high antimicrobial activities. Compounds **2**, **3** and **4** have Y groups of $COOCH_3$, $COOCH_2CH_2CH_3$ and $COOCH(CH_3)_2$, respectively. The results of the preliminary antibacterial testing of compounds **2–4** are also shown in Table 1.

The data shown in Table 1 demonstrate that 8 compounds **2**, **3** and **4** exhibited good inhibition activity. Compound **2b** exhibited larger inhibition zones against bacteria than against fungi and displayed the highest antibacterial activity against *S. aureus* but no antifungal activity against the *C. neoformans* clinical strain. Compounds **2c** and **2d** exhibited slight antimicrobial activity against *S. aureus.* Compounds **2e** and **2f** showed high to moderate antimicrobial activities against *C. neoformans*, *E. coli* and *S. aureus*, but **2g** (R = Br) did not exhibit any activity against any of the tested microorganisms. These results illustrate that a Br atom may reduce the antimicrobial activity of this series of compounds. Overall, the inhibition zones of compounds **2** against bacteria were larger than their zones against fungi. Therefore, compounds **2** exhibited better sensitivity against bacteria than against fungi. Furthermore, compounds **2** showed better activity against Gram-positive bacteria than against Gram-negative bacteria. Compounds **3a** and **3b** inhibited all of the tested microorganisms and showed an efficient broad-spectrum inhibitory effect. These compounds exhibited excellent activity against the Gram-positive bacteria *S. aureus* and *C. neoformans* clinical strain. Compound **4a** was also an efficient broad-spectrum antibiotic and exhibited excellent inhibition activities against the *C. neoformans* clinical strain and *S. aureus.* In contrast, compound **4b** had no activity against any of the tested microorganisms.

Concentration gradient

Compounds **1v**, **1w**, **2e**, **3a**, **3b** and **4a**, which exhibited remarkable antimicrobial activity, were screened for their *in vitro* antimicrobial activities using different concentration gradients against the various strains through the standard two-fold serial dilution method using agar media.[10] The changes in the inhibition zone size with the concentration of the compounds are shown in Table 2.

From Table 2, it can be observed that the antimicrobial activity of the compounds was reduced with decreasing compound concentration. Compounds **1v** and **1w** exhibited slight activity at doses <1.5625 μg per disc, but **2e**, **3a** and **3b** did not exhibit any activity at doses <25 μg per disc, which indicates that the ethoxycarbonyl ($-COOCH_2CH_3$) is more conducive to the maintenance of biological activity at low concentrations.

The antimicrobial capacity of **1v** against the tested microorganisms was in the following order: *C. neoformans* clinical strain > *C. neoformans* > *S. aureus* > *E. coli*. The antimicrobial capacity of **1w** for the tested microorganisms was in the same order as **1v**. The same order of antimicrobial capacity was observed for most of the compounds in our present study.

Table 1 Antimicrobial activity results (zone of inhibition) of the newly synthesized compounds (**1–4**)

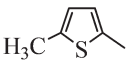
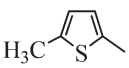
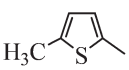
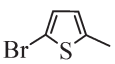
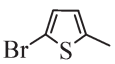
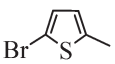
				Zone of inhibition[a] (mm), dose (200 μg per disc)				
Compd	R	X	Y	*C. n.*[b]	*C. n. C.*[b]	*C. a.*[b]	*E. c.*[b]	*S. a.*[b]
1a	H		$COOCH_2CH_3$	9.90 ± 0.20	13.03 ± 0.15	12.10 ± 0.22	6.00 ± 0.00	8.80 ± 0.10
1b	CH_3		$COOCH_2CH_3$	10.37 ± 0.15	12.73 ± 0.25	10.73 ± 0.10	8.50 ± 0.10	9.23 ± 0.15
1c	Br		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
1d	H		$COOCH_2CH_3$	7.97 ± 0.05	10.40 ± 0.06	9.03 ± 0.10	6.00 ± 0.00	8.47 ± 0.05
1e	CH_3		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
1f	Br		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
1g	H		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
1h	CH_3		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	18.73 ± 0.32	6.00 ± 0.00	6.00 ± 0.00
1i	Br		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	17.03 ± 0.25	6.00 ± 0.00	6.00 ± 0.00
1j	H		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
1k	CH_3		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	18.67 ± 0.30	6.00 ± 0.00	6.00 ± 0.00
1l	Br		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	17.47 ± 0.42	6.00 ± 0.00	6.00 ± 0.00
1m	H		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
1n	CH_3		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
1o	Br		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
1p	H		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
1q	CH_3		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
1r	Br		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
1s	H		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
1t	CH_3		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
1u	Br		$COOCH_2CH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00

Table 1 *(Contd.)*

Compd	R	X	Y	Zone of inhibition[a] (mm), dose (200 μg per disc)				
				C. n.[b]	*C. n. C.*[b]	*C. a.*[b]	*E. c.*[b]	*S. a.*[b]
1v	H		$COOCH_2CH_3$	24.27 ± 0.20	28.63 ± 0.30	15.00 ± 0.10	19.70 ± 0.25	21.17 ± 0.20
1w	CH_3		$COOCH_2CH_3$	21.00 ± 0.21	24.07 ± 0.23	14.37 ± 0.15	15.03 ± 0.19	17.10 ± 0.30
1x	Br		$COOCH_2CH_3$	12.07 ± 0.12	15.70 ± 0.25	11.70 ± 0.20	12.03 ± 0.10	13.30 ± 0.15
1y	F		$COOCH_2CH_3$	15.83 ± 0.32	15.90 ± 0.25	13.33 ± 0.15	12.67 ± 0.20	19.00 ± 0.32
2a	H		$COOCH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
2b	CH_3		$COOCH_3$	9.03 ± 0.35	6.00 ± 0.00	7.30 ± 0.20	10.27 ± 0.35	13.53 ± 0.42
2c	H		$COOCH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	8.83 ± 0.15
2d	CH_3		$COOCH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	7.83 ± 0.05
2e	H		$COOCH_3$	13.57 ± 0.26	6.00 ± 0.00	6.00 ± 0.00	17.37 ± 0.32	22.47 ± 0.43
2f	CH_3		$COOCH_3$	11.20 ± 0.10	6.00 ± 0.00	6.00 ± 0.00	14.23 ± 0.06	19.70 ± 0.23
2g	Br		$COOCH_3$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
3a	H		$COO(CH_2)_2CH_3$	11.73 ± 0.26	27.27 ± 0.32	17.10 ± 0.24	16.30 ± 0.25	28.77 ± 0.42
3b	CH_3		$COO(CH_2)_2CH_3$	16.93 ± 0.15	29.87 ± 0.35	20.53 ± 0.30	17.73 ± 0.30	26.60 ± 0.35
4a	H		$COOCH(CH_3)_2$	11.87 ± 0.23	23.87 ± 0.30	10.47 ± 0.12	13.73 ± 0.15	26.67 ± 0.32
4b	CH_3		$COOCH(CH_3)_2$	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00

[a] The values indicate the average diameters in mm (of three trials) for the zone of growth inhibition observed after 24 h of incubation at 30 °C. [b] *C. n.*: *C. neoformans* ATCC 32264; *C. n. C.*: *C. neoformans clinical strain; C. a.*: *C. albicans* ATCC 10231; *E. c.*: *E. coli* ATCC 44752; *S. a.*: *S. aureus* ATCC 25923.

Table 2 Inhibition zones at different dose levels for compounds **1v**, **1w**, **2e**, **3a**, **3b** and **4a**

	(Zone of inhibition/mm)													
	1v				**1w**		**2e**	**3a**		**3b**			**4a**	
Dose (μg per disc)	*C. n.*	*C. n. C.*	*E. c.*	*S. a.*	*C. n.*	*C. n. C.*	*S. a.*	*C. n. C.*	*S. a.*	*C. n. C.*	*C. a.*	*S. a.*	*C. n. C.*	*S. a.*
200	25.20	28.97	20.67	21.93	21.63	24.23	22.47	27.27	28.77	29.87	20.53	26.60	23.87	26.67
100	24.73	27.50	20.33	21.47	20.00	23.97	19.63	21.23	12.27	21.20	15.40	14.27	14.03	12.77
50	22.27	27.30	19.23	20.90	18.67	23.23	16.93	18.13	11.60	20.03	12.97	12.47	12.13	10.77
25	19.63	21.83	18.90	17.70	17.90	22.93	6.50	6.00	10.00	13.23	7.97	10.37	6.00	6.00
12.5	16.67	17.60	14.73	14.73	17.03	20.37	6.00	6.00	6.00	6.00	6.00	9.03	6.00	6.00
6.25	15.70	16.87	7.93	6.93	14.80	17.03	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
3.125	13.20	13.53	6.00	6.00	13.20	13.63	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
1.5625	8.37	8.77	6.00	6.00	6.00	8.20	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00

Table 3 MIC, MIC_{80} and MFC values for **1v**, **1w** and fluconazole (μg mL^{-1})

	1v			1w			Fluconazole		
Fungal strain[a]	MIC	MIC_{80}	MFC	MIC	MIC_{80}	MFC	MIC	MIC_{80}	MFC
C.n	2.0	1.0	10.0	4.0	3.0	14.0	>128.0	2.0	>128.0
C. n. C.	6.0	5.0	14.0	3.0	1.5	12.0	>128.0	2.0	>128.0

[a] *C.n.*: *C. neoformans* ATCC 32264; *C. n. C.*: *C. neoformans clinical strain*.

The MIC and MFC determination

The MIC is defined as the minimum concentration of a compound required to exhibit complete inhibitions of bacterial and fungal growth. MIC_{80} was recorded as the concentration that produced 80% growth reduction compared to wells with no compound present. The MFC is the concentration at which fungi failed to grow in the liquid nutrient medium. Fluconazole and ciprofloxacin were used as the standard drugs against fungi and bacteria, respectively.[11] Compounds **1v** and **1w** maintained biological activities even at low concentration (1.5625 μg per disc). Compounds **1v** and **1w**, which had prominent antimicrobial activities, were subjected to further pharmacological evaluation, including determining MIC, MIC_{80}, and MFC using standard techniques.[10] The MIC, MIC_{80} and MFC values of the compounds tested are listed in Table 3.

To our surprise, compounds **1v** demonstrated excellent inhibitory activity against *C. neoformans* and emerged as the most effective antifungal agents with 64 times lower MIC (2 μg mL^{-1}) and 12.8 times lower MFC (10 μg mL^{-1}) than the reference drug fluconazole (MIC > 128.0 μg mL^{-1}, MFC > 128.0 μg mL^{-1}). It should be emphasized that compounds **1w** also exhibited especially interesting inhibitory activity against *C. neoformans* with 32 times lower MIC (4 μg mL^{-1}) and 9.1 times lower MFC (14 μg mL^{-1}) than the reference drug fluconazole (MIC > 128.0 μg mL^{-1}, MFC > 128.0 μg mL^{-1}). Furthermore, compounds **1v** and **1w** showed especially higher antifungal potential than the reference drug fluconazole against *C. neoformans* clinical strain. These results indicated that compounds **1v** and **1w** may become lead molecules of antifungal drug against *C. neoformans*.

In addition, **1v** had a MIC value of 40 μg mL^{-1} against both *S. aureus* and *E. coli* and demonstrated equipotent antibacterial activity with reference drug ciprofloxacin (MIC = 28 μg mL^{-1} for *S. aureus* and MIC = 36 μg mL^{-1} for *E. coli*). In general, compounds **1v** and **1w** demonstrated better antifungal activity than antibacterial activity. The antimicrobial activity of **1v** is better than that of **1w**.

Cytotoxicities of compounds 1v and 1w

To investigate whether the antimicrobial activities of compounds **1v** and **1w** related specifically to their selective toxicity toward the tested strains, we evaluated their cytotoxicities using a BV2 cell line and a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The cytotoxicity of

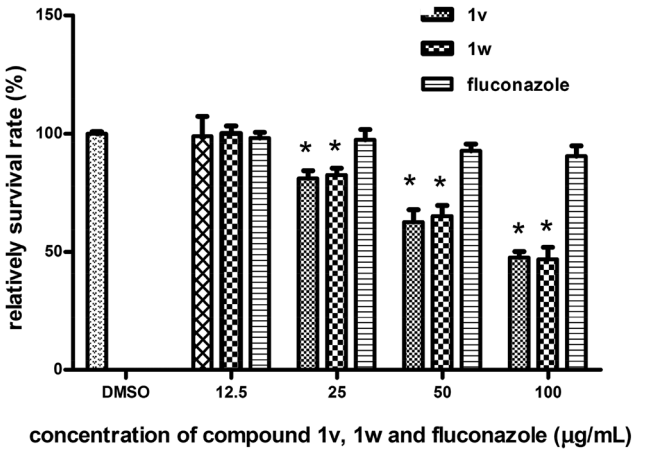


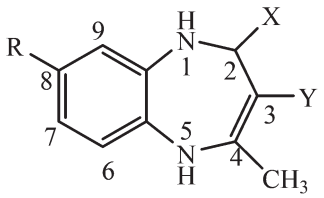
Fig. 3 Effects of compounds **1v**, **1w** and fluconazole on the survival of cells.

DMSO was used as a control. The results (Fig. 3) showed that compounds **1v** and **1w** had no cytotoxic activity at concentrations ≤12.5 μg mL^{-1} and low cytotoxicities within the range of 12.5–25 μg mL^{-1}, which was not significantly different from fluconazole. This indicated that compounds **1v** and **1w** did not affect the cell viability of the BV2 cell line at their MIC values (2 and 4 μg mL^{-1}) against the *C. neoformans* and MIC values (6 and 3 μg mL^{-1}) against the *C. neoformans* clinical strain, respectively. The compounds **1v** and **1w** also exhibited less effect on the survival of the BV2 cell line at their MIC values against the *S. aureus* and *E. coli*. But the relative survival rate of the cells significantly decreased with compounds **1v** and **1w** at concentrations ≥25.0 μg mL^{-1} ($p < 0.01$) which indicated that compounds **1v** and **1w** were cytotoxic at high concentrations. The disparity between the cytotoxicities and antibacterial activities of compounds **1v** and **1w** suggested that these compounds exhibited high *in vitro* antimicrobial activities at non-cytotoxic concentrations.

SAR

To analyze structure–activity relationships, three structural components were considered: the nature of the five-membered heterocycle nucleus (X) at the C_2 position, the length and size of the ester group chain (Y) at the C_3 position and the elec-

tronic properties of substitution (R) at the C_8 position.



The presence of a thienyl group at the C_2 position resulted in high selectivity and specificity to *C. albicans*. The introduction of the thiazole nucleus at the C_2 position also led to a significant increase of activity. The influence of X at the C_2 position was easily observed as compounds with the thiazole moiety (**1v** and **1w**) showed much superior activities over the thienyl group (**1a** and **1b**). The lowest MIC value (2.0 μg mL^{-1}) was obtained with **1v** which appeared as the most active compound against the *C. neoformans*. This value is 64 times lower than the control (fluconazole), indicating the powerful and very interesting antifungal potential of this structure on *C. neoformans*. The thiazole moiety in this type of 1,5-benzodiazepine plays a key role in enhancing the efficacy of the antimicrobial activity. Similar results have been reported in the literature.[12] Therefore, 2-thiazolyl may be a pharmacophore group in these compounds.

The types of thiophene ring (X) at the C_2 position and substituents also affected the activity of the compounds. Overall, when X = 3-thienyl, the compounds exhibited better activities compared with the activity observed when X = 2-thienyl. The introduction of an electron-donating group (CH_3) at the thiophene improved the antimicrobial activity of compounds against *C. albicans* slightly, but no improvement in the antibacterial activity of the compounds against the other tested microorganisms was observed. The role of the electron-donating group in improving antimicrobial activities has been reported previously.[13]

The ester group chain length and size at the C_3 position, on the other hand, appeared to be much more important for biological activity.

An increase in the ester group chain length [$-COOCH_3$, $-COOC_2H_5$, $-COOCH_2CH_2CH_3$] can increase the antibacterial activity and broad spectrum of the compounds. However, replacement of the small size *n*-propyl ester group ($-COOCH_2CH_2CH_3$) at the C_3 position with a bigger isoproply ester group ($COOCH(CH_3)_2$) resulted in a decrease in the antimicrobial activity.

The substituent (R = CH_3, H, F and Br) at the C_8 position plays a key role in varying the efficacy of antimicrobial activity. Compounds without a substituent (R = H) at the C_8 position provided the best antimicrobial action, which demonstrates that C_8, in the class of compounds studied, should be unsubstituted. The influence of the electronic properties of substitution (R) at the C_8 position was also important. It was noticed that compounds possessing an electron-donating group (CH_3) at the C_8 position are more active against all tested bacteria and fungi than compounds having electron-withdrawing substituents (F and Br). On the whole, the antibacterial screening results led to the assumption that the activity of the compounds was in the order R = –H > R = $-CH_3$ > R = –F > R = –Br. In addition, it seems that sizes of substituents are important. The compounds containing larger substituents (Br **1c**, **1f**) at the C_8 position are the least active. The electron-withdrawing group –Br at the C_8 position, which is larger than $-CH_3$, –H, increased the volume of the compound and lowered the electron density of the seven-membered ring, which resulted in reduced biological activity. Similar results have been reported previously.[14]

The observed antimicrobial activity of compounds could be attributed to the synergistic effect of the five-membered heterocycle nucleus, 1,5-benzodiazepine moieties and ester group as antimicrobial moieties.

Conclusions

In conclusion, 36 novel 1,5-benzodiazepine derivatives were synthesized by incorporating a five-membered S-heterocyclic group and an ester group and investigated for their antimicrobial properties and cytotoxicities with an anticipation of generating new structural leads serving as potent antimicrobial agents. Among the 36 synthesized compounds screened, compounds **1v**, **1w**, **2e**, **3a**, **3b** and **4a** exhibit remarkable antimicrobial activities at different concentrations. The results also show that most of the target compounds **1–4** with a thiazole ring exhibited considerable potency against all of the tested strains at microgram concentrations, especially compounds **1v** and **1w**. The most active compound **1v** (MIC: 2–6 μg mL^{-1} and MFC: 10–14 μg mL^{-1}) was found to be 21–64 times and 9–12.8 times more potent than fluconazole against *C. neoformans* and the *C. neoformans* clinical strain, respectively. Compound **1w** (MIC: 3–4 μg mL^{-1} and MFC: 10–14 μg mL^{-1}) was found to be 9–42.7 times more potent than fluconazole against *C. neoformans* and the *C. neoformans* clinical strain. Moreover, the MIC value (40 μg mL^{-1}) of **1v** against both *S. aureus* and *E. coli* indicates that it exhibits equipotent antibacterial activity compared to ciprofloxacin. Simultaneously, compounds **1v** and **1w** were accompanied with relatively low levels of cytotoxicity. Therefore, **1v** and **1w** are very attractive antimicrobial leads and should be excellent scaffolds for further study.

The structure–activity relationship (SAR) results indicated that compounds with a thiazole moiety at the C_2 position showed much superior activities over the thienyl group. The introduction of an electron-withdrawing group instead of an electron-donating group at the thiophene ring at the C_2 position was apparently detrimental to their activity. The variations of R at the C_8 position of the 1,5-benzodiazepine also have a great effect on the antimicrobial activity of these compounds. The activity based on different R groups against all of the microorganisms tested was in the order hydrogen (**1v**) > methyl (**1w**) > fluoro (**1y**) > bromine (**1x**). The results of the tested concentration gradients showed that $COOC_2H_5$ is the best substituent for the maintenance of biological activities at low concentrations (1.5625 μg per disc). Furthermore,

lengthening the carbon chain at the end of the ester group can increase the antibacterial activity and broaden the spectrum of the compounds. However, increasing the number of branched chains at the end may decrease or eliminate the activity of the compounds.

Findings from the SAR and toxicity studies have encouraged us to make some modifications to the basic structure of the obtained compounds **1v** and **1w** to achieve selective, more active and non-toxic derivatives in ongoing studies. In addition, the further investigations on these findings can be useful for medicinal chemists to synthesize similar compounds.

Experimental

General

The melting points (°C) were determined with a PXT-4 digital melting point apparatus. The ^{1}H NMR spectra were recorded on a Bruker AVANCE-500 MHz spectrometer; the chemical shifts (δ) are reported in ppm using $SiMe_4$ as the internal standard when measured in $CDCl_3$. The signal multiplicities are represented by s (singlet), d (doublet), t (triplet), m (multiplet), and q (quartet). Low-resolution mass spectra were recorded on a Thermo DSQ II mass spectrometer. The IR spectra (in KBr pellets) were recorded on a BIO-RAD PE-M-1730 IR spectrophotometer. The elemental analysis was performed with a Vario EL-III-CHN-0 Elemental Analyser. The structure of **1v** was further determined by single-crystal X-ray diffraction on a Bruker Smart-1000 diffractometer. Thiazol aldehyde, substituted thiophene aldehyde and *o*-PDA were purchased from Aladdin, and the other chemicals used were commercial products of analytical grade. The solvents were dried and purified according to the literature as necessary. The reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel GF254 plates.

Synthesis

Synthesis of *N*-*o*-aryl amino-β-enamino esters 5a–5j. A mixture of ethyl acetoacetate (methyl acetoacetate, acetyl propyl acetate or acetyl isopropyl acetate) (30 mmol) and various *o*-PDAs (*o*-PDA, 4-methyl-*o*-PDA or 4-bromo-*o*-PDA) (25 mmol) was stirred at room temperature for approximately 50 min. The solids were collected by filtration under reduced pressure to give the corresponding crude *N*-*o*-aryl amino-β-enamino esters **5a–5j** (~98%), which were used directly without further purification.

General procedure for the preparation of ethyl 4-methyl-2-(thiophen-2-yl, thiophen-3-yl or thiazol-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1a–1x)

Ethyl acetoacetate and *o*-PDA, 4-methyl-*o*-PDA or 4-bromo-*o*-PDA yield **5a–5c**. A mixture of compound **5a** (**5b** or **5c**) (2 mmol) and a substituted thiophene aldehyde or thiazol aldehyde (2 mmol) in dry ethanol (15 ml) was stirred at 0 °C for approximately 8 h. After the reaction was complete, the precipitate that formed was filtered, washed with ethanol and recrystallised from dry ethanol to yield **1a–1x**.

Ethyl 4-methyl-2-(thiophen-3-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1a). Yield: 82%, mp 52–54 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.49–7.09 (7H, m, $-C_6H_4$, $-C_4H_3S$), 5.96 (1H, s, –NH), 5.89 (1H, s, –CH), 4.41 (1H, s, –NH), 4.12 (2H, q, J = 7.5 Hz, $-COOCH_2$), 2.52 (3H, s, $-CH_3$), 1.21 (3H, t, J = 7.5 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.49, 25.32, 59.89, 60.46, 102.12, 110.45, 119.73, 121.31, 121.54, 122.42, 124.79, 129.23, 130.86, 136.03, 150.19, 151.31, 167.86 ppm. IR (KBr, cm^{-1}) ν = 3340 (N–H), 1674 (C═O), 1632 (C═C). MS (*m*/*z*): 315 $[M + H]^+$; $C_{17}H_{18}N_2O_2S$: found: C, 64.90; H, 5.79; N, 8.89; Calcd: C, 64.94; H, 5.77; N, 8.91.

Ethyl 4,8-dimethyl-2-(thiophen-3-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1b). Yield: 83%, mp 68–70 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.31–7.09 (6H, m, $-C_6H_3$, $-C_4H_3S$), 5.93 (1H, s, –NH), 5.87 (1H, s, –CH), 4.35 (1H, s, –NH), 4.12 (2H, q, J = 7.0 Hz, $-COOCH_2$), 2.50 (3H, s, $-CH_3$), 2.14 (3H, s, $-CH_3$), 1.22 (3H, t, J = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.64, 19.68, 25.29, 58.65, 59.62, 116.23, 119.19, 121.92, 122.31, 125.89, 126.47, 128.51, 131.15, 138.05, 150.69, 151.62, 161.91, 170.66 ppm. IR (KBr, cm^{-1}) ν = 3337 (N–H), 1672 (C═O), 1625 (C═C). MS (*m*/*z*): 329 $[M + H]^+$. $C_{18}H_{20}N_2O_2S$: found: C, 65.80; H, 6.15; N, 8.54; Calcd: C, 65.83; H, 6.14; N, 8.53.

Ethyl 8-bromo-4-methyl-2-(thiophen-3-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1c). Yield: 84%, mp 88–90 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.45–7.14 (6H, m, $-C_6H_3$, $-C_4H_3S$), 5.89 (1H, s, –NH), 5.86 (1H, s, –CH), 4.46 (1H, s, –NH), 4.13 (2H, q, J = 7.0 Hz, $-COOCH_2$), 2.50 (3H, s, $-CH_3$), 1.23 (3H, t, J = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.45, 24.87, 58.87, 59.88, 103.93, 114.81, 119.12, 120.58, 121.47, 122.11, 124.60, 129.47, 130.67, 137.06, 150.08, 151.51, 168.07 ppm. IR (KBr, cm^{-1}) ν = 3297 (N–H), 1630 (C═O), 1583 (C═C). MS (*m*/*z*): 393 $[M + H]^+$. $C_{17}H_{17}BrN_2O_2S$: found: C, 51.90; H, 4.37; N, 7.14; Calcd: C, 51.92; H, 4.36; N, 7.12.

Ethyl 4-methyl-2-(thiophen-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1d). Yield: 81.8%, mp 62–64 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.52–7.03 (7H, m, $-C_6H_4$, $-C_4H_3S$), 6.09 (1H, s, –CH), 6.04 (1H, s, –NH), 4.39 (1H, s, –NH), 4.13 (2H, q, J = 7.5 Hz, $-COOCH_2$), 2.53 (3H, s, $-CH_3$), 1.23 (3H, t, J = 7.5 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.45, 25.22, 56.41, 59.77, 103.29, 119.54, 121.46, 123.10, 124.12, 124.57, 126.24, 127.67, 132.29, 136.63, 148.46, 150.52, 167.81 ppm. IR (KBr, cm^{-1}) ν = 3341 (N–H), 1673 (C═O), 1629 (C═C). MS (*m*/*z*): 315 $[M + H]^+$. $C_{17}H_{18}N_2O_2S$: found: C, 64.98; H, 5.75; N, 8.89; Calcd: C, 64.94; H, 5.77; N, 8.91.

Ethyl 4,8-dimethyl-2-(thiophen-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1e). Yield: 82.6%, mp 74–76 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.34–7.03 (6H, m, $-C_6H_3$, $-C_4H_3S$), 6.07 (1H, s, –CH), 6.00 (1H, s, –NH), 4.33 (1H, s, –NH), 4.13 (2H, q, J = 7.5 Hz, $-COOCH_2$), 2.51 (3H, s, $-CH_3$), 2.15 (3H, s, $-CH_3$), 1.23 (3H, t, J = 7.5 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.46, 20.49, 25.33, 58.62, 59.69, 102.77, 119.07, 119.45, 121.65, 122.14, 124.08, 124.49,

128.97, 132.79, 136.38, 148.58, 152.61, 167.87 ppm. IR (KBr, cm^{-1}) ν = 3336 (N–H), 1670 (C═O), 1625 (C═C). MS (*m*/*z*): 329 $[M + H]^+$. $C_{18}H_{20}N_2O_2S$: found: C, 65.80; H, 6.12; N, 8.55; Calcd: C, 65.83; H, 6.14; N, 8.53.

Ethyl 8-bromo-4-methyl-2-(thiophen-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1f). Yield: 83.4%, mp 82–84 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.49–7.06 (6H, m, $-C_6H_3$, $-C_4H_3S$), 6.05 (1H, d, *J* = 6.0 Hz, –CH), 5.95 (1H, s, –NH), 4.42 (1H, d, *J* = 6.0 Hz, –NH), 4.14 (2H, q, *J* = 7.0 Hz, $-COOCH_2$), 2.51 (3H, s, $-CH_3$), 1.23 (3H, t, *J* = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.32, 25.01, 56.03, 59.87, 103.80, 114.94, 123.30, 124.00, 124.35, 124.59, 125.56, 126.32, 130.57, 137.88, 147.73, 150.15, 167.58 ppm. IR (KBr, cm^{-1}) ν = 3296 (N–H), 1629 (C═O), 1580 (C═C). MS (*m*/*z*): 393 $[M + H]^+$. $C_{17}H_{17}BrN_2O_2S$: found: C, 51.95; H, 4.35; N, 7.11; Calcd: C, 51.92; H, 4.36; N, 7.12.

Ethyl 4-methyl-2-(3-methylthiophen-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1g). Yield: 78.8%, mp 95–97 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.67–6.84 (6H, m, $-C_6H_4$, $-C_4H_2S$), 6.41 (1H, d, *J* = 6.5 Hz, –CH), 6.20 (1H, s, –NH), 4.31 (1H, d, *J* = 7.0 Hz, –NH), 4.08 (2H, q, *J* = 7.0 Hz, $-COOCH_2$), 2.53 (3H, s, $-CH_3$), 2.30 (3H, s, $-CH_3$), 1.17 (3H, t, *J* = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.40, 20.87, 20.91, 55.18, 59.72, 103.47, 117.98, 121.01, 121.81, 123.23, 124.67, 129.70, 132.66, 136.71, 142.18, 149.98, 151.97, 167.83 ppm. IR (KBr, cm^{-1}) ν = 3330 (N–H), 1626 (C═O), 1611 (C═C). MS (*m*/*z*): 329 $[M + H]^+$. $C_{18}H_{20}N_2O_2S$: found: C, 65.86; H, 6.16; N, 8.50; Calcd: C, 65.83; H, 6.14; N, 8.53.

Ethyl 4,8-dimethyl-2-(3-methylthiophen-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1h). Yield: 79.4%, mp 86–88 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.53–6.86 (5H, m, $-C_6H_3$, $-C_4H_2S$), 6.31 (1H, s, –CH), 6.23 (1H, s, –NH), 6.04 (1H, s, –NH), 4.09 (2H, q, *J* = 7.5 Hz, $-COOCH_2$), 2.53 (3H, s, $-CH_3$), 2.31 (3H, s, $-CH_3$), 2.18 (3H, s, $-CH_3$), 1.18 (3H, t, *J* = 7.5 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.10, 14.64, 19.68, 21.27, 50.16, 61.39, 84.82, 116.72, 117.67, 119.13, 121.72, 121.94, 123.14, 128.82, 130.98, 134.68, 148.07, 148.98, 167.12 ppm. IR (KBr, cm^{-1}) ν = 3353 (N–H), 1673 (C═O), 1620 (C═C). MS (*m*/*z*): 343 $[M + H]^+$. $C_{19}H_{22}N_2O_2S$: found: C, 66.66; H, 6.45; N, 8.15; Calcd: C, 66.64; H, 6.48; N, 8.18.

Ethyl 8-bromo-4-methyl-2-(3-methylthiophen-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1i). Yield: 80.4%, mp 58–60 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.79–6.94 (5H, m, $-C_6H_3$, $-C_4H_2S$), 6.71 (1H, d, *J* = 6.5 Hz, –NH), 6.56 (1H, d, *J* = 7.0 Hz, –CH), 6.04 (1H, s, –NH), 4.10 (2H, q, *J* = 7.0 Hz, $-COOCH_2$), 2.53 (3H, s, $-CH_3$), 2.30 (3H, s, $-CH_3$), 1.18 (3H, t, *J* = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.31, 18.15, 20.96, 54.88, 59.87, 88.39, 117.73, 120.00, 120.56, 122.27, 123.14, 124.45, 129.40, 130.58, 139.15, 143.57, 152.58, 166.17 ppm. IR (KBr, cm^{-1}) ν = 3311 (N–H), 1680 (C═O), 1633 (C═C). MS (*m*/*z*): 407 $[M + H]^+$. $C_{18}H_{19}BrN_2O_2S$: found: C, 53.05; H, 4.68; N, 6.91; Calcd: C, 53.08; H, 4.70; N, 6.88.

Ethyl 4-methyl-2-(5-methylthiophen-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1j). Yield: 80.0%, mp 90–92 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.42–6.85 (6H, m, $-C_6H_4$, $-C_4H_2S$), 6.12 (1H, s, –NH), 6.07 (1H, s, –CH), 4.30 (1H, s, –NH), 4.09 (2H, q, *J* = 7.0 Hz, $-COOCH_2$), 2.54 (3H, s, $-CH_3$), 1.20 (3H, t, *J* = 7. 0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.10, 15.97, 19.69, 50.17, 61.39, 85.17, 115.36, 117.02, 118.35, 123.35, 126.19, 127.39, 132.09, 142.27, 145.45, 150.26, 161.51, 167.12 ppm. IR (KBr, cm^{-1}) ν = 3335 (N–H), 1673 (C═O), 1638 (C═C). MS (*m*/*z*): 329 $[M + H]^+$. $C_{18}H_{20}N_2O_2S$: found: C, 65.85; H, 6.15; N, 8.55; Calcd: C, 65.83; H, 6.14; N, 8.53.

Ethyl 4,8-dimethyl-2-(5-methylthiophen-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1k). Yield: 80.2%, mp 66–68 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.43–6.89 (5H, m, $-C_6H_3$, $-C_4H_2S$), 6.40 (1H, s, –NH), 5.96 (1H, s, –CH), 4.30 (1H, s, –NH), 4.14 (2H, q, *J* = 7.0 Hz, $-COOCH_2$), 3.48 (3H, s, $-CH_3$), 2.49 (3H, s, $-CH_3$), 2.33 (3H, s, $-CH_3$), 1.23 (3H, t, *J* = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.66, 15.99, 20.76, 20.93, 50.78, 58.58, 86.48, 119.12, 119.16, 121.99, 123.77, 124.29, 125.24, 128.49, 132.78, 134.73, 149.18, 152.68, 169.89 ppm. IR (KBr, cm^{-1}) ν = 3346 (N–H), 1674 (C═O), 1646 (C═C). MS (*m*/*z*): 343 $[M + H]^+$. $C_{19}H_{22}N_2O_2S$: found: C, 66.66; H, 6.51; N, 8.16; Calcd: C, 66.64; H, 6.48; N, 8.18.

Ethyl 8-bromo-4-methyl-2-(5-methylthiophen-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1l). Yield: 81.8%, mp 60–62 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.37–6.72 (5H, m, $-C_6H_3$, $-C_4H_2S$), 5.97 (1H, s, –NH), 5.96 (1H, s, –CH), 4.28 (1H, s, –NH), 4.15 (2H, q, *J* = 7.0 Hz, $-COOCH_2$), 2.52 (3H, s, $-CH_3$), 2.21 (3H, s, $-CH_3$), 1.25 (3H, t, *J* = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.44, 15.36, 20.83, 56.22, 59.91, 103.85, 117.74, 120.03, 121.25, 123.28, 124.43, 128.33, 133.77, 138.09, 145.22, 150.09, 153.46, 167.72 ppm. IR (KBr, cm^{-1}) ν = 3296 (N–H), 1670 (C═O), 1647 (C═C). MS (*m*/*z*): 407 $[M + H]^+$. $C_{18}H_{19}BrN_2O_2S$: found: C, 53.12; H, 4.68; N, 6.90; Calcd: C, 53.08; H, 4.70; N, 6.88.

Ethyl 2-(3-bromothiophen-2-yl)-4-methyl-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1m). Yield: 81.6%, mp 132–134 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.55–6.90 (6H, m, $-C_6H_4$, $-C_4H_2S$), 6.32 (1H, s, –NH), 6.03 (1H, d, *J* = 6.0 Hz, –CH), 4.88 (1H, d, *J* = 6.0 Hz, –NH), 4.07 (2H, q, *J* = 7.0 Hz, $-COOCH_2$), 2.56 (3H, s, $-CH_3$), 1.16 (3H, t, *J* = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.37, 25.15, 56.36, 59.80, 101.68, 107.97, 119.47, 121.28, 122.08, 123.53, 123.73, 129.89, 132.49, 136.62, 143.22, 151.24, 167.58 ppm. IR (KBr, cm^{-1}) ν = 3311 (N–H), 1667 (C═O), 1627 (C═C). MS (*m*/*z*): 393 $[M + H]^+$. $C_{17}H_{17}BrN_2O_2S$: found: C, 51.90; H, 4.34; N, 7.11; Calcd: C, 51.92; H, 4.36; N, 7.12.

Ethyl 2-(3-bromothiophen-2-yl)-4,8-dimethyl-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1n). Yield: 83.4%, mp 130–132 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.36–6.93 (5H, m, $-C_6H_3$, $-C_4H_2S$), 6.06 (1H, s, –NH), 6.02 (1H, d, *J* = 5.0 Hz, –CH), 4.80 (1H, d, *J* = 5.0 Hz, –NH), 4.08 (2H, q, *J* = 7.0 Hz, $-COOCH_2$), 2.57 (3H, s, $-CH_3$), 2.13 (3H, s, $-CH_3$), 1.18 (3H, t, *J* = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.39, 20.52, 25.31, 56.21, 59.69, 101.30, 107.96, 119.29, 121.61, 122.72, 123.69, 124.34, 129.81, 133.27, 136.42, 143.30, 151.07, 167.55 ppm. IR (KBr, cm^{-1}) ν = 3296 (N–H), 1670 (C═O), 1647 (C═C). MS (*m*/*z*): 407 $[M + H]^+$. $C_{18}H_{19}BrN_2O_2S$: found: C, 53.11; H, 4.68; N, 6.90; Calcd: C, 53.08; H, 4.70; N, 6.88.

Ethyl 8-bromo-2-(3-bromothiophen-2-yl)-4-methyl-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1o). Yield: 84.8%, mp 72–74 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.84–6.95 (5H, m, $-C_6H_3$, $-C_4H_2S$), 6.70 (1H, d, J = 7.0 Hz, –NH), 6.54 (1H, d, J = 7.0 Hz, –CH), 6.01 (1H, s, –NH), 4.10 (2H, q, J = 7.0 Hz, $-COOCH_2$), 2.56 (3H, s, $-CH_3$), 1.17 (3H, t, J = 7.5 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.34, 25.08, 56.04, 59.94, 102.40, 108.27, 118.31, 120.53, 123.47, 123.89, 124.75, 129.02, 131.78, 137.93, 142.39, 150.57, 167.27 ppm. IR (KBr, cm^{-1}) ν = 3311 (N–H), 1670 (C═O), 1629 (C═C). MS (m/z): 473 $[M + H]^+$. $C_{17}H_{16}Br_2N_2O_2S$: found: C, 43.22; H, 3.43; N, 5.94; Calcd: C, 43.24; H, 3.42; N, 5.93.

Ethyl 2-(4-bromothiophen-2-yl)-4-methyl-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1p). Yield: 82.4%, mp 95–97 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.74–6.93 (6H, m, $-C_6H_4$, $-C_4H_2S$), 6.54 (1H, d, J = 7.0 Hz, –NH), 6.06 (1H, s, –CH), 6.02 (1H, s, –NH), 4.13 (2H, q, J = 7.0 Hz, $-COOCH_2$), 2.53 (3H, s, $-CH_3$), 1.22 (3H, t, J = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.45, 25.33, 56.27, 59.91, 102.48, 108.59, 119.73, 121.34, 121.63, 121.76, 123.39, 127.24, 131.26, 136.13, 149.77, 150.99, 167.61 ppm. IR (KBr, cm^{-1}) ν = 3311 (N–H), 1673 (C═O), 1627 (C═C). MS (m/z): 393 $[M + H]^+$. $C_{17}H_{17}BrN_2O_2S$: found: C, 51.90; H, 4.34; N, 7.11; Calcd: C, 51.92; H, 4.36; N, 7.12.

Ethyl 2-(4-bromothiophen-2-yl)-4,8-dimethyl-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1q). Yield: 83.7%, mp 70–72 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.35–6.93 (5H, m, $-C_6H_3$, $-C_4H_2S$), 6.02 (1H, s, –CH), 6.00 (1H, s, –NH), 4.33 (1H, s, –NH), 4.13 (2H, q, J = 7.0 Hz, $-COOCH_2$), 2.51 (3H, s, $-CH_3$), 2.17 (3H, s, $-CH_3$), 1.23 (3H, t, J = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.38, 20.52, 25.31, 56.21, 59.69, 101.30, 107.96, 119.30, 119.90, 121.61, 122.72, 123.69, 129.86, 133.27, 136.42, 143.30, 151.07, 167.55 ppm. IR (KBr, cm^{-1}) ν = 3313 (N–H), 1668 (C═O), 1621 (C═C). MS (m/z): 407 $[M + H]^+$. $C_{18}H_{19}BrN_2O_2S$: found: C, 53.08; H, 4.70; N, 6.88; Calcd: C, 53.10; H, 4.72; N, 6.90.

Ethyl 8-bromo-2-(4-bromothiophen-2-yl)-4-methyl-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1r). Yield 84.6%, mp 80–82 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.52–6.97 (5H, m, $-C_6H_3$, $-C_4H_2S$), 5.99 (1H, s, –NH), 5.98 (1H, s, –CH), 4.44 (1H, s, –NH), 4.14 (2H, q, J = 7.0 Hz, $-COOCH_2$), 2.51 (3H, s, $-CH_3$), 1.24 (3H, t, J = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.44, 25.11, 55.92, 60.12, 102.98, 108.73, 115.31, 120.94, 121.98, 123.35, 124.35, 127.35, 130.36, 137.52, 149.18, 150.95, 167.57 ppm. IR (KBr, cm^{-1}) ν = 3310 (N–H), 1672 (C═O), 1632 (C═C). MS (m/z): 475 $[M + H]^+$. $C_{17}H_{16}Br_2N_2O_2S$: found: C, 43.26; H, 3.42; N, 5.92; Calcd: C, 43.24; H, 3.42; N, 5.93.

Ethyl 2-(5-bromothiophen-2-yl)-4-methyl-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1s). Yield: 82.8%, mp 63–65 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.44–6.81 (6H, m, $-C_6H_4$, $-C_4H_2S$), 6.10 (1H, s, –CH), 5.99 (1H, s, –NH), 4.42 (1H, s, –NH), 4.15 (2H, q, J = 7.0 Hz, $-COOCH_2$), 2.53 (3H, s, $-CH_3$), 1.25 (3H, t, J = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.46, 25.32, 56.47, 59.91, 102.75, 110.51, 119.71, 121.28, 121.70, 123.41, 124.84, 129.20, 131.22, 136.23, 150.00, 150.89, 167.62 ppm. IR (KBr, cm^{-1}) ν = 3320 (N–H), 1630 (C═O), 1611 (C═C). MS (m/z): 395 $[M + H]^+$. $C_{17}H_{17}BrN_2O_2S$: found: C, 51.90; H, 4.36; N, 7.11; Calcd: C, 51.92; H, 4.36; N, 7.12.

Ethyl 2-(5-bromothiophen-2-yl)-4,8-dimethyl-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1t). Yield: 84.0%, mp 88–90 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.36–6.70 (5H, m, $-C_6H_3$, $-C_4H_2S$), 5.98 (1H, s, –NH), 5.96 (1H, s, –CH), 4.31 (1H, s, –NH), 4.14 (2H, q, J = 7.0 Hz, $-COOCH_2$), 2.50 (3H, s, $-CH_3$), 2.18 (3H, s, $-CH_3$), 1.23 (3H, t, J = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.48, 20.55, 25.34, 56.29, 59.83, 102.18, 119.68, 120.24, 121.53, 122.38, 124.78, 128.64, 129.21, 133.11, 136.03, 150.22, 151.14, 167.74 ppm. IR (KBr, cm^{-1}) ν = 3317 (N–H), 1645 (C═O), 1596 (C═C). MS (m/z): 409 $[M + H]^+$. $C_{18}H_{19}BrN_2O_2S$: found: C, 53.07; H, 4.72; N, 6.90; Calcd: C, 53.08; H, 4.70; N, 6.88.

Ethyl 8-bromo-2-(5-bromothiophen-2-yl)-4-methyl-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1u). Yield: 85.6%, mp 66–68 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.42–6.88 (5H, m, $-C_6H_3$, $-C_4H_2S$), 5.97 (1H, s, –NH), 5.94 (1H, s, –CH), 4.39 (1H, s, –NH), 4.13 (2H, q, J = 7.0 Hz, $-COOCH_2$), 2.49 (3H, s, $-CH_3$), 1.23 (3H, t, J = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.35, 24.93, 55.99, 59.99, 103.38, 110.65, 115.20, 120.84, 123.09, 124.13, 124.87, 125.80, 129.32, 137.57, 149.44, 150.76, 167.46 ppm. IR (KBr cm^{-1}) ν = 3319 (N–H), 1674 (C═O), 1648 (C═C). MS (m/z): 473 $[M + H]^+$. $C_{17}H_{16}Br_2N_2O_2S$: found: C, 43.25; H, 3.43; N, 5.92; Calcd: C, 43.24; H, 3.42; N, 5.93.

Ethyl 4-methyl-2-(thiazol-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1v). Yield: 83.8%, mp 166–168 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.58–7.64 (5H, m, $-C_6H_3$, $-C_3H_2NS$), 6.11 (1H, s, –NH), 6.15 (1H, s, –CH), 4.86 (1H, s, –NH), 4.13 (2H, q, J = 7.0 Hz, $-COOCH_2$), 2.60 (3H, s, $-CH_3$), 1.18 (3H, t, J = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.41, 25.38, 58.88, 59.85, 100.83, 118.59, 119.63, 121.64, 121.73, 123.53, 131.37, 136.71, 142.97, 152.17, 167.59, 175.08 ppm. IR (KBr cm^{-1}) ν = 3321 (N–H), 1680 (C═O), 1632 (C═C). MS (m/z): 316 $[M + H]^+$. $C_{16}H_{17}N_3O_2S$: found: C, 60.95; H, 5.44; N, 13.34; Calcd: C, 60.93; H, 5.43; N, 13.32.

Ethyl 4,8-dimethyl-2-(thiazol-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1w). Yield: 91.8%, mp 162–164 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.40–7.65 (5H, m, $-C_6H_3$, $-C_3H_2NS$), 6.16 (1H, s, –NH), 6.12 (1H, s, –CH), 4.81 (1H, s, –NH), 4.12 (2H, q, J = 7.0 Hz, $-COOCH_2$), 2.57 (3H, s, $-CH_3$), 2.12 (3H, s, $-CH_3$), 1.17 (3H, t, J = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.44, 20.44, 25.21, 58.68, 59.73, 100.16, 118.65, 119.65, 121.86, 122.36, 128.84, 133.20, 136.56, 142.89, 152.63, 167.71, 175.34 ppm. IR (KBr, cm^{-1}) ν = 3317 (N–H), 1674 (C═O), 1621 (C═C). MS (m/z): 330 $[M + H]^+$. $C_{17}H_{19}N_3O_2S$: found: C, 61.96; H, 5.83; N, 12.78; Calcd: C, 61.98; H, 5.81; N, 12.76.

Ethyl 8-bromo-4-methyl-2-(thiazol-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1x). Yield: 89.4%, mp 168–170 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.48–7.67 (5H, m, $-C_6H_3$, $-C_4H_2NS$), 6.12 (1H, s, –CH), 6.11 (1H, s, –NH), 4.91 (1H, s, –NH), 4.13 (2H, q, J = 7.0 Hz, $-COOCH_2$), 2.57 (3H, s, $-CH_3$),

1.18 (3H, t, J = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.30, 24.90, 58.70, 59.96, 102.27, 113.34, 118.64, 121.99, 122.81, 126.00, 132.65, 135.95, 143.04, 151.44, 167.34, 174.44 ppm. IR (KBr, cm^{-1}) ν = 3340 (N–H), 1670 (C═O), 1625 (C═C). MS (m/z): 394 $[M + H]^+$. $C_{16}H_{16}BrN_3O_2S$: found: C, 48.76; H, 4.10; N, 10.68; Calcd: C, 48.74; H, 4.09; N, 10.66.

Ethyl 8-fluoro-4-methyl-2-(thiazol-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (1y). Yield: 89.7%, mp 168–169 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.33–7.66 (5H, m, $-C_6H_3$, $-C_4H_2NS$), 6.16 (1H, s, –CH), 6.12 (1H, d, J = 6.5 Hz, –NH), 5.01 (1H, d, J = 6.0 Hz, –NH), 4.12 (2H, q, J = 5.0 Hz, $-COOCH_2$), 2.57 (3H, s, $-CH_3$), 1.17 (3H, t, J = 7.0 Hz, $-CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 14.39, 25.31, 58.64, 59.90, 100.52, 118.69, 120.52, 127.65, 138.25, 138.33, 143.13, 152.08, 157.93, 159.86, 167.47, 174.65 ppm. IR (KBr, cm^{-1}) ν 3356 (N–H), 1670 (C═O), 1616 (C═C). MS (m/z): 334 $[M + H]^+$. found: C, 57.67; H, 4.86; N, 12.62; Calcd: C, 57.64; H, 4.84; N, 12.60.

General procedure for the preparation of methyl 4-methyl-2-(thiophen-2-yl, thiophen-3-yl or thiazol-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (2a–2g)

Methyl acetoacetate and *o*-PDA, 4-methyl-*o*-PDA or 4-bromo-*o*-PDA yield **5d–5f**. A mixture of compound **5d** (**5e** or **5f**) (2 mmol) and thiophene aldehyde or thiazol aldehyde (2 mmol) in dry ethanol (15 ml) was stirred in an ice-salt bath for approximately 5 h. After the reaction was complete, the precipitate that formed was filtered, washed with cool ethanol and recrystallised from dry ethanol to yield **2a–2g**.

Methyl 4-methyl-2-(thiophen-3-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (2a). Yield: 80.9%, mp 78–80 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.49–7.10 (7H, m, $-C_6H_4$, $-C_4H_3S$), 6.00 (1H, s, –NH), 5.88 (1H, s, –CH), 4.49 (1H, s, –NH), 3.66 (3H, s, $-CH_3$), 2.53 (3H, s, $-CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 25.24, 51.15, 56.56, 103.06, 118.49, 119.37, 121.22, 121.48, 123.05, 131.43, 136.74, 143.55, 145.02, 150.55, 151.87, 168.53 ppm. IR (KBr, cm^{-1}) ν = 3343 (N–H), 1684 (C═O), 1638 (C═C). MS (m/z): 301 $[M + H]^+$. $C_{16}H_{16}N_2O_2S$: found: C, 63.96; H, 5.38; N, 9.36; Calcd: C, 63.98; H, 5.37; N, 9.33.

Methyl 4,8-dimethyl-2-(thiophen-3-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (2b). Yield: 81.6%, mp 107–109 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.31–7.10 (6H, m, $-C_6H_3$, $-C_4H_3S$), 5.94 (1H, s, –CH), 5.86 (1H, s, –NH), 4.37 (1H, s, –NH), 3.65 (3H, s, $-CH_3$), 2.51 (3H, s, $-CH_3$), 2.14 (3H, s, $-CH_3$) ppm. ^{13}C NMR (DMSO, 125 MHz) δ = 19.03, 20.69, 23.84, 55.20, 56.50, 101.39, 120.00, 120.36, 121.28, 125.83, 128.19, 129.17, 131.14, 138.69, 146.73, 152.72, 168.45 ppm. IR (KBr, cm^{-1}) ν = 3315 (N–H), 1673 (C═O), 1631 (C═C). MS (m/z): 315 $[M + H]^+$. $C_{17}H_{18}N_2O_2S$: found: C, 64.97; H, 5.75; N, 8.93; Calcd: C, 64.94; H, 5.77; N, 8.91.

Methyl 4-methyl-2-(thiophen-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (2c). Yield: 80.8%, mp 174–176 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.53–7.03 (7H, m, $-C_6H_4$, $-C_4H_3S$), 6.07 (1H, s, –CH), 6.05 (1H, s, –NH), 4.40 (1H, s, –NH), 3.67 (3H, s, $-CH_3$), 2.54 (3H, s, $-CH_3$) ppm. ^{13}C NMR (DMSO, 125 MHz) δ = 19.04, 23.81, 55.07, 56.50, 102.34, 120.20, 120.61, 122.67, 124.59, 124.69, 126.87, 131.65, 138.41, 150.19, 153.13, 168.14 ppm. IR (KBr, cm^{-1}) ν = 3262 (N–H), 1680 (C═O), 1632 (C═C). MS (m/z): 301 $[M + H]^+$. $C_{16}H_{16}N_2O_2S$: found: C, 64.01; H, 5.35; N, 9.36; Calcd: C, 63.98; H, 5.37; N, 9.33.

Methyl 4,8-dimethyl-2-(thiophen-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (2d). Yield: 81.4%, mp 94–96 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.34–7.03 (6H, m, $-C_6H_3$, $-C_4H_3S$), 6.06 (1H, s, –CH), 6.02 (1H, s, –NH), 4.34 (1H, s, –NH), 3.66 (3H, s, $-CH_3$), 2.52 (3H, s, $-CH_3$), 2.15 (3H, s, $-CH_3$) ppm. ^{13}C NMR (DMSO, 125 MHz) δ = 20.69, 23.87, 50.99, 54.82, 101.89, 120.23, 120.67, 120.75, 124.58, 124.63, 126.88, 129.13, 131.43, 138.22, 150.33, 153.18, 168.13 ppm. IR (KBr, cm^{-1}) ν = 3316 (N–H), 1674 (C═O), 1617 (C═C). MS (m/z): 315 $[M + H]^+$. $C_{17}H_{18}N_2O_2S$: found: C, 64.94; H, 5.76; N, 8.94; Calcd: C, 64.94; H, 5.77; N, 8.91.

Methyl 4-methyl-2-(thiazol-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (2e). Yield: 81.6%, mp 172–174 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.57–7.65 (6H, m, $-C_6H_4$, $-C_4H_2NS$), 6.27 (1H, s, –NH), 6.12 (1H, d, –CH), 4.92 (1H, d, –NH), 3.64 (3H, s, $-CH_3$), 2.59 (3H, s, $-CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 25.40, 51.27, 58.76, 100.60, 118.67, 119.67, 121.64, 121.72, 123.59, 131.22, 136.70, 143.01, 152.52, 168.03, 174.97 ppm. IR (KBr, cm^{-1}) ν = 3306 (N–H), 1693 (C═O), 1638 (C═C). MS (m/z): 302 $[M + H]^+$. $C_{15}H_{15}N_3O_2S$: found: C, 59.81; H, 5.01; N, 13.96; Calcd: C, 59.78; H, 5.02; N, 13.94.

Methyl 4,8-dimethyl-2-(thiazol-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (2f). Yield: 85.6%, mp 170–172 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.40–7.66 (5H, m, $-C_6H_3$, $-C_4H_2NS$), 6.12 (1H, s, –CH), 6.11 (1H, s, –NH), 4.81 (1H, d, –NH), 3.65 (3H, s, $-CH_3$), 2.59 (3H, s, $-CH_3$), 2.12 (3H, s, $-CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 20.45, 25.40, 51.20, 58.56, 100.04, 118.70, 119.59, 121.90, 122.39, 128.62, 133.33, 136.49, 142.97, 152.73, 168.08, 175.14 ppm. IR (KBr, cm^{-1}) ν = 3318 (N–H), 1701 (C═O), 1625 (C═C). MS (m/z): 316 $[M + H]^+$. $C_{16}H_{17}N_3O_2S$: found: C, 60.96; H, 5.40; N, 13.34; Calcd: C, 60.93; H, 5.43; N, 13.32.

Methyl 8-bromo-4-methyl-2-(thiazol-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (2g). Yield: 84.8%, mp 180–182 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.10–6.50, 7.07–7.68 (5H, m, $-C_6H_3$, $-C_4H_2NS$), 6.81 (1H, d, –CH), 6.76 (1H, s, –NH), 4.92 (1H, d, –NH), 3.66 (3H, s, $-CH_3$), 2.58 (3H, s, $-CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 25.35, 51.38, 58.49, 101.75, 118.83, 120.75, 122.00, 122.90, 123.86, 124.41, 138.00, 143.30, 152.04, 167.78, 174.31 ppm. IR (KBr, cm^{-1}) ν = 3255 (N–H), 1675 (C═O), 1638 (C═C). MS (m/z): 382 $[M + H]^+$. $C_{15}H_{14}BrN_3O_2S$: found: C, 47.40; H, 3.70; N, 11.07; Calcd: C, 47.38; H, 3.71; N, 11.05.

General procedure for the preparation of propyl 4-methyl-2-(thiazol-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (3a–3b)

Propyl acetoacetate and *o*-PDA, 4-methyl-*o*-PDA or 4-bromo-*o*-PDA yield **5g–5h**. A mixture of compound **5g** or **5h** (2 mmol) and thiazol aldehyde (2 mmol) in ethanol (15 ml) was stirred in an ice-salt bath for approximately 7 h. After the reaction was

complete, the precipitate that formed was filtered, washed with ethanol and recrystallised from ethanol to yield products **3a** and **3b**.

Propyl 4-methyl-2-(thiazol-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (3a). Yield: 82.8%, mp 154–156 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.56–7.64 (6H, m, $-C_6H_4$, $-C_3H_2NS$), 6.22 (1H, s, –NH), 6.14 (1H, d, J = 6.0 Hz, –CH), 4.90 (1H, d, J = 6.0 Hz, –NH), 4.01 (2H, t, J = 6.5 Hz, $-CH_2CH_2CH_3$), 2.59 (3H, s, $-CH_3$), 1.52–1.60 (2H, m, $-CH_2CH_2CH_3$), 0.81 (3H, t, J = 7.5 Hz, $-CH_2CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 10.39, 22.04, 25.33, 58.88, 65.50, 100.66, 118.41, 119.50, 121.58, 121.70, 123.48, 131.37, 136.59, 132.87, 151.92, 167.51, 175.02 ppm. IR (KBr, cm^{-1}) ν = 3304 (N–H), 1734 (C═O), 1668 (C═C). MS (m/z): 330 $[M + H]^+$. $C_{17}H_{19}N_3O_2S$: found: C, 61.94; H, 5.82; N, 12.78; Calcd: C, 61.98; H, 5.81; N, 12.76.

Propyl 4,8-dimethyl-2-(thiazol-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (3b). Yield: 83.6%, mp 120–122 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.38–7.64 (5H, m, $-C_6H_3$, $-C_4H_2S$), 6.17 (1H, s, –NH), 6.12 (1H, s, –CH), 4.82 (1H, s, –NH), 4.00 (2H, t, J = 6.5 Hz, $-CH_2CH_2CH_3$), 2.56 (3H, s, $-CH_3$), 2.11 (3H, s, $-CH_3$), 1.54–1.58 (2H, m, $-CH_2CH_2CH_3$), 0.81 (3H, t, J = 7.0 Hz, $-CH_2CH_2CH_3$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 10.40, 22.05, 25.17, 58.69, 65.40, 99.96, 118.47, 119.52, 121.80, 122.33, 128.84, 133.16, 136.44, 142.79, 152.39, 167.64, 175.29 ppm. IR (KBr, cm^{-1}) ν = 3343 (N–H), 1671 (C═O), 1656 (C═C). MS (m/z): 344 $[M + H]^+$. $C_{18}H_{21}N_3O_2S$: found: C, 62.98; H, 6.18; N, 12.24; Calcd: C, 62.95; H, 6.16; N, 12.23.

General procedure for the preparation of isopropyl 4-methyl-2-(thiazol-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (4a–4b)

Isopropyl acetoacetate and *o*-PDA, 4-methyl-*o*-PDA or 4-bromo-*o*-PDA yield **5i–5j**. A mixture of compound **5i** or **5j** (2 mmol) and thiazol aldehyde (2 mmol) in ethanol (15 ml) was stirred in an ice-salt bath for approximately 8 h. After the reaction was complete, the precipitate that formed was filtered, washed with ethanol and recrystallised from ethanol to yield products **4a** and **4b**.

Isopropyl 4-methyl-2-(thiazol-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (4a). Yield: 81.8%, mp 168–170 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.58–7.63 (6H, m, $-C_6H_4$, $-C_3H_2NS$), 6.19 (1H, s, –CH), 6.13 (1H, s, –NH), 4.99 (1H, t, J = 5.0 Hz, $-CH(CH_3)_2$), 4.90 (1H, s, –NH), 2.58 (3H, s, $-CH_3$), 1.21 (3H, d, J = 5.0 Hz, $-CH(CH_3)_2$), 1.06 (3H, d, J = 5.0 Hz, $-CH(CH_3)_2$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 25.12, 58.98, 66.81, 100.80, 118.43, 119.57, 121.54, 123.37, 126.47, 128.29, 131.54, 136.68, 142.78, 151.90, 167.03, 175.26 ppm. IR (KBr, cm^{-1}) ν = 3326 (N–H), 1730 (C═O), 1669 (C═C). MS (m/z): 330 $[M + H]^+$. $C_{17}H_{19}N_3O_2S$: found: C, 62.02; H, 5.83; N, 12.78; Calcd: C, 61.98; H, 5.81; N, 12.76.

Isopropyl 4,8-dimethyl-2-(thiazol-2-yl)-2,5-dihydro-1,5-benzodiazepine-3-carboxylate (4b). Yield: 82.4%, mp 150–152 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 6.37–7.73 (5H, m, $-C_6H_3$, $-C_3H_2NS$), 6.12 (1H, s, –NH), 5.59 (1H, d, –CH), 4.76 (1H, t, J = 6.0 Hz, $-CH(CH_3)_2$), 4.52 (1H, d, J = 4.0 Hz, –NH), 1.96 (3H, s, $-CH_3$), 2.21 (3H, s, $-CH_3$), 0.97 (3H, d, J = 6.5 Hz, $-CH(CH_3)_2$), 0.85 (3H, d, J = 6.5 Hz, $-CH(CH_3)_2$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 10.49, 20.44, 22.15, 25.33, 58.79, 65.51, 100.10, 118.55, 119.58, 121.91, 122.44, 128.91, 133.28, 136.52, 142.88, 152.19, 152.40, 167.74, 175.39 ppm. IR (KBr, cm^{-1}) ν = 3273 (N–H), 1667 (C═O), 1635 (C═C). MS (m/z): 344 $[M + H]^+$. $C_{18}H_{21}N_3O_2S$: found: C, 62.97; H, 6.18; N, 12.23; Calcd: C, 62.95; H, 6.16; N, 12.23.

Biological evaluations

Cytotoxicity assays was performed using an Epoch microplate spectrophotometer (BioTek, USA). Membrane permeability was measured using a DDS-307 conductivity meter.

Evaluation of cytotoxicity

The BV2 cells were cultured at 37 °C in 96-well plates at a density of 4×10^4 cells per well with 5% CO_2 in Dulbecco's Modified Eagle Medium (Invitrogen, GIBCO) supplemented with 10% fetal bovine serum (Invitrogen) for 72 h. Compounds **1v**, **1w** and fluconazole were added to each well at final concentrations of 10.0, 20.0, 50.0 and 100.0 μg mL^{-1}. An equivalent volume of DMSO without the compound was added as a control. The cells were incubated for 48 h. Subsequently, 10 μL of aqueous MTT solution (5.0 mg mL^{-1}) and the mixture were incubated at 37 °C for 4 h. The MTT solution was carefully decanted off, and 100 μL of DMSO was added to each well. The color was measured with an Epoch microplate spectrophotometer at 490 nm with the reference filter set to 620 nm. All MTT assays were repeated three times. Each measurement contained six parallel treatments (wells).

Relative survival rate = (treatment A490/negative control A620) × 100%.

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