Full Paper

Synthesis and Anti-tumor Activities of Novel Methylthio-, Sulfinyl-, and Sulfonyl-8*H*-thieno[2,3-*b*]pyrrolizin-8-oximino Derivatives

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A series of novel methylthio-, sulfinyl-, and sulfonyl-8*H*-thieno[2,3-*b*]pyrrolizin-8-oximino derivatives **7A–12P** was designed and synthesized as anti-tumor agents. Their structures were confirmed by IR, ^{1}H-NMR, MS, and elemental analysis. The anti-tumor activities of all the target compounds were tested by the MTT method *in vitro* against Bel-7402 (human liver cancer) and HT-1080 (human fibro sarcoma) cell lines. Among them, compound **11N** (IC_{50} = 18.2 μM, 8.2 μM), was the most promising compound of all synthesized molecules, it was 2.5- and 3.3-times more active than cisplatin (IC_{50} = 45.2 μM, 26.7 μM), respectively.

Keywords: Anti-tumor activities / Synthesis / Thieno[2,3-*b*]pyrrolizines

Received: March 6, 2007; accepted: June 1, 2007

DOI 10.1002/ardp.200700044

Introduction

Cancer is a growing public problem estimated to have worldwide about seven million new incidences per year. Research oriented towards the discovery of a new generation of agents useful in anticancer chemotherapy has resulted in the identification of various more potent compounds. Among them, sulfur-containing compounds, such as thiazole, thiophene, thioether, sulfonamide, sulfones, and thio-heterocyclic derivatives demonstrated excellent anti-tumor activities. They have been shown to function by different mechanisms of action, including cyclin-dependent kinase inhibition [1], DNA photocleavage [2–4], inhibition of Bcl-2 protein [5], and inhibition of tubulin polymerization [6]. It is noted that thieno[2,3-*b*]pyrrolizines, a new tripentacyclic skeleton, attract increasing interest due to their diverse pharmacological effects, for instance, as CDK/GSK-3 inhibitors [7], 5-HT_3 and/or 5-HT_{2C} agonists [8, 9], and anti-tumor agents [10]. Recently, 3-arylthieno[2,3-*b*]pyrrolizin-8-one derivatives **1** acting as anti-tubulin inhibitors were found to possess good cell-growth inhibitory activity over a large panel of human tumoral cell lines, including the resistant KB-A1 cell line [11]. These features make the thieno[2,3-*b*]pyrrolizines potential leads for new anti-tumor agents. And the structure-activity relationship (SAR) of **1** indicated that the attachment of a phenyl-bearing, small substituent in the *meta* and *para* position to position-3 on the tripentacyclic skeleton was essential for cytotoxicity.

With the aim of searching for new anti-tumor agents and understanding their SARs, we designed and synthesized a novel series of 2-alkylthio-, sulfinyl- and sulfonyl-3-aryl-8*H*-thieno[2,3-*b*]pyrrolizin-8-oximino derivatives (Fig. 1), focusing our attention on modifying position-2 and position-8 on the thieno[2,3-*b*]pyrrolizine scaffold. Alkylthio/sulfinyl/sulfonyl groups, which proved to be effective moieties for the potent inhibition of tubulin polymerization [6, 12], were introduced at position-2. While various hydrophilic aminoalkoxyimino branching chains, which were then replaced with aminoalkoxymethyloxyimino substituents for further exploring the SAR, were introduced at position-8. In light of the SAR mentioned above, a 4-nitrophenyl group was attached to position-3 for preliminary study. Anti-tumor activities of all

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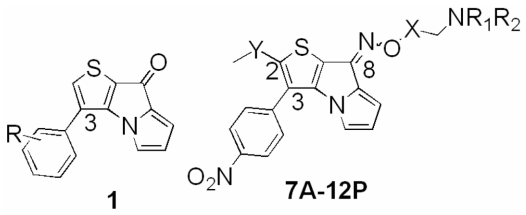


Figure 1. Structure of thieno[2,3-*b*]pyrrolizin-8-oximino derivatives **7A-12P**.

synthesized compounds were evaluated by the MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] method *in vitro*.

Results and discussion

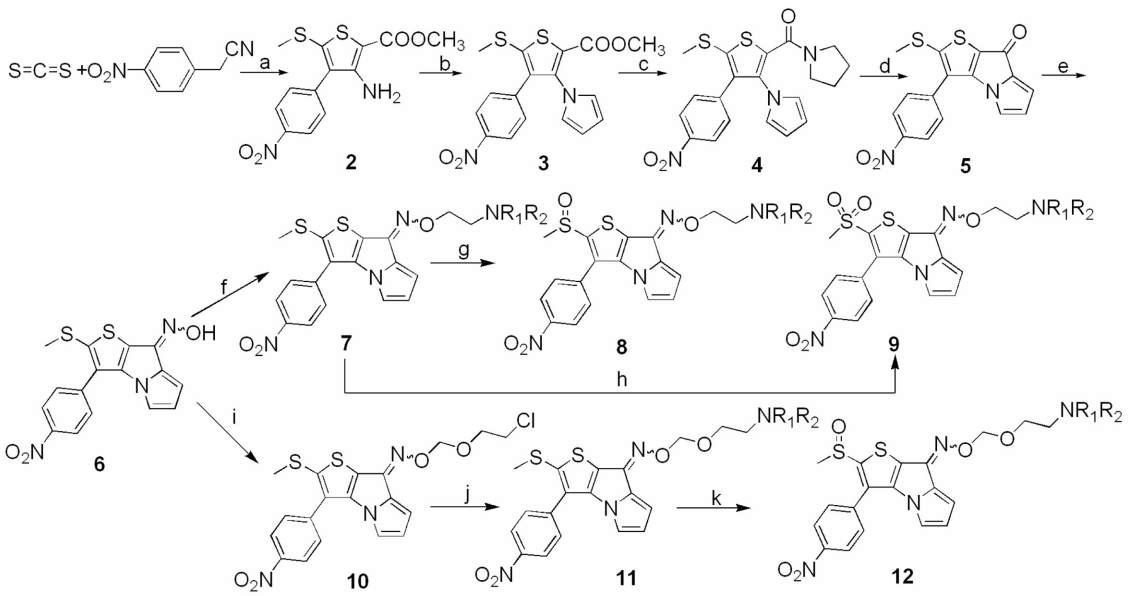
Chemistry

The title compounds methylthio-, sulfinyl- and sulfonyl-8*H*-thieno[2,3-*b*]pyrrolizin-8-oximino derivatives were obtained as described in Scheme 1. The substituents of compounds **7A-12P** are listed in Table 1.

The preparation of thiophene intermediate **2** was performed in a one-pot reaction by treatment of (4-nitrophenyl)acetonitrile with carbon disulfide in the presence of sodium hydroxide in THF, via an intermediate iminium salt, followed by successively reacting with methyl 2-chloroacetate and methyl iodide in 53% yields [13]. Treatment of **2** with 2,5-dimethoxytetrahydrofuran in acetic acid, according to the Clauson–Kaas method [14], afforded the pyrrolylthiophene carboxylate **3**, which was converted into the corresponding carboxamide **4** in pyrrolidine at 70°C. Cyclization of **4** in phosphorus oxychloride yielded an iminium chloride intermediate, which was treated with an aqueous solution of sodium hydroxide to yield the tricyclic ketone **5**. Reaction of the latter compound with hydroxylamine hydrochloride in pyridine furnished the oxime **6** generally in a mixture of *Z*- and *E*-forms without separation. By treatment of **6** with appropriate aminoalkyl chloride prepared as published [15, 16] and potassium carbonate, the oxime derivatives **7A-F** were obtained.

To further investigate the effects of the substituents at position-8 of the tricyclic core, we introduced the aminoalkoxymethyloxyimino groups by a two-step sequence involving the addition of the oxime **6** to the 1-chloro-2-(chloromethoxy)ethane in the presence of sodium methanolate to give 8-(2-chloroethyloxymethyloxyimino)-2-methylthio-3-(4-nitrophenyl)-8*H*-thieno[2,3-*b*]pyrrolizine **10** and then reaction with various secondary amines to afford the desired compounds **11K-O**.

The sulfur derivatives **7** and **11** were oxidized into the corresponding sulfoxides **8G-H** and **12P** by one equivalent of sodium perborate in acetic acid at room temperature. However, the sulfone derivatives were difficult to accomplish here with the above oxidant as well as MCPBA (m-chloroperoxybenzoic acid), because the nitrogen at position-8 would also be oxidized to form an unde-



Reagents and conditions: a) (i) NaOH / THF, r.t., (ii) $ClCH_2COOCH_3/CH_3I$, −20°C; b) dimethoxy THF/CH_3COOH, 70°C; c) pyrrolidine, 70°C; d) (i) $POCl_3$, 80°C, (ii) 10% NaOH, 60°C; e) $NH_2OH \times HCl$, pyridine, 90°C; f) $ClCH_2CH_2NR_1R_2 \times HCl/K_2CO_3$, 70°C; g) $NaBO_3 \times 4\ H_2O/CH_3COOH$, 25~30°C; h) $H_2O_2/Na_2WO_4 \times 2\ H_2O/CH_3OH$, 20°C; i) $ClCH_2OCH_2CH_2Cl/CH_3ONa$, 25°C; j) HNR_1R_2/NaOH/DMF, 60°C; k) $NaBO_3 \times 4\ H_2O/CH_3COOH$, 25~30°C.

Scheme 1. Synthesis of target compounds **7A-12P**.

Table 1. The substituents of compounds **7A-12P**.

Compound	X	Y	NR_1R_2
7A	$-CH_2-$	-S-	morpholinyl
7B	$-CH_2-$	-S-	diethylamino
7C	$-CH_2-$	-S-	pyrrolidinyl
7D	$-CH_2-$	-S-	dimethylamino
7E	$-CH_2-$	-S-	piperdinyl
7F	$-CH_2-$	-S-	4-methylpiperazinyl
8G	$-CH_2-$	-SO-	morpholinyl
8H	$-CH_2-$	-SO-	diethylamino
9I	$-CH_2-$	$-SO_2-$	morpholinyl
9J	$-CH_2-$	$-SO_2-$	diethylamino
11K	$-CH_2OCH_2-$	-S-	pyrrolidinyl
11L	$-CH_2OCH_2-$	-S-	dimethylamino
11M	$-CH_2OCH_2-$	-S-	piperdinyl
11N	$-CH_2OCH_2-$	-S-	4-methylpiperazinyl
11O	$-CH_2OCH_2-$	-S-	morpholinyl
12P	$-CH_2OCH_2-$	-SO-	morpholinyl

sired *N*-oxidized side-product. After an extensive survey of the different reagents available, we have observed that H_2O_2/Na_2WO_4 is very effective for oxidizing sulfide into sulfone to furnish the compounds **9I-J** in excellent yield and high purity.

The *Z*/*E*-isomerism of oxime **6** and oxime derivatives **7A-12P** were elucidated by ^{1}H-NMR spectroscopy [8, 9]. The most pronounced changes were observed in the chemical shifts of the H-7 and SCH_3-2 protons of the thienopyrrolizine ring in the *Z*- and *E*-isomers. (*Z*)-Oxime **6** showed a signal at 6.55 ppm, for H-7. In contrast, (*E*)-oxime **6** exhibited the H-7 signal at a lower field, 6.80 ppm, owing to the deshielding effect of the hydroxyl group [17] on the H-7. On the other hand, the chemical shift of the SCH_3-2 of (*Z*)-**6** is influenced by the hydroxyl group and the signal appeared downfield at 2.48 ppm, whereas that of the (*E*)-**6** showed the signal at 2.43 ppm. Similar results were observed in the ^{1}H-NMR of the oxime derivatives **7A-12P**. The H-7 proton of *Z*-form showed around 6.53-6.64 ppm, whereas that of the *E*-form around 6.69–6.82 ppm, in good agreement with those of oxime **6**. The *Z*/*E*-isomeric ratios were estimated from the integration of the resonance signals.

Assay for anti-tumor activity

Compounds **5-12P** were evaluated for anti-tumor activities *in vitro* by the MTT method against Bel-7402 (human liver cancer) and HT-1080 (human fibro sarcoma) cell lines using cisplatin as positive control, and the results expressed as IC_{50} were summarized in Table 2. As shown, most of the evaluated compounds exhibited good anti-tumor activities, and some were superior to cisplatin. The *O*-substituted oximino-pyrrolizines exhibited more potent anti-tumor activities than the corresponding tripentone **5** and the oxime **6** in most cases.

Table 2. The anti-tumor activities of compounds **5-12P**.

Compound	IC_{50} (μM)	
	Bel-7402	**HT-1080**
5	195.9	193.1
6	177.8	184.8
7A	124.7	198.1
7B	31.1	31.1
7C	23.1	18.4
7D	37.7	21.2
7E	59.1	28.6
7F	31.4	34.9
8G	178.9	289.3
8H	55.5	48.0
9I	263.3	276.5
9J	298.4	316.3
11K	69.6	24.4
11L	26.4	25.5
11M	27.2	68.0
11N	18.2	8.3
11O	98.3	61.8
12P	145.8	89.3
Cisplatin	45.2	26.7

Among the compounds **7A-7F**, **7C** displayed the most potent anti-tumor activity against the two cancer cell lines. Nevertheless, **7A**, which possessed the morpholino substituent at the amino alkoxy moiety of position-8, was 6- or 10-times less active than **7C**. Other molecules **7B**, **7D-F** also exhibited comparable efficacy to the positive control cisplatin. It seemed that this region could tolerate a hindered secondary amine, but the morpholino group abolished the anti-tumor activity. In another set of **11K-O**, 8-[2-(4-methylpiperazinyl)ethyloxymethyloxyimino]-2-methylthio-3-(4-nitrophenyl)-8*H*-thieno[2,3-*b*]pyrrolizine **11N** showed IC_{50} = 18.2 μM, 8.2 μM, 2.5- and 3.3-times more active than cisplatin (IC_{50} = 45.2 μM, 26.7 μM), respectively and it was the most promising compound of all the synthesized analogues. Except for **11K**, the anti-tumor activity against the Bel-7402 cell line was improved by inserting methylene oxy group into the amino alkoxy moiety (compare **11L** with **7D**, **11M** with **7E**, **11N** with **7F**, **11O** with **7A**). These might suggest that expansion of the amino alkoxy moiety with a heteroatom or carbon and introduction of suitable cyclic secondary amines, especially 4-methyl piperazine, could enhance the anti-tumor activity.

Oxidation of the sulfur atom to the sulfoxide or the sulfone was found to be detrimental to the anti-tumor activity (compounds **7A** *vs* **8G**; **7B** *vs* **8H**; **11O** *vs* **12P** and **8G** *vs* **9I**; **8H** *vs* **9J**).

In conclusion, a series of novel methylthio-, sulfinyl- and sulfonyl-8*H*-thieno[2,3-*b*]pyrrolizin-8-oximino derivatives were synthesized and most of them exhibited better anti-tumor activities than cisplatin against Bel-7402

and HT-1080 cells. Among them, compound **11N** (IC_{50} = 18.2 μM, 8.2 μM), the most promising compound of all synthesized derivatives, was 2.5- and 3.3-times more active than cisplatin (IC_{50} = 45.2 μM, 26.7 μM), respectively. A preliminary SAR led to identification of several crucial structural requirements needed to enhance the effectiveness of alkylthiothienopyrrolizin-8-oximino derivatives. In particular, our results suggest what the required determinants should be: (i) the presence of suitable dialkylamino ethyloxymethyloxyimino substituents, especially 4-methyl piperazine in the amino moiety, at position-8 of thieno[2,3-*b*]pyrrolizine scaffold; (ii) the sulfur atom in non-oxidation state. Moreover, being the first report on 2-methylthio-, sulfinyl-, and sulfonyl-8*H*-thieno[2,3-*b*]pyrrolizin-8-oximino derivatives serving as anti-tumor agents, these results propose a new sulfur-containing lead compound in the research and development of anti-tumor drugs. More work is needed to analyze the influence of other substituents, especially at position-3 on the tripentacyclic core, and studies in this sense are currently underway.

Experimental

Chemistry

All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC-MS (Agilent, Palo Alto, CA, USA). Proton (^{1}H) nuclear magnetic resonance spectroscopy were performed using Bruker ARX-300, 300MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. IR spectra (KBr disks) were recorded with a Bruker IFS 55 instrument (Bruker). Elemental analysis was determined on a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy). Unless otherwise noted all solvents and reagents were commercially available and used without further purification.

*Methyl 3-amino-5-methylthio-4-(4-nitrophenyl)thiophene-2-carboxylate **2***

2-(4-Nitrophenyl)acetonitrile (40.5 g, 0.25 mol) and sodium hydroxide (20.0 g, 0.5 mol) was added to dry THF (350 mL). After 10 min, carbon disulfide (28.5 g, 0.375 mol) was added in one portion, the mixture was stirred at room temperature for additional 30 min. Then cooled to −20°C, methyl 2-chloroacetate (27.3 g, 0.25 mol) in dry THF (50 mL) was added dropwise and after 1 h, methyl iodide (35.5 g, 0.25 mol) in dry THF (50 mL) was dropped into the solution. After stirring at −20°C for 4 h, the resulting mixture was poured into water (1000 mL) and extracted with methylene chloride. The organic phase was dried over sodium sulfate and evaporated *in vacuo* to yield a yellow oil. Recrystallization of the oil from ethyl acetate/hexane (1/1) obtained 42.9 g (53%) of **2** as pale white powder, mp. 131-132°C. MS [MH^+] (*m/z*): 325.2; IR (KBr) cm^{-1}: 3420.5 (NH), 1718.9 (C=O), 1581.6 1540.7 1487.7 (C=C, C=N), 1516.8 1347.6 (NO_2). ^{1}H-NMR (DMSO-d_6), δ: 2.48 (s, 3H, SCH_3), 3.73 (s, 3H, $COOCH_3$), 6.07 (s, 2H, D_2O exchangeable, NH_2), 7.75 (d, 2H, *J* = 8.4 Hz, Ph-2,6-2H), 8.39 (d, 2H, *J* = 8.4 Hz, Ph-3,5-2H).

*Methyl 5-methylthio-4-(4-nitrophenyl)-3-(1H-pyrrol-1-yl)thiophene-2-carboxylate **3***

To a solution of **2** (32.4 g, 0.1 mol) in acetic acid 2,5-dimethoxytetrahydrofuran (13.2 g, 0.1 mol) was added, the reaction mixture was stirred at 70°C for 4 h, cooled, poured into cold water (1000 mL), and extracted with methylene chloride. The combined organic phase was washed with water, dried over anhydrous sodium sulfate, and was evaporated *in vacuo* to yield a solid product (32.9 g, 88%), mp. 110 – 112°C. MS [MH^+] (*m/z*): 375.0; IR (KBr) cm^{-1}: 1720.8 (C=O), 1588.5 1548.7 (C=C, C=N), 1516.6 1348.5 (NO_2). ^{1}H-NMR ($CDCl_3$), δ: 2.51 (s, 3H, SCH_3), 3.79 (s, 3H, OCH_3), 6.12 (brs, 2H, pyrrol-3,4-2H), 6.52 (brs, 2H, pyrrol-2,5-2H), 7.78 (d, 2H, *J* = 8.4 Hz, Ph-2,6-2H), 8.41 (d, 2H, *J* = 8.4 Hz, Ph-3,5-2H).

*5-Methylthio-4-(4-nitrophenyl)-3-(1H-pyrrol-1-yl)thien-2-ylpyrrolidine carboxamide **4***

A solution of **3** (3.74 g, 0.01 mol) in pyrrolidine (25 mL) was stirred at 70°C for 4 h. After cooling to room temperature, the resulting mixture was poured into water, the resulting precipitate was filtered, washed with diethyl ether and then was dried; the slight-yellow solid **4** gave (3.51 g, 85%), m.p 143 – 145°C. MS [MH^+] (*m/z*): 414.3; IR (KBr) cm^{-1}: 1638.8 (C=O), 1602.0 1552.6 (C=C, C=N), 1517.4 1350.2 (NO_2). ^{1}H-NMR ($CDCl_3$), δ: 1.62 (m, 2H, pyrrolinyl-2CH), 1.76 (m, 2H, pyrrolinyl-2CH), 2.40 (s, 3H, SCH_3), 2.64 (brs, 2H, pyrrolinyl-2CH), 3.50 (m, 2H, pyrrolinyl-2CH), 6.08 (m, 2H, pyrrol-3,4-2CH), 6.48 (m, 2H, pyrrol-2,5-2CH), 7.75 (d, 2H, *J* = 8.4 Hz, Ph-2,6-2H), 8.37 (d, 2H, *J* = 8.4 Hz, Ph-3,5-2H).

*2-Methylthio-3-(4-nitrophenyl)-8H-thieno[2,3-b]pyrrolizin-8-one **5***

A solution of **4** (4.13 g, 0.01 mol) in phosphorous oxychloride (25 mL) was stirred at 80°C for 2 h. After cooling to room temperature, the resultant mixture was concentrated to give a brown solid which was filtered, washed with diethyl ether, and dried. Then, the intermediate was slowly added to a 10% aqueous sodium hydroxide solution (100 mL) and the mixture was heated at 60°C for 2 h. After cooling, the resulting precipitate was collected by filtration, washed with water, dried, and recrystallized from ethanol/methanol (2/1) to obtain **5** (2.7 g, 79%) as a pale white powder, m.p 207-209°C. MS [MH^+] (*m/z*): 343.0; IR (KBr) cm^{-1}: 2970.0 (CH_3), 1686.0 (C=O), 1587.8 1568.8 1549.0 (C=C, C=N), 1516.4, 1349.8 (NO_2). ^{1}H-NMR ($CDCl_3$), δ: 2.61 (s, 3H, SCH_3), 6.07 (m, 1H, 6-H), 6.59 (d, 1H, *J* = 2.9 Hz, 5-H), 6.74 (d, 1H, *J* = 3.3 Hz, 7-H), 7.87 (d, 2H, *J* = 8.5 Hz, Ph-2,6-2H), 8.40 (d, 2H, *J* = 8.5 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{16}H_{10}N_2O_3S_2$: C 56.13, H 2.94, N 8.18 Found: C 56.21, H 2.92, N 8.23.

*8-Hydroxyimino-2-methylthio-3-(4-nitrophenyl)-8H-thieno[2,3-b]pyrrolizine **6***

A mixture of **5** (6.84 g, 0.02 mol) and hydroxylamine hydrochloride (2.76 g, 0.04 mol) in pyridine (100 mL) was stirred at 90°C for 4 h. The mixture was cooled and then evaporated, and the residue was dissolved in methylene chloride (150 mL). The resultant solution was washed with water, dried over sodium sulfate, evaporated *in vacuo* to give the a yellow solid, purified by column

chromatography on silica gel using ethyl acetate/hexane (1/10) as eluent. Compound **6** was afforded as a yellow powder (5.14 g, 72%) in a mixture of *Z*- and *E*-isomers (*Z* : *E* = 70 : 30) not separated, mp. 148 – 151°C. MS [MH^+] (*m/z*): 358.0; IR (KBr) cm^{-1}: 3243.9 (OH), 1596.6, 1558.6 (C=C, C=N), 1518.4 1354.1 (NO_2). ^{1}H-NMR ($CDCl_3$), *Z* isomer d: 2.48 (s, 3H, SCH_3), 6.08 (m, 1H, 6-H), 6.53 (d, 1H, *J* = 2.8 Hz, 5-H), 6.55 (d, 1H, *J* = 3.3 Hz, 7-H), 7.73 (d, 2H, *J* = 8.6 Hz, Ph-2,6-2H), 8.39 (d, 2H, *J* = 8.6 Hz, Ph-3,5-2H), *E* isomer δ: 2.43 (s, 3H, SCH_3), 6.14 (m, 1H, 6-H), 6.50 (brd, 1H, 5-H), 6.80 (d, 1H, *J* = 3.3 Hz, 7-H), 7.73 (d, 2H, *J* = 8.6 Hz, Ph-2,6-2H), 8.38 (d, 2H, *J* = 8.6 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{16}H_{11}N_3O_3S_2$: C 53.77, H 3.10, N 11.76 Found: C 53.65, H 3.12, N 11.81.

General procedure for preparation of compounds 7A-F

The oxime **6** (0.5 g, 1.4 mmol) was dissolved in *N*,*N*-dimethylformamide (DMF) (10 mL) and appropriate aminoalkyl chloride hydrochloride (1.6 mmol) and potassium carbonate (0.38 g, 2.8 mol) was added to the reaction mixture, which was stirred for 5 h at 70°C. After cooling to room temperature, the resulting mixture was poured into water, extracted with methylene chloride and washed with water. The organic phase was dried over sodium sulfate, and evaporated *in vacuo* to yield a yellow oil which was taken up in diethyl ether (50 mL). Oxalic acid (0.13 g, 1.4 mmol) in diethyl ether was added to the resultant solution, and then the precipitate was filtered, washed with diethyl ether, and dried to give the desired compounds **7** in a mixture of *Z*- and *E*-isomers not separated.

7A:

(*Z* : *E* = 65 : 35) Yield: 86%; mp. 198 – 200°C; MS [MH^+] (*m/z*): 471.0; IR (KBr) cm^{-1}: 3420.4 (OH), 2921.5 (CH_2), 1719.2 (C=O), 1597.0 1558.2 (C=C, C=N), 1514.8 1346.7 (NO_2). ^{1}H-NMR ($CDCl_3$), *Z*-isomer δ: 2.47 (s, 3H, SCH_3), 2.68 (brs, 4H, morpholino-2CH_2), 2.92 (s, 2H, CH_2N), 3.79 (s, 4H, morpholino-2CH_2), 4.54 (s, 2H, OCH_2), 6.07 (m, 1H, 6-H), 6.51 (d, 1H, *J* = 2.6 Hz, 5-H), 6.55 (d, 1H, *J* = 3.5 Hz, 7-H), 7.72 (d, 2H, *J* = 8.6 Hz, Ph-2,6-2H), 8.38 (s, 2H, *J* = 8.6 Hz, Ph-3,5-2H), *E*-isomer d: 2.42 (s, 3H, SCH_3), 2.68 (brs, 4H, morpholino-2CH_2), 2.92 (s, 2H, CH_2N), 3.79 (s, 4H, morpholino-2CH_2), 4.54 (s, 2H, OCH_2), 6.11 (m, 1H, 6-H), 6.49 (d, 1H, *J* = 2.6 Hz, 5-H), 6.69 (d, 1H, *J* = 3.4 Hz, 7-H), 7.72 (d, 2H, *J* = 8.6 Hz, Ph-2,6-2H), 8.38 (s, 2H, *J* = 8.6 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{24}H_{24}N_4O_8S_2$: C 51.42, H 4.32, N 9.99 Found: C 51.32, H 4.31, N 10.12.

7B:

(*Z* : *E* = 60 : 40) Yield: 80%; mp. 143-145°C; MS [MH^+] (*m/z*): 457.1; IR (KBr) cm^{-1}: 3434.3 (OH), 2939.7 (CH_2), 1719.6 (C=O), 1597.8 1557.6 (C=C, C=N), 1516.5 1348.0 (NO_2). ^{1}H-NMR (DMSO-d_6), *Z*-isomer d: 1.13-1.24 (m, 6H, -N(CH_2CH_3)$_2$), 2.57 (s, 3H, SCH_3), 3.19 (m, 4H, N(CH_2CH_3)$_2$), 3.48 (brs, 2H, CH_2N), 4.63 (s, 2H, OCH_2), 6.12 (m, 1H, 6-H), 6.60 (d, 1H, *J* = 3.4 Hz, 7-H), 6.64 (d, 1H, *J* = 2.5 Hz, 5-H), 7.84 (d, 2H, *J* = 8.7 Hz, Ph-2,6-2H), 8.41 (d, 2H, *J* = 8.7 Hz, Ph-3,5-2H), *E*-isomer δ: 1.13 – 1.24 (m, 6H, N(CH_2CH_3)$_2$), 2.55 (s, 3H, SCH_3), 3.19 (m, 4H, N(CH_2CH_3)$_2$), 3.48 (brs, 2H, CH_2N), 4.63 (s, 2H, OCH_2), 6.18 (m, 1H, 6-H), 6.64 (d, 1H, *J* = 2.5 Hz, 5-H), 6.81 (d, 1H, *J* = 3.2 Hz, 7-H), 7.84 (d, 2H, *J* = 8.7 Hz, Ph-2,6-2H), 8.41 (d, 2H, *J* = 8.7 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{24}H_{26}N_4O_7S_2$: C 52.37, H 4.79, N 10.25 Found: C 52.22, H 4.67, N 10.31.

7C:

(*Z* : *E* = 60 : 40) Yield: 84%; mp. 151 – 153°C; MS [MH^+] (*m/z*): 455.0; IR (KBr) cm^{-1}: 3425.1 (OH), 2952.4 (CH_3), 1719.2 (C=O), 1598.1 1558.7 (C=C, C=N), 1516.7 1348.0 (NO_2). ^{1}H-NMR (DMSO-d_6), *Z*-isomer δ: 1.90 (brs, 4H, pyrrolinyl-3,4-2CH_2), 2.57 (s, 3H, SCH_3), 3.20 (brs, 2H, pyrrolinyl-2CH), 3.32 (brs, 2H, pyrrolinyl-2CH), 3.56 (brs, 2H, CH_2N), 4.60 (s, 2H, OCH_2), 6.12 (m, 1H, 6-H), 6.60 (d, 1H, *J* = 3.4 Hz, 7-H), 6.63 (m, 1H, 5-H), 7.85 (d, 2H, *J* = 8.4 Hz, Ph-2,6-2H), 8.41 (d, 2H, *J* = 8.4 Hz, Ph-3,5-2H), *E* isomer δ: 1.90 (brs, 4H, pyrrolinyl-3,4-2CH_2), 2.55 (s, 3H, SCH_3), 3.20 (brs, 2H, pyrrolinyl-2CH), 3.32 (br s, 2H, pyrrolinyl-2CH), 3.56 (brs, 2H, CH_2N), 4.60 (s, 2H, OCH_2), 6.17 (m, 1H, 6-H), 6.63 (m, 1H, 5-H), 6.82 (brd, 1H, 7-H), 7.85 (d, 2H, *J* = 8.4 Hz, Ph-2,6-2H), 8.41 (d, 2H, *J* = 8.4 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{24}H_{24}N_4O_7S_2$: C 52.93, H 4.44, N 10.29 Found: C 53.11, H 4.39, N 10.33.

7D:

(*Z* : *E* = 75 : 25) Yield: 72%; mp. 149 – 151°C; MS [MH^+] (*m/z*): 429.0; IR (KBr) cm^{-1}: 3425.9 (OH), 2920.2 (CH_2), 1719.9 (C=O), 1598.7 1557.4 1489.2 (C=C, C=N), 1517.4 1347.4 (NO_2). ^{1}H-NMR ($CDCl_3$), *Z* isomer d: 2.46 (m, 9H, SCH_3 and N(CH_3)$_2$), 2.91 (brt, 2H, CH_2N), 4.52 (t, 2H, *J* = 5.4 Hz, OCH_2), 6.07 (m, 1H, 6-H), 6.52 (m, 1H, 5-H), 6.55 (d, 1H, *J* = 3.2 Hz, 7-H), 7.72 (d, 2H, *J* = 8.5 Hz, Ph-2,6-2H), 8.38 (d, 2H, *J* = 8.5 Hz, Ph-3,5-2H), *E*-isomer δ: 2.42 (s, 3H, SCH_3), 2.46 (m, 6H, N(CH_3)$_2$), 2.91 (brt, 2H, CH_2N), 4.52 (t, 2H, *J* = 5.4 Hz, OCH_2), 6.10 (m, 1H, 6-H), 6.49 (m, 1H, 5-H), 6.71 (m, 1H, 7-H), 7.72 (d, 2H, *J* = 8.5 Hz, Ph-2,6-2H), 8.38 (d, 2H, *J* = 8.5 Hz, Ph-3,5-2H); Anal. calcd. for $C_{22}H_{22}N_4O_7S_2$: C 50.96, H 4.28, N 10.80 Found: C 51.12, H 4.35, N 10.71.

7E:

(*Z* : *E* = 70 : 30) Yield: 82%; mp. 154 – 156°C; MS [MH^+] (*m/z*): 469.1; IR (KBr) cm^{-1}: 3422.1 (OH), 2929.6 (CH_2), 1720.3 (C=O), 1598.2 1557.9 (C=C, C=N), 1518.3 1347.0 (NO_2). ^{1}H-NMR (DMSO-d_6), *Z* isomer d: 1.38 (brs, 2H, piperidinyl-4-CH_2), 1.51 (brs, 4H, piperidinyl-3,5-2CH_2), 2.53 (m, 7H, piperidinyl-2,6-2CH_2 and SCH_3), 2.72 (brs, 2H, CH_2N), 4.38 (brs, 2H, OCH_2), 6.09 (m, 1H, 6-H), 6.56 (brd, 1H, 7-H), 6.60 (brs, 1H, 5-H), 7.83 (brd, 1H, Ph-2,6-2H), 8.38 (d, 1H, *J* = 8.1 Hz, Ph-3,5-2H), *E*-isomer δ: 1.38 (brs, 2H, piperidinyl-4-CH_2), 1.51 (brs, 4H, piperidinyl-3,5-2CH_2), 2.49 (s, 3H, SCH_3), 2.53 (m, 4H, piperidinyl-2,6-2CH_2), 2.72 (brs, 2H, CH_2N), 4.38 (brs, 2H, OCH_2), 6.14 (m, 1H, 6-H), 6.60 (brs, 1H, 5-H), 6.68 (brs, 1H, 7-H), 7.83 (brd, 1H, Ph-2,6-2H), 8.38 (d, 1H, *J* = 8.1 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{25}H_{26}N_4O_7S_2$: C 53.75, H 4.69, N 10.03 Found: C 53.68, H 4.66, N 10.12.

7F:

(*Z* : *E* = 65 : 35) Yield: 75%; mp. 186 – 188°C; MS [MH^+] (*m/z*): 484.0; IR (KBr) cm^{-1}: 3422.1 (OH), 2932.7 (CH_2), 1718.4 (C=O), 1598.3 1557.7 (C=C, C=N), 1519.2 1347.2 (NO_2). ^{1}H-NMR ($CDCl_3$), *Z* isomer δ: 2.41 (s, 3H, NCH_3), 2.47 (s, 3H, SCH_3), 2.66 (brs, 4H, piperazinyl-4H), 2.76 (brs, 4H, piperazinyl-4H), 2.88 (t, 2H, *J* = 5.5 Hz, CH_2N), 4.48 (t, 2H, *J* = 5.5 Hz, OCH_2), 6.07 (m, 1H, 6-H), 6.51 (d, 1H, *J* =2.7 Hz, 5-H), 6.55 (d, 1H, *J* = 3.5 Hz, 7-H), 7.72 (d, 2H, *J* = 8.6 Hz, Ph-2,6-2H), 8.38 (d, 2H, *J* = 8.6 Hz, Ph-3,5-2H), *E* isomer d: 2.41 (s, 3H, NCH_3), 2.42 (s, 3H, SCH_3), 2.66 (br s, 4H, piperazinyl-4H), 2.76 (brs, 4H, piperazinyl-4H), 2.88 (t, 2H, *J* = 5.5 Hz, CH_2N), 4.48 (t, 2H, *J* = 5.5 Hz, OCH_2), 6.10 (m, 1H, 6-H), 6.49 (d, 1H, *J* = 2.5 Hz, 5-H), 6.69 (d, 1H, *J* = 3.4 Hz, 7-H), 7.72 (d, 2H, *J* = 8.6 Hz, Ph-2,6-2H), 8.38 (d, 2H, *J* = 8.6 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{25}H_{27}N_5O_7S_2$: C 52.34, H 4.74, N 12.21 Found: C 52.21, H 4.72, N 12.28.

General procedure for preparation of the compounds 8G-H

Compound **7** (1.0 mmol) was dissolved in glacial acetic acid (25 mL), and sodium perborate tetrahydrate (0.154 g, 1.0 mmol) was successively added into the solution which was then stirred at 25~30°C for 3~4 h. Then, the resulting solution was poured into water and extracted with methylene chloride. The organic phase was dried over sodium sulfate and evaporated *in vacuo* to yield a yellow oil which was converted into the oxalate salt according to the preparation of compounds **7**.

8G:

(*Z* : *E* = 80 : 20) Yield: 90%; mp. 187 – 189°C; MS [MH^+] (*m/z*): 486.7; IR (KBr) cm^{-1}: 3429.5 (OH), 2925.9 (CH_2), 1721.3 (C=O), 1599.5 1557.0 (C=C, C=N), 1523.0 1348.7 (NO_2). ^{1}H-NMR ($CDCl_3$), *Z*-isomer d: 2.63 (m, 4H, morpholino-3,5-2CH_2), 2.86 (brt, 2H, CH_2N), 2.90 (s, 3H, $SOCH_3$), 3.76 (t, 4H, *J* = 4.5 Hz, morpholino-2,6-2CH_2), 4.53 (t, 2H, *J* = 5.5 Hz, OCH_2), 6.13 (m, 1H, 6-H), 6.51 (d, 1H, *J* = 2.8 Hz, 5-H), 6.61 (d, 1H, *J* = 3.6 Hz, 7-H), 7.75 (d, 2H, *J* = 8.6 Hz, Ph-2,6-2H), 8.42 (d, 2H, *J* =8.6 Hz, Ph-3,5-2H), *E*-isomer δ: 2.63 (m, 4H, morpholino-3,5-2CH_2), 2.86 (brt, 2H, CH_2N), 2.85 (s, 3H, $SOCH_3$), 3.76 (t, 4H, *J* = 4.5 Hz, morpholino-2,6-2CH_2), 4.53 (t, 2H, *J* =5.5 Hz, OCH_2), 6.16 (m, 1H, 6-H), 6.49 (d, 1H, *J* = 2.7 Hz, 5-H), 6.75 (d, 1H, *J* = 3.5 Hz, 7-H), 7.75 (d, 2H, *J* = 8.6 Hz, Ph-2,6-2H), 8.42 (d, 2H, *J* =8.6 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{24}H_{24}N_4O_9S_2$: C 49.99, H 4.20, N 9.72 Found: C 50.14, H 4.18, N 9.86.

8H:

(*Z* : *E* = 40 : 60) Yield: 88%; mp. 167 – 169°C; MS [MH^+] (*m/z*): 472.9; IR (KBr) cm^{-1}: 3438.3 (OH), 2924.2 (CH_2), 1719.7 (C=O), 1599.7 1491.7 (C=C, C=N), 1522.4 1348.8 (NO_2). ^{1}H-NMR ($CDCl_3$), *Z*-isomer δ: 1.14 (t, 6H, *J* =7.0 Hz, N(CH_2CH_3)$_2$), 2.73 – 2.81 (m, 4H, N(CH_2CH_3)$_2$), 2.89 (s, 3H, $SOCH_3$), 3.01 – 3.04 (m, 2H, CH_2N), 4.54 (m, 2H, OCH_2), 6.12 (m, 1H, 6-H), 6.50 (d, 1H, *J* = 2.7 Hz, 5-H), 6.61 (d, 1H, *J* = 3.6 Hz, 7-H), 7.75 (d, 2H, *J* = 8.7 Hz, Ph-2,6-2H), 8.42 (d, 1H, *J* = 8.7 Hz, Ph-3,5-2H), *E*-isomer δ: 1.14 (t, 6H, *J* = 7.0 Hz, N(CH_2CH_3)$_2$), 2.73 – 2.81 (m, 4H, N(CH_2CH_3)$_2$), 2.87 (s, 3H, $SOCH_3$), 3.01–3.04 (m, 2H, CH_2N), 4.54 (m, 2H, OCH_2), 6.16 (m, 1H, 6-H), 6.48 (d, 1H, *J* = 2.4 Hz, 5-H), 6.76 (d, 1H, *J* = 3.6 Hz, 7-H), 7.75 (d, 2H, *J* =8.7 Hz, Ph-2,6-2H), 8.42 (d, 1H, *J* = 8.7 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{24}H_{26}N_4O_8S_2$: C 51.24, H 4.66, N 9.96 Found: C 51.36, H 4.69, N 9.89.

General procedure for preparation of the compounds 9I-J

Compound **7** (1.0 mmol) was dissolved in methanol (25 mL), sodium wolframate dihydrate (catalyst) and 30% aqueous hydrogen peroxide (1.13 g, 10 mmol) was successively dropped into the solution and stirred at 20°C for 2 h. Then the resulting solution was poured into water and extracted with methylene chloride. The organic phase was dried over sodium sulfate and evaporated *in vacuo* to yield a yellow oil which was converted into oxalate salt according to the preparation of compounds **7**.

9I:

(*Z* : *E* = 80 : 20) Yield: 79%; mp. 197 – 199°C; MS [MH^+] (*m/z*): 503.2; IR (KBr) cm^{-1}: 3424.6 (OH), 2934.2 (CH_2), 1718.6 (C=O), 1609.7 1558.9 (C=C, C=N), 1520.4 1347.3 (NO_2). ^{1}H-NMR ($CDCl_3$), *Z*-isomer d: 2.65 (m, 4H, morpholino-3,5-2CH_2), 2.87 (brt, 2H, CH_2N), 2.92 (s, 3H, SO_2CH_3), 3.74 – 3.79 (m, 4H, morpholino-2,6-2CH_2), 4.51 (t, 2H, *J* = 5.4 Hz, OCH_2), 6.01 (m, 1H, 6-H), 6.52 (d, 1H, *J* = 2.8 Hz, 5-H), 6.59 (d, 1H, *J* = 3.4 Hz, 7-H), 7.72 (d, 2H, *J* = 8.7 Hz, Ph-2,6-2H), 8.38 (d, 2H, *J* = 8.7 Hz, Ph-3,5-2H), *E*-isomer d: 2.65 (m, 4H, morpholino-3,5-2CH_2), 2.87 (brt, 2H, CH_2N), 2.89 (s, 3H, SO_2CH_3), 3.74 – 3.79 (m, 4H, morpholino-2,6-2CH_2), 4.51 (t, 2H, *J* = 5.4 Hz, OCH_2), 6.06 (m, 1H, 6-H), 6.49 (d, 1H, *J* = 2.7 Hz, 5-H), 6.68 (d, 1H, *J* = 3.3 Hz, 7-H), 7.72 (d, 2H, *J* = 8.7 Hz, Ph-2,6-2H), 8.38 (d, 2H, *J* = 8.7 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{24}H_{24}N_4O_{10}S_2$: C 48.64, H 4.08, N 9.45 Found: C 48.52, H 4.06, N 9.51.

9J:

(*Z* > 90%) Yield: 89%; mp. 185 – 187°C; MS [MH^+] (*m/z*): 488.7; IR (KBr) cm^{-1}: 3422.2 (OH), 2968.4 (CH_2), 1720.8 (C=O), 1600.5 1558.8 1491.0 (C=C, C=N), 1524.5 1348.7 (NO_2). ^{1}H-NMR ($CDCl_3$), *Z*-isomer δ: 1.12 (m, 6H, N(CH_2CH_3)$_2$), 2.71 (m, 4H, N(CH_2CH_3)$_2$), 2.92 (m, 5H, SO_2CH_3, and CH_2N), 4.52 (m, 2H, OCH_2), 6.10 (dd, 1H, J_1 = 3.6 Hz, J_2 = 2.8 Hz, 6-H), 6.25 (d, 1H, *J* = 2.8 Hz, 5-H), 6.61 (d, 1H, *J* = 3.6 Hz, 7-H), 7.81 (d, 2H, *J* = 8.7 Hz, Ph-2,6-2H), 8.42 (d, 1H, *J* = 8.7 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{24}H_{26}N_4O_9S_2$: C 49.82, H 4.53, N 9.68 Found: C 49.73, H 4.51, N 9.59.

8-(2-Chloroethyloxymethyloxyimino)-2-methylthio-3-(4-nitrophenyl)-8H-thieno[2,3-b]pyrrolizine *10*

To a solution of the oxime **6** (0.5 g, 1.4 mmol) in anhydrous DMF (10 mL), CH_3ONa (0.09 g, 1.68 mmol) in methanol (5 mL) was added dropwise at 0°C. After stirring for 10 min at room temperature, 85% aqueous 1-chloro-2-(chloromethoxy)ethane (0.32 g, 2.1 mmol) was added and the resulting solution was continuously stirred for 1 h at 25°C. Then, the mixture was poured into water and the precipitate was collected by filtration, washed with water, dried, and purified by column chromatography on silica gel using ethyl acetate/hexane (1/10) as eluent to obtain **10** as a yellow powder (0.46 g ,73%) (*Z* : *E* = 40 : 60), mp. 91 – 93°C; MS [MH^+] (*m/z*): 449.7, 451.6; IR (KBr) cm^{-1}: 2922.9 (CH_2), 1598.6 1558.1 1489.3 (C=C, C=N), 1520.2 1348.6 (NO_2). ^{1}H-NMR ($CDCl_3$), *Z*-isomer δ: 2.49 (s, 3H, SCH_3), 3.66 (m, 2H, CH_2Cl), 3.98 (m, 2H, OCH_2), 5.42 (s, 2H, OCH_2O), 6.07 (m, 1H, 6-H), 6.50 (m, 1H, 5-H), 6.60 (brd, 1H, 7-H), 7.72 (brd, 2H, Ph-2,6-2H), 8.38 (d, 2H, *J* = 8.4 Hz, Ph-3,5-2H), *E*-isomer d: 2.43 (s, 3H, SCH_3), 3.66 (m, 2H, CH_2Cl), 3.98 (m, 2H, OCH_2), 5.42 (s, 2H, OCH_2O), 6.12 (m, 1H, 6-H), 6.50 (m, 1H, 5-H), 6.75 (brd, 1H, 7-H), 7.72 (brd, 2H, Ph-2,6-2H), 8.38 (d, 2H, *J* = 8.3 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{19}H_{16}ClN_3O_4S_2$: C 50.72, H 3.58, N 9.34 Found: C 50.59, H 3.54, N 9.29.

General procedure for preparation of the compounds 11K-O

To a solution of **10** (1.08 g, 2.4 mmol) in anhydrous DMF (30 mL), appropriate alkyl amine (3.6 mmol) and sodium hydroxide (1.92 g, 4.8 mmol) was added, the resultant solution was stirred for 4 h at 60°C. After cooling the mixture was poured into water, the precipitate was collected by filtration, washed with water, and dried. The solid was converted into oxalic salt according to the preparation of compounds **7**.

11K:

(*Z* : *E* = 80 : 20) Yield: 80%; mp. 180 – 182°C; MS [MH^+] (*m/z*): 484.9; IR (KBr) cm^{-1}: 3427.8 (OH), 2922.6 (CH_2), 1719.6 (C=O), 1598.9 1558.5 1488.9 (C=C, C=N), 1519.2 1347.5 (NO_2). ^{1}H-NMR ($CDCl_3$), *Z*-isomer δ: 1.92 (brs, 4H, pyrrolinyl-3,4-2CH_2), 2.50 (s, 3H, SCH_3), 2.92 (brs, 4H, pyrrolinyl-2,5-2CH_2), 3.00 (m, 2H, CH_2N), 4.01 (brt,

2H, OCH_2), 5.40 (s, 2H, OCH_2O), 6.07 (m, 1H, 6-H), 6.52 (d, 1H, *J* = 2.2 Hz, 5-H), 6.58 (d, 1H, *J* = 3.2 Hz, 7-H), 7.72 (d, 2H, *J* = 8.7 Hz, Ph-2,6-2H), 8.38 (d, 1H, *J* = 8.7 Hz, Ph-3,5-2H), *E*-isomer d: 1.92 (brs, 4H, pyrrolinyl-3,4-2CH_2), 2.43 (s, 3H, SCH_3), 2.92 (brs, 4H, pyrrolinyl-2,5-2CH_2), 3.00 (brs, 2H, CH_2N), 4.01 (brt, 2H, OCH_2), 5.40 (s, 2H, OCH_2O), 6.10 (m, 1H, 6-H), 6.50 (m, 1H, 5-H), 6.72 (brd, 1H, 7-H), 7.72 (d, 2H, *J* = 8.7 Hz, Ph-2,6-2H), 8.38 (d, 1H, *J* = 8.7 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{25}H_{26}N_4O_8S_2$: C 52.25, H 4.56, N 9.75 Found: C 52.31, H 4.53, N 9.69.

11L:

(*Z* : *E* = 90 : 10) Yield: 77%; mp. 177 – 179°C; MS [MH^+] (*m/z*): 458.8; IR (KBr) cm^{-1}: 3432.9 (OH), 2925.1 (CH_2), 1719.4 (C=O), 1633.6 1599.2 1558.7 (C=C, C=N), 1518.4 1348.7 (NO_2). ^{1}H-NMR (DMSO-d_6), *Z* isomer d: 2.58 (s, 3H, SCH_3), 2.71 (s, 6H, $N(CH_3)_2$), 3.23 (brs, 2H, CH_2N), 3.94 (brs, 2H, OCH_2), 5.40 (s, 2H, OCH_2O), 6.13 (m, 1H, 6-H), 6.61 (d, 1H, *J* = 3.4 Hz, 7-H), 6.65 (d, 1H, *J* = 2.7 Hz, 5-H), 7.85 (d, 2H, *J* = 8.5 Hz, Ph-2,6-2H), 8.42 (d, 2H, *J* = 8.5 Hz, Ph-3,5-2H), *E*-isomer δ: 2.53 (s, 3H, SCH_3), 2.71 (s, 6H, $N(CH_3)_2$), 3.23 (brs, 2H, CH_2N), 3.94 (brs, 2H, OCH_2), 5.40 (s, 2H, OCH_2O), 6.18 (brs, 1H, 6-H), 6.65 (d, 1H, *J* = 2.7 Hz, 5-H), 6.73 (brd, 1H, 7-H), 7.85 (d, 2H, *J* = 8.5 Hz, Ph-2,6-2H), 8.42 (d, 2H, *J* = 8.5 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{23}H_{24}N_4O_8S_2$: C 50.36, H 4.41, N 10.21 Found: C 50.22, H 4.39, N 10.15.

11M:

(*Z* : *E* = 60 : 40) Yield: 79%; mp. 100 – 104°C; MS [MH^+] (*m/z*): 498.7; IR (KBr) cm^{-1}: 3426.9 (OH), 2922.3 (CH_2), 1718.8 (C=O), 1598.6 1557.5 1490.1 (C=C, C=N), 1519.2 1347.6 (NO_2). ^{1}H-NMR ($CDCl_3$), *Z* isomer d: 1.77 (brs, 6H, piperidinyl-6H), 2.50 (s, 3H, SCH_3), 2.88 (overlap, 6H, CH_2N and piperidinyl-4H), 4.04 (brs, 2H, OCH_2), 5.38 (s, 2H, OCH_2O), 6.07 (m, 1H, 6-H), 6.53 (m, 1H, 5-H), 6.58 (m, 1H, 7-H), 7.72 (d, 1H, *J* = 8.5 Hz, Ph-2,6-2H), 8.38 (d, 1H, *J* = 8.5 Hz, Ph-3,5-2H), *E* isomer δ: 1.77 (brs, 6H, piperidinyl-6H), 2.43 (s, 3H, SCH_3), 2.88 (overlap, 6H, CH_2N and piperidinyl-4H), 4.04 (brs, 2H, OCH_2), 5.38 (s, 2H, OCH_2O), 6.12 (m, 1H, 6-H), 6.51 (m, 1H, 5-H), 6.74 (m, 1H, 7-H), 7.72 (d, 1H, *J* = 8.5 Hz, Ph-2,6-2H), 8.38 (d, 1H, *J* = 8.5 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{26}H_{28}N_4O_8S_2$: C 53.05, H 4.79, N 9.52 Found: C 52.96, H 4.75, N 9.43.

11N:

(*Z* : *E* = 70 : 30) Yield: 74%; mp. 192 – 194°C; MS [MH^+] (*m/z*): 513.7; IR (KBr) cm^{-1}: 3435.5 (OH), 3014.5 (C=CH), 1725.2 (C=O), 1599.4 1558.7 (C=C, C=N), 1519.4 1349.3 (NO_2). ^{1}H-NMR (DMSO-d_6), *Z* isomer δ: 2.58 (s, 3H, SCH_3), 2.69 (brs, 7H, piperazinyl-7H), 3.08 (overlap, 6H, piperazinyl-4H and CH_2N), 3.79 (brs, 2H, OCH_2), 5.36 (s, 2H, OCH_2O), 6.12 (brs, 1H, 6-H), 6.62 (overlap, 1H, 7-H), 6.64 (overlap,1H, 5-H), 7.87 (brd, 2H, Ph-2,6-2H), 8.41 (brd, 2H, Ph-3,5-2H), *E* isomer δ: 2.53 (s, 3H, SCH_3), 2.69 (brs, 7H, piperazinyl-7H), 3.08 (overlap, 6H, piperazinyl-4H and CH_2N), 3.79 (brs, 2H, OCH_2), 5.36 (s, 2H, OCH_2O), 6.14 (brs, 1H, 6-H), 6.64 (overlap,1H, 5-H), 6.72 (brs,1H, 7-H), 7.87 (brd, 2H, Ph-2,6-2H), 8.41 (brd, 2H, Ph-3,5-2H); Anal. Calcd. for $C_{26}H_{29}N_5O_8S_2$: C 51.73, H 4.84, N 11.60 Found: C 52.03, H 4.77, N 11.52.

11O:

(*Z* : *E* = 65 : 35) Yield: 86%; mp. 113 – 115°C; MS [MH^+] (*m/z*): 500.7; IR (KBr) cm^{-1}: 3429.0 (OH), 2921.0 (CH_2), 1717.5 (C=O), 1598.7 1557.3 1489.3 (C=C, C=N), 1519.5 1347.9 (NO_2). ^{1}H-NMR ($CDCl_3$), *Z*-isomer d: 2.48 (s, 3H, SCH_3), 2.61 (brs, 4H, morpholino-3,5-2CH2), 2.73 (m, 2H, CH_2N), 3.75 (brs, 4H, morpholino-2,6-2CH_2), 3.92 (m, 2H, OCH_2), 5.39 (s, 2H, OCH_2O), 6.06 (m, 1H, 6-H), 6.51 (m, 1H, 5-H), 6.58 (d, *J* = 3.2 Hz, 1H, 7-H), 7.72 (d, 1H, *J* = 8.5 Hz, Ph-2,6-2H), 8.38 (d, 1H, *J* = 8.5 Hz, Ph-3,5-2H), *E*-isomer d: 2.43 (s, 3H, SCH_3), 2.61(brs, 4H, morpholino-3,5-2CH_2), 2.73 (m, 2H, CH_2N), 3.75 (brs, 4H, morpholino-2,6-2CH_2), 3.92 (brs, 2H, OCH_2), 5.39 (s, 2H, OCH_2O), 6.11 (m, 1H, 6-H), 6.51 (m, 1H, 5-H), 6.74 (d, *J* = 3.1 Hz, 1H, 7-H), 7.72 (d, 1H, *J* = 8.5 Hz, Ph-2,6-2H), 8.38 (d, 1H, *J* = 8.5 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{25}H_{26}N_4O_9S_2$: C 50.84, H 4.44, N 9.49 Found: C 51.02, H 4.41, N 9.55.

8-(2-Morpholinoethyloxymethyloxyimino)-2-methylsulfinyl-3-(4-nitrophenyl)-8H-thieno [2,3-b]pyrrolizine (Z : E = 45 : 55) 12P

Compound **12P** was prepared from **11O** following the procedure described for **8** and obtained in 85% yield as a yellow solid; mp. 123 – 125°C; MS [MH^+] (*m/z*): 516.6; IR (KBr) cm^{-1}: 3419.2 (OH), 2910.2 (CH_2), 1719.6 (C=O), 1599.5 1554.9 1492.6 (C=C, C=N), 1521.0 1349.3 (NO_2). ^{1}H-NMR ($CDCl_3$), *Z* isomer δ: 2.80 (overlap, 6H, morpholino-2CH_2 and CH_2N), 2.89 (s, 3H, $SOCH_3$), 3.84 (brs, 4H, morpholino-2CH_2), 4.03 (brs, 2H, OCH_2), 5.43 (s, 2H,OCH_2O), 6.13 (m, 1H, 6-H), 6.52 (m, 1H, 5-H), 6.64 (d, 1H, *J* = 3.6 Hz, 7-H), 7.75 (d, 2H, *J* = 8.5 Hz, Ph-2,6-2H), 8.42 (d, 2H, *J* = 8.5 Hz, Ph-3,5-2H), *E*-isomer d: 2.80 (overlap, 6H, morpholino-2CH_2 and CH_2N), 2.87 (s, 3H, $SOCH_3$), 3.84 (brs, 4H, morpholino-2CH_2), 4.03 (brs, 2H, OCH_2), 5.43 (s, 2H,OCH_2O), 6.18 (m, 1H, 6-H), 6.49 (m, 1H, 5-H), 6.79 (d, 1H, *J* = 3.5 Hz, 7-H), 7.75 (d, 2H, *J* = 8.5 Hz, Ph-2,6-2H), 8.42 (d, 2H, *J* = 8.5 Hz, Ph-3,5-2H); Anal. Calcd. for $C_{25}H_{26}N_4O_{10}S_2$: C 49.50, H 4.32, N 9.24 Found: C 50.06, H 4.30, N 9.13.

Pharmacology

The MTT cell proliferation assay [18] was used to test the anti-cancer activity of all synthesized compounds. The cells were seeded in RPMI-1640 medium (100 μL) in a 96-well plate at a concentration of 4000 cells per well. After cultured for 12 h at 37°C and with 5% CO_2, cells were incubated with scalar concentrations of samples for 24 h. MTT was added at a terminal concentration of 5 μg/mL and incubated with cells for 4 h. The formazane crystals were dissolved in DMSO (100 μL) each well. The optical density was measured at 492 nm (for absorbance of MTT formazane) and 630 nm (for the reference wavelength). All of the compounds were tested twice in each of the cell lines. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of two determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

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