Synthesis and Antitumor Activity of Some *N*2-(Thien-3-yl)amidrazones

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Z. Naturforsch. **2014**, *69b*, 811 – 816 / DOI: 10.5560/ZNB.2014-4062
Received March 12, 2014

A set of new *N*2-(thien-3-yl)amidrazones (**6a**–**h**) incorporating *N*-piperazines and related congeners has been synthesized by reacting the hydrazonoyl chloride **4** (derived from 3-aminothiophene-2-carboxylate) with the appropriate *sec*-cyclic amine. The antitumor activity of these compounds was evaluated on breast cancer (MCF-7) and leukemic (K562) cell lines by a cell viability assay utilizing the tetrazolium dye (MTT). The amidrazone **6d** encompassing the *N*-piperazine moiety, was the most active against MCF-7 and K562 with IC_{50} of 7.28 and 9.91 μM, respectively.

Key words: 3-Aminothiophene-2-carboxylic Ester, *N*2-(Thien-3-yl)nitrile Imine, Piperazines, *N*2-(Thien-3-yl)amidrazones, Antitumor Activity

Introduction

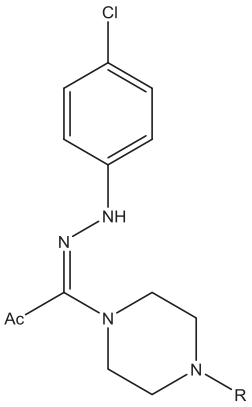
Recently, we have reported on the synthesis and antitumor activity of some *N*-aryl(piperazin-1-yl) amidrazones exemplified by **1a** and **1b** (Fig. 1) [1]. Both compounds bear significant antitumor activity against a number of human cell lines, especially leukemia, breast cancer, non-small cell lung cancer and CNS cancers. Moreover, and equally pertinent to the present investigation, *N*-(aryl)amidrazones (possessing the -C_6H_4-NH–C(COMe)=N–NH–C_6H_4 fragment) have recently been found to be a potent corticotrophin-releasing factor (CRF) receptor antagonist [2]. Several publications have also dealt with the synthesis and evaluation of thrombin inhibitors that incorporate an amidrazone functionality as a structural motif [3 – 5].

In light of the above information and based on the concept of bioisosterism interchange in drug design [6 – 10], we thought it would be worthwhile to replace the benzene ring in **1a**, **b** by the classical bioiososteric thiophene ring. Accordingly, the present work aims at the synthesis of *N*2-(thien-3-yl)amidrazones (**6a**–**h**, see Scheme 2) incorporating piperazines and their congeners for evaluation of their antitumor activity.

Results and Discussion

Synthesis

The synthetic route towards a selected set of *N*2-(thien-3-yl) amidrazone derivatives (**6a**–**h**, see Scheme 2) commenced with the preparation of methyl 3-[2-(1-chloro-2-oxopropylidene) hydrazinyl] thiophene-2-carboxylate (**4**, Scheme 1). Thus, diazotization of the amino group of methyl 3-amino-2-thiophenecarboxylate (**2**), followed by coupling of the resulting thiophene-3-diazonium salt (**2A**) with 3-chloropentane-2, 4-dione (in basic medium) gave initially the α-*chloroazo* compound (**3A**) (Scheme 1); the latter intermediate was then transformed (under the basic reaction conditions) into the respective more stable α-*chlorohydrazone* structure **4** *via*



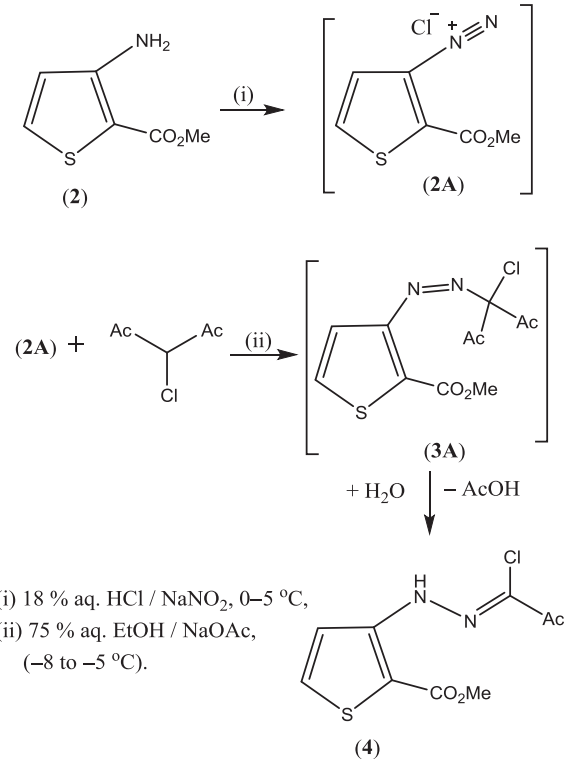
1a (R = Me); **1b** (R = Et)

Fig. 1. Molecular formulae of **1a**, **b**.

loss of the acetyl group (Japp-Klingemann reaction) [11 – 13].

Piperazines and the related *sec*-cyclic amines **5a**–**h**, acting as nitrogen nucleophiles, are expected to add readily onto *N*2-(thien-3-yl) nitrile amine (the reactive 1,3-dipolar species generated *in situ* from its corresponding hydrazonoyl chloride precursor **4** in the presence of triethylamine) to deliver the respective amidrazone adducts **6a**–**h** (Scheme 2). This mode of nucleophilic addition reaction of various nucleophiles onto 1,3-dipoles is well-documented in the literature [14 – 20].

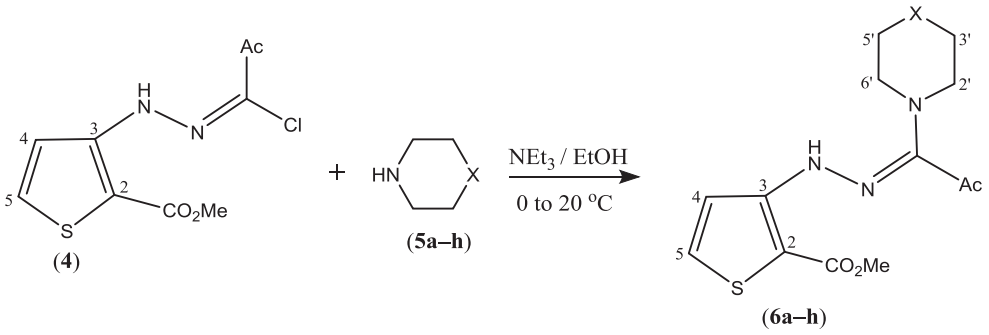
The newly synthesized compounds **4** and **6a**–**h** were characterized by elemental analyses, MS and NMR



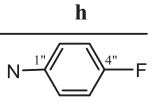
(i) 18 % aq. HCl / $NaNO_2$, 0–5 °C,
(ii) 75 % aq. EtOH / NaOAc,
(–8 to –5 °C).

Scheme 1.

spectral data. These data, detailed in the Experimental Section, are in conformity with the assigned structures. The mass spectra display the correct molecular ion peaks for which the measured high-resolution (HRMS)



compounds **5** and **6**

Entry	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**
X	CH_2	O	S	N-H	N-Me	N-CO_2Et	N-CHO	

Scheme 2.

data are in good agreement with the calculated values. DEPT and 2D (HMQS, HMBC) experiments showed correlations that helped in the ^{1}H and ^{13}C signal assignments to the different carbons and their attached, and/or neighboring hydrogens. In compound **6h**, the skeletal carbons of the fluorinated benzene ring are recognizable by their doublet signals (with varying *J* values) originating from scalar (through bond) coupling with the neighboring fluorine atom at the C-4′ locus. In HMBC experiments, distinct long-range "three-bond" correlations are observed between 5-H and each of C-2 and C-3, between 4-H and C-2, as well as between the amidrazone N–*H* and each of C-2 and the amidrazone carbon (HN–N=*C*).

Antitumor activity

The antitumor activity of the synthesized compounds was screened by conducting cell viability assay using the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Cultures of the breast cancer cell line MCF-7 and the leukemic cell line K562 were treated first at two concentrations of 12.5 and 50 μM, and the results are shown in Table 1. In the MCF-7 screening test, only compounds **6h** and **6d** showed a potential anti-MCF-7 activity at 12.5 μM concentration. At 50 μM concentration, **6f** and **6h** were able to reduce the proliferation to less than 50 %, indicating that these two compounds may have some potential anti-MCF-7 activity albeit to a lesser extent than **6d**. In the case of K562 cells, only compound **6d** illustrated a potential anti-K562 activity and again was able to reduce the viability after 72 hours to less than 50 % at both, 12.5 μM and 50 μM. Furthermore, we determined the IC_{50} values for the compounds against the MCF-7 and K562 (Table 2). Cultures of the breast cancer cell line MCF-7 and the Leukemic cell line K562 were treated with different concentrations, and the resulting IC_{50} values are listed in Table 2. From the structure-activity relationships point of view, the nature of the substituent at position X (see Scheme 2) seems to play a critical role in determining the anti-cancer activity. Clearly, different substitution at position X results in remarkable variations in the determined anti-tumor activity. For example, compounds **6e**, **6g**, **6c**, and **6b** that have *N*–Me, *N*–CHO, S or O substituents at position X showed very little, if any, anti-MCF-7 and anti-K562 activity, while compounds **6d**, **6f**, and **6h** with *N*–H, *N*–CO_2Et, and *N*–$C_6H_4F(p)$ groups showed a promising activity; in particular, compound **6d** has a very encouraging IC_{50} against both, MCF-7 and K562.

Table 2. The IC_{50} values (in μM) for compounds **6d**, **f**, **h** against MCF-7 and K562 cell lines[a].

Compound	MCF-7 IC_{50} (± SD)	K562 IC_{50} (± SD)
Doxorubicin	0.31 (0.01)	1.41 (0.31)
6d	7.26 (0.52)	9.91 (1.84)
6f	18.72 (4.60)	> 50
6h	16.28 (0.96)	> 50

[a] Doxorubicin is used as a positive control.

Experimental Section

The following chemicals were purchased from Acros and were used as received: methyl 3-amino-2-thiophenecarboxylate, 3-chloro-2,4-pentanedione, piperidine, morpholine,

Table 1. Percentage cell survival of MCF-7 and K562 following 72 h exposure to 12.5 and 50 μM of compounds **6a-h**[a].

Compound	MCF-7 % survival at 12.5μM (± SD)	MCF-7 % survival at 50μM (± SD)	K562 % survival at 12.5μM (± SD)	K562 % survival at 50μM (± SD)
Doxorubicin	29.25 (3.02)	23.60 (1.67)	19.04 (1.03)	11.90 (0.90)
6a	111.06 (31.90)	73.73 (3.68)	86.49 (13.97)	82.48 (13.27)
6b	97.98 (5.79)	90.58 (4.72)	108.64 (19.68)	66.05 (3.81)
6c	95.08 (5.15)	84.36 (2.42)	80.81 (5.99)	87.98 (20.36)
6d	36.95 (8.79)	19.80 (0.87)	44.30 (3.82)	6.76 (0.37)
6e	93.78 (2.92)	79.78 (4.46)	80.49 (3.81)	62.27 (11.28)
6f	66.04 (11.72)	45.06 (2.70)	60.08 (5.59)	56.02 (2.51)
6g	88.44 (7.45)	90.37 (7.53)	102.53 (5.60)	70.30 (8.10)
6h	61.45 (2.48)	49.94 (1.39)	85.44 (15.15)	78.80 (9.88)

[a] Doxorubicin is used as a positive control.

thiomorpholine, piperazine, 1-methylpiperazine, 1-formylpiperazine, 1-(ethoxycarbonyl)piperazine, and 1-(4-fluorophenyl)piperazine. Melting points were determined on a Gallenkamp electrothermal apparatus in open capillary tubes. Elemental analyses were performed on a Euro Vector analyzer, model EA 3000. ^{1}H and ^{13}C NMR spectra were recorded on a 500 MHz spectrometer (Bruker Advance-III) with TMS as the internal standard. Chemical shifts are expressed in δ units; J values for ^{1}H-^{1}H, ^{1}H-^{19}F and ^{13}C-^{19}F coupling constants are given in Hertz. High resolution mass spectra (HRMS) were acquired (in positive mode) using the electrospray ion trap (ESI) technique by collision-induced dissociation on a Bruker APEX-4 (7 Tesla) instrument. The samples were dissolved in acetonitrile, diluted in spray solution (methanol-water 1 : 1 v/v + 0.1 % formic acid) and infused using a syringe pump with a flow rate of 2 μL min^{-1}. External calibration was conducted using arginine clusters in a mass range $m/z = 175 - 871$.

Cell lines and cell culture

The K562 leukemia cell line was obtained from Dr. Mona Hassona (Department of Biological Sciences, The University of Jordan) and was cultured in RPMI, while the MCF-7 breast cancer cells were obtained from American Type Culture Collections (ATCC) and were cultured in RPMI. All media were supplemented with 10 % heat-inactivated fetal bovine serum (FBS) (Gibco Invitrogen), 1 % of 2 mM L-glutamine (Lonza), 50 IU per mL penicillin (Lonza), and 50 μg mL^{-1} streptomycin (Lonza), and cells were maintained at 37 °C, 5 % CO_2 in a humidified incubator.

Cell proliferation assay

MCF-7 and K562 cells were seeded at a density of 1×10^4 and 4×10^4 cells per well in 96-well plates in appropriate medium. For anti-MCF-7 and anti-K562 screening, the cells were treated with 12.5 and 50 μM concentrations of the tested compounds. For the IC_{50} determination, the cells were treated with increasing concentrations of the tested compound (1.56 – 100 μM). In all assays, the drugs were dissolved in DMSO immediately before the addition to cell cultures, and equal amounts of the solvent were added to control cells. Cell viability was assessed, after 3 days of treatment, with the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), obtained from Sigma (Dorset, UK). IC_{50} concentrations were obtained from the dose-response curves using GraphPadPrism Software 5 (San Diego, California, USA; www.graphpad.com).

*Methyl 3-[2-(1-chloro-2-oxopropylidene)hydrazinyl]-thiophene-2-carboxylate (**4**)*

The title compound was prepared by the following two steps: step (i): A cooled (0 – 5 °C) solution of methyl 3-amino-2-thiophenecarboxylate (5.5 g, 35 mmol) in 18 % aqueous HCl (60 mL) was treated dropwise with a solution of $NaNO_2$ (2.9 g, 42 mmol) in water (4 mL) under efficient stirring. Thereafter, stirring was continued for 20 – 30 min, and the resulting cold solution of the corresponding thiophene-3-diazonium chloride was used immediately as such for the following coupling reaction. step (ii): A freshly prepared cold solution of thiophene-3-diazonium chloride (35 mmol) was poured onto a cold solution (−8 to –5 °C/ice-salt bath) of 3-chloro -2,4-pentanedione (4.7 g, 35 mmol) in ethanol-water (60 mL, 3 : 1 v/v) containing sodium acetate (16 g). The resulting light-orange mixture was further stirred until a solid precipitate was formed (5 – 10 min). The reaction mixture was then diluted with cold water (200 mL), the solid product was collected by suction filtration, washed with cold water (5 × 15 mL), dried and recrystallized from dichloromethane-cyclohexane. Yield: 77 %, m. p. 158 – 160 °C. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.58 (s, 3H, C*H*$_3$–C=O), 3.94 (s, 3H, CO_2CH_3), 7.32 (d, J = 5.4 Hz, 1H, 4-H), 7.50 (d, J = 5.4 Hz, 1H, 5-H), 10.85 ppm (br s, 1H, N–*H*, exchangeable with D_2O). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 25.3 (*C*H$_3$–C=O), 52.2 (CO_2*C*H$_3$), 106.7 (C-2), 118.2 (C-4), 127.3 (-N=*C*–N-), 132.5 (C-5), 148.6 (C-3), 164.4 (*C*O$_2$Me), 188.3 ppm (O=*C*–Me). – HRMS ((+)-ESI): m/z = 261.00933 (calcd. 261.00952 for $C_9H_{10}ClN_2O_3S$, $[M+H]^+$). – $C_9H_9ClN_2O_3S$ (260.70): calcd. C 41.46, H 3.48, Cl 13.60, N 10.75; found C 41.23, H 3.39, Cl 13.42, N 10.54.

*Thiophene-3-amidrazone derivatives **6a–h**; general procedure*

To a cold (0 – 5 °C) and stirred suspension of **4** (1.3 g, 5 mmol) in ethanol (30 mL) was added a solution of the appropriate cyclic *sec*-amine **5a–h** (7 mmol) and triethylamine (5 mL) in ethanol (10 mL). Stirring was continued at 0 – 5 °C for 2 – 4 h, and at ambient temperature for additional 2 h; the reaction mixture was then poured onto cold water (200 mL). The resulting crude solid product was collected by suction filtration, washed with cold water, dried and recrystallized from dichloromethane-cyclohexane.

*Methyl 3-{2-[2-oxo-1-(piperidin-1-yl)propylidene]-hydrazinyl}thiophene-2-carboxylate (**6a**)*

Yield: 76 %, m. p. 102 – 104 °C. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.66 (m, 2H, H-4′), 1.76 (m, 4H, 3′-H$_2$/5′-H$_2$), 2.42 (s, 3H, C*H*$_3$–C=O), 3.02 (m, 4H, 2′-H$_2$/6′-H$_2$), 3.92 (s, 3H, CO_2CH_3), 7.34 (d, J = 5.4 Hz, 1H, 4-H), 7.43 (d, J = 5.4 Hz, 1H, 5-H), 10.86 ppm (s, 1H, N–*H*, exchangeable with D_2O). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 24.3 (C-4′), 26.1 (*C*H$_3$–C=O), 26.6 (C-3′/C-5′), 49.2 (C-2′/C-6′), 51.8 (CO_2*C*H$_3$), 104.6 (C-2), 118.7 (C-4), 131.8 (C-5), 146.0 (-N=*C*–N-), 150.0 (C-3), 164.0 (*C*O$_2$Me), 195.7 ppm

(O=*C*–Me). – HRMS ((+)-ESI): m/z = 310.12162 (calcd. 310.12199 for $C_{14}H_{20}N_3O_3S$, $[M+H]^+$). – $C_{14}H_{19}N_3O_3S$ (309.39): calcd. C 54.35, H 6.19, N 13.58; found C 54.08, H 6.12, N 13.44.

*Methyl 3-[2-(1-morpholino-2-oxopropylidene)hydrazinyl]-thiophene-2-carboxylate (**6b**)*

Yield: 82 %, m. p. 115 – 116 °C. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.43 (s, 3H, C*H*$_3$–C=O), 3.12 (br m, 4H, 3′-H$_2$/5′-H$_2$), 3.90 (br m, 4H, 2′-H$_2$/6′-H$_2$), 3.91 (s, 3H, CO_2CH_3), 7.33 (d, J = 5.4 Hz, 1H, 4-H), 7.44 (d, J = 5.4 Hz, 1H, 5-H), 11.06 ppm (s, 1H, N–*H*, exchangeable with D_2O). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 25.9 (*C*H$_3$-C=O), 48.2 (C-3′/C-5′), 51.8 (CO_2CH_3), 67.6 (C-2′/C-6′), 104.6 (C-2), 118.4 (C-4), 132.0 (C-5), 144.1 (-N=*C*–N-), 149.8 (C-3), 164.1 (*C*O$_2$Me), 195.3 ppm (O=*C*–Me). – HRMS ((+)-ESI): m/z = 312.10122 (calcd. 312.10125 for $C_{13}H_{18}N_3O_4S$, $[M+H]^+$). – $C_{13}H_{17}N_3O_4S$ (311.36): calcd. C 50.1, H 5.50, N 13.50; found C 50.16, H 5.38, N 13.26.

*Methyl 3-[2-(2-oxo-1-thiomorpholinopropylidene)-hydrazinyl]thiophene-2-carboxylate (**6c**)*

Yield: 78 %, m. p. 130 – 132 °C. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.42 (s, 3H, CH_3–C=O), 2.86 (m, 4H, 3′-H$_2$/5′-H$_2$), 3.30 (m, 4H, 2′-H$_2$/6′-H$_2$), 3.92 (s, 3H, CO_2CH_3),7.33 (d, J = 5.4 Hz, 1H, 4-H), 7.44 (d, J = 5.4 Hz, 1H, 5-H), 10.97 ppm (s, 1H, N–*H*, exchangeable with D_2O). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 25.8 (*C*H$_3$–C=O), 28.5 (C-3′/C-5′), 50.4 (C-2′/C-6′), 51.9 (CO_2CH_3), 104.7 (C-2), 118.5 (C-4), 132.0 (C-5), 145.2 (-N=C–N-), 149.8 (C-3), 164.1 (*C*O$_2$Me), 195.2 ppm (O=*C*–Me). – HRMS ((+)-ESI): m/z = 328.07841 (calcd. 328.07841 for $C_{13}H_{18}N_3O_3S_2$, $[M+H]^+$). – $C_{13}H_{17}N_3O_3S_2$ (327.42): calcd. C 47.69, H 5.23, N 12.83; found C 47.52, H 5.09, N 12.67.

*Methyl 3-{2-[2-oxo-1-(piperazin-1-yl)propylidene]-hydrazinyl}thiophene-2-carboxylate (**6d**)*

Yield: 61 %, m. p. 113 – 115 °C. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.42 (s, 3H, CH_3–C=O), 2.63 (s, 1H, N(4′)–H), 3.27 (m, 4H, 3′-H$_2$/5′-H$_2$), 3.30 (m, 4H, 2′-H$_2$/6′-H$_2$), 3.92 (s, 3H, CO_2CH_3), 7.33 (d, J = 5.4 Hz, 1H, 4-H), 7.45 (d, J = 5.4 Hz, 1H, 5-H), 10.97 ppm (s, 1H, N–H, exchangeable with D_2O). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 25.9 (CH_3–C=O), 45.2 (C-2′/C-6′), 47.7 (C-3′/C-5′), 51.9 (CO_2CH_3), 104.5 (C-2), 118.3 (C-4), 132.1 (C-5), 144.2 (-N=C–N-), 149.8 (C-3), 164.3 (CO_2Me), 195.4 ppm (O=C–Me). – HRMS ((+)-ESI): m/z = 311.11729 (calcd. 311.11724 for $C_{13}H_{19}N_4O_3S$, $[M+H]^+$). – $C_{13}H_{18}N_4O_3S$ (310.37): calcd. C 50.31, H 5.85, N 18.05; found C 50.12, H 5.76, N 17.84.

*Methyl 3-{2-[1-(4-methylpiperazin-1-yl)-2-oxopropyl-idene]hydrazinyl}thiophene-2-carboxylate (**6e**)*

Yield: 65 %, m. p. 113 – 114 °C. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.43 (s, 3H, C*H*$_3$–C=O), 2.59 (s, 3H, N–C*H*$_3$), 2.90 (m, 4H, 3′-H$_2$/5′-H$_2$), 3.28 (m, 4H, 2′-H$_2$/6′-H$_2$), 3.92 (s, 3H, CO_2CH_3), 7.33 (d, J = 5.4 Hz, 1H, 4-H), 7.44 (d, J = 5.4 Hz, 1H, 5-H), 10.86 ppm (s, 1H, N–*H*, exchangeable with D_2O). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 25.9 (*C*H$_3$–C=O), 45.4 (N–*C*H$_3$), 46.8 (C-2′/C-6′), 51.8 (CO_2CH_3), 55.0 (C-3′/C-5′), 104.7(C-2), 118.4 (C-4), 132.1 (C-5), 143.9 (-N=*C*–N-), 149.8 (C-3), 164.2 (*C*O$_2$Me), 195.0 ppm (O=*C*–Me). – HRMS ((+)-ESI): m/z = 325.13277 (calcd. 325.13289 for $C_{14}H_{21}N_4O_3S$, $[M+H]^+$). – $C_{14}H_{20}N_4O_3S$ (324.40): calcd C 51.83, H 6.21, N 17.27; found C 51.63, H 6.12, N 17.05.

*Ethyl 4-{1-[2-(2-(methoxycarbonyl)thiophen-3-yl)hydrazo]-2-oxopropyl}piperazine-1-carboxylate (**6f**)*

Yield: 68 %, m. p. 142 – 143 °C. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.31 (t, J = 7.1 Hz, 3H, C*H*$_3$CH$_2$O), 2.42 (s, 3H, C*H*$_3$–C=O), 3.06 (m, 4H, 3′-H$_2$/5′-H$_2$), 3.70 (br m, 4H, 2′-H$_2$/6′-H$_2$), 3.89 (s, 3H, CO_2CH_3), 4.20 (q, J = 7.1 Hz, 2H, OC*H*$_2$Me), 7.33 (d, J = 5.4 Hz, 1H, 5-H), 7.44 (d, J = 5.4 Hz, 1H, 4-H), 11.05 ppm (s, 1H, N–*H*, exchangeable with D_2O). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.7 (*C*H$_3$CH$_2$O), 25.8 (*C*H$_3$–C=O), 44.5 (C-3′/C-5′), 47.8 (C-2′/C-6′), 51.8 (CO_2CH_3), 61.4 (OCH$_2$Me), 104.7 (C-2), 118.4 (C-4), 132.0 (C-5), 144.3 (-N=*C*–N-), 149.9 (C-3), 155.6 (N-*C*O$_2$Et), 164.2 (*C*O$_2$Me), 195.2 ppm (O=*C*–Me). – HRMS ((+)-ESI): m/z = 383.13845 (calcd. 383.13837 for $C_{16}H_{23}N_4O_5S$, $[M+H]^+$), m/z = 405.12019 (calcd. 405.12031 for $C_{16}H_{22}N_4NaO_5S$, $[M+Na]^+$). – $C_{16}H_{22}N_4O_5S$ (382.43): calcd. C 50.25, H 5.80, N 14.65; found C 50.02, H 5.68, N 14.51.

*Methyl 3-{2-[1-(4-formylpiperazin-1-yl)-2-oxopropyl-idene]hydrazinyl}thiophene-2-carboxylate (**6g**)*

Yield: 71 %, m. p. 148 – 150 °C. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.42 (s, 3H, C*H*$_3$–C=O), 3.05 (m, 2H) and 3.13 (m, 2H) (3′-H$_2$/5′-H$_2$), 3.59 (m, 2H) and 3.78 (m, 2H) (2′-H$_2$/6′-H$_2$), 3.89 (s, 3H, CO_2CH_3), 7.33 (d, J = 5.4 Hz, 1H, 4-H), 7.45 (d, J = 5.4 Hz, 1H, 5-H), 8.11 (s, 1H, -C*H*O), 11.10 ppm (s, 1H, N–*H*, exchangeable with D_2O). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 25.8 (*C*H$_3$–C=O), 40.8, 46.4 (C-3′/C-5′), 47.5, 48.7 (C-2′/C-6′), 51.9 (CO_2CH_3), 104.9 (C-2), 118.3 (C-4), 132.2 (C-5), 143.8 (-N=*C*–N-), 149.8 (C-3), 161.0 (*C*HO), 164.3 (*C*O$_2$Me), 195.1 ppm (O=*C*–Me). – HRMS ((+)-ESI): m/z = 339.11232 (calcd. 339.11215 for $C_{14}H_{19}N_4O_4S$, $[M+H]^+$), 361.09356 (calcd. 361.09410 for $C_{14}H_{18}N_4NaO_4S$, $[M+Na]^+$). – $C_{14}H_{18}N_4O_4S$ (338.38): calcd. C 49.69, H 5.36, N 16.56; found C 49.61, H 5.18, N 16.28.

*Methyl 3-{2-[1-(4-(4-fluorophenyl)piperazin-1-yl)-2-oxopropylidene]hydrazinyl}thiophene-2-carboxylate (**6h**)*

Yield: 75 %, m. p. 180 – 182 °C. – ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.45$ (s, 3H, CH_3–C=O), 3.28 (m, 4H) and 3.33 (m, 4H) (2′-H_2/6′-H_2, 3′-H_2/5′-H_2), 3.88 (s, 3H, CO_2CH_3), 7.01 (m, 4H, 2″-H/6″-H, 3″-H/5″-H), 7.36 (d, $J = 5.4$ Hz, 1H, 4-H), 7.45 (d, $J = 5.4$ Hz, 1H, 5-H), 11.00 ppm (s, 1H, N–*H*, exchangeable with D_2O). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 26.0 (CH_3–C=O), 48.0 (C-2′/C-6′), 50.9 (C-3′/C-5′), 51.9 (CO_2CH_3), 104.7(C-2), 115.5 (d, $^2J_{C-F}$ = 22 Hz, C-3″/C-5″), 118.1(d, $^3J_{C-F}$ = 7.6 Hz, C-2″/C-6″), 118.2 (C-1″), 118.5 (C-4), 132.0 (C-5), 144.4 (-N=*C*-N-), 149.0 (d, $^1J_{C-F}$ = 202 Hz, C-4″), 150.0 (C-3), 164.1 (CO_2Me), 195.4 ppm (O=*C*–Me). – HRMS ((+)-ESI): m/z = 405.13914 (calcd. 405.13912 for $C_{19}H_{22}FN_4O_3S$, $[M+H]^+$). – $C_{19}H_{21}FN_4O_3S$ (404.46): calcd. C 56.42, H 5.23, N 13.85; found C 56.25, H 5.16, N 13.72.

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