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Synthesis and Antitumor Activity of Some Methyl *N*-(5-Phenylthien-3-yl)amidrazone-2-carboxylates

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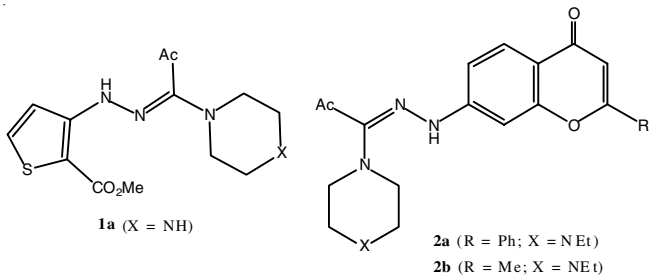
A series of new *N*-(5-phenylthien-3-yl)amidrazone-2-carboxylates (**7a-n**), incorporating *N*-substituted piperazines and related congeners, were prepared by reacting the hydrazonoyl chloride (**5**) (derived from 3-amino-5-phenyl-2-carboxylate) with the appropriate *sec*-cyclic amine. The *in vitro* antitumor activity screening of these amidrazones was performed against colon (RKO), breast (MCF-7) and cervical (HeLa) cancer cells. The *N*-alkylpiperazine derivatives **7e-g** alone exhibited good to moderate activity (IC_{50} = 5-22 μM), while the rest compounds were, however, inactive at ≤ 100 μM.

Keywords: Hydrazonoyl chlorides, 1-(Substituted)piperazines, *N*-(5-Phenylthien-3-yl)amidrazones, Antitumor activity.

INTRODUCTION

Recently, the antitumor activity of some synthetic *N*-(thien-3-yl)amidrazones was evaluated against breast cancer MCF-7 and leukemic K562 cell lines [1]. Among these, compound **1a** (Fig. 1) was the most potent with an IC_{50} in the range of 7.3-9.9 μM. Structural modifications on **1a**, as a lead compound, might produce new derivatives with improved antitumor potency. It is envisaged that incorporating a phenyl group at C-5 locus of the thiophene ring might raise the antitumor activity. This follows from the finding that the 2-phenylflavonyl-7-amidrazone (**2a**) [2] displayed much higher activity against MCF-7 and K562 cell lines as compared to the 2-methyl analog **2b** (Fig. 1).

Accordingly, the synthesis and antitumor activity of a selected set of 5-(phenyl)thiophene-3-amidrazone derivatives (**7a-n**) are reported herein.



1a (X = NH)

2a (R = Ph; X = NEt)
2b (R = Me; X = NEt)

Fig. 1. Model N-(thien-3-yl)- and N-(flavon-7-yl)amidrazones

EXPERIMENTAL

Methyl 3-amino-5-phenyl-2-thiophencarboxylate, 3-chloro-2,4-pentanedione, piperidine, 4-phenylpiperidine, morpholine, thiomorpholine, piperazine and various 1-(substitiuted)piperazines were purchased from Acros and were used as received. Melting points were determined on a Gallenkamp electrothermal melting apparatus in open capillary tubes. Elemental analyses were performed on a Euro Vector elemental analyzer, model EA 3000. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 500 MHz spectrometer (Bruker Avance-III) with TMS as the internal standard. 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 an arginine cluster in a mass range of *m/z* = 175-871.

Methyl 3-[2-(1-chloro-2-oxopropylidene)hydrazinyl]-5-phenylthiophene-2-carboxylate (5): This compound was prepared by the following two-step procedure: Step (i): Methyl 3-amino-5-phenyl-2-thiophenecarboxylate (**3**) (2.33 g, 10 mmol) was dissolved in 6 N aqueous hydrochloric acid (20 mL). To this cooled (0-4 °C) and stirred solution was added dropwise, a solution of sodium nitrite (0.83 g, 12 mmol) in water (2 mL). Stirring was then continued for 20-30 min and

the resulting fresh cold thiophene-3-diazonium chloride solution was used immediately as such for the following coupling reaction.

Step (ii): The cold thiophene-3-diazonium chloride solution, prepared above in step (i), was poured onto a cold solution (-5 °C/ice-salt bath) of 3-chloro-2,4-pentanedione (1.35 g, 10 mmol) in ethanol-water (20 mL, 3:1 v/v) containing sodium acetate (18 g). The resulting orange-coloured mixture was then diluted with cold water (200 mL), the precipitated solid product collected by suction filtration, washed with cold water (5 × 15 mL), dried and recrystallized from dichlomethane/*n*-hexane. Yield: 88 %, m.p.: 176-180 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.61 (s, 3H, O=C-CH_3), 3.95 (s, 3H, CO_2-CH_3), 7.45 (m, 3H, H-3'/H-5' + H-4'), 7.49 (s, 1H, H-4), 7.69 (d, *J* = 7.2 Hz, 2H, H-2'/H-6'), 10.89 (s, 1H, N-H, exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.4 (O=C-*C*H_3), 52.2 (CO_2-*C*H_3), 105.3 (C-2), 113.5 (C-4), 126.2 (C-2'/C-6'), 127.5 (-N=C-Cl), 129.2 (C-3'/C-5'), 129.6 (C-4'), 132.8 (C-1'), 148.8 (C-5), 150.6 (C-3), 164.4 (*C*O_2Me), 188.3 (O=*C*-Me). HRMS ((+)ESI): *m/z* = 359.02278 (calcd. 359.02276 for $C_{16}H_{13}{}^{35}ClN_2NaO_3S$, [M+ Na]$^+$); *m/z* = 361.01982 (calcd. 361.01978 for $C_{16}H_{13}{}^{37}ClN_2NaO_3S$, [M + 2 + Na]$^+$); *m/z* = 337.04064 (calcd. 337.04082 for $C_{15}H_{14}{}^{35}ClN_2O_3S$, [M + H]$^+$); *m/z* = 339.03769 (calcd. 339.03784 for $C_{15}H_{14}{}^{37}ClN_2O_3S$, [M + 2 + H]$^+$); *m/z* = 673.07407 (calcd. 673.07436 for $C_{30}H_{27}{}^{35}Cl_2N_4O_6S_2$, [2M + H]$^+$); *m/z* = 675.07065 (calcd. 675.07137 for $C_{30}H_{27}{}^{35}Cl^{37}ClN_4O_6S_2$, [2M + 2 + H]$^+$); *m/z* = 677.06670 (calcd. 677.06712 for $C_{30}H_{27}{}^{37}Cl_2N_4O_6S_2$, [2M + 4 + H]$^+$). Anal. calcd. for $C_{15}H_{13}N_2O_3SCl$ (336.79): C 53.49, H 3.89, N 8.32; found: C 53.28, H 3.77, N 8.25.

General procedure for the synthesis of amidrazones (7a-n): To a cold suspension (0-4 °C) of the hydrazonoyl chloride (**4**) (0.3 g, 0.9 mmol) in ethanol (2 mL) was added, with stirring, a solution of the appropriate secondary cyclic amine (1.0 mmol) and triethylamine (2 mL) in ethanol (5 mL). Stirring was continued at 0-4 °C for 2-3 h and at ambient temperature for additional 2 h, then the reaction mixture poured onto cold water (120 mL). The resulting solid product was collected by suction filtration, washed with water, dried and recrystallized from dichloromethane/cyclohexane.

Methyl 3-{2-[2-oxo-1-(piperidin-1-yl)propylidene]-hydrazinyl}-5-phenylthiophene-2-carboxylate (7a): Yield: 93 %, m.p.: 144-146 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.65 (m, 2H, H_2-4''), 1.77 (m, 4H, H_2-3''/H_2-5''), 2.46 (s, 3H, O=C-CH_3), 3.04 (m, 4H, H_2-2''/H_2-6''), 3.93 (s, 3H, CO_2-CH_3), 7.44 (m, 3H, H-3'/H-5' + H-4'), 7.54 (s, 1H, H-4), 7.70 (d, *J* = 7.2 Hz, 2H, H-2'/H-6'), 10.89 (s, 1H, N-H, exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.3 (C-4''), 26.2 (O=C-*C*H_3), 26.6 (C-3''/C-5''), 49.3 (C-2''/C-6''), 51.8 (CO_2-*C*H_3), 103.1 (C-2), 114.2 (C-4), 126.1 (C-2'/C-6'), 129.1 (C-3'/C-5'), 129.2 (C-4'), 133.3 (C-1'), 146.1 (-N-C=N-), 149.7 (C-5), 150.2 (C-3), 164.0 (*C*O_2Me), 195.7 (O=*C*-Me). HRMS ((+)ESI): *m/z* = 386.15343 (calcd. 386.15329 for $C_{20}H_{23}N_3O_3S$, [M+H]$^+$); *m/z* = 408.13538 (calcd. 408.13523 for $C_{20}H_{23}N_3NaO_3S$, [M + Na]$^+$); *m/z* = 793.28234 (calcd. 793.28125 for $C_{40}H_{46}N_6O_6S_2Na$, [2M + Na]$^+$).Anal. calcd. for $C_{20}H_{23}N_3O_3S$ (385.48): C 62.32, H 6.01, N 10.90; found: C 62.08, H 5.92, N 10.81.

Methyl 3-[2-(1-morpholino-2-oxopropylidene)hydrazinyl]-5-phenylthiophene-2-carboxylate (7b): Yield: 94 %, m.p.: 178-180 °C (dec.). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.47 (s, 3H, O=C-CH_3), 3.13 (m, 4H, H_2-2''/H_2-6''), 3.92 (m, 4H, H_2-3''/H_2-5''), 3.93 (s, 3H, CO_2-CH_3), 7.44 (m, 3H, H-3'/H-5' + H-4'), 7.54 (s, 1H, H-4), 7.70 (d, *J* = 7.1 Hz, 2H, H-2'/H-6'), 11.10 (s, 1H, N-H, exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.1 (O=C-*C*H_3), 48.2 (C-2''/C-6''), 51.8 (CO_2-*C*H_3), 67.6 (C-3''/C-5''), 103.5 (C-2), 113.9 (C-4), 126.1 (C-2'/C-6'), 129.1 (C-3'/C-5'), 129.3 (C-4'), 133.2 (C-1'), 144.2 (-N-C=N-), 149.9 (C-5), 150.1 (C-3), 164.1 (*C*O_2Me), 195.3 (O=*C*-Me). HRMS ((+)ESI): *m/z* = 388.13265 (calcd. 388.13255 for $C_{19}H_{22}N_3O_4S$, [M + H]$^+$); *m/z* = 410.11449 (calcd. 410.11450 for $C_{19}H_{21}N_3NaO_4S$, [M + Na]$^+$); *m/z* = 797.24128 (calcd. 797.23977 for $C_{38}H_{42}N_6O_8S_2Na$, [2M + Na]$^+$).Anal. calcd. for $C_{19}H_{21}N_3O_4S$ (387.45): C 58.90, H 5.46, N 10.85; found: C 59.06, H 5.42, N 10.67.

Methyl 3-[2-(2-oxo-1-thiomorpholinopropylidene)-hydrazinyl]-5-phenylthiophene-2-carboxylate (7c): Yield: 91 %, m.p.: 215-217 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.45 (s, 3H, O=C-CH_3), 2.88 (m, 4H, H_2-3''/H_2-5''), 3.32 (m, 4H, H_2-2''/H_2-6''), 3.94 (s, 3H, CO_2-CH_3), 7.44 (m, 3H, H-3'/H-5' + H-4'), 7.53 (s, 1H, H-4), 7.70 (d, *J* = 7.1 Hz, 2H, H-2'/H-6'), 11.01 (s, 1H, N-H, exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.0 (O=C-*C*H_3), 28.5 (C-3''/C-5''), 50.5 (C-2''/C-6''), 51.9 (CO_2-*C*H_3), 103.5 (C-2), 113.9 (C-4), 126.1 (C-2'/C-6'), 129.1 (C-3'/C-5'), 129.3 (C-4'), 133.2 (C-1'), 145.3 (-N-C=N-), 149.9 (C-5), 150.0 (C-3), 164.1 (*C*O_2Me), 195.2 (O=*C*-Me). HRMS ((+)ESI): *m/z* = 404.10977 (calcd. 404.10971 for $C_{19}H_{22}N_3O_3S_2$, [M + H]$^+$); *m/z* = 426.09142 (calcd. 426.09165 for $C_{19}H_{21}N_3NaO_3S_2$, [M + Na]$^+$); *m/z* = 829.19422 (calcd. 829.19409 for $C_{38}H_{42}N_6O_6S_4Na$, [2M = Na]$^+$).Anal. calcd. for $C_{19}H_{21}N_3O_3S_2$ (403.52): C 56.55, H 5.25, N 10.41; found: C 56.32, H 5.10, N 10.33.

Methyl 3-{2-[2-oxo-1-(piperazin-1-yl)propylidene]-hydrazinyl}-5-phenylthiophene-2-carboxylate (7d): Yield: 78 %, m.p.: 220-222 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.47 (s, 3H, O=C-CH_3), 2.78 (m, 4H, H-3''/H-5''), 3.15 (m, 4H, H-2''/H-6''), 3.18 (s, 1H, N(4'')-H, exchangeable with D_2O), 3.95 (s, 3H, CO_2Me), 7.44 (m, 3H, H-3'/H-5' + H-4'), 7.54 (s, 1H, H-4), 7.70 (d, *J* = 7.2 Hz, 2H, H-2'/H-6'), 10.92 (s, 1H, N-H, exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.2 (O=C-*C*H_3), 48.7 (C-2''/C-6''), 51.8 (CO_2*C*H_3), 52.0 (C-3''/C-5''), 103.3 (C-2), 114.2 (C-4), 126.1 (C-2'/C-6'), 129.1 (C-3'/C-5'), 129.2 (C-4'), 133.3 (C-1'), 145.1 (-N-C=N-), 149.7 (C-5), 150.1 (C-3), 163.9 (*C*O_2Me), 195.4 (O=*C*-Me). HRMS ((+)ESI): *m/z* = 387.14902 (calcd. 387.14854 for $C_{19}H_{23}N_4O_3S$, [M + H]$^+$); *m/z* = 409.13100 (calcd. 409.13048 for $C_{19}H_{22}N_4O_3SNa$, [M + Na]$^+$). Anal. calcd. for $C_{19}H_{22}N_4O_3S$ (386.47): C 59.05, H 5.74, N 14.50; found: C 58.82, H 5.58, N 14.26.

Methyl 3-{2-[1-(4-methylpiperazin-1-yl)-2-oxopropylidene]hydrazinyl}-5-phenylthiophene-2-carboxylate (7e): Yield: 90 %, m.p.: 142-144 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.42 (s, 1H, N-CH_3), 2.46 (s, 3H, O=C-CH_3), 2.64 (m, 4H, H-3''/H-5''), 3.16 (m, 4H, H-2''/H-6''), 3.94 (s, 3H, CO_2CH_3), 7.43 (m, 3H, H-3'/H-5' + H-4'), 7.53 (s, 1H, H-4), 7.69 (d, *J* = 7.1 Hz, 2H, H-2'/H-6'), 10.89 (s, 1H, N-H, exchangeable with

D_2O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.1 (O=C-*C*H_3), 46.4 (N-CH_3), 47.8 (C-2"/C-6"), 51.9 (CO_2*C*H_3), 55.7 (C-3"/C-5"), 103.3 (C-2), 114.1 (C-4), 126.1 (C-2'/C-6'),129.1 (C-3'/C-5'), 129.3 (C-4'), 133.2 (C-1'), 144.8 (-N-C=N-), 149.8 (C-5), 150.1 (C-3), 164.0(*C*O_2Me), 195.3 (O=*C*-Me). HRMS ((+)ESI): *m/z* = 401.16431 (calcd. 401.16419 for $C_{20}H_{25}N_4O_3S$, $[M + H]^+$); *m/z* = 423.14618 (calcd. 423.14613 for $C_{20}H_{24}N_4NaO_3S$, $[M + Na]^+$); *m/z* = 823.30357 (calcd. 823.30304 for $C_{40}H_{48}N_8O_6S_2Na$, $[2M + Na]^+$).Anal. calcd. for $C_{20}H_{24}N_4O_3S$ (400.49): C 59.98, H 6.04, N 13.99; found: C 59.62, H 6.15, N 13.76.

Methyl 3-{2-[1-(4-ethylpiperazin-1-yl)-2-oxopropylidene]-hydrazinyl}-5-phenylthiophene-2-carboxylate (7f): Yield: 95 %, m.p.: 140-142 °C. ^1H NMR (500 MHz, $CDCl_3$): δ 1.17 (t, *J* = 7.2 Hz, 3H, C*H*$_3$CH$_2$-) 2.46 (s, 3H, O=C-CH$_3$), 2.56 (q, *J* = 7.2 Hz, 2H, C*H*$_2$Me), 2.67 (m, 4H, H$_2$-3"/H$_2$-5"), 3.17 (m, 4H, H$_2$-2"/H$_2$-6"), 3.94 (s, 3H, CO_2CH_3), 7.43 (m, 3H, H-3'/H-5' + H-4'), 7.54 (s, 1H, H-4), 7.69 (d, *J* = 7.1 Hz, 2H, H$_2$-2'/H$_2$-6'), 10.90 (s, 1H, N-H, exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.0 (*C*H$_3$CH$_2$-), 26.1 (O=C-*C*H$_3$), 47.9 (C-2"/C-6"), 51.8 (CO_2*C*H$_3$), 52.5 (-*C*H$_2$Me), 53.4 (C-3"/C-5"), 103.3 (C-2), 114.1 (C-4), 126.1 (C-2'/C-6'), 129.1 (C-3'/C-5'), 129.3 (C-4'), 133.2 (C-1'), 144.9 (-N-C=N-), 149.8 (C-5), 150.1 (C-3), 164.0 (*C*O_2Me), 195.3 (O=*C*-Me). HRMS ((+)ESI): *m/z* = 415.18000 (calcd. 415.17984 for $C_{21}H_{27}N_4O_3S$, $[M + H]^+$); *m/z* = 437.16196 (calcd. 437.16178 for $C_{21}H_{26}N_4NaO_3S$, $[M + Na]^+$); *m/z* = 851.33534 (calcd. 851.33434 for $C_{42}H_{52}N_8O_6S_2Na$, $[2M + Na]^+$).Anal. calcd. for $C_{21}H_{26}N_4O_3S$ (414.52): C 60.85, H 6.32, N 13.52; found: C 60.59, H 6.14, N 13.41.

Methyl 3-{2-[1-(4-(2-hydroxyethyl)piperazin-1-yl)-2-oxopropylidene]-hydrazinyl}-5-phenylthiophene-2-carboxylate (7g): Yield: 86 %, m.p.: 192-194 °C (dec.). ^1H NMR (500 MHz, $CDCl_3$): δ 2.47 (s, 3H, O=C-CH$_3$), 2.70 (t, *J* = 5.3 Hz, 2H, HOCH$_2$C*H*$_2$N), 2.75 (m, 4H, H$_2$-3"/H$_2$-5"), 3.16 (m, 4H, H$_2$-2"/H$_2$-6"), 3.68 (t, *J* = 5.3 Hz, 2H, HOC*H*$_2$CH$_2$N), 3.93 (s, 3H, CO_2CH_3), 7.44 (m, 3H, H-3'/H-5' + H-4'), 7.54 (s, 1H, H-4), 7.69 (d, *J* = 7.1 Hz, 2H, H-2'/H-6'), 10.94 (s, 1H, N-H, exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.1 (O=C-*C*H$_3$), 47.9 (C-2"/C-6"), 51.8 (CO_2*C*H$_3$), 53.4 (C-3"/C-5"), 57.7 (-CH$_2$OH), 59.3 (N-*C*H$_2$CH$_2$OH), 103.3 (C-2), 114.0 (C-4), 126.1 (C-2'/C-6'), 129.1 (C-3'/C-5'), 129.3 (C-4'), 133.2 (C-1'), 144.7 (-N-C=N-), 149.9 (C-5), 150.1 (C-3), 164.1 (*C*O_2Me), 195.4 (O=*C*-Me). HRMS ((+)ESI): *m/z* = 431.17475 (calcd. 731.17475 for $C_{21}H_{27}N_4O_4S$, $[M + H]^+$); *m/z* = 453.15658 (calcd. 453.15670 for $C_{21}H_{26}N_4O_4SNa$, $[M + Na]^+$).Anal. calcd. for $C_{21}H_{26}N_4O_4S$ (430.52): C 58.59, H 6.09, N 13.01; found: C 58.52, H 6.16, N 12.88.

Methyl 3-{2-[1-(4-formylpiperazin-1-yl)-2-oxopropylidene]hydrazinyl}-5-phenylthiophene-2-carboxylate (7h): Yield: 86 %, m.p.: 178-180 °C. ^1H NMR (500 MHz, $CDCl_3$): δ 2.47 (s, 3H, O=C-CH$_3$), [3.07 (t, *J* = 5 Hz, 2H) and 3.16 (t, *J* = 4.7 Hz, 2H) (H$_2$-2" / H$_2$-6")], [3.61 (t, *J* = 5 Hz, 2H) and 3.81 (br s, 2H) (H$_2$-3"/H$_2$-5")], 3.93 (s, 3H, CO_2CH_3), 7.45 (m, 3H, H-3'/H-5' + H-4'), 7.53 (s, 1H, H-4), 7.70 (d, *J* = 7 Hz, 2H, H-2'/H-6'), 8.13 (s, 1H, -CHO), 11.15 (s, 1H, N-H, exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.9 (O=C-*C*H$_3$), 40.8 and 46.5 (C-3"/C-5"), 47.5 and 48.7 (C-2"/C-6"), 51.9 (CO_2*C*H$_3$), 103.7 (C-2), 113.7 (C-4), 126.2 (C-2'/C-6'), 129.1 (C-3'/C-5'), 129.4 (C-4'), 133.1 (C-1'), 143.9 (-N-C=N-), 150.0 (C-5), 150.2 (C-3), 161.1 (-CHO), 164.3 (*C*O_2Me), 195.1 (O=*C*-Me). HRMS((+)ESI): *m/z* = 437.12504 (calcd. 437.12540 for $C_{20}H_{22}N_4NaO_4S$, $[M + Na]^+$); *m/z* = 851.26228 (calcd. 851.26157 for $C_{40}H_{44}N_8NaO_8S_2$, $[2M + Na]^+$). Anal. calcd. for $C_{20}H_{22}N_4O_4S$ (414.48): C 57.96, H 5.35, N 13.52; found: C 58.17, H 5.23, N 13.36.

Ethyl 4-{1-[2-(2-(methoxycarbonyl)-5-phenylthiophen-3-yl)hydrazono]-2-oxopropyl}piperazine-1-carboxylate (7i): Yield: 87 %, m.p.: 178-180 °C. ^1H NMR (500 MHz, $CDCl_3$): δ 1.31 (t, *J* = 7.1 Hz, 3H, C*H*$_3$CH$_2$O), 2.47 (s, 3H, O=C-CH$_3$), 3.06 (m, 4H, H-2"/H-6"), 3.71 (m, 4H, H-3"/H-5"), 3.91(s, 3H, CO_2CH_3), 4.20 (q, *J* = 7.1 Hz, 2H, OC*H*$_2$Me), 7.44 (m, 3H, H-3'/H-5' + H-4'), 7.53 (s, 1H, H-4), 7.69 (d, *J* = 7.2 Hz, 2H, H-2'/H-6'), 11.09 (s, 1H, N-H, exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.7 (*C*H$_3$CH$_2$O), 26.0 (O=C-*C*H$_3$), 44.6 (C-3"/C-5"), 47.8 (C-2"/C-6"), 51.9 (CO_2*C*H$_3$), 61.5 (O*C*H$_2$Me), 103.5 (C-2), 113.8 (C-4), 126.1 (C-2'/C-6'), 129.1 (C-3'/C-5'), 129.3 (C-4'), 133.2 (C-1'), 144.4 (-N-C=N-), 150.0 (C-5), 150.1 (C-3), 155.6 (N-*C*O_2Et), 164.2(*C*O_2Me), 195.2 (O=*C*-Me). HRMS ((+)ESI): *m/z* = 459.16954 (calcd. 459.16967 for $C_{22}H_{27}N_4O_5S$, $[M + H]^+$); *m/z* = 481.15118 (calcd. 481.15161 for $C_{22}H_{26}N_4O_5SNa$, $[M + Na]^+$); *m/z* = 939.31358 (calcd. 939.31400 for $C_{44}H_{52}NaO_{10}S_2$, $[2M + Na]^+$).Anal. calcd. for $C_{22}H_{26}N_4O_5S$ (458.53): C 57.63, H 5.72, N 12.22; found: C 57.28, H 5.59, N 12.03.

Methyl 3-{2-[2-oxo-1-(4-phenylpiperazin-1-yl)-propylidene]hydrazinyl}-5-phenylthiophene-2-carboxylate (7j): Yield: 86 %, m.p.: 216-218 °C. ^1H NMR (500 MHz, $CDCl_3$): δ 2.50 (s, 3H, O=C-CH$_3$), 3.31 (m, 4H, H$_2$-2"/H$_2$-6"), 3.43 (m, 4H, H-3"/H-5"), 3.89 (s, 3H, CO_2-CH$_3$), 6.92 (t, *J* =7.2 Hz, 1H,H-4'''), 7.04 (d, *J* = 8.0 Hz, 2H, H-2'''/H-6'''), 7.34 (pseudo t, 2H, H-3'''/H-5'''), 7.45 (m, 3H, H-3'/H-5' + H-4'), 7.56 (s, 1H, H-4), 7.71 (d, *J* = 7.2 Hz, 2H, H-2'/H-6'), 11.05 (s, 1H, N-H, exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.1 (O=C-*C*H$_3$), 48.1 (C-2"/C-6"), 49.9 (C-3"/C-5"), 51.9 (CO_2-*C*H$_3$), 103.4 (C-2), 114.0 (C-4), 116.3 (C-2'''/C-6'''), 119.7 (C-4'''), 126.1 (C-2'/C-6'), 129.1 (C-3'/C-5'), 129.2 (C-3'''/C-5'''), 129.3 (C-4'), 133.2 (C-1'), 144.6 (-N-C=N-), 149.8 (C-5), 150.1 (C-3), 151.6 (C-1'''), 164.0 (*C*O_2Me), 195.4 (O=*C*-Me). HRMS ((+)ESI): *m/z* = 463.17963 (calcd. 463.17984 for $C_{25}H_{27}N_4O_3S$, $[M+H]^+$); *m/z* = 485.16136 (calcd. 485.16178 for $C_{25}H_{26}N_4NaO_3S$, $[M + Na]^+$); *m/z* = 947.33458 (calcd. 947.33434 for $C_{50}H_{52}N_8NaO_6S_2$, $[2M + Na]^+$).Anal. calcd. for $C_{25}H_{26}N_4O_3S$ (462.56): C 64.91, H 5.67, N 12.11; found: C 64.75, H 5.62, N 11.94.

Methyl 3-{2-[1-(4-(4-fluorophenyl)piperazin-1-yl)-2-oxopropylidene]-hydrazinyl}-5-phenylthiophene-2-carboxylate (7k): Yield: 89 %, m.p.: 212-214 °C. ^1H NMR (500 MHz, $CDCl_3$): δ 2.50 (s, 3H, O=C-CH$_3$), 3.30 (m, 4H, H$_2$-3"/H$_2$-5"), 3.33 (m, 4H, H$_2$-2"/H$_2$-6"), 3.89 (s, 3H, CO_2-CH$_3$), 7.00 (m, 4H, H-2'''/H-6''' + H-3'''/H-5'''), 7.46 (m, 3H, H-3'/H-5' + C-4'), 7.55 (s, 1H, H-4), 7.70 (d, *J* = 8.5 Hz, 2H, H-2'/H-6'), 11.03 (s, 1H, N-H, exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.1 (O=C-*C*H$_3$), 48.0 (C-2"/C-6"), 50.9 (C-3"/C-5"), 51.9 (CO_2-*C*H$_3$), 103.5 (C-2), 114.0 (C-4), 115.5 (d, $^2J_{C\text{-}F}$ = 21.8 Hz, C-3'''/C-5'''), 118.1 (d, $^3J_{C\text{-}F}$ = 7 Hz, C-2'''/C-6'''), 126.1 (C-2'/C-6'), 129.1 (C-3'/C-5'), 129.3

(C-4'), 133.2 (C-1'), 144.5 (-N-C=N-), 148.2 (d, $^4J_{C-F}$ = 2 Hz, C-1'''), 149.9 (C-5), 150.1 (C-3), 157.2 (d, $^1J_{C-F}$ = 238 Hz, C-4'''), 164.0 (*C*O_2Me), 195.4 (O=*C*-Me). HRMS ((+)ESI): *m/z* = 481.17004 (calcd. 481.17042 for $C_{25}H_{26}FN_4O_3S$, $[M+H]^+$); *m/z* = 503.15144 (calcd. 503.15236 for $C_{25}H_{25}FN_4O_3SNa$, $[M + Na]^+$). Anal. calcd. for $C_{25}H_{25}FN_4O_3S$ (480.55): C 62.48, H 5.24, N 11.66; found: C 62.57, H 5.28, N 11.46.

Methyl 3-{2-[2-oxo-1-(4-(pyridin-2-yl)piperazin-1-yl)-propylidene]hydrazinyl}-5-phenylthiophene-2-carboxylate (7l): Yield: 83 %, m.p.: 202-204 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.48 (s, 3H, O=C-CH_3), 3.24 (m, 4H, H_2-2''/H_2-6''), 3.79 (m, 4H, H_2-3''/H_2-5''), 3.88 (s, 3H, CO_2-CH_3), 6.66 (dd, *J* = 6.9, 5.1 Hz, 1H, H-5'''), 6.73 (d, *J* = 8.6 Hz, 1H, H-3'''), 7.44 (m, 3H, H-3'/H-5' + H-4'), 7.52 (ddd, *J* = 8.6, 6.9, 1.3 Hz, 1H, H-4'''), 7.55 (s, 1H, H-4), 7.70 (d, *J* = 7.1 Hz, 2H, H-2'/H-6'), 8.24 (dd, *J* = 5.1, 1.3 Hz, 1H, H-6'''), 11.10 (s, 1H, N-H, exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.1 (O=C-*C*H_3), 46.0 (C-2''/C-6''), 47.9 (C-3''/C-5''), 51.9 (CO_2-*C*H_3), 103.5 (C-2), 107.2 (C-3'''), 113.3 (C-5'''), 114.0 (C-4), 126.1 (C-2'/C-6'), 129.1 (C-3'/C-5'), 129.3 (C-4'), 133.2 (C-1'), 137.5 (C-4'''), 144.6 (-N-C=N-), 148.0 (C-6'''), 149.9 (C-5), 150.1 (C-3), 159.9 (C-2'''), 164.0 (*C*O_2Me), 195.3 (O=*C*-Me). HRMS ((+)ESI): *m/z* = 464.17590 (calcd. 464.17509 for $C_{24}H_{26}N_5O_3S$, $[M+H]^+$); *m/z* = 486.15614 (calcd. 486.15703 for $C_{24}H_{25}N_5NaO_3S$, $[M + Na]^+$); *m/z* = 949.32407 (calcd. 949.32484 for $C_{48}H_{50}N_{10}NaO_6S_2$, $[2M + Na]^+$).Anal. calcd. for $C_{24}H_{25}N_5O_3S$ (463.55): C 62.18, H 5.44, N 15.11; found: C 61.95, H 5.52, N 14.89.

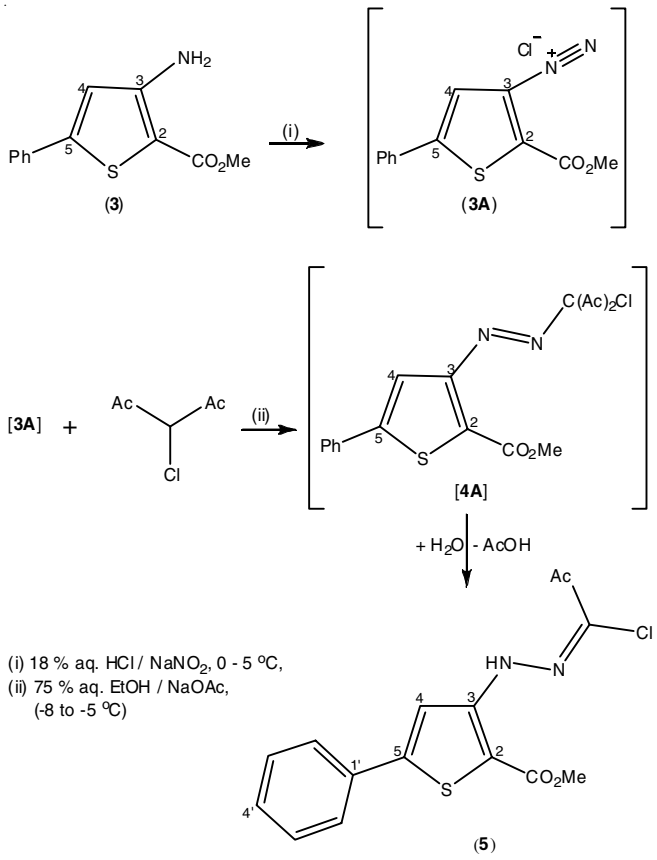
Methyl 3-{2-[2-oxo-1-(4-(pyrimidin-2-yl)piperazin-1-yl)propylidene]hydrazinyl}-5-phenylthiophene-2-carboxylate (7m): Yield: 81 %, m.p.: 178-180 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.48 (s, 3H, O=C-CH_3), 3.18 (m, 4H, H_2-2''/H_2-6''), 3.90 (s, 3H, CO_2-CH_3), 4.08 (m, 4H, H_2-3''/H_2-5''), 6.53 (t, *J* = 4.7 Hz, 1H, H-5'''), 7.44 (m, 3H, H-3'/H-5' + H-4'), 7.55 (s, 1H, N-H, H-4), 7.70 (d, *J* = 7.1 Hz, 2H, H-2'/H-6'), 8.35 (d, *J* = 4.7 Hz, 2H, H-4'''/H-6'''), 11.17 (s, 1H, N-H, exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.0 (O=C-*C*H_3), 44.5 (C-3''/C-5''), 47.9 (C-2''/C-6''), 51.9 (CO_2-*C*H_3), 103.5 (C-2), 109.9 (C-5'''), 113.9 (C-4), 126.2 (C-2'/C-6'), 129.1 (C-3'/C-5'), 129.3 (C-4'), 133.2 (C-1'), 144.7 (-N-C=N-), 149.9 (C-5), 150.1 (C-3), 157.7 (C-4'''/C-6'''), 161.7 (C-2'''), 164.1 (*C*O_2Me), 195.3 (O=*C*-Me). HRMS ((+)ESI): *m/z* = [487.15175 (calcd. 487.15228 for $C_{23}H_{24}N_6NaO_3S$, $[M + Na]^+$); *m/z* = 465.17016 (calcd. 465.17034 for $C_{23}H_{25}N_6O_3S$, $[M + H]^+$).Anal.Clacd for $C_{23}H_{24}N_6O_3S$ (464.54): C 59.47, H 5.21, N 18.09; found: C 59.41, H 5.13, N 17.92.

Methyl 3-{2-[2-oxo-1-(4-phenylpiperidin-1-yl)propylidene]hydrazinyl}-5-phenylthiophene-2-carboxylate (7n): Yield: 88 %, m.p.: 216-218 °C (dec.).^{1}H NMR (500 MHz, $CDCl_3$): δ 1.95 (m, 2H) and 2.10 (m, 2H, H_2-3''/H_2-5''), 2.49 (s, 3H, O=C-CH_3), 2.75 (m, 1H, H-4''), 3.01 (m, 2H) and 3.39 (m, 2H, H_2-2''/H_2-6''), 3.96 (s, 3H, CO_2-CH_3), 7.26 (t, *J* = 7.1 Hz, 1H, H-4'''), 7.40 (m, 4H, H-2'''/H-6''' + H-3'''/H-5'''), 7.47 (m, 3H, H-3'/H-5' + H-4'), 7.56 (s, 1H, H-4), 7.71 (d, *J* = 7.1 Hz, 2H, H-2'/H-6'), 11.12 (s, 1H, N-H, exchangeable with D_2O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.1 (O=C-*C*H_3), 34.1 (C-3''/C-5''), 42.5 (C-4''), 49.1 (C-2''/C-6''), 51.7 (CO_2-*C*H_3), 103.2 (C-2), 114.0 (C-4), 126.1 (C-2'/C-6'), 126.2 (C-4'''), 127.1 (C-2'''/6'''), 128.4 (C-3'''/5'''), 129.1 (C-3'/C-5'), 129.2 (C-4'), 133.3 (C-1'), 146.0 (-N-C=N-), 146.6 (C-1'''), 149.8 (C-5), 150.3 (C-3), 164.0 (*C*O_2Me), 195.6 (O=*C*-Me). HRMS ((+)ESI): *m/z* = 462.18505 (calcd. 462.18459 for $C_{26}H_{28}N_3O_3S$, $[M+H]^+$); *m/z* = 484. 16707 (calcd. 484.16653 for $C_{26}H_{27}N_3NaO_3S$, $[M+Na]^+$); *m/z* = 945.34597 (calcd. 945.34385 for $C_{52}H_{54}N_6O_6S_2Na$, $[2M + Na]^+$).Anal. calcd. for $C_{26}H_{27}N_3O_3S$ (461.58): C 67.65, H 5.90, N 9.10; found: C 67.52, H 5.84, N 8.95.

Cytotoxicity assay (MTT assay): Breast cancer (MCF-7), cervical cancer (HeLa) and colon cancer (RKO) cell lines were obtained as a generous gift from Dr. Ahmad Al-Jada (King Saud Bin Abdulaziz University for Health Sciences (KSAU-HS), KSA). All plastic consumables were obtained from Corning, USA. Chemicals and reagents, used in this assay, were obtained from Invitrogen, USA (unless otherwise stated). All tested compounds **7a-n** were initially dissolved in DMSO (Sigma-Aldrich, UK) at 100 µM stock concentration and stored in -20 °C until required. Cells were routinely maintained in RPMI medium containing 10 % fetal calf serum (FCS), 2 mM L-glutamine and 1 mM sodium pyruvate and incubated at 37 °C in a CO_2 enriched atmosphere (5 %). Once cells reach 70-80 % confluence, cells were seeded into 96-well plates (2×10^3 per well) and left to adhere overnight. Cells were then exposed to each compound at different ranges of concentration for 96 h at 37 °C in a CO_2 enriched atmosphere (5 %). DMSO concentration was ≤ 0.1 % in all wells. After the time course is completed, MTT assay was performed as described previously by Mossman [3]. The supernatant was washed out and replaced with fresh medium containing the tetrazolium dye MTT (0.5 mg/mL, Sigma-Aldrich, UK) and incubated in the dark for 4 h at 37 °C in a CO_2 enriched atmosphere (5 %) to allow the formation of formazan salt. In order to dissolve the reduced formazan salt crystals, the supernatant was removed carefully and 150 µL of DMSO were added and mixed. The absorbance was then read immediately at 540 nm using a Multiscan spectrophotometer (BioTek, USA). Background absorbance (average absorbance of wells containing DMSO, but no cells) was subtracted from the average absorbance of test wells. The average absorbance in the control wells was taken as 100 % survival and the IC_{50} values were defined as the compound concentrations that inhibited the cell growth by 50 % after 96 h exposure.

RESULTS AND DISCUSSION

The hydrazonoyl chloride (**5**), required in this study, is prepared *via* direct coupling of 5-phenylthiophene-3-diazonium chloride (**3A**) [accessible by diazonation of the respective methyl 3-amino-5-phenylthiophene-2-carboxylate (**3**)] with 3-chloro-2,4-pentanedione in aqueous ethanolic sodium acetate (**Scheme-I**). The resulting intermediate azo-compound (**4A**) suffers a loss of an acetyl group, under the prevailing basic reaction conditions, to deliver the corresponding hydrazone structure **5** (Japp-Klingemann reaction) [4-7]. Piperazine, 1-substituted piperazines and the related cyclic *sec*-amine congeners **6a-n** acting as nitrogen nucleophiles, are expected to add readily onto *N*-(thien-3-yl) nitrile imine (the reactive 1,3-dipole generated *in situ* from its hydrazonoyl chloride precursor **5** in the presence of triethylamine) to produce the

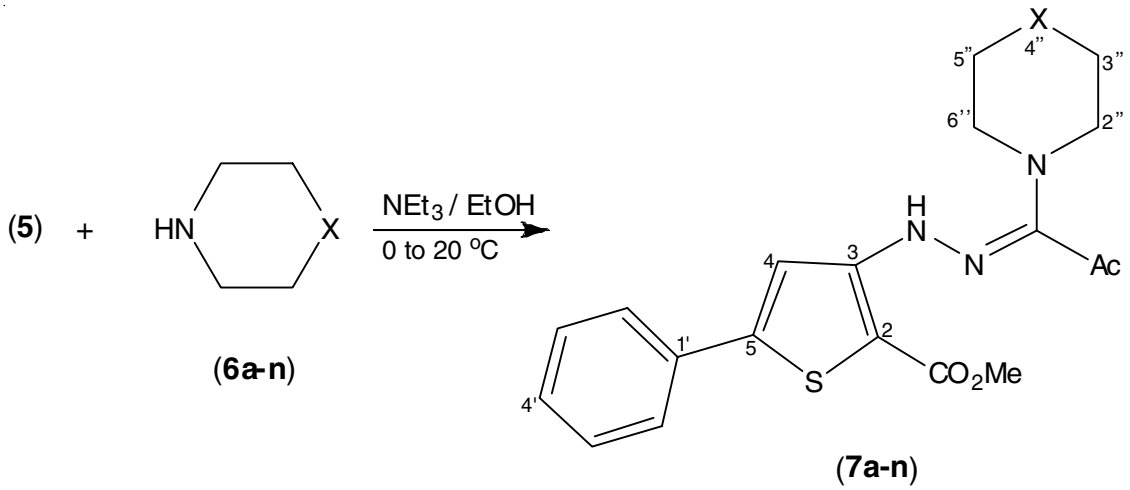


Scheme-I: Synthetic route for compound **5**

respective amidrazone adducts **7a-n** (**Scheme-II**). This mode of nucleophilic addition of various nucleophiles onto 1,3-dipolar species is well-documented in the literature [8-13] and several related amidrazone adducts were obtained from the reaction of amines with hydrazonoyl chlorides [1,2,14-17].

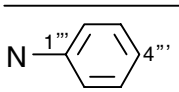
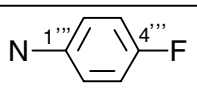
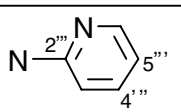
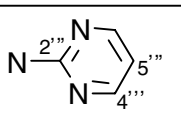
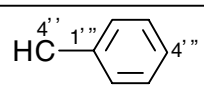
The newly synthesized compounds **5** (**Scheme-I**) and **7a-n** (**Scheme-II**) were characterized by MS, NMR spectral data and elemental analysis (C, H, N). These data, detailed in the experimental section, are in accordance with the assigned structures. Thus, the mass spectra display the correct molecular ion peaks for which the measured high-resolution (HRMS) data are in good agreement with the calculated values. DEPT and 2D (COSY, HMQC, HMBC) experiments showed correlations that helped in the ^{1}H and ^{13}C signal assignments to the different carbons and their attached and/or neighbouring hydrogens. In HMBC experiments, distinct long range "three-bond" (^{1}H and ^{13}C) correlations are observed between H-4 and each of C-1' and C-2, between H-3'/H-5' and C-1',as well as between H-2'/H-6' and C-5. Likewise, correlations are also observes between the amidrazone N-*H* and each of C-2 and the amidrazone carbon (-*C*=N-NH).

Antitumor activity: The antitumor activity of the synthesized amidrazones (**7a-n**) was screened by conductivity cell viability assay using the tetrazolium dye [MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. Cultures of the breast cancer (MCF-7), cervical cancer (HeLa) and colon cancer (RKO) cell lines were treated with the target compounds **7a-n** at one concentration of 100 μM; among these,



(Compounds **6** and **7**)

Entry	a	b	c	d	e	f	g	h	i
X	CH_2	O	S	N-H	N-Me	N-Et	N-CH_2CH_2OH	N-CHO	N-CO_2Et

Entry	j	k	l	m	n
X					

Scheme-II: Synthetic route for compounds **7a-n**

only compounds **7e-g** showed appreciable antitumor activity. The IC_{50} values were determined for each of these three potential compounds against MCF-7, HeLa and RKO cancer cells (Table-1). From the view point of structure-activity relationship, the nature of substituent appended at the *N*(4)-position of the piperazine moiety, seems to play a crucial role in determining the antitumor activity. As can be seen from Table-1, only the *N*(4)-alkyl substituents (Me, Et and CH_2CH_2OH groups in compounds **7e, 7f, 7g,** respectively) enhance considerably the antiproliferative activity of this series. On the other hand, the phenyl group, located at C-5 of the thiophene ring, appears to have no critical contribution in this regard.

TABLE-1
IC_{50} VALUES (μM) FOR COMPOUNDS **7a-n**
AGAINST RKO, MCF-7 AND HeLa CELL LINES

Compound	Colon cancer RKO	Breast cancer MCF-7	Cervital cancer HeLa
7e	5.47 ± 0.03	20.19 ± 1.53	10.36 ± 0.55
7f	12.26 ± 0.19	22.67 ± 3.44	16.42 ± 0.24
7g	20.38 ± 5.47	21.13 ± 2.60	17.05 ± 2.58
7a-d and **7h-n**	Inactive at ≤ 100	Inactive at ≤ 100	Inactive at ≤ 100

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REFERENCES

1. M.M. Abadleh, M.M. El-Abadelah, S.S. Sabri and H.H. Mohammed, *Z. Naturforsch*, **69b**, 811 (2014).
2. M.N. Abu-Aisheh, M.S. Mustafa, M.M. El-Abadelah, R.G. Naffa, S.I. Ismail, M.A. Zihlif, M.O. Taha and M.S. Mubarak, *Eur. J. Med. Chem.*, **54**, 65 (2012).
3. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983).
4. R.R. Phillips, *Org. React.*, **10**, 143 (1959).
5. H.C. Yao and P. Resnick, *J. Am. Chem. Soc.*, **84**, 3514 (1962).
6. G.C. Barrett, M.M. El-Abadelah and M.K. Hargreaves, *J. Chem. Soc. C*, 1986 (1970).
7. V.M. Neplyuev, I.M. Bazavova and M.O. Lozinskii, *Zh.Org.Khim.*, **25**, 2225 (1989).
8. R.N. Butler and F.L. Scott, *Chem. Ind. (London)*, 1216 (1970).
9. A.F. Hegarty, J.B. Aylward and F.L. Scott, *J. Chem. Soc. C*, 2587 (1967).
10. V.A. Galishev, V.N. Chistokletov and A.A. Petrov, *Zh. Obshch. Khim.*, **45**, 1695 (1975).
11. A.S. Shawali and C. Párkanyi, *J. Heterocycl. Chem.*, **17**, 833 (1980).
12. H.M. Hassaneen, H.A.H. Mousa, N.M. Abed and A.S. Shawali, *Heterocycles*, **27**, 695 (1988).
13. T. Benincori and F. Sannicolo, *J. Org. Chem.*, **53**, 1309 (1988).
14. R.J. Abdel-Jalil, E.Q. El-Momani, M. Hamad, W. Voelter, M.S. Mubarak, B.H. Smith and D.G. Peters, *Monatsh. Chem.*, **141**, 251 (2010).
15. E.D. Awad, M.M. El-Abadelah, S. Matar, M.A. Zihlif, R.G. Naffa, E.Q. Al-Momani and M.S. Mubarak, *Molecules*, **17**, 227 (2012).
16. M.M. Abadleh, M.M. EL-Abadelah, S.S. Sabri, Q.M.A. Abdallah, A. Nasr and M.F. EL-Badawy, *Lett. Org. Chem.*, **11**, 664 (2014).
17. A.Y. Habashneh, M.M. El-Abadelah, M.A. Zihlif, A. Imraish and M.O. Taha, *Arch. Pharm. Chem. Life Sci.*, **347**, 415 (2014).