Received: 11 April 2020 | Revised: 12 May 2020 | Accepted: 14 May 2020

DOI: 10.1002/jhet.4041



WILEY

ARTICLE

# Synthesis and in vitro anticancer activity of some novel cyclohepta[*b*]thiophene-3-carboxamides bearing pyrazole moiety

**Mohammed Al-Ghorbani**[1,2] | **Moustafa A. Gouda**[1,3]

[1]Department of Chemistry, Faculty of Science and Arts, Ulla, Taibah University, Medina, Saudi Arabia

[2]Department of Chemistry, Faculty of Education, Thamar University, Dhamar, Yemen

[3]Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, Egypt

**Correspondence**
Moustafa A. Gouda, Department of Chemistry, Faculty of Science and Arts, Ulla, Taibah University, Medina, Saudi Arabia.
Email: dr_mostafa_chem@yahoo.com

**Abstract**

2-Aminothiophene **3** was achieved through the one-pot multicomponent reaction of cycloheptanone, cyanoacetamide, elemental sulfur, and morpholine in ethanol. Diazotization of 2-aminothiophene **3** with $NaNO_2$/HCl gave the corresponding diazonium salt **4**, that combined with the appropriate active methylene components; **5a**, **5b**, **7**, **11**, **13**, **16**, **18**, **21**, **9**, **19**, **22a**, and **22b** in pyridine (AcONa/EtOH) to form the corresponding hydrazones **6a**, **6b**, **8**, **10**, **14**, **15**, **17**, **20**, **23**, **24**, **25a**, and **25b**, respectively. Heating of compound **8** with malononitrile **9** in ethanol gave the thiazole **10**. Treatment of compound **10**, **25a**, and **25b** with hydrazine hydrate achieve the pyrazoles **12**, **27a**, and **27b**, respectively. Hydrazinolysis of compound **14** with hydrazine hydrate, followed by condensation of the obtained hydrazide **15** with acetylacetone **19** gave the pyrazole **20**. The recently orchestrated thiophenes were assessed for their cytotoxic action. The result revealed that compound **12** indicated comparable and better action towards HePG2, HCT-116, MCF-7, and PC3 cancer cell lines than Doxorubicin.

## 1 | INTRODUCTION

The chemistry of 2-aminothiophenes has received much attention; due to their convenient availability through the versatile synthesis introduced by Gewalled.[1] Furthermore, they were discovered to have diverse organic programs, which include, *N*-(4-methoxyphenyl)-*N*,2,5-trimethylthieno [2,3-days]pyrimidin-4-amine (I), a potent apoptosis indu cer[2]; *N*-(4-chlorophenethyl)-2,5-dimethylthieno[2,3-days] pyrimidin-4-amine (II), a potential anti-inflammatory and anti-osteoporosis agent[3]; (2-amino-4,5-dimethylthiophen-3-yl)(3-[trifluoromethyl]phenyl)methanone (III), an agonist of allosteric enhancers (AE)[4] (Figure 1).

Moreover, cyclohepta[*b*]thiophene possesses a huge spectrum of pharmacological activities, including: antimicrobial,[5–8] antiviral,[9,10] anti-inflammatory,[11,12] and antitumor activities.[7,13–17] Moreover, pyrazole derivatives are a distinctive lead nucleus that represents a basic framework of a large number of anticancer agents.[18–21] AT9283 is a powerful inhibitor of Aurora A kinase and of different cancers-associated kinases, PHA-739358 is likewise by and by in Stage II of clinical preliminary, basically for the cure of leukemia.[22,23] The 2-aminothiophene bearing pyrazole nucleus was hardly ever acknowledged.[24–30] Therefore, our research deals with the effective use of 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide in the synthesis of novel pyrazoles incorporating thiophene nucleus to assess their antitumor activity.

## 2 | RESULTS AND DISCUSSION

### 2.1 | Chemistry

The aminopyrazoles **6a**,**b** had been prepared in multi-step reaction sequences as shown in Scheme 1. Treatment of cycloheptanone **1** with cyanoacetamide **2** in ethanol and

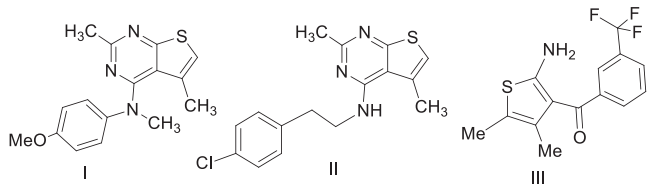


**FIGURE 1** The biological applications of some 2-aminothiophene derivatives

in the presence of morpholine and sulfur, resulted the corresponding 2-aminothiophene-3-carboxamide **3**. Compounds **3** was ultimately subjected to diazotization with sodium nitrite in AcOH/HCl to afford the corresponding diazonium salt **4**, that combined with 3-amino-5-(cyano methyl)-1*H*-pyrazole-4-carbonitriles **5a**,**b**[31] in pyridine to give the preferred cyclohepta[*b*]thiophene-3-carboxamide**s** **6a**,**b** (Scheme 1).

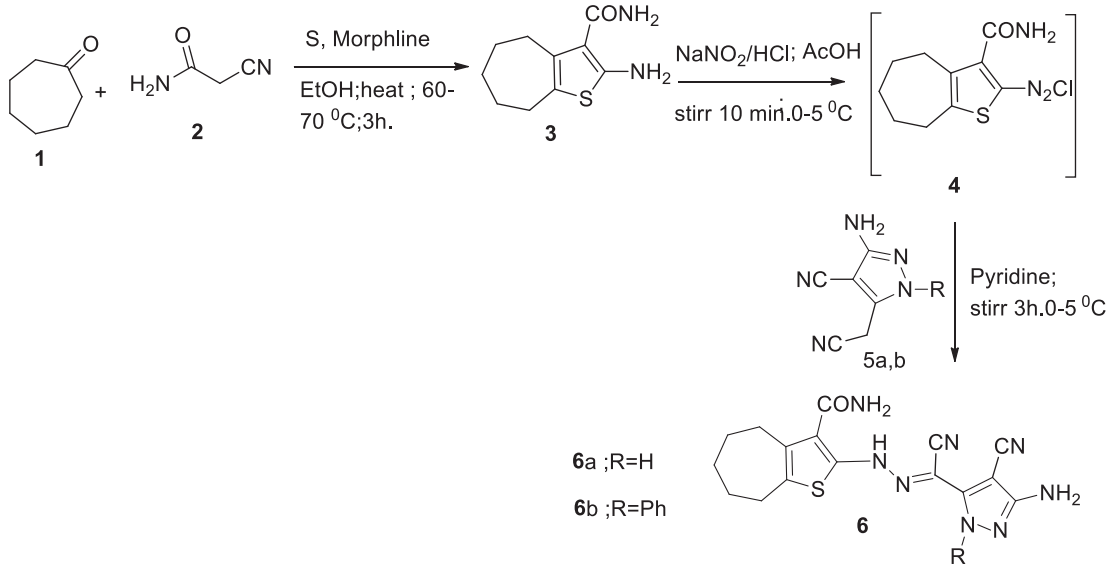
Moreover, the intermediate **4** was coupled with 1-phenyl-2-thiocyanatoethanone **7**[32] in pyridine giving the corresponding hydrazo **8** which, underwent ring closure with malononitrile **9** in DMF in the presence of piperidine to achieve the thiazole **10**. Furthermore, compound **10** was prepared through interaction of **4** with substituted thiazole **11**[33] in pyridine. Cyclization of malononitrile derivative **10** with hydrazine hydrate in DMF achieved the 3,5-diaminopyrazole **12** (Scheme 2).

In addition, the diazonium salt **4** was coupled with various compounds specifically: ethyl 2-cyanoacetate **13** or 2-cyanoacetohydrazide **14**[34] or 5-amino-1*H*-pyrazol-3-ol **16**, or 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile **19**[34] in ethanol in the presence sodium acetate or pyridine to gain the corresponding hydrazones **14**, **15**, **17**, and **20**. Compound **15** may be also prepared through heating of compound **14** with hydrazine hydrate. Cyclization of compound **15** under basic condition gave also, the aminopyrazole **17**. Compound **15** was further converted into 3,5-dimethylpyrazole **20** via the reaction with acetylacetone in EtOH/DMF (Scheme 3).

Finally, the intermediate **4** was reacted with malononitrile **9**, acetylacetone **19**, or 3-methyl-1-*H*-pyrazol-5(4-*H*)-one **20a**,**b**[35,36] in ethanol/sodium acetate (pyridine) to afford the corresponding hydrazone **23**, **24**, **25a**, and **25a**,**b**, respectively. Compounds **23** and **24** cyclized with hydrazine hydrate in ethanol to give the 3,5-diaminopyrazole **26** and 3,5-dimethylpyrazole **27**, respectively (Scheme 4).

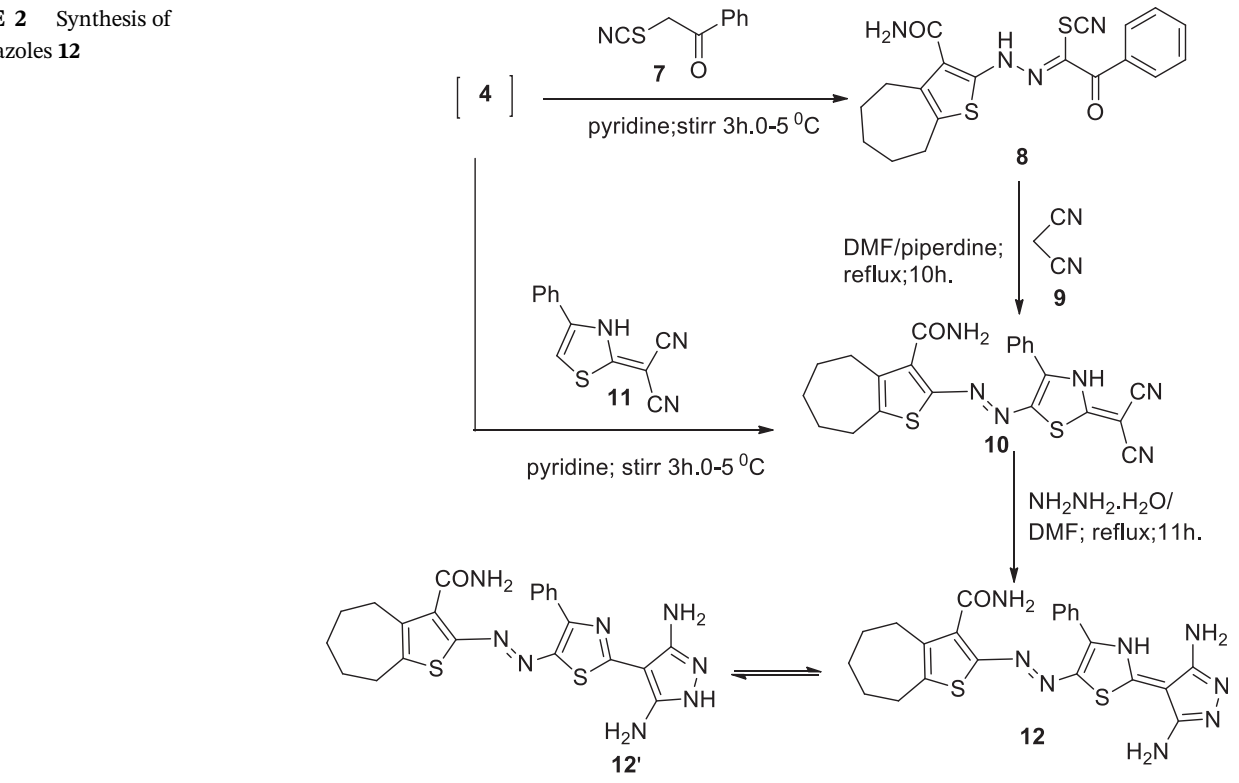
## 2.2 | In vitro anticancer activity

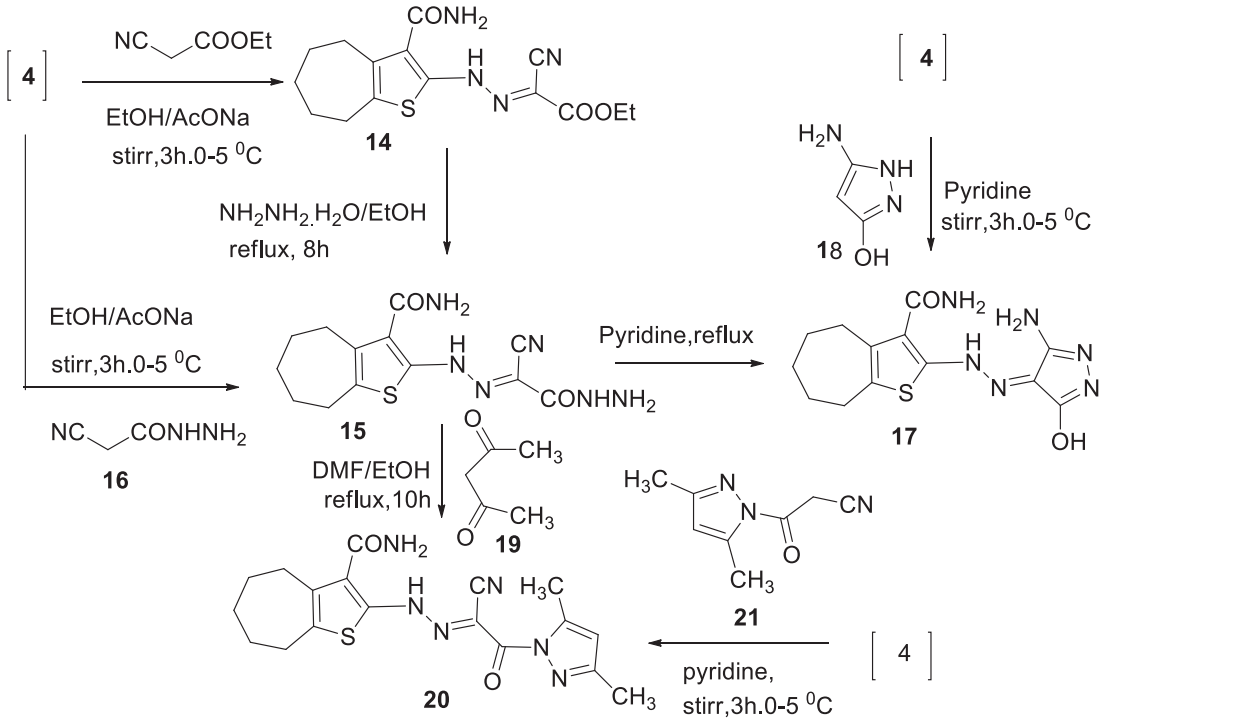
The anticancer activity of 15 novel pyrazoles were evaluated in vitro against a liver (HePG2), breast (MCF-7), colon (HCT-116), and prostate (PC3 human) cancer cell lines, using the standard MTT method.[37] All tested compounds were screened for cytotoxic activity with imply 50% inhibition concentration ($IC_{50}$). MTT assay is measuring cell growth by colorimetric changes. It is utilized to decide cytotoxicity of capacity therapeutic specialists and other dangerous materials. In summary, yellow MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bro mide) is decreased to purple formazan by mitochondrial dehydrogenases of living cells. An appropriate solvent is used to dissolve purple formazan product. The absorbance of this colored solution might be evaluated at a definite wavelength. At the point when the measure of




**SCHEME 1** Synthesis of thiophene-3-carboxamides **6a**,**b**

**SCHEME 2** Synthesis of diaminopyrazoles **12**

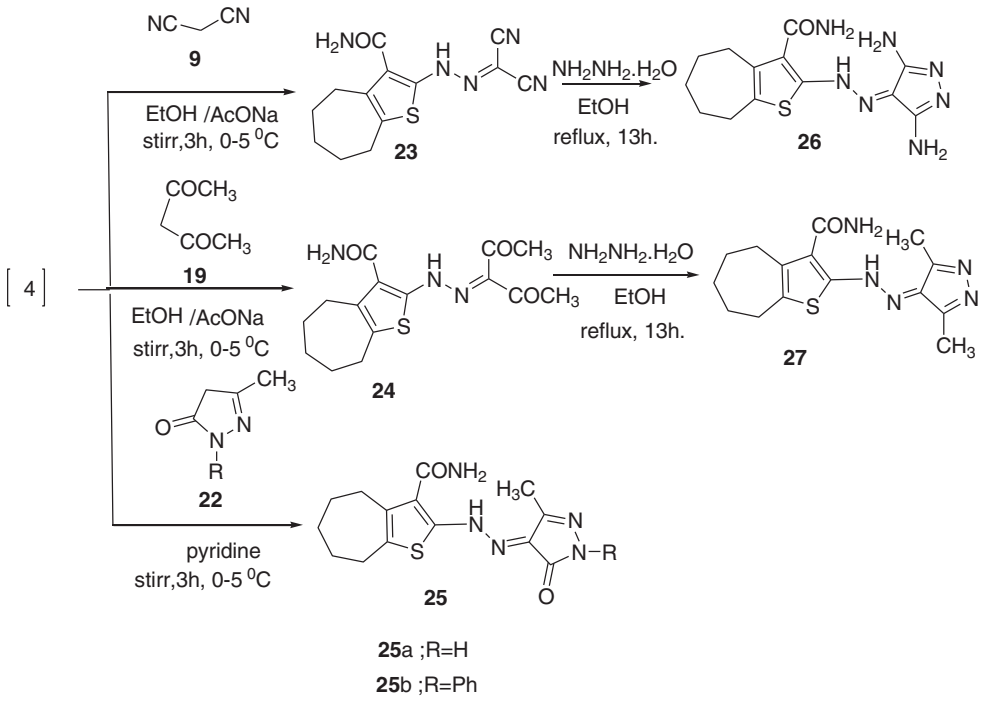



**SCHEME 3** Synthesis of pyrazoles **17** and **20**

purple formazan created by cells treated with an agent is compared with that produced by unreacted control cells, the viability of the agent in causing death of cells can be deduced, through the production of a dose response curve.[38]

The cytotoxicity consequences Table 1, found out that compounds, **12** was the first-rate inhibitory activity in opposition to HCT-116, HePG2, PC3, and MCF-7 cancer cell lines. Whereas, compounds **8** and **25a** are the vulnerable inhibitory activity towards the selected cancer cell lines. As




**SCHEME 4** Synthesis of pyrazoles **25**-**27**

**TABLE 1** Cytotoxic activity of pyrazole derivatives against four human tumor cells

| Compound no. | In vitro cytotoxicity IC50 (μg/mL)[a] | | | |
|---|---|---|---|---|
| | HePG2 | HCT-116 | MCF-7 | PC3 |
| Doxorubicin | 4.50 ± 0.2 | 5.23 ± 0.3 | 4.17 ± 0.2 | 8.87 ± 0.6 |
| **5** | 20.36 ± 1.8 | 38.52 ± 3.2 | 32.73 ± 2.5 | 32.73 ± 2.5 |
| **6** | 59.50 ± 3.8 | 76.60 ± 4.6 | 63.84 ± 4.2 | 82.33 ± 4.9 |
| **8** | 81.29 ± 4.3 | 78.63 ± 4.6 | 69.93 ± 4.5 | 93.82 ± 5.3 |
| **10** | 8.94 ± 0.7 | 20.13 ± 1.8 | 11.65 ± 1.0 | 28.31 ± 2.8 |
| **12** | 3.81 ± 0.2 | 5.85 ± 0.4 | 4.92 ± 0.3 | 9.70 ± 0.8 |
| **14** | 45.49 ± 3.2 | 62.41 ± 4.1 | 53.41 ± 3.8 | 79.39 ± 4.8 |
| **15** | 15.40 ± 1.4 | 35.76 ± 2.9 | 24.55 ± 2.0 | 45.94 ± 3.7 |
| **17** | 6.14 ± 0.4 | 16.84 ± 1.4 | 7.82 ± 0.6 | 27.39 ± 2.4 |
| **20** | 47.23 ± 3.4 | 71.42 ± 4.5 | 56.90 ± 3.8 | 89.43 ± 5.1 |
| **23** | 48.13 ± 3.6 | 66.05 ± 4.1 | 58.76 ± 3.9 | 76.29 ± 4.8 |
| **24** | 7.58 ± 0.6 | 13.76 ± 1.2 | 9.39 ± 0.8 | 20.78 ± 1.7 |
| **25a** | 92.65 ± 5.1 | 85.35 ± 4.8 | 73.18 ± 4.7 | >100 |
| **25b** | 25.08 ± 2.0 | 43.78 ± 3.5 | 35.18 ± 2.7 | 59.04 ± 3.9 |
| **27** | 14.32 ± 1.3 | 31.12 ± 2.7 | 22.69 ± 1.9 | 41.45 ± 3.6 |

[a]IC50 (μg/mL): 1-10 (very strong); 11-20 (strong); 21-50 (moderate); 51-100 (weak); and above 100 (non-cytotoxic).

for hobby in the competition to HepG2 cell line, the very highest cytotoxic activity has become showed with the aid of compound **10**, **17**, and **24**, that displayed the proportion viability IC50 at 8.94, 6.14, and 7.58 mg/mL, respectively, while, the best cytotoxic activity was hobby changed into displayed by compound **5**, **15**, and **27** which, confirmed the proportion viability IC50 at 20.36, 15.40, and 14.32 mg/mL respectively. The MCF-7, and HCT-116 cell lines showed moderate activity towards compounds **5**, **15**, **25b**, and **27** which have IC50 at 38.52 (32.73), 35.76 (24.55), 31.12 (22.69), and 43.18 (35.18) mg/mL, respectively. However, compound **5** and **15** showed slight activity, while, compounds **6**, **12**, **22**, and **23** displayed weak cytotoxic activities against MCF-7, PC3, and HCT-116 cancer cell lines.

Compound **12** showed comparable and better activity in opposition to HCT-116, MCF-7, PC3, and HePG2 cancer cell lines than Doxorubicin.

## 3 | EXPERIMENTAL

### 3.1 | Materials and instrumentation

All melting points were decided on Gallenkamp electronic melting point equipment. The infrared (IR) spectra were recorded on a Mattson 5000 Fourier transform-IR (FT-IR) spectrophotometer ($\lambda$, $cm^{-1}$). The $^1$H-NMR (DMSO-d) spectra were determined on a Bruker AV 300 MHz, using TMS as internal standard. Mass spectra were obtained on GCMS/QP1000 Ex mass spectrometer at 70 eV. Elemental analyses (C, H, and N) had been done at the Microanalytical Center of Cairo University, Giza, Egypt. The biological activities were carried in Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

### 3.2 | Chemistry

Coupling of the diazonium salt of 2-aminothiophene derivative **4** with 3-amino-5-(cyanomethyl)-1-*H*-pyrazole-4-carbonitrile **5a** or 3-amino-5-(cyanomethyl)-1-phenyl-1-*H*-pyrazole-4-carbonitrile **5b** or 1-phenyl-2-thiocyanato ethanone 7 or 2-(4-phenylthiazol-2[3*H*]-ylidene) malononitrile **10** or 3-methyl-1*H*-pyrazol-5(4*H*)-one **22a**, or 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one **22b**.

### 3.3 | General procedure

A solution of 2-aminothiophene derivative **3** (10 m*M*; 2.10 g) in 5 mL acetic acid, and 3 mL conc. HCl was stirred and cooled in ice bath. Then, a solution of sodium nitrite (0.8 g, 1.15 m*M*) in 8 mL water was brought dropwise for 10 min. The cold diazonium solution become delivered slowly to a properly stirred solution of 3-amino-5-(cyanomethyl)-1*H*-pyrazole-4-carbonitrile (1.47 g, 10 m*M*) **5a**, 3-amino-5-(cyanomethyl)-1-phenyl-1*H*-pyrazole-4-carbonitrile (2.23 g, 10 m*M*) **5b**, 1-phenyl-2-thiocyanato ethanone **7** (1.77 g, 10 m*M*), 2-(4-phenylthiazol-2-[3*H*]-ylidene)malononitrile **10** (2.25 g, 10 m*M*), 3-methyl-1*H*-pyrazol-5(4*H*)-one **22a** (0.98 g, 10 m*M*), or 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one **22b** (1.74 g, 10 m*M*) in pyridine (40 mL). Then, the reaction mixture was stirred for 3 hours, and the formed precipitate was filtered off, dried, and recrystallized from ethanol/benzene to afford **6a**,**b**, **10**, **12**, and **25a**,**b**.

#### 3.3.1 | (*Z*)-2-(2-((3-amino-4-cyano-1*H*-pyrazol-5-yl)(cyano)methylene)hydrazinyl) 5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*] thiophene-3-carboxamide (6a)

Yield 83%; reddish brown powder; mp > 320°C. IR (KBr) $\nu_{max}/cm^{-1}$ = 3424, 3314, 3214, 3171 (2$NH_2$, 2NH), 2923, 2853 (C-aliph), 2215 (2CN), 1649 (CO), 1596 (C═C), 1526 (C═N). $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.50-1.68 (m, 4H, cycloheptane, $CH_2$), 1.75-1.81 (m, 2H, cycloheptane, $CH_2$), 2.66-2.78 (m, 4H, cycloheptane, $CH_2$), 7.17 (br, 2H, $NH_2$), 7.34 (br, 2H, $CONH_2$), 7.65 (br, 1H, $NH_{pyrazole}$), 12.54 (br, 1H, $NH_{hydrazo}$); ms: (m/z, %): 368 (M+, 22.23), 329.27 (61.16), 317.94 (100), 282.34 (76.65), 272.01 (67.84), 254.61 (97.11), 227.27 (40.08), 194.30 (46.51), 131.51 (38.68), 94.82 (51.91), 69.86 (44.49). Anal. Calcd for $C_{16}H_{16}N_8OS$: C, 52.16; H, 4.38; N, 30.41. Found: C, 51.99; H, 4.15; N, 30.22%.

#### 3.3.2 | (*Z*)-2-(2-((3-amino-4-cyano-1-phenyl-1*H*-pyrazol-5-yl)(cyano) methylene) hydrazin-yl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide (6b)

Yield 69%; reddish brown powder; mp > 320°C. IR (KBr) $\nu_{max}/cm^{-1}$ = 3428 (br), 3332, 3224, 3185 (2$NH_2$, NH), 2924, 2854 (C-aliph), 2208 (2CN), 1634 (CO), 1628 (C═C), 1528 (N═N). $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.50-1.73 (m, 4H, cycloheptane, $CH_2$), 1.75-1.81 (m, 2H, cycloheptane, $CH_2$), 2.63-2.80 (m, 4H, cycloheptane, $CH_2$), 6.85 (br, 2H, $NH_2$), 7.20-7.67 (m, 9H, ArH, $CONH_2$), 7.93 (br, 1H, $NH_{pyrazol}$), 12.50 (br, 1H, $NH_{hydrazo}$). Anal. Calcd for $C_{22}H_{20}N_8OS$; C, 59.44; H, 4.54; N, 25.21. Found: C, 59.26; H, 4.29; N, 25.17%.

#### 3.3.3 | (*Z*)-2-(2-[2-oxo-2-phenyl-1-thiocyanatoethylidene] hydrazinyl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*] thiophene-3-carboxamide (10)

Yield 90%; pale yellow powder; mp > 320°C. IR(KBr) $\nu_{max}/cm^{-1}$ = 3433 (br), 3300, 3185 ($NH_2$, NH), 2924, 2854 (C-aliph), 2203 (CN), 1662 (2CO), 1599 (C═C), 1571 (N═N). $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.50-1.62 (m, 4H, cycloheptane $CH_2$), 1.81-1.89 (m, 2H, cycloheptane, $CH_2$), 2.59-2.92 (m, 4H, cycloheptane, $CH_2$), 7.18-7.84 (m, 7H, ArH, $CONH_2$,), 13.05 (br, 1H, NH); ms: (m/z, %): 400 (M + 2, 22.23), 398.76 ($M^+$, 7.61), 368.82 (100), 351.08 (36.97), 229.14 (49.30), 228 (58.63), 200.01 (40.08), 144.93 (54.73). Anal. Calcd for $C_{19}H_{18}N_4O_2S_2$; C, 57.27; H, 4.55; N, 14.06. Found: C, 57.09; H, 4.26; N, 14.13%.

### 3.3.4 | (*E*)-2-(2-[dicyanomethylene]-4-phenyl-2,3-dihydrothiazol-5-yl) diazenyl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*] thiophene-3-carboxamide (10)

Yield 83%; green powder; mp > 320°C. IR (KBr) $\nu_{max}/cm^{-1}$ = 3445, 3363, 3170 ($NH_2$, NH), 2920, 2847 (C-aliph), 2214, 2201 (2CN), 1649 (CO), 1593 (C═C), 1543 (N═N). $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.50-1.69 (m, 4H, cycloheptane, $CH_2$), 1.75-1.81 (m, 2H, cycloheptane, $CH_2$), 2.63-2.75 (m, 4H, cycloheptane $CH_2$), 7.33-7.84 (m, 8H, Ar-H, $NH_2$, NH); ms: (m/z, %): 447 (M + 1, 22.33), 446 (M+, 19.49), 400.79 (40.58), 373.31 (34.33), 303.48 (56.07), 271.96 (32.54), 175.35 (31.06), 140.17 (40.89), 126.96 (55.51), 92.39 (100), 73.42 (59.09), 53.30 (43.02). Anal. Calcd for $C_{22}H_{18}N_6OS_2$; C, 59.17; H, 4.06; N, 18.82. Found: C, 58.94; H, 4.13; N, 18.66%.

Compound **10** was additionally, prepared via heating of (*Z*)-2-(2-[2-oxo-2-phenyl-1-thiocyanatoethyli dene] hydrazinyl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thi ophene-3-carbox-amide **8** (1.78 g, 10 m*M*), malononitrile **9** (0.66 g, 10 m*M*) and piperidine (0.85 g, 10 m*M*) in ethanol 50 mL for 10 hours. The reaction mixture became cooled and the fashioned precipitate became filtered to give compound **9**. Yield 80%; green powder.

## 3.4 | Synthesis of (*E*)-2-([2-3,5-diamino-4*H*-pyrazol-4-ylidene]-4-phenyl-2,3-dihydrothia-zol-5-yl)diazenyl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*] thiophene-3-carboxamide (12)

A suspension of **10** (0.446 g, 1 m*M*), and hydrazine hydrate (0.26 g, 4 m*M* mol) in ethanol (40 mL) was refluxed for 11 hours. The formed precipitate after cooling was filtered off and crystallized from DMF/EtOH to yield compound **12**. Yield 66%; brown crystals; mp > 320°C. IR (KBr) $\nu_{max}/cm^{-1}$ = 3431, 3376, 3328, 3271 ($3NH_2$, NH), 2922, 2851 (C-aliph), 2198 (CN), 1648 (CO), 1592 (C═C), 1546 (N═N). $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.50-1.69 (m, 4H, cycloheptane, $CH_2$), 1.75-1.81 (m, 2H, cycloheptane, $CH_2$), 2.63-2.75 (m, 4H, cycloheptane, $CH_2$), 7.34-7.84 (m, 12H, Ar-H, $3NH_2$, NH); ms: (m/z, %): 479 (M + 1, 0.81), 478 (M+, 9.47), 386.58 (100), 341.28 (54.64), 216.28 (16.02), 187.11 (54.61), 156.08 (36.07), 129.25 (36.56), 68.22 (19.26). Anal. Calcd for $C_{22}H_{22}N_8OS_2$; C, 55.21; H, 4.63; N, 23.41. Found: C, 55.13; H, 4.55; N, 23.53%.

### 3.4.1 | (*E*)-2-(2-(3-methyl-5-oxo-1*H*-pyrazol-4[5*H*]-ylidene) hydrazinyl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*] thiophene-3-carboxamide (25a)

Yield 93%; scarlet red crystal; mp > 320°C. IR(KBr) $\nu_{max}/cm^{-1}$ = 3423, 3357, 3195 ($NH_2$, NH), 2924, 2854 (C-aliph), 1690, 1659 (2CO), 1587 (N═N); $^1$H NMR (DMSO-$d_6$) ($\delta$/ppm): 1.50-1.68 (m, 4H, cycloheptane, $CH_2$), 1.73-1.84 (m, 2H, cycloheptane, $CH_2$), 2.62-2.83 (m, 4H, cycloheptane, $CH_2$), 2.91 (s, 3H, $CH_3$), 7.58 (br, 2H, $NH_2$), 8.55 (br,1H, $NH_{pyrazolone}$), 12.50 (br, 1H, $NH_{hydrazo}$); ms: (m/z, %): 320 (M + 1, 22.44), 319 (M+, 32.12), 304.23 (64.13), 276.98 (69.42), 250.35 (74.27), 231.07 (78.16), 204.47 (67.68), 190.35 (69.76), 150.27 (67.25), 122.04 (100), 67.55 (60.34); Anal. for $C_{14}H_{17}N_5O_2S$; C, 52.65; H, 5.37; N, 21.93. Found: C, 52.33; H, 5.13; N, 22.05%.

### 3.4.2 | (*E*)-2-(2-(3-methyl-5-oxo-1-phenyl-1*H*-pyrazol-4[5*H*]-ylidene)hydrazinyl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*] thiophene-3-carboxamide (25b)

Yield 90%; scarlet red crystal; mp > 320°C. IR(KBr) $\nu_{max}/cm^{-1}$ = 3422, 3313, 3195 ($NH_2$, NH), 2919, 2849 (C-aliph), 1671, 1631 (2CO), 1542 (N═N). $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.50-1.66 (m, 4H, cycloheptane, $CH_2$), 1.73-1.84 (m, 2H, cycloheptane, $CH_2$), 2.57 (s, 3H, $CH_3$), 2.65-2.81 (m, 4H, cycloheptane $CH_2$), 6.80-7.65 (m, 7H, ArH, $NH_2$), 9.12 (br, 1H, $NH_{hydrazo}$); ms: (m/z, %): 395(M+, 14.97), 235.17 (100), 217.30 (71.30), 177.28 (18.28), 126.93 (46.29), 89.16 (60.17), 74.59 (56.19), 64.18 (67.33), 62.91 (64.25), 55.40 (46). Anal. Calcd for $C_{20}H_{21}N_5O_2S$; C, 60.74; H, 5.35; N, 17.71. Found: C, 60.63; H, 5.42; N, 17.49%.

Reaction of the diazonium salt of 2-aminothiophene derivative **4** with ethyl 2-cyanoacetate, 2-cyanoaceto hydrazide **16**, 5-amino-1*H*-pyrazol-3-ol **18**, 3-(3,5-dimethyl-*1-H*-pyrazol-1-yl)-3-oxopropanenitrile **21**, malononitrile **9**, or acetylacetone **19**.

## 3.5 | General procedure

A solution of 2-aminothiophene **3** (2.10 g, 10 m*M*) in 5 mL AcOH, and 3 mL conc. HCl was stirred and cooled in ice bath. Then, a solution of sodium nitrite (0.8 g, 1.15 10 m*M*) in 8 mL water was added dropwise for 10 minutes. The cold diazonium salt was added slowly to a nicely stirred cold solution of ethyl 2-cyanoacetate (1.13 g, 10 m*M*), 2-cyanoacetohydrazide **16** (1.00 g, 10 m*M*),

5-amino-1-*H*-pyrazol-3-ol **18** (1.00 g10 m*M*), 3-(3,5-dimethyl-1-*H*-pyrazol-1-yl)-3-oxopropanenitrile **21** (1.63 g, 10 m*M*), malononitrile **9** (0.66 g, 10 m*M*), or acetylacetone **19** (1.00 g, 10 m*M*) in EtOH (60 mL) containing AcONa (4 g, 50 m*M*). The reaction mixture was stirred for 3 hours. The formed precipitate was filtered off, dried, and recrystallized from EtOH/benzene to give **14**, **15**, **17**, **20**, **23**, and **24**.

### 3.5.1 | (*E*)-Ethyl 2-(2-(3-carbamoyl-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*] thiophen-2-yl) hydrazono)-2-cyanoacetate (14)

Yield 81%; reddish brown powder; mp = 315°C. IR(KBr) $\nu_{max}/cm^{-1}$ = 3428, 3385, 3185 ($NH_2$, NH), 2924, 2853 (C-aliph), 2191 (CN), 1661 (br, 2CO), 1542 (N═N). $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.14 (t, 3H, $CH_3$, J = 6.9), 1.50-1.69 (m, 4H, cycloheptane, $CH_2$), 1.75-1.81 (m, 2H, cycloheptane, $CH_2$), 2.63-2.75 (m, 4H, cycloheptane, $CH_2$), 4.12 (q, 2H, $CH_2$, J = 6.9), 7.44 (br, 2H, $NH_2$), 12.52 (br, 1H, $NH_{hydrazo}$) ms: (m/z, %): 334(M+, 7.47), 331.37 (33.37), 309.60 (56.89), 268.28 (41.99), 249.99 (26.59), 193.20 (21.13), 152.31 (49.89), 94.56 (30.07), 69.29 (100), 53.39 (52.31). Anal. Calcd for $C_{15}H_{18}N_4O_3S$; C, 53.88; H, 5.43; N, 16.75. Found: C, 53.53; H, 5.29; N, 16.68%.

### 3.5.2 | (*E*)-2-(2-(3-carbamoyl-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*] thiophen-2-yl) hydrazono)-2-cyanoacetohydrazide (15)

Yield 79%; reddish brown powder; mp > 320°C. IR(KBr) $\nu_{max}/cm^{-1}$ = 3385, 3333, 3242, 3185 ($2NH_2$, 2NH), 2923, 2851 (C-aliph), 2221 (CN), 1649 (br, 2CO), 1519 (N═N ms: (m/z, %): 320 ($M^+$, 6.93), 311.27 (34.89), 278.28 (15.73), 268.08 (16.67), 229.37 (16.10), 165.21 (30.68), 120.15 (39.59), 85.17 (63.57), 76.18 (57.55), 52.22 (34.97) .$^1$H-NMR (DMSO-$d_6$) $\delta$ 1.55-1.72 (m, 4H, cycloheptane, $CH_2$), 1.75-1.81 (m, 2H, cycloheptane, $CH_2$), 2.63-2.75 (m, 4H, cycloheptane, $CH_2$), 3.85 (br, 2H, $NH_2$), 7.29 (br, 2H, $CONH_2$), 9.23 (br, 1H, $NH_{hydrazon}$) 12.15 (br, 1H, NHCO) ms: (m/z, %): 322 (M + 2, 2.15), 320 (M+, 4.38), 242.30 (100), 226.49 (67.77), 197.96 (46.80), 184.80 (69), 146.40 (86.99), 130.16 (54.77), 104.19 (71.05), 84.27 (68.02), 70.37 (59.72), 52.21 (30.18). Anal. Calcd for $C_{13}H_{16}N_6O_2S$; C, 48.74; H, 5.03; N, 26.23. Found: C, 48.54; H, 4.87; N, 26.08%.

Also, compound **15** can be prepared via refluxing a suspension of (*E*)-ethyl 2-(2-(3-carbamoyl-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophen-2-yl)hydrazono)-2-cyanoacetate **14** (0.344 g, 1 m*M*) and hydrazine hydrate (0.26 g, 4 m*M*) in ethanol (40 mL) for 8 hours. The response mixture was cooled and the formed precipitate was filtered to offer compound **15**; Yield 79%; mp >320°C.

### 3.5.3 | (*E*)-2-([5-amino-3-hydroxy-1*H*-pyrazol-4-yl] diazenyl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide (17)

Yield 80%; black powder; mp > 320°C. IR(KBr) $\nu_{max}/cm^{-1}$ = 3421, 3414, 3357, 3285, 3185 ($2NH_2$, NH, OH), 2923, 2852 (C-aliph), 2205 (CN), 1635 (CO), 1513 (N═N). $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.50-1.62 (m, 4H, cycloheptane, $CH_2$), 1.81-1.89 (m, 2H, cycloheptane, $CH_2$), 2.59-2.92 (m, 4H, cycloheptane, $CH_2$), 6.95 (br, 2H, $NH_2$), 7.43 (br, 2H, $CONH_2$), 10.35 (br, 1H, NH), 11.25 (br, 1H, OH); MS (rel.int.%) (70 ev, %): 322 (M + 2, 6.11), 320 (M+, 15.46), 294.72 (13.45), 227.53 (11.25), 209.40 (17.17), 134.60 (14.12), 109.48 (16.05), 76.13 (100), 59.17 (35.53). Anal. Calcd for $C_{13}H_{16}N_6O_2S$; C, 48.74; H, 5.03; N, 26.23. Found: C, 48.53; H, 4.89; N, 26.11%.

Compound **17** may be prepared via heating a suspension of acetohydrazide **15** (0.32 g, 1 m*M*) in ethanol (20 mL) containing pyridine (5 mL) for 12 hours. The reaction mixture was cooled and the formed precipitate after neutralization the reaction mixture with dilute HCl, was filtered to form compound **17**. Yield 82%; mp > 320°C.

### 3.5.4 | (*E*)-2-(2-(1-cyano-2-[3,5-dimethyl-1*H*-pyrazol-1-yl]-2-oxoethylidene) hydrazinyl)-5, 6, 7, 8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide (20)

Yield 67%; red powder; mp > 320°C. IR (KBr) $\nu_{max}/cm^{-1}$ = 3431, 3342, 3185 ($NH_2$, NH), 2926, 2855 (C-aliph), 2207 (CN), 1671 (br, 2CO), 1515 (N═N). $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.50-1.69 (m, 4H, cycloheptane, $CH_2$), 1.75-1.81 (m, 2H, cycloheptane, $CH_2$), 2.63-2.75 (m, 4H, cycloheptane, $CH_2$), 3.05 (s, 3H, $CH_3$), 3.15 (s, 3H, $CH_3$), 6.66 (s, 1H, CH, pyrazole), 7.44 (br, 2H, $NH_2$), 12.52 (br, 1H, $NH_{hydrazo}$) ms: (m/z, %): 386 (M + 2, 17.87), 384 ($M^+$, 21.24), 347.34 (49.38), 328.44 (23.19), 250.85 (37.77), 202.23 34.83), 169.33 (35.34), 160.19 (39.82), 115.78 (55.06), 106.13 (87.86), 104.26 (100), 93.09 (67.95), 74.72 (41.90), 66.13 (12.80). Anal. Calcd for $C_{18}H_{20}N_6O_2S$; C, 56.23; H, 5.24; N, 21.86. Found: C, 56.17; H, 5.33; N, 21.75%.

Compound **20** can be also, prepared via refluxing a mixture of acetohydrazide **15** (0.32 g, 1 m*M*) and acetylacetone **19** (0.1 g, 1 m*M*) in ethanol (20 mL) containing dimethylformamide (5 mL) was refluxed for 10 hours. The reaction mixture was cooled and the obtained precipitate after pouring the reaction mixture in

ice cooled water was filtered to achieve compound **20**. Yield 67%; mp > 320°C.

### 3.5.5 | 2-(2-hydrazonoemalonyl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*] thiophene-3-carboxamide (23)

Yield 70%; reddish brown powder; mp > 320°C. IR(KBr) $\nu_{max}/cm^{-1}$ = 3434, 3342, 3192 ($NH_2$, NH), 2927, 2855 (C-aliph), 2201 (br, 2CN), 1659 (CO), 1588 (N═N). $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.50-1.69 (m, 4H, cycloheptane, $CH_2$), 1.75-1.91 (m, 2H, cycloheptane, $CH_2$), 2.60-2.90 (m, 4H, cycloheptane, $CH_2$), 7.48 (br, 2H, $CONH_2$), 13.23 (br, 2H, NH═N) ms: (m/z, %): 287 ($M^+$, 26.63), 266.05 (47.26), 225.61 (39.26), 170.24 (95.05), 139.64 (44.85), 133.61 (100), 113.82 (50.15), 105.13 (92.61), 101.63 (73.71), 95.42 (32.62), 65.02 (40.54). Anal. Calcd for $C_{13}H_{13}N_5OS$; C, 54.34; H, 4.56; N, 24.37. Found: C, 54.12; H, 4.37; N, 24.39%.

### 3.5.6 | 2-(2-[2, 4-Dioxopentan-3-ylidene] hydrazinyl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide (24)

Yield 73%; pale yellow powder; mp > 320°C. IR (KBr) $\nu_{max}/cm^{-1}$ = 3373, 3342, 3190 ($2NH_2$, NH), 2923, 2852 (C-aliph), 2208 (CN), 1670 (br, 2CO), 1638 ($CONH_2$), 1551 (N═N). $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.50-1.62 (m, 4H, cycloheptane, $CH_2$), 1.81-1.89 (m, 2H, cycloheptane, $CH_2$), 2.34 (s, 3H, $CH_3$), 2.36 (s, 3H, $CH_3$), 2.59-2.81 (m, 4H, cycloheptane, $CH_2$), 7.60 (br, 2H, $CONH_2$), 15.02 (br, 1H, $NH_{hydrazo}$) ms: (m/z, %): 321.00 ($M^+$, 2.8), 245.19 (24.15), 220.41 (26.64), 126.28 (22.59), 115.48 (24.45), 105.75 (39.89), 96.23 (46.19), 88.31 (32.17), 77.19 (100), 74.28 (48.80), 69.19 (33.55), 63.17 (44.55). Anal. Calcd for $C_{15}H_{19}N_3O_3S$; C, 56.06; H, 5.96; N, 13.07. Found: C, 56.19; H, 6.03; N, 13.34%.

## 3.6 | Synthesis of 2-(2-[3,5-diamino-4*H*-pyrazol-4-ylidene]hydrazinyl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*] thiophene-3-carboxamide (26) and 2-(2-[3,5-dimethyl-4*H*-pyrazol-4-ylidene] hydrazinyl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thioph-ene-3-carboxamide (27)

### 3.6.1 | General procedure

A mixture of dicyanide **23** (0.287 g, 1 m*M*) or 2-(2-[2, 4-dioxopentan-3-ylidene]hydrazinyl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thioph-ene-3-carboxamide **24** (0.321 g, 1 m*M*) and hydrazine hydrate (0. 26 g, 4 m*M*) in EtOH (45 mL) was refluxed for 13 hours. The reaction mixture was cooled and the formed precipitate was filtered to afford compounds **26** and **27**.

### 3.6.2 | Compound 26

Yield 78%; black powder; mp > 320°C. IR(KBr) $\nu_{max}/cm^{-1}$ = 3424, 3357, 3192 ($3NH_2$, NH), 2922, 2852 (C-aliph), 1641 (CO), 1528 (N═N). $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.50-1.73 (m, 4H, cycloheptane, $CH_2$), 1.75-1.81 (m, 2H, cycloheptane, $CH_2$), 2.63-2.80 (m, 4H, cycloheptane, $CH_2$), 5.75 (br, 4H, $2NH_2$), 7.17 (br, 2H, $CONH_2$), 12.54 (br, 1H, $NH_{hydrazo}$); ms: (m/z, %): 321 (M + 2, 26.63), 319 (M+, 5.54), 245.19 (24.15), 236.33 (21.83), 220.41 (26.64), 190.23 (18.70), 164.99 (19.67), 126.28 (22.59), 105.20 (4.26), 96.23 (46.19), 77.19 (100), 64.10 (55.78), 51.15 (27.70); Anal. for $C_{13}H_{17}N_7OS$; C, 48.89; H, 5.36; N, 30.70. Found: C, 48.77; H, 5.23; N, 30.55%.

### 3.6.3 | Compound 27

Yield 75%; black powder; m.p 320°C. IR (KBr) $\nu_{max}/cm^{-1}$ = 3402, 3314, 3214, 3192 ($NH_2$, NH), 2922, 2851 (C-aliph), 1655 (CO), 1585 (N═N). $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.50-1.74 (m, 4H, cycloheptane, $CH_2$), 1.81-1.89 (m, 2H, cycloheptane, $CH_2$), 2.59-2.92 (m, 4H, cycloheptane, $CH_2$), 3.02 (s, 3H, $CH_3$), 3.05 (s, 3H, $CH_3$), 7.42 (br, 2H, $CONH_2$), 13.23 (br, 1H, NH═N); ms: (m/z, %): 333 ($M^+$, 14.19), 304.23 (64.13), 250.35 (74.27), 217.07 (30.78), 204.47 (67.68), 150.27 (67.25), 122.04 (100), 113.95 (26.90), 78.36 (35.61), 67.55 (60.34), 58.42 (31.30); Anal. for $C_{15}H_{19}N_5OS$; C, 56.76; H, 6.03; N, 22.06. Found: C, 56.34; H, 6.12; N, 22.02%.

## 3.7 | Biological activity

The reagents RPMI-1640 medium (Sigma Co., St. Louis) Fetal Bovine serum (GIBCO, UK) and the human cell lines HepG2, HCT-116, MCF-7, and PC3 were obtained from ATCC. The cell lines cited above have been used to decide the inhibitory effects of compounds on cell growth using the MTT assay. Extra information about the MTT assay, cytotoxicity activity and $IC_{50}$ for each compound was presented in literature [39].

## 4 | CONCLUSION

The thiophene moiety is presence in natural and synthetic heterocyclic compounds with an extensive spectrum of biological activities. Inside the presence have a look at the synthesis and anticancer evaluations of some pyrazole derivatives bearing tetrahydro-4*H*-cyclohepta[*b*] thiophene-3-carboxamide moiety. The consequences discovered that compound **12** confirmed comparable and higher interest in opposition to MCF-7, HePG2, PC3, and HCT-116 cancer cell lines than Doxorubicin.

## ORCID

*Mohammed Al-Ghorbani* https://orcid.org/0000-0002-9079-5788
*Moustafa A. Gouda* https://orcid.org/0000-0002-9508-8089

## REFERENCES

[1] K. Gewald, M. Gruner, U. Hain, G. Süptitz, *Monatsh. Chem.* **1988**, *119*, 985.
[2] W. Kemnitzer, N. Sirisoma, C. May, B. Tseng, J. Drewe, S. X. Cai, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3536.
[3] J. Katada, K. Iijima, M. Muramatsu, M. Takami, E. Yasuda, M. Hayashi, M. Hattori, Y. Hayashi, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 797.
[4] R. Narlawar, J. R. Lane, M. Doddareddy, J. Lin, J. Brussee, A. P. IJzerman, *J. Med. Chem.* **2010**, *53*, 3028.
[5] D. K. Sureja, K. R. Vadalia, *J. Saudi Chem. Soc.* **2018**, *22*, 248.
[6] H. N. Hafez, H. A. Hussein, A. R. B. El-Gazzar, *Eur. J. Med. Chem.* **2010**, *45*, 4026.
[7] K. M. Al-Taisan, H. Al-Hazimi, S. S. Al-Shihry, *Molecules* **2010**, *15*, 3932.
[8] F. M. Junior, R. G. Lima-Neto, T. B. de Oliveira, R. S. de Araujo, R. P. Neves, *Lat. Am. J. Pharm.* **2011**, *30*, 1492.
[9] M. A. El-Sherbeny, M. B. El-Ashmawy, H. I. El-Subbagh, A. A. El-Emam, F. A. Badria, *Eur. J. Med. Chem.* **1995**, *30*, 445.
[10] T. Masaoka, S. Chung, P. Caboni, J. W. Rausch, J. A. Wilson, H. Taskent-Sezgin, J. A. Beutler, G. Tocco, S. F. Le Grice, *J. Med. Chem.* **2013**, *56*, 5436.
[11] K. Madhusudana, B. Shireesha, V. Naidu, S. Ramakrishna, B. Narsaiah, A. Rao, P. Diwan, *Eur. J. Pharmacol.* **2012**, *678*, 48.
[12] K. M. Khan, Z. Nullah, M. A. Lodhi, S. Jalil, M. I. Choudhary, A. U. Rahman, *J. Enzyme Inhib. Med. Chem.* **2006**, *21*, 139.
[13] K. A. Sharkawy, M. M. Said, G. Dardas, *Bulg. Chem. Commun.* **2014**, *46*, 691.
[14] Y. D. Wang, S. Johnson, D. Powell, J. P. McGinnis, M. Miranda, S. K. Rabindran, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3763.
[15] S. A. Abdel-Rahman, N. S. El-Gohary, E. R. El-Bendary, S. M. El-Ashry, *J. Am. Sci.* **2016**, *12*, 55.
[16] S. B. Salib, O. M. Khalil, M. M. Kamel, Y. El-Dash, *Open Access Library J.* **2016**, *3*, 1.
[17] K. Bozorov, J. Y. Zhao, B. Elmuradov, A. Pataer, H. A. Aisa, *Eur. J. Med. Chem.* **2015**, *102*, 552.
[18] D. Dewangan, T. Kumar, A. Alexander, K. Nagori, D. K. Tripathi, *Curr. Pharm. Res.* **2011**, *1*, 369.
[19] M. S. Christodoulou, S. Liekens, K. M. Kasiotis, S. A. Haroutounian, *Bioorg. Med. Chem. Lett.* **2010**, *18*, 4338.
[20] H. Raju, S. Chandrappa, M. K. Ramakrishna, T. S. Nagamani, H. Ananda, S. M. Byregowda, K. S. Rangappa, *J. Cancer Ther.* **2010**, *1*, 1.
[21] A. Balbi, M. Anzaldi, C. Macciò, C. Aiello, M. Mazzei, R. Gangemi, P. Castagnola, M. Miele, C. Rosano, M. Viale, *Eur. J. Med. Chem.* **2011**, *46*, 5293.
[22] G. Nitulescu, C. Draghici, O. Olaru, *Int. J. Mol. Sci.* **2013**, *14*, 21805.
[23] W. Qi, X. Liu, L. S. Cooke, D. O. Persky, T. P. Miller, M. Squires, D. Mahadevan, *Int. J. Cancer* **2012**, *130*, 2997.
[24] R. Lin, P. J. Connolly, Y. Lu, G. Chiu, S. Li, Y. Yu, S. Huang, X. Li, S. L. Pesquera, S. A. Emanuel, R. H. Middleton, M. Gruninger, A. R. Adams, L. M. Fuentes-Greenberger, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4297.
[25] G. M. Nitulescu, C. Draghici, A. V. Missir, *Eur. J. Med. Chem.* **2010**, *45*, 4914.
[26] J. Li, Y. F. Zhao, X. L. Zhao, X. Y. Yuan, P. Gong, *Arch. Pharm.* **2006**, *339*, 593.
[27] Y. Xia, Z. W. Dong, B. X. Zhao, X. Ge, N. Meng, D. S. Shin, J. Y. Miao, *Bioorg. Med. Chem.* **2007**, *15*, 6893.
[28] A. M. Farag, A. S. Mayhoub, S. E. Barakat, A. H. Bayomi, *Bioorg. Med. Chem.* **2008**, *16*, 4569.
[29] Y. Xia, C. D. Fan, B. X. Zhao, J. Zhao, D. S. Shin, J. Y. Miao, *Eur. J. Med. Chem.* **2008**, *43*, 2347.
[30] M. A. Gouda, H. F. Eldien, M. M. Girges, M. A. Berghot, *Indian J. Heterocy. Ch.* **2018**, *28*, 401.
[31] E. C. Taylor, K. S. Hartke, *J. Am. Chem. Soc.* **1959**, *81*, 2456.
[32] L. Arapides, *Liebigs Ann. Chem.* **1888**, *249*, 7.
[33] A. M. Salah El-Din, *Sulfur. Lett.* **2003**, *26*, 35.
[34] N. Y. Gorobets, B. H. Yousefi, F. Belaj, C. O. Kappe, *Tetrahedron* **2004**, *60*, 8633.
[35] W. T. Li, W. H. Wu, C. H. Tang, R. Tai, S. T. Chen, *ACS Comb. Sci.* **2011**, *13*, 72.
[36] C.-J. Xu, Y.-Q. Shi, *J. Chem. Crystallogr.* **2011**, *41*, 1816.
[37] F. Denizot, R. Lang, *J. Immunol. Methods* **1986**, *89*, 271.
[38] S. Bondock, S. Adel, H. A. Etman, F. A. Badria, *Eur. J. Med. Chem.* **2012**, *48*, 192.
[39] M. A. Metwally, M. A. Gouda, A. N. Harmal, A. M. Khalil, *Eur. J. Med. Chem.* **2012**, *56*, 254.

**How to cite this article:** Al-Ghorbani M, Gouda MA. Synthesis and in vitro anticancer activity of some novel cyclohepta[*b*]thiophene-3-carboxamides bearing pyrazole moiety. *J Heterocyclic Chem*. 2020;1–9. https://doi.org/10.1002/jhet.4041