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KADİR DOĞANAY

ÜNZİLE KELEŞTEMUR

SEVGİ BALCIOĞLU

BURHAN ATEŞ

ALİYE ALTUNDAŞ

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Synthesis, reaction, and evaluation of the anticancer activity of 6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]selenopheno[2,3-*d*]pyrimidine derivatives

Kadir DOĞANAY[1], Unzile KELEŞTEMUR[2], Sevgi BALCIOĞLU[2], Burhan ATEŞ[2], Aliye ALTUNDAŞ[1,*]
[1]Department of Chemistry, Faculty of Science, Gazi University, Ankara, Turkey
[2]Department of Chemistry, Faculty of Science and Arts, İnönü University, Malatya, Turkey

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Abstract: The cyclocondensation of 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*] selenophene-3-carbonitrile (**1**) with formic acid and formamide gave the selenophenopyrimidine **15** and selenophenopyrimidone **6** derivatives. The reaction of **6** with phosphorus oxychloride produced 4-chloro-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5] seleno[2,3-*d*]pyrimidine (**12**), the key compound for our nucleophilic substitution reactions. The hydrazinoselenophenopyrimidine **19** obtained from the reaction of **12** with hydrazine hydrate was converted to its tetrazoloselenophenopyrimidine **21** and triazoloselenophenopyrimidine **26** derivatives. Moreover, the chloropyrimidine derivative was reacted with pyrrolidine and morpholine to afford 4-(1-pyrrolidinyl)-6,7,8,9-tetrahydro-5*H*-cylohepta[4,5]selenopheno[2,3-*d*]pyrimidine (**27**) and 4-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]selenopheno[2,3-*d*]pirimidin-4-yl)morpholine (**28**). Anticancer activities of the synthesized compounds were investigated against the MCF-7 breast cancer cell line and the IC_{50} values of these compounds were in the range of 70.86–250.06 μM.

Key words: Selenophene, selenophenopyrimidine, organoselenium, anticancer activity

1. Introduction

Molecules containing oxygen or sulfur heteroatoms have undergone extensive study over the years and their chemistry heavily investigated compared to selenium.

The inimitable redox properties of selenium are influential in the catalytic and biological activities of organoselenium compounds, especially among compounds in which selenium is a part of the heterocyclic ring. However, there are several differences in organoselenium compounds despite the similarities between the molecules containing sulfur and their selenium congeners. They can be used in nucleophilic and electrophilic as well as in radical reactions.[1–3] Therefore, many syntheses of heterocyclic compounds based on selenium have been developed into standard procedures in organic chemistry.[1–3] The synthesis of heterocyclic compounds containing sulfur and selenium has been reported within the past several years because of their chemical properties,[4,5] biological activity, and pharmaceutical potential.[6,7] Some organoselenium compounds, for example oligoselenophenes, are known organic semiconductors, particularly in the application to thin-film transistors (TFT).[8] 2-β-D-ribofuranosylselenazole-4-carboxamide was found to be effective against Lewis lung carcinoma in mice.[9] Selenium-containing bicyclic heterocycles having activity as D-amino acid oxidase (DAO) inhibitors are useful in the treatment of neurodegenerative and psychiatric disorders and diseases.[10] In addition, pyrim-

*Correspondence: aaltundas@gazi.edu.tr

idine is considered one of the most important pharmacophoric rings, exhibiting remarkable pharmacological activities since it is one of the essential building blocks of nucleic acids, DNA, and RNA. Thienopyrimidines fused heteroaromatic ring systems, however, have a special importance as they serve as structural analogues for biogeneric purines and potential nucleic acid antimetabolites.[11]

In the literature, several studies have been reported on thienopyrimidine derivatives; however, studies on selenium-containing derivatives are not abundant and some of them are related to 4-(aryl amino)selenophene pyrimidine derivatives having antitumor effects on proliferative disorders, particularly cancer.[12,13]

Our interest focuses in particular on the synthesis of new polyheterocyclic seven-membered cycloalkane-fused selenophene derivatives and their further screening for anticancer activity.

2. Results and discussion

2.1. Chemistry

The synthetic pathways for all target molecules are illustrated in Schemes 1–4. 2-Amino-3-cyanoselenophene **1** was prepared from the classical, multicomponent Gewald reaction.[14–17] A mixture of metallic selenium, cycloheptanone, and malononitrile in ethanol in the presence of morpholine was refluxed and **1** was isolated in good yield.

Heating **1** with formic acid and a catalytic amount of concentrated acid gave **6**, which on treatment with phosphorus oxychloride afforded 4-chloro-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]selenopheno[2,3-*d*]pyrimidine (**3**) (Scheme 1).[18] The IR spectrum of **6** indicated the presence of absorption bands at 3216 and 1652 cm^{-1} corresponding to NH and C=O groups, respectively. ^{1}H NMR spectra revealed the disappearance of signals at δ 4.97 ppm of **1**, whereas appearance of the exchangeable signal at δ 12.36 ppm and one singlet at δ 7.95 ppm indicated the NH and C-2 proton of selenopyrimidine, respectively. 4-Chloroselenopyrimidine **12** showed the disappearance of signals at δ 12.36 ppm and signals at δ 7.95 ppm indicated the NH and CH protons, respectively.

Furthermore, 6,7,8,9-tetrahydro-5H-cyclohepta[4,5]selenopheno[2,3-*d*]pyrimidin-4-amine (**15**) was obtained by treating the 2-aminocyanoselenophene with excess formamide (Scheme 2).

The IR spectrum of **15** showed the disappearance of the CN group absorption band of the precursor **1** at 2200 cm^{-1}. ^{1}H NMR spectra revealed the presence of the signal due to NH_2 protons in the aromatic region in addition to the singlet at δ 8.11 ppm corresponding to the pyrimidine ring CH proton.

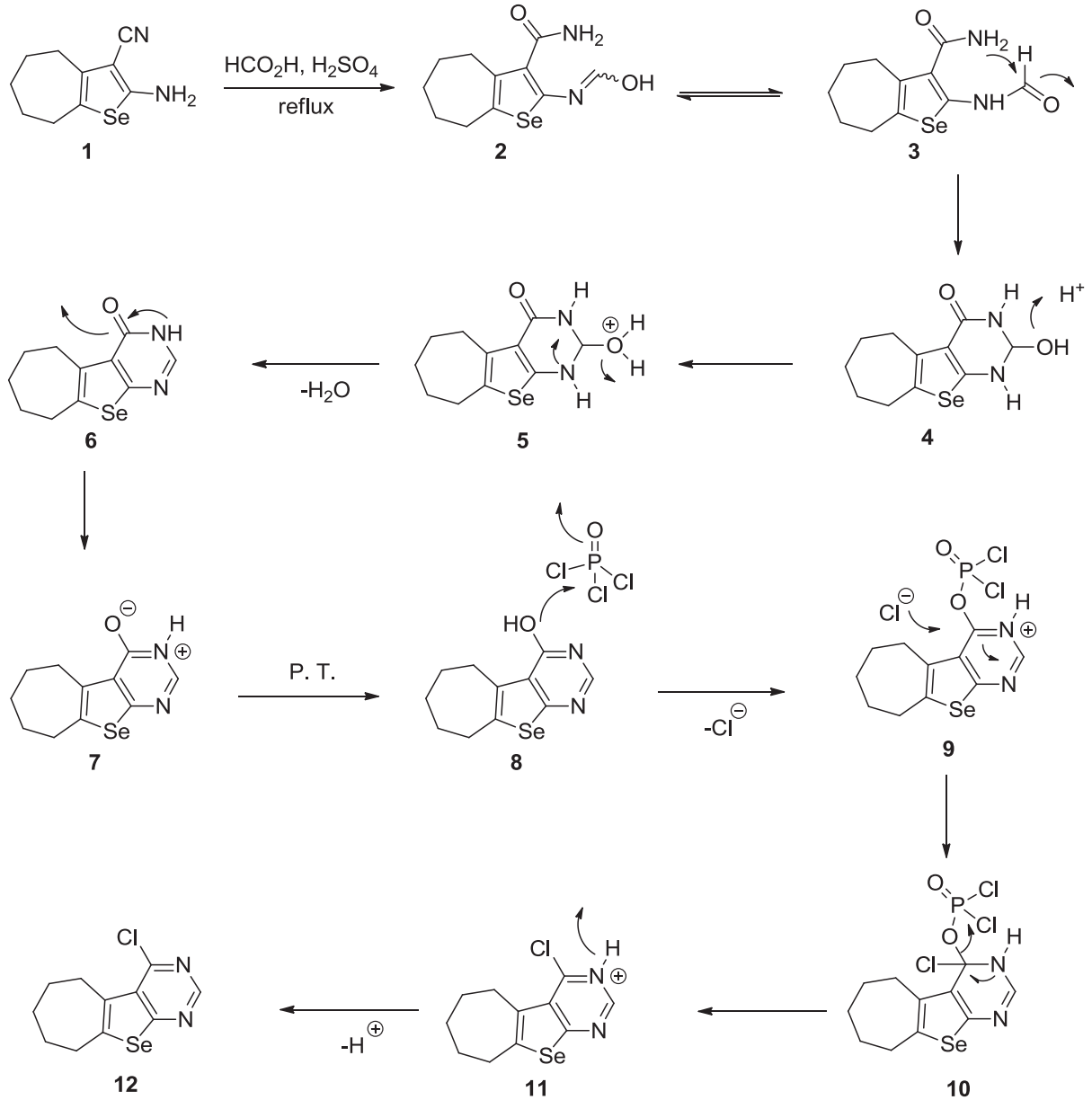
4-Chloro-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]selenopheno[2,3-*d*]pyrimidine (**12**) was converted into the corresponding 4-hydrazinyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]selenopheno[2,3-d]pyrimidine (**19**) by treatment with hydrazine hydrate in dioxane. The IR spectra of the hydrazinylselenophenopyrimidine compound indicated the presence of new absorption bands at 3228–3461 cm^{-1} corresponding to the NH and NH_2 groups. In the ^{1}H NMR spectrum of **19**, new signals seen resonating at δ 8.086 and at δ 8.30 ppm indicate the NH and NH_2 groups, respectively (Scheme 3).

The preparation of 9,10,11,12-tetrahydro-8*H*-cyclohepta[4,5]selenopheno[3,2-*e*]-[1,2,4-]triazolo[4,3-*c*] pyrimidine **26**, in the present study, was achieved via a one-pot reaction of the 4-hydrazinyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]selenopheno[2,3-d]pyrimidine **19** with trimethyl orthoformate.

In the ^{13}C NMR spectrum of **26** a new signal appeared belonging to the triazole ring carbon atom at 136.8 ppm. The synthesis of 9,10,11,12-tetrahydro-8*H*-cyclohepta[4,5]selenopheno[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine **21** was realized with the reaction of the hydrazinylselenophenopyrimidine derivative **19** with $NaNO_2$ in acetic

acid (Scheme 3). In the ^{1}H NMR spectrum of the tetrazoloselenophenopyrimidine derivatives, we observed the disappearance of signals at δ 8.08 and 8.30 ppm belonging to the NH and NH_2 protons in the precursor.

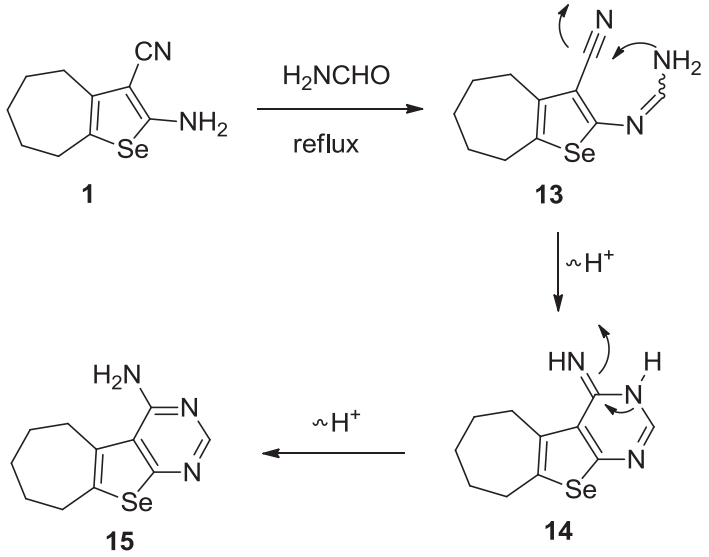
Nucleophilic reaction of the chloro-substituted pyrimidine derivative **12** with the appropriate amine in Et_3N and heating under reflux led to the compounds **27** and **28** (Scheme 3). The products were isolated as colorless solids. The ^{1}H NMR spectra of **27** and **28** with peaks between δ 1.88 and 3.86 ppm belong to the methylenic protons.



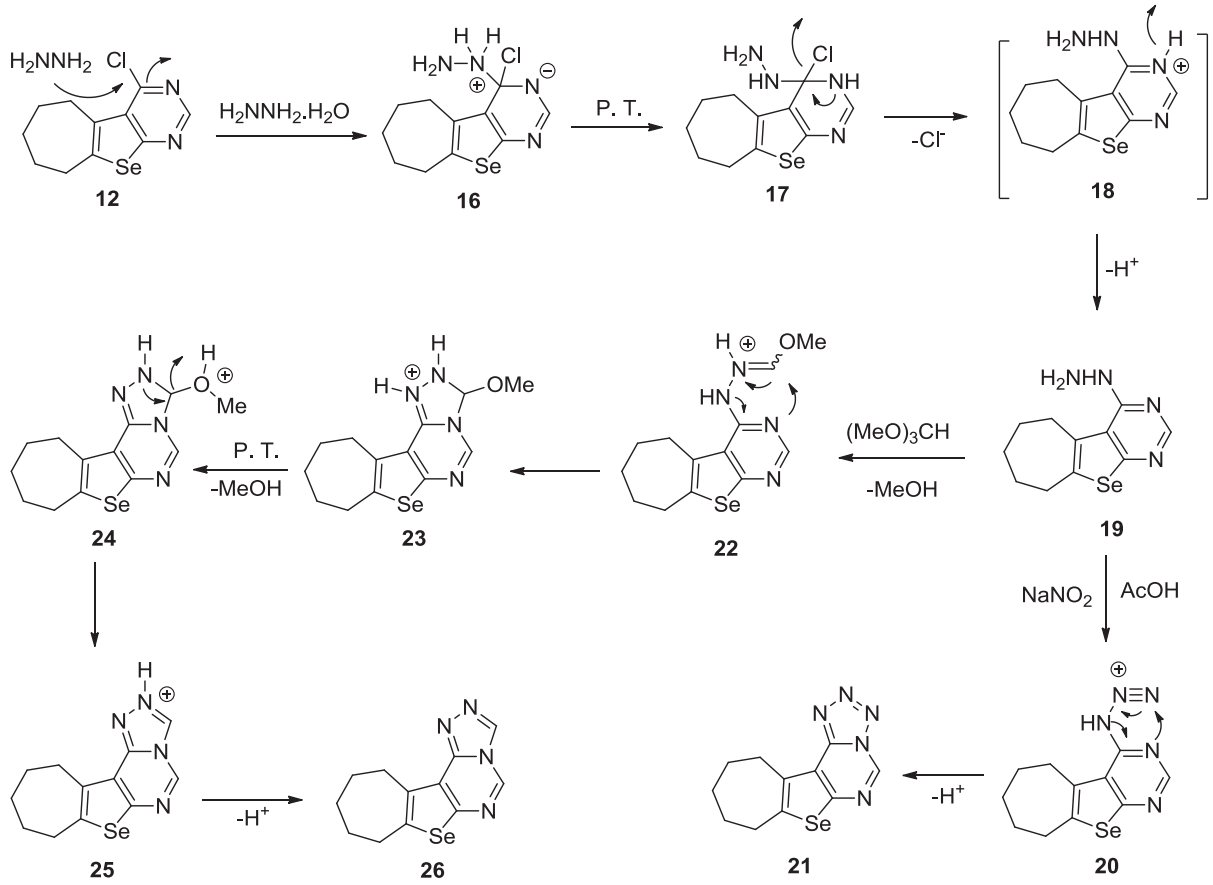
Scheme 1. Synthesis of selenophenopyrimidinone **6** and chloroselenophenopyrimidine **12**.

The structures of the synthesized compounds were determined on the basis of spectroscopic and analytical data. The IR spectra of **27** and **28** showed C–N bands at 1115–1126 cm^{-1} that were not present in the precursor. In the ^{1}H NMR spectra of **27** and **28**, which were synthesized via the substitution of the chlorine atom of **12**

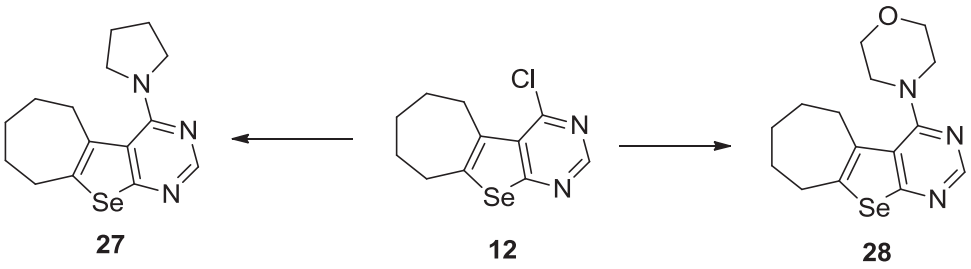
with pyrrolidine and morpholine, the C=NH signals showed peaks at δ 8.31, 8.82, and 8.52 ppm, respectively. In the precursor, the C=NH signals was at δ 8.66 ppm.



Scheme 2. Synthesis of 4-aminoselenophenopyrimidine **15**.



Scheme 3. Synthesis of polyheterocyclic selenophenes.



Scheme 4. Substitution reaction of 4-chloro-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5] selenopheno[2,3-*d*]pyrimidine (**12**) with nucleophiles.

2.2. Anticancer activity

The compounds were evaluated at a different concentration for anticancer activity against the MCF-7 cell line. The IC_{30} and IC_{50} values of the compounds were in the range of 29.60–133.73 and 70.86–227.58 μM, respectively (Table). The potent compounds were **1**, **12**, **21**, and **26** with IC_{30} and IC_{50} values in the range of 29.60–71.30 and 70.86–101.78 μM, respectively. On the basis of IC_{30} and IC_{50} values, it is obvious that **1**, **12**, **21**, and **26** have a moderate effect and **19**, **15**, **27**, and **28** have a low effect on the MCF-7 cell line. In addition, **1**, **12**, **21**, and **26** showed two times the anticancer activity compared with the other synthesized compounds. Moreover, **1**, **12**, **21**, and **26** exhibited more anticancer activity on the MCF-7 cell line compared with other compounds in the presence of the amino-cyano group, chlorine group, and tetrazol and triazol rings, respectively. **6** did not show anticancer activity in the concentration range of 5–300 μM. The IC_{50} value of doxorubicin, a cancer drug in breast cancer, was measured at 0.056 μM against the MCF-7 cell line.[16] Although the anticancer activity results demonstrated that the in vitro anticancer effect of the synthesized compounds are mainly moderate and low, it seems that this structure may be used as a novel anticancer scaffold for the further modification and design of novel potent compounds.[17]

Table. IC_{50} values of synthesized compounds on MCF-7 cancer cell lines. Each value is the average of triplicate experiments with standard deviation (ND: Not detected).

Samples	IC_{30} (μM)	IC_{50} (μM)
1	49.64	70.86
6	ND	ND
12	67.13	101.78
19	133.73	203.44
21	71.33	100.30
26	29.60	94.77
15	135.78	250.06
27	125.53	190.09
28	103.09	175.84

3. Experimental

3.1. Chemistry

All the reagents for syntheses were commercially available and used without further purification or purified by standard methods prior to use. Melting points were determined using an Electrothermal 9100 apparatus (Thermo Fisher Scientific Inc., UK), uncorrected. All NMR spectra were recorded on a Bruker 400 (^{1}H: 400

MHz, ^{13}C: 100 MHz) NMR spectrometer (Bruker Corporation, Germany), in $CDCl_3$ or DMSO-d_6. Chemical shifts were reported in ppm relative to TMS as an internal standard, J in Hz. FTIR spectra were recorded on a Mattson 1000 spectrometer (Mattson Instruments, Baton Rouge, LA, USA) using KBr pellets. Elemental analyses (C, H, N, and S) were performed using a vario MICRO V 1.5.7 Elemental Analyzer. The progress of reactions was monitored by TLC using Silufol UV-254 plates from Merck (Germany).

3.1.1. Synthesis of 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]selenophene-3-carbonitrile (1)

2-Aminoselenophene-3-carbonitrile (**1**) was prepared according to the procedure described previously.[18–20] To a stirred solution of 5.61 g (0.05 mol) of cycloheptanone, 3.30 g (0.05 mol) of malononitrile, and 3.95 g (0.05 mol) of powdered metallic selenium in 50 mL of anhydrous ethanol was added 4.36 g (0.05 mol) of morpholine dropwise at room temperature. Then the reaction was refluxed for 48 h. After completion, the mixture was filtrated and then poured into ice-water. The resulting solid was collected and recrystallized from ethanol.

Yield 71%; mp 100–102 °C. IR (KBr), ν (cm^{-1}): 3425–3333 (NH_2), 2192 (CN). ^{1}H NMR ($CDCl_3$) δ (ppm) = 1.59–1.65 (m, 4H, CH_2), 1.76–1.81 (m, 2H, CH_2), 2.55–2.58 (m, 2H, CH_2), 2.58–2.62 (m, 2H, CH_2), 4.97 (bs, 2H, NH_2). ^{13}C NMR ($CDCl_3$) δ (ppm) = 27.2, 28.2, 30.6, 31.1, 32.1, 93.6 (C–CN), 117.2 (CN), 128.3, 138.0, 163.6 (C–NH_2).

Anal. calcd. for $C_{10}H_{12}N_2Se$ (240.02); C, 50.22; H, 5.06; N, 11.71; found: C, 50.85; H, 4.76; N, 11.74%.

3.1.2. Synthesis of 3,5,6,7,8,9-hexahydro-4H-cyclohepta[4,5]selenopheno[2,3-d]pyrimidin-4-one (6)

A solution of 2-amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]selenophene-3-carbonitrile (0.24 g, 0.001 mol) in excess of formic acid (10 mL) and a catalytic amount of H_2SO_4 was refluxed for 12 h. After the reaction was completed the mixture was cooled to room temperature and poured into a water-ice bath. The resulting solid was filtered off and washed with cold ethanol. The crude product was recrystallized from ethanol.

Yield 63%; mp 204–205 °C. IR (KBr), ν (cm^{-1}): 3216 (NH), 3083 (CH), 1658 (C=O), 1593 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm) = 1.57–1.63 (m, 4H, CH_2), 1.80–1.81 (m, 2H, CH_2), 2.86–2.90 (m, 2H, CH_2), 3.26–3.34 (m, 2H, CH_2), 7.95 (s, 1H, CH), 12.36 (bs, 1H, NH). ^{13}C-APT NMR (DMSO-d_6) δ (ppm) = 27.1, 27.6, 28.5, 31.2, 32.4, 126.6, 139.0, 142.9, 144.1 ($CH_{prymidone}$), 159.1, 167.6 (C=O).

Anal. calcd. for $C_{11}H_{12}N_2OSe$ (267.19); C, 49.45; H, 4.53; N, 10.48; found: C, 49.14; H, 4.39; N, 10.50%.

3.1.3. Synthesis of 4-chloro-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]seleno[2,3-*d*]pyrimidine (12)

To a stirred solution of 3,5,6,7,8,9-hexahydro-4H-cyclohepta[4,5]selenopheno[2,3-d]pyrimidin-4-one (**6**) (2.67 g, 0.01 mol) in dioxane (30 mL) was added $POCl_3$ (7 mL) dropwise at room temperature. The mixture was refluxed for 4 h. After this time it was cooled and poured dropwise into a water-ice bath. The resulting solid was filtered off and dried in vacuo. The solid product was recrystallized with diethyl ether.

Yield 87%; mp 68–70 °C. IR (KBr), ν (cm^{-1}): 3105 (CH), 1620 (C=N). ^{1}H NMR ($CDCl_3$) δ (ppm) = 1.73–1.82 (m, 4H, CH_2), 1.92–1.99 (m, 2H, CH_2), 3.06–3.27 (m, 2H, CH_2), 3.35–3.39 (m, 2H, CH_2), 8.67 (s, 1H, $CH_{prymidine}$). ^{13}C-APT NMR ($CDCl_3$) δ (ppm) = 26.1, 26.8, 29.1, 31.9, 32.2, 132.4, 134.7, 149.1, 150.4 ($C_{prymidine}$), 153.9, 173.2 (C–Cl).

Anal. calcd. for $C_{11}H_{11}ClN_2Se$ (285.63); C, 46.25; H, 3.88; N, 9.81; found: C, 46.82; H, 3.88; N, 9.67%.

3.1.4. Synthesis of 4-hydrazinyl-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]selenopheno[2,3- 4 d]pyrimidine (19)

To a solution of 4-chloro-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]seleno[2,3-d] pyrimidine (1.43 g, 0.005 mol) in anhydrous dioxane (15 mL) was added hydrazine hydrate (0.0075 mol) dropwise. The mixture was refluxed for 2 h. After this time the mixture was cooled to room temperature and poured into a water-ice bath. The resulting solid was filtered off and dried in vacuo. The solid product was recrystallized with chloroform.

Yield 74%; mp 170–172 °C. IR (KBr), ν (cm^{-1}): 3416–3228 (NH, NH_2), 3057 ($CH_{prymidine}$), 1623 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm) = 1.58–1.69 (m, 4H, CH_2), 1.77–1.86 (m, 2H, CH_2), 2.86–2.92 (m, 2H, CH_2), 3.0–3.05 (m, 2H, CH_2), 4.46 (bs, 2H, NH_2), 8.08 (bs, 1H, NH), 8.30 (s, 1H, $CH_{prymidine}$). ^{13}C-APT NMR ($CDCl_3$) δ (ppm) = 26.1, 27.3, 30.7, 30.8, 31.2, 120.0, 132.8, 143.4, 151.5 ($C_{prymidine}$), 159.6, 169.1 (C–N).

Anal. calcd. for $C_{11}H_{14}N_4Se$ (281.22); C, 46.98; H, 5.02; N, 19.92; found: C, 47.54; H, 4.94; N, 20.11%.

3.1.5. Synthesis of 9,10,11,12-tetrahydro-8*H*-cyclohepta[4,5]selenopheno[3,2-*e*]-tetrazolo[1,5-*c*] pyrimidine (21)

4-Hydrazinyl-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]selenopheno[2,3-4d]pyrimidine (**19**) (0.28 g, 0.001 mol) was dissolved in acetic acid (3 mL) at room temperature. A solution of $NaNO_2$ (0.10 g, 0.0015 mol) in 1 mL of water was added dropwise to the mixture. The mixture was stirred additionally for 30 min at room temperature. After completion, the crude solid product was filtered off and washed with water and then was crystallized from ethanol.

Yield 77%; mp 151–153 °C. IR (KBr), ν (cm^{-1}): 3084 ($CH_{prymidine}$), 1600 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm) = 1.66–1.75 (m, 4H, CH_2), 1.86–1.95 (m, 2H, CH_2), 3.08–3.70 (m, 2H, CH_2), 3.49–3.54 (m, 2H, CH_2), 10.02 (s, 1H, $CH_{prymidine}$). ^{13}C-APT NMR (DMS-d_6) δ (ppm) = 27.1, 27.7, 29.6, 32.5, 32.8, 120.1, 131.6, 143.9, 150.8 ($C_{prymidine}$), 159.8, 169.5 (C–N).

Anal. calcd. for $C_{11}H_{11}N_5Se$ (292.20); C, 45.22; H, 3.79; N, 23.97; found: C, 45.23; H, 3.73; N, 23.82%.

3.1.6. Synthesis of 9,10,11,12-tetrahydro-8*H*-cyclohepta[4,5]selenopheno[3,2-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidine (26)

The reaction mixture of 4-hydrazinyl-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]selenopheno[2,3- 4 d]pyrimidine (**19**) (0.28 g, 0.001 mol) and trimethylorthoformate (5 mL) was refluxed for 3 h. After completion, the mixture was cooled to room temperature and the solid was precipitated. The resulting solid product was filtered, washed with cold ethanol (5 mL), and recrystallized from ethanol.

Yield 73%; mp 316–318 °C. IR (KBr), ν (cm^{-1}): 3098, 3074 (=CH), 1596 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm) = 1.63–1.72 (m, 4H, CH_2), 1.88–1.96 (m, 2H, CH_2), 3.02–3.08 (m, 2H, CH_2), 3.53–3.58 (m, 2H, CH_2), 8.81 (s, 1H, $CH_{triazole}$), 8.91 (s, 1H, $CH_{prymidine}$). ^{13}C NMR (DMSO-d_6) δ (ppm) = 26.9, 27.5, 29.1, 32.2, 32.4, 122.7, 134.4, 136.8 ($C_{triazol}$) 147.4, 156.9, 151.82 ($C_{prymidine}$).

Anal. calcd. for $C_{12}H_{12}N_4Se$ (291.21); C, 49.49; H, 4.15; N, 19.24; found: C, 49.86; H, 4.12; N, 18.91%.

3.1.7. Synthesis of 6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]seleno[2,3-*d*]pyrimidin-4-amine (15)

A mixture of 2-amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]selenophene-3-carbonitrile (0.24 g, 0.001 mol) and excess formamide (10 mL) was refluxed for 2 h. Then the reaction mixture was cooled and poured into ice-water. The resulting solid was filtered off, washed with water, dissolved in ethanol, and boiled with charcoal. Then the mixture was filtered, the excess ethanol removed by rotary evaporation, and the product recrystallized from ethanol.

Yield 80%; mp 247–248 °C. IR (KBr), ν (cm^{-1}): 3407–3334 (NH_2), 3076 (=CH), 1647 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm) = 1.59–1.70 (m, 4H, CH_2), 1.77–1.85 (m, 2H, CH_2), 2.88–2.93 (m, 2H, CH_2), 2.95–2.99 (m, 2H, CH_2), 6.81 (bs, 2H, NH_2), 8.11 (s, 1H, $CH_{prymidine}$). ^{13}C-APT NMR (DMSO-d_6) δ (ppm) = 26.6, 27.1, 39.9, 30.7, 31.1, 119.5, 134.9, 140.4, 152.3 ($C_{prymidine}$) 159.3, 169.9 (C–NH_2).

Anal. calcd. for $C_{11}H_{13}N_3Se$. 11(Hexanes) (266.20); C, 50.84; H, 5.24; N, 15.26; found: C, 50.55; H, 5.48; N, 14.87%.

3.1.8. Synthesis of 4-(1-pyrrolidinyl)-6,7,8,9-tetrahydro-5*H*-cylohepta[4,5] selenopheno[2,3-*d*] pyrimidine (27)

A solution of 4-chloro-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]seleno[2,3-d]pyrimidine (**12**) (0.28 g, 0.001 mol) and pyrrolidine (0.11 g, 0.0015 mol) in triethylamine (5 mL) was refluxed for 2 h. Then the mixture was cooled to room temperature and poured into water. The mixture was extracted with ethyl acetate (2 × 30 mL), the organic phase dried over with $MgSO_4$, and ethyl acetate removed by rotary evaporation. The resulting solid product was recrystallized from ethanol.

Yield 70%; mp 112–114 °C. IR (KBr), ν (cm^{-1}): 3076 (=CH), 2918. ^{1}H NMR ($CDCl_3$)δ (ppm) = 1.58–1.65 (m, 2H, CH_2), 1.69–1.77 (m, 2H, CH_2), 1.86–1.92 (m, 6H, CH_2), 2.91–2.98 (m, 4H, CH_2), 3.56–3.62 (t, J: 4.5 Hz, 4H, $CH_{2pyrrolidiny}$), 8.67 (s, 1H, $CH_{prymidine}$). ^{13}C-APT NMR ($CDCl_3$) δ (ppm) = 25.2 ($C_{pyrrolidiny}$), 27.3, 27.8, 30.7, 32.2, 32.7, 50.5 ($C_{pyrrolidnyl}$) 122.1, 135.2, 141.3, 150.2 ($C_{prymidine}$) 150.5, 170.2.

Anal. calcd. for $C_{15}H_{19}N_3Se$ (320.29); C, 56.25; H, 5.98; N, 13.12; found: C, 56.12; H, 5.93; N, 12.96%.

3.1.9. Synthesis of 4-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]selenopheno[2,3-*d*]pirimidin-4-yl) morpholine (28)

A solution of 4-chloropyrimidine derivative **12** (0.28 g, 0.001 mol) and morpholine (0.13 g, 0.0015 mol) in triethylamine (5 mL) was refluxed for 2 h. After completion, the mixture was cooled to room temperature and poured into water. The mixture was extracted with ethyl acetate (2 × 30 mL), the organic phase dried over with $MgSO_4$, and the ethyl acetate removed by rotary evaporation. The resulting solid product was recrystallized from ethanol.

Yield 78%; mp 132–134 °C. IR (KBr), ν (cm^{-1}): 3084 (=CH), 2916. ^{1}H NMR ($CDCl_3$) δ (ppm) = 1.59–1.64 (m, 2H, CH_2), 1.70–1.78 (m, 2H, CH_2), 1.90–1.98 (m, 2H, CH_2), 2.95–2.99 (m, 2H, CH_2), 3.06–3.11 (m, 2H, CH_2), 3.36 (bs, 4H, $CH_{2morpholine}$), 3.86 (bs, 4H, $CH_{2morpholine}$), 8.49 (s, 1H, $CH_{prymidine}$). ^{13}C-APT NMR ($CDCl_3$) δ (ppm) = 27.1, 27.5, 29.1, 32.3, 32.7, 50.6 ($C_{morpholine}$), 66.5 (C–O), 123.9, 134.5, 144.4, 162.2, 150.6 ($C_{prymidinyl}$), 171.5.

Anal. calcd. for $C_{15}H_{19}N_3OSe$ (336.29); C, 53.57; H, 5.69; N, 1250; found: C, 53.24; H, 5.59; N, 12.12.33%.

3.2. Anticancer activity assay

3.2.1. Cell culture

MCF-7s (breast cancer cells) were maintained in 25-mL plastic flasks in RPMI 1640 supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin. Cells were kept incubated in a CO_2 incubator at 37 °C under a humidified atmosphere of 5% CO_2. The medium was replaced three times a week. For enumeration, 100 μL of cells concentration were stained with trypan blue (0.4%) and the cells were counted using a hemocytometer.

3.2.2. MTT assay

MCF-7 (5×10^3 cells) was seeded in each well of a 96-well plate, using 100 μL of culture media. They were allowed to attach for 24 h in a CO_2 incubator. The medium was aspirated and adherent cells were exposed to the medium containing varying concentrations of the compounds (5, 10, 25, 50, 100, and 200 μM) for 24 h. After exposure, 10 μL of the solubilized MTT (stock solution of 12 mM MTT was prepared by adding 1 mL of sterile PBS to one 5-mg vial of MTT) was added to each well, and the cultures were incubated for an additional 4 h. The medium was carefully discarded. For solubilization of formazan crystals (MTT formazan), 100 μL of dimethylsulfoxide (DMSO) was added to each well. The absorbance at 540 nm was determined in each well with a microplate reader. The growth of the treated cells was compared with that of the untreated cells and calculated using the formula: (absorbance treated wells/absorbance untreated wells) $\times$ 100.[21]

4. Conclusion

In this work, eight pyrimidone, chloropyrimidine, triazole, and tetrazol functionalized cycloalkylselenophene derivatives were prepared after several steps starting from 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]selenophene-3-carbonitrile. Anticancer activity was studied against MCF-7 (breast cancer cells) in vitro. The results show that compounds **1**, **12**, **21**, and **26** have more anticancer activity out of all the synthesized compounds. Among them, IC_{50} values of compounds **1** and **26** were 70.86 and 94.77 μM, respectively. With the results of the anticancer activity studies in hand, we observed that the structures of the compounds showing more activity include compounds **1**, **21**, and **26** containing a five-membered heteroaromatic moiety, except for compound **12**, which consists of a chloropyrimidine moiety.

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