Design, Synthesis, and Biological Evaluation of Some Cyclohepta[*b*]Thiophene and Substituted Pentahydrocycloheptathieno[2,3-*d*]Pyrimidine Derivatives

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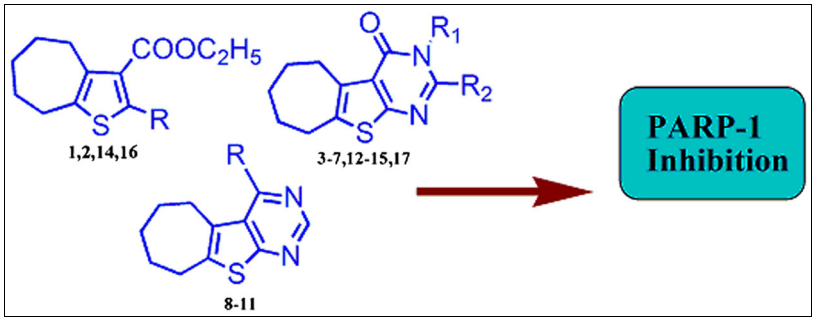


This investigation describes the design of a series of cycloheptathieno[2,3-d]pyrimidines along with their synthetic strategy. The target compounds were screened for their PARP-1 inhibitory activity. The modeling study declared that most of the docked compounds showed the same interactions as 3-aminobenzamide, where Gly 894, His 862, Tyr 896, Arg 878, and Ser 864 were the main residues involved in hydrogen bond formation. Compounds eliciting the top ranked docking results were screened for their PARP-1 inhibitory activity giving promising results, and three representative compounds were tested for their cytotoxic activity using Doxorubicin as a reference standard. The target compounds **1–17** including cyclohepta[b]thiophene and pentahydrocycloheptathieno[2,3-d]pyrimidine cores were designed, prepared, and tested for their PARP-1 inhibitory activity. Compounds **16** (R: —NHC(S)NH2) and **11** (R: —C=S) were the most potent ones.

INTRODUCTION

Poly(ADP-ribose)polymerases are a family of enzymes that uses NAD+ as a substrate. They tend to cause chromatin decondensation in response to DNA damage so as to enhance the repair of the damaged DNA binding protein [1]. DNA damage may be induced by oxidative stress, eroded telomers, genotoxic medication, irradiation, depurination, reactive nitrogen species, alkylating agents, and lipid peroxidation products [2]. The activated PARP enzymes in response to DNA damage cause cleavage of nicotinamide from ADP-ribose, the latter is then polymerized on glutamic residues onto the nuclear acceptor protein, the resulted polymer is used to repair DNA at the damaged site [3] (Fig. 1). Eventually ADP-ribose polymers are rapidly hydrolyzed by the action of PARG (poly ADP-ribose glucohyrolase) [4]. However, severe DNA damage may lead to PARP over activation causing ATP depletion eventually causing several inflammatory pathogenesis [5,6]. In parallel to the repair of DNA damaged protein, activation of cell cycle check points at the lesion site must be coordinated otherwise accumulation of DNA damage would display causing oncogenic chromosomal translocation and eventually to cancer [7]. An inspection of the available PARP–ligand complex structures [8] showed that the NAD+ binding pocket is made up of three parts: a base formed by β-sheet 3 and a loop connecting to α-helix 3, walls formed by β-sheet 4 and α-helix 4, and a lid formed by a loop between α-helix 3 and β-sheet 4 (the D-loop).

Because PARP activation plays a role in DNA repair and cell survival as well; therefore, PARP inhibition has a cytotoxic effect [9]. Accordingly, PARP inhibitors may be used either in combination with anticancer drugs or in potentiating their effects [10] as exemplified by the use of Rucaparib (AG014699) (Fig. 2a) which is a known PARP inhibitor in combination with Temozolomide [11] (Fig. 2b). Several chemical classes of PARP-1 inhibitors have been reported including the NAD structurally similar compound 3-aminobenzamide [12,13] which is mostly used to investigate both *in-vivo* and *in-vitro* effects of PARP inhibition because of its availability commercially and apparent less toxicity (Fig. 3a). Different isoquinolinones and dihydroisoquinolinones were also described as effective

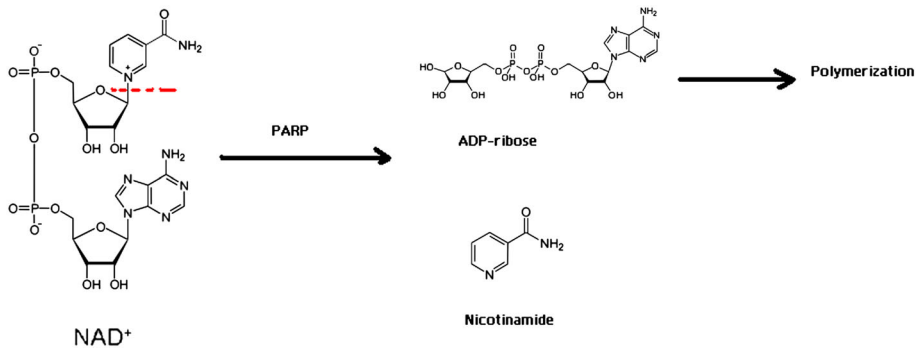


Figure 1. PARP enzyme in response to DNA damage.

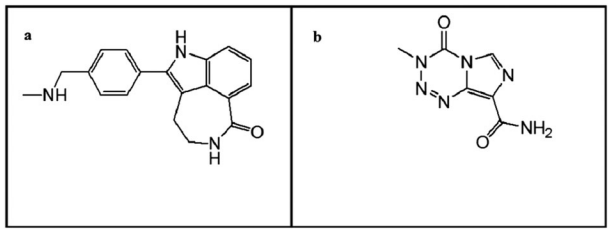


Figure 2. **a**: Rucaparib (structure). **b**: Temozolamide (structure).

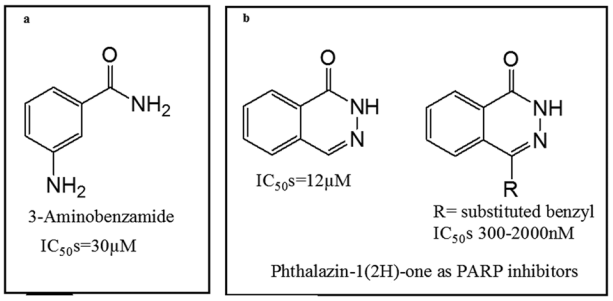


Figure 3. **a**: 3-Aminobenzamide. **b**: Phthalazin-1(2H)-one.

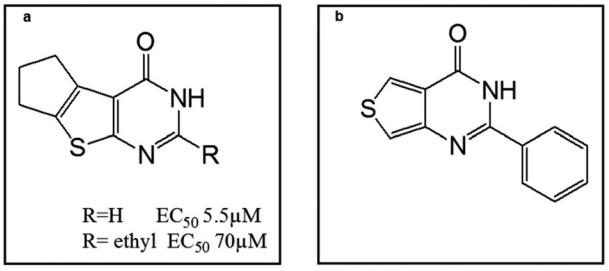


Figure 4. Some thienopyrimidines as PARP inhibitors.

PARP inhibitors [14,15]. Phthalazin-1(2*H*)-one was cited to have IC_{50} of 12 μM [16], (Fig. 3b). Moreover, Pfizer (Warner-Lambert) disclosed 5-methoxy-4-methyl-1(2) phthalizinone with an IC_{50} of 80 nM [14,15].

Furthermore, some thienopyrimidines were reported as selective PARP-1 inhibitors showing selectivity for PARP-1

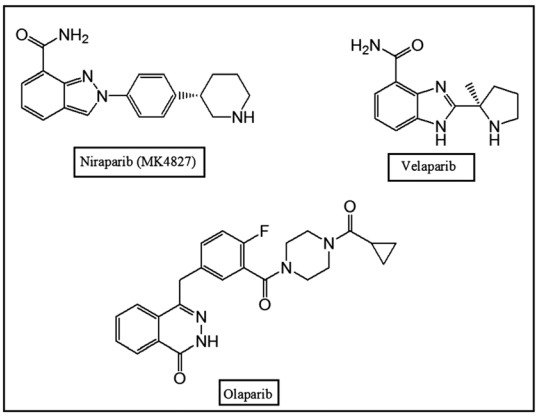


Figure 5. MK4827, Olaparib, and Velaparib.

over PARP-2 at R=H with EC_{50}s of 5.5 and 41 μM respectively [17] (Fig. 4a), the thieno[3,4-*d*]pyrimidine derivative (Fig. 4b) expressed 93% inhibition of PARP at a dose of 10 μM [18]. Additionally, many PARP-1 inhibitors were declared to be used in clinical trials as: MK4827 [19] (Fig. 5), Olaparib (Fig. 5) + Dacarbazine [20], and also Velaparib (Fig. 5) + Cyclophosphamide or Topotecan [21,22].

Based on the aforementioned facts, the search for simple readily accessible compounds that act as PARP inhibitors prompted our interest to synthesize a series of cycloheptathieno[2,3-*d*]pyrimidines, and screen their *in-vitro* PARP-1 inhibitory activity.

RESULTS AND DISCUSSION

Molecular modeling and docking studies. Molecular docking study of the thienopyrimidine scaffold with PARP-1 crystal structure revealed that both Gly 894 and Tyr 896 residues were involved in both hydrogen bond and hydrophobic interactions, respectively. The molecular surface of the active site of PARP-1 (pdb code = 1 UK1) was determined by using MOE 2012.10 [23] in order to

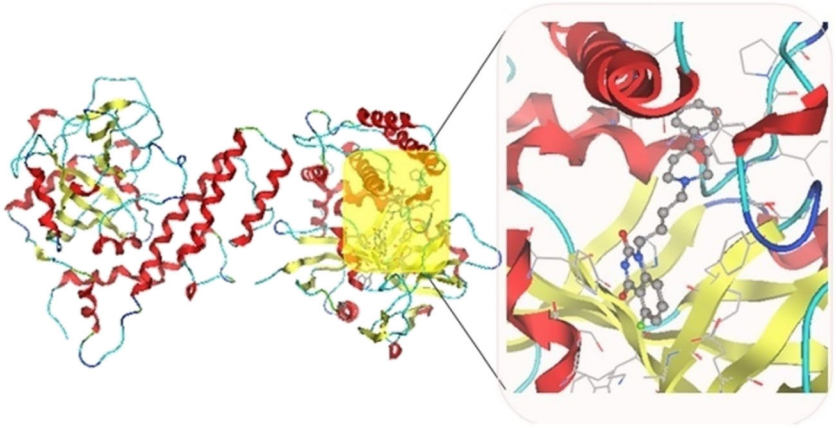


Figure 6. Binding site identification of PARP-1 enzyme. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

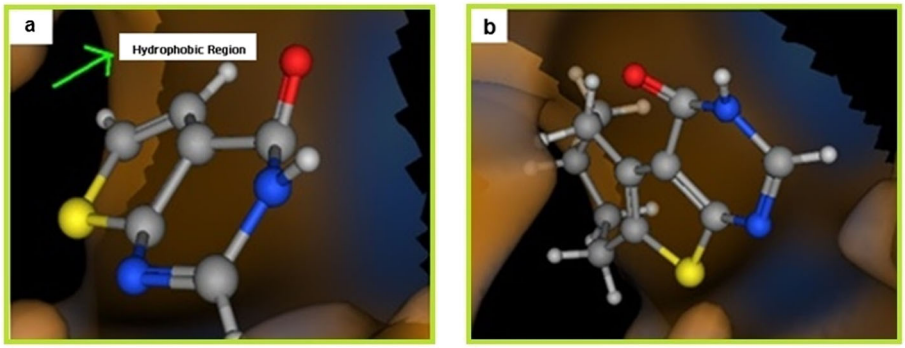


Figure 7. **a**: Docking of thienopyrimidine scaffold inside the PARP-1 active site. **b**: Docking of cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-one scaffold inside PARP active site. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

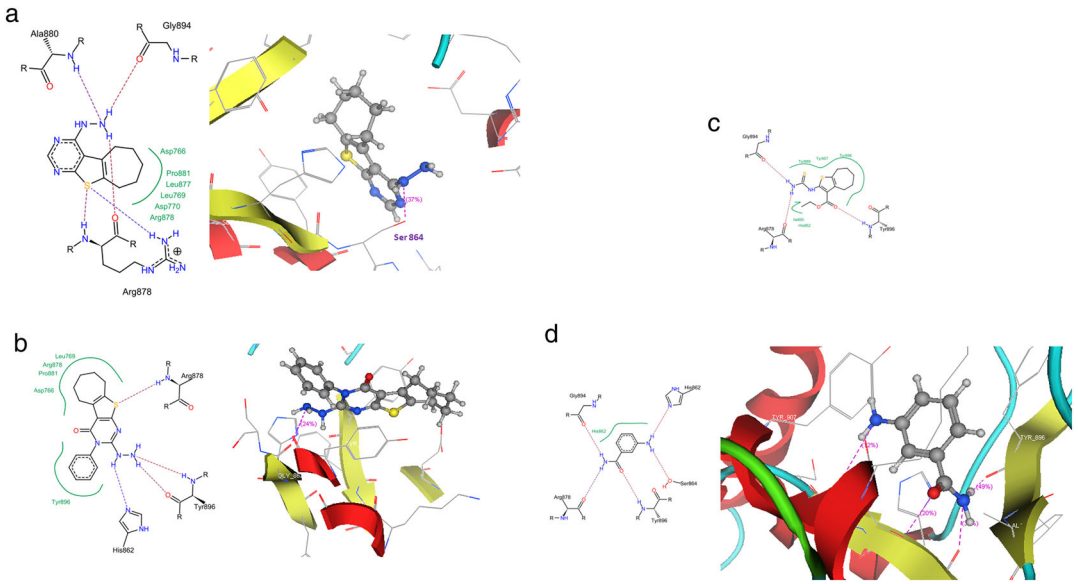


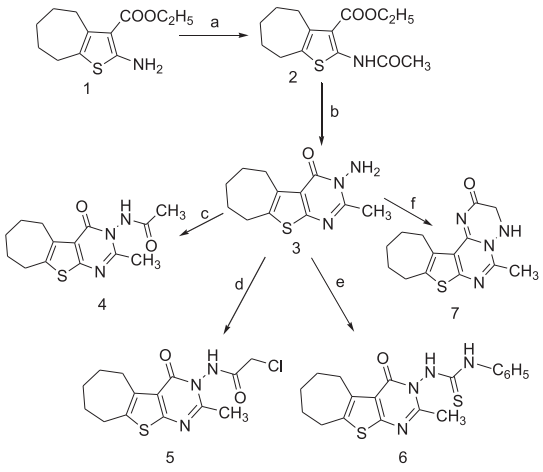
Figure 8. **a**: Docking of compound **10** inside PARP-1 active site. **b**: Docking of compound **13** inside PARP-1 active site. **c**: Docking of compound **16** inside PARP-1 active site. **d**: Docking of 3-Aminobenzamide inside PARP-1 active site. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 1

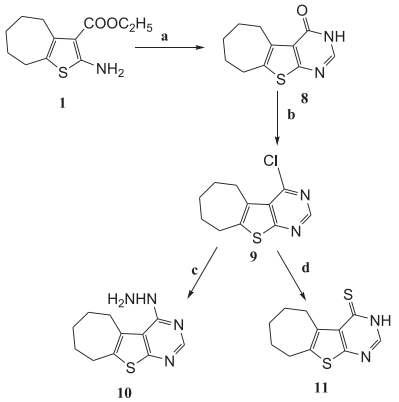
Docking results of the synthesized compounds.

Compound no.	Affinity by **MOE** kcal/mol	Residues involved in interactions using **MOE**	Affinity by **Leadit** kcal/mol	Residues involved in interactions using **Leadit**
1	−3.64	Ser 864	−13.66	Arg 878, Tyr 896, His 862
2	−3.58	Arg 865, Ser 864	−13.60	Gly 863
3	−3.70	Gly 863	−13.55	Arg 878, Tyr 896, Gly 894
4	−3.98	Ser 864	−12.74	Arg 878
5	−3.60	Ser 864	−14.11	Tyr 896
6	−3.50	Gly 863	−13.33	Gly 863
7	−4.00	Tyr 907, Glu 988	−16.74	Gly 863, Ala 898
8	−3.55	His 862, Ser 864	−13.14	Gly 863
10	−4.50	Ser 864	−19.86	Ala 880, Arg 878, Gly894
11	−4.56	His 864	−18.57	Gly 863
12	−3.85	Gly 863	−18.20	Gly 863
13	−3.90	Gly 863	−15.22	Arg 878, Tyr 896, His 862
15	−3.51	His 862	−13.12	Arg 878, Asp 770, Asp 766
16	−4.57	Ser 864	−21.91	Arg 878, Tyr 896, Gly 894
17	−3.80	Gly 862	−18.36	Gly 863, Glu 988
3-Amino-benzamide	−3.75	Arg 878, Ser 864	−15.86	Gly 894, Arg 878, Tyr 896, Ser 864, His 862

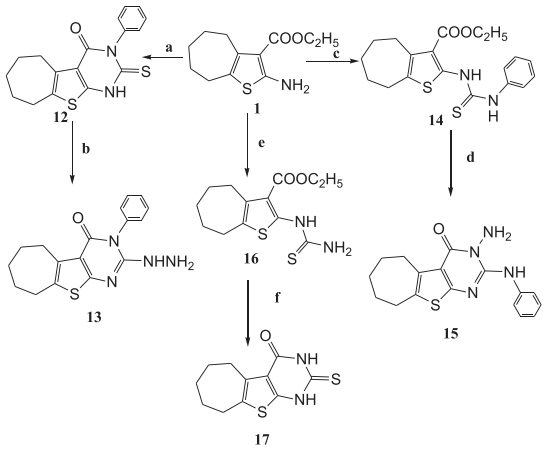
Scheme 1. Synthesis of compounds **1** to **7**.



Scheme 2. Synthesis of compounds **8** to **11**.



Scheme 3. Synthesis of compounds **12** to **17**.



get the hydrophobic regions of the site (Fig. 6). By computing the molecular surface, we were able to determine the large hydrophobic space in the binding site that required to be filled. As a result, a cycloheptane ring was introduced to the thienopyrimidine scaffold, and the designed structure was then re-docked in the PARP-1 active site. It was a successful trial where the hydrophobic space was filled and the main interactions with the conserved residues were not affected (Fig. 7a, 7**b**). Derivatives of the cyclohepta[4,5]thieno[2,3-*d*] pyrimidine scaffold were subjected to molecular docking study to predict their affinity against PARP-1 enzyme. The interactions of the docked compounds were compared to 3-aminobenzamide. Most of the docked compounds showed the same interactions of 3-

Table 2

IC_{50} values, docking results, and % inhibition of PARP-1 activity of the tested compounds.

Compound no.	Affinity by **MOE** kcal/mol	Affinity by **Leadit** kcal/mol	**IC_{50}** μM Means ± SD (n = 3)	% inhibition at min conc. 50 μM	% inhibition at max conc. 200 μM
1	−3.64	−13.66	80.007	73.399	63.644
2	−3.58	−13.60	102.516	55.515	68.756
3	−3.70	−13.55	86.744	73.473	58.042
4	−3.98	−12.74	105.126	58.228	63.803
5	−3.60	−14.11	79.275	72.647	66.326
6	−3.50	−13.33	92.235	56.041	74.175
7	−4.00	−16.74	74.379	74.214	68.231
8	−3.55	−13.14	108.438	64.790	51.850
10	−4.50	−19.86	64.782	69.200	79.457
11	−4.56	−18.57	58.206	75.304	78.903
12	−3.85	−18.20	72.952	70.516	74.443
13	−3.90	−15.22	75.242	67.189	75.762
15	−3.51	−13.12	94.934	67.648	78.454
16	−4.57	−21.91	50.392	77.491	82.458
17	−3.80	−18.36	73.563	79.132	60.137

aminobenzamide where Gly 894, His 862, Tyr 896, Arg 878, and Ser 864 were found to be the main residues involved in hydrogen bond formation (Fig. 8a–**d**).

The docking score was calculated by MOE 2012.10, and all affinities were calculated in kcal/mol (Table 1). According to the docking outputs, 15 compounds were selected to be tested for their PARP-1 inhibitory activity. Another molecular docking process was accomplished by Leadit 2.1.2 software [24]. The ranking of the docking outputs in both cases was nearly consistent. Docking with Leadit 2.1.2 software added some binding modes and suggested more residues that could be a good explanation for the biological activities.

Chemistry. The synthetic strategy to synthesize the target thienopyrimidines **1–17** is depicted in Schemes 1–3. The aminothiophene ester [25] **1** was readily available through Gewald reaction [26] using cycloheptanone, ethyl cyanoacetate, sulfur, and a secondary amine. Reaction of the aminothiophene ester **1** with acetic anhydride afforded the corresponding acetylated derivative **2** [25]. Refluxing **2** with hydrazine hydrate in ethanol gave the 3-amino-2-methyl thienopyrimidine derivative **3** [25] which acted as a key intermediate in (Scheme 1). Reaction of the amino derivative **3** with excess acetic anhydride afforded the acetamido **4**.^{1}H-NMR spectrum revealed a singlet signal at δ 2.30 ppm corresponding to the CH_3 protons of the acetamido group, and also a singlet signal at δ 11.00 ppm representing the NH proton that disappeared upon deuteration. Furthermore, mass spectrum showed $M^{+\cdot}$ at m/z 291. Yet intermediate **3** reacted with chloroacetyl chloride upon stirring in dry benzene at room temperature overnight to give the chloroacetamido derivative **5**.^{1}H-NMR spectrum indicated a singlet signal at δ 4.30 ppm corresponding to CH_2 protons of the chloroacetamido moiety, and a singlet signal at δ 11.32 ppm equivalent to NH proton that disappeared upon deuteration. In addition, ^{13}C-NMR spectrum confirmed a signal at δ 60.67 ppm corresponding to the CH_2 carbons of the chloroacetamido group. Furthermore, mass spectrum disclosed [M^+Cl^{35}] at m/z 325 and [M^+Cl^{37}] at m/z 327.

Heating the 3-amino derivative **3** with phenyl isothiocyanate in dichloromethane at 70°C on a water bath gave the thiosemicarbazide derivative **6**.^{1}H-NMR spectrum indicated the presence of a multiplet representing the aromatic protons at δ 7.12–7.45 ppm and two singlet signals at δ10.83 and δ 11.50 ppm assigned for the two NH protons, which disappeared upon deuteration. Moreover, ^{13}C-NMR spectrum disclosed a signal at δ 175.64 ppm attributed to the C=S carbon and signals equivalent to the aromatic ring carbons at the range δ 124.23–139.40 ppm. The condensed triazinothienopyrimidine derivative **7** was prepared by refluxing the key compound **3** with chloroacetamide in DMF.^{1}H-NMR spectrum illustrated a singlet signal at δ 3.23 ppm attributed to the exchangeable NH proton and a singlet signal at δ 5.79 ppm equivalent to CH_2 protons of the triazine ring (Scheme 1).

Refluxing the aminothiophene ester **1** with excess formamide as reported [20] gave the cycloheptathieno [2,3-*d*]pyrimidin-4(3*H*)-one derivative **8** which afforded the corresponding 4-chloro derivative **9** upon treatment with thionyl chloride as described in literature [11]. Refluxing **9** with hydrazine hydrate in dioxane or ethanol produced the 4-hydrazino **10**.^{1}H-NMR spectrum of **10** disclosed two exchangeable singlet signals at δ 3.81 and δ12.28 ppm attributed to NH_2 and NH protons, respectively, whereas the ^{13}C-NMR spectrum indicated two signals at δ 158.99 ppm and 169.21 ppm corresponding to C-2 and C-4 of the pyrimidine ring respectively. The 4-thioxo derivative **11** was obtained via

refluxing the 4-chloro derivative **9** with thiourea in ethanol and in the presence of TEA. The structure of **11** was established by IR spectrum that revealed the presence of a band equivalent to NH group at 3396 cm^{-1}, and a band for C=S at 2020 cm^{-1}. Furthermore, ^{1}H-NMR spectrum disclosed a singlet signal at δ 12.15 ppm assigned for the NH proton which was exchanged upon deuteration, in addition to the lack of exchangeable signal of NH_2 protons; moreover, ^{13}C-NMR spectrum showed a signal at δ 158.10 ppm assigned for the C=N and a signal attributed to C=S at δ 173.78 ppm. Also, mass spectrum of **11** indicated the molecular ion peak at m/z 236 corresponding to the molecular weight of the 4-thioxo derivative (Scheme 2).

Refluxing the aminothiophene ester **1** with phenyl isothiocyanate in ethanol or acetonitrile on a water bath at 90°C for 15 h gave 3-phenyl-2-thioxothienopyrimidine derivative **12**; ^{1}H-NMR spectrum disclosed the presence of multiplet at δ 7.19–7.47 ppm representing the aromatic protons, and a singlet signal at δ 13.59 ppm assigned to the exchangeable NH proton. Mass spectrum revealed $M^{+\cdot}$ at m/z 328. Decreasing the reflux time to 2 h in ethanol afforded the ethyl (3-phenylthioureido)cycloheptathiophene carboxylate derivative **14**.^{1}H-NMR spectrum indicated multiplet corresponding to the aromatic protons that appeared at δ 7.12–7.49 ppm and two exchangeable singlet signals at δ 10.83 and δ 11.50 ppm representing the two NH protons. The two previously mentioned derivatives **12** and **14** were allowed to react with hydrazine hydrate, the former in pyridine and the latter in ethanol under reflux to afford the thienopyrimidine derivatives **13** and **15** respectively.^{1}H-NMR spectrum of **13** indicated the two singlet signals at δ 4.28 ppm and δ 6.80 ppm assigned for NH_2 protons and NH proton, respectively, that disappeared upon deuteration; moreover, ^{13}C-NMR spectrum revealed signals corresponding to the aromatic carbons at δ 124.23–130.03 ppm and signals at δ 161.13 and 164.84 ppm equivalent to C-4 and C-2 of the pyrimidine ring, respectively. Also, mass spectrum of **13** showed $M^{+\cdot}$ at m/z 326. The ^{1}H-NMR spectrum of **15** indicated two exchangeable singlet signals at δ 2.60 ppm and at δ 13.85 ppm equivalent to NH_2 and NH protons, respectively (Scheme 3).

Yet, treatment of the aminothiophene ester **1** with potassium thiocyanate in the presence of HCl 10% afforded the ethyl-2-thioureido-cycloheptathiophene-3-carboxylate **16**, which was cyclized into 2-thioxocycloheptathieno[2,3-*d*] pyrimidine **17** upon refluxing with sodium ethoxide. ^{1}H-NMR spectrum of the thioureido **16** showed the presence of two exchangeable singlet signals at δ 8.30 and δ 11.00 ppm assigned to NH_2 and NH protons, respectively; moreover, mass spectrum of **16** showed $M^{+\cdot}$ at m/z 298 (11.19%), whereas, IR spectrum of 2-thioxo **17** confirmed the absence of the forked band of NH_2 and elicited the presence of a band at 3416 cm^{-1} representing the NH group.^{1}H-NMR spectrum of **17** revealed two singlet signals at δ12.23 and δ13.21 ppm assigned to the two NH protons that were exchanged upon deuteration.^{13}C-NMR spectrum of **17** disclosed two signals at δ 157.37 ppm and δ 172.46 ppm assigned for C=O and C=S respectively (Scheme 3).

Enzyme specific assay against PARP-1. The designed compounds were screened as PARP-1 inhibitors at different concentrations, and the PARP-1 inhibitory activity reflected by their IC_{50} was expressed as %inhibition values and provided in Table 2.

Biological discussion. The results presented in Table 2 indicate that the most active compounds reflected by their IC_{50} values are the bicyclic 2-thioureidocyclohepta[*b*] thiophene **16** (IC_{50} 50.392 μM) followed by the 4-thioxothienopyrimidine derivative **11** (IC_{50} 58.206 μM), then the 4-hydrazinocycloheptathienopyrimidine **10** (IC_{50} 64.782 μM).

Regarding Scheme 1, compounds bearing 2-methyl cycloheptathienopyrimidine skeleton, the 3-chloroacetylated derivative **5** and the rigid triazino derivative **7**, exhibited IC_{50}s 79.275 and 74.379 μM respectively. On the other hand, out of the 4-substituted thienopyrimidine series (Scheme 2), 4-hydrazino **10** and 4-thioxocycloheptathienopyrimidine **11** derivatives afforded distinguished elevation in activity recording IC_{50}s 64.782 and 58.206 μM with respect to their former congener **8** (IC_{50} 108.438 μM).

Concerning Scheme 3, the most potent compound was the 2-thioureidothiophene derivative **16** (IC_{50} 50.392 μM) whose activity decreased upon cyclization into the thioxo derivative **17** (IC_{50} 73.563 μM), while, the 2-thioxothienopyrimidine **12** (IC_{50} 72.952 μM) was more active than its 2-hydrazino congener **13** (IC_{50} 75.242 μM).

Cytotoxic activity against human Caucasian breast adenocarcinoma (MCF-7). The Cytotoxic activity against Human Caucasian breast adenocarcinoma (MCF-7) of three compounds were tested in order to see if they possess anticancer effect against breast cancer cell line or not. The examined compounds were compound **11** as a representative compound for thioxothienopyrimidines, compound **15** representing the thienopyrimidine derivatives, and compound **16** on behalf of the thiophene derivatives. According to the results, compound **11** showed 22.9% of inhibition at 50 μM/mL and compound **15** showed 20.8% of inhibition at 50 μM/mL the thiophene derivative **16** showed 30.65% of inhibition at 50 μM/mL, and were compared to **Doxorubicin** that showed 45.02% of inhibition at 50 μM/mL.

CONCLUSION

The previous enzyme specific assay demonstrated that, all the designed compounds were found to be moderately

potent and efficacious as PARP-1 inhibitors with IC_{50} values (50.392–108.438 μM), when compared to 3-aminobenzamide as a positive control. The highest activity was associated with the 2-thioureidothiophene derivative **16** (IC_{50} 50.392 μM). The least potent one was the cycloheptathienopyrimidin-4-one **8** (IC_{50} 108.438 μM).

In general, it should be noted that almost all structural modifications on thiophene derivative **1** concerning the synthesis of mono- or di-substituted cycloheptathienopyrimidine at positions 2-, 2, 3-, or 4, were found to produce compounds with promising PARP-1 inhibitory activity. Interestingly, the biological activity profile of these compounds for both the enzyme inhibition and the cytotoxic activity assays was nearly consistent with the docking results.

EXPERIMENTAL

Molecular modeling study. ***Preparation of macromolecule.*** Protein data bank (www.pdb.org) was used for downloading the crystal structure of PARP-1 enzyme with pdb code = 1UK1 along with a potent quinazolinone derivative. The active site of the enzyme was determined, and important pharmacophores were identified.

Preparation of ligands. All compounds were built using MOE 2012.10 builder, minimized, and saved as mol2 format. The gradient for energy minimization was set to be =0.05, and the force field was selected as "MMFF94X" to be suitable for organic molecules. All bonded, Van der Waals, electrostatics, and restraints were enabled. Partial charges were calculated.

Molecular docking using Leadit 2.1.2. *Preparation of receptor.* The crystal structure of PARP-1 was prepared using Leadit 2.1.2 software. Quinazolinone derivative inhibitor was selected as a reference ligand.

Leadit docking. Docking strategy was selected to be Enthalpy and Entropy (Hybrid approach). Scoring was set as default. Clash factor was set to be medium; maximum number of solution per iteration was =200.

Chemistry. ***General methods.*** All melting points were measured using electro thermal IA9100 apparatus (Shimadzu, Japan). Infrared spectra were recorded on Bruker FT-IR spectrophotometer and were expressed as wave number (cm^{-1}) using potassium bromide discs, at the Micro-analytical center, Faculty of Science, Cairo University. The ^{1}H-NMR spectra were determined on Varian Mercury VX-300 NMR spectrophotometer at 300 MHZ at the Faculty of Science, Cairo University. The ^{13}C-NMR spectra were recorded on Varian Mercury VX-300 NMR spectrophotometer and were run at 75.446 MHZ in (DMSO-d6), at main defense chemical laboratories. Mass spectra were recorded on 70 eV EL Ms-QP 1000 EX (Shimadzu-Japan), Faculty of Science Cairo University, Cairo, Egypt. Elemental analyses were carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University, and the results were within the accepted range (±0.40) of the calculated values.

The starting materials, namely, ethyl-2-amino-4,5,6,7,8-pentahydrocyclohepta[*b*]thiophene-3-carboxylate **1**, ethyl 2-acetamido-4,5,6,7,8-pentahydrocyclohepta[*b*]thiophene-3-carboxylate **2**, and 3-amino-2-methyl-5,6,7,8,9-pentahydro cyclohepta[4,5]thieno-[2,3-*d*] pyrimidin-4(3*H*)-one **3** were prepared following the corresponding literature procedures [25] in (Scheme 1). 5,6,7,8,9-Pentahydro-cyclohepta[4,5] thieno[2,3-*d*]pyrimidin-4-one **8** and 4-chloro-5,6,7,8,9-pentahydrocyclo-hepta[4,5]thieno[2,3-*d*]pyrimidine **9** were synthesized according to the reported methods [20] (Scheme 2).

N-(2-methyl-4-oxo-5,6,7,8,9-pentahydrocyclohepta[4,5] thieno[2,3-d]pyrimidin-3(4H)-yl) acetamide (4). A mixture of 3-aminothienopyrimidine **3** (2.49 g, 10 mmol) and acetic anhydride (15 mL) was refluxed for 3 h. The mixture was then left at room temperature for 24 h, where a solid product was separated, filtered, and recrystallized from absolute ethanol to give yellow needles. Yield: 71%, m.p. (110–111°C). IR (KBr disc) (cm^{-1}): 3413 (NH), 2920 (CH_2), 2851 (CH_3), 1738 (C=O), 1669 (C=O). ^{1}H-NMR (DMSO) δ (ppm): 1.21 (s, CH_3 at C-2 of pyrimidine), 2.30 (s, CH_3 acetamido group), 1.26–3.34 (m, 10H cycloheptane), 11.00 (s, NH, D_2O exchangeable).^{13}C NMR (DMSO-d_6) δ ppm: 20.39, 20.76, 26.67, 26.93, 27.20, 28.91, 31.76, 120.93, 136.39, 136.85, 155.76, 155.87, 158.99, 169.21. m/z (%): $M^{+\cdot}$: 291 (17.2), 43 (100). *Anal.* for $C_{14}H_{17}N_3O_2S$ (291.369), Calcd %: C, 57.71; H, 5.88; N,14.42 Found %: C, 57.42; H, 5.63; N, 14.21.

2-Chloro-N-(2-methyl-4-oxo-5,6,7,8,9-pentahydrocyclohepta [4,5]thieno[2,3-d] pyrimidin-3(4H)-yl) acetamide (5). Chloroacetylchloride (1.12 mL, 10 mmol) was added drop wise to 3-aminothienopyrimidine **3** (2.49 g, 10 mmol) in DMF (10 mL), and stirring was continued overnight at room temperature. The reaction mixture was then poured onto ice/water. The separated solid was filtered, left to dry then recrystallized from absolute ethanol. Yellowish white crystals were resulted in a yield of 67%, m.p. (140–141°C). IR (KBr disc) (cm^{-1}): 3337 (NH), 1705, 1655 (C=O), 2922 (CH-aliphatic).^{1}H-NMR (DMSO) δ (ppm), 1.26–3.34 (m, 3H, C_2—H, 10H of cycloheptane), 4.30 (s, 2H, $COCH_2$), 11.32 (s, 1H, NH D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ ppm: 13.97, 20.60, 26.50, 27.81, 28.92, 31.74, 60.67, 120.89, 136.34, 138.08, 155.53, 158.90, 164.69. m/z (%): 325 (M ^{35}Cl, 10.85), 327 (M ^{37}Cl, 4.01),77 (100). *Anal.* for $C_{14}H_{16}N_3O_2S$ (325.81) Calcd %: C, 51.61; H, 4.95; N, 12.90. Found %: C, 51.46; H, 4.74; N, 12.74

1-(2-Methyl-4-oxo-5,6,7,8,9-pentahydrocyclohepta[4,5]thieno [2,3-d]pyrimidin-3(4H)-yl)-3-phenylthiourea (6). Phenyl isothiocyanate (5.6 mL, 40 mmol) was added to 3-

aminothienopyrimidine **3** (2.49 g, 10 mmol) in dichloromethane (20 mL). The mixture was heated at (70°C) on water bath for 12 h, and excess solvent was removed under reduced pressure. The remained solid was collected, dried, and recrystallized from absolute ethanol. Light brown powder was obtained in a yield of 73%, m.p. (138–140°C). IR (KBr disc) (cm^{-1}): 3340 (NH broad), 3090 (CH aromatic), 1656 (C=O), 2922 (CH-aliphatic). ^{1}H-NMR (DMSO), δ (ppm), 1.22 (s, 1H, CH_3, 1.26–2.98 (m, 10 H of cycloheptane ring), 7.12–7.45 (m, 5H of aromatic ring), 10.83 (s, 1H, NH, D_2O exchangeable), 11.50 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ ppm: 14.20, 26.52, 27.49, 27.90, 29.10, 31.76, 120.81, 123.51, 124.23, 127.90, 130.03, 136.34, 137.68, 139.40, 155.36, 161.13, 165.37, 175.64, m/z (%): 384 $M^{+\cdot}$ (62.76). *Anal*. for $C_{19}H_{20}N_4OS_2$ (384.52) Calcd %: C, 59.35; H, 5.24; N, 14.57. Found %: C, 59.48; H, 5.46; N, 14.88.

3,4-Dihydro-6-methyl[1,2,4]triazino[2,3c]9,10,11,12,13 pentahydrocyclohepta [4,5]thieno[3,2-e]pyrimidin-2-one (7). An equimolar amount of 3-aminothienopyrimidine derivative **3** (2.49 g, 10 mmol) and chloroacetamide (~1 g, 10 mmol) in DMF (10 mL) was heated under reflux for 12 h, left to cool then poured onto ice/water. The formed solid was filtered, dried, and recrystallized from ethanol/DMF. Brownish powder was obtained in a yield of 65%, m.p. (188–190°C). IR (KBr disc) (cm^{-1}): 3420 (NH), 2922 (C-aliphatic), 1667 (C=O), 1641 (C=N). ^{1}H-NMR (δppm) (DMSO), 1.26–3.34 (m, 3H of C-2 pyrimidine ring, 10H of cycloheptane), 3.23 (s, 1H, NH triazine ring, D_2O exchangeable), 5.79 (s, 2H, CH_2 of triazine ring), m/z (%); 288 (M^+, 0.56), 249(100). *Anal*. for $C_{14}H_{16}N_4OS$ (288.37) Calcd %: C, 58.31; H:, 5.59; N, 19.43. Found %: C, 58.42; H, 5.57; N, 19.75.

4-Hydrazino-5,6,7,8,9-pentahydrocyclohepta[4,5]thieno[2,3-d]pyrimidine (10). A mixture of 4-chlorothienopyrimidine derivative **9** (2.4 g, 10 mmol) and hydrazine hydrate (~2 mL, 20 mmol) was refluxed in dioxane (10 mL) for 4 h. The separated solid was filtered off, dried, and recrystallized from dioxane. Reddish brown powder was resulted in a yield of 58%, m.p. (145–147°C). IR (KBr) (cm^{-1}): 3410 (NH_2), 3387 (NH), 3109 (CH-aromatic), 1554 (C=C).^{1}H-NMR (DMSO) δ (ppm) 1.21–2.90 (m, 10H cycloheptane) 3.81 (s, 2H, NH_2, D_2O exchangeable), 7.98 (s, 1H, CH pyrimidine), 12.28 (s, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ ppm: 26.42, 27.36, 28.22, 27.17, 31.76, 123.17, 136.37, 144.47, 158.99, 169.21. m/z (%): 234 ($M^{+\cdot}$, 1.05), 64 (100). *Anal*. for $C_{11}H_{14}N_4S$ (234.321), Calcd %: C, 56.38; H, 6.02; N, 23.91. Found %: C, 56.44; H, 6.09; N, 24.04

4-Thioxo-5,6,7,8,9-pentahydrocyclohepta[4,5]-thieno[2,3-d]pyrimidine (11). A mixture of 4-chloro thienopyrimidine derivative **9** (2.4 g, 10 mmol), thiourea (0.76 g, 10 mmol) in ethanol (10 mL), and in the presence of 3 drops of TEA was refluxed for 4 h. The solvent was evaporated under reduced pressure, and the obtained solid was recrystallized from methanol. Dark brown powder was obtained in a 57% yield, m.p. (150–151°C). IR (KBr) (cm^{-1}): 3396 (NH), 3100 (CH-aromatic), 1500 (C=C). ^{1}H-NMR (DMSO) δ (ppm), 1.21–2.90 (m, 10H cycloheptane), 8.00 (s, 1H, CH pyrimidine), 12.15 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ ppm: 26.78, 27.23, 27.79, 29.07, 31.88, 123.17, 136.38, 143.20, 144.39, 158.10, 173.78. m/z (%): 236 ($M^{+\cdot}$, 5.21), 220 (100). *Anal*. for $C_{11}H_{12}N_2S_2$ (236.356), Calcd %: C, 55.90; H, 5.12; N, 11.85; S, 27.13. Found %: C, 55.96; H, 5.10; N, 12.02; S, 27.22.

3-Phenyl-2-thioxo-5,6,7,8,9-pentahydrocyclohepta[4,5]thieno[2,3-d]pyrimidin-4(1H)one (12). Equimolar amounts of aminothiophene **1** (2.40 g, 10 mmol), phenyl isothiocyanate (1.36 mL, 10 mmol), and anhydrous potassium carbonate (1.4 g, 10 mmol) in acetonitrile (20 mL) were refluxed on a water bath for 15 h. The reaction mixture was then cooled, filtered, and neutralized with 2 M hydrochloric acid. The separated product was filtered, washed with water, dried, and recrystallized from acetic acid. Yellowish white crystals were obtained in a yield of 81%, m.p. >250°C. IR (KBr disc) (cm^{-1}): 3416 (NH), 3109 (CH-aromatic), 1656 (C=O). ^{1}H-NMR (DMSO), δ (ppm) 1.54–3.07 (m, 10H cycloheptane ring), 7.19–7.47 (m, 5H of aromatic ring), 13.59 (s, NH, D_2O exchangeable), m/z (%): 328 ($M^{+\cdot}$, 64.04), 252 (100). *Anal*. for $C_{17}H_{16}N_2OS_2$ (328.452), Calcd %: C, 62.16; H, 4.91; N, 8.53. Found %: C, 62.31; H, 4.88; N: 8.35.

3-Phenyl-2-hydrazino-5,6,7,8,9-pentahydrocyclohepta[4,5]thieno[2,3-d]pyrimidin-4-one (13). A mixture of 2-thioxo **12** (3.2 g, 10 mmol) and hydrazine hydrate (~2 mL, 20 mmol) was refluxed in pyridine (10 mL) for 15 h, and the reaction mixture was then poured onto ice/water. The solid product was filtered, washed with ethanol, and recrystallized from ethanol. Yellow crystals were obtained in a yield 74%, m.p. (205–208°C). IR (KBr) (cm^{-1}): 3425 (NH_2), 3336 (NH), 1679 (C=O), 3103 (CH-aromatic).^{1}H-NMR (DMSO) δ (ppm): 1.29–3.09 (m, 10 H cycloheptane), 4.28 (s, 2H, NH_2 D_2O exchangeable), 6.80 (s, 1H, NH, D_2O exchangeable), 7.00–8.00 (m, 5H Ar—H). ^{13}C NMR (DMSO-d_6) δ ppm: 26.78, 27.19, 27.23, 28.94, 31.87, 117.12, 119.24, 129.63, 136.55, 141.20, 144.31, 150.53, 155.49, 157.37, 161.13, 164.84. m/z (%): 326 ($M^{+\cdot}$, 60.04), 77 (100). *Anal*. for $C_{17}H_{18}N_4OS$ (326.416), Calcd %: C, 62.55; H, 5.56; N, 17.16. Found %: C, 62.63; H, 5.95; N, 17.31.

Ethyl-2-(3-phenylthioureido)-4,5,6,7,8-pentahydrocyclohepta[b]thiophene-3-carboxylate (14). 2-Aminothiophene derivative **1** (2.40 g,10 mmol) was dissolved in hot ethanol (10 mL), then phenyl isothiocyanate (~2 mL, 15 mmol) was added drop wise while stirring. The reaction mixture was heated under reflux on a water bath for 2 h and left to stand overnight at room temperature. The separated solid was

filtered, washed with ethanol, and recrystallized from ethanol. Yellow crystals were obtained in a yield of 96%, m.p. (150–153°C). IR (KBr disc) (cm^{-1}): 3330 (NH), 2223 (—S=C=N—), 1702 (C=O). ^{1}H-NMR (DMSO) δ (ppm) 1.22 (t, 3H, CH_3), 1.34–2.97 (m, 10H cycloheptane) 4.22 (q, 2H, $C\underline{H}_2$— CH_3), 7.12–7.49 (m, 5H, aromatic H), 10.83 (s, 1H, NH, D_2O exchangeable), 11.50 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ ppm: 14.29, 26.53, 27.41, 27.79, 27.90, 31.50, 60.45, 113.87, 118.87, 123.53, 124.23, 128.33, 136.81, 146.61, 155.21, 161.13, 175.64. m/z (%): 374 ($M^{+\cdot}$, 62.76), 193 (100). *Anal*. for $C_{19}H_{22}N_2O_2S_2$ (374.52), Calcd %: C, 60.93; H, 5.92; N, 7.48. Found %: C, 60.68; H; 5.89; N, 7.71.

3-Amino-2-phenylamino-5,6,7,8,9-pentahydrocyclohepta [4,5]thieno[2,3-d]pyrimidin-4(3H)-one (15). A mixture of the thioureido derivative **14** (3.70 g, 10 mmol) and hydrazine hydrate (~1 mL, 20 mmol) in ethanol (15 mL) was refluxed for 8 h and left to cool. The separated solid was filtered, washed with water, and recrystallized from ethanol/DMF. Yellow powder was obtained in a yield of 64%, m.p. (200–203°C). IR (KBr) (cm^{-1}): 3324 (NH_2), 3310 (NH), 3105 (CH-aromatic), 1690 (C=O). ^{1}H-NMR (DMSO) δ (ppm): 1.29–2.99 (m, 10H cycloheptane), 2.60 (s, 2H, NH_2, D_2O exchangeable), 7.16–7.49 (m, 5H, Ar—H), 13.85 (s, 1H, NH, D_2O exchangeable). m/z (%): 326 ($M^{+\cdot}$, 100). *Anal*. for $C_{17}H_{18}N_4OS$ (326.416), Calcd %: C, 62.55; H, 5.56; N, 17.16. Found %: C, 62.61; H, 5.58; N, 17.29.

Ethyl-2-thioureido-4,5,6,7,8-pentahydrocyclohepta[b]thiophene-3-carboxylate (16). A mixture of 2-aminothiophene **1** (2.40 g, 10 mmol) and potassium thiocyanate (~0.15 g, 15 mmol) was refluxed in 10% HCl (10 mL) for 12 h. The reaction mixture was allowed to stand overnight at room temperature. The formed solid was filtered, washed with ethanol, dried, and recrystallized from ethanol. Brown powder was obtained in a yield of 59% m.p. (180–182°C). IR spectrum: 3430 (NH_2), 2982 (CH-aliphatic), 2259 (—N=C=S), 1661 (C=O). ^{1}H-NMR spectrum (DMSO) δ (ppm): 1.22–3.40 (m, 3H, CH_3, and 10H cycloheptane), 4.26 (q, 2H, CH_2), 8.30 (s, 2H, NH_2, D_2O exchangeable), 11.00 (s, 1H, NH, D_2O exchangeable). m/z (%): $M^{+\cdot}$: 298 ($M^{+\cdot}$, 11.19), 60 (100). *Anal*. for $C_{13}H_{18}N_2O_2S_2$ (298.42), Calcd %: C, 52.32; H, 6.08; N, 9.39. Found %: C, 52.46; H, 6.13; N, 9.52.

2-Thioxo-5,6,7,8,9-pentahydrocyclohepta[4,5]thieno[2,3-d] pyrimidin-4[1H]-one (17). A solution of thiourea derivative **16** (2.60 g, 10 mmol) and sodium ethoxide (0.23 g of sodium metal in ethanol 5 mL) was refluxed in ethanol (15 mL) for 6 h and left to cool. The reaction mixture was neutralized with 10% HCl where a solid was formed, collected by filtration, washed with water, dried, and recrystallized from ethanol/DMF. Light brown powder was obtained in a yield of 78%, m.p. (230–232°C). IR (KBr) (cm^{-1}): 3416 (NH), 1662 (C=O), 1560 (C=C). ^{1}H-NMR (DMSO) δ (ppm) 1.29–3.40 (m, 10H (cycloheptane)), 12.23 (s, 1H, NH, D_2O exchangeable), 13.21 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ ppm: 26.59, 27.19, 28.28, 31.67, 116.90, 131.94, 136.27, 148.27, 157.37, 172.43. m/z (%): 252 ($M^{+\cdot}$, 4.05), 80 (100). *Anal*. for $C_{11}H_{12}N_2OS_2$ (252.356), Calcd %: C, 52.35; H, 4.79; N, 11.10. Found %: C, 52.42; H, 4.81; N, 10.23.

Inhibition of PARP-1 activity. The enzyme specific assay was carried out at the National Research Centre, Dokki, Giza, Egypt.

Materials and methods. Na_2HPO_4, NaH_2PO_4, and NaCl were purchased from Sigma Chem. Co. (St. Louis, MO, USA). The investigated compounds were dissolved in DMSO and diluted at specified concentrations (from 50 to 200 μM). The PARP inhibitory activities of the investigated compounds were determined using Trevigen's HT Universal 96-well PARP Assay Kits (Trevigen, Inc., Gaithersburg, Maryland, USA) to measure the incorporation of biotinylated poly (ADP-ribose) onto histone proteins in a 96-well strip well format. This assay is ideal for the screening of PARP inhibitors and the determination of IC_{50} values. The measurements were performed according to the manufacturer's protocol. The decrease in absorbance of each solution was measured at 450 nm using an ELISA micro plate reader (Model 550, Bio-Rad Laboratories Inc., California, USA). Absorbance of blank sample containing the same amount of DMSO was also prepared and measured. A negative control without PARP was prepared to determine background absorbance. 3-Aminobenzamide was used as a positive control for PARP inhibitory activity with a reported IC_{50} of 30 μM [27] (Table 2).

Statistical analysis. All experiments were conducted in triplicate ($n = 3$), and all the values were represented as mean ± SD. Significant differences between the means of parameters as well as IC_{50} values were determined by probit analysis using SPSS software program (SPSS Inc., Chicago, IL) (Table 2).

Cytotoxic effect on human Caucasian breast adenocarcinoma (MCF7). Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [28].

The experiment was done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in RPMI 1640 medium for MCF7. The media are supplemented with 1% antibiotic–antimycotic mixture (10 000 U/mL potassium penicillin, 10 000 μg/mL streptomycin sulfate, and 25 μg/mL amphotericin B), 1% L-

glutamine, and 10% fetal bovine serum and kept at 37°C under 5% CO_2. Cells were batch cultured for 10 days, then seeded at concentration of 10×10^3 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37°C for 24 h under 5% CO_2 using a water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added, and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (100–50–25–12.5–6.25–3.125–0.78 and 1.56 μM/mL). After 48 h of incubation, medium was aspirated, 40-μL MTT salt (2.5 μg/mL) was added to each well and incubated for further 4 h at 37°C under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200 μL of 10% sodium dodecyl sulfate (SDS) in deionized water was added to each well and incubated overnight at 37°C. A positive control which composed of 100 μM/mL was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions [29].

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent *t*-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts, and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula:

$$((\text{Reading of extract} / \text{Reading of negative control}) - 1) \times 100.$$

A probit analysis was carried for IC_{50} determination using SPSS 11 program.

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