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Naphthoquinone–thiazole hybrids bearing adamantane: Synthesis, antimicrobial, DNA cleavage, antioxidant activity, acid dissociation constant, and drug-likeness

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ABSTRACT: In this study, four novel naphthoquinone–thiazole hybrids bearing adamantane were synthesized by reaction of naphthoquinone–aroylthiourea derivatives with 1-adamantyl bromomethyl ketone in 75-85% yield and were characterized using $^{1}H/^{13}C$ NMR, FT-IR, and HRMS techniques. Various biological activities of the synthesized compounds, such as antibacterial, antifungal, DNA cleavage, and antioxidant activities, were screened. The compounds showed antibacterial activity against *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus hirae*, and *Legionella pneumophila subsp. pneumophila* strains with MIC values in the range of 4–64 µg/mL and antifungal activity against *Candida albicans* strains with MIC values in the range of 16–64 µg/mL. The compounds had DNA cleavage activity at 250 and 500 µg/mL. Additionally, the antioxidant activity of the compounds was assessed based on the radical scavenging effect of the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical and the compounds exhibited acceptable antioxidant activity. The acid dissociation constants (pK_a) of the compounds were determined potentiometrically in 30% (v/v) dimethyl sulfoxide–water at an ionic background of 0.1 mol L^{-1} NaCl, at 25 ± 0.1 °C, and the HYPERQUAD computer program was used to calculate the pK_a values from the data obtained from potentiometric titrations. Prediction of the drug-likeness properties of the compounds was performed with the use of the MolSoft website, and the compounds had promising drug-likeness model scores within a range of 1.09–1.56.

KEYWORDS: Naphthoquinone; thiazole; adamantane; antimicrobial; antioxidant; DNA cleavage; acid dissociation constant; drug-likeness.

1. INTRODUCTION

Many compounds containing naphthoquinone scaffolds occur naturally and play an active role in biological processes of various fungi, bacteria, and plants [1]. Naphthoquinones are considered to be an important pharmacophore in pharmaceutical chemistry because many biologically active compounds, such as atovaquone, doxorubicin, buparvaquone, and lapachol, contain a naphthoquinone scaffold in their molecular structures (Figure 1) [2-4]. It is known that synthetic or natural products of 1,4-naphthoquinone derivatives exhibit a wide range of biological activities, such as antibacterial, antifungal [5-7], antimalarial [8], antioxidant [9, 10], and anticancer [11-13], as well as tyrosinase [14] and trypanothione reductase inhibition [15]. Moreover, naphthoquinones have been considered as active components of many herbs used in traditional medicine, such as *Lithospermum erythrorhizon* and *Onosma hookeri Clarke var. longiforum Duthie* [16].

The thiazole scaffold is one of the pharmacophores attracting the most interest recently and numerous bioactive compounds, such as tiazofurin, ravuconazole, dasatinib, and dabrafenib, contain a thiazole core (Figure 2) [17-20]. Additionally, a great number of thiazole hybrids exhibit a broad spectrum of biological activity, including antibacterial, antifungal [21-23], antimycobacterial [22-24], antiviral [25], and anticancer [26-28] activities, as well as lanosterol 14-α demethylase [29] and xanthine oxidase [30] inhibition.

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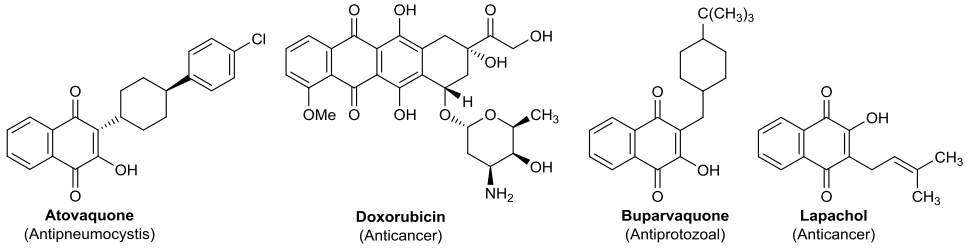

Figure 1. Some pharmaceuticals containing naphthoquinone core.

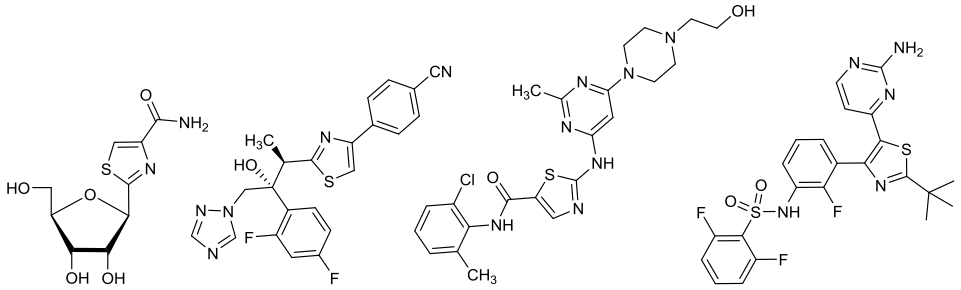

Figure 2. Some pharmaceuticals containing thiazole core.

Whether isolated from plants as natural products or produced synthetically, many derivatives of adamantane, which is a highly symmetric polycyclic cage molecule, show various pharmacological properties [31]. Adamantane is now commonly included in biologically active compounds to increase their lipophilicity and improve their pharmacological properties [32].

The pK_a value is a critical parameter of a substance because it gives information about acidity/basicity, hydrogen-bonding capacity, and solubility, which are useful for understanding the nature of compounds. Knowing the pK_a value(s) of a biologically active compound is essential in drug research because pK_a values determine the solubility, absorption, distribution, metabolism, and elimination of molecules. Furthermore, the pK_a value is also a useful parameter to optimize separation procedures [33-37]. Potentiometric and spectrophotometric titration methods are the oldest and most widely used methods to determine pK_a values [38, 39].

In this study, the synthesis and investigation of the broad biological activities of four compounds including naphthoquinone, thiazole, and adamantane moieties are reported. Additionally, determination of the pK_a values and calculation of the drug-likeness properties of the products have also been performed.

2. RESULTS AND DISCUSSION

Naphthoquinone–thiazole hybrids bearing adamantane (**6a–d**) were synthesized by reaction of 1,4-naphthoquinone *N*-aroylthioureas **4a–d** and 1-adamantyl bromomethyl ketone **5** under reflux temperature with 75-85% yield (Figure 5). The 1,4-naphthoquinone *N*-aroylthioureas **4a–d** were prepared in three steps using 2,3-dichloronaphthalene-1,4-dione **1** as the starting compound [40]. Molecular structures of **6a–d** were characterized using FT-IR, ^{1}H NMR, ^{13}C NMR and HRMS techniques. In the FT-IR spectra of **6a–d**, strong aliphatic C-H stretching peaks were observed in the range of 2960–2846 cm^{-1}. Additionally, the bands observed in the range of 1652–1650 cm^{-1} were attributed to the C=O groups. In the ^{1}H NMR spectra of **6a–d**, the singlet peak observed in the range of 6.79–6.68 ppm was attributed to the proton of the thiazole ring. Moreover, multiple peaks in the ranges of 1.91–1.79 ppm and 1.64–1.51 ppm whose integrals corresponded to 9 protons and 6 protons, respectively, indicated that the adamantane moiety was present in the molecular structure of the products. In the ^{13}C NMR spectra of **6a–d**, peaks related to the C=O groups were observed in the range of 181.3–171.3 ppm. The peak observed in the range of 171.1–170.6 ppm was attributed to the carbon of the C=N group formed after cyclization. There are four types of carbon atoms with different chemical environments in the adamantane moiety, and their associated peaks were observed at approximately 40.2 ppm, 36.0 ppm, 35.6 ppm, and 27.7 ppm.

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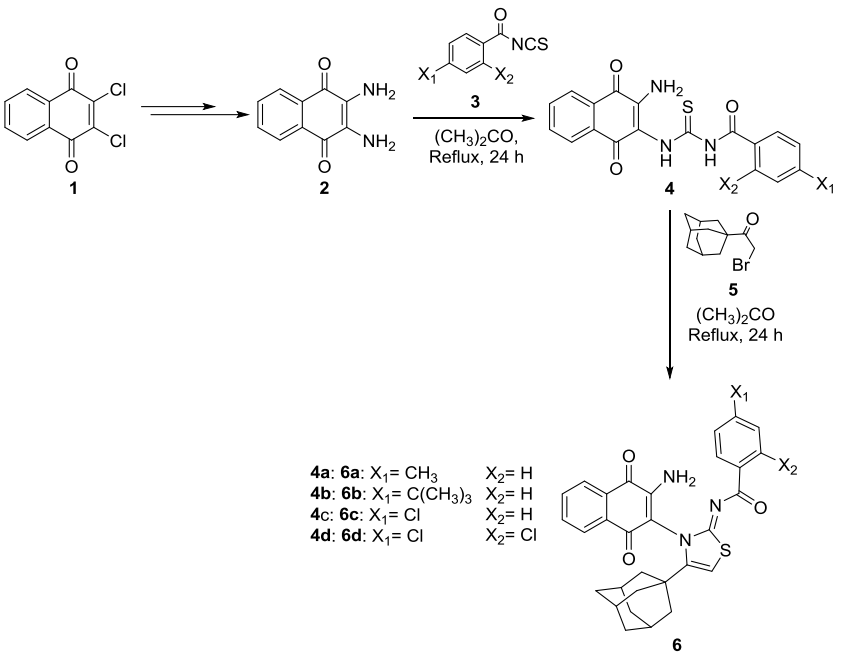
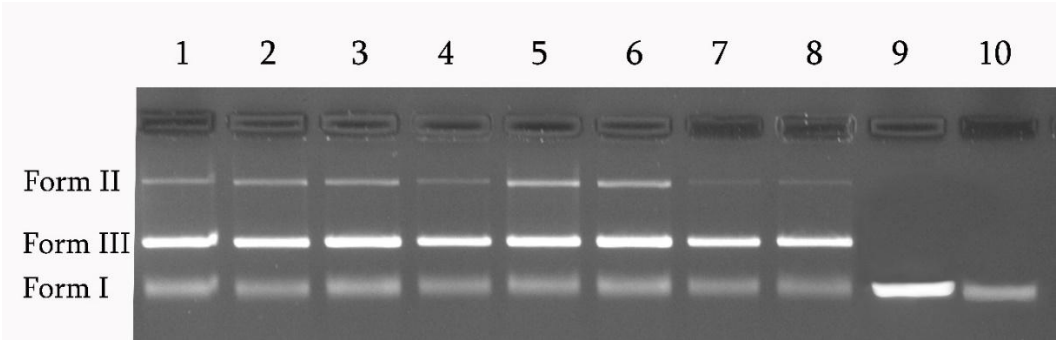


Figure 3. Synthesis of the naphthoquinone–thiazole hybrids bearing adamantane (**6a–d**).

Antibacterial activity studies of **6a–d** molecules were performed against six bacterial strains. The two-fold serial broth dilution process was applied. The naphthoquinone–thiazole hybrids bearing adamantane (**6a–d**) inhibited the growth of bacteria at minimum inhibitory concentrations (MICs) of 4 to 64 µg/mL and the reference drug ampicillin exhibited antibacterial activity with MIC values in the range of 0.5–1 µg/mL against the same bacteria strains (Table 1). Compound **6a**, containing the methyl group as a substituent at the para position of the phenyl ring, showed antibacterial activity against *E. coli* strains with a MIC value of 16 µg/mL. For the same bacterial strain, **6b** containing tert-butyl as X_1, **6c** containing a chlorine atom as X_1, and **6d** containing chlorine atoms as X_1 and X_2 exhibited antibacterial activity with MIC values of 32, 32, and 64 µg/mL, respectively. Compound **6a** showed antibacterial activity against *B. cereus* and *P. aeruginosa* strains with a MIC value of 8 µg/mL, and replacement of the methyl group with other substituents (*tert*-butyl or chlorine atom) in compounds **6b–d** caused a decrease in antibacterial activity against *B. cereus* and *P. aeruginosa*. Although **6a** and **6c** showed antibacterial activity with a MIC value of 8 µg/mL against *S. aureus*, the best antibacterial activity against *S. aureus* was obtained by compound **6b** with a MIC value of 4 µg/mL. Compound **6b** also showed antibacterial activity against *E. hirae* with a MIC value of 8 µg/mL, while compounds **6a** and **6c** showed antibacterial activity with a MIC value of 4 µg/mL against the same strain. Compound **6c** showed the best antibacterial activity against *L. pneumophila* subsp. *pneumophila* with a MIC value of 4 µg/mL, while compounds **6a**, **6b**, and **6d** showed antibacterial activity with MIC values of 16, 64, and 32 µg/mL, respectively, against the same bacterial strain. Thus, these naphthoquinone–thiazole hybrids bearing adamantane (**6a–d**) possessed a broad spectrum of antibacterial activity. It can be said that compounds **6a–d** showed better antibacterial activity against the *S. aureus* and *E. hirae* strains than the other bacterial strains and compounds **6a–d** can be considered as good starting points for further development of antibacterial agents.

Antifungal activity study of **6a–d** was performed against *C. albicans* and it was found that these compounds exhibited antifungal activity with MIC values in the range of 16–64 µg/mL. Meanwhile, the reference drug fluconazole exhibited antifungal activity with a MIC value of 0.5 µg/mL against *C. albicans*. Consequently, it can be said that compounds **6a–d** showed moderate antifungal activity against *C. albicans*.

The chemical nuclease activities of **6a–d** were screened using the agarose gel electrophoresis method. This method was applied using two different concentrations (250 and 500 µg/mL) of compounds **6a–d**. Plasmid DNA and DMSO-treated plasmid DNA were used as controls. After gel electrophoresis, as can be clearly seen in Figure 4, all compounds had nuclease activity and showed double-strained plasmid DNA cleaving ability (lanes 1–8), whereas control studies showed that untreated DNA and DNA treated with DMSO alone did not demonstrate any significant DNA cleavage (lanes 9, 10). Based on the results of this study, it can be said that **6a–d** may have promising effects in several types of medical treatment and also in cancer therapy after further clinical studies.



Lane 1, pBR 322 DNA + 250 µg/mL of **6a**; Lane 2, pBR 322 DNA + 250 µg/mL of **6b**; Lane 3, pBR 322 DNA + 250 µg/mL of **6c**; Lane 4, pBR 322 DNA + 250 µg/mL of **6d**; Lane 5 pBR 322 DNA + 500 µg/mL of **6a**; Lane 6, pBR 322 DNA + 500 µg/mL of **6b**; Lane 7, pBR 322 DNA + 500 µg/mL of **6c**; Lane 8, pBR 322 DNA + 500 µg/mL of **6d**; Lane 9, pBR 322 DNA; Lane 10, pBR 322 DNA + DMSO

Figure 4. DNA cleavage activities of test compounds.

Table 1. The MIC values (µg/mL) of **6a–d** against bacterial and fungal strains.

	Microorganisms*						
Compounds/Standards	**EC**	**BC**	**SA**	**PA**	**EH**	**LPP**	**CA**
6a	16	8	8	8	4	16	16
6b	32	32	4	32	8	64	64
6c	32	64	8	64	4	4	32
6d	64	64	32	64	64	32	32
Fluconazole	-	-	-	-	-	-	0.5
Ampicillin	0.5	1	1	0.5	0.5	0.5	-

* EC: *Escherichia coli* (ATCC 10536); BC: *Bacillus cereus*; SA: *Staphylococcus aureus* (ATCC 6538); PA: *Pseudomonas aeruginosa* (ATCC 9027); EH: *Enterococcus hirae* (ATCC 10541); LPP: *Legionella pneumophila* subsp. *pneumophila* (ATCC 33152); CA: *Candida albicans*.

The antioxidant activity of the naphthoquinone–thiazole hybrids bearing adamantane (**6a–d**) was evaluated against DPPH radical. The impact of antioxidant agents on DPPH is thought to be due to their hydrogen-donating activity. Free radical scavenging abilities are very significant to prevent the harmful actions of radicals in various diseases, such as cancer. As can be seen in Figure 5(a), the radical scavenging effect of compounds **6a–d** increased with increasing concentrations. Compounds **6a–d** exhibited DPPH scavenging ability of 30.5%, 26.6%, 21.8%, and 15.8% in the order of **6a** > **6c** > **6d** > **6b**, respectively, at a concentration of 250 mg/L. When the concentrations of **6a–d** increased from 250 mg/L to 500 mg/L, the scavenging activities increased from 54.6% to 66.3%, from 39.2% to 46.6%, from 57.7% to 62.4%, and from 41.7% to 46.2%, respectively. Acceptable effects were recorded at the 500 mg/L concentration for compounds **6a** and **6c** as 66.3% and 62.4%, respectively. Ascorbic acid and Trolox, used as reference compounds, had a 100% radical scavenging effect at the same concentrations. Finally, it can be said that for **6a** and **6c**, modification of the substituents (X_1= –CH_3 and –Cl) in the structure of the naphthoquinone–thiazole hybrids bearing adamantane caused a significant change in free radical scavenging activity.

The emergence of most reactive oxygen species is due to the electron transport system and other metabolic activities, and reactive oxygen species are produced by metal-catalysed oxidation reactions [41]. Ferrozine forms a complex with ferrous ions and a magenta colour appears. In the presence of any chelating agents, the formation of the iron–ferrozine complex is inhibited, and, as a result, the magenta colour's intensity is reduced. The metal chelating abilities of **6a–d** were determined by applying the ferrozine test. The results are presented in Figure 5(b). The reducing abilities on Fe^{2+} were in the following order at 100 mg/L: **6b** > **6a** > **6c** > **6d**. The ferrous chelating activities were also determined at 250 mg/L and **6a**, **6b**, **6c**, and **6d** showed respective activities of 38.4%, 45.3%, 27.4%, and 20.9%, respectively. The highest Fe^{2+} reducing ability was obtained as 57.9% with compound **6b**. The evaluation of the antioxidant activity revealed that tested compounds **6a** and **6b** exhibited acceptable metal chelating ability compared with the positive control, EDTA. The variability of the metal chelating ability can be attributed to modifications of the substituents in the structure of the naphthoquinone–thiazole hybrids bearing adamantane.

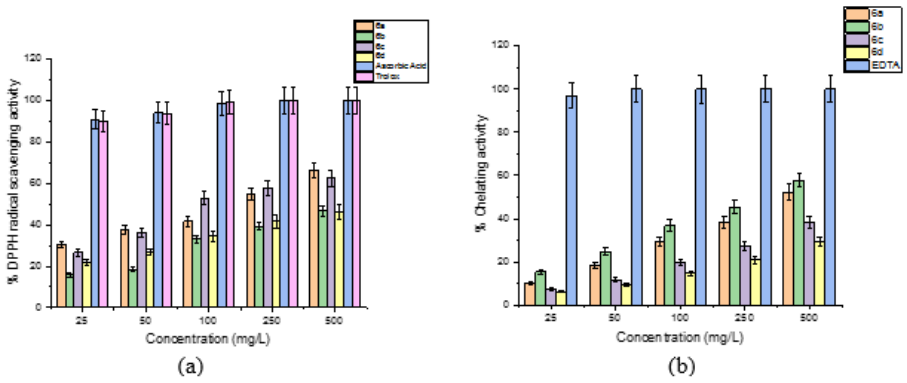


Figure 5. % Radical scavenging (a) and chelating (b) activity of **6a–d**.

The pK_a values of compounds **6a–d** were determined in a DMSO–water mixture because they have low solubility in water. DMSO–water mixtures have various advantages, such as compatibility with the standard glass electrode and a large acidity range, and are therefore among the most widely used solvent systems for potentiometric determination of pK_a values [42, 43]. The pK_a values of **6a–d** were determined potentiometrically in an acidic medium at 25.0 ± 0.1 °C and 0.1 M NaCl ionic strength in 30% (v/v) DMSO–water hydro-organic solvent. As shown in Figure 7, we expect such compounds to be protonated by seven different groups. In this study, we determined four pK_a values each for **6a** and **6d** and three pK_a values each for **6b** and **6c** using the HYPERQUAD computer program to calculate the pK_a values from the data obtained from potentiometric titrations. Potentiometric titration and distribution curves of **6a–d** obtained from potentiometric titrations are given in Figure 6.

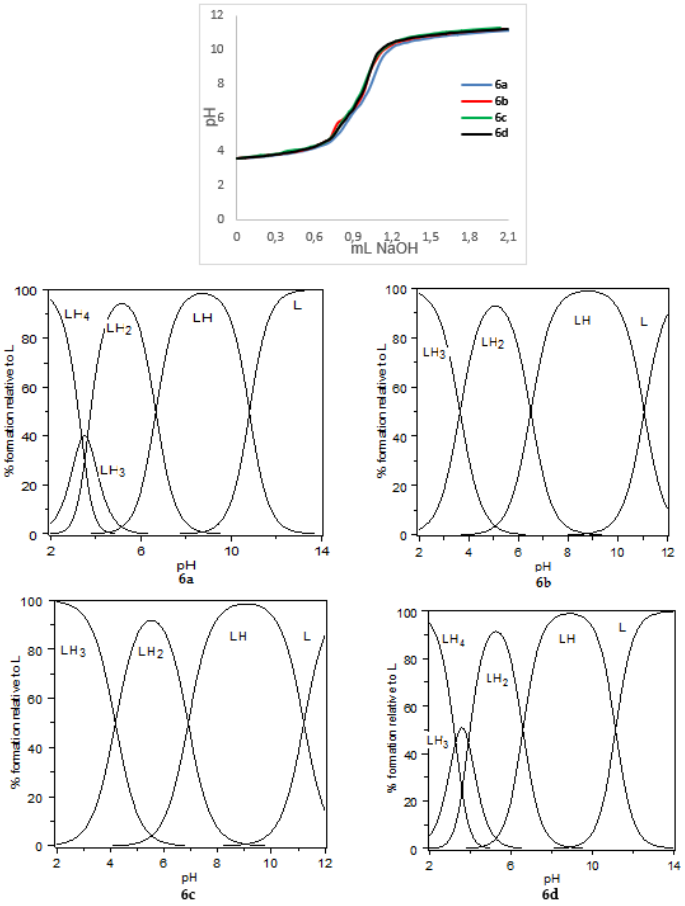


Figure 6. Potentiometric titration and distribution curves of **6a–d** in acidic medium (25.0 ± 0.1 °C, 0.1 M NaCl in 30% (v/v) DMSO–water).

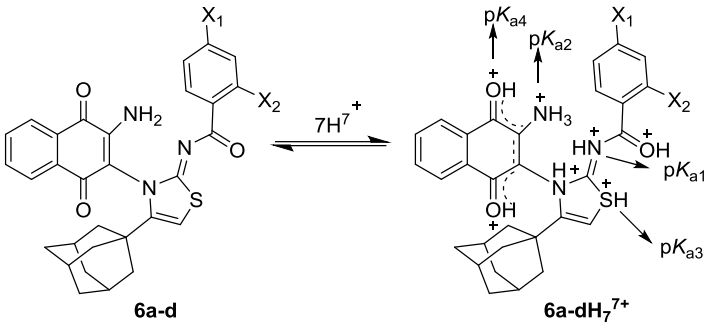


Figure 7. Full protonated form of the ligands **6a–d**H_7^{7+}.

There are three nitrogen atoms in the molecular structure of compounds **6a–d** and all of the nitrogen atoms have conjugation. Altun *et al*. [44] reported that the pK_a values of the imine nitrogen atom, the nitrogen atom on the thiazole ring, and the phenolate oxygen atom were obtained in the ranges of 2.46–2.85, 2.86–4.92, and 8.89–11.61, respectively, in a 60% dioxane–water mixture. Gemili *et al*. [40] reported that the pK_a values of the amine nitrogen atom bound to the 1,4-naphthoquinone ring –SH and –OH groups that formed after protonation of the thiocarbonyl group and carbonyl group on the 1,4-naphthoquinone ring in acidic medium were obtained in the ranges of 4.79–5.64, 6.21–6.48, and 8.95–9.54, respectively, in a 50% DMSO–water mixture.

The pK_{a1}, pK_{a2}, pK_{a3}, and pK_{a4} values of **6a** and **6d** were found as 3.37 ± 0.10 and 3.26 ± 0.11, 3.63 ± 0.12 and 3.90 ± 0.11, 6.67 ± 0.07 and 6.58 ± 0.08, and 10.82 ± 0.04 and 11.13 ± 0.04, respectively (Table 2). We propose that the pK_{a1}, pK_{a2}, pK_{a3}, and pK_{a4} values are related to the imine nitrogen atom (C=NH^+), the amine nitrogen atom bound to the 1,4-naphthoquinone ring (NH_3^+), the sulphur atom on the thiazole ring, and one of the oxygen atoms of the carbonyl groups on the 1,4-naphthoquinone ring, respectively. However, the pK_a values of the other three protonated species could not be determined. The pK_{a1}, pK_{a2}, and pK_{a3} values of **6b** and **6c** were found as 3.65 ± 0.13 and 4.18 ± 0.13, 6.49 ± 0.09 and 6.90 ± 0.11, and 11.06 ± 0.07 and 11.22 ± 0.05, respectively (Table 2). We suggest that the pK_{a1}, pK_{a2}, and pK_{a3} values are related to the amine nitrogen atom bound to the 1,4-naphthoquinone ring (NH_3^+), the sulphur atom on the thiazole ring, and one of the oxygen atoms of the carbonyl groups on the 1,4-naphthoquinone ring, respectively. In addition to the three protonated species that were in the molecular structures of **6a** and **6d** and whose pK_a values could not be determined, the pK_a values related to the imine nitrogen atom (C=NH^+) for **6b** and **6c** could not be determined.

Table 2. The pK_a values of **6a–d** in acidic medium (25.0 ± 0.1 °C, 0.1 M NaCl in 30% (v/v) DMSO–water).

Compound	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}
6a	3.37± 0.10	3.63 ± 0.12	6.67 ± 0.07	10.82 ± 0.04
6b	3.65± 0.13	6.49 ± 0.09	11.06 ± 0.07	
6c	4.18± 0.13	6.90 ± 0.11	11.22 ± 0.05	
6d	3.26± 0.11	3.90 ± 0.11	6.58 ± 0.08	11.13 ± 0.04

Many drug candidates are eliminated in the further stages of drug research because of their weak pharmacokinetic properties. Determination of pharmacokinetic properties of compounds in the first step avoids the loss of time and money. The "drug-likeness" concept was proposed to eliminate molecules that do not have suitable properties at the initial stage to minimize losses in later stages of drug research. pK_a values, lipophilicity, number of hydrogen bond donors and recipients, polar surface area, and percentage absorption are useful parameters that help determine the pharmacokinetic properties of drug candidate molecules. There are many statistical programs developed to estimate the drug-likeness properties of compounds [45, 46].

In this study, to estimate the pharmacokinetic and drug-likeness properties of compounds **6a–d**, a theoretical study was performed using the MolSoft website. The MolSoft programme uses many estimated parameters such as number of hydrogen bond donors (HBDs) and acceptors (HBAs), topological polar surface area (TPSA), molecular volume, etc. and calculates a drug-likeness model score (DLS), which is a parameter that predicts the extent to which a compound will possess the properties of drug molecules [47]. Some of the parameters obtained are summarized in Table 3. The percentage of absorption (%ABS) was calculated using the formula %ABS = 109 - (0.345 × TPSA) as given in the literature [48].

Table 3. Drug-likeness calculations of compounds **6a–d**.

Comp.	HBA[a]	HBD[b]	TPSA[c] (A^2)	Volume[d] (A^3)	%ABS[e] = 109 - (0.345 × TPSA)	DLS
6a	5	2	72.14	556.83	84.11	1.19
6b	5	2	72.14	620.50	84.11	1.09
6c	5	2	72.14	553.08	84.11	1.56
6d	5	2	72.14	568.48	84.11	1.14

[a] = Number of hydrogen bond acceptors, [b] = Number of hydrogen bond donors, [c] = Topological polar surface area, [d] = Molecular volume, [e] = Percentage absorption

The numbers of HBDs and HBAs for each compound were found to be 2 and 5, respectively. DLS values ranged in the order of **6b < 6d < 6a < 6c**, which means that **6c** has the best pharmacokinetic properties according to MolSoft calculations. **6c**, containing a chlorine atom in the para position of the phenyl ring as a substituent, had the maximum DLS value of 1.56 (Figure 8). Binding the chlorine atom to both the ortho position and the para position of the phenyl ring causes a decrease in the DLS value. As a result, although **6c** has the highest DLS, considering Figure 6, the DLS values of **6a–d** show that all of the compounds have good pharmacokinetic properties with DLS values in the range of 1.09–1.56.

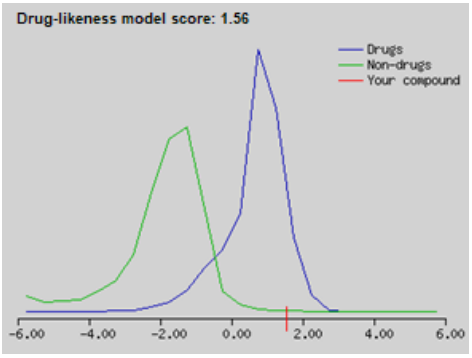


Figure 8. Calculated drug-likeness model score for **6c** using the MolSoft website.

3. CONCLUSION

In summary, we have demonstrated the synthesis of naphthoquinone–thiazole hybrids bearing adamantane by the reaction of 1,4-naphthoquinone N-aroylthiourea derivatives and 1-adamantyl bromomethyl ketone. These compounds have three very important pharmacophore groups that are desired to be found in the molecular structure of the compounds in pharmaceutical studies are reported. The compounds exhibited antibacterial activity with MIC values in the range of 4–64 µg/mL and their antibacterial activities are promising for future studies. Furthermore, the compounds exhibited moderate antifungal activity with MIC values in the range of 16–64 µg/mL. All of the compounds showed DNA cleavage activity and these results indicate that the compounds can be considered as good starting points for further anticancer studies. The compounds showed moderate antioxidant and ferrous chelating activities. The pK_a values, which are critical for the further study of the compounds, were determined potentiometrically using the HYPERQUAD computer program in 30% (v/v) DMSO–water hydro-organic solvent. Furthermore, all of the compounds had valuable drug-likeness model scores within a range of 1.09–1.56, and these results indicate that these naphthoquinone–thiazole hybrids bearing adamantane have good pharmacokinetic properties for further studies.

4. MATERIALS AND METHODS

4.1. Synthesis

High-purity precursor chemicals were purchased from Merck or Aldrich. A PerkinElmer spectrophotometer for Fourier-transform infrared spectroscopy (FTIR) spectra and a Bruker Ultrashield Plus Biospin at 400 MHz for nuclear magnetic resonance (NMR) spectra and decoupling experiments were utilized. Chemical shifts are given in parts per million (δ) downfield from TMS (tetramethylsilane, internal standard). Dimethyl sulfoxide-d_6 was used in obtaining spectra. Abbreviations used are as follows: s: singlet, d: doublet, dd: doublet of doublets, td: triplet of doublets, m: multiplet. An Agilent 6224 TOF LC-ESI-MS was used for

recording high-resolution mass spectra (HRMS). A Mettler Toledo MP90 device was used for determination of melting points. A Titroline 7000 automated titrator with SI-Analytics combined with a glass electrode, which could be controlled by a computer and had an automatic micro-burette, was used for pH-metric titrations.

*4.1.1. Synthesis of 1,4-naphthoquinone N-aroylthioureas (**4a–d**)*

The 1,4-naphthoquinone *N*-aroylthioureas **4a–d** were prepared as established in our previous study [40] by reacting 2,3-diaminonaphthalene-1,4-dione (**2**), which was prepared from 2,3-dichloronaphthalene-1,4-dione **1** and the corresponding aroylisothiocyanates **3**. A solution of the corresponding aroyl chloride (2 mmol) in acetone (20 mL) was added to a stirred solution of potassium thiocyanate (2 mmol) in acetone (20 mL), and then the reaction mixture was stirred at reflux temperature for 2 h. After 2 h, a solution of 2,3-diaminonaphthalene-1,4-dione (1 mmol) in acetone (10 mL) was added dropwise to the reaction medium and heated at reflux temperature for a further 22 h. When the reaction was determined to be complete, the solvent was evaporated using a rotary evaporator. The crude product was washed with deionized water and then several times with diethyl ether. The structures of **4a–d** were confirmed by $^{1}H/^{13}C$ NMR spectra.

*4.1.2. General procedure for the synthesis of the naphthoquinone–thiazole hybrids bearing adamantane (**6a–d**)*

1-Adamantyl bromomethyl ketone (1.5 mmol) solution in acetone (20 mL) was added drop by drop to a stirred solution of 1,4-naphthoquinone *N*-aroylthioureas (1 mmol) in acetone (30 mL) and the formed reaction mixture was stirred for 24 h under reflux temperature. The solvent was evaporated under reduced pressure after the reaction was completed, and then ethyl acetate was used for extraction. Purification of the crude mixture was performed by column chromatography (ethyl acetate:hexane, 1:3)

N-(3-(3-Amino-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-4-(p-tolyl)thiazol-2(3H)-ylidene)adamantane-1-carboxamide (**6a**)

Orange powder. Yield 0.39 g, 75%; m.p.: >300 °C; Rf = 0.32 (EtOAc:hexane / 1:3); IR (cm^{-1}): υ_{max} 3329, 3182, 3071, 2902, 2850, 1652, 1614. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.16 (dd, 1H, *J* = 7.5 Hz, 0.8 Hz, Ar-H), 7.99 (dd, 1H, *J* = 7.5 Hz, 0.8 Hz, Ar-H), 7.93-7.84 (m, 3H, N-H, Ar-H), 7.76–7.72 (m, 3H, N-H, Ar-H), 7.08 (d, 2H, *J* = 8.0 Hz, Ar-H), 6.68 (s, 1H, C-H thiazole), 2.24 (s, 3H, CH_3), 1.89–1.79 (m, 9H, C-H adamantane), 1.62–1.51 (m, 6H, C-H adamantane). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 181.3 (C=O), 177.2 (C=O), 172.7 (C=O), 170.6 (C=N), 147.3, 147.2, 141.1, 135.6, 134.6, 133.0, 132.2, 130.1, 128.6 (2 x C), 128.4 (2 x C), 126.5, 126.1, 114.3, 104.1, 40.2 (3 x CH_2), 36.0 (3 x CH_2), 35.6, 27.7 (3 x *C*H), 20.9 (CH_3). HRMS (ESI-TOF-MS): calcd. for $C_{31}H_{29}N_3O_5S$ [M+Na] 546.1827; found 546.1828.

N-(3-(3-Amino-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-4-(4-(tert-butyl)phenyl)thiazol-2(3H)-ylidene)adamantane-1-carboxamide (**6b**)

Orange powder. Yield, 0.44 g, 78%; m.p.: >300 °C; Rf = 0.53 (EtOAc:hexane / 1:3); IR (cm^{-1}): υ_{max} 3443, 3292, 3148, 2960, 2907, 2851, 1651, 1626. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.17 (dd, 1H, *J* = 7.6 Hz, 1.2 Hz, Ar-H), 8.00 (dd, 1H, *J* = 7.6 Hz, 1.2 Hz, Ar-H), 7.94 (s, 1H, N-H), 7.92 (td, 1H, *J* = 7.5 Hz, 1.4 Hz, Ar-H), 7.86 (td, 1H, *J* = 7.5 Hz, 1.4 Hz, Ar-H), 7.81–7.79 (m, 2H, Ar-H), 7.75 (s, 1H, N-H), 7.31–7.29 (m, 2H, Ar-H), 6.69 (s, 1H, C-H thiazole), 1.89–1.79 (m, 9H, C-H adamantane), 1.62–1.51 (m, 6H, C-H adamantane), 1.21 (s 9H, C(CH_3)$_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 181.3 (C=O), 177.1 (C=O), 172.7 (C=O), 170.7 (C=N), 154.1, 147.2 (2 x C), 135.7, 134.5, 133.0, 132.2, 130.1, 128.3 (2 x C), 126.5, 126.1, 124.8 (2 x C), 114.3, 104.2, 40.2 (3 x CH_2), 36.0 (3 x CH_2), 35.6, 34.6 (C, *C*(CH_3)$_3$), 30.9 (3 x C, C(CH_3)$_3$), 27.7 (3 x *C*H). HRMS (ESI-TOF-MS): calcd. for $C_{34}H_{35}N_3O_3S$ $[M+H]^+$ 566.2477; found 566.2468.

N-(3-(3-Amino-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-4-(4-chlorophenyl)thiazol-2(3H)-ylidene)adamantane-1-carboxamide (**6c**)

Orange powder. Yield, 0.46 g, 85%; m.p.: >300 °C; Rf = 0.45 (EtOAc:hexane / 1:3); IR (cm^{-1}): υ_{max} 3436, 3227, 3149, 2913, 2852, 1651, 1622 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.18 (dd, 1H, *J* = 7.5 Hz, 1.0 Hz, Ar-H), 8.02 (dd, 1H, *J* = 7.5 Hz, 1.0 Hz, Ar-H), 7.96 (s, 1H, N-H), 7.94 (td, 1H, *J* = 7.5 Hz, 1.4 Hz, Ar-H), 7.90–7.86 (m, 3H, Ar-H), 7.80 (s, 1H, N-H), 7.41–7.38 (m, 2H, Ar-H), 6.77 (s, 1H, C-H thiazole), 1.91–1.81 (m, 9H, C-H adamantane), 1.64–1.53 (m, 6H, C-H adamantane). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 181.2 (C=O), 177.1 (C=O), 171.7 (C=O), 171.1 (C=N), 147.7, 147.2, 136.1, 136.0, 135.7, 133.0, 132.1, 130.1 (3 x C), 128.2 (2 x C), 126.5, 126.1, 114.1, 104.7, 40.2 (3 x CH_2), 35.9 (3 x CH_2), 35.6, 27.7 (3 x *C*H). HRMS (ESI-TOF-MS): calcd. for $C_{30}H_{26}ClN_3O_3S$ [M+Na] 566.1277; found 566.1281.

N-(3-(3-Amino-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-4-(2,4-dichlorophenyl)thiazol-2(3H)-ylidene)adamantane-1-carboxamide (**6d**)

Orange powder. Yield, 0.47 g, 81%; m.p.: 296-298 °C (decomp.); Rf = 0.41 (EtOAc:hexane / 1:3); IR (cm^{-1}): υ_{max} 3369, 3180, 2903, 2846, 1650, 1610. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.10 (dd, 1H, *J* = 7.2 Hz, 0.9 Hz, Ar-H), 7.98 (dd, 1H, *J* = 7.2 Hz, 0.9 Hz, Ar-H), 7.93 (s, 1H, N-H), 7.89 (td, 1H, *J* = 7.5 Hz, 1.3 Hz, Ar-H), 7.82 (td, 1H, *J* = 7.5 Hz, 1.3 Hz, Ar-H), 7.75 (s, 1H, N-H), 7.71 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.47 (d, 1H, *J* = 2.0 Hz, Ar-H), 7.34 (dd, 1H, *J* = 8.4 Hz, 2.0 Hz, Ar-H), 6.79 (s, 1H, C-H thiazole), 1.89–1.79 (m, 9H, C-H adamantane), 1.62–1.51 (m, 6H, C-H adamantane). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 181.2 (C=O), 177.0 (C=O), 171.3 (C=O), 171.1 (C=N), 147.7, 147.3, 135.6, 135.4, 135.0, 133.0 (2 x C), 132.7, 132.2, 130.1 (2 x C), 126.9, 126.4, 126.1, 114.0, 105.0, 40.1 (3 x CH_2), 35.9 (3 x CH_2), 35.6, 27.7 (3 x *C*H). HRMS (ESI-TOF-MS): calcd. for $C_{30}H_{25}Cl_2N_3O_3S$ $[M+H]^+$ 578.1072; found 578.1064.

4.2. Determination of antimicrobial activity

Antimicrobial activities of **6a–d** were investigated by two-fold serial dilution method against sensitive organisms such as *Pseudomonas aeruginosa* (ATCC 9027), *Legionella pneumophila* subsp. *pneumophila* (ATCC 33152), and *Escherichia coli* (ATCC 10536) as Gram-negative bacteria; *Enterococcus hirae* (ATCC 10541), *Bacillus cereus*, and *Staphylococcus aureus* (ATCC 6538) as Gram-positive bacteria; and *Candida albicans* as a microfungus. Ampicillin and fluconazole were chosen as reference drugs. Model microorganisms (10^8–10^9 CFU/mL) were inoculated in medium containing the desired concentrations of **6a–d** in 96-well microplates. After incubation at 37 °C and stirring at 120 rpm for 24 hours, microplates were read at 600 nm.

4.3. Determination of DNA cleavage activity

The agarose gel electrophoresis method was utilized for the determination of DNA cleavage activity of compounds **6a–d**. Test solution (5 µL; 250 and 500 µg/mL) was added to 5 µL of pBR 322 plasmid DNA sample (0.1 mg/mL). The mixture was diluted with Tris buffer (50 mM) and incubated for 1 h at 37 °C. Samples were then loaded on agarose gel containing ethidium bromide as the dye. Constant electricity (80 V) was applied for 90 min. A UV transilluminator was used to monitor the bands.

4.4. Determination of DPPH scavenging activity

Antioxidant activity, based on the radical scavenging effect of the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical, was studied for compounds **6a–d**, ascorbic acid, and Trolox. Stock solutions were prepared by dissolving 5 mg of each compound in 5 mL of DMSO. Working solutions were prepared by dilution to desired concentrations, and then 0.5 mL of each solution of **6a–d** and 2 mL of DPPH radical solution were mixed and incubated for 30 min in the dark. The blank samples contained all reagents except the tested compounds. Absorbance values were measured at a wavelength of 517 nm. The following equation was used for the calculation of free radical scavenging ability (eq. 1):

$$\%\text{Inhibition Activity} = [(A_0 - A_1)/A_0] \times 100 \qquad \text{Eq. 1}$$

A_0: Control absorbance; A_1: the absorbance value of the solution containing compounds and DPPH after 30 min.

4.5. Determination of chelating activity

The metal chelating activities of **6a–d** and EDTA were studied on the basis of the ferrozine method [49]. One millilitre of compound solutions at the desired concentration in DMSO, 0.1 mL of $FeCl_2.H_2O$ (2 mM), and 0.2 mL of ferrozine-1,2,4-triazine (5 mM) were mixed in a test tube. The final volume was completed to 5 mL with ethanol. A blank reaction without compound was conducted under the same conditions. After incubation for 10 min at room temperature, activities were determined at 562 nm. The chelating ability of the compounds for Fe^{2+} was calculated using the following equation 2:

$$\text{Chelating Activity (\%)} = (A_{control} - A_{sample})/A_{control} \times 100 \qquad \text{Eq. 2}$$

$A_{control}$: Absorbance of the control reaction; A_{sample}: the absorbance obtained in the presence of compounds or EDTA.

4.6. Determination of acidic dissociation constants

Solutions of **6a–d** were prepared using DMSO at a concentration of 1.10^{-3} M. Stock solutions of NaOH (0.025 M), HCl (0.1 M), and NaCl (1.0 M) were prepared in deionized water. pK_a value determination was carried out based on our previously described method [22]. The Titroline 7000 automated titrator procedure [50] was used for calibrating the electrode system. A double-walled glass titration cell at 25.0 ± 0.1 °C equipped with a thermostat was used for potentiometric titrations. The titration cell was stirred at a constant rate using a magnetic stirrer during titration. pK_a value determination was performed for compounds **6a–d** in 30% (v/v) DMSO–water by addition of solutions of ligands **6a–d** (10 mL), HCl (1 mL), and NaCl (5 mL) to the titration cell. DMSO (5 mL) and deionized water were added to the titration cell to finalize the volume of the solution to 50.00 mL.

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Appendix A. Supplementary Material

Supplementary material related to this article can be accessed at https://dx.doi.org/10.29228/jrp.20 .

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