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ORIGINAL ARTICLE

Bioactive Fluorenes. Part II. Unprecedented biologically active thiazole derivatives based-2,7-dichlorofluorene as competent DHFR inhibitors: Design, synthesis, and molecular docking approaches

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Abstract In this study, a new series of (4-(2,7-dichloro-9*H*-fluoren-4-yl)thiazol-yl)acetamide derivatives was synthesized, and the new heterocycles were completely characterized, evaluated for their antimicrobial activity, and screened for cytotoxic activity against human lung carcinoma (A-549) and human breast carcinoma (MCF-7) cell lines. A molecular docking study was undertaken to identify the possible mode of action of the synthesized compounds, which suggested binding interactions with the dihydrofolate reductase (DHFR) active sites.

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DHFR inhibitors

Most of the synthesized compounds displayed meaningful activity against A-549 and MCF-7 cell lines when compared to 5-fluorouracil (5-FU), which was used as a reference drug. Furthermore, some of the prepared compounds exhibited potent antibacterial and antifungal activities. The highly pronounced biological activities of the compounds under investigation offer such species as promising future drug prospects which may find applications in the fields of biological and medicinal sciences.

1. Introduction

In the last decades, fluorene and its derivatives have experienced extensive and effective uses as precursors in a wide range of of synthetic applications (Gualtieri et al., 1985). For example, in medical applications, 2,7-dichloro-4-(chloroacetyl)fluorene is considered as a key intermediate for the synthesis of the antimalarial agent known as benflumetol (Fun et al., 2010). On the other hand, in optical applications, 2,7-dichloro fluorenyldihydroindolizines (DHIs) represent multiaddressable photochromic properties in both solution, polymer materials and in solid state thin films (Ahmed et al., 2016, 2017). Indeed, fluorene derivatives display remarkable photophysical and spectroscopic properties (El Guesmi et al., 2017b). Fluorene-based fluorophores were significantly utilized for the improvement of dyes absorption properties (El Guesmi et al., 2017a) and fluorophore sensors (El Guesmi et al., 2018).

Nitrogen-based heterocycles containing sulfur atoms are an imperative class of compounds due to their broad applications in medicinal chemistry. The thiazole ring is a core structural feature found in a variety of biologically and medicinally active molecules. Furthermore, the thiazole ring is a structural constituent of natural compounds such as thiamine (vitamin B1) and penicillin. Additionally, thiazole derivatives demonstrate an extensive spectrum of medicinal and biological activities, such as antifungal, antibacterial, (Bharti et al., 2010) antiviral (Spector et al., 1998), anti-inflammatory (Yang et al., 2010), anti-HIV (Bell et al., 1995), antimalarial (Diego et al., 2011) and anticancer (Dos Santos et al., 2016; Silva et al., 2017; de Santana et al., 2018) activities.

One particular class of thiazole derivatives, the heterocyclic 2-aminothiazoles and their derivatives, are notable as fundamental intermediates for the synthesis of numerous biologically active compounds which include sulfur drugs, fungicides, biocides and intermediates in the synthesis of antibiotics, where many 2-aminothiazoles have been substituted with dissimilar groups for pharmaceutical applications purposes (Geronikaki et al., 2009; Papadopoulou et al., 2005; Kreutzberger and Tantawy, 1981).

Moreover, compounds incorporating the acetamide linkage exhibit a wide range applications and are well-recognized as chemotherapeutic agents (McCarthy et al., 2009; Liu et al., 2012). It's known that the acetamide functional group is responsible for inhibition of platelet aggregation (Xiang et al., 2018), and strong antimicrobial (Berest et al., 2011; Shams et al., 2011), anti-inflammatory (Dogruer et al., 2004; Raghavendra et al., 2012), antioxidant (Autore et al., 2010; Ley and Bertram, 2001) and urease inhibitory activities (Gull et al., 2016).

In cellular functions, dihydrofolate reductase (DHFR) is an enzyme that catalyzes the NADPH-dependent reduction of 7,8-dihydrofolate (DHF) to 5,6,7,8-tetrahydrofolate (THF): DHF + NADPH + H^+ → THF + $NADP^+$, which is the precursor of the co-factors required for the biosynthesis of purine nucleotides, thymidine (precursor for DNA replication) and numerous amino acids (Blakley, 1984). Thus, inhibition of dihydrofolate reductase can lead to the disruption of DNA synthesis and the rapid death of proliferating cells (Blakley, 1984; Brown and Kraut, 1992). Additionally, bacteria also need DHFR to grow and multiply and therefore inhibitors with high specificity and ability to discriminate between bacterial and host DHFR have found usage as antibacterial agents (Hawser et al., 2006). These two noteworthy features render the DHFR enzyme as a key target for both antimicrobial and antitumor drug design (Hussein et al., 2019a; Hussein et al., 2019b).

Based on these previous findings, we projected that combining the 2,7-dichlorofluorene moiety with the versatile thiazole ring bearing acetamide pharmacophores into a single chemical entity could produce competent antimicrobial and anticancer hybrids with highly enhanced biological activities. Thus, in continuation of our previous studies on the synthesis, antimicrobial and anticancer properties of bioactive organic molecules (Hussein et al., 2019a, 2019b, 2015a, 2015b, 2016), herein we report the synthesis of some novel (4-(2,7-dichloro-9*H*-fluoren-4-yl)thiazol-yl)acetamide derivatives and evaluate their cytotoxic activities against human lung carcinoma (A-549) and human breast carcinoma (MCF-7) cell lines. In addition, antimicrobial evaluations and molecular docking studies for the synthesized compounds have been carried out.

2. Experimental

2.1. Chemistry

2.1.1. General methods

All Chemicals and solvents used were purchased from Sigma-Aldrich (spectroscopic grade) and were used without further purifications. Melting points were determined on a Stuart SMP3 melting point apparatus and are uncorrected. FT-IR spectra were recorded on a Shimadzu IR-3600 FT-IR spectrometer using KBr pellets. NMR spectra were acquired on a Bruker Avance 500 instrument (500 MHz for ^{1}H, 125 MHz for ^{13}C) in DMSO d_6 solutions, using residual solvent signals as internal standards. The starting materials, 2,7-dichloro-9*H*-fluorene (**2**) and 2-chloro-1-(2,7-dichloro-9*H*-fluoren-4-yl) ethanone (**3**) were prepared according to our previously reported method (Hussein et al., 2019b). 2-chloro-*N*-arylacetamides **7a-j** were prepared according to our previously reported method (Hussein et al., 2019a).

2.1.2. Synthesis of 4-(2,7-dichloro-9H-fluoren-4-yl)thiazol-2-amine (4)

A mixture of chloroacetyl derivative **3** (12.45 g, 40 mmol) and thiourea (3.80 g, 50 mmol) in ethanol (200 mL) was refluxed for 3 h. The reaction mixture was cooled and neutralized with saturated aqueous solution of sodium bicarbonate. The obtained solid product was filtered off, washed with cold water (3 × 50 mL), then with cold ethanol (3 × 10 mL), dried and recrystallized from ethanol to afford 12.90 g (97%) of pure 2-aminothiazole derivative **4** as pale yellow crystals, mp 199–200 °C. FT-IR (KBr): ν (cm^{-1}) 3282, 3106 (NH_2), 1639 (C═N); ^{1}H NMR (DMSO d_6): δ 7.66 (s, 1H, Flu-H), 7.63 (s, 1H, Flu-H), 7.55 (d, 1H, J = 5.5 Hz, Flu-H), 7.37 (s, 1H,

Flu-H), 7.31 (d, 1H, J = 5.5 Hz, Flu-H), 7.18 (s, 2H, NH_2), 6.77 (s, 1H, thiazolyl-H), 4.00 (s, 2H, CH_2); ^{13}C NMR (DMSO d_6): δ 168.8 (C═N), 148.9 (C), 146.7 (C), 146.3 (C), 139.1 (C), 136.9 (C), 133.5 (C), 131.9 (C), 131.2 (C), 128.7 (CH), 126.9 (CH), 125.3 (CH), 125.1 (CH), 124.9 (CH), 105.5 (thiazole-CH), 36.7 (CH_2).

*2.1.3. Synthesis of 2-chloro-N-(4-(2,7-dichloro-9H-fluoren-4-yl)thiazol-2-yl)acetamide (**5**)*

A mixture of 2-aminothiazole derivative **4** (10.0 g, 30 mmol) and chloroacetyl chloride (2.5 mL, 30 mmol) in DMF (10 mL) was stirred at room temperature for 3 h. The reaction mixture was poured onto ice–water. The solid obtained was filtered off, dried and recrystallized from ethanol to give 11.40 g (93%) of chloroacetyl derivative **5** as pale yellow crystals, mp 190–192 °C. FT-IR (KBr): v (cm^{-1}) 3190 (NH), 1670 (C═O), 1619 (C═N); ^{1}H NMR (DMSO d_6): δ 9.34 (s, 1H, NH), 7.96 (s, 1H, Flu-H), 7.78 (s, 1H, Flu-H), 7.52 (s, 1H, Flu-H), 7.39 (d, 1H, J = 6.5 Hz, Flu-H), 7.21 (d, 1H, J = 6.5 Hz, Flu-H), 7.05 (s, 1H, thiazolyl-H), 4.44 (s, 2H, C$\underline{H_2}$CO), 4.02 (s, 2H, CH_2); ^{13}C NMR (DMSO d_6): δ 170.3 (C═O), 162.8 (C═N), 146.7 (C), 146.3 (C), 146.2 (C), 139.1 (C), 138.0 (C), 137.5 (C), 135.1 (C), 131.9 (C), 127.8 (CH), 126.5 (CH), 125.3 (CH), 125.1 (CH), 113.3 (CH), 107.2 (thiazole-CH), 43.5 (CH_2), 36.9 (CH_2).

*2.1.4. Synthesis of N-(4-(2,7-dichloro-9H-fluoren-4-yl)thiazol-2-yl)-2-(arylamino)acetamides **6a-j***

A mixture of chloroacetyl derivative **5** (0.41 g, 1 mmol) and different aryl amines (1 mmol) in absolute ethanol (15 mL) was refluxed for 5–6 h. The reaction mixture was concentrated under reduced pressure, the solid obtained was filtered off, washed with n-hexane (3 × 10 mL), dried and recrystallized from ethanol to give the title products **6a-j**.

*2.1.4.1. N-(4-(2,7-dichloro-9H-fluoren-4-yl)thiazol-2-yl)-2-(phenylamino)acetamide (**6a**).* Pale yellow crystals, yield 93%, mp 117–119 °C; FT-IR (KBr): ν (cm^{-1}) 3349 (NH), 3197 (NH), 3054 (CH arom.), 2923 (CH aliph.), 1685 (C═O), 1602 (C═N); ^{1}H NMR (DMSO d_6): δ 7.70 (s, 1H, Flu-H), 7.65 (s, 1H, Flu-H), 7.59 (s, 1H, NH), 7.43 (s, 1H, Flu-H), 7.40 (d, J = 5.5 Hz, 1H, Flu-H), 7.25 (d, J = 5.5 Hz, 1H, Flu-H), 7.13–7.11 (m, 2H, Ph-H), 7.00 (s, 1H,NH), 6.63–6.61 (m, 3H, Ph-H), 6.06 (s, 1H, thiazolyl-H), 4.07 (s, 2H, CH_2), 4.01 (s, 2H, CH_2); ^{13}C NMR (DMSO d_6): δ 170.5 (C═O), 158.2 (C═N), 148.5 (C), 147.5 (C), 146.7 (C), 146.2 (C), 138.8 (C), 137.0 (C), 132.7 (C), 132.2 (C), 131.6 (C), 129.4 (CH), 129.2 (CH), 127.0 (CH), 125.5 (CH), 124.6 (CH), 119.5 (CH), 116.9 (CH), 112.7 (CH), 46.5 (CH_2), 36.9 (CH_2).

*2.1.5. Synthesis of 2-(4-(2,7-dichloro-9H-fluoren-4-yl)thiazol-2-ylamino)-N-arylacetamides **8a-j***

A mixture of aminothiazole 4 (0.33 g, 1 mmol) and 2-chloro-N-arylacetamides **7a-j** (1 mmol) and triethylamine (0.1 mL) in absolute ethanol (15 mL) was refluxed for 6–9 h. The reaction mixture was cooled to room temperature, the solid obtained was filtered off, washed with cold ethanol, dried and recrystallized from ethanol to give the title products **8a-j**.

*2.1.5.1. 2-(4-(2,7-Dichloro-9H-fluoren-4-yl)thiazol-2-ylamino)-N-phenylacetamide (**8a**).* Pale pink crystals, yield 81%, mp 160–162 °C; FT-IR (KBr): ν (cm^{-1}) 3267 (NH), 3199 (NH), 3084 (CH arom.), 2930 (CH aliph.), 1671 (C═O), 1636 (C═N); ^{1}H NMR (DMSO d_6): δ 10.2 (s, 1H, NH), 7.64–7.62 (m, 3H, Flu-H), 7.51 (d, J = 10.0 Hz, 1H, Flu-H), 7.40–7.35 (m, 3H, Ph-H), 7.31 (d, J = 10.0 Hz, 1H, Flu-H), 7.19–7.17 (m, 2H, Ph-H), 6.77 (s, 1H, thiazolyl-H), 4.28 (s, 2H, CH_2), 3.99 (s, 2H, CH_2), 3.51 (s, 1H, NH); ^{13}C NMR (DMSO d_6): δ 168.9 (C═O), 165.2 (C═N), 148.6 (C), 146.8 (C), 146.1 (C), 139.1 (C), 137.9 (C), 136.9 (C), 133.5 (C), 131.6 (C), 131.4 (C), 129.2 (CH), 128.5 (CH), 127.3 (CH), 126.9 (CH), 125.8 (CH), 125.0 (CH), 122.8 (CH), 121.3 (CH), 105.6 (CH), 43.9 (CH_2), 36.8 (CH_2).

2.2. Antimicrobial screening

The antimicrobial activity was evaluated by the disc-agar diffusion method (Hay et al., 2000; Wilson, 2000; Saintigny et al., 2002), at 50-μg/disk concentration. Samples under testing were dissolved in dimethyl sulfoxide (DMSO) to 10 mg/mL. The disc (6 mm in diameter) containing the sample at certain concentration, are placed on the agar medium, which was seeded previously with 0.2 mL of broth culture of each organism for 18 h. Discs were incubated at 37 °C for 24 h for bacteria (and at 22 °C for 48 h; for fungi) and the zones of inhibition (ZI) determined in mm.

Efficacy of the novel compounds was evaluated against different strains of bacteria either gram-positive such as *St. aureus (RCMB010010), and B. subtilis RCMB 015 (1) NRRL B-543 or* gram-negative such as *E. coli (RCMB 010052) ATCC 25955, and P. vulgaris RCMB 004 (1) ATCC 13315* (the reference drug is *Gentamycin*) as well as fungal strains include *Aspergillus fumigatus (RCMB 002008), and Candida albicans RCMB 005003 (1) ATCC 10231* (the reference drug is Ketoconazol).

2.3. In vitro anticancer screening

The newly synthesized compounds were screened against human lung carcinoma (A-549) and breast carcinoma (MCF-7) by the Regional Center for Mycology and Biotechnology, Al-Azhar University as reported previously (Skehan et al., 1990). Cells were seeded for 24 h in ninety-six well plate (1×10^4 cells/well) in growth medium with 100-μL concentration. Then, fresh medium, which involve test sample with various concentrations, was added. Moreover, serial two-fold dilutions of the tested compound were added, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette that incubated for 48 h at 37 °C and in presence of 5% carbon dioxide. Test samples were not added to the control cells. After incubation, different concentrations of sample (500, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 2 & 1 μg/L) were added, and continued the incubation for 48 h and viable cells were determined by a colorimetric method in which crystal violet solution (1%) was added to every well for 30 min. The excess stain was removed using tap water. After that, addition of well-mixed 30% glacial acetic acid, followed by gentle shaking on Microplate reader (TECAN, Inc.) and by using test

wavelength of 490 nm, the absorbance of the plates was measured. Experiments were carried out in triplicates. The concentration of the compound that inhibits the tumor cell growth by 50% was determined (IC_{50} in μM/L) and the results summarized in Table 3. The reference control was 5-Fluorouracil (5-FU).

2.4. Docking assay

The study was performed in the Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy (Girls), Al-Azhar University, Egypt, with computational software using Protein Data Bank, 5-Fluorouracil (PDB ID: 1BID) while dihydrofolate reductase (DHFR) (PDB ID: 4DFR) to predict the anticancer activity of the newly tested compounds.

3. Results and discussion

3.1. Chemistry

The current study deals with the synthesis of some acetamide derivatives incorporating different aryl substituents (tails) conjugated with biologically active 2,7-dichlorofluorene and thiazole moieties in order to assess their combined influence on the antimicrobial, anticancer activities, and study their structure–activity relationship (SAR).

As the DHFR inhibition is considered one of the most prominent mechanism responsible for antimicrobial and anticancer activities (Bush et al., 1982; Rao and Venkatachalam, 1999; Patel et al., 2017), the synthesized compounds were designed in order to fulfill the following criteria: (i) inclusion of pharmacophores that may act as DHFR inhibitors; (ii) bearing of hydrophilic and hydrophobic moieties that can interact with the hydrophilic and hydrophobic regions of the DHFR active site, respectively, as presented in Fig. 1.

The main strategy for the synthesis of the target compounds **6a-j** and **8a-j** is outlined in Scheme 1. The synthetic approach begins with a simple and convenient approach to prepare 2,7-dichlorofluorene (**2**) which involves direct chlorination of fluorene (**1**) with NCS in a mixture of acetic acid AcOH and hydrochloric acid (HCl). Chloroacetylation of **2** is then accomplished in moderate to excellent yields by adding a solution of **2** in dichloromethane (DCM) at 0–5 °C to a suspension of chloroacetyl chloride and aluminum chloride in dichloromethane according to our previously reported method (Hussein et al., 2019b) after minor modification. As for the preparation of 2-aminothiazoles, and on account of their importance, numerous synthetic strategies have been formerly described. Hantzsch reaction of α-halocarbonyl compounds with thiourea affords an advantageous method for the synthesis of thiazoles (Hantzsch and Weber, 1887). Thus, 4-(2,7-Dichloro-9*H*-fluoren-4-yl)thiazol-2-amine (**4**) has been assembled through Hantzsch reaction of 2-chloro-1-(2,7-dichloro-9H-fluoren-4-yl)ethanone (**3**) with thiourea in refluxing ethanol. The aminothiazole derivatives **4** were converted to the corresponding chloroacetamide derivatives (**5**) in excellent yield by reaction with chloroacetyl chloride in dimethylformamide (DMF) at room temperature. The target compounds *N*-(4-(2,7-dichloro-9*H*-fluoren-4-yl)thiazol-2-yl)-2-(arylamino) acetamides **6a-j** formed in good to excellent yields (67–93%) by refluxing chloroacetamide derivative **5** with arylamines (namely; aniline, 4-methylaniline, 4-chloroaniline, 4-bromoaniline, 4-methoxyaniline, 4-nitroaniline, 4-aminobenzoic acid, ethyl 4-aminobenzoate, 1-naphthyl amine and 2-naphthyl amine) in absolute ethanol for 4–7 h.

On the other hand, 2-chloro-*N*-arylacetamides **7a-j** were promptly prepared by the reaction of arylamines (namely; aniline, 4-methylaniline, 4-chloroaniline, 4-bromoaniline, 4-methoxyaniline, 4-nitroaniline, 4-aminobenzoic acid, ethyl 4-aminobenzoate, 1-naphthyl amine and 2-naphthyl amine) with

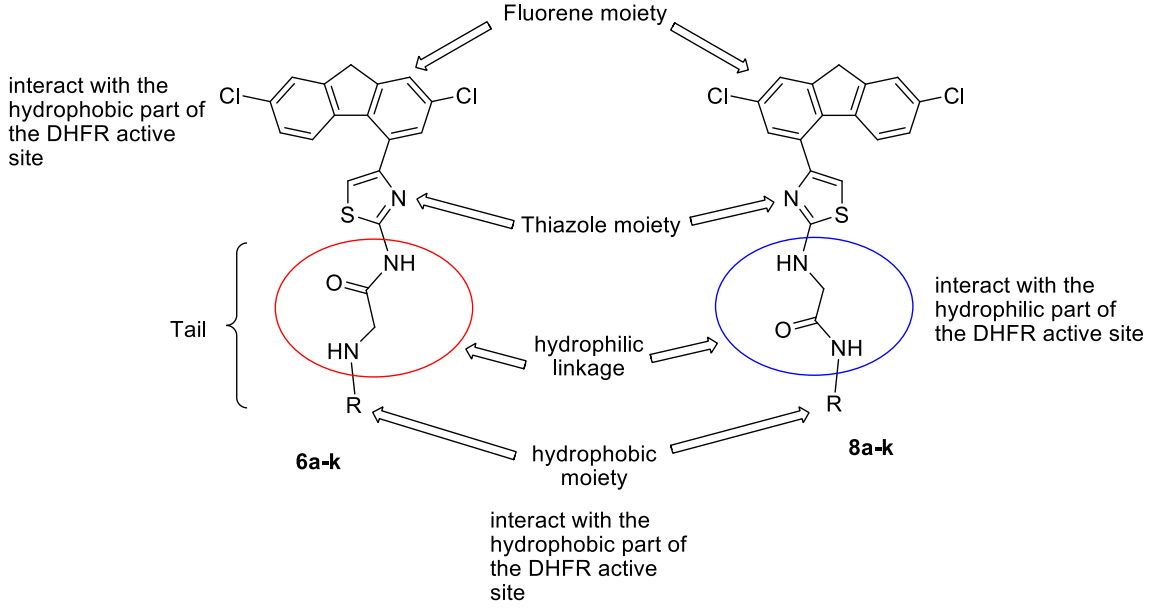
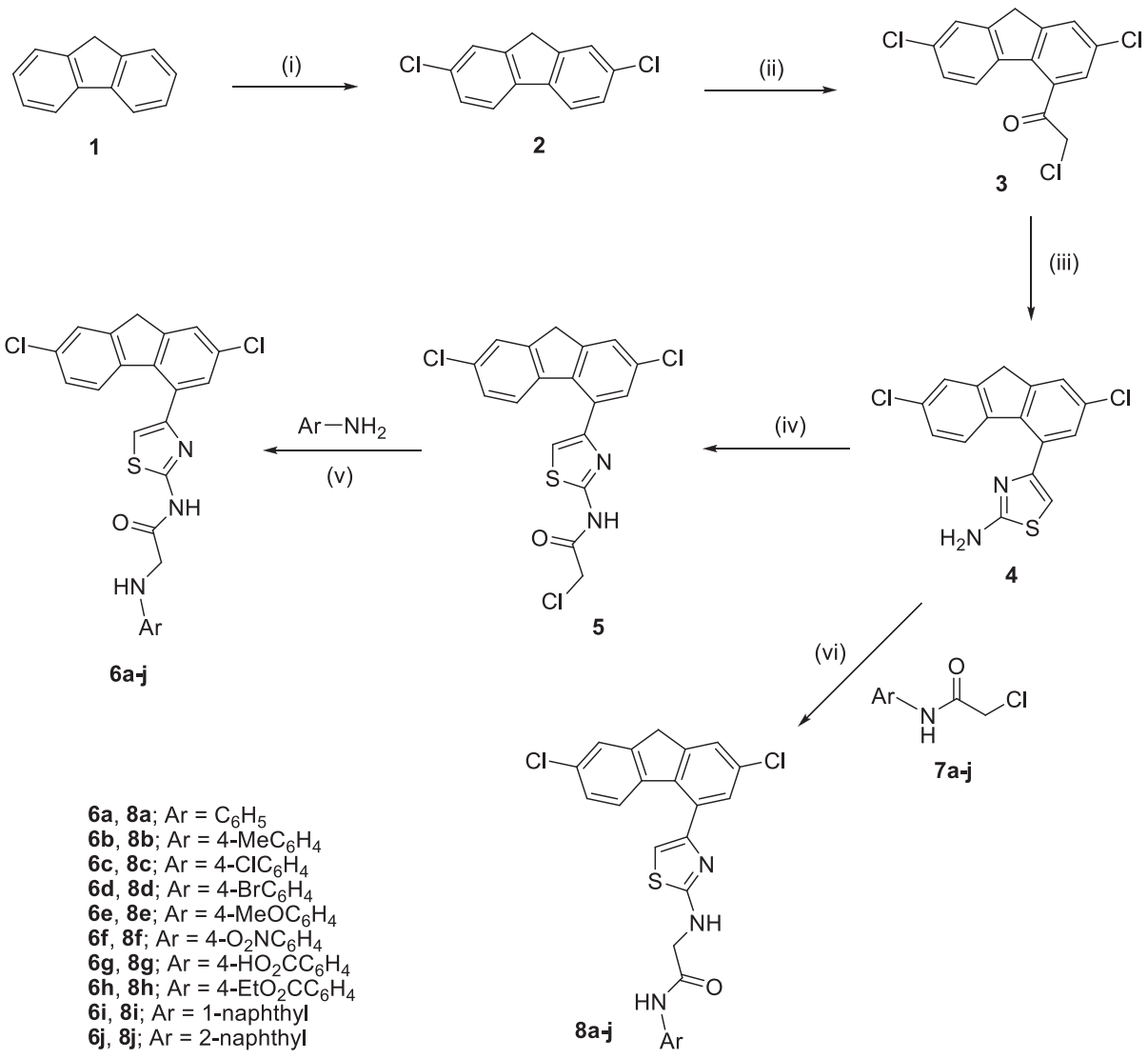


Fig. 1 Structural elements of DHFR inhibitors in the DHFR enzymatic active site.



Scheme 1 Synthetic routes to the target compounds **6a-j** and **8a-j**. Reagents and conditions: (i) NCS, AcOH/ HCl, rt; (ii) $ClCH_2COCl$, $AlCl_3$, DCM, 0–5 °C; (iii) thiourea, EtOH, reflux, then aq. $NaHCO_3$; (iv) $ClCH_2COCl$, DMF, rt; (v) EtOH, reflux; (vi) EtOH/ Et_3N, reflux.

chloroacetyl chloride in dimethylformamide at room temperature.

Reaction of 2-chloro-*N*-arylacetamides **7a-j** with 4-(2,7-dichloro-9*H*-fluoren-4-yl)thiazol-2-amine (**4**) in ethanol under refluxing conditions yielded the target compounds 2-(4-(2,7-dichloro-9H-fluoren-4-yl)thiazol-2-ylamino)-*N*-arylacetamides **8a-j** in excellent yields (72–92%). The reactions were activated by addition of catalytic amount of triethylamine (TEA) as a basic catalyst with a reaction period of 5–7 h. The chemical structures of all synthesized compounds **4**, **5** and **6a-j** and **8a-j** were well-established on the basis of spectroscopic data such as FT-IR, ^{1}H NMR, ^{13}C NMR, and DEPT-135 data (*c.f. experimental section and supporting information*).

The FT-IR spectra of compounds **6a-j** showed the characteristic absorption bands at 3376–3343 and 3203–3191 cm^{-1} for the two NH groups, 1689–1672 cm^{-1} for the C═O groups, and 1632–1600 cm^{-1} for the C═N groups. Compounds **6g** and **6h** showed additional absorption bands at 1690 and 1744 cm^{-1} corresponding to the carbonyl of the carboxylic and the ester groups, respectively. Furthermore, to fully elucidate the chemical structures of all products, intensive 1D (^{1}H, ^{13}C, and DEPT-135) NMR studies were conducted in DMSO d_6. As a representative example, the ^{13}C and ^{13}C-DEPT-135 NMR spectra of **6a** showed 22 signals (10 aromatic quaternary carbons, 9 aromatic CH's, two methylene carbons, and one carbonyl carbon). Its ^{1}H NMR spectrum showed three singlets at δ 7.70, 7.65, and 7.43 ppm, two doublets at 7.40 and 7.25 ppm (J = 5.5 Hz) for five protons of the fluorene moiety. Two multiplets at δ 7.13–7.11 and 6.63–6.61 ppm are corresponding to five protons of the phenyl ring. The thiazole ring proton appeared as a singlet at δ 6.06 ppm. In addition, two singlets appeared at 7.59 and 7.00 ppm for the two NH protons, and two other singlets corresponding to the two methylene protons resonated at δ 4.07 and 4.01 ppm.

Furthermore, the FT-IR spectra of compounds **8a-j** displayed characteristic absorption bands at 3317–3257 and

3204–3119 cm^{-1} for two NH groups, 1694–1665 cm^{-1} for (C═O) groups, and 1638–1624 cm^{-1} for C═N groups. As a typical example, NMR analysis of the ^{13}C and ^{13}C-DEPT-135 NMR spectra of **8h** indicated the presence of 25 signals (11 aromatic quaternary carbons, 8 aromatic CH's, 3 methylene carbons, 2 carbonyl carbons, and one methyl carbon). Its ^{1}H NMR spectrum showed two singlets at δ 4.07 and 4.01 ppm (NCH_2 protons) and δ 8.18, 7.28 ppm for two fluorene protons, two multiplets at δ 7.61–7.59 and δ 7.34–7.32 ppm for three fluorene and four phenyl protons. A singlet appearing at δ 6.75 ppm corresponds to a proton of the thiazole ring. In addition, two NH protons appeared as two singlets at δ 6.12 and 4.31 ppm. Additionally, two singlet at δ 3.98 and 3.84 ppm correspond to two methylene protons. The ester group protons appeared as a quartet and a triplet signals at δ 4.70 and 2.82 ppm (J = 7.5 Hz), respectively.

3.2. Biological activity

3.2.1. Antimicrobial activity

The target compounds presented in this investigation were evaluated for antimicrobial activity against different strains of bacteria, either Gram-positive (St. aureus *(RCMB010010)*, and B. subtilis *RCMB 015 (1) NRRL B-543*) or Gram-negative (E. coli *(RCMB 010052) ATCC 25955, and* P. vulgaris *RCMB 004 (1) ATCC 13315* (the reference drug is *Gentamycin*)), as well as fungal strains which include *Aspergillus fumigatus (RCMB 002008)*, and *Candida albicans RCMB 005003 (1) ATCC 10231* (the reference drug is Ketoconazol).

The results of the antimicrobial assay of the synthesized compounds is given in Table 1 and Fig. 2. It is noted that some compounds have proven highly effective inhibitors of different types of bacteria and fungi. Acetamide derivatives **8c** and **8i** exhibited promising activity against *P. vulgaris* with zone of inhibition (ZI) value of 18 mm, while, compound **6i** bearing naphthyl-1-amino acetamide moiety is approximately equipotent to the reference drug against *C. Albicans*. However, compounds **6i**, **6j**, **8c**, **8a**, **8h**, **8e**, **8i** and **8b** showed excellent activity against *A. fumigatus*. From the previous results it is noteworthy that both compounds **8c** and **8i** are active as antimicrobial agents (have higher antibacterial and antifungal activities), while compounds **6i**, **6j**, **8a**, **8b**, **8e** and **8h** have only antifungal activity. This underscores the selectivity of the synthesized compounds towards bacteria and fungi which bestows significant importance to these compounds for medicinal applications.

3.2.2. In vitro anticancer activity

According to the tabulated results listed in Table 2, the high efficiency of the synthesized target compounds displayed against cancer cell lines such as human lung (A-549 and MCF-7) it's a noteworthy observation. For example, compound **6j** having naphthalen-2-amino acetamide moiety exhibited IC_{50} 7.67 μM and 9.53 μM, respectively, and compound **8a** bearing phenylacetamido group produced IC_{50} values of 11.43 μM and 13.34 μM, respectively. While, compound **6h** incorporating 4-(ethoxycarbonyl)phenylamino acetamide moiety displayed IC_{50} values of 13.00 μM and 27.49 μM, respectively. Furthermore, compound **8b** having *p*-tolylacetamide moiety showed IC_{50} values of 13.30 μM and 15.02 μM, respectively. On the other hand, compound **6f** having 4-nitrophenyl amino acetamide moiety showed IC_{50} values of 19.75 μM and 39.31 μM, respectively. Compound **8c** having 4-chlorophenyl acetamide moiety showed IC_{50} values of 23.47 μM and

Table 1 Antimicrobial zone of inhibition of newly synthesized compounds **6a-j** and **8a-j** (tested at 10 μg/ mL).

Comp. No.	Gram (+ve) Bacteria		Gram (-ve) Bacteria		Fungi	
	S. aureus	*B. subtilis*	*E. coli*	*P. vulgaris*	*A. fumigatus*	*C. albicans*
6a	—	—	—	—	—	—
6b	—	—	—	—	—	—
6c	—	—	—	—	12	—
6d	—	—	—	—	13	—
6e	—	—	—	—	11	—
6f	—	—	—	—	15	—
6g	—	—	—	—	—	—
6h	—	—	—	—	—	—
6i	14	15	12	17	30	17
6j	—	8	—	12	27	—
8a	—	15	14	17	22	10
8b	—	16	13	16	18	12
8c	12	20	16	18	25	11
8d	—	12	—	—	—	—
8e	—	16	13	14	20	12
8f	—	14	—	—	—	—
8g	—	—	—	—	—	—
8h	—	19	12	16	22	10
8i	10	17	11	18	20	12
8j	15	17	12	—	—	—
St[a]	24	26	30	25	17	20

[a] Standard control for the microorganisms are "*Gentamycin*" (10 μg/ mL) for the Gram-positive and Gram-negative bacteria, and "*Ketoconazol*" (10 μg/ mL) for the Fungi.

— No activity.

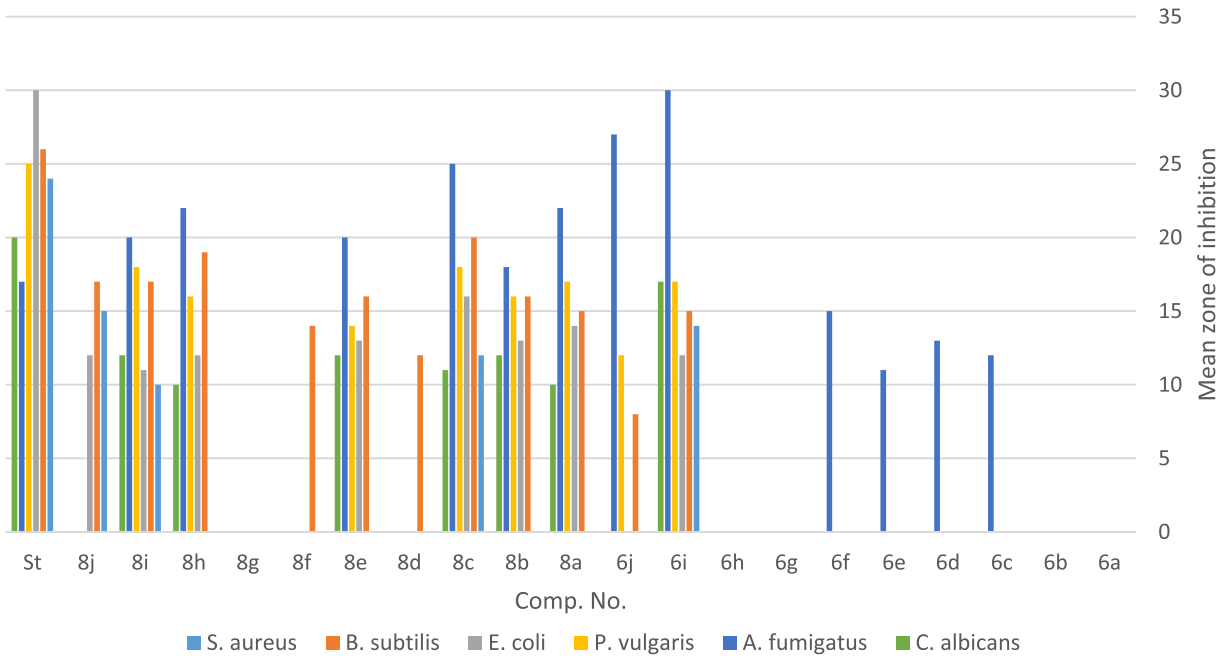


Fig. 2 Comparison of the antimicrobial activity of the newly synthesized compounds **6a-j** and **8a-j**.

Table 2 IC_{50} of the synthesized compounds **6a-j** and **8a-j** against human lung carcinoma (A-549) and human breast carcinoma (MCF-7).

Comp.	A-549		MCF-7	
	IC_{50}[a] (μg/mL)	IC_{50}[a] (μM)	IC_{50}[a] (μM)	IC_{50}[a] (μM)
6a	247	529.61	229	491.02
6b	234	487.08	228	474.59
6c	114	227.63	188	375.38
6d	62	113.70	107	196.23
6e	23.5	47.34	33.6	67.69
6f	10.1	19.75	20.1	39.31
6g	99.6	195.14	82.4	161.45
6h	7	13.00	14.8	27.49
6i	24	46.47	29.4	56.93
6j	3.96	7.67	4.92	9.53
8a	5.49	11.43	6.41	13.34
8b	6.66	13.30	7.52	15.02
8c	12.8	23.47	10.2	18.71
8d	90.3	181.91	61.1	123.08
8e	18.3	35.79	27.1	52.99
8f	> 500	1036.53	> 500	1036.53
8g	202	375.16	234	434.59
8h	14.9	28.85	25.2	48.80
8i	14.1	27.30	29.8	57.70
8j	371	772.26	> 500	1040.78
5FU	43.9	337.48	27.8	213.71

IC_{50} value: concentration causing 50% inhibition of cell viability.
[a] Mean of three results obtained from three experiments.

18.71 μM, respectively. Furthermore, compound **8i** having 1-naphthyl acetamide moiety revealed IC_{50} values of 27.30 μM and 57.70 μM, respectively. While, compound **8h** having 4-(ethoxycarbonyl)phenyl acetamide moiety showed IC_{50} values of 28.85 μM and 48.80 μM, respectively.

However, compound **8e** bearing 4-methoxyphenyl acetamide moiety showed relatively higher IC_{50} values of 35.79 μM and 52.99 μM, respectively. Compound **6i** having naphthalen-1-amino acetamide moiety showed IC_{50} values of 46.47 μM and 56.93 μM, respectively. Compound **6e** having

4-methoxyphenyl amino acetamide moiety showed IC_{50} values of 47.34 μM and 67.69 μM, respectively. Moreover, compound **6d** having 4-bromophenyl amino acetamide moiety showed IC_{50} values of 113.70 μM and 196.23 μM, respectively. Compound **8d** having 4-bromophenyl acetamide moiety showed IC_{50} values of 181.91 μM and 123.08 μM, respectively. Finally, compound **6g** having 4-carboxyphenyl amino acetamide moiety showed IC_{50} values of 195.14 μM and 161.45 μM, respectively.

Based on the tabulated results shown in Table 2, it is noted that, the cytotoxic activity of the investigated compounds against human lung carcinoma (A-549) follow the order: **6j** > **8a** > **6h** > **8b** > **6f** > **8c** > **8i** > **8h** > **8e** > **6i** > **6e** > **6d** > **8d** > **6g** > **6c** > **5-FU** > **8g** > **6b** > **6a** > **8j** > **8f** (Fig. 3). However, the cytotoxic activity of the tested compounds against Human breast carcinoma cell line (MCF-7) followed the order: **6j** > **8a** > **8b** > **8c** > **6h** > **6f** > **8h** > **8e** > **6i** > **8i** > **6e** > **8d** > **6g** > **6d** > **5-FU** > **6c** > **8g** > **6b** > **6a** > **8f** > **8j** (Fig. 3). The preceding data concerning the biological screening for the synthesized compounds helped identify the relevant structural motifs as necessary features required in the investigated compounds to produce optimum enhancement in anticancer properties and render such species effective anticancer agents. Further, it is worth highlighting the biological screening results obtained with compounds **8c** and **8i** which clearly exhibit dual biological activities as they are active as antimicrobial and anticancer agents.

3.2.3. Docking and molecular modeling study

The most prominent enzymes used for the development of anticancer and antimicrobial agents are thymidylate synthase and dihydrofolate reductase (DHFR) (Rao and Venkatachalam, 1999; Du et al., 2013). Molecular modeling is considered as an outstanding way to study molecular interactions and binding site. In the present work, Molecular Operating Environment (MOE) module was adapted to verify the cytotoxic potency of the tested compounds (Vilar et al., 2008). Moreover, study of molecular docking aids in explaning how compounds behave through their reaction with the enzyme's active sites. Twenty active anticancer compounds **6a-j** and **8a-j** were subjected to docking using Molecular Operating Environment (MOE) program on the 3D structure of dihydrofolate reductase (DHFR). Furthermore, it was used to demonstrate the enzyme interactions with its substrate doxorubicin (DOX). Binding free energy data obtained after the docking procedure revealed that the above compounds exhibit favorable docked complexes with the target. Docking was performed for the synthesized compounds **6a-j** and **8a-j** on DHFR to predict their activity as anticancer agents (*c.f. SI file*). The tested compounds garnered interactions with the DHFR active sites to different extents. Compounds **8h**, **6g**, **8g**, **8b**, **6h** and **6j** produced cytotoxic activity by inhibiting the active sites of DHFR (Table 3). Docking score energy of the tested compounds found to follow this order: **8h** > **6g** > **8g** > **8b** > **6h** > **6j** > **6c** > **6d** > **6e** > **8f** > **6f** > **8j** > **8e** > **8d** > **6b** > **8i** > **6i** > **8c** > **8a** > **6a**, as shown in Fig. 4.

3.2.3.1. Docking study of 6j and 8a in the DHFR active sites. The active site docking studies of **6j** having naphthalen-2-amino acetamido group showed that (N) atom acted as a H-bond donor with Ser 49 (3.26 Å) with energy −1.7 kcal/mol. However, there are several hydrophobic interactions with: Phe 31, Leu 28, Leu54, Lys 32, Ile 50, Ala 19, Met 16, His 45, Glu 17, Thr 46, Asn 18, Gly 15, Gly 96, Ala 6, Ile 14, tyr 100, Ala 7, Leu 8, Met 20, Arg 52.

However, the active site docking studies of **8a** bearing phenylacetamido group showed that (O) atom of (C═O) group acted as a H-bond acceptor with Arg 52 (3.14 Å) having energy −1.1 kcal/mol. Moreover, it showed an arene-H interaction between the phenyl ring and Thr 46 (3.88 Å) having energy −0.6 kcal/mol. Additionally, there are various

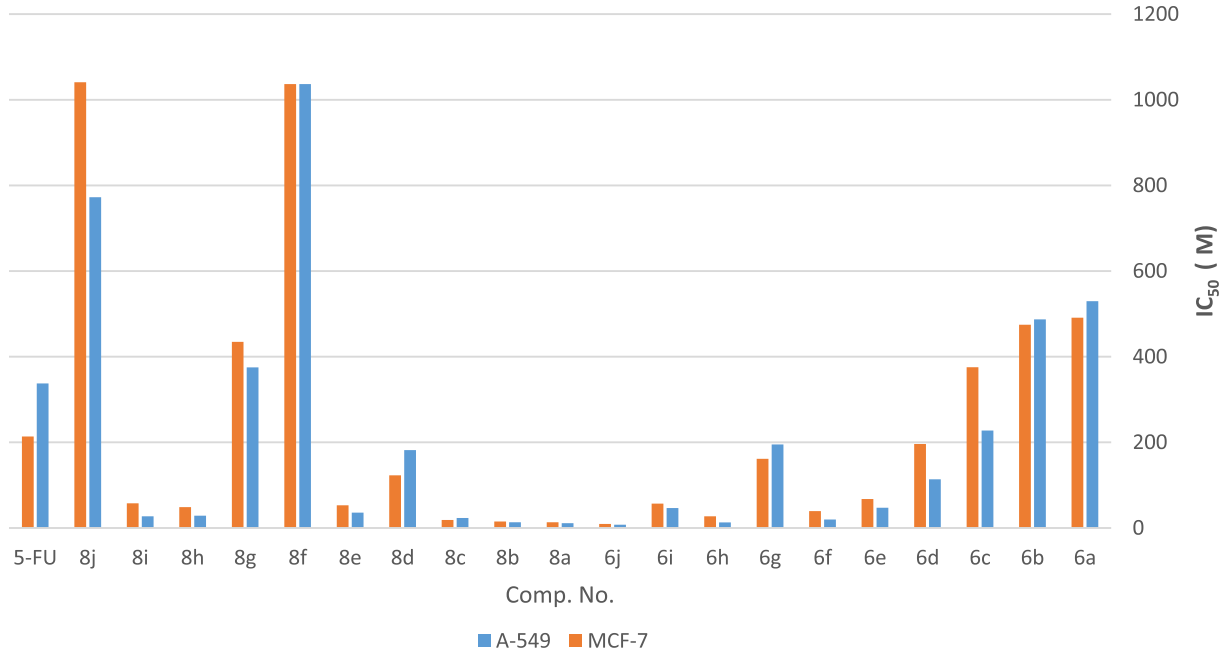


Fig. 3 Comparison of the cytotoxic activity of the newly synthesized compounds **6a-j** and **8a-j** against human lung carcinoma (A-549) and human breast carcinoma (MCF-7).

Table 3 Docking score energy of the synthesized compounds **6a-j** and **8a-j**.

Comp. No.	Score	E_conf	E_place	E_score1	E_score2	E_refine
6a	−7.2787	11.8659	−86.1684	−9.8410	−7.2787	−44.9553
6b	−7.5857	19.1284	−54.1995	−10.3997	−7.5857	−34.3523
6c	−7.7689	17.0702	−56.2519	−10.7120	−7.7689	−38.0747
6d	−7.7625	24.7109	−94.0857	−9.4051	−7.7625	−48.2892
6e	−7.7217	17.8309	−76.4011	−10.4083	−7.7217	−47.0829
6f	−7.6817	56.2074	−76.6499	−10.8973	−7.6817	−39.0149
6g	−8.2413	−51.8593	−84.6688	−12.7100	−8.2413	−56.2588
6h	−7.8777	25.0914	−38.1129	−10.3773	−7.8777	−36.7423
6i	−7.5599	38.3409	−70.3623	−11.1607	−7.5599	−45.3069
6j	−7.8543	20.4799	−47.9571	−10.9165	−7.8543	−40.1227
8a	−7.3022	−50.3011	−74.7956	−10.3966	−7.3022	−38.7188
8b	−7.9480	−56.3332	−40.1580	−10.7623	−7.9480	−39.5560
8c	−7.4879	−58.3923	−63.1983	−9.7065	−7.4879	−46.3605
8d	−7.5962	−46.4119	−66.0624	−10.5870	−7.5962	−37.5610
8e	−7.6346	−58.2125	−42.5277	−10.8762	−7.6346	−45.6290
8f	−7.7121	−17.3436	−51.2697	−11.6343	−7.7121	−36.2258
8g	−8.2263	−114.3670	−52.3371	−13.8340	−8.2263	−46.2320
8h	−8.2943	−36.7831	−44.7879	−10.8018	−8.2943	−42.2573
8i	−7.5817	−42.5465	−66.4027	−10.4089	−7.5817	−46.4740
8j	−7.6730	−56.4827	−60.1864	−10.8810	−7.6730	−45.5533

Score: lower scores are more favorable. The unit for scoring functions is kcal/mol. **E-conf:** energy of the conformer. **E-place:** Score from the placement stage. **E-score 1:** Score from the first rescoring stage. **E-score 2:** Score from the second rescoring stage. **E-refine:** Score from the refinement stage.

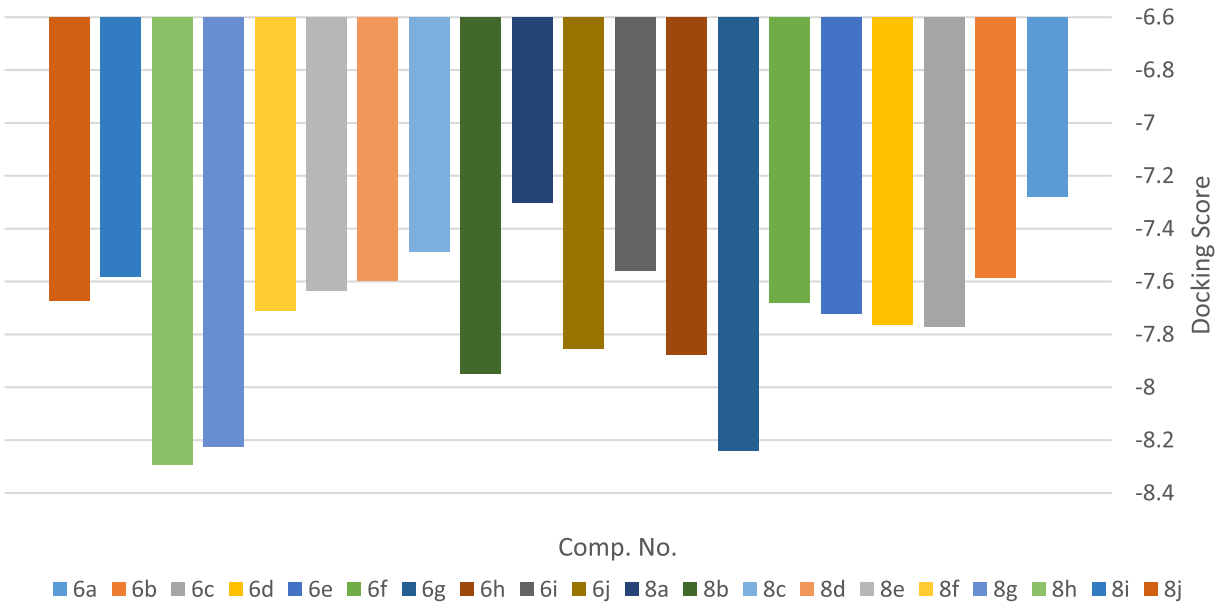


Fig. 4 Docking Score energy of the tested compounds **6a-j** and **8a-j**.

hydrophobic interactions with; Pro 55, Lys 32, Ile 5, Phe 31, Leu 28, Ala 6, Ala 7, Tyr 100, Ile 94, Gly 15, Ser 49, Met 16, Ile 50, His 45, Asp 27, Leu 54 (Fig. 5).

4. Conclusion

In this report, the synthesis and characterization of a novel series of (4-(2,7-dichloro-9*H*-fluoren-4-yl)thiazol-yl)acetamide derivatives have been achieved. Most of the newly synthesized compounds exhibited potent anticancer activity against human lung carcinoma (A-549) and human breast carcinoma (MCF-7) cell lines compared with 5-Fluorouracil which used as a reference drug in this study. Furthermore, the synthesized compounds were evaluated as antimicrobial agents where several showed remarkable activity as antibacterial and antifungal agents. A dual biological activity of some investigated compounds against microbial strains and cancer cell lines has been observed. The results indicated that 2-(4-(2,7-dichloro-9*H*-fluoren-4-yl)thiazol-2-ylamino)-*N*-arylacetamides such as 2-(4-(2,7-Dichloro-9*H*-fluoren-4-yl)thiazol-2-ylamino)-*N*-

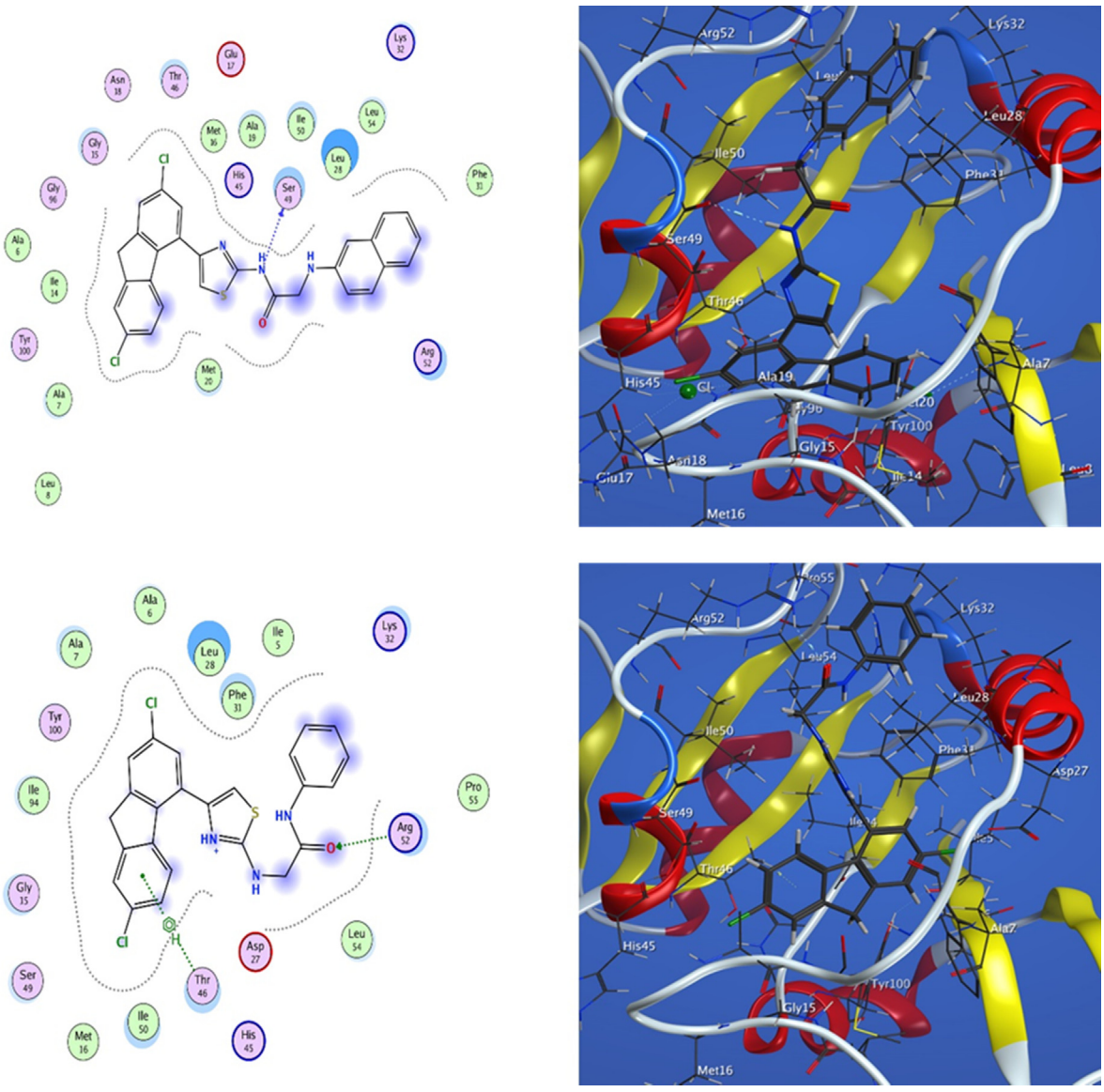


Fig. 5 Docking of compounds **6j** and **8a** into DHFR active sites.

phenylacetamide (**8a**), 2-(4-(2,7-Dichloro-9*H*-fluoren-4-yl) thiazol-2-ylamino)-*N*-*p*-tolylacetamide (**8b**), and *N*-(4-chlorophenyl)-2-(4-(2,7-dichloro-9*H*-fluoren-4-yl)thiazol-2-ylamino) acetamide (**8c**) are more efficacious antimicrobial and anticancer agents compared to *N*-(4-(2,7-dichloro-9*H*-fluoren-4-yl)thiazol-2-yl)-2-(arylamino)acetamide derivatives. The findings described in this work will open a new alternative area in the field of medicinal chemistry of fluorene derivatives that can be considered as multi-addressable pharmacophores.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2020.03.024.

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