Heliyon 5 (2019) e01982

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.heliyon.com

Bioactive fluorenes. part I. Synthesis, pharmacological study and molecular docking of novel dihydrofolate reductase inhibitors based-2,7-dichlorofluorene

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ARTICLE INFO

Keywords:
Organic chemistry
Fluorene
Anti-cancer
Antimicrobial
DHFR inhibitors
Molecular docking

ABSTRACT

In this study, a new series of 2,7-dichloro-4-(2-substituted-amino acetyl)fluorene derivatives were synthesized, characterized and evaluated for their antimicrobial activity and screened for cytotoxic activity against human lung carcinoma (A-549) and human breast carcinoma (MCF-7) cell lines. Most of the synthesized compounds displayed significant activity against A-549 and MCF-7 cell lines when compared to 5-fluorouracil (5-FU), which was used as a reference drug. In addition, some of these reported novel compounds exhibited promising antibacterial and antifungal properties. A molecular docking study was performed to identify the mechanism of action of the synthesized compounds, which suggested binding interactions with the active sites of dihydrofolate reductase (DHFR).

1. Introduction

Fluorene and its derivatives are versatile reagents which are used in wide range of synthetic applications [1]. Fluorene-based aromatic ketones are of profound interest as building blocks for the production of drugs, pharmaceuticals and industrial fine chemicals [2, 3] particularly in the production of lubricating and thermosetting plastic materials. Furthermore, fluorene-based polymers and copolymers are of interest owing to their unusual optical and electrical properties and therefore are usually used in organic light-emitting diodes, flat panel displays and in solar cells [4, 5, 6].

Benflumetol is a racemic fluorene derivative, which mimics the structure and reactivity of the arylamino alcohol group of antimalarial drugs such as quinine, mefloquine, and halofantrine (Fig. 1) and is used as antimalarial agent in combination treatment with artemether [7]. In the synthesis of benflumetol, 2,7-dichloro-4-(chloroacetyl)fluorene is an important intermediate [8]. Interestingly, *N*-arylaminoacetyl, *N*-alkylaminoacetyl, and *N*-dialkylaminoacetyl derivatives are reported to exhibit a wide spectrum of pharmacological activities such as antitumor, anti-inflammatory, antibacterial, antiviral, antitubercular and anti-arrhythmic activity [9, 10, 11].

In cellular functions, dihydrofolate reductase (DHFR) is an enzyme that reduces 7,8-dihydrofolate (DHF) to 5,6,7,8-tetrahydrofolate (THF) using NADPH as electron donor: $DHF + NADPH + H^{+} \rightarrow THF + NADP^{+}$, which is the precursor of the co-factors required for the biosynthesis of purine nucleotides, thymidine (precursor for DNA replication) and several amino acids [12]. Thus, inhibition of DHFR can lead to the disruption of DNA synthesis and the death of the cancer cells [12, 13]. In addition to this, bacteria also need DHFR to grow and multiply and hence inhibitors selective for bacterial vs. host DHFR have found application as antibacterial agents [14]. These two important factors make the enzyme a key target for both antibacterial and antitumor drug design in cancer

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[1] This work is a part of Ph. D thesis of R.I. Alsantali.

https://doi.org/10.1016/j.heliyon.2019.e01982
Received 2 May 2019; Received in revised form 18 May 2019; Accepted 19 May 2019

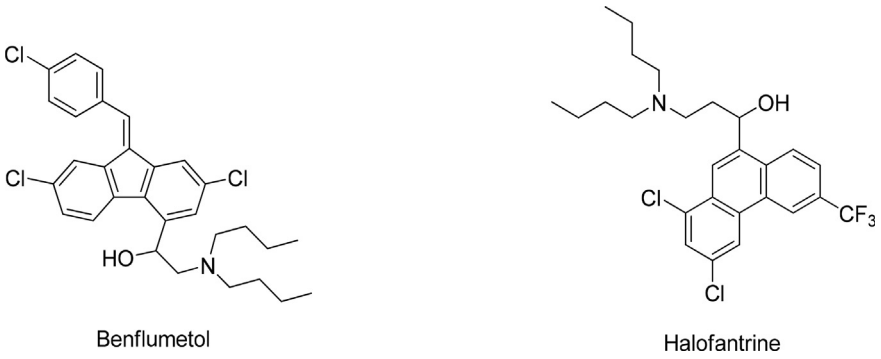


Fig. 1. Structure of antimalarial drugs Benflumetol and Halofantrine.

chemotherapy.

Based on these previous findings, we envisaged that the hybridization of the versatile 2,7-dichlorofluorene moiety with *α-N*-aryl-, *N*-alkyl, *N*-dialkylamino acetyl pharmacophores into a single chemical entity could be effective in enhancing its anticancer and antimicrobial properties. To achieve this, our previous experience in the synthesis of new antimicrobial and anticancer agents was helpful [15, 16, 17]. In the present work, we have synthesized some new 2,7-dichloro-4-(2-substitutedamino acetyl)fluorenes and studied their cytotoxic activity against human lung carcinoma (A-549) and human breast carcinoma (MCF-7) cell lines followed by antimicrobial and molecular docking studies.

2. Results and discussion

2.1. Chemistry

In this present work, our goal deals with the design and synthesis of some new 2,7-dichloro-4-(2-substituted-amino acetyl)fluorene derivatives combining different *N*-aryl/alkyl-amino acetyl groups with the biologically active 2,7-dichlorofluorene moiety on the antimicrobial and antitumor activities. In addition, a structure-activity relationship (SAR) study with different aromatic and aliphatic substituents was also carried out. As the DHFR inhibition is regarded as one of the most outstanding mechanism in exerting antimicrobial and antitumor activities [18, 19, 20], the synthesized compounds were designed to: (a) mimic the pharmacophores that may act as DHFR inhibitors; (b) possess hydrophilic and hydrophobic moieties that can interact with the hydrophilic and hydrophobic parts of the DHFR active site, respectively (Fig. 2).

The present strategy for the synthesis of the target compounds **4a-u** is outlined in Schemes 1 and 2. It starts with a convenient and straightforward approach to 2,7-dichlorofluorene (**2**) involving direct chlorination of fluorene (**1**) by means of *N*-chlorosuccinimide (NCS) in acetic acid in the presence of hydrogen chloride [21]. The chloroacetylation of **2** is

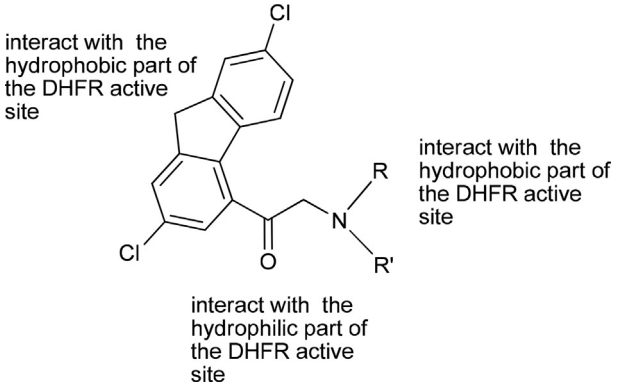


Fig. 2. Structural elements of DHFR inhibitors in the DHFR enzymatic active site.

accomplished by adding a solution of **2** in dichloromethane at 0–5 °C to a suspension of aluminum chloride and chloroacetyl chloride in dichloromethane (DCM). The reaction mixture is then quenched with diluted hydrochloric acid, after phase separation and washing, dichloromethane is evaporated under reduced pressure and cooled ethanol was added. The corresponding 2,7-dichloro-4-(chloroacetyl)fluorene (**3**) is isolated in 95% yield by filtration and drying (Scheme 1) [22].

The target 2,7-dichloro-4-(2-substituted-amino acetyl)fluorenes **4a-u** are obtained in moderate-excellent yields (58–96%) by simple nucleophilic substitution reaction of the chloroacetyl derivative **3** with different primary and secondary aryl-, alkyl-amines in dimethylformamide as reaction medium (Scheme 2).

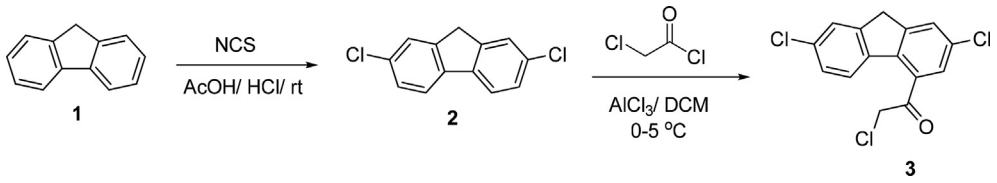
The structures of all synthesized compounds **4a-u** were confirmed on the basis of FT-IR, ^{1}H-NMR, ^{13}C-NMR, and DEPT-135 data (*c.f.* experimental section). The FT-IR spectra showed the presence of characteristic absorption bands at 3429-3370 cm^{-1} for NH groups, 1720-1660 cm^{-1} for (C=O) groups. Furthermore, to fully establish the chemical structures of the products, intensive 1D (^{1}H, ^{13}C, and DEPT-135) NMR spectroscopic analysis were conducted. For example, analysis of the ^{13}C and ^{13}C-DEPT-135 NMR spectra of **4c** indicated the presence of 19 signals representing the nineteen of nonequivalent carbons (9 aromatic quaternary carbons, 7 aromatic CH's, 2 methylene carbons, and one carbonyl carbon). Its ^{1}H-NMR spectrum showed three singlet signals at 7.95, 7.23, 7.07 ppm, two doublets at 6.98 and 6.88 (J = 7.0 Hz) for five protons of the fluorene moiety. Two doublets at 7.85 and 6.80 ppm (J = 8.5 Hz) appeared for the protons of 4-chlorophenyl moiety. In addition to this, NH proton appeared as a singlet signal at 5.27 ppm and the two singlet signals at 3.97 and 3.91 ppm corresponded to the two methylene protons.

2.2. Biological activity

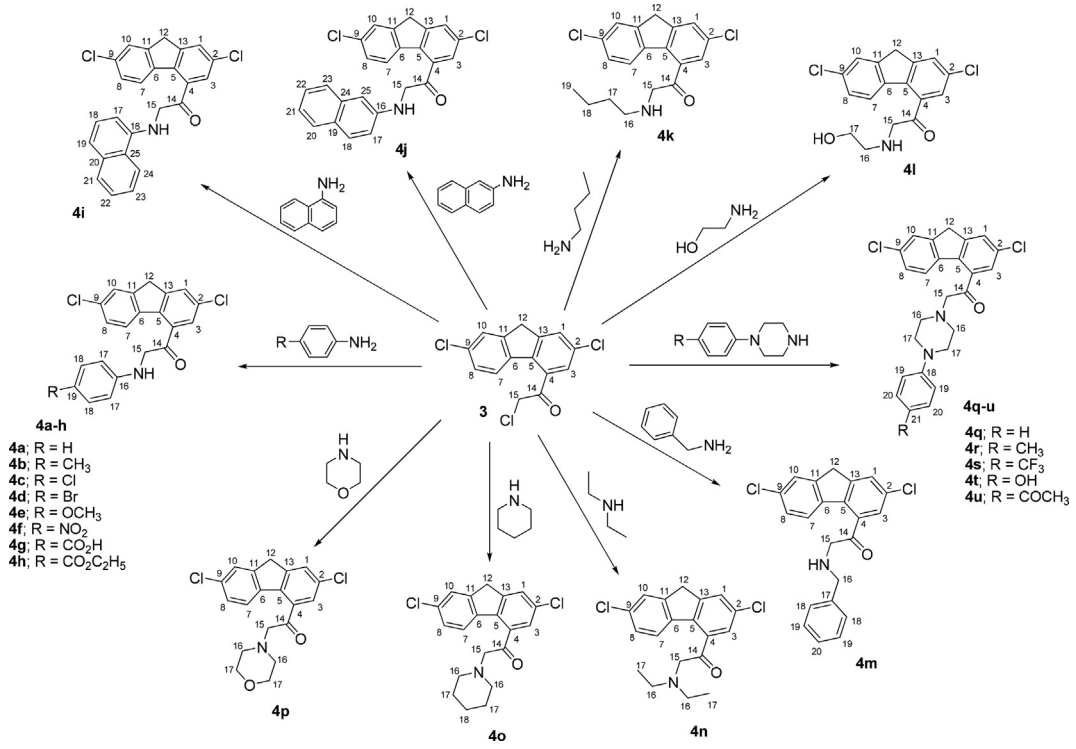
2.2.1. Antimicrobial activity

The novel compounds were assessed for antimicrobial activity against two strains of Gram + ve bacteria namely *S. aureus* (RCMB010010), and *B. subtilis* RCMB 015 (1) NRRL B-543 as well as two strains Gram -ve bacteria namely *E. coli* (RCMB 010052) ATCC 25955, and *P. vulgaris* RCMB 004 (1) ATCC 13315. The strains *A. fumigatus* (RCMB 002008), and *C. albicans* RCMB 005003 (1) ATCC 10231 were employed in assessment of antifungal activity.

The result of the antimicrobial assay of the synthesized compounds is given in Table 1 and Fig. 3. It is observed that some of the compounds showed increased antimicrobial activity when compared to the reference drugs. These compounds have given the best results in the inhibition of different types of bacteria and fungi; compound **4m** against the *S. aureus* and *B. Subtilis* with zone of inhibition (ZOI) values 18 and 17, respectively, compounds **4h** and **4m** against *E. coli*, ZOI value 14, compound **4u** against *P. vulgaris* ZOI value 15, compounds **4h**, **4r** and **4u** against *A. fumigatus*, ZOI values 22, 24 and 20, respectively, compound **4h** against *C. albicans*, ZOI value 18. From the previous results we observed that, both compounds **4h** and **4u** are active as antimicrobial agents (have



Scheme 1. Synthesis 2,7-dichloro-4-(chloroacetyl)fluorene (**3**).



Scheme 2. Synthetic routes to the target compounds **4a-u**.

Table 1
Anti-microbial activity of newly synthesized compounds **4a-u** (tested at 10 mg/ml) and expressed as the mean inhibition zone in mm ± standard deviation.

Comp. No.	Gram (+ve) Bacteria		Gram (-ve) Bacteria		Fungi	
	S. aureus	*B. subtilis*	*E. coli*	*P. vulgaris*	*A. fumigatus*	*C. albicans*
4a	-	-	-	-	10.1 ± 0.7	-
4b	12.3 ± 0.5	-	10 ± 0.4	7.8 ± 0.4	16.4 ± 0.8	-
4c	14.1 ± 0.8	-	11.2 ± 0.7	9.8 ± 0.6	-	-
4d	-	15.2 ± 0.8	11.9 ± 0.6	10.6 ± 0.8	-	-
4e	-	-	-	-	-	-
4f	-	11.2 ± 0.6	-	-	-	-
4g	-	9.9 ± 0.7	-	-	-	-
4h	15.2 ± 0.6	16.3 ± 0.7	14.5 ± 0.9	12.9 ± 0.7	22.3 ± 1.4	18.3 ± 1.2
4i	11.3 ± 0.9	15.2 ± 0.8	12.4 ± 0.8	10.1 ± 0.4	-	-
4j	11.8 ± 0.9	13.7 ± 0.9	11.1 ± 0.6	11.8 ± 0.6	-	-
4k	-	14.3 ± 0.9	9.8 ± 0.7	11.2 ± 0.7	-	-
4l	-	11.2 ± 0.4	10.1 ± 0.5	-	-	-
4m	18.2 ± 1.4	17.1 ± 0.8	14.3 ± 0.9	13.4 ± 0.6	-	-
4n	-	-	-	-	-	-
4°	-	-	-	-	-	-
4p	-	-	-	-	-	-
4q	13.1 ± 0.8	15.4 ± 0.8	-	-	15.5 ± 0.6	14.3 ± 0.9
4r	15.4 ± 0.7	16 ± 0.9	-	12.5 ± 0.7	24.2 ± 0.8	12.1 ± 0.7
4s	-	-	-	-	14.3 ± 0.9	-
4t	-	-	-	-	-	-
4u	16.2 ± 1.4	13.5 ± 0.8	10.2 ± 0.6	14.8 ± 0.6	20.1 ± 1.3	17.2 ± 0.7
St[a]	24.3 ± 0.9	26.2 ± 1.4	30.4 ± 1.2	25.3 ± 0.9	17.2 ± 0.8	20.1 ± 0.8

[a] Standard controls for the microorganisms are "*Gentamycin*" for the Gram-positive and Gram-negative bacteria, and "*Ketoconazol*" for the Fungi.

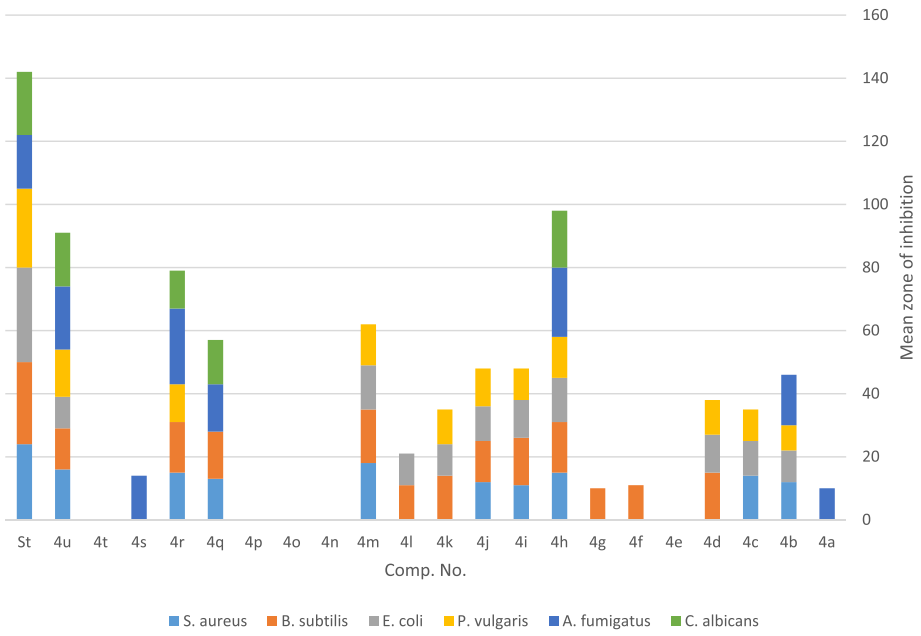


Fig. 3. Comparison of the antimicrobial activity of the newly synthesized compounds 4a-u.

antibacterial and antifungal activities), while compound **4r** and **4m** exhibited only antifungal and antibacterial activity, respectively.

2.3. *In vitro* anticancer activity

The tested compounds were screened for their cytotoxic activity against human lung carcinoma (A-549) and breast carcinoma (MCF-7). Their IC_{50} value, the concentration which can inhibit 50% of viable cells, was determined and data are showed in Tables 2 and 3. The reference control in this study is 5-Fluorouracil (5-FU).

From the obtained results in Tables 2 and 3, we can observe the effect of the following compounds on A-549 and MCF-7 cancer cell lines, respectively. Compound **4k** possessing butylamino moiety showed IC_{50} values of 4.94 μM and 5.57 μM and compound **4r** possessing 4-(*p*-tolyl) piperazino moiety showed IC_{50} values of 7.53 μM and 11.54 μM against A-549 and MCF-7 cancer cell lines, respectively. While, compound **4h** having ethyl benzoate moiety exhibited IC_{50} values of 14.24 μM and 12.47 μM, respectively.

Furthermore, compound **4j** having naphthalen-2-ylamino moiety showed IC_{50} values of 16.76 μM and 20.8 μM, respectively. On the other hand, compound **4q** having 4-phenylpiperazine moiety showed IC_{50} values of 19.46 μM and 22.64 μM respectively. Compound **4i** having naphthalen-1-ylamino moiety exhibited IC_{50} values of 43.75 μM and 69.09 μM, respectively. Moreover, compound **4u** possessing 4-acetylphenylpiperazine moiety exhibited IC_{50} values of 47.14 μM and 59.24 μM respectively. While, compound **4d** having 4-bromophenyl moiety showed IC_{50} values of 55.46 μM and 59.71 μM, respectively.

However, compound **4s** having 4-(trifluoromethyl)phenylpiperazine

Table 2
In vitro anticancer screening of the synthesized compounds against human lung carcinoma cell line (A-549).

Comp. No.	Validity for sample Conc.											IC_{50}[a] (μg/mL)	IC_{50}[a] (μM)
	500	250	125	62.50	31.25	15.60	7.80	3.90	2	1	0		
4a	15.86	29.43	42.78	69.21	84.06	94.18	99.76	100	100	100	100	108.00	293.27 ± 21.95
4b	2.98	6.75	16.54	30.81	45.17	61.82	74.56	87.59	95.62	99.81	100	26.70	69.8 4 ± 6.32
4c	37.84	58.46	76.53	91.48	99.51	100	100	100	100	100	100	353.00	876.58 ± 41.26
4d	5.65	12.87	24.91	36.76	45.24	56.92	70.81	85.17	94.08	98.74	100	24.80	55.46 ± 2.72
4e	13.89	26.54	36.87	49.01	67.8	85.41	96.38	99.86	100	100	100	60.90	152.91 ± 3.57
4f	16.41	33.96	48.3	74.82	90.67	98.14	100	100	100	100	100	121.00	292.80 ± 4.64
4g	75.49	92.63	99.46	100	100	100	100	100	100	100	100	>500	>500
4h	2.36	4.17	6.98	14.51	23.49	34.65	46.8	54.97	62.91	74.03	100	6.27	14.24 ± 0.62
4i	4.97	9.12	17.54	26.95	38.67	52.36	65.01	76.98	89.42	95.03	100	18.30	43.75 ± 1.59
4j	1.87	3.94	7.51	15.42	23.88	35.63	47.28	60.79	74.2	83.69	100	7.01	16.76 ± 0.88
4k	1.38	2.97	5.86	9.34	18.26	26.93	33.84	39.71	47.53	56.49	100	1.72	4.94 ± 0.42
4l	16.86	29.47	48.42	69.76	88.04	97.51	100	100	100	100	100	120.00	356.92 ± 29.74
4m	38.09	56.81	78.42	92.34	99.76	100	100	100	100	100	100	341.00	892.02 ± 61.46
4n	24.83	41.68	60.95	78.4	91.43	98.72	100	100	100	100	100	196.00	562.78 ± 39.52
4o	13.74	21.92	36.5	49.82	56.78	73.81	89.56	97.14	100	100	100	61.70	171.26 ± 20.43
4p	29.46	46.31	67.52	87.9	93.74	98.23	100	100	100	100	100	228.00	629.40 ± 43.18
4q	2.41	5.89	11.24	19.67	28.59	37.4	51.26	64.15	76.34	83.06	100	8.51	19.46 ± 1.72
4r	1.82	3.74	6.95	12.36	23.67	30.14	36.89	46.95	57.13	65.82	100	3.40	7.53 ± 0.61
4s	8.74	18.63	27.41	38.96	51.37	65.4	81.93	92.46	98.65	100	100	34.70	68.66 ± 1.92
4t	10.21	19.3	31.76	48.35	61.74	78.92	90.41	98.73	100	100	100	58.60	129.26 ± 2.38
4u	3.45	8.62	17.38	26.31	38.96	59.04	76.13	90.67	98.52	100	100	22.60	47.14 ± 0.86
5-FU	10.28	19.45	25.39	39.48	57.21	70.82	86.19	94.36	99.25	100	100	43.9	337.48 ± 22.82

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

Table 3
In vitro anticancer screening of the synthesized compounds against human breast carcinoma cell line (MCF-7).

Comp. No.	Validity for sample Conc.											IC_{50}[a] (μg/mL)	IC_{50}[a] (μM)
	500	250	125	62.50	31.25	15.60	7.80	3.90	2	1	0		
4a	12.78	26.91	38.52	60.84	81.27	96.83	100	100	100	100	100	92.90	252.27 ± 29.41
4b	3.45	8.62	15.3	28.56	41.89	62.94	78.51	90.67	98.23	100	100	25.20	65.92 ± 2.34
4c	34.62	63.08	88.74	98.16	100	100	100	100	100	100	100	365.00	906.38 ± 67.16
4d	6.71	15.29	26.43	38.17	46.08	59.74	72.36	88.4	97.21	100	100	26.70	59.71 ± 1.23
4e	11.27	20.95	31.74	43.82	58.16	70.89	86.25	94.06	98.77	100	100	49.00	123.03 ± 6.85
4f	13.67	29.53	42.76	67.54	84.06	94.38	99.72	100	100	100	100	107.00	258.92 ± 27.84
4g	63.74	88.41	97.62	100	100	100	100	100	100	100	100	>500	>500
4h	2.53	4.81	8.59	12.34	19.56	28.17	39.72	57.08	71.43	85.12	100	5.49	12.47 ± 0.89
4i	5.84	12.36	19.7	31.82	47.53	64.19	79.56	90.64	97.08	100	100	28.90	69.09 ± 1.47
4j	2.41	4.59	6.98	13.67	21.96	34.82	51.97	68.4	82.95	91.47	100	8.70	20.80 ± 0.96
4k	1.56	3.44	6.21	10.87	17.59	24.86	35.04	41.82	49.27	62.31	100	1.94	5.57 ± 0.25
4l	20.48	36.02	64.18	79.53	94.76	99.58	100	100	100	100	100	188.00	559.17 ± 41.89
4m	31.97	46.25	70.46	89.02	97.13	100	100	100	100	100	100	231.00	604.27 ± 49.31
4n	31.76	48.53	70.42	87.15	96.28	100	100	100	100	100	100	242.00	694.86 ± 61.24
4°	9.82	18.07	28.96	41.27	62.39	80.95	91.43	98.76	100	100	100	49.60	137.67 ± 7.81
4p	23.75	40.89	57.23	71.4	87.92	93.16	99.74	100	100	100	100	180.00	496.89 ± 40.23
4q	2.88	6.95	14.81	20.39	26.74	39.56	54.08	71.23	85.16	92.47	100	9.90	22.64 ± 0.52
4r	1.69	3.86	6.79	11.24	19.85	30.72	41.93	54.07	63.88	79.12	100	5.21	11.54 ± 0.38
4s	7.41	13.25	20.64	29.74	43.86	69.42	86.03	94.16	99.71	100	100	27.50	54.42 ± 1.12
4t	8.74	15.49	27.82	40.64	68.52	81.49	92.75	99.61	100	100	100	52.00	114.70 ± 4.26
4u	3.82	9.71	18.54	31.78	46.23	67.42	79.14	92.75	99.84	100	100	28.40	59.24 ± 1.18
5-FU	9.18	17.84	28.01	35.39	47.13	60.35	71.82	86.97	95.23	98.12	100	27.80	213.71 ± 18.53

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

moiety exhibited IC_{50} values of 68.66 μM and 54.42 μM, respectively. Compound **4b** having 4-methylphenyl moiety showed IC_{50} values of 69.84 μM and 65.92 μM, respectively. Compound **4t** having 4-hydroxyphenyl piperazine moiety gave IC_{50} values of 129.26 μM and 114.7 μM, respectively. Furthermore, compound **4e** having 4-methoxyphenyl moiety IC_{50} values of 152.91 μM and 123.03 μM, respectively, while compound **4o** having piperidine moiety exhibited IC_{50} values of 171.26 μM and 137.67 μM, respectively. Compound **4f** having 4-nitrophenyl moiety showed IC_{50} values of 292.8 μM and 258 μM, respectively. Finally, compound **4a** having phenyl moiety, showed IC_{50} values of 293.27 μM and 252 μM, respectively. It is clear from the data as illustrated in Fig. 4, the order of the cytotoxic activity of the tested compounds against human lung carcinoma (A-549) followed the order **4k** > **4r** > **4h** > **4j** > **4q** > **4i** > **4u** > **4d** > **4s** > **4b** > **4t** > **4e** > **4o** > **4f** > **4a** > 5-fluorouracil > **4l** > **4g** > **4n** > **4p** > **4c** > **4m**. However, the potency of cytotoxicity of the tested compounds against human breast carcinoma cell line (MCF-7) has following order **4k** > **4r** > **4h** > **4j** > **4q** > **4s** > **4u** > **4d** > **4b** > **4i** > **4t** > **4e** > **4o** > 5-fluorouracil > **4a** > **4f** > **4p** > **4g** > **4l** > **4m** > **4n** > **4c**. The results of this biological screening for the synthesized compounds may lead improvement of the anticancer agents. Over all, from the biological evaluation results, it can be clearly concluded that the synthesized compounds **4g**, **4r**, **4u** and **4m** have dual activities (they are active as both antimicrobial and anticancer agents).

2.4. Docking and molecular modeling study

Literature survey [19, 23] proved that thymidylate synthase and dihydrofolate reductase (DHFR) were used in the development of anticancer and antimicrobial agents. In the present investigation, Molecular Operating Environment (MOE) module was accomplished to justify the cytotoxic potency of the tested compounds [24]. Furthermore, study of the Molecular docking help in well knowing the action of any compound via their different interactions with the active sites of DHFR. Docking was performed for the compounds **4a-4u** on DHFR to predict their action as anticancer agents (*c.f. SI file*). All the tested compounds show different interactions with the active site of DHFR. Especially, the tested

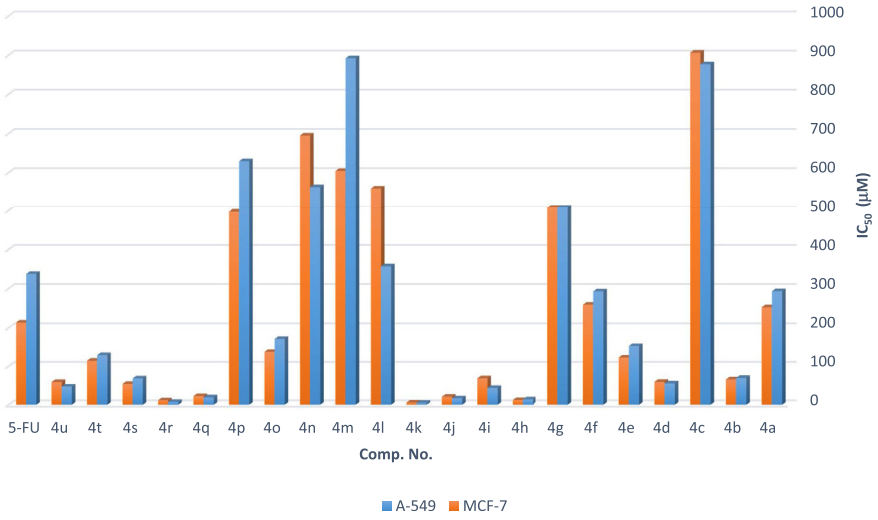


Fig. 4. Comparison of the cytotoxic activity of the tested compounds 4a-u against human lung carcinoma (A-549) and human breast carcinoma (MCF-7).

compounds **4r**, **4u**, **4s**, **4e**, **4q** and **4f**, exert their anticancer action via inhibition of DHFR active sites (Table 4 in SI file). The docking score energy for the newly synthesized compounds follows the order: **4r** > **4u** > **4s** > **4e** > **4q** > **4f** > **4t** > **4h** > **4i** > **4c** > **4b** > **4j** > **4m** > **4g** > **4a** > **4d** > **4k** > **4n** > **4o** > **4p** > **4l** (Fig. 5).

2.4.1. Docking study of *4k* and *4r* in the DHFR active site

Methotrexate (PDB ID: 4DFR) used as a template with dihydrofolate reductase co-crystallized in MOE docking studies of the inhibitors. The active site docking studies of **4k** showed that nitrogen atom acted as a hydrogen bond donor with amino acid residue Ile 94 (3.07 Å) with binding energy of -7.3 kcal/mol. This is besides several hydrophobic interactions with various amino acid residues: Tyr 100, Phe 31, Thr 113, Asp 27, Leu 28, Trp 30, Trp 22, Met 20, Ser 49, Ala 19, Thr 46, His 45, Asn 18, Glu 17, Met 16, Gly 15, Ile 5, Ala 7, Ala 6.

In addition, the active site docking studies of **4r** showed that oxygen atom of carbonyl group acted as a hydrogen bond acceptor with Met 20 (2.98 Å) with binding energy of -3.4 kcal/mol. Furthermore, it indicated an arene interaction between the phenyl ring and Gly 15 (3.72 Å) with binding energy of -0.9 kcal/mol. This is besides several hydrophobic interactions with different amino acid residues: Ile 5, Trp 30, Ala 6, Ala 7, Thr 113, Gly 96, Tyr 100, Leu 8, Ser 49, Ile 14, Met 16, Thr 46, Glu 17, Asn 18, His 45, Ala 19, Asp 27, Ile 94, Leu 28, Phe 31 (Fig. 6).

3. Conclusion

We report herein the synthesis of a new series of 2,7-dichloro-4-(2-substituted-amino acetyl)fluorene derivatives. Most of these new compounds exhibited significant anticancer activity against human lung carcinoma (A-549) and human breast carcinoma (MCF-7) cell lines, when compared to 5-Fluorouracil as a reference drug. In addition, on the antimicrobial evaluation; some of these synthesized compounds showed acceptable activity as antibacterial and antifungal agents. To the best of our knowledge, these multi-addressable properties of the new synthesized 2,7-dichlorofluorene derivatives described in this work will open a new alternative era in the field of medicinal chemistry and can be considered as pharmacophores.

4. Experimental

4.1. Chemistry

4.1.1. General methods

All Chemicals and solvents used purchased from Sigma-Aldrich are spectroscopic grade and 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 in 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.

4.2. Synthesis of 2,7-dichloro-9H-fluorene (2)

16.6 g of fluorine (0.1 mol), 26.6 g of *N*-chlorosuccinimide (0.2 mol), 150 mL of glacial acetic acid, and 10 mL of concentrated HC1 added over about 30 min. Without addition of water the white solid was filtered after 3 days, washed with ethanol to give 9.35 g (40%) of the dichlorofluorene as white crystals, mp 127–128 °C (lit [21]. m. p. 125–126 °C).

4.3. Synthesis of 2-chloro-1-(2,7-dichloro-9H-fluoren-4-yl)ethanone (3)

In two necked flask, a suspension of chloroacetyl chloride (4 g, 0.036 mol) and $AlCl_3$ (6 g, 0.045 mol), in dichloromethane (20 mL) was cooled to -5 °C. A solution of **2** (6 g) in dichloromethane (50 mL) was added dropwise over about 1 h. After 10 h, the deep-red reaction mixture was quenched with aqueous hydrochloric acid (100 mL). After phase separation, the organic layer was washed three times with distilled water. Dichloromethane was distilled off under reduced pressure. Cold ethanol was added and the crystallized product was filtered-off to give 7.65 g (95%) of pure chloroacetyl derivative **3** as colorless crystals, mp 122–123 °C (lit [8]. m. p. 124–125 °C). ^{1}H-NMR (DMSO-d_6): δ 7.95 (s, 1H, C_3–H), 7.85 (s, 1H, C_1–H), 7.71 (d, J = 6.0 Hz, 1H, C_7–H), 7.66 (s, 1H, C_{10}–H), 7.42 (d, J = 6.0 Hz, 1H, C_8–H), 5.26 (s, 2H, C_{15}–H), 3.98 (s, 2H, C_{12}–H); ^{13}C-NMR (DMSO-d_6): δ 195.6 (C_{14}), 147.8 (C_{11}), 146.8 (C_{13}), 137.5 (C_6), 136.7 (C_4), 133.4 (C_9), 133.0 (C_2), 131.7 (C_5), 128.9 (C_1), 127.2 (C_7), 127.1 (C_{10}), 125.6 (C_3), 125.1 (C_8), 49.9 (C_{15}), 36.7 (C_{12}).

4.4. Synthesis of 2,7-dichloro-4-(2-substituted-amino acetyl)fluorenes 4a-u

A mixture of chloroacetyl derivative **3** (0.001 mol) and different aryl/alkyl amine (0.001 mol) in dimethylformamide (10 mL) was refluxed for 4–6 h. The reaction mixture was cooled to room temperature and poured onto ice/water mixture, the obtained solid product was filtered, washed with cold ethanol then with *n*-hexane, dried and recrystallized from ethanol to give the titled products **4a-u**.

4.5. 1-(2,7-Dichloro-9H-fluoren-4-yl)-2-(phenylamino)ethanone (4a)

Pale yellow crystals, yield 61%, mp 116–117 °C; FT-IR (KBr): ν (cm^{-1}) 3374 (NH), 3060 (CH arom.), 2925 (CH aliph.), 1683 (C=O); ^{1}H-NMR (DMSO-d_6): δ 8.28 (s, 1H, C_3–H), 7.95 (s, 1H, C_1–H), 7.59 (s, 1H, C_{10}–H), 7.44 (d, J = 6.5 Hz, 1H, C_7–H), 7.33–7.30 (m, 2H, C_{18}–H), 6.87 (d, J = 6.5 Hz, 1H, C_8–H), 6.66 (s, 1H, NH), 7.56–7.54 (m, 3H, C_{17}–H, C_{19}–H), 4.05 (s, 2H, C_{15}–H), 3.98 (s, 2H, C_{12}–H); ^{13}C-NMR (DMSO-d_6): δ 162.7 (C_{14}), 160.0 (C_{16}), 146.7 (C_{11}), 139.0 (C_{13}), 138.7 (C_6), 137.3 (C_4), 136.9 (C_9), 135.5 (C_2), 129.3 (C_1), 128.5 (C_5), 127.2 (C_7), 124.0 (C_{10}), 120.8 (C_3), 119.5 (C_8), 117.9 (C_{18}), 116.0 (C_{19}), 114.3 (C_{17}), 49.8 (C_{15}),

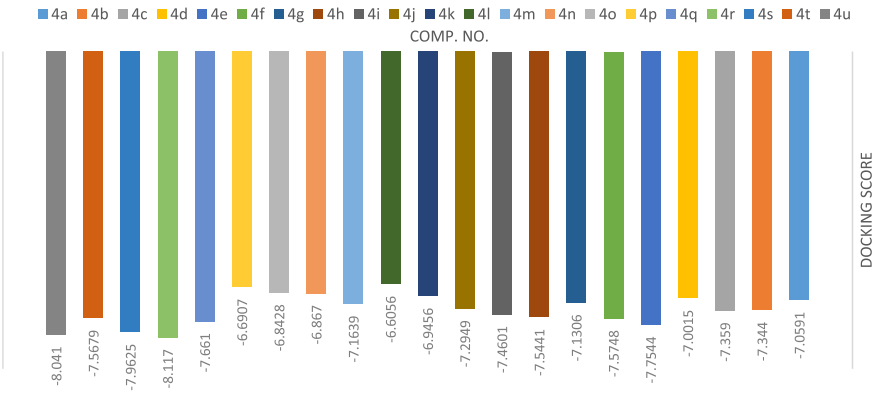


Fig. 5. Docking Score energy of the tested compounds 4a-u.

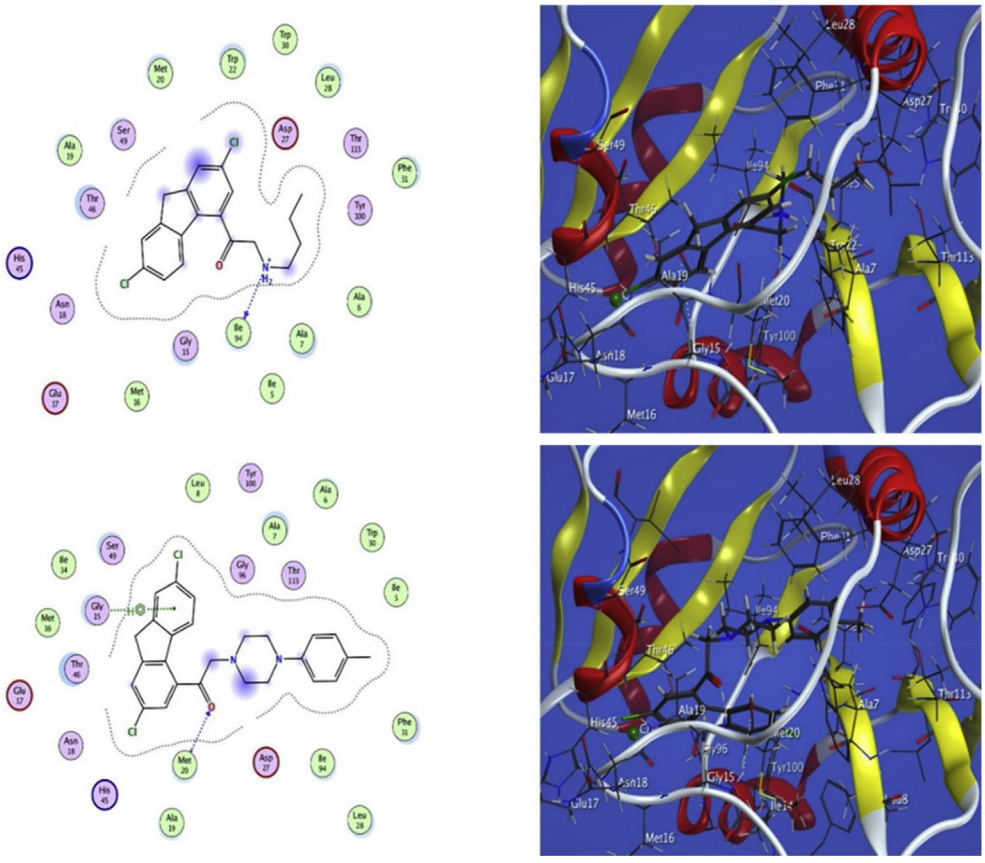


Fig. 6. Docking of compounds 4k and 4r into DHFR.

36.7 (C_{12}). Anal. Calcd. for $C_{21}H_{15}Cl_2NO$ (368.26): C, 68.49; H, 4.11; N, 3.80. Found: C, 68.21; H, 4.00; N, 3.58%.

4.6. 1-(2,7-Dichloro-9H-fluoren-4-yl)-2-(p-tolylamino)ethanone (4b)

Orange crystals, yield 67%, mp 89–91 °C; FT-IR (KBr): ν (cm^{-1}) 3370 (NH), 3077 (CH arom.), 2927 (CH aliph.), 1708 (C=O); ^{1}H-NMR (DMSO-d_6): δ 8.03 (s, 1H, C_3–H), 7.95 (s, 1H, C_1–H), 7.84 (s, 1H, C_{10}–H), 7.71 (d, J = 8.0 Hz, 2H, C_{18}–H), 7.64 (d, J = 7.0 Hz, 1H, C_7–H), 7.41 (d, J = 7.0 Hz, 1H, C_8–H), 6.86–6.74 (m, 1H, C_{17}–H), 6.62–6.60 (m, 1H, C_{17}–H), 5.26 (s, 1H, NH), 4.05 (s, 2H, C_{15}–H), 3.97 (s, 2H, C_{12}–H), 2.73 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6): δ 162.8 (C_{14}), 156.1 (C_{16}), 147.8 (C_{11}), 146.8 (C_{13}), 137.5 (C_6), 136.7 (C_4), 136.0 (C_{19}), 133.4 (C_9), 133.0 (C_2), 131.7 (C_5), 128.9 (C_1), 126.8 (C_7), 125.1 (C_{10}), 122.5 (C_3), 120.8 (C_8), 119.4 (C_{18}), 112.1 (C_{17}) 49.9 (C_{15}), 36.8 (C_{12}), 21.3 (CH_3). Anal. Calcd. for $C_{22}H_{17}Cl_2NO$ (382.28): C, 69.12; H, 4.48; N, 3.66. Found: C, 69.23; H, 4.30; N, 3.50%.

4.7. 2-(4-Chlorophenylamino)-1-(2,7-dichloro-9H-fluoren-4-yl)ethanone (4c)

Beige crystals, yield 65%, mp 130–132 °C; FT-IR (KBr): ν (cm^{-1}) 3394 (NH), 3066 (CH arom.), 2926 (CH aliph.), 1672 (C=O); ^{1}H-NMR (DMSO-d_6): δ 7.95 (s, 1H, C_3–H), 7.85 (d, J = 8.5 Hz, 2H, C_{18}–H), 7.23 (s, 1H, C_1–H), 7.07 (s, 1H, C_{10}–H), 6.98 (d, J = 7.0 Hz, 1H, C_7–H), 6.88 (d, J = 7.0 Hz, 1H, C_8–H), 6.80 (d, J = 8.5 Hz, 2H, C_{17}–H), 5.27 (s, 1H, NH), 3.97 (s, 2H, C_{15}–H), 3.91 (s, 2H, C_{12}–H); ^{13}C-NMR (DMSO-d_6): δ 162.3 (C_{14}), 160.1 (C_{16}), 147.8 (C_{11}), 146.8 (C_{13}), 137.6 (C_6), 136.7 (C_4), 133.4 (C_9), 133.0 (C_2), 131.5 (C_5), 129.2 (C_{18}), 128.9 (C_1), 127.4 (C_7, C_{10}), 125.6 (C_{19}), 122.4 (C_3), 121.1 (C_8), 119.4 (C_{17}), 49.9 (C_{15}), 36.8 (C_{12}). Anal. Calcd. for $C_{21}H_{14}Cl_3NO$ (402.70): C, 62.63; H, 3.50; N, 3.48. Found: C, 62.49; H, 3.14; N, 3.26%.

4.8. 2-(4-Bromophenylamino)-1-(2,7-dichloro-9H-fluoren-4-yl)ethanone (4d)

Orange crystals, yield 83%, mp 152–153 °C; FT-IR (KBr): ν (cm^{-1}) 3410 (NH), 3068 (CH arom.), 2856(CH aliph.), 1685 (C=O); ^{1}H-NMR (DMSO-d_6): δ 7.95 (s, 1H, C_3–H), 7.56 (s, 1H, C_1–H), 7.42 (d, J = 8.5 Hz, 2H, C_{18}–H), 7.30 (s, 1H, C_{10}–H), 7.17 (d, J = 6.5 Hz, 1H, C_7–H), 7.02 (d, J = 6.5 Hz, 1H, C_8–H), 6.81 (d, J = 8.5 Hz, 2H, C_{17}–H), 6.58 (s, 1H, NH), 4.00 (s, 2H, C_{15}–H), 3.92 (s, 2H, C_{12}–H); ^{13}C-NMR (DMSO-d_6): δ 162.9 (C_{14}), 160.2 (C_{16}), 148.1 (C_{11}), 146.9 (C_{13}), 136.9 (C_6), 136.5 (C_4), 132.6 (C_9), 133.6 (C_2), 131.0 (C_5), 129.6 (C_{18}), 128.2 (C_1), 127.1 (C_7, C_{10}), 125.7 (C_{19}), 121.7 (C_3), 120.9 (C_8), 119.2 (C_{17}), 49.9 (C_{15}), 36.9 (C_{12}). Anal. Calcd. for $C_{21}H_{14}BrCl_2NO$ (447.15): C, 56.41; H, 3.16; N, 3.13. Found: C, 56.30; H, 3.15; N, 3.00%.

4.9. 1-(2,7-Dichloro-9H-fluoren-4-yl)-2-(4-methoxyphenylamino) ethanone (4e)

Pale brown crystals, yield 94%, mp 180–182 °C; FT-IR (KBr): ν (cm^{-1}) 3398 (NH), 3069 (CH arom.), 2929 (CH aliph.), 1667 (C=O); ^{1}H-NMR (DMSO-d_6): δ 8.20 (s, 1H, C_3–H), 7.94 (d, J = 7.5 Hz, 2H, C_{18}–H), 7.68 (s, 1H, C_1–H), 7.53 (s, 1H, C_{10}–H), 7.05 (s, 1H, NH), 6.87 (d, J = 6.5 Hz, 1H, C_7–H), 6.65 (d, J = 6.5 Hz, 1H, C_8–H), 6.53 (d, J = 7.5 Hz, 2H, C_{17}–H), 3.81 (s, 2H, C_{15}–H), 3.76 (s, 2H, C_{12}–H), 3.62 (s, 3H, OCH_3); ^{13}C-NMR (DMSO-d_6): δ 162.7 (C_{14}), 151.1 (C_{19}), 147.5 (C_{11}), 142.7 (C_{13}), 137.6 (C_6), 136.7 (C_4), 134.1 (C_{16}), 133.1 (C_9), 132.8 (C_2), 131.5 (C_5), 129.1 (C_1), 127.9 (C_7), 127.1 (C_{10}), 125.3 (C_3), 121.0 (C_8), 119.4 (C_{17}), 114.9 (C_{18}), 55.7 (C_{15}), 36.2 (C_{12}), 31.2 (OCH_3). Anal. Calcd. for $C_{22}H_{17}Cl_2NO_2$ (398.28): C, 66.34; H, 4.30; N, 3.52. Found: C, 66.20; H, 4.26; N, 3.40%.

4.10. 1-(2,7-Dichloro-9H-fluoren-4-yl)-2-(4-nitrophenylamino)ethanone (4f)

Orange crystals, yield 88%, mp 260–262 °C; FT-IR (KBr): ν (cm^{-1}) 3378 (NH), 3065 (CH arom.), 2922 (CH aliph.), 1720 (C=O); ^{1}H-NMR (DMSO-d_6): δ 8.24 (s, 1H, C_3–H), 7.95 (s, 1H, C_1–H), 7.65 (d, J = 8.0 Hz, 2H, C_{18}–H), 7.48 (s, 1H, C_{10}–H), 7.10 (d, J = 7.0 Hz, 1H, C_7–H), 6.74 (d, J = 7.0 Hz, 1H, C_8–H), 6.45 (d, J = 8.0 Hz, 2H, C_{17}–H), 4.94 (s, 1H, NH), 3.97 (s, 2H, C_{15}–H), 3.89 (s, 2H, C_{12}–H); ^{13}C-NMR (DMSO-d_6): δ 162.9 (C_{14}), 159.7 (C_{16}), 146.3 (C_{11}, C_{13}), 146.1 (C_{19}), 136.2 (C_6, C_4), 132.9 (C_9), 132.1 (C_2), 130.2 (C_5), 129.6 (C_1), 129.5 (C_{18}), 125.4 (C_7), 124.9 (C_{10}), 119.5 (C_3), 118.1 (C_8), 113.1 (C_{17}), 51.2 (C_{15}), 36.8 (C_{12}). Anal. Calcd. for $C_{21}H_{14}Cl_2N_2O_3$ (413.25): C, 61.03; H, 3.41; N, 6.78. Found: C, 60.90; H, 3.20; N, 6.55%.

4.11. 4-(2-(2,7-Dichloro-9H-fluoren-4-yl)-2-oxoethylamino)benzoic acid (4g)

Orange crystals, yield 74%, mp 180–181 °C; FT-IR (KBr): ν (cm^{-1}) 3373–3210 (br, OH, NH), 3068 (CH arom.), 2927 (CH aliph.), 1707, 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 8.36 (s, 1H, C_3–H), 8.20 (d, J = 9.5 Hz, 2H, C_{18}–H), 7.95 (s, 1H, C_1–H), 7.70 (s, 1H, C_{10}–H), 7.61 (d, J = 9.5 Hz, 2H, C_{17}–H), 7.41 (d, J = 7.0 Hz, 1H, C_7–H), 7.28 (d, J = 7.0 Hz, 1H, C_8–H), 6.87 (s, 1H, OH), 5.68 (s, 1H, NH), 3.97 (s, 2H, C_{15}–H), 3.81 (s, 2H, C_{12}–H); ^{13}C-NMR (DMSO-d_6): δ 167.7 (C=O), 162.7 (C_{14}), 160.5 (C_{16}), 153.6 (C_{19}), 152.4 (C_{11}), 146.8 (C_{13}), 141.7 (C_6), 138.1 (C_4), 132.5 (C_2, C_9), 131.7 (C_5), 131.0 (C_1), 130.4 (C_7), 127.3 (C_{10}), 125.3 (C_3), 125.0 (C_8), 124.0 (C_{18}), 112.8 (C_{17}), 51.1 (C_{15}), 36.2 (C_{12}). Anal. Calcd. for $C_{22}H_{15}Cl_2NO_3$ (412.27): C, 64.09; H, 3.67; N, 3.40. Found: C, 64.20; H, 3.50; N, 3.30%.

4.12. Ethyl 4-(2-(2,7-dichloro-9H-fluoren-4-yl)-2-oxoethylamino) benzoate (4h)

Yellow crystals, yield 60%, mp 95–97 °C; FT-IR (KBr): ν (cm^{-1}) 3374 (NH), 3068 (CH arom.), 2900 (CH aliph.), 1696 (C=O), 1660 (C=O); ^{1}H-NMR (DMSO-d_6): δ 7.98 (s, 1H, C_3–H), 7.79 (s, 1H, C_1–H), 7.64 (s, 1H, C_{10}–H), 7.42 (d, J = 8.5 Hz, 2H, C_{18}–H), 7.25 (d, J = 7.5 Hz, 1H, C_7–H), 6.85 (d, J = 7.5 Hz, 1H, C_8–H), 6.58 (d, J = 8.5 Hz, 2H, C_{17}–H), 5.52 (s, 1H, NH), 4.19 (q, J = 6.5 Hz, 2H, CH_2CH_3), 4.00 (s, 2H, C_{15}–H), 3.89 (s, 2H, C_{12}–H), 1.26 (t, J = 6.5 Hz, 3H, CH_3CH_2); ^{13}C-NMR (DMSO-d_6): δ 194.9 (C=O), 166.3 (C_{14}), 153.7 (C_{16}), 146.3 (C_{11}), 146.0 (C_{13}), 145.8 (C_6), 135.6 (C_4), 133.1 (C_9), 132.0 (C_2), 131.4 (C_5), 129.2 (C_1), 128.5 (C7, C_{10}), 125.9 (C_3), 124.6 (C_8), 123.8 (C_{18}), 116.6 (C_{19}), 113.2 (C_{17}), 64.8 (CH_2CH_3), 59.9 (C_{15}), 36.8 (C_{12}), 14.8 (CH_3CH_2). Anal. Calcd. for $C_{24}H_{19}Cl_2NO_3$ (440.32): C, 65.47; H, 4.35; N, 3.18. Found: C, 65.30; H, 4.22; N, 3.00%.

4.13. 1-(2,7-Dichloro-9H-fluoren-4-yl)-2-(naphthalen-1-ylamino) ethanone (4i)

Pale brown crystals, yield 75%, mp 101–103 °C; FT-IR (KBr): ν (cm^{-1}) 3412 (NH), 3059 (CH arom.), 2900 (CH aliph.), 1691 (C=O); ^{1}H-NMR (DMSO-d_6): δ 8.10 (s, 1H, C_3–H), 7.78–7.76 (m, 2H, C_1–H, C_{24}–H), 7.60–7.53 (m, 4H, C_{10}–H, C_{21}–H, C_{22}–H, C_{23}–H), 7.46–7.37 (m, 2H, C_{18}–H, C_{19}–H), 7.29 (d, J = 7.0 Hz, 1H, C_7–H), 7.13 (d, J = 7.0 Hz, 1H, C_8–H), 6.85 (d, J = 7.5 Hz, 1H, C_{17}–H), 5.54 (s, 1H, NH), 3.89 (s, 2H, C_{15}–H), 3.57 (s, 2H, C_{12}–H); ^{13}C-NMR (DMSO-d_6): δ 194.9 (C_{14}), 146.7 (C_{11}), 146.0 (C_{13}), 145.8 (C_{16}), 145.0 (C_{20}), 144.3 (C_{25}), 143.7 (C_6), 137.7 (C_4), 136.7 (C_2, C_9), 134.4 (C_5), 128.5 (C_1), 126.7 (C_7, C_{10}), 125.8 (C_3, C_8), 124.7 (C_{21}), 122.7 (C_{18}), 119.7 (C_{22}), 116.6 (C_{23}, C_{24}), 113.1 (C_{19}), 104.1 (C_{17}), 56.5 (C_{15}), 36.7 (C_{12}). Anal. Calcd. for $C_{25}H_{17}Cl_2NO$ (418.31): C, 71.78; H, 4.10; N, 3.35. Found: C, 71.52; H, 3.99; N, 3.17%.

4.14. 1-(2,7-Dichloro-9H-fluoren-4-yl)-2-(naphthalen-2-ylamino) ethanone (4j)

Pale yellow crystals, yield 68%, mp 130–132 °C; FT-IR (KBr): ν (cm^{-1}) 3398 (NH), 3052 (CH arom.), 2905 (CH aliph.), 1688 (C=O); ^{1}H-NMR (DMSO-d_6): δ 7.99 (s, 1H, C_3–H), 7.84 (s, 1H, C_1–H), 7.69–7.65 (m, 2H, C_{10}–H, C_{20}–H), 7.58 (d, J = 7.0 Hz, 1H, C_7–H), 7.45 (d, J = 7.0 Hz, 1H, C_8–H), 7.34–7.30 (m, 1H, C_{18}–H), 7.18–7.14 (m, 1H, C_{22}–H), 7.05–7.02 (m, 2H, C_{23}–H, C_{25}–H), 6.84 (d, J = 7.5 Hz, 1H, C_{17}–H), 6.65 (m, 1H, C_{21}–H), 5.52 (s, 1H, NH), 3.99 (s, 2H, C_{15}–H), 3.62 (s, 2H, C_{12}–H); ^{13}C-NMR (DMSO-d_6): δ 194.9 (C_{14}), 146.9 (C_{11}), 146.7 (C_{13}), 145.8 (C_{16}), 145.5 (C_{19}), 145.0 (C_{24}), 144.1 (C_6), 135.0 (C_4), 134.0 (C_2, C_9), 132.0 (C_5), 129.1 (C_1), 128.0 (C_7), 127.7 (C_{10}), 126.5 (C_3, C_8), 125.9 (C_{18}), 123.7 (C_{20}), 123.5 (C_{22}), 122.3 (C_{23}), 119.4 (C_{21}), 118.7 (C_{17}), 108.9 (C_{25}), 64.8 (C_{15}), 36.8 (C_{12}). Anal. Calcd. for $C_{25}H_{17}Cl_2NO$ (418.31): C, 71.78; H, 4.10; N, 3.35. Found: C, 71.50; H, 3.81; N, 3.10%.

4.15. 2-(Butylamino)-1-(2,7-dichloro-9H-fluoren-4-yl)ethanone (4k)

Orange crystals, yield 89%, mp 165–167 °C; FT-IR (KBr): ν (cm^{-1}) 3429 (NH), 3068 (CH arom.), 2929 (CH aliph.), 1668 (C=O); ^{1}H-NMR (DMSO-d_6): δ 7.88 (s, 1H, C_3–H), 7.72 (d, J = 9.5 Hz, 1H, C_7–H), 7.61 (s, 1H, C_1–H), 7.36 (d, J = 9.5 Hz, 1H, C_8–H), 7.16 (s, 1H, C_{10}–H), 6.92 (s, 1H, NH), 3.79 (s, 2H, C_{12}–H), 3.68 (s, 2H, C_{15}–H), 2.45 (t, J = 13.5 Hz, 2H, C_{16}–H), 1.55–1.47 (m, 2H, C_{17}–H), 1.08–1.03 (m, 2H, C_{18}–H), 0.66 (t, J = 22.5 Hz, 3H, C_{19}–H); ^{13}C-NMR (DMSO-d_6): δ 162.7 (C_{14}), 147.8 (C_{11}), 145.5 (C_{13}), 139.3 (C_6), 136.3 (C_4), 132.1 (C_2, C_9), 131.8 (C_5), 128.3 (C_1), 127.4 (C_7), 123.8 (C_{10}), 122.0 (C_3), 118.9 (C_8), 45.3 (C_{15}), 36.7 (C_{16}), 32.9 (C_{12}), 28.2 (C_{17}), 21.3 (C_{18}), 18.4 (C_{19}). Anal. Calcd. for $C_{19}H_{19}Cl_2NO$ (348.27): C, 65.53; H, 5.50; N, 4.02. Found: C, 65.41; H, 5.23; N, 3.85%.

4.16. 1-(2,7-Dichloro-9H-fluoren-4-yl)-2-(2-hydroxyethylamino) ethanone (4l)

Green crystals, yield 87%, mp 175–177 °C; FT-IR (KBr): ν (cm^{-1}) 3395–3200 (br, OH, NH), 3071 (CH arom.), 2928 (CH aliph.), 1664 (C=O); ^{1}H-NMR (DMSO-d_6): δ 7.95 (s, 1H, C_3–H), 7.81 (s, 1H, C_1–H), 7.71 (d, J = 6.5 Hz, 1H, C_7–H), 7.51 (s, 1H, C_{10}–H), 7.40 (d, J = 6.5 Hz, 1H, C_8–H), 5.70 (s, 1H, OH), 4.41 (s, 1H, NH), 3.98 (s, 2H, C_{12}–H), 3.91 (s, 2H, C_{15}–H), 3.44–3.42 (m, 2H, C_{17}–H), 2.20–2.16 (m, 2H, C_{16}–H); ^{13}C-NMR (DMSO-d_6): δ 162.7 (C_{14}), 146.2 (C_{11}), 145.1 (C_{13}), 139.0 (C_6), 136.3 (C_4), 132.7 (C_2, C_9), 132.1 (C_5), 128.6 (C_1), 127.0 (C_7), 122.9 (C_{10}), 122.1 (C_3), 118.7 (C_8), 60.2 (C_{17}), 43.1 (C_{15}), 36.2 (C_{12}), 31.2 (C_{16}). Anal. Calcd. for $C_{17}H_{15}Cl_2NO_2$ (336.21): C, 60.73; H, 4.50; N, 4.17. Found: C, 60.60; H, 4.35; N, 4.00%.

4.17. 2-(Benzylamino)-1-(2,7-dichloro-9H-fluoren-4-yl)ethanone (4m)

Dark gray crystals, yield 95%, mp 175–177 °C; FT-IR (KBr): ν (cm^{-1}) 3390 (NH), 3062 (CH arom.), 2927 (CH aliph.), 1665 (C=O); ^{1}H-NMR (DMSO-d_6): δ 7.95 (br s, 2H, C_1–H, C_3–H), 7.78 (s, 1H, C_{10}–H), 7.67 (d, J = 6.5 Hz, 1H, C_7–H), 7.54 (d, J = 6.5 Hz, 1H, C_8–H), 7.48 (m, 2H, C_{19}–H), 7.30 (m, 2H, C_{18}–H), 7.16 (m, 1H, C_{20}–H), 4.76 (s, 1H, NH), 4.32 (s, 2H, C_{16}–H), 4.03 (s, 2H, C_{12}–H), 3.96 (s, 2H, C_{15}–H); ^{13}C-NMR (DMSO-d_6): δ 162.7 (C_{14}), 161.5 (C_{17}), 139.4 (C_1, C_{13}), 129.4 (C_4, C_6), 129.3 (C_9), 129.1 (C_2), 128.8 (C_5), 128.7 (C_1), 128.4 (C_{19}), 128.3 (C_{18}), 128.1 (C_{20}), 127.6 (C_7), 127.6 (C_{10}), 127.4 (C_3), 127.3 (C_8), 64.4 (C_{16}), 36.2 (C_{15}), 31.2 (C_{12}). Anal. Calcd. for $C_{22}H_{17}Cl_2NO$ (382.28): C, 69.12; H, 4.48; N, 3.66. Found: C, 68.90; H, 4.29; N, 3.45%.

4.18. 1-(2,7-Dichloro-9H-fluoren-4-yl)-2-(diethylamino)ethanone (4n)

Green crystals, yield 90%, mp 194–195 °C; FT-IR (KBr): ν (cm^{-1}) 3069 (CH arom.), 2930 (CH aliph.), 1666 (C=O); ^{1}H-NMR (DMSO-d_6): δ 7.95 (br s, 2H, C_1–H, C_3–H), 7.63–7.58 (s, 1H, C_7–H), 7.36–7.34 (s, 1H,

C_{10}–H), 6.67 (d, $J = 7.5$ Hz, 1H, C_8–H), 4.01 (s, 2H, C_{12}–H), 3.95 (s, 2H, C_{15}–H), 3.59 (q, $J = 10.0$ Hz, 4H, C_{16}–H), 1.15 (t, $J = 10.0$ Hz, 6H, C_{17}–H); ^{13}C-NMR (DMSO-d_6): δ 162.7 (C_{14}), 146.5 (C_{11}, C_{13}), 138.3 (C_6), 136.3 (C_4), 132.1 (C_9), 130.1 (C_2, C_5), 128.3 (C_1), 127.4 (C_7), 125.9 (C_{10}), 119.1 (C_3), 113.1 (C_8), 65.7 (C_{16}), 46.4 (C_{15}), 36.2 (C_{12}), 11.5 (C_{17}). Anal. Calcd. for $C_{19}H_{19}Cl_2NO$ (348.27): C, 65.53; H, 5.50; N, 4.02. Found: C, 65.40; H, 5.59; N, 4.10%.

4.19. 1-(2,7-Dichloro-9H-fluoren-4-yl)-2-(piperidin-1-yl)ethanone (4°)

Green crystals, yield 84%, mp 293–294 °C; FT-IR (KBr): ν (cm^{-1}) 3069 (CH arom.), 2859 (CH aliph.), 1669 (C=O); ^{1}H-NMR (DMSO-d_6): δ 8.41 (d, $J = 7.5$ Hz, 1H, C_3–H), 7.95 (s, 2H, C_1–H, C_{10}–H), 7.69–7.62 (m, 2H, C_7–H, C_8–H), 3.99 (s, 2H, C_{12}–H), 3.94 (s, 2H, C_{15}–H), 3.34–3.26 (m, 4H, C_{16}–H), 1.64–1.62 (m, 2H, C_{18}–H), 1.47–1.38 (m, 4H, C_{17}–H); ^{13}C-NMR (DMSO-d_6): δ 162.7 (C_{14}), 147.0 (C_{11}, C_{13}), 138.4 (C_4, C_6), 133.2 (C_2, C_9), 131.0 (C_5), 128.3 (C_1), 127.4 (C_7), 126.4 (C_{10}), 125.2 (C3, C_8), 46.2 (C_{15}), 44.0 (C_{16}), 36.2 (C_{12}), 31.2 (C_{17}), 22.1 (C_{18}). Anal. Calcd. for $C_{20}H_{19}Cl_2NO$ (360.28): C, 66.67; H, 5.32; N, 3.89. Found: C, 66.49; H, 5.25; N, 3.70%.

4.20. 1-(2,7-Dichloro-9H-fluoren-4-yl)-2-morpholinoethanone (4p)

Green crystals, yield 86%, mp 190–191 °C; FT-IR (KBr): ν (cm^{-1}) 3069 (CH arom.), 2923 (CH aliph.), 1663 (C=O); ^{1}H-NMR (DMSO-d_6): δ 8.40 (s, 1H, C_3–H), 8.02 (s, 1H, C_1–H), 7.95 (s, 1H, C_{10}–H), 7.65–7.63 (m, 2H, C_7–H, C_8–H), 4.01 (s, 2H, C_{12}–H), 3.96 (s, 2H, C_{15}–H), 3.76–3.74 (m, 4H, C_{17}–H), 3.08–3.06 (m, 4H, C_{16}–H); ^{13}C-NMR (DMSO-d_6): δ 162.8 (C_{14}), 147.7 (C_{11}), 145.5 (C_{13}), 139.3 (C_6), 136.3 (C_4), 132.1 (C_9), 131.8 (C_2, C_5), 128.3 (C1), 127.4 (C7), 123.8 (C10), 122.0 (C3), 119.7 (C8), 66.2 (C_{17}), 64.0 (C_{16}), 45.5 (C_{15}), 34.7 (C_{12}). Anal. Calcd. for $C_{19}H_{17}Cl_2NO_2$ (362.25): C, 63.00; H, 4.73; N, 3.87. Found: C, 62.80; H, 4.56; N, 3.70%.

4.21. 1-(2,7-Dichloro-9H-fluoren-4-yl)-2-(4-phenylpiperazin-1-yl) ethanone (4q)

Green crystals, yield 90%, mp 119–120 °C; FT-IR (KBr): ν (cm^{-1}) 3071 (CH arom.), 2900 (CH aliph.), 1697 (C=O); ^{1}H-NMR (DMSO-d_6): δ 8.34 (d, $J = 7.5$ Hz, 1H, C_3–H), 8.08 (s, 1H, C_1–H), 7.84–7.83 (m, 1H, C_7–H), 7.76 (m, 1H, C_8–H), 7.67 (s, 1H, C_{10}–H), 7.54–7.44 (m, 2H, C_{20}–H), 7.24–7.21 (m, 1H, C_{21}–H), 6.99–6.80 (m, 2H, C_{19}–H), 4.00 (s, 2H, C_{12}–H), 3.51 (s, 2H, C_{15}–H), 3.14–3.08 (m, 4H, C_{17}–H), 2.76–2.71 (m, 4H, C_{16}–H); ^{13}C-NMR (DMSO-d_6): δ 168.3 (C_{14}), 151.3 (C_{18}), 147.6 (C_{11}), 146.8 (C_{13}), 138.0 (C_4, C_6), 132.8 (C_2, C_9), 131.5 (C_5), 129.5 (C_{20}), 128.5 (C_1), 127.4 (C_7), 127.3 (C_{10}), 126.4 (C_3, C_8), 120.0 (C_{21}), 116.7 (C_{19}), 49.9 (C_{15}), 48.7 (C_{16}), 45.0 (C_{17}), 36.8 (C_{12}). Anal. Calcd. for $C_{25}H_{22}Cl_2N_2O$ (437.36): C, 68.65; H, 5.07; N, 6.41. Found: C, 68.42; H, 4.90; N, 6.26%.

4.22. 1-(2,7-Dichloro-9H-fluoren-4-yl)-2-(4-p-tolylpiperazin-1-yl) ethanone (4r)

Orange crystals, yield 93%, mp 125–126 °C; FT-IR (KBr): ν (cm^{-1}) 3068 (CH arom.), 2922 (CH aliph.), 1678 (C=O); ^{1}H-NMR (DMSO-d_6): δ 8.34 (s, 1H, C_3–H), 8.06 (s, 1H, C_1–H), 7.76 (d, $J = 7.5$ Hz, 1H, C_7–H), 7.65 (s, 1H, C_{10}–H), 7.42 (d, $J = 7.5$ Hz, 1H, C_8–H), 7.01–6.76 (m, 4H, C_{19}–H, C_{20}–H), 3.97 (s, 2H, C_{12}–H), 3.93 (s, 2H, C_{15}–H), 3.32–3.30 (m, 4H, C_{17}–H), 3.04–2.97 (m, 4H, C_{16}–H), 2.18 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6): δ 168.3 (C_{14}), 149.2 (C_{18}), 147.2 (C_{11}), 146.7 (C_{13}), 137.8 (C_4, C_6), 132.8 (C_9), 132.0 (C_2), 129.9 (C_5), 128.5 (C_1), 128.2 (C_{21}), 127.7 (C_7), 126.4 (C_{10}), 125.9 (C_3), 125.3 (C_8), 124.9 (C_{20}), 116.9 (C_{19}), 50.4 (C_{15}), 49.2 (C_{16}), 45.1 (C_{17}), 36.8 (C_{12}), 20.5 (CH_3). Anal. Calcd. for $C_{26}H_{24}Cl_2N_2O$ (451.39): C, 69.18; H, 5.36; N, 6.21. Found: C, 69.00; H, 5.15; N, 6.00%.

4.23. 1-(2,7-Dichloro-9H-fluoren-4-yl)-2-(4-(4-(trifluoromethyl)phenyl) piperazin-1-yl)ethanone (4s)

Orange crystals, yield 96%, mp 170–171 °C; FT-IR (KBr): ν (cm^{-1}) 3065 (CH arom.), 2922 (CH aliph.), 1701 (C=O); ^{1}H-NMR (DMSO-d_6): δ 8.10 (s, 1H, C_3–H), 8.03–8.00 (s, 1H, C_1–H), 7.86–7.78 (m, 1H, C_7–H), 7.65 (s, 1H, C_{10}–H), 7.50 (d, $J = 8.5$ Hz, 2H, C_{20}–H), 7.40 (m, 1H, C_8–H), 7.17–7.06 (m, 2H, C_{19}–H), 3.98 (s, 2H, C_{12}–H), 3.94 (s, 2H, C_{15}–H), 3.55–3.49 (m, 4H, C_{17}–H), 3.30–3.19 (m, 4H, C_{16}–H); ^{13}C-NMR (DMSO-d_6): δ 168.2 (C_{14}), 153.4 (C_{18}), 147.0 (C_{11}), 146.7 (C_{13}), 137.9 (C_4, C_6), 137.6 (C_{21}), 131.8 (C_2, C_9), 131.7 (C_5), 129.1 (C_{20}), 128.5 (C_1), 127.7 (C_7), 126.4 (C_{10}), 125.4 (C_3), 125.3 (C_8), 124.9 (CF_3), 115.3 (C_{19}), 51.8 (C_{15}), 48.4 (C_{16}), 44.6 (C_{17}), 36.7 (C_{12}). Anal. Calcd. for $C_{26}H_{21}Cl_2F_3N_2O$ (505.36): C, 61.79; H, 4.19; N, 5.54. Found: C, 61.58; H, 3.92; N, 5.25%.

4.24. 1-(2,7-Dichloro-9H-fluoren-4-yl)-2-(4-(4-hydroxyphenyl)piperazin-1-yl)ethanone (4t)

Orange crystals, yield 88%, mp 135–137 °C; FT-IR (KBr): ν (cm^{-1}) 3400 (OH), 3072 (CH arom.), 2930 (CH aliph.), 1709 (C=O); ^{1}H-NMR (DMSO-d_6): δ 8.35 (s, 1H, C_3–H), 8.02 (s, 1H, C_1–H), 7.94 (s, 1H, C_{10}–H), 7.82 (d, $J = 7.5$ Hz, 1H, C_7–H), 7.72–7.57 (m, 2H, C_{20}–H), 7.42–7.38 (m, 1H, C_8–H), 6.72 (d, $J = 9.5$ Hz, 2H, C_{19}–H), 5.80 (s, 1H, OH), 4.00 (s, 2H, C_{12}–H), 3.95 (s, 2H, C_{15}–H), 3.55–3.49 (m, 4H, C_{17}–H), 3.29–3.18 (m, 4H, C_{16}–H); ^{13}C-NMR (DMSO-d_6): δ 168.3 (C_{14}), 148.3 (C_{11}), 147.7 (C_{21}), 147.5 (C_{18}), 146.7 (C_{13}), 137.5 (C_6), 136.7 (C_4), 133.4 (C_9), 133.0 (C_2), 131.7 (C_5), 128.9 (C_1), 127.2 (C_7), 127.1 (C_{10}), 125.5 (C_3), 125.1 (C_8), 124.9 (C_{20}), 116.0 (C_{19}), 49.9 (C_{15}), 49.8 (C_{16}), 40.6 (C_{17}), 36.7 (C_{12}). Anal. Calcd. for $C_{25}H_{22}Cl_2N_2O_2$ (453.36): C, 66.23; H, 4.89; N, 6.18. Found: C, 66.00; H, 4.61; N, 5.97%.

4.25. 2-(4-(4-Acetylphenyl)piperazin-1-yl)-1-(2,7-dichloro-9H-fluoren-4-yl)ethanone (4u)

Pale yellow crystals, yield 88%, mp 118–119 °C; FT-IR (KBr): ν (cm^{-1}) 3069 (CH arom.), 2925 (CH aliph.), 1702 (C=O), 1666 (C=O); ^{1}H-NMR (DMSO-d_6): δ 8.31 (s, 1H, C_3–H), 8.09 (s, 1H, C_1–H), 7.99–7.91 (m, 1H, C_7–H), 7.79 (s, 1H, C_{10}–H), 7.66–7.65 (m, 2H, C_{20}–H), 7.42–7.40 (m, 1H, C_8–H), 7.04–6.96 (m, 2H, C_{19}–H), 4.00 (s, 2H, C_{12}–H), 3.97 (s, 2H, C_{15}–H), 3.58–3.50 (m, 4H, C_{17}–H), 3.47–3.37 (m, 4H, C_{16}–H), 2.44 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6): δ 196.1 (C=O), 168.3 (C_{14}), 153.4 (C_{18}), 147.2 (C_{11}), 146.8 (C_{13}), 137.5 (C_6), 137.2 (C_4), 132.8 (C_9), 132.6 (C_2), 131.5 (C_5), 130.5 (C_{21}), 130.1 (C_{20}), 128.2 (C_1), 127.2 (C_7, C_{10}), 125.5 (C_3), 125.4 (C_8), 114.3 (C_{19}), 67.8 (C_{15}), 46.8 (C_{16}), 44.3 (C_{17}), 36.8 (C_{12}), 26.5 (CH_3). Anal. Calcd. for $C_{27}H_{24}Cl_2N_2O_2$ (479.40): C, 67.65; H, 5.05; N, 5.84. Found: C, 67.49; H, 4.90; N, 5.62%.

4.26. Antimicrobial screening

All microbial strains were provided from culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The antimicrobial activities were evaluated by disc-agar diffusion method [25, 26, 27]. The efficacy of the novel compounds was examined against different strains of gram-positive and gram-negative bacteria, also evaluated against different fungal strains. The Gram-positive organisms that were used for culture sensitivity include *S. aureus* (RCMB010010), and *B. subtilis RCMB* 015 (1) NRRL B-543, on the other hand, the Gram-negative organisms that were used for culture sensitivity include *E. coli* (RCMB 010052) ATCC 25955, and *P. vulgaris* RCMB 004 (1) ATCC 13315. The fungal strains that were used include *Aspergillus fumigatus* (RCMB 002008), and *Candida albicans* RCMB 005003 (1) ATCC 10231. Different antibiotics were used as a reference for evaluating the antimicrobial activity of novel compounds. Gentamycin and Ketoconazol were used as a reference antibiotic for assessing the antimicrobial activity of the novel compounds against bacterial and fungal strains respectively.

4.27. In vitro anticancer screening

The cell lines were purchased from the American Type Culture collection and their accession number as follows: A-549 (ATCC CCL-185™) lung carcinoma cell line and MCF-7 (ATCC HTB-22™) breast adenocarcinoma cell line. Cytotoxic activity screening was performed at Regional Center for Mycology and Biotechnology, Al-Azhar University, according to the suggested method of Skehan *et al.* [28]. Cells were plated in 96-multiwell micro titer plates (10^4 cells/well) for 24 h before treatment with the compound(s) to allow attachment of cells to the wall of the plate. Test compounds dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compound under test (500, 250, 125, 62.50, 31.25, 15.60, 7.8, 3.9, 2, 1 and 0 μg/mL) were added to the cell monolayer. The cytotoxicity was estimated by IC_{50} in μM; the concentration that inhibits 50% of growth of cancer cell was presented in Tables 2 and 3.

4.28. Docking assay

Docking were done in the Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy (Girls), Al-Azhar University, Egypt, as computational software using Protein Data Bank. 5-Fluorouracil (5-FU) [act as a potent inhibitor] (PDB ID: 1BID) bound to dihydrofolate reductase (PDB ID: 4DFR).

Docking on the Active Site of Dihydrofolate Reductase (DHFR).

Improvement of a model of the dihydrofolate reductase-binding site was done by selection of the 3D structure of DHFR complexed with the 5-Fluorouracil as a potent inhibitor (PDB ID: 4DFR).

Declarations

Author contribution statement

Saleh A. Ahmed, Essam M. Hussein: Conceived and designed the experiments, performed the experiments, analyzed and interpreted the data and wrote the paper.

Reem I. Alsantali: Performed the experiments, contributed reagents, materials, analysis tools or data.

Shimaa M. Abd El-Galil, Mohamed A. S. Abourehab: Analyzed and interpreted the data.

Rami J. Obaid, Ahmed Alharbi: Contributed reagents, materials, analysis tools or data.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2019.e01456.

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