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Evaluation of the anticancer activity of monoamidinic arylthiophenes

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Abstract: Hepatocellular carcinoma (HCC) is the sixth most common cancer and the second leading cause of cancer deaths worldwide. The anticancer drugs being used are losing their effectiveness due to development of anticancer-drug resistance and have severe side effects, poor therapeutic index and high cost. This work was designed to study the anticancer effect of new monocationic arylthiophene derivatives against HCC. The cell viability was initially determined by treating Huh-7 cells with 50 µM concentration of each tested arylthiophene compound. Subsequently, the IC_{50} values for the most active compounds were determined using MTT assay. The monocationic derivative **2j** exhibit the highest cytotoxicity (IC_{50} = 1.47 ± 0.25 µM) in comparison to the positive control cisplatin (IC_{50} = 24.9 ± 1.4 µM). The selectivity index of compound **2j** (SI = 7.25 ± 0.11) proved the safety to normal lung fibroblast cells (WI-38) *vs* Huh-7 cells.

keywords: Hepatocellular carcinoma, cationic compounds, arylthiophenes, cytotoxicity, selectivity index.

1.Introduction

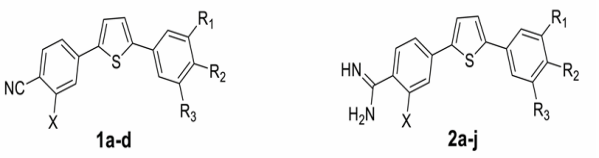
Hepatocellular carcinoma (HCC) is the most common form of primary malignancy of liver [1]. HCC is the sixth most common cancer and the second leading cause of cancer deaths worldwide [1]. In Egypt, HCC is the fourth most common form of cancer and is the second leading cause of cancer-related deaths [2,3]. Egypt has the highest incidence of hepatitis C virus (HCV) which is the major cause of HCC development followed by aflatoxin exposure, and chronic alcohol consumption [4].

Sulfur-containing molecules are well known for having a broad spectrum of biological activities. For example, thiophene containing molecules proved to be antimicrobial [5-7], antitumor [8-10], analgesic and anti-inflammatory [11], antihypertensive [12], anti-diabetes [13], inhibitory activity of cholesterol [14], anti-allergic [15], insecticide [16] and antioxidant [17]. Introduction of fluorine atom can alter the biological and chemical properties of the compounds due to the high electronegativity of fluorine atom which alters the electron distribution in the molecules affecting their absorption, distribution, and metabolism [18]. Also, fluorine atom introduction increases lipophilicity and bio-availability [19]. Recently, some cationic heterocyclic compounds including chalcophene derivatives have been reported for their anticancer activity [20, 21]. Recently, many compounds of monocationic arylthiophenes have been reported for their potent antiproliferative activity against a panel of 60 human cancer cell lines (representing nine types of cancer) [22]. The present work aims to examine these cationic arylthiophene derivatives for possible use as anticancer agents against HCC.

2. Materials and Methods

The tested arylthiophenes (Fig 1) were provided by Ismail, M.A. [22], and were dissolved in dimethyl sulfoxide (DMSO, Cat. No. 20385.01) then stored at -20°C tell use. Cisplatin was obtained from Sigma-Aldrich (St Louis, MO, USA). 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT, Cat. No. 002039501) [SERVA Electrophores GmbH, Germany]. Trypsin-Versene (EDTA, Cat. No. 17-161E) and

Dulbecco's Modified Eagle's Medium (DMEM, Cat. No. 12-604F) were purchased from Lonza BioWhittaker™. The culture medium was supplemented with 10% Fetal Bovine Serum (Cat. No. 14-801EV, Life Science Group L, UK, origin Brazil), 1% antibiotic (penicillin (1000 IU/ml) and streptomycin (1000µg/ml), Cat. No. 17-602E) from Lonza BioWhittaker™. Huh-7 and WI-38 cell lines were obtained from VACSERA, Holding company for biological products and vaccines, Cairo, Egypt.



Code:	X	R_1	R_2	R_3
1a	H	H	$N(Me)_2$	H
b	H	H	Cl	H
c	F	OCH_3	H	OCH_3
d	F	H	$N(Me)_2$	H
e	H	H	OCH_3	H
f	F	H	H	H

Code:	X	R_1	R_2	R_3
2a	F	H	H	H
b	F	H	OCH_3	H
c	F	OCH_3	H	OCH_3
d	F	H	$N(Me)_2$	H
e	F	H	Cl	H
f	H	H	OCH_3	H
g	H	OCH_3	H	OCH_3
h	H	H	$N(Me)_2$	H
i	H	H	Cl	H
j	H	Cl	H	Cl

Fig 1: The tested 2,5-diarylthiophene derivatives.

Anticancer activity

2.1. Initial screening

This experiment aims to investigate the anticancer effect of the tested compounds against Huh-7 cell line. The initial screening was performed by seeding Huh-7 cells at a density of 2×10^4 cells/ml in a 96-well plate (100 µl/well). After incubation overnight at 5% CO_2 and 37°C, the cells were treated with 50 µM of each of the tested compounds then the cells were incubated for 48 hours at 5% CO_2 and 37°C. After treatment, cells were stained with 0.5% crystal violet/0.25% methanol for 10 minutes then washed with water until no more stain was seen in the washing solution. Thereafter, 200 µl of methanol was added to the stained cells and the plate was shaken before reading the absorbance at λ_{570} nm by Biotek microplate reader. Cisplatin was used as a positive control and 0.5% DMSO was used as a negative control. Data are the average of triplicate analyses.

2.2. Cytotoxicity assay

After the *in vitro* initial screening on Huh-7 cells, the most active compounds were selected for the determination of IC_{50}, the concentration that kills 50% of the cells [23]. Huh-7 cells were seeded in a 96-well plate at a density of 5×10^4 cells/ml (100 µl/well) and incubated overnight at 37°C and 5% CO_2. After that, different concentrations (50, 25, 12.5, 6.25, 3.125 & 1.56 µM) of each of the tested arylthiophenes were added to the cells and incubated for 48 hours at 37°C and 5% CO_2. After treatment, 10 µl of MTT solution (5 mg/ml in 1X PBS) was added and incubated for 4 hours. Then, the purple formazan crystals formed were dissolved by adding 100 µl of 10% SDS solution (10% SDS/0.01 N HCl in 1X PBS). After 14 hours of incubation at 37°C and 5% CO_2, the absorbance was measured at λ_{570} nm using Biotek microplate reader. The cells were monitored and the images were acquired by Gx microscopes (GXMGXD202 Inverted Microscope) (10x Eyepiece). Positive control was treated with cisplatin. Negative control was treated with 0.5% DMSO. Data are represented as the average of triplicate independent experiments.

2.3. Selectivity index determination

The selectivity of the most active compounds towards cancer cells was evaluated by incubating serial dilutions (50, 25, 12.5, 6.25, 3.125 & 1.56 µM) of the compounds with the normal lung fibroblast cells (WI-38). After 48 hours of incubation, the viability of normal cells was quantified by MTT assay as mentioned above. The selectivity index was calculated [24-26].

3. Results

3.1. Anticancer activity

The cytotoxic activity of sixteen compounds was tested initially on Huh-7 cell line. The results showed that ten compounds out of sixteen were cytotoxic (the viable cells were less than 50%) (Table 1) and the majority of the active cytotoxic compounds are cationic derivatives (2a-j) and one active carbonitrile derivative 1a. The IC_{50} values of the compounds were determined and presented in Table 2. Compound 3,5-dichlorophenyl derivative 2j retain the highest cytotoxicity against Huh-7 cells with IC_{50} value of 1.47 ±

0.25 µM (Fig 2) while the IC_{50} for the positive control cisplatin was 24.9 ± 1.4 µM. Fig 3 and Fig 4 showed the microscopic pictures of Huh-7 cells treated with compounds 2j and 2h respectively, where all the cells are died at concentrations higher than the IC_{50} values. Seven monocationic derivatives of the tested arylthiophenes exhibited a good anticancer activity against Huh-7 cells with IC_{50} values lower than 6 µM. The carbonitrile compound **1a** showed the least activity with IC_{50} value of 10.1 ± 0.8 µM which prove the reported the waek anticancer activity of carbonitrile derivatives when compared to their cationic counterparts [20,27].

Table 1: Cell Viability (%) of Huh-7 cells treated with the tested compounds with a concentration of 50 µM. Cisplatin was used as a positive control. ± is the standard deviation obtained from triplicate experiment.

Carbonitrile derivatives1a-f		**Cationic derivatives 2a-h**	
Compd #	Percentage of viable cells (%)	Compd #	Percentage of viable cells (%)
cisplatin	8.9 ± 0.22.	**2a**	26.9 ± 0.46
1a	16.5 ± 0.9	**2b**	20.68 ± 0.52
1b	100 ± 1.6	**2c**	27.8 ± 0.66
1c	79 ± 1.3	**2d**	12.4 ± 0.35
1d	100 ± 1	**2e**	39.3 ± 0.55
1e	100 ± 1.8	**2f**	16 ± 0.37
1f	100 ± 1.53	**2g**	36.5 ± 0.84
		2h	17.2 ± 0.4

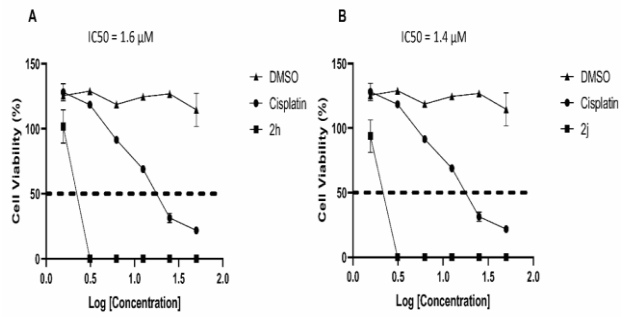


Fig 2: Cytotoxicity of the tested compounds against Huh-7 cells. The Huh-7 cells were cultured and incubated overnight then treated with different concentrations of arylthiophene derivatives and incubated for 48 hours. After incubation, MTT solution was added and incubated for another 4 hours, then, 100 µl of 10% SDS solution was used to dissolve the purple formazan crystals. The absorbance was measured at λ_{570} nm. Cisplatin was used as a positive control. DMSO (0.5%) was used as a negative control. A) IC_{50} for the compound **2h**. B) IC_{50} for the compound **2j**.

Table 2: IC_{50} (µM) for the tested arylthiophene compounds. Cisplatin was used as a positive control. ± is the standard deviation obtained from triplicate experiment.

Compd#	**IC_{50} on Huh-7 (µM)**
Cisplatin	24.9 ± 1.4
1a	10.1 ± 0.8
2a	7.5 ± 1
2b	5.76 ± 0.42
2c	6 ± 0.36
2d	2.59 ± 0.27
2e	3.55 ± 0.67
2f	2.33 ± 0.54
2g	5.5 ± 0.5
2h	1.6 ± 0.23
2j	1.47 ± 0.25

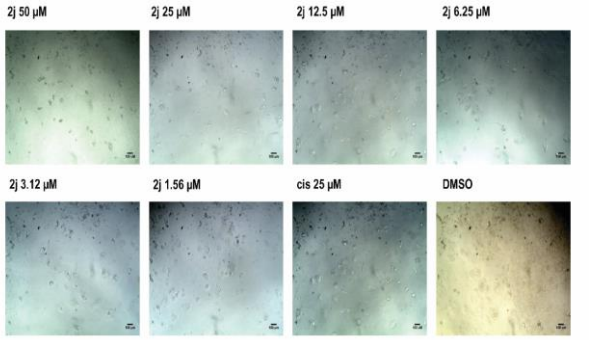


Fig 3: Microscopic pictures of Huh-7 cells treated with different concentrations (50, 25, 12.5, 6.25, 3.12 & 1.56 µM) of the monocationic arylthiophene 2j (IC_{50} = 1.47 ± 0.25 µM). Cisplatin (IC_{50} = 24.9 ± 1.4 µM) was used as a positive control. DMSO (0.5%) was used as a negative control. The images were processed by ImageJ software.

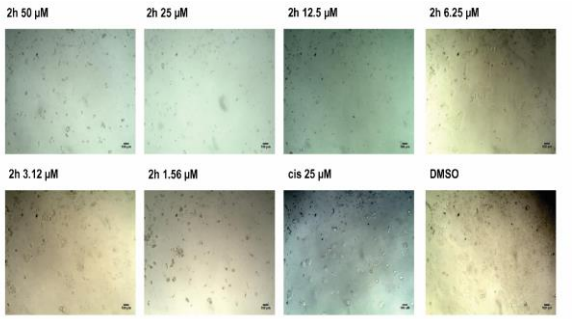


Fig 4: Microscopic pictures of Huh-7 cells treated with different concentrations (50, 25, 12.5, 6.25, 3.12 & 1.56 µM) of the monocationic arylthiophene **2h** (IC_{50} = 1.6 ± 0.23 µM). Cisplatin (IC_{50} = 24.9 ± 1.4 µM) was used as a positive control. DMSO (0.5%) was used as a negative control. The images were

processed by ImageJ software.

3.2. Selectivity index

To test the selectivity of the investigated arylthiophenes toward the liver cancer cells rather than normal cells, the most active compounds were incubated with normal cells (WI-38) and the viability was determined by MTT assay. The tested arylthiophenes **2e**, **2f** , **2h** & **2j** have selectivity indices higher than 2 (4.69 ± 0.13, 4.18 ± 0.02, 3.125 ± 0.03 & 7.25± 0.11, respectively) (Table 3) which indicates that these compounds are safe and selective toward cancer cells rather than normal cells and worth further investigation for their mechanism of action and *in vivo* studies.

Table 3: In vitro cytotoxicity of tested arylthiophenes against human lung fibroblasts (WI-38) and Huh-7 cell lines. The selectivity index was calculated. ± is the standard deviation obtained from triplicate experiment.

Compound	IC_{50} on Huh-7 (µM)	IC_{50} on WI-38 (µM)	Selectivity Index
Cisplatin	24.9 ± 1.4	10 ± 1.5	0.4 ± 0.03
2a	7.5 ± 1	4.43 ± 0.66	0.59 ± 0.008
2b	5.76 ± 0.15	8.2 ± 0.32	1.42 ± 0.02
2c	6 ± 0.36	6 ± 0.6	1 ± 0.03
2d	2.59 ± 0.27	5 ± 0.57	1.93 ± 0.08
2e	3.55 ± 0.28	16.66 ± 1.8	4.69 ± 0.13
2f	2.33 ± 0.18	9.75 ± 0.8	4.18 ± 0.02
2h	1.6 ± 0.13	5 ± 0.46	3.125 ± 0.03
2j	1.47 ± 0.1	10.66 ± 0.9	7.25 ± 0.11

4. Discussion

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the second leading cause of deaths related to cancer worldwide. The anticancer drugs being used are losing their effectiveness due to the development of drug resistance and have severe side effects, poor therapeutic index and high cost. In this study, the new monocationic 2,5-diarylthiophenes were tested for their anticancer activity against Huh-7 cell line. The tested compounds exhibited a potent anticancer effect against hepatocellular carcinoma Huh-7 cell line. Moreover, the most active cationic arylthiophene derivatives showed selectivity towards the cancer cells rather than normal cells. The potent activity of these tested compounds against hepatocellular carcinoma Huh-7 cell line was in agreement with the reported anticancer activity against the tested NCI-60 Human cancer cell lines [22]. In conclusion, a group of monocationic arylthiophenes was tested for their anticancer activity against hepatocellular carcinoma. The investigated cationic derivatives showed a good cytotoxic activity against Huh-7 cell line and these derivatives were safe to normal cells. The data reported herein confirmed the anticancer activity of the tested cationic arythiophenes and recommends for further *in vitro* and *in vivo* studies

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