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Determination of anticancer and antibacterial activities of disubstituted tacrine derivatives

Salih Ökten*[1], Ali Aydin[2], Ahmet Tutar[3]

Abstract

The present study describes the biological features of disubstituted tacrine derivatives using cell proliferation and cell cytotoxicity assays. The abilities of tacrine derivatives to inhibit microbial growth and to interact with DNA were also investigated. Here, the tested compounds (**1**-**4**) exhibited selective antiproliferative activity against the cancer cell lines (IC_{50} values 1.1 – 38.9 µg/mL) and showed a similar non-toxic property to cells such as positive control (percent cytotoxicity 7% - 27%). Studies on human pathogenic bacteria showed that the novel tacrine analogues exhibited significant antimicrobial activities between concentrations of 31.25 µg/mL and 250 µg/mL. The data show that they can bind to DNA with the groove binding mode with K_b range of 7.4×10^4 - 2.9×10^4 M^{-1}. As a result, the preliminary data showed that disubstituted tacrine derivatives exhibited effective pharmacological properties.

Keywords: tacrine; anticancer; cytotoxcity; antibacterial

1. INTRODUCTION

Cancer is an enormous threatening problem for the human health in the World due to lead to uncontrolled growth and division of cells to invade other tissues and organs by spreading to the body through blood [1].

Tacrine (**1**, 9-amino-1,2,3,4-tetrahydroacridine) and its derivatives, the class of well-known bioactive compounds, have been commonly used as antimalarial and antibacterial agents [2-4]. Also tacrine has reversibly inhibited the acetylcholinesterase enzyme and was the first approved cholinesterase inhibitor drug, tested clinically for the treatment of Alzheimer's disease (AD) [5-8]. However, tacrine was withdrawn due to its hepatotoxicity and serious side effects on Alzheimer's patients. [9-13]. In recent years, some studies have been focused on determining the anticancer effects of tacrines due to that its derivatives can inhibit topoisomerase emzyme and block the transcription of DNA [4].

Tacrine is similar to the planar acridine moiety [14-15] and has a cyclohexyl-fused quinoline structure. Its pharmacophore is known ability of to interact with DNA [16] and its platinum(II) complexes might be potential telomerase inhibitors [17-20] and have been reported to trigger cell senescence and apoptosis [21-25]. Some reports showed different substituted tacrines bearing penta or hepta hydrocycle have antitumor activity. For example, a pentacyclic acridine, RHPS4 (3,11-difluoro-6,8,13-trimethyl(8H)-quino[4,3,2-kl]acridinium methylsulfate) was reported to induce tumor cell apoptosis via inhibiting the telomerase activity [22-23]. Tacrine is a quindoline derivative with a large planar aromatic conjugated system [26-27]. In addition, the natural product quindolines were determined as a potent telomerase inhibitor [24,28].

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In our recent studies, a series of disubstituted tacrine derivatives were prepared by Friedlander reaction and metal induced substitution reaction [29] and we reported their cholinesterase and carbonic anhydrase enzymes' inhibition activities [30-31]. In this study, prepared disubstituted tacrines bearing six and seven membered hydrocycle were tested for anticancer activities against HT29 (Human colorectal adenocarcinoma), HeLa (Human cervix adenocarcinoma), MCF7 (Human breast adenocarcinoma), A549 (Human lung carcinoma), and Hep3B (Human hepatocellular carcinoma) cancer cell lines and FL (Human amnion cells) normal cell line by MTT cell proliferation assay and also determined their antibacterial activities by microdilution assay.

2. MATERIALS AND METHODS

2.1. Synthesis of compounds (1-4)

This study was carried out with disubstituted tacrine derivatives (**1-4**) according to our previous paper [29].

2.2. MTT Cell Proliferation Assay

HT29 (Human colorectal adenocarcinoma), HeLa (Human cervix adenocarcinoma), MCF7 (Human breast adenocarcinoma), A549 (Human lung carcinoma), and Hep3B (Human hepatocellular carcinoma) cancer cell lines and FL (Human amnion cells) normal cell line were maintained in a suitable medium containing fetal bovine serum and antibiotic solution. A Cell suspension was adjusted 1×10^6 cells in 10 mL and transferred 100 µL into each well of culture plates. The compounds were dissolved in sterile DMSO at final concentrations of 10-200 µg/mL and transferred the cells at 37 °C with 5% CO_2 for overnight. The antitumor activities of the compounds were determined using MTT cell proliferation assay. In MTT assay, the percent inhibitions of test and control molecules were determined. The percent inhibition was equal % inhabitations with following formula;

$$1- \frac{\text{Absorbance of Treatments}}{\text{Absorbance of DMSO}} \times 100$$

The IC_{50} values (half maximal inhibitory concentration) of the compounds were obtained by using Excel software and noted in µg/mL at 95 % confidence intervals. The dose response parameters (GI_{50}, TGI, LC_{50}) were calculated according to the following formulas using an Excel software. Growth inhibition of 50% (GI_{50}) was calculated from following equation:

$$\frac{(Ti-Tz)}{(C-Tz)} \times 100 = 50$$

This formula is the drug concentration resulting in a 50% reduction in the net growth increase in control cells during the drug incubation. The total growth inhibition (TGI) was calculated from Ti = Tz. The LC_{50} indicating a net loss of treated cells was calculated from following equation:

$$\frac{(Ti-Tz)}{Tz} \times 100 = -50$$

2.3. Cytotoxicity Assay

The cytotoxic potentials of the compounds were determined by cytosolic LDH measurement kit according to manufacturer's procedures. Briefly, 5×10^3 cells were conveyed into each well as triplicates and exposed with IC_{50} concentrations of the compounds at 37 °C with 5% CO_2 for overnight. The percentage cytotoxicities were obtained by using the following equation,

$$\frac{(\text{experimental value - low control})}{(\text{high control - low control})} \times 100$$

where experimental value is the cells treated with test compound, high control (maximum LDH release) is Triton X-100 treated cells, low control (spontaneous LDH release) is the untreated cells.

2.4. DNA binding studies

The binding constants (K_b) against calf thymus DNA and physiological interactions of disubstituted tacrine derivatives were examined by using UV–visible absorption spectroscopy technique. To prepare stock calf thymus DNA solution, a 2.5 mg DNA was dissolved in 10.0 mL Tris–HCl buffer (20 mM Tris–HCl, 20 mM NaCl, pH 7.0) and store at +4 °C for up to seven days. DNA concentration in solution was calculated by using Ɛ value (6600 M^{-1} cm^{-1} at 260 nm) that belong to DNA. In addition, the purity of calf thymus DNA solution was controlled with the help of change of absorbance obtained from the ratio of A260/A280. Since the value was equal to 1.87, the DNA was considered to be sufficiently pure. To obtain 25 µM of working solution, disubstituted tacrine derivatives were diluted with Tris–HCl buffer and then all of the compounds were incubated at 24 °C for 30 min before the measurement. To ensure sufficient solubility in solution throughout measurement were prepared a special solvent system (1/9 DMSO/Tris–HCl buffer). Eight measurement points at room temperature for disubstituted tacrine derivatives were

recorded by using 1-cm-path quartz cuvettes. The amount of disubstituted tacrine derivatives was kept constant while increasing the CT-DNA concentrations (6.5-800 μM) in the UV absorption titrations.

2.5. Microdilution assay

The minimal inhibitory concentration (MIC) values of disubstituted tacrine derivatives towards some human bacterial strains were examined with the help of a micro-well dilution method. According to this method, inocula of bacteria were obtained using 12 h LB broth cultures. The optical density at 600 nm (OD600) was adjusted to 0.08–0.1 and 0.5 McFarland bacterial suspensions were obtained. Each disubstituted tacrine derivative was dissolved in dimethyl sulfoxide (20 mg/mL). A concentration gradient range from 7.81–1000 μg/mL in uncovered microplate wells containing nutrient broth was made by using serial two-fold dilutions of these compounds. This plate was inoculated with bacteria and incubated at 35 °C for 24 hours. At the end of this period, the growth of microorganisms was determined visually and the point where no visible growth was accepted as the MIC.

2.6. Statistical Analysis

For the statistical analysis, SPSS (Statistical Package for Social Sciences) for Windows computer program was used and standard deviation, *P value*, using means, one-way analysis of variance (ANOVA) followed by Tukey test.

3. RESULTS AND DISCUSSION

3.1. Antiproliferative activities of the compounds

Many anticancer drug candidates have been withdrawn from market due to their serious side effects, loss of sensitivity to drugs, and limited use for many cancer types. In the present study, the disubstituted tacrine derivatives (Table 1) were prepared according to reported procedure by our research group [29] and investigated for their anticancer and cytotoxicities against A549, HeLa, Hep3B, HT29, MCF7 and FL cell lines according to the MTT protocol. Growth inhibition (GI_{50}), total growth inhibition (TGI), and lethal concentration (LC_{50}) parameters of the compounds were evaluated according to NCI screening method and half-maximal inhibitory concentration (IC_{50}) of these molecules were calculated using Four-Parameter Logistic Function, as well. When TGI and IC_{50} values of the compounds were examined, it was found that tested compounds caused selective antitumor properties against all tested cell lines (Table 1 and 2). Although compound **2** has antiproliferative effect (IC_{50} values between 4.5 and 20.7 μg/mL; TGI values between 4.4 and 20.9 μg/mL) against FL, HeLa and Hep3B cell lines (Table 1 and 2), compound **4** showed high antitumoral properties (IC_{50} values between 7.8 and 38.9 μg/mL; TGI values between 7.9 and 40.1 μg/mL) against FL, HeLa, HT29, and MCF7 cell lines (Table 1 and 2). Compound **1** depicted significant antiproliferative effect (IC_{50} value 1.1 and TGI value 1.1 μg/mL) against FL cells (Table 1). In Hep3B cells, compound **1** (IC_{50} value 10.4 and TGI value 10.6 μg/mL) and **2** (IC_{50} value 9.5 and TGI value 9.6 μg/mL) showed a potent antitumor effect (Table 2). When the IC_{50} and TGI values of all the above-mentioned compounds are considered, effective ones have better antiproliferative effects compared to the positive control group, cisplatin and 5-FU (Tables 1 and 2). In addition, the active compounds can be used in advanced pharmacological studies when the low GI_{50} values (~1 - 2 μg/mL) and the high LC_{50} values (~40 - 400 μg/mL) are considered (Table 1 and 2). Overall, the GI_{50}, TGI and LC_{50} parameters of the respective molecules are at the desired level and meet the NCI criteria.

3.2. Cytotoxic activity of compounds.

That the toxic effect against normal cells should be minimal is important for a substance. For this reason, antitumor and cytotoxic properties of these compounds should be compared in order to find the forward pharmacological capacity of each. The cytotoxicities of the compounds in cells were tested by the LDH cytotoxicity assay, indirectly demonstrating membrane damage. When cytoplasmic LDH activity measurement results are evaluated for these compounds, it has been found that the compounds **1**-**4** for A549 and Hep3B cell lines, compounds **1** and **4** for MCF7 and HeLa cell, compounds **1** and **3** for HT29 cell lines, and compound **2** and **4** for FL cell lines causes approximately 7% to 27% membrane damage at their IC_{50} concentration (Tables 3). If the compounds are compared to controls (5-FU and cisplatin) for this evaluation, the toxicity of molecules above-mentioned is very close to the cytotoxicity values of 5-FU and cisplatin. Therefore, they may be suitable for advanced pharmacological assays (Table 3).

3.3. Antibacterial activities of the compounds

The effects of the compounds on some pathogenic bacteria causing disease in the human body have been

Table 1. GI_{50}, TGI, LC_{50} and IC_{50} of test compounds against A549, FL, and HeLa cell line

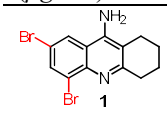
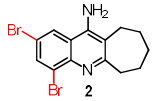
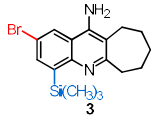
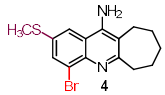
Compounds (*μg/mL*)	A549				FL				HeLa			
	GI_{50}	**TGI**	**LC_{50}**	**IC_{50}**	**GI_{50}**	**TGI**	**LC_{50}**	**IC_{50}**	**GI_{50}**	**TGI**	**LC_{50}**	**IC_{50}**
 1	7.4	>1000	>1000	>1000	1.0	1.1	4.4	1.1	5.7	>1000	>1000	>1000
 2	12.1	>1000	>1000	>1000	4.5	20.9	452.8	20.7	2.1	4.4	22.1	4.5
 3	8.8	>1000	>1000	>1000	4.6	>1000	>1000	>1000	4.2	461.1	>1000	416.9
 4	593.7	>1000	>1000	>1000	3.6	40.1	>1000	38.9	2.8	7.9	61.8	7.8
Cisplatin				60.49				52.79				50.29
5FU				69.79				59.09				61.59

Table 2. GI_{50}, TGI, LC_{50} and IC_{50} of test compounds against Hep3B, HT29, and MCF7 cell

Compounds (*μg/mL*)	Hep3B				HT29				MCF7			
	GI_{50}	**TGI**	**LC_{50}**	**IC_{50}**	**GI_{50}**	**TGI**	**LC_{50}**	**IC_{50}**	**GI_{50}**	**TGI**	**LC_{50}**	**IC_{50}**
1	2.7	10.6	243.2	10.4	1.6	>1000	>1000	>1000	1.6	135.3	>1000	106.9
2	2.8	9.6	127.3	9.5	3.5	229.4	>1000	100.2	3.5	>1000	>1000	>1000
3	4.3	>1000	>1000	>1000	9.9	>1000	>1000	>1000	1.5	>1000	>1000	>1000
4	3.9	192.8	>1000	176.6	3.8	17.6	465.1	15.2	2.4	32.1	>1000	29.6
Cisplatin				48.69				40.39				63.79
5FU				62.89				65.19				74.19

Table 3. % Cytotoxicity of these compounds and positive controls against A549, Hep3B, MCF7, HeLa, HT29, and FL at IC_{50} concentrations

Comp.	**A549**	**Hep3B**	**MCF7**	**HeLa**	**HT29**	**FL**
1	19.5	21.8	24.8	25.1	20.3	24.7
2	19.1	26.9	29.2	34.3	38.3	17.1
3	19.3	26.9	31.7	30.7	15.8	25.3
4	16.2	24.7	17.4	27.1	38.5	14.6
Cisplatin	8.63	8.46	10.71	9.85	11.23	8.33
5FU	9.19	9.67	7.69	8.83	7.91	8.44

Table 4. Minimum-inhibitory concentrations (MIC, in μg/mL) of these compounds

Compounds	*E. faecalis* ATCC 19433	*E.faecalis* ATCC 29212	*S. aureus* ATCC 25923	*S. aureus* ATCC 29213	*S. aureus* ATCC 46300	*E. coli* ATCC 25922	*E. coli* ATCC 35218	*P. eruginosa* ATCC 27853
1	250	250	62.50	125	125	500	500	500
2	62.50	500	31.25	125	62.50	500	500	500
3	500	>1000	62.50	>1000	1000	125	500	500
4	>1000	>1000	>1000	250	250	250	250	250
SCF	250	62.5	250	62.5	250	15.62	31.25	250

SCF. sulbactam (30 μg) + cefoperazone (75 μg), as a positive control

evaluated using the Minimum Inhibition Concentration (MIC) method. We considered our test molecules to be antibacterial at 250 μg/mL and below the MIC values.

The MIC values of the compounds were compared with the values of antimicrobial drugs used as positive controls. When the MIC values of recently prepared molecules shows on Gram (+) bacteria were tested, the antibacterial effect of compounds **1** and **2** against *E. faecalis* (VRE) ATCC 19433 (62.5 – 250 μg/mL), compound **1** against *E. faecalis* ATCC 29212 (250 μg/mL), compounds **1-3** against *S. aureus* ATCC 25923 (31.25 – 62.50 μg/mL), compounds **1**, **2** and **4** against *S. aureus* (MSSA) ATCC 29213 (125 – 250 μg/mL), and compounds **1**, **2** and **4** against *S. aureus* MRSA ATCC 46300 (62.50 – 250 μg/mL) are more or similar to the antibiotic used as a positive control (SCF) (Table 4). According to the MIC values exhibited by the recently synthesized molecules on Gram (-) bacteria, the antibacterial effect of compounds **3** and **4** against the *E. coli* ATCC 25922

(125 – 250 μg/mL), compound **4** against *E. coli* ATCC 35218 (250 μg/mL), and compound **4** against *P. aeruginosa* ATCC27853 (250 μg/mL) strain are more or similar to the SCF (positive control) (Table 4).

As a result, it is obvious that compounds **1** and **2** have promising activity for future studies. In general, tacrines bearing seven membered hydrocycle had higher activity than tacrines bearing six membered hydrocycle in our previous work [30]. Especially, dibromo tacrine bearing seven membered hydrocycle **2** was more active against microorganisms than its silyl **3** and thiomethyl **4** derivatives.

3.4. DNA binding properties of the compounds

DNA binding properties of the compounds were determined using UV-Vis spectrophotometer. Binding type and the binding constants of the compounds were tried to be explained below. These compounds have no clear redshifts or blue shifts at their maximum absorption peak. When CT-DNA was added to the reaction mixture, the decreasing in the absorption intensity of compounds **2** and **4** showed hypochromic effect and the increasing in the absorption intensity of compounds **2** and **4** caused hyperchromic appearance. The binding constants (K_b), showing the affinity of the complex to DNA, of the compounds with the aid of the Benesi-Hildebrand equation. When the binding constants given in Table 5 are evaluated, it can be seen that the K_b values of the compounds are between 2.9 x 10^4 and 7.4 x 10^4 M^{-1}. The binding constants of the compounds are ordered from large to small as follows: **2** > **4** > **3**. According to data in Table, the compound 3 bind DNA much more strongly than others. However, the binding constants of the compound 1 could not be calculated using UV-Vis spectrophotometric method.

Table 5. The binding constants (K_b) of these compounds

Compound	K_b (M^{-1})
2	2.9 x 10^4
3	7.4 x 10^4
4	4.6 x 10^4

4. CONCLUSION

Recently synthesized disubstituted tacrine bearing six or seven membered hydrocycle were tested for their antibacterial and anticancer activities *in vitro*. We have showed that disubstituted tacrines have significant potential as being antitumor and antibacterial agents. The test results describe both a good antiproliferative effect and a low cytotoxic effect, depending on substitution group on tacrine ring. The *in vitro* studies also displayed that mono silyl substituted seven membered tacrine analogue **3** was found to bind the DNA of cancer cells. According to our results, it is suggested that four disubstituted tacrine derivatives are promising anticancer and antibacterial drug candidates but further pharmacological tests should be worked.

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