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## Antimicrobial, antitumor and side effects assessment of a newly synthesized tamoxifen analogue

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### Abstract

Tamoxifen citrate is a very prevalent drug marketed under several trade names like Apo-Tamox, Nolvadex, Tamec, Tamizam and Tamoplex. This molecule is approved by the FDA for breast cancer treatment. Some studies have shown that tamoxifen has anti-tuberculosis and anti-parasitic activities. Like any drug tamoxifen possesses side effects, more or less dangerous. Basically, this work is a comparative study which aims to: Primarily compare the

27 antimicrobial and antitumor activities of tamoxifen and a newly synthesized tamoxifen  
28 analogue. Secondly, determine the molecule with lesser side effects. Three groups of mice  
29 were injected with tamoxifen citrate and compound **2** (1,1-bis[4-(3-  
30 dimethylaminopropoxy)phenyl]-2-phenyl-but-1-ene dihydrochloride) at doses corresponding  
31 to C1 (1/10), C2 (1/50), C3 (1/100) to compound **2** lethal dose ( $LD_{50} = 75$  mg/kg)  
32 administered for adult mice. A group of not injected mice has served as a study control.  
33 Experimental results suggest that compound **2** have better antitumor and antimicrobial  
34 activity than tamoxifen citrate besides its lower toxicity effects.

35 **Keywords:** tamoxifen citrate, 1,1-bis[4-(3-dimethylaminopropoxy)phenyl]-2-phenyl-but-1-  
36 ene dihydrochloride, antimicrobial activity, antitumor, toxicity, mice.

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## 40 **1. Introduction**

41 Nowadays humans are exposed to a variety of diseases, with a degree of dangerousness and  
42 virulence that continues to grow each year. This effect can be detected by the emergence of  
43 diseases that are resistant to treatments previously discovered and approved by agencies such  
44 as the Food and Drug Administration (FDA). This resistance is underlined in the cases of  
45 infectious diseases, cancer and several other diseases. Bacteria[1, 2], viruses[3] and malignant  
46 cancer cells adapt to therapies and find ways to escape the treatment[4, 5].

47 Many organizations like United Nations (UN) and WHO sounded the alarm on the  
48 inefficiency of older treatments against new emerging bacterial strains, which can be  
49 catastrophic because it can lead to a human powerlessness against a possible outbreak if  
50 emergency measures are not taken, like the discoveries of new active molecules and new  
51 treatments to combat multidrug-resistant strains[6].

52 For this purpose, research laboratories and pharmaceutical companies have focused on this  
53 subject. Several research strategies have been developed to search for effective products:  
54 natural way, by analysis of plant extracts, or synthetic way. In this paper, we will focus about  
55 chemically synthesized molecule [7, 8].

56 Tamoxifen (ICI46,474) is an example of “magical” molecule, with a very remarkable story.  
57 Essentially synthesized by Imperial Chemical Industries ICI (AstraZeneca) as part of a  
58 fertility control program, this selective estrogen receptor modulator has failed on the goal in  
59 which it was designed. After the establishment of links between estrogen and hormone  
60 dependent breast cancer in 1960, tamoxifen has been clinically tested for breast cancer disease  
61 where it has shown encouraging results[9].

62

63 In 1973, tamoxifen has been marketed in the UK as a breast cancer treatment and in 1975 as  
64 an ovulation inductor. The FDA approved tamoxifen as treatment of metastatic breast cancer  
65 in 1977[10].

66 Thereafter, in 1986 it was approved as adjuvant together with chemotherapy. Later between  
67 1988 and 1990 it was recognized and allowed to be used alone for patients with: node-  
68 positive post-menopausal and pre or post-menopausal node-negative estrogen positive  
69 disease. In the same period, tamoxifen was approved as a risk reducer for high-risk women  
70 for breast cancer. Three years after, men with breast cancer was treated with it (1993). In  
71 2000, tamoxifen treatment *in situ* of ductal carcinoma (DCIS) was approved[11].

72  
73 Like any drug tamoxifen has side effects, more or less dangerous. The percentage of  
74 occurrence, frequency and the manifestation of these effects are variable, and depend mostly  
75 on two factors: the dose administered and the subject receiving the treatment. According to  
76 the guide of use and labels approved by FDA for tamoxifen commercialization and some  
77 others studies, with tamoxifen the incidence of uterine malignancies (uterus-endometrial  
78 cancer and uterine sarcoma) is increased by 50% [12,13]. Tamoxifen also causes gynecologic  
79 symptoms like pelvic pain, menstrual irregularities, and vaginal bleeding[14]. The thrombo  
80 embolic events were reported (pulmonary embolism and deep vein thrombosis) [15]. Liver  
81 can be affected with tamoxifen and some severe hepatic abnormalities including fatty changes  
82 in the liver, cholestasis, hepatitis, hepatic necrosis (some fatal) and liver cancer were reported  
83 [12]. Hypercalcemia, visual disturbance, nausea, depression in addition of abnormality in  
84 hematology and lipid profiles are mentioned [12].

85  
86 Recent innovative studies have shown that tamoxifen has activity against bacteria even those  
87 that present a problem of resistance such as tuberculosis; In addition to the activity against  
88 leishmaniosis that has been indicated [16, 17].

89 Ferrocenyl or phenyl derivatives of diphenyl butene series, that are tamoxifen analogs, are  
90 known for their anti-proliferative activity on breast cancer cells and leukemia, as well as for  
91 their excellent antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*,  
92 *Staphylococcus aureus*, *Enterococcus hirae*, *P. savastanoi* pv. *Savastanoi*, *A. tumefaciens*, *F.*  
93 *Solani*, *Listeria monocytogenes* and *Salmonella enterica* [18-24].

94

95 The focus of this study is based on the comparison of two molecules: tamoxifen citrate and  
96 newly synthesized Compound **2**. This comparison is made on two parts: a microbiological  
97 aspect corresponding to the test of the activities of these two molecules against Gram-  
98 negative and Gram-positive bacteria as first part, and the evaluation of toxicological effects  
99 on mice as second part. Compound **2** is the dihydrochloride version of compound **1** and was  
100 used in place of **1** in order to make it soluble into water.

## 101 **2. Materials and methods**

### 102 **2.1. Chemical compounds**

103 Tamoxifen citrate was purchased from Sigma Chemical Company (Dorset, UK). 1,1-bis[4-(3-  
104 dimethylaminopropoxy)phenyl]-2-phenyl-but-1-ene dichloride(compound **2**) was prepared  
105 (Figure 1). Compound **1** [25] (1.15 g, 2.36 mmol) was dissolved in 150 mL of diethyl ether  
106 and stirred. A 2 M solution of hydrochloric acid in diethyl ether (2.6 mL, 5.2 mmol) was  
107 added drop-wise into the solution. A white precipitate was immediately formed. After stirring  
108 for 20 min, the mixture was filtered under argon and the obtained white solid was washed  
109 with 3 x 5 mL of diethyl ether and dried under vacuum, giving compound **2** in quantitative  
110 yield. Crystals contain traces of diethyl ether that can't be removed even after several hours  
111 under high vacuum, making elemental analysis not relevant. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 0.88 (t, *J*  
112 = 7.1 Hz, 3H, CH<sub>3</sub>), 2.03-2.30 (m, 4H, CH<sub>2</sub>), 2.37-2.50 (m, 2H, CH<sub>2</sub>), 2.75 (d, *J* = 4.4 Hz,

113 6H, NMe<sub>2</sub>), 2.80 (d, *J* = 4.4 Hz, 6H, NMe<sub>2</sub>), 3.09-3.32 (m, 4H, CH<sub>2</sub>N), 3.94 (d, *J* = 5.1 Hz,  
114 2H, CH<sub>2</sub>O), 4.11 (d, *J* = 5.1 Hz, 2H, CH<sub>2</sub>O), 6.63 (d, *J* = 8.4 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 6.76 (d, *J* = 8.4  
115 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 6.98 (d, *J* = 8.4 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.07-7.29 (m, 7H, C<sub>6</sub>H<sub>4</sub> + C<sub>6</sub>H<sub>5</sub>), 10.84-11.22  
116 (m, 2H, NH<sup>+</sup>). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) : δ 14.3 (CH<sub>3</sub>), 24.7 (CH<sub>2</sub>), 24.8 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 42.8  
117 (NMe<sub>2</sub>), 42.9 (NMe<sub>2</sub>), 54.8 (CH<sub>2</sub>N), 54.9 (CH<sub>2</sub>N), 65.6 (CH<sub>2</sub>O), 65.8 (CH<sub>2</sub>O), 114.3 (2 CH  
118 C<sub>6</sub>H<sub>4</sub>), 115.1 (2 CHC<sub>6</sub>H<sub>4</sub>), 127.0 (CHC<sub>6</sub>H<sub>5</sub>), 128.9 (2CH<sub>arom</sub>), 130.3 (2CH<sub>arom</sub>), 131.1  
119 (2CH<sub>arom</sub>), 132.4 (2CH<sub>arom</sub>), 136.4 (C), 136.7 (C), 138.4 (C), 141.4 (C), 142.9 (C), 157.1 (C),  
120 157.9 (C). IR (KBr, ν cm<sup>-1</sup>): 3026, 2961, 2688, 2512, 2474 (CH, CH<sub>2</sub>, CH<sub>3</sub>).

## 121 **2.2. Bacterial strains and growth conditions**

122 *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 6538, *Escherichia coli*  
123 ATCC 10536, *Escherichia coli* K12ATCC 23716 were used as indicator strains. Bacteria  
124 were grown aerobically in LB broth (Difco laboratories, Sparks, MD, USA) at 30°C for 24 h.

## 125 **2.3. Determination of the antimicrobial activity**

126 Micro dilution method on 96 well plates was used to estimate the antimicrobial effects of  
127 molecules. Tamoxifen citrate and compound **2**, were dissolved in double-distilled sterile  
128 water, the levofloxacin was dissolved in dimethyl sulfoxide (DMSO). A serial of two half  
129 dilutions were performed for each molecule to be tested in a final volume of 100μL and every  
130 well was inoculated with 100μL bacterial culture (10<sup>6</sup> CFU/mL). The final concentrations of  
131 each molecule were 100μg/mL, 50μg/mL, 25μg/mL, 12.5μg/mL, 6.25μg/mL, 3.1μg/mL,  
132 1.55μg/mL and 0.75μg/mL. A set of wells containing 200μL of LB medium was used as  
133 negative control and a blank for reading the optical density at 600nm. The positive control  
134 contained 100μL of LB medium inoculated with 100μL of culture (10<sup>6</sup> CFU/mL) obtained  
135 after overnight culture of 16h shaking at 30 °C. The MIC (Minimal Inhibitory Concentration)  
136 was determined as the minimal concentration of molecule where no visible growth was noted.

137 MTT, (3-(4,5dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), was used to determine  
138 the MIC. A sample from each well was plated on solid LB medium and maintained overnight  
139 at 30°C to determine the MBC as the lowest concentration of molecule corresponding to the  
140 inhibition of 95% of cells growth. The IA was calculated according to the formula:

$$IA(\%) = 100 * \left(1 - \frac{[OD600(M) - OD600(i)]}{[OD600(C) - OD600(i)]}\right)$$

141 Where M is OD with molecule and C is OD of control culture.

#### 142 **2.4. Cell viability assay**

143 MCF7 cells ( $5 \times 10^4$ ) were seeded in 98-well plates and left to grow overnight. The following  
144 day, cells were treated with increasing concentrations of Tamoxifen citrate and compound **2**  
145 (0.1µM to 1mM) in triplicate. After 24 h, cell viability was measured using the Cell  
146 Proliferation kit (MTT). Results were representative of at least three independent  
147 experiments, and were expressed as inhibition rate in reference to the cell growth without  
148 drug treatment.

#### 149 **2.5. Animals**

150 Three-to-five-week-old swiss mice ( $\approx 20g$ ) were purchased from Central Pharmacy (SIPHAT,  
151 Tunisia), quarantined for more than 1 week before starting the experiments, kept under  
152 pathogen-free conditions, and handled according to the Guidelines of the “Institute Ethical  
153 Committee Guidelines” for the care and use of laboratory animals [26], as provided by the  
154 Ethical Committee of Faculty of Sciences at University of Sfax, which approved the present  
155 animal experiments.



## 156 **2.6. Experimental design**

157 Mice were accommodated in polycarbonate cages and were daily provided with water and  
158 standard pellet diet (SNA, Sfax, Tunisia). Cages are kept in an air-conditioned room,  
159 temperature  $22\pm 3$  °C and 40% of relative humidity. After intraperitoneal injection, the lethal  
160 dose of compound **2** and tamoxifen citrate resulting in 50% mortality ( $LD_{50}$ ) were found as  
161 75mg/kg and 218mg/kg body weight (bw), respectively. In this study, we used the  $LD_{50}$  of  
162 compound **2** (75mg/kg) for both molecules in order to compare their effects for the same  
163 dose. Mice were divided into seven groups, counting seven mice per each. The first group was  
164 used as control groups 2, 3, and 4 were injected by tamoxifen citrate and groups 5, 6, and 7 by  
165 compound **2**. Tamoxifen citrate and compounds **2** doses ( $C1=1/10$ ,  $C2=1/50$ ,  $C3=1/100$  of  
166  $LD_{50}$  of compound **2** (75mg/kg) were not lethal to mice. Both molecules were dissolved in  
167 water and daily intraperitoneally injected to mice. Food, water intake and body weights of  
168 mice were daily registered. All mice were killed by cervical decapitation after 15 days of  
169 treatment. Heparinized tubes were used to collect the blood, and after centrifugation for 10  
170 min at  $2200 \times g$ , plasma samples were collected and stored at  $-80$  °C for biochemical analysis.

## 171 **2.7. Clinical signs feed intake and body weight measurement**

172 The general health appearance of each mouse was daily monitored using the following score  
173 system: 1 (no sign of discomfort); 2 (slightly ruffled fur, active); 3 (ruffled fur and back  
174 circumflexed, active); 4 (ruffled fur, back circumflexed, inactive, buprenorphine was  
175 administered); 5 (ruffled fur, inactive, hunched posture, and closed or sunken eyes, with or  
176 without ocular secretions). Mice were weighed every three days and expressed as average for  
177 each group throughout the experiments.

## 178 **2.8. Biochemical assays**

### 179 **2.8.1. Hemogram**

180 Also called CBC (Complete Blood Count), it is an automated way to obtain quickly a set of  
181 quantitative data about red blood cell (erythrocytes) parameters, white blood cell (leukocytes)  
182 count and platelets, including leukocyte formula. Blood was collected in tubes with heparin,  
183 and CBC was determined just after animal sacrifice, this service is assumed by the  
184 hematology service of Habib Bourguiba Hospital.

### 185 **2.8.2. Biochemical markers in plasma**

186 Plasma lipid parameters including total cholesterol (TC), triglycerides (TG) and high-density  
187 lipoprotein cholesterol (HDL) were determined using commercial kits (#20111, #20131 and  
188 #20113) purchased from Biomaghreb (Ariana, Tunisia). The low-density lipoprotein  
189 cholesterol (LDL) was determined according to Friedewald equation [27]:

$$LDL = \frac{TC - (TG - HDL)}{HDL}$$

## 190 **2.9. Statistical analyses**

191 Data are presented as mean values with their standard errors (SEM). Data were subjected to  
192 ANOVA using the GLM procedure of SAS (SAS Institute, 2003). The means were compared  
193 with LSD test. A probability of  $P < 0.05$  was required for statistical significance. Treatments  
194 with different letters are significantly different ( $p < 0.05$ ).

## 195 **3. Results and Discussion**

196 In a previous published work, several diaryl-butene analogues and derivatives of tamoxifen  
197 were tested against pathogenic Gram-positive and Gram-negative bacteria. The idea came  
198 from the fact that other research teams have found that tamoxifen citrate has an inhibitory  
199 power as bactericidal against the pathogenic germs. Several diaryl-butene have shown some

200 very interesting activities. However, compound **2** have not been tested. To urge this query and  
201 keen interest about the question that whether compound **2** has a potential for tumor and  
202 bacterial inhibition? and to compare its activity versus tamoxifen citrate.

203 We evaluated cytotoxicity of compound **2** compared to tamoxifen citrate on MCF7 breast  
204 cancer cells. As the compound **2** is an analogue of tamoxifen, those cells seem to be a good  
205 choice given their invasive appearance. The results appearing in the figure 2 shown that both  
206 of the two molecules have an antiproliferative activity against MCF7 cells, however, we noted  
207 significant effects differences that favor the compound **2**. Indeed, cell's death caused by this  
208 compound compared to tamoxifen citrate is equivalent to twice at the concentration of 10  $\mu\text{M}$ .  
209 Our results showed that in the case of MCF7 cells, based on the  $\text{LD}_{50}$  value, compound **2** is  
210 five times more toxic than the tamoxifen citrate with respective  $\text{LD}_{50}$ s of 5  $\mu\text{M}$  and 25  $\mu\text{M}$ .

211 Inhibition test was applied with two types of *E. coli* as gram-negative, and *Staphylococcus*  
212 *aureus*, *Listeria monocytogenes* as gram-positive bacterial strains. The antibiotic levofloxacin  
213 was used as a control. The MIC and MBC for the different molecules are shown in the table 1.

214 Compound **2** and tamoxifen citrate show a better potential against gram-positive than against  
215 gram-negative. The inhibition profiles obtained for tamoxifen citrate and compound **2** are  
216 different, a difference that manifested by a better inhibitory activity for compound **2** than for  
217 tamoxifen citrate, whether for the Gram-positive or Gram-negative (Figure 3). Compound **2**  
218 and tamoxifen citrate have a ratio MBC/MIC less than or equal to 4, sign of bacteriostatic and  
219 not bactericidal activity. MBC of compound **2** for *Staphylococcus aureus* is 6.25  $\mu\text{g/mL}$ , this  
220 value corresponds to the MIC of tamoxifen citrate. These results point out that compound **2** is  
221 much better and more active than tamoxifen citrate, and also has the potential to be used  
222 against gram-positive strains especially against *Staphylococcus aureus*.

223 Following 15 days of intraperitoneal injection to the mice with tamoxifen citrate and  
224 compound **2**, a hematologic study was conducted. The findings obtained shows a disturbance  
225 in the blood count for different groups injected with tamoxifen citrate and with compound **2**,  
226 compared to the control groups without being injected as depicted in Figure 4. In the scientific  
227 literature tamoxifen citrate, as part of these secondary effects, is described as disruptive of  
228 blood parameters. In this context, our work has shown a decline in red blood cell count  
229 (Figure4A), and variance statistical study on different doses used for each molecule  
230 ( $LD_{50}/100, LD_{50}/10$ ) showed no significant differences. However, variance becomes  
231 significant by comparing each molecule to the control group. Although the two molecules to  
232 be tested reduce the number of red blood cells, a phenomenon already described for tamoxifen  
233 and its active derivatives like hydroxytamoxifen known as factors causing erythrocyte  
234 hemolysis [28,29]. Compound **2** shows that having a similar effect of reducing the number of  
235 these cells, but with a less acute form (Figure 4A). The mean corpuscular volume (MCV)  
236 reflecting the size of red blood cells also showed a fall, a decline that seems not to be  
237 statistically significant compared to the control group (Figure 4B). However, comparing the  
238 effect of the two molecules on the MVC value, the rates of significance become very strong,  
239 with a relative decline less important than compound **2**. One of the most relevant and  
240 interesting things that was revealed by this study is the difference in effect of the molecules  
241 tested on platelets. In contrast to tamoxifen citrate which decreases dramatically the number  
242 of platelets in the blood, compound **2** has an opposite effect by a slight increase in platelet  
243 count relative to the control (Figure 4D). Detrimental effect of Tamoxifen on blood platelets  
244 is already well known [30]. The reason might be explained by the fact that tamoxifen causes  
245 mini injury into blood vessel, which explains the appearance of hematomas in some organs  
246 such as the liver. Due to their role in cicatrization these thrombosis injuries can occur  
247 anywhere in the body (lungs, heart and even the brain). Thrombosis risk increase may lead to

248 dangerous consequences, like sudden death. Our results also show a significant drop in the  
249 number of leukocytes for tamoxifen citrate (Figure 4C). This important downfall for  
250 tamoxifen citrate compared to compound **2**, can induce a mess and declining reliability of the  
251 immune system, making the body vulnerable for attack by pathogens [31].

252 In summary comparison between tamoxifen citrate and compound **2** as well as their effects on  
253 hematological parameters demonstrates that these two molecules cause anemia, but the  
254 severity of the decline is greater for tamoxifen (Figure4A). Compound **2**, unlike tamoxifen  
255 citrate, does not cause leukopenia and thrombocytopenia (Figure 4C, and 4D). In nut shell,  
256 compound **2** found to be far less toxic and better than tamoxifen citrate.

257 The analysis of blood biochemical parameters following tamoxifen citrate and compound **2**  
258 injections shows majorly changes in triglycerides, High-Density Lipoprotein Cholesterol  
259 (HDL) and other cholesterol profiles. Statistical analysis of the dose effect for tamoxifen are  
260 not significant, therefore all doses are equivalent (Figure 5). The same thing was observed for  
261 the different doses of compound **2** used, except for triglycerides and Low-Density Lipoprotein  
262 Cholesterol (LDL) where it seems that the dose is related to the effect of the product (Figure  
263 5).

264 Outcome of the study suggested that the two molecules alter in a clear manner the serum  
265 concentration of cholesterol and HDL (Figure 5B, and 5C). However, compound **2** alters the  
266 serum concentration of triglycerides less than tamoxifen citrate and does not register a  
267 significant difference with the control (Figure 5A). Although this change, the statistical scale  
268 seems to be not significant for the two molecules for LDL (Figure 5D). Cholesterol decrease  
269 in the blood during tamoxifen citrate treatment is something that is well known [32].  
270 However, the problem for the triglyceride levels in the blood following administration of  
271 tamoxifen citrate is controversial. The majority of studies carried out leads to the fact that

272 blood triglycerides level increases following administration of tamoxifen citrate. This increase  
273 is due to inhibition of hepatic enzymes involved in lipid metabolism which increases the rate  
274 of cell and serum triglycerides [33].Some studies, like our results, founds that tamoxifen  
275 citrate causes a drop in plasma lipids cholesterol, triglycerides, LDL and HDL [34-37].

## 276 **4. Conclusion**

277 The results emerged from the present study confirmed the antitumor and antimicrobial effect  
278 of tamoxifen citrate and its hematological side effects. Compound **2** seems to be more  
279 effective than tamoxifen citrate for antitumor and antimicrobial treatment while having less  
280 hematological side effects and less disruption of the blood biochemical parameters. These  
281 findings encourage us to push further studies for compound **2** and test it for other therapeutic  
282 usage for which tamoxifen was effective.

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401 **Figure legends**

402 **Figure 1.**Preparation of compound **2** and structures of the compounds object of the study

403 **Figure2.** Inhibition of MCF7cells in presence of tamoxifen citrate or compound **2**.

404 **Figure 3.** Growth inhibition of *Listeria monocytogenes*ATCC 7644 (A) and *Escherichia coli*  
405 K12 ATCC 23716 (B) in presence of tamoxifen citrate (square) or compound **2** (triangle).

406 Non-linear regression equation:  $Y=Bottom + (Top-Bottom)/(1+10^{((LogEC_{50}-$   
407  $X)*HillSlope))$ .

408 **Figure 4.** Effect of tamoxifen citrate and compound **2** at (A) Erythrocytes enumeration; (B)  
409 VCM; (C) Leucocytes enumeration and (D) platelets.

410 **Figure 5.** Effect of tamoxifen citrate and compound **2** on (A) TG; (B) Cholesterol; (C) HDL  
411 and (D) LDL profile in blood.

412

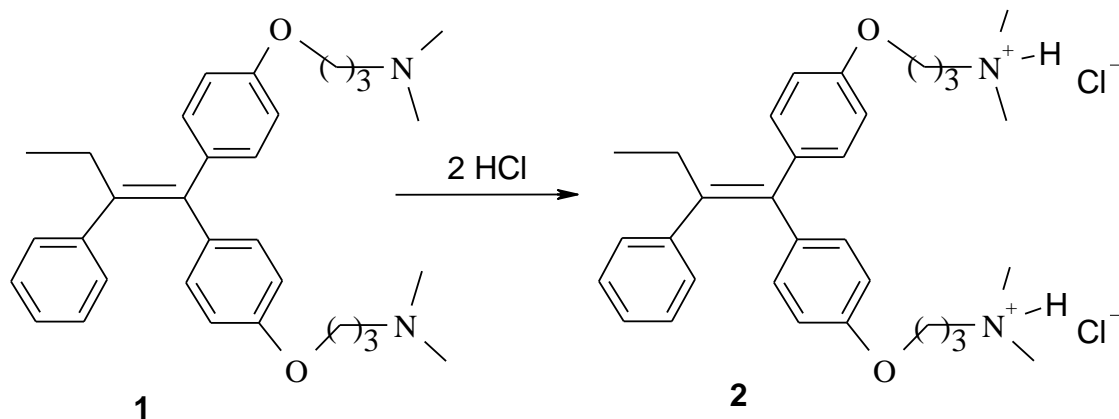
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414 **Table 1:** Determination of MIC and MBC of Levofloxacin, tamoxifen citrate and compound 2

Bacterial strains	Compound concentration ( $\mu\text{g/mL}$ )					
	Levofloxacin		Tamoxifen citrate		Compound 2	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>Listeria monocytogenes</i> ATCC 7644	6.25	>100	12.5	25	6.25	12.5
<i>Staphylococcus aureus</i> ATCC 6538	12.5	25	12.5	25	6.25	12.5
<i>Escherichia coli</i> ATCC 10536	<0.7	<0.7	> 100	>100	100	>100
<i>Escherichia coli</i> K12 ATCC 23716	<0.7	<0.7	> 100	>100	25	50

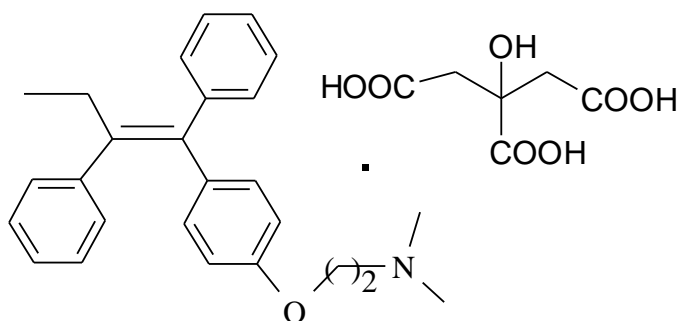
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1,1-Bis[4-(3-dimethylaminopropoxy)phenyl]-2-phenyl-but-1-ene

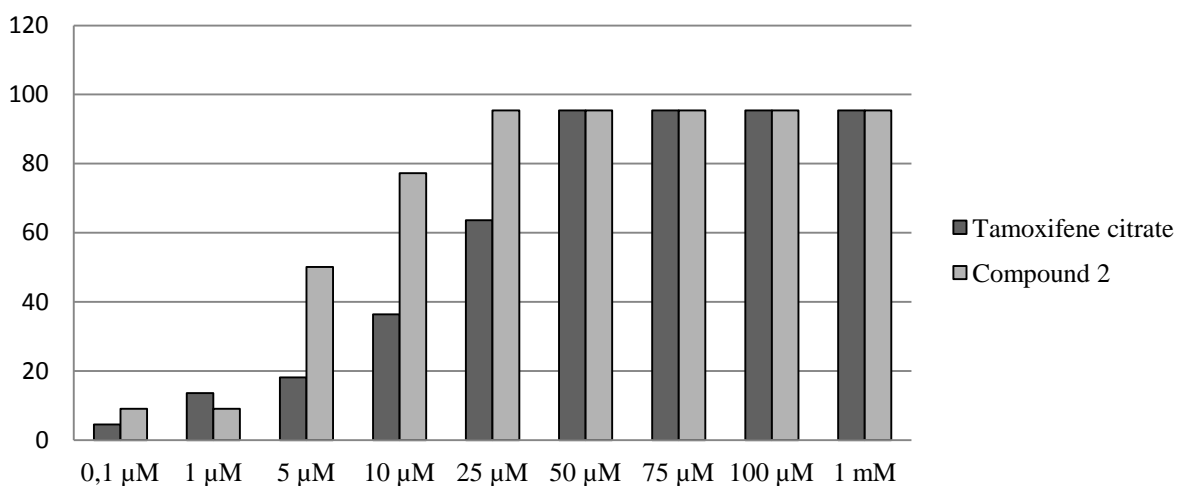
1,1-Bis[4-(3-dimethylaminopropoxy)phenyl]-2-phenyl-but-1-ene dihydrochloride



Tamoxifen citrate

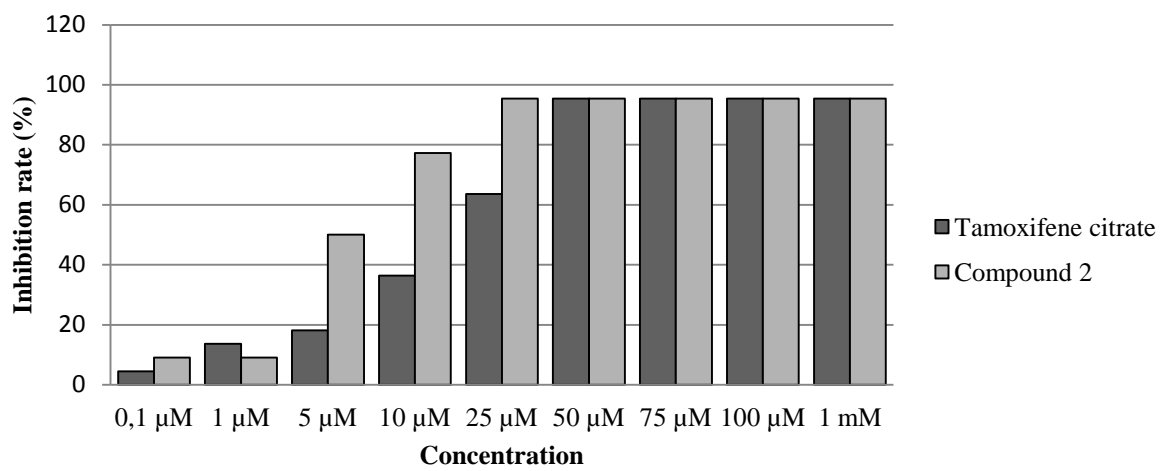
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**Figure 1**



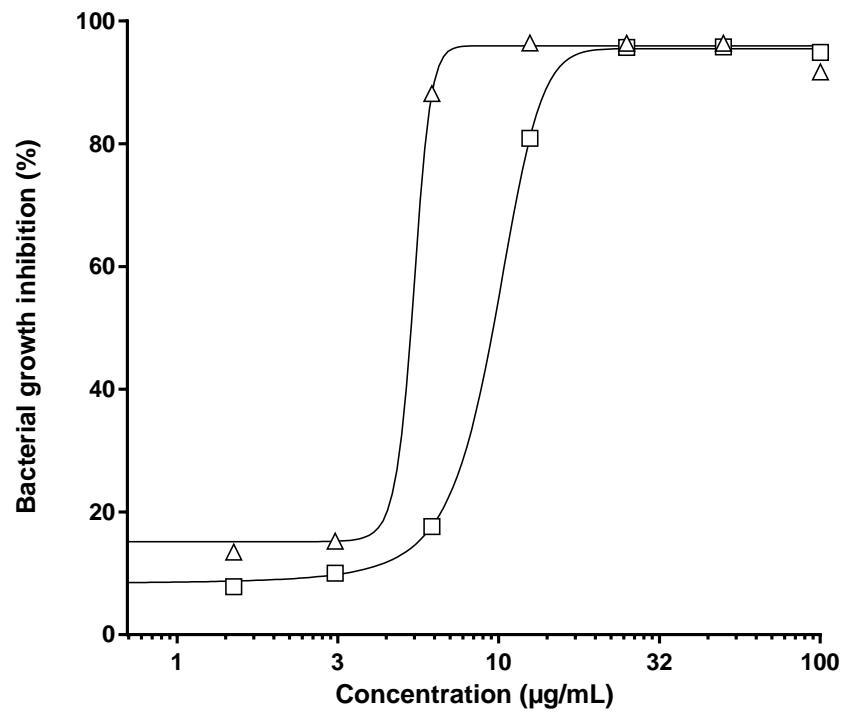
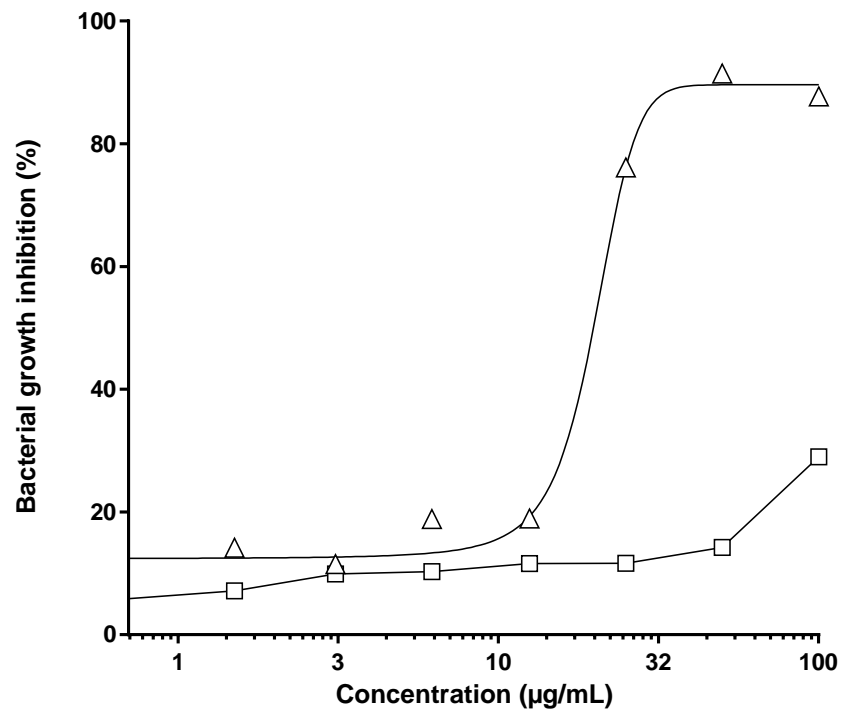
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424 **Figure 2**

**A****B**

425 **Figure 3**

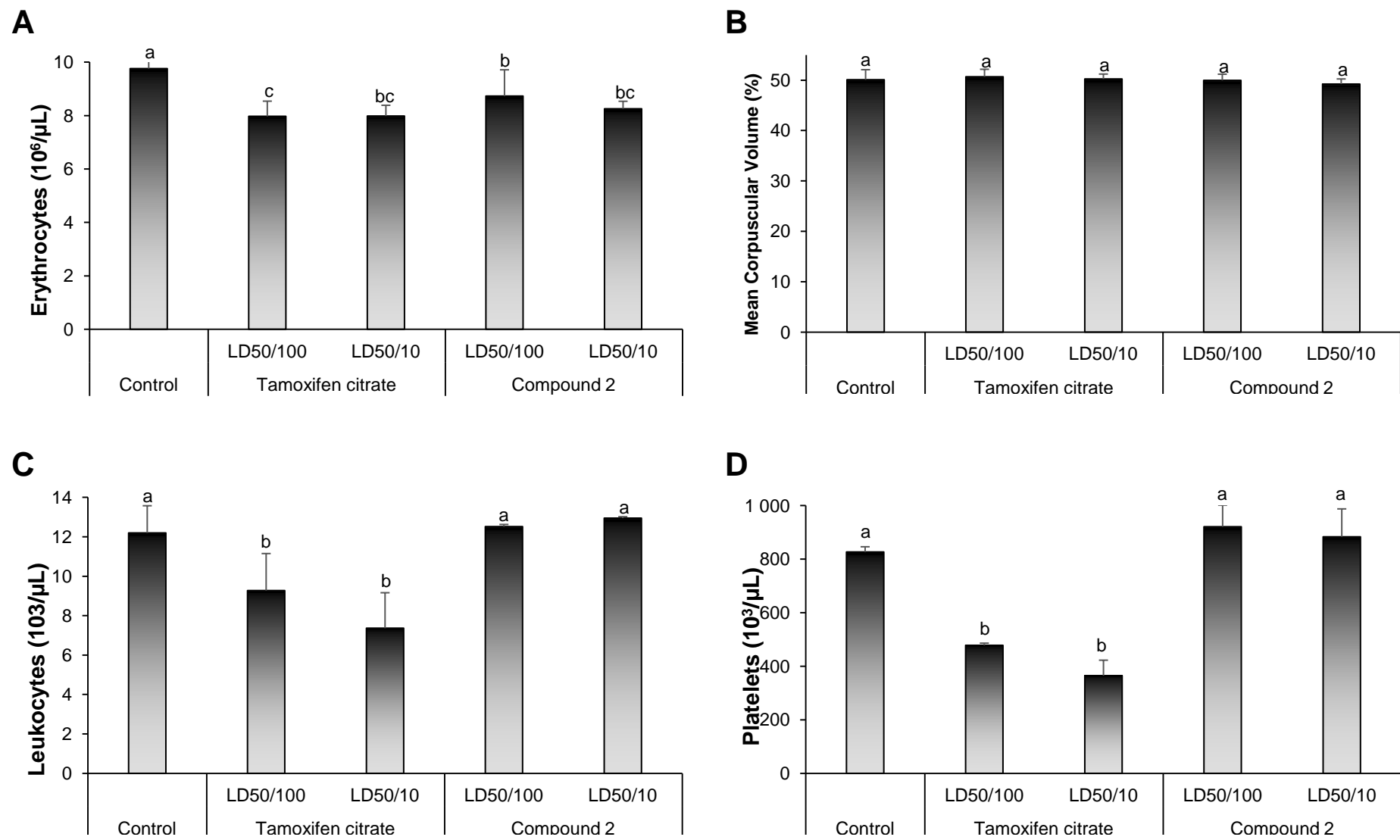
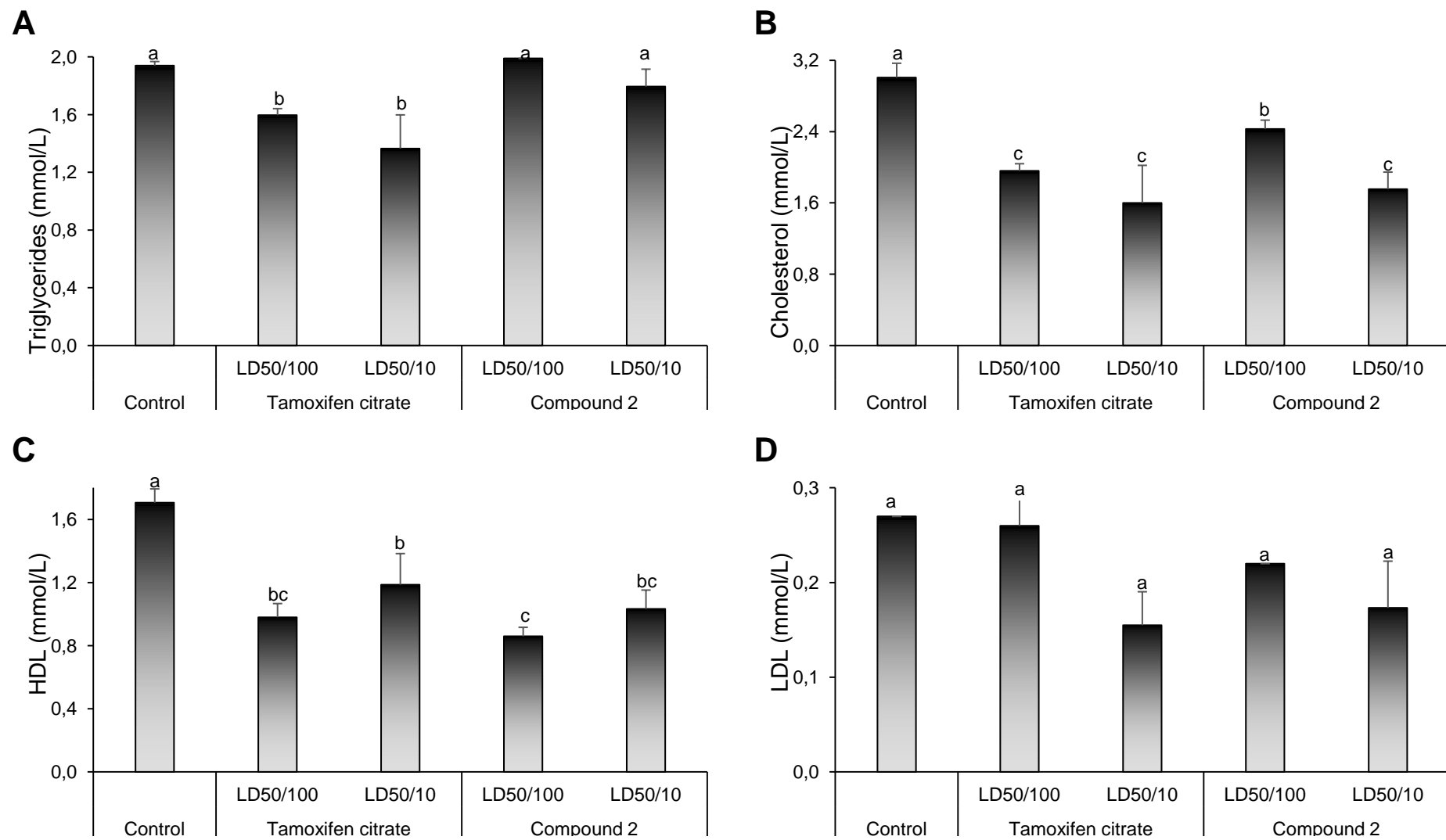


Figure4



**Figure 5**