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Biological and in silico studies of methyl 2-(2-methoxy-2-oxoethyl)-4-methylfuran-3-carboxylate as a promising antimicrobial agent

Khudaverdi Ganbarov[1,2], Alakbar Huseynzada[2,3,*], Gaoussou Binate[1], Koray Sayin[4], Nurlana Sadikhova[5], Valeh Ismailov[5], Niftali Yusubov[5], Gamze Tuzun[4], Mustafa Demiralp[6] and Abdallah Ahmed Khalil Algherbawi[7]

[1]Research Laboratory of Microbiology and Virology, Baku State University, Baku, Azerbaijan. [2]Islamic World Educational, Scientific and Cultural Organization Biomedical Materials Department, Baku State University, Z. Khalilov 33, Baku, AZ 1148, Azerbaijan. [3]Industrial Chemistry Research Laboratory, Baku State University, Z. Khalilov 33, Baku, AZ 1148, Azerbaijan. [4]Chemistry Department, Faculty of Science, Sivas Cumhuriyet University, Sivas, 58140, Türkiye. [5]Department of Organic Chemistry, Baku State University, Baku, Azerbaijan. [6]Advanced Technology Application and Research Center, Sivas Cumhuriyet University, Sivas, Türkiye. [7]Chemical Engineering Department, Faculty of Engineering, Sivas Cumhuriyet University, Sivas, 58140, Türkiye. *Author for correspondence. E-mail: alakbar.huseynzada1117@gmail.com

ABSTRACT. Herein, we report the biological and in silico investigations of synthesized furan derivative as a promised antimicrobial agent. The biological activity of synthesized targeted compound was investigated against opportunistic gram-positive (*Bacillus mesentericus, B. subtilis* and *Staphylococcus aureus*) and gram-negative (*Acinetobacter baumannii, Escherichia coli, Klebsiella pneumonia*e and *Pseudomonas aeruginosa*) bacteria, as well as yeast of genus *Candida* (*C. albicans, C. guillermondii* and *C. tropicalis*). The studied substance inhibited the growth of all bacteria and fungi at concentrations of 0.3-0.05%, whereas MIC in relation to the test organisms varied between 62.5 and 15.6 µg mL showing the lowest value for *S. aureus* and *A. baumannii*. The obtained results were also compared with the activity of pristine antibiotics (gentamicin and fluconazole), which revealed the more potent activity of the targeted compound than that of antibiotics. Computational analyses of the studied compound are performed at M06-2X/6-31+G(d,p) level in the water. Molecular docking calculations revealed 2CCG (TMK) and 4FUV (CarO) proteins as target proteins in the case of *S. aureus* and *A. baumannii* respectively, whereas p450 cytochrome analyses demonstrated the inhibition of CYP2C9 protein. ADME properties and MM-GBSA analyses showed that the studied compound exhibits better results than pristine antibiotic as in the case of experimental analysis.

Keywords: Methyl 2-(2-methoxy-2-oxoethyl)-4-methylfuran-3-carboxylate; antimicrobial agent; gram-positive and gram-negative bacteria; fungi of genus *Candida*; MIC.

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Introduction

The spread of antimicrobial resistance is the most serious and ongoing threat to human existence and requires a continuous search for new antimicrobials to successfully treat infectious diseases (Ahmad, 2015; Zaman et al., 2017; Althagafi et al., 2019). More than 30 species of pathohenic bacteria that are resistant to more than one drug grow at an exceptional rate. These microorganisms are a major obstacle in the treatment and eradication of infectious diseases. A limited number of antibacterial drugs are available against Gram-positive and Gram-negative bacteria, as bacteria have become resistant to almost all available antimicrobial agents (Saleh et al., 2019; Zaman et al., 2019; Elkhalifa et al., 2021; Shui et al., 2021; Deepa & Jain, 2015; Lagemaat et al., 2022]. The World Health Organization has emphasized the need for large-scale action against multidrug-resistant pathogens by developing new antimicrobials with a unique mode of action (Theuretzbacher, 2013; Jebli et al., 2020; Yu et al., 2021).

The shortage of new antimicrobials and the rapid development of antimicrobial resistance pose a major challenge to health systems. In this regard, scientists are exploring new compounds of various origins as new antimicrobial substances. There is currently growing interest in the development and evaluation of organics as economical, feasible and likely future antimicrobial agents (Rani & Ravindranath, 2016; Bielawski et al., 2017; Ismiev et al., 2020; Mehrabani et al., 2020; Obi et al., 2020; Huseynzada et al., 2021). Various classes of organic compounds can be used, among which oxygen-containing heterocycles occupy a special position. The second most frequent form of heterocycles to show up as structural elements in drugs licensed by the US Food

and Drug Administration (FDA) are oxygen heterocycles. A review of the medication database (through 2017) found that 311 pharmaceuticals—or 27% of authorized small molecules and 15% of all approved drugs—have an oxygen-containing heterocycle in their moiety. Among these drugs, 95% of all aromatic representatives are derivatives of furane (Delost et al., 2018). Various investigations demonstrate that furan derivatives have antibacterial, antifungal (Loğoğlu et al., 2010), anti-inflammatory (Alizadeh et al., 2020), antidepressant, anti-anxiolytic, analgesic, muscle relaxant, antihypertensive, antiarrhythmic, antiglaucoma, steroidal, anti-ulcer, antidiuretic, anorectic, inhibition of sickle cell formation, antiageing, antiparkinsonian, antihistaminic, anticholinergic, antineoplastic and insecticidal activities (Banerjee et al., 2015). All the mentioned demonstrate the importance of furan derivatives in the preparation of novel drugs.

Taking into account the fact that there is an acute need in search for new antimicrobial drugs caused by the acquisition of pathogenic microorganisms resistance to most antibiotics, furan derivative, viz. methyl 2-(2-methoxy-2-oxoethyl)-4-methylfuran-3-carboxylate was synthesized by alkylation of dimethyl 3-oxo-pentadioate with 1,2,3-tribromopropane in the presence of potassium carbonate in DMSO and it's antimicrobial properties was investigated against opportunistic gram-positive (*Bacillus mesentericus, B. subtilis* and *Staphylococcus aureus*) and gram-negative (*Acinetobacter baumannii, Escherichia coli, Klebsiella pneumonia*e and *Pseudomonas aeruginosa*) bacteria, as well as the yeast of genus *Candida* (*C. albicans, C. guillermondii* and *C. tropicalis*). The outcomes were also compared to the efficacy of pure antibiotics (gentamicin and fluconazole). In order to explain the biological activity of the promised studied compound, molecular docking analysis was performed and the target protein in the case of *S. aureus* and *A. baumannnii* is 2CCG (TMK) and 4FUV (CarO) respectively. In addition, P450 cytochrome, ADME and MM-GBSA studies were also performed.

Material and methods

General information

All the solvents and reagents were purchased from commercial suppliers and were of analytical grade and used without further purification. The control of the reaction's progress and the determination of the synthesized compound purity were done by thin-layer chromatography (TLC) which was visualized under UV light. Elemental analysis was performed on the Carlo Erba 1108 analyzer (Huseynzada et al., 2023).

NMR experiments

The NMR experiments were performed on a BRUKER FT NMR spectrometer AVANCE 300 (Bruker, Karlsruhe, Germany; 300 MHz for ^{1}H and 75 MHz for ^{13}C with a BVT 3200 variable temperature unit in 5mm sample tubes using Bruker Standard software (TopSpin 3.1). Chemical shifts were given in ppm (δ) and were referenced to internal tetramethyl silane (TMS). Multiplicities are declared as follows: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet). Coupling constants *J* are given in Hz. The experimental parameters for ^{1}H are as follows: digital resolution = 0.23 Hz, SWH = 7530 Hz, TD = 32 K, SI = 16 K, 90^{0} pulse-length = 10 ms, PLI = 3 dB, ns = 1, ds = 0, d1 = 1s and for ^{13}C as follows: digital resolution = 0.27 Hz, SWH = 17985 Hz, TD = 64 K, SI = 32 K, 90^{0} pulse-length = 9 ms, PL1 = 1.5 dB, ns = 300, ds = 2, dI =3 s. The NMR-grade $CDCl_3$ was used for the solution of the synthesized compound.

Experimental part

Synthesis of methyl 2-(2-methoxy-2-oxoethyl)-4-methylfuran-3-carboxylate

The synthesis of methyl 2-(2-methoxy-2-oxoethyl)-4-methylfuran-3-carboxylate was carried out by the known in literature method (Ismailov et al., 2027), viz. alkylation of dimethyl 3-oxo-pentadioate with 1,2,3-tribromopropane in the presence of potassium carbonate in DMSO (Figure 1).

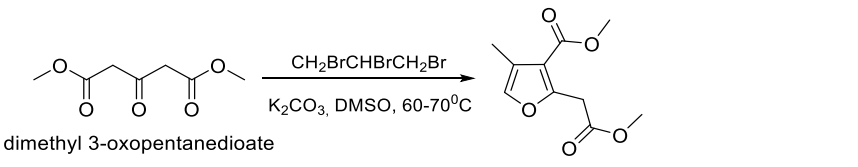

Figure 1. Alkylation reaction of dimethyl 3-oxopentadioate with 1,2,3-tribromopropane.

For this purpose, 7.3 g of dimethyl 3-oxopentadioate and 12 g of K_2CO_3 were added to 50 mL of DMSO followed by the dropwise addition of 11.7 g of 1,2,3-tribromopropane during 2 hours at room temperature. Afterwards, the reaction mixture was stirred for an additional 3 hours at room temperature and 10 hours at 60-70°C. Subsequently, the formed solution was cooled, treated with water and extracted with diethyl ether. The extract was dried over $MgSO_4$, the solvent was distilled off, and the residue was purified under vacuum with the receiving of yellowish methyl 2-(2-methoxy-2-oxoethyl)-4-methylfuran-3-carboxylate. Yield 68.7%, b.p. 120-122°C (2 mm Hg), n_d^{20} = 1.4283. ^{1}H NMR spectrum of methyl 2-(2-methoxy-2-oxoethyl)-4-methylfuran-3-carboxylate (Figure 2): ($CDCl_3$, δ, ppm), 2.01-2.04 d (3H, CH_3, J=9 Hz), 3.58-3.89 m (8H, $2OCH_3$+CH_2), 7.02-7.05 d (1H, =CH, J=9 Hz). ^{13}C NMR spectrum of methyl 2-(2-methoxy-2-oxoethyl)-4-methylfuran-3-carboxylate (Figure 3): ($CDCl_3$, δ, ppm), 9.93 (CH_3), 34.33 (CH_2), 51.39 (OCH_3), 52.46 (OCH_3), 115.78 (C), 121.64 (C), 139.58 (CH), 155.40 (C), 164.60 (COO), 169.64 (COO). Found, %: C 56.67; H 5.79. $C_{10}H_{12}O_5$. Calculated, %: C 56.60; H 5.70.

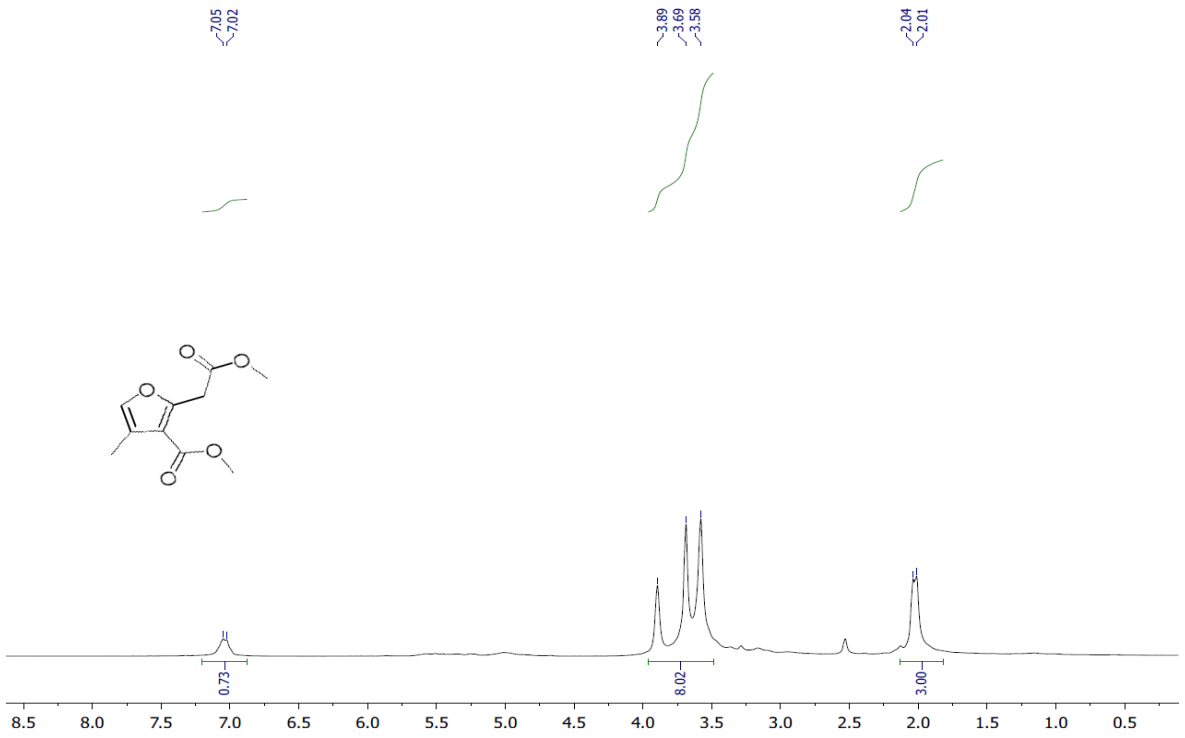

Figure 2. ^{1}H NMR spectrum of methyl 2-(2-methoxy-2-oxoethyl)-4-methylfuran-3-carboxylate.

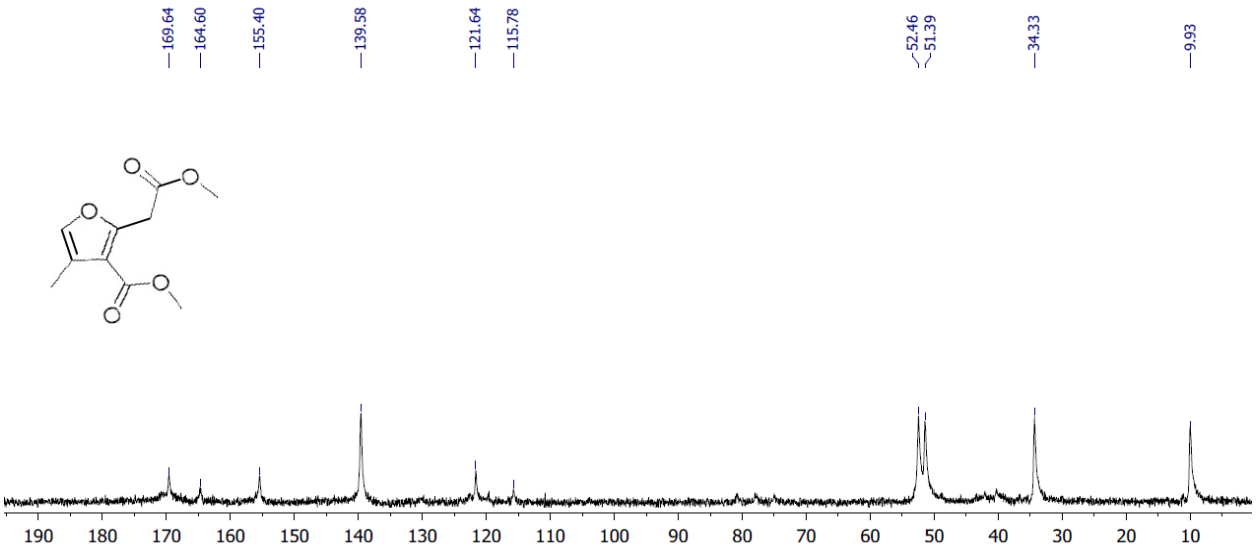

Figure 3. ^{13}C NMR spectrum of methyl 2-(2-methoxy-2-oxoethyl)-4-methylfuran-3-carboxylate.

Culture test and growing conditions

All bacterial and fungal cultures were taken from the culture collection of Baku State University. Gram-positive (*Bacillus mesentericus* BDU-54, *B.subtilis* BDU-50 and *Staphylococcus aureus* BDU-23) and Gram-negative (*Acinetobacter baumannii* BDU-32, *Escherichia coli* BDU-12, *Klebsiella pneumoniae* BDU-44 and *Pseudomonas aeruginosa* BDU-49) bacteria and opportunistic fungi of genus *Candida* (*C. albicans* BDU-XD45, *C. guillermondii* BDU-217 and *C. tropicalis* BDU-LK30) were used as test cultures. Bacteria were grown on Nutrient agar (Liofilchem), whereas fungi were grown on Sabouraud CAF agar (Liofilchem). Fresh 24 hours old cultures were used in all experiments.

Determination of antimicrobial activity and MIC

The antimicrobial activity of methyl 2-(2-methoxy-2-oxoethyl)-4-methylfuran-3-carboxylate was *in-vitro* determined by the standard agar diffusion method (Balouiri et al., 2016). A microbial suspension of test culture (density 0.5 McFarland) in an amount of 0.1 mL was added to the surface of the dense medium and smeared over the entire surface with a sterile spatula. Then, a hole with a diameter of 8 mm was opened with a sterile glass hole punch. A solution of test substance in DMSO was added to the hole in an amount of 150 µL. The effect of the substance was tested at three concentrations: 0.3, 0.1 and 0.05%. Bacterial cultures were incubated at 37°C for 24 hours, and fungal cultures at 30°C for 48-60 hours. The zone of inhibition (the clear zone around the holes) was measured with a ruler in mm. DMSO was used as a negative control. Gentamicin and Fluconazole were used as a positive control for bacteria and fungi, correspondingly. All experiments were performed in 4 replicates and statistically processed.

The minimum inhibitory concentration (MIC) of methyl 2-(2-methoxy-2-oxoethyl)-4-methylfuran-3-carboxylat was determined from the ratio of all test cultures in the concentration range of 1000-7.8 µg mL^{-1}. Serial twofold dilutions of the test compound were prepared.

In silico analysis

GaussView 6.0.16 (GaussView, 2009) and Gaussian 16 ES64L-G16RevA.03 (Gaussian 09, 2009) package programs were used to do computational analyses at the M06-2X/6-31+G(d,p) level. In these calculations, the water phase was taken into consideration via the IEF-PCM method. No imaginary frequency was encountered in the optimized structure. Electronic properties of the studied compounds were investigated by the illustration of the contour plot of frontier molecular orbitals (HOMO and LUMO) and molecular electrostatic potential (MEP) maps. For easier interpretation of the in silico results, the gentamicin was optimized at the related calculation level and included in the molecular docking calculation. In molecular docking analyses, 2 bacteria, viz. *Staphylococcus aureus* and *Acinetobacter baumannii*, were chosen as targets due to the fact that according to the experimental results the studied compound showed the lowest MIC against the mentioned bacteria. TMK and CarO proteins were selected as targets for the *S. aureus* and *A. baumannii*, respectively. Related proteins were downloaded from the Protein Data Bank web tool (https://www.rcsb.org/). Furthermore, ADME properties, MM-GBSA and p450 cytochrome analyses of the studied compound were performed. Maestro 12.8 software was used for *in silico* analyses (Friesner et al., 2006; Halgren et al., 2004; Friesner et al., 2004; Schrödinger Release, 2021a; 2021b; 2021c).

Results and discussion

Synthesis and biological investigations

The targeted methyl 2-(2-methoxy-2-oxoethyl)-4-methylfuran-3-carboxylate was synthesized by the known in literature method (Ismailov et al., 2017), viz. alkylation of dimethyl 3-oxo-pentadioate with 1,2,3-tribromopropane in the presence of potassium carbonate in DMSO (Figure 1). The structure of the obtained product was established by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis. In the ^{1}H NMR spectrum, the presence of signals in the region of 3.58-3.89 ppm was assigned to methoxy and methylene groups, whereas the methyl group was observed at 2.01-2.04 ppm, respectively. In addition, the sp^2 hybridized CH-group was observed at 7.02-7.05 ppm. Looking at the ^{13}C NMR spectrum, the signals at 9.93, 34.33, 51.39 and 52.46 ppm correspond to methyl, methylene and methoxy groups respectively, whereas the position of sp^2 hybridized CH-group is 139.58 ppm. In addition, carboxyl group signals are observed at 164.60 and 169.64 ppm respectively (Figures 2-3).

Afterwards, the antimicrobial activity of the investigated compound was studied against gram-positive (*Bacillus mesentericus* BDU-54, *B.subtilis* BDU-50 and *Staphylococcus aureus* BDU-23) and gram-negative (*Acinetobacter baumannii* BDU-32, *Escherichia coli* BDU-12, Klebsiella pneumoniae BDU-44 and *Pseudomonas aeruginosa* BDU-49) bacteria and opportunistic fungi of genus *Candida* (*C. albicans* BDU-XD45, *C. guillermondii* BDU-217 and *C. tropicalis* BDU-LK30). The results of the antimicrobial activity of the tested compound are presented in Table 1. As it can be seen from the table, all tested bacteria and fungi were sensitive to this substance at concentrations of 0.3, 0.1 and 0.05%. In the case of gram-negative bacteria, the greatest inhibition was observed in *A. baumannii* and *K. pneumoniae*, in which the zone of inhibition at a concentration of 0.3% was 1.2-1.3 times larger than that of *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. In the case of gram-positive bacteria, the greatest inhibition was observed in *Bacillus mesentericus*, where the zone of growth inhibition was 1.2 and 1.3 times larger than that of *B. subtilis* and *Staphylococcus aureus* respectively. A decrease in the concentration of the substance by 6 times (from 0.3 to 0.05%) did not significantly reduce its inhibitory activity. It was found that in the case of gram-negative bacteria, the decrease in inhibitory activity was 1.3–1.6 times, whereas in the case of gram-positive bacteria, it was 1.4–1.9 times. In addition, the activity of the targeted compound was compared with the activity of the pristine antibiotic Gentamicin. It was found that the activity of the compound in most cases was higher than that of pristine antibiotic. Only in the case of *Ps.aeruginosa* and *S.aureus* the activity of the compound was equal to the activity of Gentamicin (Figures 4-5).

Antimicrobial activity tests also demonstrated that the substance significantly inhibited the growth of yeast fungi. The zone of inhibition in the case of both *C. tropicalis* and *Candida guillermondii* was 1.2 times larger than in the case of *Candida albicans*. In addition, it was found that the activity of the targeted compound against all three fungi was higher than that of the pristine antibiotic Fluconazole (Figure 6).

Furthermore, the MIC of the investigated substance was also determined. It was found that it varied within 62.5–15.6 μg mL^{-1} (Table 1). However, all three fungal species, *E.coli* and *B.mesentericus* had the same MIC (62.5 μg mL^{-1}). The lowest MIC (15.6 μg mL^{-1}) was observed in the case of *A.baumannii* and *S.aureus*. The variation of the MIC of the substance with respect to bacteria can apparently be explained by the difference in the mechanism of action of the inhibition of different species of bacteria.

Table 1. Antimicrobial activity of methyl 2-(2-methoxy-2-oxoethyl)-4-methylfuran-3-carboxylate.

Test cultures		Diameter of inhibition zone (mm), M ± m				MIC (μg mL^{-1}) of the studied compound
		Antibiotic 0.3%	The concentration of the test compound			
			0.3%	0.1%	0.05%	
		Gentamicin				
Gram-negative bacteria	*Acinetobacter baumannii*	19.0±0.7	25.2±1.2	20.7±1.0	18.5±0.7	15.6
	Escherichia coli	18.0±0.6	21.0±1.1	18.0±0.6	16.8±0.5	62.5
	Klebsiella pneumoniae	20.0±0.1	25.0±1.2	20.7±1.1	15.8±0.4	31.25
	Pseudomonas aeruginosa	20.0±0.1	20.0±1.0	18.5±0.6	14.3±0.3	31.25
Gram-positive bacteria	*Bacillus mesentericus*	26.0±1.3	30.0±1.5	23.5±1.2	15.7±0.4	62.5
	Bacillus subtilis	24.0±1.1	25.0±1.2	18.0±0.6	16.0±0.4	31.25
	Staphylococcus aureus	24.0±1.1	24.0±1.0	19.7±0.8	17.7±0.6	15.6
		Fluconazole				
Fungi	*Candida albicans*	23.0±1.1	26.2±1.3	21.8±1.1	19.0±0.8	62.5
	Candida guilliermondii	26.0±1.3	30.2±1.5	24.3±1.2	16.2±0.4	62.5
	Candida tropicalis	25.0±1.2	31.2±1.5	28.0±1.4	19.7±08	65.5

Due to the fact that compounds dissolved in the solvent, which is able to demonstrate antimicrobial activity, viz. DMSO, the activity of the mentioned solvent was also studied. The tests showed no effect of DMSO on test cultures.

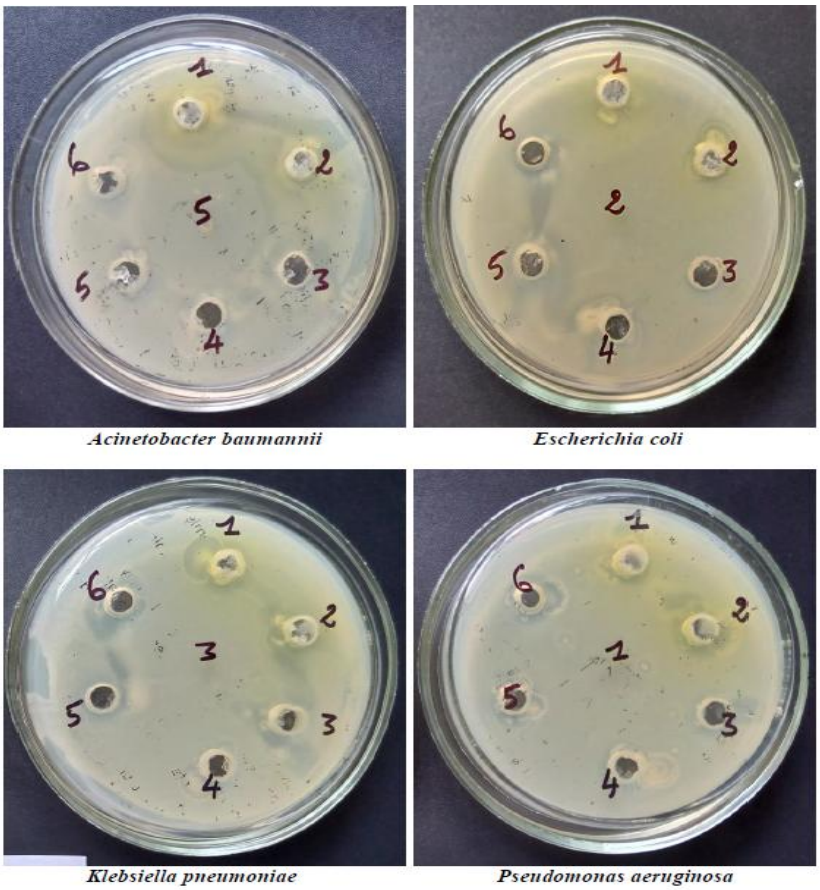

Figure 4. Antibacterial activity of the targeted compound against gram-negative bacteria.

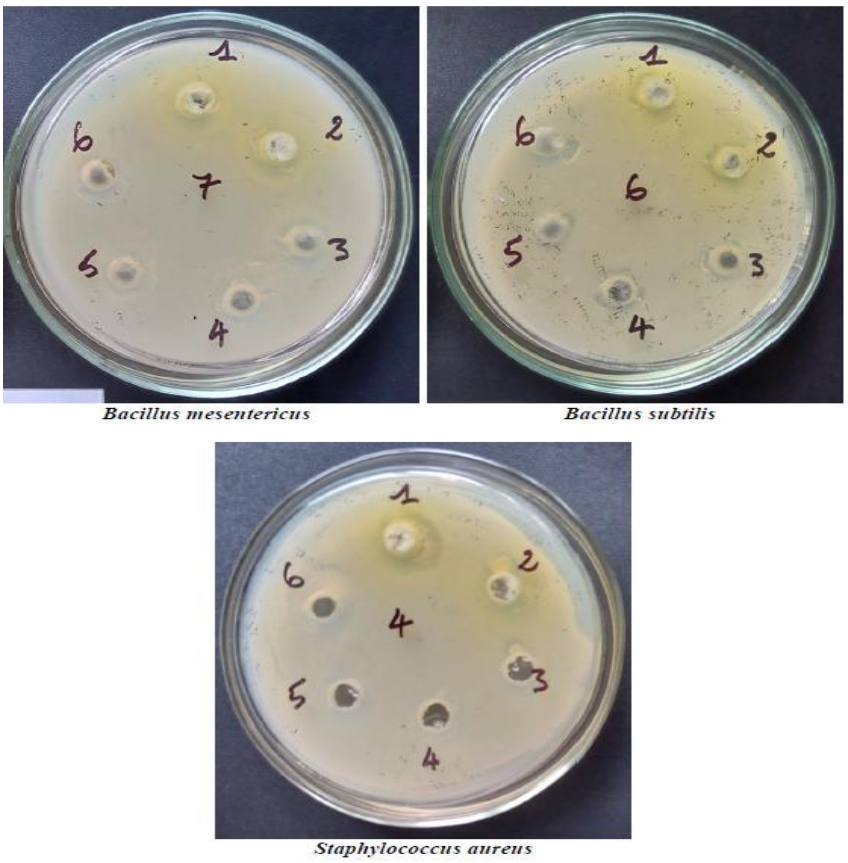

Figure 5. Antibacterial activity of the targeted compound against gram-positive bacteria.

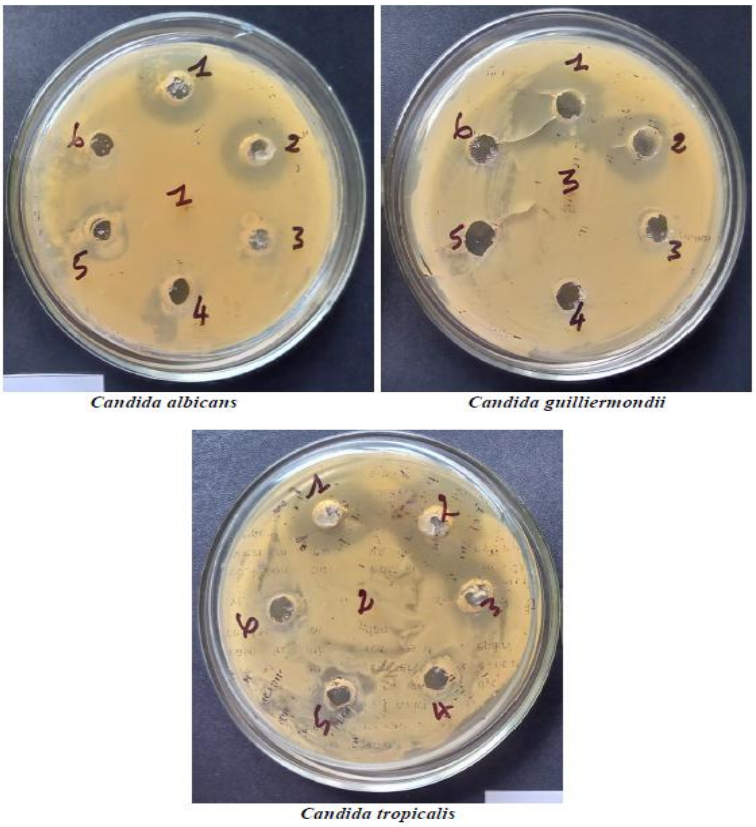

Figure 6. Antifungal activity of the targeted compound.

In silico analysis

The studied compound is optimized at M06-2X/6-31+G(d,p) level in the water, which is represented in Figure 7.

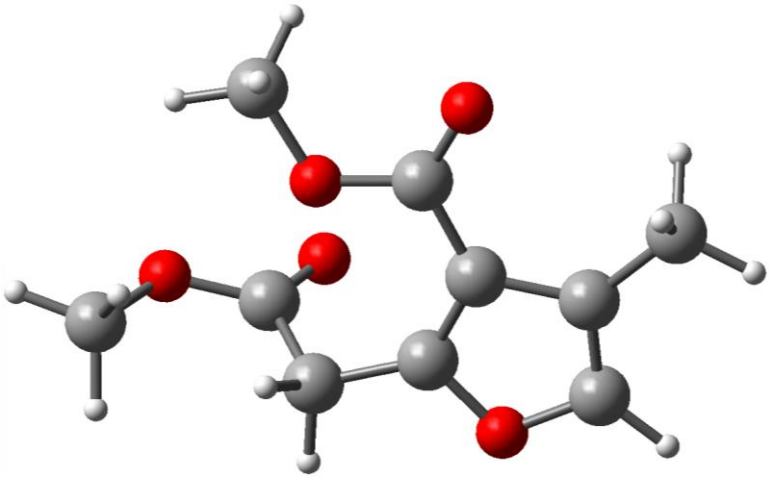

Figure 7. Optimized structure of related compound.

In the result of the optimization calculation, no imaginary frequency is observed. It implies that the obtained optimized structure is at the ground state level. Electronic properties of the studied compound were investigated by contour plot of frontier molecular orbitals (FMOs) which are HOMO and LUMO, MEP map, and Fukui functions. The main goal is to determine the active sites of the molecule. The contour plots of HOMO and LUMO are represented in Figure 8.

According to Figure 8, electrons in the HOMO are mainly delocalized on the furan ring and oxygen atoms on the structure. Accepted electrons will be located on LUMO and it seems that the accepted electrons will be mainly delocalized on the whole structure. The MEP map of the studied compound is calculated and represented in Figure 9.

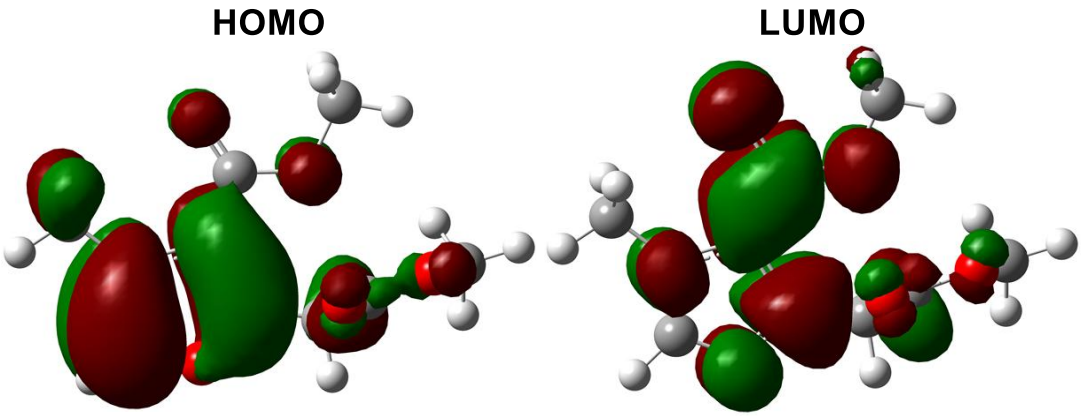

Figure 8. Contour plots of FMOs.

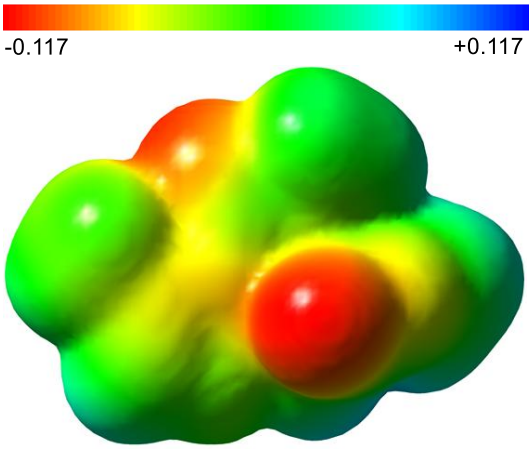

Figure 9. MEP map of the studied compound.

According to Figure 9, the green colour is the dominant one on the whole structure. The red colour represents the most electron-dense region and it is seen that this is around the oxygens in the carbonyl group, while the yellow colour dominates around the carbon atom in the same functional group. To determine the electrophilic (f^-) and nucleophilic (f^+) attack sites, Fukui functions are calculated and represented in Figure 10. As it can be seen from the mentioned figure, especially carbonyl group seems appropriate for the nucleophilic attack while the furan ring is more appropriate for the electrophilic attack.

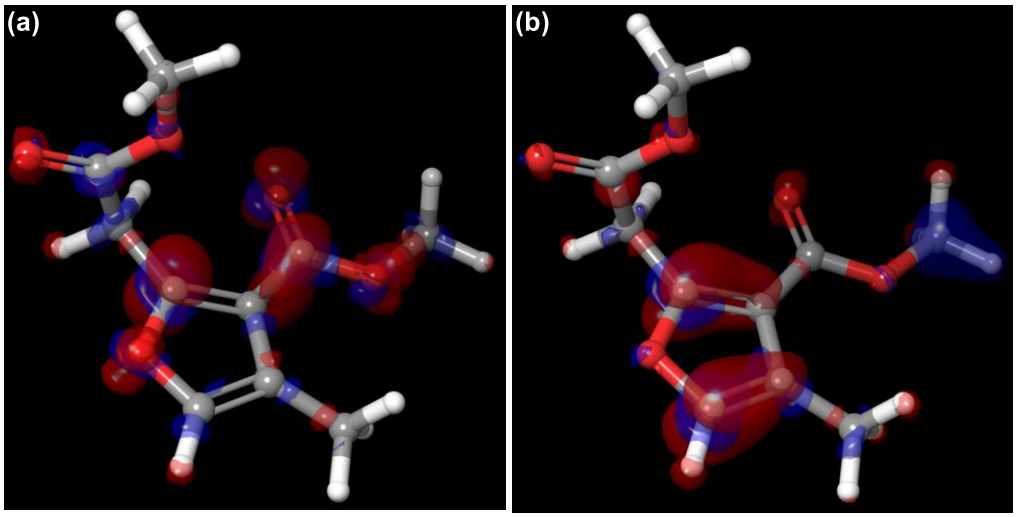

Figure 10. Fukui functions, viz f^+ (a) and f^- (b), of the studied compound.

Molecular docking, "absorption, distribution, metabolism, and excretion" (ADME), p450 cytochrome analysis, and MM-GBSA calculations are also performed for the studied compound. In molecular docking, MM-GBSA and ADME calculations, gentamicin is used as the comparison compound. Firstly, ADME calculations of related compounds were performed and selected ADME parameters were given in Table 2.

Table 2. Calculated QikProp parameters[a] of studied compound and gentamicin.

Compound	Stars	Amine	rtvFG	SASA	FOSA	FISA	PISA
Studied Compound	0	0	1	464.001	307.908	94.422	61.67
Gentamicin	5	5	2	794.679	549.533	245.145	0
RV[b]	0-5	0-1	0-2	300.0-1000.0	0.0-750.0	7.0-330.0	0.0-450.0
Compound	WPSA	donorHB	AccptHB	QPpolrz	QPPCaco	QplogBB	QPPMDCK
Studied Compound	0	0	4.5	22.547	1260.353	-0.467	635.287
Gentamicin	0	11	16.95	45.207	0.728	-2.012	0.272
RV[b]	0.0-175.0	0.0-6.0	2.0-20.0	13.0-70.0	<25 poor >500 great	-3 - 1.2	<25 poor >500 great
Compound	QPlogKp	Metab	QPlogKhsa	Percent Human-Oral Absorption	PSA	Rule Of Five	Rule Of Three
Studied Compound	-2.754	3	-0.534	90.748	79.654	0	0
Gentamicin	-8.915	8	-1.101	0	188.583	2	2
RV[b]	-8.0 - -1.0	1 – 8	-1.5 - 1.5	<25 poor >500 great	7.0 - 200.0	Max is 4	Max is 3

[a] Stars: Number of property or descriptor values that fall outside the 95% range of similar values for known drugs; Amine: Number of non-conjugated amine groups; rtvFG: Number of reactive functional groups; SASA: Total solvent accessible surface area; FOSA: Hydrophobic component of the SASA; FISA: Hydrophilic component of the SASA; PISA: π (carbon and attached hydrogen) component of the SASA; WPSA: Weakly polar component of the SASA; donorHB: Estimated number of hydrogen bonds that would be donated; AccptHB: Estimated number of hydrogen bonds that would be accepted; QPpolrz: Predicted polarizability in cubic angstroms; QPPCaco: Predicted apparent Caco-2 cell permeability in nm/sec; QPlogBB: Predicted brain/blood partition coefficient; QPPMDCK: Predicted apparent MDCK cell permeability in nm/sec; QPlogKp: Predicted skin permeability; metab: Number of likely metabolic reactions; QPlogKhsa: Prediction of binding to human serum albumin; PercentHuman-OralAbsorption: Predicted human oral absorption on 0 to 100% scale; PSA: Van der Waals surface area of polar nitrogen and oxygen atoms; RuleOfFive: Number of violations of Lipinski's rule of five; RuleOfThree: Number of violations of Jorgensen's rule of three.[b] RV: Recommended Value

According to Table 2, the calculated parameters are in good agreement with the recommended value for the studied compound. Gentamicin shows some out-of-reference values. Therefore, it is determined that the ADME properties of the studied compound are better than that of pristine antibiotic.

Further, molecular docking calculations were performed for the studied compound and the obtained docking results are given in Table 3. Molecular docking calculations were done against thymidine kinase (TMK) in *Staphylococcus aureus* and CarO (outer membrane protein) in *Acinetobacter baumannii*. The reason for choosing the mentioned bacteria is caused by the fact that according to the experimental results, the studied compound showed the lowest MIC against these mentioned bacteria (Table 1). 2CCG and 4FUV are selected targets for the TMK and CarO, respectively.

Table 3. The molecular docking results of the studied compound and gentamicin against 2CCG and 4FUV.

Compounds	DS[a,b]	E_{vdW}[a,b]	E_{Coul}[a,b]	E_{Total}[a,b]
	2CCG			
Studied Compound	-2.926	-24.810	-2.617	-27.427
Gentamicin	-0.480	-21.899	-31.049	-52.948
	4FUV			
Studied Compound	-2.574	-19.797	-1.341	-21.139
Gentamicin	-1.856	-23.474	-12.792	-36.266

[a] in kcal mol^{-1}; [b] DS (Docking score), E_{vdW} (van der Walls energy), E_{Coul} (Coulomb interaction energy), E_{Total} (Total interaction energy)

According to Table 3, key-lock harmony between the inhibitor (studied compound) and protein is better than that of gentamicin while interaction energy of gentamicin is better than that of the studied compound. However, it can be said that the studied compound exhibits better results because the key lock compatibility between the inhibitor and protein is more important.

So, what can the targeting of TMK and CarO lead to?

Being a member of the special reaction chain that introduces thymidine into the DNA, TMKs play a crucial role in DNA synthesis and, by extension, cell division (Topolcan et al., 2008). Thus, targeting TMK can lead to stalling DNA synthesis or lessions, that generate single-stranded and double-stranded DNA breaks (Berdis, 2017).

From the other side, CarO is an Acinetobacter baumannii outer membrane protein (porin), which is a β-barrel-shaped monomeric structure with a molecular weight of 25-29 kDa. It forms multimeric channels and

is mostly responsible for the influx of small molecules (water, ions and so on), peptides, and some antibiotics. Several investigations demonstrated that CarO is implicated in carbapenem resistance (Mussi et al., 2007; Poirel & Nordmann, 2006; Simo Tchuinte et al., 2019). Carbapenems are a class of antibiotics that cannot be inactivated by the majority of Ambler class A, C, and D β-lactamases and are often saved for known or suspected multidrug-resistant bacterial infections. As a result, medicines such as carbapenems are often utilized as a last option in the treatment of infections brought on by Acinetobacter spp. It was found that carbapenems target CarO which allows them to diffuse through multimeric channels inside bacterial cells (Sariyer, 2022). Due to the fact that the studied compound also targets CarO, it is possible to assume that the mechanism of antibacterial activity of furan derivative against *A. baumannii* occurs by the same way as in the case of carbapenem class of antibiotics. Thus, it is possible to suppose that the synthesized furan derivative is able to mimic the carbapenems (towards activity against *A. baumannii)*.

Furthermore, MM-GBSA calculations were performed to determine the binding energy of the studied compound and gentamicin. While the interaction energy and Docking score play an important role, the binding energy of the ligand to the protein in the complex structure is very important in determining the efficiency of the ligand. The binding energy of the studied compound and gentamicin is calculated as -46.985 and -43.949 kcal mol^{-1} for 2CCG; -32.056 and -23.646 kcal mol^{-1} for 4FUV. These results show that the studied compound exhibits better results in comparison with pristine antibiotic.

Subsequently, p450 cytochrome calculation was done for the studied compound to predict the excretion of the drug to be taken into the body. If the excretion of the drug from the body is late, drug accumulation will occur, which will lead to the interaction with other drugs taken into the body and as a result will show toxicity. Therefore, p450 cytochrome analysis of the compound under investigation plays an important role in this context. As it was observed from p450 cytochrome calculations, the studied compound inhibits the CYP2C9 enzyme protein (Figure 11), which is essential for the oxidation of both xenobiotics, such as pharmaceuticals, and endogenous substances, such as fatty acids, in the body. Thus, all this mentioned makes CYP2C9 a crucial cytochrome P450 enzyme.

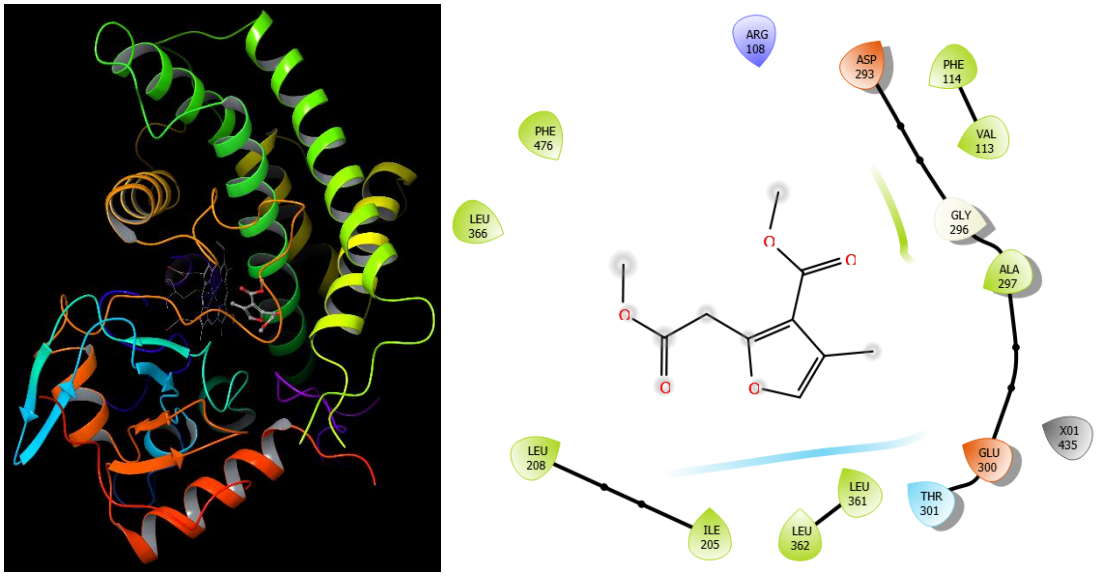

Figure 11. Interaction between studied compounds and CYP2C9.

The docking score and total interaction energy are calculated as -4.179 and -25.934 kcal mol^{-1}, respectively. Due to the fact that liver tissue shows the highest expression of CYP2C9 (Furuya et al., 1991) the inhibition of this enzyme by the studied compound will lead to retention and accumulation in the liver of furan derivative. As a result, it may cause toxicity if taken together with different drugs.

Conclusion

The biological activity investigations of methyl 2-(2-methoxy-2-oxoethyl)-4-methylfuran-3-carboxylate, which was synthesized by alkylation of dimethyl 3-oxopentadioate with 1,2,3-tribromopropane, revealed a

wide range of antimicrobial activity in comparison with pristine antibiotics against gram-positive and gram-negative bacteria, as well as yeasts of genus *Candida.* MIC investigations demonstrated that the lowest value, viz. 15.6 μg mL^{-1}, was observed in the case of *S. aureus* and *A. baumannii*. The target proteins for the aforementioned bacteria were determined using molecular docking calculations, which revealed 2CCG and 4FUV for the TMK (S. aureus) and CarO (A. baumannii), respectively. Taking into account the targeting of CarO by synthesized compound it is possible to assume that the furan derivative is able to mimic the carbapenem class of antibiotics. In order to predict the excretion of the drug to be taken into the body, p450 cytochrome analysis was performed and revealed that the studied compound inhibits the CYP2C9 enzyme protein, which plays a significant role in the metabolism by oxidation of both xenobiotic and endogenous compounds. Additionally, the examined molecule displays superior outcomes than pristine antibiotic as in the case of the experimental study, according to ADME characteristics and MM-GBSA studies.

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