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Original Article

Allomaltol derivatives as Antimycobacterial agents: *In vitro* and *in silico* evaluations with potential protein targets

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ABSTRACT

Background and Aims: Mycobacterium species cause life-threatening pulmonary and extrapulmonary diseases in humans. This study aimed to evaluate the potential antimycobacterial activity of allomaltol derivatives in the Mannich base structure *in vitro* and *in silico.*

Methods: The antimycobacterial activity of each compound against *Mycobacterium tuberculosis* and *Mycobacterium avium* was tested using a resazurin microplate assay, and cytotoxicity was assessed using human MRC-5 and He-La cells. Using the SwissTarget tool, Rip1 protease, the metallo-beta-lactamase (MBL) superfamily protein, the serine protease Rv3671c, and zinc metalloprotease 1 (ZMP1) were identified as potential targets. Blind docking was performed for compound 14 using CB-Dock to identify and assess the most probable binding sites on the target proteins. Defined docking was performed with Flare to determine the best binding pose at the predicted binding pocket. The druglikeness of hit compounds, including the partition coefficient, number of hydrogen bond donors/acceptors, molecular refractivity, topological polar surface area (PSA), and gastrointestinal and blood-brain barrier absorption, were evaluated using the SwissADME tool.

Results: Compounds with methyl-substituted piperidine groups were found to have antimycobacterial activity (MICs: 2 µg/mL) against M. avium, which was as potent as the clinically used drugs ethambutol and streptomycin. The predicted physicochemical properties of the four hit compounds were satisfactory. According to the docking results, the binding energies of compound 14, which showed the best overall antimycobacterial activity, ranged from -8.14 to -5.97 kcal/mol, with ZMP1 showing the lowest binding energy.

Conclusion: The results of this study provide evidence that allomaltol derivatives are promising antimycobacterial agents with satisfactory drug profiles.

Keywords: Allomaltol, Tuberculosis, Molecular Docking, Druglikeness

INTRODUCTION

Tuberculosis (TB), a life-threatening disease caused by *Mycobacterium tuberculosis*, is one of the most deadly respiratory bacterial diseases and the second leading cause of death from infectious agents, following COVID-19. War, immigration, social status, poverty, gender, HIV infection, and homelessness are significant risk factors for tuberculosis, as well as other public health problems (Amiri, Siami, & Khaledi, 2018). Moreover, the development of drug resistance and the presence of comorbidities, such as acquired immunodeficiency syndrome (AIDS) and diabetes mellitus, increase the morbidity and mortality of the disease (Venugopala et al., 2021). Non-tuberculous mycobacteria (NTM) are opportunistic pathogens that can be found in natural water and soil, posing a serious threat to human health, particularly for patients who are immunocompromised or with pre-existing lung diseases. Among these, *Mycobacterium avium* is the most clinically significant pathogen. The substantial levels of inherent drug resistance in NTM contribute to unsatisfactory treatment outcomes, necessitating the development of novel drugs and therapeutic regimens (Portell-Buj et al., 2019; O. Falkinham, 2018). Therefore, *Mycobacterium* spp. have attracted the attention of medicinal chemists, encouraging them to search for novel compounds with improved bioactivities.

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Kojic acid (5-hydroxy-2-hydroxymethyl-4*H*-pyran-4-one), is a natural secondary metabolite produced by different types of fungi during aerobic fermentation. Owing to the unique structure of its scaffold, many synthetic derivatives have been prepared and investigated for different biological activities (Brtko, 2022; Zilles et al., 2022; Saeedi, Eslamifar, & Khezri , 2019). Since 3-Hydroxy-4(1*H*)-pyrones and their analogues are extensively utilised as foundational components for biologically active substances, allomaltol (5-hydroxy-2-methyl-4H-pyran-4-one) has drawn the attention of researchers (Milyutin et al., 2023; Ercan et al., 2020; Kandioller et al., 2011).

Nitrogen-containing heterocyclic compounds such as piperazine and piperidine rings have been widely used for the development of many drugs and other bioactive substances, including several of the currently used antitubercular drugs (Girase et al., 2021). Mannich reactions, also known as aminomethylation reactions, facilitate the synthesis of chemical entities with basic nitrogen atoms, making them valuable tools for drug discovery (Roman, 2022). Our research group has previously reported the anticonvulsant effects of several Mannich bases of allomaltol with piperazine moieties (Aytemir, Çaliş, & Özalp 2004; M. Aytemir & Çalış, 2006) (Figure1). In another study, Mannich bases of kojic acid and allomaltol were used to synthesize piperazine and piperidine analogues. These derivatives were examined for their effects on seizures induced by scMet and MES (maximal electroshock) (Aytemir & Çalış, 2010). The results show that piperidine-containing allomaltol derivatives have enhanced activity compared with piperazine-containing derivatives.

The primary objective of drug discovery is to develop bioactive compounds that are effective, selective, and have low toxicity. Most of the compounds bearing 3-Hydroxy-4(1*H*)-pyrone-containing Mannich bases designed and synthesized by our research group exhibited important bioactivities (Karakaya et al., 2019; Karakaya et al., 2019; Oncul et al., 2019). Because nitrogen-containing heterocyclic derivatives of allomaltol exhibit a wide spectrum of biological activities, our current goal is to use these derivatives to further develop bioactive compounds, particularly those with anticancer and antimicrobial properties, that do not harm healthy cells. These compounds have shown promising and uniform results and can be used as leads for drug development in the future.

Therefore, in the present study, we aimed to investigate the activities of 19 allomaltol derivatives against *M. tuberculosis* and *M. avium* and their cytotoxic effects on healthy (MRC-5) and carcinogenic (He-La) cell lines. Molecular docking studies were conducted with these compounds against several potential antimycobacterial drug targets. In addition, the drug-likeness properties of the most promising compounds were predicted in silico.

MATERIALS AND METHODS

Antimicrobial agents

All the compounds were dissolved in dimethylsulphoxide (3%) or d-H_2O at a final concentration of 512 µg /ml, sterilised by filtration using 0.22 µm Millipore (MA 01730, USA), and used as stock solutions. The standard antimycobacterial agents were isoniazid, ethambutol, and streptomycin. All compounds were purchased from Sigma Chemical Co. The stock solutions of the agents were prepared in accordance with the guidelines for the preparation of solutions outlined by the CLSI (Clinical and Laboratory Standards Institute) (Ozcelik et al. 2013; Aytemir & Özçelik, 2011; CLSI 2006).

Antimycobacterial Activity

The antimycobacterial activities of the compounds against M. tuberculosis and M. avium were determined using the Resazurin Microtiter Assay (REMA). M. tuberculosis H37Rv ATCC 27294 reference strain and M. avium ATCC 15769 strains were maintained on Lowenstein–Jensen medium, subcultured on Middlebrook 7H11 agar (Becton Dickinson), and resuspended in 7H9-S broth medium supplemented with 10% OADC (0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase), 0.2% glycerol, and 0.1% bactocasitone (Difco). Suspensions were prepared in 0.04% (v/v) Tween 80–0.2%+bovine serum albumin and adjusted to McFarland tube number 1. This was diluted to 1:20, and a 100-L aliquot was used as the inoculum. 100 µL of Middlebrook 7H9 broth (10% OADC, 0.5% glycerol, 0.1% casitone, Becton-Dickinson) was dispensed in each well of a sterile flat-bottom 96-well plate, and serial two-fold dilutions (256-0.06 µg /mL) of each compound were prepared. 100 µL of inoculum was added to each well. Growth and sterile control were also included for each isolate. Sterile water was added to prevent evaporation. The plate was then covered and incubated at 37 °C under a normal atmosphere. After 7 days of incubation, 10 µg /mL of resazurin solution was added, and the plate was reincubate overnight. Any change in colour from blue to pink indicated the growth of bacteria, and the minimum inhibitory concentration (MIC) was defined as the lowest concentration of the drug that prevented change in colour (Ozcelik et al. 2013; Aytemir & Özçelik, 2011).

Cytotoxicity assay

MRC-5 and He-La cell cultures were grown in Eagle's Minimal Essential Medium (Seromed; Biochrom; Berlin; Germany) enriched with 10% foetal calf serum (Biochrom, Germany). Streptomycin at a concentration of 100 mg/ml and penicillin at a concentration of 100 IU/ml in a humidified atmosphere with 5% CO_2 at 37 °C. The cells were harvested using trypsin solution (BibcoLife Technologies, UK). The Maximum non-toxic

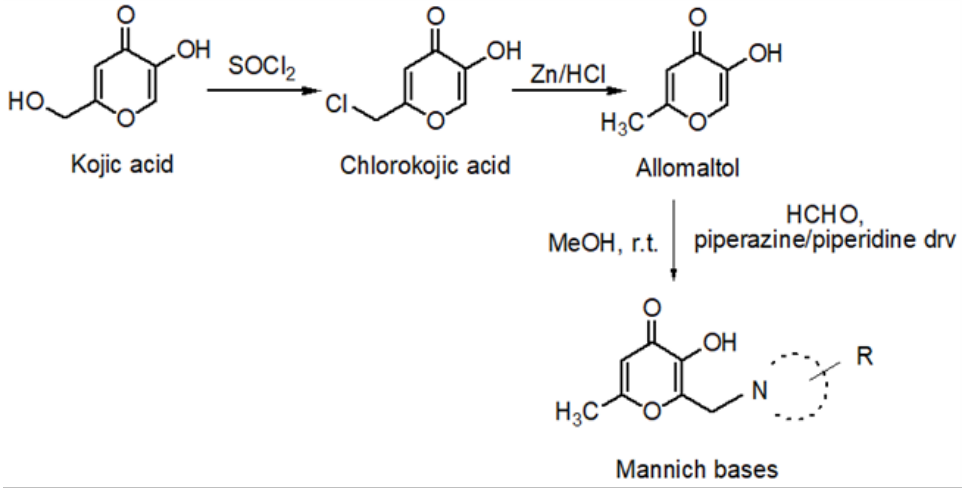


Figure 1. Synthesis and chemical structure of allomaltol derivatives.

concentrations (MNTCs) were determined on the basis of cellular morphologic alteration (Aytemir, Özçelik, & Karakaya, 2013; Karakaya et al., 2013). Several concentrations of each sample were placed in contact with confluent cell monolayers and incubated in 5% CO2, at 37 °C for 48 h. After incubation, non-toxic drug concentrations were evaluated and compared with those of untreated cells. The rows causing cell damage were evaluated as toxicity. In addition, maximum drug concentrations that did not affect the cells were evaluated as nontoxic concentrations. The MNTCs of the compounds were determined by comparing treated and untreated cultures.

In silico Studies

The structures were downloaded from the protein data bank (https://www.rcsb.org) and processed with UCSF Chimaera software. The structures of the compounds were drawn using ChemSketch. First, blind docking was performed using the online tool CB-Dock, which was developed using AutoDock Vina. This approach was used to identify and assess the most probable binding sites on target proteins via cavity detection (CurPocket) (Liu et al., 2020). Next, we performed defined docking using Flare version 6.0 software (Cresset, UK) to determine the best binding pose at the predicted binding pocket. Hydrogen atoms were included, and the optimal ionisation states were assigned to each residue. The chemical structure of compound 14 was uploaded in SDF format and processed using default settings. The grid was selected to include the binding site, which was determined using blind docking. The binding poses of each protein with the best scores and analysed for its interaction with compound 14.

RESULTS AND DISCUSSION

Chemistry

All of the compounds that were evaluated for their antimycobacterial activity in this study have been synthesised and characterised in our previous studies (Aytemir & Çalış, 2010; Aytemir, 2007; Aytemir & Çalış, 2006; Aytemir et al., 2004). Kojic acid was used as the starting compound in a two-step reaction including chlorination of kojic acid and subsequently reduction with zinc dust in conc. HCl, to gain allomaltol.

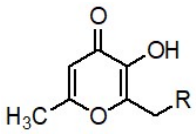
In vitro antimycobacterial effects of the compounds

Infectious diseases, particularly tuberculosis, pose a serious public health threat, and the emergence of antimicrobial resistance has limited clinical treatment options (Lv et al. 2024). Therefore, the discovery of new anti-TB agents is required.

Herein, REMA was performed to determine the antimycobacterial activity of all compounds against *M. tuberculosis* H37Rv and *M. avium* ATCC 15769)using isoniazid and ethambutol as control agents. The mechanism of action of ethambutol is not fully understood, whereas isoniazid inhibits the formation of the mycobacterial cell wall. A bacterial enzyme called "KatG" activates the drug, and the complex product inhibits the synthesis of mycolic acid, which is an essential cell wall component (Suarez et al., 2009). MICs (Table 1) of studied compounds against *M. tuberculosis* and *M. avium* were in the range of 2–128 µg/ml.

All 21 compounds exhibited antimycobacterial activity, including the synthesis starting materials. Among these compounds, 3, 10, 11 and 19 were found to be equally effective against both species, whereas the other compounds were more effective against *M. avium*.

Table 1. Anti-Mycobacterium activities coupled with the cytotoxicity of the compounds as MICs and MNTCs (µg/mL).



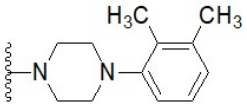
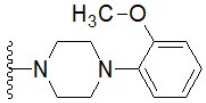
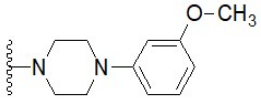
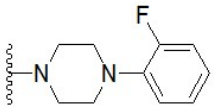
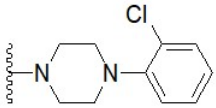
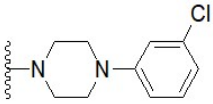
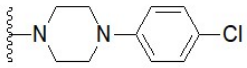
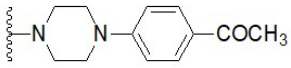
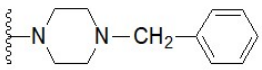
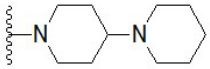
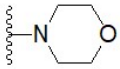
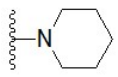
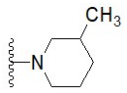
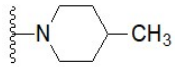
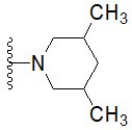
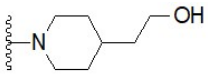
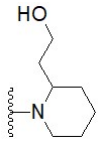
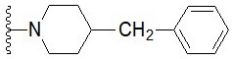
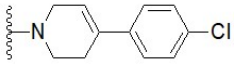
Comp. No.	R	MICs		Cytotoxicity (MNTCs)	
		M. tuberculosis ATCC 27294	*M.avium* ATCC15769	MRC-5	He-La
1[a]		64	16	≥512	≥512
2[b]		64	16	≥512	≥512
3[b]		16	16	≥512	≥512
4[b]		64	16	≥512	≥512
5[b]		64	16	≥512	≥256
6[a]		64	16	≥512	≥512
7[b]		64	16	≥512	≥256
8[b]		64	16	≥256	≥256
9[b]		64	16	≥256	≥256
10[a]		16	16	≥256	≥256
11[a]		16	16	≥256	≥256
12[c]		32	16	≥256	≥256

Table 1. continued

13[c]		**16**	**2**	≥256	≥256
14[c]		**16**	**2**	≥256	≥256
15[c]		**32**	**2**	≥256	≥256
16[c]		**8**	**4**	≥256	≥256
17[c]		64	128	≥256	≥256
18[c]		64	16	≥256	≥256
19[d]		16	16	≥256	≥256
Kojic acid		32	16	≥256	≥256
Allomaltol		32	16	≥256	≥256
Isoniazide		0.125	0.125		
Ethambutol		2	2		
Streptomycin		1	2		

a: (M. Aytemir & Çalış, 2006), b:(M. D. Aytemir et al., 2004), c:(Aytemir, M. D., Çalış, Ü., 2007), d: (M. D. Aytemir & Çalış 2010); MNTCs: Maximum non-toxic concentrations

Compound 16, which includes a 4-(2-hydroxyethyl) piperidine-1-yl group, was the most effective compound against *M. tuberculosis* (MIC = 8 µg/ml) and was four times more active than kojic acid and allomaltol (MIC = 32 µg/ml). However, compounds 12 and 15 showed similar activities to those of kojic acid and allomaltol.

Since it is known that bioactivity is not the only parameter required for a good drug candidate, drug-like properties were improved by switching to Mannich bases, which will be discussed in detail in the next section.

All compounds except compound 17 were more active against *M. avium*. In particular, compounds 13, 14, and 15 were the best derivatives in the series (MIC = 2 µg/ml) and were eight times more active than kojic acid and allomaltol (MIC = 16 µg/ml). More importantly, these three compounds were found to have the same antimycobacterial activity as the reference drug ethambutol and streptomycin. Compound 16 also showed good activity against *M. avium* (MIC = 4 µg/ml).

Correlating the chemical structures of compounds with their bioactivities is an important area of medicinal chemistry. The chemical structures of the compounds tested in this study differ according to the type of amine groups added by the Mannich reaction. The molecular modification was performed by adding secondary amine groups. Hence, the synthesised derivatives can be categorised into those bearing substituted phenyl piperazine and piperidine groups.

According to the electronic characteristics of the substituents in the compounds bearing the phenylpiperazine group, there was no difference between the groups in terms of electron-donating or electron-withdrawing properties. In addition, the position of the substituent does not have any effect, which can be easily observed from the lack of difference in the activities of compounds 5, 6, and 7, which are positional isomers with respect to chlorine atoms. Furthermore, the introduction of a methylene bridge between the piperazine and phenyl groups, as in the structure of compound 9, did not alter the activity. The antimycobacterial activity of compound 18, an isomer with

a piperidine ring instead of a piperazine ring based on the structure of compound 9, remained the same.

By evaluating all activity results, we found that the most potent compounds in the series were piperidine derivatives bearing -CH3 at different structural positions. Although the MIC values of compounds 13, 14, and 15 bearing 3-CH_3, 4-CH_3, and 3,5-diCH_3, respectively, were 16 µg/ml, 16 µg/ml, and 32 µg/ml, respectively, against *M. tuberculosis*, their activity against *M. avium* was the same, with an MIC of 2 µg/ml, similar to the reference drugs ethambutol and streptomycin. By comparing the antimycobacterial activity of compound 12, a nonsubstituted piperidine derivative without a methyl group, it can be assumed that the methyl group, which provides electrons to the ring inductively, significantly contributes to the bioactivity.

In contrast to the piperazine derivatives, the positions of the hydroxyethyl groups in the piperidine derivatives significantly affected their bioactivity. In this case, the hydroxyethyl group at the 4-position resulted in the second most active compound in the series, whereas the hydroxyethyl group at the 2-position resulted in the weakest compound.

Cytotoxicity of the compounds

Allomaltol derivatives were evaluated for their cytotoxic effects against normal MRC-5 (human lung fibroblast) and cancer HeLa (Human cervix epithelial carcinoma) cells using a previously described method (M. D. Aytemir et al., 2013; Karakaya et al., 2013). The MNTCs were determined based on cellular morphologic alterations and were designated as either ≥128 or ≥512 µg/mL. According to the cytotoxicity results, we observed no selectivity between the two groups. All compounds were bioactive at non-toxic concentrations (≥256 µg/mL). Except for compounds 8 and 9, piperazine containing Mannich bases had higher MNTCs (512 µg/mL) than kojic acid, allomaltol, and piperidine analogues (MNTCs: ≥256 µg/mL).

In silico studies

Target Prediction Analysis Results

The protein groups with a high likelihood of binding, as determined by the SwissTarget prediction analysis, a web-based tool, are depicted in the graph below (Figure2) (Daina et al., 2019). This tool predicts the protein targets with which the investigated compound may interact. In this study, Rip1 protease, metallo-beta-lactamase (MBL) superfamily protein, serine protease Rv3671c, and zinc metalloprotease 1 (ZMP1) were predicted as potential targets of compound 14 and were selected for molecular docking. Rip1 is a metalloprotease and an important virulence factor in mycobacteria. It cleaves anti-sigma factors K, L, and M, which negatively regulate the corresponding sigma factors SigK, SigL, and SigM, respectively. These sigma factors are involved in activating the expression of several other virulence factors (Schneider, Sklar, & Glickman, 2014). The MBL superfamily includes a group of enzymes that inactivate a broad spectrum of -lactam antibiotics, except for monobactam. They are not affected by most beta-lactamase inhibitors, such as clavulanate, sulbactam, and tazobactam (Boyd et al. 2020). Rv3671c is a serine protease that enables mycobacteria to persist within phagolysosome (Biswas et al. 2010). Zinc metalloprotease-1 (Zmp1) is an important target and virulence factor in mycobacteria. It is a zinc-containing peptidase that interferes with phagosome maturation in macrophages, possibly by inhibiting the secretion of caspase-1/interleukin-1β and suppressing the formation of inflammasome and phagolysosomes (Ferraris et al. 2011). Cyclic di-GMP (c-di-GMP) phosphodiesterase (CDP) hydrolyses c-di-GMP to yield two GMP molecules. It regulates biofilm formation, cell motility, and virulence in mycobacteria (Hull et al. 2012). Therefore, these five enzymes represent attractive therapeutic targets for mycobacteria, and their inhibition may attenuate virulence.

Molecular Docking Results

According to the literature, only a few publications have reported the targets and mechanism of action of synthetic antimycobacterium compounds. The use of computational chemistry accelerates the discovery and design of new compounds with improved potency while also reducing the synthesis cost. Molecular docking simulation is a crucial tool for structure-based drug design (SBDD) because it can predict the binding affinity as well as the binding pose of the ligand with the active sites of a target (Abdullahi et al., 2020). It is an optimisation process in which the main goal is to find the most stable binding position of the ligand with the target molecule. The binding affinity of each possible orientation of the ligand within the active sites forming the complex was calculated by sampling the 3D coordinate space of the binding site on the target. Thus, the pose with the lowest binding energy is predicted as the most stable conformation of the protein-ligand complex.

In this study, we subjected compound 14 to two rounds of molecular docking. The first round was a blind docking approach used to identify and assess the most probable binding sites on the target proteins. The second round was a defined docking approach used to determine the best binding pose at the predicted binding pocket. The binding energies, shown in Table 2, ranged from -8.14 to -5.97, kcal/mol, with ZMP1 exhibiting the lowest binding energy. These results suggest that compound 14 binds with high affinity to each of the predicted targets. The predicted binding interactions showed that compound 14 formed one hydrogen bond with ALA 631 in ZMP1, two hydrogen bonds with SER 442 and GLN 445 in CDP, one hydrogen bond with SER 397 in the serine protease Rv3671c, one hydrogen bond with ASP 76 and one aromatic interaction with PHE 175 in the MBL superfamily protein, and two hy-

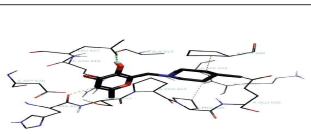
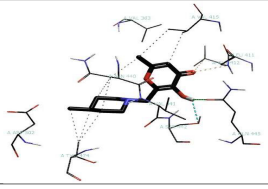
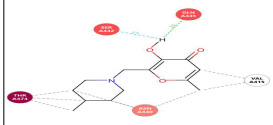
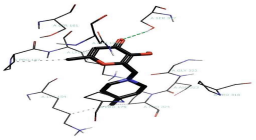
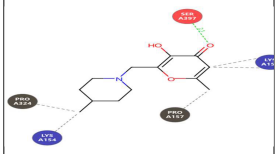
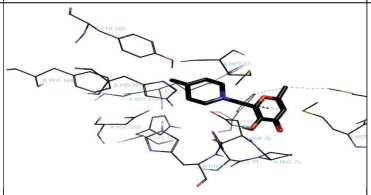
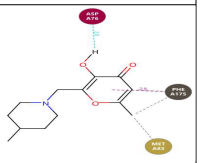
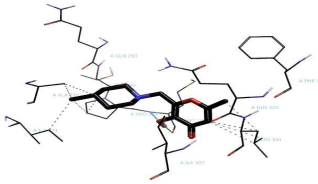
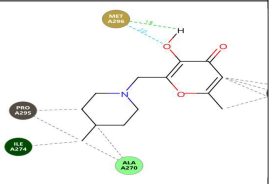
Target	Docked view of the target active site	2D Interactions
ZINC METALLOPROTEASE ZMP1 (PDB: 3ZUK)		
Cyclic diguanylate phosphodiesterase (EAL) domain protein (A0A0H2ZTL9)		
Serine protease Rv3671c **(P9WHR8)**		
Metallo-beta-lactamase superfamily protein (P96924)		
Zinc metalloprotease Rip1 (Q9CBU4)		

Figure 2. 2D and 3D binding poses of compound 14

Table 2. Docking scores for compound 14 for predicted targets

Protein	**UniProt Acc. (No.**	**Docking Score (kcal/mol)**
Zinc metalloprotease Rip1 (PDB:3ZUK)	Q9CBU4	-6.83
Metallo-beta-lactamase superfamily proteins	P96924	-5.97
Serine protease Rv3671c	P9WHR8	-6.77
Zinc metalloprotease ZMP1 (PDB:6XLY)	O53649	-8.14
c-di-GMP phosphodiesterase	A0H2ZTL9	-6.16

Table 3. Predicted ADME and physicochemical parameters of the compounds

Comp. no	MW (g/mol)	Consensus logP	HBD	HBA	MR	TPSA (Å2)	GI Absorption	BBB Permanent
13	237.29	1.68	1	4	70.47	53.68	High	Yes
14	237.29	1.67	1	4	70.47	53.68	High	Yes
15	251.32	1.91	1	4	75.28	53.68	High	Yes
16	267.32	1.19	2	5	76.44	73.91	High	No

Table 4. Drug-likeness filters of the compounds

Comp. no	Lipinski	Those	Veber	Egan	Muegge	Bioavailability score
	MW ≤ 500; Mean logP ≤ 4.15; HBA ≤ 10; HBD ≤ 5	60 ≤ MW ≤ 480; − 0.4 ≤ WlogP ≤ 5.6; 40 ≤ MR ≤ 130; 20 ≤ atoms ≤ 70	Rotatable Bonds ≤ 10; TPSA ≤ 140 Å2	WLOGP ≤ 5.88; TPSA ≤ 131.6	200 ≤ MW ≤ 600, − 2 ≤ XLOGP ≤ 5; TPSA ≤ 157; HBA ≤ 10; HBD ≤ 5; RB ≤ 15; Number of rings ≤ 7; Number of carbons > 4; Number of heteroatoms > 1	Probability of F > %10 in rats
13	Yes	Yes	Yes	Yes	Yes	0.55
14	Yes	Yes	Yes	Yes	Yes	0.55
15	Yes	Yes	Yes	Yes	Yes	0.55
16	Yes	Yes	Yes	Yes	Yes	0.55

drogen bonds with MET 296 in Rip1. Our molecular docking results provide further evidence that compound 14 might exert its anti-TB effect by inhibiting all five enzymes, particularly ZMP1. However, further in vitro studies are required to confirm this result.

Druglikeness of the compounds

In principle, the optimisation of lead compounds into drug candidates should address their potency and selectivity and improve their ADME profiles. Insufficient ADME and physicochemical properties led to the withdrawal of drug candidates from preclinical studies. Therefore, it is useful to evaluate the ADME properties of the compounds in the first step. Compounds 13, 14, 15, and 16 were selected to predict ADME properties because they exhibited the highest antimycobacterial activity.

The druglikeness of a molecule is determined by balancing hydrophobicity, electronic distribution, size, and flexibility. These properties influence the bioavailability, toxicity, metabolic stability, affinity to proteins, and transport properties of compounds in living organisms.

In a previous study, 1271 synthetic anti-tubercular compounds were evaluated based on their physicochemical properties (Motamen & Quinn, 2020). Calculated partition coefficient (clog P), molecular weight (MW), and polar surface area (PSA) as the three important properties arising from the analysis. In addition, a new TB space with more appropriate values of MW ≤ 500, -4 ≤ clog P ≤ 3, and 30 ≤ PSA ≤ 140 Å is proposed which may be a useful guide for designing new compounds against *Mycobacterium* species.

In this study, the drug-likeness properties of the selected derivatives were evaluated using the SwissADME web-based tool (Daina et al., 2017). In silico prediction of physicochemical and pharmacokinetic properties such as hydrogen bond donor/acceptor (HBD/HBA), molecular refractivity (MR), partition coefficient (logP), topological PSA (tPSA) val-

ues, blood–brain barrier (BBB) transport, and gastrointestinal (GI) absorption are presented in Table 3.

The logP value, a valuable indicator of drug permeability, was estimated using different models, i.e., iLOGP, XLOGP3, MLOGP, SILICOS-IT, WLOGP, and the arithmetic mean of the logP values. The MR and tPSA polarity parameters indicate the transport of compounds in the body. Drug-likeness filters are also presented with their limitations in Table 4. All selected Mannich bases obeyed all considered filters.

CONCLUSION

The allomaltol derivatives tested in this study were effective against M. tuberculosis and M. avium and relatively non-toxic against human cells. In silico studies predicted that these compounds exhibit excellent drug-likeness and satisfactory physicochemical properties. In particular, compounds bearing methyl-substituted piperidine groups were found to be as effective as the drugs ethambutol and streptomycin, which are clinically used in these treatments. Furthermore, Rip1, MBL superfamily proteins Rv3671c, and ZMP1 were predicted to be potential targets of these compounds, with high binding affinity. Therefore, allomaltol derivatives are promising antimycobacterial agents that can be used to develop novel therapeutic agents for the treatment of tuberculosis and other mycobacterial infections with high safety profiles.

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REFERENCES

Abdullahi, M., Uzairu, A., Shallangwa, G. A., Arthur, D. E., Umar, B. A., & Ibrahim, M. T. (2020). Virtual molecular docking study of some novel carboxamide series as new anti-tubercular agents. *European Journal of Chemistry, 11*(1), 30–36. https://doi.org/10.5155/eurjchem.11.1.30-36.1955

Amiri, M. R. J., Siami, R., & Khaledi, A. (2018). Tuberculosis Status and Coinfection of Pulmonary Fungal Infections in Patients Referred to Reference Laboratory of Health Centers Ghaemshahr City during 2007-2017. *Ethiopian Journal of Health Sciences, 28*(6). https://doi.org/10.4314/ejhs.v28i6.2

Aytemir, M. D., & Çalış, Ü. (2007). Synthesis of Some Novel Mannich Bases Derived From Allomaltol and Evaluation of Their Anticonvulsant Activities. *Hacettepe University Journal of the Faculty of Pharmacy 27*(1), 1-10.

Aytemir, M.D. & Çalış, Ü. (2006). Synthesis of some new hydroxypyranone derivatives and evaluation of their anticonvulsant activities. *FABAD Journal of Pharmaceutical Sciences* 31, 23-29.

Aytemir, M. D., Çaliş, Ü., & Özalp, M. (2004). Synthesis and Evaluation of Anticonvulsant and Antimicrobial Activities of 3-Hydroxy-6-methyl-2-substituted 4H-Pyran-4-one Derivatives. *Archiv Der Pharmazie, 337*(5), 281–288. https://doi.org/10.1002/ardp.200200754

Aytemir, M. D., & Çalış, Ü. (2010). Anticonvulsant and Neurotoxicity Evaluation of Some Novel Kojic Acids and Allomaltol Derivatives. *Archiv Der Pharmazie, 343*(3), 173–181. https://doi.org/10.1002/ardp.200900236

Aytemir, M. D., & Özçelik, B. (2011). Synthesis and biological activities of new Mannich bases of chlorokojic acid derivatives. *Medicinal Chemistry Research, 20*(4), 443–452. https://doi.org/10.1007/s00044-010-9338-x

Aytemir, M. D., Özçelik, B., & Karakaya, G. (2013). Evaluation of bioactivities of chlorokojic acid derivatives against dermatophytes couplet with cytotoxicity. Bioorganic & Medicinal Chemistry Letters, 23(12), 3646–3649. https://doi.org/10.1016/j.bmcl.2013.03.098

iswas, T., Small, J., Vandal, O., Odaira, T., Deng, H., Ehrt, S., & Tsodikov, O. V. (2010). Structural insight into serine protease Rv3671c that Protects M. tuberculosis from oxidative and acidic stress. *Structure* (London, England: 1993), 18(10), 1353–1363. https://doi.org/10.1016/j.str.2010.06.017

Brtko, J. (2022). Biological functions of kojic acid and its derivatives in medicine, cosmetics, and food industry: Insights into health aspects. *Archiv Der Pharmazie, 355*(10), 2200215. https://doi.org/10.1002/ardp.202200215

Boyd, S. E., Livermore, D. M., Hooper, D. C., & Hope, W. W. (2020). Metallo-β-Lactamases: Structure, Function, Epidemiology, Treatment Options, and the Development Pipeline. Antimicrobial Agents and Chemotherapy, 64(10), e00397-20. https://doi.org/10.1128/AAC.00397-20

Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) (2006) National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing, 16th informational supplement. CLSI document M7-A7, 940 West Valley Road, Wayne, Pennsylvania 19087

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports, 7*(1), 42717. https://doi.org/10.1038/srep42717

Daina, A., Michielin, O., & Zoete, V. (2019). SwissTargetPrediction: Updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Research, 47*(W1),

W357–W364. https://doi.org/10.1093/nar/gkz382

Ercan, A., Oncul, S., Karakaya, G., & Aytemir, M. (2020). An allomaltol derivative triggers distinct death pathways in luminal a and triple-negative breast cancer subtypes. Bioorganic Chemistry, 105, 104403. https://doi.org/10.1016/j.bioorg.2020.104403

Falkinham, J.O. (2018). Mycobacterium avium complex: Adherence as a way of life. *AIMS Microbiology, 4*(3), 428–438. https://doi.org/10.3934/microbiol.2018.3.428

Ferraris, D. M., Sbardella, D., Petrera, A., Marini, S., Amstutz, B., Coletta, M., Sander, P., & Rizzi, M. (2011). Crystal structure of Mycobacterium tuberculosis zinc-dependent metalloprotease-1 (Zmp1), a metalloprotease involved in pathogenicity. *The Journal of Biological Chemistry,* 286(37), 32475–32482. https://doi.org/10.1074/jbc.M111.271809

Girase, P. S., Dhawan, S., Kumar, V., Shinde, S. R., Palkar, M. B., & Karpoormath, R. (2021). An appraisal of anti-mycobacterial activity with structure-activity relationship of piperazine and its analogues: A review. *European Journal of Medicinal Chemistry, 210*, 112967. https://doi.org/10.1016/j.ejmech.2020.112967

Kandioller, W., Kurzwernhart, A., Hanif, M., Meier, S. M., Henke, H., Keppler, B. K., & Hartinger, C. G. (2011). Pyrone derivatives and metals: From natural products to metal-based drugs. *Journal of Organometallic Chemistry, 696*(5), 999–1010. https://doi.org/10.1016/j.jorganchem.2010.11.010

Karakaya, G., Aytemir, M. D., Özçelik, B., & Çalış, Ü. (2013). Design, synthesis and in vivo / in vitro screening of novel chlorokojic acid derivatives. *Journal of Enzyme Inhibition and Medicinal Chemistry, 28*(3), 627–638. https://doi.org/10.3109/14756366.2012.666538

Karakaya, G., Ercan, A., Oncul, S., & Aytemir, M. D. (2019). Synthesis and Cytotoxic Evaluation of Kojic Acid Derivatives with Inhibitory Activity on Melanogenesis in Human Melanoma Cells. *Anti-Cancer Agents in Medicinal Chemistry, 18*(15), 2137–2148. https://doi.org/10.2174/1871520618666180402141714

Karakaya, G., Türe, A., Ercan, A., Öncül, S., & Aytemir, M. D. (2019). Synthesis, computational molecular docking analysis and effectiveness on tyrosinase inhibition of kojic acid derivatives. *Bioorganic Chemistry, 88*, 102950. https://doi.org/10.1016/j.bioorg.2019.102950

Hull, T. D., Ryu, M. H., Sullivan, M. J., Johnson, R. C., Klena, N. T., Geiger, R. M., Gomelsky, M., & Bennett, J. A. (2012). Cyclic Di-GMP phosphodiesterases RmdA and RmdB are involved in regulating colony morphology and development in Streptomyces coelicolor. *Journal of Bacteriology*, 194(17), 4642–4651. https://doi.org/10.1128/JB.00157-12

Liu, Y., Grimm, M., Dai, W., Hou, M., Xiao, Z.-X., & Cao, Y. (2020). CB-Dock: A web server for cavity detection-guided protein–ligand blind docking. *Acta Pharmacologica Sinica, 41*(1), 138–144. https://doi.org/10.1038/s41401-019-0228-6

Milyutin, C. V., Komogortsev, A. N., Lichitsky, B. V., & Melekhina, V. G. (2023). Acid-catalyzed recyclization of spiroindolinepyrano[3,2- b]pyrans to 3,4-dihydroquinolinone derivatives containing allomaltol fragment. *Journal of Heterocyclic Chemistry, 60*(8), 1427–1436. https://doi.org/10.1002/jhet.4690

Motamen, S., & Quinn, R. J. (2020). Analysis of Approaches to Anti-tuberculosis Compounds. ACS *Omega, 5*(44), 28529–28540. https://doi.org/10.1021/acsomega.0c03177

Oncul, S., Karakaya, G., Dilsiz Aytemir, M., & Ercan, A. (2019). A kojic acid derivative promotes intrinsic apoptotic pathway of hepatocellular carcinoma cells without incurring drug resistance. *Chemical Biology & Drug Design, 94*(6), 2084–2093. https://doi.org/10.1111/cbdd.13615

Ozcelik, B., Sheikh, J., Orhan, I. E., Juneja, H., Bennani, B., Kerbal, A., ... Ben Hadda, T. (2013). Outstanding effect of the conformational restriction of isoquinolines: Hints for the development of optimized antimicrobial agents. *Research on Chemical Intermediates, 39*(7), 2955–2962. https://doi.org/10.1007/s11164-012-0808-2

Portell-Buj, E., Vergara, A., Alejo, I., López-Gavín, A., Monté, M. R., San Nicolás, L., González-Martín, J., & Tudó, G. (2019). In vitro activity of 12 antimicrobial peptides against Mycobacterium tuberculosis and Mycobacterium avium clinical isolates. *Journal of Medical Microbiology, 68*(2), 211–215. https://doi.org/10.1099/jmm.0.000912

Roman, G. (2022). Anticancer Activity of Mannich Bases: A Review of Recent Literature. *ChemMedChem, 17*(16), e202200258. https://doi.org/10.1002/cmdc.202200258

Saeedi, M., Eslamifar, M., & Khezri, K. (2019). Kojic acid applications in cosmetic and pharmaceutical preparations. *Biomedicine & Pharmacotherapy*, 110, 582–593. https://doi.org/10.1016/j.biopha.2018.12.006

Schneider, J. S., Sklar, J. G., & Glickman, M. S. (2014). The Rip1 protease of Mycobacterium tuberculosis controls the SigD regulon. *Journal of Bacteriology, 196*(14), 2638–2645. https://doi.org/10.1128/JB.01537-14

Suarez, J., Ranguelova, K., Jarzecki, A. A., Manzerova, J., Krymov, V., Zhao, X., ... Magliozzo, R. S. (2009). An Oxyferrous Heme/Protein-based Radical Intermediate Is Catalytically Competent in the Catalase Reaction of Mycobacterium tuberculosis Catalase-Peroxidase (KatG). *Journal of Biological Chemistry, 284*(11), 7017–7029. https://doi.org/10.1074/jbc.M808106200

Venugopala, K. N., Chandrashekharappa, S., Deb, P. K., Tratrat, C., Pillay, M., Chopra, D., ... Mailavaram, R. (2021). Antitubercular activity and molecular docking studies of indolizine derivatives targeting mycobacterial InhA enzyme. *Journal of Enzyme Inhibition and Medicinal Chemistry, 36*(1), 1471–1486. https://doi.org/10.1080/14756366.2021.1919889

Zilles, J. C., Dos Santos, F. L., Kulkamp-Guerreiro, I. C., & Contri, R. V. (2022). Biological activities and safety data of kojic acid and its derivatives: A review. *Experimental Dermatology, 31*(10), 1500–1521. https://doi.org/10.1111/exd.14662

Lv, H., Zhang, X., Zhang, X., Bai, J., You, S., Li, X., ... Xu, Y. (2024). Global prevalence and burden of multidrug-resistant tuberculosis from 1990 to 2019. *BMC infectious diseases, 24*(1), 243. https://doi.org/10.1186/s12879-024-09079-5

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