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In-silico design and Synthesis of Quercetin derivatives as potent Antimicrobial and Anti-inflammatory drugs.

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Abstract:

The advent of multidrug-resistant bacteria has drawn attention to the need for innovative antimicrobials to treat life-threatening infections. The current study investigates the in-silico design and synthesis of new quercetin derivatives in order to synthesise potent antibacterial and anti-inflammatory medicines. A naturally occurring flavonoid called quercetin has demonstrated encouraging therapeutic potential against microbial infections and inflammatory conditions. However, the creation of derivatives with better pharmacological characteristics is required due to their restricted bioavailability and metabolic stability. Quercetin, a polyphenolic compound, which are found in large quantities in a variety of fruits, vegetables, and medicinal plants, have drawn a lot of interest due to their possible medical applications, especially in the areas of antibacterial and anti-inflammatory properties. However, enhancing these activities through structural modification can lead to the development of more efficacious derivatives. In-silico design approaches such as molecular docking and computational chemistry were utilised to predict the binding affinity of the proposed quercetin derivatives to important microbial and inflammatory targets. To optimise its interactions with target proteins, the Quercetin scaffold underwent key structural changes. The designed compounds were subjected to virtual screening against selected microbial targets, as well as anti-inflammatory targets, including cytokines and enzymes involved in inflammatory pathways. The results reveal promising interactions between the designed Quercetin derivatives and the selected targets, suggesting their potential as antimicrobial and anti-inflammatory agents. For antimicrobial activity (PDB ID: 4Q2W and PDB ID: 9LYZ) which demonstrated potential binding affinity between -8.4 kcal/mol to -9.4kcal/mol and -8.2 kcal/mol to -8.7 kcal/mol respectively while compared with Gentamycin as a reference drug which showed binding affinity -7.2 kcal/mol and -7.3 kcal/mol respectively and for anti-inflammatory activity PDB ID: 1CX2 demonstrated potential binding affinity between -8.4 kcal/mol to -9.4 kcal/mol compared with Ibuprofen as reference drug which showed binding affinity -7.8 kcal/mol. Furthermore, ADMET properties (Absorption, Distribution, Metabolism, Excretion and Toxicity) of the derived molecules had been anticipated in order to assess their drug-likeness and safety profiles. This in-silico method reveals insightful information about the possible medical uses of new Quercetin compounds. The synthesized compounds further evaluated for their biological properties and gives promising results comparing with standard drugs.

Keywords: In-silico design, Molecular Docking, Menthol, Antimicrobial & Anti-inflammatory activity.

Introduction: In recent years, the rise of antimicrobial resistance and the increasing prevalence of inflammatory diseases have underscored the urgent need for the development of novel therapeutic agents with enhanced efficacy and safety profiles [1]. Natural compounds, particularly flavonoids, have garnered significant interest due to their diverse pharmacological activities, including antimicrobial and anti-inflammatory properties [2]. Among these, quercetin, a flavonoid abundantly found in various plant sources, has emerged as a promising candidate for drug development. One of the six subclasses of flavonoid chemicals is quercetin, which is classified as a flavonol. Originating from quercetum (oak forest) and named after Quercus, the word has been in use since 1857. Quercetin is chemically 3, 3′, 4′, 5, 7-pentahydroxyflvanone, or its synonym 3, 3′, 4′, 5, 7-pentahydroxy-2-phenylchromen-4-one. This indicates that the OH groups on quercetin are connected at positions 3, 5, 7, 3′, and 4′. (Figure1) [3]. Quercetin exhibits notable antimicrobial activity against a wide range of pathogens, including bacteria, fungi, and viruses [4]. Additionally, it possesses potent anti-inflammatory effects by modulating key inflammatory pathways and reducing the production of pro-inflammatory mediators [5]. However, its clinical utility is hindered by challenges such as limited bioavailability and metabolic instability [6]. To overcome these limitations and harness the therapeutic potential of quercetin, an in-silico driven approach for the design and synthesis of quercetin derivatives has gained traction. Insilico methods, including computational modelling and molecular docking, offer a cost-effective and time-efficient means [7] to explore the structure-activity relationship (SAR) of quercetin derivatives, predict their pharmacological properties, and elucidate their mechanisms of action. This approach allows for the rational modification of quercetin's chemical structure to enhance its antimicrobial and anti-inflammatory activities while optimizing its pharmacokinetic properties. By leveraging computational techniques, researchers can design quercetin derivatives with improved target specificity, binding affinity, and stability, thereby facilitating the development of potent and selective antimicrobial and anti-inflammatory drugs.

Conventional New Drug Development comprises lead compound exploration, target validation and selection, and optimisation. It is a laborious, risky, and reasonably cost-effective approach [8]. Furthermore, in addition to excessive toxicity and subpar drug pharmacokinetics, a high failure rate in clinical trials is frequently linked to elements like low therapeutic efficacy, low binding affinity, off-target effects, or physicochemical properties like stability or solubility[9]. Typical in vivo and in vitro procedures were used on a regular basis to assess medication safety, including toxicity and adverse effects, but these methods are still costly, labor-intensive, and time-consuming[10]. The development of rapid, low-cost, accurate target identification and prediction techniques has gained more attention due to limitations in outputs, accuracy, efficacy, and cost. This has led to the creation of computational techniques that are frequently used in the drug discovery process, such as silico structure prediction, refinement, modelling, and target validation [11]. The word "in-silico" describes simulation or experimentation supported by a computer [12]. Learning about specific chemical reactions in the target organism or body is the first stage in the In-silico drug design process. From there, combinations of these reactions can be tailored to fit a therapeutic profile [13]. The goal of the drug development process is to find compounds that can be rapidly transformed into medicines that address unmet medical needs and effectively treat endogenous and external disorders.

Computational approaches provide the advantage of facilitating the faster and more affordable transportation of novel drug candidates. By eliminating toxic and useless chemical molecules from the mix, in-silico methods can reduce the evolutionary parameters and increase the rate of success [14,15]. The novel medicine candidate could be semi-synthetic, synthetic, sourced from plants, or a modified version of an already approved medication. If the structure is readily available, both molecular docking and structure-based virtual screening are possible.

Material and Methods:

Apparatus, Chemicals and Analysis

All the chemicals were purchased from Loba Chem Pvt Ltd in Mumbai. Other materials were directly used and belonged to the analytical grade. Precoated silica gel plates (Merck Kiesegel GF254, Germany) were used for TLC analysis. The results were seen using an Ultra-Violet light chamber or iodine vapour.

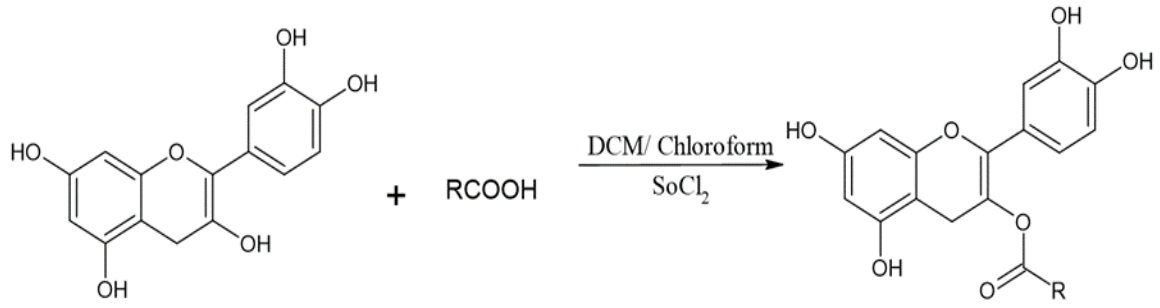
Tools for computation PyMOL: Three-dimensional, cross-platform visualisation of micromolecules is achieved. It makes molecular docking, virtual screening, and binding site analysis possible [16]. (https://pymol.org/2/)

Swiss ADME: This web service, which requires no login fees, offers dependable models for pharmacokinetics, drug analogies, and therapeutic chemical compatibility prediction. BOILED-Egg, bioavailability radar, and iLOGP[17]. (http://www.switzerlandadme.ch)

Discovery Studio: To examine and simulate molecular structures, sequences, and other pertinent data, researchers in the life sciences employ a comprehensive software package known as Discovery Studio [18]. Creating an interactive environment where users may view and work with data such as sequences, X-ray reflections, scripts, and molecular structures is the aim[19]. (https://find.3ds.com/download-discovery-studio-visualizer)

Quercetin Derivatives Preparation:

Designed scheme for derivatization of Quercetin.



2-(3,4-dihydroxyphenyl)-4*H*-1-benzopyran-3,5,7-triol

Table 1. Substituted compounds

Compounds	**1**	**2**	**3**	**4**
R				

Figure1: scheme for synthesis of Quercetin Derivative

Procedure for synthesis of new compounds from Quercetin

After dissolving the substituted acid in 5 ml of Chloroform or DCM, meq of of SOCl2 was added. At 50C temperature, the mixture was swirled for 15 minutes on ice bath. At a low temperature, mEq of quercetin was eventually added. The reaction mixture was continuously agitated for 45 minutes at a temperature of 5 to 8^0C. The reaction was monitored through TLC technique. After the completion of the reaction, the solvent was purged. Methanol was used to recrystallize the solid mass.

Result and Discussion

In Silico Lipophilicity (Anti-microbial)

The log p (n-butanol/water partition coefficient) for each planned structure was computed using the iLOG descriptor [20]. All derivatives were shown to have larger n-butanol/water partition coefficients than the reference, ranging from 1.27 to 3.06.

Table 2. Lipophilicity for designed compounds

Sr. No	Parameters	Properties	Compound 1	Compound 2	Compound 3	Compound 4	Gentamycin
1.	Lipinski's rule	Molecular weight g/mol	460.39	412.39	457.39	484.45	250.33
		Log P	1.27	2.15	2.06	3.06	1.95
		H-bond Acceptor	11	8	10	3	3
		H-bond donor	7	4	4	0	1
		Rotatable bond	3	3	4	4	5
2.	Medicinal chemistry	Synthetic accessibility	2.55	3.10	2.73	2.18	2.10
3.	Toxicity	AMES toxicity	yes	NO	NO	NO	NO
		hERG inhibition	NO	NO	NO	NO	NO

Molecular Docking

PyRx software has been utilized in molecular docking studies and is now frequently employed to find powerful treatments for certain drug targets in lethal infections. Due to its complicated structure with the inhibitor, PyMOL was used to clean up the crystal structure of PDB ID: 4Q2W before molecular docking. After unwanted molecules like water and ions were eliminated, it was docked to designed inhibitors to study the binding energy and interaction of amino acids. Grid box parameters were adjusted to a size of resolution 1.65 for protein (PDB ID: 4Q2W) and a size of resolution 1.95 for protein (PDB ID: 9LYZ). The interaction of the ligand molecule with the viral proteins was visualized and investigated using Pymol and Discovery Studio. All of these recently created inhibitors were used in molecular docking, and the scoring function was used to forecast how the aforementioned ligands and inhibitors would

interact. The PyRx was used for molecular docking and the results are shown in Table 3 and Table 4.

Table 3. Molecular Docking showing binding affinity with amino acid using PDB ID: 4Q2W, and Gentamycin as reference drug

Compound	Binding Affinity	Amino Acid Residue	Distance [Hydrogen bond]
PDB ID: 4Q2W			
Designed Inhibitor			
Compound 1	-9.3	TYR A148, VAL A447, HIS A 388	ASN A382, ALA A 202 GLN A 459
Compound 2	-8.4	ASN A 277. GLY A282, VAL 272	ASN A 277. GLY A282, VAL 272
Compound 3	-8.6	TYR A122, LEU A 472, PRO A 479	ARG A 44, ASN A43, LEU A60
Compound 4	-9.4	TYR A 212, PHE A 210, ALA A 450	GLN A 454, ASN A382, HIS A207
Reference drug:			
[Gentamycin]	-6.2	GLNA:153, SERA:97, HISA:96, ASNA:102, GLYA:98, TYRA:74, HISA:63, TRPA:69, LEUA:67, HISA:149	GLNA:153, ALAA:1107, SERA:97, ASNA:102, GLYA:98: 2.56

Table 4. Molecular Docking showing binding affinity with amino acid using PDB ID: 9LYZ, and Gentamycin as reference drug.

Compound	Binding Affinity	Amino Acid Residue	Distance [Hydrogen bond]
PDB ID: 9LYZ			
Designed Inhibitor			
Compound 1	-8.2	ARG A112, GLNA59, ARG A61	ALA A107. ASN A59, ASN A46
Compound 2	-8.2	LEU A59, TRP A108	TRP A63, GLN A 57
Compound 3	-8.4	ARG A 61, ASN A59, TRP A 108	ASN A 46, TRP A62
Compound 4	-8.7	ALA A110, LEU A59, TRP A108	TRP A63, GLN A 57

[Gentamycin]	-7.3	TRP A108, VAL A109, ILE A58	ALA A107, ASP A52, TRP A62

This study employed molecular docking to investigate the possible inhibition of selected derived compounds at the reported binding site of the 4Q2W and 9LYZ. Out of these synthesised compounds, compound 4 was found to have a high binding affinity to 4Q2W (-9.4 kcal/mol), creating a typical hydrogen bond with GLN A 454, ASN A: 382, HIS A: 207 (Fig 1). Similarly Compound 4 has an excellent binding affinity to 9LYZ (-8.7kcal/mol), developing a conventional hydrogen bond with TRP A:63, GLN A:57. Compounds 2, and 3 showed significant binding affinity to 4Q2W at -9.3 kcal/mol, -8.6 kcal/mol, respectively. Also compounds 3 and 1 showed binding affinity against 9LYZ as -8.4 kcal/mol, and -8.2 kcal/mol correspondingly

Figure 2. Interaction between Ligand (Compound 4) and Target Molecule (PDB ID:4Q2W). The specific amino acid interaction force applied to bind the ligand molecules with the target, van de wall force attached with TYR A: 212, PHE A: 210, ALA A: 450etc, other amino acids attached to the ligand side are shown in the figure.

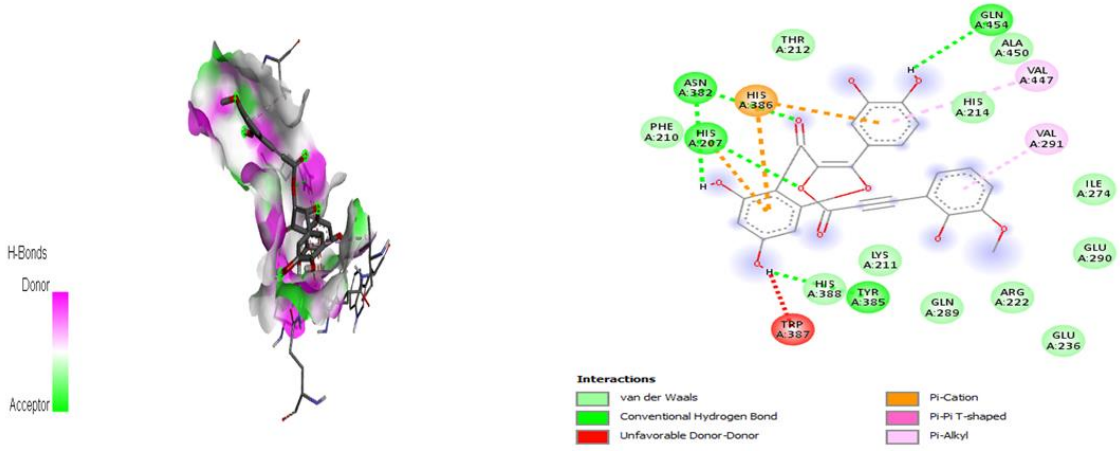


Figure3. Interaction between Ligand (Compound 4) and Target Molecule (PDB ID:9LYZ). The specific amino acid interaction force applied to bind the ligand molecules with the target, van de wall force attach with ALA A:110, LEU A:59, TRP A:108 etc, other amino acids attach to the ligand side shown in the figure.

Evaluation Of Antimicrobial Potential

A disc diffusion experiment was used to assess the proposed compounds' antibacterial properties [21], which was carried out using different selected microbial strains. Nutrient agar plates using the agar well diffusion method with Gentamycin (10µg/ml) assisted as a positive control[22]. Inhibition zones were measured for all the contents and activity was compared as shown in Figure 4 and Table 5.

From Table 5, it can be seen that the designed compounds exhibit antimicrobial properties against the microbes tested i.e., S. aureus (Gram-positive) and E. coli (Gram-negative)

Figure 4. (a) Inhibition zone of S. aureus (b) Inhibition zone of E. coli.

Table 5. Inhibition measurements (diameter in mm) for the various compounds

Compound	S.aureus	E.Coli
Compound 1	11mm	10mm
Compound 2	13mm	13 mm
Compound 3	13mm	14 mm
Compound 4	14mm	14 mm
Gentamycin	15 mm	14 mm

In Silico Lipophilicity (anti-inflammatory agents)

The log p (n-butanol/water partition coefficient) log P for each planned structure was computed using the iLOG descriptor. All derivatives were shown to have larger n-butanol/water partition coefficients than the reference, ranging from 2.29 to 3.59.

Table 6. Lipophilicity for designed compounds

Sr. No	Parameters	Properties	Compound 1	Compound 2	Compound 3	Compound 4	Ibuprofen
1.	Lipinski's rule	Molecular weight g/mol	460.39	412.39	457.39	484.45	250.33
		Log P	1.27	2.15	2.06	3.06	1.95
		H-bond Acceptor	11	8	10	3	3
		H-bond donor	7	4	4	0	1
		TPSA	3	3	4	4	5
		Rotatable bond	2.55	3.10	2.73	2.18	2.10
2.	Medicinal chemistry	Synthetic accessibility	Yes	NO	NO	NO	NO
3.	Toxicity	AMES toxicity	NO	NO	NO	NO	-
		hERG inhibition	NO	NO	NO	NO	NO

Molecular Docking

PyRx software has been utilized in molecular docking studies and is now frequently employed to find powerful treatments for certain drug targets in lethal infections due to its complicated structure with the inhibitor, PyMOL was used to clean up the crystal structure of PDB ID: 1CX2 before molecular docking. After unwanted molecules like water and ions were eliminated, it was docked to designed inhibitors to study the binding affinity and interface of amino acids. The interaction of the ligand molecule with the viral proteins was visualized and investigated using Pymol and Discovery Studio. All of these recently created inhibitors were used in molecular docking, and the scoring function was used to forecast how the ligand above and the inhibitors would interact. The docking of molecules has been carried out using the PyRx, and the results obtained were given in Table 7.

Table7. Molecular Docking showing binding affinity with amino acid using PDB ID: 1CX2, and Ibuprofen as reference drug

Compound	Binding Affinity	Amino Acid Residue	Distance [Hydrogen bond]
PDB ID: 1CX2			
Designed Inhibitor			
Compound 1	-9.3	TYR A148, VAL A447, HIS A 388	ASN A382, ALA A 202

			GLN A 459
Compound 2	-8.4	ASN A 277. GLY A282, VAL 272	ASN A 277. GLY A282, VAL 272
Compound 3	-8.6	TYR A122, LEU A 472, PRO A 479	ARG A 44, ASN A43, LEU A60
Compound 4	-9.4	TYR A 212, PHE A 210, ALA A 450	GLN A 454, ASN A382, HIS A207
		Reference drug:	
[Ibuprofen]	-7.8	HIS A 388, ALA A 199, LEU A 390	TYR A 385

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This study employed molecular docking to investigate how designed drug could inhibit the 1CX2 binding site. Among the synthesised compounds, 4had the highest binding affinity (-9.4 kcal/mol) and formed typical hydrogen bonds with GLN A: 454, ASN A:382, HIS A:207 (Fig 5). Compounds 1 and 3 had bind significantly to 1CX2 at -9.3 kcal/mol, and -8.6 kcal/mol, respectively.

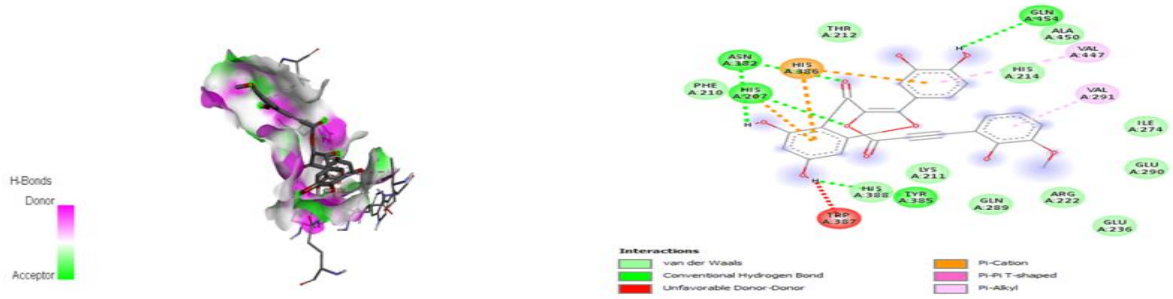

Figure 5. Interaction between Ligand (Compound 4) and Target Molecule (PDB ID:1CX2).

EVALUATION OF ANTI-INFLAMMATORY POTENTIAL:

Human red blood cell (HRBC) membrane stabilization assay:

The HRBC membrane stabilization approach is used to evaluate invitro anti-inflammatory effectiveness of drug.[23] As a control, NSAIDs were employed, and anti-inflammatory activity was evaluated as a percentage of RBC lysis. The HRBC membrane performs similarly to the lysosomal membrane.[24] If the substance is employed to stabilise it, it will also stabilise the lysosomal membrane. A spectrophotometer with a 560 nm wavelength was used to determine the haemoglobin concentrated in suspension. A healthy human volunteer donated blood, and any usage of NSAIDs within the previous two weeks of the trial was considered the key exclusion criterion. Na-oxalate was used to prevent the blood from clotting. Before use, all blood samples were stored for 24 hours at 4°C. The supernatant was removed by centrifugation at 2500 rpm for 5 minutes.

Washing was performed using a sterile saline solution (0.9% w/v NaCl) and centrifugation for 5 min at 2500 rpm. The process of extracting supernatant was repeated three times with the packed cell volume determined each time. A suspension of 40% (v/v) mixed with phosphate-buffered saline (10 mM, pH 7.4) in 1 ml of distilled water. NaH2PO4. 2H2O- 0.26 g; Na2HPO4- 1.15 g; NaCl- 9 g was used for cellular component reconstitution. To create derived drug samples (100 to 1000 µg/ml), add 0.5 ml of HRBC suspension, 2 ml of hyposaline, and 1 ml of Phosphate buffer to each concentration. Distilled was used. After 30 minutes of incubation at 37 °C, it was centrifuged at 3000 rpm for 20 minutes. The haemoglobin concentration in the supernatant solution was determined using spectrophotometry at 560 nm. Ibuprofen (1000 µg/ml) was utilised as the reference standard, whereas a control was created by omitting the drug sample. The percentage suppression of haemolysis or membrane stabilization was estimated using modified approach [25] and displayed in Table 8.

Table 8: Percentage inhibition of derived compounds

Concentration in µg/ml	% inhibition of Derived Compound			
	Compound 1	Compound 2	Compound 3	Compound 4
100	11.26	8.31	7.68	11.45
200	21.35	17.62	11.23	18.65
400	39.17	32.87	29.19	26.79
800	60.93	51.22	45.31	46.87
1000	73.19	74.34	67.24	78.65
Ibuprofen 1000 µg/ml	83.33%			

Discussion:

The in-silico design and synthesis of new menthol compounds as antibacterial and anti-inflammatory medicines show considerable promise. The rational design technique, target selection, molecular docking, and ADMET investigations lay a solid framework for future research. The study examined the antibacterial and anti-inflammatory properties of derive compounds. The impact can be demonstrated using the disc diffusion method for antibacterial and the human red blood cells (HRBC) membrane stabilisation assay for anti-inflammatory properties. Both the mechanisms are concentration dependent, with greater protection as the concentration of test sample increases. The disc diffusion method worked on the principle that an antibiotic -impregnated disc placed on agar that has already been infected with the test bacterium absorbs moisture and the antibiotic diffuses outward radially through the agar medium, resulting in an antibiotic concentration gradient. The antibiotic concentration is higher along the disk's edge and gradually decreases as the distance from the disc increases until it is no longer inhibitory for the organism, which then develops freely. During incubation, a clear zone or ring appears around an antibiotic disc if it inhibits bacterial growth. In contrast, the hypotonic solution's hemolytic action is caused by an excessive accumulation of fluid inside the cell, which causes the membrane to rupture. Injury to the red cell membrane exposes the cell to further damage produced by free radical-induced lipid peroxidation.[26] When

inflammatory mediators increase permeability, membrane stabilization limits fluids and serum protein leakage into organs.

Conclusion:

Quercetin derivatives were produced utilising acids. Quercetin and its derivatives have a possible binding affinity for PDB IDs 4Q2W and 9LYZ when compared to the reference medication Gentamycin. Antibacterial activity was examined. Compound 4 had the highest antibacterial affinity for S. aureus and E. coli. Further research will be conducted to investigate antibacterial and broader spectrum activity. Furthermore, these menthol compounds demonstrated binding affinity with PDB ID: 1CX2 when compared to the reference medication Ibuprofen and were tested for in vitro anti-inflammatory activity. Compound 4 has the best anti-inflammatory potential, with a 78% inhibition rate at 1000µg/ml compared to 83% for Ibuprofen. Since human red blood cell membranes are the same as lysosomal membrane components, the avoidance of hypotonicity-induced HRBC membrane lysis was utilised to assess a drug's anti-inflammatory effectiveness.

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