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Leveraging machine learning and molecular docking techniques to predict novel melanocortin-4 receptor agonists

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Abstract

Introduction: Monogenic obesity caused by mutations in the melanocortin-4 receptor (MC4R) gene remains a significant health challenge, despite numerous efforts to discover effective treatments. The MC4R has emerged as a promising target for drug development due to its role in energy homeostasis and adipose tissue formation.

Aim: The present study explores the hybridization of machine learning and in silico techniques to propose natural compounds that potentially act as agonists against the obesity-related MC4R.

Materials and methods: Specifically, a predictive model was developed to classify molecules based on their activity against the human MC4R (hMC4R). Additionally, a comprehensive molecular docking study was conducted on 2,000 compounds derived from natural sources to predict their binding affinity to the hMC4R.

Results: The subsequent analysis of the docking results identified five natural compounds that have the potential to act as hMC4R agonists and contain a flavone chemical scaffold. Integration of the predictive model with molecular docking simulations reinforced these findings, illustrating the complementary roles of data-driven insights and structural assessments in pinpointing viable hit compounds.

Conclusion: The study's findings suggest that the flavone chemical scaffold could serve as a template for designing novel MC4R agonists.

Keywords

cheminformatics, computational techniques, drug design, virtual screening, therapeutics, machine learning

Introduction

Obesity is recognized as one of the most pervasive chronic health problems on a global scale, often associated with an elevated risk of adverse health outcomes. According to statistical studies conducted by the World Health Organization (WHO) in 2022, it is estimated that 43% of adults are classified as overweight, while 16% are characterized as obese, indicating a more than twofold increase over a period of 3 decades globally.[1] Moreover, it is estimated that excess weight is responsible for over 1.2 million deaths per year in Europe.[2] It is critical to note that obesity is direct-

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ly associated with an increased risk of developing diabetes mellitus, cardiovascular diseases including myocardial infarction and arterial hypertension, deterioration of both bone and reproductive health, and various types of can- cer. [3,4] To date, scientific research reinforces the notion that obesity is a multifactorial disease as environmental, behavioral, and biological factors contribute to the establishment of a positive energy balance.[5,6]

The melanocortin-4 receptor (MC4R), a class of G-protein coupled receptors, plays a pivotal role in regulating appetite, maintaining energy balance, and influencing the formation of adipose tissue in the body.[7] Therefore, it is a key focus for the pharmacological treatment of obe- sity. [8] MC4R is expressed in the hypothalamus, brainstem, and other regions of the nervous system, where it performs a critical role in regulating food intake and energy expenditure.[9]

In 2020, the Food and Drug Administration (FDA) approved setmelanotide, also known as RM-49 or commercially as Imcivree, as the first pharmaceutical treatment targeting obesity.[10] This cyclic peptide exhibits 20 times greater selectivity for hMC4R compared to the natural ligand α-MSH. In addition, as indicated by Hammad et al., setmelanotide displays a higher binding affinity for hMC4R in comparison to the natural ligand α-MSH.[11] Unlike previous clinically tested agonists, setmelanotide does not exhibit cardiovascular side effects such as tachycardia or increased blood pressure, underscoring its safety profile for therapeutic use.[10] Recently, considerable progress has been made in developing both peptide-based and non-peptide MC4R agonists, offering improved specificity, poten- cy, and reduced adverse effects compared to earlier li-gands. [12-14] Specifically, novel non-peptide ligands demonstrate promising pharmacokinetics, indicating significant potential for clinical translation.[14]

However, the widespread adverse effects associated with existing MC4R ligands underline the necessity for the development of more selective ligands, to address the escalating medical and socioeconomic challenges posed by the global rise in obesity.[10,15] Therefore, there are ongoing challenges in identifying ligands with long-term stability and specificity, which are essential for developing novel approaches to comprehensive obesity management. Within this context, leveraging machine learning and molecular docking, as pursued in our study, aligns well with these contemporary efforts by systematically identifying potent natural compounds, particularly flavone derivatives, as potential MC4R agonists.

Aim

Towards this direction, the present study attempts to employ a combinatorial methodology, including machine learning (ML) and in silico techniques, in an effort to identify potential hMC4R ligands, with a specific focus on non-peptidic agonists.

Materials and methods

Generation of descriptors for the development of machine learning predictive model

The ChEMBL database (https://www.ebi.ac.uk/chembl/), the largest open-access source of bioactive molecules, was utilized to retrieve 2,177 compounds that were tested against hMC4R (ChEMBL259) at half-maximal inhibitory concentration (IC50) values.

A curated dataset of 1,906 compounds was created using an IC50 threshold filter of 10 μM. Consequently, 825 of these compounds were categorized as "Active" and 1,081 as "Not Active". RDKit (https://www.rdkit.org/) was used to generate features for machine learning models. Particularly, 208 molecular descriptors were calculated, including several categories such as constitutional, topological, fragment-based, charge, and shape descriptors, using simplified molecular input line specification (SMILES) of the examined compounds.[16]

Machine learning system design

Pre-processing step
To ensure the functionality of the machine learning classifiers, a preprocessing step was implemented. This step included the utilization of 11 classifiers from the scikit-learn library (https://scikit-learn.org/stable/), balancing between the studied compounds, and data normalization, to compensate against unbalanced class-data sizes and uneven ranges of descriptor-data values that distort classifiers' performance.

The implementation of a single classifier was employed in each instance, with values being scaled to enhance classifier performance. Normalization was achieved through the implementation of the *preprocessing.normalize* function from the scikit-learn Python library (https://scikit-learn.org/stable/). The dataset was balanced by augmenting the size of the smallest class to match the largest class using the Synthetic Minority Over-sampling Technique (SMOTE), which was implemented via the imbalanced-learn Python library (https://imbalanced-learn.org/stable/). The *model.feature_importance_* attribute, where the refers to the classifier used, from the scikit-learn Python library (https://scikit-learn.org/stable/) was then employed to rank descriptors according to their importance. The ranking procedure was executed 50 times, with each execution generating a ranked list of features based on their frequency of occurrence. The top 10 descriptors with the highest occurrence across the 50 repetitions were selected for further processing.

Data splitting
The dataset was segmented into two subsets to ensure effective model training and evaluation. Specifically, 70% of the data was allocated for training the machine learning models, while the remaining 30% was reserved for testing and

assessing model performance. To enhance reliability, the data-splitting process was repeated 10 times. This iterative approach reduces the impact of random variations in data selection. Each iteration provides a distinct combination of training and testing sets, facilitating the evaluation of the consistency and generalizability of the model.

Optimal ML-model design process

The machine learning system underwent a systematic optimization process for each of the ten train-test splits. Accordingly, for each train-test dataset split, the training dataset was utilized to design the classifier, using a combination of descriptors, amongst the top 10 descriptors, identified as of high importance in the preprocessing step. The designed classifier was evaluated by the repeated K-Fold cross-validation method, using the **RepeatedKFold** function from scikit-learn Python library (https://scikit-learn.org/stable/). This process was systematically repeated for all possible descriptor combinations (of 2 to up to 10 descriptors per combination), and the best model design for the particular training dataset was the classifier with the least number of descriptors that produced the highest accuracy in classifying correctly compounds into active and non-active. That best-performing model (classifier/features-combination) was then applied to the left-out test dataset to evaluate its final classification accuracy and overall effectiveness. This process was repeated for ten train-test data splits and the average performance of the particular classifier and the involved features were recorded.

Finally, the whole classifier/features combination design process described above was repeated for all 11 classifiers so as to identify the best-performing machine learning designed system that produced the highest classification accuracy **(Fig. 1)**. The performance of each classifier was assessed by the overall compounds' classification accuracy, the non-active compounds' accuracy, the active compounds' accuracy, the area under the curve (AUC) of the receiver operating characteristic (ROC) curves. The clas-

sifier with the highest classification accuracy, sensitivity, specificity, and area under the ROC curve was selected as the optimal model.

Molecular docking studies

In parallel, molecular docking studies were subjected to explore the potential binding affinity of a series of natural compounds into the hMC4R. For this scope, the electron microscopy structure of hMC4R complexed with the agonist setmelanotide[10] (PDB:7PIU, resolution: 2.58 Å) was downloaded from the Protein Data Bank (https://www.rcsb.org) and prepared by performing the Protein Preparation Wizard[17] of the Maestro interface.[18] All missing residues and hydrogen atoms were added, bond orders were assigned, and finally the complex was minimized, using the OPLS3 force field. Simultaneously, 2,000 natural compounds, a subset of ZINC database (https://zinc.docking.org/), were prepared at pH=7.0±0.5, using LigPrep[19] of the Maestro interface.[18]

In continuation, a grid box centroid on setmelanotide with the dimensions x=10Å, y=10Å, and z=10Å was created and molecular docking studies were carried out on all examined natural compounds, using the Standard Precision (SP) mode of Glide.[20] The maximum number of docking poses was set equal to 10, and all poses were visually inspected and analyzed.

Combination of machine learning and molecular docking

For this part of the study, MetaboAnalyst 6.0, a freely accessible platform for comprehensive metabolomics data analysis and interpretation, was employed. Initially, the RDKit molecular descriptors of the natural compounds that were selected as potential ligands of hMC4R were calculated. Then, the data were uploaded to MetaboAnalyst's Biomarker Analysis module, offering the ROC curve-based evalu-
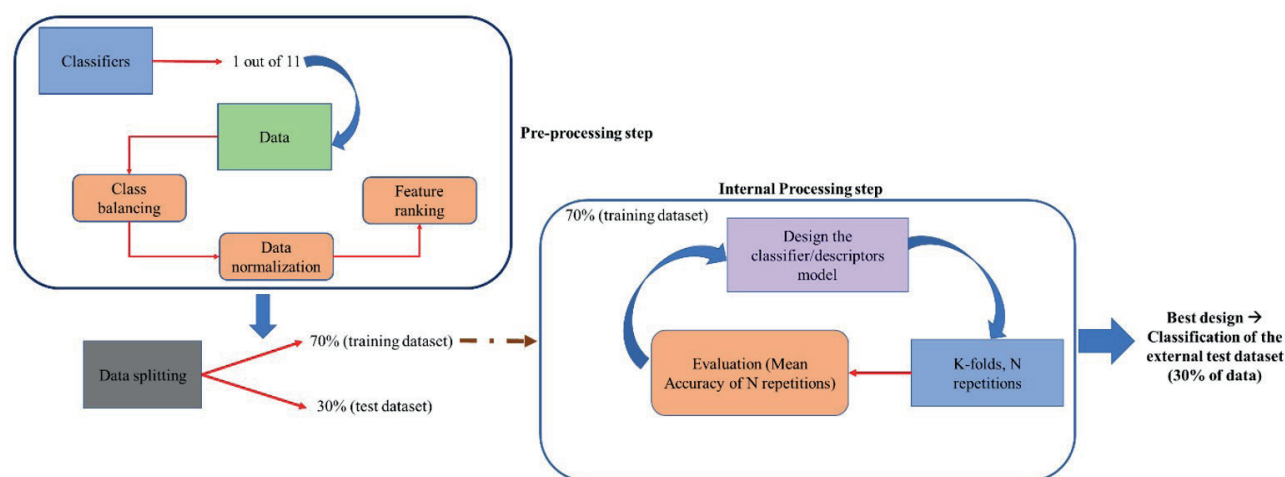
Figure 1. The procedure followed in the machine learning model design. The procedure was utilized for each one of the 11 classifiers employed.

ation approach to potential biomarkers identification and model performance evaluation. Auto Scaling algorithm was selected for data normalization, and a Random Forest classifier based on the best combination of biomarkers, was selected to predict the inhibition of the tested compounds.

Results and discussion

Machine learning results

Optimal feature combination
Among the 10 descriptors identified through the feature importance process, a combination of 7 RDKit molecular descriptors proved to be the most effective for building a robust machine learning model. **Table 1** lists the molecular descriptors that achieved optimal model performan- ce.[21-23] The classifier that achieved the highest efficiency was the Random Forest Classifier, using the default parameters from the scikit-learn library (https://scikit-learn.org/stable/).

ML-model validation
Table 2 presents the average performance metrics of the test set evaluated over 10 data-split repetitions for the selected classifier. Furthermore, **Fig. 2** illustrates the ROC curve for the final results across the 10 data-split repetitions, providing a visual representation of the performance.

Statistical analysis

The statistical analysis results for the molecular descriptors detailed in **Table 1**, indicated that 4 descriptors (MaxAbsEstateIndex, PEOE_VSA8, Kappa_2 and BCUT2D_MR-LOW) presented statistically significant differences. The boxplots of the selected descriptors are illustrated in **Fig. 3.**

The MaxAbsEStateIndex molecular descriptor uses E-State index and surface area contributions. E-State is a concept developed by Kier and Hall[24] that corresponds to an Electrotopological State index, which describes the electronic environment and topology of atoms within a molecule. Thus, the E-State index is a measure of the electronic accessibility of a specific atom and can be interpreted as a probability of interaction with another molecule. However, this type of descriptors is not considered electronic, but descriptors of atom polarity and steric accessibility.[23]

The Van der Waals surface area (VSA) is a value obtained by considering the shape of each atom to be a sphere with a radius equal to that of Van der Waals. At this point, it is important to note that the surface area of an atom in a molecule is the amount of surface area of that atom not contained in any other atom of the molecule.[25] The atomic partial charge is calculated using the Partial Equalization of Orbital Electronegativity (PEOE) method, which was developed by Marsili and Gasteiger[26] through a topological iterative approach. In other words, partial charges are assigned to atoms of a molecule based on their electronegativities and neighboring atoms. As evident, the PEOE_VSA descriptors are numerical values that correspond to the electron density distribution across molecular surface areas.

The Kier alpha-modified shape or kappa descriptors are a group of molecular descriptors which are associated with the different shape contribution of heteroatoms and hybridization states. As a result, they offer a way to describe the structural characteristics of molecules, which is crucial for drug design.[24,25]

**Table 1.** Optimal feature combination and a brief interpretation

RDKit Descriptor	Brief Interpretation
VSA_EState6	The 6th of the 10 VSA_EState molecular descriptors. They quantify the surface area contributions of different types of atoms or bonds within a molecule.
MaxAbsEStateIndex	It refers to the maximum absolute value of the E-State indices across all atoms in the molecule.
PEOE_VSA8	The 8th of the 14 PEOE_VSA molecular descriptors. They intend to capture the direct electrostatic interactions within a certain range of atomic partial charges of $0 \leq x \leq 0.5$.
Kappa2	The 2nd of the three kappa shape indexes. It is a measure of molecular branching and connectivity.
MolMR	The Molecular Weight of a molecule expressed in Da.
BCUT2D_MRLOW	The lowest eigenvalue weighted by Crippen Molar Refractivity (Crippen MRR)
Kappa3	The 3rd of the three kappa shape indexes. It involves complex connectivity information and is influenced by the presence of rings or cyclic structures.

Table 2. Average performance metrics over 10 data-split repetitions for the Random Forest Classifier

Metrics	Mean	Std. Dev.
Active compounds accuracy	0.93	0.87
Non-active compounds accuracy	0.95	0.89
Overall accuracy	0.94	0.44
AUC	0.98	0.003
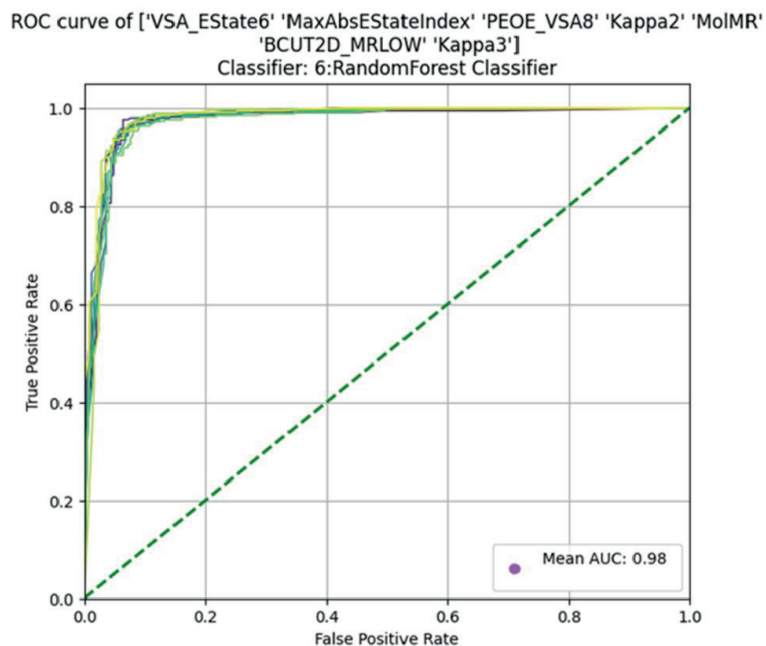
Figure 2. ROC curves of the optimal features combination, using the Random Forest Classifier over 10 data-split repetitions (AUC=0.98).
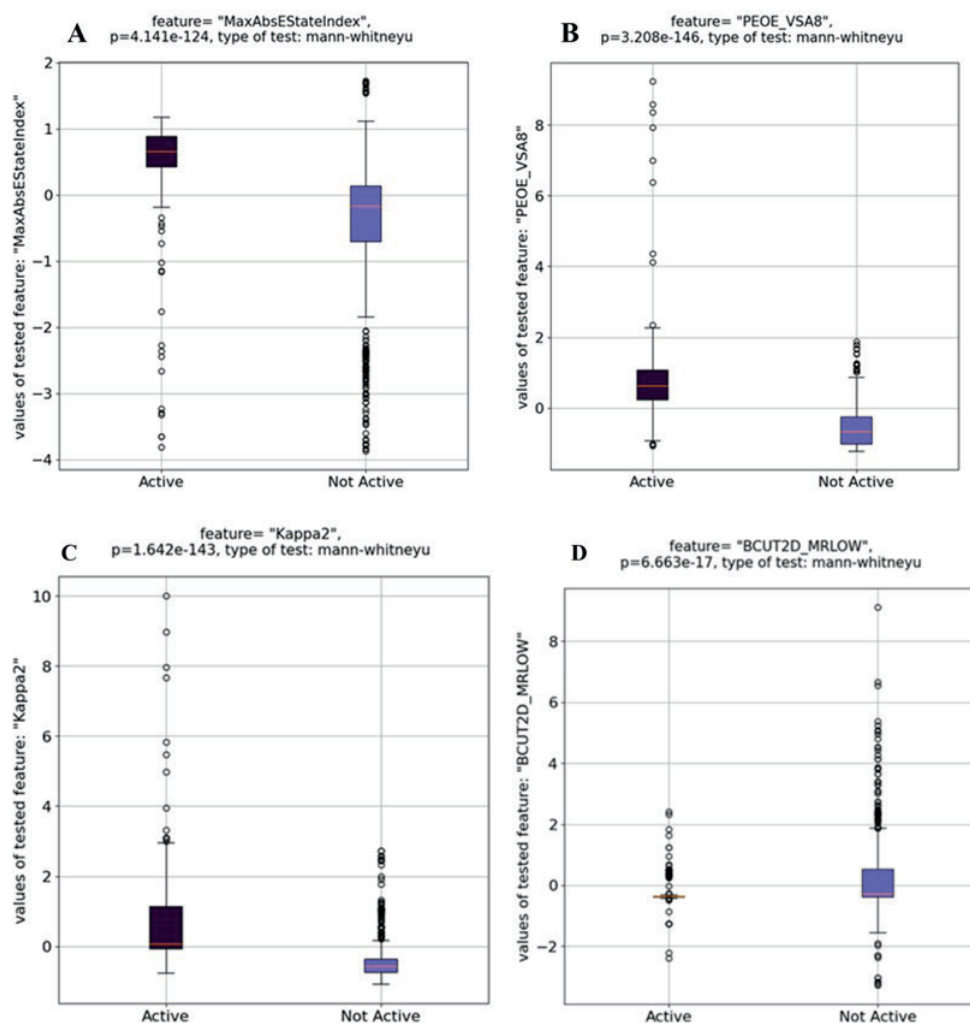
Figure 3. Boxplots of the statistically significant descriptors **(A)** MaxAbsEstateIndex, **(B)** PEOE_VSA8, **(C)** Kappa_2, and **(D)** BCU-T2D_MRLOW.

Finally, Burden-Cas-University of Texas eigenvalues (BCUT) are based on the Burden approach, considering three matrices whose diagonal elements correspond to **I)** atomic charge-related values, **II)** atomic polarizability-related values, and **III)** atomic H-bond abilities. The BCUT2D descriptors are a specific type of BCUT descriptors calculated based on a 2D representation of the molecular structure.[23]

Molecular docking results

In a further step, 2,000 natural compounds were docked into the hMC4R and the results evaluation was based on a) the predictive binding affinity, represented as docking score and b) the interaction pattern of the examined compounds, compared to the co-crystallized ligand setmelanotide. Therefore, 5 compounds **(Fig. 4)** were predicted as the most promising hMC4R agonists and were used for further analysis. The docking score and the interaction pattern of the setmelanotide agonist and the five selected natural compounds are illustrated in **Table 3**.

The docking results analysis revealed that the selected natural compounds are stabilized into the binding pocket of MC4R via the formation of a rich interaction pattern, including hydrogen bonds, pi-pi interactions and metal coordination **(Table 3, Fig. 5)**. All compounds present common interactions compared to the known agonist setmelanotide, highlighting the validity of our selection. Especially, all compounds develop a hydrogen bond with Asp122, similarly to setmelanotide. Also, Compounds 2 and 3 may coordinate the calcium metal ion, offering additional stabilization into the examined receptor. Representative binding poses of the proposed compounds are depicted in **Fig. 5**.

It is critical to note that the proposed molecules are flavonoids and specifically categorized into flavones. This category of compounds is abundant in plants, fruits and vegetables and according to epidemiological studies, randomized controlled trials, in vivo and in vitro assays contribute positively on weight management and obesity control.[27-29] Therefore, the aforementioned studies reinforce our results. Nevertheless, clear evidence is still lacking regarding the effectiveness of flavones in regulating obesity related to central nervous system gene mutations.

Combination of machine learning and molecular docking results

A step further, in order to predict the inhibition of the molecular docking proposed compounds, the machine learning tested dataset was used as input in the Biomarker Analysis module of Metaboanalyst 6.0.

Briefly, the dataset was subjected to ROC curve-based model evaluation, to calculate **a)** the model performance and validation, **b)** the diagnostic power of the model, and c) the prediction ability of the model. For this reason, a training test of 1906 compounds was created based on the 4 statistically significant descriptors (MaxAbsEstateIndex, PEOE_VSA8, Kappa_2, and BCUT2D_MRLOW) extracted from the machine learning model. The molecular docking proposed compounds were assessed as a test set and Linear SVM was utilized for the calculations.

ROC curve analysis showed that the model including the 4 biomarkers had a strong diagnostic power (with an AUC of 0.911) in discriminating Active from Not Active
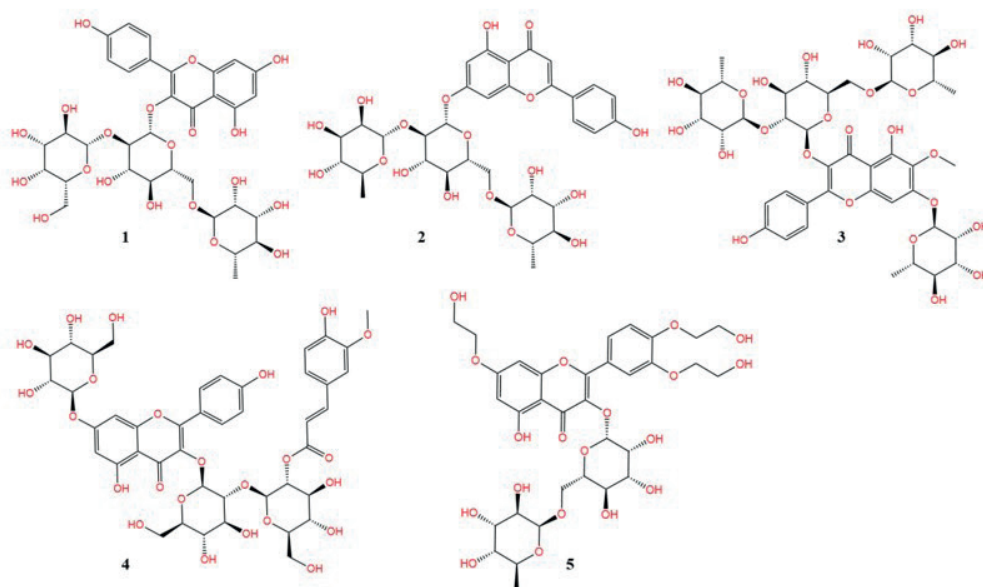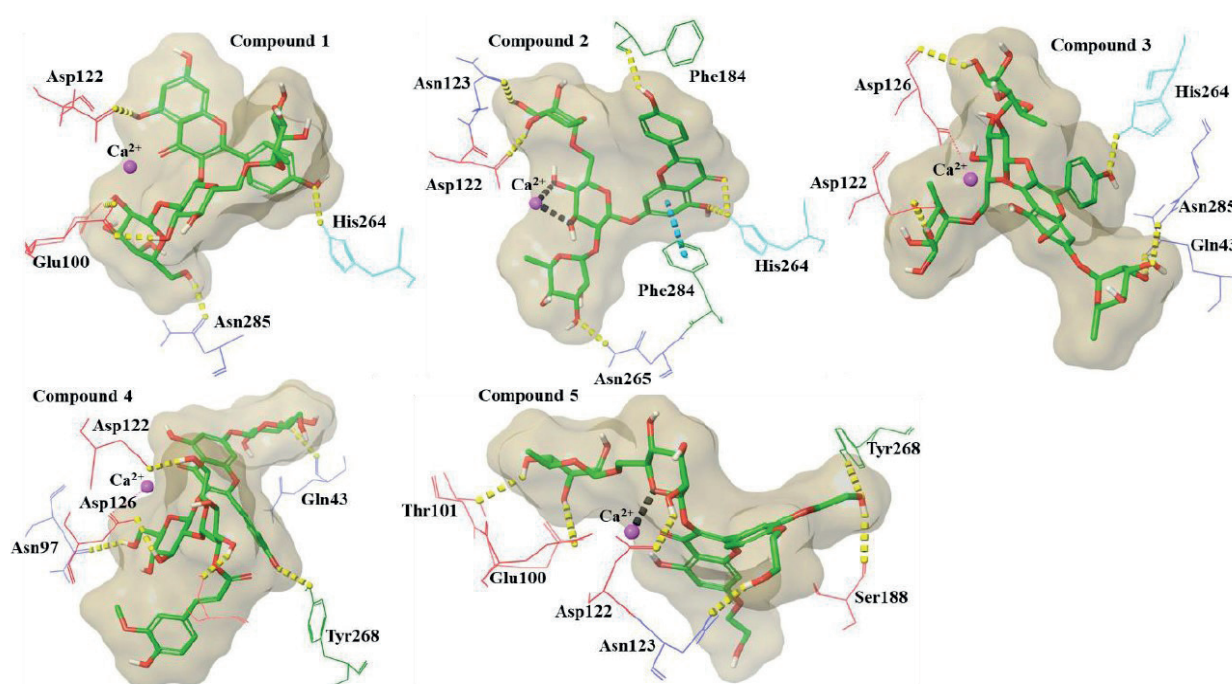

Figure 4. Chemical scaffolds of the 5 selected natural compounds, Compound 1: ZINC000169302042, Compound 2: ZINC000169724085, Compound 3: ZINC000253389129, Compound 4: ZINC000255260827, Compound 5: ZINC000299817569, derived from molecular docking studies into MC4R.

Table 3. The docking score and the interaction pattern of setmelanotide and selected natural compounds into hMC4R (PDB:7PIU). The common interactions among selected compounds and setmelanotide are marked in bold font

Compounds	Docking score kcal·mol⁻¹	Interactions
Setmelanotide	−11.12	[1]HB: Glu100, Thr101, Asp122, Asn123, Asp126, Ser188, His264
Compound 1 ZINC000169302042	−7.78	[1]HB: **Glu100, Asp122, His264,** Asn285
Compound 2 ZINC000169724085	−7.55	[1]HB: **Asp122, Asn123,** Phe184, **His264,** Asn285 & [2]pi-pi Phe284 & [3]mc
Compound 3 ZINC000253389129	−6.16	[1]HB: Gln43, **Asp122, Asp126, His264,** Asn285
Compound 4 ZINC000255260827	−8.41	[1]HB: Gln43, Asn97, **Asp122, Asp126, Ser188,** Tyr268
Compound 5 ZINC000299817569	−7.08	[1]HB: **Glu100, Thr101, Asp122, Asn123, Ser188,** Tyr268 & [3]mc

[1] HB: hydrogen bond; [2] pi-pi: pi-pi stacking; [3] mc: metal-coordination with Ca^{2+}

Figure 5. Representative binding poses of Compound 1: ZINC000169302042, Compound 2: ZINC000169724085, Compound 3: ZINC000253389129, Compound 4: ZINC000255260827, Compound 5: ZINC000299817569, derived from molecular docking studies into hMC4R. Hydrogen bonds are depicted with dashed yellow lines, pi-pi stacking with dashed blue lines, and Ca^{2+}-coordination with black lines.

compounds **(Fig. 6a)**. A step further, cross-validation with the random forest algorithm showed an average prediction accuracy of 0.889, while by performing the permutation tests (n=1,000), none of the results was better than the original one (*p*<0.001) **(Fig. 6b)**.

Finally, the prediction of inhibition of the tested compounds, based on the above model, is presented in **Table 4.**

The molecular docking results provide a robust foundation for interpreting the calculated inhibition probabilities presented in **Table 4**. Among the five selected natural compounds, the docking scores ranged from −6.16 to −8.41 kcal·mol⁻¹, reflecting moderate to high binding affinities compared to the reference agonist, setmelanotide (−11.12

kcal·mol⁻¹). Notably, all five compounds formed key interactions within the binding pocket of the hMC4R, particularly hydrogen bonds with Asp122, a critical residue for ligand stabilization as observed in setmelanotide.

The binding affinities correspond well with the predicted activity probabilities derived from the biomarker analysis. For example, Compound 4 (ZINC000255260827), which exhibited the most favorable docking score (−8.41 kcal·mol⁻¹), also achieved the highest predicted activity (87.2%). This compound forms hydrogen bonds with a series of residues, including Asp122, Asp126, and Ser188, and engages Tyr268, contributing to enhanced binding stability.

Similarly, Compound 3 (ZINC000253389129) showed a docking score of −6.16 kcal·mol^{-1} and a high predicted activity probability of 85.1%. Although its docking score is less favorable compared to others, its ability to coordinate with Ca^{2+} ions offers additional stabilization, aligning with its high classification probability as "Active."

The shared interaction patterns among the compounds, such as hydrogen bonding with Asp122 and interactions with His264 and Asn123, highlight the validity of these molecules as potential hMC4R agonists. The structural insights provided by molecular docking also support the observed trend of activity probabilities, suggesting that these common interactions likely contribute to functional outcomes.

Interestingly, Compound 2 (ZINC000169724085 demonstrated a moderate docking score (−7.55 kcal·mol^{-1}) but retained a strong activity probability (64.3%). This compound forms unique interactions, including π-π stacking
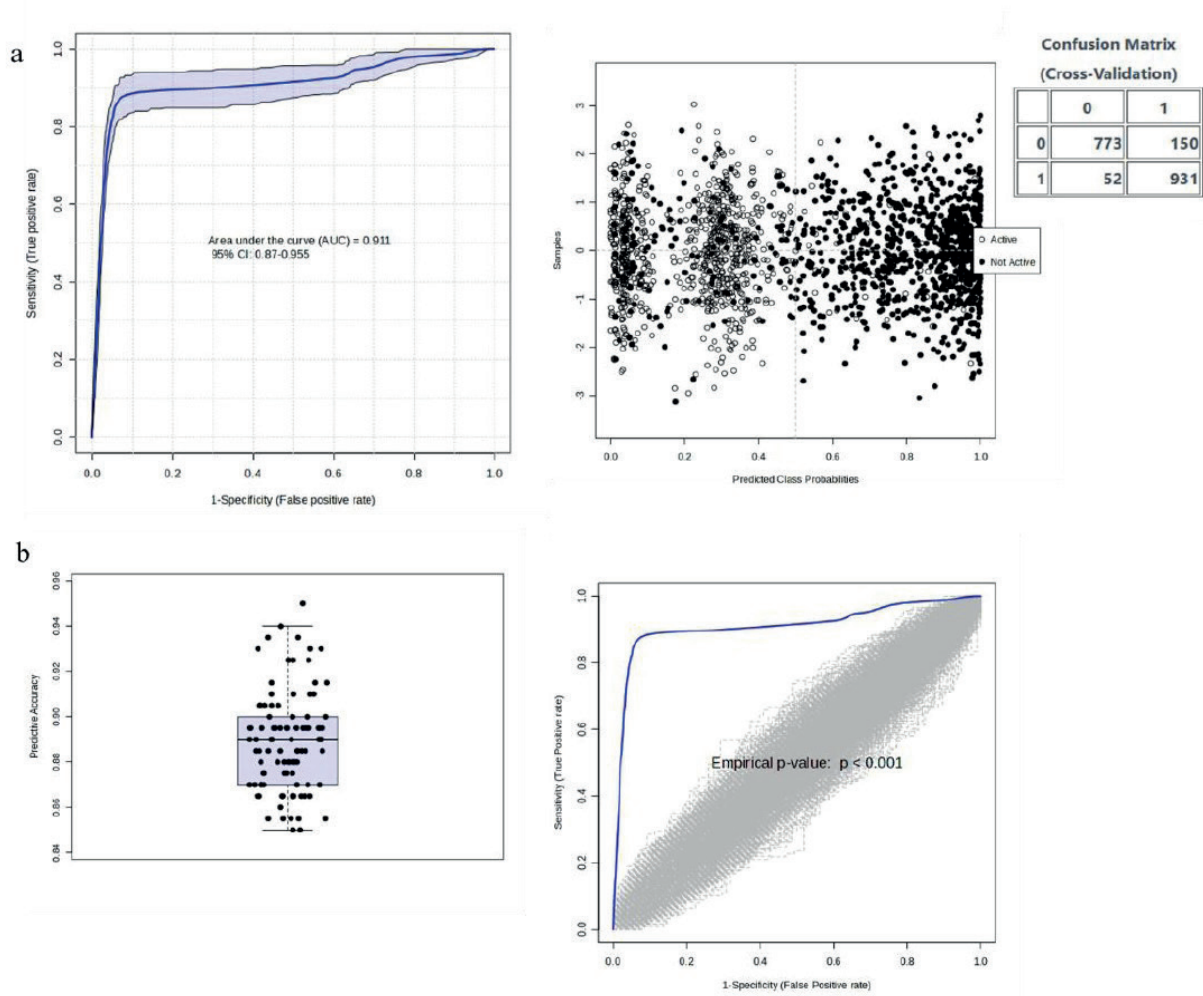
Figure 6. Biomarker analysis performed by Metaboanalyst 6.0. **a)** ROC-curve evaluation of the created predicted model based on Linear SVM algorithm; **b)** Model's cross validation alongside with permutation test based on 1000 permutations.

Table 4. The calculated probability for the classification (Active / Not Active) based on biomarker analysis.

Compounds	Probability	Class
Compound 1 ZINC000169302042	73.2%	Active
Compound 2 ZINC000169724085	64.3%	Active
Compound 3 ZINC000253389129	85.1%	Active
Compound 4 ZINC000255260827	87.2%	Active
Compound 5 ZINC000299817569	78.9%	Active

with Phe284 and metal coordination with Ca^{2+}, which may enhance its functional relevance despite the lower binding affinity compared to Compound 4.

Finally, Compound 5 (ZINC000299817569) displayed a docking score of -7.08 kcal·mol^{-1}, coupled with a predicted activity of 78.9%. Its interaction pattern closely mimics that of setmelanotide, forming hydrogen bonds with Glu100, Thr101, Asp122, and Asn123. This overlap in interactions suggests a comparable mechanism of action, validating its classification as "Active."

The alignment between the docking scores, interaction profiles, and activity probabilities underscores the complementary nature of molecular docking and machine learning-based biomarker analysis. The flavonoid scaffold, prevalent among the selected compounds, further supports their potential as therapeutic agents due to its established roles in weight management and obesity control.

Conclusions

This study demonstrates the successful combination of machine learning and molecular docking techniques to identify potential hMC4R agonists for obesity treatment. The predictive model, built using key molecular descriptors, achieved high accuracy in distinguishing active from inactive compounds. Molecular docking validated five flavone-based natural compounds as promising candidates, highlighting their strong binding affinities and critical interactions with hMC4R, similar to the reference agonist setmelanotide. The integration of machine learning predictions with molecular docking results underscores the synergy of data-driven and structural approaches in drug discovery. These findings propose the flavone scaffold as a promising template for developing selective MC4R agonists. Future in vitro and in vivo validation is essential to confirm their therapeutic potential.

Limitations

Despite the promising findings, our study has some limitations. Firstly, the absence of experimental validation, including biochemical assays or in vivo evaluations, means that further research is necessary to verify the biological efficacy of the identified compounds. Additionally, the use of SMOTE in our machine learning model could introduce synthetic bias, potentially affecting the model's real-world performance. Future studies should consider experimental validations or independent datasets to confirm and enhance model robustness.

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Competing Interests

The authors have declared that no competing interests exist.

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