Research Article

In silico Study of Molecular Properties, Bioactivity, and Toxicity of 2-(Substituted Benzylidene)Succinic Acids and some selected Anti-Inflammatory Drugs

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ABSTRACT

Succinic acid and its derivatives have many important uses, especially in pharmaceutical and polymer industries. The 2-(substituted benzylidene)succinic acids are also known as substituted phenylitaconic acids that are utilized in the synthesis of some lignans, lignanamides, and renin inhibitors. In view of this, the present in silico study aimed to calculate the molecular properties, bioactivity score, and toxicity of several benzylidene succinic acids, as well as, some selected anti-inflammatory drugs by computational methods. The study revealed that all the compounds obeyed Lipinski’s rule of five, indicating drug-likeness properties. The bioactivity data revealed that the 2-(substituted benzylidene)succinic acids were active as nuclear receptor ligands, enzyme inhibitors, G-protein coupled receptors (GPCR) ligands, and ion channel modulators. Among all, 2-(3,5-di-tert-butyl-4-hydroxybenzylidene)succinic acid was predicted as non-toxic with better in silico molecular properties and bioactivity as nuclear receptor ligand, enzyme inhibitor, GPCR ligand, ion channel modulator, and protease inhibitor compared to some of the predicted anti-inflammatory drugs.

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INTRODUCTION

Succinic acid is the simplest organic dicarboxylic acid recognized as an intermediate in the citric acid cycle. It is primarily used as a food additive, dietary supplement, and as an excipient in pharmaceutical products. It is also used as a precursor for various industrially important starting materials, such as, γ-butyrolactone, tetrahydrofuran, N-methylyprollidone, 1,4-butanediol, adipic acid, and linear aliphatic acids. Succinic acid is extensively used in polymer industry as the butanediol acid-derived polymer is biodegradable in nature. Itaconic acid or methylidene succinic acid, a succinic acid derived as a co-monomer in the production of acrylonitrile butadiene styrene and acrylate latexes with application in paper and architectural coating industry. Some of the itaconic acid derivatives are known to possess anti-influenza activity. Phenylitaconic acid or benzylidene succinic acid, a derivative of itaconic acid obtained naturally from Artemisia argyi. Further, the literature survey revealed that various substituted phenylitaconic acids or 2-(substituted benzylidene)succinic acids are utilized in the synthesis of some lignans, lignanamides, and renin inhibitors.

Discovering or developing a new drug has been considered as an expensive and time-consuming process. Advancing the research for the effective treatment of various diseases depend on the complex financial ecosystem. The estimated time for the development of a new drug and its release into the market was nearly 15 to 25 years. Developing non-toxic drugs having...
greater efficiency and selectivity within short period of
time and with less Research and Development (R & D)
investments are worrying factors for the growth of
the pharmaceutical industry. The use of scientific and
technological innovations as a research tool combining
multidisciplinary informatics, biotechnology, chemistry,
and biology knowledge is essential for optimizing time and
reducing cost in the drug design. Thus, the integration of
these in silico techniques makes it possible to search for
new drugs with better pharmacokinetic and toxicological
profiles compared to commercially used drugs.[11] Many
counter-based software are available through internet,
which helps to understand the molecular properties,
bioactivity, and toxicity of generated structures. Hence,
the present investigation aimed to design and identify
bioactive 2-(substituted benzylidene)succinic acids with
good pharmacokinetic and safety profile by computational
methods. The main objective of the study is to compare
the in silico molecular properties and toxicity profile of
the most bioactive 2-(substituted benzylidene)succinic
acid [(E)-2-(3,5-di-tert-butyl-4-hydroxybenzylidene)
succinic acid] with some selected anti-inflammatory drugs
possessing similar substituent fragments.

MATERIALS AND METHODS

Generation of Molecular Structures and
Nomenclature of 2-(Substituted Benzylidene)
Succinic Acids

ChemDraw, a powerful chemical drawing tool, became
the leading chemically-intelligent solution for multiple
disciplines from specialty chemistry to pharmaceutical
drug discovery. ChemDraw provides chemists with a
rich set of easy to use tools for creating scientifically
meaningful drawings of chemical structures and reactions.
The software also used to find the nomenclature and
stereochemistry of compounds. The chemical structures
and nomenclature of some selected benzylidenesuccinic
acids were developed using ChemBioDraw Ultra 11.0.

Calculation of Molecular Properties of
2-(Substituted Benzylidene)Succinic Acids and
some selected Anti-Inflammatory Drugs

Drug-likeness is a qualitative concept indicated by the
molecular properties that affect absorption, distribution,
metabolism, and excretion (ADME) of a compound.
Molinspiration online molecular property calculation
toolkit (http://www.molinspiration.com) was used
to evaluate the in silico pharmacokinetic properties of
2-(substituted benzylidene)succinic acids and some
selected anti-inflammatory drugs based on Lipinski’s
rule of five. The rule specifies that the molecules with
good membrane permeability have log $P \leq 5$, molecular
weight $\leq 500$, number of hydrogen bond acceptors $\leq 10$,
and number of hydrogen bond donors $\leq 5$. Other rules
which are important in computational prediction of drug-
likeness, include number of rotatable bonds, the molecular
volume, and topological polar surface area (TPSA). The
number of rotatable bonds indicates conformational
flexibility of a compound and ultimately binding with
receptors or ion channels. Molecular volume determines
transport characteristics of molecules, such as, intestinal
absorption or blood-brain barrier penetration. TPSA
also recognized as a good indicator of drug absorption
in intestine (TPSA $< 140$ angstroms squared ($Å^2$)) and its
penetration through blood-brain barrier (TPSA $< 60$ $Å^2$).
The magnitude of absorption can be expressed by
percentage of absorption ($\%$ ABS), which can be calculated
using equation $\%$ ABS = 109 - (0.345 x TPSA).[12]

Calculation of Bioactivity Score of 2-(Substituted
Benzylidene)Succinic Acids and some selected
Anti-Inflammatory Drugs

Miscreen, a molinspiration virtual screening engine
(http://www.molinspiration.com), used to calculate the
bioactivity score of 2-(substituted benzylidene)succinic
acids and some selected anti-inflammatory drugs. Virtual
screening or in silico screening utilizes computational
chemistry techniques to analyze large chemical databases
in order to identify possible new drug candidates. This
computational tool is used to find ligands modulating GPCR,
ion channels, nuclear receptors, and to identify kinase
inhibitors, protease inhibitors, and enzyme inhibitors.

The molinspiration bioactivity tool, miscreen engine
first analyzes a training set of active structures and
compares it with inactive molecules by using sophisticated
Bayesian statistics. The analysis generates a fragment-
based model (Bayesian statistical model, table of
fragments with their activity contributions) and used
to calculate the bioactivity score of screened molecules
as sum of activity contribution of fragments in these
molecules. For this kind of analysis, only SMILES and
SDfile structures of active molecules are necessary but
not the information about active site or binding mode.
This computational tool is particularly useful in projects
where structure-based approach cannot be applied due to
unavailability of information about 3D receptor structure.
Another advantage of this virtual screening tool is to learn
general structural requirements, which are sufficient for
the bioactivity and to identify new active structure classes
(scaffold hopping).

Prediction of Toxicity of 2-(Substituted
Benzylidene)Succinic Acids

Osiris Property Explorer (www.organic-chemistry.
org/prog/peo/) an online cheminformatics tool used
to determine the toxicity potential of 2-(substituted
benzylidene)succinic acids. The in silico toxicity properties
estimated are mutagenicity, tumorigenicity, irritant,
and reproductive effects. The virtual toxicity results are
color-coded either in green or red. The toxicity properties
shown in green indicate the molecules are safe and non-toxic. If they are expressed in red, indicates high risk of undesired effects. This computational tool also used to estimate the molecular properties, drug-likeness, and drug score. The molecular properties assessed are ClogP, solubility, molecular weight, and TPSA. The drug-likeness calculated using an equation summing up the score values of those fragments that are present in the molecule under investigation. The overall drug score of molecules estimated with an equation utilizing the values of molecule’s drug-likeness, toxicity, ClogP, solubility, and molecular weight.

**RESULTS AND DISCUSSION**

Computer-aided drug design plays a vital role in drug discovery and development and has become an indispensable tool in the pharmaceutical industry. Computational medicinal chemists can take advantage of all kinds of software and resources in computer-aided drug design fields for the purpose of discovering and optimizing biologically active compounds. Optimizing the chemical structure of lead candidates with respect to ADME processes has become an integral part of the current drug discovery process.

In the present research investigation, free online computational programs Molinspiration Cheminformatics and Osiris Property Explorer were used to estimate molecular properties, bioactivity, and toxicity profile of 2-(substituted benzylidene)succinic acids. Initially, the molecular structure and nomenclature of unsubstituted benzylidenesuccinic acid and twelve different 2-(substituted benzylidene)succinic acids were generated using ChemBioDraw Ultra 11.0 and the data presented in Table 1. The general molecular structure of computationally generated 2-(substituted benzylidene)succinic acid shown in Fig. 1. Among the generated structures, seven compounds (2, 4, 8, 10, 11, 12, and 13) are new and not existed or synthesized until now.

The molecular properties of all the generated structures were calculated using Molinspiration Cheminformatics, and the data presented in Table 2. The drug-likeness of all these compounds was evaluated by Lipinski’s rule of five that deals with four simple physicochemical parameters (log P ≤ 5, molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10, number of hydrogen bond donors ≤ 5). The log P measurement used to understand the substance solubility behavior and hence, its oral absorption and bioavailability. The in silico study revealed that the log P values of all the 2-(substituted benzylidene)succinic acids lie between 0.40 and 4.41 (within acceptable range ≤ 5). All the phenolic substituted benzylidenesuccinic acids have low log P value except compound 13 [(E)-2-(3,5-di-tert-butyl-4-hydroxybenzylidene)succinic acid]. The log P value of compound 13 was 4.41, indicating its high lipophilicity or hydrophobicity. Thus, specify better distribution of compound 13 in the body after its absorption. All the 2-(substituted benzylidene)succinic acids have molecular weight within the acceptable range ≤ 500. Low molecular weight compounds are easily absorbed, diffused, 

![General structure of 2-(substituted benzylidene)succinic acid](image)

**Table 1: Nomenclature and molecular formula of 2-(substituted benzylidene)succinic acids**

| Compound No. | Nomenclature                                      | Molecular formula       | R₁  | R₂  | R₃  |
|--------------|--------------------------------------------------|-------------------------|-----|-----|-----|
| 1            | (E)-2-benzylidenesuccinic acid                   | C₁₁H₁₀O₄                 | H   | H   | H   |
| 2            | (E)-2-(4-hydroxybenzylidene)succinic acid       | C₁₁H₁₀O₅                 | OH  | H   | H   |
| 3            | (E)-2-(4-methoxybenzylidene)succinic acid       | C₁₂H₁₂O₅                 | OCH₃| H   | H   |
| 4            | (E)-2-(3,4-dihydroxybenzylidene)succinic acid   | C₁₁H₁₀O₆                 | OH  | OH  | H   |
| 5            | (E)-2-(4-hydroxy-3-methoxybenzylidene)succinic acid | C₁₂H₁₂O₆             | OH  | OCH₃| H   |
| 6            | (E)-2-(3,4-dimethoxybenzylidene)succinic acid   | C₁₃H₁₄O₆                 | OCH₃| OCH₃| H   |
| 7            | (E)-2-(benzo[d][1,3]dioxol-5-ylmethylene)succinic acid | C₁₂H₁₀O₆             | -O=CH₂-O-  | H |
| 8            | (E)-2-(4-hydroxy-3,5-dimethoxybenzylidene)succinic acid | C₁₃H₁₄O₇             | OH  | OCH₃| OCH₃|
| 9            | (E)-2-(3,4,5-trimethoxybenzylidene)succinic acid | C₁₄H₁₆O₇                 | OCH₃| OCH₃| OCH₃|
| 10           | (E)-2-(4-hydroxy-3,5-dimethylbenzylidene)succinic acid | C₁₃H₁₄O₅             | OH  | CH₃ | CH₃|
| 11           | (E)-2-(3,5-diethyl-4-hydroxybenzylidene)succinic acid | C₁₅H₁₉O₅             | OH  | C₂H₅| C₂H₅|
| 12           | (E)-2-(4-hydroxy-3,5-diisopropylbenzylidene)succinic acid | C₁₇H₂₂O₅             | OH  | CH(CH₃)₂| CH(CH₃)₂|
| 13           | (E)-2-(3,5-di-tert-butyl-4-hydroxybenzylidene)succinic acid | C₁₉H₂₆O₅             | OH  | C(CH₃)₃| C(CH₃)₃|
and transported as compared to compounds with high molecular weight greater than 500.\textsuperscript{13} The 2-(substituted benzylidene) succinic acids also possess an adequate number of hydrogen bond acceptors and hydrogen-bond donors, ensuring efficient interaction with hydrogen bonding groups of an intractable receptor. The number of rotatable bonds explains the flexibility and conformational changes of molecules for binding to the receptors. It has been accepted that the number of rotatable bonds should be \leq 10 to pass the oral bioavailability.\textsuperscript{14} All the predicted compounds possess 4 to 7 rotatable bonds and, therefore, exhibit optimum conformational flexibility. TPSA is a very useful physicochemical parameter of molecules that gives information about the polarity of compounds. It is used to predict the transport properties of compounds, such as, intestinal absorption and blood-brain barrier penetration.\textsuperscript{15} It was observed that the TPSA values of all the predicted molecules were found between 74.60 and 115.05. Using these values, % ABS were calculated and presented in Table 2. The data indicated that the benzylidene succinic acid and various 2-(substituted benzylidene) succinic acids exhibited good percent absorption ranging from 69.31 to 83.26. Furthermore, none of the 2-(substituted benzylidene) succinic acids violated Lipinski’s parameters, making them promising drug-like molecules.

The bioactivity scores of the 2-(substituted benzylidene) succinic acids were calculated by Molinspiration Cheminformatics software and the data presented in Table 3. The bioactivity score gives information about the binding cascade of the molecules with different protein

### Table 2: Molecular properties of 2-(substituted benzylidene)succinic acids

| Compound         | milog P | M. wt | HBA | HBD | Volume | n violations | n rotb | TPSA | % ABS |
|------------------|---------|-------|-----|-----|--------|--------------|--------|------|-------|
| 1                | 1.36    | 206.2 | 4   | 2   | 182.26 | 0            | 4      | 74.6 | 83.26 |
| 2                | 0.89    | 222.2 | 5   | 3   | 190.28 | 0            | 4      | 94.83| 76.28 |
| 3                | 1.42    | 236.22| 5   | 2   | 207.81 | 0            | 5      | 83.83| 80.08 |
| 4                | 0.4     | 238.19| 6   | 4   | 198.3  | 0            | 4      | 115.05| 69.31 |
| 5                | 0.7     | 252.22| 6   | 3   | 215.83 | 0            | 5      | 104.06| 73.1  |
| 6                | 1.01    | 266.25| 6   | 2   | 233.36 | 0            | 6      | 93.07| 76.9  |
| 7                | 1.25    | 250.21| 6   | 2   | 206.19 | 0            | 4      | 93.07| 76.9  |
| 8                | 0.72    | 282.25| 7   | 3   | 241.37 | 0            | 6      | 113.29| 69.91 |
| 9                | 0.99    | 296.27| 7   | 2   | 258.9  | 0            | 7      | 102.3 | 73.71 |
| 10               | 1.90    | 250.25| 5   | 3   | 223.4  | 0            | 4      | 94.83| 76.28 |
| 11               | 2.83    | 278.3 | 5   | 3   | 257.01 | 0            | 6      | 94.83| 76.28 |
| 12               | 3.08    | 306.36| 5   | 3   | 290.18 | 0            | 6      | 94.83| 76.28 |
| 13               | 4.41    | 334.41| 5   | 3   | 322.66 | 0            | 6      | 94.83| 76.28 |
| BHT              | 5.43    | 220.36| 1   | 1   | 241    | 1            | 2      | 20.23| 102.02|
| SA               | -0.66   | 118.09| 4   | 2   | 100.24 | 0            | 3      | 74.60| 83.26 |
| Darbufelone      | 4.18    | 332.47| 4   | 3   | 312.02 | 0            | 3      | 76.94| 82.46 |
| Prifelone        | 6.34    | 316.47| 2   | 1   | 305.54 | 1            | 1      | 37.3 | 96.13 |
| Tazofelone       | 4.63    | 321.49| 3   | 2   | 313.54 | 0            | 4      | 49.33| 91.98 |
| Tebufelone       | 5.71    | 300.44| 2   | 1   | 316.08 | 1            | 6      | 37.3 | 96.13 |

### Table 3: Bioactivity score of 2-(substituted benzylidene)succinic acids

| Compound         | GPCR ligand | Ion channel modulator | Kinase inhibitor | Nuclear receptor ligand | Protease inhibitor | Enzyme inhibitor |
|------------------|-------------|-----------------------|-----------------|------------------------|-------------------|------------------|
| 1                | -0.1        | 0.07                  | -0.73           | -0.04                  | -0.42             | 0.23             |
| 2                | 0.01        | 0.14                  | -0.56           | 0.22                   | -0.34             | 0.32             |
| 3                | -0.04       | 0                     | -0.57           | 0.09                   | -0.34             | 0.21             |
| 4                | 0.05        | 0.12                  | -0.49           | 0.23                   | -0.3              | 0.33             |
| 5                | 0.03        | 0.03                  | -0.43           | 0.18                   | -0.33             | 0.27             |
| 6                | 0.04        | -0.02                 | -0.4            | 0.16                   | -0.24             | 0.23             |
| 7                | 0.06        | -0.02                 | -0.51           | 0.06                   | -0.27             | 0.25             |
| 8                | 0.09        | 0.03                  | -0.28           | 0.23                   | -0.18             | 0.31             |
| 9                | 0.1         | -0.02                 | -0.27           | 0.15                   | -0.15             | 0.23             |
| 10               | 0.05        | 0.05                  | -0.45           | 0.31                   | -0.24             | 0.3              |
| 11               | 0.2         | 0.14                  | -0.36           | 0.42                   | -0.07             | 0.37             |
| 12               | 0.22        | 0.11                  | -0.3            | 0.47                   | -0.02             | 0.34             |
| 13               | 0.26        | 0.18                  | -0.19           | 0.53                   | 0.04              | 0.35             |
| BHT              | -0.34       | 0                     | -0.48           | -0.08                  | -0.57             | -0.07            |
| SA               | -2.74       | -2.45                 | -3.37           | -2.68                  | -2.76             | -2.38            |
| Darbufelone      | -0.33       | -0.5                  | -0.05           | -0.53                  | -0.48             | -0.03            |
| Prifelone        | 0.01        | 0.01                  | -0.02           | 0.25                   | -0.25             | 0.07             |
| Tazofelone       | 0.07        | -0.28                 | -0.57           | 0.2                    | 0.01              | -0.02            |
| Tebufelone       | 0.16        | 0.28                  | -0.16           | 0.45                   | 0.05              | 0.41             |
In silico Study of 2-(Substituted Benzylidene)Succinic Acids

structures, and it is used for the identification of new functional drugs with increased binding selectivity profile and less undesirable effects. It is well documented that, if bioactivity score is more than 0, the molecules have better biological activity. If bioactivity score is -0.5 to 0, the molecules have moderate activity, and less than -0.5, the molecules have no biological activity.\[16\] The results of bioactivity data indicated that all the benzylidenesuccinic acids were highly active as enzyme inhibitors, nuclear receptor ligands, ion channel modulators, GPCR ligands, and moderately active as protease inhibitors. Only some compounds were predicted as moderately active kinase inhibitors, and few compounds (1, 2, 3, and 7) were inactive as kinase inhibitors. Among all the 2-(substituted benzylidene) succinic acids, the phenolic derivative exhibited greater bioactivity score as enzyme inhibitors and nuclear receptor ligands. The bioactivity data revealed that the modification of the phenolic hydroxyl group to methoxy group cause reduction in their bioactivity score. Introduction of electron releasing alkyl substituents ortho to phenolic hydroxyl group (compounds 10, 11, 12, and 13) increased their bioactivity score as nuclear receptor ligands, but does not show much variation as enzyme inhibitors.

The calculated bioactivity data revealed that the (E)-2-((3,5-di-tert-butyl-4-hydroxybenzylidene) succinic acid (compound 13) was excellent nuclear receptor ligand, enzyme inhibitor, and GPCR ligand with highest bioactivity score 0.53, 0.35, and 0.26, respectively. The data also indicated that compound 13 was a good ion channel modulator and protease inhibitor with bioactivity score of 0.18 and 0.04, respectively. The greater bioactivity score of compound 13 was attributed to its high log P value, molecular volume, and also due to its good TPSA and % ABS values. This observation was supported by previous reports, the anti-inflammatory activity and quantitative structure activity relationship (Q SAR) study of substituted 3,5-di-tert-butyl-4-hydroxy styrene.\[17,18\] Therefore, the molecular properties and bioactivity score of compound 13 was compared with its structural components, butylated hydroxy toluene (BHT), and succinic acid. The compared results revealed that compound 13 obeyed all the Lipinski’s rules with zero number of violations and possess much greater bioactivity score than the antioxidant BHT and succinic acid. The better bioactivity of compound 13 was attributed to its higher number of rotatable bonds, number of hydrogen bond acceptors, and number of hydrogen bond donors responsible for conformational flexibility and receptor interaction when compared with BHT and succinic acid.

The better molecular properties and bioactivity score exhibited by (E)-2-((3,5-di-tert-butyl-4-hydroxybenzylidene)succinic acid gave an impetus to estimate and compare the molecular properties and bioactivity score of some selected anti-inflammatory drugs, such as, darbufelone, prifelone, tazofelone, and tebufelone possessing similar structural fragment 3,5-di-tert-butyl-4-hydroxyphenyl ring. The generated in silico data presented in Tables 1 and 2. The data revealed that the above anti-inflammatory drugs obeyed Lipinski’s rule of five, as it states that an orally active drug generally has no more than one violation.\[19\] The calculated TPSA and % ABS values of these drugs indicated good oral bioavailability. The bioactivity data revealed that the 2-(substituted benzylidene) succinic acids showed higher bioactivity scores as enzyme inhibitors than the darbufelone, prifelone, and tazofelone, but showed lower bioactivity than tebufelone. The scores of 2-(substituted benzylidene)succinic acids range from 0.21 to 0.37 depending upon the substituents they possess. The unsubstituted benzylidenesuccinic acid also possesses good bioactivity score than the darbufelone, prifelone, and tazofelone. This observation may indicate the importance of phenylitaconic acid structure to exhibit enzyme inhibition. The estimated bioactivity score of phenolic benzylidenesuccinic acids as nuclear receptor ligands was good and appeared almost equipotent or more potent with the evaluated anti-inflammatory drugs except for darbufelone. It is observed that the bioactivity score as nuclear receptor ligands increases when phenolic benzylidenesuccinic acids substituted with one or two electron releasing substituents ortho to phenolic

### Table 4: Toxicity and risk assessment of substituted benzylidene succinic acids using osiris property explorer

| Compound No. | Toxicity and risk | ClogP | Solubility | Molecular weight | TPSA | Drug likeness | Drug score |
|--------------|------------------|-------|------------|------------------|------|---------------|------------|
| 1            | Safe             | 1.26  | -1.87      | 206              | 74.6 | -0.69         | 0.64       |
| 2            | Reproductive effect | 0.91  | -1.57      | 222              | 94.83 | 0.64         | 0.48       |
| 3            | Reproductive effect | 1.19  | -1.89      | 236              | 83.83 | 0.76         | 0.48       |
| 4            | Safe             | 0.57  | -1.28      | 238              | 115  | 1.5           | 0.87       |
| 5            | Safe             | 0.84  | -1.59      | 252              | 104  | 1.14         | 0.84       |
| 6            | Reproductive effect | 1.12  | -1.9       | 266              | 93.06 | 2.82         | 0.55       |
| 7            | Safe             | 1.37  | -2.58      | 250              | 93.06 | 0.73         | 0.77       |
| 8            | Safe             | 0.77  | -1.16      | 282              | 113.2 | 1.84         | 0.88       |
| 9            | Reproductive effect | 1.05  | -1.92      | 296              | 102.2 | 3.94         | 0.55       |
| 10           | Safe             | 1.6   | -2.26      | 250              | 94.83 | -4.03        | 0.47       |
| 11           | Mutagenic        | 2.43  | -2.26      | 278              | 94.83 | -4.76        | 0.27       |
| 12           | Safe             | 3.29  | -3.31      | 306              | 94.83 | -9.14        | 0.41       |
| 13           | Safe             | 4.08  | -3.89      | 334              | 94.83 | -15.08       | 0.35       |
hydroxyl group. The other substituted benzylidene succinic acids also exhibited good bioactivity scores as nuclear receptor ligands (> 0). These observations indicating the importance of not only the phenolic group, but also the other electron releasing substituents present on the benzylidene succinic acid.

The bioactivity prediction revealed that the prifelone, tazofelone, and tebufelone were active as GPCR ligands, but the scores were less than compounds 11, 12, and 13. The benzylidene succinic acid and phenolic benzylidene succinic acids were active as ion channel modulators, the bioactivity scores of these compounds were greater than the prifelone and lesser than the tebufelone. The bioactivity data revealed that the predicted anti-inflammatory drugs were either moderately active or inactive kinase inhibitors. The study also revealed that tazofelone, tebufelone, and compound 13 were active as protease inhibitors, indicating the importance of 3,5-di-tert-butyl-4-hydroxyphenyl ring system.

The toxicity of 2-(substituted benzylidene)succinic acids was assessed using Osiris Property Explorer and the data presented in Table 4. This computational tool also used to calculate ClogP, solubility, molecular weight, TPSA, drug-likeness, and drug score. The study revealed that the unsubstituted benzylidene succinic acid and the phenolic compounds were predicted as safe and non-toxic except compounds 2 and 11. The nonphenolic derivatives (compounds 3, 6, and 9) were predicted to possess toxic reproductive effects, whereas compound 7 was identified as safe and non-toxic molecule. The calculated ClogP values of all the benzylidene succinic acid were similar to that of molig P values. TPSA calculation performed by this software was found same as that of Molinspiration Cheinformatics software. The calculated drug-likeness was positive only for compounds 2 to 9. The calculated drug scores, based on ClogP, solubility, molecular weight, drug-likeness, and toxicity risks, greatly varied due to the substituents on benzylidene succinic acid.

**Conclusions**

The *in silico* study concluded that all the 2-(substituted benzylidene)succinic acids were drug-like molecules as they satisfy Lipinski’s rule. The bioactivity results indicated that these compounds were identified as better enzyme inhibitors, nuclear receptor ligands, and good GPCR ligands and ion channel modulators. Among all, 2-(3,5-di-tert-butyl-4-hydroxybenzylidene)succinic acid (compound 13) appeared as the most potent bioactive and non-toxic molecule compared to its structural components BHT and succinic acid. This observation specifies the importance of phenylitaconic acid or benzylidene succinic acid structure with 3,5-di-tert-butyl-4-hydroxy group. Therefore, an effort has been made to compare the molecular properties and bioactivity score of 2-(3,5-di-tert-butyl-4-hydroxybenzylidene) succinic acid with some selected anti-inflammatory drugs, darbufelone, prifelone, tazofelone, and tebufelone, which contain the structural fragment 3,5-di-tert-butyl-4-hydroxyphenyl ring. The study indicated that the 2-(3,5-di-tert-butyl-4-hydroxybenzylidene) succinic acid has better *in silico* molecular properties and bioactivity score as nuclear receptor ligand and GPCR ligand than darbufelone, prifelone, tazofelone, and tebufelone. The study also indicated that the 2-(3,5-di-tert-butyl-4-hydroxybenzylidene) succinic acid has improved bioactivity as enzyme inhibitor than darbufelone, prifelone, and tazofelone. Hence, further research is required to synthesize and evaluate the toxic and pharmacological properties of 2-(3,5-di-tert-butyl-4-hydroxybenzylidene) succinic acid using appropriate procedures.

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