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Preliminary investigation of drug impurities associated with the anti-influenza drug Favipiravir – An insilico approach

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A B S T R A C T

The role of repurposed or modified antiviral drugs has become more significant during the current global pandemic of SARS Covid-19. In the present study, four structurally analogous impurity molecules of antiviral drug Favipiravir are selected for preliminary computational investigation for assessing the structure-activity relationship. The optimized geometry and the electronic structures of the compounds are computed using Density Functional Theory as a precursor to evaluating their physical, chemical and spectral properties. The frontier orbitals analysis is performed to obtain global reactivity parameters namely, the chemical potential, absolute electronegativity, global softness, global hardness, electrophilicity, etc. The natural Bond Orbital (NBO) analysis and Mulliken analysis provided an understanding of the charge-transfer interactions of molecules. The possibilities of intermolecular interactions of the drug systems with the receptors are also visualized using the electrostatic potential maps (MEP) derived from the DFT computations. The physiochemical properties are assessed computationally using SwissADME webtool to correlate the structural aspects of the compounds with their biological responses. Useful parameters namely flexibility, lipophilicity, size, polarity, solubility and saturation were also computed to evaluate the therapeutic activity or drug-likeness.

1. Introduction

Favipiravir (FVPR), chemically 6-fluoro-3-hydroxypyrazine-2-carboxamide, is an antiviral drug cleared for treating pandemic infections in 2014 [1]. It has demonstrated uses in treating viral infections like Ebola, Yellow fever, West Nile virus, Foot and Mouth disease, Rift-valley fever, etc. [2,3]. Viruses causing seasonal influenza like H1N1, H2N2, H3N2 [2] and influenza A (H5N1) were also found to be vulnerable against FVPR [4,5]. Further, FVPR was found to be potent against viruses that have shown resistance against conventional drugs like oseltamivir and amantadine [6]. The current global health crisis of SARS COVID-19 has necessitated the exploratory usage of many re-purposed potential drug systems including FVPR. FVPR is a synthetic derivative of pyrazinamide with a marked activity towards RNA-based virus. In one of the comprehensive literature scrutiny that covered over 2600 studies by Ahmed Wadaa-Alla et al., both FVPR and HCQ demonstrated effectiveness in clearing the viral load, shortening the recovery time, and improving pneumonia [7]. The study also envisaged that FVPR and Remdesivir stand better chances as COVID-19 drugs [8] with the FVPR showing a hal-maximal response of 61.88 μM [9].

FVPR shows keto-enol tautomerism via an intramolecular proton transfer demonstrating the switching of structures between a ketone form and an alcohol form. The proton transfer is bound to cause changes in the structural and electronic properties thereby affecting the biological activities. DFT studies by L. Antonov showed that FVPR is present predominantly in the enol form in the neutral state. However, the predominant structure switches to the keto form in an acidic medium as the carboxamide group easily gets protonated [10].

Upon administration, FVPR gets converted into FVPR-RMP (favipiravir ribofuranosyl-5-monophosphate) derivative by phosphoribosylation by an enzyme [11] and this is later metabolized to the active form of FVPR-RTP (favipiravir-ribofuranosyl triphosphate) as seen in Fig. 1. While the FVPR-RMP showed mild inhibitory action, FVPR-RTP showed antiviral effects on influenza viruses by having marked inhibitory action on the RNA-dependent RNA polymerase (RdRp) [12].

As FVPR is metabolized by the enzyme aldehyde oxidase and is not influenced by the cytochrome isoenzymes, it is expected to show minimum drug interactions [13]. Further, the cardiovascular side effects of FVPR are assessed to be minimum [14]. Even though FVPR appears to be relatively safer without many serious adverse effects, there is a possibility of altered serum urate levels in patients. Also, certain aspects such as teratogenic implications and altered QTc intervals etc need to be assessed further for the long-term usage of FVPR [15]. It is observed that there is a dearth of clinical safety data in the literature for the use of FVPR in Covid-19 treatment.

Drug impurities are compounds that are formed during synthesis and formulation and stay remain active along with the ‘active pharmaceutical ingredients’. They might also form during storage by

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The term impurity often assumes a negative connotation due to its possible harmful impacts on the safety and efficacy of the drugs. Regulatory bodies stipulate that all the impurities above the critical concentration levels must be detected, qualified and reported. However, it has been observed that at times the drug impurities can be useful as their structural and clinical exploration might lead us to better drug molecules. Especially, during pressing situations such as a pandemic, they offer a plethora of lead molecules to work upon in pursuit of novel and efficient medication. 6-flouro-3,5-dihydroxypyrazine-2-carboxamide (FVPR-1), 3-hydroxypyrazine-2-carboxamide (FVPR-2), 3,5-dihydroxypyrazine-2-carboxamide (FVPR-3) and 6-flouro-3-hydroxy-5-carboxamide (FVPR-9) are some of the molecules that have been studied for their potential therapeutic effects.

**Fig. 1.** Phosphoribosylation of FVPR into FVPR-RTP

**Fig. 2.** Structure of FVPR-1, FVPR-2, FVPR-3 and FVPR-9.
(FVPR-2), 6-flouro-4-hydroxy-3-oxo-3,4-dihydroxypyridine-2-carboxamide (FVPR-3) and 6-flouro-3-hydroxypyrazine-2-carboxamide (FVPR-9) are process-related impurities of FVPR.

In the present study, the four impurity molecules of FVPR namely FVPR-1, FVPR-2, FVPR-3 and FVPR-9 (Fig. 2) are subjected to structural, spectral and physiochemical studies computationally to assess the drug-likeness and medicinal chemistry friendliness for potential use in antiviral treatments including COVID-19. Among the impurity molecules studied, FVPR-1, also known as hydroxyl favipiravir (T-705M1), is an established metabolite of FVPR [16]. Though FVPR-1 will be in its inactive state, its presence in plasma may lead to elevated uric acid concentration in blood [17] leading to hyperuricemia especially in patients with renal complications. Another impurity molecule FVPR-2, often designated as compound T-1105, has been explored for its potential application in antiviral treatments by Toyama Chemical Co., Ltd [18]. In another study [19], FVPR-2 was found to be effective against Zika virus replication. Further, Hutching et al [20] demonstrated that the favipiravir-ribonucleoside complex which plays a crucial role in the drug action is not very stable and easily gets decomposed by the nucleophilic displacement of Fluorine by the hydroxyl group. On the contrary, the non-fluorine analog of favipiravir, namely FAVPR-2 (T-1105) will form its ribonucleoside complex which is more stable in the given circumstances. Studies also revealed that FAVPR-2 could be a more potent antiviral drug for inhibiting influenza viruses [21].

Table 1
Major optimization parameters.

| Parameters                  | FAVPR-1 | FAVPR-2 | FAVPR-9 | FAVPR-9 |
|-----------------------------|---------|---------|---------|---------|
| Global minimum energy (a.u.)| -682.7  | -508.2  | -666.6  | -627.3  |
| Polarizability              | 84.07   | 76.66   | 84.57   | 74.01   |
| Dipole moment (D)           | 1.81    | 4.6     | 2.5     | 1.62    |
| Total Thermal energy (Kcal/mol) | 71.6   | 73.1    | 78.2    | 59.8    |
| Heat Capacity (cal/mol K)   | 37.0    | 30.6    | 39.2    | 31.9    |
| Entropy (cal/mol K)         | 95.7    | 89.2    | 101.5   | 91.1    |

Fig. 3. Optimized geometries of [1] FVPR-1; [2] FVPR-2; [3] FVPR-3 and [4] FVPR-9 computed using DFT at B3LYP 6-311g.
interface GaussView 6.0.

2.2. ADME Studies

The input for the geometry optimization was constructed and the results were interpreted using the graphical analysis at suitable basis sets. The ADME properties including absorption, distribution, metabolism, and excretion were evaluated with the help of the SwissADME web tool [23,24]. The graphical output of the programme namely the bioavailability radar has been utilized for forecasting important physicochemical parameters which determine the preliminary appropriateness of the lead molecule for drug development.

2.3. Results and Discussions

Table 2
Calculated net charges by Mulliken Population Method and Natural Bond orbital analysis.

| Atom   | Natural Charge | Mulliken Charge |
|--------|----------------|-----------------|
| C1     | 0.46375        | 0.333           |
| C2     | 0.51487        | 0.407           |
| N3     | -0.42895       | -0.327          |
| C4     | -0.02231       | -0.131          |
| C5     | 0.52846        | 0.492           |
| N6     | -0.48407       | -0.323          |
| O7     | -0.63449       | -0.543          |
| H8     | 0.50318        | 0.408           |
| O9     | -0.63103       | -0.546          |
| H10    | 0.48931        | 0.401           |
| F11    | -0.32669       | -0.287          |
| C12    | 0.62122        | 0.664           |
| O13    | -0.6661        | -0.491          |
| N14    | -0.74397       | -0.772          |
| H15    | 0.40314        | 0.35            |
| H16    | 0.41367        | 0.366           |

2.4. Materials and Methods

2.4.1. DFT Calculations

Gaussian 09 software package [22] has been used for the computational modeling to yield the optimized structures, geometrical parameters, vibrational frequencies, frontier orbital and Mulliken charge analysis at suitable basis sets. The input for the geometry optimization was constructed and the results were interpreted using the graphical interface GaussView 6.0.

2.4.2. ADME Studies

The ADME properties including absorption, distribution, metabolism, and excretion were evaluated with the help of the SwissADME web tool [23,24]. The graphical output of the programme namely the bioavailability radar has been utilized for forecasting important physicochemical parameters which determine the preliminary appropriateness of the lead molecule for drug development.

3. Results and Discussions

3.1. Structural optimizations

The most optimized structures corresponding to the global minimum energy states were visualized using DFT at the basis set B3LYP/6-311 and the same have been presented in Fig. 3 with atom labeling. The global minimum energies of FVPR-1, FVPR-2, FVPR-3 and FVPR-9 are computed at −682.7, −508.2, −666.6 and −627.3 a.u. respectively indicating the most stable states both thermally and dynamically [25].

The summary of major optimization parameters computed is appended in Table 1. The parameters such as global minimum energy and polarizability are useful indicators in the prospective docking studies while the dipole moment of the molecules envisages drug-receptor interactions [26].

The polarizability provides the overall sum of partial charge distribution across the atoms and the dipole moment is the product of the sum of the individual atomic charges, and this can be further corroborated by performing the Natural bond orbitals (NBO) analysis. The NBO analysis provides an understanding of the charge-transfer interactions of molecules [28]. Another parameter that describes the electronic charge distributions in molecules is the Mulliken charge. In the Mulliken charge analysis, the sites with maximum negative charges will undergo electrophilic attack and the sites with maximum positive charges will be more prone to nucleophilic attack.
The natural charges computed by the NBO analysis and Mulliken charges of FVPR-1, FVPR-2, FVPR-3, and FVPR-9 are appended in Table 2.

The computed results demonstrate that the most positive site in all the four compounds is the carboxamide carbon (0.62122 at C12 in FVPR-1; 0.60472 at C9 in FVPR-2; 0.59434 at C8 in FVPR-3 and 0.75715 at C11 in FVPR-9) and hence will be liable for an attack by the nucleophiles. Conversely, the most negative site in FVPR-1, FVPR-2, and FVPR-3 was found at the amino nitrogen (−0.74397 at N14, −0.7762 at N11 and −0.76237 at N10 respectively) while the same in FVPR-9 was seen at the hydroxyl oxygen of the carboxylic acid group (−0.62933 at O13). These sites will be susceptible to electrophilic attacks. The propensity of intermolecular interactions of the drug systems with the receptors can also be studied with the help of electrostatic potential maps (MEP) derived from the DFT computations. These plots are generated by plotting the electrostatic potentials onto the fixed electronic density surfaces [28]. The MEP is a useful tool in assessing the reactivity of drug systems towards electrophilic and nucleophilic attacks [29] by using a color gradient in which the most electropositive and negative sites are represented in blue and red respectively. The MEP plots visualized computationally are presented in Fig. 4.

The MEP plots show that the hydrogen atoms attached to the amino group and the carbonyl oxygen are more susceptible to nucleophilic and electrophilic attacks, respectively.

3.2. Frontier orbital analysis

The Highest Occupied (HOMO) and Lowest Unoccupied (LUMO) Molecular Orbitals, commonly known as the frontier orbitals, often provide valuable information regarding the electron-donating and electron-gaining tendencies of the drug molecules. This will in turn, help us to assess the drug-receptor biochemical interactions, thermodynamical stability and chemical reactivity [30,31].

The HOMO-LUMO energy gap further reveals the scope of electron excitation between the highest occupied and lowest unoccupied orbitals, thus describing the overall stability. The frontier orbitals of the lead

![Fig. 4. MEP plots of [1] FVPR-1; [2] FVPR-2; [3] FVPR-3 and [4] FVPR-9.](image)

| Table 3: The global reactivity parameters of FVPR-1, FVPR-2, FVPR-3, and FVPR-9. |
|-----------------|---|---|---|---|
| Parameter       | FVPR-1 | FVPR-2 | FVPR-3 | FVPR-9 |
| E_HOMO (eV)     | −7.338 | −6.858 | −7.397 | −7.824 |
| E_HOMO−1 (eV)   | −7.973 | −7.278 | −7.908 | −8.066 |
| E_LUMO (eV)     | −2.656 | −2.407 | −4.046 | −3.280 |
| ΔE (eV)         | −1.463 | −1.316 | −1.668 | −1.702 |
| Ionization energy (I) | 7.338 | 6.858 | 7.397 | 7.824 |
| Electron affinity (A) | 2.656 | 2.407 | 4.046 | 3.280 |
| Global hardness (η) | 2.341 | 2.225 | 1.675 | 2.272 |
| Global softness (S) | 0.214 | 0.225 | 0.298 | 0.220 |
| Absolute electro negativity (χ) | 4.977 | 4.633 | 5.722 | 5.552 |
| Chemical potential (μ) | −4.977 | −4.633 | −5.722 | −5.552 |
| Electrophilicity (ω) | 5.291 | 4.822 | 9.771 | 9.298 |
| Maximum charge transfer index | 2.126 | 2.082 | 3.416 | 2.443 |

The natural charges computed by the NBO analysis and Mulliken charges of FVPR-1, FVPR-2, FVPR-3, and FVPR-9 are appended in Table 2.
molecules visualized via DFT studies can be used to obtain several useful
global reactivity parameters namely, the chemical potential, absolute
electroegativity, global softness, global hardness, electrophilicity, etc.
These descriptors are calculated using the following Eqs. (1)-(8).

Ionization potential \( (I) = -E_{\text{HOMO}} \)  \hspace{1cm} (1)  
Electron affinity \( (A) = -E_{\text{LUMO}} \)  \hspace{1cm} (2)  
Absolute electronegativity \( (\chi) = \frac{I + A}{2} \)  \hspace{1cm} (3)  
Chemical potential \( (\mu) = -\chi \)  \hspace{1cm} (4)  
Global hardness \( (\eta) = \frac{E_{\text{LUMO}} - E_{\text{HOMO}}}{2} \)  \hspace{1cm} (5)  
Global softness \( (S) = \frac{1}{2\eta} \)  \hspace{1cm} (6)  

Electrophilicity \( (\omega) = \frac{\mu^2}{2\eta} \)  \hspace{1cm} (7)  
Maximum charge transfer index \( (\Delta N_{\text{max}}) = -\frac{\mu}{\eta} \)  \hspace{1cm} (8)  

The results in Table 3 showed that FVPR-2 has the highest HOMO
energy and FVPR-3 has the lowest LUMO energy making them the most
nucleophilic and electrophilic systems, respectively. The HOMO-LUMO
energy difference of FVPR-1, FVPR-2 and FVPR-9 are relatively higher
and comparable while that of FVPR-3 is relatively smaller at 3.351 eV. In
general, a relatively higher \( \Delta E \) represents higher stability and a lower
\( \Delta E \) indicates more chemical reactivity.

FVPR-9 and FVPR-2 demonstrated the highest and lowest ionization
energies respectively. It is noted that systems with a high Electrophilicity
index \( (\omega) \) will be less reactive and act as electrophiles. Going by
this thumb rule, FVPR-3 will be more stable and nucleophilic in nature.
Conversely, FVPR-2 will be more reactive and electrophilic during drug-
receptor interactions. Further FVPR-2 shows the lowest absolute elec-
tronegativity \( (\chi) \) and thereby making it the most basic of the lot. This
could be an important marker that governs the intracellular interactions
of the drug.

The HOMO and LUMO contour plots of the molecules are visualized
using DFT are presented below (Fig. 5). These plots have red and blue
regions representing the negative and positive phases of the wave
function, respectively. In FVAPR-1, the HOMO is populated throughout
the molecule except on the pyrazine nitrogens. The hydroxyl oxygen, the
O and N on the carboxamide and the fluorine atoms contribute to the
HOMO. The pyrazine nitrogens, O and N on the carboxamide, mainly
contribute to the LUMO with no population over the fluorine atom.

In FVPR-2, the HOMO has contributions from the carbonyl oxygen,
pyrazine nitrogens and hydroxyl oxygen atoms. The LUMO is highly
localized over the pyrazine nitrogens and the carboxamide moiety with
no population over the OH groups. In FVPR-3, the HOMO is mainly due
to the O and N of carboxamide and pyrazine nitrogens whereas the
LUMO is mainly due to carbonyl oxygen attached to the ring and pyr-
azine nitrogens. Again, there is no involvement from the fluorine atoms.
In FVPR-9, the HOMO contributions involve the pyrazine nitrogen hy-
donated oxygen and fluorine atoms. The LUMO is primarily due to the
COOH and the ring with no population over the fluorine atom.

Of the molecules studied, FVPR-1, FVPR-2, and FVPR-9 showed
comparable HOMO-LUMO band gap with FVPR-3 showing a relatively
lower value at 3.351 eV. It is attributed to the smaller aromatic system in
FVPR-3. The comparatively shorter energy gaps of the molecules suggest
the feasibility of electron transfer and increased chemical reactivity. The
HOMO-LUMO energies were also assessed by using the Density of States
(DOS) plots in which the occupied and virtual orbitals are illustrated
(Fig. 6). The HOMO-LUMO distribution along with the electrostatic
potential plots often provide suggestive inputs for the potential docking
studies as the HOMO of the ligand molecule is expected to bind with the
LUMO of the receptor molecule. Therefore, it is envisaged that higher
HOMO energy of the lead molecule favors docking [32].

3.3. Geometrical Parameters

Geometrical parameters including the bond lengths and bond angles
of FVPR-1, FVPR-2, FVPR-3, and FVPR-9 compared against the experi-
mental values [33]. In general, the computed and experimental results
showed necessary consilience (Table 4). It is expected that all three
molecules except FVPR-3 show keto-enol tautomerism. The noticeable
reduction in the bond lengths involving the OH group is attributed to the
possibilities of hydrogen bonding involving these atoms. The same trend

\[4.682 \text{ eV} \]  
\[4.451 \text{ eV} \]  
\[3.351 \text{ eV} \]  
\[4.544 \text{ eV} \]
is also noticed among the N—H bond lengths of carboxamide moieties, again owing to the hydrogen bonding. Intramolecular hydrogen bonding is visualized at O7—H8—O13 (2.727 Å) for FVPR-1 and at O8—H9—O12 (2.787 Å) for FVPR-9. All the molecules are capable of intermolecular hydrogen bonding that involves the NH$_2$. The compounds FVPR-1, FVPR-2 and FVPR-9 are structurally planar including the fluorine as evident from their torsional angles whereas, in FVPR-3 the carboxamide moiety is placed at a dihedral angle of 20 Å.

3.4. FTIR Studies

Even though FTIR data are often overlooked during the structural and geometrical optimization, it can substantiate the optimized structure corresponding to the global minimum energy by having all positive force constant values. All the characteristic wave numbers are identified and present in the consolidated FTIR plot (Fig. 7). The trademark twin peaks of amino group corresponding to the asymmetric and stretching modes are computed at 3700 and 3500 cm$^{-1}$ respectively for FVPR-1, FVPR-2, and FVPR-3. The OH stretching modes are computed at around 3600 cm$^{-1}$ for all four compounds. In FVPR-9, the stretching vibrations involving the—OH of the carboxylic acid group are visualized at 3350 and 3640 cm$^{-1}$ respectively. The other significant peaks such as NH$_2$ scissoring and C=O stretching are computed at 1680 and 1630 cm$^{-1}$ respectively. As expected, the vibrations involving C—O, C—N, C—F, N—H, C—H, O—H and the ring stretch dominate the fingerprint region.

3.5. Absorption-Distribution-Metabolism-Excretion (ADME) studies

The ADME studies are conducted computationally to evaluate the extent of absorption of the drug, its distribution in the body, various metabolic processes it undergoes, and finally the elimination of the drugs from the body. These parameters also give us a preclinical assessment of the overall toxicity of the lead molecules. ADME-based filtering studies help us to handpick the best druglike molecules and also help to minimize potential failures at an advanced stage, by avoiding the unsuitable candidates initially [34,35]. For better pharmacological efficacy, the selected drugs are expected to present inside the body in the right quantity for the right duration of time before getting excreted. Useful parameters namely flexibility, lipophilicity, size, polarity, solubility and saturation and their functional limits are computed and presented using the bioavailability radar (Fig. 8) function of the program. Further, important clinical aspects such as gastrointestinal absorption (HIA), blood-brain-barrier (BBB) permeability,
Table 4
Geometrical parameters of FVPR-1, FVPR-2, FVPR-3, and FVPR-9 compared against the experimental results.

| Bond angles | FVPR-1 | FVPR-2 | FVPR-3 | FVPR-9 | Expt. |
|-------------|--------|--------|--------|--------|-------|
| C1–C2       | 1.41   | C1–C2  | 1.35   | C1–C2  | 1.34  |
| C1–N6       | 1.33   | C1–C2  | 1.39   | C1–C13 | 1.50  |
| C1–O9       |       |        |        |        | 1.34  |
| C2–N3       | 1.30   | N6–C5  | 1.35   | C2–N3  | 1.39  |
| C2–F11      | 1.38   | C2–F7  | 1.39   | C2–F10 | 1.39  |
| N3–C4       | 1.36   | C5–C4  | 1.41   | N3–C4  | 1.30  |
| C4–C5       | 1.41   | C4–N3  | 1.30   | C4–C5  | 1.50  |
| C4–C12      | 1.47   | C4–C9  | 1.50   | C4–C8  | 1.50  |
| C5–N6       | 1.35   | N3–C2  | 1.39   | C5–C13 | 1.52  |
| C5–O7       | 1.35   |        |        | C5–O6  | 1.24  |
| O7–H8       | 1.00   |        |        |        |       |
| O9–H10      | 0.98   | O14–H15| 0.98   | O14–H15| 0.98  |
| C12–O13     | 1.27   | C9–O10 | 1.25   | C8–O9  | 1.25  |
| C12–N14     | 1.35   | N9–C11 | 1.36   | C8–N10 | 1.36  |
| N14–H15     | 1.00   | N11–H12| 1.00   | N10–H11| 1.01  |
| N14–H16     | 1.01   | N11–H13| 1.01   | N10–H12| 1.01  |

Fig. 7. Consolidated FTIR spectra of FVPR-1, FVPR-2, FVPR-3, and FVPR-9.

The results showed that all four compounds show more or less similar physiochemical properties as compared to Favipiravir. All the compounds are free from any Lipinski violations, suggesting good drug-like properties. Among the compounds studied, FVPR-1 has 6 hydrogen bond acceptors and 3 donors indicating more reactivity than others with similar physiochemical properties as compared to Favipiravir. All the compounds are comparable to that of the parent drug.

topological polar surface area (TPSA) etc. of the compounds were also derived (Table 5).

The SwissADME tool also provides a graphics output called the BOILED-Egg plot which outlines the gastrointestinal or CNS absorption rates of the potential drugs. In the BOILED-Egg plot, the molecules that are present in the egg’s white will undergo GI absorption, while those in the yolk would be more prone to CNS absorption (Fig. 9). It is also noted
Fig. 8. Bio-availability radars of the metabolites and parent drugs
that if the molecule is present in the grey area, they are expected to show poor GI and CNS absorption rates. The results showed that FVPR and its impurities are present in the ‘white’ region demonstrating good GI absorption properties. In general, all the compounds studied showed good GI absorption and poor BBB permeation suggesting almost no effect on the CNS system.

4. Conclusions

Four structurally analogous impurity molecules of FVPR are investigated computationally to reveal the structural, spectral and electronic properties. The geometrical parameters computed showed a satisfactory concordance with the experimental results. Our results showed that FVPR-2 has a relatively very high dipole moment of 4.6D indicating high probabilities of intramolecular charge transfer interactions. The most positive site (liable for nucleophilic attack) in all the four compounds is the carboxamide carbon, whereas the most negative site (liable for electrophilic attack) in FVPR-1, FVPR-2, and FVPR-3 was found at the amino nitrogen. The same for FVPR-9 was seen at the hydroxyl oxygen of the carboxylic acid group. The MEP plots further revealed that the hydrogen atoms attached to the amino group and the carbonyl oxygen are more susceptible to nucleophilic and electrophilic attacks respectively. Further, the assessment of ADME parameters revealed that all the lead molecules possessed very low logP values indicating very minimal toxicity and hydrophilicity which are hallmarks of good drug systems. FVPR-1 has 6 hydrogen bond acceptors and 3 donors indicating more reactivity with the receptor systems and showed relatively higher values of TPSA due to the presence of an additional polar hydroxyl group.

FVPR-9 and FVPR-2 demonstrated the highest and lowest ionization energies, respectively. Going by the thumb rule of Electrophilicity index ($\omega$), FVPR-3 will be more stable and nucleophilic while FVPR-2 will be more reactive and electrophilic in nature during drug-receptor interactions. Further FVPR-2 shows the lowest absolute electronegativity ($\chi$) and thereby making it the most basic of the lot. This could be an important marker that governs the intracellular interactions of the drug.

| Table 5 | The physiochemical properties of lead molecules compared against the parent molecules. |
|---------|----------------------------------------------------------------------------------|
| Molecule | FVPR | FVPR-1 | FVPR-2 | FVPR-3 | FVPR-9 |
| Molecular weight (g) | 157.1 | 173.1 | 139.11 | 172.11 | 158.09 |
| No. of heavy atoms | 11 | 12 | 10 | 12 | 11 |
| No of rotatable bonds | 1 | 1 | 1 | 1 | 1 |
| No. of H-bond acceptors | 4 | 6 | 4 | 5 | 6 |
| No. of H-bond donors | 2 | 3 | 2 | 2 | 2 |
| Molar refractivity | 32.91 | 34.13 | 32.15 | 39.7 | 30.97 |
| TPSA, $\text{Å}^2$ | 88.84 | 109.33 | 89.1 | 92.75 | 83.31 |
| Consensus Log P | -0.27 | -0.42 | -0.6 | -0.71 | 0.19 |
| ESOL Log S | -0.8 | -1.17 | -0.78 | -0.46 | -1.54 |
| Solubility | Very soluble | Very soluble | Very soluble | Very soluble | Very soluble |
| GI absorption | High | High | High | High | High |
| BBB permeant | No | No | No | No | No |
| Pgp substrate | No | No | No | No | No |
| CYP1A2 inhibitor | No | No | No | No | No |
| CYP2C19 inhibitor | No | No | No | No | No |
| CYP2C9 inhibitor | No | No | No | No | No |
| CYP2D6 inhibitor | No | No | No | No | No |
| CYP3A4 inhibitor | No | No | No | No | No |
| log Kp (cm/s) | -7.66 | -7.41 | -7.49 | -7.78 | -6.83 |
| Lipinski #violations | 0.55 | 0.55 | 0.55 | 0.55 | 0.56 |
| Bioavailability Score | 0 | 0 | 0 | 0 | 0 |
| PAINS #alerts | 0 | 0 | 0 | 0 | 0 |
| Brenk #alerts | 0 | 0 | 0 | 1 | 0 |
| Leadlikeness #violations | 1 | 1 | 1 | 1 | 1 |
| Synthetic Accessibility | 2.08 | 2.3 | 1.77 | 3.38 | 2.12 |

Fig. 9. BOILED-Egg plot for the metabolites and parent drug molecules.
All these listed parameters could be useful indicators in assessing binding interactions of the lead molecules with protein receptors in the potential docking studies.

**CRediT authorship contribution statement**

**S. Anil Kumar:** Conceptualization, Methodology, Software, Data curation, Writing – original draft. **B.L. Bhaskar:** Visualization, Supervision, Writing - review & editing.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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