In silico studies for the effect of fluorination of Hydroxypyridin-4-ones on their toxicity profile in comparison to Deferiprone

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Abstract:

The vast advancement in pharmaceutical analysis technology, and the evolutionary rise in molecular Biology following the milestone coding of the human genome and the splendid utilisation of computational chemistry and bioinformatics, have called for urgent employment of knowledge, tools and skills to optimise therapies and clinical management. Oral iron chelators have long been in the market but nevertheless require a quality booster to parallel the overall upgrade in all life aspects let alone clinical advances, drugs, and therapeutics.

The implementation for such development lies in the hands of medicinal chemists and experts in drug design and discovery. Using bioinformatic approaches, this study aims to predict an ideal structural configuration capable of producing a more effective compound than the long-used oral iron chelator, Deferiprone.

In pursuit of such an aspiration, a range of fluorinated hydroxypyridinones (the backbone structure for Deferiprone) have been investigated. Fluorination of drugs in general, has recently been shown to bring about many desired features such as metabolic stability. ADMET lab 2.0 along with few other software that are used in molecular docking; have been brought into play to unfold an ideal, more potent fluorinated hydroxypyridinone than Deferiprone particularly with regards to the toxicity profile.

Keywords: Deferiprone, Hydroxypyridin-4-ones, oral iron chelators, molecular docking

دراسات حاسوبية لتأثير فلورة هيدروكسي بيريدينونات على ملف سميتها بالمقارنة مع ديفيريبرون

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****كنكز كوليج لندن****

الخلاصة:

لقد استدعت التطورات الهائلة في تقنيات التحليل الصيدلاني، والنهضة التطورية في علم الأحياء الجزيئي في أعقاب إنجاز فك شفرة الجينوم البشري والاستخدام المتميز للكيمياء الحاسوبية والمعلوماتية الحيوية، حاجة ملحة لتوظيف المعارف والأدوات والمهارات لتحسين العلاجات والإدارة السريرية.

وعلى الرغم من وجود عوامل مخلبية للحديد فموية في الأسواق منذ فترة طويلة، إلا أنها ما تزال بحاجة إلى تعزيز نوعي لمواكبة التطوير الشامل في جميع جوانب الحياة، ناهيك عن التقدم في المجال السريري والأدوية والعلاجات.

يقع تنفيذ مثل هذا التطوير على عاتق خبراء الكيمياء الصيدلانية والمتخصصين في تصميم الأدوية واكتشافها. تهدف هذه الدراسة، باستخدام المنهجيات المعلوماتية الحيوية، إلى التنبؤ بتكوين بنيوي مثالي قادر على إنتاج مركب أكثر فعالية من عامل تخليب الحديد الفموي المستخدم منذ فترة طويلة، الديفيريبرون.
وفي سعي لتحقيق هذا الهدف، تم دراسة مجموعة من مركبات هيدروكسي البيريدينون المفلورة (التي تمثل البنية الأساسية للديفيريبرون). حيث أظهرت الأبحاث الحديثة أن إدخال الفلور إلى الأدوية بشكل عام يمكن أن يمنحها العديد من الخصائص المرغوبة مثل الثبات الاستقلابي.
بالإضافة إلى عدد محدود من البرامج الأخرى المستخدمة في الإرساء الجزيئي، وقد تم استخدام برنامج
" ADMET lab ,"
للكشف عن مركب هيدروكسي بيريدينون مفلور مثالي وأكثر فعالية من الديفيريبرون، خاصة فيما يتعلق بملف السمية.

الكلمات المفتاحية: ديفيريبرون، هيدروكس يبيريدينونات ، مخالب الحديد الفموية، الالتحام الجزيئي

Introduction

For over three decades, the oral iron chelator deferiprone has been at the forefront of iron overload treatment due to its high efficacy 1. Its therapeutic profile could be considered nearly flawless if not for its most serious adverse effects—neutropenia and agranulocytosis 2.
While the exact mechanism behind these side effects remains unclear, several studies have proposed immunological and genetically linked pathways. However, the hypothesis of direct myelotoxicity caused by the chelation of iron and other metals essential for haematopoiesis, holds substantial scientific support 3, 4, 5.
Patients prescribed Deferiprone require care under specialist clinicians, along with regular monitoring to ensure treatment safety and efficacy. However, treatment interruptions are not uncommon due to adverse effects. Fortunately, these side effects typically diminish or resolve completely upon discontinuation, as their occurrence is dose dependent. This means the risk of severe side effects increases with higher doses 6.
Toxicity profile for a candidate compound often contributes a major factor for its acceptance or dismissal. Indeed, toxicity of a compound is directly linked to the 'selectivity' of that compound; a major contributing element for a successful lead compound in drug design; Figure 1.

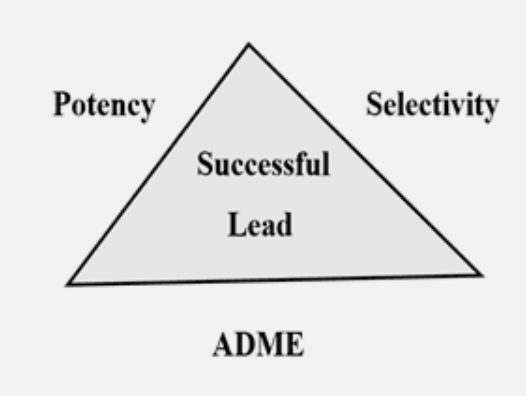


Figure 1: The connection between the main three elements that need to be optimised for a successful lead

For example, before Deferiprone was approved, CP94—a structurally similar compound—demonstrated greater potency than Deferiprone. However, further development of CP94 as a lead candidate was halted entirely because its toxicity profile proved unacceptable.

As previously stated, toxicity is dose dependent. Notably, deferiprone exhibits less than 10% protein binding. This characteristic results in a higher proportion of the drug remaining in its free, pharmacologically active form, thereby elevating the risk of toxicity. Furthermore, the unbound drug undergoes more rapid metabolism, necessitating larger administered doses to maintain therapeutic efficacy. This, in turn, increases systemic exposure and the potential for tissue toxicity.

The latter aspect holds particular significance for the objectives of this study.

The current study adopts a dual-pronged design strategy to optimize Deferiprone's therapeutic profile. The primary approach involves targeted fluorination of the molecule while retaining its structural backbone and iron-chelating pharmacophore.

Introducing fluorine into organic molecules induces strategic physicochemical modifications that are highly beneficial for pharmaceutical development. Fluorination gained prominence around two decades ago for several key reasons, including:

1. Enhanced metabolic stability – The strong C-F bond (compared to C-H) increases resistance to metabolism, particularly oxidative degradation by cytochrome P450 enzymes.
2. Bioisosteric replacement – Fluorine or CF_3 can serve as effective substitutes for -OH, -H, or -CH_3, often improving binding affinities despite differing electronic properties.
3. Improved binding interactions – Due to its high electronegativity, fluorine facilitates hydrogen bonding and dipole-dipole interactions with target proteins.
4. Synthetically speaking, there are many available fluorinating agents as well as fluorine synthons, making the fluorination process more readily accessible. 7, 11, 12, 13, 14

As a secondary strategy, the aim is to modulate the compound's pharmacokinetics through enhanced plasma protein binding. This modification is anticipated to extend systemic circulation time and duration of action, potentially enabling lower dosing requirements while maintaining therapeutic efficacy.

To achieve this goal, albumin was identified as the target protein, as it could theoretically alter the candidate compound's metabolism by enhancing its binding to albumin. This would increase the proportion of protein-bound drug, making it less susceptible to metabolism and elimination.

Albumin was chosen as the target for several reasons, chief among them being its abundance—it constitutes roughly 60% of plasma proteins 7. Given that deferiprone is less than 10% protein-bound, the objective is to increase the candidate drug's protein binding, thereby reducing metabolism and elimination while extending its half-life and duration of action. This would ultimately allow for a lower administered dose, minimizing toxicity and side effects. 8, 9

Ultimately, stronger interaction with albumin (as an intact protein) is expected to reduce metabolism, prolong the drug's activity, and decrease the total required dose of the iron chelator.

The strategic incorporation of fluorine atoms into the candidate compound is anticipated to enhance binding affinity to albumin's target pockets. This effect is mediated through fluorine's high electronegativity, which facilitates stronger hydrogen bonding interactions and favourable dipole-dipole effects with the protein 8, 9.

In this study, a range of readily synthesised fluorinated hydroxypyridin-4-ones (HPOs) have been subjected to in silico tools to assist in predicting the effect of fluorination of HPOs, specifically, on the toxicity of the compounds. 10

Very conveniently, all the structures have been readily screened for their potency (efficacy in iron chelation), in addition to being characterised in relation to some of their physicochemical properties that are believed to have an effect on their chelation activity, such as their average pka, distribution coefficient and iron affinities. This advantage has made it possible to compare the obtained in-silico values to their corresponding in vitro determined ones.

The software used for assessing the pharmacokinetic profile of the flourinated HPOs offers the advantage of predicting not only the physicochemical properties of the compounds but also the toxicity profile, in which the latter is resembled by parameters such as protein binding. The ratio of protein binding is in turn related to and has an effect on, the distribution of the drug which consequently, determines its selectivity and potential side effects.

Drawing together the conclusions of the work of Yongmin Ma et al ; collectively with basic understanding of the factors that play a role in the selectivity (and hence the emergence of side effects), the plan for this in silico study was established. The fluorinated HPOs showed a reduction in phase 1 metabolism of the compounds when compared to Deferiprone. This is a one highly important contributing factor to the toxicity profile of the mainstream drug, Deferiprone; because the prominent influence of this is to reduce the total dose administered and therefore the potential of side effects. 10

Methodology

Aim and general approach

The aim is to initially produce a set of data using the ADMET lab2.0 software for the main drug Deferiprone and all the other fluorinated derivatives of this drug. Based on the results obtained from the application of the ADMET software, three fluorinated Hydroxypyridin-4-one forms will be nominated for molecular docking analysis and compared to the original drug Deferiprone. Naturally, these forms must have the optimum features in regard to the ADMET analysis.

There are various ADME analysis software programmes available, such as Swiss ADME, additional to many molecular docking programs, the like of GOLD. 15, 16, 24, 25

While tools like SwissADME are valuable for comparative analysis of ADMET properties, ADMET Lab 2.0 offers distinct advantages for the purpose of this study through its comprehensive toxicity profiling capabilities, which include specialized endpoints (e.g., hERG inhibition, CYP450 interactions) not covered by SwissADME. This enhanced toxicity assessment provides critical insights for this research objectives.

Choices of software for analysing ADMET and for molecular docking were based on the training that was made available at the institution where this study was carried out. Nevertheless, the parameters discussed in all the applications are similar and comparisons can be made.

At this step, a particular attention will be paid for the analysis of the ADMET lab 2.0 ‘predicted’ toxicity. The latter will be analysed separately and once a ‘favourable’ fluorinated HPO structure/structures is/are identified, molecular docking against Albumin protein will be undertaken, (see below).

As mentioned earlier, the nominated compounds will undergo molecular docking with human serum albumin (PDB ID 1ao6)

using DockThor. Human serum albumin is used as it is a ubiquitous serum protein and has a major role in drug distribution and delivery. PDB ID 1ao6 was selected out of the PDB enteries for human serum albumin due to the completeness of data and hence its reliability, clear resolution of its structure, and also the fact that its crystallised form is in the apo/unbound form (not bound to any ligands). For this purpose, the compounds will have to be converted from their 2D structure to an optimized 3D structure using Biomodel. Finally, the data will be analyzed using Chimera and LigPlot; and the effects of the different fluorinated derivates will be compared with the original Deferiprone.

Table 1 below shows all the studied structures with the fluorinated forms. Figure 2 shows the general structure of the Hydroxypyridin-4-one backbone.

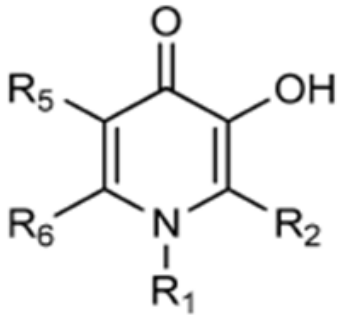


Figure 2: showing the Hydroxypyridin-4-one's backbone with the positions R_1, R_2, R_5 and R_6 that are modified with fluorinated or another moiety.

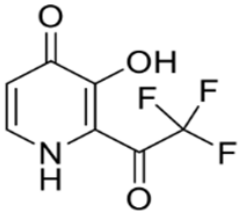
Table 1 [Adapted from work of Yoming Ma, reference 10], showing all compounds that are fluorinated and subjected for ADMET analysis.

Compound	R_1	R_2	R_5	R_6
DFP	Me	Me	H	H
CP94	Et	Et	H	H
1a	H	$CH(OH)CF_3$	H	H
1b, Fluorinated form 3, (D)	H	$COCF_3$	H	H
Fluorinated form 1 (B)	Me	$COCF_3$	H	H
Fluorinated form 2 (C)	Et	$COCF_3$	H	H
1c	Me	F	H	Me
1d	H	F	H	H
1e	Me	F	H	H
1f	Et	F	H	H
1g	n_Pr	F	H	H
1h	i_Pr	F	H	H
1i	n_Bu	F	H	H
1j	H	F	H	Me
1k	H	F	Me	H
1l	H	F	Me	Me
1m	Me	F	Me	H
1n	H	Me	F	H
1o	Me	Me	F	H
1p	H	H	F	Me
1q	H	H	F	H

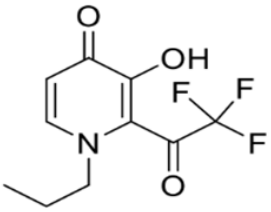
1r	Me	H	F	H
1s	Et	H	F	H
1t	n_Pr	H	F	H
1u	i_Pr	H	F	H
1v	H	F	F	F
1w	H	F	H	F
1x	H	CF_3	H	H
1y	Me	CF_3	H	H
1z	H	COOMe	Me	H

Note that the fluorinated form 1 and 2, in the table above are direct derivatives of compound 1b or fluorinated form 3 (D) such that R_1 is a methyl in fluorinated form 1 (B) and R_1 is a propyl group in fluorinated form 2 (C).

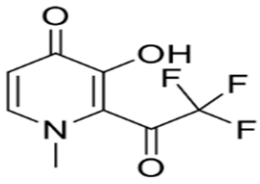
The structures for the derivatives of fluorinated form 3 (D) or **1b**, namely fluorinated form 1 or (B) and fluorinated form 2 or (C) are shown below along with compound 1b or fluorinated form 3 (D).



Fluorinated form 1 (B)



Fluorinated form 2 (C)



Fluorinated form 3 (D) or 1b

ADMET methods and calculations

The ADMET calculations were performed using the ADMET lab2.0. The online web address is as follows: https://admetmesh.scbdd.com/ .15,16

The 2D drug structures were converted to 3D structures through optimization of bond length and bond angles using the online "biomodel" link as follows: https://biomodel.uah.es/en/DIY/JSME/draw.en.htm. 17

The 2D to 3D conversion was done using the steps shown in Figure 3.

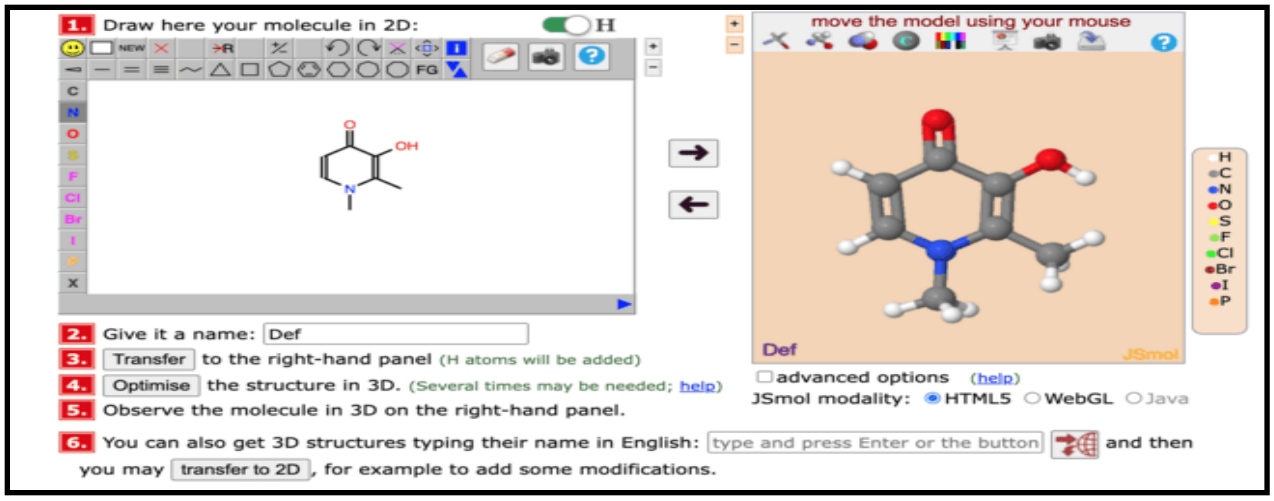


Figure 3: Steps for the conversion of 2D structures to 3D structures.

Molecular Docking

The molecular docking program 'DockThor' was used to assess the binding of Deferiprone and its fluorinated derivatives to human serum albumin (PDB ID 1ao6). The emphasis on the DockThor output and the ranking of the compounds was placed on affinity prediction values (Kcal/mol) of the four compounds in virtual screening experiments considering the top-energy pose (according to Total Energy) of each compound. 18, 19, 20, 21

All compound PDB files were initially converted from mol. files to pdb files using UCSF Chimera (version 1.17.3) before being imported into DockThor for docking studies. While Chimera excels at visualizing molecular poses with exceptional clarity, it doesn't provide the detailed residue interaction analysis that LigPlot+ specializes in displaying. 22, 23

Blind docking in DockThor included grid centre values of 25.32, 6.69 and 22.07 for the x, y and z axes, respectively, and grid size of 40 for all axes (Figure 4). x,y,z axis selection is based on DockThor default references.

These were the default parameters for the blind docking jobs. All other parameters were also used at their default values. There is no ligand preparation step as the DockThor program readily performs it. In addition, DockThor automatically does the preparation step for the target protein, Albumin. 21

HSA 1AO6 (Human serum Albumin released in 1997) was chosen for docking because the more recent structures of human serum albumin are not in the apo/ uncompleted form and are instead in complex with a compound. This affects the native structure of human serum albumin, which is not wanted for this study. As this was 'blind docking', there was no need to locate binding sites or any region of the HAS 1AO6 structure and the program is allowed to freely select the binding sites.

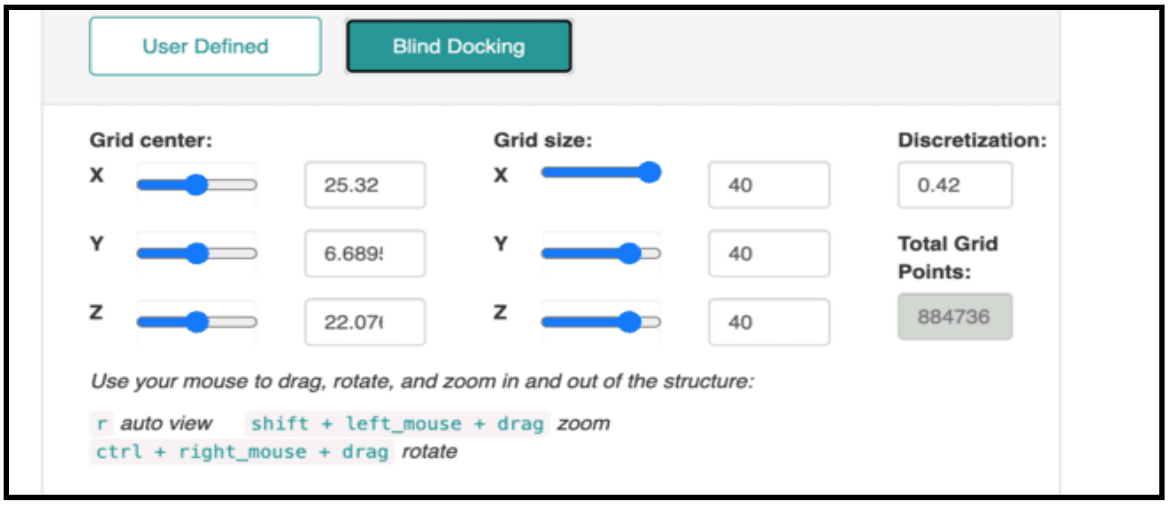


Figure 4: Grid center and grid size information for Blind docking using DockThor.

The ligand binding mode (whether bound via hydrogen bonding or hydrophobic interactions) in each structure was then analysed by Ligplot; and the common residues of human serum albumin involved in binding to the compounds were assessed. 23

Figure5: Ligplot^{+} program layout. 22

Validation of docking results through Redocking

Validation of the docking protocol was performed using DockThor. Initially the ligand was omitted from the file containing the complex information and used as the 'protein file'. The best ranking ligand poses with RMSD of 0.0 obtained in the initial docking was then used as the 'ligand 'and re-docking performed. The final result containing the best ranking ligand pose was compared with the reference conformation (the pose of the ligand upon initial docking), leading to the calculated RMSD values.

Results and Discussion

ADMET analysis

The fluorinated compounds including, Deferiprone and the three fluorinated derivatives were all evaluated using the ADMETlab2.0 online program. 15, 16

ADMET Lab 2.0 serves as an optimal computational tool for this investigation, particularly due to its robust toxicity

prediction capabilities for candidate compounds. The software comprehensively evaluates pharmacokinetic parameters (ADME properties) of potential small-molecule oral therapeutics. Specifically, it assesses drug-likeness through established pharmaceutical rules including (and not limited to), Lipinski's Rule of Five, GSK's 4/400 guidelines, and the Golden Triangle rule.

The application of these predictive rules is particularly relevant for our iron chelator study, as these guidelines were developed through systematic analysis of thousands of orally active compounds. While these rules primarily evaluate oral bioavailability parameters, they indirectly provide toxicity insights since increased bioavailability often correlates with potential toxicity endpoints. This secondary toxicity prediction emerges because compounds with optimal absorption and distribution profiles may consequently exhibit greater biological activity - including potential toxic effects.

A summary of some of the calculated parameters for each ligand are given in Table 3. Table 4 contains the same information as Table 3 but limited to the three fluorinated derivatives with the addition of red and green colour shades to highlight acceptance or dismissal of the values. The three fluorinated derivatives and Deferiprone itself satisfied all the three rules. The fluorinated form 3 compound satisfied most of the factors relating to the ADMET analysis, followed by the fluorinated form 1 compound, and then the fluorinated form 2 compound. Deferiprone ranked lowest in satisfying the factors relating to the ADMET analysis. The golden triangle (where a molecular weight of between 50 or less and 200 or more, with a logD of less than or equal to 5 indicated a more favourable ADMET profile) revealed that all three fluorinated derivates, excluding Deferiprone itself, satisfied the criteria.

Only after analysing the ADME results and selecting the ones with the most favourable features, particularly the toxicity parameters, the compounds that will be undergoing molecular docking with albumin shall be determined. Looking at the fluorinated HPO structures and comparing their ADMET lab 2 profiles to that of Deferiprone, CP94 and fluorinated form 1 and fluorinated form2, there are many aspects that need to be addressed. It should be noted that fluorinated form 3 is the same as compound 1b and fluorinated form 1 and 2 are in fact two variants of fluorinated form 3 or 1b. The decision to put forward fluorinated form 1, 2 and 3 (1b) for molecular docking was based on the inconclusive results that were obtained from the results of the ADMET lab 2 especially when comparing the toxicity parameters of all the fluorinated structures with those of Deferiprone and CP94. It is worth remembering that CP94 had an excellent potency profile (iron chelation power) that exceeded Deferiprone but was rejected early on because of its toxicity profile. Due to the above “mixed” picture, only the fluorinated forms that are variants of compound 1b were selected for molecular docking.

A more detailed scrutiny of the ADMET results relating specifically to the predicted toxicity of all the compounds including DFP (Deferiprone) and CP94, one will notice that there seem to be no direct determining parameter that can confidently secure a decision of which compound has better A than Deferiprone.

This can be seen more clearly when comparing compound 1b or fluorinated form 3 to Deferiprone and CP94.

Table 3 showing ADMET results for all the structures in Table 2.

Compound	MR g/mol	Log S	LogD	Log P	CACO	MDCK	PPB%	VD	Fu	CL
1a	209	-1.960	-0.00	-0.213	-4.614	$8.2e^{-05}$	18.36	0.620	47.0	3.41
CP94	167	-8.898	0,800	0.405	4.473	$3e^{-05}$	22.765	1.087	70.98	7.407
1b	207.0	-1.705	0.471	0.676	4.60-	$1.2e^{-05}$	64.85	0.665	26.91	5.790
1d	129	-1.432	-0.27	-0.281	-4.450	$1.9e^{-05}$	45.99	0.804	56.97	7.95
1c	235	-1.406	1.124	0.758	-4.805	$1.6e^{-05}$	74.526	1.408	16.811	5.805
1e	143	-0.974	-0.19	-0.427	4.74-	$3.4e^{-05}$	21.55	1.609	74.87	8.07
1f	157	-1.163	0.158	-0.001	-4.417	$3.4e^{-05}$	19.96	1.376	73.3	8.306
1g	171	-1.149	0.709	0.422	-4.422	$3.4e^{-05}$	37.5	1.523	65.3	8.596
1h	171	-1.34	0.538	0.368	-4.45	$1.3e^{-05}$	40.66	1.11	54.6	9.031
1I	185	-1.704	1.06	0.922	4.65-	$3.1e^{-05}$	39.503	1.448	63.6	8.036
1J	143	-1.255	0.154	-0.09	-4.4	1.7^{-05}	57.8	0.864	35.51	7.23
1K	143	-1.309	0.070	0.230	-4.59	$1.3e^{-05}$	60.0	0.666	42.8	6.383
1L	157	-1.445	0.54	0.187	-4.59	$1.4e^{-05}$	72.3	0.822	20.6	5.28
1M	157	-1.278	0.015	0.00	-4.55	$2.7e^{-05}$	39.0	1.333	63.0	7.16
1N	143	-0.71	-0.39	-2.30	-4.63	$1e^{-05}$	61.0	0.788	40.0	6.54
1O	157	-0.45	-0.01	-0.26	-4.47	2.3^{e-05}	29.7	1.508	70.6	6.68
1P	143	-1.45	-0.09	-0.172	-4.429	$1.1e^{-05}$	60.9	0.897	42.6	6.34
1Q	143	-0.64	-0.51	-0.318	-4.485	$1.1e^{-05}$	350	0.806	69.2	
1R	143	-0.43	-0.38	-0.468	-4.33	$2.6e^{-05}$	17.8	1.509	85.0	9.248
1S	157	-0.31	0.004	0.039	-4.348	$3e^{-05}$	16.9	1.414	85.3	10.01
1T	171	-376	0.501	0.465	-4.363	$3.1e^{-05}$	22.1	1.554	80.9	9.40
1U	171	-0.57	0.50	0.47	-4.48	$2.5e^{-05}$	18.1	1.303	83.0	10.0
1V	165	-1.82	0.541	0.46	-4.43	$3.1e^{-05}$	69.3	0.940	13.3	5.57
1W	147	-1.52	0.176	0.042	-4.45	2.3^{e-05}	57.6	0.88	27.7	7.651
1X	179	-1.24	0.607	0.363	-4.635	$1.3e^{-05}$	64.0	0.821	22.6	7.00
1Y	193	-1.27	0.696	0.336	-4.57	$2.3e^{-05}$	37.3	1.88	54.6	6.33
1Z	183	-1.73	0.096	0.746	-4.57	$97e^{-05}$	45.0	0.525	52.5	6.85
DFP	139	-0.21	-0.10	-0.453	-4.78	$2e^{-05}$	19.5	1.575	75.6	8.772

Table 4: ADMET Lab 2.0 analysis of Deferiprone and the three fluorinated derivatives of compound 1b.

Compound	**Mol. Weight g/mol**	**Physicochemical property**			**Absorption (Permeability)**		**Distribution**			**Metabolism CYP1A2 inhibitor**	**Excretion CL** mL/min/kg
		logS **LogP**	**LogD pH7.4**		**Caco** Log unit	**MDCK** cm/s	**PPB** **Fu %**	**VD** L/kg			
Deferiprone	139.06	-0.214	-0.107	-0.453	-4.779	2E-05	19.54	1.575	75.65	0.31	8.272
Fluorinated form 1 (B)	221.03	-1.467	0.865	0.531	-4.84	2.1E-05	58.08	1.845	51.34	0.38	7.311
Fluorinated form 2 (C)	249.06	-1.851	1.401	1.419	-4.583	2.1E-05	78.58	1.617	26.96	0.673	6.814
Fluorinated form 3 (D)	207.01	-1.705	0.471	0.676	-4.6	1.2E-05	64.84	0.665	26.91	0.432	5.79

LogS: Log of the aqueous solubility. Optimal -4to0.5 log mol/L.
LogP: Log of the octanol/water partition coefficient. Optimal 0 to 3.
LogD: LogP at physiological pH 7.4. Optimal: 1 to 3.
Caco-2 permeability: Optimal: higher than -5.15 Log unit.
MDCK permeability: low: <2x10^{-6} cm/s; medium: 2-20x 10^{-6} cm/s; high passive permeability: >20x10^{-6} cm/s.
PPB: Plasma protein binding; optimal: <90%. VD: Volume distribution; optimal: 0.04-20L/kg. Fu: The fraction unbound in plasma; Low: <5%; Middle: 5-20%; High: >20%. CYP1A2 inhibitor: The output value is the probability of being inhibitor. CL: Clearance; High: >15 mL/min/kg; moderate: 5-15 mL/min/kg; low: <5 mL/min/ kg

The predicted toxicity profile that resulted from the ADMET analysis are given separately in Table 5 below.
All comparisons to Deferiprone and compound CP94 can be shown clearly. It can be seen here that the predicted results for the toxicity of the compound had no clear pattern, ***bearing in mind that compound CP94 was rejected due to its toxicity.*** So, in comparison the red indicators in table 5 were showing almost equal distribution amongst Deferiprone DFP, CP94 (the rejected compound) and compounds B, C and D. More importantly compound D or 1b has an established n vitro superiority over Deferiprone. Hence the decision to put forward for molecular docking compounds B, C and D alongside the original compound DFP was taken.

Table 5 showing ADMET Lab 2.0 results with regard to toxicity of all the compounds.

Compound	Carcinogenicity	Oral toxicity in rats	Drug Induced Liver injury	Human Hepatotoxicity	AMES toxicity
1a	++	- - -	+	- - -	- - -
Cp94	++	+++	-	- -	++
1b	- -	+++	++	++	- -
Flourinated form 2 (C)	+	--	++	--	++
Fluorinated form 1 (B)	+	++	++	-	- -
1c	- -	+++	-	- -	-
1d	+		+	++	- - -
1e	++	++	-	-	-
1f	+++	+	+	-	++
1g	++	-	+	-	++
1h	++	-	- -	++	-
1I	++	- -	+	++	++
1J	-	++	+	++	- -
1k	- - -	+ ++	-	++	- - -
1L	-	+ ++	+	++	- - -
1M	++	+ +	- -	++	-
1N	_ _ _	+ + +	- -	+	- - -
1O	+	+ +	- -	-	- -

1R	- - -	+++	- -	+	- -
1S	- - -	+++	-	- -	- -
1T	+ +	++	+	- -	-
1U	+ + +	++	++	- -	++
1V	+ +	- -	++	++	++
1W	_	+++	+	++	- -
1X	- -	+++	+++	- -	- -
1Y	+	+++	+	- -	- -
1Z	- - -	+ +	-	-	- - -
DFP	++	- -	-	+	- -

In the table above the colours red, yellow, and green, indicates the degree of toxicity such that red is toxic, yellow is moderately toxic and green is non-toxic. Also, the number of + and – indicate the extent of toxicity.

It's important to recognize the distinction between Drug-Induced Liver Injury (DILI) and Human Hepatotoxicity, as they may seem similar but are not the same. In fact, DILI is a specific subcategory within the broader assessment of Human Hepatotoxicity.

While Human Hepatotoxicity refers to *any* liver toxicity in humans—including damage from environmental toxins, industrial chemicals, or non-drug substances—*DILI* specifically describes liver injury caused by medications, herbs, or xenobiotics. DILI is further classified into two types.

Looking at the results of the ADMET lab 2.0 program and comparing the values obtained for the synthesised structures against the properties that were obtained from the running of the ADMET lab 2.0 software, there seems to be a wide range of differences between the two results namely the Log $D_{7.5}$ and the Log P parameter.

More importantly, the predicted toxicity of the compounds had no straightforward pattern that may predict or determine whether the compound will produce an acceptable toxicity profile, or it will be rejected. So based on these results solely, one cannot fully predict the structure of the fluorinated compound that will have a better toxicity profile than that of Deferiprone.

For this reason, it was more meaningful to run the molecular docking software only the promoted fluorinated structures, Fluorinated form 1, 2 and 3 as well as the main drug, Deferiprone for comparison. The decision was made simply based on the in vitro metabolism results for those fluorinated forms; which were carried out in Yongmin Ma et al study (above) 6.

Molecular docking results

The top-energy pose (according to Total Energy) of each compound is presented (with an RMSD of 0.0). The DockThor molecular docking program was used for analysis of interactions between Deferiprone (A), fluorinated form 1 (B), fluorinated form 2 (C) and fluorinated form 3 (D), with human serum albumin, using default settings.

Table 6. Molecular docking results of Deferiprone and its three fluorinated derivatives with human serum albumin, ased on affinity of binding.

Rank	File ID	Compound	Affinity	Total Energy	vdW Energy	Elec. Energy
1	da8186b804	ligand 1	-6.398	0.768	-2.649	-25.915

Rank	File ID	Compound	Affinity	Total Energy	vdW Energy	Elec. Energy
1	136e993bc	ligand 1	-6.582	17.410	-6.678	-20.861

Rank	File ID	Compound	Affinity	Total Energy	vdW Energy	Elec. Energy
1	8ba0de1c99	ligand 1	-6.401	18.157	-9.834	-15.458

Rank	File ID	Compound	Affinity	Total Energy	vdW Energy	Elec. Energy
1	405aca400f	ligand 1	-5.819	10.789	6.122	-31.451

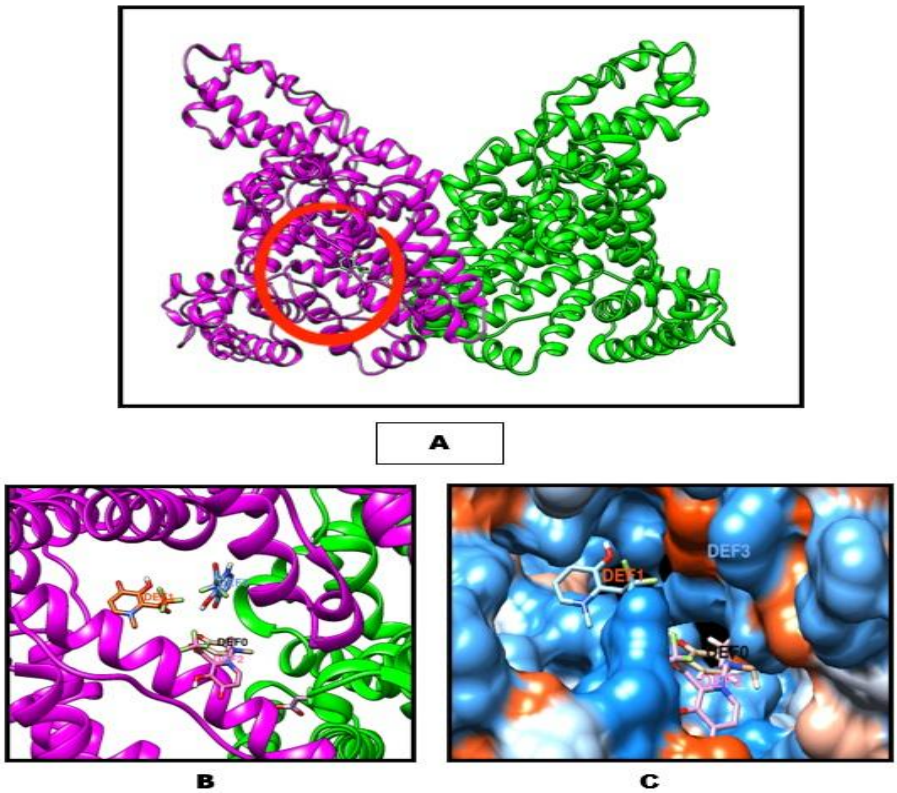


Figure 6: Ribbon and Hydrophobic surface representation of Deferiprone and fluorinated derivatives bound to the human serum albumin. (A) Ribbon representation of human serum albumin chain A (in green) and chain B (in magenta). The red circle shows the common site where the ligands have bound. (B) Ribbon representation of human serum albumin with bound ligands. The four complexes are superposed. (C) Hydrophobic surface representation of the four superposed complexes (human serum albumin in complex with the compounds). The figure was generated using Chimera.

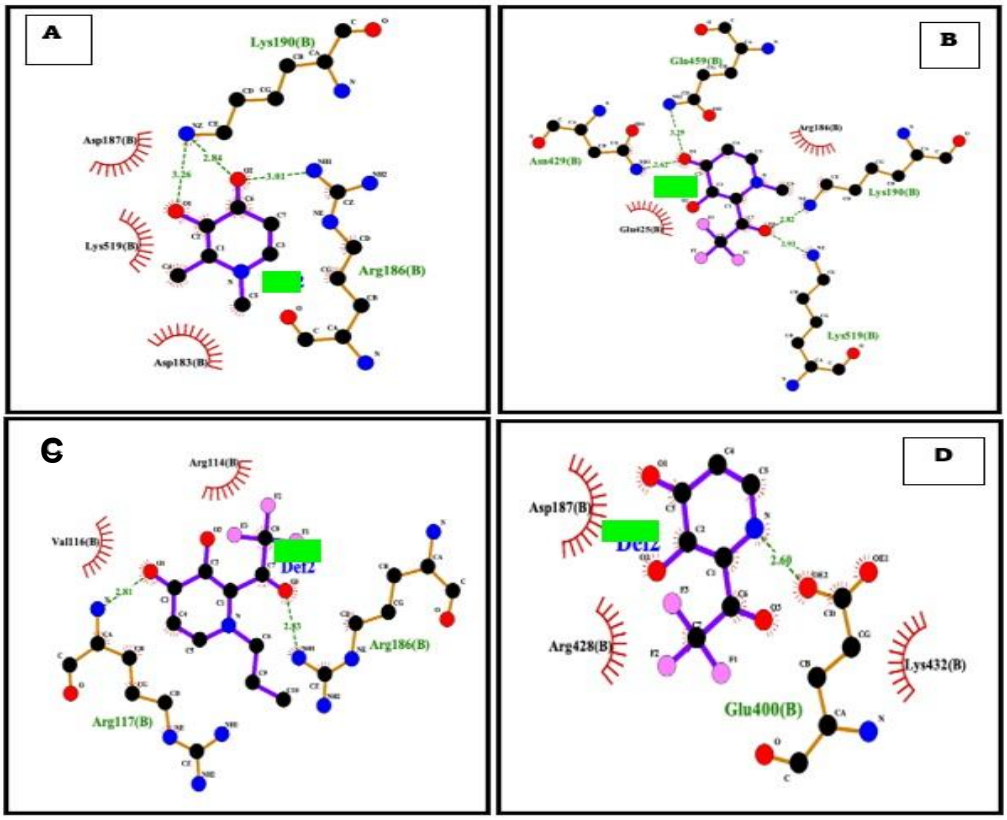


Figure 7: Ligplot analysis of Deferiprone and fluorinated derivatives bound to the human serum albumin. Ligplots for Deferiprone (A), flourinated form 1 (B), fluorinated form 2 (C) and fluorinated form 3 (D), bound to human serum albumin are shown in this figure.

Table 7. Summary of Ligplot analysis of Deferiprone and its three fluorinated derivatives with human serum albumin showing hydrogen bonding and hydrophobic interactions. 23

Compounds	**Residues involved in hydrogen bonding (Å) Including which chain**	**Residues involved in hydrophobic interactions Including which chain**	**Total number of amino acids involved in hydrogen bonding and hydrophobic interactions**
Deferiprone	1-Lys190-NZ: O2-Def (2.84) Chain B 2. Lys190-NZ: O1-Def (3.26) Chain B	Asp183 Asp187 Lys519 Chain B	6

D

Compounds	**Residues involved in hydrogen bonding (Å) Including which chain**	**Residues involved in hydrophobic interactions Including which chain**	**Total number of amino acids involved in hydrogen bonding and hydrophobic interactions**
	3. Arg186-NH1: O2-Def (3.01) Chain B		
Flourinated form 1	1- Gln459-NE2 O1-Def F1 (3.29) Chain B 2- Asn429-ND2 O1-Def F1 (2.62) Chain B 3- Lys190-NZ O3-Def F1 (2.82) Chain B 4- Lys519-NZ O3-Def F1 (2.93) Chain B	Glu425 Arg186 Chain B	6
Flourinated form 2	Arg186-NH1 O3-Def F2 (2.83) Chain B	Arg114 Val116 Arg117 Chain B	4
Flourinated form 3	Glu400-OE2 N-Def F3 (2.6) Chain B	Asp187 Arg428 Lys432 Chain B	4

The table above shows all the interactions of the fluorinated HPOs that were derived from compound 1b or fluorinated form 3 (in the table above). As the Hydrogen bonding interaction is considered the strongest intermolecular forces of attraction; it is therefore considered the best compound and so consequently, it will correspond to the lowest binding energy results in Table 3 above (i.e. -6.582). It should be noted here that the more negative is the number, the more stable is the interaction and the better is binding between the compound and the HSA protein. For

Re-docking as a means of validating molecular docking results

As a means of gaining more confidence in the results from molecular docking, re-docking of the best pose for the fluorinated deferiprone form 1, which gave the lowest energy binding and hence the best affinity for binding, was performed in DockThor and results revealed a valid docking result with a RMSD value of 0.943 Å (Fig 8). RMSD

values less than 2.0 Å represent good re-docking results and validate the ligand to protein binding structure that was initially suggested via molecular docking.

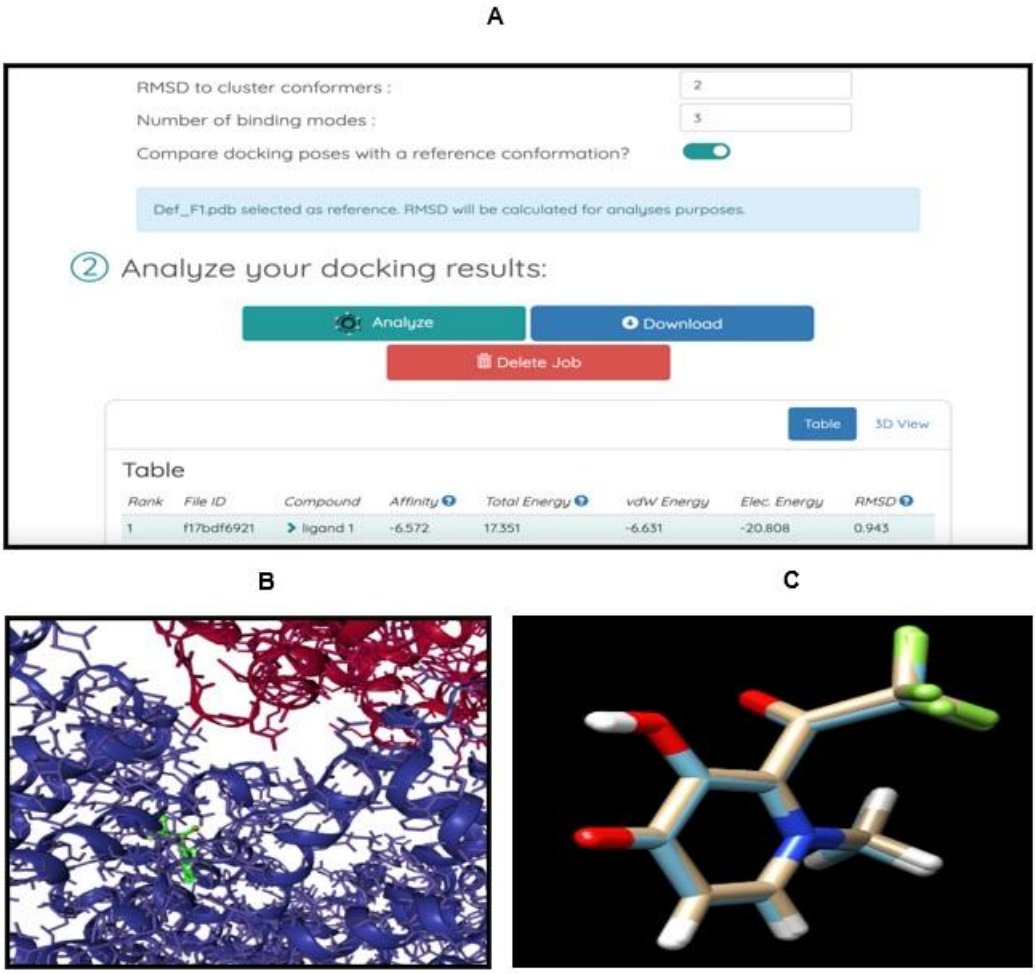


Figure 8: Re-docking of the molecular docking result for the binding of fluorinated deferiprone form 1 to albumin. (A) DockThor was used to compare the re-docked result of fluorinated deferiprone form 1 bound to albumin with that of the initial molecular docking pose of the compound. (B) Re-docked fluorinated deferiprone form 1 bound to chain B of albumin. (C) Superposition of the initial best pose of the ligand and the re-docked pose using Chimera, showing very close match, with RMSD value of 0.943 Å, representing a valid structural interaction as proposed by molecular docking.

Conclusion

This *in silico* study demonstrates that fluorination of hydroxypyridinones (HPOs) exhibits promising indicators of an enhanced toxicity profile compared to the current standard, deferiprone. While the ADMET Lab 2.0 predictions were not unequivocally conclusive, the molecular docking results revealed a pronounced affinity for serum albumin—supporting our secondary strategy of prolonging circulatory residence time. By mitigating rapid metabolism, fluorinated HPOs (particularly **Fluorinated form 1, Structure B**) may reduce dosing frequency and cumulative exposure, thereby

diminishing the likelihood of dose-dependent toxicity. Collectively, these computational insights suggest that fluorinated derivatives hold potential as safer alternatives to deferiprone, warranting further experimental validation.

Future Work

To further validate and expand upon the findings of this study, the following investigations are proposed:

1. Comparative ADMET Analysis Conduct a systematic comparison between ADMET Lab 2.0 predictions and those generated by alternative *in silico* tools (e.g., SwissADME) to assess consistency and identify potential software-specific biases.
2. Molecular Dynamics Simulations Investigate the interactions of fluorinated compounds using specialized programs such as GOLD, which offers the unique advantage of incorporating metal ions in its simulations—a highly relevant feature for studying metalloenzyme interactions. 24, 25
3. Experimental Validation Perform *in vitro* pharmacokinetic assays (e.g., metabolic stability, permeability) to empirically verify computational predictions and evaluate the translational reliability of the *in-silico* models.

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