POST-SYNTHETIC DATA-VISUALIZATION AND THEORETICAL ANALYSIS USING WEB SERVERS *PROTOX-II, SWISSADME* AND *PKCSM* PHARMACOKINETIC COMPUTATIONAL METHODS ON COVALENT ORGANIC FRAMEWORK OF DIFFERENTLY SUBSTITUTED PHENYL ACETATES.

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ABSTRACT:
Exploratory research output for the purpose of post-synthetic data-visualization grouping technique and theoretical analysis scheduled to design a degradable pharmaceutical ingredient on covalent organic framework like differently substituted phenyl acetates **(DSPA: 2.1-2.17)** is validated in all possible way using web servers ***ProTox-II, SwissADME*** **and** ***pkCSM*****.** The substituent behaviors towards the physicochemical parameters of the **DSPA: 2.1-2.17** have been investigated by dividing them into three different groups; (i) Alkoxy group is varied by keeping the phenyl acetate unit as constant, (ii) Substitution on phenyl ring is varied by keeping the Alkoxy group constant and (iii) Number of hydrogen bond acceptor is increased in the phenyl ring by increasing methoxy groups. *In-silico* investigational toxicity studies like oral acute toxicity, organ toxicity, immunotoxicity, genetic toxicity endpoints, nuclear receptor signalling, and stress response pathways of **DSPA: 2.1-2.17** using ***ProTox-II*** webserver have been widely explored to assess the safety profile of drug candidates and the computational studies categorized as safer chemical compound to mammal. The title compounds **DSPA: 2.1-2.17** are subjected to predict pharmacokinetic properties using graph-based signatures called ***pkCSM*** and the results of every category of the compounds marvelously complies the Lipinski's rule of five without even a single violation and exhibited good drug-likeness scores. However, a dissimilar linearity observed in the BOILED-Egg graph plotted by ***SwissADME*** web server based on the categories (i)-(iii) of the compounds listed. The topological polar surface area values attained by ***SwissADME*** are almost similar for the (i) and (ii) cases, the substituent behaviors (alkoxy groups or substitution on phenyl ring) towards the physicochemical parameters of those compounds are not much deviated. Hence, this is directed to synthesis compounds **2.1-2.12** (having a highly valuable consensus Log Po/w values ≤ 3) by introducing varies alkoxy group of the ester and also by increasing the number of hydrogen bond acceptor in phenyl ring by increasing methoxy groups for further studies.

KEYWORDS: Physicochemical Parameters, ***ProTox-II, SwissADME***, BOILED-Egg graph and ***pk CSM*****.**

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INTRODUCTION:

Post-synthetic approaches to the assessment of toxicity in the pharmaceutical[1-3], pesticide[4-5], cosmetic[6] or plastic industry[7] for a target molecule using *in-silico* toxicology are economical as well as time saving than the animal models or any other prototypes. In-fact, the aim of *in-silico* modeling to complement *in-vitro* and *in-vivo* toxicity tests to hypothetically diminish the necessity for animal testing, reduce the cost and time of toxicity tests, and also improve toxicity prediction and safety assessment.[8]. ***ProTox-II*** one among the software available in the literature used for the development of physicochemical parameters and toxicology optimization by *in-silico* modeling and then the lead compounds are taken to *in-vitro* and *in-vivo* assay predictions, being focus in the recent years.[9-11] ***pkCSM*** [12-13] is also one of software used for *in-silico* ADMET forecasts to increase our ability in order to predict and represent the most important pharmacokinetic, metabolic, and toxicological endpoints, since computational experiments accomplished by computer simulations (*in-silico*) are related to know the biological degradability interactions namely *in-vivo* and *in-vitro* actions.

SwissADME a popular and open access free *in-silico* model is widely used to predict various factors like physicochemical factors, lipophilicity, water-solubility, pharmacokinetics, drug-likeness, and other factors related to medicinal chemistry of the small molecules during the process of drug discovery and development.[14] Moreover, optimized pharmacokinetic and toxicity properties of new drug candidates like small organic molecules,[15-16] ferrocene-bisphosphonates hybrid drug molecules,[17] herbal formulation[18] and mononuclear metal complexes[19] predicted by ***SwissADME*** are well documented recent sources for medicinal and pharmaceutical chemist. BOILED-Egg, an in-built graphical output method of ***SwissADME*** to predict the two key ADME parameters (HIA and BBB) simultaneously is actually a plot of WLOGP (lipophilicity) versus TPSA (topological polar surface area).[20] BOILED-Egg graph by its shape is divided into 3 parts including a yellow yolk region (i.e. the physicochemical space for highly probable BBB permeation), an albumen region (i.e. the physicochemical space for highly probable HIA absorption) and the outside grey region stands for molecules with properties implying predicted low absorption and limited brain penetration.

Hence, with the aim of selectively synthesizing the novel chemical entities of **DSPA: 2.1-2.17** based on the computer simulation (*in-silico*) results, we have scrutinized the title compounds using three different software viz. ***ProTox-II, SwissADME* and *pkCSM*.** In-silico investigational toxicity studies of **DSPA: 2.1-2.17** using ***ProTox-II*** webserver have been widely explored to assess the safety profile of drug candidates and the computational studies categorized as safer chemical compound. The title compounds are also subjected to predict pharmacokinetic properties using ***pkCSM*** and the results are found to be marvelously complies the Lipinski's rule of five without even a single violation and exhibited good drug-likeness scores. A dissimilar linearity observed in the BOILED-Egg graph plotted by ***SwissADME*** web server based on the substituent behaviors were used for the categorical discussion. The computational investigations are helpful in finding roots based on important pharmacokinetic and toxicology outputs for the aim proposed.

EXPERIMENTAL/COMPUTATIONAL DETAILS:

2.1. ADME/T Predictions

***SwissADME*:**

Required two-dimensional (2D) structure/SMILES (Simplified Molecular Input Line-Entry System) of the differently substituted phenyl acetates (DSPA: 2.1-2.17) were obtained from the PubChem database or drawn using ***SwissADME*** (http://www.swissadme.ch) web server.

The input zone comprises molecular sketcher based on ChemAxon's Marvin JS (http://www.chemaxon.com) is used to import, draw and edit a 2D chemical structure, and also transferred it to a list of molecules on the right-hand side of the submission page, which exploited as input for computation. The list was made to contain one input molecule per line, defined by a SMILES and on computation the results were appeared in 1 to 5 seconds for a drug-like molecule. The computed parameter values then appeared as groups in the different sections of the one-panel-par-molecule output were extracted in a table form and used for further analysis. The bioavailability radar appeared with 2D structure has been extracted and enables a first glance at the drug-likeness of a molecule. Similarly, statistical performance of SVM classification for substrate and statistical performance of synthetic accessibility (SA) scores on two external test sets data were also collected. Finally, the BOILED-Egg

graph generated was allowed an instinctual evaluation of passive gastrointestinal absorption (HIA) and brain penetration (BBB) in function of the position of the molecules in the WlogP versus TPSA referential.

***pkCSM* platform for ADMET properties prediction:**

SMILES of the DSPA: 2.1-2.17 were used as extracted from molecular sketcher based on ChemAxon's Marvin JS (http://www. chemaxon.com) on ***SwissADME*** web server for the computation of ADME properties using small-molecule pharmacokinetics ***pkCSM*** (https://biosig.lab. uq.edu.au/pkcsm/prediction)[21], to rapid evaluation of pharmacokinetic and toxicity properties. The given input molecule in the ***pkCSM*** workflow were provided two main sources of information and the resulted compound general properties including molecular properties, toxicophores and pharmacophore being analyzed.

***ProTox-II* PLATFORM:**

Tox-Prediction user interface of the ProTox-II (https://tox-new.charite.de/protox_II/index.php?site=compound_input), a self-explanatory was used to predict potential toxicities associated with a chemical structure. Name of the compound, SMILES or possible chemical structure drawn with the help of the chemical editor (https://www.chemdoodle.com/) being used as inputs. Furthermore, the integrated PubChem search (https:/pubchem.ncbi.nlm.nih.gov/) were also used to search a chemical structures using the compound name for inputs.

The predicted acute toxicity, toxicity targets, median lethal dose (LD_{50}) in mg/kg weight, toxicity class and prediction accuracy were generated instantly against the input, as well as average similarity along with three most similar toxic compounds from the dataset with the known rodent oral toxicity value. The predicted toxicity targets information, if available will be shown with the name of the target as well as the average fit and similarity of the input compound with the pharmacophore and known ligands of the respective targets. Furthermore, from an additional model, the prediction outcomes with confidence score for each model in a table resulted page appeared. The prediction results are also displayed a toxicity radar plot comparing the average confidence score of the active compounds in the training set of each model, to that of the input compound. This plot being assessed using the 'Open Toxicity Radar Chart' link and appeared on the result page, once the computation was completed.

OSIRIS structure editor was used for generating ADME properties in DataWarrior computational software.

RESULT AND DISCUSSION:

***In-silico* Experimental Toxicity Predictor *ProTox-II*:**

In-silico experimental toxicity studies to detect oral acute toxicity, organ toxicity, immunotoxicity, genetic toxicity endpoints, nuclear receptor signalling, and stress response pathways of common synthetic drug candidates using ProTox-II webserver are one of impressive contemporary objective of in the observances within scientific communities to integrate molecular resemblances. ProTox-II webserver helps faster screening of large numbers of compounds within short duration by running down the animal testing is used for toxicological assessment in organism, organs, cell and gene level along with molecular mechanisms of toxicity. In sense, the webserver **useful in pharmaceutical industry to assess the safety profile of a drug candidate**[22-24] and also **in agricultural pesticide industry to classify a chemical compound being pesticides encompasses to eradicate pests**[25-26] from crops;

That is, helps to categorize a safer chemical compound to mammal and toxic to non-mammals. *In silico* toxicity computations through ProTox-II webserver was very first established by Drwal et al.[27] in the year of 2014 and the parameters such as rat oral acute toxicity with special reference to median lethal dose (LD_{50}) as mg/Kg, organ toxicity especially hepatotoxicity, immunotoxicity, genetic toxicity endpoints especially cytotoxicity, mutagenicity and carcinogenicity, nuclear receptor signalling (AhR, AR, AR-LBD, ER, ER-LBD and PPARGamma), and stress response pathways (nrf2/ARE, HSE, MMP, p53 and ATAD5) were predicted for the conventional **DSPA: 2.1-2.17** as per a practice followed by Banerjee et al.[2] and tabulated in **Tables 1-5**.

Table 1: Classification of Different Types of Toxicity.

Classification	Target	Shorthand	Type
Organ toxicity	Hepatotoxicity	dili	**A**
Toxicity end points	Carcinogenicity	carcino	**B**
	Immunotoxicity	immuno	**C**
	Mutagenicity	mutagen	**D**
	Cytotoxicity	cyto	**E**
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	**F**
	Androgen Receptor (AR)	nr_ar	**G**
	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	**H**
	Aromatase	nr_aromatase	**I**
	Estrogen Receptor Alpha (ER)	nr_er	**J**
	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	**K**
	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	**L**
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	**M**
	Heat shock factor response element (HSE)	sr_hse	**N**
	Mitochondrial Membrane Potential (MMP)	sr_mmp	**O**
	Phosphoprotein (Tumor Supressor) p53	sr_p53	**P**
	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	**Q**

Table 2: Prediction and Probability Values of Different Types of Target Toxicity for compounds 2.1.-2.12.

Type	Prediction												Probability											
	2.1.	**2.2.**	**2.3.**	**2.4.**	**2.5.**	**2.6.**	**2.7.**	**2.8.**	**2.9.**	**2.10.**	**2.11.**	**2.12.**	**2.1.**	**2.2.**	**2.3.**	**2.4.**	**2.5.**	**2.6.**	**2.7.**	**2.8.**	**2.9.**	**2.10.**	**2.11.**	**2.12.**
A	Inactive	**Active**	Inactive		**Active**	Inactive							0.72	**0.51**	0.54	0.67	**0.52**	0.72	0.50	0.55	0.52	0.70	0.74	0.74
B	**Active**	Inactive											**0.51**	0.63	0.62	0.67	0.67	0.64	0.66	0.65	0.57	0.65	0.62	0.67
C	Inactive								**Active**	Inactive		**Active**	0.99	0.97	0.98	0.95	0.93	0.94	0.84	0.91	0.91	**0.80**	0.94	**0.60**
D	Inactive												0.98	0.83	0.83	0.91	0.76	0.82	0.83	0.78	0.83	0.81	0.84	0.75
E													0.79	0.68	**1.00**	0.75	0.85	0.86	0.76	0.80	0.80	0.80	0.81	0.82
F													**1.00**	0.95	0.75	0.96	0.96	0.93	0.97	0.86	0.97	0.93	0.95	0.93
G													0.99	0.99	0.98	0.99	0.99	0.98	0.98	0.98	0.98	0.99	0.99	0.99

H		1.00	0.98	0.98	0.99	0.99	0.99	0.98	0.97	0.99	0.99	0.99	0.99
I		1.00	0.99	0.99	0.99	0.99	0.99	0.99	0.98	0.96	0.99	0.98	0.99
J		0.89	0.86	0.75	**0.83**	0.91	0.93	0.92	0.90	0.87	0.94	0.83	0.93
K		0.99	0.93	0.86	0.92	0.98	0.97	0.96	0.92	0.91	0.95	0.92	0.94
L		0.99	0.99	0.99	0.99	0.98	0.99	0.99	0.98	0.99	0.98	0.98	0.98
M		0.97	0.97	0.97	0.97	0.97	0.86	0.96	0.94	0.96	0.90	0.94	0.91
N		0.97	0.97	0.97	0.97	0.97	0.86	0.96	0.94	0.96	0.90	0.94	0.91
O		0.99	0.91	0.85	0.87	0.91	0.91	0.91	0.82	0.78	0.86	0.86	0.85
P		0.99	0.98	0.98	0.99	0.97	0.96	0.97	0.95	0.93	0.94	0.93	0.87
Q		0.99	0.99	0.97	0.91	0.99	0.99	0.99	0.98	0.97	0.96	0.94	0.87

Table 3: Prediction and Probability Values of Different Types of Target Toxicity of Ref-1, Ref-2 and Compounds 2.13.-2.17.

Type	Prediction									Probability								
	2.1.	**2.4.**	**2.13.**	**2.14.**	**2.15.**	**2.16.**	**2.17.**	**Ref-1**	**Ref-2**	**2.1.**	**2.4.**	**2.13.**	**2.14.**	**2.15.**	**2.16.**	**2.17.**	**Ref-1**	**Ref-2**
A	Inactive								**Active**	0.72	0.67	0.72	0.76	0.76	0.76	0.73	0.51	0.74
B	**Active**	Inactive	**Active**	Inactive						0.51	0.67	0.51	0.53	0.52	0.50	0.61	0.86	0.51
C	Inactive									0.99	0.95	0.99	0.96	0.98	0.97	0.97	0.99	0.99
D	Inactive						**Active**	Inactive		0.98	0.91	0.98	0.91	0.91	0.85	0.91	0.97	0.90
E	Inactive									0.79	0.75	0.79	0.73	0.76	0.65	0.77	0.94	0.82
F										1.00	0.96	1.00	0.98	0.98	0.97	0.78	0.99	0.90
G										0.99	0.99	0.99	0.92	0.95	0.85	0.99	0.99	1.00
H										1.00	0.99	1.00	1.00	1.00	0.99	0.99	1.00	1.00
I										1.00	0.99	1.00	0.99	0.99	0.99	0.91	1.00	1.00
J										0.89	0.83	0.89	0.92	0.94	0.92	0.77	0.98	0.94
K										0.99	0.92	0.99	0.99	0.99	0.99	0.97	0.99	0.99
L										0.99	0.99	0.99	0.97	0.97	0.94	0.97	0.99	1.00
M										0.97	0.97	0.97	0.98	0.97	0.98	0.87	0.99	0.98
N										0.97	0.97	0.97	0.98	0.97	0.98	0.87	0.99	0.98

O		0.99	0.87	0.99	0.87	0.92	0.87	0.74	0.97	0.96
P		0.99	0.99	0.99	0.99	0.99	0.99	0.96	0.98	0.95
Q		0.99	0.91	0.99	0.99	0.99	0.97	0.84	0.99	0.97

Table 4: Oral toxicity prediction results for compounds 2.1.-2.12.

Entry	**2.1.**	**2.2.**	**2.3.**	**2.4.**	**2.5.**	**2.6.**	**2.7.**	**2.8.**	**2.9.**	**2.10.**	**2.11.**	**2.12.**
Molweight	206.28	208.25	222.28	236.31	210.23	224.25	238.28	252.31	252.31	266.33	266.33	296.36
Number of hydrogen bond acceptors	20	19	21`	23	18	20	22	24	24	26	26	29
Number of hydrogen bond donors	0	0	0	0	0	0	0	0	0	0	0	0
Number of atoms	33	31	34	37	29	32	35	38	38	41	41	45
Number of bonds	33	31	34	37	29	32	35	38	38	41	41	45
Number of rotable bonds	6	5	6	7	5	6	6	7	6	8	8	9
Molecular refractivity	61.54	58.41	63.22	68.03	55.29	60.1	64.91	69.71	69.75	74.52	74.52	81.01
Topological Polar Surface Area	26.3	35.53	35.53	35.53	44.76	44.76	44.76	44.76	44.76	44.76	44.76	53.99
Octanol/water partition coefficient (logP)	2.82	2.19	2.58	2.83	1.42	1.81	2.2	2.59	2.59	2.84	2.84	2.84
LD50 (mg/kg)	5000	1550	1630	2000	1400	1400	1400	1630	1400	1630	1123	1630
Toxicity Class	5	4	4	4	4	4	4	4	4	4	4	4
Toxicity classes color												
Average similarity (%)	100	84.03	81.12	78.46	75.96	73.44	73.44	71.49	73.44	69.04	69.47	65.46
Prediction accuracy (%)	100	70.97	70.97	69.26	69.26	69.26	69.26	69.26	69.26	68.07	68.07	68.07
Prediction accuracy color												

Table 5: Oral toxicity prediction results for Ref-1, Ref-2 and Compounds 2.13.-2.17.

Entry	**2.1**	**2.4**	**2.13**	**2.14**	**2.15**	**2.16**	**2.17**	**Ref - 1**	**Ref - 2**
Molweight	206.28	236.31	220.31	224.27	240.73	285.18	251.28	180.16	151.16
Number of hydrogen bond acceptors	20	23	22	19	19	19	20	12	12
Number of hydrogen bond donors	0	0	0	0	0	0	0	1	2

Number of atoms	33	37	36	33	33	33	35	21	20
Number of bonds	33	37	36	33	33	33	35	21	20
Number of rotable bonds	6	7	6	6	6	6	7	3	2
Molecular refractivity	61.54	68.03	66.5	61.49	66.55	69.24	70.36	44.9	42.78
Topological Polar Surface Area	26.3	35.53	26.3	26.3	26.3	26.3	72.12	63.6	49.33
Octanol/water partition coefficient (logP)	2.82	2.83	3.13	2.96	3.47	3.58	3.25	1.31	1.42
LD50 (mg/kg)	5000	2000	5000	5000	5000	5000	5000	250	338
Toxicity Class	5	4	5	5	5	5	5	3	4
Toxicity classes color									
Average similarity (%)	100	78.46	95.45	77.45	77.94	77.45	63.61	100	100
Prediction accuracy (%)	100	69.26	72.90	69.26	69.26	69.26	68.07	100	100
Prediction accuracy color									

Theoretically predicted organ toxicity and toxicity end points of **DSPA: 2.1-2.17.** are found to be inactive for most of the cases (Table 1 and 2) except a few, the toxic end points namely hepatotoxicity of compounds **2.2., 2.5.** and **Ref-2**, carcinogenicity of compounds **2.1.** and **2.3.,** immunotoxicity of compounds **2.9.** and **2.12.** and mutagenicity of compound **2.17.,** on behalf of which the predictions are active. Interestingly, though the hepatotoxicity of compounds **2.2., 2.5.** and **Ref-2**, carcinogenicity of compounds **2.1.** and **2.3.,** immunotoxicity of compounds **2.9.** and **2.12.** and mutagenicity of compound **2.17.** are found to be active, accompanying a reasonable probability values of ≥ 0.51 (Table 1 and 2) may support the compounds for being as a bio-materials than pesticides. However, the predicted target toxicity of Tox21-Nuclear receptor signalling and Tox21-Stress response pathways ranges 0.80-1.00 for compounds **2.1.-2.12** and the same for compounds **2.13.-2.17** appeared in a lower range of probability ranges from 0.63 to 1.00. This data visualization is in-fact supporting the compounds presented in Table 1 for being as good bio-materials than the compounds presented in **Table 2**.

The median lethal dose (LD_{50})[28-31] describes acute oral toxicity against single oral dose usually used to forecast the death of 50% of the test animals are commonly presented as milligrams of compound per kilogram of body weight of the animal (mg/kg). Estimated LD50 for the **DSPA: 2.1-2.17.** using ProTox II (Table 4 and 5) is found with high flexibility from 1123 (compound **2.11.)** to 5000 (many compounds in **Table 5**). As per the literature availability, a compound with an oral LD_{50} of 0−50 mg/kg are distinguished out as highly toxic, although drug candidates with an LD_{50} of greater than 2000 mg/kg are dignified as a little toxicant output or higher in medicinal values. However, if a compound LD50 is in between 500 and 2000 mg/kg, the approximate lethal dose required for an average adult to be in between an ounce and pint of the drug candidate. The predicted LD50 values for the compounds **2.1., 2.4., 2.13.-2.17.** are > 2000 mg/kg and the remaining compounds are displayed in between 1123 and 1630 mg/kg, shows that the compounds possess > 2000 mg/kg can act as bio-materials. Even though, as per Drwal et al.,[15] classification the LD50 values are classified into five sub-classes as follows:

- ❖ Class I: If the LD50 ≤ 5, **a toxican**t material is always **leads to death** after swallowing;
- ❖ Class II: For conditions like 5< LD50≤50 is also **results death** after swallowing like class I;
- ❖ Class III: A range 50< LD50≤300 is only **toxic** after swallowing;
- ❖ Class IV: **A harmful material** after swallowing if the range is 300<LD50≤2000;
- ❖ Class V: 2000<LD50≤5000 range **may be harmful** after swallowing and
- ❖ Class VI: If LD50>5000 is non-toxic can be bio-materials.

According to the above predictions the experimental compounds are found to fall in class IV, V and VI, clearly shows that the compounds are not going to be toxic but a few of may be harmful after swallowing or can act as good bio-materials. A review published by Keith et al., on the LD_{50} and its current role in hazard communication[16] has also supporting the above theoretical observations.

Toxicity Class, average similarity as well as prediction accuracy estimated for the **DSPA: 2.1-2.17.** using ProTox II are also with good agreement

to the literature values. All the title compounds are reported with toxicity classes 4 or 5 for acute oral toxicity with LD_{50} value ranging 1123 and 5000 mg/kg, with an average similarity of ~ 70.00-100.00% and prediction accuracy of ~ 65.00-100.00%. Furthermore, a few new data computational methods will be added to the existing ProTox II model and new endpoints like genotoxicity, nephrotoxicity, neurotoxicity, and cardiotoxicity as well as in detail physicochemical parameters will be added.

***pkCSM* - a Graph Based ADME-TOX Descriptive Analysis:**

Innovative molecular structure development in drug discovery is not depend only the chemical synthesis, but also *in-silico* calculation of physicochemical parameters from the point of view of bioavailability and pharmacokinetics becoming more relevant for the development of effective potential drug candidate.[25] The requirement of comprehensive databases that accumulate high-quality and up-to-date data are essential for constructing regulatory components for *in-silico* drug-likeness evaluation and ADME/Tox resources provide useful guidelines to extract rational compounds that match the desirable pharmacokinetics and toxicity properties or to filter compounds that are not likely to be drugs. Hence, the freely accessible two web servers SwissADME and pkCSM pharmacokinetic computational methods to calculate the ADME and Tox-related descriptors like BIOFACQUIM[18] (a Mexican compound database of natural products) have been used for *in-silico* drug-likeness evaluation and extensively validated with experimental figures.

Lipophilicity,[21] a critical parameter describes the partition coefficient of uncharged molecules responsible for the efficacy of a newly synthesized drug candidate is the basic criteria for its formulation and dosage. Customarily, the hydrophilic compounds with higher affinity for the aqueous phase is giving a constant negative LogP value, lipophilic compounds display higher affinity for the lipid/organic solvent phase is with a constant positive value and zero value for compounds which partition equally between lipid and aqueous phases. If a newly synthesized drug candidate possess the LogP value around 2 (compound is 20 times more in lipid/organic solvent phase compared to aqueous phase) capable of crossing the blood-brain barrier as CNS targeting drugs and also drugs developed for sub-lingual absorption the expected LogP value is >5. Interestingly, the results obtained for the title compounds **DSPA: 2.1-2.17** occasioned in three different sequences of data based on its substitutional performances towards the Lipophilicity of the drug candidates have been investigated and analyzed. In the first case, the alkyl group variation by keeping the phenyl acetate unit as constant the resultant Log P value is around 2 (**Table 6**: 1.4193-2.827), substitution on phenyl ring is varied by keeping the alkyl group constant in case two ensued Log P value is moving > 3 (**Table 7**: 2.7266-3.5809) and thirdly number of hydrogen bond acceptor increased in the phenyl ring by increasing methoxy groups is also resulted the Log P value is around 2 (**Table 8**: 2.8184-2.8442). Even though, the overall Log P value is widely ranges from 1.4193-3.5809 for the title compounds 2.1-2.17, a convincing results obtained for compounds listed in the **Table 6** (1.4193-2.827; 2.1-2.10) and Table 3 (2.8184-2.8442; 2.1, 2.4, 2.13, 2.14, 2.15, 2.16 and 2.17) which denotes that the halogen substitution at para position less pronounced than the other two types of substitution.

The Lipinski's Rule-of-Five:

From the past two-decades scientific community able to classify a chemical compound very easy way using *in-silico* screening approaches with an optimistic pharmacological or biological activity, those properties that would make it a probable orally active drug in man. Lipinski's Rule-of-Five[26] one among the methods appropriately defines the potentiality of a drug candidate based on the origin of absorption/permeation is more likely when the molecule has more than 5 H-bond donors, more than 10 H-bond acceptors, its molecular weight is over 500 and its LogP is over 5. The title compounds 2.1-2.17 are subjected to predict pharmacokinetic properties using graph-based signatures called pkCSM, a freely accessible web server and modern high throughput drug discovery approach. The data of the ADME and Tox-related descriptors have been extracted and tabulated (**Table 6-8**).

Table 6: Molecular properties of different alkyl group substituted phenylacetates (2.1-2.10).

Descriptor	Value											Lipinski's Rule
	2.1	**2.2**	**2.3**	**2.4**	**2.5**	**2.6**	**2.7**	**2.8**	**2.9**	**Ref 1**	**Ref 2**	
Molecular Weight	206.285	208.257	222.284	236.311	210.229	224.256	238.283	252.31	252.31	180.159	151.165	< 500
LogP	2.8184	2.1893	2.5794	2.827	1.4193	1.8094	2.1979	2.588	2.588	1.3101	1.3506	< 5
#Rotatable Bonds	5	4	5	6	4	5	5	6	4	2	1	< 10
#Acceptors	2	3	3	3	4	4	4	4	4	3	2	< 10
#Donors	0	0	0	0	0	0	0	0	0	1	2	< 5
Surface Area	91.261	90.010	96.375	102.740	88.758	95.123	101.488	107.853	107.853	74.757	64.667	-

Table 7: Molecular properties of substituted *iso*-amyl aryl acetates (2.1, 2.4, 2.13, 2.14, 2.15, 2.16 and **2.17).**

Descriptor	Value									Lipinski's Rule
	2.1	**2.4**	**2.13**	**2.14**	**2.15**	**2.16**	**2.17**	**Ref 1**	**Ref 2**	
Molecular Weight	206.285	236.311	220.312	224.275	240.73	285.181	251.282	180.159	151.165	< 500
LogP	2.8184	2.827	3.12682	2.9575	3.4718	3.5809	2.7266	1.3101	1.3506	< 5
#Rotatable Bonds	5	6	5	5	5	5	6	2	1	< 10
#Acceptors	2	3	2	2	2	2	4	3	2	< 10
#Donors	0	0	0	0	0	0	0	1	2	< 5
Surface Area	91.261	102.740	97.626	95.427	101.564	105.129	105.914	74.757	64.667	-

Table 8: Molecular properties of methoxy groups substitution on *iso*-amyl aryl acetates (2.1, 2.4, 2.13, 2.14, 2.15, 2.16 and **2.17).**

Descriptor	Value							Lipinski's Rule
	2.1	**2.4**	**2.10**	**2.11**	**2.12**	**Ref 1**	**Ref 2**	
Molecular Weight	206.285	236.311	266.337	266.337	296.363	180.159	151.165	< 500
LogP	2.8184	2.827	2.8356	2.8356	2.8442	1.3101	1.3506	< 5
#Rotatable Bonds	5	6	7	7	8	2	1	< 10
#Acceptors	2	3	4	4	5	3	2	< 10
#Donors	0	0	0	0	0	1	2	< 5
Surface Area	91.261	102.740	114.218	114.218	125.697	74.757	64.667	-

Traffic light scheme for absorption:

According to Lobell et al.,[27] the collective calculated values of physicochemical properties namely aqueous solubility ($logS_{aq}$), octanol-water partition coefficient (ClogP), molecular weight (MW), polar surface area (PSA) and the number of rotatable bonds (RotB) resolved for good gastrointestinal absorption is follows a scheme "traffic light" as given bellow:

- Green: $logS_{aq}$ @ 50; ClogP @ 3; MW @ 400; PSA @ 120; RotB @ 7;
- Yellow: $logS_{aq}$: 10-50; ClogP: 3-5; MW: 400-500; PSA: 120-140 RotB: 8-10; and
- Red: $logS_{aq}$ < 10; ClogP > 5; MW > 500; PSA > 140; RotB @ 11

A green traffic light combination of physicochemical properties is always a low level in silico oral PhysChem score had better be preferable for an oral drug candidate and enhance its chances to make it all the way to market approval.

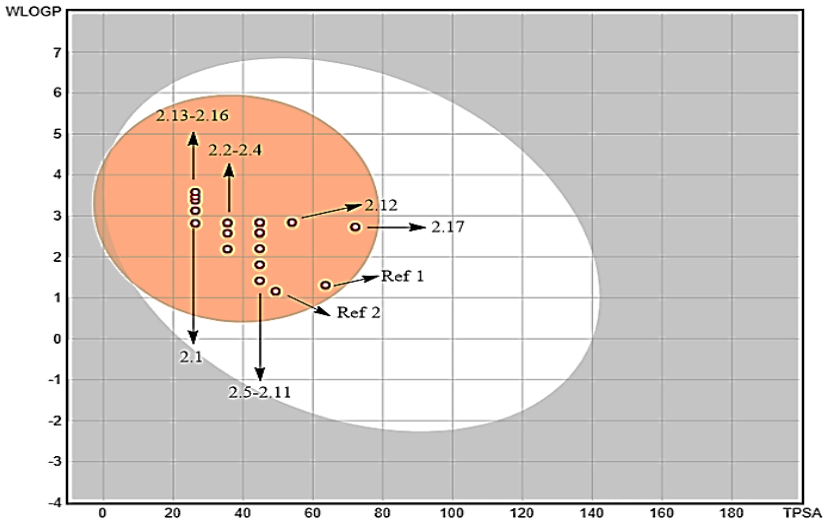
The substituent behaviors towards the physicochemical parameters of the **DSPA: 2.1-2.17** have been examined by isolating them into three different groups as Alkyl group variant, phenyl ring variant and number of hydrogen bond acceptor variant. The results of every category of the compounds **2.1-2.17** marvelously complies the Lipinski's rule of five without even a single violation and exhibited good drug-likeness scores and also encouraging fulfillers of traffic light scheme for absorption.

SwissADME-Pharmacokinetic Computational Method:

Human gastrointestinal absorption (HIA) and blood brain barrier (BBB) are two pharmacokinetic behaviors crucial to estimate at various stages of the drug discovery processes. To this end, the Brain or IntestinaL EstimateD permeation method (BOILED-Egg) is proposed as an accurate predictive model that works by computing the lipophilicity and polarity of small molecules. BOILED-Egg, an in-built graphical output method of SwissADME to predict the two key ADME parameters (HIA and BBB) simultaneously is actually a plot of WLOGP (lipophilicity) versus TPSA (topological polar surface area). BOILED-Egg graph by its shape is divided into 3 parts including a yellow yolk region (i.e. the physicochemical space for highly probable BBB permeation), an albumen region (i.e. the physicochemical space for highly probable HIA absorption) and the outside grey region stands for molecules with properties implying predicted low absorption and limited brain penetration.

This BOILED-Egg graph are also used to predict whether they are substrates of P-glycoprotein (PGP) or not by denoting the color of dots appeared in that graph. In that, red dots (PGP–) represent compounds that are not substrates of the PGP CNS efflux transporter, while, blue dots (PGP+) represent compounds that are substrates of PGP and predicted to pass through the CNS. As revealed in **Figure 1**, aspirin, paracetamol and compounds 2.1-2.17 are represented by red dots and not substrates of the PGP CNS efflux transporter. Therefore, all the compounds passed the pharmacokinetic requirements for drug-like compound behavior, suggesting that these compounds had good oral bioavailability.

Figure 1: The Total Boiled-Egg Plot of **Aspirin, Paracetamol** and **DSPA: 2.1-2.17.**



Subsequently, a dissimilar linearity observed in the BOILED-Egg graph, the substituent behaviors towards the physicochemical parameters of the compounds 2.1-2.17 have been investigated by dividing them into three different groups; (i) Alkyl group is varied by keeping the phenyl acetate unit as constant, (ii) Substitution on phenyl ring is varied by keeping the alkyl group constant and (iii) Number of hydrogen bond acceptor is increased in the phenyl ring by increasing methoxy groups.

(i) Alkyl group is varied by keeping the phenyl acetate unit as constant:

In the case of different alkyl group substitution, the consensus Log Po/w values are found to increase with the increase in number of carbon atoms in chain and an almost constant topological polar surface area values obtained for the same. In the BOILED-Egg graph, red dots for those compounds appeared in the yellow yolk region is a clearcut intimation for the physicochemical cosmos for highly probable BBB permeation. As revealed in Figure 1, aspirin, paracetamol and compounds 2.5, 2.6, 2.7, 2.8, 2.9 and 2.10 are represented by red dots and not substrates of the PGP CNS efflux transporter. Therefore, all the compounds passed the pharmacokinetic requirements for drug-like compound behaviour, suggesting that these compounds had good oral bioavailability tableted in **Table (9-11).**

Table 9: Consensus Log Po/w and Topological Polar Surface Area (TPSA) Values for BOILED-Egg graph.

Lipophilicity	**Ref 1**	**Ref 2**	**CH_3**	**CH_2CH_3**	**$CH(CH_3)_2$**	**$CH(CH_3)$ CH_2CH_3**	**$C(CH_3)_3$**	**$CH_2CH_2CH(CH_3)_2$**
Log $P_{o/w}$ (iLOGP)	1.30	1.21	2.50	2.37	3.01	3.14	3.15	3.41
Log $P_{o/w}$ (XLOGP3)	1.19	0.46	1.28	1.65	2.08	2.61	2.27	2.97
Log $P_{o/w}$ (WLOGP)	1.31	1.16	1.42	1.81	2.20	2.59	2.59	2.84
Log $P_{o/w}$ (MLOGP)	1.51	0.91	1.38	1.66	1.93	2.20	2.20	2.45
Log $P_{o/w}$ (SILICOS-IT)	1.10	0.89	2.11	2.50	2.72	3.12	2.96	3.52
Consensus Log $P_{o/w}$	**1.28**	**0.93**	**1.74**	**2.00**	**2.39**	**2.73**	**2.63**	**3.04**
TPSA (Å²)	**63.60**	**49.33**	**44.76**	**44.76**	**44.76**	**44.76**	**44.76**	**44.76**

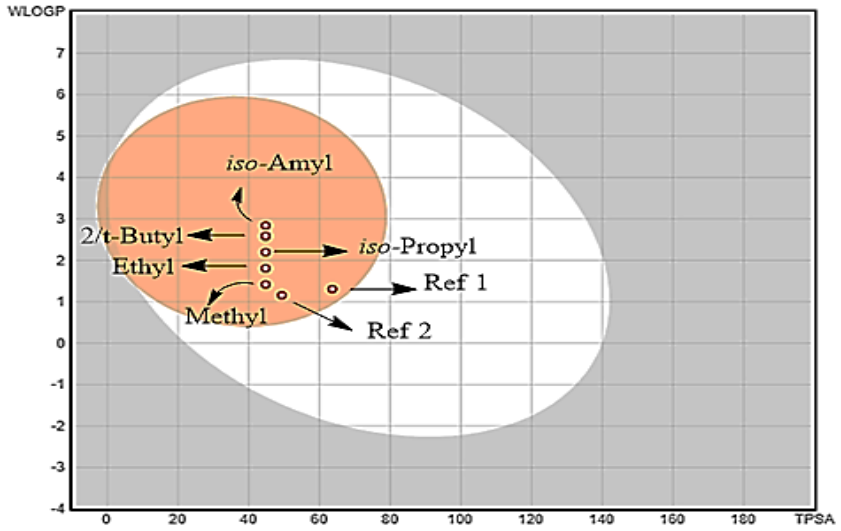
Table 10: Consensus Log Po/w and Topological Polar Surface Area (TPSA) Values for BOILED-Egg graph.

Lipophilicity	**Ref 1**	**Ref 2**	**H**	**4-OCH_3**	**4-CH_3**	**4-F**	**4-Cl**	**4-Br**	**4-NO_2**
Log $P_{o/w}$ (iLOGP)	1.30	1.21	2.98	2.80	3.23	3.06	3.25	3.23	2.71
Log $P_{o/w}$ (XLOGP3)	1.19	0.46	3.60	3.37	3.76	3.50	4.03	4.09	3.40
Log $P_{o/w}$ (WLOGP)	1.31	1.16	2.82	2.83	3.13	3.38	3.47	3.58	2.73
Log $P_{o/w}$ (MLOGP)	1.51	0.91	3.13	2.78	3.39	3.53	3.66	3.79	2.04
Log $P_{o/w}$ (SILICOS-IT)	1.10	0.89	3.34	3.42	3.86	3.78	4.00	4.03	1.24
Consensus Log $P_{o/w}$	**1.28**	**0.93**	**3.17**	**3.04**	**3.47**	**3.45**	**3.68**	**3.74**	**2.42**
TPSA (Å²)	**63.60**	**49.33**	**35.53**	**26.30**	**26.30**	**26.30**	**26.30**	**26.30**	**72.12**

Table 11: Consensus Log Po/w and Topological Polar Surface Area (TPSA) Values for BOILED-Egg graph.

Lipophilicity	**Ref 1**	**Ref 2**	**H**	**4-OCH_3**	**3,4-$(OCH_3)_2$**	**3,5-$(OCH_3)_2$**	**3,4,5-$(OCH_3)_3$**
Log $P_{o/w}$ (iLOGP)	1.30	1.21	2.98	2.80	3.41	3.56	3.51
Log $P_{o/w}$ (XLOGP3)	1.19	0.46	3.60	3.37	2.97	3.34	3.31
Log $P_{o/w}$ (WLOGP)	1.31	1.16	2.82	2.83	2.84	2.84	2.84
Log $P_{o/w}$ (MLOGP)	1.51	0.91	3.13	2.78	2.45	2.45	2.14
Log $P_{o/w}$ (SILICOS-IT)	1.10	0.89	3.34	3.42	3.52	3.52	3.62
Consensus Log $P_{o/w}$	**1.28**	**0.93**	**3.17**	**3.04**	**3.04**	**3.14**	**3.09**
TPSA (Å²)	**63.60**	**49.33**	**26.30**	**35.53**	**44.76**	**44.76**	**53.99**

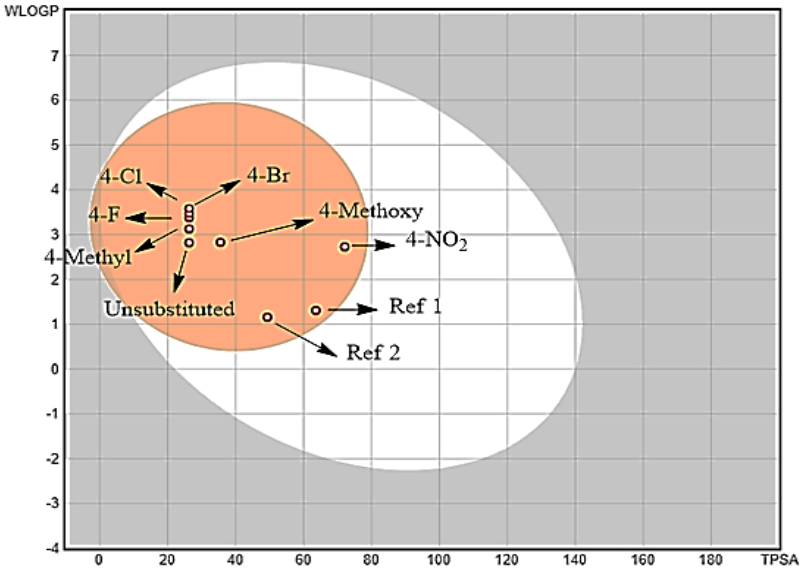
Figure 2: The Total Boiled-Egg Plot of Aspirin, Paracetamol and compounds 2.5, 2.6, 2.7, 2.8, 2.9 and .10.



(ii) Substitution on phenyl ring is varied by keeping the alkyl group constant:

However, the same trend of the above is found with phenyl ring substitution, the compounds 2.4 and 2.17 are found to have different topological polar surface area values, may due to the presence of additional hydrogen bond acceptor in those compounds. In the BOILED-Egg graph, red dots for those compounds appeared in the yellow yolk region is a clearcut intimation for the physicochemical cosmos for highly probable BBB permeation. As revealed in **Figure (2-4)**, aspirin, paracetamol and compounds 2.1, 2.4, 2.13, 2.14, 2.15, 2.16 and 2.17 are represented by red dots and not substrates of the PGP CNS efflux transporter. Therefore, all the compounds passed the pharmacokinetic requirements for drug-like compound behavior, suggesting that these compounds had good oral bioavailability.

Figure 3: The Total Boiled-Egg Plot of Aspirin, Paracetamol and compounds 2.1, 2.4, 2.13, 2.14, 2.15, 2.16 and 2.17.

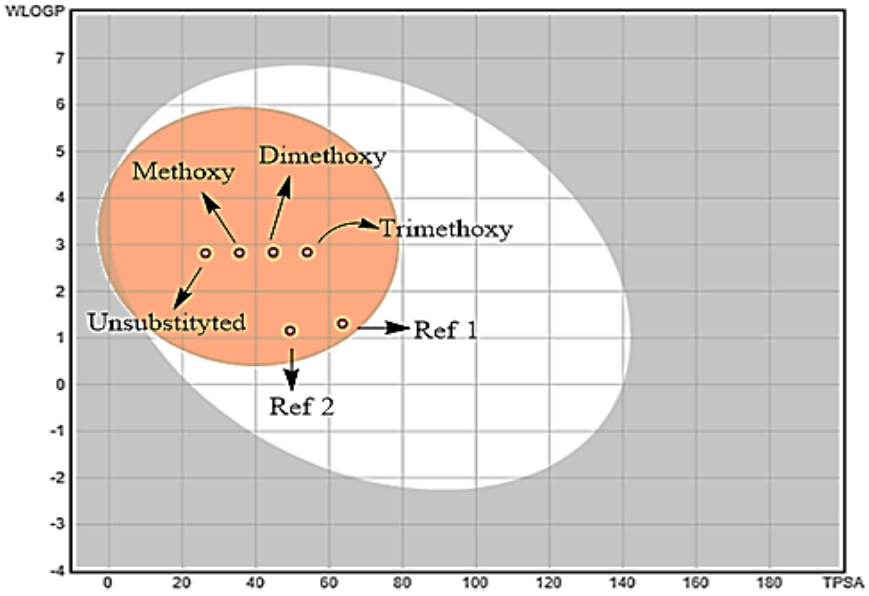


(iii) Number of hydrogen bond acceptor is increased in the phenyl ring by increasing methoxy groups:

Even though almost constant topological polar surface area values attained for the previse two sequences of cases, the number of hydrogen bond acceptor increased in the phenyl ring by increasing methoxy groups is generating remarkable increase in the topological polar surface area values against the number of methoxy groups. In the BOILED-Egg graph, red dots for those compounds appeared in the yellow yolk region is a clear cut intimation for the physicochemical cosmos for highly probable BBB permeation. As revealed in Figure

3, aspirin, paracetamol and compounds 2.1, 2.4, 2.10, 2.11 and 2.12 are represented by red dots and not substrates of the PGP CNS efflux transporter. Therefore, all the compounds passed the pharmacokinetic requirements for drug-like compound behavior, suggesting that these compounds had good oral bioavailability.

Figure 4: The Total Boiled-Egg Plot of Aspirin, Paracetamol and compounds 2.1, 2.4, 2.10, 2.11 and 2.12.



Herewith, this is concluded that the topological polar surface area values attained are almost similar for the (i) and (ii) cases, the substituent behaviors (Alkyl groups or Substitution on phenyl ring) towards the physicochemical parameters of those compounds not going to be much deviated. Hence, this is directed to synthesis compounds 2.1-2.12 (having a highly valuable consensus Log Po/w values ≤ 3) by introducing varies alkyl group of the ester and also by increasing the number of hydrogen bond acceptor in phenyl ring by increasing methoxy groups for further studies.

CONCLUSION:

In conclusion, seventeen (**DSPA: 2.1-2.17**) efficient drug candidates designed using post-synthetic data-visualization grouping technique and then the twelve vital analogs categorized by *in-silico* investigation of differently substituted

phenyl acetate (**DSPA: 2.1-2.12**) have been forwarded to synthesis and bio-logical studies. The validation of compounds **DSPA: 2.1-2.17** by all possible way using web servers ***ProTox-II,***

SwissADME and ***pkCSM*** in order to know the substituent behaviors towards the physicochemical parameters have been investigated by dividing them into three different groups; (i) Alkoxy group is varied by keeping the phenyl acetate unit as constant, (ii) Substitution on phenyl ring is varied by keeping the Alkoxy group constant and (iii) Number of hydrogen bond donor is increased in the phenyl ring by increasing methoxy groups.

In-silico toxicity studies like oral acute toxicity, organ toxicity, immunotoxicity, genetic toxicity endpoints, nuclear receptor signalling, and stress response pathways of **DSPA: 2.1-2.17** using ***ProTox-II*** webserver have been widely explored to assess the safety profile of drug. The title compounds **DSPA: 2.1-2.17** are subjected to predict pharmacokinetic properties using graph-based signatures called ***pkCSM*** and the results of every category of the compounds marvelously complies the Lipinski's rule of five without even a single violation and exhibited good drug-likeness scores.

A dissimilar linearity observed in the BOILED-Egg graph plotted by ***SwissADME*** web server based on the categories (i)-(iii) of the compounds listed. The topological polar surface area values attained by ***SwissADME*** are almost similar for the (i) and (ii) cases, the substituent behaviors (alkoxy groups or substitution on phenyl ring) towards the physicochemical parameters of those compounds are not much deviated. Hence, this is directed to synthesis compounds **DSPA: 2.1-2.12** (having a highly valuable consensus Log Po/w values ≤ 3) by introducing varies alkoxy group of the ester and also by increasing the number of hydrogen bond

donor in phenyl ring by increasing methoxy groups for further studies.

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