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Detection of aroma compound's binding mode conformations on anticancer target DNA topoisomerase II

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Abstract

Cancer is one of the most common, devastating class of disease affecting millions of people causing large number of death every year. It is therefore considered to be the second leading cause of death in developing countries next to cardiovascular diseases. Recent molecular studies have focussed on the most targeted gene for cancer i.e. DNA topoisomerase II (DNA TOP2), an enzyme that controls and alters the topological states of DNA during transcription. DNA TOP2 is also a target of known anticancer drugs like etoposide, doxorubicin, daunorubicin, amsacrine, amrubicin, and many others. These drugs have toxic properties and possess adverse side effects like cardiomyopathy, leading to congestive heart failure. Due to this reason, more active and safe drug are being searched worldwide. In this study, we performed virtual screening of natural compounds (small molecules) for the exploration of best fit compounds against DNA TOP2 through in silico molecular docking experiment and also evaluated the bioavailability and safety concern through electronic pharmacokinetics parameters (absorption, distribution, metabolism, excretion) compliance check-up and toxicity risk studies by using standard drugs. More than 1300 aroma compounds from AromaDb database were virtually docked on DNA TOP2 (PDB:1ZXM) for detection of the binding site, best fit binding mode conformation & their binding side residues by using FlexX docking software. Leads based on docking scores better than approved/investigational anticancer drugs targeting DNA TOP2 were prioritised. Total eight approved and five investigational anticancer drugs were used reported to bind with DNA TOP2. Results showed best fit 50 compounds with docking score (-44.05 to -26.85 kJ/mol), higher to standard drugs -16.88 to -38.27 kJ/mol and revealed four binding sites on DNA TOP2. This study suggest that virtually screened top 50 compounds may be used for preparation of aroma based herbal products with anticancer activity.

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INTRODUCTION

DNA topoisomerase is an enzyme which is used to increase or decrease the extent of DNA underwinding or relaxing the supercoiled of DNA during the replication, transcription and mitosis. There is two class of DNA topoisomerase Type I topoisomerase act as breaking one of the two DNA strands, and Type II topoisomerase breaks both DNA strand [1]. The reports reveals that in most human solid tumours, the topoisomerase II is higher in concentration. The Topoisomerase II (TOP2) has been identified as important and primary cellular target for some antitumor drugs currently being used in cancer therapies [2]. The studies showed that most clinically active cancer drugs target TOP2. Some approved drugs like Etoposide, Doxorubicin, Pirarubicin, Daunorubicin, Amrubicin, Gatifloxacin, Topotecan, Irinotecan and Amsacrine; whereas some investigational drugs like Voreloxin, Amonafide and Phenoxodiol were used in their studies [3]. There are two class of drugs which act on DNA TOP2 class I compounds lead to increase in the levels of TOP2 DNA covalent complexes, these drugs generate lesions that include DNA strand breaks and protein covalently bound to DNA, and they have been termed as DNA TOP2 poisons [4]. Another class of compounds inhibits TOP2 catalytic activity and do not increase the level of TOP2 covalent complexes [5]. Agenst of the second class kills cells through the elimination of special enzymatic activity of the TOP2 and term as TOP2 catalytic inhibitors [6].

All the present drugs that target DNA TOP2 and are available in the market for treatment of cancer orally or chemotherapically showed effective results, but these drugs have severe long-term side effect, like cardiac arrest leading to heart failure and are highly toxic in nature [7]. This raises a question that instead of having a number of anti-cancer drugs do we need more drug for study which targets DNA TOP2. The answer is yes. In the clinical studies, it was seen that TOP2 targeting drugs not only binds with the target and showed activity, but mainly show empirical anti-tumour activity. Therefore we need to identify new potent and specific TOP2 targeting drug-like compounds through rational screening. It will be better if we discover improved and more responsive TOP2 targeting drugs.

Natural drugs have always been a good solution towards the treatment of cancer as they have many functional abilities to repair the damage of DNA and to induce apoptosis [8]. The range of new compounds has been discovered with the ability to inhibit the DNA topoisomerase and act as promising anticancer agents. These natural compounds include alkaloids, terpenoids and flavonoids. All the class of compounds have different properties: alkaloids are large and diverse group of low molecules weight nitrogenous compounds found in several plant species. Alkaloids are derived as allcaline metabolite that can be easily isolated as salt and that is why they had important pharmaceutical properties for designing of drugs [9]. Alkaloid like camptothecin is most popular and widely used anticancer drug which is originated from the plant, which inhibits DNA topoisomerase, these compounds initiate the discovery of new alkaloid which targets topoisomerase for anticancer properties.

Plants derived terpenoids which are low molecular weight volatiles are also used as an anticancer drug that inhibit the cancer growth. Some compounds like Ganoderic acid, Fomitellic acid B and Ursolic acid are reported to binds with DNA topoisomerase I and IIá [10,11]. Another class of anti neoplastic natural compounds are flavonoids that are low molecular weight polyphenolic compounds [12]. These compounds are universal in the plant kingdom and present in vegetables, grains, fruits, leaves, flowers, root, seeds etc (13). This diverse group of compound possess pleasant aroma and flavour that is why they are highly used in beverage, pharmaceuticals and cosmetics industries. Flavonoids like baicalein, myricetin and quercetin were studied for their anticancer property and found that they strongly inhibit DNA topoisomerase II. The above prior studies reveal that using natural compounds for the treatment of cancer is a good way to reduce the toxic effect on human health and we need to screen more potent natural compounds which can inhibit DNA

topoisomerase II and stop the proliferation cancerous cells.

MATERIALS AND METHODS

Retrieval of aroma compounds and library preparation

Plant-based aroma compounds list was retrieved from the AromaDb (http://bioinfo.cimap.res.in/aromadb/) [14]. The 1321 three dimensional compound structures were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in sdf format. Market available drugs with proven efficiency for targeting DNA Topoisomerase II as its inhibitor were also searched from PubMed literature, GeneCards (https://www.genecards.org) and ClinicalTrials.gov databases (https://clinicaltrials.gov), where 13 drugs were selected among which eight drugs was approved and five under investigation. All the drugs and aroma compounds were downloaded from the PubChem database in sdf file format and converted them into mol2 file format using OpenBabel 2.3.2 version. Further, all downloaded compounds were prepared for docking studies by adding H-bonds. After compounds preparation all the compounds were clubbed into a single mol2 library file.

Protein structure preparation and molecular docking

The x-ray crystallographic protein 3D structure of DNA topoisomerase IIa was retrieved from RCSB PDB (www.rcsb.org/) with PDB ID: 1ZXM in the PDB file format [15]. The 3D structure showed good resolution of 1.87 Å and crystallised from Homo sapiens. The downloaded structures were further prepared for docking studies using Discovery Studio 3.5 (Accelrys USA) (DS); all the hetero atoms (water molecules and bound ligands) were removed from the protein 3D structure, and H-bonds were added to protonate the protein. The molecular docking studies were performed by FlexX docking program available in LeadIT 2.1.8 version of (BioSolveIT Germany) [16]. The blind docking was performed by keeping all ligands, and protein structure flexible using by default docking parameters (Figure 1). FlexX uses a fast algorithm for flexible docking of

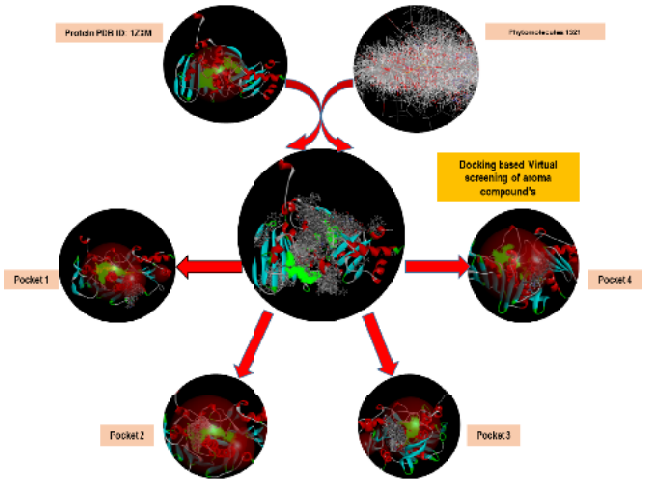

Fig. 1: Representation of ligand-based virtual screening workflow applied in this study for detection of aroma compound's binding mode confirmation.

small molecules into the protein binding site using an incremental construction process. The 2D visualization of the top pose of docked compounds generated by FlexX was selected and saved. Further binding pocket mode conformation of compounds and drugs within DNA TOP2 was defined and visualized by DS 3.5 visualizer.

Pharmacokinetic screening using in-silico method

The pharmacokinetic (PK) studies of standard drugs and virtually screened aroma compounds was performed by the Discovery Studio v3.5

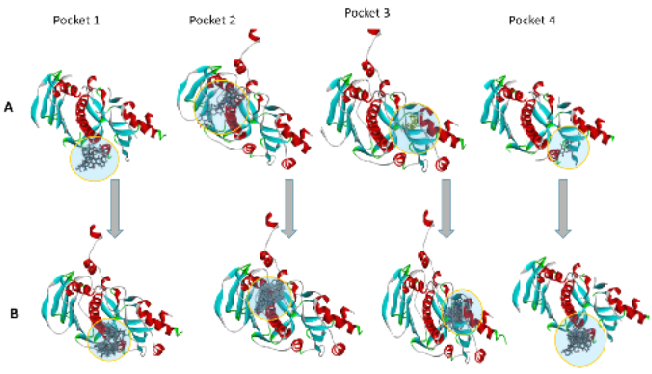

Fig. 2: Binding pocket mode conformation representation of aroma molecules and FDA approved and investigational drugs: A shows the binding conformation of drugs within the pocket of DNA TOP2 and reveals four Pockets. B shows that the aroma compounds also share the same binding pockets where drugs bind and also shows higher docking score.

Table 1. Llist of 8 FDA approved and five investigational drugs (highlighted in bold) with their docking score and binding mode pocket.

S. No.	Drugs name	Docking score (kJ/mol)	Pocket
1	Amsacrine	-38.2775	Pocket 2
2	Doxorubicin	-30.4937	Pocket 1
3	**Pirarubicin**	-30.0961	Pocket 3
4	Daunorubicin	-30.2032	Pocket1
5	Amrubicin	-28.3019	Pocket 4
6	**Voreloxin**	-27.5785	Pocket 3
7	**Amonafide**	-24.6833	Pocket 2
8	**Phenoxodiol**	-20.8902	Pocket 2
9	**Ellipticine**	-20.1464	Pocket 2
10	Gatifloxacin	-20.1295	Pocket 1
11	Topotecan	-19.9659	Pocket 2
12	Irinotecan	-19.5298	Pocket 2
13	Etoposide	-16.8895	Pocket 1

software (Accelrys, USA) TOPKAT module. Lipinski's rule of five was also calculated to know the bioavailability of aroma compounds. These PK calculations include Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties which cover Aqueous solubility, Blood-Brain Barrier Penetration, CYP2D6 Binding, Hepatotoxicity, Intestinal Absorption, and Plasma Protein Binding descriptors. The calculation of ADMET properties are essential in the early drug development process to avoid the elimination of compounds with unfavourable ADMET characteristics. If this process was not practiced early, then it will be very cost intensive and time consuming for new drug development.

RESULTS AND DISCUSSIONS

Virtual screening of aroma compounds

The total of 1321 compounds retrieved from AromaDb were run under Ligand-based virtual screening against DNA Topoisomerase II. To set a cutoff value range for docking studies, we docked all 13 FDA approved and investigational drugs such as doxorubicin, amsacrine, pirarubicin, daunorubicin, amrubicin, voreloxin, amonafide, phenoxodiol, ellipticine, gatifloxacin, topotecan, irinotecan, and etoposide using FlexX docking software. The results showed that Amsacrine has the highest binding affinity towards DNA TOP2 with a docking score -38.28 kJ/mol and the lowest docking energy was shown by etoposide with a docking score of -16.89 kJ/mol, rest of the drugs shown docking score with in this range (Table 1). The aroma compounds showing docking score above-16.89 kJ/mol were included in virtual screening results for consideration of better lead compounds.

Table 2. Number of aroma compounds binding in the different binding pockets of DNA TOP2.

S. No	Aroma compounds	Pockets
1	36	Pocket 1
2	537	Pocket 2
3	132	Pocket 3
4	20	Pocket 4
Total	**725**	

Binding mode conformation analysis of aroma compounds

Docking results reveal the binding mode conformation on anticancer target DNA topoisomerase II. Docking pose analysis of all 13 drugs with higher docking score was selected. On the basis of binding mode confirmation and higher binding energy, all the aroma compounds were screened, and the protein structure with their docking pockets was divided into four binding pockets namely Pocket 1, 2, 3, and Pocket 4. According to the binding pocket; four drugs bound in Pocket 1, six in Pocket 2, two in Pocket 3, and one drug bind in Pocket 4 (Table 1). After screening by docking score cutoff value, the aroma compounds were further screened according to the docking mode conformation or binding pockets. We selected those aroma compounds which binds in the specific binding pocket where the FDA drugs

bind. Applying this two screening methods the results reveals that 36 aroma compounds interestingly binds in Pocket 1, 537 in Pocket 2, 132 in Pocket 3, and 20 aroma compounds binds in Pocket 4 as shown in Table 2 and Figure 2.

Screening of aroma compounds by docking energy

In this study, docking results determine the better compound compared to the available drugs. According to the results we screened 411 aroma compounds which showed higher docking score than the FDA approved drug Etoposide which shows the docking score of -16.8895 kJ/mol (Table 3). As shown in this table we only selected top 50 aroma compounds which showed higher docking score than the existing drugs and share the same binding pocket although rest also showed higher docking score. It was observed that approved drugs and screened aroma compounds bind in the specific binding pocket and also shared maximum number of same amino acid, which reveals that the selected aroma compound will show a higher inhibitory effect when bound to the target.

Pharmacokinetic parameters screening by in silico methods

Pharmacokinetics (PK) is a branch in which pharmacological parameters of substance were checked like xenobiotic such as pharmaceutical drugs, pesticides, food additives, cosmetics etc. Most of the product or drugs fail during their initial clinical trials if they do not have good PK. These PK consist of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity). These all parameters influence the drug levels and kinetics of drug exposure to the tissue and hence influence the performance and pharmacological activity of a compound as a drug. The PK values were calculated for drug compounds and top 50 shortlisted aroma compounds by DS software. In the study PK results showed that all 13 drug showed FALSE (Non inhibition) binding with Cytochrome (CYP-2D) which shows that all the drugs will be easily metabolised in the body whereas out of selected 50 aroma compounds only six compounds *i.e.,* Curcumin, Hydroxybenzaldehy,

Table 3. Top 50 results of aroma compounds screened by docking virtual screening method, with higher docking score.

S. No	Number of docked aroma compounds	Docking score (kJ/mol)
1	1- O-Caffeoylquinic Acid	-44.05
2	2-Benzamidobenzoic Acid	-42.59
3	Ferulic Acid	-41.95
4	Caffeic Acid	-40.90
5	Camptothecin	-40.89
6	Lycorine	-39.19
7	Vanillic Acid	-38.11
8	Trans Cinnamic Acid	-37.51
9	Protocatechuic Acid	-37.41
10	Densiflorol	-36.63
11	P-Coumaric Acid	-36.35
12	Curcumin	-36.9
13	Phenylpropanoic Acid	-34.28
14	Perillic Acid	-34.15
15	Catechins	-34.12
16	Chlorigenic Acid	-33.63
17	Epicatechin Gallate	-33.62
18	Piperine	-33.61
19	Quercetin	-33.59
20	Myricitrin	-33.42
21	Cinnamic Acid	-33.41
22	Anisyl Formate	-32.39
23	Geranic Acid	-32.38
24	Oct 2 Enoic Acid	-30.83
25	Myristicin	-29.57
26	Biflorin	-29.46
27	Methylbutyric Acid	-29.40
28	Isovaleric Acid	-29.40
29	Musk Ambrette	-29.27
30	Velutin	-29.26
31	Morachalcone A	-29.22
32	Genkwanin	-29.21
33	Butyric Acid	-29.21
34	Methyl Pyrazine	-28.79
35	Hydroxybenzaldehy	-28.71
36	Safrole	-28.66
37	Luteolin	-28.64
38	Berberine	-28.62
39	Tiglic Acid	-28.55
40	Decanoic Acid	-28.48
41	Octanoic Acid	-28.36
42	Heptanoic Acid	-28.34
43	Nonanoic Acid	-28.12
44	Methyl Cinnamate	-27.73
45	Cinnamyl Formate	-27.57
46	Epicatechin	-27.32
47	Ellagic Acid	-27.23
48	Cadambine	-26.98
49	Benzyl_Formate	-26.86
50	Bakuchicin	-26.85

Table 4. Compliance of standard drugs with the standard range of computational pharmacokinetic parameters (ADMET).

S. No	Number of docked aroma compounds	Aqueous solubility level	BBB	CYP-2D	Hepato-toxicity	Intestinal absorption	PPB
1	Amsacrine	2	2	FALSE	TRUE	0	TRUE
2	Doxorubicin	2	4	FALSE	TRUE	3	FALSE
3	**Pirarubicin**	2	4	FALSE	TRUE	3	FALSE
4	Daunorubicin	2	4	FALSE	TRUE	3	FALSE
5	Amrubicin	2	4	FALSE	TRUE	3	FALSE
6	**Voreloxin**	3	3	FALSE	TRUE	0	FALSE
7	**Amonafide**	3	3	FALSE	TRUE	0	TRUE
8	**Phenoxodiol**	3	2	FALSE	TRUE	0	FALSE
9	**Ellipticine**	1	1	FALSE	TRUE	0	TRUE
10	Gatifloxacin	3	3	FALSE	TRUE	0	FALSE
11	Topotecan	3	3	FALSE	TRUE	0	FALSE
12	Irinotecan	2	4	FALSE	TRUE	0	FALSE
13	Etoposide	3	4	FALSE	TRUE	3	TRUE

Note: (**Solubility Level**: 1= very low; 2= low; 3= good); (**Intestinal Absorption level**: 0=Good; 1=Moderate; 2=Low; 3 =Very low 4=very low); (**Blood Brain Barrier (BBB)**: 0= very high; 1= high; 2= medium; 3= low 4=very low); (**Hepato-toxicity**: TRUE=bounded, FALSE=Poorly bounded); (**CYP-2D**: TRUE=inhibitor, FALSE=non inhibitor); (**Protein Plasma Binding (PPB)**: TRUE=bounded, FALSE=poorly bounded)

Berberine, Epicatechin, Ellagic Acid, and Cadambine shows TRUE (inhibitor) result otherwise remaining 44 aroma compound showed FALSE (Non inhibitor) results. The enzyme CYP-2D involved in the metabolism and activation of some drugs in the human body. Drugs that inhibit CYP-2D activity are likely to increase the plasma concentrations of certain medications.

On the other hand, evaluation through standard ADMET parameters aqueous solubility level showed differently, *i.e.,* seven aroma compounds have low, 22 showed good and rested 21 aroma compounds showed very good aqueous solubility level whereas standard drug compounds showed very low, low and good aqueous solubility level. The Blood-Brain Barrier (BBB) penetration level and intestinal absorption level showed mixed results, for BBB aroma compounds showed maximally high and medium, whereas intestinal absorption showed maximally good absorption except for few compounds.

The hepato-toxicity (liver toxicity) predictions of standard drug compounds and studied top 50 aroma compounds were also done, and the results showed that all 13 drugs have hepato-toxicity with true value, whereas aroma compounds showed a mixed form of toxicity results (Table 4 and 5). Most of the aroma compounds showed high plasma-protein binding (PPB), thus seems to be easily distributed in the body, as results showed that only 19 aroma compounds showed poor binding with plasma protein and 31 aroma compounds showed good binding, whereas out of 13 standard drugs maximum 9 drugs showed poor binding, and 4 drugs showed good binding with plasma protein. These comparative results of aroma compounds showed that the screened compounds represent good results in all pharmacokinetics aspect when compared to standard anti-cancer drugs which bound to DNA topoisomerase II.

CONCLUSION

In this study, we tried to identify the new potent anti neoplastic aroma compounds and detected their binding conformation on DNA topoisomerase II enzyme. The virtual screening based molecular docking studies reveal that short listed aroma compounds show higher binding energy when compared to standard anticancer drugs. Our study also reveals that the selected aroma compounds showed less toxicity comparable to standard drugs and also share same binding conformation where

Table 5. Predicted ADMET parameters of top 50 aroma compounds.

S. No.	Number of docked aroma compounds	Aqueous solubility level	BBB	CYP-2D	Hepato-toxicity	Intestinal absorption	PPB
1	1-O-Caffeoylquinic Acid	4	4	FALSE	FALSE	3	FALSE
2	2-Benzamidobenzoic Acid	3	1	FALSE	FALSE	0	TRUE
3	Ferulic Acid	3	3	FALSE	TRUE	0	TRUE
4	Caffeic Acid	4	3	FALSE	FALSE	0	FALSE
5	Camptothecin	3	3	FALSE	TRUE	0	FALSE
6	Lycorine	4	4	FALSE	TRUE	1	FALSE
7	Vanillic Acid	4	3	FALSE	FALSE	0	FALSE
8	Trans Cinnamic Acid	4	2	FALSE	FALSE	0	TRUE
9	Protocatechuic Acid	3	4	FALSE	TRUE	1	FALSE
10	Densiflorol	3	4	FALSE	TRUE	1	TRUE
11	P-Coumaric Acid	3	1	FALSE	FALSE	0	TRUE
12	Curcumin	2	1	TRUE	TRUE	0	TRUE
13	Phenylpropanoic Acid	3	1	FALSE	FALSE	0	TRUE
14	Perillic Acid	4	2	FALSE	FALSE	0	TRUE
15	Catechins	4	4	FALSE	FALSE	3	FALSE
16	Chlorigenic Acid	4	2	FALSE	FALSE	0	TRUE
17	Epicatechin Gallate	4	3	FALSE	FALSE	0	TRUE
18	Piperine	4	3	FALSE	TRUE	0	FALSE
19	Quercetin	3	1	FALSE	TRUE	0	TRUE
20	Myricitrin	3	1	FALSE	FALSE	0	TRUE
21	Cinnamic Acid	3	1	FALSE	FALSE	0	TRUE
22	Anisyl Formate	4	2	FALSE	FALSE	0	TRUE
23	Geranic Acid	4	2	FALSE	FALSE	0	TRUE
24	Oct-2-enoic acid	4	1	FALSE	FALSE	0	TRUE
25	Myristicin	3	1	FALSE	FALSE	0	TRUE
26	Biflorin	4	4	FALSE	TRUE	3	FALSE
27	Methylbutyric Acid	2	3	FALSE	TRUE	0	TRUE
28	Isovaleric Acid	3	3	FALSE	TRUE	0	TRUE
29	Musk Ambrette	3	1	FALSE	TRUE	0	TRUE
30	Velutin	3	3	FALSE	TRUE	0	TRUE
31	Morachalcone A	2	4	FALSE	TRUE	3	FALSE
32	Genkwanin	3	1	FALSE	FALSE	0	TRUE
33	Butyric Acid	4	2	FALSE	FALSE	0	FALSE
34	Methyl Pyrazine	2	4	FALSE	TRUE	1	TRUE
35	Hydroxybenzaldehy	3	4	TRUE	TRUE	0	FALSE
36	Safrole	3	3	FALSE	TRUE	0	FALSE
37	Luteolin	3	1	FALSE	FALSE	0	TRUE
38	Berberine	2	1	TRUE	TRUE	0	TRUE
39	Tiglic Acid	4	2	FALSE	TRUE	0	TRUE
40	Octanoic Acid	4	3	FALSE	FALSE	0	FALSE
41	Decanoic Acid	4	2	FALSE	FALSE	0	FALSE
42	Heptanoic Acid	4	2	FALSE	FALSE	0	TRUE
43	Nonanoic Acid	3	1	FALSE	FALSE	0	TRUE
44	Methyl Cinnamate	4	2	FALSE	TRUE	0	TRUE
45	Cinnamyl Formate	3	3	FALSE	FALSE	0	TRUE
46	Epicatechin	3	4	TRUE	TRUE	0	FALSE
47	Ellagic Acid	2	4	TRUE	TRUE	3	FALSE
48	Cadambine	2	4	TRUE	TRUE	3	FALSE
49	Benzyl Formate	4	2	FALSE	FALSE	0	TRUE
50	Bakuchicin	3	2	FALSE	TRUE	0	FALSE

Note: (Solubility Level: 1= very low; 2= low; 3= good, 4=very good); (Intestinal Absorption level: 0=Good; 1=Moderate; 2=Low; 3 =Very low); (Blood Brain Barrier (BBB): 0= very high; 1= high; 2= medium; 3= low); (Hepato-toxicity: TRUE=bounded, FALSE=Poorly bounded); (CYP-2D: TRUE=inhibitor, FALSE=non inhibitor); (Protein Plasma Binding (PPB): TRUE=bounded, FALSE=poorly bounded).

these drugs bind. This also reveals that studied aroma compounds will share same amino acid residues but with a higher binding affinity which may act as inhibition of DNA TOP2 enzyme to stop the cell proliferation and induce apoptosis. These results suggest that the virtually screened and selected top 50 aroma compounds may be further used for anticancer activity and will show good results.

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