Physicochemical, Druggable, ADMET Pharmacoinformatics and Therapeutic Potentials of Azadirachtin - a Prenol Lipid (Triterpenoid) from Seed Oil Extracts of Azadirachta indica A. Juss.

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Abstract

Azadirachtin (AZA) is the most abundant bioactive secondary metabolite (BASM) in neem seed oil extract (NSOE) of Azadirachta indica A. Juss. AZA is localised in different parts of the plant [seeds, fruits, flowers, leaves, stem, bark and root] however, with varying degree of concentration. It has been documented that maximum concentration of AZA is present to the tune of 48000 μg g⁻¹ in the seeds. It has been established that the environmental conditions determines the overall content and composition of BASM in different parts of the plant. Neem plant parts are most commonly used as therapeutic agents in remote villages in India for its ethnomedicinal therapeutic potentials; however, its physicochemical, druggable and pharmacological properties inadequately described. In the present study an attempt has been made to evaluate the physicochemical, druggable and pharmacological properties of Azadirachtin in NSOE of A. indica from ADMET perspectives.

Keywords: NEEM; Azadirachta indica; Azadirachtin; Pharmacoinformatics; ADMET; Drug-Likeness; Toxicology

INTRODUCTION

Azadirachta indica A. Juss commonly known as Neem or Margosa belongs to the family Meliaceae1-3. Popular as Miracle tree it is a natural store-house of phyto-drugs since the dawn of civilization4,5. This tree is one of the most versatile plant across the country and elsewhere known for its use in various Indigenous/ Traditional Systems of Medicine. A. indica has its origin from India and is commonly distributed in the South East Asian (SEA) Region (Bangladesh, Sri Lanka, Bhutan, Myanmar, Pakistan, and Nepal)6; however, it has been disseminated world over, in particular the tropical and sub-tropical regions7.

Neem is a perennial, small to medium-sized (10 - 15 m) and fast-growing tree and grows well in locations with temperature to a maximum of 48-50 °C, the plant needs low annual rainfall (400 – 800 mm/annum). Furthermore, the plant grows well in poor/ degraded/ mined soils. However, growth is affected by low temperature (poor growth below 14 °C) and frosts. Being the storehouse/ repository of wide array of BASM, Neem tree remains the ideal target of interest for research. As most of the BASM are localised in the leaves and seeds, destruction of whole plant is not required for the isolation/ extraction of bioactive principles. Furthermore, being perennial, annual replenishment of leaves and seeds prevents whole-plant harvest. BASM of Neem contains high proportion of water-soluble substances that favours DIY extraction and application in folklore medicine. Moreover, majority of these metabolites are eco-friendly bioactive compounds that are biodegradable in nature, adhere to GRAS standards, therefore harmless to man and environment8.

A. indica shows therapeutic potential in healthcare and management due to rich source of BASM9-11. The most important active constituent is azadirachtin, while others
include nimbonin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin. Leaves contain BASM such as nimbin, nimbanene, 6-desacyetylomninibenene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylegedunin, 17-hydroxyazadiradione, and nimbol[12-14]. Quercetin and β-sitosterol, polyphenolic flavonoids, obtained from fresh leaves have significant antibacterial and antifungal properties; while seeds are comparably rich in azadirachtin[14].

Since antiquity all parts of the plant, including root, stem, bark, leaves, fruits, and seeds are used to cure various ailments in humans and domestic animals therefore, Neem has been considered as a multi-purposes village dispensary[15-25]. In fact, therapeutic applications attributed to Neem include abortive, analgesic, antibacterial, anticancer, antiidiabetic, anti-fungal, anti-helminthic, anti-hyperglycemic, anti-inflammatory, antimalarial, antipyretic, antispasmodic, anti-spermagenic, antiviral, diuretic, hyper-choleremic, immuno-modulatory, mouth-wash, contraception, dental plaque, head lice, heart disease, insect repellent, malaria, pesticide, psoriasis, skin diseases, wound healing, gastrointestinal ailments[26-55].

Neem is influenced by a myriad of factors, namely geographic area, climate, genetic variability, agronomic conditions, plant morphology and physiology, collection and storage of plant material which determines the therapeutic potential. Further, this variation affects the development processes as regulation of secondary metabolite synthesis is directly linked to gene expression. This boils down to the fact that growth of Neem plant and the biochemical composition of the active principle is significantly influenced by external parameters. Kaushik et al[56], and Tomar et al[57], independently, analysed trees from different regions of India and observed significant difference in the AZA content of seeds collected in different regions. Furthermore, Kaushik et al[58] evaluated the effect of climatic conditions in the AZA content of seeds and indicated that AZA values of samples from semi-arid regions with mild winters were different from values observed in hot sub-humid, hot arid and hot semi-arid with cold winter regions. Similarly, Zheng et al[59] pointed out that season and ecosystem properties significantly affect neem seed oil yield and, in a less extent, AZA content. In fact, AZA quantity obtained in seed was significantly influenced by precipitation, with lower values observed in rainy season. Likewise, the procedure and time of collection of the plant material also influences AZA concentration in the seeds. In the case of seeds, AZA concentration is maximized when clean and healthy seeds are collected[59,60].

Indeed, it has been reported that mechanical damage, insect infestation and fungal infection of seeds significantly affect quantity and quality of AZA content. Since its isolation for the first time in 1968, AZA has been the subject of intense research, particularly of biological, synthetic and structural studies[61]. Azadirachtin - limonoid group of compound is a bioactive secondary metabolite present in neem seeds[12,13,26,27,56]. It is a highly oxidized tetrnorirtrpeneroid that asserts a plethora of oxygen-bearing functional group which includes an enol-ether, acetal, hemiacetal, tetrasubstituted epoxide structure with variety of carboxylic esters (Fig. 1). Increasing interest in AZA is mainly due to the unique biomolecular properties, including broad spectrum of activity even in trace amounts, no or low toxicity to mammals. Its complex structure makes its synthesis a daunting task. Biological activities attributed to AZA include application as a bioinsecticide, biopesticide, insect-pest repellent as it is non-toxicity to humans. Azadirachtin has been identified as potential inhibitor of SARS-CoV-2 main protease[62-64] and is expected to play a major role in the management of COVID-19. Furthermore, pharmacological characterization is expected to validate Azadirachtin as novel drug lead[65-68].

**MATERIALS AND METHODS**

| Class          | Equisetopsida C. Agardh |
|----------------|--------------------------|
| Subclass       | Magnoliidae Novák Ex Takht. |
| Superorder     | Rosanae Takht. |
| Order          | Sapindales Juss. Ex Bercht. |
| Family         | Meliaceae Juss. |
| Genus          | Azadirachta A. Juss. |
| Species        | Azadirachta indica A. Juss. |
| Common Name    | Neem |
| Vernacular Name| Vempu (Tamil) |

**Botanical Description:** Tree, up to 15 m tall. Branches glabrous; Leaves imparipinnate, pubinus at the base; leaflets alternate to opposite, 2.5 - 7.0 cm long, 1.5 - 4.0 cm broad; ovate, subelliptic, acuminate; Flowers white, sweet-scented; Sepals obovate, 1.5 mm long, puberulous, imbricate. Petals 6 mm long, obovate to oblong, white, margin ciliate; Staminal tube 5 mm long, puberulous, 10-striate, 10-toothed; teeth 2-lobed; anthers oblong, basifixed; Ovary sub-globose; style linear 2.5 mm long; stigma trifid. Fruit: Drupe oblong, 1.3 - 2.0 cm long, greenish-yellow, Seed: 1-seeded. Plants were collected from the fields in the wild Palani Hills, Western Ghats, INdia as described previously[33].

**GC-MS Analysis**

Neem Seed Oil Extracts of *A. indica* was obtained from the seed samples collected from the foothills of Alagar Hills, Alagarkovil Reserve Forest, Dindigul District, Tamil Nadu, India. Phyto-components were identified using GC-MS...
detection system as described previously, however with modification, whereby portion of the extract was analysed directly by headspace sampling. GC-MS analysis was accomplished using an Agilent 7890A GC system set up with 5975C VL MSD (Agilent Technologies, CA, USA). Capillary column used was DB-5MS (30×0.25 mm, film thickness of 0.25 μm; J&W Scientific, CA, USA). Temperature program was set as follows: initial temperature 50°C held for 1 min, 5°C per min to 100°C, 9°C per min to 200°C held for 7.89 min, and the total run time was 40 min. The flow rate of helium as a carrier gas was 0.811851 mL/min. MS system was performed in electron ionization (EI) mode with Selected Ion Monitoring (SIM). The ion source temperature and quadruple temperature were set at 230°C and 150°C, respectively. Identification of phytocomponents was performed by comparison of their retention times and mass with those of authentic standards spectra using computer searches in NIST 08L and Wiley 7n.l libraries.3,33

**ADMET Prediction**

PubChem database was applied to get the smiles structures of the natural compounds, and was further used for the ADMET prediction. The qualitative assessment of pharmacokinetics viz; absorption, distribution, metabolism, excretion and toxicity (ADMET) profile of selected compounds were predicted computationally by using SwissADME and toxicity prediction using TOPKAT (Accelrys, Inc., USA). QikProp develops and employs QSAR/QSPR models using partial least squares, principal component analysis and multiple linear regression to predict physicochemical significant descriptors.68-70.

**RESULTS AND DISCUSSION**

**Chemical kingdom**: Organic compounds  
**Super class**: Lipids and lipid-like molecules  
**Class**: Prenol lipids  
**Subclass**: Triterpenoids  
**PubChem Identifier**: 102146586  
**Synonyms**: Azadirachtin  
**Canonical SMILES**: CC(=O)O[C@@H]1O[C@@H]10C@@[C@@H][C@H](OC(=O)C)C@@[C@H]2OC(=O)C[C@@H]2C(C@@[C@H](OC(=O)C)C[C@H]2)C]C(C@@)(C)C(1C)C(C@@)2(1C)C1CCOC1C)C0  
**InChI Key**: YQSSXRVYCLMRM-SIUABMRBSA-N

**Physicochemical Properties**: The molecular weight of AZA was 720.72 (g/mol); the calculated LogP value was -0.20; LogD - 0.14; LogSw - -4.34. The total number of stereocenters in the molecule was 16; the stereo-chemical complexity of the molecule was 0.457; the calculated Ps3 value of AZA was 0.771; The overall calculated Topological polar surface area of AZA was 215.34(Å2). Likewise the calculated number of hydrogen bond donors in the molecule was 3; whereas the number of hydrogen bond acceptors was 16; the number of smallest set of smallest rings (SSSR) in the molecule analyzed was 2; the size of the biggest system ring in the molecule was 15; similarly, the total number of rotatable bonds in the molecule was 6; the number of rigid bonds was 38; the number of charged groups was 0; similarly the total charge of the compound was 0; the number of carbon atoms in the molecule was 35; whereas the number of heteroatoms in AZA was calculated as 16; the number of heavy atoms in the molecule was calculated as 51; the ratio between the number of non-carbon atoms and the number of carbon atoms in the compound was 0.46 (Fig. 2.3).

**Druggability Properties**: Lipinski’s rule of 5 violations of the molecule was 2; Veber rule was Low for the molecule; similarly Egan rule for the molecule was also Low; the Oral PhysChem score (Traffic Lights) for the molecule was recorded as 5; GSX’s 4/40.0 score for the molecule was Good; Pfizer’s 3/75 score for the molecule was Good; Weighted quantitative estimate of drug-likeness (QEDw) score for the molecule was 0.164; Solubility Forecast Index was Good and the solubility score was 9441.49.

**ADMET Properties**: Only when the ADME/Tox properties of a drug like compound are of high quality, and when the target has been validated, the compound could be developed into a pharma-drug. In silico drug-likeness evaluation of Azadirachtin for Human Intestinal Absorption (HIA+) value had a probability of 0.890; Blood Brain Barrier (BBB-) value for the molecule had a probability of 0.773; Caco-2 permeable (Caco2-) value for the molecule had a probability of 0.711 (Fig. 4); P-glycoprotein substrate (Substrate) value for the molecule had a probability of 0.835; P-glycoprotein inhibitor I (Inhibitor) value for the molecule had a probability of 0.672; P-glycoprotein inhibitor II (Non-inhibitor) value for the molecule had a probability of 0.534. CYP450 2C9 substrate (Non-substrate) value for the molecule had a probability of 0.857; CYP450 2D6 substrate (Non-substrate) - 0.872; CYP450 3A4 substrate (Substrate) - 0.714; CYP450 1A2 inhibitor (Non-inhibitor) - 0.887; CYP450 2C9 inhibitor (Non-inhibitor) - 0.845; CYP450 2D6 inhibitor (Non-inhibitor) - 0.944; CYP450 2C19 inhibitor (Non-inhibitor) - 0.833; CYP450 3A4 inhibitor (Non-inhibitor) - 0.770; CYP450 inhibitory promiscuity (Low CYP Inhibitory Promiscuity) - 0.886; Ames test (Non AMES toxic) - 0.756; Carcinogenicity (Non-carcinogens) - 0.946; Biodegradation (Not ready biodegradable) - 1.000; Rat acute toxicity (4.349 LD50, mol/kg) - PNA; hERG inhibition (predictor I) (Weak inhibitor) - 0.992; hERG inhibition (predictor II) (Non-inhibitor) - 0.569 respectively. Computational methods for analysing and estimating the toxicity of natural bioactive compounds are considered as useful tool for validation as it provides in-depth understanding of toxicogenomics. Therefore, determining the toxicity of BASM in-silico is warranted to identify their potential harmful effects on humans, animals, plants, besides the environment as in-vivo animal tests are constrained by time, ethical considerations, and financial burden. Data pertaining to the descriptors viz., Toxicity, Environmental toxicity, Tox21 pathway and Toxicophore Rules for Azadirachtin are summarized in Table
2. Furthermore, GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor, enzyme inhibitor score for AZA were calculated as -0.71; -1.51; -1.46; -0.67; -0.35 and -0.71 respectively (Fig. 3). Swiss Target Prediction towards Macrophage migration inhibitory factor, Heat shock protein (HSP 90-alpha), Kappa Opioid receptor, Mu opioid receptor, Delta opioid receptor, Thrombin, Squalene synthetase, Glycogen synthase kinase-3 beta, Glycogen synthase kinase-3 alpha, Protein kinase C alpha, Apoptosis regulator Bcl-X, HMG-CoA reductase, Zinc finger protein GLI1, Proto- oncogene c-JUN, Vanilloid receptor for the compound has been provided in Table 4. Chemical and biological investigations on Azadirachta indica bioactive compounds indicates that the compound is safe for use as a drug molecule3,7,12,22.

CONCLUSION

The present study is an example to insights into the broad scope of pharmacoinformatics to plant based natural product research with an emphasis on drug discovery. The study indicates that plant based natural products still possess an extraordinary challenge that has to be solved before taken for drug development. However, it is anticipated that as more quality data on natural product research, such as bioactivity, biomolecularinformatics, cheminformatics, toxicoinformatics integrated together with new algorithms and machine learning techniques to accelerate natural product based drug discovery. Furthermore, online databases serve as attractive sources for identifying novel natural product scaffolds with promising drug-like properties in NPs which is expected to accelerate the pace of Drug Discovery.

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The page contains numerous references and citations, which are extracted from the text. The references are cited in the text, and the list of references is structured in a bibliographic format. The text is dense with scientific information, indicating a thorough review or study on the subject. The references cover a wide range of topics, from immunomodulatory effects of neem to antidermatophytic activity of neem, and from traditional medicinal uses to modern molecular bases and therapeutic effects. The references are from a variety of journals and sources, indicating a comprehensive approach to the subject matter.
Table 1: Physicochemical, Medicinal Chemistry and ADMET properties of Aza

| Property            | Value         | Comment                          |
|---------------------|---------------|----------------------------------|
| Molecular Weight    | 720.26        | Contain hydrogen atoms. Optimal:100~600 |
| Volume              | 67.0289       | Van der Waals volume             |
| Density             | 1.075         | Density = MW / Volume             |
| nHA                 | 16            | Number of hydrogen bond acceptors. Optimal:0~12 |
| nHD                 | 3             | Number of hydrogen bond donors. Optimal:0~7 |
| nRot                | 10            | Number of rotatable bonds. Optimal:0~11 |
| nRing               | 8             | Number of rings. Optimal:0~6     |
| MaxRing             | 14            | Number of atoms i The biggest ring. Optimal:0~18 |
| nHet                | 16            | Number of heteroatoms. Optimal:1~15 |
| fChar               | 0             | Formal charge. Optimal:-4 ~4    |
| nRig                | 38            | Number of rigid bonds. Optimal:0~30 |
| Flexibility         | 0.263         | Flexibility = nRot / nRig        |
| Stereo Centers      | 16            | Optimal: £ 2                     |
| TPSA                | 215.34        | Topological Polar Surface Area. Optimal:0~140 |
| logS                | -3.837        | Log of the aqueous solubility. Optimal: -4~0.5 log mol/L |

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### 2. Medicinal Chemistry

| Property    | Value  | Comment |
|-------------|--------|---------|
| logP        | 1.306  | Log of the octanol/water partition coefficient. Optimal: 0~3 |
| logD        | 1.493  | logP at physiological pH 7.4. Optimal: 1~3 |

#### Property Value Comment

| QED         | 0.14   | A measure of drug-likeness based on the concept of desirability; Attractive: > 0.67; unattractive: 0.49~0.67; too complex: < 0.34 |
| SAscore     | 7.579  | Synthetic accessibility score is designed to estimate ease of synthesis of drug-like molecules. n SAscore > 6, difficult to synthesize; SAscore <6, easy to synthesize |
| Fsp3        | 0.771  | The number of sp3 hybridized carbons / total carbon count, correlating with melting point and solubility. n Fsp3 >0.42 is considered a suitable value. |
| MCE-18      | 215.065 | MCE-18 stands for medicinal chemistry evolution. n MCE-18 >34S is considered a suitable value. |
| NPscore     | 3.457  | Natural product-likeness score. n This score is typically i The range from -5 to 5. The higher the score is, the higher the probability that the molecule is a NP. |
| Lipinski Rule | Rejected | MW £ 500; logP £ 5; Hacc £ 10; Hdon £ 5 n If two properties are out of range, a poor absorption or permeability is possible, one is acceptable. |
| Pfizer Rule | Accepted | logP > 3; TPSA < 75 Compounds with a high log P (>3) and low TPSA (<75) are likely to be toxic. |
| GSK Rule    | Rejected | MW £ 400; logP £ 4 n Compounds satisfying the GSK rule may have a more favourable ADMET profile |
| Golden Triangle | Rejected | 200 £ MW £ 50; -2 £ logD £ 5 n Compounds satisfying the Golden Triangle rule may have a more favourable ADMET profile |
| PAINS       | 0 alerts | Pan Assay Interference Compounds, frequent hitters, Alpha-screen artifacts and reactive compounds. |
| ALARM NMR   | 1 alerts | Thiol reactive compounds. |
| BMS         | 0 alerts | Undesirable, reactive compounds. |
| Chelator Rule | 0 alerts | Chelating compounds. |

### 3. Absorption

| Property  | Value  | Comment |
|-----------|--------|---------|
| Caco-2 Permeability | -5.261 | Optimal: higher than -5.15 Log unit |
| MDCK Permeability     | 0.000138 | low permeability: < 2 × 10^-6 cm/s n medium permeability: 2~20 × 10^-6 cm/s n high passive permeability: > 20 × 10^-6 cm/s |
| Pgp-inhibitor        | 1      | Category 1: Inhibitor; Category 0: Non-inhibitor; The output value is the probability of being Pgp-inhibitor |
| Pgp-substrate        | 0.975  | Category 1: substrate; Category 0: Non-substrate; The output value is the probability of being Pgp-substrate |
| HIA                   | 0.66   | Human Intestinal Absorption Category 1: HIA+ (HIA < 30%); Category 0: HIA- (HIA < 30%); The output value is the probability of being HIA+ |
| F20%                  | 0.649  | 20% Bioavailability Category 1: F20%+ (bioavailability < 20%); Category 0: F20%- (bioavailability > 20%); The output value is the probability of being F20%+ |
| F30%                  | 0.905  | 30% Bioavailability Category 1: F30%+ (bioavailability < 30%); Category 0: F30%- (bioavailability > 30%); The output value is the probability of being F30%+ |

### 4. Distribution

| Property | Value  | Comment |
|----------|--------|---------|
| PPB      | 38.52% | Plasma Protein Binding n Optimal: < 90%. Drugs with high protein-bound may have a low therapeutic index. |
| VD       | 1.581  | Volume Distribution n Optimal: 0.04-20L/kg |
**BBB Penetration** 0.246  Blood-Brain Barrier Penetration Category 1: BBB+; Category 0: BBB-; The output value is the probability of being BBB+

**Fu** 37.25%  The fraction unbound in plasms n Low: <5%; Middle: 5~20%; High: > 20%

## 5. Metabolism

| Property       | Value | Comment                                                |
|----------------|-------|--------------------------------------------------------|
| CYP1A2 inhibitor | 0     | Category 1: Inhibitor; Category 0: Non-inhibitor; The output value is the probability of being inhibitor. |
| CYP1A2 substrate | 0.993 | Category 1: Substrate; Category 0: Non-substrate; The output value is the probability of being substrate. |
| CYP2C19 inhibitor | 0.017 | Category 1: Inhibitor; Category 0: Non-inhibitor; The output value is the probability of being inhibitor. |
| CYP2C19 substrate | 0.724 | Category 1: Substrate; Category 0: Non-substrate; The output value is the probability of being substrate. |
| CYP2C9 inhibitor | 0.014 | Category 1: Inhibitor; Category 0: Non-inhibitor; The output value is the probability of being inhibitor. |
| CYP2C9 substrate | 0.005 | Category 1: Substrate; Category 0: Non-substrate; The output value is the probability of being substrate. |
| CYP2D6 inhibitor | 0.003 | Category 1: Inhibitor; Category 0: Non-inhibitor; The output value is the probability of being inhibitor. |
| CYP2D6 substrate | 0.107 | Category 1: Substrate; Category 0: Non-substrate; The output value is the probability of being substrate. |
| CYP3A4 inhibitor | 0.719 | Category 1: Inhibitor; Category 0: Non-inhibitor; The output value is the probability of being inhibitor. |
| CYP3A4 substrate | 0.857 | Category 1: Substrate; Category 0: Non-substrate; The output value is the probability of being substrate. |

## 6. Excretion

| Property | Value | Comment |
|----------|-------|---------|
| CL       | 1.748 | Clearance n High: >15 mL/min/kg; moderate: 5-15 mL/min/kg; low: <5 mL/min/kg |
| T1/2     | 0.016 | Category 1: long half-life; Category 0: short half-life; long half-life: >3h; short half-life: <3h The output value is the probability of having long half-life. |

## 7. Toxicity

| Property            | Value | Comment                                                                 |
|---------------------|-------|-------------------------------------------------------------------------|
| hERG Blockers       | 0.03  | Category 1: active; Category 0: inactive; The output value is the probability of being active. |
| H-HT                | 0.342 | Human Hepatotoxicity Category 1: H-HT positive (+); Category 0: H-HT negative (-); The output value is the probability of being toxic. |
| DILI                | 0.32  | Drug Induced Liver Injury. Category 1: drugs with a high risk of DILI; Category 0: drugs with no risk of DILI. The output value is the probability of being toxic. |
| AMES Toxicity       | 0.844 | Category 1: Ames positive (+); Category 0: Ames negative (-); The output value is the probability of being toxic. |
| Rat Oral Acute Toxicity | 0.978 | Category 0: low-toxicity; Category 1: high-toxicity; The output value is the probability of being highly toxic. |
| FDAMDD              | 0.961 | Maximum Recommended Daily Dose Category 1: FDAMDD (+); Category 0: FDAMDD (-); The output value is the probability of being positive. |
| Skin Sensitization  | 0.005 | Category 1: Sensitizer; Category 0: Non-sensitizer; The output value is the probability of being sensitizer. |
Carcinogen city 0.976 Category 1: carcinogens; Category 0: non-carcinogens; The output value is the probability of being toxic.

Eye Corrosion 0.003 Category 1: corrosives; Category 0: non-corrosives The output value is the probability of being corrosives.

Eye Irritation 0.01 Category 1: irritants; Category 0: non-irritant The output value is the probability of being irritants.

Respiratory Toxicity 0.963 Category 1: respiratory toxicants; Category 0: respiratory non-toxicants The output value is the probability of being toxic.

8. Environmental toxicity

| Property                | Value | Comment                                                                 |
|-------------------------|-------|-------------------------------------------------------------------------|
| Bioconcentration Factors| 0.985 | Bioconcentration factors are used for considering secondary poisoning potential and assessing risks to human health via the food chain. The unit is $-\log_{10}\left(\frac{mg/L}{(1000*MW)}\right)$ |
| IGC50                   | 3.752 | Tetrahymena pyriformis 50 percent growth inhibition concentration The unit is $-\log_{10}\left(\frac{mg/L}{(1000*MW)}\right)$ |
| LC50FM                  | 5.88  | 96-hour fathead minnow 50 percent lethal concentration The unit is $-\log_{10}\left(\frac{mg/L}{(1000*MW)}\right)$ |
| LC50DM                  | 5.549 | 48-hour daphnia magna 50 percent lethal concentration The unit is $-\log_{10}\left(\frac{mg/L}{(1000*MW)}\right)$ |

9. Tox21 pathway

| Property | Value | Comment                                                                 |
|----------|-------|-------------------------------------------------------------------------|
| NR-AR    | 0.022 | Androgen receptor Category 1: actives; Category 0: inactives; The output value is the probability of being active. |
| NR-AR-LBD| 0.816 | Androgen receptor ligand-binding domain Category 1: actives; Category 0: inactives; Output value is probability of being active. |
| NR-AhR   | 0.014 | Aryl hydrocarbon receptor Category 1: actives; Category 0: inactives; The output value is the probability of being active. |
| NR-Aromatase | 0.773 | Category 1: actives; Category 0: inactives; The output value is the probability of being active. |
| NR-ER    | 0.209 | Estrogen receptor Category 1: actives; Category 0: inactives; The output value is the probability of being active. |
| NR-ER-LBD| 0.778 | Estrogen receptor ligand-binding domain Category 1: actives; Category 0: inactives; The output value is the probability of being active. |
| NR-PPAR-gamma | 0.923 | Peroxisome proliferator-activated receptor gamma Category 1: actives; Category 0: inactives; The output value is the probability of being active. |
| SR-ARE   | 0.711 | Antioxidant response element Category 1: actives; Category 0: inactives; The output value is the probability of being active. |
| SR-ATAD5 | 0.976 | ATPase family AAA domain-containing protein 5 Category 1: actives; Category 0: inactives; The output value is the probability of being active. |
| SR-HSE   | 0.769 | Heat shock factor response element Category 1: actives; Category 0: inactives; The output value is the probability of being active. |
| SR-MMP   | 0.968 | Mitochondrial membrane potential Category 1: actives; Category 0: inactives; The output value is the probability of being active. |
| SR-p53   | 0.999 | Category 1: actives; Category 0: inactives; The output value is the probability of being active. |

10. Toxicophore Rules

| Property                     | Value | Comment                                                                 |
|------------------------------|-------|-------------------------------------------------------------------------|
| Acute Toxicity Rule          | 0 alerts | 20 substructures; acute toxicity - oral administration                  |
| Genotoxic Carcinogenicity Rule | 8 alerts | 117 substructures; carcinogenicity or mutagenicity                      |
| Non-Genotoxic Carcinogenicity Rule | 1 alerts | 23 substructures; carcinogenicity through non-genotoxic mechanisms       |
| Skin Sensitization Rule      | 5 alerts | 155 substructures; skin irritation                                       |
Table 2 Summary of Physicochemical, Druggability, ADMET of AZA

| PROPERTY                                                                 | VALUE                      |
|-------------------------------------------------------------------------|----------------------------|
| **Physicochemical Properties**                                          |                            |
| Molecular weight                                                        | 720.72 g/mol               |
| LogP                                                                    | -0.20                      |
| LogD                                                                    | 0.14                       |
| LogSw                                                                   | -4.34                      |
| Number of stereo-centers                                                | 16                         |
| Stereocchemical complexity                                              | 0.457                      |
| Fsp3                                                                    | 0.771                      |
| Topological polar surface area                                          | 215.34 Å²                 |
| Number of hydrogen bond donors                                         | 3                          |
| Number of hydrogen bond acceptors                                      | 16                         |
| Number of smallest set of smallest rings (SSSR)                         | 2                          |
| Size of the biggest system ring                                         | 15                         |
| Number of rotatable bonds                                              | 6                          |
| Number of rigid bonds                                                  | 38                         |
| Number of charged groups                                               | 0                          |
| Total charge of the compound                                           | 0                          |
| Number of carbon atoms                                                 | 35                         |
| Number of heteroatoms                                                  | 16                         |
| Number of heavy atoms                                                  | 51                         |
| Ratio between The number of non-carbon atoms and the number of carbon atoms | 0.46                  |
| **Druggability Properties**                                            |                            |
| Lipinski’s rule of 5 violations                                         | 2                          |
| Veber rule                                                             | Low                        |
| Egan rule                                                              | Low                        |
| Oral PhysChem score (Traffic Lights)                                   | 5                          |
| GSK’s 4/400 score                                                      | Good                       |
| Pfizer’s 3/75 score                                                   | Good                       |
| Weighted quantitative estimate of drug-likeness (QEDw) score           | 0.164                      |
| Solubility                                                             | 9441.49                    |
| Solubility Forecast Index                                              | Good                       |
| **ADMET Properties**                                                   |                            |
| Property                                                                | Value                      | Probability   |
| Human Intestinal Absorption                                            | HIA+                       | 0.890         |
| Blood Brain Barrier                                                    | BBB-                       | 0.773         |
The physicochemical properties were computed using FAF-Drugs4 (28961788) and RDKit open-source cheminformatics platform. The druggability scoring schemes were computed using FAF-Drugs4 (28961788) and FAF-QED (28961788) open-source cheminformatics platform. ADMET features were predicted using admetSAR (23092397) open-source tool.

Table 3 Molecular Properties and of Bioactivity Score of AZA

| Property                        | Score |
|---------------------------------|-------|
| miLogP                          | 1.42  |
| TPSA                            | 215   |
| n-atoms                         | 51    |
| MW                              | 721   |
| n-ON                            | 16    |
| n-OHNH                          | 3     |
| n-violations                    | 2     |
| n-rotb                          | 10    |
| Volume                          | 612   |
| GPCR ligand                     | -0.71 |
| Ion channel modulator           | -1.51 |
| Kinase inhibitor                | -1.46 |
| Nuclear receptor ligand         | -0.67 |
| Protease inhibitor              | -0.35 |
| Enzyme inhibitor                | -0.71 |
| Target                                | Common name                                           | Uniprot ID | ChEMBL ID      | Target Class                              | Probability* | Known actives (3D/2D) |
|--------------------------------------|-------------------------------------------------------|------------|----------------|-------------------------------------------|--------------|----------------------|
| Macrophage migration inhibitory factor | MIF                                                   | P14174     | CHEMBL 2085    | Enzyme                                    | 0.06613      | 0 / 1                |
| Heat shock protein HSP 90-alpha      | HSP90AA1                                             | P07900     | CHEMBL 3880    | Other cytosolic protein                    | 0.06613      | 0 / 2                |
| Kappa Opioid receptor                | OPRK1                                                | P41145     | CHEMBL 237     | Family A G protein-coupled receptor        | 0.00         | 0 / 128              |
| Mu opioid receptor                   | OPRM1                                                | P35372     | CHEMBL 233     | Family A G protein-coupled receptor        | 0.00         | 0 / 35               |
| Delta opioid receptor                | OPRD1                                                | P41143     | CHEMBL 236     | Family A G protein-coupled receptor        | 0.00         | 0 / 21               |
| Thrombin                             | F2                                                   | P00734     | CHEMBL 204     | Protease                                  | 0.00         | 0 / 2                |
| Squalene synthetase (by homology)   | FDFT1                                                | P37268     | CHEMBL 3338    | Enzyme                                    | 0.00         | 0 / 28               |
| Glycogen synthase kinase-3 beta     | GSK3B                                                | P49841     | CHEMBL 262     | Kinase                                    | 0.00         | 0 / 1                |
| Glycogen synthase kinase-3 alpha    | GSK3A                                                | P49840     | CHEMBL 2850    | Kinase                                    | 0.00         | 0 / 1                |
| Protein kinase C alpha               | PRKCA                                                | P17252     | CHEMBL 299     | Kinase                                    | 0.00         | 0 / 1                |
| Apoptosis regulator Bcl-X           | BCL2L1                                               | Q07817     | CHEMBL 4625    | Other ion channel                         | 0.00         | 0 / 1                |
| HMG-CoA reductase                    | HMGCR                                                | P04035     | CHEMBL 402     | Oxidoreductase                            | 0.00         | 0 / 1                |
| Zinc finger protein GLI1             | GLI1                                                 | P08151     | CHEMBL 5461    | Transcription factor                      | 0.00         | 0 / 1                |
| Proto-oncogene c-JUN                 | JUN                                                  | P05412     | CHEMBL 4977    | Transcription factor                      | 0.00         | 0 / 2                |
| Vanilloid receptor (by homology)    | TRPV1                                                | Q8NER1     | CHEMBL 4794    | Voltage-gated ion channel                 | 0.00         | 0 / 1                |

Figure 1: Structure of Azadirachtin (AZA) molecule
Figure 2: Phytocompound physiochemical properties of Azadirachtin; a) 3D; b) 2D; c) Upper Limit Radar Map; d) Lower Limit Radar Map; e) Compound Properties Radar Map; f) Cumulative Radar Map.
Figure 3: Bioactivity properties and percentage distribution chart for Azadirachtin

Figure 4: Boiled Egg Model for Azadirachtin as drug candidate