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# INSILICO ANALYSIS WITH PHYTOQUINOLINES IN ANTI-INFLAMMATORY DRUG DISCOVERY

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## ABSTRACT

Non-steroidal anti-inflammatory drugs are used as analgesics and antipyretic. They aggravate many side effects, that should be focussed to give safety, by reducing their adverse effects. Naturally occurring quinoline compounds are compared for their physico-chemical properties, and their interactions identified with chosen biological targets. Study indicates favourable target binding and pharmacological properties. Phytocompounds are found potent as drugs, and comparable to standard drugs in target interactions. They will offer natural substituents for commonly used drugs, and help avert their side effects.

**Keywords: Inflammation, ligand, phytoquinoline, drug**

## INTRODUCTION

Immune system produces many pro-inflammatory and anti-inflammatory molecules as cytokines, the interleukin, to combat changing intrinsic biological conditions. [1,2]

NSAID (Non-steroidal anti-inflammatory drugs), are generally used to treat the cardinal signs of inflammation, as pain. They block the production of pain-signaling molecules. They interact with Cyclooxygenase (COX-2) type of intermediate molecules, in the pathway and relieve pain in joints, muscles and other soft tissues. The drugs which are non-specific to COX-2, also block related enzyme COX-1 but since they have an active protecting role in stomach lining, interaction of NSAIDs increases the risk of stomach ulcers, and gastrointestinal (GI)

bleeding. [3]

NSAIDs are specific or non-specific and include tropical and oral drugs. Time bound studies show difference in adverse effects, caused by NSAIDs. They are differentiated as non-selective, selective or partly selective NSAIDs, and can cause adverse effects in biological system. [4]

NSAID intake analyzed, based on automated prescription records among a population of 8423 participants showed, intake of these drugs increased the risk, than while refraining from their intake over a time. [5] The relative risk of developing Alzheimer's was found to decrease with increased time of usage, but the use of these drugs did not alter the risk of vascular dementia. [6]

NSAIDs inhibit prostaglandin synthesis and produce gastric erosion. Their intake causes a high risk of mucosal injury, through inhibition of COX, reversibly or irreversibly by Aspirin. This results in decreased mucosal Prostaglandins, which actively take part in maintaining gastric mucosal defense system. [7] The inhibition of prostaglandin synthesis from arachidonic acid by non-specific blocking of the enzyme cyclooxygenase, leads to vasoconstriction and reversible mild renal impairment in volume contracted states. If unopposed, this is found to lead to acute tubular necrosis and acute renal failure. Interstitial nephritis in presence or absence of nephritic syndrome, progressing to acute or chronic renal failure, is discovered. [8] The drugs used in rheumatological disorders, as analgesics and antipyretics, exhibit hepatotoxicity. Although uncommon, the condition appears to be more common with diclofenac and sulindac in particular. [9]

Two signalling pathways are found to be involved in functioning of Interleukine, the cytokines which involve in metabolic, regenerative and neural process. Classic signalling involve, simulation of target cells through membrane bound interleukine-6 recepor, activating Janus kinases, and tyrosine residues subsequently get phosphorylated. This pathway is identified to be involved in anti-inflammatory activities of interleukine-6. Soluble form of interleukine-6 receptor, help in widening of spectrum of interleukin-6 target cells through trans-signaling. [10]

The serine/threonine kinase, p38 Mitogen-Activated Protein (MAP) kinase, actively take part in inflammatory process. The á-form of p38 actively participate in biosynthesis of pro-inflammatory cytokines as interleukine (IL) or Tumor Necrosis Factor (TNF- á) at both the transcriptional and translational levels. Adequete modulation of production of map kinases, is found to provide effective therapies, in chronic inflammatory diseases. [11] Secondary

metabolites from Toddalia aculeata [12] were analyzed, for their binding characteristics in comparison to standard NSAIDs, used in inflammatory conditions.

Quinoline compounds and its derivatives belong to class of alkaloids which show pharmacological properties. [13-16] Quinoline derivaties are identified and extracted from Toddalia aculeata (Lopez root). Qualitative and quantitative analysis carried out, identified the compound to be predominantly present among the other phytochemical compounds obtained from the source. Toxicity studies show, the compounds exhibit no toxicity during the in vivo analysis of hepatotoxicity. [17] QSAR (Quantitative Structure Activity Relationship) based analysis, identified quinoline compounds as potential pharmacophores. [18]

These phytochemical compounds, are compared with the standard inflammatory drugs to determine their suitability, as potential anti-inflammatory compound. The interaction of compounds with targets are found favourable, in comparison to interaction of other drug molecules.

**MATERIALS AND METHODS**

Biological activity is balanced, through activation of pathways involving pro-inflammatory and anti-inflammatory molecules, as cytokines. Biological targets, Interleukine-6 (IL-6) and p38MAP kinase are chosen for interaction analysis. Target molecules are taken as three dimensional coordinates from PDB.

The solution structure of recombinant human interleukine-6 (1IL6) determined through NMR spectroscopy, and p38 MAP Kinase (1KV1), which plays critical role in regulating production of pro-inflammatory cytokines as tumor necrosis factor and interleukine-1, are taken. The macromolecules are separated from their ligands, and structures energy minimized for further analysis.

COX-2 plays critical role in inflammatory diseases and cancer. COX enzyme converts arachidonic acid to common intermediate, during the synthesis of prostaglandins and thromboxane. Prostaglandins, derived from arachidonic acid is identified to sustain homeostatic functions, and takes part in pathogenic mechanisms, including inflammatory responses. Their biosynthesis is blocked by NSAIDs, which include those that are selective in inhibiting COX-2. Prostaglandins, are found to be involved in the system as pro-inflammatory and anti-inflammatory molecule. [19]

Anti-inflammatory molecules, NSAIDs were chosen from drug database. [20] They contribute to about half of the used analgesics, in treating pain and inflammation. Their long-term use cause adverse side effects that lead to stomach ulcers

etc. From among the drugs many anti-inflammatory drugs, which are administered or were in use, are screened. [21,22]

Quinoline derivatives are identified to have anti-inflammatory properties and analgesic properties. They posses medicinal properties in comparison to characteristics studied, against available drug compounds. [23-25] Phytoquinolines from Toddalia aculeata (TA), are focussed in the study. [18] The structure of the compounds are modelled, [26] and ligand database prepared consisting of these derivatives along with standard drugs, for inflammatory diseases. The structural determinants are identified for the compounds. [27,28].

Molecular weight, hydrogen bonds, polar surface area, molar volume are determined. Lipophilic measurement is compared for different compounds at a pH of 5.5 and 7.4. Polar surface area and solvent accessible surface area are determined, for all compounds. General characteristics as mass, vapour pressure, enthalpy of vaporization molar refractivity are compared, notable differences among compounds identified, in comparison to standard drugs used for inflammation. The different filters computed include Lipinski's rule, Ghose filter, Lead likeness, Muegge filter and Veber filter. Pharmacokinetics based on bioavailability is compared among compounds.

Biochemical properties are determined, through analysis of interaction of compounds with biological targets of inflammation. [29] The stability in complex formation, are compared based on binding energy. Change in free energy, exhibited during complexation of phytoquinoline compounds, in comparison to the NSAID complexes are determined. Interface interactions are analysed and interface components compared, including interaction between ligands and biological targets. Investigation of the number of atomic contacts between ligands and target is done, and number of interactions within a distance of 1 Angstrom is determined, to get the contacts that effect interaction to produce biological response. [30-33]

Results indicate a significant variation based on their structural and interaction parameters, for the compounds.

**RESULTS AND DISCUSSION**

Plant extract contain two phytoquinolines that exhibited druggable properties, comparable with standard inflammatory drugs as:

- N-methyl-4-hydroxy-7-methoxy-3-(2,3-epoxy-3-methylbutyl)-1H-quinolin-2-one (TA QCpd 1)
- 3-(2,3-Dihydroxy-3-methylbutyl)-4,7-dimethoxy-1- methyl-1H-quinolin-2-one (TAQCpd2).

Physico-chemical properties of compounds are

high with respect to mass, enthalpy of vaporizaiton, molecular refractivity. However, compounds like celecoxib, diclofenac, esomeprazole, indomethacin, and prioxicam also show equal or higher mass. Only few drug molecules as diclofenac, nabumetone have relatively lower mass. The enthalpy of vaporization is higher for compounds celecoxib, esomeprazole, etodolac, indomethacin, ketorolac, piroxicam in comparison to phytoquinolines. Molecular refractivity is relatively high in case of celecoxib, esomeprazole, etodolac, indomethacin and piroxicam. Celecoxib, esomeprazole, indomethacin and piroxicam, are taken for their similarity to phytoquinolines based on the physico-chemical properties. Their properties are comparable with relatively high mass, enthalpy of vaporization and molecular refractivity, in relation to the other compounds. These compounds show no violation of the Lipinski's rule of drug-likeness. [Table1]

**Table 1: Structural-properties of compounds taken in complexation**

| | ASPIRIN | CELECOXIB | DICLOFENAC | ESOMEPRAZOLE | ETODOLAC | IBUPROFEN | INDOMETHACIN | KETOROLAC | NABUMETONE | NAPROXEN | PIROXICAM | SALSALATE | TAQ.Cpd1 | TAQ.Cpd2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Average mass (Da) | 180.16 | 381.37 | 296.15 | 345.42 | 287.35 | 206.28 | 357.79 | 255.27 | 228.29 | 230.26 | 331.35 | 258.23 | 289.33 | 321.37 |
| Density (g/cm3) | 1.3±0.1 | 1.4±0.1 | 1.4±0.1 | 1.4±0.1 | 1.2±0.1 | 1.0±0.1 | 1.3±0.1 | 1.3±0.1 | 1.1±0.1 | 1.2±0.1 | 1.5±0.1 | 1.4±0.1 | 1.3±0.1 | 1.3±0.1 |
| Enthalpy of Vaporization (kJ/mol) | 59.5±3.0 | 80.4±3.0 | 70.1±3.0 | 89.3±3.0 | 81.9±3.0 | 59.3±3.0 | 80.8±3.0 | 80.1±3.0 | 61.8±3.0 | 69.1±3.0 | 89.8±3.0 | 78.8±3.0 | 72.2±3.0 | 83.3±3.0 |
| Molar Refractivity (cm3) | 44.5±0.3 | 91.9±0.5 | 76.5±0.3 | 94.0±0.4 | 82.0±0.3 | 60.8±0.3 | 94.6±0.5 | 70.5±0.5 | 69.6±0.3 | 66.5±0.3 | 85.4±0.5 | 66.7±0.3 | 77.5±0.3 | 85.6±0.4 |

Environmental properties of molecules, as polarizability, polar surface area, solvent accessible surface area, log P, log D done, show phytoquinoline compound 2 from Toddalia aculeata(TAQCpd2) exhibit lowest logP value, which is lesser than logP of aspirin molecule. Esomeprazole, ketorolac, nabumetone, naproxen, piroxicam, TAQCpd1 had optimum values for oral bioavailability. Aspirin, TAQCpd2 has lower partition coefficient, which is lesser than that of the other compounds but greater than 1.

Lipophilicity distribution is same, at different pH in case of TAQCpd2 and nabumetone. TAQCpd1 shows the same amount of variation of lipophilicity distribution as diclofenac. Other compounds as etodolac, naproxen, ibuprofen and ketorolac, also exhibit equal change in lipophilic distribution. These characteristics would enable good interaction of compounds with the targets. [Table 2]

**Table 2: Physico-chemical of compounds taken in complexation**

| | ASPIRIN | CELECOXIB | DICLOFENAC | ESOMEPRAZOLE | ETODOLAC | IBUPROFEN | INDOMETHACIN | KETOROLAC | NABUMETONE | NAPROXEN | PIROXICAM | SALSALATE | TA Q.Cpd1 | TA Q.Cpd2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ACD/LogP | 1.19 | 4.21 | 4.06 | 2.17 | 3.59 | 3.72 | 3.11 | 2.08 | 2.82 | 3.00 | 2.23 | 3.05 | 2.15 | 1.14 |
| ACD/LogD (pH 5.5) | -0.60 | 3.24 | 3.14 | 2.03 | 2.23 | 2.25 | 2.47 | 1.34 | 2.92 | 2.24 | 0.65 | 1.18 | 1.10 | 1.39 |
| ACD/LogD (pH 7.4) | -1.69 | 3.23 | 1.37 | 2.08 | 0.44 | 0.45 | 0.75 | -0.44 | 2.92 | 0.45 | 0.46 | 0.44 | -0.67 | 1.39 |
| Polar Surface Area (Å2) | 64 | 86 | 49 | 96 | 62 | 37 | 69 | 59 | 26 | 47 | 111 | 84 | 62 | 79 |
| Polarizability (10-24cm3) | 17.7±0.5 | 36.4±0.5 | 30.3±0.5 | 37.3±0.5 | 32.5±0.5 | 24.1±0.5 | 37.5±0.5 | 28.0±0.5 | 27.6±0.5 | 26.4±0.5 | 33.8±0.5 | 26.4±0.5 | 30.7±0.5 | 33.9±0.5 |

Complexes are prepared from the minimized structures of ligand and receptor, by docking small molecules from the ligand dataset, with interleukine and MAP kinase proteins. Docking was found to be stable for all complexes. Free energy of binding (dG) and full fitness score were calculated and the most stable docking conformation for each ligand complex was obtained. An estimate of free energy of binding when low, indicate higher affinity in binding.

Free energy of binding (dG) and Full fitness score were taken for complexes, and lowest energy conformation of the ligands docked to receptors, are obtained. Binding energy based on complexes formed by each ligand chosen, show least change in free energy for complexes formed by interleukine-6 (IL6 ie 1IL6) for salsalate, celecoxib, ibuprofen and naproxen. Among MAP kinase complexes, iburprofen formed the most stable complex. However stability of interaction, was comparable to stability in binding, exhibited by all other MAP kinase complexes. Interleukine complexes formed by Toddalia compounds, were found to be more stable in complex than Aspirin.

Although TAQCpd1 exhibits stability in interaction in complex with IL-6, and less change in free energy, the interaction of TAQCpd2 is found better and comparable, to the most stable interactions among compounds.

Among the p38 MAP kinase complexes (1KV1), the maximum change in free energy was exhibited by complexes formed by etodolac. The change in free energy is found maximum for complexes diclofenac, ibuprofen. TAQCpd1 exhibited relatively high energy in comparison to other compounds while interacting with MAP kinase target. The interaction is however stable, and change in free energy is high in comparison to change exhibited by other molecules.

TAQCpd2 obtained from the source, exhibites similarity in interaction, and energy of formation of complex with MAP kinase and free energy change exhibited during complexation, is comparable to that exhibited by other drug molecules among the dataset.

**Table 3: Energy differences of complexes formed with interleukine-6 and MAPKinase receptors**

| Ligands | 1IL6 | | 1KV1 | |
|---|---|---|---|---|
| | FullFitness (kcal/mol) | Estimated ΔG (kcal/mol) | FullFitness (kcal/mol) | Estimated ΔG (kcal/mol) |
| Aspirin | -1456.78 | -5.74 | -1882.1 | -7.16 |
| Salsalate | -1480.37 | -10.53 | -1862.84 | -7.88 |
| Celecoxib | -1502.06 | -9.79 | -1898.86 | -7.65 |
| Quinoline compound1 | -1216.65 | -5.25 | -1651.17 | -7.44 |
| Quinoline compound2 | -1431.69 | -7.87 | -1839.3 | -7.72 |
| Diclofenac | -1456.42 | -8.95 | -1843.87 | -8.37 |
| Esomeprazole | -1462.7 | -8.16 | -1854.79 | -7.26 |
| Etodolac | -1499.48 | -8.28 | -1910.79 | -8.14 |
| Ibuprofen | -1509.3 | -10.31 | -1881.58 | -8.82 |
| Indomethacin | -1444.53 | -8.55 | -1833.82 | -8.01 |
| Ketorolac | -1450.24 | -8.37 | -1840.45 | -7.28 |
| Nabumetone | -1465.24 | -7.55 | -1860.89 | -7.12 |
| Naproxen | -1478.21 | -9.99 | -1866.81 | -6.61 |
| Piroxicam | -1435.72 | -6.32 | -1861.04 | -8.05 |

The interface of complex formed by interleukine and p38MAP kinase contain, ligand interaction with basic residues as lysine and arginine, Imino group proline and also threonine. Glutamic acid interactions, were present in complexes formed by interleukine. Based on binding site interaction, complexes were differentiated.

**Interleukine interaction for phytochemical (TAQCpd1) from Toddalia is through glutamic acid at 173rd position, and the binding site interaction is similar as that of compound esomeprazole. The phytoquinoline2 (TAQCpd2) interacted at the same binding site through the target residue arginine, at 169th position.** MAPkinase complexes of TAQCpd1 shared same binding site with celecoxib, and nabumetone. The ligand protein interaction was through lysine 165 in the plant compound (TAQCpd1) and nabumetone. Celecoxib interacted with interleukine through proline 352 at the binding site of target. TAQCpd2 shared same binding position of naproxen, piroxicam, indomethacin, ibuprofen and salsalate. TAQCpd2 interacted with target through arginine residue at 94th position, while piroxicam interaction involved threonine residue at 91st position. All other compounds interacted through arginine residue

at 5th position in the binding site. [Fig 1(a-f)]

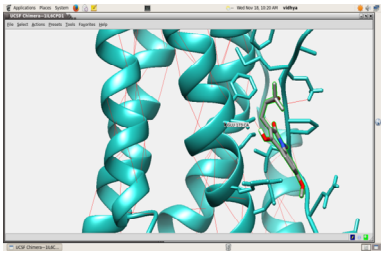

Fig 1(a)

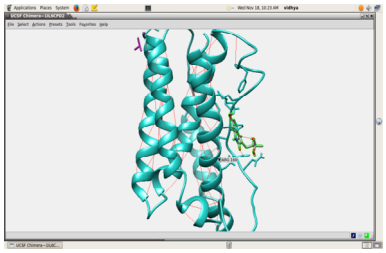

Fig 1(b)

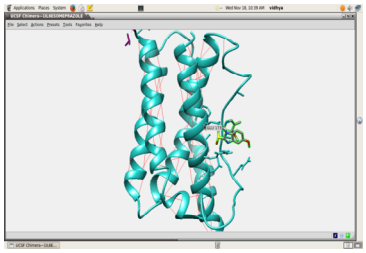

Fig1(c)

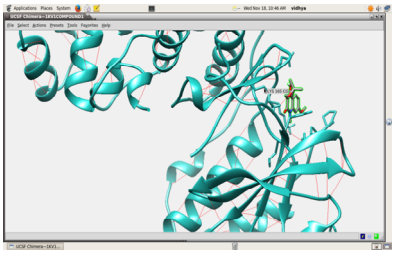

Fig 1(d)

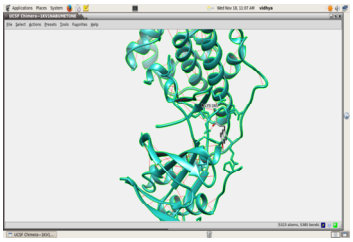

Fig1(e)

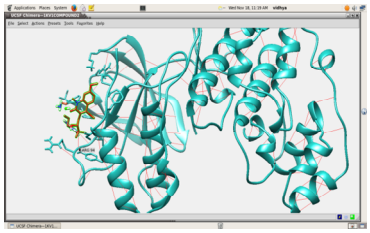

Fig 1(f)

**Fig 1: a) TAQCpd1 and 1IL6-Glu173, b) TAQCpd2 and 1IL6- Arg169 c) Esomeprazole and 1IL6-Glu173, d) TAQCp1 and 1KV1- Lys165 e) Nabumetone and 1KV1- Lys165 f) TAQCpd2 and 1KV1- Arg94**

## CONCLUSION

Resources obtained from natural sources provide a variety of application, whether in medicine or other purpose. Plant secondary metabolites used as medicine, are obtained from various plants. Quinoline derivatives present in Toddalia aculeata studied for molecular characteristics, show potential to give effective alternative to standard anti-inflammatory NSAIDs. Structural properties and various physico-chemical properties of these molecules are found to be in accordance, with the rules to decide drug-likeness of the molecules. Interaction of molecules with chosen biological targets, are found stable and atomic interactions formed, are comparable to interactions formed by standard drug molecules. Parametric analysis of compounds, identify quinolines from Toddalia aculeata, to be successful in binding to inflammatory targets, in comparison to standard drug compounds. The adverse effects of currently used medicines can be averted, with the use of these derivatives, which are identified with anti-inflammatory properties.

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**CONFLICT OF INTEREST**

Conflicts of interest declared none.

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