QSAR, ADME and docking guided semi-synthesis and *in vitro* evaluation of 4-hydroxy- α -tetralone analogs for anti-inflammatory activity

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Abstract

The compound 4-hydroxy- α -tetralone (**1**) is major bioactive secondary metabolite of genus *Ammannia* (Family- Lythraceae). The compound **1** and its various derivatives are reported to possess anti-tubercular, anti-diabetic, anti-leishmanial and bioenhancing activities. A quantitative structure activity relationship (QSAR) model for predicting anti-inflammatory activity of 4-hydroxy- α -tetralone derivatives against the tumour necrosis factor (TNF)- α , a pro-inflammatory cytokine was developed by non-linear model using artificial neural network (ANN). The regression coefficient (r^2) and the leave-one-out cross-validation regression coefficient (LOO r_{CV}^2) of the QSAR model were 0.6976 and 0.4016, respectively, while the regression coefficient (r^2) for the external set of experimental compounds was 0.835. The 4-hydroxy- α -tetralone virtual derivatives, which showed significant inhibition of TNF- α were subjected to docking and *in-silico* absorption, distribution, metabolism and excretion (ADME) studies and the results showed similar binding affinity and bioavailability in compliance with the standard drug, Diclofenac. Finally, in order to validate the developed QSAR model, the most and least active virtual derivatives were semi-synthesized, characterized on the basis of their ¹H and ¹³C NMR spectroscopic data and *in vitro* tested for concentration dependent inhibition of TNF- α . The experimental results obtained, agreed well with the predicted values.

Keywords QSAR · Docking · Anti-inflammatory · TNF- α · *Ammannia baccifera* · *In vitro* studies

1 Introduction

Inflammation is a natural defence mechanism caused by a variety of stimuli including microbial invasion, physical damage, ultra-violet irradiation and immune reactions [1,

2]. Inflammation cascades can lead to variety of diseases like chronic asthma, rheumatoid arthritis, osteoarthritis, multiple sclerosis, inflammatory bowel disease (IBD), and psoriasis prevailing in aging society of entire world [3–5]. Osteoarthritis, a chronic inflammatory disease is

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characterized by loss of joint cartilage leading to pain and loss of function, primarily in the knees and hips [6, 7]. It is one of the most common types of arthritis affecting 9.6% of men and 18% of women aged more than 60 years [7]. WHO projects osteoarthritis to be the fourth leading cause of disability in aging population of the world by the year 2020 [8]. Moreover, it is now globally accepted that chronic inflammation is one of the major risk factors for development of various types of cancer [3, 9]. Diverse reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as superoxide anion (O_2^-), hydroxyl radical (OH), hydrogen peroxide (H_2O_2), nitric oxide (NO), and singlet oxygen (1O_2) generated by inflammatory cells, injure cellular biomolecules including nucleic acids, proteins and lipids, causing cellular and tissue damage, which in turn augments the state of inflammation [2, 5, 10]. Immune cells are particularly important in inflammation because they play the key role in release of several mediators of defence process such as enzymes, cytokines, prostaglandins and nitric oxide [2, 5]. However, uncontrolled production of these mediators is associated with tissue damage due to oxidative stress [11, 12]. Tumor necrosis factor (TNF)- α is a cytokine, involved in systemic inflammation that stimulate the acute phase reaction. Primarily, TNF- α is produced by immune cells such as monocytes and macrophages in response to immunological challenges like microorganisms, microbial products (viz. endotoxins), lipopolysaccharide (LPS) and lymphokines [12, 13]. TNF- α play important role in antitumor activity, immune modulation, inflammation, anorexia, cachexia, septic shock, viral replication and hematopoiesis [13–15]. Thus, TNF- α is a central regulator of inflammation, and TNF- α antagonists may be effective in treating inflammatory disorders [15, 16]. LPS is a major component of the cell wall of gram-negative bacteria, known to stimulate the macrophage secretion of nitric oxide and inflammatory cytokines such as TNF- α and interleukin-1-beta (IL-1-beta) [17, 18]. Both steroidal and non-steroidal class of drugs are popularly used for treatment of inflammation. The common nonsteroidal anti-inflammatory drugs (NSAIDs), such as Aspirin, Diclofenac, Ibuprofen, Indomethacin etc. as well as corticosteroids are associated with several adverse effects [4, 19, 20]. Corticosteroids are common treatment for rheumatoid arthritis and IBD's, but their long time use cause some serious adverse effects, including hypertension, hyperglycemia, muscular weakness, increased susceptibility to infection, osteoporosis, glaucoma, psychiatric disturbances, and growth arrest [20, 21].

The natural product based remedy, mainly plants has been used since the beginning of human civilization as folklore or as orderly-regulated systems of indigenous medicines viz. traditional Chinese medicine, Ayurveda, Kampo, traditional Korean medicine and Unani [22, 23]. Due to existence of diverse range of pharmacologically

active chemical entities, plants are still of continued interest to modern pharma scientists because compounds existing in plants have better compatibility to human body, hence lesser side effects [22–24]. There are several records in traditional medicine on use of medicinal plants in case of pain and inflammation, which was further verified by isolation of anti-inflammatory drugs from plants like aspirin [4, 25]. Several other molecules of plant origin are either being used as a drug or in clinical trials as promising targets to treat inflammatory diseases. Some of them are Epigallocatechin-3-gallate from *Camellia sinensis*, Triptolide from *Tripterygium wilfordii*, Boswellic acid from *Boswellia serrata*, Andrographolid from *Andrographis paniculata*, Curcumin from *Curcuma longa*, colchicines from *Colchicum autumnale*, capsaicin from *Capsicum* species and Honokiol from various *Magnolia species* [4, 5, 25]. The plant secondary metabolites mainly phenolics act as reducing agents either by donating hydrogen atom and/or by quenching singlet oxygen to remove reactive oxygen species (ROS) from living system, justifying their antioxidant activities [11]. Further, these natural antioxidants regulate the production of pro-inflammatory mediators TNF- α , leukocyte adhesion, and nitric oxide (NO) playing a crucial role in immune-inflammatory response [14, 15]. Thus, natural product based novel leads offer a great hope for the development of valid alternative to existing drugs with cost-effectiveness and lesser side effects for treating inflammatory diseases.

QSAR, ADME and docking have become inexorably embedded as essential tools in the pharmaceutical industry, from lead discovery, optimization to the lead development and computer-aided drug designing [26]. A growing trend is to use these modern techniques in early stage of drug discovery process as a screening and enrichment tool to eliminate the compounds lacking drug like properties from further development process [26, 27]. The structurally related properties of a compound can be determined by computational mean much more efficiently in quicker and easier mode than its biological activity ($LD_{50}/IC_{50}/ED_{50}$) using *in vivo* or *in vitro* approaches. A statistically validated QSAR model is capable of predicting the biological activity of a new compound within the same series many times faster than the intensive processes of chemical synthesis and biological evaluation. When applied judiciously, QSAR, ADME and docking can save a substantial amount of time, money and human resources [28].

The compound 4-hydroxy- α -tetralone (**1**) and its glucoside (**2**) have been isolated as a major bioactive compound from various species of *Ammannia* (Lythraceae) [29–31]. In continuation of our efforts to explore the biological potential of 4-hydroxy- α -tetralone derivatives [32–34], the compounds (**1** & **2**) were isolated from *A. baccifera*; a plant of high medicinal value used in herbal

formulations in cancer, spinal disease, human female infertility, chronic tonsillitis, pelvic inflammatory disease, treatment of bladder stones, urinary tract infections, dermatitis. The isolated tetralone derivatives (**1** & **2**) showed significant inhibition of TNF- α , suggesting for the possible use of **1** and its various analogs as anti-inflammatory drugs. This prompted us to develop and validate a QSAR model for predicting anti-inflammatory activity of 4-hydroxy- α -tetralone derivatives against the TNF- α . A total of 28 virtual analogs of 4-hydroxy- α -tetralone were screened using this developed QSAR model, and the model predicted that a few analogues possess high, while others possess low anti-inflammatory activity. To validate the predictions made by the model, semi synthesis of few highly and low active analogs was carried out in the wet lab, and experimentally evaluated there *in vitro* anti-inflammatory activity by quantification of TNF- α . In this way, 4-hydroxy- α -tetralone analogs can be designed with enhanced anti-inflammatory activity using QSAR model and *in silico* pharmacokinetics compliance (ADME).

2 Materials and methods

2.1 *In silico* studies

2.1.1 Molecular descriptors and structure cleaning

The 2D structures of various tetralone derivatives drawn with ChemDraw-Ultra, were transformed into 3D and geometry was cleaned using ChemBioDraw-Ultra-v12.0 (<http://www.cambridgesoft.com>). Energy minimization was performed with MM2/MM3 molecular mechanics parameter until achieving the lowest stable energy (<0.001 kcal/mol) [35]. The 3D chemical structure of standard drug Diclofenac (DB00586) was retrieved from the PubChem compound database at NCBI (<http://www.pubchem.ncbi.nlm.nih.gov>). Crystallographic 3D structures of target protein TNF- α (PDB: 2AZ5) was retrieved from the Brookhaven Protein Databank (<http://www.pdb.org>). Molecules for dataset of QSAR model were collected from Niwayama et al. 1996 [38] with molecule IDs: ChEMBL468, ChEMBL11440, ChEMBL88935, ChEMBL99900, ChEMBL105402, ChEMBL275009, ChEMBL319245, ChEMBL319803 and ChEMBL330174 as known inhibitors of TNF- α . The PaDEL descriptor software (National University of Singapore) was used to calculate molecular structure descriptors, which contain CDK-library to calculate descriptors.

2.1.2 ANN based non-linear QSAR modelling

In the present study, percentage inhibition activity pattern against TNF- α was captured in the form of QSAR model. The molecular properties and their respective bioactivity values have been fitted into the polynomial equation map. A series of data generated from the equations has been used as input and output value for analysis in the back propagation neural network (BPNN) model. In order to build reliable and predictive QSAR model, we adopted the ANN technique, which has been proven to have outstanding non-linear approximation ability [36, 37]. In ANN architecture, the neurons are arranged in layers (input, hidden and output), and unidirectional connections. The adjacent layers are fully connected but no connections exist between the neurons within the same layer. The signals are propagated from the input neurons through the hidden layer to the output neuron, and then the error is calculated and back propagated to iteratively adjust weights and biases in order to minimize the error in prediction; this is the most distinct character of typical back propagation (BP) algorithm. The ANN program used was the neural network software package of MATLAB v2012 developed by the MathWorks, USA (<http://www.mathworks.com/>). Some fully connected 3-layer BP neural networks with sigmoid transfer function were constructed. The number of neurons in the input layer equalled the number of selected descriptors. Before the network training, all of the input and output values were normalized to between 0.1 and 0.9, and the outputs were transferred back to the same units as the original outputs for comparison purpose. The Levenberg–Marquardt algorithm was adopted to optimise weights and biases because it was significantly faster than other algorithms based on gradient descent [36, 37]. The experimental dataset as well as independent external testing set was used to evaluate the predictive ability of the models. Performance of the model has been evaluated by the regression coefficients as: R^2 for training data, R^2 for LOO cross validation, R^2 for optimization set, R^2 for external set experimental compounds.

2.1.3 Molecular interaction and bioavailability study

Docking study was performed with Autodock Vina v0.8 (Molecular Graphics Lab at The Scripps Research Institute, La Jolla, CA 92037, USA) with Lamarckian Genetic algorithm for grid based searching of most optimal binding pose of tetralones and Diclofenac with anti-inflammatory target TNF- α following earlier described procedures [12, 35]. Bioavailability was screened through absorption, distribution, metabolism and excretion (ADME) parameters [12, 35].

2.2 Chemistry

2.2.1 General experimental procedures

The 300 MHz NMR (Avance, Bruker, Switzerland) was used to record ^1H and ^{13}C NMR with tetramethylsilane (TMS) as internal standard. Hyphenated LC-PDA-MS (Prominence LC and mass MS-2010EV, Shimadzu, Japan) was used for mass spectra. TLC plates (silica gel 60F₂₅₄, Merck) were first examined under UV illumination at 254 and 365 nm and then sprayed with vanillin-sulphuric acid (1: 5, w/v) solution in ethanol followed by heating at 95 °C for 5 min. The solvents used in extraction and fractionation were purchased from by Merck India Pvt. Ltd. and other chemicals were purchased from Sigma-Aldrich Pvt. Ltd., India.

2.2.2 Isolation and chemical transformation of compounds

The samples of *A. baccifera* (CIMAP Herbarium voucher No.-9460) were collected from local areas near the institute. The shade dried powdered plant material was extracted with methanol. The dried methanol extract was dissolved in hot water and then fractionated using *n*-hexane, chloroform and *n*-butanol. The compounds **1** and **2** (Fig. 1) were isolated through repeated chromatographic separations from chloroform and *n*-butanol fractions respectively [29,

30]. Further, compound **1** was chemically transformed in to its two semi-synthetic aryl ester derivatives (**1b** & **1d**) following earlier described procedures [32–34]. In brief, for each reaction, the compound **1** (50 mg) was dissolved in dry pyridine and benzoyl/3,4,5-trimethoxybenzoyl chloride (1.5 molar ratio), catalytic amount of 4-dimethylaminopyridine (DMAP) was added and left overnight at room temperature (30–35 °C). Progress of the reaction was monitored by TLC. Crushed ice was added to the reaction mixture, extracted with chloroform and the solvent removed from chloroform extract. Further purification of organic extract by column chromatography applying gradient elution with solvents of increasing polarity using hexane and chloroform in various proportions afforded the respective derivatives, **1b** & **1d** in 90 & 85% yields respectively. The spectroscopic data of compounds are given in supplementary file.

2.3 In vitro studies

2.3.1 In vitro anti-inflammatory activity

Isolation of murine peritoneal macrophage cells was carried out as described previously [39]. The viable cells (5×10^5 cells/mL) were suspended in Dulbecco's Modified Eagle's medium (DMEM, Sigma-Aldrich, USA) containing

Fig. 1 Structure of the isolated compounds and derivatives

10% heat-inactivated fetal bovine serum (Gibco, USA) and incubated in a culture plate at 37 °C in 5% CO₂ in an incubator. Cells were pre-treated with 5, 10 and 20 µg/mL of test compounds and standard anti-inflammatory drug, Diclofenac sodium (Sigma Aldrich, USA) separately, at 10 µg/mL for 30 min. Then after, the cells were stimulated with lipopolysaccharide (LPS, 1 µg/mL. After incubation with LPS for 18 h, supernatants were collected and immediately frozen at –80 °C. The harvested supernatants were tested for quantification of pro-inflammatory mediator (TNF-α) by EIA kits (BD Biosciences, USA) following the manufacturer's protocol and earlier described procedure [40]. The percentage TNF-α inhibition was calculated as follows:

$$\% \text{inhibition} = 100 \times (\text{OD of control} - \text{OD of sample}) / \text{OD of control}$$

where control indicates cells treated in LPS alone.

2.3.2 Cell viability assay

The inhibitory effects of test compounds on peritoneal macrophage cell growth were tested using the 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyltetrazolium bromide (MTT) [41]. Cells were seeded at a density of 1×10^6 cells/well in a 96-well plate and incubated for 24 h at 37 °C and 5% CO₂. Cells were then treated with test compounds and incubated for another 24 h, after which the MTT solution at 2 mg/mL was added for 1 h. The MTT containing medium was carefully removed and the cells were solubilised in DMSO (100 µL) for 10 min. The extent of the reduction of MTT to formazon within cells was quantified by measuring the optical density at 570 nm using Molecular Device microplate reader (Spectramax; Molecular Devices, USA). The average optical density formed in control cells were taken as 100% viability and the results of treatments were expressed as a percentage of the control.

2.3.3 Statistical analysis

The data collected were compiled and analyzed using GraphPad Prism 4. The ANOVA followed by Bonferroni post-test were used to assess the statistical significance of various groups. Results are presented as the means ± SE. Differences with a P value <0.05 were considered significant.

3 Results and discussion

3.1 Non-linear QSAR modelling

In order to virtually screen the derivatives of 4-hydroxy-α-tetralone (**1**), an ANN based QSAR model was built with the help of other molecules possessing similar structural as well as functional properties. The ANN (using feed forward back propagation method) based non-linear QSAR model was developed for percentage inhibition as dependent variable and derived independent variables which are the basic requirement for model development. Firstly, all compounds were prepared for QSAR, and then the biological activities were specified as dependent property. Concentration along with molecular descriptors was processed for calculation of independent variables for model building. Diclofenac was taken as positive control for similar activity. Molecular descriptors with least interdependencies were used for QSAR modelling. In present study, 13 orthogonal components derived from 2D molecular descriptors along with concentration of lead molecules were used for QSAR model development. These orthogonal components provide pattern in high dimension feature space for mapping of consensus diversification of active compounds. The model was optimised for tetralone pharmacophore. The regression coefficient (r^2) and LOO cross-validation regression coefficient (LOO rCV^2) of the QSAR model were 0.6976 and 0.4016, respectively. The other model evaluation parameters were as: R^2 for optimization set: 0.733, R^2 for external set experimental compounds: 0.835. Architecture for ANN-QSAR model is as: Input, hidden, output = 13, 4, 1. Learning of model was processed for one thousand epochs with momentum of 0.555556 and learning rate of 0.222222. Initial weights of the neural network were ranging between ±0.5, while data normalization was done between 0.1 and 0.9. The training data contains optimization set molecules, which were used to mould the tendency of model for prediction of specific pharmacophores (Fig. 2).

3.2 Screening of 4-hydroxy-α-tetralone virtual derivatives through the developed QSAR model

For predicting anti-inflammatory activity by the developed QSAR model, 28 new derivatives of 4-hydroxy-α-tetralone (**1**) were designed and screened for their TNF-α inhibiting potential along with **1**, its glucoside (**2**) and the positive control, Diclofenac. Out of 28 derivatives, only 12 were in the applicability domain of the model and the results of their respective percentage inhibition

Fig. 2 Regression plot of ANN-QSAR model

of TNF- α at 10 $\mu\text{g/mL}$ concentration is given in Table 1 below, while the results of rest of the virtual derivatives are given in supplementary file.

From the above table, it is evident that the most active virtual derivative was **1b** (34.78% inhibition) followed by compound **1** (29.85% inhibition). The increased TNF- α inhibitory activity of **1b** is plausibly due to its greater hydrophobic interaction with aromatic amino acids of the pro-inflammatory cytokine, TNF- α .

3.3 Docking study

The aim of the molecular docking study was to elucidate whether 4-hydroxy- α -tetralone derivatives modulate the anti-inflammatory target and also to identify the binding site against well-known human anti-inflammatory molecular target TNF- α . The docking was performed on **1** and its virtually active derivatives (**1b** & **1d**) with Diclofenac for their binding affinity with TNF- α . The results (Fig. 3) indicate that these compounds have similar range of binding affinity as that of Diclofenac sharing common residues at binding pockets: TYR-119C, GLY-121C, LEU-120C, TYR-59C & TYR-151C from chain-C and TYR-59D & TYR-119D from chain-D of TNF- α . The derivative **1b** showed highest binding affinity (-6.7 kcal/mol) even better than the positive control, Diclofenac.

3.4 Bioavailability study

The drug likeliness of 4-hydroxy- α -tetralone (**1**), its glucoside (**2**) and semi-synthetic analogues **1b** and **1d** was assessed through Lipinski's rule of five and the results showed (Table 2) that none of the active molecules violated the Lipinski's rule and showed compliance with positive control Diclofenac.

3.5 e-Toxicity study

The e-toxicity prediction for the experimentally validated molecules **1**, **2**, **1b**, **1d** and the positive control, Diclofenac was performed by pancreatic cancer cell (PaCa2) cellular uptake, which provides the response of cancerous cells for the molecules under study. The results are given in Table 3 below.

From Table 5, it is evident that experimentally tested molecules **1** and **1b** shows compliance with control and possess positive attitude of cellular uptake. These predictions have been made with 80% confidence. The most potent derivative **1b** did not show any hepatotoxicity, while the positive control Diclofenac showed hepatotoxicity.

3.6 Experimental validation of ANN-QSAR based predictions

The isolation of 4-hydroxy- α -tetralone (**1**) and its glucoside (**2**) was carried out from the chloroform and *n*-BuOH fractions of *A. baccifera* following earlier described procedures. Further, the most (**1b**) and least (**1d**) active virtual analogs of **1** were semi-synthesized and characterized on the basis of their ^1H and ^{13}C NMR spectroscopic data (Fig. 1) [32–34]. The anti-inflammatory potential of compounds **1**, **2**, **1b** and **1d** was assessed *in vitro* in terms of quantification of TNF- α inhibiting potential at 5, 10 and 20 $\mu\text{g/mL}$ concentrations. The results indicate that **1**, its glucoside (**2**) and the semi-synthetic analogues **1b** and **1d** possess moderate to significant TNF- α inhibiting property in a concentration dependent manner (Table 4).

The TNF- α inhibiting potential increased by increasing the concentration of the test compounds. Among the tested compounds, the benzoyl derivative (**1b**) showed maximum inhibition of TNF- α (23.57%, 38.75% and 44.46%) followed by **1** (18.02%, 27.58% and 37.33%)

Table 1 Prediction for % inhibition of TNF- α by 4-hydroxy- α -tetralone derivatives

Molecule	Structure	% inhibition of TNF- α at 10 μ g/mL
1		29.85045253
2		13.35833
1a		15.69261156
1b		34.7757394

Table 1 (continued)

Molecule	Structure	% inhibition of TNF- α at 10 μ g/mL
1d		5.368668896
1g		21.75470378
1p		20.07469366
1q		17.71798101

Table 1 (continued)

Molecule	Structure	% inhibition of TNF- α at 10 $\mu\text{g}/\text{mL}$
1r		18.43627147
1t		17.81445132
1u		22.22238246
1x		13.97499973

Table 1 (continued)

Molecule	Structure	% inhibition of TNF- α at 10 $\mu\text{g}/\text{mL}$
1aa		4.414170791
1ab		5.391318637

at 5, 10 and 20 $\mu\text{g}/\text{mL}$ concentrations respectively. It would be worth mentioning that the benzoyl derivative of 4-hydroxy- α -tetralone (**1b**) at 20 $\mu\text{g}/\text{mL}$ concentration was equally active to the standard drug, Diclofenac. Further, on comparing the results, it is evident that the predictions made as percentage inhibition of TNF- α for the molecules **1**, **2**, **1b** and **1d** by the ANN-QSAR based model showed very high similarity with those obtained from *in-vitro* results (Table 5). These results validate our developed QSAR model.

3.7 *In vitro* cytotoxicity of tetralone derivatives

In order to ascertain safety profile of compounds, the cell viability was determined using MTT assay. The isolated compounds, 4-hydroxy- α -tetralone (**1**) and its glucoside

Fig. 3 Compliance of 4-hydroxy- α -tetralone (**1**), its derivative (**1b** & **1d**) and Diclofenac for binding with TNF- α

(**2**) did not show any cytotoxicity in murine peritoneal macrophage cells at 20, 50 and 100 μ g/mL concentrations (Fig. 4). From the results, it may be concluded that the decrease in TNF- α concentration was not due to the death of cells.

4 Conclusion

ANN based non-linear QSAR model has been developed for *in silico* prediction of TNF- α inhibition potential of 4-hydroxy- α -tetralone (**1**) and other structurally related virtual derivatives. The regression coefficient (r^2) and LOO

Table 2 Screening of newly designed molecules through Lipinski's rule of five

Molecule	ALogP	Lipoaffinity Index	nHBAcc	nHBDOn	nRotB	Lipinski Failures	Topo-PSA	MW
1	−0.0155	0.015523	2	0	0	0	17.07	151.9898
2	−1.7624	0.080061	7	0	3	0	35.53	303.9644
1b	1.5615	0.11048	3	0	3	0	43.37	251.9847
1d	0.0657	0.472751	6	0	6	0	71.06	335.9695
Diclofenac	1.8296	0.103383	3	0	4	0	17.07	283.9306

Table 3 e-toxicity prediction for the experimentally validated molecules

Compound	PaCa ₂ cellular uptake	Confidence (PaCa ₂ -positive)	Confidence (PaCa ₂ -negative)	Hepatotoxic
1	Positive	1	0	Positive
2	Negative	0.4	0.6	Positive
1b	Positive	1	0	Negative
1d	Positive	0.8	0.2	Positive
Diclofenac	Positive	1	0	Positive

Table 4 *In vitro* anti-inflammatory activity of tetralone derivatives

Treatment	Concentration (μg/mL)	TNF-α (pg/mL) Mean ± SE	% Inhibition of TNF-α
Normal ^a	–	140.88 ± 4.86	NA
Vehicle ^b	–	1162.60 ± 155.28	00.00
1	5	953.08 ± 109.28	18.03
	10	841.98 ± 67.62	27.59
	20	728.60 ± 59.72	37.34
1b	5	888.54 ± 94.10	23.58
	10	712.04 ± 54.00	38.76
	20	645.73 ± 70.38	44.47
1d	5	1051.28 ± 55.91	9.58
	10	1026.43 ± 67.31	11.72
	20	998.31 ± 12.99	14.13
2	5	1101.28 ± 54.22	5.28
	10	1101.43 ± 37.76	5.26
	20	1073.31 ± 79.32	7.68
Diclofenac	10	648.65 ± 52.26	44.22

^aNeither treated with LPS, nor with any test compound^bTreated with LPS (1 μg/mL) only

cross-validation regression coefficient (LOO rCV²) of the QSAR model were 0.6976 and 0.4016, respectively. Screening of virtual analogs of **1** using the derived QSAR model showed that benzoyl derivative (**1b**) possesses potent TNF-α inhibiting activity. This analogue on docking studies showed similar binding affinity as that of Diclofenac

Table 5 Experimental validation of ANN-QSAR based prediction

Molecule	% Inhibition of TNF-α at 10 μg/mL conc.	
	Predicted	Experimental
1	29.85	27.58
1b	34.78	38.75
1d	5.36	5.26
2	13.36	11.71

(positive control) with common residues at binding pocket. Based on bioavailability, drug likeness, ADMET and toxicity risk assessments studies, we concluded that compound **1b** possesses anti-inflammatory potential similar to that of Diclofenac. In order to validate the developed QSAR model, most and least virtually active derivatives were semi-synthesized and *in vitro* tested for TNF-α inhibition potential. The results obtained agreed well with the predicted values. The analog **1b** showed 44.47% inhibition of TNF-α at 20 μg/mL concentration which was better than standard drug Diclophenac (44.22% inhibition) showing at 10 μg/mL concentration. Further, *in vitro* cytotoxicity study using MTT assay revealed that parent compound (**1**) and its glucoside (**2**) did not show any cytotoxicity in murine peritoneal macrophage cells up to 100 μg/mL concentrations. These results offer a useful reference for QSAR and docking guided molecular design of TNF-α inhibitors with improved activity for development of anti-inflammatory leads.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Fig. 4 *In vitro* cell viability assessment of compounds (**1** & **2**) by MTT assay

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