Screening of flavonoids for antitubercular activity and their structure–activity relationships

ARTICLE *in* MEDICINAL CHEMISTRY RESEARCH · JUNE 2012

Impact Factor: 1.4 · DOI: 10.1007/s00044-012-0268-7

Screening of flavonoids for antitubercular activity and their structure–activity relationships

Akhilesh K. Yadav, Jayprakash Thakur, Om Prakash, Feroz Khan, Dharmendra Saikia & Madan M. Gupta

Medicinal Chemistry Research

ISSN 1054-2523

Med Chem Res
DOI 10.1007/s00044-012-0268-7

Springer

Med Chem Res
DOI 10.1007/s00044-012-0268-7

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Screening of flavonoids for antitubercular activity and their structure–activity relationships

Akhilesh K. Yadav · Jayprakash Thakur · Om Prakash · Feroz Khan · Dharmendra Saikia · Madan M. Gupta

Received: 28 February 2012 / Accepted: 29 September 2012

Abstract The antitubercular activity of selected flavonoids and their structure–activity relationships were studied against *Mycobacterium tuberculosis* $H_{37}Rv$ strain radiometrically by BACTEC 460 assay. Present study led to the identification of five flavonoids, viz., luteolin, baicalein, quercetin, myricetin and hispidulin with MIC 25–100 μg ml^{-1}, as new antitubercular templates. Rest flavonoids were found inactive against *M. tuberculosis* at a concentration of 100 μg ml^{-1}. A possible structure–activity relationship (SAR) was also drawn to determine the specific structural requirements of flavonoids toward antitubercular activity. The hydroxyl substitution at position 5 and 7 provides no activity, whereas the hydroxyl substitutions at 5, 6, 7 (trihydroxy) or 3′, 4′ (dihydroxy) are of particular importance for antitubercular activity of a flavonoid. The *O*-methylation or glycosylation at any of di- or trihydroxyl substitutions inactivates the antitubercular potential of the flavonoids. We have also predicted the activity of studied flavonoids through QSAR model. A multiple linear regression QSAR mathematical model was developed for activity prediction that successfully and accurately (noting the corresponding experimental activities) predicted the antituberculosis activities of studied flavonoid compounds that had the basic pharmacophore, namely luteolin, baicalein, quercetin, myricetin, and hispidulin, with experimental and predicted *n* log MIC (μg ml^{-1}) of 3.2189 & 2.583, 3.912 & 2.433, 3.912 & 2.433, 3.912 & 3.529, and 4.6052 & 2.703, respectively. The structure–activity relationship denoted by the QSAR model yielded a very high activity-descriptor relationship accuracy of 87 % referred by regression coefficient ($r^2 = 0.870533$) and a high activity prediction accuracy of 81 % ($rCV^2 = 0.81423$). These compounds may represent novel leads toward the development of pharmacologically acceptable antitubercular agent/agents.

Keywords Flavonoids · Isolation · *Mycobacterium tuberculosis* · Structure–activity relationships

A. K. Yadav · M. M. Gupta (✉)
Analytical Chemistry Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow 226015, India
e-mail: guptammg@rediffmail.com

J. Thakur · D. Saikia (✉)
Molecular Bio-Prospection Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow 226015, India
e-mail: d.saikia@cimap.res.in

O. Prakash · F. Khan
Metabolic and Structural Biology Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow 226015, India

Introduction

The infectious killer disease, tuberculosis (TB), is the leading cause of death worldwide from a single human pathogen. About one third of world's population is currently infected with *Mycobacterium tuberculosis* (Zumla, 1998). Worldwide resurgence of TB is due to two major problems: the acquired immunodeficiency syndrome (AIDS) epidemic, which started in the mid-1980s, and the outbreak of multidrug-resistant tuberculosis (MDR-TB). Emergence of drug-resistant strains of *M. tuberculosis* has lead to increase the concern on current chemotherapy regimes. Worldwide increase in the incidence of morbidity and mortality from tuberculosis prompted WHO to declare this disease a global emergency in the early 1990s (De Souza, 2006; Ballell *et al.*, 2005; Janin, 2007).

Mycobacterium tuberculosis infection is at higher risk for progression if they are coinfected with HIV (Steinbrook,

Published online: 11 October 2012

2007). Tuberculosis is the most common HIV-related opportunistic infection and treatment of both the diseases at a time is a major public health challenge as certain antituberculosis drugs may interact adversely with medications commonly used to treat HIV-infections. To overcome the complexities of treatment in consideration, the identification of anti-HIV flavonoids as antitubercular agent may be of great therapeutic importance. Moreover, the development of new anti-TB drugs from known compounds, the therapeutic use of which has already shown safety and efficacy along the years, is an attractive strategy from the economical, pharmaceutical, and clinical point of view.

Flavonoids are ubiquitous in photosynthesizing cells and are commonly found in fruits, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis, and honey. For centuries, preparations containing these compounds as the principal physiologically active constituents have been used to treat human diseases. Increasingly, this class of natural products is becoming the subject of anti-infective research, and many groups have isolated and identified the structures of flavonoids possessing antifungal, antiviral, and antibacterial activities. Flavonoids have been known for a long time to exert diverse biologic effects (bioflavonoids), and in particular to act as preventive agents against cancer (Harborne and Williams, 2000). Flavonoids have also been reported to possess multiple biologic activities such as antioxidant, anti-inflammatory, hypoglycemic, hypertensive, hepatoprotection, hypodynamic, and vasoactive effects (Oh *et al.*, 2004; Wang and Ng, 1999; Occuhiuto *et al.*, 1990; Calderone *et al.*, 2004).

Many flavonoids have been identified to possess antitubercular activity (Lin *et al.*, 2002; Murillo *et al.*, 2003; Gu *et al.*, 2004; Yenjai *et al.*, 2004; Chinworrungsee *et al.*, 2004; Sivakumar *et al.*, 2007). Here, we have examined the in vitro ability of 15 flavonoids to inhibit the growth of *Mycobacterium tuberculosis* $H_{37}Rv$ strain radiometrically by BACTEC 460 assay. A possible SAR of tested flavonoids has also been drawn.

Materials and methods

Flavonoids

Luteolin, myricetin, galangin, hesperidin, apigenin-7-O-glucoside, apigenin, and kaempferol were obtained from Sigma-Aldrich Inc., Germany. Oroxylin A, chrysin, baicalein, hispidulin (*Oroxylum indicum*), kaempferol-3-O-rhamnoside, quercetin (*Pithecellobium dulce*), rutin (*Desmodium gangeticum*), and casticin (*Vitex trifolia*) were isolated and characterized in the laboratory. Authenticated plant materials for the isolation of flavonoids were collected from the Central Institute of Medicinal and Aromatic Plants, Lucknow. Authentication of the plant was established by the Taxonomy and Pharmacognosy Department of the institute.

In vitro antimycobacterial assay by BACTEC radiometric susceptibility assay

The BACTEC TB medium (12B) is an enriched Middlebrook 7H9 broth base. Mycobacteria utilize a ^{14}C-labeled substrate (fatty acid) present in the medium and release $^{14}CO_2$ into the atmosphere above the medium. When the vials are tested on the BACTEC 460 TB System instrument, the gas is aspirated from the vial and the $^{14}CO_2$ radioactivity is determined quantitatively in terms of numbers on a scale from 0 to 999. These numbers are designated as the growth index (GI). The GI numbers are displayed using the BACTEC 460 TB System instrument and are also printed along with the identifying rack and bottle numbers (100 GI units are approximately equal to 0.025 μCi). The daily increase in the GI is directly proportional to the rate and amount of growth in the medium.

If an inhibitory agent is introduced into the medium, inhibition of metabolism is indicated by reduced production of $^{14}CO_2$ when compared to a control having no inhibitory agent. This basic principle is applied for drug susceptibility testing.

Mycobacterium tuberculosis $H_{37}Rv$ (ATCC 27294) used in this screening was obtained from the National JALMA Institute for Leprosy and other Mycobacterial Diseases, Agra, India, and maintained on Löwenstein-Jansen media slant at 37 °C. A test protocol as described by Siddiqi was followed (Siddiqi, 1996). After 21 days of incubation, bacterial cells were scraped from slants and transferred in 1.0 ml of BACTEC diluting fluid and made complete homogenized suspension by vortexing with glass beads (2-mm diameter). The suspension was allowed to stand for a few minutes to permit sedimentation of the bacterial clumps if any. The turbidity of the homogenous suspension was adjusted to McFarland standard 1.0 with diluting fluid. A BACTEC 12B vial (Becton–Dickinson) was injected with 0.1 ml of this suspension. This vial was used as primary inoculum after the growth index (GI) reached a value of about 500 (approximately 1×10^6 cfu ml^{-1}).

Briefly, 0.1 ml of bacterial suspension from the primary inoculum culture vial (GI 500) was injected into test compound-containing vials using 1.0 ml insulin syringe. To comply with 1 % proportion method, 0.1 ml of primary inoculum was added to 9.9 ml BACTEC diluting fluid to obtain 1:100 dilution. From this, 0.1 ml was injected into two 12B media vials and used as control. Vials were incubated at 37 °C and the GI was recorded every 24 h in a BACTEC 460 TB instrument (Becton–Dickinson). Once the GI of the control vial (1:100) reached 30 then the GI values of the vials containing test compounds were compared with that of

control based on difference in growth (ΔGI). The result was interpreted as follows: If the difference (ΔGI) of current GI from previous day GI in the case of drug/test compound-containing vials is lower than the ΔGI of 1:100 control vial for the same period then the test compound is termed as active against MTB or otherwise inactive.

Twofold serial dilution technique was used to assess the minimum inhibitory concentration (MIC) of test compounds. Only broth culture was used as positive control and media as a negative control. The positive control having broth culture only was used to insure that a viable amount of mycobacterium was inoculated into each test vial. The negative control was used to insure that the observed GI values were not due to spontaneous release of $^{14}CO_2$ from the medium. All the experiments were performed in triplicates.

Geometry cleaning, energy optimization, and parameterization of compounds

Drawing and geometry cleaning of the series of compounds was performed through ChemBioDraw-Ultra-v12.0 (http://www.cambridgesoft.com/). The 2D structures were transformed into 3D structures by means of converter module of ChemBioDraw. The 3D structures were then subjected to geometry optimization and energy minimization, which was performed in two steps, in the first step energy minimized using molecular mechanics-2 (MM2) until the root mean square (RMS) gradient value became smaller than 0.100 kcal/mol Å and then in second step minimized MM2 (dynamics) compounds were subjected to re-optimization through MOPAC (Molecular Orbital Package) method until the RMS gradient attained a value smaller than 0.0001 kcal/mol Å.

Structure retrieval and docking parameters

The chemical structures of small molecules and drugs were retrieved from PubChem compound database (NCBI) (http://www.pubchem.ncbi.nlm.nih.gov). Crystallographic structure of *M. tuberculosis* target protein was taken from PDB database (http://www.pdb.org). The valency and hydrogen bonding of compounds and target protein was checked through Scigress Explorer v.7.7.0.47 (previously, CAChe) software (Fujitsu Ltd., Poland; http://www.fqs.pl/). Hydrogen atoms were added to protein so that to achieve the correct ionization and tautomeric states of amino acid residues such as His, Asp, Ser, and Glu. Molecular docking of drugs and the studied compounds against antituberculosis target was achieved by Autodock Vina software (Trott and Olson, 2010). To perform the automated docking of compounds into the protein active sites, we used a Lamarckian genetic algorithm and an empirical binding free energy function with a simplified docking scoring scheme in kcal/mol (Trott and Olson, 2010; Morris *et al.*, 1998).

Parameters for QSAR model development

Initially, antituberculosis drugs/compounds with reported activities were used as training data set compounds while developing the quantitative structure activity relationship (QSAR) models. The antituberculosis activity was in the form of minimum inhibitory concentration (MIC in $\mu g\ ml^{-1}$). Later, various chemical descriptors were calculated for each compound. While selecting the best subset of chemical descriptors, highly correlated descriptors were excluded by correlation matrix approach (co-variance analysis) or principal component analysis. To narrow down the false positive predictions of QSAR model, for the first time two step screening process for antituberculosis activity of flavonoids was studied. In the first step, QSAR model-1 screened the untested compounds for being active or non-active against *M. tuberculosis* $H_{37}Rv$. The activity value more than zero indicates active compounds and less than zero value indicates non-active compounds. Second, QSAR model-2 predicts the antituberculosis activity in terms of MIC value of active compounds (flavonoids) against *M. tuberculosis* $H_{37}Rv$.

The QSAR models were developed by multiple linear regression (MLR) method. The derived QSAR models showed a high regression coefficient. The model was validated successfully using the test dataset of known antituberculosis compounds. The models were evaluated for the robustness of its predictions through cross-validation coefficient and leave-one-out (LOO) method was used for validating QSAR models.

Screening through pharmacokinetic properties

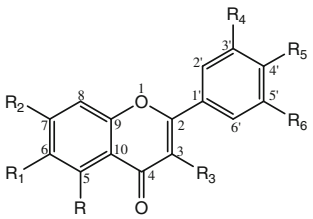
As we know that most of drugs in discovery process fail to cross clinical trials because of poor pharmacokinetics (PK). The properties of PK depend on chemical descriptors of compounds such as absorption, distribution, metabolism, excretion, and toxicity (ADMET). Some descriptors correlate well with PK properties, e.g., primary determinant of fractional absorption referred as polar surface area (PSA) and low molecular weight (MW—for oral absorption). The compound distribution in human depends on factors, e.g., blood–brain barrier (log BB), permeability (apparent Caco-2 and MDCK permeability, log Kp for skin permeability), the volume of distribution and plasma protein binding refer by log Khsa for serum protein binding. The octanol–water partition coefficient (log P) has been implicated in log BB penetration and permeability studies. Process of excreting the compound from human body depends on MW and log P. Likewise, rapid renal clearance is associated with

small and hydrophilic compounds. Metabolism of most drugs is associated with large and hydrophobic compounds in liver. Higher compound lipophilicity leads to increased metabolism and poor absorption. The descriptor values of 90 % orally active drugs follows Lipinski's rule. The bioavailability was evaluated by topological polar surface area (TPSA) calculated by ChemAxon's MarvinView 5.2.6, PSA plugin. The number of rotatable bonds is also a topological parameter as a measure of molecular flexibility (cut-off $\leq$ 10) and oral bioavailability (Kalani *et al.*, 2012; Yadav *et al.*, 2012; Meena *et al.*, 2011).

Results and discussion

Antitubercular activity of 15 flavonoids (Table 1) was evaluated against *M. tuberculosis* $H_{37}Rv$ strain radiometrically by BACTEC 460 assay as above using certain fixed concentration of test compounds starting with 100, 50, 25, 12.5 μg ml^{-1}, etc. The results were calculated and expressed as minimum inhibitory concentrations (MIC) μg ml^{-1}. Out of the tested fifteen flavonoids, five flavonoids, viz., luteolin, quercetin, baicalein, myricetin, and hispidulin, were found active against *M. tuberculosis* in the MIC range of 25–100 μg ml^{-1}, whereas rest flavonoids were found inactive at concentration of 100 μg ml^{-1} (Table 1). The concentration of drug/test compound at which there was no detectable/suppressive growth of MTB as compared to control is determined as the MIC (based on difference in ΔGI). Flavonoids, luteolin, quercetin, baicalein, and myricetin are previously reported to exhibit inhibitory activity against HIV-1 RT (Stavri *et al.*, 2005; Lin *et al.*, 1997; Mahmood *et al.*, 1993). Keeping in consideration the role of HIV infections in the resurgence of TB, the identification of anti-HIV flavonoids, viz., luteolin, quercetin, baicalein, and myricetin as antitubercular agent may be of great therapeutic importance.

Table 1 Tested flavonoids including their structural features and the MIC values of *M. tuberculosis* inhibition



S. no.	Name	R	R1	R2	R3	R4	R5	R6	MIC (μg ml^{-1})
1	Luteolin	OH	H	OH	H	OH	OH	H	25
2	Quercetin	OH	H	OH	OH	OH	OH	H	50
3	Baicalein	OH	OH	OH	H	H	H	H	50
4	Myricetin	OH	H	OH	OH	OH	OH	OH	50
5	Hispidulin	OH	OCH_3	OH	H	H	OH	H	100
6	Chrysin	OH	H	OH	H	H	H	H	Inactive[a]
7	Apigenin	OH	H	OH	H	H	OH	H	Inactive[a]
8	Galangin	OH	H	OH	OH	H	H	H	Inactive[a]
9	Kaempferol	OH	H	OH	OH	H	OH	H	Inactive[a]
10	Oroxylin A	OH	OCH_3	OH	H	H	H	H	Inactive[a]
11	Casticin	OH	OCH_3	OCH_3	OCH_3	OCH_3	OH	H	Inactive[a]
12	Apigenin-7-O-glu	OH	H	O-glu	H	H	OH	H	Inactive[a]
13	Kaempferol-3-O-rha	OH	H	OH	O-rha	H	OH	H	Inactive[a]
14	Hesperidin	OH	H	O-g-r[b]	H	OCH_3	OH	H	Inactive[a]
15	Rutin	OH	H	OH	O-g-r[b]	OH	OH	H	Inactive[a]
16	Streptomycin								2
17	Rifampicin								2

[a] At a concentration of 100 μg ml^{-1}

[b] Glucose–rhamnose (1→6)

Luteolin with 3′,4′ di-hydroxy groups inhibited the growth of *M. tuberculosis* at MIC 25 μg ml^{-1} (Fig. 1) whereas, on addition of an extra OH group at position 3 in luteolin (i.e., quercetin) activity decreases to the MIC value of 50 μg ml^{-1}. Glycosylation of quercetin at position 3 (i.e., rutin) totally diminishes the antitubercular activity. Addition of an extra hydroxyl group at position 5′ in quercetin (i.e., myricetin) showed no change in the activity. Flavonoid baicalein (5,6,7-trihydroxy) also showed the antitubercular activity at MIC 50 μg ml^{-1} (Fig. 1). Methylation of hydroxyl group in baicalein at position 6 (i.e., oroxylin A) completely inactivates the antitubercular potential. Oroxylin A (5,7-dihydroxy-6-methoxy) was inactive against *M. tuberculosis* at a concentration of 100 μg ml^{-1}. However, it was interesting to note that the addition of an extra hydroxyl group at position 4′ in oroxylin A (i.e., hispidulin) induces the antitubercular activity at MIC 100 μg ml^{-1}.

Molecular docking of flavonoids

The objective of docking studies was to explore the binding affinity of studied flavonoids against selected antituberculosis target 2-*trans*-enoyl-ACP (acyl carrier protein) reductase enzyme (PDB ID: 2B37) (InhA or ENR) from *Mycobacterium tuberculosis* $H_{37}Rv$ strain and to study their possible mechanisms of action. The results of docking studies suggest that studied compounds inhibit the activity of InhA by binding on it strongly, as indicated by the docking energy. In the studied work, we explored the orientations and binding affinities (in terms of the docking energy in kcal mol^{-1}) of flavonoids toward antituberculosis target InhA or ENR (EC number: 1.3.1.9) from *M. tuberculosis* $H_{37}Rv$ strain (PDB: 2B37).

The binding affinity obtained in the docking study allowed the activity of the studied flavonoids to be compared to that of the standard antituberculosis drugs Rifampicin and Streptomycin. All the studied flavonoid compounds showed high binding affinities (low docking energies) for InhA. When we compared how the binding pocket residues of InhA interacted with the flavonoids, we found that compounds myricetin, luteolin, baicalein, quercetin, and hispidulin showed interaction with conserved amino acid residues, thus leading to more stability and potency in these cases. The docking results for the active flavonoids showed that compound myricetin, luteolin, baicalein, quercetin, and hispidulin docked onto InhA enzyme of *M. tuberculosis* $H_{37}Rv$ with significant docking energy of −7.1, −7.0, −7.0, −7.0, and −6.9 kcal mol^{-1}, respectively, comparable to the co-crystallized antituberculosis ligand (8PS_300) bound to InhA target enzyme of *M. tuberculosis* (PDB: 2B37), streptomycin (PubChem CID_19649), and rifampicin (PubChem CID_6842115) with standard docking energy of −6.8, −6.0, and −8.2 kcal mol^{-1}. Results of molecular docking indicate similar binding site pocket residues for most favorable conformations of predicted active compounds against InhA (Fig. 2).

Predicting antituberculosis activity with the QSAR model

Prior studies related to structure–activity relationship of flavonoids showed promising role in the development of novel antituberculosis drugs (Sutthivaiyakit *et al.*, 2009). Following so, in the present work, we have predicted the activity of studied flavonoids through QSAR model. The structure–activity relationship denoted by the QSAR model yielded a very high activity–descriptor relationship accuracy of 87 % referred by regression coefficient ($r^2 = 0.870533$) and a high activity prediction accuracy of 81 % ($rCV^2 = 0.81423$) (Fig. 3). Four chemical descriptors were found to be applicable to the antituberculosis activity. The QSAR equation indicated that chemical descriptors namely, dipole moment, dipole vector_X, ether group count, and the heat of formation correlated well with activity. The QSAR model equation is given below, showing the relationship between experimental activity in vitro [*i.e.*, natural log of minimum inhibitory concentration (MIC) in μg ml^{-1}] as the dependent variable and four independent variables (chemical descriptors or properties):

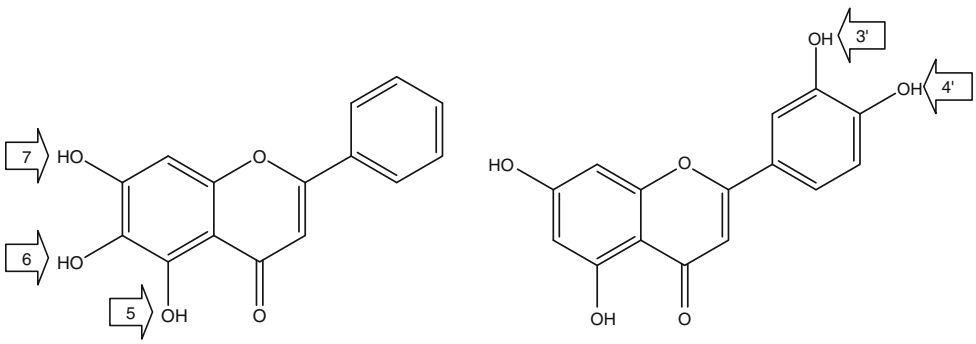


Fig. 1 Specific structural requirements for antitubercular activity in tested flavonoids

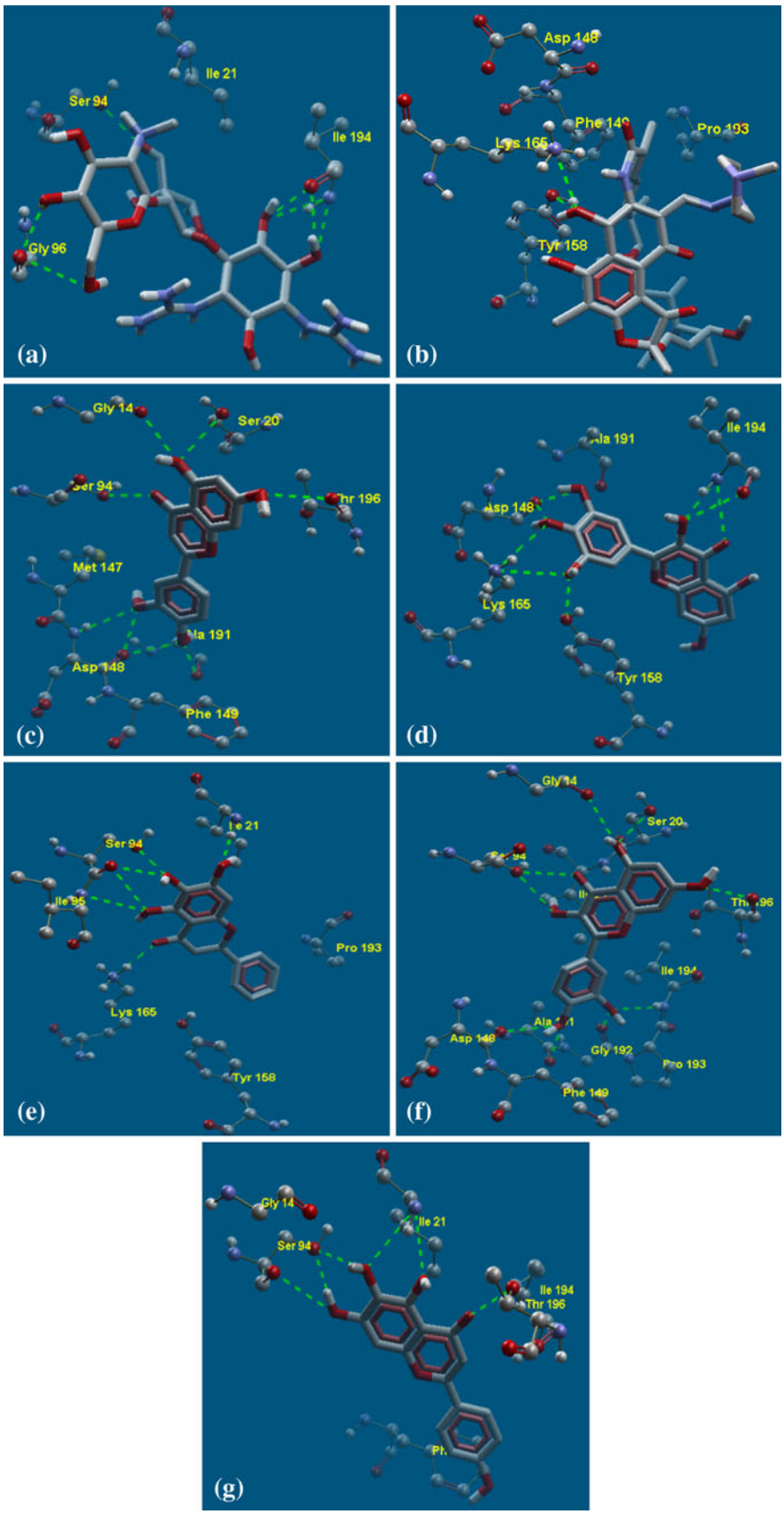


Fig. 2 Molecular docking poses of standard antituberculosis drugs and studied active flavonoids showing interacting binding site amino acid residues of InhA (PDB: 2B37) of *M. tuberculosis* $H_{37}Rv$. **a** Streptomycin docked with docking score of -6.0 kcal mol^{-1}, **b** rifampicin docked with docking score of -8.2 kcal mol^{-1}, **c** luteolin docked with docking score of -7.0 kcal mol^{-1}, and **d** myricetin docked with docking score of -7.1 kcal mol^{-1}, **e** baicalein docked with docking score of -7.0 kcal mol^{-1}, **f** quercetin docked with docking score of -7.0 kcal mol^{-1}, **g** hispidulin docked with docking score of -6.9 kcal mol^{-1}

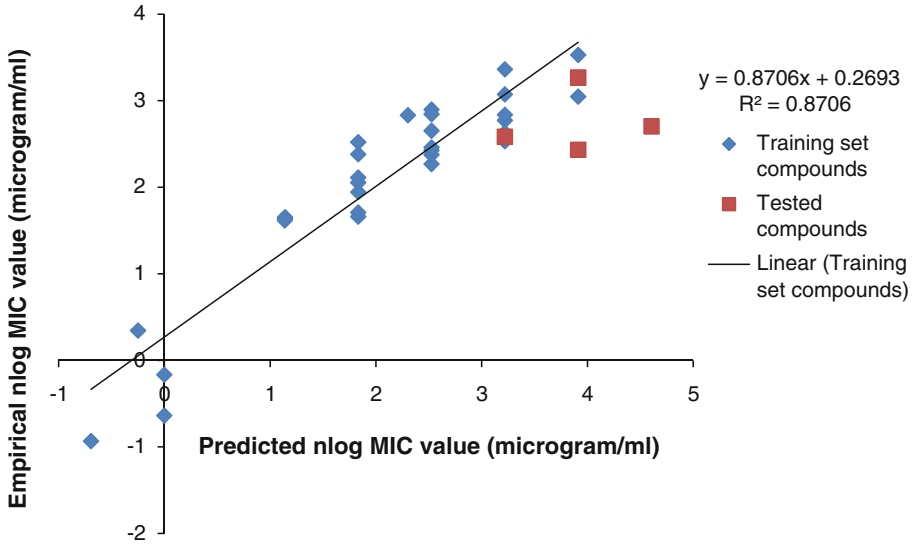


Fig. 3 Regression plot of QSAR model developed for virtual screening of flavonoids against antitubercular compounds

Step-1 (QSAR model-1): Pre-screening

Activity prediction or classification of untested/unknown compounds through QSAR model-1:

$$\begin{aligned}\text{Activity (anti-TB)} &= +2.16745 \times \text{(Dipole moment)} \\ &+ 1.55169 \times \text{(Dipole vector X)} \\ &- 0.0512673 \times \text{(Heat of formation)} \\ &- 13.8041\end{aligned}$$

Activity >0 indicates active compounds against *M. tuberculosis* $H_{37}Rv$ and may be used further for prediction of antituberculosis activity in terms of MIC value through QSAR model-2. Activity <0 indicates non-active compounds against *M. tuberculosis* $H_{37}Rv$.

Step 2 (QSAR model-2): Activity screening

Antituberculosis activity value prediction in terms of *n* log MIC (in μg ml^{-1}) of Step-1 predicted active compounds through QSAR model-2:

$$\begin{aligned}&\text{Anti-TB activity } \left(\text{in n log MIC, unit } \mu g\ ml^{-1}\right) \\ &= -0.256707 \times \text{(Dipole moment)} \\ &\quad - 0.2711 \times \text{(Dipole vector X)} \\ &\quad + 0.210235 \times \text{(Group count ether)} \\ &\quad - 0.00293715 \times \text{(Heat of formation)} \\ &\quad + 2.59368\end{aligned}$$

$$\left(rCV^2 = 0.81423, \text{ and } r^2 = 0.870533\right)$$

Here, rCV^2 (the cross-validation regression coefficient) = 0.81, which indicates that the newly derived QSAR model has a prediction accuracy of 81 %, and r^2 (regression coefficient) = 0.87, which indicates that the correlation between the activity (dependent variable) and the chemical descriptors (independent variables) for the training data set compounds was 87 %. Thus, we successfully developed a predictive QSAR model for in vitro antituberculosis activity of flavonoids. A multiple linear regression QSAR mathematical model was developed for activity prediction which successfully and accurately (noting the corresponding experimental activities) predicted the antituberculosis activities of studied flavonoid compounds that had the basic pharmacophore namely, myricetin with experimental and predicted *n* log MIC (μg ml^{-1}) of 3.912 and 3.529, respectively, baicalein with experimental and predicted *n* log MIC (μg ml^{-1}) of 3.912 and 2.433, respectively, hispidulin with experimental and predicted *n* log MIC (μg ml^{-1}) of 4.6052 and 2.703, respectively, luteolin with experimental and predicted *n* log MIC (μg ml^{-1}) of 3.2189 and 2.583, respectively, and quercetin with experimental and predicted *n* log MIC (μg ml^{-1}) of 3.912 and 3.268, respectively (Table 2). The QSAR model quantified the activity-dependent chemical descriptors and predicted the minimum inhibitory concentration (*n* log MIC) of each flavonoids, thus indicating the potential range of inhibition. Results of QSAR showed that the predicted activities were comparable with those obtained experimentally. Results showed that the predicted activity of compounds luteolin has comparatively more antituberculosis activity than myricetin, baicalein, quercetin, and hispidulin.

Assessment through pharmacokinetic parameters

The results revealed that all the studied flavonoid compounds followed Lipinski's rule of five (Table 3). All compounds showed calculated log P values less than 5 so

these will be soluble in aqueous solution and hence able to gain access to membrane surfaces. All the compounds have polarities which enabled better permeation and absorption, as revealed by the number of H-bond donors and H-bond acceptors. Similarly, the ADME parameters were calculated for the active compounds. The calculated values of these parameters also showed close correspondence with those of standard drugs and fell within the standard range of values exhibited by 95 % of all known drugs.

Toxicity risk assessment

In the studied work, we have calculated the toxicity risk parameters, for example, mutagenicity, tumorigenicity, irritation, and reproductive or developmental toxicities of the studied active flavonoids. The toxicity risk predicting software locates fragments within a molecule that indicate a potential toxicity risk. Toxicity screening results showed that none of the compounds, viz., luteolin, quercetin,

Table 2 Predicted antituberculosis activity of studied active flavonoids against InhA target of *M. tuberculosis*

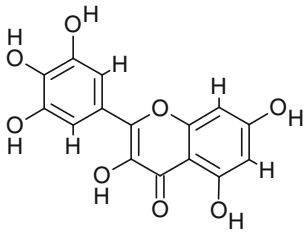
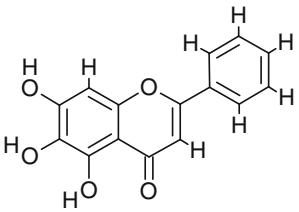
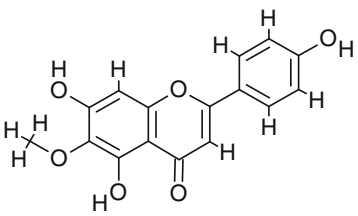
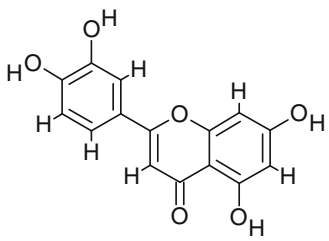
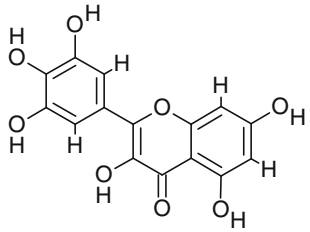
Chemical sample	Experimental activity (n log MIC)[a] (μg ml^{-1})	Dipole moment (debye)	Dipole vector *X* (debye)	Group count (ether)	Heat of formation (kcal/mol)	Predicted activity (n log MIC) (μg ml^{-1})	Residual (absolute error)
 Myricetin	3.912	1.477	−1.22	1	−263.245	3.529	0.383
 Baicalein	3.912	5.418	−2.361	1	−129.417	2.433	1.479
 Hispidulin	4.6052	4.06	−0.873	2	−168.293	2.703	1.9022
 Luteolin	3.2189	6.198	−3.132	1	−177.542	2.583	0.6359

Table 2 continued

Chemical sample	Experimental activity (n log MIC)[a] (μg ml^{-1})	Dipole moment (debye)	Dipole vector *X* (debye)	Group count (ether)	Heat of formation (kcal/mol)	Predicted activity (n log MIC) (μg ml^{-1})	Residual (absolute error)
 Quercetin	3.912	3.625	−2.769	1	−219.316	3.268	0.644

[a] Experimental nlog MIC calculated as natural logarithm value of experimental MIC (in μg ml^{-1})

Table 3 Compliance of studied active flavonoids to electronic parameters of bioavailability and drug likeness properties

S. no	Compounds	miLog P	TPSA (Å^2)	*n* atoms	MW	*n* ON (H-bond acceptor)	*n* OHNH (H-bond doner)	*n* violations (rule of five)	*n* rotb	Volume (Å^3)
1	Luteolin	1.974	111.12	21.0	286.23	6	4	0	1	232.06
2	Quercetin	1.683	131.35	22.0	302.23	7	5	0	1	240.08
3	Baicalein	2.682	90.89	20.0	270.24	5	3	0	1	224.04
4	Myricetin	1.392	151.57	23.0	318.23	8	6	1	1	248.10
5	Hispidulin	2.479	100.12	22	300.26	6	3	0	2	249.59
6	Streptomycin	−5.35	336.45	40.0	581.58	19	16	3	9	497.24
7	Rifampicin	2.096	216.66	59.0	822.95	16	6	3	5	756.01

miLog P octanol/water partition coefficient, *TPSA* topological polar surface area, *n* atoms number of atoms, *MW* molecular weight, *n* ON number of hydrogen acceptor, *n* OHNH number of hydrogen donor, *n* violations violations from Lipinski's rule, *n* rotb number of rotatable bonds, *volume* volume of molecule

baicalein, myricetin, and hispidulin indicate no risk of irritation, but indicate high mutagenicity at high doses or long term therapeutic use comparable to standard antituberculosis drugs streptomycin and rifampicin. Hispidulin indicates high, while luteolin, quercetin, and myricetin indicate mild and baicalein indicates no sign of reproductive or developmental toxicity. Luteolin, quercetin, and myricetin indicate mild and baicalein and hispidulin indicates no predictive sign of tumorigenicity toxicity at high doses or long term use (Table 4).

Conclusions

Present study led to the identification of five flavonoids (luteolin, baicalein, quercetin, myricetin, and hispidulin) as new antitubercular templates. The development of new antitubercular drugs from known compounds could be an attractive strategy from the economical, pharmaceutical, and clinical point of view because of already available safety and efficacy data. A possible structure–activity relationship concluded that the presence of hydroxyl groups at the 5, 6, and 7 positions (baicalein), or the 3′, 4′ positions (luteolin, quercetin, and myricetin) or the 4′ position in 5,7-dihydroxy-6-methoxy arrangement (hispidulin) were the prerequisites for the inhibition of growth of *M. tuberculosis.* The increase in the number of hydroxyl substitution at random positions showed no effect on the antitubercular activity of tested flavonoids. This structure–activity relationship study demonstrates that two hydroxyl groups at adjacent positions are required for antitubercular activity. However, they become inactive if hydroxyl group is methylated or glycosylated (e.g., casticin, rutin, and hesperidin). Further, results of QSAR showed that the predicted activities were comparable with those obtained experimentally. Based on experimental and in silico molecular modeling studies, such as oral bioavailability, drug likeness, and eADMET studies, we concluded that

Table 4 Compliance of the studied active flavonoids with the standard intervals for electronic toxicity risk parameters

Toxicity risk and drug likeness parameters (Osiris)	Luteolin	Quercetin	Baicalein	Myricetin	Hispidulin	Streptomycin	Rifampicin
Mutagenic toxicity	High	High	High	High	High	No risk	No risk
Tumorigenic toxicity	Medium	Medium	No risk	Medium	No risk	No risk	No risk
Irritation toxicity	No risk	No risk	No risk	No risk	No risk	Medium	No risk
Reproductive toxicity	Medium	Medium	No risk	Medium	High	No risk	No risk
Clog P	2.4	1.8	2.7	1.51	2.59	−7.51	3.77
Solubility	−2.56	−2.49	−2.86	−2.2	−2.87	−0.9	−5.04
Molecular weight	286	302	270	318	300	567.0	870
Drug likeness	1.9	1.6	0.75	0.75	1.11	1.07	9.86
Drug score	0.32	0.32	0.44	0.29	0.27	0.45	0.33

CLog P octanol/water partition coefficient

compound luteolin has comparatively more antituberculosis activity than myricetin, baicalein, quercetin, and hispidulin. Finally, results of toxicity risk screening suggest that none of the studied flavonoid compounds, viz., luteolin, quercetin, baicalein, myricetin, and hispidulin, indicate no risk of irritation but indicate high mutagenicity at high doses or long term therapeutic uses comparable to standard antituberculosis drugs. viz., streptomycin and rifampicin.

Acknowledgments We are thankful to Director, CIMAP, Lucknow for fundamental facilities of research. We are also thankful to Director, National JALMA Institute for Leprosy and other Mycobacterial Diseases, Agra for providing *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294). Authors AKY and JPT are thankful to CSIR for the award of fellowships.

References

Ballell L, Field RA, Duncan K, Young RJ (2005) New small-molecule synthetic antimycobacterials. Antimicrob Agents Chemother 49:2153–2163

Calderone V, Chericoni S, Martinelli C, Testai L, Nardi A, Morelli I, Breschi MC, Martinotti E (2004) Vasorelaxing effects of flavonoids: investigation on the possible involvement of potassium channels. Naunyn-Schmiedeberg's Arch Pharmacol 370:290–298

Chinworrungsee M, Kittakoop P, Isaka M, Maithip P, Supothins S, Thebtaranonth Y (2004) Isolation and structure elucidation of a novel antimalarial macrocyclic polylactone, menisporopsin A, from the fungus *Menisporopsis theobromae*. J Nat Prod 67:689–692

De Souza MVN (2006) Current status and future prospects for new therapies for pulmonarytuberculosis. Curr Opin Pulm Med 12:167–171

Gu J-Q, Wang Y, Granzblau SG, Montenegro G, Yang D, Timmermann BN (2004) Antitubercular constituents of *Valeriana laxiflora*. Planta Med 70:509–514

Harborne JB, Williams CA (2000) Advances in flavonoid research since 1992. Phytochemistry 55:481–504

Janin YL (2007) Antituberculosis drugs: ten years of research. Bioorg Med Chem 15:2479–2513

Kalani K, Yadav DK, Khan F, Srivastava SK, Suri N (2012) Pharmacophore-, QSAR-, and ADME-based semisynthesis and in vitro evaluation of ursolic acid analogs for anticancer activity. J Mol Model 18:3389–3413

Lin YM, Anderson H, Flavin MT, Pai YHS (1997) In vitro anti-HIV activity of biflavonoids isolated from *Rhus succedanea* and *Garcinia multiflora*. J Nat Prod 60:884–888

Lin YM, Zhou Y, Flavin MT, Zhou LM, Niea W, Chen FC (2002) Chalcones and flavonoids as anti-tuberculosis agents. Bioorg Med Chem 10:2795–2802

Mahmood N, Pizza C, Aquino R, Colman S, Burke A, Hay A (1993) International conference on AIDS. Int Conf AIDS 9:467

Meena A, Yadav DK, Srivastava A, Khan F, Chanda D, Chattopadhyay SK (2011) In silico exploration of anti-inflammatory activity of natural Coumarinolignoids. Chem Biol Drug Des 78:567–579

Morris G, Goodsell D, Halliday R, Huey R, Hart W, Belew R, Olson A (1998) Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem 19:1639–1662

Murillo JI, Encarnacion DR, Malmstrom J, Christophersen C, Franzblau SG (2003) Antimycobacterial flavones from *Haplopappus sonorensis*. Fitoterapia 74:226

Occuhiuto F, Circosta C, de Pasquale A, Briguglio F (1990) Comparative haemodynamic effects of the falvonoids rhoifolin and vitexin in the dog. Phytother Res 4:118–120

Oh H, Kim DH, Cho JH, Kim YC (2004) Hepatoprotective and free radical scavenging activities of phenolic petrosins and flavonoids isolated from *Equisetum arvense*. J Ethnopharmacol 95:421–424

Siddiqi SH (1996) BACTEC 460 system, product and procedure manual, MA 0029, revision E, May 1996. Becton Dickinson and Company, USA

Sivakumar PM, Geetha Babu SK, Mukesh D (2007) QSAR studies on chalcones and flavonoids as anti-tuberculosis agents using genetic function approximation (GFA) method. Chem Pharm Bull 55:44–49

Stavri M, Ford CHJ, Bucar F, Streit B, Hall ML, Williamson RT, Mathew KT, Gibbons S (2005) Bioactive constituents of *Artemisia monosperma*. Phytochemistry 66:233–239

Steinbrook R (2007) Tuberculosis and HIV in India. N Engl J Med 356:1198–1199

Sutthivaiyakit S, Thongnak O, Lhinhatrakool T, Yodchun O, Srimark R, Dowtaisong P, Chuankamnerdkarn M (2009) Cytotoxic and antimycobacterial prenylated flavonoids from the roots of Eriosema Chinense. J Nat Prod 72:1092–1096

Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem 31:455–461

Wang HX, Ng TB (1999) Natural products with hypoglycemic, hypotensive, hypocholesterolemic, antiatherosclerotic and antithrombotic activities. Life Sci 65:2663–2677

Yadav DK, Khan F, Negi AS (2012) Pharmacophore modeling, molecular docking, QSAR, and in silico ADMET studies of gallic acid derivatives for immunomodulatory activity. J Mol Model 18:2513–2525

Yenjai C, Prasanphen K, Daodee S, Wongpanich V, Kittakoop P (2004) Bioactive flavonoids from *Kaempferia parviflora*. Fitoterapia 75:89–92

Zumla A, Grange J (1998) Clin Rev Tuberc Br Med J 316:1962–1964