Med Chem Res
DOI 10.1007/s00044-015-1401-1

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antitubercular activity, and molecular modeling studies of analogues of isoliquiritigenin and liquiritigenin, bioactive components from *Glycyrrhiza glabra*

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Received: 10 July 2014 / Accepted: 29 June 2015

Abstract Isoliquiritigenin (ISL, **1**) and liquiritigenin (LTG, **2**) were isolated from the rhizomes of *Glycyrrhiza glabra*. In an attempt to develop potent and selective antituberculosis agents, a series of ISL analogues were synthesized mainly via acid- and base-catalyzed Claisen–Schmidt condensation reaction for their antitubercular activity. Compared to ISL (MIC = 25 μg/mL), analogues **5**, **8**, and 1**0** showed similar antitubercular activity, but, interestingly, **6**, **7**, and **15** exhibited twofold higher activity (MIC = 12.5 μg/mL) over ISL, against *Mycobacterium tuberculosis*. Among the LTG derivatives, LTG 4′-acetate and LTG-oxime were found to be as active (MIC = 25 μg/mL) as LTG. It is the first report on antimycobacterial activity of these ISL- and LTG-based derivatives. Molecular docking and in silico ADME studies revealed that compounds **6**, **7**, and **15** are potent inhibitors of *M. tuberculosis* H_{37}Rv alanine dehydrogenase and showed compliance with standard parameters of drug likeness.

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Keywords *Glycyrrhiza glabra* · Isoliquiritigenin · Liquiritigenin · Derivative · *Mycobacterium tuberculosis*

Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* infects <30 % of the global population. Nearly two million people die with a global case fatality rate of 23 % and reaching >50 % in some African countries. The worldwide resurgence of TB is due to two major problems, first by the acquired immunodeficiency syndrome (AIDS) epidemic which started in the mid-1980s, and second, the outbreak of multidrug-resistant tuberculosis (MDR-TB). Emergence of drug-resistant strains of *M. tuberculosis* has led to increased concern on current chemotherapy regimens. A 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).

Although plant species have not yielded any antibacterial compounds of comparable potency to the antibiotics produced by microorganisms so far, many plant extracts have been tested for activity against microorganisms in anticipation that highly active compounds will be found because natural products are a proven templates for the development of new scaffolds of drugs (Cragg *et al.*, 1997; Newmann *et al.*, 2000). Another important aspect that strengthens the search of antimicrobial compounds from natural products is the recent report about presence of compounds that do not have antimicrobial activity per se, but act as potent synergistic agents together with other antimicrobials (Stermitz *et al.*, 2000; Lechner *et al.*, 2008). Moreover, advances in identifying new antibiotics from

Published online: 09 July 2015

plant sources and expanding antibiotic chemical diversity are providing crucial leads for new drugs.

Licorice is the name applied to the roots and stolons of some *Glycyrrhiza* species. It is one of the oldest traditional Chinese medicines, used as a tonic, anticoagulative, expectorant, antitussive agent (Tang and Eisenbrand, 1992), in the treatment of gastric disease (Krausse *et al.*, 2004; Chin *et al.*, 2007), and also used worldwide as a natural sweetener as well as a flavoring additive in various preparations. Glycyrrhizin, a major compound of *Glycyrrhiza glabra,* is responsible for the sweetness and various biological properties of the roots (Nomura and Fukai, 1998). The plant contains a variety of compounds including bioactive triterpenoids and phenols, which have mainly antimicrobial, antioxidant, antiulcer, anti-inflammatory, and antifungal activity. Antimicrobial activities of flavonoids, glabridin, and several glabridin analogues obtained from *G. glabra* against *Staphylococcus aureus*, *Mycobacterium smegmatis*, *M. tuberculosis* have been reported (Mitscher *et al.*, 1980; Gupta *et al.*, 2008). Phenolics, isoliquiritigenin (ISL, **1**), and liquiritigenin (LTG, **2**) were previously reported from the roots of *G. glabra* (Gaur *et al.*, 2010, 2014).

Chalcones (1,3-diaryl-2-propen-1-ones) with an enone system between two aromatic rings (Fig. 1a) constitute an important class of natural products which are considered as precursors for the preparation of various flavonoids and exhibit interesting pharmacological activities (Asl and Hosseinzadeh, 2008; Gaur *et al.*, 2014, 2015, Hans *et al.*, 2010; Lin *et al.*, 2002). This prompted us to synthesize derivatives of ISL and LTG in order to study their antimicrobial effects. The indoles are widely distributed in nature, and they possess a variety of significant biological activities (Andreani *et al.*, 2008; Biswal *et al.*, 2012;

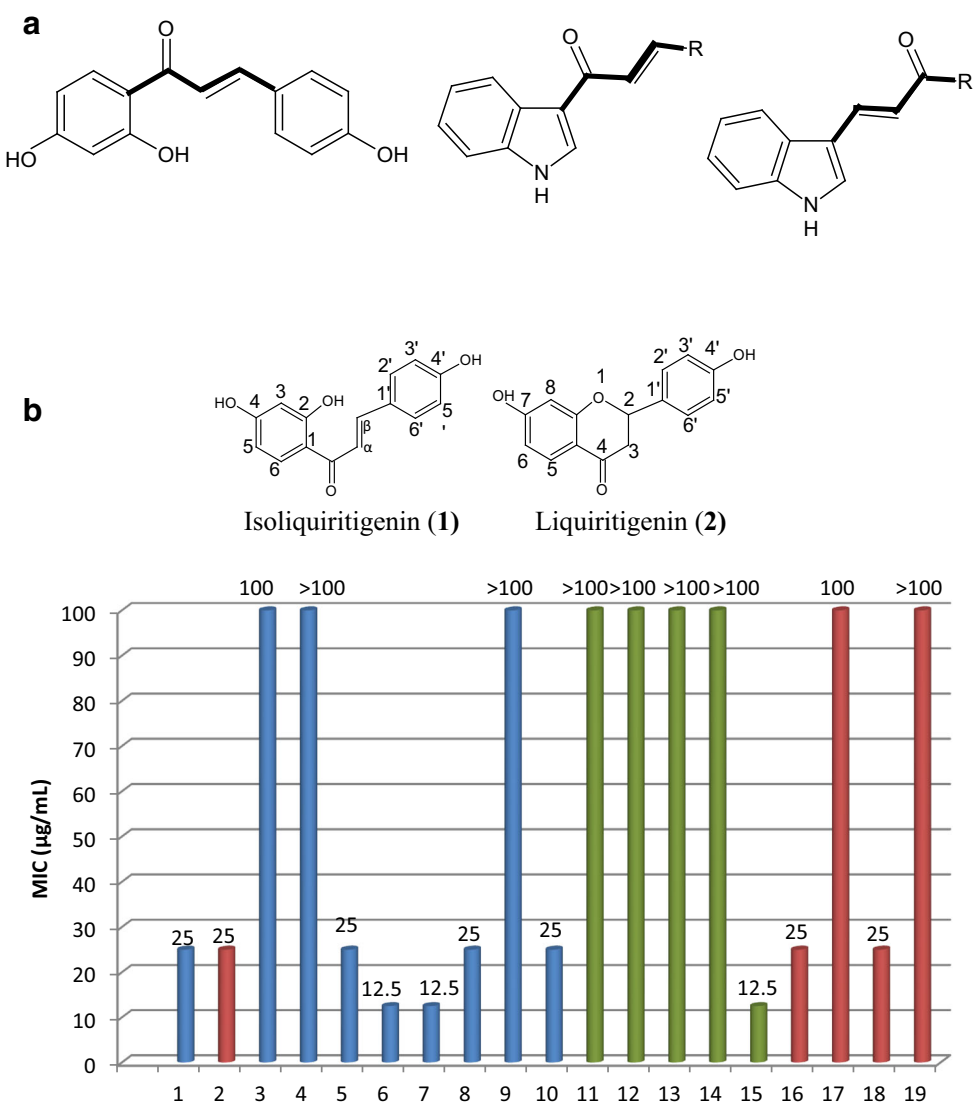


Fig. 1 **a** Basic skeleton of chalcone derivatives responsible for their biological activity. **b** Antimycobacterial activity of phenolics **1**, **2**, their analogues, and indole-based chalcones

Grugni *et al.*, 2006). So we have made changes in the structure of ISL by substituting with indole moiety for value addition.

In continuation to our previous studies on bioactive compounds from *G. glabra* (Gaur *et al.*, 2010, 2014, 2015), we tried to synthesize and explore the antimicrobial potential of ISL and LTG derivatives (**3–19**) supported by their in silico and in vitro activity studies (Fig. 1b) in the present study.

Materials and methods

Extraction and isolation

Rhizomes of *G. glabra* L. (Fabaceae) were collected from CIMAP research farm, Lucknow, India, in March 2011. The plant was identified by a taxonomist at Botany and Pharmacognosy Department, CIMAP, Lucknow, where a voucher specimen #7401 was deposited. ISL and LTG were isolated according to reported procedure (Gaur *et al.*, 2014).

HPLC analysis

HPLC analysis was performed using a Shimadzu LC-10AD liquid chromatography equipped with two LC-10A pumps controlled by a CBM-10 interface module, SPD-M10A VP diode array detector, and a SIL-10ADVP auto injector. Data were collected and analyzed using a Class LC-10 Work Station. The samples were analyzed by using reverse-phase chromatography on Waters Spherisorb ODS2 (250 × 4.6 mm i.d., 10 mm) column using binary gradient elution with acetonitrile and water containing 0.1 % TFA mobile phase (30:70) at a flow rate of 0.6 mL/min, a column temperature of 25 °C, and UV detection at λ 254 nm.

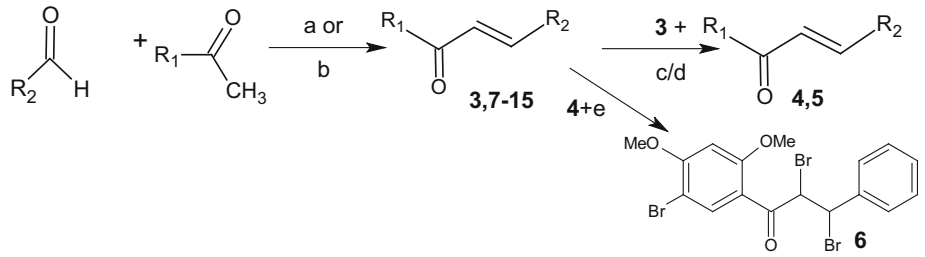
Synthesis of ISL analogues

Because of poor yield of ISL of the plant, its derivatives (**3–6**, **8–15**) were chemically synthesized (Scheme 1) according to the reported procedure (Guantai *et al.*, 2011; Bianco *et al.*, 2003, 2004; Gaur *et al.*, 2014; Ohkatsu and Satoh, 2008) and their physical properties are described in Table 1.

*Spectral data of compounds (**5**, **7**, and **10**)*

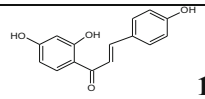
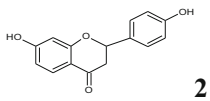
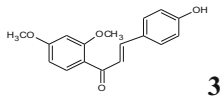
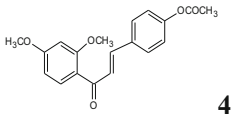
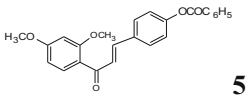
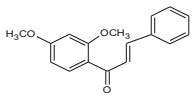
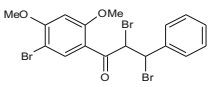
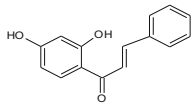
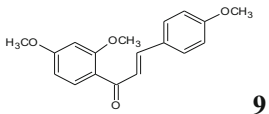
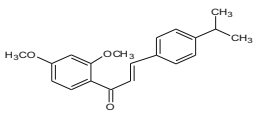
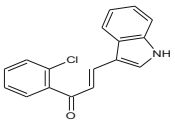
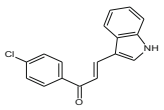
4-Benzoyloxy-2′,4′-dimethoxychalcone (**5**) IR ν^{max} (KBr): 1739 (ester CO), 1649 (chalcone CO), 1610, 1558, 1452, 1328, 1250, 1209, 1058 (aromatic) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.85, 3.89 (s, 3H each, 2× OCH_3), 6.50 (1H, *d*, $J = 1.8$ Hz, H-3′), 6.57 (1H, *dd*, $J = 8.7$, 1.8 Hz, H-5′), 7.25 (2H, *d*, $J = 8.7$ Hz, H-3, H-5), 7.49 (1H, *d*, $J = 15.9$ Hz, H-α), 7.56(2H, *d*, $J = 7.8$ Hz, H-4″, H-6″), 7.64 (H-2, *d*, $J = 8.7$ Hz, 2H, H-6), 7.67 (1H, m, H-5″), 7.69 (1H, *d*, $J = 15.9$ Hz, H-β), 7.77 (1H, *d*, $J = 8.7$ Hz, H-6′), 8.20 (2H, *d*, $J = 7.8$ Hz, H-3′, H-7′); ^{13}C NMR, DEPT ($CDCl_3$, 75 MHz): δ 55.98 (OCH_3), 56.21 (OCH_3), 99.07 (CH, C-3′), 105.69(CH, C-5′), 122.61 (CH, C-3, CH, C-5, C, C-1′), 127.83 (CH, C-α), 129.05 (CH, C-2, CH, C-6), 129.69 (C, C-2″), 129.86 (CH, C-4″, CH, C-6″), 130.63 (CH, C-3″, CH, C-7″), 133.35 (CH, C-6′), 133.75 (C, C-1), 134.19 (CH, C-5″), 141.25 (CH, C-β), 152.55(C, C-4), 160.89 (C, C-2′), 164.70 (C, C-4′), 165.3 (C = O, C-1″), 190.73 (C = O); ESI–MS, MeOH (positive): *m/z* 389 $[M + H]^+$, 411 $[M + Na]^+$ (negative): *m/z* 387 $[M - H]^-$, $C_{24}H_{20}O_5$. CAS Registry Number: 619313-51-8.

1-(5″-Bromo-2″, 4″-dimethoxyphenyl)-2,3-dibromo-3-phenyl-propan-1-one (**7**) A solution of bromine (0.03 mol, 1.5 mL) and chloroform (2 mL) was added drop wise with continuous stirring at room temperature to a solution of **6** (0.268 g, 1 mmol) in chloroform (20 mL) in 30 min. Evaporation of solvent afforded a semisolid, which was washed with water several times, and triturated with light petroleum ether 40–60 °C to give solid product, which was crystallized in ethylacetate–hexane to yield **7** (in 90 % yields). IR ν^{max} (KBr): 3433, 1666 (CO), 1589, 1468, 1396, 1271, 1215 (aromatic), 698 (C–Br) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.98 (3H, s, OCH_3), 4.05 (3H, s,



Scheme 1 General routes used for the chalcone synthesis. Reagents and conditions: (*a*) $SOCl_2$–EtOH, RT, 2 h, 87 %; (*b*) NaOH–EtOH, RT, 2–4 h, 58–93 %; (*c*) Ac_2O–C_5H_5N, RT, 0.5 h, 91 %; (*d*) PhCOCl–C_5H_5N, RT, 0.5 h, 96 %; (*e*) Br_2, CH_3COOH, RT

Table 1 Antimycobacterial activity of ISL, LTG, and their analogues against *M. tuberculosis* $H_{37}Rv$ strain by BACTEC-460 assay

Structure of compounds	MIC (μg/mL)	Physical data	MP (°C)	Yield (%)
 **1**	25	Yellow powder	208–210	0.00056
 **2**	25	Cream powder	206–208	0.0006
 **3**	n.a.	Orange crystals	91–92	87
 **4**	100	Light orange crystals	70–71	91
 **5**	25	Dark orange crystals	90–91	96
 **6**	12.5	Yellow solid	65–66	93
 **7**	12.5	White shiny crystals	118–119	90
 **8**	25	Yellow powdery crystals	145–146	8
 **9**	n.a.	Yellow crystals	50–51	92
 **10**	25	Yellow viscous	Viscous	83
 **11**	n.a.	Yellow fluffy crystals	180–181	60
 **12**	n.a.	Yellow shiny crystals	110–111	85

OCH_3), 5.63 (1H, *d*, $J = 11.4$ Hz, H-3).6.13(1H, *d*, $J = 11.4$ Hz, H-2), 6.51 (1H, s, H-3″), 7.39–7.49 (5H, m, H-2′, H-3′, H-4′, H-5′, H-6′), 8.22 (1H, s, H-6″); ^{13}C and DEPT NMR (75 MHz, $CDCl_3$): δ 50.90 (CH, C-3), 52.02 (CH, C-2), 56.89 (OCH_3), 57.02 (OCH_3), 96.62 (CH, C-3″), 104.33 (C, C-5″), 118.86 (C, C-1″), 128.72 (CH, C-3′, C-5′), 129.21 (CH, C-2′, C-6′), 129.51 (CH, C-4′), 137.05 (CH, C-6″), 139.15 (C, C-1′), 160.85 (C, C-2″), 161.69 (C, C-4″), 190.01 (C, C-1); HMBC correlations (Fig. 2): C-1 with H-2/H-3/H-6″, C-2 with H-3, C-3 with H-2/H-2′/H-6′, C-1′ with H-2/H-3/H-3′, H-5′, C-1″ with H-3″/H-6″, C-3″ with 2× OCH_3, C-5″ with H-3″/H-6″; ESI–MS, MeOH (positive): *m/z* 504 $[M + H]^+$, 526 $[M + Na]^+$ (negative): *m/z* 503 $[M - H]^-$, $C_{15}H_{15}O_3Br_3$.

4-Isopropyl-2′,4′-dimethoxychalcone (**10**) IR ν^{max} (KBr): 1653 (chalcone CO), 1605, 1463, 1327, 1254 (aromatics), 1212, 1162 (isopropyl) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.26 (6H, *d*, $J = 6.9$ Hz, H_3-8, H_3-9), 2.93 (1H, m, H-7), 3.86, 3.89 (s, 3H each, 2× OCH_3), 6.49 (1H, *d*, $J = 2.1$ Hz, H-3′), 6.56 (1H, *dd*, $J = 8.4$, 2.1 Hz, H-5′), 7.28 (2H, *d*, $J = 6.0$ Hz, H-3, H-5), 7.48 (1H, *d*, $J = 15.9$ Hz, α-H), 7.53 (2H, *d*, $J = 7.8$ Hz, H-2, H-6), 7.67 (1H, *d*, $J = 15.9$ Hz, β-H), 7.75 (1H, *d*, $J = 8.4$ Hz, H-6′); ^{13}C NMR, DEPT ($CDCl_3$, 75 MHz): δ 24.22, 24.44 (CH_3, C-8/C-9), 34.26 (CH, C-7), 55.83 (OCH_3), 56.12 (OCH_3), 99.02 (CH, C-3′), 105.65 (CH, C-5′), 122.68 (C, C-1′), 126.75 (CH, C-α), 127.66 (CH, C-2, C-6), 128.86 (CH, C-3, C-5), 133.08 (CH, C-6′), 133.50 (C, C-1), 142.59 (CH, C-β), 151.70 (C, C-4), 160.81 (C, C-2′), 164.56 (C, C-4′), 191.06 (CO); ESI–MS, MeOH (positive): *m/z* 311 $[M + H]^+$, 333 $[M + Na]^+$ (negative): *m/z* 309 $[M - H]^-$, $C_{20}H_{22}O_3$. CAS Registry Number: 100246032-3.

General method for the synthesis of indole chalcones 1-(2″-Chlorophenyl)-3-indolyl-2-propen-1-one (**11**) and 1-(4″-chlorophenyl)-3-indolyl-2-propen-1-one (**12**). Chalcones **11** and **12** were obtained by condensing indole-3-carboxaldehyde (1.45 g, 0.01 mol) with 2-chloroacetophenone (1.54 g, 0.01 mol) and 4-chloroacetophenone (1.54 g, 0.01 mol), respectively, by dissolving in methanol (35 ml) and 40 % NaOH–methanol (5 ml) at RT with constant stirring (Scheme 1). After completion of the reaction, it was poured on ice (100 g) and neutralized with dilute hydrochloric acid, which provided **11**, **12** (Manna *et al.*, 1999).

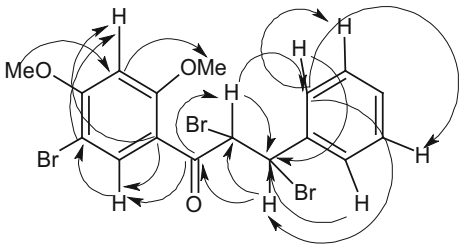


Fig. 2 HMBC correlation diagram of analogue of ISL derivative (**7**)

General method for the synthesis of indole chalcones (**13–15**) Chalcones **13**, **14**, and **15** were obtained by reacting 3-acetylindole (1.59 g, 0.01 mol) and 2, 4-dimethoxyaldehyde (1.66 g, 0.01 mol), 4-hydroxyaldehyde (1.22 g, 0.01 mol), and o-tolualdehyde (1.20 g, 0.01 mol), respectively, by dissolving in methanol (35 ml), and 1.0 mL $SOCl_2$ was added at RT with constant stirring. After completion of the reaction, it was poured on ice (100 g). The reaction was completed in about 2 h, and the solid obtained was separated by filtration, dried, and crystallized in ethyl acetate hexane to provide respective chalcones **13–15** (Manna *et al.*, 1999; Kumar *et al.*, 2010; Gaur *et al.*, 2015).

Derivatization of LTG derivatives

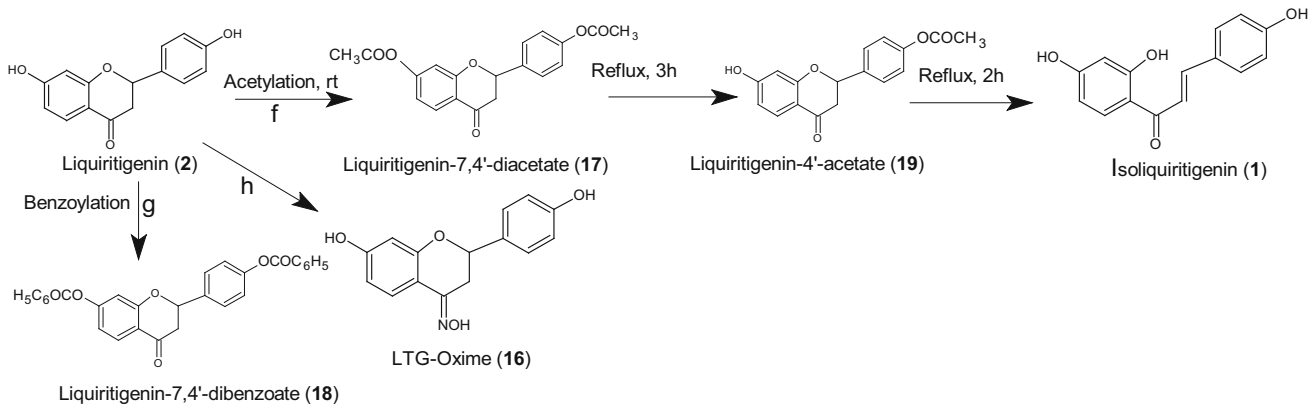
The plant derived LTG was derivatized to four analogues as per Scheme 2. LTG-oxime (**16**), LTG 7,4′-diacetate (**17**), LTG 4′-acetate (**18**), and LTG 7,4′-dibenzoate (**19**) were obtained by acetylation and benzoylation of LTG according to reported procedure (Gaur *et al.*, 2010, 2014, 2015).

Bioevaluation

BACTEC radiometric susceptibility assay

Mycobacterium tuberculosis H_{37}Rv (ATCC 27294) used in this screening was obtained from the National JALMA Institute for Leprosy and other Mycobacterial Diseases (ICMR), Agra, India, and maintained on Löwenstein–Jensen media slant at 37 °C. After 21 days of incubation, bacterial cells were scraped from slants and transferred in 1.0 mL of BACTEC diluting fluid and made the 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 1.0×10^6 cfu/mL).

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 dilutions. From this, 0.1 mL was injected into two 12B media vials and used as control. The



Scheme 2 Reagents and conditions: (*f*) Ac_2O-C_5H_5N, RT, 0.5 h, (*g*) PhCOCl-C_5H_5N, RT, 0.5 h, (*h*) $NH_2OH{\cdot}HCl$, CH_3COONa, EtOH, reflux

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 test (compounds containing) vials were compared with that of control vials based on difference in growth (ΔGI). The result was interpreted as follows: If the difference (called as ΔGI) of the current GI from previous day GI in the case of drug 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. The twofold serial dilution technique was used to assess the minimum inhibitory concentration (MIC) of a test compound.

Molecular docking studies

To find the possible bioactive conformations of chalcone derivatives, the Sybyl X 1.3 software (Certara, USA) interfaced with Surflex–Dock program was used to dock the compounds into the active site of the known antitubercular target protein alanine dehydrogenase (PDB ID: 2VOE) (Tripathi and Ramachandran, 2008) due to availability of experimental 3D protein crystallographic structural data in the RCSB Protein Data Bank (PDB; www.rcsb.org/). The program automatically docks ligand into the binding pocket of a target protein by using a protocol-based algorithm and empirically produced scoring function. The protocol is a very important and necessary factor for docking algorithm and works as a computational representation of proposed ligand that interacts into the binding site. Surflex–Dock scoring function has several factors that play an important role in the legend–receptor interaction, in terms of hydrophobic, polar, repulsive, entropies, and salvation, and it is a worldwide well-established and recognized method. The most standard docking protocols have ligand flexibility in the docking process, while counting the protein as a rigid structure. During the docking process, all of the other parameters were assigned their default values (Yadav *et al.*, 2012, 2013a, b).

Results and discussion

Chemistry

ISL (**1**) and LTG (**2**) were isolated from the chloroform/ethyl acetate fraction of rhizomes, identified by spectral analysis of their IR, ^1H, ^{13}C NMR, DEPT-135 and electrospray ionization mass spectroscopy and comparison with the data reported in literature (Gaur *et al.*, 2010, Gaur *et al.*, 2014). Because of low yield of ISL (**1**) in the plant, we followed a direct synthetic approach for other analogues (Scheme 1). 2′,4′-Dimethoxy-4-hydroxychalcone (**3**) was synthesized via acid-catalyzed Claisen–Schmidt condensation by adding thionyl chloride to a mixture of 2, 4-dimethoxyacetophenone and 4-hydroxybenzaldehyde in ethanol at room temperature (Jaypal *et al.*, 2010). The resulted chalcone **3** was further acetylated to 4-acetoxy-2′,4′-dimethoxychalcone (**4**) and benzoylated to derivative, 4-benzoyloxy-2′,4′-dimethoxychalcone (**5**). 2′,4′-Dimethoxychalcone (**6**) and 2′,4′-dihydroxychalcone (**8**) were obtained by reacting 2, 4-dimethoxyacetophenone/2, 4-dihydroxyacetophenone and benzaldehyde in the presence of ethanolic NaOH. 2′,4′-Dimethoxychalcone (**6**) gave a tribromo product **7** by electrophilic substitution reaction in the benzene ring and addition reaction at the chalcone double bond by adding bromine in acetic acid at room temperature (Scheme 1).

The chalcone **8**, reported from *G. astragalin*, has shown its presence (TLC) in the ethyl acetate fraction of *G. glabra* roots. The ISL derivative, 4-isopropyl-2′,4′-dimethoxychalcone (**10**) was synthesized by reacting 2, 4-dimethoxyacetophenone and 4-isopropylbenzaldehyde. Indole-based chalcones **11–15** were synthesized using respective aldehydes and ketones in acidic and basic conditions (Scheme 1). The new active chalcones **7** and **15** were

characterized based on their 1D (^{1}H, ^{13}C, DEPT), 2D (COSY, HSQC, HMBC) NMR correlations, IR, and mass.

The present study deals with the isolation, identification of ISL and LTG from *G. glabra,* and synthesis of their derivatives for antituberculer evaluation. It is the first report on antimycobacterial activity of ISL derivatives **3–10**, indole-based chalcones **11–15**, and LTG derivatives **16–19**. 2′,4′-Dimethoxychalcone (**6**) and new chalcones 1-(5″-bromo-2″,4″-dimethoxyphenyl)-2,3-dibromo-3-phenylpropan-1-one (**7**) and *trans*-1-indolyl-3-(2′-methylphenyl)-2-propen-1-one (**15**) possessed two times better activities over the parent compound (**1**).

The in vitro antimycobacterial activity of ISL, LTG, and their derivatives was evaluated against *M. tuberculosis* H37Rv strain by BACTEC-460 radiometric susceptibility assay. Table 1 shows the minimal inhibitory concentration (MIC) values of all 19 test compounds. The MICs of ISL and its derivatives, 4-benzoyloxy-2′,4′-dimethoxychalcone (**5**), 2′,4′-dihydroxychalcone (**8**), and 4-isopropyl-2′,4′-dimethoxychalcone (**10**), were 25 μg/mL, whereas chalcone 2′,4′- dimethoxychalcone (**6**) (MIC, minimal inhibitory concentrations = 12.5 μg/mL) and its new derivative 1-(5″-bromo-2″,4″-dimethoxyphenyl)-2,3-dibromo-3-phenylpropan-1-one (**7**) (MIC = 12.5 μg/mL) were approximately twice as active as ISL (**1**) (MIC = 25 μg/mL). Derivative **4** was moderately active with MIC value 100 μg/mL. No activity was detected at 100 μg/mL concentrations in derivatives 2′,4′-dimethoxy 4-hydroxychalcone (**3**) and 4, 2′,4′-trimethoxychalcone (**9**). This shows that ether groups in ISL analogues **5**, **8**, and **10** had a minimal effect on antitubercular activity, but the presence of methoxy groups at position 2′,4′ and absence of OH group (deoxygenation) at position 4 in ISL analog **8** and a bromine derivative of ISL (**6**) showed enhancement in the activity. Among indole-based chalcones (**11–15**), *trans*-1-indolyl-3-(2′-methylphenyl)-2-propen-1-one (**15**) possessed antitubercular activity twofold higher (MIC = 12.5 μg/mL) over the parent chalcone ISL (**1**). In the indole chalcones **11–15**, the deoxygenation and methylation in the phenyl ring might be responsible for the activity in *trans*-1-indolyl-3-(2′-methylphenyl)-2-propen-1-one (**15**). A recent study reported that ISL (**1**) possessed MIC value 25 μg/mL (Chokchaisiri *et al.*, 2009) in agreement with the data of this study.

Among LTG (**2**) and its derivatives, LTG-oxime (**16**) and LTG 4′-acetate (**18**) were found to be as active as the parent flavonoid **2** (MIC = 25 μg/mL). LTG 7,4′-diacetate (**17**) was moderately active (MIC = 100 μg/mL), whereas LTG 7,4′-dibenzoate (**19**) showed no activity at 100 μg/mL concentration. Rifampicin was used as a positive control.

ISL and LTG were also previously reported as anti-inflammatory (Kobayashi *et al.*, 1995; Lin *et al.*, 2002), hepatoprotective (Gaur *et al.*, 2010), antimycobacterial, and antitumor agents (Jaypal *et al.*, 2010). However, this is the first report on antimycobacterial activity of their derivatives.

Molecular docking revealed high binding affinity

The aim of the molecular docking study was to show the binding site interactions and active conformation and to elucidate the possible mechanism of action. In the studied work, the orientation and binding affinity (in terms of Sybyl total docking score) of active compounds **6**, **7**, and **15** on known tuberculosis target alanine dehydrogenase (PDB: 2VOE) were explored. The results of docking suggest that active compounds **6**, **7**, and **15** bind well to the target alanine dehydrogenase. The molecular interactions were measured in terms of Sybyl total docking score and

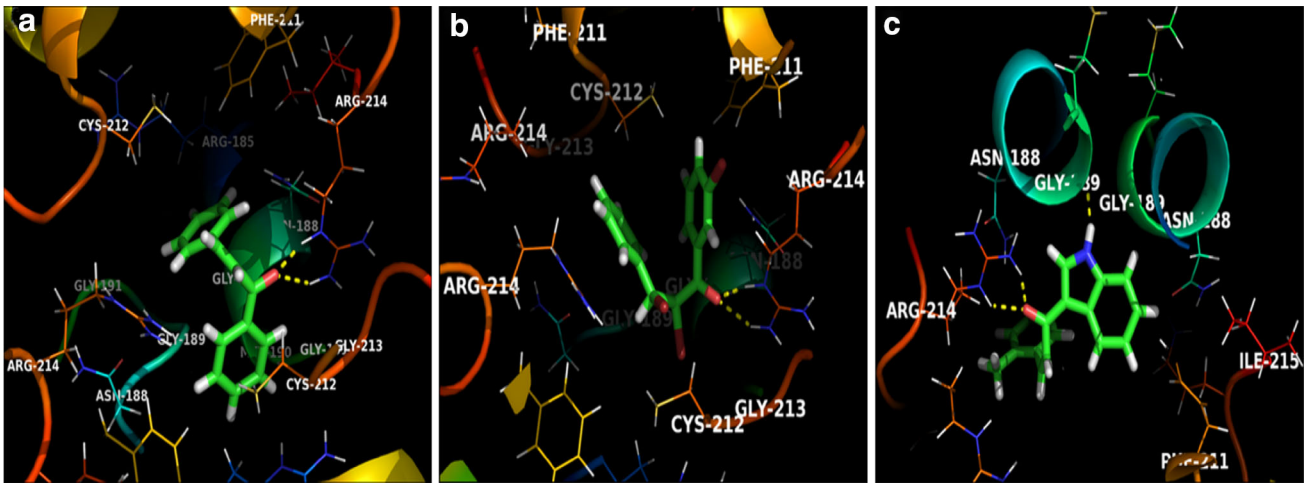


Fig. 3 Molecular docking studies of active compounds against antituberculosis target alanine dehydrogenase of *M. tuberculosis H37Rv* (PDB ID: 2VOE). Compounds **6**, **7**, and **15** docked well on alanine dehydrogenase with total docking score of 5.5193 (**a**), 4.9948 (**b**), and 4.1852 (**c**)

Table 2 Docking scores (total score) of ISL (**1**), LTG (**2**) and their analogues against antituberculosis target alanine dehydrogenase (PDB ID: 2VOE)

Compound	Total score[a]	Binding site amino acids within 4 Å	H-bond forming residue	Length of H-bond Å	No. of H-bond
1	4.8134	CYS-212, GLY-213, ARG-185, ASN-188, GLY-189, GLY-191, ARG-214, ARG-185, ILE-186, ASN-188, GLY-189M MET190, GLY-191, PHE-211, ARG-214, ILE-215	GLY-189 ASN-188 ARG-185	2.0 1.9 1.9	3
2	4.8439	GLY-189, ARG-214, ARG-185, ILE-186, ASN-188, GLY-189, PHE-211, ILE-215, CYS-212, GLY-213	ASN-188 ARG-185	1.9 1.9	2
6	5.5193	CYS-212, GLY-213, ARG-185, ASN-188, GLY-189, GLY-191, PHE-211, ARG-214, ARG-185, ILE-186, ASN-188, GLY-189, MET-190, GLY-191, PHE-211, ARG-214	ARG-214 ARG-214	1.9 2.2	2
7	4.9948	CYS-212, GLY-213, GLY-189, GLY-191, ARG-214, ARG-185, ASN-188, GLY-189, PHE-211, ARG-214	ARG-214 ARG-214	2.0 2.2	2
15	6.7638	PHE-211, CYS-212, GLY-213, ARG-214, ARG-185, ASN-188, GLY-189, PHE-211, ILE-215, CYS-212, ASN-188, GLY-189, GLY-191, PHE-211, ARG-214	ARG-214 ARG-214 GLY-189	2.1 1.7 2.0	3
Rifampcin	4.1852	GLU-210, PHE-211, CYS-212, PHE-211, CYS-212, GLY-213, ARG-214, ASN-188, GLY-189, PHE-211, CYS-212, GLY-213, ARG-214, ASN-188, CYS-212, ARG-214, ASN-188, GLY-189, ARG-214, ASN-188, PHE-211, CYS-212, ARG-214	ARG-214	2.4 2.5	2

[a] Surflex–Dock scores (total scores) were expressed in $-\log_{10}$ (Kd) units to represent binding affinities

revealed the binding site interacting amino acid residues of the target (Fig. 3).

For comparison of binding affinity and docking score, known antituberculosis drug rifampicin was selected for docking simulation. The docking results of rifampicin with that of targetalanine dehydrogenase of *M. tuberculosis H37Rv* showed a significant binding affinity as indicated by docking score 4.1852 (Table 2). This docking result was considered as positive control parameter and later was used to compare the significance level of active compounds **6**, **7**, and **15** on the basis of their docking scores. Parent compounds ISL (**1**) and LTG (**2**) also showed a high binding affinity as indicated by docking score of 4.8134 and 4.8439, respectively, and revealed the formation of hydrogen bond between the ligand and receptor. The docking results of compound **6** showed a high docking score indicated by total score of 5.5193 and formed the two hydrogen bonds of length 1.9 and 2.2 Å to the binding site basic residue arginine (Arg-214) (Fig. 3a). Similarly, docking results of compound **7** also showed a high binding affinity as indicated by total docking score 4.9948 and formed the two hydrogen bonds of length 2.0 and 2.2 Å to the basic residue arginine (Arg-214) (Fig. 3b). Likewise, compound **15** docking results also showed higher binding affinity than rifampicin, as indicated by total docking score of 6.7638 and formed three hydrogen bonds of length 2.1, 1.7, and 2.0 Å to the basic residue arginine (ARG-214) and glycine (GLY-189) (Fig. 3c). Docking results showed that studied compounds **6**, **7**, and **15** binds well with the *M. tuberculosis* H37Rv target alanine dehydrogenase through hydrogen and electrostatic bonds and this could be the reason for higher molecular stability and activity (Table 2).

Assessment through electronic pharmacokinetic parameters

The pharmacokinetics parameters such as ADME/Tox (absorption, distribution, metabolism, excretion, and toxicity) are important descriptors for human therapeutic use of drugs. The ADME descriptors were calculated by Qikprop v.3.2 software (Schrödinger, USA, trial version) and evaluated for compliance with their standard ranges. Screening of active parent compounds, namely ISL (**1**) and LTG (**2**), and their analogues, namely compounds **6**, **7**, and **15** for compliance with standard electronic ADME parameters, e.g., log S for aqueous solubility, log IC50 for HERG (the human Ether-à-go-go-Related Gene) K+ Channel (potassium ion channel) blockage, apparent Caco-2 (colon cancer cell line) permeability, log BB for brain/blood barrier, apparent MDCK (Madin–Darby canine kidney) membrane permeability, log Kp for skin permeability, no. of metabolic reactions, log Khsa serum protein binding, topological polar surface area (TPSA), predicted percent (%) of human oral absorption, and predicted human oral absorption through qualitative model, was evaluated. The compound distribution in human was evaluated by

Table 3 Compliance of ISL (**1**), LTG (**2**), and their analogues with the standard range of computational pharmacokinetic parameters (ADME)

Comp. no.	log S for aqueous solubility	Log IC50 for HERG K+ channel blockage	Apparent caco-2 permeability (nm/s)	log BB for brain/ blood barrier	Apparent MDCK permeability (nm/s)	log Kp for skin permeability	No. of metabolic reactions	log Khsa serum protein binding	TPSA	% of human oral absorption	Qual. model for human oral absorption
1	−3.755	−5.255	194.56	−1.489	84.314	−3.113	3	−0.146	87.651	79.336	HIGH
2	−3.536	−4.924	307.242	−0.959	138.158	−3.31	4	−0.121	80.497	80.726	HIGH
6	−3.287	−5.469	3705.547	−0.07	2038.037	−0.56	0	0.082	26.422	100	HIGH
7	−8.357	−5.379	6527.796	0.625	10,000	−0.429	3	0.545	20.818	100	HIGH
15	−5.058	−5.653	2301.089	−0.289	1217.747	−1.067	1	0.713	37.759	100	HIGH
Rifampicin	−0.673	−5.897	13	−4.770	−1.784	−6.446	2	−0.258	214.087	22	Low
Stand. range[a]	(−6.5/0.5)	(Concern <−5)	(<25 poor, >500 great)	(−3.0/1.2)	(<25 poor, >500 great	(−8.0 to −1.0, Kp in cm/h)	(1.0/8.0)	(−1.5/1.5)	(7.0/200.0)	(<25 % is poor)	(>80 % is high)

[a] For 95 % of known drugs, based on –Qikprop v.3.2 (Schrödinger, USA, 2012) software results

following factors, e.g., log BB, permeability (apparent Caco-2 and MDCK permeability, and log Kp for skin permeability), the volume of distribution and plasma protein binding was evaluated by log Khsa. ADME results showed that compound **7** violates the standard range for aqueous solubility logs. Compounds **6**, **7**, and **15** showed Caco-2 permeability and log BB for brain/blood barrier. Compounds **6**, **7**, and **15** showed apparent MDCK permeability and underlimit TPSA value for oral bioavailability (Table 3).

Conclusions

The present study has shown that the ISL-type chalcones as well as LTG-derived flavonoids could be used as possible leads for the control of TB infections. Therefore, plants are excellent sources or information centers of diverse molecules to be modified for the discovery of human therapeutics.

Acknowledgments The authors are grateful to the, Director, CIMAP for providing necessary facilities for this work. Financial support from the National Medicinal Plant Board (NMPB), New Delhi, is duly acknowledged. We acknowledge the Council of Scientific and Industrial Research (CSIR), New Delhi, for financial support through networking project GENESIS (BSC0121) at CSIR-CIMAP, Lucknow.

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