J Korean Soc Appl Biol Chem (2015) 58(4):581–585
DOI 10.1007/s13765-015-0079-x

Online ISSN 2234-344X
Print ISSN 1738-2203

ARTICLE

Antimicrobial and docking studies of (−)-catechin derivatives

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Received: 3 April 2015 / Accepted: 27 April 2015 / Published online: 16 May 2015

Abstract Antimicrobial activities of (−)-catechin derivatives were assayed for their antibacterial and antifungal activities against gram positive, gram negative bacteria, and fungi. Most of the compounds significantly active among which Compounds **1a**, **1b**, and **1c** showed excellent antibacterial for both gram negative and gram positive bacteria, these compounds also exhibited excellent antifungal activity more than the standard drug. Molecular docking studies of Compounds **1a** and **1b** established good binding affinity with ATP-binding pocket of DNA gyrase and are in favor of the observed biological activity. These data collectively suggest that Compounds **1a** and **1b** could serve as a novel antimicrobial agent.

Keywords Antibacterial activities · Antifungal activities · (−)-catechin · Neomycin · Miconazole

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Introduction

Increasing resistance of microorganisms and multidrug-resistance (MDR) against the available microbial drug is one of the major concerns among researchers and clinicians across the world which led to an increasing search for new antibiotics (Paul et al. 2010). In short periods of time, new and multidrug resistance against the microbes is occurring rapidly (Alanis 2005). New drugs development with increased antimicrobial activity with no adverse effects against MDR bacteria is urgently needed for the human kind worldwide (Fernandes et al. 2013). It is also expected to develop drugs that can reverse the resistance observed overturning the actual bacterial profile, and search for new drug with potent activity is always needed.

Green tea, a widely consumed beverage next to water worldwide is obtained from the leaves of *Camellia sinensis* (Graham 1992). Catechins and its derivatives showed various pharmacological activities viz antimutagenic (Paschka et al. 1998), antioxidant (Gadow et al. 1997), antibacterial (Hu et al. 2001; Stapleton et al. 2004; Park and Cho 2010), anticancer activities (Uesato et al. 2000; Kumar et al. 2015), and prevention of atherosclerosis (Miura et al. 2001; Chyu et al. 2004). Catechins face one of the major limitations. They have low bioavailability, and low stability in neutral or slightly alkaline solutions and inability to cross cellular membranes (Hong et al. 2002). Thus, derivatives of catechins are of great interest, and have become targets for synthetic chemists and biologists. 3-*O*-alkyl and acyl analogs of (−)-epicatechin with moderate alkyl chains, exhibited greater improvements in their activity and also prevented from enzymatic or non-enzymatic cleavage (Park et al. 2004). Introduction of moderate-sized aliphatic chain at the C-3 hydroxyl position

(lipophilic substituent) significantly improved the inhibitory effect of microbial growth (Park and Cho 2010). In view of this, we synthesized the derivatives of (−)-catechins and studied their activities of particular interest and, in continuation of our studies, on biologically active chemical entities (Husain et al. 2012; Xie et al. 2014; Kumar et al. 2015). Herein we report the antimicrobial activities and docking studies of (−)-catechin analogs (**1a–l**) with an optimal alkyl chain length for their enhanced activities.

Materials and methods

Chemistry

Series of (−)-catechin derivatives (Fig. 1) were synthesized and characterized according to our reported procedure (Kumar et al. 2015).

Antimicrobial activity

The antimicrobial activity of the synthesized catechin derivatives was determined using well diffusion method (Amsterdam 1996) against different pathogenic bacterial and *Candida* reference strains procured from the Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The pathogenic bacterial and *Candida* reference strains were seeded on the surface of the media petri plates, containing Muller-Hinton agar with 0.1 mL of previously prepared microbial suspensions individually containing 1.5×10^8 cfu mL^{-1} (equal to 0.5 McFarland). Wells of 6.0 mm diameter were prepared in the media petri plates using a cork borer and the synthesized compounds at a dose range of 300–1.17 μg $well^{-1}$ were added in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solution of Neomycin and Miconazole at a dose

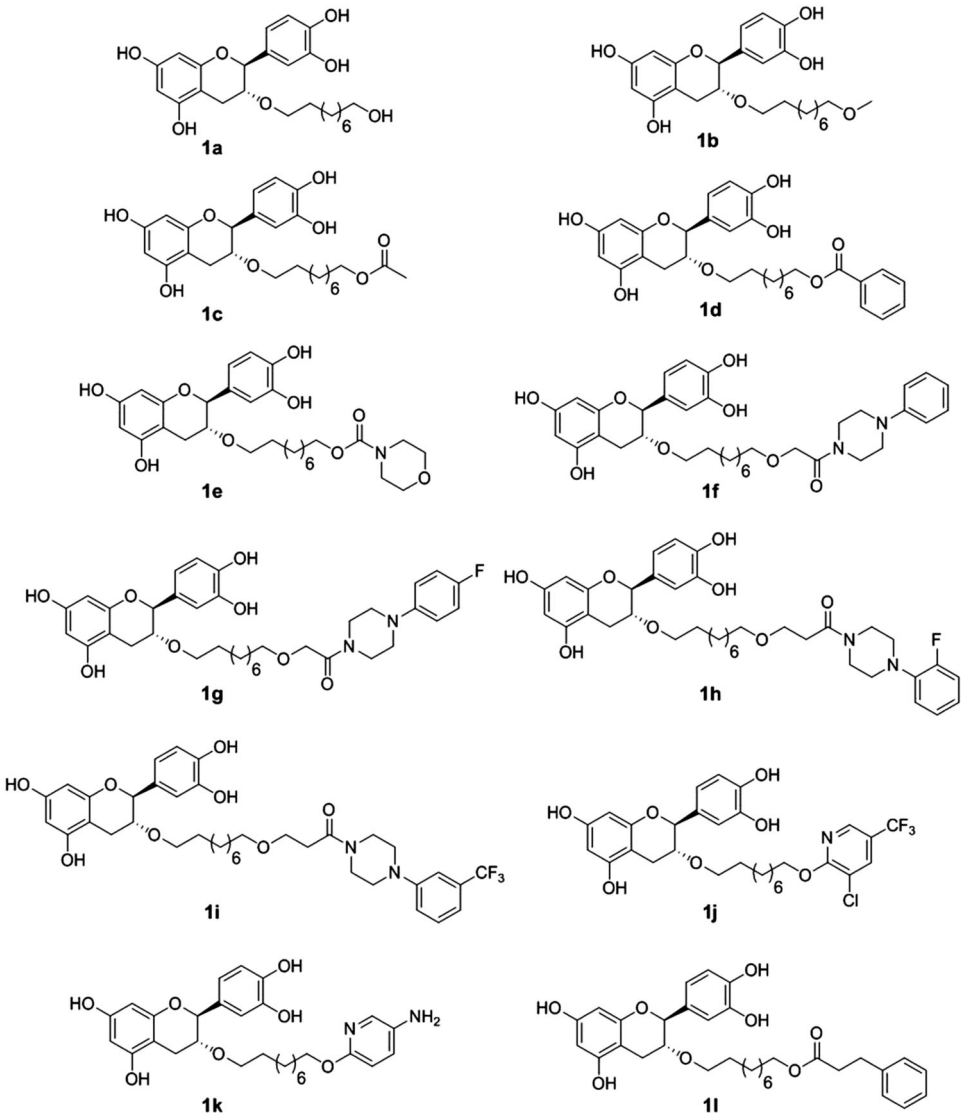

Fig. 1 Synthesized (−)-catechin derivatives

range of 300–1.17 μg well^{-1} and the well containing methanol served as positive and negative controls, respectively. The plates were incubated for 24 h at 37 and 30 °C for bacterial and *Candida* strains respectively, and the well containing the least concentration showing the inhibition zone was considered as the minimum inhibitory concentration. All experiments were carried out in duplicates and mean values are represented.

Docking studies

DNA gyrase (PDB code: 1AJ6) was selected as the receptor for docking simulation in ATP-binding pocket (Holdgate et al. 1997; Gradisar et al. 2007). After removing the ligand and solvent molecules, hydrogen atoms and Kollman charges were added to each protein atom. Coordinates of each compound were generated using Chemdraw11 followed by MM2 energy minimization. Docking was carried out by AutoDock4 in ATP-binding pocket (Morris et al. 2009). Grid map in Autodock that defines the interaction of protein and ligands in binding pocket was defined. The grid map was used with 60 points in each *x*, *y*, and *z* direction, equally spaced at 0.375 Å. Docking was performed using the Lamarckian genetic algorithm. Each docking experiment was performed 100 times, yielding 100 docked conformations. Parameters used for the docking were as follows: population size of 150; random starting position and conformation; maximal mutation of 2 Å in translation and 50° in rotations; elitism of 1; mutation rate of 0.02 and crossover rate of 0.8; and local search rate of 0.06. Simulations were performed with a maximum of 1.5 million energy evaluations and a maximum of 50,000 generations. Final docked conformations were clustered using a tolerance of 1.0 Å root mean square deviation. The best model was picked based on the best stabilization energy.

Results and discussion

Twelve derivatives of (−)-catechin (Fig. 1) were synthesized and characterized according to our reported procedure from the starting material 2,4,6-trihydroxyacetophenone and 3,4-bis(benzyloxy)benzaldehyde in 10 steps with an yields of 75–86 % (Kumar et al. 2015). The antimicrobial activity of the synthesized (−)-catechin derivatives was determined using well diffusion method. All the synthesized compounds were screened for their antibacterial activity against *Staphylococcus aureus (S. aureus* MTCC 96, MLS16, MTCC 2940), *Micrococcus luteus* (*M. luteus* MTCC 2470), *Bacillus subtilis* (*B. subtilis* MTCC 121), *Klebsiella planticola (K. planticola* MTCC 530), *Escherichia coli* (*E. coli* MTCC 739), and *Pseudomonas aeruginosa* (*P. aeruginosa* MTCC 2453) bacterial species. Standard antibiotic solution of Neomycin at a dose range of 300–1.17 μg well^{-1} and the well containing methanol served as positive and negative controls, respectively. The plates were incubated for 24 h at 37 and 30 °C for bacterial and *Candida* strains respectively, the well containing least concentration showing the inhibition zone which was considered as the minimum inhibitory concentration.

The antimicrobial screening data showed that Compounds **1a**, **1b**, and **1c** exhibited excellent activity against *Bacillus subtilis* (*B. subtilis* MTCC 121), with MIC 2.34 μg mL^{-1} and moderate activity against *S. aureus* MTCC 96 2940 with MIC-75 μg mL^{-1} and it showed a significant activity toward other gram positive bacteria. Other compounds showed weak activity against *Micrococcus luteus* MTCC 2470 (Fig. 2). Among all tested Compounds **1a**, **1b**, and **1c** showed good antibacterial activity. The cLog *P* values of all catechin analogs were calculated using ACD/ChemSketch software version 12.01 (listed in Table 1) and were used for structure–activity relationship (SAR) studies. SAR analyses indicate that the Compounds **1a**, **1b**, and **1c** showed excellent activity when compared to other analogs. The increase in the activity may be attributed due to ester-linked analogs. However, other derivatives did not show good activity, which means that replacement of other substituent is not necessary. This shows that ether or ester link to hydroxyl group or simply hydroxyl group is necessary for the activity.

The antifungal activity of all Compounds **1a**–**1l** was evaluated against *Candida albicans* (*C. albicans* MTCC 1962) in which Compounds **1a**, **1b**, and **1c** exhibited excellent activity as shown in Fig. 3. Compounds **1a**, **1b**, and **1c** were evaluated for other strains of fungi such as *C. albicans* MTCC 183 (C. A1), *C. albicans* MTCC 854 (C.

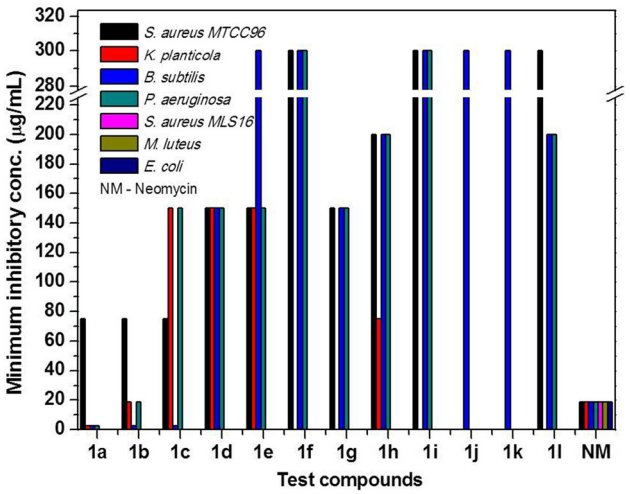

Fig. 2 Antibacterial activities of Compounds **1a–l**

Table 1 cLog *P* values of (−)-catechin derivatives

1a	1b	1c	1d	1e	1f
3.39	4.50	4.34	6.27	3.67	5.66
1g	**1h**	**1i**	**1j**	**1k**	**1l**
5.97	6.18	7.04	7.10	5.87	6.43

A2), *C. albicans* MTCC 1637 (C. A3), *C. albicans* MTCC 3018 (C. A4), *C. albicans* MTCC 4748 (C. A5), *C. albicans* MTCC 7315 (C. A6), *C. albicans* MTCC 3019 (C. A7), *C. albicans* MTCC (C.A8), *Candida aaseri (C. aaseri* MTCC 1962), *Issatchenkia hanoiensis* (*I. hanoiensis* MTCC 4755), *Candida glabrata* (*C. glabrata* MTCC 3019), *Issatchenkia orientalis* (*I. orientalis* MTCC 3020), *Candida parapsilosis (C. parapsilosis MTCC* 1744), and they showed excellent activity against all type of fungi, which was found to be more than the standard drug. Compounds **1a**, **1b**, and **1c** showed very high inhibitory activity (MIC = 2.4 μg mL^{-1}) when compared with the standard drug, which is illustrated in Fig. 4. Introduction of lipophilic group at C-3 position influences the pharmacokinetic properties.

Docking of catechin derivatives in ATP-binding pocket of DNA gyrase B was performed by Autodock4 (Holdgate et al. 1997; Gradisar et al. 2007; Morris et al. 2009). Two hydroxyl groups of chroman-5,7-diol participate in hydrogen bond with main chains of Val97 and Gly117, and the side chain of Ser121. The 9-alkoxynonan-1-ol group of Compound **1a** makes hydrogen bond with Asp73, while methoxynonyloxy of **1b** not involving in hydrogen but it can accommodate in ATP-binding pocket (Fig. 5A, B). Compound **1c** has nonyl acetate group which is difficult to

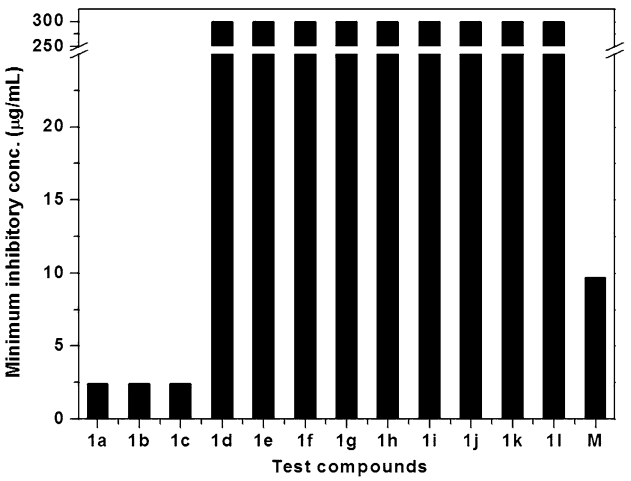

Fig. 3 Antifungal activity of the (−)-catechin derivatives against *candida* strain

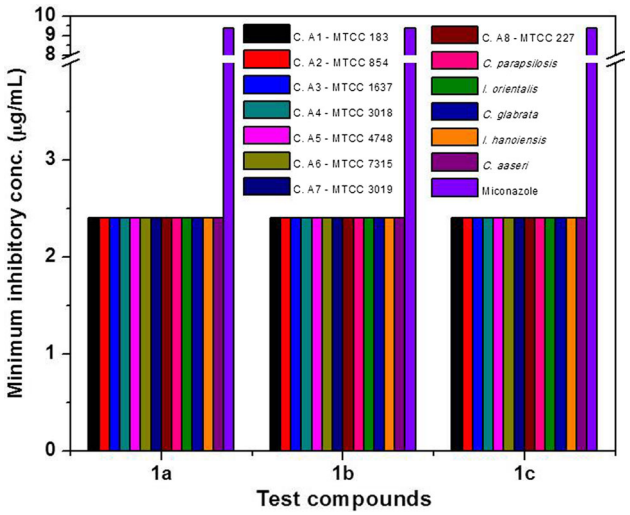

Fig. 4 Antifungal activities of **1a**, **1b**, and **1c** against different strains of fungus

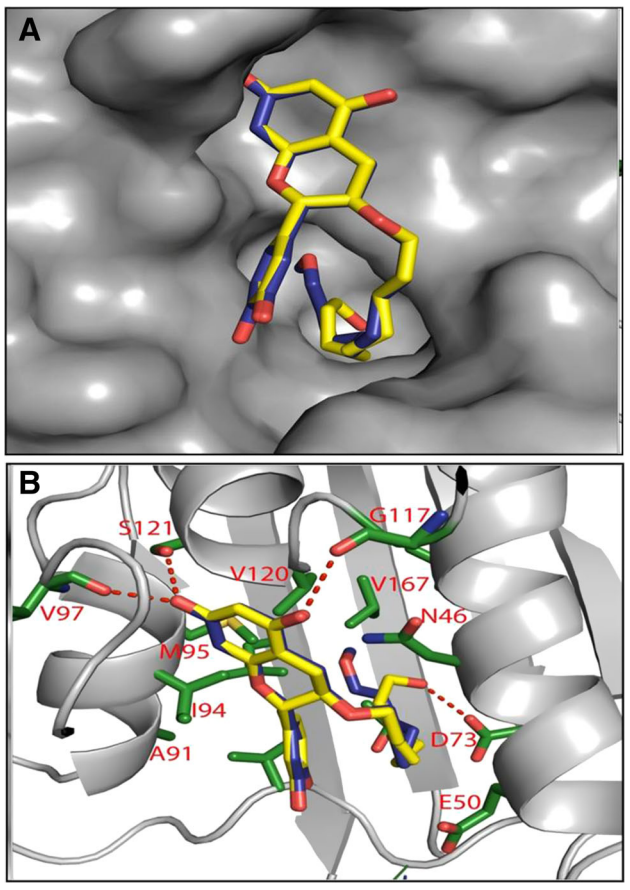

Fig. 5 **A** Docking pose of **1a** and **1b** on ATP-binding pocket of DNA gyrase B (PDB code: 1AJ6). *Panel A* represents overall binding of compounds. **B** Docking pose of **1a** and **1b** on ATP-binding pocket of DNA gyrase B (PDB code: 1AJ6). *Panel B,* Stick representation of selected amino acids of DNA gyrase B interacting with Compounds **1a** and **1b**. *Red dot line* represents hydrogen bonds

accommodate in ATP-binding pocket. Compounds **1d**, **1e**, **1f**, **1g**, **1h**, and **1i** have alkyl chain containing bulky group that is difficult to fit in ATP-binding pocket of DNA gyrase. The presence of small hydroxy and methoxy groups in Compounds **1a** and **1b** makes it easy to be accommodated in ATP-binding pocket of DNA gyrase, which might be a reason for good activity against microbial strain.

In summary, antimicrobial and docking studies of (−)-catechin derivatives have been studied. Compounds **1a**, **1b**, and **1c** showed excellent antibacterial activity for gram negative and gram positive bacteria. These compounds also exhibit excellent antifungal activity more than the standard drug "neomycin, miconazole," respectively. This indicates that (−)-catechin derivatives can be considered as potential antimicrobial agents. Molecular docking studies also exhibited good binding affinity of Compounds **1a** and **1b** which have hydroxyl and methoxy group respectively. Both groups are small and easy to accommodate in ATP-binding pocket of DNA gyrase. Finally, it is conceived that further derivation of such compounds could serve as new templates for antibacterial drugs.

Acknowledgments This work was supported by the Grants from Ministry of Environment (KME, 412-111-008) and National Research Foundation (NRF-2009-0094063), Korea.

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