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Inhibition of *Candida rugosa* lipase by saponins, flavonoids and alkaloids

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Abstract

Lipase inhibitors have generated a great interest because they could help in the prevention or the therapy of lipase-related diseases. Therefore, the aim of the work was to evaluate by HPLC, and using *Candida rugosa* lipase as model, the inhibitory effect of several saponins: β-aescin, digitonin, glycyrrhizic acid (GA) and *Quillaja* saponin (QS); flavonoids: 3-hydroxyflavone, 5-hydroxyflavone, (±)-catechin and kaempferol; and alkaloids: aspidospermine, papaverine, physostigmine, pilocarpine, raubasine, rescinnamine, reserpine and trigonelline.

The inhibition produced by most of these compounds is described here for the first time. Saponins appeared very active, being β-aescin and digitonin the most active compounds (IC_{50} = 0.8–2.4 × 10^{-5} M). The inhibitory activity of flavonoids was lower than that of saponins (except GA), and (±)-catechin and kaempferol were the most active. Alkaloids was the most heterogeneous group assayed, varying from rescinnamine, with an IC_{16} similar to that of digitonin, to papaverine and others which showed almost no inhibition.

In conclusion, β-aescin, digitonin, kaempferol or (±)-catechin, strong lipase inhibitors with a low toxicity and present herbal drugs used for lipase-related diseases such as acne or ulcer, are promising candidates for the prevention or the treatment of these diseases.

Keywords: *Candida rugosa* lipase; Inhibition; Saponins; Flavonoids; Alkaloids

1. Introduction

Lipases and esterases are glycerol ester hydrolases (E.C. 3.1.1.–) acting on acylglycerols to liberate fatty acids and glycerol. Several lipases produced by microbial pathogens play an important role in infective diseases. Indeed, *Propionibacterium acnes* lipase and its inhibition by antiacne compounds have been studied because the fatty acids produced by *P. acnes* lipase activity on sebaceous triglycerides induce severe inflammation [1]. It has also been described that *Helicobacter pylori* lipase activity can weaken the barrier properties of mucus by hydrolyzing endogenous lipids [2,3], and it is inhibited by sucralfate and other antiulcer drugs [4,5]. Furthermore, lipase-producing fungal dermatophytes can efficiently colonize the keratinized layers of the skin producing cutaneous diseases [6]. Therefore, research on new lipase inhibitors for the therapy of these diseases and also for other pathologies like obesity, has generated a great interest.

On the other hand, lipolytic enzymes are currently attracting an increasing attention because of their biotechnological potential [7,8]. Inhibition studies on lipases could contribute to better understand their mechanism of action in order to design novel substrate specificities for improving the biotechnological applications of these enzymes [9].

Plant secondary metabolites present in herbal drugs and food have shown to be very useful in the prevention and treatment of many diseases [10]. Among these metabolites, saponins, flavonoids and alkaloids are a promising source of lipase inhibitors since they are present in high concentrations in plant extracts capable of inhibiting porcine pancreatic lipase activity [11]. Furthermore, these compounds are also present in several plant extracts that have been used for the treatment of diseases in which lipases could play an important role like ulcer [12] and acne [1]. Recent studies have also demonstrated that saponins like platycodin D [13], flavonoids like quercetin [14] and alkaloids like berberine and sanguinarine [15] are

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good lipase inhibitors. However, further studies are necessary to elucidate the effect of other purified plant metabolites on lipolytic enzymes in order to select the most suitable ones for therapeutic or preventive pharmacological treatments.

Here, we report the inhibitory effect of several saponins, flavonoids and alkaloids (Fig. 1) on *Candida rugosa* lipase (Crl). This enzyme is well known and widely used in biotechnology [7] and the analysis of its activity and inhibition could help in controlling and increasing its effectiveness in a wide range of biotechnological processes. Moreover, Crl could serve as a model for fungal pathogenic lipases, and its inhibition could be of interest in the treatment against *C. rugosa* strains refractory to antifungal therapy involved in veterinary mycology and in emerging pathogenesis on immunocompromised patients [16]. For these reasons, Crl has been recently used as model for lipase inhibition assays [14,15,17].

2. Experimental

2.1. Reagents

Methanol, acetonitrile and water for HPLC were purchased from Lab-Scan (Dublin, Ireland). Trifluoroacetic acid and ethyl acetate (EA) were obtained from Aldrich (Milwaukee, WI, USA). β-Naphthyl laurate (β-NL), β-naphthol and kaempferol were purchased from Fluka (Buchs, SG, Switzerland). Raubasine, rescinnamine and aspidospermine were obtained from Simes Spa (Milan, Italy). Digitonin was purchased from ICN Biomedicals Inc. (Irvine, CA, USA). *C. rugosa* lipase (cat. No. L-1754), β-aescin, glycyrrhizic acid (GA), *Quillaja* saponin (QS), (±)-catechin, 3-hydroxyflavone and 5-hydroxyflavone, reserpine, pilocarpine, physostigmine and the other reagents were from Sigma (St. Louis, MO, USA). All reagents were used without further purification.

2.2. Evaluation of lipase inhibition by HPLC

Lipase inhibition assays by HPLC were performed as previously described [15]. Essentially, the substances under evaluation were dissolved at their maximum solubility in a proper solvent such as water (trigonelline, papaverine and pilocarpine), acetone (3-hydroxyflavone and 5-hydroxyflavone), dimethyl sulfoxide (DMSO; GA and kaempferol), or methanol (the rest of substances evaluated). Then, 2020 μl reaction mixtures containing varying inhibitor concentrations (from 0 to their maximum solubility), 3.75% inhibitor solvent, 0.46 mM β-naphthyl laurate (β-NL), 1.25% acetone, 1 mM sodium taurocholate, 3.5 mM NaCl, 1.5 mM $CaCl_2$, 50 mM Tris–HCl buffer (pH 7.4 at 22 °C) and 10 μg ml^{-1} of Crl, were incubated for 30 min at 37 °C under gentle mixing. Then, β-naphthol was extracted with 2 ml of ethyl acetate, and 500 μl of the organic phase were withdrawn, evaporated at room temperature under a nitrogen stream and redissolved in 1 ml methanol. Aliquots of 50 μl were analyzed at room temperature using a C-18 reversed-phase column (4.6 mm × 250 mm; 5 μm particle size, 90 Å pore size; Beckman) equilibrated at a flow rate of 1 ml min^{-1}, with a mobile phase consisting of 40% (v/v) acetonitrile in water, containing 0.1% trifluoroacetic acid. The eluate was monitored at a wavelength of 230 nm with a sensitivity of 0.8 A.U.F.S. The chromatographic system consisted of a precision pump (Waters, model 515) and a variable wavelength monitor (Waters, model 2487). The area under the chromatographic peak was measured using the Millennium 32 chromatography manager 4.0 software package for Windows®. β-NL unspecific hydrolysis was subtracted performing proper blanks.

2.3. Statistical analysis

Lipase inhibition was calculated from the residual activity detected in the presence of the compound under assay with respect to that of untreated samples (without inhibitor but prepared and analyzed under the same conditions than the inhibitor-treated samples, and including the inhibitor solvent to take into consideration the effect of each solvent in Crl activity). The concentrations yielding a lipase inhibition of 16% (IC_{16}) and 50% (IC_{50}) were calculated from the inhibition rate versus inhibitor concentration curves by regression analysis performed using the software Sigma-Plot 8.0 (SPSS, Chicago, IL, USA). Three or more replicates of regression curves with *R*-square coefficients higher than 0.99 were used for IC calculations, being each replicate the result of an independent HPLC assay performed in duplicate.

3. Results and discussion

The effect of several saponins, flavonoids and alkaloids on *C. rugosa* lipase was analyzed to evaluate their potential as antilipase drugs. Among the compounds tested, saponins were very active, flavonoids displayed a lower inhibition, and alkaloids, the most heterogeneous group, showed a wide inhibition range (Table 1; Fig. 2). The different inhibition produced by these substances is probably related to their different structure (Fig. 1) and physicochemical properties, as explained below.

3.1. Effect of saponins on C. rugosa lipase

Saponins are glycosidic compounds containing a steroid or triterpenoid sapogenin nucleus. They are characterized by antimicrobial, hypocholesterolemic, anti-inflammatory and other activities and by a low toxicity [10,18,19]. They also have a certain activity against ulcer [12] and human pathogenic *Candida* spp. [20]. In vitro inhibition by saponin-containing formulations on *P. acnes* lipase [21] and by purified saponins (platycodin D and dioscin) on pancreatic lipases [13,22] has also been reported recently. However, saponins from *Medicago sativa* activated pancreatic lipase [23].

Here, we have analyzed the effect of β-aescin, digitonin, glycyrrhizic acid (GA) and *Quillaja* saponin (QS) on Crl using an HPLC assay (Table 1; Fig. 2). All saponins showed a high inhibition on Crl, being the most active group of inhibitors in general terms. Both digitonin and β-aescin were the most active compounds (IC_{50} = 0.8–2.4 × 10^{-5} M). β-Aescin was the most active inhibitor at concentrations below 2–5 × 10^{-5} M, and digitonin displayed the highest inhibition above this concentration.

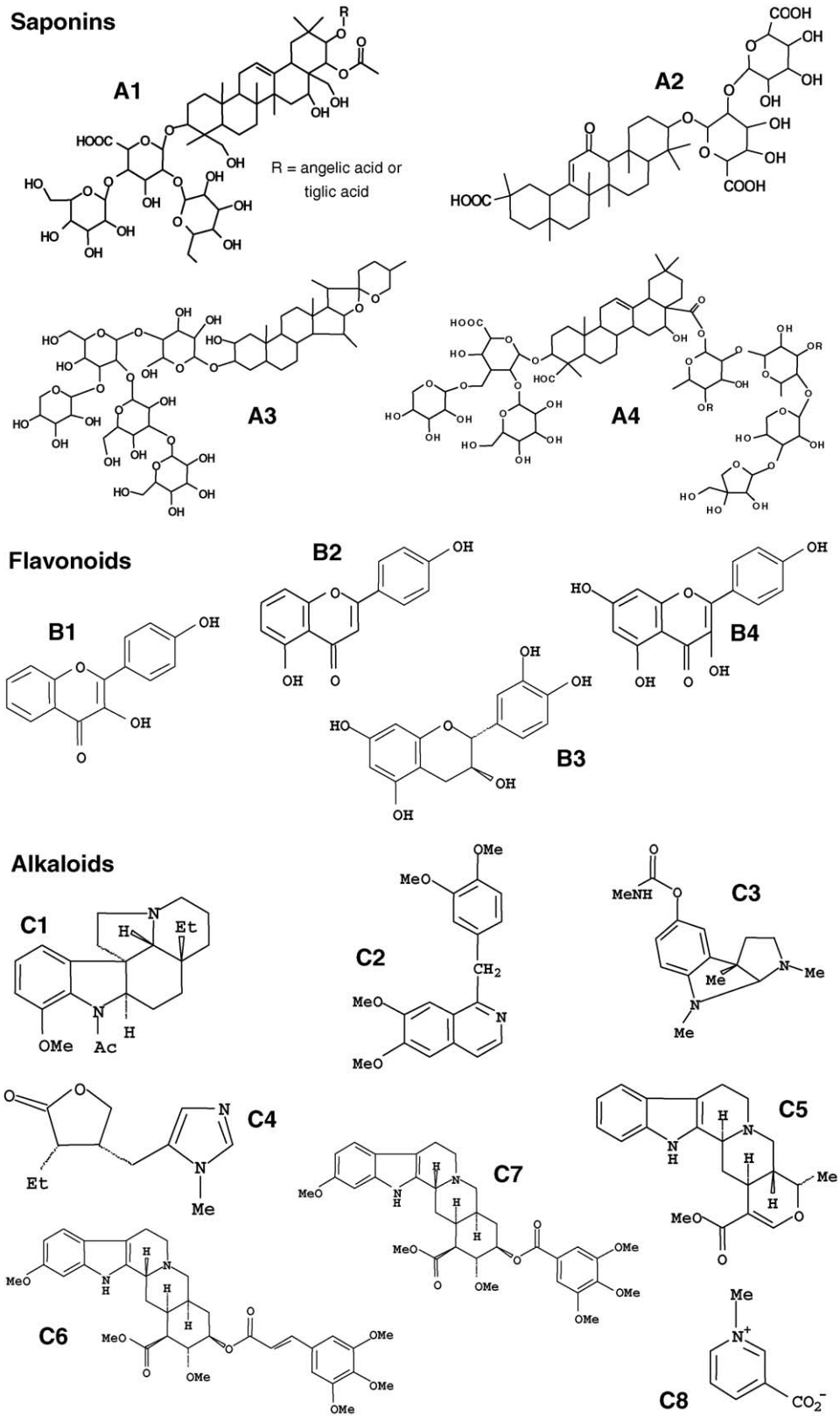

Fig. 1. Natural substances assayed on *Candida rugosa* lipase. (A) Saponins: (1) β-aescin, (2) glycyrrhizic acid, (3) digitonin, (4) *Quillaja* saponin; (B) flavonoids: (1) 3-hydroxyflavone, (2) 5-hydroxyflavone, (3) (±)-catechin, (4) kaempferol; (C) alkaloids: (1) aspidospermine, (2) papaverine, (3) physostigmine, (4) pilocarpine, (5) raubasine, (6) rescinnamine, (7) reserpine, (8) trigonelline.

Table 1
Effect of natural substances on *Candida rugosa* lipase evaluated by HPLC assay

Substance	S_{max}[a] (M)	IC_{16} (M)[b]	IC_{50} (M)[b]
(I) Saponins			
β-Aescin	4.0×10^{-4}	7.5×10^{-7}	8.0×10^{-6}
Digitonin	1.5×10^{-4}	5.9×10^{-6}	2.4×10^{-5}
Glycyrrhizic acid	2.0×10^{-3}	8.5×10^{-4}	$>S_{max}$
Quillaja saponin	1.4×10^{-3}	1.0×10^{-4}	5.5×10^{-4}
(II) Flavonoids			
3-Hydroxyflavone	1.5×10^{-3}	1.1×10^{-3}	$>S_{max}$
5-Hydroxyflavone	2.5×10^{-3}	4.5×10^{-4}	$>S_{max}$
(±)-Catechin	3.0×10^{-2}	8.3×10^{-4}	6.3×10^{-3}
Kaempferol	1.4×10^{-2}	2.8×10^{-4}	7.5×10^{-3}
(III) Alkaloids			
Aspidospermine	2.5×10^{-3}	6.3×10^{-4}	1.0×10^{-3}
Papaverine	2.0×10^{-3}	Inactive	
Physostigmine	3.8×10^{-2}	Inactive	
Pilocarpine	1.0×10^{-2}	Inactive	
Raubasine	1.2×10^{-3}	Inactive	
Rescinnamine	8.0×10^{-4}	2.3×10^{-6}	1.6×10^{-4}
Reserpine	4.5×10^{-4}	1.1×10^{-4}	4.0×10^{-4}
Trigonelline	5.0×10^{-2}	6.8×10^{-3}	2.0×10^{-2}

[a] Highest concentration at which each substance was tested expressed as mol/l (M).
[b] Inhibitory concentrations (IC) 16% and 50% calculated from the inhibition vs. inhibitor concentration curves.

On the other hand, GA produced the lower inhibition among the saponins evaluated ($IC_{16} = 8.5 \times 10^{-4}$ M). QS showed an increasing inhibition at concentrations up to $1.5–2.2 \times 10^{-4}$ M (about the IC_{50}). However, above these concentrations the inhibition by QS remained stable between 50 and 60%, in contrast to the other saponins which always produced a higher inhibition when increasing concentrations were assayed (Table 1; Fig. 2).

Direct inhibition of a lipase by these saponins is described here for the first time. Only inhibition of the hydrolysis of acetyl fluorescein (substrate of lipases and esterases) by β-aescin on several bacterial strains [24], inhibition of a cholesterol esterase by digitonin [25], inhibition of pancreatic lipase by GA-containing plant extracts [11], and the presence of GA and QS in plant formulations that inhibited *P. acnes* lipase [21] have been reported before.

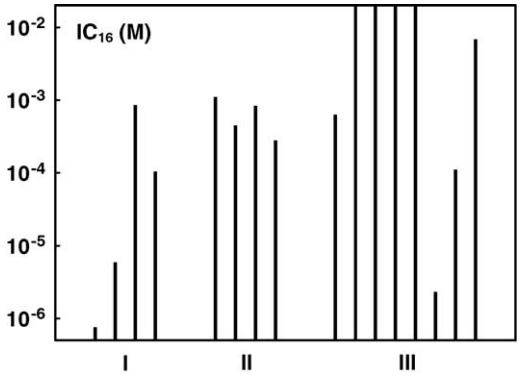

Fig. 2. Comparison of the effect of natural substances on *Candida rugosa* lipase. The effect of several saponins, flavonoids and alkaloids on Crl was evaluated by HPLC assay. The concentrations of these substances that produced a 16% inhibition (IC_{16}) on Crl activity are compared, since the IC_{50} was not reached in some of them. The compounds assayed are plotted in the same order as reported in Table 1. (I) Saponins; (II) flavonoids; (III) alkaloids. The compounds being inactive are plotted as vertical bars reaching the top of the graphic.

Crl displays complex substrate-binding site, since the polypeptide chain folds over this site forming a deep tunnel penetrating towards the centre of the molecule. The tunnel has an unusual "L" shape, it is 25 Å long and it has a diameter of 4 Å, thus it can only accommodate a single acyl chain not longer than 18 carbon atoms, whereas the positions of the other acyl chains of the substrate would be located in hydrophobic patches under the tunnel entrance. A phenylalanine-rich area near the tunnel entrance contains the catalytic serine, whereas the tunnel walls are lined with hydrophobic residues [33–35]. The fact that the aglicone nucleus of saponins is too large to enter into the tunnel, together with the higher inhibition produced by the saponins with larger and more branched carbohydrate side chains (β-aescin and digitonin) seems to indicate that the carbohydrate chains of saponins could enter, at least partially, into the tunnel of Crl, being thus the responsible for Crl inhibition, although further experiments are necessary to confirm this hypothesis.

3.2. Effect of flavonoids on Candida rugosa lipase

Flavonoids are benzo-γ-pyrone derivatives that can be grouped according to the presence of different substituents and the degree of benzo-γ-pyrone ring saturation. They are capable of modulating the activity of many enzymes and cell systems producing, among others, antitumoral, cardioprotective or anti-inflammatory effects [27]. Flavonoids are also components of antiacne formulations [1], and some of them, like kaempferol, have shown antiulcer activity [28]. Furthermore, some flavonoids such as quercetin [14] and hesperidin or neohesperidin [29] are known lipase inhibitors.

Here, we have evaluated the inhibition produced by (±)-catechin, kaempferol, 5-hydroxyflavone and 3-hydroxyflavone on Crl (Table 1; Fig. 2). All of them produced a lower inhibition than saponins (except GA). All flavonoids displayed a similar IC_{16} (from 2.8×10^{-4} to 1.1×10^{-3} M), whereas the IC_{50} was only achieved for (±)-catechin and kaempferol (IC_{50} of 6.3×10^{-3} and 7.5×10^{-3} M, respectively). At low concentrations kaempferol was the most active flavonoid, although (±)-catechin was the most efficient at high concentrations. 5-Hydroxyflavone showed an intermediate inhibition at low concentrations, halfway between (±)-catechin and kaempferol. However, 3-hydroxyflavone caused an inhibition more than two-fold lower than its isomer 5-hydroxyflavone (Table 1; Fig. 2).

It has previously been reported that catechin-rich extracts or catechin-related compounds like (−)-epicatechin inhibit pancreatic or gastric lipases and rat adipose tissue-derived lipoprotein lipase, although they do not inhibit hormone-sensitive lipase [11,30,31]. Inhibition of pancreatic lipase by kaempferol, a compound similar to quercetin, or by extracts of *Thea sinensis* (rich in kaempferol) has also been reported [11,32]. However, microbial lipase inhibition by (±)-catechin and kaempferol, and lipase inhibition by 5-HF and 3-HF, are described here for the first time.

The mechanism of enzyme inhibition by these compounds is unclear. The flavonoids analyzed have a similar structure,

and are small enough to enter in the tunnel of Crl [33–35]. However, (±)-catechin and kaempferol produced a higher inhibition than 5-hydroxyflavone, which in turn is more active than 3-hydroxyflavone. These results suggest that a higher number hydroxyl groups, as well as their disposition (mainly the presence of the hydroxyl group at position 5 of ring A, which is missing in the less active flavonoid 3-hydroxyflavone, as well as the presence of the hydroxyl group at position 7 of ring A, only present in the most active flavonoids (±)-catechin and kaempferol), would be the responsible for the different inhibition produced by these compounds on Crl. The mechanism of action of these compounds and the importance of their hydroxyl groups will be analyzed in the future, although the requirement of polyhydroxylated flavonoids for the inhibition of Crl is in agreement with the inhibition of the H^+, K^+-ATPase by polyhydroxylated flavonoids [36].

3.3. Effect of alkaloids on Candida rugosa lipase

Alkaloids are very diverse natural substances (and their related synthetic compounds) that contain nitrogen, usually as a part of a cyclic system. They are active on many enzymes and biological systems, like the central nervous system, producing a wide range of effects [37]. Moreover, berberine and other alkaloids are known lipase inhibitors [15].

The inhibition obtained by HPLC assays on Crl by trigonelline and several heterocyclic alkaloids was very different among the compounds assayed, even for those belonging to the same structural group. Among the indole-benzopyrrole alkaloids, rescinnamine was the most active, displaying an IC_{16} of 2.3×10^{-6} M, similar to those of digitonin and β-aescin. However, this alkaloid was less active than the mentioned saponins at high concentrations ($IC_{50} = 1.6 \times 10^{-4}$ M). Reserpine was the second most active alkaloid, showing an inhibition higher than flavonoids. Aspidospermine displayed an inhibition rate similar to most flavonoids at low concentrations (IC_{16}), but it was more active than flavonoids at high concentrations ($IC_{50} = 1.0 \times 10^{-3}$ M), and physostigmine and raubasine caused almost no inhibition of CRL (Table 1; Fig. 2). Among the non-indole-benzopyrrole alkaloids, only trigonelline (pyridine and piperidine group) was active, but it caused a low inhibition, whereas papaverine (isoquinoline group) and pilocarpine (imidazole or glyoxaline group) did not inhibit Crl (Table 1; Fig. 2).

Lipase inhibition by the mentioned alkaloids is reported here for the first time, except for reserpine and physostigmine. Among other effects, reserpine increased lipoprotein lipase activity of heart tissue [38] and inhibited epididymal hormone-sensitive lipase but not that of other tissues [39]. Physostigmine, a known esterase inhibitor, inhibited also lipases like bile-salt stimulated lipase [40] or pancreatic lipase [41], but not lipolytic liver extracts [42]. Moreover, some isoquinoline-group alkaloids similar to papaverine like palmatine were inactive on Crl, although berberine and others inhibited Crl [15].

Inhibition by alkaloids is difficult to explain as they have very different structures. Except trigonelline, most of them have a large structure with several rings that makes difficult or not possible their entering into the narrow tunnel of Crl [33–35], which could explain the lack of inhibition of some of them. However, further considerations are possible with respect to the indole-benzopyrrole alkaloids. Raubasine (inactive) and rescinnamine or reserpine (the most active) share the same indole nucleus (Fig. 1: C5–C7). Thus, it is clear that the inhibition produced by rescinnamine and reserpine is caused by their lateral groups. As we suggested for saponins, the lateral groups probably enter into the active site of Crl interfering with the activity of the enzyme, whereas the indole nucleus remains out of the tunnel, since it is too large to enter. However, further assays are in progress to establish more accurately the mechanism of action of these compounds.

3.4. Conclusions and clinical implications

Saponins, (±)-catechin and kaempferol, produced a high Crl inhibition, and some of them have been described to be active against acne, ulcer or pancreatic lipase, as explained before. Moreover, almost all of them have a low toxicity. For example, β-aescin is currently used in the treatment of peripheral vascular diseases as it displays more effectiveness and tolerability than the conventional therapy [19]. At high concentrations, (±)-catechin produces toxic effects like haemolysis, and kaempferol is genotoxic. Nevertheless, they are some of the most occurring compounds among the approximately 1 g of flavonoids that contains the daily human diet [27]. Therefore, these inhibitors are promising candidates for the prevention (creams, shampoos, etc.) and the therapy of diseases in which lipases play an important role like acne, *H. pylori*-associated ulcers, obesity, or fungal diseases. In fact, further assays in our research group have demonstrated that some of these inhibitors are also effective on *P. acne*s lipase and other lipases involved in virulence (unpublished data). However, further studies are necessary to confirm their pharmacological potential. On the contrary, the potential use of rescinnamine and reserpine to treat lipase-related diseases is more limited by their other marked pharmacological activities [43].

In conclusion, knowledge about the effect on Crl of these compounds will be helpful with respect to their application in pharmacology, in the designing of new lipase inhibitors, and in the evolution of the active site of the enzyme in order to carry out new biotechnological processes.

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References

[1] S. Higaki, J. Mol. Catal. B Enzym. 22 (2003) 377.
[2] A. Slomiany, B.L. Slomiany, J. Physiol. Pharmacol. 43 (1992) 115.

[3] D.T. Smoot, Gastroenterology 113 (1997) S31.
[4] B.L. Slomiany, J. Piotrowski, A. Slomiany, Am. J. Gastroenterol. 87 (1992) 595.
[5] B.L. Slomiany, V.L. Murty, J. Piotrowski, A. Slomiany, Gen. Pharmacol. 25 (1994) 833.
[6] T.M. Muhsin, A.H. Aubaid, A.H. al-Duboon, Mycoses 40 (11–12) (1997) 465.
[7] S. Benjamin, A. Pandey, Yeast 14 (1998) 1069.
[8] K.-E. Jaeger, B.W. Dijkstra, M.T. Hertz, Annu. Rev. Microbiol. 53 (1999) 315.
[9] J.W. Simons, R.C. Cox, M.R. Egmond, H.M. Verheij, Biochemistry 38 (1999) 6346.
[10] B. Singh, T.K. Bhat, B. Singh, J. Agric. Food Chem. 51 (2003) 5579.
[11] S. Shimura, W. Tsuzuki, S. Kobayashi, T. Suzuki, Biosci. Biotechnol. Biochem. 56 (9) (1992) 1478.
[12] F. Borrelli, A. Izzo, Phytother. Res. 14 (2000) 581.
[13] L.K. Han, Y.N. Zheng, B.J. Xu, H. Okuda, Y. Kimura, J. Nutr. 132 (8) (2002) 2241.
[14] M.T. Gatto, S. Falcocchio, E. Grippa, G. Mazzanti, L. Battinelli, G. Nicolosi, D. Lambusta, L. Saso, Bioorg. Med. Chem. 10 (2) (2002) 269.
[15] E. Grippa, R. Valla, L. Battinelli, G. Mazzanti, L. Saso, B. Silvestrini, Biosci. Biotechnol. Biochem. 63 (1999) 1557.
[16] A.L. Colombo, A.S. Melo, A.F. Crespo Rosas, R. Salomao, M. Briones, R.J. Hollis, S.A. Messer, M.A. Pfaller, Diagn. Microbiol. Infect. Dis. 46 (2003) 253.
[17] C. Ruiz, S. Falcocchio, E. Xoxi, F.I.J. Pastor, P. Diaz, L. Saso, Biochim. Biophys. Acta 1672 (2004) 184.
[18] J. Milgate, D.C.K. Roberts, Nutr. Res. 15 (8) (1995) 1223.
[19] C.R. Sirtori, Pharmacol. Res. 44 (3) (2001) 183.
[20] G. Bader, M. Seibold, K. Tintelnot, K. Hiller, Pharmazie 55 (1) (2000) 72.
[21] S. Higaki, T. Kitagawa, M. Kagoura, M. Morohashi, T. Yamagishi, J. Dermatol. 27 (10) (2000) 635.
[22] C.S. Kwon, H.Y. Sohn, S.H. Kim, J.H. Kim, K.H. Son, J.S. Lee, J.K. Lim, J.S. Kim, Biosci. Biotechnol. Biochem. 67 (7) (2003) 1451.
[23] Z. Sroka, M. Jurzysta, J. Tylcz, H. Rzadkowska-Bodalska, Z. Naturforsch [C] 52 (3–4) (1997) 235.
[24] R.M. Zablotowicz, R.E. Hoagland, S.C. Wagner, Adv. Exp. Med. Biol. 405 (1996) 83.
[25] I.M. Karmanskii, Vop. Med. Khim. 29 (1) (1983) 66.
[27] G. Di Carlo, N. Mascolo, A.A. Izzo, F. Capasso, Life Sci. 65 (4) (1999) 337.
[28] A.A. Izzo, G. Di Carlo, N. Mascolo, G. Autore, F. Capasso, Phytother. Res. 6 (1994) 179.
[29] K. Kawaguchi, T. Mizuno, K. Aida, K. Uchino, Biosci. Biotechnol. Biochem. 61 (1) (1997) 102.
[30] C. Juhel, M. Armand, Y. Pafumi, C. Rosier, J. Vandermander, D. Lairon, J. Nutr. Biochem. 11 (1) (2000) 45.
[31] M. Yoshikawa, H. Shimoda, N. Nishida, M. Takada, H. Matsuda, J. Nutr. 132 (7) (2002) 1819.
[32] S. Shimura, Y. Itoh, A. Yamashita, A. Kitano, T. Hatano, T. Yoshida, T. Okuda, Nippon Shokuhin Kogyo Gakkaishi 41 (11) (1994) 847.
[33] M. Cygler, J.D. Schrag, Methods Enzymol. 284 (1997) 3.
[34] J. Pleiss, M. Fischer, R.D. Schmid, Chem. Phys. Lipids 93 (1998) 67.
[35] M. Cygler, J. Schrag, Biochim. Biophys. Acta 1441 (1999) 205.
[36] S. Murakami, M. Muramatsu, K. Tomisawa, J. Enzyme Inhib. 14 (2) (1999) 151.
[37] W.C. Evans, in: W.C. Evans (Ed.), Trease and Evans' Pharmacognosy, Saunders cop., London, 1996, p. 559 (Chapter: Alkaloids).
[38] I. Heinroth, W. Forster, Acta Biol. Med. Ger. 39 (8–9) (1980) 961.
[39] Y. Matsuara, Nippon Naibunpi Gakkai Zasshi 46 (2) (1970) 163.
[40] L.A. Ellis, M. Hamosh, Lipids 27 (11) (1992) 917.
[41] D.A. Hall, Biochem. J. 78 (1961) 491.
[42] S. Katz, Am. J. Physiol. 189 (2) (1957) 311.
[43] J. La Barre, J. Physiol. 52 (1960) 138.