Med Chem Res
DOI 10.1007/s00044-011-9750-x

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Antifungal activity of a novel quercetin derivative bearing a trifluoromethyl group on *Candida albicans*

Tomas C. Tempesti · M. Gabriela Alvarez · Marcelo Francisco de Araújo · Francisco Eduardo Aragão Catunda Júnior · Mário Geraldo de Carvalho · Edgardo N. Durantini

Received: 8 May 2010 / Accepted: 8 July 2011

Abstract In this study, a novel quercetin derivative bearing a trifluoromethyl group was synthesized by the nucleophilic aromatic substitution reaction between rutin and 2-chloro-5-(trifluoromethyl)-1,3-dinitrobenzene in basic medium. This synthetic quercetin showed antifungal activity against *Candida albicans* cultures. The results indicated a remarkable increase in the biological activity of this compound as compared to rutin.

Keywords Antifungal activity · Flavonoid · Rutin · Quercetin · *Candida albicans*

Introduction

Resistance to antimicrobial agents has become an increasingly important and pressing global problem. Antimicrobial resistance complicates the treatment of important nosocomial and community-acquired infections. Candidiasis is a significant infection in patients being treated with chemotherapy and radiotherapy for cancer, and in patients who are immunocompromised because of HIV infection and AIDS. *Candida albicans* causes hematogeneous disseminated candidiasis and local infections, such as thrush and vaginitis. *C. albicans* is the most common fungal pathogen and has developed an extensive array of recognized virulent mechanisms, which allows successful colonization and infection of the host under suitable predisposing conditions (White *et al.*, 2002). In the past few years, resistance of *C. albicans* is increasing against traditional antifungals, such as fluconazole (Goldman *et al.*, 2004; Briona *et al.*, 2007; Ribeiro and Rodrigues 2007).

Increasingly, flavonoids are becoming the subject of medical research. They have been reported to possess many useful properties, including anti-inflammatory, estrogenic, antimicrobial, antiallergic, antioxidant, vascular, and cytotoxic antitumor activities and enzyme inhibition (Cushnie and Lamb 2005). Owing to the widespread ability of flavonoids to inhibit spore germination of plant pathogens, they have been proposed for use against fungal pathogens of human beings (Harborne and Williams 2000). Extracts and natural products from plants containing flavonoids offer an alternative for the treatment of oral candidiasis. Extracts of the leaves and tips of the twigs of *Dodonaea viscosa* var. *angustifolia*, an indigenous South African plant, are traditionally used as a gargle for sore throats and oral candidiasis (Patel and Coogan 2008).

Rutin (quercetin-3-rutinoside, Fig. 1) is a polyphenolic flavonoid widely present in foods of plant origin, such as buckwheat, parsley, and tomatoes (Guardia *et al.*, 2001; Erlund *et al.*, 2000). Rutin is known as one of the common and naturally occurring flavonoids with a variety of biochemical and pharmacological activities. Recent reports indicate that rutin scavenges free radicals (Duthie and Dobson 1999), suppresses cellular immunity (Middleton *et al.*, 2000), and has anti-carcinogenic activity (Rotelli *et al.*, 2003) as well as anti-inflammatory effect (Guardia *et al.*, 2001). More recent reports show that rutin also has antimicrobial activity (Pereira *et al.*, 2007). A prenylated flavanone isolated from the shrub *Eysenhardtia texana*,

T. C. Tempesti · M. G. Alvarez · E. N. Durantini (✉)
Departamento de Química, Facultad de Ciencias Exactas Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Agencia Postal Nro 3, X5804BYA Rio Cuarto, Argentina
e-mail: edurantini@exa.unrc.edu.ar

M. F. de Araújo · F. E. A. Catunda Júnior · M. G. de Carvalho
Departamento de Química, Instituto de Ciências Exatas, Universidade Federal Rural do Rio de Janeiro, BR 465, Km 7, 23890-000 Seropédica, RJ, Brazil

Published online: 19 July 2011

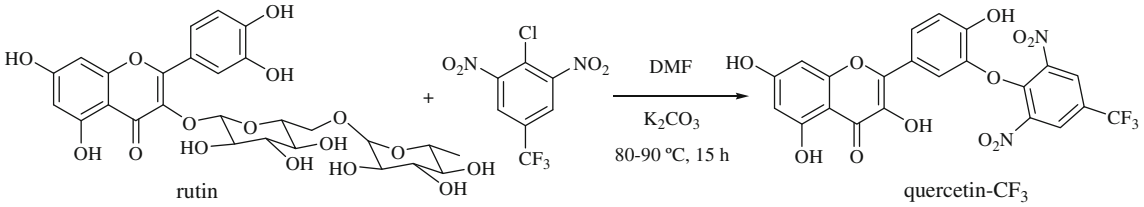

Fig. 1 Synthesis of quercetin-CF_3 derivative

identified as 4′,5,7-trihydroxy-8-methyl-6-(3-methyl-[2-butenyl])-(2S)-flavanone, has been shown to possess activity against the opportunistic pathogen *C. albicans* (Wachter *et al.*, 1999). The flavonoid 7-hydroxy-3′,4′-(methylenedioxy)flavan, isolated from *Terminalia bellerica* fruit rind, was active against *C. albicans* (Valsaraj *et al.*, 1997). Recently, rutin was proposed to exert a therapeutic effect on septic arthritis caused by *C. albicans* (Yongmoon 2009). However, a disadvantage of rutin is its poor solubility in aqueous media. This is the reason for its poor bioavailability. Therefore, it imposes some restraints to further pharmaceutical use, especially for oral administration (Mauludin *et al.*, 2009). Also, it is generally considered that when flavonols are supplied as glycosides in the diet, they are first hydrolyzed by the digestive microflora before they are absorbed (Manach *et al.*, 1997).

On the other hand, novel quercetin-3-*O*-amino acid-esters were discovered as a new class of Src tyrosine kinase inhibitors (Huang *et al.*, 2009). Quercetin disappears immediately from the plasma when administered intravenously to rodents. This suggests that quercetin is metabolized rapidly and excreted into the urine with no accumulation in tissues and biological fluids (Murakami *et al.*, 2008).

In order to discover a new rutin prodrug with improved bioavailability, in the present investigation, a novel derivative bearing a trifluoromethyl group was synthesized (quercetin-CF_3, Fig. 1). The influence of the trifluoromethyl group in biologically active molecules is often associated with the increased lipophilicity that this substituent imparts (McClinton and McClinton 1992; Ando and Kumadaki 1999). The antifungal activity of this synthetic compound was compared to that of rutin on *C. albicans* cultures. The results showed a remarkable increase in the biological activity of this compound.

Materials and methods

General

UV–visible spectra were recorded at 25.0 ± 0.5°C using 1-cm path length quartz cells on a Shimadzu UV-2401PC spectrometer. NMR spectra were recorded on a FT-NMR Bruker Advance 400 and 200 MHz spectrometer. Mass spectra were captured using a Varian 1200L MS-APCI interface. The HPLC experiments were performed in a Waters 1525 liquid chromatograph equipped with a Varian 2550 UV–visible variable-wavelength detector and Phenomenex Luna C18 (5 µm, 150 × 4.60 mm) column. The separation was performed using a mobile phase composed of 49% water/49% methanol/2% acetic acid (flow: 0.5 ml/min). Semiempirical molecular orbital calculations (AM1) were carried out using HyperChem software. All chemicals from Aldrich were used without further purification. Silica gel thin-layer chromatography (TLC) plates of 250 microns from Aldrich (Milwaukee, WI, USA) were used.

Synthesis of rutin derivative (quercetin-CF_3)

A solution of 50 mg (0.08 mmol) of rutin and 11 mg (0.04 mmol) of 2-chloro-5-(trifluoromethyl)-1,3-dinitrobenzene in 5 ml the *N,N*-dimethylformamide (DMF) was stirred with K_2CO_3 (100 mg) for 15 h at 80–90°C. Then, the solution was extracted with ethyl acetate (3 × 15 ml) and water (20 ml). The organic phase was separated, and the water phase was washed three times with ethyl acetate. Combined organic phase was evaporated in a rotary evaporator, and the solid was dried under reduced pressure. The product (quercetin-CF_3) was obtained with 90% yield. M.p. 143–145°C. ^{1}H-NMR (DMSO-d_6, TMS) δ [ppm] 6.17 (d, 1H, J = 2.0 Hz, C-6), 6.39 (d, 1H, J = 2.0 Hz, C-8), 6.87 (d, 1H, J = 8.3 Hz, C-5′), 7.53 (dd, 1H, J = 2.0, 8.3 Hz, C-6′), 7.66 (d, 1H, J = 2.0 Hz, C-2′), 8.1 (s, 2H, C-3″ and C-5″). ^{13}C-NMR (DMSO-d_6, TMS) 93.8 (C-8), 98.6 (C-6), 103.5 (C-10), 115.5 (C-2′), 116.1 (C-5′), 120.4 (C-6′), 122.1 (CF_3), 122.4 (C-1′), 126.8 (C-4″), 126.9 (C-3″ and C-5″), 136.2 (C-3), 143.1 (C-1″), 145.5 (C-3′), 147.3 (C-4′), 148.2 (C-2″ and C-6″), 156.6 (C-9), 157.1 (C-2), 161.2 (C-5), 164.3 (C-7), 176.3 (C-4). APSI-MS [*m*/*z*] 575 $(M + K)^+$ (575.00 calculated for $C_{22}H_{11}F_3N_2O_{11}K$).

Solutions

Stock solutions of rutin or quercetin-CF_3 (10 mg/ml) were prepared by dissolution in 5 ml of water. Cloves of garlic were peeled and pressed through a garlic presser into eight layers of cheesecloth, and then squeezed to extract garlic

juice. The liquid was then centrifuged with a filter at 13000 rpm for 5 min. The liquid was then weighed, and a stock concentration of 10 mg/ml was prepared in water. Stock solution of fluconazole (500 μg/ml) was prepared by dissolution in 2 ml of water.

Partition coefficient measurements

1-Octanol/water partition coefficients (*P*) were determined at 25°C using equal volumes of water (2 ml) and 1-octanol (2 ml). Typically, a solution of each flavonoid (~100 μM) was stirred in a thermostat after equilibrium had been reached (8 h). An aliquot (200 μl) of aqueous and organic phases were dissolved in 2 ml of methanol and the final flavonoid concentration determined by absorption spectroscopy (Scalise and Durantini 2004).

Microorganism and growth conditions

Candida albicans strain PC31, recovered from human skin lesion, was previously characterized and identified according to conventional procedures (Cormick *et al.*, 2008, 2009). Primary classification of colonies from plates was based on colony characteristics (pigmentation and shape), mode of vegetative reproduction, formation of pseudohyphae, and ascopore production. Identification of the yeast isolates to species level was done using the API 20C AUX (BioMérieux, Marcy l'Etoile, France) system of carbohydrate assimilation profiles. The strain of *C. albicans* was grown aerobically overnight in Sabouraud (Britania, Buenos Aires, Argentina) broth (4 ml) at 37°C to stationary phase. Viable *C. albicans* cells were monitored, and the number of colony-forming units (CFU) was determined on Sabouraud agar plates after ~48-h incubation at 37°C. This procedure produces ~10^7 CFU/ml after an overnight incubation.

Antifungal activity

After overnight incubation, the cells were appropriately diluted to obtain ~10^4 CFU/ml in Sabouraud broth. In all the experiments, 2 ml of the cell suspensions in Pirex brand culture tubes (13 × 100 mm) was used, and the flavonoid derivatives were added from a stock solution ~10 mg/ml in DMF:water (1:1). The minimal inhibitory concentration (MIC) values were defined as the lowest concentration of flavonoid derivatives, which inhibits the visible growth of *C. albicans* after 48-h incubation at 37°C (range 0.06–2.50 mg/ml). Thus, no turbidity after incubation was indicative of growth inhibition. Before the assays, it was verified that the loading solvent, *N,N*-dimethylformamide:water (1:1), was completely inactive against the test organisms under the assay conditions. Also, control experiments were carried out under the same conditions in the absence of extracts. Each experiment was repeated separately three times.

Effect on growth of *C. albicans*

Cultures of *C. albicans* cells were grown overnight as described above. A portion (1 ml) of this culture was transferred to 20 ml of fresh Sabouraud broth medium. The suspension was homogenized, and aliquots of 2 ml were incubated with different concentrations of the flavonoid derivatives at 37°C. The culture grown was measured by turbidity at 660 nm using a Tuner SP-830 spectrophotometer. In all the cases, control experiments were carried out in the absence of the extracts. Each experiment was repeated separately three times.

Statistical analysis

All the data were presented as the mean ± standard deviation of each group. Variation between groups was evaluated using the Students *t*-test, with a confidence level of 95% ($P < 0.05$) considered statistically significant.

Results and discussion

Synthesis

Coupling of rutin and 2-chloro-5-(trifluoromethyl)-1,3-dinitrobenzene by an ether bond allows for the formation of the new flavonoid derivate quercetin-CF_3, as shown in Fig. 1. To synthesize this compound, the nucleophilic aromatic substitution reaction was performed in DMF at 80–90°C for 15 h. The basic medium was employed to activate the –OH group to nucleophilic reaction with the chlorine aromatic substrate. This procedure was also accompanied by the hydrolysis of the sugar group. The quercetin-CF_3 product was isolated with 90% yield.

The quercetin-CF_3 bears a highly lipophilic trifluoromethyl group, which increases the amphiphilic character of the structure. The influence of the trifluoromethyl group in biologically active molecules is often associated with the increased lipophilicity that this substituent imparts (McClinton and McClinton 1992; Ando and Kumadaki 1999).

The product was purified for HPLC. As expected for its lipophilicity, the retention time (t_R) of the compound quercetin-CF_3 ($t_R = 18$ min) was longer than that of rutin ($t_R = 5$ min). NMR spectroscopic studies confirm the suggested structure (Fig. 2). The comparison of ^{1}H-NMR spectra (in DMSO-d_6) of rutin and quercetin-CF_3 shows that the signals in the region of 7.5–7.6 ppm of rutin,

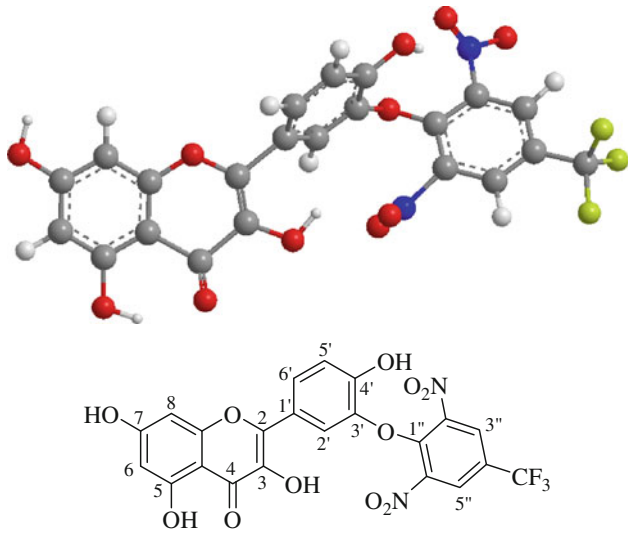

Fig. 2 Structure of quercetin-CF_3

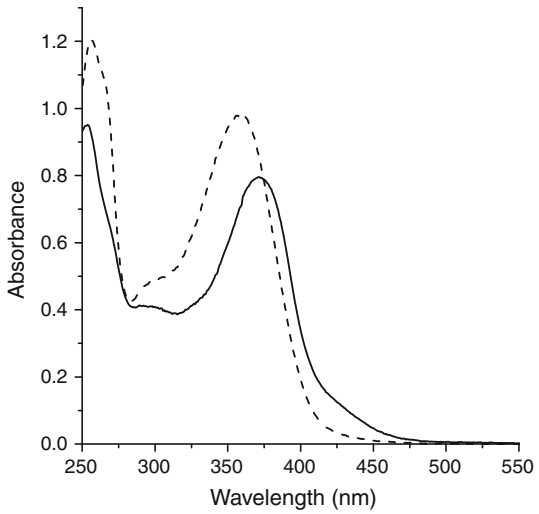

Fig. 3 Absorption spectra of quercetin-CF_3 (*solid line*) and rutin (*dashed line*) in methanol

corresponding to the *ortho* protons and the hydroxy group in the benzene ring, suffer a splitting in the compound quercetin-CF_3 as defined by two signals, one at 7.5 ppm and the other at 7.7 ppm, being the effect of the substituent. The COSY and HMQC spectra were recorded to establish the specific assignment of protons. It also notes the disappearance of signals corresponding to the sugars of the routine and the emergence of a new signal at 8.1 ppm which is correlated with the protons of the substrate containing the $-CF_3$ group.

To evaluate the effect produced by the distribution of different polarity groups upon the intramolecular polarity, the dipole moments of the porphyrins were estimated. The semi-empirical method for molecular modeling (AM1) was used in structure geometry optimization calculations (Fig. 2). A value of 5.2 D was found for quercetin-CF_3. This value is higher than that found for the corresponding rutin derivative without substitution by amino groups (3.8 D). As expected, the presence of a lipophilic group in the periphery of quercetin structure enhances the dipole moment. The combination of hydrophobic and hydrophilic substituents in the molecular structure results in an intramolecular polarity axis, which can facilitate membrane penetration and produces a better accumulation in subcellular compartments, enhancing the effective antifungal agent.

UV–visible absorption studies and lipophilic properties

The UV–visible absorption spectra of rutin and quercetin-CF_3 in methanol are compared in Fig. 3. The flavonoid derivative shows the typical band I around 370 nm and band II at 260 nm, according to the molecular structure. Maximum wavelength of band I of quercetin-CF_3 derivative presents a bathochromic shift of $\sim$12 nm respect to rutin. Similar behavior has previously been observed for quercetin in different media (Le Nest *et al.*, 2004).

The *n*-octanol/water partition coefficients (*P*) of flavonoids were evaluated at 25°C ($P = [\text{porphyrin}]_o/[\text{porphyrin}]_w$). As can be observed in Table 1, results indicate that the lipophilic character increased in the quercetin-CF_3 structure with respect to rutin because of the presence of a trifluoromethyl group.

Antimicrobial activity of flavonoid derivatives on *C. albicans*

The biological activity of flavonoid derivatives was investigated on *C. albicans* cellular suspension in Sabouraud medium. The extracts were evaluated in a range of 0.060–2.5 mg/ml. The antimicrobial activities on *C. albicans* are summarized in Table 2. From these results, MIC values of 2.20 ± 0.10 and 0.60 ± 0.10 mg/ml were calculated for rutin and quercetin-CF_3, respectively. As can be observed, quercetin-CF_3 showed about fourfold higher antifungal activity than rutin. On the other hand, the susceptibility of this yeast was evaluated using fluconazole and garlic extract. Azole derivatives are standard active antifungals established to eradicate *C. albicans* (Soysa *et al.*,

Table 1 UV-visible absorption characteristics in methanol and 1-octanol/water partition coefficients (*P*) of rutin and quercetin-CF_3

Compound	λ (nm) (ε M^{-1} cm^{-1})	λ (nm) (ε M^{-1} cm^{-1})	Log *P*
Rutin	256 (14100)	360 (12200)	0.31
Quercetin-CF_3	254 (18800)	372 (17100)	0.62

Table 2 Antifungal activity of flavonoid derivatives on *C. albicans*

Compound	Concentration (mg/ml)												
	2.50	2.20	2.10	1.90	1.60	1.30	1.25	0.60	0.50	0.40	0.30	0.15	0.06
Rutin	+	+	−	−	−	−	−	−	−	−	−	−	−
Quercetin-CF_3	+	+	+	+	+	+	+	+	−	−	−	−	−

+ inhibition of visible growth, − no inhibition

2004). Also, garlic oil has shown to have anti-*Candida* activity at higher concentrations, indicating that they may be useful in the topical treatment of superficial *Candida* infections (Iwalokun *et al.*, 2004). The MIC values obtained were 4 μg/ml and 2.0 mg/ml for fluconazole and garlic extract, respectively. Thus, natural antimycotic remedies, such as rutin and garlic extract, are likely not to be as effective as azole antifungal agents, and may require higher doses to produce similar results. However, chemical modification of rutin to produce quercetin-CF_3 was accompanied by a considerable increase in the antifungal activity.

Taking into account these results, growth delay of *C. albicans* cultures produced by flavonoids was carried out in Sabouraud medium. Thus, different amounts of flavonoids were added to fresh cultures of *C. albicans* reaching the log phase, and the flasks were incubated at 37°C. Three concentrations of rutin and quercetin-CF_3, ranging above and below the MIC values, were analyzed under these conditions. As can be observed in Fig. 4, growth was suppressed when *C. albicans* cultures were

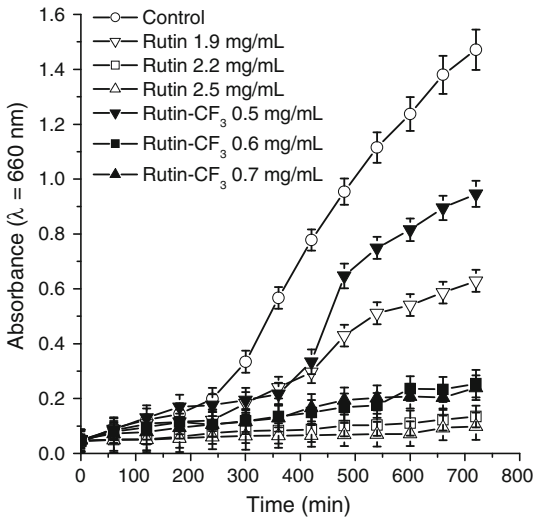

Fig. 4 Growth delay curves of *C. albicans* cells incubated with different concentrations of rutin [(*open down triangle*) 1.9 mg/ml, (*open square*) 2.2 mg/ml, (*open up triangle*) 2.5 mg/ml] and quercetin-CF_3 [(*filled down triangle*) 0.5 mg/ml, (*filled square*) 0.6 mg/ml and (*filled up triangle*) 0.7 mg/ml] in Sabouraud broth at 37°C. Control cultures: cells without addition of flavonoid derivatives (*open circle*). Values represent mean ± standard deviation of three separate experiments

treated with both extracts using concentration identical to or higher than MIC. After 30 min of incubation in the presence of 2.2 or 2.5 mg/ml of rutin, the cells no longer appeared to be growing as measured by turbidity at 660 nm. Under these conditions, the effect of quercetin-CF_3 was more efficient than that of rutin, because it was effective between 0.6 and 0.7 mg/ml. On the other hand, *C. albicans* cells incubated with a lower concentration of flavonoid (less than MIC value) showed only a small growth delay compared with controls. Under this condition, Lag phase was increased with respect to the control. Therefore, with the greater than MIC values , the growth did not occur, and at concentrations less than the MIC values, growth occurred but with a prolonged lag phase. These data illustrate that the observed growth delay is due to the antimicrobial effect of the quercetin-CF_3 on the *C. albicans* cells.

The results obtained in the present study indicate that a stronger antifungal activity is produced by quercetin-CF_3 in comparison with that exerted by rutin. Also, compound quercetin-CF_3 has a higher log *P* value. In previous studies, it had been observed that glycosylation of quercetin reduced the vasodilator activity (Chen *et al.*, 2004). In general, in this kind of compounds, a stronger biological activity is accompanied by an increase of log *P* values.

Conclusions

A new rutin derivative was synthesized by the nucleophilic aromatic substitution reaction between rutin and 2-chloro-5-(trifluoromethyl)-1,3-dinitrobenzeneound in basic medium. The reaction conditions also produced the loss of sugar structure from the flavonoid, to obtain quercetin-CF_3 with a high yield. The potential antifungal activities of rutin and quercetin-CF_3 were compared against the strains of *C. albicans*. Quercetin-CF_3 was more effective than rutin against *C. albicans*. Therefore, this study indicates that quercetin-CF_3 is an interesting antifungal agent with considerably higher activity than rutin.

Acknowledgments The authors are grateful to the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina for financial support. M.G.A. and E.N.D. are Scientific Members of CONICET. T.C.T. thanks CONICET for a research fellowship.

References

Ando A, Kumadaki I (1999) Progress on the syntheses of fluorine analogs of natural porphyrins potentially useful for the diagnosis and therapy of certain cancers. J Fluor Chem 100:135–146

Briona LP, Ukoa SE, Goldmanb DL (2007) Risk of resistance associated with fluconazole prophylaxis: systematic review. J Infect 54:521–529. doi:10.1016/j.jinf.2006.11.017

Chen Z, Hu Y, Wu H, Jiang H (2004) Synthesis and biological evaluation of flavonoids as vasorelaxant agents. Bioorg Med Chem Lett 14:3949–3952. doi:10.1016/j.bmcl.2004.05.061

Cormick MP, Rovera M, Durantini EN (2008) Synthesis, spectroscopic properties and photodynamic activity of a novel Zn(II) phthalocyanine substituted by fluconazole groups. J Photochem Photobiol A Chem 194:220–229. doi:10.1016/j.jphotochem.2007.08.013

Cormick MP, Alvarez MP, Rovera M, Durantini EN (2009) Photodynamic inactivation of *Candida albicans* sensitized by tri- and tetra-cationic porphyrin derivatives. Eur J Med Chem 44:1592–1599. doi:10.1016/j.ejmech.2008.07.026

Cushnie TPT, Lamb AJ (2005) Antimicrobial activity of flavonoids. Int J Antimicrob Agents 26:343–356. doi:10.1016/j.ijantimicag.2005.09.002

Duthie SJ, Dobson VL (1999) Dietary flavonoids protect human colonocyte DNA from oxidative attack in vitro. Eur J Nutr 38:28–34. doi:10.1007/s003940050043

Erlund I, Kosonen T, Alfthan G, Mäenpää J, Perttunen K, Kenraali J, Parantainen J, Aro A (2000) Pharmacokinetics of quercetin from quercetin aglycone and rutin in healthy volunteers. Eur J Clin Pharmacol 56:545–553

Goldman GH, da Silva Ferreira ME, dos Reis Marques E, Savoldi M, Perlin D, Park S, Godoy Martinez PC, Goldman MHS, Colombo AL (2004) Evaluation of fluconazole resistance mechanisms in *Candida albicans* clinical isolates from HIV-infected patients in Brazil. Diagn Microbiol Infect Dis 50:25–32. doi:10.1016/j.diagmicrobio.2004.04.009

Guardia T, Rotelli AE, Juarez AO, Pelzer LE (2001) Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. Farmaco 56:683–687

Harborne JB, Williams CA (2000) Advances in flavonoid research since 1992. Phytochem 55:481–504. doi:10.1016/S0031-9422(00)00235-1

Huang H, Jia Q, Ma J, Qin G, Chen Y, Xi Y, Lin L, Zhu W, Ding J, Jiang H, Liu H (2009) Discovering novel quercetin-3-O-amino acid-esters as a new class of Src tyrosine kinase inhibitors. Eur J Med Chem 44:1982–1988. doi:10.1016/j.ejmech.2008.09.051

Iwalokun BA, Ogunledun A, Ogbolu DO, Bamiro SB, Jimi-Omojola J (2004) In vitro antimicrobial properties of aqueous garlic extract against multidrug-resistant bacteria and Candida species from Nigeria. J Med Food 7:327–333. doi:10.1093/jac/dkg476

Le Nest G, Caille O, Woudstra M, Roche S, Guerlesquin F, Lexa D (2004) Zn-polyphenol chelation: complexes with quercetin, (+)-catechin, and derivatives: I optical and NMR studies. Inorg Chim Acta 357:775–784. doi:10.1016/j.ica.2003.09.014

Manach C, Morand C, Demigné C, Texier O, Régérat F, Rémésy C (1997) Bioavailability of rutin and quercetin in rats. FEBS Lett 409:12–16. doi:10.1016/S0014-5793(97)00467-5

Mauludin R, Müller RH, Keck CM (2009) Development of an oral rutin nanocrystal formulation. Int J Pharm 370:202–209. doi:10.1016/j.ijpharm.2008.11.029

McClinton MA, McClinton DA (1992) Trifluoromethylations and related reactions in organic chemistry. Tetrahedron 48:6555–6666

Middleton E, Kandaswami C, Theoharides TC (2000) The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 52:673–751

Murakami A, Ashida H, Terao J (2008) Multitargeted cancer prevention by quercetin. Cancer Lett 269:315–325. doi:10.1016/j.canlet.2008.03.046

Patel M, Coogan MM (2008) Antifungal activity of the plant *Dodonaea viscosa* var angustifolia on Candida albicans from HIV-infected patients. J Ethnopharmacol 118:173–176. doi:10.1016/j.jep.2008.03.009

Pereira AP, Ferreira IC, Marcelino F, Valentão P, Andrade PB, Seabra R, Estevinho L, Bento A, Pereira JA (2007) Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. Cv. Cobrançosa) leaves. Molecules 12:1153–1162. doi:10.3390/12051153

Ribeiro MA, Rodrigues CP (2007) Up-regulation of ERG11 gene among fluconazole-resistant *Candida albicans* generated in vitro: is there any clinical implication? Diagn Microbiol Infect Dis 57:71–75. doi:10.1016/j.diagmicrobio.2006.04.019

Rotelli AE, Guardia T, Juárez AO, de la Rocha NE, Pelzer LE (2003) Comparative study of flavonoids in experimental models of inflammation. Pharmacol Res 48:601–606

Scalise I, Durantini EN (2004) Photodynamic effect of metallo 5-(4-carboxyphenyl)-10, 15, 20-tris(4-methylphenyl) porphyrins in biomimetic media. J Photochem Photobiol A Chem 162:105–113. doi:10.1016/S1010-6030(03)00317-4

Soysa NS, Samaranayake LP, Ellepola ANB (2004) Cytotoxic drugs, radiotherapy and oral candidiasis. Oral Oncol 40:971–978

Valsaraj R, Pushpangadan P, Smitt UW, Adsersen A, Christensen SB, Sittie A, Nyman U, Nielsen C, Olsen CE (1997) New anti-HIV-1, antimalarial, and antifungal compounds from *Terminalia bellerica*. J Nat Prod 60:739–742

Wachter GA, Hoffmann JJ, Furbacher T, Blake ME, Timmermann BN (1999) Antibacterial and antifungal flavanones from *Eysenhardtia texana*. Phytochem 52:1469–1471. doi:10.1016/S0031-9422(99)00221-6

White TC, Holleman S, Dy F, Mirels LF, Stevens DA (2002) Resistance mechanisms in clinical isolates of *Candida albicans*. Antimicrob Agents Chemother 46:1704–1713

Yongmoon H (2009) Rutin has therapeutic effect on septic arthritis caused by *Candida albicans*. Int Immunopharmacol 9:207–211. doi:10.1016/j.intimp.2008.11.002