**Synthesis and *in vitro* Evaluation of Antifungal Properties of Some**

**4-Aryl-3-Methyl-1,2,3,4-Tetrahydroquinolines Derivatives**

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**Recibido: xx-xx-2014 Aprobado:**

**Abstract**

Two series of 4-aryl-3-methyl-1,2,3,4-tetrahydroquinoline derivatives were efficiently synthesized according to a two-step synthesis and evaluated as potential antifungal agents. The first and key step involved the formation of the corresponding *N*-benzyltetrahydroquinolines 5 via three-component cationic imino Diels-Alder cycloaddition. The second step consisted in their catalytic debenzylation to obtain the respective *N*-unprotected tetrahydroquinolines 6. The purity of the products and the composition of the reaction mixtures were monitored by thin layer chromatography (TLC), and products were isolated and purified by column chromatography. Substances were characterized using nuclear magnetic resonance (NMR) and mass spectrometry (MS). All compounds were tested *in vitro* against standardized clinically important fungi, including yeasts, hialohyphomycetes, and dermatophytes. These studies showed that between the tetrahydroquinoline (THQ) series tested, compounds 6f and 6g showed antifungal activity, specifically against dermatophytes. The compound 6-methoxy-4-(4-hydroxi-3-methoxyphenyl)-3-methyl-1,2,3,4-tetrahydroquinoline 6g exhibited the best *in vitro* activity (MIC 32-65 μg/mL). Results indicated that removed benzyl group from the *N*-benzyltetrahydroquinolines derivatives and the introduction of hydroxyl group in the 4-aryl substituent caused an important improvement of the antifungal activity. These results, were supported by the *in silico* prediction, that showed the high bioavailability, high drugs score and little potential risk for most tetrahydroquinolines evaluated.

**Keywords -** Antifungal activity; tetrahydroquinolines; cationic Imino Diels-Alder reaction; Lipinski’s rule; propenylbenzenes.

**Resumen**

**Síntesis y evaluación *in vitro* de las propiedades antifúngicas de algunos derivados de 4-aril-3-metil-1,2,3,4-tetrahidroquinolinas**

Dos series de derivados de las 4-aril-3-metil-1,2,3,4-tetrahidroquinolinas fueron sintetizadas eficientemente de acuerdo con una metodología sintética de dos pasos y fueron evaluados como potenciales agentes antifúngicos. El primer y paso clave involucró la formación de las correspondientes *N*-bencil tetrahidroquinolinas 5 a través de una reacción de cicloadición imino Diels-Alder catiónica de tres componentes. El segundo paso consistió en la obtención de las respectivas tetrahidroquinolinas *N*-desprotegidas 6 vía una desbencilación catalítica. La pureza de los productos y la composición de las mezclas de reacción fueron monitoreadas por cromatografía en capa fina (CCD). Los productos fueron aislados y purificados usando cromatografía en columna. Las sustancias fueron identificadas usando resonancia magnética nuclear (RMN) y espectrometría de masas (EM). Todos los compuestos fueron evaluados *in vitro* frente a cepas estandarizadas de hongos y clínicamente relevantes, incluyendo levaduras, hialohifomicetes y dermatofitos. Estos estudios mostraron que entre las series de tetrahidroquinolinas (THQ) ensayadas, los compuestos 6f y 6g mostraron actividad antifúngica, específicamente frente a dermatofitos. El compuesto 6-metoxi-4-(4-hidroxi-3-metoxifenil)-3-metil-1,2,3,4-tetrahidroquinolina 6g exhibió la mejor actividad *in vitro* (MIC 32-65 μg/mL). Los resultados obtenidos indican que la remoción el grupo bencilo y la introducción de un grupo hidroxilo en el sustituyente aril de las N-bencil tetrahidroquinolinas, produjo un importante mejoramiento de la actividad antifúngica. Estos resultados, fueron soportados por predicciones *in silico*, los cuales mostraron la alta biodisponibilidad, los altos “drug score” y poco riesgo potencial de la mayoría de las tetrahidroquinolinas evaluadas.

**Palabras clave -** Actividad antifúngica; tetrahidroquinolinas; reacción imino Diels-Alder catiónica, regla de Lipinski, propenilbencenos.

**Resumo
Síntese e avaliação *in vitro* das propriedades antifúngicas de alguns derivados de 4-aril-3-metil-1,2,3,4-tetra-hidroquinolina**
Duas séries de derivados de 4-aril-3-metil-1,2,3,4-tetrahidroquinolina foram eficientemente sintetizado de acordo com um método de síntese em duas etapas e foram avaliados como potenciais agentes antifúngicos. O primeiro e fundamental passo envolveu a formação dos correspondentes *N*-bencil tetrahidroquinolinas 5 através de uma reacção de cicloadição de imino Diels-Alder catiónica de três componentes. O segundo passo foi a obter as respectivas *N*-tetrahidroquinolinas desprotegidas 6 através de uma desbenzilação catalítica. A pureza do produto e a composição das misturas reaccionais foram monitorizadas por cromatografia em camada fina (CCD). Os produtos foram isolados e purificados utilizando cromatografia em coluna. As substâncias foram identificados por ressonância magnética nuclear (RMN) e espectrometria de massa (EM). Todos os compostos foram testados in vitro contra as estirpes padrão e os fungos clinicamente importantes, incluindo as leveduras, hialohifomicetes e dermatófitos. Estes estudos mostraram que entre a série de tetrahidroquinolinas (THQ) testaram os compostos 6f e 6g mostraram atividade antifúngica, particularmente contra dermatófitos. O composto 6-metoxi-4-(4-hidroxi-3-metoxifenil)-3-metil-1,2,3,4-tetrahidroquinila 6g mostrou melhor actividade *in vitro* (MIC 32-65 μg/mL). Os resultados indicam que a remoção do grupo benzilo e a introdução de um grupo hidroxilo no substituinte arilo do N-benzil-hidroquinolina, houve um aumento significativo da actividade antifúngica. Estes resultados foram apoiados por previsões in silico, que mostraram alta biodisponibilidade, alta "drugs score" e pouco risco potencial de a maioria dos tetrahidroquinolinas avaliados.

**Introduction**

Fungal infections constitute a common and serious problem of public health, that even threat to human life, particularly to immunocompromised patients, having worldwide remarkable impact on morbidity and mortality especially in tropical and subtropical developing countries (Brown et al. 2012, Mathew & Nath 2009, Pappas 2011). The majority of fungal infections are caused by opportunistic pathogens, which may be endogenous or acquired from the environment. *Candida*, *Cryptococcus* and *Aspergillus* infections are the most common invasive mycotic diseases that affect the humans (Pfaller et al. 2006, Senet et al. 2012). However, besides these known fungal species, new fungal pathogens have emerged as a risk in a global scale and their incidence continues to increase (Galimberti et al. 2012, Seebacher et al. 2008). Particularly, some forms of dermatomycoses caused by fungal microorganisms are the cause of a high morbidity, that affect quality of life of patients with AIDS, patients receiving anticancer chemotherapy, or those undergoing organ transplants (Hsu et al. 2011, Ramos-e-Silva et al. 2012). The high prevalence of superficial mycotic infections shows that 20% of the world’s population has skin mycoses, making these one of the most frequent forms of infection (Havlickova et al. 2008, Woodfolk 2005). Superficial mycoses are produced by dermatophytes fungi of the genera *Trichophyton*, *Epidermophyton*, and *Microsporum*. This fungi group infects specifically the keratin tissues of the body, causing superficial infections of the skin, hair, or nails, thanks to the ability of these fungi to obtain nutrients from keratinized material (Grappel et al. 1974, Weitzman & Summerbell 1995). Important progresses have been made in the development and evaluation of new antifungal agents, especially by superficial mycoses (Kathiravan et al. 2012, Rotta et al. 2012). Although imidazole compounds such as clotrimazole and miconazole have proven to be an effective treatment for dermatomycoses (Boucher et al. 2004, Chai et al. 2011), these fungal infections are frequently very difficult to eradicate (Li et al. 1995, Zacchino et al. 1999). The clinical efficacy of the currently used antifungal drugs have been limited due to the emergence of drug resistance, high risk of toxicity, insufficiencies in their antifungal activity and undesirable side effects (Marr et al. 1997, Sanglard 2002). Therefore, there is still a need to develop new, safe and efficient chemotherapeutic agents with potent antifungal activity. Consequently, it is urgent to develop new broad-spectrum antifungal agents with less toxicity and a better pharmacokinetic profile.

In this context, natural and synthetic quinoline compounds and their partially reduced derivatives tetrahydroquinoline are heterocycles with great importance in medicinal chemistry, due to their remarkable pharmacological applications (Chen et al. 2007, Singer et al. 2005, Wallace et al. 2013). These kind of compound have displayed different biological activities (Jaquemond-Collet et al. 2002, Houghton et al. 1999), therefore they are considered attractive scaffolds to develop new antifungal agents. Including some molecules such as, a relatively simple heterocyclic compound with significant antibiotic properties, called helquinoline 1 (Asolkar et al. 2004); the antifungal agent (*N*-tetrahydroquinoline) urea derivative 2 that displayed an MIC value of 12.5 µg/mL against *A. Niger* and *T. Rubrum*, respectively (Zheng et al. 2010); the 2-(4-pyridinyl)-1,2,3,4-tetrahydroquinoline derivative 3 that is highly active against *Aspergillus* spp. (MIC = 31.2 µg/mL) and mainly against dermatophytes (MIC = 8 µg/mL), and more recently, Gutierrez and coworkers (2012) reported the 2-(furan-2-yl)-1,2,3,4-tetrahydroquinoline 4 that showed interesting phytopathogenic fungi bioactivity against *Cladosporium cladosporoides* with MIC values of 13.75 µg/mL.



**Fig. 1**. Tetrahydroquinolines derivatives with remarkable antifungal activity

In the course of our screening program for novel and selective bioactive compounds, we have previously reported the antiparasitic and antifungal activity of some substituted *N*-heterocycles including some (tetrahydro)quinolines (Fonseca et al. 2013, Romero et al. 2012, Kouznetsov et al. 2012, Vargas et al. 2003). Keeping in mind the above facts and continuing our program on the search of new antifungal agents, we decided to prepare different polyfuntionalized 1,2,3,4-tetrahydroquinolines and test its biological properties. Herein, in this paper, we report the synthesis and *in vitro* antifungal activity of several 4-aryl-3-methyl tetrahydroquinoline derivatives (5a-h) and (6a-h) against standardized, as well as clinically important fungi including *Cryptococcus neoformans*, several species of *Candida* and *Aspergillus* genus, and dermatophytes. These THQs compounds were prepared through the three-components cationic imino Diels-Alder cycloaddition methodology and then a respective catalytic reduction (Romero & Kouznetsov 2010) provided access to the *N*-benzyl- or *N*-H-1,2,3,4-tetrahydroquinoline derivatives with high structural diversity.

**Materials and methods**

***In silico* pharmacological properties study**

Preliminary molecular design for 4-aryl-3-methyl-1,2,3,4-tetrahydroquinoline compounds series 5 and 6 were achieved based on structure-activity relationship studies, and virtual screening analysis reported in literature. Five physicochemical parameters of Lipinki’s rule were calculated using the Molinspiration virtual platform (http://www.molinspiration.com/services/). The toxicity risk profile assessment was accomplished using the OSIRIS program (http://www.organic-chemistry.org/prog/peo). The OSIRIS and Molinspiration Property Explorers, shown in these web-pages, are an integral part of some pharmaceutical companies’ *in-house* substance registration system. They allow drawing chemical structures and calculating on-the-fly numerous drug-relevant properties whenever a structure is valid.

**Chemistry**

The melting points (uncorrected) were determined on a Fisher-Johns melting point apparatus. IR spectra were recorded on a Lumex Infralum FT-02 spectrophotometer in KBr. 1H and 13C NMR spectra were measured on a Bruker AM-400 spectrometer (400 MHz 1H NMR and 100 MHz 13C NMR), using CDCl3 as solvent. TMS was used as internal standard. *J* values are reported in Hz, chemical shifts are reported in ppm (δ) relative to the solvent peak (CHCl3 in CDCl3 at 7.26 ppm for protons). Signals are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublets of doublets; t, triplet; td, triplet of doublets; q, quartet; m, multiplet; br., broad. A Hewlett Packard 5890a Series II Gas Chromatograph interfaced to an HP 5972 Mass Selective Detector with an HP MS ChemStation Data system was used for MS identification at 70 eV using a 60 m capillary column coated with HP-5 [5% phenylpoly(dimethylsiloxane)]. Elemental analyses were performed on a Perkin-Elmer 2400 Series II analyzer, and were within ±0.4 of theoretical values. The reaction progress was monitored using thin layer chromatography on a Silufol UV 254 TLC aluminum sheets. Column chromatography was carried out using Silica gel (230-400 mesh). All reagents were purchased from Sigma and Aldrich Chemical Co and used without further purification.

*General Procedure for the synthesis of N-Benzyl-4-aryl-3-methyltetrahydroquinoline derivatives*

The general procedure was previously described by Romero and Kouznetsov(2010) and performed as follows: A mixture of *N*-benzylaniline (1 mmol) and formaldehyde (37% in MeOH, 1.1 mmol) in MeCN (10 mL) was stirred at r.t. for 10 min. The system was cooled to 0 °C and BF3·OEt2 (154.27 mL, 1.1 mmol) was added dropwise into the mixture. After 30 min, dienophile reagent (*trans*-anethole or *trans*-isoeugenol; 1.1 mmol) was added over 5 min to the reaction mixture. The resulting mixture was stirred at 70 °C for 8 h. After completion of the reaction as indicated by TLC, the reaction mixture was extracted with EtOAc (3 × 15 mL). The organic layer was separated and dried (Na2SO4), concentrated *in vacuo* and the crude product was purified by column chromatography using silica gel (60-120 mesh; petroleum ether-EtOAc) to afford pure tetrahydroquinoline 5a-h.

*Selected Spectral Data*

***trans*-*N*-Benzyl-3,6-dimethyl-4-(4-methoxyphenyl)-1,2,3,4-tetrahydroquinoline (5b)**: This compound was isolated as a white solid; with Melting point (Mp) 88-90ºC (Uncorrected) and the molecular characterization carried out with infrared (IR) spectroscopy showed the following characteristic signals: 3024, 2962, 1620, 1512, 1250 cm-1; Mass Spectrometry (MS) gave a molecular ion peak *m/z* = 357 (65, M+•); Nuclear Magnetic Resonance on protons 1H NMR (400 MHz, CDCl3 Me4Si) showed δ (ppm) to be: 0.92 (3H, d, *J* = 6.7 Hz, 3-CH3), 2.08 (3H, s, 6-CH3), 2.19 (1H, m, H-3), 3.03 (1H, dd, *J* = 11.4, 7.9 Hz, H-2ax), 3.27 (1H, dd, *J* = 11.4, 3.7 Hz, H-2eq), 3.62 (1H, d, *J* = 7.8 Hz, H-4), 3.80 (3H, s, 4’-OCH3), 4.49 (2H, s, N-CH2-Ph), 6.49 (2H, m, H-8 and H-5), 6.80 (1H, dd, *J* = 8.5, 1.5 Hz, H-7), 6.84 (2H, d, *J* = 8.6 Hz, 2’-ArH), 7.01 (2H, d, *J* = 8.6 Hz, 3’-ArH), 7.22-7.34 (5H, m, PhH); Nuclear Magnetic Resonance on Carbons 13C-NMR (100 Hz, CDCl3 Me4Si), δ (ppm), presents the following data: 157.9, 143.2, 139.2, 137.9, 131.0, 129.9 (2C), 128.5 (2C), 127.8, 126.7 (2C), 126.6, 125.0, 124.5, 113.6 (2C), 111.0, 55.6, 55.2, 54.5, 50.7, 34.8, 20.3, 18.2.

***trans*-*N*-Benzyl-3,6-dimethyl-4-(4-hydroxy-3-methoxyphenyl)-1,2,3,4-tetrahydroquinoline (5f)**: This compound was isolated as a white solid; with Melting point (Mp) 127-129ºC (Uncorrected) and the molecular characterization that was carried out with infrared (IR) spectroscopy showed the following characteristic signals: 3433, 3024, 2916, 1612, 1512, 1273 cm-1; Mass Spectrometry (MS) gave a molecular ion peak *m/z* = 373 (77, M+•); Nuclear Magnetic Resonance on protons 1H NMR (400 MHz, CDCl3 Me4Si) showed δ (ppm) to be: 0.99 (3H, d, *J* = 6.7 Hz, 3-CH3), 2.15 (3H, s, 6-CH3), 2.27 (1H, m, H-3), 3.12 (1H, dd, *J* = 11.3, 8.2 Hz, H-2ax), 3.36 (1H, dd, *J* = 11.4, 3.8 Hz, H-2eq), 3.67 (1H, d, *J* = 8.1 Hz, H-4), 3.87 (3H, s, 3’-OCH3), 4.55 (2H, br. d, *J* = 7.2 Hz, N-CH2-Ph), 5.58 (1H, s, 4’-OH), 6.57 (1H, br. d, *J* = 8.4 Hz, H-8), 6.59 (1H, br. s, H-5), 6.69 (2H, br. d, *J* = 8.3, Hz, 2’-ArH y 6’-ArH), 6.86 (1H, dd, *J* = 8.2, 1.4 Hz, H-7), 6.88 (1H, d, *J* = 8.0 Hz, 5’-ArH), 7.26-7.40 (5H, m, PhH). Nuclear Magnetic Resonance on Carbons 13C-NMR (100 Hz, CDCl3 Me4Si), δ (ppm), presents the following data: 146.5, 143.9, 143.2, 139.2, 137.6, 130.9, 128.5 (2C), 127.8, 126.7, 126.7 (2C), 125.1, 124.5, 122.2, 114.0, 111.1, 110.9, 55.9, 55.6, 54.7, 51.3, 34.7, 20.2, 18.3.

*General procedure synthesis of N-H-4-Aryl-3-methyltetrahydroquinoline derivatives*

The general procedure also was previously described by Romero and Kouznetsov(2010) and performed as follows: A mixture of tetrahydroquinoline 5a-h (1 mmol), wet 10% Pd/C (cat.) and MeOH-CH2Cl2 (3:1, 20 mL) was stirred under H2 (1atm) at r.t. for 14-16 h. The reaction mixture was filtered and the filtrate was concentrated *in vacuo* and extracted with CH2Cl2 (3 × 15 mL). The organic layer was separated and dried (Na2SO4), concentrated again *in vacuo* and the resulting product was purified by flash column chromatography (silica gel; petroleum ether-EtOAc) to afford the respective pure tetrahydroquinoline 6a–h.

*Selected Spectral Data*

 ***trans*-3,6-Dimethyl-4-(4-methoxyphenyl)-1,2,3,4-tetrahydroquinoline (6b)** : This compound was isolated as a yellow solid; with Melting point (Mp) 71-73ºC (Uncorrected) and the molecular characterization that was carried out with infrared (IR) spectroscopy showed the following characteristic signals: 3412, 2962, 1604, 1504 cm-1; Mass Spectrometry (MS) gave a molecular ion peak *m/z* = 267 (65, M+•); Nuclear Magnetic Resonance on protons 1H NMR (400 MHz, CDCl3 Me4Si) showed δ (ppm) to be: 0.91 (3H, d, *J* = 6.7 Hz, 3-CH3), 2.08 (4H, br. s, 6-CH3 and H-3), 2.98 (1H, dd, *J* = 11.1, 8.5 Hz, H-2ax), 3.24 (1H, dd, *J* = 11.2, 3.5 Hz, H-2eq), 3.56 (1H, d, *J* = 8.2 Hz, H-4), 3.80 (3H, s, 4’-OCH3), 6.46 (2H, br. d, *J* = 8.1 Hz, H-8 y H-5), 6.79 (1H, dd, *J* = 8.1, 1.4 Hz, H-7), 6.83 (2H, d, *J* = 8.6 Hz, 2’-ArH), 7.03 (2H, d, *J* = 8.6 Hz, 3’-ArH).Nuclear Magnetic Resonance on Carbons 13C-NMR (100 Hz, CDCl3 Me4Si), δ (ppm), presents the following data: 157.9, 142.3, 138.0, 131.0, 130.0 (2C), 127.6, 126.3, 124.3, 114.1, 113.6 (2C), 55.2, 50.4, 47.2, 35.3, 20.4, 18.0.

***trans*-3,6-Dimethyl-4-(4-hydroxy-3-methoxyphenyl)-1,2,3,4-tetrahidroquinoline (6f)**: This compound was isolated as a white solid; with Melting point (Mp) 139-141ºC (Uncorrected) and the molecular characterization that was carried out with infrared (IR) spectroscopy showed the following characteristic signals: 3548, 3425, 2924, 1612, 1512 cm-1; Mass Spectrometry (MS) gave a molecular ion peak *m/z* = 283 (59, M+•); Nuclear Magnetic Resonance on protons 1H NMR (400 MHz, CDCl3 Me4Si) showed δ (ppm) to be: 0.93 (3H, d, *J* = 6.6 Hz, 3-CH3), 2.12 (4H, br. s, H-3 and 6-CH3), 3.02 (1H, dd, *J* = 10.7, 9.2 Hz, H-2ax), 3.29 (1H, dd, *J* = 11.1, 3.1 Hz, H-2eq), 3.56 (1H, d, *J* = 8.5 Hz, H-4), 3.82 (3H, s, 3’-OCH3), 4.64 (1H, br. s, 4’-OH), 6.49 (2H, br. d, *J* = 8.0 Hz, H-5 y H-8), 6.63 (1H, br. s, 2’-ArH), 6.66 (1H, dd, *J* = 8.8 Hz, 6’-ArH), 6.82 (1H, dd, *J* = 7.7 Hz, H-7), 6.86 (1H, d, J = 7.9 Hz, 5’-ArH).Nuclear Magnetic Resonance on Carbons 13C-NMR (100 Hz, CDCl3 Me4Si), δ (ppm), presents the following data: 146.5, 144.0, 142.2, 137.6, 130.9, 127.5, 126.4, 124.4, 122.3, 114.2, 113.9, 111.1, 55.9, 51.1, 47.5, 35.3, 20.4, 18.0.

 **Biology**

*Microorganisms and media*

For the antifungal evaluation, standardized strains from the American Type Culture Collection (ATCC), Rockville, MD, USA, and CEREMIC (CCC), Centro de Referencia en Micología, Facultad

de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531-(2000)-Rosario, Argentina were used in a first instance of screening: *Candida albicans* ATCC 10231, *Candida tropicalis* CCC 131, *Saccharomyces cerevisiae* ATCC 9763, *Cryptococcus neoformans* ATCC 32264, *A. fumigatus* ATTC 26934, *A. niger* ATCC 9029, *Trichophyton rubrum* CCC 110, *T. mentagrophytes* ATCC 9972 and *M. gypseum* CCC 115. Strains were grown on Sabouraud-chloramphenicol agar slants for 48 h at 30ºC, maintained on slopes of Sabouraud-Dextrose agar (SDA, Oxoid) and sub-cultured every 15 days to prevent pleomorphic transformations. Inocula of cell or spore suspensions were obtained according to reported procedures and adjusted to 1-5x10-3 cells/spores with colony forming units (CFU)/mL (CLSI 2008 a,b).

*Antifungal susceptibility testing*

Minimum Inhibitory Concentration (MIC) of each compound was determined by using broth microdilution techniques according to the guidelines of the National Committee for Clinical Laboratory Standards for yeasts (M27-A3)(CLSI 2008 a) and for filamentous fungi (M 38-A2)(CLSI 2008 b). MIC values were determined in RPMI-1640 (Sigma, St. Louis, Mo, USA) buffered to pH 7.0 with MOPS. Microtiter trays were incubated at 30 ºC for yeasts and species of Aspergillus and at 28-30 ºC for dermatophytes in a moist, dark chamber. MICs were visually recorded at 48 h for yeasts, and at a time according to the control fungus growth, for the rest of fungi.

For the assay, stock solutions of pure compounds were twofold diluted with RPMI from 250-0.98 µg/mL (final volume = 100 µl) and a final DMSO concentration ≤ 1%. A volume of 100 µl of inoculums suspension was added to each well with the exception of the sterility control (sterile water was added instead). Ketoconazole (Sigma Chem. Co.) and terbinafine (Novartis, Bs. As.) were used as positive controls. Endpoints recorded in Table 4 were defined as the lowest concentration of drug resulting in total inhibition (MIC100) of visual growth compared to the growth in the control wells containing no antifungal. All tests were made by duplicate and compounds with MICs >250 µg/mL were considered inactive.

**Results**

***In silico* chemoinformatics tools**

The pre-screening for the identification of synthetic compounds libraries hits was based on the oral bioavailability using the Lipinski’s rules concepts (Lipinski et al. 1997). In this case, we have performed the analysis of the rule of five, employing the Molisnpiration free software (Table 1).

**Table 1**. Calculated Lipinski’s rule of five parameters for the 4-aryl-3-methyl-1,2,3,4- tetrahydroquinolines (5, 6) assayed

|  |  |  |  |
| --- | --- | --- | --- |
| Comp. | Mol. formula*a* | MW, g/mol | Parameters |
| Log P | TPSA, Å | nNO*b* | nOHNH*c* | RBN*d* | Violations |
| **5a** | C24H25NO | 347.47 | 5.619 | 12.472 | 2 | 0 | 4 | 1 |
| **5b** | C25H27NO | 357.50 | 6.044 | 12.472 | 2 | 0 | 4 | 1 |
| **5c** | C25H27NO2 | 373.50 | 5.652 | 21.706 | 3 | 0 | 5 | 1 |
| **5d** | C24H24ClNO | 377.92 | 6.273 | 12.472 | 2 | 0 | 4 | 1 |
| **5e** | C24H25NO2 | 359.47 | 4.902 | 32.700 | 3 | 1 | 4 | 0 |
| **5f** | C25H27NO2 | 373.50 | 5.326 | 32.700 | 3 | 1 | 4 | 1 |
| **5g** | C25H27NO3 | 389.50 | 4.934 | 41.934 | 4 | 1 | 5 | 0 |
| **5h** | C24H24ClNO2 | 393.91 | 5.556 | 32.700 | 3 | 1 | 4 | 1 |
| **6a** | C17H19NO | 253.34 | 3.976 | 21.261 | 2 | 1 | 2 | 0 |
| **6b** | C18H21NO | 267.37 | 4.400 | 21.261 | 2 | 1 | 2 | 0 |
| **6c** | C18H21NO2 | 283.37 | 4.009 | 30.495 | 3 | 1 | 3 | 0 |
| **6d** | C17H18ClNO | 287.79 | 4.630 | 21.261 | 2 | 1 | 2 | 0 |
| **6e** | C17H19NO2 | 269.34 | 3.258 | 41.489 | 3 | 2 | 2 | 0 |
| **6f** | C18H21NO2 | 283.37 | 3.683 | 41.489 | 3 | 2 | 2 | 0 |
| **6g** | C18H21NO3 | 299.34 | 3.291 | 50.723 | 4 | 2 | 3 | 0 |
| **6h** | C17H18ClNO2 | 303.79 | 3.912 | 41.489 | 3 | 2 | 2 | 0 |
| **Ket** | C26H28Cl2N4O4 | 531.44 | 3.772 | 69.075 | 8 | 0 | 7 | 1 |
| **Terb** | C21H25N | 291.43 | 5.719 | 3.238 | 1 | 0 | 4 | 1 |

*a* Confirmed by elemental analysis with ± 0.5% of calculated values, *b* number of hydrogen bond acceptors, *c* number of hydrogen bond donors, *d* number of rotatable bonds, Reference drug: Ketoconazole (Ket) and terbinafine (Terb).

The principal molecular properties, including the number of hydrogen donors (nNHOH), number of hydrogen acceptors (nNO), number of rotables bonds (nRB) and molecular weight (MW) were calculated. Another recognized parameter for membrane permeation, prerequisite for the bioavailability, the topological polar surface area (TPSA) was also considered (Table 1). Other molecular properties explorers for the potential risk assessment and associated to some fragments of the synthesized tetrahydroquinoline compounds, were evaluated employing the Osiris software (Table 2).

**Table 2**. Toxicity risk, drug-likeness and drug-score parameters obtained for the 4-aryl-3-methyl-1,2,3,4- tetrahydroquinolines (5,6) assayed

|  |
| --- |
| Potential Risk *a, b* |
| Comp. | Mut. | Tum. | Irr. | Rep. Eff. | Drug-likeness | Drug-Score |
| **5a** |  |  |  |  | 4.78 | 0.57 |
| **5b** |  |  |  |  | 3.31 | 0.50 |
| **5c** |  |  |  |  | 4.54 | 0.57 |
| **5d** |  |  |  |  | 4.79 | 0.45 |
| **5e** |  |  |  |  | 4.87 | 0.62 |
| **5f** |  |  |  |  | 3.35 | 0.55 |
| **5g** |  |  |  |  | 4.58 | 0.61 |
| **5h** |  |  |  |  | 4.83 | 0.48 |
| **6a** |  |  |  |  | 2.87 | 0.79 |
| **6b** |  |  |  |  | 1.33 | 0.68 |
| **6c**  |  |  |  |  | 2.60 | 0.62 |
| **6d** |  |  |  |  | 2.88 | 0.68 |
| **6e** |  |  |  |  | 2.98 | 0.82 |
|  |  |  |  |  |  |  |
| **6f** |  |  |  |  | 1.38 | 0.72 |
|  |  |  |  |  |  |  |
| **6g** |  |  |  |  | 2.65 | 0.65 |
| **6h** |  |  |  |  | 2.93 | 0.72 |
| **Terb** |  |  |  |  | -3.84 | 0.17 |
| **Ket**  |  |  |  |  | 0.17 | 0.63 |

*a* Colors code for potential risk: drug-conform,  middle risk, undesired effects

The data acquired from this topological analysis, allowed us to get information about fragment with possible mutagenic (Mut.), tumorigenic (Tum.), irritant (Irr.) and reproductive effective (Rep. Eff.) effects. Prediction results were valued and colour coded. Properties with middle risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in light orange. While, the green color indicates that the substance evaluated has a behavior like drugs. Final molecular properties calculated in this work were the drug-score and the drug-likeness (Table 2). The molecular fragments with frequent occurrence in trade drugs yield positives drug-likeness values.

**Chemistry**

Two series of 4-aryl-3-methyl-1,2,3,4-tetrahydroquinolines derivatives were easily and efficiently synthesized according to a two-step synthesis. The first and key step involves the formation of the corresponding *N*-benzyltetrahydroquinolines 5 via the protocol of a “one pot” three-component cationic imino Diels-Alder cycloaddition starting from readily available *N*-benzylanilines, inexpensive formalin (37% formaldehyde in methanol), and the commercially available *trans*-anethole (and isoeugenol) in the presence of BF3.OEt2 at 70 ºC in acetonitrile, according to the literature procedure (Romero & Kouznetsov 2010). The second step consisted on their catalytic debenzylation using hydrogen gas and Pd/C as catalyst to obtain the respective *N*-unprotected tetrahydroquinolines 6 (Figure 2).



**Fig. 2**. Preparation of substituted 3-methyl-4-aryl-1,2,3,4-tetrahydroquinolines (5,6): Reagents and conditions: (*i*) BF3.OEt2 (1 mmol), dry acetonitrile, reflux (70ºC), 6-8 h.; (*ii*) wet 10% Pd/C (cat.), MeOH/CH2Cl2 (3:1), H2 (1 atm.), r.t., 14-16 h.

Both series of compounds were obtained in good yields after column chromatography purification on silica gel (Table 3). The spatial structure and stereochemistry of all these tetrahydroquinoline compounds synthesized (5,6) were determined by IR and MS and confirmed by 1H and 13C NMR spectroscopy and supported by inverse-detected 2D NMR experiments (COSY, HSQC and HMBC spectra).

**Table 3**. Physico-chemical data of the 4-aryl-3-methyl-1,2,3,4-tetrahydroquinolines (5,6)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Comp. | R1 | R2 | R3 | m.p., ºCa | Yield, %b | Comp. | R1 | R2 | R3 | m.p., ºCa | Yield, %b |
| **5a** | H | CH3 | H | 89-91 | 59 | **6a** | H | CH3 | H | 73-75 | 94 |
| **5b** | CH3 | CH3 | H | 88-90 | 61 | **6b** | CH3 | CH3 | H | 71-73 | 92 |
| **5c** | OCH3 | CH3 | H | 113-115 | 67 | **6c** | OCH3 | CH3 | H | Sticky oil | 95 |
| **5d** | Cl | CH3 | H | 125-127 | 76 | **6d** | Cl | CH3 | H | 116-118 | 93 |
| **5e** | H | H | OCH3 | 167-169 | 49 | **6e** | H | H | OCH3 | 118-120 | 96 |
| **5f** | CH3 | H | OCH3 | 127-129 | 76 | **6f** | CH3 | H | OCH3 | 139-141 | 96 |
| **5g** | OCH3 | H | OCH3 | 121-123 | 60 | **6g** | OCH3 | H | OCH3 | 118-120 | 98 |
| **5h** | Cl | H | OCH3 | 136-138 | 77 | **6h** | Cl | H | OCH3 | 112-114 | 97 |

a Isolated yield after column chromatography; b Melting point Uncorrected.

**Antifungal assays**

The *in vitro* antifungal activity of the 4-aryl-3-methyl-1,2,3,4-tetrahydroquinolines series 5a-h and 6a-h was evaluated againststandardized clinically important fungi, including yeasts, hialohyphomycetes, and dermatophytes (Table 4). To carry out the antifungal evaluation, concentrations of compounds up to 250 µg/mL were incorporated to growth media according to the CLSI standardized procedures (CLSI 2008 a,b). Terbinafine (Terb), and ketoconazole (Ket) were used as positive controls under the same assay conditions. Table 4 summarizes the minimum concentration of compounds that completely inhibited the growth (MIC100) obtained by nine opportunistic pathogenic fungi including yeasts (*Candida albicans*, *C. neoformans*, *Saccharomyces cerevisiae*), hialohyphomycetes (*Aspergillus spp*.) as well as dermatophytes (*Microsporum* and *Trichophyton spp*.). The structure of each tetrahydroquinoline derivative is included in Table 4, to allow an easier analysis of the results.

**Table 4**. Minimum inhibitory concentrations (MIC in g/mL) of *N*-benzyl-4-aryl-3-methyl -1,2,3,4-tetrahydroquinoline (5) and *N*-H-4-aryl-3-methyl -1,2,3,4-tetrahydroquinoline derivatives (6)

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Compd. | Structure | *Ca* | *Ct* | *Sc* | *Cn* | *Afu* | *Ani* | *Mg* | *Tr* | *Tm* |
| **5a** |  | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 |
| **5b** |  | >250 | >250 | >250 | >250 | >250 | >250 | 250 | 250 | 250 |
| **5c** |  | >250 | >250 | >250 | >250 | >250 | >250 | 250 | 250 | 250 |
| **5d** |  | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 |
| **5e** |  | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 |
| **5f** |  | >250 | >250 | >250 | >250 | >250 | >250 | 250 | 250 | 250 |
| **5g** |  | >250 | >250 | >250 | >250 | >250 | >250 | 250 | 250 | 250 |
| **5h** |  | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 |
| **6a** |  | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 |
| **6b** |  | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 |
| **6c** |  | >250 | >250 | >250 | >250 | >250 | >250 | 125 | 125 | 125 |
| **6d** |  | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 |
| **6e** |  | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 |
| **6f** |  | >250 | >250 | >250 | >250 | >250 | >250 | 31,25 | 62,5 | 62,5 |
| **6g** |  | >250 | >250 | >250 | >250 | >250 | >250 | 62,5 | 62,5 | 62,5 |
| **6h** |  | >250 | >250 | >250 | >250 | >250 | >250 | 250 | 250 | 250 |
| **Ket** |  | 0.49 | 0.49 | 0.25 | 0.25 | 0.49 | 0.25 | 0.06 | 0.03 | 0.03 |
| **Terb** |  | 1.95 | 3.90 | 0.49 | 0.25 | 0.98 | 1.96 | 0.04 | 0.01 | 0.03 |

*Ca*: *Candida albicans* ATCC 10231, *Ct*: *Candida tropicalis* CCC 131, *Sc*: *Saccharomyces cerevisiae* ATCC 9763, *Cn*: *Cryptococcus neoformans* ATCC 32264, *Afu*: *Aspergillus fumigatus* ATCC 26934, *An*: *Aspergillus niger* ATCC 9029, *Mg*: *Microsporum gypseum* CCC 115, *Tr*: *Trichophyton rubrum* CCC 113, *Tm*: *Trichophyton mentagrophytes* ATCC 9972. CCC = CEREMIC (Centro de Referencia en Micologia). ATCC: American Type Culture Collection. MIC ≥ 250 g/mL is indicative that the compound is inactive, Reference drug: ketoconazole (Ket) and terbinafine (Terb).

**Discussion**

With the obvious need to combat diseases caused by pathogenic fungi, there has been significant progress towards the development and implementation of strategies to access new and more potent antifungal agents. In this regard, search effective and safer drug against pathogens fungi is urgent and priority. Based on the above and taking into account that the tetrahydroquinoline ring is a key structural scaffold in many bioactive heterocyclic compounds, including some antifungal agents. Here, we want to report the synthesis, characterization and results of the antifungal assays of the two series of 3-methyl-4-aryl-1,2,3,4-tetrahydroquinolines 5a-h and 6a-h.

**Chemistry**

The general route for the synthesis of the *N*-benzyl-3-methyl-4-aryl-1,2,3,4-tetrahydroquinoline derivatives 5 and its debenzylated analogous THQs 6, including as a key-step a cycloaddition reaction, and is outlined in figure 2. Base on the BF3.OEt2-catalyzed multicomponent cationic imino Diels-Alder reaction among substituted *N*-benzylanilines, formalin (37% formaldehyde in methanol), and propenylbenzenes (*trans*-anethole and isoeugenol) at 70 ºC in CH3CN for 6-8 hours. The catalytic debenzylation (10mol% Pd/C) using hydrogen gas (H2) at room temperature and over nigh allowed to prepare the corresponding 3-methyl-4-aryl-1,2,3,4-tetrahydroquinoline derivatives 6. Both series of tetrahydroquinolines was majority obtained as stable solid substances with defined melting points in 49-77% (5a-h) and 93-98% (6a-h) yields after their chromatography purification (table 3). All studied tetrahydroquinolines were isolated and purified by column chromatography (on silica gel) using petroleum ether/ethyl acetate mixtures with gradient of polarity. The stereochemistry of all THQ compounds was confirmed by 1D and 2D NMR experiments. 1H NMR analysis indicated that the structure of the unique diastereoisomers obtained for 5 and 6 were *trans*-configured with respect to the 3-CH3/4-Ar substituents (Figure 2).

***In silico* chemoinformatics tools**

In this work, the most significant theoretical pharmacokinetic parameters for all tetrahydroquinoline derivatives synthesized (5a-h and 6a-h) were obtained using chemoinformatics platforms. Molinspiration Software allowed obtaining five properties associated with the Lipinski’ rules (Lipinski et al. 1997) that indicates whether a chemical can be orally active in humans (drug-likeness), furthermore of the TPSA parameters (Chohan et al. 2010) that has proven to be very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability and blood brain barrier penetration. We found that tetrahydroquinoline derivatives 5a-h did not present more than one violation of the Lipinski’ rules, while that their debenzylated derivatives 6a-h doesn’t present any violation. The obtained calculations demonstrate that all analyzed tetrahydroquinoline compounds showed high bioavailability properties, similar and comparable them with structures of reference drugs (ketoconazole and terbinafine), largely fulfilling all the parameters set by this rule (Molecular weight = 253.34-393.91, log P = 3.26-6.27, nON = 2-4, and nOHNH = 0-2). Prediction results by TPSA parameters for the compounds 5a-h and 6a-h (Table 1) showed TPSA values between 12.5 and 50.7 Å2, confirming that this pharmacokinetic parameter is a relevant property in the drug design. It should be noted that TPSA values less than 60 Å2 are defined for compounds with good membrane permeability, as well a good penetration of the blood-brain barrier.

In order to assess the possible pharmacological properties and predict the compounds drug-score of the tetrahydroquinolines 5a-h and 6a-h, a toxicity profile evaluation was performed employing the OSIRIS software. Exploring virtually the potential risk associated to some fragments of the synthesized tetrahydroquinoline compounds, it can be noted that all obtained and evaluated products presented low biological risks and have few negative effect. The methoxy group in the 6c and 6g compounds on the C-6 position of the tetrahydroquinoline scaffolds showed a moderate mutagenic risk. Nevertheless, the fragments and topology of the reference compound present higher potential risk than all the tetrahydroquinoline molecules evaluated. Performing calculations on drug-likeness and drug-score parameters, we could note that both series afforded favorable numbers, especially for the molecular structures 6a-h. Compared with the antifungal activity of the most active molecules (6f and 6g), the drug-likeness calculation showed good correlation and confirm the importance of remove of the benzyl group and introduction of hydroxyl group in the 4-aryl substituent of the tetrahydroquinoline structure (Table 3). Furthermore, the drug-score obtained for each analyzed molecule showed an important relationship with the *in vitro* antifungal evaluation for tetrahydroquinolines 6f and 6g, whose values were above the average of all calculated compounds.

**Antifungal assays**

All compounds were tested *in vitro* against standardized clinically important fungi, including yeasts, hialohyphomycetes, and dermatophytes. The obtained results for antifungal activities of all the title compounds were summarized in the Table 3, in which ketoconazole and terbinafine were used as the controls. The structure of each tetrahydroquinoline derivative was included in table 3, for easier the analysis of results. These studies showed that between these two tetrahydroquinoline series tested, *N*-H-tetrahydroquinoline derivatives 6a-h showed moderate inhibition activities, while that the *N*-benzyl-tetrahydroquinoline series 5a-h did not show antifungal activity, which was evidenced by the fact that their MICs were all over 250 μg/mL.

Compounds 6c, 6f and 6g showed antifungal activity, particularly versus all strains dermatophytes assayed. The 6-methoxy-4-(4-hydroxy-3-methoxyphenyl)-3-methyl-1,2,3,4-tetrahydroquinoline 6g exhibit the best *in vitro* antifungal activity, particularly against to dermatophytes*,* including *Microsporum gypseum* (MIC 31.25 μg/mL), *Trichophyton rubrum* (MIC 62.5 μg/mL) and *Trichophyton mentagrophytes* (MIC 62.5 μg/mL).

In a search for structural parameters that might enhance the antifungal activity, we introduced chlorine, methoxy, and methyl substituent’s onto the 2,4-diaryl 1,2,3,4-tetrahydroquinoline ring, furthermore we used two different dienophiles for the cycloaddition reaction (anethole and isoeugenol) and finally we performed the N-debenzylation of the tetrahydroquinoline derivatives 5a-h. Antifungal results indicated that presence of the methoxy group in the compounds 6a-h showed an interesting performance. Besides, results inhibition activities obtained indicated that remove the benzyl group and introduction of hydroxyl group and the 4-aryl substituent from the *N*-benzyltetrahydroquinolines derivatives caused an important improvement of the antifungal activity of this series (Table 3).

**Conclusion**

An easy and efficient synthetic route was employed for the preparation of two series of 4-aryl-3-methyl-1,2,3,4-tetrahydroquinoline derivatives. Key step involves a three-component cationic imino Diels- Alder reaction. These studies showed that between the tetrahydroquinolines (THQ) series tested, compounds 6f and 6g showed antifungal activity, specifically against dermatophytes. The compound 6-methoxy-4-(4-hydroxy-3-methoxyphenyl)-3-methyl-1,2,3,4-tetrahydroquinoline 6g exhibit the best in vitro activity (MIC 32-65 μg/mL). Results indicated that removed benzyl group from the *N*-benzyltetrahydroquinolines derivatives and the introduction of hydroxyl group in the 4-aryl substituent caused an important improvement of the antifungal activity. These results, were supported by the in silico prediction, that showed the high bioavailability, high drugs score and little potential risk for most tetrahydroquinolines evaluated. More complete structure–activity relationship studies, in order to increase the in vitro antifungal activity, are currently pursued in our laboratory.

**Acknowledgments**

The authors appreciate the financial support by the Universidad Industrial de Santander (VIE-UIS, project 5714). ARRB thanks COLCIENCIAS for the PhD studies scholarship (2005-2010).

The authors declare that no conflicts of interest exist in relation to this work.

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