Quinolines: Microwave-assisted Synthesis and their Antifungal, Anticancer and Radical Scavenger Properties

Natália Aparecida Liberto, Juliana Baptista Simões, Sarah de Paiva Silva, Cristiane Jovelina da Silva, Luzia Valentina Modolo, Ângelo de Fátima, Luciana Maria Silva, Marcos Derita, Susana Zacchino, Omar Miguel Portilla Zuñiga, Gustavo Pablo Romanelli, Sergio Antonio Fernandes

PII: S0968-0896(16)30783-0
DOI: http://dx.doi.org/10.1016/j.bmc.2016.12.023
Reference: BMC 13450

To appear in: Bioorganic & Medicinal Chemistry

Received Date: 21 September 2016
Revised Date: 12 December 2016
Accepted Date: 19 December 2016

Please cite this article as: Aparecida Liberto, N., Baptista Simões, J., de Paiva Silva, S., Jovelina da Silva, C., Valentina Modolo, L., de Fátima, A., Maria Silva, L., Derita, M., Zacchino, S., Miguel Portilla Zuñiga, O., Pablo Romanelli, G., Antonio Fernandes, S., Quinolines: Microwave-assisted Synthesis and their Antifungal, Anticancer and Radical Scavenger Properties, Bioorganic & Medicinal Chemistry (2016), doi: http://dx.doi.org/10.1016/j.bmc.2016.12.023

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Quinolines: Microwave-assisted Synthesis and their Antifungal, Anticancer and Radical Scavenger Properties

Natália Aparecida Liberto, Juliana Baptista Simões, Sarah de Paiva Silva, Cristiane Jovelina da Silva, Luzia Valentina Modolo, Ângelo de Fátima, Luciana Maria Silva, Marcos Derita, Súsana Zacchino, Omar Miguel Portilla Zuñiga, Gustavo Pablo Romanelli and Sergio Antonio Fernandes

Grupo de Química Supramolecular e Biomimética (GQSB), Departamento de Química, Universidade Federal de Viçosa, Viçosa, MG, 36570-900, Brazil.
Grupo de Estudos em Bioquímica de Plantas (GEBioPlan), Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil.
Grupo de Estudos em Química Orgânica e Biológica (GEQOB), Departamento de Química, ICEx, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil.
Laboratório de Biologia Celular, Fundação Ezequiel Dias, Belo Horizonte, MG, Brazil.
Centro de Investigación y Desarrollo en Ciencias Aplicadas ‘Dr. Jorge J. Ronco’ (CINDECA-CONICET-CCT-La Plata), Universidad Nacional de La Plata, Calle 47 No. 257, B1900AJK La Plata.

Corresponding authors: santonio@ufv.br or sefernandes@gmail.com

Graphical Abstract

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Quinoline
Antifungal
Anticancer
Antioxidant
Calix[n]arenes

ABSTRACT

An efficient method for the synthesis of quinolines using microwave irradiation was developed providing 28 quinolines with good yields. The reaction procedures are environmentally friendly, convenient, mild and of easy work-up. Quinolines were evaluated for their antifungal, anticancer and antioxidant properties and exhibited high activities in all tests performed.

1. Introduction

The quinolic core is repeatedly found in various natural and synthetic products, including several important clinically used drugs and pharmaceutical candidates. Some 2,4-disubstituted
quinones have been reported as anti-tuberculosis agents and also effective for the treatment of leishmaniasis, a protozoan disease widespread in tropical areas of South America. Functionalized aminoquinolines and 2,3/benzo- and 2,6/di/benzo-heteroarylquinolines have been reported as potential antifungal agents. Synthesized amidino and ureido quinoline derivatives substituted with 2-N-methylamido-pyridin-4-yl-oxy group effectively inhibited the proliferation of renal carcinoma, ovarian and lung cancer cells with potency higher than that of sorafenib, a reference drug. Diarylureas containing a 4-aryl-8-amino(acetamido)quinoline moiety are notable for their activities against melanoma cells as they exhibited concentration necessary to inhibit cell proliferation by 50% (IC50) in the order of nanomolar. Some quinoline hybrids with chalcogenide, ferrocene or coumarins were shown to be good antioxidants.

Due to the broad range of applications in medicinal, industrial and bioorganic processes and in the synthetic organic chemistry area, there has been an increasing interest in developing efficient methods for the synthesis of quinolines. A variety of synthetic methods have been developed for obtaining 2,4-disubstituted quinolines, in which the three-component Povarov reaction has been proved to be the most empowering and versatile approach. Indeed, the three-component Povarov reaction is as convenient as the classical synthesis for this purpose in terms of efficacy, speed and atom economy. Then, the Povarov reaction, based on an inverse electron-demand aza-Diels–Alder reaction between an aniline, aldehyde and an electron-rich alkene to afford a tetrahydroquinoline, quinoline or julolidine, has long attracted the interest of synthetic chemists. This three-component reaction is reported to be catalyzed by BF3/Et2O, lanthanide(III) triflates, molecular iodine, SnCl2, TMSCl, TEMPO salt (T*BF3), fluorinated alcohols, AG®50W-X2 resin, cellulose sulphonic acid and triphenylmethyl cation. Our group and others have used calix[n]arenes as organocatalysts in multicomponent reactions (MRCs), including a Povarov reaction designed for the synthesis of julolidines. Recently, we have reported the direct synthesis of 2,4-disubstituted quinolines using the Povarov reaction among anilines, benzaldehyde and styrene followed by in situ oxidation assisted by the catalyst p-sulfonic acid calix[4]arene (CX4SO3H). Despite the good yields obtained from a single step reaction (38–71%), the formation of 2,4-disubstituted quinolines occurred after long periods of reaction incubation (12 h) in the presence of acetonitrile as the solvent.

Microwave-assisted organic synthesis has become an interesting tool in organic synthesis for obtaining desired products from environmentally-friendly reactions based on the use of catalysts and free of solvents. Atom-economical processes have collectively contributed to promote the school of modern synthesis. To the best of our knowledge, the use of microwave irradiation to promote the Povarov reaction is still underexplored. Additionally, the design of an efficient and green approach based on the application of a reusable catalyst for the synthesis of 2,4-disubstituted quinolines is of great interest.

In this work, we investigated a simple and efficient protocol for the microwave-assisted synthesis of a series of 2,4-disubstituted quinolines under solvent-free conditions, with potential to inhibit the growth of fungal of clinical interest and proliferation of cancer cells and to scavenge free radicals.

2. Results and Discussion

Synthesis of quinolines

A series of solvents as well as solvent-free conditions (Table 1) were tested to determine the best condition for obtaining quinolones in good yields. For this purpose, a model reaction constituted of 4-bromoaniline (1a), benzaldehyde (2a) and styrene (3) was chosen and carried out in the presence of p-sulfonic acid calix[4]arene (CX4SO3H) as catalyst (1 mol%) under microwave irradiation. The use of ethanol or water as protic solvents yielded the quinoline Q1 in only 8% and 5%, respectively, in which the imine II was isolated as the major product (up to 27% yield) and the amine A1 in up to 15% yield, respectively (Table 1, entries 1 and 2).

Table 1 Optimization of the reaction conditions

| Entry | Solvent | CX4SO3-H (mol%) | Q1 yield (%) | II yield (%) | A1 yield (%) |
|-------|---------|-----------------|-------------|-------------|-------------|
| 1     | ethanol | 1.0             | 8           | 20          | 15          |
| 2     | water   | 1.0             | 5           | 27          | 11          |
| 3     | acetonitrile | 1.0      | 15          | 13          | 12          |
| 4     | DCM     | 1.0             | 15          | 47          | -           |
| 5     | none    | 1.0             | 64          | 20          | -           |
| 6     | none    | 0.5             | 43          | 15          | -           |
| 7     | none    | 2.0             | 65          | 16          | -           |
| 8     | none    | 5.0             | 58          | 17          | -           |
| 9     | none    | 0.0             | 0           | 30          | 20          |

*Reagents and conditions: 4-bromoaniline 1a (1.0 mmol), benzaldehyde 2a (1.2 mmol) and styrene 3 (1.5 mmol) for 20 min under power of 50 W in a sealed tube. **Under reflux. %Isolated yield. DCM, dichloromethane.

The Q1 was also obtained in low yield when aprotic solvents, such as acetonitrile or dichloromethane were used, conditions that yielded II in almost 50% and A1 in 12% in reactions performed with the former solvent (Table 1, entries 3 and 4). Under solvent-free conditions, the quinoline Q1 was isolated in 64% yield along with imine II in 20% yield. Once determined that a solvent-free reaction was the best condition to obtain Q1, we further investigated the minimum amount of catalyst (CX4SO3H) required to achieve the maximum reaction yield. The Q1 yield decreased to 43% when the amount of CX4SO3H was diminished from 1.0 mol% to 0.5 mol% (Table 1, entry 6). No further yield increment was achieved when the concentration of CX4SO3H was increased from 1 to 2 mol% (Table 1, entry 7). The use of CX4SO3H at 5 mol% provided Q1 at 58% (Table 1, entry 8). No detectable amount of Q1 was observed in reaction devoid of catalyst, a condition that yielded the imine II at 30% (Table 1, entry 9). Overall, the use of solvent-free conditions and 1 mol% CX4SO3H as catalyst provided the highest Q1 yield (64%) from Povarov reaction in which 20% of the substrate aniline was converted to some byproducts (Table 1, entry 5).

By using the optimal reaction conditions, it was found that the incubation of reactions at 150, 200 or 250 °C for 5 or 10 min yielded undetectable amounts of Q1 (Table 2, entries 1-6). Similar results was achieved when reaction were incubated at 150 °C for 15 min (Table 2, entry 7). The Q1 yield of up to 35% was verified from incubation of reactions at 200 to 250 °C.
for 15 min (Table 2, entries 8 and 9). Longer periods of reaction incubation (20 to 25 min) at the maximum temperature of 200 °C provided the best yields for Q1 (Table 2, entries 11 and 14).

Notably, the highest yield was obtained for the model reaction carried out in an open vessel in the presence of 1 mol% CX4SO$_3$H, under acetonitrile for 12 h and oil bath (80 °C) (Table 2, entry 16).$^{13a}$

Once determined the best mild conditions to synthesize quinolines from the Povarov reaction, we next examined the scope of this reaction by varying the aldehydes employed as shown in Fig. 1. The classical thermal method using acetonitrile under 12 h reflux$^{13a}$ was adopted for obtaining Q1-Q28 for comparison purpose (Fig. 1).

Table 2 Effect of temperature and incubation time on the production of quinolines$^a$

| Entry | Temperature (°C) | Time (min) | Yield Q1 (%)$^b$ | Yield Q1 (%)$^c$ |
|-------|------------------|------------|------------------|------------------|
| 1     | 150              | 5          | -                | 20               |
| 2     | 200              | 5          | -                | 24               |
| 3     | 250              | 5          | -                | 29               |
| 4     | 150              | 10         | -                | 33               |
| 5     | 200              | 10         | -                | 45               |
| 6     | 250              | 10         | -                | 44               |
| 7     | 150              | 15         | -                | 36               |
| 8     | 200              | 15         | 30               | 30               |
| 9     | 250              | 15         | 35               | 20               |
| 10    | 150              | 20         | 24               | 47               |
| 11    | 200              | 20         | 64               | 20               |
| 12    | 250              | 20         | 36               | 34               |
| 13    | 150              | 25         | 26               | 45               |
| 14    | 200              | 25         | 65               | 16               |
| 15    | 250              | 25         | 42               | 26               |
| 16$^a$| 80               | 720        | 65               | 13               |

$^a$Reagents and conditions: 4-bromoaniline/benzaldehyde/styrene (molar ratio of 1:0.1:2:1.5) in sealed tube$^{13a}$; Isolated yield. *Conventional heating (80 °C) in the presence of acetonitrile.

With respect to the reactions using 4-bromoaniline, similar yields were observed for obtaining Q1-Q13 using either the conventional heating or microwave approaches regardless of the aldehyde employed. However, the use of aromatic aldehydes bearing –OH or –NO$_2$ provided the corresponding quinoline in poor yields (< 20%). Additionally, cyclohexane-carboxaldehyde furnished the corresponding quinoline (Q7) in yields equal to or lower than 35% (Fig. 1).

The use of microwave irradiation and different aniline substrates led to the formation of quinolines in better yields as attested by the results obtained for the synthesis of Q14-Q28 (Fig. 1). Indeed, the microwave irradiation was determined to be in the range from 11% to 19% more efficient than conventional heating for obtaining the quinolines Q16, Q18, Q21 and Q25 while the yield of Q26 using the former approach was twice as high as that of the latter ones (Fig. 1). Reactions under microwave irradiation furnished the desired quinolines more rapidly when compared with those under thermal heating and solvent-free conditions.

![Scheme 1](image)

Scheme 1 - Mechanistic proposal for the synthesis of quinolines from Povarov reaction.

The ion exchange capacity of the catalyst was determined by acid-base titration$^{29}$ using 5 mM NaOH (aq). A total of 8.6
mmol of H⁺ g⁻¹ were necessary to completely titrate CX4SO₂H. This result is consistent as the catalyst bears eight strong acid sites (four –SO₂H and four –OH groups).

The potential of CX4SO₂H to be reused in the reaction to obtain quinolines was also investigated. The CX4SO₂H could be successfully used up to five successive reactions without significant loss of catalytic power (Fig. 2). The catalyst was easily recovered from the reaction by liquid-liquid extraction with water and dichloromethane followed by evaporation of aqueous phase under reduced pressure. As a result, the reaction model constituted of 4-bromoaniline (Ia), benzaldehyde (2) and styrene (3) could still afford quinoline Q1 in good yield even after five cycles (Fig. 2).

Antifungal activities

Because of the need to develop new structures with antifungal activity and considering that other quinoline-bearing structures have shown promising antifungal activities, the 2,4-disubstituted quinolines synthesized were tested against some fungi of clinical interest such as Candida albicans and Cryptococcus neoformans. The C. albicans is the etiological agent of many opportunistic infections in immunocompromised hosts while C. neoformans causes cryptococcal meningitis, one of the most important HIV-related fatal opportunistic mycosis that has killed to date over 650,000 immunocompromised patients worldwide. Although the incidence of HIV tends to decrease in countries with highly active anti-retroviral therapy, fungal infections is influenced by several factors in which the design of compounds with anticytotoxic activity is very desirable.

To better explore the results of MIC values for inhibition of fungal growth, the quinolines synthesized were divided into three categories: i) group I, comprised of non-substituted or 2-position-substituted benzene ring quinolines; ii) represented by Q7 that bears a 2-cyclohexyl ring and iii) represented by Q13 that bears a 2-furyl substituent at 2-position of the quinoline ring (Table 3). Group I was further subdivided in I.1, in which it was grouped quinolines (Q1-Q6 and Q8-Q12) that possess a –Br substituent at 6-position (R₃) of quinoline skeleton and a variety of substitution at 2-position (R₁, R₂ and/or R₄) of the benzene ring. The subgroup I.2, on the other hand, includes compounds (Q14-Q28) that present a phenyl group at 2-position of the quinoline ring with a variety of substituents at positions 5 (R₅), 6 (R₆) and 7 (R₇) of this same core.

The MIC₀ and MIC₅₀ values shown in Fig. 3 were determined from experiments of fungal growth inhibition in the presence of quinolines or amphotericin B (Amph B; reference drug) in the range from 3.9 to 250 µg mL⁻¹ (Table S1; Supplementary material). The MIC₀ and MIC₅₀ values correspond to the minimum concentration of a compound-test necessary to inhibit the fungus growth by 80% and 50%, respectively. Such values are well accepted in the literature as representative of the in vitro activity of target compounds.

Quinolines Q1-Q3, Q6-Q8, Q13-Q28 inhibited C. albicans or/and C. neoformans growth at different extents (Table 3). The C. neoformans, however, was shown to be more sensitive to the quinolines tested (MIC₅₀ values lower than 250 µg mL⁻¹) than did C. albicans.

### Table 3 Effect of quinolines on the growth of Candida albicans ATCC 10231 and Cryptococcus neoformans ATCC 32264

| Compounds | Category | R₁ | R₂ | R₃ | R₄ | R₅ | R₆ | R₇ | C. albicans | C. neoformans |
|-----------|----------|----|----|----|----|----|----|----|-------------|--------------|
| Q1        | I        | H  | H  | H  | H  | H  | H  | H  | 250.0       | 125.0        |
| Q2        | I        | H  | H  | H  | Cl | H  | H  | H  | 250.0       | 125.0        |
| Q3        | I        | H  | H  | H  | F  | H  | H  | H  | 250.0       | 125.0        |
| Q4        | I        | H  | H  | H  | OMe| H  | H  | H  | 250.0       | 125.0        |
| Q5        | I        | H  | H  | H  | Me | H  | H  | H  | 250.0       | 125.0        |
| Q6        | I.1      | H  | H  | H  | CH₃| H  | H  | H  | 125.0       | 62.5         |
| Q7        | I.1      | H  | H  | H  | CH₃| OCH₃| H  | H  | 250.0       | 125.0        |
| Q8        | I.1      | H  | H  | H  | OCH₃| H  | H  | H  | 250.0       | 125.0        |
| Q9        | I.1      | H  | H  | H  | H  | OH | H  | H  | 250.0       | 125.0        |
| Q10       | I.1      | H  | H  | H  | H  | NO₂| H  | H  | 250.0       | 125.0        |
| Q11       | I.1      | H  | H  | H  | H  | H  | NO₂| H  | 250.0       | 125.0        |
| Q12       | I.1      | H  | H  | H  | H  | CN | H  | H  | 250.0       | 125.0        |
| Q13       | I.1      | H  | H  | H  | H  | CN | H  | H  | 250.0       | 125.0        |
| Q14       | I.2      | H  | H  | OH | H  | H  | H  | H  | 250.0       | 15.6         |
| Q15       | I.2      | H  | OH | H  | H  | H  | H  | H  | 250.0       | 125.0        |
| Q16       | I.2      | H  | F  | H  | H  | H  | H  | H  | 250.0       | 125.0        |
| Q17       | I.2      | H  | NO₂| H  | H  | H  | H  | H  | 250.0       | 125.0        |
| Q18       | I.2      | H  | CH₃| H  | H  | H  | H  | H  | 125.0       | 31.2         |
| Q19       | I.2      | H  | Cl | H  | H  | H  | H  | H  | 250.0       | 15.6         |
| Q20       | I.2      | H  | SCH₃| H  | H  | H  | H  | H  | 250.0       | 15.6         |
| Q21       | I.2      | H  | CN | H  | H  | H  | H  | H  | 250.0       | 125.0        |
| Q22       | I.2      | H  | CO₂H| H  | H  | H  | H  | H  | 250.0       | 125.0        |
| Q23       | I.2      | H  | CH₂CH₂| H  | H  | H  | H  | H  | 15.6       | 3.9          |
| Q24       | I.2      | H  | H  | NO₂| H  | H  | H  | H  | 250.0       | 125.0        |
| Q25       | I.2      | H  | Cl | H  | H  | H  | H  | H  | 250.0       | 125.0        |
| Q26       | I.2      | H  | OCH₃| OCH₃| H  | H  | H  | H  | 125.0       | 15.6         |
| Q27       | I.2      | H  | OCH₃| OCH₃| H  | H  | H  | H  | 125.0       | 31.2         |
| Q28       | I.2      | H  | OCH₃| H  | H  | H  | H  | H  | 250.0       | 31.2         |
position of quinolic ring boosts the antifungal activity of quinolines.

When considering all the other quinolines synthesized, almost all compounds belonging to the subgroup I.1 were determined to be poor antifungal against C. albicans. On the other hand, some of them exhibited relatively low MIC values activity against C. neoformans. Indeed, the most active compounds (MIC values lower than 16 µg mL⁻¹) possess –F (Q3) or –CH₃ (Q5) as R₃ group (Table 3). Compound Q6, which presents a –p-OCH₃ as R₂ group and two other –OCH₃ groups as R₄ and R₅, exhibited moderate activity (MIC₅₀ lower than 65.0 µg mL⁻¹). Notably, the presence of –Cl, –OCH₃, –NO₂, and –CN as R₂ group and –NO₂ as R₅ group negatively affected the activity of quinolines as attested by the results of MIC values obtained for Q2, Q4, Q10, Q11 and Q12 (Table 3). On the other hand, compounds belonging to subgroup I.2 that bear −NO₂ or −CF₃ groups, either at 6- (R₂; Q17) or 7-position (R₃; Q24) presented similar effects on C. neoformans (Table 3). Quinolines presenting –Cl, –SCH₃, –C(CH₃)₃, –OCH₃O or –OCH₃ at 6-position (Q19, Q20, Q23, Q26, Q27 and Q28) were very active against C. neoformans. Compound Q26 was also identified as the only effective against C. albicans (Table 3). The presence of −OH at 7-position (Q14) enhances 8-fold the antifungal activity against C. neoformans when compared with a non-substituted quinoline at similar position (Q15).

Overall, C. neoformans was more susceptible than C. albicans to 2,4-disubstituted quinolines and their derivatives. The Q13 was the most active among the 2-substituted compounds (bearing 2-phenyl, 2-cyclohexyl or 2-furan) containing −Br at 6-position. Then, this opens an opportunity for the synthesis of a new series of potential antifungal quinolines bearing a furan ring. Within 6-brominated compounds (I.1 category), the most active were those solely substituted at the 2-phenyl group (Q3 with R₅ = F and Q5 with R₅ = CH₃). Likewise, the 3′,4′,5′-OCH₃ derivative (Q6) was more active than the corresponding monosubstituted one (Q4).

**Antiproliferative assays**

Cell proliferation was determined using the MTT assay with absorbance measurements at 540 nm. All synthesized quinolines (Q1-Q28) were evaluated in vitro against the following cancer cell lines: NCI-H226 (lung), TOV-21G (ovary) and Hep-2c (Hela contaminant). Compounds were also tested against a non-cancerous human cell line (CCD 19-Lu; lung fibroblast) to determine the selective index (SI). Data (Fig. 4) were submitted to Analysis of variance (ANOVA) one factor post-hoc analysis using the Least Significant Difference (LSD) method, which showed statistically difference only between the control and concentrations of 1,000 and 1,500 µg mL⁻¹.

The concentrations of quinolines that caused cell growth inhibition by 50% (IC₅₀ values) and the selectivity indexes are summarized in Table 4. The selectivity index compares the cytotoxicity of a compound against tumor cells and non-tumor cells. The calculation provides the ratio of IC₅₀ values obtained for the non-tumor cell line to the IC₅₀ value obtained for the tumor cell line. Regarding the analysis of selectivity ratios, there is no consensus in the literature on the threshold values for tumor cell lines. However, it was found that values greater than 5 are good indicators of selectivity.

The IC₅₀ values indicated that Tov21G cells were more resistant and that NCI-H226 cells were more susceptible to all compounds tested (Fig. 5). According to the TP53 database of the International Agency of cancer (http://p53.iarc.fr/CellLines.aspx), the NCI-H226 cell line is TP53 mutated while the TOV21G is wild-type. This mutation could explain the differences of cell survival observed for the tumor lines studied when incubated with quinolines, as TP53 gene encodes for a tumour suppressor protein that is responsible for regulating the expression of target genes related to DNA repair.

**Fig. 2** Comparative antifungal activities of Q1, Q7 and Q13 against C. albicans ATCC 10231 (a) and C. neoformans ATCC 32264 (b).

**Fig. 4** Tumor and non-tumor cell survival after treatment with quinolines. The y = 0 indicates the 50% survival limit, in which compounds furnishing values higher than zero show cytostatic effect while those providing values lower than zero are considered cytotoxic. Differences between groups were analyzed using a non-parametric test (Kruskal Wallis; P < 0.05).
Table 4 Concentration of quinolines required to inhibit the proliferation of tumor cells by 50% (IC<sub>50</sub>) and respective selective index (SI)

| Quinolines | IC<sub>50</sub> (µM) | SI (µM) |
|------------|----------------------|---------|
|            | TOV-21G | CCD19-Lu | NCI-H226 | Hep-2C | TOV-21G | NCI-H226 | Hep-2C |
| Q1         | 530.7   | 608.7    | 45.4     | 128.1  | 1       | 15       | 5       |
| Q2         | ND      | 528.1    | 1.58     | 269.0  | ND      | ND       | ND      |
| Q3         | 570.4   | ND       | ND       | 5.0    | ND      | ND       | ND      |
| Q5         | ND      | ND       | ND       | 175.0  | ND      | ND       | ND      |
| Q6         | 492.6   | 104.5    | 61       | ND     | 0       | 1        | ND      |
| Q7         | ND      | 489.5    | 91       | ND     | ND      | 5        | ND      |
| Q8         | ND      | 104.2    | 8        | 104.8  | ND      | 13       | 0       |
| Q9         | ND      | ND       | 8.5      | ND     | ND      | ND       | ND      |
| Q10        | ND      | ND       | ND       | 98.8   | ND      | ND       | ND      |
| Q13        | 876.8   | 631.1    | 30       | 101.8  | 0       | 21       | 6       |
| Q14        | ND      | ND       | ND       | ND     | ND      | ND       | ND      |
| Q24        | ND      | ND       | 558.9    | ND     | ND      | ND       | ND      |
| Q27        | ND      | 384.1    | 40       | 951.1  | ND      | 8        | 0       |
| Q28        | 110.5   | 924.9    | 57       | 495.5  | 8       | 16       | 1       |

Nd: not determined. The IC<sub>50</sub> value was calculated using the non-linear regression obtained by OriginPro 7.0.

An examination of the association between survival and IC<sub>50</sub> values highlighted the compounds that had the most promising antitumour activities (Fig. 6). The normal cell line CCD19-Lu proved to be the most susceptible to cytotoxicity with quinolines Q7, Q8, Q27 and Q28, while all were cytotoxic for NCI-H226 cells (susceptible), and none were cytotoxic for TOV21G (resistant). The NCI-H226 line was selectively susceptible for the majority of quinolines and presented the best selective indices. The NCI-H226 line is an epithelial lung cancer derived from a metastatic site. It is one of the more aggressive cancer types with a high incidence and prevalence. These compounds proved to be good sources of antitumoural molecules for lung cancer. The TOV21G cells (resistant) were selectively susceptible only to the Q1, Q3 and Q28 quinolines. The Tov21G cell line is from an epithelial ovary cancer that is classified as aggressive and very deadly among women. Thus, these compounds proved to be good agents for improving effectiveness in this cancer type. Interestingly, Q1 and Q3 were not cytotoxic against NCI-H226, suggesting that there are different biological pathways between them. The Hep2c line was susceptible to the majority of compounds as was NCI-H226, but not in a selective manner. The results for these cells were meaningless once it was shown to be a HeLa-contaminated cell line.

Fig. 6 Association between IC<sub>50</sub> values and viability of human tumor cells (Tov21G, Hep2 and NCI-H226) and non-tumor (CCD19-Lu) cells by 50% (IC<sub>50</sub>). Asterisks indicate significant difference by ANOVA post hoc LSD test (P < 0.05).

Fig. 7 Values of IC<sub>50</sub> for quinolines as a function of substituents. The asterisk shows the substituents that present lower IC<sub>50</sub> values as a function of position. The dotted lines show the 50% survival limit, in which values are under these lines are indicative of compound cytotoxicity. Compounds whose data are under the dotted line are considered the most promising antitumor agents.

The quinoline with lower IC<sub>50</sub> values was the one containing a 4-methoxyphenyl group, which indicates that methoxyl group at 4-position improved the antiproliferative activity against lung cancer. The most effective substituents on tumor cells (except for Hep2c cancer cell line) were 4-fluorophenyl, 4-nitrophenyl, 3-nitrophenyl and cyclohexane (Fig. 7). The presence of activating groups on the aromatic ring provided promising anticancer activity.

Scavenging of reactive nitrogen species (RNS)

The ability of the synthesized quinolines to scavenge reactive species with unpaired electrons on nitrogen atoms (RNS) was investigated using the 2,2-diphenyl-1-
picrylhydrazyl (DPPH) method. An initial screening was performed to select potential ROS scavengers. Compounds were tested at 160 μM against 100 μM DPPH using the plant natural product resveratrol (Resv) as a positive control. Under our experimental conditions, quinolines Q14, Q15, Q21 and Q26 were determined to be the best DPPH scavengers because they were able to scavenge 70% to 85% of the free radicals. These results are comparable to those obtained for Resv, a known free radical scavenger (Fig. 8). Quinolines Q1, Q2, Q5, Q7, Q13, Q17, Q18 and Q28 were also found to be promising DPPH-capturing compounds; they scavenged free radicals by 45% to 55% (Fig. 8). The other quinolines, except for Q11 that was almost inactive, sequestered DPPH by approximately 35-40% (Fig. 8). Overall, no significant changes were observed in the DPPH scavenging ability for compounds with structural alterations in both the quinoline core and the two phenyl rings. However, some structure-activity relationships can be addressed. For instance, the presence of a hydroxy group at C6 (compound Q15) or C7 (compound Q14) on the quinoline moiety was determined to be essential for scavenging of DPPH. In contrast, a hydroxyl group on the phenyl ring bound to the C2 of the quinoline core is not mandatory for ROS scavenging, as Q1 (which is devoid of the corresponding –OH) is as effective as compounds Q8 and Q9. It is noteworthy that the presence of an m-NO₂ group on the phenyl ring bound to the C2 of the quinoline core compromises the ability of quinoline Q11 to scavenge DPP radicals. Contrary to Q11, Q10, which bears a p-NO₂ group instead, is able to scavenge DPPH radicals by approximately 40% (Fig. 8).

The concentration of the compound tested necessary to scavenge a free radical by 50% was determined varying the concentration of quinolines (SC50; Table 5). Compounds Q21 and Q26 were the most efficient DPPH scavengers, as they exhibited SC50 values lower than 20 μM. The Q13 was also able to scavenge 50% of the DPPH present in the reaction medium when applied at 82.7 μM (Table 5). The order of increasing potency for the other quinolines is Q17 > Q7 > Q28 > Q2 > Q5 > Q18 > Q6.

Table 5 Concentrations of promising quinolines that are necessary to scavenge DPPH or O₂⁻ radicals by 50% (SC50 in μM).

| Compounds | DPPH (SC50 μM) | O₂⁻ (SC50 μM) |
|-----------|---------------|---------------|
| Q1        | >250.0        | >250.0        |
| Q2        | 180.3         | >250.0        |
| Q5        | 197.1         | 104.3         |
| Q6        | 428.1         | >250.0        |
| Q7        | 118.5         | >250.0        |
| Q9        | >250.0        | 121.4         |
| Q13       | 82.7          | >250.0        |
| Q15       | >250.0        | >250.0        |
| Q17       | 104.5         | >250.0        |
| Q18       | 260.6         | >250.0        |
| Q21       | 19.5          | 220.2         |
| Q23       | >250.0        | >250.0        |
| Q26       | 18.9          | >250.0        |
| Q28       | 168.5         | >250.0        |
| Resveratrol (Resv) | 8.2          | 255.0         |

Scavenging of reactive oxygen species (ROS)

A screening was also performed with the synthesized quinolines to evaluate the ability of such compounds to capture the reactive oxygen species (ROS) superoxide anion (O₂⁻). Again, quinolines were used at 160 μM in a reaction medium in which O₂⁻ was artificially generated. Quinolines Q5, Q8, Q9, Q15, Q17, Q20, Q21, Q23, Q26 and Q27 were much more efficient in the scavenging of ROS than Resv, with a positive control (Fig. 9). The efficiency of quinolines Q3, Q4, Q7, Q10, Q12 and Q22 was comparable to that of Resv (Fig. 9). In contrast to the observations with DPPH scavenging, considerable variations in the efficiency of compounds were observed toward O₂⁻. The presence of a p-tolyl substituent at C2 boosted the activity of compound Q5 compared to the corresponding quinoline that bears a phenyl substituent at this same position (compound Q1). The positive effect observed for Q7 (Fig. 9) is likely due to the presence of benzyl hydrogens, which may favor the formation of benzyl radicals, known to be very stable. Electron-withdrawing groups (–NO₂ and –CN; Q17 and Q21, respectively) at C6 on the quinoline moiety, except for a 3-fluoromethyl group (–CF₃; Q18), increased the activity of quinolines towards O₂⁻ (Fig. 9). In contrast, the presence of electron-withdrawing groups at C7 (–CO₂H and –NO₂; Q22 and Q24, respectively) and/or C6 (6-CI and 7-CF₃; Q25) negatively affected the potential of quinolines to scavenge O₂⁻ species (Fig. 9). The presence of a hydroxyl group (–OH) at C6 (compounds Q14 and Q15) contributes to the O₂⁻ scavenging activity of quinolines. However, a m-OH group on the phenyl ring bound to the C2 of the quinoline core renders the compound twice as active as the one bearing a p-OH at the same position (Q9 and Q8, Fig. 9). The quinoline containing a thiomethyl group (–SCH₃) at C7 (Q20) was 2-fold more active than the corresponding derivative bearing an –OCH₃ group (Q28) (Fig. 9). The presence of more than one –OCH₃ group (compound Q26) or a methylenedioxy group (compound Q27), however, boosted the activity of such quinolines toward O₂⁻. Notably, quinoline Q23, which bears a tert-butyl substituent at C6, was able to scavenge 50% of the O₂⁻ artificially generated in the medium. All quinolines bearing halogen atoms (Br, F and Cl; Q1, Q16 and Q19, respectively) and unsubstituted phenolic rings demonstrated a lower potential for scavenging ROS (< 10% scavenging) (Fig. 9).
The remaining quinolines were found to be poor $O_2^-$ scavengers (Fig. 9). The most promising quinolines were used further to assess the concentration of compounds necessary to scavenge ROS by 50% (SC50). Indeed, quinolines Q5 and Q9 were twice as potent as Resv towards $O_2^-$ (Table 5).

Fig. 9 Effect of quinolines on reactive oxygen species. The reaction medium included artificially-generated superoxide anion ($O_2^-$) and compounds tested at 160 μM. Resveratrol (Resv) was used as a positive control. Standard deviations (SD) were lower than 17.0%. Data are from three independent experiments, each done in triplicate.

Conclusions

A new and efficient method using microwave irradiation and p-sulfonic acid calix[4]arene as catalyst was developed for the synthesis of 28 2,4-disubstituted quinolines. Some advantages of the approach described herein include the use of solvent- and metal-catalyst-free conditions, short reaction times, recycling catalyst besides the catalyst tolerance towards a wide range of functional groups. Quinolines excelled, as they have good to excellent free radical scavenging activity being notable as good antifungal and antiproliferative candidates. The 6-Br-2,4-disubstituted quinolines, in particular, are attractive for use as templates for the development of new antifungal and antitumor agents.

3. Experimental Section

3.1. General methods and materials

All starting materials were obtained from commercially available sources with high-grade purity and were used without further purification. The p-sulfonic acid calix[4]arene (CX4SO3H) was prepared according to known procedures.35 Reactions did not require anhydrous conditions. The melting points (uncorrected) of synthesized quinolines were determined using a MQAPF-301 Microquímica micromelting device. Infrared spectra were obtained on a FT-IR Varian 660 Fourier Transform Infrared spectrometer. The $^1$H and $^{13}$C-NMR spectra were obtained on a Varian Mercury spectrometer at 300 MHz for $^1$H and 75 MHz for $^{13}$C, in CDCl$_3$. Coupling constants (J) are reported in Hertz (Hz). High resolution mass spectra (HRMS) were obtained on a Shimadzu LC-IT-TOF Prominence system.

3.2. General procedure for the synthesis of 2,4-disubstituted quinolines

Microwave (MW) approach: A mixture of an aniline (1 mmol), aldehyde (1.2 mmol), styrene (1.5 mmol) and p-sulfonic acid calix[4]arene (1 mol%) was stirred at 200 °C for 20 min.

Conventional heating (CH): A mixture of an aniline (1 mmol), aldehyde (1.2 mmol), styrene (1.5 mmol), p-sulfonic acid calix[4]arene and acetonitrile (1 mol%) was stirred at 80 °C for 12 h.

Treatments (MW and CH): The reaction was quenched by addition of water (10 mL) and the product was extracted with dichloromethane (3 x 10 mL). The solvent was removed under reduced pressure in a rotary evaporator. The obtained solid was obtained was purified by silica gel column chromatography (hexane/dichloromethane/acetone 8:2:8:0.1) to afford the quinolines Q1-Q28 in high purity (Fig. 1). All quinolines were characterized by $^1$H and $^{13}$C NMR, IR, HRMS (ESI) and melting points.

3.3. Antifungal Activity

Microorganisms and media

For the evaluation of antifungal activity, standardized strains of C. albicans ATCC 10231 and C. neoformans ATCC 32264 from the American Type Culture Collection (ATCC, Rockville, MD, USA) were used. Strains were grown on Sabouraud-chloramphenicol agar slants for 48 h at 30 °C and then maintained on slopes of Sabouraud-dextrose agar (SDA, Oxoid). Strains were sub-cultured every 15 days to prevent pleomorphic transformations. Inocula were obtained according to reported procedures39 and adjusted to 1-5 x 10$^8$ colony forming units (CFU)/mL.

3.4. Fungal growth inhibition

Yeast broth microdilutions (technique M27-A3 of CLSI) were performed in 96-well microtiter plates. For the assay, compound-test wells (CTWs) were prepared with stock solutions of each compound in DMSO (concentration ≤ 1%) and diluted with RPMI-1640 to final concentrations in the range from 3.9 to 250.0 μg mL$^{-1}$. An inoculum suspension (100 μL) was added to each well (final volume in the well = 200 μL). A growth control well (GCW) (containing medium, inoculum and the same amount of DMSO used in a CTW) and a sterility control well (SCW) (sample, medium and sterile water) were included for each fungus tested. Microtiter trays were incubated in a moist, dark chamber at 30 °C for 48 h for both yeasts. Microtiter plates were read in a VERSA Max microplate reader (Molecular Devices, Sunnyvale, CA, USA). Amphotericin B was used as a positive control. Tests were performed in triplicate. Reduction of growth for each compound concentration was calculated as it follows: % inhibition = 100 - (OD 405 CTW – OD 405 SCW)/(OD 405 GCW – OD 405 SCW). The means ± standard deviations (SD) were used for plotting dose-response curves representing % inhibition versus concentration of each compound using SigmaPlot 11.0 software.

MIC$_{50}$ and MIC$_{90}$ determinations

Two endpoints were defined from the experiments described above. Minimum Inhibitory Concentrations 80 (MIC$_{80}$) and 50 (MIC$_{50}$) were defined as the minimum concentration that inhibits 80% or 50% of the fungal growth, respectively.
3.5. Antiproliferative assay

Human tumor cell lines CCD-19Lu (ATCC® CCL-210™), WI-26 VA4 (ATCC® CCL-95™), HEp-2C (ATCC® CCL-23™) and NCI-H226 (ATCC® CL-5826™) were cultured in MEN-NEA, EEMEM and RPMI media, respectively (Sigma-Aldrich Co. LLC.) supplemented with 10% foetal bovine serum and 1% L-glutamine in a 37 °C humid atmosphere enriched with 5% CO2. Cells were culture on 96 well plates (1 x 10^5 cells/well) in the presence or absence of synthesized quinolines at concentration in the range from 1.0 to 1,000 μg mL^-1. After 24 h, the samples were sensitized in a MTT assay, and their absorbances (at 540 nm) measured 3 h later using a Molecular Devices Spectramax M5E. Values, corresponding the cell viability, were used to calculate the ICso values for statistical analysis purposes.

3.6. Scavenging of reactive nitrogen species

The ability of quinolines Q1-Q28 to scavenge a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, a reactive nitrogen species (RNS), was determined according to Guelin^39, with modifications. The screening of potential candidates was performed by incubating each compound-test (200 μg mL^-1) in an ethanolic medium containing 100 μM DPPH. The systems were maintained under stirring and absence of light for 30 min and the absorbance was measured at 517 nm. Those compounds with potential scavenging activities were then tested in the range from 0 to 160 μg mL^-1 to determine the concentration necessary to scavenge DPPH radicals by 50% (ICso). The results presented are from three independent experiments, each performed in triplicate.

3.7. Scavenging of reactive oxygen species

The capacity of quinolines to scavenge superoxide anions (O2^-) was evaluated in 50 mM phosphate buffer (pH 7.8) containing 13 mM L-methionine, 75 μM nitroblue tetrazolium, 100 μM EDTA, 2 μM riboflavin and compound-test at 0-200 μg mL^-1. Reaction mixtures were incubated for 10 min at 25 °C in the presence of fluorescent light to induce O2^- formation. Controls consisted of reaction mixtures maintained at 25 °C for 10 min in the absence of light. The percentage of O2^- scavenged by each compound-test was determined at 575 nm. The results presented are from three independent experiments, each performed in triplicate.

Acknowledgements

Authors are thankful for the financial support provided by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). AdF, LVM and SAF are supported by Research Fellowships from CNPq and MD is a member of the CONICET Researcher Career. SZ acknowledges ANPCyT PICT 2014 1170.

This work was supported by the National Program for Academic Cooperation (PROCAD) of CAPES/Brazil.

References and notes

[1] (a) R. C. Bernotas, R. R. Singhaus, D. H. Kaufman, J. M. Trivins, J. W. Ulrich, R. Unwalla, E. Quinet, M. Evans, P. Nambi, A. Olland, B. Kauppi, A. Wilhelmsen, A. Goos-Nilsson, J. Wrobel, 4-((3-Aryloxyaryl)quinoline sulfones are potent liver X receptor agonists, Bioorg. Med. Chem. Lett. 20 (2010) 209-212; (b) S. Vandezekerchev, M. D’Hooghe, Quinoline-based antimalarial hybrid compounds, Bioorg. Med. Chem. 23 (2015) 5098-5119; (c) R. A. Jones, S. S. Panda, C. D. Hall, Quinone conjugates and quinine analogues as potential antimalarial agents, Eur. J. Med. Chem. 97 (2015) 335-355; (d) P.-Y. Chung, Z.-X. Bian, H.-Y. Pan, D. Chan, A. S.-C. Chan, C.-H. Chui, J.-C. Oo, Tang, K.-H. Lam, Recent advances in research of natural and synthetic bioactive quinolones, Future Med. Chem. 7 (2015) 947-967; (e) O. AlFaiz, S. Kumar, M. R. Haider, M. R. Ali, R. Kumar, M. Jaggi, S. Bawa, A review on anticaner potential of bioactive heterocycle quinoline, Eur. J. Med. Chem. 97 (2015) 871-910; (f) J. B. Bharate, R. A. Vishvakarma, S. B. Bharate, Metal-free domino one-pot protocols for quinoline synthesis, RSC Adv. 5 (2015) 42020-42053.

[2] (a) S. R. Patel, R. Gangwal, A. T. Sangamwar, R. Jain, Synthesis, biological evaluation and 3D QSAR study of 2,4-disubstituted quinolines as anti-tuberuculos agents, Eur. J. Med. Chem. 93 (2015) 511-522; (b) S. R. Patel, R. Gangwal, A. T. Sangamwar, R. Jain, Synthesis, biological evaluation and 3D QSAR study of hydrazide, semicarbazide and thiosemicarbazide derivatives of 4-(adamantan-1-yl)quinoline as anti-tuberculosis agents, Eur. J. Med. Chem. 85 (2014) 255-267.

[3] (a) S. Rossiter, J. M. Pérus, P. J. Whitfield, K. Jones, Synthesis and antimalaric properties of aryquinolines with activity against drug-resistant nematodes, Bioorg. Med. Chem. Lett. 15 (2005) 4806-4808; (b) A. Fournet, B. Vagneur, P. Richomme, J. Bruneton, Aeryl-2 et akyl-2 quinolines nouvelles isolées d’une Rutaceae bolivienne: Galipea longiflora Can. J. Chem. 67 (1989) 2116-2118.

[4] R. Devakaram, D. S. Black, N. Kumar, An efficient synthesis of novel 2,4-disubstituted tetrahydroquinolines and quinolines, Tetrahedron Lett. 53 (2012) 2269-2272.

[5] (a) S. Vanderkercchev, S. V. Herreweghe, J. Willems, B. Danneels, T. Desmet, C. Kock, P. J. Smith, K. Chibale, M. D’hooghe, Synthesis of functionalized 3-, 5-, 6- and 8-aminoquinolines via intermediate (3-pyrrolin-1-yl)- and (2-oxopyrrolidin-1-yl)quinolines and evaluation of their antiplasmodial and antifungal activity, Eur. J. Med. Chem. 92 (2015) 91-102; (b) S. Vanderkercchev, H. G. Tran, T. Desmet, M. D’hooghe, Corriganium to “Evaluation of 4-(aminobutylatoxy)quinolines as a novel class of antifungal agents”, Bioorg. Med. Chem. Lett. 23 (2013) 4641-4643.

[6] (a) I. Almansour, N. Arunugam, R. S. Kumar, J. C. Menéndez, H. A. Ghobbour, H. K. Fun, R. R. Kumar, Straightforward synthesis of pyrrol[3,4-f]quinolines through intramolecular Povarov reactions, Tetrahedron Lett. 49 (2015) 6900-6903.

[7] A. K. El-Damasy, S. H. Neo, N-C. Cho, S. B. Kang, A. N. Pae, K-S. Kim, G. Keum, Design, synthesis, in-vitro antiproliferative activity and kinase profile of new picolimamide based 2-amido and ureido quinoline derivatives, Eur. J. Med. Chem. 101 (2015) 754-768.

[8] E. J. Koh, M. I. E. Gamal, C. Oh, S. H. Lee, T. Sim, G. Kim, H. S. Choi, J. H. Hong, S. Lee, K. H. Yoo, New diarylamides and diarylureas possessing 8-aminoadamantquinoline scaffold: Synthesis, antiproliferative activities against melanoma cell lines, kinase inhibition, and in silico studies, Eur. J. Med. Chem. 70 (2013) 10-21.

[9] L. Savegnago, A. I. Vieira, N. Neus, B. S. Goldani, M. R. Castro, E. J. Lenardão, D. Alves, Synthesis and antioxidant properties of novel quinoline-chalconeogen compounds, Tetrahedron Lett. 1 (2013) 40-44.

[10] G. L. Xi, Z. Q. Liu, Solvent-free Povarov reaction for synthesizing ferrocenyll quinolines: Antioxidant abilities deriving from ferrocene moity, Eur. J. Med. Chem. 86 (2014) 759-768.

[11] J. Greff, J. Joubert, S. F. Malan, S. Dyk, Antioxidant properties of 4-quinolines and structurally related flavones, Bioorg. Med. Chem. 2 (2012) 809-818.

[12] (a) M. J. Sandelier, P. de Shon, Reductive Cyclization of o-Nitrophenyl Propargyl Alcohols: Facile Synthesis of Substituted Quinolines, Org. Lett. 9 (2007) 3209-3212; (b) R. P. Korivi, C. H. J. Cheng, Nickel-Catalyzed Cyclization of 2-Iodoamines with Arylalkynes: An Efficient Route for Quinoline Derivatives, Org. Lett. 71 (2006) 7079-7082; (c) A. Arcadia, F. Marinelli, E. Rossi, Synthesis of functionalised quinolines through tandem addition/annulation reactions of
β-(2-aminophenyl)-α,β-ynones. Tetrahedron 55 (1999) 13233-13250; (d) R. Devakaram, D. S. Black, N. Kumar, An efficient synthesis of novel 2,4-disubstituted tetrahydroquinolines and quinolines, Tetrahedron Lett. 53 (2012) 2269-2272; (e) J. Tang, L. Wang, D. Mao, W. Wang, L. Zhang, S. Wu, Y. Xie, Yterbium Pentafluorobenzonate as a novel fluorous Lewis acid catalyst in the synthesis of 2,4-disubstituted quinolines, Tetrahedron, 67 (2011) 8465-8469; (f) K. Cao, F. M. Zhang, Y. Q. Tu, X. T. Zhao, C. A. Fan, Iron(III)-Catalyzed and Air-Mediated Tandem Reaction of Aldehydes, Alkynes and Amines: An Efficient Approach to Substituted Quinolines, Chem. Eur. J. 15 (2009) 6332-6334; (g) S. Rotzoll, B. Willy, J. Schönhaber, F. Rominger, T. J. J. Müller, Regiospecific Three-Component Access to Fluorescent 2,4-Disubstituted Quinolines via One-Pot Coupling-Addition-Cyclocondensation-Sulfur Exudation Sequence, Eur. J. Org. Chem. 18 (2010) 3516-3524.

[13] (a) J. B. Simões, A. de Fátima, A. A. Sabino, L. C. A. Barbosa, S. A. Fernandes, Efficient synthesis of 2,4-disubstituted quinolines: calix[4]arene-catalyzed Povarov-hydrogen-transfer reaction cascade, RSC Adv. 4 (2014) 18612-18615; (b) A. Kulkarni, B. Tórók, Microwave-assisted multimulticomponent dimono cyclization-aromatization: an efficient approach for the synthesis of substituted quinolines, Green Chem. 12 (2010) 875-878.

[14] L. S. Povarov, sf-Unsaturated ethers and their analogues in reactions of diane synthesis, Russ. Chem. Rev. 36 (1967) 656-670.

[15] R. A. Batey, P. D. Simoncic, D. Lin, R. P. Smy, A. J. Lough, A three-component coupling protocol for the synthesis of substituted hexahydropyrolo[3,2-c]quinolines, Chem. Commun., (1999) 651-652.

[16] M. Xia, Y. Lu, Molecular Iodine-Catalyzed Imino-Diels-Alder Reactions: Efficient One-Pot Synthesis of Pyranol[3,2-c]quinolines, Synlett (2005) 2357-2361.

[17] R. Suresh, S. Muthusubramanian, R. Senthilkumar, G. Manickam, SnCl2-catalyzed selective atom economic imino Diels-Alder reaction: synthesis of 2-(1H-pyrol-2,3-b-3-pyridin-3-yl)quinolines, J. Org. Chem. 77 (2012) 1468-1476.

[18] S. V. More, M. N. V. Sasty, C.-F. Yao, TMSCl-Catalyzed Aza-Diels-Alder Reaction: A Simple and Efficient Synthesis of Pyranol- and Furanoquinolines, Synlett (2006) 1399-1403.

[19] H. Richter, O. Mancheño, TEMPO Oxoammonium Salt-Mediated Dehydrogenative Povarov/Oxidation Tandem Reaction of N-Alkyl Anilines, Org. Lett. 13 (2011) 6606-6609.

[20] K. De, J. Legros, B. Crousse, S. Chandrasekaran, D. Bonnet-Delpon, Synthesis of substituted 8-aminquinolines and phenanthrolines through a Povarov approach, Org. Biomol. Chem. 9 (2011) 347-350.

[21] L. Chen, C.-J. Li, Domino reaction of anilines with 3,4-dihydro-2H-pyran catalyzed by cation-exchange resin in water: an efficient synthesis of 1,2,3,4-tetrahydroquinoline derivatives, Green Chem. 5 (2003) 627-629.

[22] A. Kumar, S. Sivaslava, G. Gupta, V. Chaturvedi, S. Sinha, R. Srivastava, Natural Product Inspired Diversity Oriented Synthetic Synthesis of Tetrahydroquinoline Scaffolds as Antitubercular Agent, ACS Comb. Sci. 13 (2011) 65-71.

[23] Y. Huang, C. Qiu, Z. Li, W. Feng, H. Gan, J. Liu, K. Guo, Tritylium Cation as Low Loading Lewis Acid Organicocatalyst in Povarov Reactions, ACS Sustainable Chem. Eng. 4 (2016) 47-52.

[24] (a) J. V. de Assis, P. A. S. Abranches, I. B. Braga, O. M. P. Zaniga, A. G. Sathiog, G. P. Romanelli, A. G. Sato, S. A. Fernandes, p-Sulfonic acid calix[4]arene-functionalized alkyl-bridged organosilica in esterification reactions, RSC Adv. 6 (2016) 24285-24289; (b) V. Palermo, A. Sathiog, N. Liberto, S. Fernandes, P. Langer, J. Jios, G. Romanelli, Calix[4]arenes: active organocatalysts for the synthesis of densely functionalized piperidines by one-pot multicomponent procedure, Tetrahedron Lett. 56 (2015) 2049-2054; (c) Y. F. Rego, C. M. da Silva, D. L. da Silva, J. G. da Silva, A. L. T. G. Ruiz, J. E. de Carvalho, S. A. Fernandes, A. de Fátima, Phthalazine-triones: Calix[4]arene-assisted synthesis using green solvents and their anticancer activities against human cancer cells, Arabian J. Chem. (2016). doi: http://dx.doi.org/10.1016/j.arabjc.2016.04.007; (d) C. R. Borel, L. C. A. Barbosa, C. R. A. Malhas, S. A. Fernandes, A facile one-pot synthesis of 2-(2-pyridyl)quinolines via Povarov reaction, Tetrahedron Lett. 56 (2015) 662-665; (e) D. L. da Silva, B. S. Terra, M. R. Lage, A. L. T. G. Ruiz, C. C. da Silva, J. E. de Carvalho, J. W. M. Carneiro, F. T. Martins, S. A. Fernandes, A. de Fátima, Xanthone derivatives: calixarenes-catalyzed syntheses, anticancer activity and QSAR studies, Org. Biomol. Chem. 13 (2015) 3280-3287; (f) R. Natalino, E. V. V. Varela, M. J. da Silva, A. L. Cardoso, S. A. Fernandes, p-Sulfonic acid calix[4]arenes: the most active and water tolerant organocatalysts for esterification reactions, Catal. Sci. Technol. 4 (2014) 1369-1375; (g) J. B. Simões, D. L. da Silva, A. de Fátima, S. A. Fernandes, Calix[4]arenes in Action: Useful Host-Guest Catalysis in Organic Chemistry,Curr. Org. Chem. 16 (2012) 949-971; (h) S. A. Fernandes, R. Natalino, M. J. da Silva, C. F. Lima, A comparative investigation of palladium acid esterification over p-sulfonic acid calix[4]arene and sulfuric acid catalyats via 1H NMR spectroscopy, Catal. Commun. 26 (2012) 127-131; (i) S. A. Fernandes, R. Natalino, P. A. R. Gazzola, M. J. da Silva, G. N. Jham, p-Sulfonic acid calix[4]arenes as homogeneous and recyclable organocatalysts for esterification reactions, Tetrahedron Lett. 53 (2012) 1630-1633; (j) D. L. da Silva, S. A. Fernandes, A. A. Sabino, A. de Fátima, p-Sulfonic acid calixarenes as efficient and reusable organocatalysts for the synthesis of 3,4-dihydropyrimidin-2(1H)-ones/thiones, Tetrahedron Lett. 52 (2011) 6328-6330.

[25] J. B. Simões, A. de Fátima, A. A. Sabino, F. J. T. de Aquino, D. L. da Silva, L. C. A. Barbosa, S. A. Fernandes, Organocatalysis in the three-component Povarov reaction and investigation by mass spectrometry, Org. Biomol. Chem. 11 (2013) 5069-5073.

[26] (a) M. K. Taur, A. Gupta, A. Sharma, A. green, catalyst-free, solvent-free, high yielding one step synthesis of functionalized benzof[4,5,6,7]-[3,4-c]-[1,2,3]tetrahydroquinoline and furano[3,2-c][4,5,6,7]-[3a,4,5,9b]tetrahydroquinoline derivatives, Green Chem. 5 (2003) 1316-1318; (b) Y. F. Rego, C. M. da Silva, D. L. da Silva, J. G. da Silva, A. L. T. G. Ruiz, J. E. de Carvalho, S. A. Fernandes, Phthalazine-triones: Calix[4]arene-assisted synthesis using green solvents and their anticancer activities against human cancer cells, Arabian J. Chem. (2016). doi: http://dx.doi.org/10.1016/j.arabjc.2016.04.007; (c) O. Reiser, A Catalytic
Multicomponent Approach for the Stereoselective Synthesis of cis-4,5-Disubstituted Pyrrolidinones and Tetrahydro-3H-pyrorol(3,2-c)quinolines, Angew. Chem. Int. Ed. 51 (2012) 4722-4725; (g) C-H Chen, G. S. Yello, P-T. Lin, C-M. Sun, Base-Catalyzed Povarov Reaction: An Unusual [1,3] Sigmatropic Rearrangement to Dihydropyrimidobenzimidazoles, Org. Lett. 13 (2011) 5120-5123; (h) L. S. Astudillo, M. Gutierrez, H. Gaete, V. V. Kouznetsov, C. M. Melendez, J. A. Palenzuela, G. Valles, Solvent-Free Microwave-Assisted Synthesis of New 2-Aryl-Tetrahydroquinolines Using Three-Component Povarov Reaction, Lett. Org. Chem. 6 (2009) 208-212; (i) X. L. Xing, J. L. Wu, W. M. Dai, Acid-mediated three-component azadiels–Alder reactions of 2-aminophenols under controlled microwave heating for synthesis of highly functionalized tetrahydroquinolines, Part 9: Chemistry of aminophenols, Tetrahedron 62 (2006) 11200-11206; (j) D. Duvelroy, U. Perrio, O. Parisel, M. C. Lasne, Rapid synthesis of quinoline-4-carboxylic acid derivatives from arylamines and 2-substituted acrylates or acrylamides under indium(III) chloride and microwave activations. Scope and limitations of the reaction, Org. Biomol. Chem. 3 (2005) 3794-3804.

[28] V. V. Kouznetsov, Recent synthetic developments in a powerful imino Diels–Alder reaction (Povarov reaction): application to the synthesis of N-polyheterocyclics and related alkaloids, Tetrahedron 65 (2009) 2721–2750.

[29] J. J. Martínez, E. Nohe, R. Rojas, M. H. Brijaldolo, F. Passos and G. Romanelli, Reductive ammination of furfural over Me/SiO2–SO2–H (Me: Pt, Ir, Au) catalysts, J. Mol. Catal. A: Chem., 392 (2014) 235-240.

[30] (a) B. Mathew, M. Nath, Recent Approaches to Antifungal Therapy for Invasive Mycoses, ChemMedChem. 4 (2009) 310-323; (b) S. Chen, E. Playford, T. Sorrell, Antifungal therapy in invasive fungal infections, Curr. Opin. Pharmacol. 10 (2010) 522-530; (c) A. Butts, D. Krysan, Antifungal Drug Discovery: Something Old and Something New, PLOS Pathog. 8 (2012) e1002870.

[31] (a) V. Kouznetsov, G. C. Meléndez, M. Douta, L. Syvet, E. del Olmo, S. A. Zacchino, Synthesis and antifungal activity of diverse C-2 pyridyl and pyridinylvinyl substituted quinolines, Bioorg. Med. Chem. 20 (2012) 6506-6512; (b) L. Vargas, S. A. Zacchino, V. V. Kouznetsov, Synthesis of New 4-Methyl-2-(4-pyridyl)-1,2,3,4-tetrahydroquinolines as Potent Antifungal Compounds, J. Braz. Chem. Soc. 21 (2010) 105-111; (c) C. Meléndez, V. Kouznetsov, M. Sorin, S. Álvarez, S. A. Zacchino, In vitro antifungal activity of polyfunctionalized 2-(hetero)arylquinolines prepared through imino Diels–Alder reactions, Bioorg. Med. Chem. 16 (2008) 7908-7920.

[32] M. A. Pfaller, D. J. Diekema, Epidemiology of invasive candidiasis: a persistent public health problem, Clin. Microbiol. Rev. 20 (2007)133-163.

[33] A. Trpkovic, M. Pekmezovic, A. Barac, L. C. Radovic, V. A. Arsenijevic, In vitro antifungal activities of amphotericin B, 5-fluorocytosine, fluconazole anditraconazole against Cryptococcus neoformans isolated from cerebrospinal fluid and blood from patients in Serbia, J. Mycol. Med. 22 (2012) 243-248.

[34] E. J. Ernst, E. E. Roling, C. R. Petzold, D. J. Keel, M. E. Klepser, In vitro activity of micafungin (FK-463) against Candida spp.: microdilution, time-kill, and postantifungal-effect studies, Antimicrob. Agents Chemother. 46 (2002) 3846-3853.

[35] W. Mahavorasirikul, V. Vyanant, W. Chaiauroenkul, A. Itharat, K. Na-Bangchang, Cytotoxic activity of Thai medicinal plants against human cholangiocarcinoma, laryngeal and hepatocarcinoma cells in vitro, BMC Complement Altern. Med. 10 (2010) 55-62.

[36] D. L. da Silva, F. S. Reis, D. R. Muniz, A. L. T. G. Ruiz, J. E. de Carvalho, A. A. Sabino, L. V. Modolo, A. de Fátima, Free radical scavenging and antiproliferative properties of Biginelli adducts, Bioorg. Med. Chem. 20 (2012) 2645-2650.