



**HAL**  
open science

## New 3-substituted-2,1-benzisoxazoles: Synthesis and antimicrobial activities

Aida Chaker, Ennaji Najahi, Olivier Chatriant, Alexis Valentin, Nathan Téné,  
Michel Treilhou, Fakher Chabchoub, Françoise Nepveu

► **To cite this version:**

Aida Chaker, Ennaji Najahi, Olivier Chatriant, Alexis Valentin, Nathan Téné, et al.. New 3-substituted-2,1-benzisoxazoles: Synthesis and antimicrobial activities. *Arabian Journal of Chemistry*, 2017, 10 (Supplement 2), pp.S2464-S2470. 10.1016/j.arabjc.2013.09.011 . hal-02417861

**HAL Id: hal-02417861**

**<https://hal.science/hal-02417861v1>**

Submitted on 18 Dec 2019

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License



## ORIGINAL ARTICLE

# New 3-substituted-2,1-benzisoxazoles: Synthesis and antimicrobial activities



Aida Chaker <sup>a,d</sup>, Ennaji Najahi <sup>a,b,\*</sup>, Olivier Chatriant <sup>a</sup>, Alexis Valentin <sup>a,b</sup>, Nathan Téné <sup>c</sup>, Michel Treilhou <sup>c</sup>, Fakher Chabchoub <sup>d</sup>, Françoise Nepveu <sup>a,b</sup>

<sup>a</sup> University of Toulouse III, UPS, UMR 152 PHARMA-DEV, 118 Route de Narbonne, F-31062 Toulouse cedex 9, France

<sup>b</sup> IRD, UMR 152, F-31062 Toulouse cedex 9, France

<sup>c</sup> University of Toulouse III, CUFR JF Champollion, EA 4357, Place de Verdun, F-81012 Albi, France

<sup>d</sup> University of Sfax, Faculty of Science, Laboratory of Applied Chemistry, 3000 Sfax, Tunisia

Received 22 May 2013; accepted 7 September 2013

Available online 19 September 2013

## KEYWORDS

2,1-Benzisoxazole;  
Anthranil;  
Antiplasmodial;  
Antimicrobial

**Abstract** A new series of 3-substituted-2,1-benzisoxazoles (anthranils) were prepared by different methods and characterized by spectroscopic methods and mass spectrometry. These 2,1-benzisoxazoles were tested *in vitro* for their antiplasmodial activity on a chloroquine-resistant strain of *Plasmodium falciparum* (*P.f.*) (FcB1), and for antimicrobial activity against representative bacterial and fungal strains, as well as for cytotoxicity on MCF7 human breast cancer cells. Given the log  $P_{calc}$  and selectivity index values (cytotoxicity/antiplasmodial activity ratio), the benzo[*c*]isoxazol-3-ylmethylene-phenyl-amine (**11**) (imino-benzisoxazole) was identified as the best hit against *P.f.* (FcB1), and the benzo[*c*]isoxazol-3-yl-phenyl-methanone (**3**) (3-acyl-2,1-benzisoxazole) against *P.f.* and the *Geotrichum candidum* fungal strain.

© 2013 Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/3.0/>).

## 1. Introduction

The interest in heterocyclic compounds is basically because of their biological activities and pharmacological properties.

\* Corresponding author at: Université de Toulouse III, UPS, UMR 152 PHARMADEV, 118 Route de Narbonne, F-31062 Toulouse cedex 9, France. Fax: +33 562259802.

E-mail addresses: [ennaji.najahi@univ-tlse3.fr](mailto:ennaji.najahi@univ-tlse3.fr), [najahimco@yahoo.fr](mailto:najahimco@yahoo.fr) (E. Najahi).

Peer review under responsibility of King Saud University.



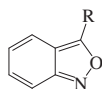
Production and hosting by Elsevier

Benzisoxazoles (Boduszek et al., 1997; Sulikowski and Makosza, 2009; Shelke et al., 2009; Chauhan and Fletcher, 2012) are a promising class of heterocyclics which possess significant pharmacological and biological activities such as anticonvulsant (Stiff and Zemaitis, 1990), antimicrobial (Priya et al., 2005), antipsychotic (Hrib et al., 2003), anticancer (Jain and Kwon, 2003), and affinity for serotonergic and dopaminergic receptors (Nuhlich et al., 1996). In particular, 3-(*N*-benzylpiperidinylolethyl)-1,2-benzisoxazoles inhibit acetyl cholinesterase, making them suitable candidates for the palliative treatment of Alzheimer's disease (Villalobos et al., 1994). 2,1-Benzisoxazoles (anthranils) are fused heteroaromatic systems applied as versatile synthons in fine organic synthesis. This is due to the easy ring opening at the labile *N*–*O* bond and the ability of 2,1-benzisoxazoles to transform into other

<http://dx.doi.org/10.1016/j.arabjc.2013.09.011>

1878-5352 © 2013 Production and hosting by Elsevier B.V. on behalf of King Saud University.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/3.0/>).



**Figure 1** Structure of 3-substituted-2,1-benzisoxazoles **1–13**.

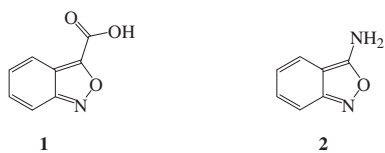
**Table 1** Structure, antiplasmodial and cytotoxic activities of 2,1-benzisoxazoles **1–12**.

Compd.	Structure	M (g mol <sup>-1</sup> )/Log <i>P</i> <sub>calc</sub> <sup>a</sup>	IC <sub>50</sub> (μmol L <sup>-1</sup> ) FcB1	CC <sub>50</sub> (μmol L <sup>-1</sup> ) <sup>b</sup> MCF7	Selectivity index <sup>c</sup> MCF-7/FcB1
1		163.13/1.49	61.3	> 613	> 10
2		134.13/1.54	70.1	> 480.5	> 10
3		223.22/3.25	15.0	103	6.9
4		257.67/3.75	12.8	50.5	3.9
5		253.25/3.12	12.4	63.2	5.1
6		237.25/3.35	13.9	63	4.5
7		238.24/3.11	55.0	25.2	0.5
8		218.25/2.28	130.6	155.8	1.2
9		268.26/3.08	69.9	37.3	0.53
10		147.13/1.36	68.0	> 680	> 10
11		222.25/3.69	16.4	> 450	> 27.4
12		342.35/3.59	> 29.2	15.8	< 0.54
	Chloroquine	319/5.28	0.2	19.4	96
	Sodium artesunate	406/2.29	0.006	9.8	1633

<sup>a</sup> Log *P*<sub>calc</sub>: calculated with VCCLAB (<http://www.virtuallaboratory.org/lab/alogps/start.html>).

<sup>b</sup> The drug concentration needed to cause a 50% decrease in cell viability; The IC<sub>50</sub> SD were always lower than 20% and were discarded for maximum lisibility.

<sup>c</sup> Cytotoxicity/antiplasmodial activity ratio.



**Figure 2** Structure of 2,1-benzisoxazole-3-carboxylic acid (1) and 3-amino-2,1-benzisoxazole (2).

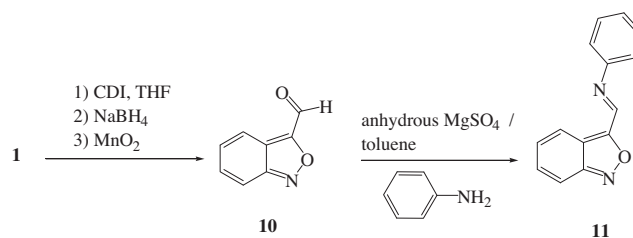
heterocyclic systems, for instance, into acridinones (Ogata et al., 1969). The acridinones are used extensively in the production of dyes and pharmaceuticals (Ogata et al., 1969), and also as biologically active compounds acting as anti-inflammatory agents (Walsh, 1987) and glucosylase inhibitors (Farr and Peet, 1992). We were interested by the wide variety of pharmacological properties of benzisoxazoles and so we designed the synthesis of a new series of 3-substituted-2,1-benzisoxazole in a continuing search for new alternative drugs for malaria and infectious diseases (Nepveu et al., 2010; Najahi et al., 2013). In this paper we report the antiparasitodal and antimicrobial activities of a series of 3-substituted-2,1-benzisoxazoles 1–13 (See Fig. 1).

## 2. Results and discussion

### 2.1. Chemistry

The series of 3-substituted-2,1-benzisoxazoles (anthranils) (Table 1) were prepared from 2,1-benzisoxazole-3-carboxylic acid 1 (Eckroth and Cochran, 1970) and 3-amino-2,1-benzisoxazole 2 (Musso and Schröder, 1965) (Fig. 2).

Different synthetic pathways were considered and successfully used in this work, which led to the desired 3-substituted-2,1-benzisoxazoles. Scheme 1 shows the synthesis of anthranils starting from 2,1-benzisoxazole-3-carboxylic acid 1. The Friedel-Crafts reaction of the 2,1-benzisoxazole-3-carboxylic acid 1 with benzene or substituted benzene (chlorobenzene, anisole or toluene) in dry benzene (96–98%) in the presence of oxalyl chloride and aluminium chloride as catalyst



**Scheme 2** Synthesis of the 2,1-benzisoxazole 11.

yielded the expected 2,1-benzisoxazoles of type 3 (Benjamin and Theodorus, 1993; Benjamin et al., 1991) or (4–6) in acceptable yields (45–61%).

The 2,1-benzisoxazoles 7–9 were prepared starting from compound 1 with aniline, diethylamine and 4-methoxyphenylamine, respectively, in the presence of oxalyl chloride in anhydrous toluene in low yield (Scheme 1).

The reduction of the 2,1-benzisoxazole 1 via the benzylic alcohol provided the anthranil 10 (Jackie et al., 2007), which was transformed to the 3-imino-2,1-benzisoxazole 11 in an acceptable yield by condensation with aniline (Scheme 2).

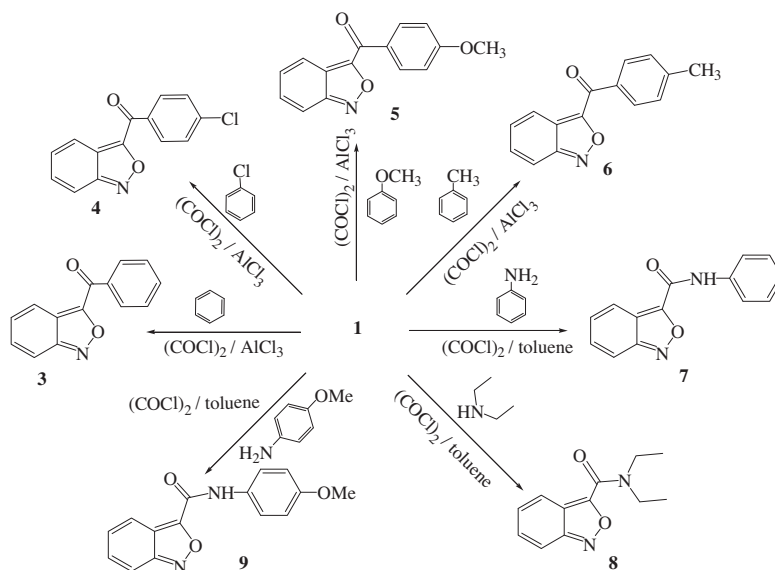
The 3-amino-2,1-benzisoxazole 2 condensed with benzyl chloride in a solution of toluene and triethylamine gave the anthranil 12 at a 22% yield. Approximately 7% of the compound 2 was converted to the 2,1-benzisoxazole 13 as determined by <sup>1</sup>H NMR of the crude material (Scheme 3).

The lipophilicity of the synthesized derivatives 1–12 was expressed in terms of their Log *P*<sub>calc</sub> values that were calculated with the VCCLAB software. As shown in Table 1, higher Log *P*<sub>calc</sub> values were observed for compounds 3–7 and 11–12 (3.11–3.69), compared to the 2,1-benzisoxazoles 1, 2 and 10 (1.36–1.49). For the former, the values were comparable to those of the antimalarials sodium artesunate and of chloroquine.

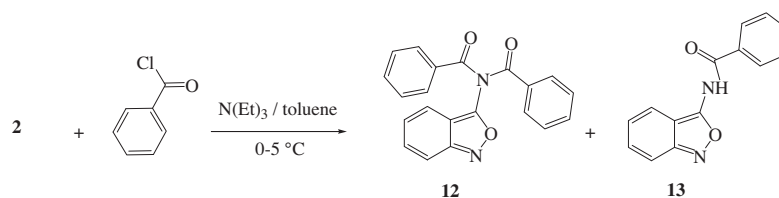
### 2.2. Pharmacology

#### 2.2.1. Antiplasmodial and cytotoxic activities

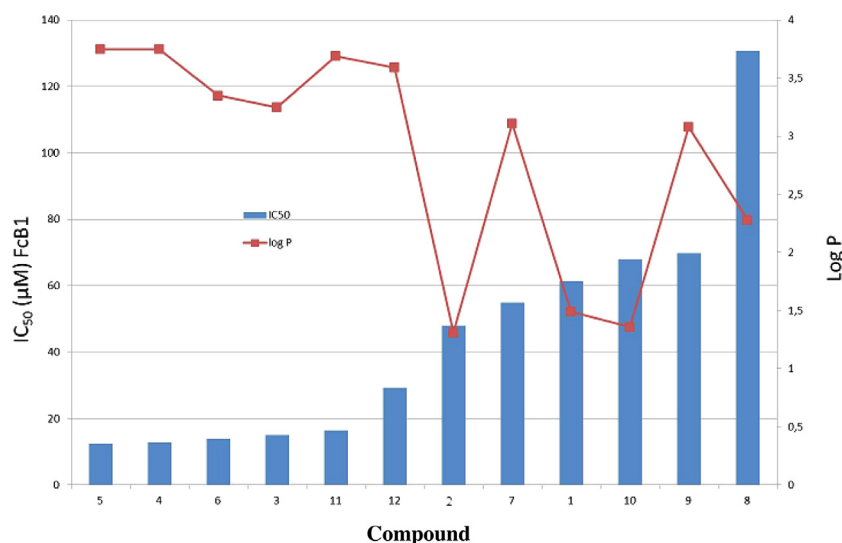
Antiplasmodial and cytotoxic activities were performed as previously described (Nepveu et al., 2010; Najahi et al., 2013). The



**Scheme 1** Synthesis of 3-substituted-2,1-benzisoxazoles 3–9 starting from the 2,1-benzisoxazole 1.



**Scheme 3** Synthesis of 3-substituted-2,1-benzisoxazoles **12–13** starting from the 2,1-benzisoxazole **2**.



**Figure 3** Relationships between  $P.f.$   $IC_{50}$  values (blue) and  $\log P_{calc}$  in the series (red).

antiplasmodial activities of the 2,1-benzisoxazoles **1–12** expressed as the  $IC_{50}$  values, ranged between 12.4 and  $130.6\ \mu\text{mol L}^{-1}$  (Table 1). The 2,1-benzisoxazole-3-carboxylic acid **1** where  $R^3 = \text{COOH}$  had the weakest activities with  $IC_{50}$  values higher than  $60\ \mu\text{mol L}^{-1}$ . Groups with higher lipophilicity ( $\log P > 3$ ) at position  $R^3$  (acyl/amide/imine) improved the antiplasmodial activity ( $IC_{50}/\mu\text{mol L}^{-1} \approx 12\text{--}16$ ) (**3–6, 11**) while groups (aldehyde) at  $R^3$  with lower lipophilicity ( $\log P < 3$ ) led to a significant decrease in the activity ( $IC_{50}/\mu\text{mol L}^{-1}$ : **10** = 68). Fig. 3 illustrates the inverse relation between  $\log P_{calc}$  and the  $IC_{50}$  values.

Cytotoxicity was assayed on the mammalian MCF7 cell line and was higher than  $15\ \mu\text{mol L}^{-1}$  with  $CC_{50}$  values ranging from 15.8 to  $680\ \mu\text{mol L}^{-1}$  (Table 1).

### 2.2.2. In vitro evaluation of antimicrobial activity

The synthesized 2,1-benzisoxazoles **1–12** were evaluated for their *in vitro* antibacterial activity against representative Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *Cronobacter sakazakii*) and against two fungi (*Geotrichum candidum*, *Candida albicans*) using reported techniques (Najahi et al., 2013). Minimum inhibitory concentrations (MICs) were calculated in order to express the antimicrobial activity and were defined as the concentration of the compound required to give complete inhibition of the bacterial growth. From the 12 tested compounds, only three presented antimicrobial activities. Their MICs values are reported in Table 2 with those of reference drugs. The best activities were observed for compound **3**

**Table 2** Antimicrobial activity of 2,1-benzisoxazoles.

Compound	<i>Pseudomonas aeruginosa</i>	<i>Cronobacter sakazakii</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Geotrichum candidum</i>	<i>Candida albicans</i>
	MIC <sup>a</sup>	MIC	MIC	MIC	MIC	MIC
<b>3</b>	b	b	b	b	44.8	b
<b>7</b>	839.5	b	b	b	b	b
<b>12</b>	584.2	b	b	b	b	b
Tetracycline	56.5	17.9	71.2	2.3	b	b
Ampicillin	71.9	14.3	14.5	0.6	b	b
Amphotericin <sup>c</sup>	b	b	b	b	6.8	0.3

<sup>a</sup> MICs: minimum inhibitory concentration ( $\mu\text{mol L}^{-1}$ ).

<sup>b</sup> Inactive.

<sup>c</sup> Ref. (Sharma et al., 2011).

(MIC = 44.8  $\mu\text{mol L}^{-1}$ ) against *G. candidum*, and for compounds **7** and **12** (MIC = 839.5 and 584.2  $\mu\text{mol L}^{-1}$ , respectively).

### 3. Conclusions

We report a new approach to the synthesis of 3-substituted-2,1-benzisoxazoles starting from 2,1-benzisoxazole-3-carboxylic acid and 3-amino-2,1-benzisoxazole. Although representatives of this new series do not exhibit strong antimicrobial activities, it should be noted that compound **11**, with an IC<sub>50</sub> value around the 10  $\mu\text{molar}$  level against *P.f.*, had however the best selectivity index in the series. This was achieved without any substituent effect on either ring. Thus, there are actually opportunities of pharmaco-modulation for this compound by introducing groups on both rings to try to reach an IC<sub>50</sub> value in the nanomolar range and then multiplying the selectivity index by a factor 10<sup>3</sup>. Compounds **3–6** also showed interesting antiplasmodial activities with an initial small pharmaco-modulation effect of the substituent in the para position of the phenyl ring. Working on both rings by introducing new substituents may give good to very good antiplasmodial activities. Compound **3** gave a good result on *G. candidum* but other similar derivatives (**4–6**) were inactive leaving little scope for pharmacological modulation.

### 4. Experimental

#### 4.1. General

Commercially available reagent grade chemicals were used as received without additional purification. All reactions were followed by TLC (E. Merck Kieselgel 60 F-254), with detection by UV light at 254 nm. Column chromatography was performed on silica gel (60–200 mesh E. Merck). IR spectra were recorded on a Perkin-Elmer PARAGON 1000 FT-IR spectrometer. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on an AC Bruker spectrometer at 300 MHz (<sup>1</sup>H) and 75 MHz (<sup>13</sup>C) using (CD<sub>3</sub>)<sub>2</sub>SO as solvent with (CD<sub>3</sub>)<sub>2</sub>SO ( $\delta_{\text{H}}$  2.5) or (CD<sub>3</sub>)<sub>2</sub>SO ( $\delta_{\text{C}}$  39.5). Chemical shifts ( $\delta$ ) are reported in parts per million (ppm) relative to tetramethylsilane (0 ppm). High-resolution mass spectra (DCI in CH<sub>4</sub>) were recorded on a Bruker Maxis spectrometer (Service Commun Toulouse, France). Melting points were determined with an Electrothermal 9300 capillary melting point apparatus and are uncorrected. The purity of all compounds was determined by LC–PDA–MS methods and was found to be in the range 96–99%.

#### 4.2. Synthesis of 2,1-benzisoxazoles **3–6**

This procedure was used for compounds **3–6**. For example, for compound **3** oxalyl chloride ( $m = 2.5$  g, 20 mmol) was added to benzisoxazole-3-carboxylic acid (0.150 g, 1 mmol) in dry benzene (4 mL), and the reaction mixture was stirred at 80 °C under a nitrogen atmosphere for 4 h. The solvent was vacuum evaporated and the resultant residue dissolved in dry benzene (4 mL), and AlCl<sub>3</sub> (0.392 g, 2.94 mmol) was added and the reaction mixture stirred at 80 °C for 1 h. This reaction mixture was then poured into ice-cold water and 37% aqueous HCl and extracted with ethyl acetate (3  $\times$  100 mL) and the combined organic phase subsequently dried over anhydrous

MgSO<sub>4</sub> and vacuum evaporated to afford the crude product. This was further purified by column chromatography using ethyl acetate-petroleum ether ( $v/v = 20/80$ ) and recrystallized from methanol-H<sub>2</sub>O ( $v/v = 60/40$ ).

#### 4.2.1. Benzo[*c*]isoxazol-3-yl-phenyl-methanone (**3**)

Yellow powder, yield: 49%, mp: 96–98 °C. IR (KBr, cm<sup>-1</sup>): 1651, 1627, 1189. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 8.14 (d,  $J = 6$  Hz, 2H), 7.98 (d,  $J = 9$  Hz, 1H), 7.91 (d,  $J = 9$  Hz, 1H), 7.76 (d,  $J = 7.5$  Hz, 1H), 7.60 (m, 3H), 7.44 (d,  $J = 7.8$  Hz, 1H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 181.8 (C=O), 157.3 (C), 154.3 (C), 136.3 (C), 134.4 (CH), 132.5 (CH), 130.2 (2CH), 129.6 (CH), 129.4 (2CH), 121.4 (CH), 121 (C), 116.2 (CH). HRMS (DCI, CH<sub>4</sub>)  $m/z$  calcd for C<sub>14</sub>H<sub>10</sub>NO<sub>2</sub> [M + H]<sup>+</sup> 224.0712. Found 224.0721.

#### 4.2.2. Benzo[*c*]isoxazol-3-yl-(4-chloro-phenyl)-methanone (**4**)

Yellow powder, yield: 43%, mp: 127–129 °C. IR (KBr, cm<sup>-1</sup>): 1712, 1640, 1185, 753. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 8.20 (d,  $J = 8.4$  Hz, 2H), 8.02 (d,  $J = 8.7$  Hz, 1H), 7.93 (d,  $J = 9$  Hz, 1H), 7.76 (d,  $J = 8.7$  Hz, 2H), 7.61 (dd,  $J = 9.3$  Hz,  $J = 5.4$  Hz, 1H), 7.45 (dd,  $J = 8.7$  Hz,  $J = 6.6$  Hz, 1H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta_{\text{C}}$  180 (C=O), 156.7 (C), 153.3 (C), 138.9 (C), 134.4 (C), 132.1 (CH), 131.6 (2CH), 129.2 (CH), 129 (2CH), 120.9 (CH), 120.6 (C), 115.7 (CH). HRMS (DCI, CH<sub>4</sub>)  $m/z$  calcd for C<sub>14</sub>H<sub>9</sub>ClNO<sub>2</sub> [M + H]<sup>+</sup> 258.0322. Found 258.0328.

#### 4.2.3. Benzo[*c*]isoxazol-3-yl-(4-methoxy-phenyl)-methanone (**5**)

Yellow powder, yield: 13%, mp: 125–127 °C. IR (KBr, cm<sup>-1</sup>): 1637, 1600, 1240, 1179. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 8.21 (d,  $J = 9$  Hz, 2H), 8.01 (d,  $J = 8.4$  Hz, 1H), 7.90 (d,  $J = 9$  Hz, 1H), 7.57 (dd,  $J = 9$  Hz,  $J = 6$  Hz, 1H), 7.41 (dd,  $J = 8.7$  Hz,  $J = 6.6$  Hz, 1H), 7.21 (d,  $J = 9$  Hz, 2H), 3.91 (s, 3H). HRMS (DCI, CH<sub>4</sub>)  $m/z$  calcd for C<sub>15</sub>H<sub>12</sub>NO<sub>3</sub> [M + H]<sup>+</sup> 254.0817. Found 254.0826.

#### 4.2.4. Benzo[*c*]isoxazol-3-yl-*p*-tolyl-methanone (**6**)

White powder, yield: 9%, mp: 105–107 °C. IR (KBr, cm<sup>-1</sup>): 1647, 1617, 1235. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 8.10 (d,  $J = 8.4$  Hz, 2H), 8.00 (d,  $J = 8.7$  Hz, 1H), 7.92 (d,  $J = 9$  Hz, 1H), 7.59 (dd,  $J = 9$  Hz,  $J = 6.6$  Hz, 1H), 7.44 (m, 3H), 2.46 (s, 3H). HRMS (DCI, CH<sub>4</sub>)  $m/z$  calcd for C<sub>15</sub>H<sub>12</sub>NO<sub>2</sub> [M + H]<sup>+</sup> 238.0868. Found 238.0875.

#### 4.3. Synthesis of 2,1-benzisoxazoles **7–9**

This procedure was used for compounds **7–9**. For example, for compound **7** oxalyl chloride ( $m = 2.5$  g, 20 mmol) was added to benzisoxazole-3-carboxylic acid (0.150 g, 0.919 mmol) in dry toluene (4 mL), and the reaction mixture was stirred at 80 °C under nitrogen for 16 h. The solvent was vacuum evaporated and the resultant residue dissolved in dry toluene (2 mL). A solution of aniline ( $m = 0.207$  g, 2.22 mmol) in dry toluene (2 mL) was added drop-wise and the reaction mixture was stirred at room temperature for 1 h. The mixture was treated with aqueous HCl (1 M) and then extracted with ethyl acetate (3  $\times$  100 mL), and the combined organic phase quenched with NaOH (1 M). Subsequently, the organic phase



was washed with brine (1 × 100 mL), dried over anhydrous MgSO<sub>4</sub>, and evaporated in vacuum to afford the crude product. This was further purified by column chromatography using ethyl acetate-petroleum ether (v/v = 5/95).

#### 4.3.1. Benzo[*c*]isoxazole-3-carboxylic acid phenylamide (**7**)

Yellow powder, yield: 39%, mp: 161–163 °C. IR (KBr, cm<sup>-1</sup>): 3270, 1676, 1617, 1152. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ: 11.12 (s, 1H, NH), 8.03 (d, *J* = 9 Hz, 1H), 7.85 (m, 3H), 7.55 (dd, *J* = 9 Hz, *J* = 6.3 Hz, 1H), 7.36 (m, 3H), 7.18 (m, 1H). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ: 157.5 (C=O), 157.2 (C), 154.9 (C), 138.3 (C), 132.5 (CH), 129.2 (2CH), 127.2 (CH), 125.1 (CH), 121.3 (CH), 121.2 (2CH), 119.3 (C), 115.7 (CH). HRMS (DCI, CH<sub>4</sub>) *m/z* calcd for C<sub>14</sub>H<sub>11</sub>N<sub>2</sub>O<sub>2</sub> [M + H]<sup>+</sup> 239.0821. Found 239.0826.

#### 4.3.2. Benzo[*c*]isoxazole-3-carboxylic acid diethylamide (**8**)

Yellow powder, yield: 34%, mp: 168–170 °C. IR (KBr, cm<sup>-1</sup>): 1672, 1612, 1151. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ: 7.83 (d, *J* = 8.7 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.49 (dd, *J* = 9 Hz, *J* = 6.3 Hz, 1H), 7.24 (dd, *J* = 8.7 Hz, *J* = 6.3 Hz, 1H), 3.56 (m, 4H), 1.23 (m, 6H). HRMS (DCI, CH<sub>4</sub>) *m/z* calcd for C<sub>12</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub> [M + H]<sup>+</sup> 219.1134. Found 219.1121.

#### 4.3.3. Benzo[*c*]isoxazole-3-carboxylic acid (4-methoxyphenyl)-amide (**9**)

Yellow powder, yield: 27%, mp: 172–174 °C. IR (KBr, cm<sup>-1</sup>): 3335, 1672, 1629, 1206, 1189. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ: 11.01 (s, 1H, NH), 8.02 (d, *J* = 9 Hz, 1H), 7.79 (m, 3H), 7.55 (dd, *J* = 9 Hz, *J* = 6.3 Hz, 1H), 7.43 (dd, *J* = 8.7 Hz, 6.6 Hz, 1H), 6.97 (d, *J* = 9 Hz, 2H), 3.76 (s, 3H). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ: 157 (C=O), 156.9 (C), 156.1 (C), 154.1 (C), 132 (CH), 130.8 (C), 127.2 (CH), 122.2 (2CH), 120.9 (CH), 118.7 (C), 115.1 (CH), 113.8 (2CH), 55.2 (OCH<sub>3</sub>). HRMS (DCI, CH<sub>4</sub>) *m/z* calcd for C<sub>15</sub>H<sub>13</sub>N<sub>2</sub>O<sub>3</sub> [M + H]<sup>+</sup> 269.0926. Found 269.0921.

#### 4.4. Benzo[*c*]isoxazol-3-ylmethylene-phenyl-amine (**11**)

2,1-Benzisoxazole-3-carbaldehyde (**10**) (0.08 g, 0.543 mmol), prepared according to the literature [16], was added to a vigorously stirring solution of aniline (0.051 g, 0.547 mmol) and anhydrous MgSO<sub>4</sub> (0.262 g, 2.175 mmol) in dry toluene (1 mL) under a nitrogen atmosphere at 0–5 °C. The reaction mixture was stirred for an additional 15 min at 0–5 °C. The heterogeneous mixture was stirred vigorously for 4 h at room temperature, at which point it cleared and TLC analysis indicated complete consumption of the reactants. The mixture was filtered and the filtrate was evaporated in a vacuum to afford a yellow oil. This was further purified by column chromatography using ethyl acetate-petroleum ether (3:97). Yellow oil, yield: 63%. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ: 9.19 (s, 1H, HC=N), 8.20 (d, *J* = 8.7 Hz, 1H), 7.80 (d, *J* = 9 Hz, 1H), 7.53 (m, 5H), 7.36 (m, 2H). HRMS (DCI, CH<sub>4</sub>) *m/z* calcd for C<sub>14</sub>H<sub>11</sub>N<sub>2</sub>O [M + H]<sup>+</sup> 223.0871. Found 223.0887.

#### 4.5. *N*-Benzo[*c*]isoxazol-3-yl-*N*-benzoyl-benzamide (**12**)

Et<sub>3</sub>N (0.045 g, 0.447 mmol) was added to 3-amino-2,1-benzisoxazole (m = 0.060 g, 0.447 mmol) in dry toluene (6 mL), and

stirred at 0–5 °C for 10 min and then benzoyl chloride (0.069 g, 0.492 mmol) was added drop-wise. The reaction mixture was then stirred at 0–5 °C for 2 h. The mixture was treated with 5% aqueous HCl (1 × 10 mL) and then extracted with ethyl acetate (3 × 25 mL), and the combined organic phase quenched with aqueous NaHCO<sub>3</sub> (1 × 25 mL). The organic phase was subsequently washed with brine (1 × 25 mL), dried over anhydrous MgSO<sub>4</sub>, and vacuum evaporated to afford the crude product. This was further purified by column chromatography using toluene-acetone (v/v = 80/20). Yellow powder, yield: 22%, mp: 105.4 °C. IR (KBr, cm<sup>-1</sup>): 1683, 1630, 1447, 1190. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ: 8.01 (m, 3H), 7.78 (m, 3H), 7.91 (m, 5H), 7.51 (d, *J* = 6 Hz, 1H), 7.44 (m, 2H). HRMS (DCI, CH<sub>4</sub>) *m/z* calcd for C<sub>21</sub>H<sub>15</sub>N<sub>2</sub>O<sub>3</sub> [M + H]<sup>+</sup> 343.1083. Found 343.1097.

#### 4.6. Methodology for *in vitro* biological evaluation

##### 4.6.1. *In vitro* antipalmodial and cytotoxicity assays

The *in vitro* antipalmodial assay was carried out against the chloroquine-resistant strain (FcB1) of *Plasmodium falciparum* and the *in vitro* cytotoxicity was determined on human breast cancer cells (MCF7) (Cachet et al., 2009; Ribaut et al., 2008). All these assays were performed as previously reported (Nepveu et al., 2010; Najahi et al., 2013) and details are given in the supplementary data.

##### 4.6.2. Antimicrobial assays

All microorganisms were grown in broth medium with continuous shaking (200 rpm). *Bacillus subtilis* (CIP 5262), *Staphylococcus aureus* (CIP 53156) were used as Gram-positive strains. The Gram-negative strains used were *Pseudomonas aeruginosa* (CIP 82118), *Cronobacter sakazakii* (CIP 103183). Fungal strains used were *Candida albicans* (CIP 4872) and *Geotrichum candidum* (ATCC 204307). The minimal inhibitory concentrations (MICs) were performed as previously reported (Najahi et al., 2013) and details are given in the supplementary data.

#### Acknowledgement

This work was supported by the European Commission (FP6-LSH-20044-2.3.0-7, STREP no. 018602, Redox antimalarial Drug Discovery, READ-UP). We thank J.-P. Nallet, S. Petit and IDEALP-PHARMA for their scientific contributions.

#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.arabjc.2013.09.011>.

#### References

- Benjamin, S., Theodorou, V.E., 1993. Production of 3-benzoyl-2,1-benzisoxazoles, 2-phenyl-4*H*-3,1-benzoxazin-4-ones, and novel quinolinone derivatives from 2-phenylquinolin-4(1*H*)-ones and sodium dichloroisocyanurate. *J. Chem. Soc. Perkin Trans. I: Org. Bioorg. Chem.* 4, 511–516.

- Benjamin, S., Theodorus, V.E., David, G.B., Jan, C.A.B., 1991. Novel production of 3-benzoyl-2,1-benzisoxazoles from 2-phenyl-quinolin-4(1*H*)-ones. *J. Chem. Soc. Chem. Commun.* 14, 927–928.
- Boduszek, B., Halama, A., Zofi, J., 1997. The cleavage of 1-amino-2'-nitrobenzylphosphonates in a basic medium. Formation of the 3-amino-2,1-benzisoxazole derivatives. *Tetrahedron* 53, 11399–11410.
- Cachet, N., Hoakwie, F., Bertani, S., Bourdy, G., Deharo, E., Stien, D., Houel, E., Gornitzka, H., Fillaux, J., Chevalley, S., Valentin, A., Jullian, V., 2009. Antimalarial activity of simalikalactone E, a new quassinoid from *Quassia amara* L. (Simaroubaceae). *Antimicrob. Agents Chemother.* 53, 4393–4398.
- Chauhan, J., Fletcher, S., 2012. One-pot synthesis of 2,1-benzisoxazoles (anthranils) by a stannous chloride-mediated tandem reduction–heterocyclization of 2-nitroacylbenzenes under neutral conditions. *Tetrahedron Lett.* 53, 4951–4954.
- Eckroth, D.R., Cochran, T.G., 1970. Mechanism of the conversion of 2-nitrobenzyl systems into 2,1-benzisoxazoles. *J. Chem. Soc.* 6, 2660–2661.
- Farr, R.A., Peet, N.P., 1992. Novel glucosyltransferase inhibitors useful as antidiabetic agents. WO Patent 011867.
- Hrib, N.J., Jurcak, J.G., Burgher, K.L., Conway, P.G., Hartman, H.B., Kerman, L.L., Roehr, J.E., Woods, A.T., 2003. Benzisoxazole and benzisothiazole-3-carboxamides as potential atypical antipsychotic agents. *J. Med. Chem.* 37, 2308–2314.
- Jackie, D.K., Gordon, W.R., Raphael, F., Claire, M., William, A.D., Elaine, S.M., Bruce, C.B., Claire, C., Shaun, P.J., Peter, R.S., 2007. Synthesis, biological evaluation and molecular modelling of sulfonohydrazides as selective PI3K p110 $\alpha$  inhibitors. *Bioorg. Med. Chem.* 15, 7677–7687.
- Jain, M., Kwon, C.H., 2003. 1,2-Benzisoxazole phosphorodiamidates as novel anticancer prodrugs requiring bioreductive activation. *J. Med. Chem.* 46, 5428–5436.
- Musso, H., Schröder, H., 1965. Mechanism of the oxygen transfer in the hydrogenation of 2-nitrobenzotrile to 2-aminobenzamide. *Chem. Ber.* 98, 1562–1576.
- Najahi, E., Valentin, A., Téné, N., Treilhou, M., Nepveu, F., 2013. Synthesis and biological evaluation of new *bis*-indolone-*N*-oxides. *Bioorg. Chem.* 48, 16–21.
- Nepveu, F., Kim, S., Boyer, J., Chatriant, O., Ibrahim, H., Reybier, K., Monje, M.C., Chevalley, S., Perio, P., Lajoie, B.H., Bouajila, J., Deharo, E., Sauvain, M., Tahar, R., Basco, L., Pantaleo, A., Turini, F., Arese, P., Valentin, A., Thompson, E., Vivas, L., Petit, S., Nallet, J.P., 2010. Synthesis and antiplasmodial activity of new indolone *N*-oxide derivatives. *J. Med. Chem.* 53, 699–714.
- Nuhrich, A., Varache-Lembege, M., Vercauteren, J., Dokhan, R., Renard, P., Devaux, G., 1996. Synthesis and binding affinities of a series of 1,2-benzisoxazole-3-carboxamides to dopamine and serotonin receptors. *Eur. J. Med. Chem.* 31, 957–964.
- Ogata, M., Matsumoto, H., Kanō, H., 1969. Organic photochemical reactions-V: photorearrangement of anthranils into azepines. *Tetrahedron* 25, 5205–5215.
- Priya, B.S., Swamy, S.N., Rangappa, K.S., 2005. Synthesis and characterization of novel 6-fluoro-4-piperidinyl-1,2-benzisoxazole amides and 6-fluoro-chroman-2-carboxamides: antimicrobial studies. *Bioorg. Med. Chem.* 13, 2623–2628.
- Ribaut, C., Berry, A., Chevalley, S., Reybier, K., Morlais, I., Parzy, D., Nepveu, F., Benoit-Vical, F., Valentin, A., 2008. Concentration and purification by magnetic separation of the erythrocytic stages of all human *Plasmodium* species. *Malar. J.* 7, 45.
- Sharma, M., Joshi, P., Kumar, N., Joshi, S., Rohilla, R.K., Roy, N., Rawat, D.S., 2011. Synthesis, antimicrobial activity and structure activity relationship study of *N,N*-dibenzyl-cyclohexane-1,2-diamine derivatives. *Eur. J. Med. Chem.* 46, 480–487.
- Shelke, K.F., Sapkal, S.B., Shitole, N.V., Shingate, B.B., Shingare, M.S., 2009. Microwave-assisted synthesis of 1,2-benzisoxazole derivatives in ionic liquid. *Org. Commun.* 2, 72–78.
- Stiff, D.D., Zemaitis, M.A., 1990. Metabolism of the anticonvulsant agent zonisamide in the rat. *Drug Metab. Dispos.* 18, 888–894.
- Sulikowski, D., Makosza, M., 2009. Synthesis of 3-phenyl-2,1-benzisoxazoles *via* conversion of diethyl  $\alpha$ -(*o*-nitroaryl)benzylphosphonates. *Acta Chim. Slov.* 56, 680–683.
- Villalobos, A., Blake, J.F., Biggers, C.K., Butler, T.W., Chapin, D.S., Chen, Y.L., Ives, J.L., Jones, S.B., Liston, D.R., 1994. Novel benzisoxazole derivatives as potent and selective inhibitors of acetylcholinesterase. *J. Med. Chem.* 37, 2721–2734.
- Walsh, D.A., 1987. Antiinflammatory derivatives of 3-aryl-2,1-benzisoxazole. European Patent 0260924.