



ELSEVIER

Contents lists available at ScienceDirect

Comptes Rendus Chimie

www.sciencedirect.com



Preliminary communication/Communication

A synthetic route to novel 3-substituted-2,1-benzisoxazoles from 5-(2-nitrobenzylidene)(thio)barbiturates

João L. Serrano^a, Eunice Cavalheiro^a, Sónia Barroso^b, Maria J. Romão^b, Samuel Silvestre^{a, c}, Paulo Almeida^{a, *}^a CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal^b Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal^c CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Rua Larga, 3004-517 Coimbra, Portugal

ARTICLE INFO

Article history:

Received 8 September 2017

Accepted 2 October 2017

Available online xxxx

Keywords:

2,1-Benzisoxazoles

Anthranils

Barbiturates

Single crystal X-ray

Xanthine oxidase inhibition

ABSTRACT

2,1-Benzisoxazoles, also called anthranils, are one of the two types of aromatic bicyclic heterocycles having a benzene ring fused with an isoxazole, which are particularly recognized as valuable intermediates in organic synthesis. Nevertheless several methods can be found in the literature to prepare 2,1-benzisoxazoles, we herein report a new, efficient, simple, mild, and alternative procedure to prepare 3-substituted-2,1-benzisoxazoles from 5-(2-nitrobenzylidene)barbiturates in moderate to good yields (51–82%). All the novel benzisoxazoles showed spectral data fully consistent with the assigned structures, which were unequivocally confirmed by single crystal X-ray analysis. A possible mechanism of the reaction is proposed. In addition, a screening of the bioactivity of these benzisoxazoles as xanthine oxidase inhibitors, antioxidants, and cytotoxic compounds was performed. The benzisoxazole formed from barbituric acid revealed moderate xanthine oxidase inhibitory effects ($IC_{50} = 22.10 \mu M$).

© 2017 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.

1. Introduction

Benzisoxazoles are aromatic ring heterocycles containing one oxygen atom adjacent to one nitrogen atom in a five-membered ring fused with a benzo moiety. There are two types of benzisoxazoles, namely 1,2-benzisoxazoles (indoxazene) and 2,1-benzisoxazoles (2,1-benzoisoxazoles or anthranil). The first one is more widely used due to its high therapeutic interest [1]. The second is more useful in the preparation of biologically active compounds. Indeed, several important heterocycles such as quinolones, acridines, or quinazolines can be prepared directly from 2,1-benzisoxazoles or via amino-arylketones easily obtained by treatment with iron in acetic acid [2].

Since the first described synthesis of 2,1-benzisoxazoles by reductive cyclization of *ortho*-nitrobenzaldehydes by Friedlander, in 1882, which wrongly assigned the resulting product as an anthranil lactam [3], several other methods have been described (see [Supplementary information](#)) and recently reviewed [2,4].

To the best of our knowledge, 2,1-benzisoxazoles containing an *exo* double bond at 3-position have been scarcely described [5]. In this context, in the reinvestigation of the synthesis of indoles from Baylis–Hillman adducts [5a], three new 2,1-benzisoxazoles possessing an *exo* double bond were obtained as secondary products. In fact, the reduction of *ortho*-nitro adducts of nitrobenzaldehyde using tin(II) chloride in refluxing 1,4-dioxane originated these 2,1-benzisoxazoles in 26% yield [5b]. Later, the intramolecular $FeBr_2$ -catalyzed N–O bond formation from an aryl azide possessing an *ortho*-isopropylcarbonyl group

* Corresponding author.

E-mail address: paulo.almeida@ubi.pt (P. Almeida).

affording the corresponding 1*H*-benzisoxazole in 72% yield after SiO₂ purification was also described [5c].

2. Results and discussion

2.1. Synthesis and structural characterization

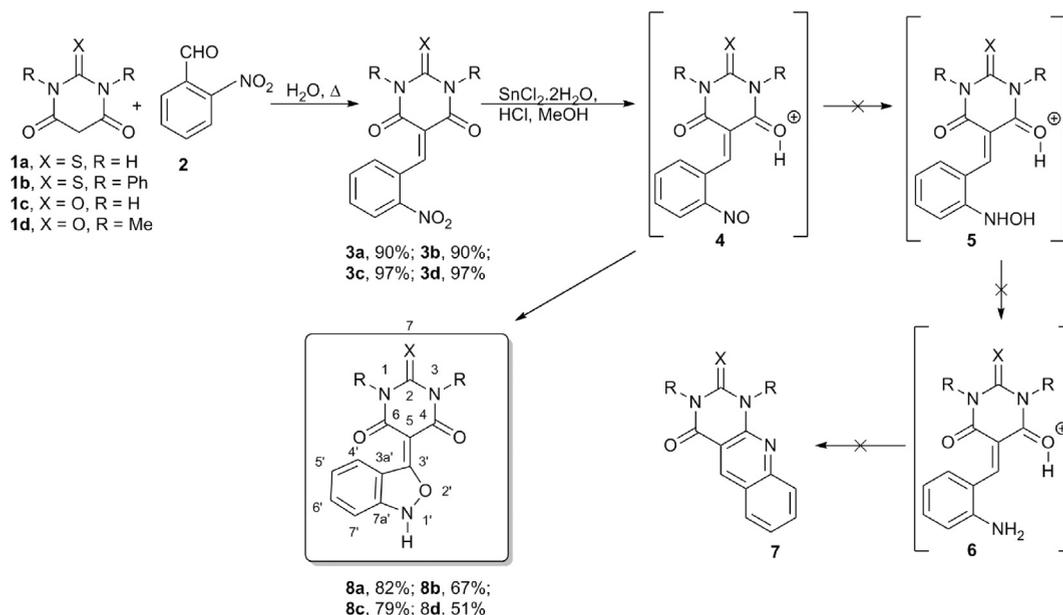
As a part of our ongoing research on the development of new barbiturate[4,5-*b*]quinolones **7** as potential xanthine oxidase (XO) inhibitors and antitumor agents, we herein report a new route to prepare 3-substituted-2,1-benzisoxazoles **8** from 5-(2-nitrobenzylidene)barbiturates **3** (Scheme 1).

The starting materials thiobarbituric acid (**1a**), barbituric acid (**1c**), *N,N*-dimethylbarbituric acid (**1d**), and *ortho*-nitrobenzaldehyde were purchased from sigma aldrich, whereas *N,N*-diphenylthiobarbituric acid (**1b**) was prepared as described [6]. (2-Nitrobenzylidene)barbiturates **3a–d** were prepared in a simple and mild way based on the method described by Deb and Bhuyan [7], using water as the solvent, in 90–97% yield. Surprisingly after refluxing for 2 h the methanolic acid suspension containing the respective 5-(2-nitrobenzylidene)barbiturate **3** and tin chloride dihydrate, the 5-benzo[*c*]isoxazolbarbiturates **8** were easily obtained by a direct filtration of the solid formed, in moderate to good yields (51–82%), instead of the expected barbiturate[4,5-*b*]quinolones **7** (Scheme 1).

Apart from precursors, all 5-benzo[*c*]isoxazolbarbiturates **8a–d** prepared have not hitherto been described. Consequently, their full spectroscopic characterization was performed, including ¹H and ¹³C NMR shift assignments, which were established with the aid of distortionless enhancement by polarization transfer 135, heteronuclear simple quantum coherence, and heteronuclear multiple bond correlation experiments. In the ¹H NMR spectrum of 5-benzo[*c*]isoxazolbarbiturates **8a,c** a characteristic signal

at 11.31 or 10.69 ppm can be found, corresponding to the proton bond to the nitrogen of the pyrimidine scaffold suggesting the symmetry of this ring. In addition, four signals observed for all **8a–d** in the range of 7.90–6.71 ppm indicated an *ortho*-substituted benzene ring. In the **8a–d** ¹³C NMR spectrum, the signals at 178.6–174.5, 162.7–150.8, and 85.4–82.5 ppm were attributed to carbons directly attached to a sulfur atom, to an oxygen atom, and to a methylenic carbon, respectively. Within these, the signal at 162.7–159.6 ppm was associated with two carbonyls, confirming the symmetry of this pyrimidine scaffold. The ¹³C NMR spectrum of the benzisoxazoles **8a–d** presented two signals at 167.4–163.6 and 156.4–155.9 ppm related to 3' and 7a' isoxazole ring carbon atoms. The remaining aromatic carbon atoms can be found between 141.8 and 112.9 ppm. In addition, attenuated total reflectance infrared (ATR-IR) spectroscopy and high-resolution mass spectrometry (HRMS) were also performed. In conclusion, all the novel compounds showed spectral and analytical data fully consistent with the assigned structures. In addition, single crystal X-ray data clearly confirmed the structure of **8d** (Fig. 1 and Supplementary information).

On the basis of the results mentioned previously and described in Scheme 1, a possible reaction mechanism for the formation of **8** is proposed considering an intramolecular attack of a nucleophilic intermediate in the reduction of the nitro group, specifically a nitroso [8] compound (Scheme 2). At first, the nitrosoarene **4** formed by the partial reduction of the nitro group behaves as a nucleophile capable to attack the C-5 of the (thio)barbiturate in a typical Michael addition reaction. Moreover, this cyclization path is activated by carbonyl acid catalysis. Several mechanisms proposed for the 2,1-benzisoxazole cyclization also involved radical anion nitroso or nitroso as nucleophilic (methods D, E G, Scheme S1) [2] or electrophilic (method F, Scheme S1) [2b] species. Besides,



Scheme 1. Attempt to prepare (thio)barbiturate[4,5-*b*]quinolones **7** with the formation of 2,1-benzisoxazoles **8**.

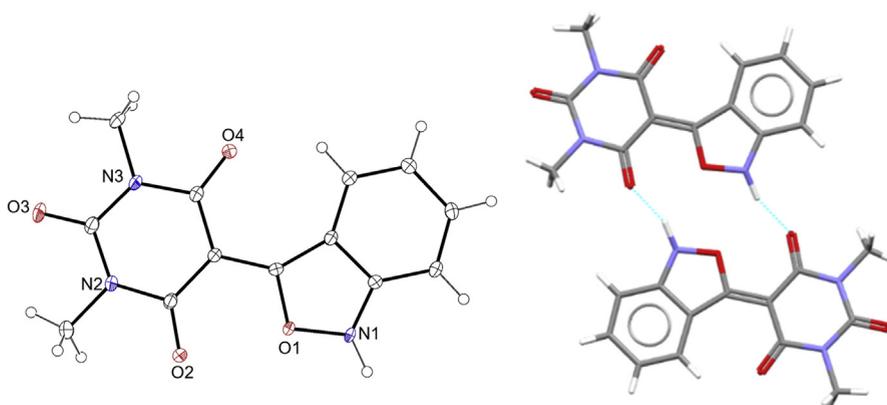
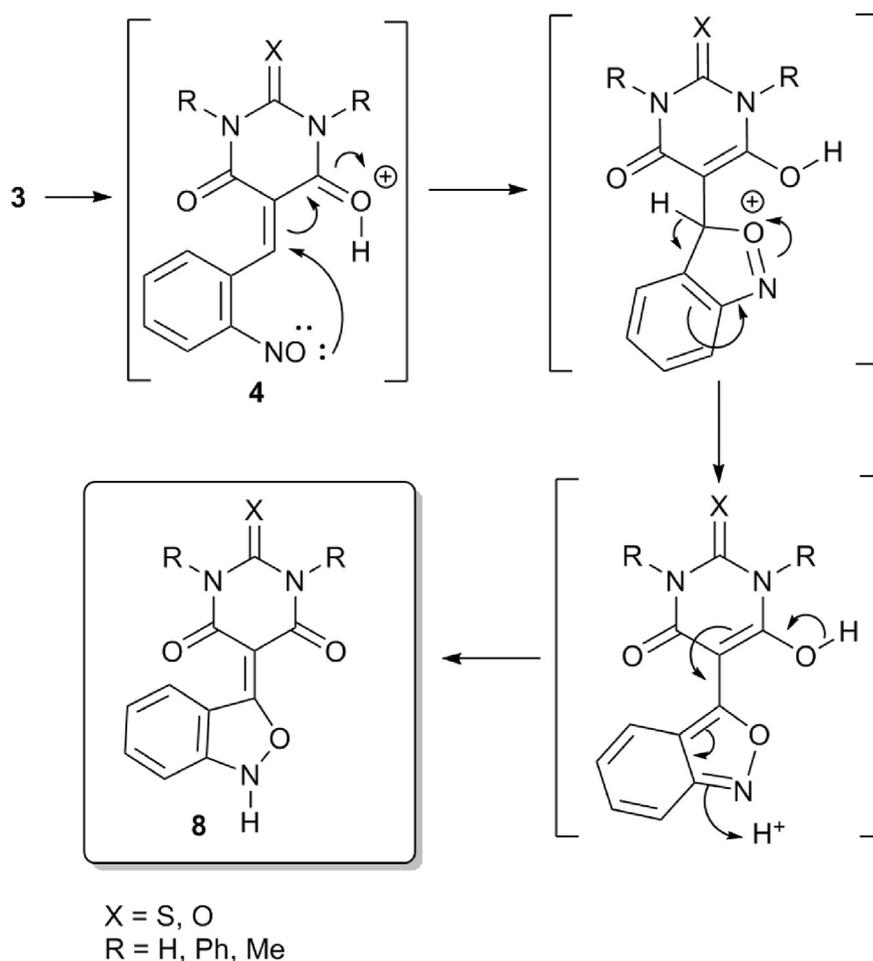


Fig. 1. Molecular structure of 5-benzo[c]isoxazolbarbiturate **8d**. (Left) ORTEP-3 plot (30% thermal ellipsoids). (Right) Packing showing N1–H1...O2 hydrogen bonds (dashed light blue lines).



Scheme 2. Proposed mechanism for the formation of 2,1-benzisoxazoles.

considering the local reactivity descriptors applied on the nitroso group of the nitrosobenzene, it has been shown that both N and O nitroso atomic centers can be suitable nucleophilic reactive sites in aqueous solutions [9]. Because

tin chloride is a rather effective reducing agent [10], the unlikely partial reduction of the nitroarene to nitrosoarene can be explained by the intramolecular trapping of this unstable species by the reaction with the β -keto

unsaturated system. Finally, elimination of a proton and the conversion of the enol form to the corresponding ketone leads to the formation of 2,1-benzisoxazole **8**. Therefore, a novel system and associated reactivity different from all of those previously described for the preparation of 2,1-benzisoxazoles are reported in the present work. In addition, the simplicity and mildness of the reaction, the easy access to the starting materials, and the straightforward workup and good yields of the products obtained clearly are interesting advantages of this new method.

2.2. Biological evaluation

Following our initial aim of study and considering the growing interest on (thio)barbiturates by medicinal chemists because of their increasingly wide range of biological activities [11], in addition to their most important and classical use as sedative hypnotics [12], these new benzisoxazole–barbiturate systems were also screened as XO inhibitors, radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavengers, and cytotoxic compounds (Table 1).

From the results of Table 1, it is important to stress the moderate XO inhibition of the isoxazolbarbiturate **8c** ($IC_{50} = 22.10 \mu\text{M}$). In addition, the thiobarbiturates **8a** and **8b** have some DPPH radical scavenger effects, especially for compound **8b**. Moreover, a marked reduction in MCF-7 cell proliferation was observed with the benzisoxazole **8c**. Therefore, these structures can be considered in further studies concerning the development of antioxidants and/or XO inhibitors.

3. Conclusions

A new, efficient, simple, and mild method to prepare bioactive 5-benzo[c]isoxazolbarbiturates from 5-(2-nitrobenzylidene)barbiturates has been developed and for the first time described. Single crystal X-ray data unequivocally confirmed the presence of this ring system, whose formation can involve a nitroso intermediate in the reduction of the nitroaromatic group. In addition, the isoxazolbarbiturate **8c** has potential to be a starting point in the development of new and improved XO inhibitors.

Table 1

XO inhibitory, antioxidant, and antiproliferative effects on MCF-7 cells of 5-benzo[c]isoxazolbarbiturates **8a–d**.^a

Compound	XO inhibition (IC_{50} , μM)	DPPH scavenging (IC_{50} , μM)	Relative MCF-7 cell proliferation
8a	12.65	24.62 (70.91)	72.79
8b	15.50	57.28 (46.99)	58.06
8c	52.26 (22.10)	5.39	43.48
8d	29.89	–0.64	64.82
Allopurinol	80.41 (3.17)	n.d. ^b	n.d. ^b
Trolox	n.d. ^b	70.35 (35.93)	n.d. ^b
5-FU	n.d. ^b	n.d. ^b	11.88

^a The screening was effected at a concentration of 30 μM , and the results are presented as % of XO inhibition, % of DPPH reduction, and % of the negative control in cell proliferation, respectively. For the most relevant compounds, dose–response curves to determine the half-medium inhibitory concentrations (IC_{50}) were then performed and presented within parentheses.

^b n.d., Not determined.

4. Materials and methods

Reagents and solvents were purchased from standard sources and were of analytical grade. Melting points were measured in open capillary tubes in a Büchi B-540 apparatus and were uncorrected. ATR-IR spectra were collected using a Thermo-scientific Nicolet iS10: smart iTR, equipped with a diamond ATR crystal using OMNIC 8.2 software. For ATR data acquisition the sample was placed onto the crystal and the spectrum was recorded, using an air spectrum as a reference in absorbance calculations. The sample spectra were collected at room temperature in the 4000–600 cm^{-1} range by averaging 32 scans with a spectral resolution of 4 cm^{-1} . NMR spectra were acquired at room temperature using a Bruker Avance III 400 MHz spectrometer (^1H NMR at 400.13 MHz and ^{13}C NMR at 100.62 MHz) and were processed with the software MestReNova 11.0.3 (trial). Deuterated dimethyl sulfoxide ($\text{DMSO-}d_6$) and chloroform (CDCl_3) were used as solvents and as an internal standard. The ^{13}C NMR chemical shifts were attributed based on distortionless enhancement by polarization transfer 135, heteronuclear multiple bond correlation, and heteronuclear simple quantum coherence experiments. Electrospray ionization (ESI) HRMS was performed by the microanalysis service using a QSTAR XL instrument (Salamanca, Spain).

4.1. Synthesis

4.1.1. 1,3-Diphenylthiourea [13]

To a stirred solution of phenyl isothiocyanate (1 mmol; 135 mg) in dichloromethane (0.5 mL) at room temperature was added dropwise a solution of aniline (1 mmol; 93 mg) in dichloromethane (0.5 mL). The reaction was followed by thin-layer chromatography (dichloromethane) and completed in 3 h. The product so formed in suspension was filtered and washed with ethyl ether; 217 mg (95%); white crystals; mp 142–144 °C (lit. [14], 140–142 °C). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) 9.79 (s, 2H), 7.48 (d, $J = 7.4$ Hz, 4H), 7.33 (t, $J = 8.2$ and 7.6 Hz, 4H), 7.12 (t, $J = 7.4$ Hz, 2H); ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ (ppm) 179.6, 139.5, 128.5, 124.4, 123.7.

4.1.2. 1,3-Diphenylthiobarbituric acid (**1b**) [6]

A stirred solution of 1,3-diphenylthiourea (1 mmol; 228 mg), malonic acid (1.3 mmol; 135 mg), and acetyl chloride (3 mmol; 235 mg; 214 μL) was heated at 60 °C for 30 min. The solid product so obtained was divided into finer pieces, filtered, washed with water, and recrystallized from acetic acid; 282 mg (95%); needle yellow crystals; mp 252–253 °C (lit. [6], 258–259 °C). ^1H NMR (400 MHz, CDCl_3) δ (ppm) 7.55 (m, 6H), 7.21 (d, $J = 7.3$ Hz, 4H), 4.10 (s, 2H); ^{13}C NMR (101 MHz, CDCl_3) δ (ppm) 181.6, 163.3, 138.8, 129.7, 129.2, 128.6, 41.2.

4.1.3. 5-Benzylidenepyrimidines **3a–d** [7]

A stirred mixture of (thio)barbituric acid **1a–d** (1 mmol) and 2-nitrobenzaldehyde (**2**) (1 mmol) in water (5 mL) was refluxed for 2 h. The product so formed after cooling was filtered, washed with water, ethanol, and ethyl ether to afford the following 5-benzylidenepyrimidines.

5-(2-Nitrobenzylidene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (**3a**), from thiobarbituric acid (**1a**); 250 mg (90%); pale yellow solid; mp 239–241 °C (lit. [15], 246–250 °C). ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 12.56 (s, 1H), 12.33 (s, 1H), 8.63 (s, 1H), 8.24 (dd, *J* = 8.3 and 1.0 Hz, 1H), 7.80 (td, *J* = 7.6 and 1.1 Hz, 1H), 7.72–7.66 (m, 1H), 7.62 (dt, *J* = 7.7 and 1.2 Hz, 1H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm) 179.1, 160.6, 159.2, 153.3, 146.3, 133.8, 131.6, 130.5, 130.4, 124.1, 120.6.

5-(2-Nitrobenzylidene)-1,3-diphenyl-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (**3b**), from 1,3-diphenylthiobarbituric acid (**1b**); 387 mg (90%); pale orange solid; mp 235 °C decomposes (lit. [16], 232 °C). ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 8.80 (s, 1H), 8.19 (d, *J* = 8.2 Hz, 1H), 7.71 (t, *J* = 7.5 Hz, 1H), 7.59 (t, *J* = 7.9 Hz, 2H), 7.45 (t, *J* = 7.6 Hz, 2H), 7.40–7.28 (m, 6H), 7.20 (d, *J* = 7.7 Hz, 2H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm) 181.4, 160.3, 158.8, 155.0, 146.2, 140.0, 139.7, 134.0, 131.8, 130.4, 130.2, 129.1, 128.9, 128.8, 128.7, 128.3, 128.1, 124.1, 121.3.

5-(2-Nitrobenzylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**3c**), from barbituric acid (**1c**); 254 mg (97%); white solid; mp 274–275 °C (lit. [17], 274–276 °C). ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 11.49 (s, 1H), 11.24 (s, 1H), 8.60 (s, 1H), 8.23 (d, *J* = 7.9 Hz, 1H), 7.79 (t, *J* = 7.4 Hz, 1H), 7.68 (t, *J* = 7.7 Hz, 1H), 7.57 (d, *J* = 7.7 Hz, 1H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm) 162.4, 161.2, 152.5, 150.3, 146.3, 133.8, 131.7, 130.4, 130.2, 124.1, 120.5.

1,3-Dimethyl-5-(2-nitrobenzylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**3d**), from 1,3-dimethylbarbituric acid (**1d**); 281 mg (97%); white solid; mp 158–159 °C (lit. [16], 159–161 °C). ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 8.71 (s, 1H), 8.26 (dd, *J* = 8.3 and 1.2 Hz, 1H), 7.80 (td, *J* = 7.6 and 1.3 Hz, 1H), 7.69 (t, *J* = 7.7 Hz, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 3.25 (s, 3H), 3.06 (s, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm) 161.2, 160.0, 153.7, 151.1, 146.2, 133.9, 132.0, 130.1, 130.0, 124.1, 120.1, 28.4, 27.8.

4.1.4. 3-Substituted-2,1-benzisoxazoles **8a–d**

A stirred suspension of related 5-benzylidenepyrimidine **3a–d** (1 mmol), tin chloride dihydrate (2 mmol), and concentrated hydrochloric acid (1.1 mL) was refluxed in methanol (35 mL) for 2 h. The obtained hot mixture was filtered. The solid was washed with ethanol and ethyl ether to afford the following 3-substituted-2,1-benzisoxazoles.

5-(Benzo[*c*]isoxazol-3(1*H*)-ylidene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (**8a**), from 5-(2-nitrobenzylidene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (**3a**); 215 mg (82%); yellow solid; mp 207–208 °C decomposes. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.05 (s, 2H, 1- and 3-NH), 7.88 (d, *J* = 8.8 Hz, 1H, 4'-ArCH), 7.34 (d, *J* = 9.0 Hz, 1H, 7'-ArCH), 7.22 (dd, *J* = 9.0 and 6.3 Hz, 1H, 6'-ArCH), 6.73 (dd, *J* = 8.8 and 6.3 Hz, 1H, 5'-ArCH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm) 174.5 (2-CS), 166.1 (3'-C), 161.0 (4 and 6-CO), 156.3 (7a'-ArC), 130.3 (6'-ArCH), 125.4 (4'-ArCH), 119.3 (5'-ArCH), 114.2 (3a'-C), 113.1 (7'-ArCH), 85.0 (5-C); IR ν_{\max} (cm⁻¹) 3200–2200 (br), 3381, 2879, 2709, 1664, 1607, 1506, 1466, 1357, 1282, 1251, 1164, 1005, 939, 907, 785, 754, 714; HMRS (ESI-TOF) *m/z*: (M⁺+H) calcd for C₁₁H₈N₃O₃S 262.0281, found 262.0281.

5-(Benzo[*c*]isoxazol-3(1*H*)-ylidene)-1,3-diphenyl-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (**8b**), from 5-(2-nitrobenzylidene)-1,3-diphenyl-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (**3b**); 278 mg (67%); yellow solid; mp 255–256 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 7.80 (d, *J* = 8.8 Hz, 1H, 4'-ArCH), 7.40 (t, *J* = 7.7 Hz, 4H, 3''- and 5''-ArCH), 7.35 (d, *J* = 8.9 Hz, 1H, 7'-ArCH), 7.29 (t, *J* = 7.4 Hz, 2H, 4''-ArCH), 7.24–7.19 (m, 5H, 2''-, 6''- and 6'-ArCH), 6.71 (dd, *J* = 8.9 and 6.2 Hz, 1H, 5'-ArCH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm) 178.6 (2-CS), 166.3 (3'-C), 159.6 (4 and 6-CO), 156.1 (7a'-ArC), 141.8 (1''-ArC), 130.2 (6'-ArCH), 129.6 (2''- and 6''-ArCH), 128.4 (3''- and 5''-ArCH), 126.9 (4''-ArCH), 125.3 (4'-ArCH), 119.3 (5'-ArCH), 114.3 (3a'-ArC), 113.1 (7'-ArCH), 85.4 (5-C); IR ν_{\max} (cm⁻¹) 3200–2200 (br), 3041, 2791, 2718, 1684, 1614, 1592, 1467, 1412, 1347, 1297, 1246, 1183, 955, 902, 764, 741, 684, 603; HMRS (ESI-TOF) *m/z*: (M⁺+H) calcd for C₂₃H₁₆N₃O₃S 414.0907, found 414.0908.

5-(Benzo[*c*]isoxazol-3(1*H*)-ylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**8c**), from 5-(2-nitrobenzylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**3c**); 195 mg (79%); yellow solid; mp 266–268 °C decomposes. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.69 (s, 1H), 7.77 (dt, *J* = 8.8 and 1.7 Hz, 1H, 4'-ArCH), 7.46 (dt, *J* = 9.1 and 1.7 Hz, 1H, 7'-ArCH), 7.32 (ddd, *J* = 9.1, 6.4, and 0.8 Hz, 1H, 6'-ArCH), 6.89 (ddd, *J* = 8.8, 6.4, and 0.8 Hz, 1H, 5'-ArCH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm) 163.6 (3'-C), 162.7 (4 and 6-CO), 156.4 (7a'-ArC), 150.8 (2-CO), 131.8 (6'-ArCH), 124.1 (4'-ArCH), 122.0 (5'-ArCH), 116.2 (3a'-C), 113.7 (7'-ArCH), 82.7 (5-C); IR ν_{\max} (cm⁻¹) 3200–2200 (br), 3174, 2960, 2813, 1722, 1666, 1648, 1601, 1440, 1403, 1360, 1320, 1286, 1238, 1142, 906, 841, 766, 752; HMRS (ESI-TOF) *m/z*: (M⁺+H) calcd for C₁₁H₈N₃O₄ 246.0509, found 246.0511.

5-(Benzo[*c*]isoxazol-3(1*H*)-ylidene)-1,3-dimethylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**8d**), from 1,3-dimethyl-5-(2-nitrobenzylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**3d**); 140 mg (51%); yellow solid; yellow crystals by crystallization in dichloromethane/ethanol; mp 211–213 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.90 (dt, *J* = 8.8 and 1.1 Hz, 1H, 4'-ArCH), 7.37 (dt, *J* = 8.9 and 1.0 Hz, 1H, 7'-ArCH), 7.29 (ddd, *J* = 8.9, 6.2, and 1.1 Hz, 1H, 6'-ArCH), 6.80 (ddd, *J* = 8.8, 6.3, and 0.9 Hz, 1H, 5'-ArCH), 3.17 (s, 6H, 1- and 3-NCH₃); ¹³C NMR (101 MHz, DMSO-*d*₆) δ (ppm) 167.4 (3'-C), 160.9 (4- and 6-CO), 155.9 (7a'-ArC), 152.3 (2-CO), 131.1 (6'-ArCH), 125.5 (4'-ArCH), 120.0 (5'-ArCH), 114.9 (3a'-C), 112.9 (7'-ArCH), 82.5 (5-C), 27.7 (1 and 3-NCH₃); IR ν_{\max} (cm⁻¹) 3200–2200 (br), 2922, 2852, 2715, 1707, 1644, 1604, 1445, 1353, 1230, 1175, 1142, 1068, 899, 760, 749, 683; HMRS (ESI-TOF) *m/z*: (M⁺+H) calcd for C₁₃H₁₂N₃O₄ 274.0822, found 274.0827.

4.2. X-ray analysis

Crystals suitable for single-crystal X-ray analysis were grown from a mixture of ethanol and dichloromethane. Selected crystals were covered with polyfluoroether oil (Fombling, Sigma Aldrich) and mounted on a nylon loop. The data were collected at 110(2) K using a Bruker D8 Venture diffractometer equipped with a Photon 100 detector and an Oxford Crystem Cooler, using graphite monochromated Mo K α radiation (λ = 0.71073 Å). Table S1

(Supplementary information) contains crystallographic experimental data and structure refinement parameters. CCDC 1557900 contains the supplementary crystallographic data for this article. The data can be obtained free of charge from the Cambridge Crystallographic Data Centre via <http://www.ccdc.cam.ac.uk/structures>.

4.3. Biological evaluation

Preparation of sample solutions and experimental procedures are presented in [Supplementary information](#).

Acknowledgments

This work is supported by FEDER funds through the POCI—COMPETE 2020—Operational Programme Competitiveness and Internationalisation in Axis I—Strengthening research, technological development and innovation (Project POCI-01-0145-FEDER-007491) and National Funds by FCT—Foundation for Science and Technology (Project UID/Multi/00709/2013). The SCXR determinations were performed at UCIBIO/FCT-NOVA, financed by national funds from FCT/MEC (UID/Multi/04378/2013), and cofinanced by the ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007728).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.crci.2017.10.002>.

References

- [1] (a) M.A. Barmade, P.R. Murumkar, M.K. Sharma, M.R. Yadav, *Curr. Top. Med. Chem.* 16 (2016) 2863–2883;
- (b) Y. Uto, *Expert Opin. Ther. Pat.* 25 (2015) 643–662;
- (c) R.A. Shastri, *Chem. Sci. Trans.* 5 (2016) 8–20;
- (d) S.Y. Kwan, Y.C. Chuang, C.W. Huang, T.C. Chen, S.B. Jou, A. Dash, *CNS Neurosci. Ther.* 21 (2015) 683–691;
- (e) J.H. Cox, S. Seri, A.E. Cavanna, *Adv. Ther.* 31 (2014) 276–288.
- [2] (a) M. Wiclaw, M. Bobin, A. Kwast, R. Bujok, Z. Wrobel, K. Wojciechowski, *Mol. Divers.* 19 (2015) 807–816 and references cited therein;
- (b) A.V. Budruev, D.Y. Dzhons, *Chem. Heterocycl. Compd.* 52 (2016) 441–443 and references therein.
- [3] P. Friedländer, R. Henriques, *Ber. Dtsch. Chem. Ges.* 15 (1882) 2105–2110.
- [4] A.D. Kotov, M.A. Prokaznikov, E.A. Antonova, A.I. Rusakov, *Chem. Heterocycl. Compd.* 50 (2014) 647–657 and references cited therein.
- [5] (a) K.Y. Lee, J.M. Kim, J.N. Kim, *Bull. Korean Chem. Soc.* 23 (2002) 1493–1495;
- (b) K.Y. Lee, H.S. Lee, J.N. Kim, *Bull. Korean Chem. Soc.* 28 (2007) 333–335;
- (c) B.J. Stokes, C.V. Vogel, L.K. Urnezis, M. Pan, T.G. Driver, *Org. Lett.* 12 (2010) 2884–2887.
- [6] H. Schulte, *Chem. Ber.* 87 (1954) 820–824.
- [7] M.L. Deb, P.J. Bhuyan, *Tetrahedron Lett.* 46 (2005) 6453–6456.
- [8] H. Faustino, R.M. El-Shishtawy, L.V. Reis, P.F. Santos, P. Almeida, *Tetrahedron Lett.* 49 (2008) 6907–6909.
- [9] V. Pilepić, S. Ursić, *J. Mol. Struct. (Theochem.)* 538 (2001) 41–49.
- [10] L.F. Fieser, M. Fieser, *Reagents for Organic Synthesis*, Wiley, New York, 1967, pp. 1113–1116.
- [11] (a) R.M. Shaker, E.A. Ishak, Z. Naturforsch. 66b (2011) 1189–1201;
- (b) G.M. Ziarani, F. Aleali, N. Lashgari, *RSC Adv.* 6 (2016) 50895–50922;
- (c) K.M. Khan, M. Khan, A. Karim, M. Taha, N. Ambreen, A. Gojavey, S. Perveen, M.I. Choudhary, *J. Chem. Soc. Pak.* 35 (2013) 495–498.
- [12] (a) F. López-Muñoz, R. Ucha-Udabe, C. Alamo, *Neuropsychiatric Dis. Treat.* 1 (2005) 329;
- (b) T.L. Lemke, D.A. Williams, *Wolters Kluwer Health* (2012) 485–507.
- [13] A. Lu, Z. Wang, Z. Zhou, J. Chen, Q. Wang, *J. Agric. Food Chem.* 63 (2015) 1378–1384.
- [14] F. Wang, P. Zhao, C. Xi, *Tetrahedron Lett.* 52 (2011) 231–235.
- [15] M. Kuanar, B.K. Mishra, *Indian J. Chem., Sect. A: Inorg., Bio-inorg., Phys., Theor. Anal. Chem.* 34 (1995) 721–723.
- [16] N. Seyyedi, F. Shirini, M.S. Nikoo Langarudi, *RSC Adv.* 6 (2016) 44630–44640.
- [17] M.K. Haldar, M.D. Scott, N. Sule, D.K. Srivastava, S. Mallik, *Bioorg. Med. Chem. Lett.* 18 (2008) 2373–2376.