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Synthesis of polysubstituted benzofuran derivatives as novel inhibitors of parasitic growth

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ABSTRACT

A series of polysubstituted benzofuran derivatives was easily and rapidly prepared using a tandem Sonogashira coupling/cyclization reaction. Subsequent acylation afforded a small library of 39 new compounds that were assayed in cellulo on *Plasmodium falciparum* and *Trypanosoma brucei* parasites. Some of them exhibited good inhibitory activity on *T. brucei* proliferation.

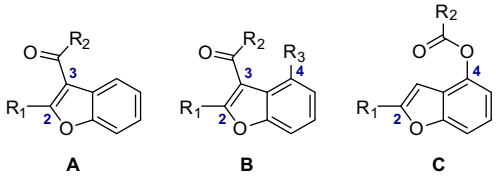
1. Introduction

According to WHO reports, *Plasmodium falciparum* is the major cause of malaria in humans, with 200–300 million individuals affected annually, causing 200–250 thousand deaths,[1] while *Trypanosoma brucei*, responsible for the African sleeping sickness, concerns 30 thousand persons and is lethal in absence of any treatment.[2] Currently resistance phenomena to established therapeutics against these diseases has dramatically developed. Hence new efficient antiparasitic agents are deeply needed.

In the course of our search for new active molecules, we synthesized intermediate benzofuran derivatives that displayed good antiparasitic activity. Benzofuran containing molecules have already been described to possess many biological properties as antiinflammatory,[3,4] antivasoconstriction,[5] antimicrobial[6] or antifungal.[7,8] However, to the best of our knowledge, few authors studied their potential antiparasitic activity.[9–13] From the chemical standpoint, many syntheses of molecules possessing a benzofuran motif have been developed. Recently rapid metal-catalyzed methods yielding benzofurans from phenols were described.[14–17] As well, Singh and Wirth published an efficient metal-free cyclization of *o*-hydroxystilbenes to benzofurans catalyzed by hypervalent iodine.[18] Nevertheless 2,3-, 2,4- and 2,3,4-trisubstituted benzofurans herein reported have rarely been studied. For all these reasons we decided to create a library of polysubstituted benzofuran derivatives to evaluate their antiproliferative effects on *P. falciparum* and on *T. brucei*.

Thus we synthesized benzofuran derivatives substituted at position 2 by an aryl or cyclopropyl group. These compounds were either *C*-acylated at position 3 (**A** and **B**, Fig. 1) or *O*-acylated at position 4 (**C**, Fig. 1), the acyl substituent R_2 being an aryl moiety bearing various substituents. In order to increase the molecular diversity in the *C*-acylated series, R_3 at position 4 is either a hydrogen atom (**A** series), a hydroxyl group or a methoxy substituent (**B** series).

Herein, we describe an easy and rapid synthesis of polysubstituted benzofuran derivatives along with their biological evaluation on *P. falciparum* and *T. brucei* proliferation.



R_1 = aryl, cyclopropyl
R_2 = aryl
R_3= OH, OMe

Figure 1. General structures of benzofuran derivatives.

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2. Chemistry

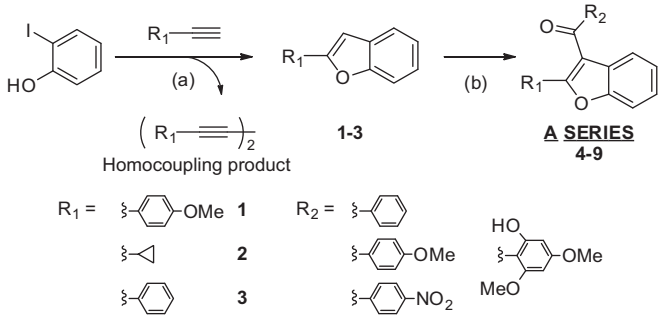
2.1. Tandem Sonogashira coupling/cyclization reaction

2-Substituted benzofurans **1**–**3** and **11**–**14** were synthesized via a tandem Sonogashira coupling/cyclization reaction from the commercially available 2-iodophenol (Scheme 1) and from the prepared 2-iodoresorcinol (Scheme 2), respectively. The substituent R_1 (aryl or cyclopropyl) was brought about by acetylene derivatives. Though this same Sonogashira coupling reaction has already been reported with 2-iodoresorcinol,[19] it was only obtained with hexyl or phenyl R_1 substituent in 50% and 60% yield, respectively. Therefore we tried to optimize this coupling reaction. Our major aim was to avoid the Glaser coupling affording the dimer products from terminal alkynes.[20–22] Thus we found that a perfectly degassed solvent by using the freeze-pump-thaw method could minimize this homocoupling reaction.

These conditions combined with the use of 3 equiv of alkynes allowed to significantly increase the reaction yield up to 77% for benzofuran-4-ol **11** (Scheme 2). Afterwards they were applied for the formation of all 2-substituted derivatives. Thus synthesis of benzofurans **1**–**3** (Scheme 1) was performed with moderate to high yield (50–88%) as well as benzofuran-4-ols **11**–**13** (Scheme 2) (70–77%). Only compound **14** possessing a xylyl group (Scheme 2) was obtained in low yield (12%). Formation of benzofurans required diluted conditions in order to minimize homocoupling reaction. Therefore, faced with a scale-up issue for derivatives **1**–**3**, we decided to use conditions described by Alami's group[23] allowing the cyclization into benzofurans from 2-iodoanisole in two steps (Scheme 3). Thus through this method, multigram scale reactions have been carried out giving 5- to 10-fold greater amounts of desired product. Moreover, formation of benzofuran **1** substituted by a 4-methoxyphenyl group was performed in quantitative yield, whereas a 50% yield was obtained using Sonogashira coupling reaction. On the contrary, much better yields were observed with the tandem reaction for the synthesis of compounds **2** and **3** with 88% and 70% yields, respectively, compared to 65% and 16% obtained in two steps, respectively.

2.2. Friedel–Crafts acylation

A standard Friedel–Crafts acylation using tin(IV) chloride was then carried out to form compounds **4**–**9** (**A** series), **19**–**25** and **26**–**31** (**B** series) bearing a hydrogen atom, a hydroxyl group and a methoxy substituent at position 4 of benzofuran motif, respectively (Scheme 2 and Table 1). The acyl chlorides used in this reaction were generally commercially available except di- and trisubstituted derivatives that were prepared just before the acylation reaction from the corresponding acid and thionyl chloride. Few derivatives of the **A** series were prepared because they early displayed low antiparasitic activity (see Table 3). Acylation yields for **A** series (Table 1) were low with *p*-nitro- and trisubstituted benzoyl chlorides (compounds **5**, **6** and **9**) and the 2-cyclopropyl-3-(*p*-nitrobenzoyl)benzofuran could not be obtained under these conditions. However, moderate to excellent yields were reached with benzoyl chloride (compounds **4**, **7** and **8**).



Scheme 1. Reagents and conditions: (a) $PdCl_2(PPh_3)_2$ (5 mol %), CuI (10 mol %), Et_3N (10 equiv), MeCN, 60 °C, overnight, **1**: 50%, **2**: 88%, **3**: 70%; (b) R_2–COCl (1.2 equiv), $SnCl_4$ (1.2 equiv), DCM, rt, 1–5 h, 13–86% (see Table 1).

In order to avoid esterification of the 4-OH group in the **B** series, protection of benzofuran-4-ols **11**–**14** was first carried out (Scheme 2). Hence introduction of a benzyl or a methyl group was performed yielding derivatives **15**–**18** with excellent yields (81–92%). Under acylation conditions, the benzyl protecting group was hydrolyzed affording the direct formation of 2,3,4-trisubstituted analogues **19**–**25** (Scheme 2 and Table 1). It should be noticed that only traces of 2-phenyl-3-acylbenzofurans were obtained in **B** series. Indeed, acylation of 2-phenyl derivatives was unsuccessful probably because many positions of the 2-phenyl ring were also acylated underFriedel–Crafts conditions, leading to a complex mixture where the expected derivative was generally formed as a minor product. Likewise, all reactions performed with *p*-nitrobenzoyl chloride failed leading to many side products. On the other hand, acylation of compound **18** (R_1 = *p*-methoxyphenyl, R_3 = Me) afforded benzofurans **26**–**31** (Table 1) in better yields (up to 41%). Only acylation with disubstituted benzoyl chloride was less efficient (27% yield). The *p*-methoxyphenyl group was the only R_1 substituent used in this series because of our preliminary biological results (see Table 4).

Though our first goal was to obtain rapidly a small library of polysubstituted benzofurans, we tried to optimize the acylation reaction. Thus we found that using titanium(IV) chloride or aluminium(III) chloride instead of tin(IV) chloride as Lewis acid significantly increased the yield reaction. Indeed 82% yield was obtained instead of 67% for the optimized acylation performed to get compound **26** (Table 1).

2.3. *O*-Acylation

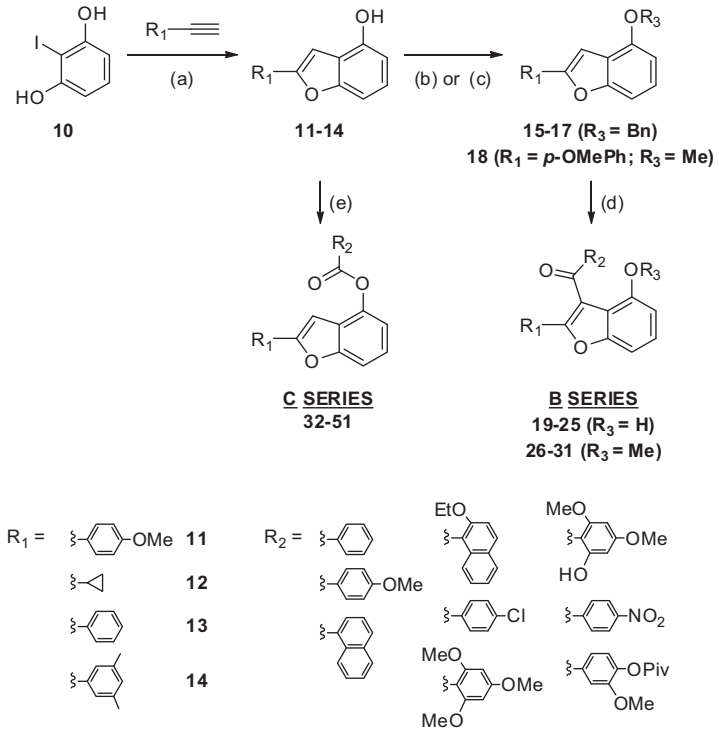
Further *O*-acyl benzofuran derivatives **32**–**51** (**C** series) were prepared from compounds **11**–**14** bearing a free hydroxyl group, according to Scheme 2. Obtained yields (Table 2) were moderate (39–54%) for 2-phenyl-benzofuran derivatives **45**–**50**. Only acylation with *p*-chlorobenzoyl chloride afforded expected compound **49** in good yield (71%). This reaction proved to be more efficient on 2-*p*-methoxyphenyl-benzofuran leading to compounds **32**–**38** in good yield but the best results were obtained on 2-cyclopropylbenzofuran leading to compounds **39**–**44** in good to excellent yields (69–96%) except with 2-ethoxynaphthaloyl chloride. This reaction led to a modest yield only with the trisubstituted benzoyl chloride that was synthesized just before acylation (11% for compound **34**, Table 3).

3. Biology

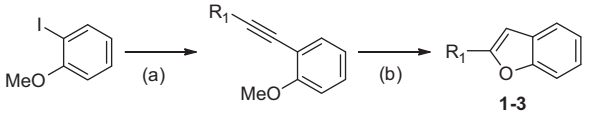
All these benzofuran derivatives were assayed on the intra-erythrocytic stages of *P. falciparum*,[24–26] responsible for malaria and the bloodstream forms of *T. brucei gambiense*, the pathogenic agent of African sleeping sickness.[27–29] Results are summarized in Tables 3–5.

None of the 2,3-disubstituted benzofurans of the **A** series (Table 3) were active against *P. falciparum*. Their activity against *T. brucei* was moderate when R_2 was a phenyl group (compounds **4**, **7** and **8**) and compounds bearing a *p*-nitrobenzoyl moiety at position 3 (compounds **4** and **9**) were inactive. Only compound **6** where R_2 was a trisubstituted phenyl (IC_{50} = 6.7 μM) displayed a better activity.

Like 2,3-disubstituted benzofurans, **B** series derivatives (Table 4) displayed no activity against *P. falciparum*. On the other hand, better results were obtained on *T. brucei*. Almost all derivatives displayed



Scheme 2. Reagents and conditions: (a) $PdCl_2(PPh_3)_2$ (5 mol %), CuI (10 mol %), Et_3N (10 equiv), MeCN, 60 °C, overnight, **11**: 77%, **12**: 73%, **13**: 76%, **14**: 12%; (b) BnBr (2 equiv), K_2CO_3 (2 equiv), acetone, reflux, 4–10 h, 81–86%; (c) MeI (2 equiv), K_2CO_3 (2 equiv), DMF, rt, 19 h, 92%; (d) R_2-COCl (1.2 equiv), $SnCl_4$ or $TiCl_4$ (1.2 equiv), DCM, rt, 1–5 h, 6–83% (see Table 1); (e) R_2-COCl (1.2 equiv), Et_3N (1.2 equiv), DCM, 0 °C to rt, 2–5 h, 27–96% (see Table 2).



Scheme 3. Reagents and conditions: (a) $PdCl_2(PPh_3)_2$ (2 mol %), CuI (1 mol %), Et_3N, rt, overnight; (b) PTSA·H_2O, EtOH, MW (130 °C, 1 h) or sealed tube, 130 °C, 24 h, **1**: 100%, **2**: 65%, **3**: 16% (in two steps).

Table 1
C-Acylation results in the **A** and **B** series

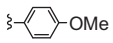
Compd	R_1	R_2	R_3	Yields (%)
4		Phenyl	/	86
5		4-Nitro phenyl	/	27
6		2-Hydroxy-4,6-dimethoxyphenyl	/	17
7		Phenyl	/	53
8		Phenyl	/	83
9		4-Nitro phenyl	/	13
19		Phenyl	H	17
20		4-Methoxyphenyl	H	26
21		1-Naphthyl	H	25
22		2-Ethoxy-1-naphthyl	H	12
23		4-Methoxyphenyl	H	6
24		1-Naphthyl	H	35
25		2-Ethoxy-1-naphthyl	H	28
26		Phenyl	Me	67 (82)[a]
27		4-Methoxyphenyl	Me	67
28		1-Naphthyl	Me	55
29		4-Chlorophenyl	Me	41
30		3-Methoxy-4-*O*-pivaloyl- phenyl	Me	27
31		2,4,6-Trimethoxyphenyl	Me	67

[a] Yield obtained under optimized conditions ($TiCl_4$ 1.2 equiv, DCM, rt, 1 h).

IC_{50} value below 10 μM and only compound **31** was found inactive. The presence of a 2,4,6-trisubstituted phenyl group at position 3 seemed to be detrimental to the inhibitory activity contrary to what was observed in the **A** series. 4-Hydroxybenzofuran derivatives (**19**–**21**) were slightly more active than their corresponding methoxy analogues (**26**–**28**). Replacement of the methoxyphenyl group at position 2 by a cyclopropyl ring did not modify much the inhibitory activity (compare **20**–**22** to **23**–**25**) suggesting that this position is not crucial for activity. Finally compound **22** was found to be the most active derivative of this series on *T. brucei* proliferation (IC_{50} = 1.5 μM). By comparison with the **A** series, 4-position substitution seemed to be beneficial for trypanocidal activities.

Contrary to **A** and **B** series derivatives, some 4-*O*-acylated benzofurans in the **C** series (**33**, **34** and **51**) (Table 5) demonstrated some activity against *P. falciparum*. Two of them (**33** and **34**) bearing a *p*-methoxyphenyl group at position 2 and at least one methoxy substituent in *para* position of the aroyl group displayed IC_{50} values of 11.2 and 7.9 μM, respectively. The most potent antimalarial compound of the series was the benzofuran derivative **51** showing an IC_{50} of 6.0 μM. Therefore the 2-xylyl group seemed to significantly improve the antiplasmodial activity. Better inhibitory activities against *T. brucei* were obtained in the **C** series ($1.4 < IC_{50} < 20$ μM) except for compound **34** bearing a trisubstituted phenyl moiety at position 4. As observed in the **B** series, trisubstituted R_2 group seemed to abolish trypanocidal activity. Generally the R_1 substituent had little influence on the activity. However, for the *O*-benzoylbenzofurans **32**, **39**, **45** and **51** (Table 5), we observed that inhibition increased with the steric hindrance of the 2-substituent, *m*-xylyl compound **51** being more active than *p*-methoxyphenyl **32**, phenyl **45** and cyclopropyl **39**, successively. Furthermore, when R_2 was a 4-chlorophenyl or 4-nitrophenyl group lower IC_{50} values were obtained with the cyclopropyl derivatives.

Table 2
O-Acylation results in the **C** series

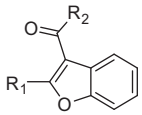
Compd	R_1	R_2	Yields (%)
32		Phenyl	77
33		4-Methoxyphenyl	68
34		2-Hydroxy-4,6-dimethoxyphenyl	11
35		1-Naphthyl	60
36		2-Ethoxy-1-naphthyl	54
37		4-Chlorophenyl	70
38		4-Nitrophenyl	62
39		Phenyl	96
40		4-Methoxyphenyl	88
41		1-Naphthyl	69
42		2-Ethoxy-1-naphthyl	19
43		4-Chlorophenyl	86
44		4-Nitrophenyl	78
45		Phenyl	49
46		4-Methoxyphenyl	45
47		1-Naphthyl	48
48		2-Ethoxy-1-naphthyl	39
49		4-Chlorophenyl	71
50		4-Nitrophenyl	48
51		Phenyl	56

Finally, we have noticed that *O*-acylated benzofurans **32**–**51** mainly possessed a little higher activities than their *C*-acyl homologues **19**–**30** (compounds **20** and **23** compared to derivatives **33** and **40**, respectively), except when these latters bear a naphthoyl group substituted or not. In that case, better inhibitory effects were observed for *C*-acyl benzofurans (compounds **21**, **22**, **24**, **25** in Table 4 compared to benzofurans **35**, **36**, **41**, **42** in Table 5 respectively). It should be noted that the best inhibitory activity on *T. brucei* proliferation (IC_{50} <2 μM) was observed in the **B** series with the 2-ethoxynaphthoyl derivative **22** and in the **C** series with *p*-substituted esters of the 2-aryl-4-hydroxybenzofuran (**33**, **37**, **38**, **46**, **49** and **50**).

4. Conclusion

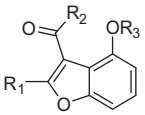
To conclude, we have reported the easy and rapid synthesis of a novel series of 39 polysubstituted benzofuran derivatives. Indeed 2 and 3 steps were only necessary to obtain 4-*O*-acyl and 3-*C*-acyl benzofurans, respectively, via a tandem Sonogashira coupling/cyclization reaction. All these compounds were then assayed for their antiparasitic effects. Most of them displayed good inhibitory activities on *T. brucei* proliferation possessing IC_{50} values below 10 μM. The most potent trypanocidal inhibitors of this novel class with micromolar IC_{50} values include one 3-*C*-acyl-2-substituted benzofuran-4-ol and six 4-*O*-acyl-2-substituted benzofurans. The

Table 3
Inhibitory activity of 3-acyl-2-substituted benzofuran derivatives **4**–**9** (**A** series)



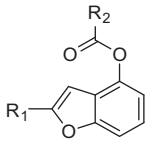
Compd	R_1	R_2	IC_{50} (μM)	IC_{50} (μM)
			P. f	*T. b*
4	4-Methoxy phenyl	Phenyl	>50	21.2 ± 0.9
5	4-Methoxy phenyl	4-Nitro phenyl	>50	>50
6	4-Methoxy phenyl	2-Hydroxy-4,6-dimethoxyphenyl	>25	6.7 ± 0.5
7	Cyclopropyl	Phenyl	>50	20.9 ± 2.0
8	Phenyl	Phenyl	>50	15.5 ± 1.9
9	Phenyl	4-Nitro phenyl	>50	>50

Table 4
Inhibitory activity of 2,3,4-trisubstituted benzofuran derivatives **19**–**31** (**B** series)



Compd	R_1	R_2	R_3	IC_{50} (μM)	IC_{50} (μM)
				P. falciparum	*T. brucei*
19	4-Methoxyphenyl	Phenyl	H	>25	6.5 ± 0.6
20	4-Methoxyphenyl	4-Methoxyphenyl	H	>25	7.7
21	4-Methoxyphenyl	1-Naphthyl	H	>50	4.2 ± 0.5
22	4-Methoxyphenyl	2-Ethoxy-1-naphthyl	H	>25	1.5 ± 0.2
23	Cyclopropyl	4-Methoxyphenyl	H	>50	3.7 ± 0.7
24	Cyclopropyl	1-Naphthyl	H	>50	4.6 ± 0.1
25	Cyclopropyl	2-Ethoxy-1-naphthyl	H	>25	2.0 ± 0.2
26	4-Methoxyphenyl	Phenyl	Me	>50	11.8 ± 2.6
27	4-Methoxyphenyl	4-Methoxyphenyl	Me	>25	8.3 ± 1.3
28	4-Methoxyphenyl	1-Naphthyl	Me	>50	12.4 ± 1.8
29	4-Methoxyphenyl	4-Chlorophenyl	Me	>50	5.5 ± 1.2
30	4-Methoxyphenyl	3-Methoxy-4-*O*-pivaloyl-phenyl	Me	>50	8.4 ± 1.2
31	4-Methoxyphenyl	2,4,6-Trimethoxyphenyl	Me	>50	>50
Chloroquine				0.072 ± 0.0074	
Pentamidine					0.011 ± 0.0017

Table 5
Inhibitory activity of 4-*O*-acyl benzofuran derivatives **32**–**51** (**C** series)



Compd	R_1	R_2	IC_{50} (μM)	IC_{50} (μM)
			P. falciparum	*T. brucei*
32	4-Methoxyphenyl	Phenyl	>25	3.2 ± 0.3
33	4-Methoxyphenyl	4-Methoxyphenyl	11.2 ± 0.1	1.8 ± 0.1
34	4-Methoxyphenyl	2-Hydroxy-4,6-dimethoxyphenyl	7.9 ± 0.3	>50
35	4-Methoxyphenyl	1-Naphthyl	>50	14.9 ± 6.2
36	4-Methoxyphenyl	2-Ethoxy-1-naphthyl	>50	7.7 ± 1.0
37	4-Methoxyphenyl	4-chlorophenyl	>25	1.5 ± 0.2
38	4-Methoxyphenyl	4-Nitrophenyl	>50	1.7 ± 0.1
39	Cyclopropyl	Phenyl	>50	14.7 ± 1.0
40	Cyclopropyl	4-Methoxyphenyl	>50	2.7 ± 0.2
41	Cyclopropyl	1-Naphthyl	>50	12.6 ± 0.8
42	Cyclopropyl	2-Ethoxy-1-naphthyl	>25	6.4 ± 0.4
43	Cyclopropyl	4-Chlorophenyl	>50	7.1 ± 0.4
44	Cyclopropyl	4-Nitrophenyl	>50	12.2 ± 1.0
45	Phenyl	Phenyl	>25	14.0
46	Phenyl	4-Methoxyphenyl	>25	1.7 ± 0.1
47	Phenyl	1-Naphthyl	>25	21.3 ± 0.7
48	Phenyl	2-Ethoxy-1-naphthyl	>50	6.7 ± 0.2
49	Phenyl	4-Chlorophenyl	>25	1.6 ± 0.0
50	Phenyl	4-Nitrophenyl	>25	1.8 ± 0.1
51	*m*-Xylyl	Phenyl	6.0 ± 0.9	2.0 ± 0.1

highest activities were found when there is a 4-methoxyphenyl group at position 2, though modification at this position is possible, and with a *p*-substituted acyl group. On the other hand, benzofuran derivative **51** substituted by a xylyl group was found to be the most potent compound on *P. falciparum* with IC_{50} = 6.0 μM. Accordingly these promising results are paving the way for further investigations on neglected antiparasitic activity of polysubstituted benzofuran derivatives. For instance we envision to add amidine or guanidine substituents that proved beneficial for antiparasitic activities as reported by Tidwell's group.[10]

5. Experimental section

5.1. General experimental procedures

All commercial reagents were used without any further purification. Analytical thin-layer chromatography was carried out on precoated silica gel aluminium plates (SDS TLC plates, silica gel $60F_{254}$). Column chromatography was performed prepacked Redisep columns. Preparative TLC (PLC) was performed on Merck TLC with silica gel $60F_{254}$. NMR spectra, including ^1H, ^{13}C (HMQC and HMBC) experiments, were recorded on a Brucker Avance 300 (300 MHz) and Avance 500 (500 MHz) spectrometers. Chemical shifts (δ) are given in ppm relative to $CDCl_3$ (7.26 ppm; 77.2 ppm), CD_3OD (3.34 ppm; 49.9 ppm), acetone-d_6 (2.05 ppm; 30.5 ppm) or DMSO-d_6 (2.50 ppm; 39.5 ppm). Splitting patterns are designed as: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and combinations thereof. Coupling constants *J* are reported in hertz (Hz). IR spectra were recorded on a Perkin–Elmer Spectrum BX. Mass spectra were recorded on Thermoquest AQA Navigator with a TOF detection (ESI-HRMS) and on MALDI (matrix: DCTB). UHPLC analyses were realized on Waters Acquity UPLC. Melting points were measured on Büchi b-450 and are uncorrected.

The purity of all target compounds was measured using reversed-phase UHPLC (HSS C-18, 2.1 × 50 mm column): compounds were eluted with 95:5 A/B for 0.5 min then with a gradient of 5–100% B/A for 3.5 min followed by 0:100 isocratic for 1 min at a flow rate of 0.6 mL/min, where solvent A was 0.1% formic acid in H_2O and solvent B was 0.1% formic acid in CH_3CN. Purity was determined on TAC (total absorbance current from 200 to 400 nM).

5.2. Preparation of 2-substituted benzofurans 1–3 and 2-substituted benzofuran-4-ols (11–14)

To a well-degazed MeCN (90 mL) via a 3 cycles of freeze-pump-thaw, phenol derivative (1 mmol), $PdCl_2(PPh_3)_2$ (5 mol %), degazed Et_3N (10 mmol), terminal alkyne (3 mmol) and CuI (10 mol %) were successively added under argon. The reaction mixture was allowed to stir overnight at 60 °C. The resulting solution was concentrated under reduced pressure and the residue obtained was dissolved in HCl 1 N solution and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with a saturated aq. NaCl solution, dried over anhydrous $MgSO_4$ and concentrated under vacuum. The crude product was then purified by flash chromatography on silica gel.

5.2.1. 2-(4-Methoxyphenyl)benzofuran (1)

Flash chromatography using as eluent: gradient heptane to heptane/AcOEt 9:1 (v/v) in 25 min. The product was obtained as a white amorphous solid (50%): ^1H NMR ($CDCl_3$) δ 7.83 (d, 2H, *J* = 9.0 Hz), 7.60–7.52 (m, 2H), 7.29–7.24 (m, 2H), 7.00 (d, 2H, *J* = 8.7 Hz), 6.91 (s, 1H), 3.88 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 160.3, 156.4, 155.0, 129.8, 126.7, 124.7, 124.1, 123.2, 120.9, 114.6, 111.3, 100.0, 55.7; MS (ESI^+, MeOH+CH_2Cl_2) *m*/*z* 224.8 $[M+H]^+$.

5.2.2. 2-Cyclopropylbenzofuran (2)

Flash chromatography using as eluent: isocratic heptane. The product was obtained as a colorless oil (88%): ^1H NMR (Acetone-d_6) δ 7.49–7.46 (m, 1H), 7.40–7.37 (m, 1H), 7.20 (td, 1H, *J* = 7.2, 1.8 Hz), 7.16 (td, 1H, *J* = 7.2, 1.8 Hz), 6.48 (s, 1H), 2.15–2.04 (m, 1H), 1.05–0.90 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 161.5, 155.2, 130.1, 123.8, 123.4, 120.9, 111.2, 101.0, 100.9, 9.81, 7.62; MS (MALDI) *m*/*z* 158.06 $[M]^+$; HRMS calcd for $C_{11}H_{10}O^+$ $[M]^+$ 158.0732, found

158.0723; IR (neat, cm^{-1}) 1604, 1455, 1176, 1043, 1025; UPLC method (H_2O/MeCN): rt, 4.62 min, 100%.

5.2.3. 2-Phenylbenzofuran (3)

Flash chromatography using as eluent: isocratic heptane. The product was obtained as a white amorphous solid (70%): ^1H NMR (Acetone-d_6) δ 7.94 (d, 2H, J = 7.5 Hz), 7.64 (dd, 1H, J = 7.5, 1.5 Hz), 7.57 (dd, 1H, J = 7.5, 1.5 Hz), 7.50 (td, 2H, J = 7.5 Hz), 7.40 (td, 1H, J = 8.1, 1.5 Hz), 7.31 (td, 1H, J = 7.5, 1.5 Hz), 7.29 (s, 1H), 7.25 (td, 1H, J = 7.5, 1.5 Hz); ^{13}C NMR (Acetone-d_6) δ 156.7, 155.7, 131.3, 130.2, 129.8, 129.6, 125.6, 125.4, 124.0, 122.0, 111.9, 102.5; MS (MALDI) m/z 195.1 $[M]^+$; HRMS calcd for $C_{14}H_{10}O_3^+$ 194.0732, found 194.0729; IR (neat, cm^{-1}) 1456, 1259, 1170, 1020; mp: 120–121 °C; UPLC method (H_2O/MeCN): rt, 5.16 min, 100%.

5.2.4. 2-(4-Methoxyphenyl)benzofuran-4-ol (11)

Flash chromatography using as eluent: gradient toluene to toluene/EtOAc 95:5 (v/v) in 25 min. The product was obtained as a brown amorphous solid (77%): ^1H NMR ($CDCl_3$) δ 7.78 (d, 2H, J = 9.0 Hz), 7.12–7.07 (m, 2H), 6.98 (d, 2H, J = 9.0 Hz), 6.95 (s, 1H), 6.62 (dd, 1H, J = 6.3, 2.1 Hz), 5.20 (s, 1H), 3.86 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 160.0, 156.4, 155.2, 149.0, 126.4, 124.5, 123.4, 114.4, 112.1, 108.1, 104.4, 96.5, 55.5; MS (ESI, MeOH+CH_2Cl_2) m/z 241.1 $[M+H]^+$; HRMS calcd for $C_{15}H_{11}O_3^-$ 239.0708, found 239.0704; IR (neat, cm^{-1}) 3452, 1177, 1076 cm^{-1}; mp: 159–160 °C; UPLC method (H_2O/MeCN): rt, 4.13 min, 95%.

5.2.5. 2-Cyclopropylbenzofuran-4-ol (12)

Flash chromatography using as eluent: gradient heptane to heptane/EtOAc 8:2 (v/v) in 25 min. The product was obtained as a brown oil (73%): ^1H NMR ($CDCl_3$) δ 7.03 (t, 1H, J = 8.0 Hz), 6.99 (d, 1H, J = 8.0 Hz), 6.57 (d, 1H, J = 8.0 Hz), 6,40 (s, 1H), 4.93 (s, 1H), 2.03–1.99 (m, 1H), 1.00–0.92 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 159.6, 156.0, 148.3, 123.7, 118.1, 107.9, 104.1, 96.9, 9.39, 7.35; MS (MALDI) m/z 174.0 $[M]^+$; HRMS calcd for $C_{11}H_{10}O_2^+$ $[M]^+$ 174.0681, found 174.0681; IR (neat, cm^{-1}) 2963, 1258, 1013, 794; UPLC method (H_2O/MeCN): rt, 3.58 min, 100%.

5.2.6. 2-Phenylbenzofuran-4-ol (13)

Flash chromatography using as eluent: gradient heptane to heptane/EtOAc 8:2 (v/v) in 25 min. The product was obtained as a beige amorphous solid (76%): ^1H NMR ($CDCl_3$) δ 7.86 (d, 2H, J = 7.5 Hz), 7.45 (t, 2H, J = 7.5 Hz), 7.35 (t, 1H, J = 7.5 Hz), 7.15 (d, 1H, J = 7.0 Hz), 7.14 (t, 1H, J = 7.0 Hz), 7.10 (s, 1H), 6.64 (d, 1H, J = 7.0 Hz), 5.06 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 157.0, 155.3, 149.2, 130.5, 128.9, 128.6, 125.1, 125.0, 118.6, 108.1, 104.6, 98.1; MS (MALDI) m/z 210.05 $[M]^+$ HRMS calcd for $C_{14}H_{10}O_2^+$ $[M]^+$ 210.0680, found 210.0680; IR (neat, cm^{-1}) 3272, 1603, 1251, 1028; mp: 163–164 °C; UPLC method (H_2O/MeCN): rt, 4.17 min, 100%.

5.2.7. 2-(3,5-Dimethylphenyl)benzofuran-4-ol (14)

Flash chromatography using as eluent: gradient heptane to heptane/EtOAc 8:2 (v/v) in 25 min. The product was obtained as a brown oil (12%): ^1H NMR ($CDCl_3$) δ 7.49 (s, 2H), 7.15 (t, 1H, J = 8.0 Hz), 7.12 (d, 1H, J = 8.0 Hz), 7.07 (s, 1H), 7.00 (s, 1H), 6.64 (dd, 1H, J = 8.0, 1.5 Hz), 5.22 (s, 1H), 2.39 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 156.5, 155.4, 149.1, 138.5, 130.5, 130.3, 124.9, 122.8, 118.7, 108.1, 104.5, 97.9, 21.5; MS (MALDI) m/z 238.08 $[M]^+$ HRMS calcd for $C_{16}H_{14}O_2^+$ [M]+. 238.0994, found 238.0980; IR (neat, cm^{-1}) 3260, 1605, 1254, 1084, 1031.

5.3. Benzylation of benzofuran-4-ol 11–14

To a solution of benzofuran-4-ol (1 mmol) in acetone (3 mmol), K_2CO_3 (2 mmol) and BnBr (2 mmol) were successively added at room temperature. The reaction mixture was then refluxed until the reaction was complete. The cooled resulting solution was diluted with water and extracted three times with EtOAc. The combined organic layers were washed two times with a saturated aq. NaCl solution, dried over anhydrous $MgSO_4$ and concentrated under vacuum. The crude product was then purified by flash chromatography on silica gel.

5.3.1. 4-(Benzyloxy)-2-(4-methoxyphenyl)benzofuran (15)

Flash chromatography using as eluent: gradient heptane to heptane/EtOAc 9:1 (v/v) in 25 min. The product was obtained as a white amorphous solid (93%). ^1H NMR ($CDCl_3$) δ 7.79 (d, 2H, J = 9 0 Hz), 7.52–7.35 (m, 5H), 7.17 (d, 2H, J = 4,0 Hz), 7.05 (s, 1H), 6.98 (d, 2H, J = 9.0 Hz), 6.73 (t, 1H, J = 4.0 Hz), 5.23 (s, 2H), 3.86 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 159.8, 155.9, 154.8, 152.5, 137.2, 128.6, 128.0, 127.5, 126.3, 124.4, 123.4, 120.1, 114.3, 104.8, 104.6, 97.4, 70.3, 55.4; MS (ESI, MeOH+CH_2Cl_2) m/z 401.1 $[M+H]^+$; HRMS calcd for $C_{22}H_{19}O_3^+$ $[M+H]^+$ 331.1334, found 331; 1320; IR (neat, cm^{-1}) 1610, 1244, 1072, 1021; mp: 113–114 °C; UPLC method (H_2O/MeCN): rt, 5.84 min, 100%.

5.3.2. 4-(Benzyloxy)-2-cyclopropylbenzofuran (16)

Flash chromatography using as eluent: isocratic heptane. The product was obtained as a colorless oil (81%): ^1H NMR ($CDCl_3$) δ 7.49–7.31 (m, 5H), 7.09 (t, 1H, J = 8.1 Hz), 7.03 (d, 1H, J = 8.1 Hz), 6.68 (d, 1H, J = 8.1 Hz), 6.49 (s, 1H), 5.18 (s, 2H), 2.06–1.97 (m, 1H), 1.01–0.90 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 159.4, 155.8, 152.0, 137.4, 128.7, 128.0, 127.5, 123.6, 119.6, 104.9, 104.5, 97.9, 70.4, 9.36, 7.30; MS (ESI, MeOH+CH_2Cl_2) m/z 265.1 $[M+H]^+$; HRMS calcd for $C_{18}H_{17}O_2^+$ $[M+H]^+$ 265.1228, found 265.1211; IR (neat, cm^{-1}) 1594, 1277, 1071, 1054; UPLC method (H_2O/MeCN): rt, 5.49 min, 96%.

5.3.3. 4-(Benzyloxy)-2-phenylbenzofuran (17)

Flash chromatography using as eluent: gradient heptane to heptane/EtOAc 95:5 (v/v) in 25 min. The product was obtained as a white amorphous solid (86%): ^1H NMR ($CDCl_3$) δ 7.89 (d, 2H, J = 7.5 Hz), 7.54 (d, 2H, J = 7.2 Hz), 7.49–7.19 (m, 6H), 7.23–7.19 (m, 2H), 7.21 (s, 1H), 6.76 (dd, 1H, J = 8.7, 3.3 Hz), 5.25 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 156.2, 154.7, 152.6, 137.1, 130.6, 128.8, 128.6, 128.3, 128.0, 127.5, 125.0, 124.8, 120.0, 104.8, 104.8, 99.1, 70.3; MS (ESI, MeCN+CH_2Cl_2) m/z 301.1 $[M+H]^+$; HRMS calcd for $C_{21}H_{17}O_2^+$ $[M+H]^+$ 301.1228, found 301.1235; IR (neat, cm^{-1}) 1601, 1245, 1070, 1025; mp: 96–97 °C; UPLC method (H_2O/MeCN): rt, 5.92 min, 100%.

5.4. Methylation of benzofuran 11

To a solution of benzofuran **11** (1 mmol) in anhydrous DMF (3 mL), K_2CO_3 (2 mmol) and methyl iodide (2 mmol) (stirring 20 min beforehand) at room temperature. The reaction mixture was allowed to stir under argon at room temperature overnight. The resulting solution was diluted with water and extracted three times with EtOAc. The combined organic layers were washed three times with a saturated aq. NaCl solution, dried over anhydrous $MgSO_4$ and concentrated under vacuum. The crude product was then purified by flash chromatography on silica gel using an eluent gradient heptane to heptane/EtOAc 9:1 (v/v) in 25 min.

5.4.1. 4-Methoxy-2-(4-methoxyphenyl)benzofuran (18)

The product was obtained as a white amorphous solid (92%): ^1H ($CDCl_3$) NMR δ 7.78 (d, 2H, J = 8.5 Hz), 7.20–7.13 (m, 2H), 6.99 (s, 1H), 6.98 (d, 2H, J = 8.5 Hz), 6.66 (d, 1H, J = 7.5 Hz), 3.96 (s, 3H), 3.86 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 159.9, 156.0, 154.9, 153.4, 126.4, 124.5, 123.6, 119.9, 114.4, 104.5, 103.5, 97.3, 55.8, 55.5; MS (ESI, MeCN+CH_2Cl_2) m/z 255.1 $[M+H]^+$; HRMS calcd for $C_{16}H_{15}O_3^+$ $[M+H]^+$ 255.1021,

found 255,0947; IR (neat, cm^{-1}) 1607, 1248, 1095, 1021; mp: 99–100 °C; UPLC method (H_2O/MeCN): rt, 5.08 min, 100%.

5.5. Friedel–Crafts acylation of benzofurans 1–3, 15–17 and 18

To a solution of benzofuran (1 mmol) and acyl chloride (1.2 mmol) in anhydrous CH_2Cl_2 (2 mL), $SnCl_4$ or $AlCl_3$ (1.2 mmol) was added under argon. The mixture was then stirred at room temperature under argon until the reaction was complete (30 min–3 h). The reaction was quenched by addition of crushed ice and the mixture was stirred for one more hour. The resulting solution was diluted with water and extracted three times with CH_2Cl_2. The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under vacuum. The crude product was then purified by flash chromatography on silica gel.

5.5.1. (2-(4-Methoxyphenyl)benzofuran-3-yl)(phenyl)methanone (4)

Flash chromatography using as eluent: heptane/EtOAc 95:5; second flash chromatography using as eluent: toluene. The product was obtained as a yellow oil (86%): ^{1}H NMR (Acetone-d_6) δ 7.83 (d, 2H, J = 8.7 Hz), 7.65 (d, 3H, J = 8.7 Hz), 7.55 (t, 1H, J = 8.7 Hz), 7.54 (d, 1H, J = 8.7 Hz), 7.43–7.37 (m, 3H), 7.29 (td, 1H, J = 8.7, 0.9 Hz), 6.90 (d, 2H, J = 8.7 Hz), 3.80 (s, 3H); ^{13}C NMR (Acetone-d_6) δ 192.4, 162.0, 158.8, 154.5, 139.1, 133.9, 130.9, 130.4, 129.5, 129.4, 126.0, 124.7, 122.7, 121.9, 115.7, 114.8, 112.0, 55.8; MS (MALDI) m/z 328.1 $[M]^+$ HRMS calcd for $C_{22}H_{16}O_3^+$ $[M]^+$ 328.1099, found 328.1089; IR (neat, cm^{-1}) 1724, 1608, 1253, 1176, 1027; UPLC method (H_2O/MeCN): rt, 5.30 min, 100%.

5.5.2. (2-(4-Methoxyphenyl)benzofuran-3-yl)(4-nitrophenyl) methanone (5)

Flash chromatography using as eluent: heptane/EtOAc 95:5 (v/v) in 25 min. The product was obtained as a white amorphous solid (27%): ^{1}H NMR (Acetone-d_6) δ 8.18 (d, 2H, J = 9.0 Hz), 7.98 (d, 2H, J = 9.0 Hz), 7.73 (d, 1H, J = 8.1 Hz), 7.67 (d, 1H, J = 8.1 Hz), 7.59 (d, 2H, J = 9.0 Hz), 7.44 (td, 1H, J = 8.1, 1.2 Hz), 7.35 (td, 1H, J = 8.1, 1.2 Hz), 6.88 (d, 2H, J = 9.0 Hz), 3.78 (s, 3H); ^{13}C NMR (Acetone-d_6) δ 190.9, 162.4, 161.2, 154.7, 150.8, 144.4, 131.7, 131.5, 128.9, 126.4, 125.1, 124.2, 122.3, 122.2, 115.4, 114.8, 112.1, 55.8; MS (MALDI) m/z 373.1 $[M]^+$ HRMS calcd for $C_{22}H_{15}NO_5^+$ $[M]^+$ 373.0950, found 373.0945; IR (neat, cm^{-1}) 1638, 1602, 1518, 1342, 1249, 1071, 1033; mp: 155–156 °C; UPLC method (H_2O/MeCN): rt, 5.23 min, 100%.

5.5.3. (2-Cyclopropylbenzofuran-3-yl)(phenyl)methanone (6)

Flash chromatography using as eluent: heptane/EtOAc 95:5 (v/v) in 25 min. The product was obtained as a white amorphous solid (53%): ^{1}H NMR (Acetone-d_6) δ 7.87 (d, 2H, J = 8.1 Hz), 7.67 (td, 1H, J = 8.1, 1.2 Hz), 7.56 (td, 2H, J = 8.1, 1.2 Hz), 7.47 (dd, 2H, J = 7.5, 1.2 Hz), 7.29 (td, 1H, J = 7.5, 1.2 Hz), 7.23 (td, 1H, J = 7.5, 1.2 Hz), 2.27–2.18 (m, 1H), 1.27–1.21 (m, 2H), 1.12–1.05 (m, 2H); ^{13}C NMR (Acetone-d_6) δ 191.9, 166.5, 153.8, 140.8, 133.3, 129.8, 129.4, 128.4, 125.2, 124.5, 121.7, 117.1, 111.6, 11.0, 9.89; MS (ESI, MeOH+CH_2Cl_2) m/z 263.1 $[M+H]^+$; HRMS calcd for $C_{18}H_{15}O_2^+$ $[M+H]^+$ 263.1072, found 263,1076; IR (neat, cm^{-1}) 1630, 1567, 1270, 1049, 1027; mp: 111–114 °C; UPLC method (H_2O/MeCN): rt, 5.06 min, 93%.

5.5.4. Phenyl(2-phenylbenzofuran-3-yl)methanone (7)

Flash chromatography using as eluent: heptane/EtOAc 95:5 (v/v) in 25 min.The product was obtained as a white amorphous solid (83%): ^{1}H NMR (Acetone-d_6) δ 7.83 (d, 2H, J = 8.7 Hz), 7.70–7.67 (m, 3H), 7.55 (td, 2H, J = 8.7, 1.5 Hz), 7.44 (td, 1H, J = 8.7, 1.5 Hz), 7.42–7.29 (m, 6H); ^{13}C NMR (Acetone-d_6) δ 192.4, 158.2, 154.7, 138.9, 134.0, 130.7, 130.4, 129.4, 129.3, 129.2, 126.5, 124.8, 122.1, 117.0, 112.1; MS (ESI, MeOH+CH_2Cl_2) m/z 299.1 $[M+H]^+$; HRMS calcd for $C_{21}H_{15}O_2^+$ $[M+H]^+$ 299.1072, found 299,1062; IR (neat, cm^{-1}) 1650, 1578, 1243, 1060; mp: 94–96 °C; UPLC method (H_2O/MeCN): rt, 5.34 min, 100%.

5.5.5. (4-Nitrophenyl)(2-phenylbenzofuran-3-yl)methanone (8)

Flash chromatography using as eluent: heptane/EtOAc 95:5 (v/v) in 25 min; second flash chromatography using as eluent: isocratic toluene. The product was obtained as a yellow amorphous solid (13%): ^{1}H NMR (Acetone-d_6) δ 8.17 (d, 2H, J = 8.7 Hz), 8.00 (d, 2H, J = 8.7 Hz), 7.77 (d, 1H, J = 8.1 Hz), 7.72 (d, 1H, J = 8.1 Hz), 7.64 (dd, 2H, J = 9.0, 1.5 Hz), 7.49 (td, 1H, J = 8.1, 1.2 Hz), 7.39 (td, 1H, J = 8.1, 1.2 Hz), 7.34 (m, 3H); ^{13}C NMR (Acetone-d_6) δ 190.9, 160.8, 155.0, 150.9, 144.2, 131.5, 131.2, 130.0, 129.8, 129.3, 128.7, 128.0, 126.8, 125.2, 124.2, 122.4, 112.2; MS (MALDI) m/z 343.1 $[M]^+$ HRMS calcd for $C_{21}H_{13}NO_4^+$ $[M]^+$ 343.0845, found 343.0845; IR (neat, cm^{-1}) 1640, 1603, 1521, 1346, 1070; mp: 134–135 °C; UPLC method (H_2O/MeCN): rt, 5.26 min, 100%.

5.5.6. (2-hydroxy-4,6-dimethoxyphenyl)(2-(4-methoxyphenyl) benzofuran-3-yl)methanone (9)

Flash chromatography using as eluent: heptane/EtOAc 8:2 (v/v) in 25 min. The product was obtained as a yellow oil (17%): ^{1}H NMR ($CDCl_3$) δ 13.1 (s, 1H), 7.76 (d, 2H, J = 8.5 Hz), 7.52 (d, 1H, J = 7.5 Hz), 7.40 (d, 1H, J = 7.5 Hz), 7.29 (t, 1H, J = 7.5 Hz), 7.21 (t, 1H, J = 7.5 Hz), 6.92 (d, 2H, J = 8.5 Hz), 6.19 (d, 1H, J = 2.5 Hz), 5.76 (d, 1H, J = 2.5 Hz), 3.87 (s, 3H), 3.85 (s, 3H), 3.18 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 193.2, 167.2, 162.7, 160.9, 155.7, 153.6, 129.4, 128.6, 124.6, 123.6, 122.9, 120.4, 118.6, 114.3, 111.3, 107.5, 93.8, 91.5, 56.0, 55.7; MS (ESI, MeOH+CH_2Cl_2) m/z 427.1 $[M+Na]^+$; HRMS calcd for $C_{24}H_{20}O_6Na^+$ $[M+Na]^+$ 427.1158, found 427.1168; IR (neat, cm^{-1}) 1676, 1626, 1576, 1248, 1177, 1151, 1020, 827; UPLC method (H_2O/MeCN): rt, 5.59 min, 94%.

5.5.7. (4-Hydroxy-2-(4-methoxyphenyl)benzofuran-3-yl) (phenyl)methanone (19)

Flash chromatography using as eluent: isocratic toluene. The product was obtained as a yellow oil (17%): ^{1}H NMR (Acetone-d_6) δ 9.56 (s, 1H), 7.70 (d, 2H, J = 8.0 Hz), 7.42 (t, 1H, J = 8.0 Hz), 7.41 (d, 2H, J = 8.5 Hz), 7.30 (t, 1H, J = 8.5 Hz), 7.25 (t, 1H, J = 8.0 Hz), 7.12 (d, 1H, J = 8.5 Hz), 6.79 (d, 2H, J = 8.5 Hz), 6.76 (d, 1H, J = 8.5 Hz), 3.77 (s, 3H); ^{13}C NMR (Acetone-d_6) δ 196.6, 162.1, 159.5, 156.2, 153.0, 139.0, 133.4, 132.1, 130.6, 128.9, 128.1, 122.7, 116.3, 114.6, 110.8, 103,1, 55.8; MS (ESI, MeCN+CH_2Cl_2) m/z 345.1 $[M+H]^+$; HRMS calcd for $C_{22}H_{17}O_4^+$ $[M+H]^+$ 345.1127, found 345.1141; IR (neat, cm^{-1}) 3060, 1736, 1592, 1277, 1034; UPLC method (H_2O/MeCN): rt, 5.12 min, 92%.

5.5.8. (4-Methoxy-2-(4-methoxyphenyl)benzofuran-3-yl) (phenyl)methanone (26)

Flash chromatography using as eluent: isocratic toluene. The product was obtained as a pale yellow amorphous solid (82%): ^{1}H NMR (Acetone-d_6) δ 7.89 (d, 2H, J = 7.5 Hz), 7.71 (d, 2H, J = 9.0 Hz), 7.62 (t, 1H, J = 7.5 Hz), 7.49 (t, 2H, J = 7.5 Hz), 7.32 (t, 1H, J = 8.0 Hz), 7.25 (d, 1H, J = 8.0 Hz), 6.98 (d, 2H, J = 9.0 Hz), 6,76 (d, 1H, J = 8.0 Hz), 3.82 (s, 3H), 3.54 (s, 3H); ^{13}C NMR (Acetone-d_6) δ 193.5, 161.6, 155.3, 154.4, 139.4, 134.1, 132.1, 130.0, 129.4, 129.0, 128.1, 127.0, 123.0, 119.4, 115.1, 105.5, 105.0, 55.9, 55.7; MS (ESI, MeCN+CH_2Cl_2) m/z 359.1 $[M+H]^+$; HRMS calcd for $C_{23}H_{19}O4^+$ $[M+H]^+$ 359.1283, found 359,1308; IR (neat, cm^{-1}) 1669, 1598, 1246, 1090, 1020; mp: 161–164 °C; UPLC method (H_2O/MeCN): rt, 5.23 min, 100%.

5.6. Preparation of 4-*O*-acyl benzofurans 32–51

To a solution of 2-substituted benzofuran-4-ol (1 mmol) and Et_3N (1.2 mmol) in CH_2Cl_2 (2 mL), chloride acid (1.2 mmol) was

added at 0 °C under argon. The mixture was allowed to stir 1-3 h until the reaction was complete.

5.6.1. 2-(4-Methoxyphenyl)benzofuran-4-yl benzoate (32)

Flash chromatography using as eluent: gradient heptane to heptane/EtOAc 8:2 (v/v) in 25 min. The product was obtained as a white amorphous solid (77%): ^{1}H NMR (Acetone-d_6) δ 8.27 (d, 2H, J = 8.1 Hz), 7.90 (d, 2H, J = 9.0 Hz), 7.78 (t, 1H, J = 8.1 Hz), 7.65 (t, 2H, J = 8.1 Hz), 7.52 (d, 1H, J = 8.1 Hz), 7.37 (t, 1H, J = 8.1 Hz), 7.52 (d, 1H, J = 8.1 Hz), 7.19 (s, 1H), 7.05 (d, 2H, J = 9.0 Hz), 3.86 (s, 3H); ^{13}C NMR (Acetone-d_6) δ 165.1, 161.5, 157.2, 156.7, 144.7, 134.7, 130.9, 130.4, 129.7, 127.4, 125.0, 124.5, 123.4, 116.7, 115.3, 109.6, 98.0, 55.7; MS (ESI, MeCN+CH_2Cl_2) m/z 345.1 $[M+H]^+$; HRMS calcd for $C_{22}H_{16}O_4^+$ $[M+H]^+$ 345,1127, found 345,1134; IR (neat, cm^{-1}) 1731, 1614, 1250, 1025; mp: 121–123 °C; UPLC method (H_2O/MeCN): rt, 5.59 min, 100%.

5.7. Biological assays

5.7.1. Assay for in vitro inhibition of *P. falciparum* growth

The chloroquine-resistant strain FcB1/Colombia of *P. falciparum* was maintained in vitro on human erythrocytes in RPMI 1640 medium supplemented by 8% (v/v) heat-inactivated human serum, at 37 °C, under an atmosphere of 3% CO_2, 6% O_2, and 91% N_2. In vitro drug susceptibility assays was measured by [^{3}H]-hypoxanthine incorporation as described.[24–26] Drugs were prepared in DMSO at a 10 mM concentration. Compounds were serially diluted two-fold with 100 μL culture medium in 96-well plates. Asynchronous parasite cultures (100 μL, 1% parasitaemia and 1% final hematocrite) were then added to each well and incubated for 24 h at 37 °C prior to the addition of 0.5 μCi of [^{3}H]-hypoxanthine (GE Healthcare, France, 1–5 Ci·mmol/mL) per well. After a further incubation of 24 h, plates were frozen and thawed. Cell lysates were then collected onto glass-fiber filters and counted in a liquid scintillation spectrometer. The growth inhibition for each drug concentration was determined by comparison of the radioactivity incorporated in the treated culture with that in the control culture maintained on the same plate. The concentration causing 50% growth inhibition (IC_{50}) was obtained from the drug concentration–response curve and the results were expressed as the mean values ± standard deviations determined from several independent experiments. Chloroquine was used as antimalarial drug control.

5.7.2. Assay for in vitro inhibition of *T. brucei gambiense* growth

Bloodstream forms of *T. brucei gambiense* strain Feo were cultured in HMI9 medium supplemented with 10% FCS at 37 °C under an atmosphere of 5% CO_2.[28,29] In all experiments, log-phage cell cultures were harvested by centrifugation at 3000*g* and immediately used. Drug assays were based on the conversion of a redox-sensitive dye (resazurin) to a fluorescent product by viable cells.[30] Drug stock solutions were prepared in pure DMSO. *T. b. gambiense* bloodstream forms (3×10^4 cells/ml) were cultured as described above in 96-well plates (200 μL per well) either in the absence or in the presence of different concentrations of inhibitors and with a final DMSO concentration that did not exceeded 1%. After a 72-h incubation, resazurin solution was added in each well at the final concentration of 45 μM. Fluorescence was measured at 530 nm excitation and 590 nm emission wavelengths after a further 4-h incubation. Each inhibitor concentration was tested in triplicate and the experiment repeated twice. The percentage of inhibition of parasite growth rate was calculated by comparing the fluorescence of parasites maintained in the presence of drug to that of in the absence of drug. DMSO was used as a control. IC_{50}s were determined from the dose–response curves with drug concentrations ranging from 100 μM to 50 nM. IC_{50} value is the mean +/− the standard deviation of three independent experiments. Pentamidine was used as antitrypanosomal drug control.

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Supplementary data

Supplementary data (procedures for the preparation of starting materials, spectral and analysis results for the other compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.07.002.

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