Journal of the Iranian Chemical Society
https://doi.org/10.1007/s13738-020-01877-3

ORIGINAL PAPER

Synthesis of *N*-glycoside compounds from phthalimide and 5-nitrobenzimidazole via 1,2-*O*-sulfinyl derivatives and in vitro cytotoxic activity

Abdelhafid Benksim[1,2] · Said Elhizazi[2] · Brahim Lakhrissi[3] · Mohamed Amine[4] · Mohamed Cherkaoui[1] · Abdessamad Tounsi[5] · Anne Wadouachi[6]

Received: 21 November 2018 / Accepted: 12 February 2020

Abstract
An efficient synthesis of *1,2-trans-N*-glycosylated derivatives from phthalimide and nitrobenzimidazole via 1,2-*O*-sulfinyl monosaccharides has been established. Such S_N2-type displacements at the anomeric center are stereospecific, giving a single anomer. The reaction was optimized using a polar aprotic solvent and K_2CO_3 at 80 °C. Phthalimide and nitrobenzimidazole 1,2-*trans-N*-glycoside derivatives were easily obtained with a free hydroxyl at C-2, which can be further functionalized. The compounds **3d–e** displayed a significant cytotoxic activity exceeding 50% of inhibition at 125 µg/Ml against the human cancer cell line (Hep-2).

Keywords Glycoside · Cyclic sulfite · Phthalimide · 5-Nitrobenzimidazole · Cytotoxic activity

Introduction

Heterocycles are a major class of organic compounds characterized by their physical and chemical properties. The interesting biological and pharmaceutical properties of heterocyclic derivatives made these compounds very attractive for organic synthesis. These structures are present in several natural products such as nucleic acids, coenzymes, alkaloids, and glyco-conjugated compounds [1]. Nowadays, a large number of well-known drugs are heterocycles, such as quinoline antimalarials [2], lactam antibiotics [3], indoles [4], pyrimidines [5], imidazole derivatives [6], as well as other important derivatives of considerable pharmaceutical interest [7–9]. For this reason, the development of stereoselective synthesis of novel derivatives, their structural characterizations, and their biological properties is a topic of increasing interest for a large number of research groups. The synthesis of compounds containing the subunit phthalimide and nitrobenzimidazole has been described as scaffolding to design new drug candidates with different biological activities [1]. Phthalimide is a non-aromatic bicyclic nitrogen heterocycle, and its derivatives are an interesting class of compounds having a wide range of applications [10]. These compounds are lipophilic and neutral compounds and can also easily cross biological membranes in vivo. These derivatives have been used for their pharmacological activities, such as hypolipidemic [11], antimicrobial [12], anti-inflammatory [13], hypoglycemic [14], anticonvulsant [15], antitumor [16], and also antiviral [17].

Benzimidazole compounds are nitrogen heterocycles which play a key role in medicinal and pharmaceutical discoveries as biologically active compounds, such as antitumor [18], antifungal [19], antiprotozoal [20], anticonvulsant [21], antihypertension [22], and antibacterial agents [23]. The therapeutic significance of these clinically active

✉ Abdelhafid Benksim
benksima@gmail.com

1 Laboratory of Human Ecology, Cadi Ayyad University, Marrakesh, Morocco

2 Direction régionale de la santé, Marrakech-Safi (ISPITS de Marrakech), Marrakech, Morocco

3 Laboratoire de Chimie des Agroressources, Université Ibn Tofail, Kénitra, Morocco

4 Epidemiology Laboratory, Faculty of Medicine and Pharmacy, Marrakesh, Morocco

5 Polydisciplinary Faculty, Moulay Slimane University, Béni-Mellal, Morocco

6 Laboratoire de Glycochimie des Antimicrobiens et Agroressources, Université de Picardie Jules Verne, Amiens, France

Published online: 24 February 2020

drugs particularly in the treatment of infectious diseases has promoted the development of potent compounds in combination with monosaccharides. Carbohydrates, as chiral pools, have been intensively studied for stereospecific and selective reactions in order to develop several families of compounds of interest. The presence of functionalized and stereo-controlled centers on sugar can offer many possibilities to synthesize a new generation of biologically active compounds, for example, for the inhibition of cell proliferation [24–26]. Our goal was to synthesize new glycoside derivatives of phthalimide and nitrobenzimidazole for better cell penetration and therefore to determine their in vitro antitumor activities on human cancer cell line of Hep-2.

Results and discussion

Chemistry

In our previous works, we have described an easy process leading to *C*-glycosyl cyanides and *O*-glycosylation of diethyl oxoglutarate via 1,2-*O*-sulfinyl monosaccharides [27–29]. Furthermore, we synthesized two new water-soluble benzimidazolone derivatives from 1,2-*cis* cyclic sulfite [30]. This approach made it possible to introduce an anion such as the heterocyclic nitrogen onto the anomeric center. To achieve our objective, various tests were carried out for optimizing the *N*-glycosylation reaction. Due to its symmetry, phthalimide by its nitrogen between two carbonyl functions was chosen as a perfect nucleophile for this *N*-glycosylation [9].

The *N*-glycosylation reaction was first studied with 3,4,6-tri-*O*-benzyl-1,2-*O*-sulfinyl-α-D-glucofuranose **1** [27] in the presence of phthalimide and NaH or n-BuLi. Unfortunately, these conditions only led to the hydrolysis of the cyclic sulfite and obtaining of the corresponding 1,2-diol glucofuranose tribenzylated. In literature, enantiopure vicinal aminoalcohols bearing a phthalimide moiety were prepared by reaction of non-carbohydrate cyclic sulfites with phthalimide using NaH in DMF, and no hydrolysis was observed [31]. Under an argon atmosphere, an efficient synthetic route was optimized by changing the base. In the presence of a weak base such as K_2CO_3, in DMF at 80 °C, the *N*-(3,5,6-tri-*O*-benzyl-β-D-glucofuranosyl)-phthalimide **1a** was obtained in 94% yield in shorter reaction time (Table 1, Scheme 1). The phthalimide derivative **1a** was already synthesized from 2,3,4,6-tetra-*O*-benzyl-α-D-glucosyl iodide, the nucleophilic displacement of the α-iodide anomeric atom by the phthalimide anion gave the corresponding phthalimido β-glycoside derivative in 66% yield [32].

To the best of our knowledge, there are few references relating to the nucleophilic substitution of cyclic sulfites with nitrogen heterocyclic anion at anomeric carbon [33].

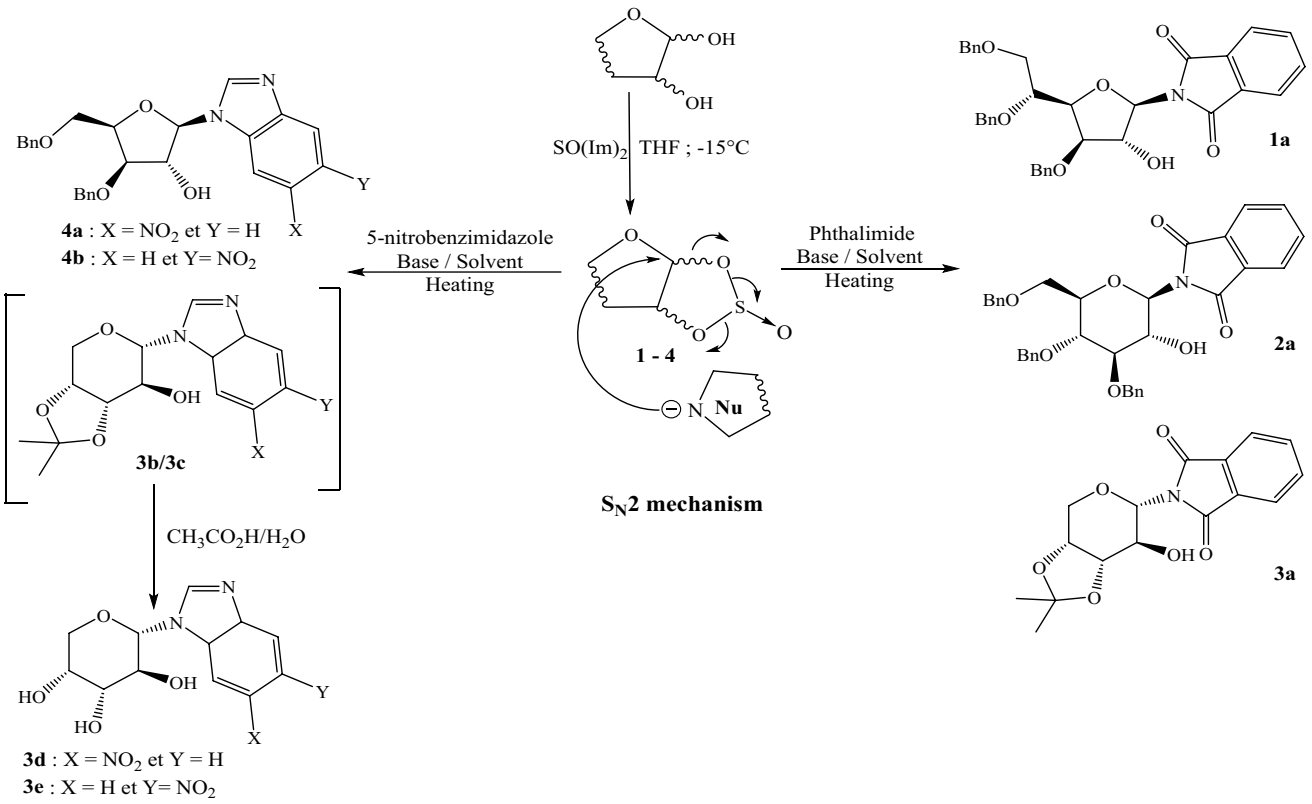
Table 1 Optimization of *N*-glycosylation reaction via cyclic sulfite **1** and phthalimide

Entry	Solvent	Base (3.1 eq.)	Reaction time	Yield%
1	DMF	NaH	6 h	Hydrolysis
2	DMF	*n*-Butyllithium	8 h	Hydrolysis
3	DMF	K_2CO_3	30 min	94
4	DMF	K_2CO_3	3 h	60
4	HMPA/DMAC	K_2CO_3	1 h	Hydrolysis

Reaction conditions: cyclic sulfite 1 (1 mmol), phthalimide (3 mmol), base (3.1 mmol), and solvent (35 mL)

Nucleophilic opening of the cyclic sulfite derivative **1** using phthalimide anion is regio- and stereospecific at the anomeric center through a S_N2-type mechanism (Scheme 1). The structure of *N*-(3,5,6-tri-*O*-benzyl-β-D-glucofuranosyl)-phthalimide **1a** was confirmed by NMR and MS analyses (Table 2) with the presence of phthalimide moiety at the anomeric position of the tetrabenzylated glucose. The [13] C NMR spectrum showed the signal of the anomeric carbon atom of compound **1a** highly shielded by about 20 ppm at 85.18 ppm and a chemical shift value of 167.80 ppm corresponding to both carboxyl functions. ^{1}H NMR spectrum showed anomeric proton of compound **1a** at δ 5.82 ppm with a coupling constant $J_{1,2} = 0$ Hz, which confirmed the furanose form and indicating the 1,2-*trans* configuration, and the signals between 7.87 and 7.49 ppm correlated to four aromatic protons proved the introduction of phthalimide part. Infrared spectrum showed an absorption band corresponding to the hydroxyl on the C-2 at 3398 cm^{-1}. ESI–MS analysis showed an ion at m/z 618.34 $[M+K]^+$. In the same way, the glucopyranoside **2a** and arabinopyranoside **3b** derivatives were prepared from cyclic sulfites compounds **2** and **3,** respectively, and characterized by NMR analysis. The anomeric protons appeared between δ 5.00 and 5.77 ppm and coupling constants $J_{1,2}$ between 9.77 and 9.99 Hz, respectively, indicated the pyranose derivatives. ^{13}C NMR spectra showed the anomeric carbon signal at δ 86.6 and 74.2 ppm for compounds **2a** and **3b**, respectively.

The nitrobenzimidazole derivatives of α-D-arabinopyranose **3d–e** and β-D-xylofuranose **4a–b** were prepared from the corresponding 1,2-*O*-sulfinyl sugars using the same protocol (Table 2). Compounds **3d** and **3e** were prepared after highly efficient and selective deprotection [34] of **3b–c** with 90% yield, and the ratio between **3d/3e** was evaluated at 8/2. HRMS data of the two diasteroisomers showed one ion at *m/z* 318.0705 $[M+Na]^+$. The latter compounds were characterized by ^{1}H NMR spectroscopy showing two signals at 5.50 and 5.51 ppm with a coupling constant $J_{1,2} = 9.22$ Hz corresponding to the D-arabinopyranoside derivatives **3d–e** (Table 2). ^{13}C NMR spectra showed the peaks between δ 69.2 and 73.8 ppm correlated to three



Scheme 1 General pathway to obtain nitrobenzimidazole and phthalimide derivatives

free hydroxyls (C-1, C-3, and C-4). Cyclic sulfite **4** was treated with 5-nitrobenzimidazole anion freshly prepared, in DMF and in the presence of K_2CO_3 at 80 °C for giving two isomers **4a** and **4b** with 90% yield. Mass spectrometry analysis showed a single ion at *m/z* 514.23 $[M+K]^+$, corresponding to both isomers, which cannot be separated by silica gel chromatography. The β-D-xylofuranoses **4a** and **4b** were characterized by NMR spectroscopy, which showed two signals for the anomeric proton between δ 5.88 and δ 6.01 ppm. In ^{13}C NMR spectrum, the peaks at δ 146.6, 145.5, 144.1, 142.7, and 147.8 confirmed the introduction of nitrobenzimidazole moiety and two signals corresponding to the anomeric carbon at δ 92.4 and δ 91.9 ppm for compounds **4a** and **4b.** This introduction has been reported in previous studies [19].

These provided isomers can be explained by intramolecular mesomeric equilibrium between two nitrobenzimidazole anions **a** and **b** (Scheme 2). In this case, the NO_2 group leads to the stabilization of anion **b**. It can be hypothesized that anion **a** was more nucleophilic than anion **b**. Therefore, compounds (**4a–b**) cannot be separated by column chromatography and the ratio between isomers: **4a**/**4b** was also evaluated at 8/2 (Scheme 2). Based on these results, we have developed an efficient method for the synthesis of new 1.2-*trans*-*N*-glycosides from phthalimide and nitrobenzimidazole via 1,2-*O*-sulfinyl monosaccharides as starting materials in polar aprotic solvent. This new method will be adopted for studying the novel complex glycosides in which the C-2 position could be functionalized by other derivatives of potential biological interest.

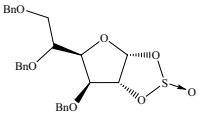
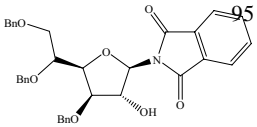
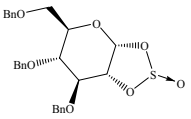
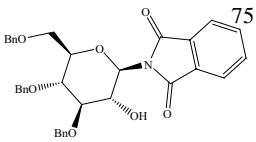
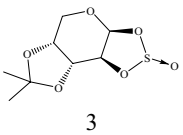
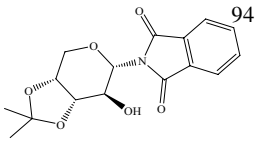
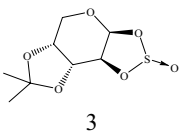
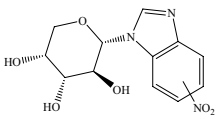
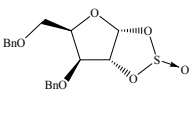
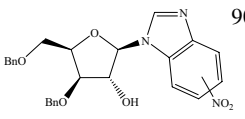
Cytotoxic evaluation

Although the advances in oncology are leading to effective treatments and cure, the cancer is a leading cause of human death in the worldwide [35]. Therefore, laryngeal cancer is one of the most common types of head and neck cancers. It accounts for about 2.7% and 2.1% of all cancers and deaths due to it, respectively [35].

The aim of this investigation was to screen the cytotoxic activity of *N*-glycoside derivatives from phthalimide and nitrobenzimidazole against human cancer cell lines of Hep-2 in vitro, using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay [35].

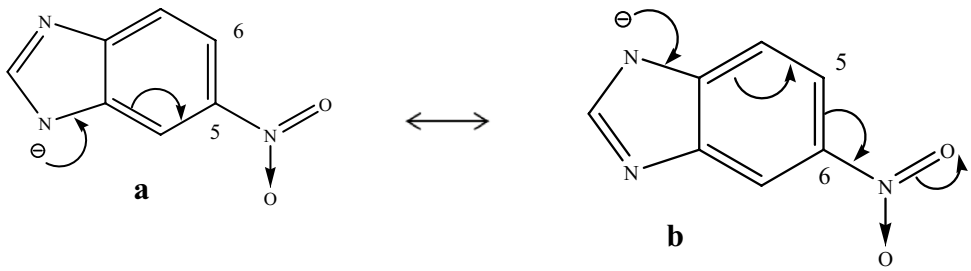
Cytotoxic index was calculated: %IC = [1 − (Mean absorbance of treated group/Mean absorbance of negative control)] × 100. The absorbance values obtained per treatment were converted to percentage proliferation [36].

Table 2 Synthesis of *N*-glycoside derivatives of phthalimide and 5-nitrobenzimidazole

1,2-*O*-sulfinyl derivatives	*N*-glycoside derivatives	% Yield	Reaction time (min)	δ Anomeric proton	$J_{1,2}$ (Hz)	δ Anomeric carbon	δ CO	δ C-NO_2
 1	 **1a**	95	30	5.82	0	85.2	167.8	–
 2	 **2a**	75	60	5.70	9.77	86. 6	167.3	–
 3	 **3a**	94	30	5.05[b]	9.99	74.2	167.8	–
 3	 **3d/3e**	90	40	5.50/5.51[b]	9.22[b]	87.3/87.3	–	144.3/142.7
 4	 **4a/4b**	90	100	5.88/6.01	2.42/2.37	92.4/91.9	–	144.1/142.7

NMR spectra were recorded at 300 MHz in $CDCl_3$ or CD_3OD

Scheme 2 Isomers of nitrobenzimidazole



After 48 h of incubation, cytotoxic activity of each molecule tested on the tumor cell lines Hep-2 is summarized in Fig. 1.

To our knowledge, it seems that very little data on cytotoxic activities of *N*-glycoside derivatives from phthalimide and nitrobenzmidazole exist in literature. *In vitro* cytotoxicity of compounds **3a**, **3d–e** and phthalimide against human cancer cell lines of carcinoma (Hep-2) were evaluated after 48 h of exposure ($n = 6$). Among the various compounds tested, we found that the compound **3d–e** can inhibit more than 50% of the number of viable cells at 125 µg/mL, which proves that the cytotoxic effects were suitable against Hep-2 cell line. A positive antitumor effect of nitrobenzimidazole derivatives was observed against breast cancer (MCF7) [37]. Besides, the antitumor effect of benzimidazole nucleosides against the human laryngeal carcinoma Hep-2 cell line was reported [38]. Antitumor activity of these compounds may be assigned to their abilities to inhibit Ser/Thr kinase CK2

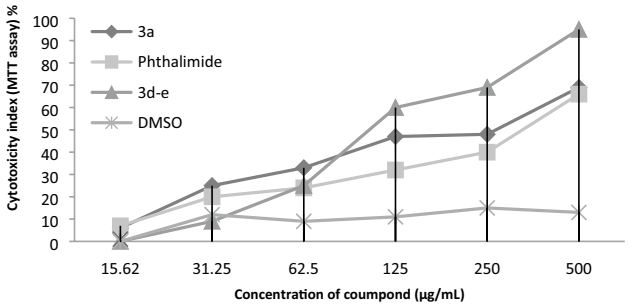


Fig. 1 Cytotoxic activity against human cancer cell line Hep-2 after 48 h of exposure ($n=6$)

that acts as a regulator of cell apoptosis and carcinogenesis [38]. However, the compounds such as phthalimide **3a** showed the modest antiproliferative activities at 500 µg/mL with only 60% of inhibition. In some reported studies, 2-phthalimide-1-(4-fluoro-phenyl) ethanone showed the best inhibition of human MDAMB-231 breast carcinoma and SKHep-1 hepatoma cell lines [9]. In this study, the promising cytotoxicity of compounds **3d–e** will encourage further researches to explore their potential as chemopreventive and chemotherapeutic agents against cancer.

Conclusion

In summary, the tested compounds exhibiting antiproliferative activity showed interesting results which would promote their use as lead compounds on further studies for the development of the new anticancer agents. This observation can open the new perspectives for in-depth studies of these molecules. The study of the "structure–activity" relationship and the mechanism of action of the active molecule should lead to a better valorization of these compounds as potential anticancer agents.

Experimental

General methods and materials

All chemical reagents were purchased from Aldrich or Acros (France), and solvents (analytical grade) were provided by Prolabo. Reactions were monitored by thin-layer chromatography (TLC) performed on silica gel 60 F254 (Merck) plates with visualization by UV light (254 nm) and/or by charring with a vanillin-H_2SO_4 or cerium molybdate reagent. Column chromatography was performed with Merck silica 60 (230 mesh). Concentrations were realized on a rotary evaporator at a temperature below 40 °C. ^{1}H and ^{13}C NMR spectra were recorded on a BRUKER DMX300 spectrometer (at 300 and 75 MHz, respectively). Spectra were recorded in $CDCl_3$ or MeOD. Assignments of ^{1}H and ^{13}C signals were determined from decoupling experiments, COSY spectra and HSQC spectra. HRMS spectra in the positive ion mode were obtained on a Q-TOF Ultima Global hybrid quadrupole/time-of-flight instrument, equipped with a pneumatically assisted electrospray (Z-spray) ion source and an additional sprayer (Lock Spray) for the reference compound. Optical rotations were measured on a Perkin Elmer 241 polarimeter: concentrations are given in g/100 mL. The starting 5-nitrobenzimidazole was prepared in the laboratory, while phthalimide was purchased already prepared by chemical companies.

General procedure of *N*-glycosylation via 1,2-*O*-sulfinyl monosaccharides

Under an argon atmosphere, solution of the 1,2-*O*-sulfinyl derivatives (1 mmol) in DMF (15 mL) was added by a solution of phthalimide or 5-nitrobenzimidazole (3 eq.) previously stirred at 65 °C for 2 h with K_2CO_3 (3.1 eq.) in DMF (10 mL). The reaction mixture was stirred at 80 °C and then the solvent was removed under reduced pressure (0.1 bar/0.1 mm) at 70 °C and filtered. The filtrate was extracted with Et_2O-H_2O (3/1) (3 × 50 mL). After that, the combined organic layers were concentrated and the crude product is purified by silica gel chromatography (Hexane/EtOAc: 8/2), giving the corresponding *N*-glycosides (**1a, 2a, 3a, 4a–b, 3b–c**).

***N*-(3,5,6-tri-*O*-benzyl-β-D-glucofuranosyl) phthalimide 1a** Yellow syrup; $[\alpha]_D^{20}$: −40,6 (c 0,56 CH_2Cl_2); ^{1}H NMR ($CDCl_3$, δppm): 5.82 (s, 1H, H1, $J_{1,2}=0$ Hz); 4.15–4.48 (m, 2H, $H_{2,4}$); 3,65 (dd, 1H, H_3, $J_{2,3}=5.21$ Hz), 3.85 (d, 1H, H_5, $J_{5,6}=1.99$ Hz), 3.90 (m, 2H, $H_{6\text{-}6'}$), 4.23/4.69 (m, 6H, CH_2-Ph), 7.25/7.43 (m, 15H, CH_2Ph), 7.87/7.49 (dd, 4H, Ph-$_{Phthalimide}$); 13C NMR ($CDCl_3$, δppm): 85.18 (C-1), 84.55 (C-2), 78.12 (C-3), 74.80 (C-4), 76.59 (C-5), 70.68 (C-6), 72.40/72.95/73.70 (CH_2-Ph), 127.70/128.33 (CH_2-Ph), 139.24/139.03/138.39 (C_{ipso}), 167.80 (CO), 132.11/124.04 (Ph-$_{Phthalimide}$), 134.82 (Cipso); IR (ATR) cm^{-1}: 3398 (OH), 1781 (CO), 2918/2850 (C_{arom}); LRMS (ESI) m/z calcd for $C_{35}H_{33}NO_7K$ $[M+K]^+$ 618.19, found 618.34.

***N*-(3,5,6-tri-*O*-benzyl-β-D-glucopyranosyl) phthalimide 2a** Yellow syrup; $[\alpha]_D^{20}$: +65.2 (c 0,46 MeOH); ^{1}H NMR ($CDCl_3$, δppm): 5.70 (d, 1H, H_1, $J_{1.2}=9.77$ Hz), 3.60–4.90 (m, 6H, $H_{2\text{-}6/6'}$), 5.00/5.30 (m, 6H, CH_2-Ph), 7.20/7.50 (m, 15H, CH_2Ph), 7.80/8.23 (m, 4H, Ph-$_{Phthalimide}$); ^{13}C NMR ($CDCl_3$, δppm): 86.56 (C-1), 69.36 (C-2), 80.55 (C-3), 78.50 (C-4), 78.10 (C-5), 68.99 (C-6), 75.75/75.38/73.89 ($\underline{C}H_2$Ph), 129.01/128.83/128.22 (CH_2-Ph), 135.58/135.31/138.97 (C_{ipso}), 167.28 (CO), 134.73/124.05 (Ph-$_{Phthalimide}$), 133.36

(C_{ipso}); IR (ATR) cm^{-1}: 3487 (OH), 1774 (CO), 2925/2847 (C-$_{arom}$); HRMS (ESI) m/z calcd for $C_{35}H_{33}NO_7Na$ $[M+Na]^+$ 602.2149, found 602.2726.

***N*-(3,4-*O*-isopropylidène-α-D-arabinopyranosyl)-phthalimide 3a** Colorless crystals: melting at 121–130′; $[\alpha]_D^{20}$: +36,4 (c 1,90 MeOH; ^{1}H NMR ($CDCl_3$), δ (ppm): 5.70 (d, 1H, H_1, $J_{1.2}=9.99$ Hz), 3.35–4.50 (m, 3H, $H_{2\text{-}4}$), 3.95/4.05 (m, 2H, $H_{5,5'}$), 1.25/1.60 (2 s, 6H, $(CH_3)_2$-C), 3.29 (s, 1H, OH).^{13}C NMR ($CDCl_3$): 74.22 (C_1), 68.31 (C_2), 80.15 (C_3), 80.09 (C_4), 65.82 (C_5), 27.37/25.54 ($\underline{C}H_3)_2$-C), 110.01 ($(CH_3)_2$-$\underline{C}$), 167.84 (CO), 134.35/134.82/123.09/123.49 (Ph-$_{Phthalimide}$), 131.94 (C_{ipso}). IR (ATR) cm^{-1}: 3509 (OH), 1777 (CO), 2918/2850 (C_{arom}). HRMS (ESI) m/z calcd for $C_{16}H_{17}NO_6Na$ $[M+Na]^+$ 342.0954, found 342.0933.

3-*N*-(α-D-arabinopyranosyl)-6-nitrobenzimidazole 3d and 3-*N*(α-D-arabinopyranosyl)-5-nitrobenzimidazole 3e Yellowish solid melting at 125–130′. $[\alpha]^{20}_{D\ mixture}$: + 72,4 (c0,67MeOH). ^{1}HNMR (CD_3OD), (δppm): 5.50 (d, 1H, H_1, $J_{1.2}=9.22$ Hz), 3.78 (t, 1H, H_2, $J_{2.3}=3.33$ Hz), 3.86 (m, 1H, H_3), 3.96 (d, 1H, H_4, $J_{4.5}=6.40$ Hz), 4.165 (dd, 1H, H_5, $J_{4.5'}=2.30$ Hz), 4.22 (t, 1H, $H_{5'}$, $J_{5.5'}=9.47$ Hz), 8.15 (q, 1H, CHN_2), 8.74–7.75 (m, 3H, Ph). ^{13}C NMR (CD_3OD): 87.32/87.28 (C_1), 70.10/70.00 (C_2), 73.84 (C_3), 69.24 (C_4), 69.68 (C_5), 147.50 (NC_{IV}), 147.32/146.46 (CHN_2); 144.31/142.66 (CNO_2); 137.34/132.56 (NC_{ipso}), 119.54/118.69/118.32/115.53/112.87/109.39 (CH de Ph); IR (ATR) cm^{-1}: 3494 (OH), 2986 (C_{arom}). HRMS (ESI) m/z calcd for $C_{13}H_{17}N_3O_6Na$ $[M+Na]^+$318.0697, found 318.0705.

3-*N*-(3,5-di-*O*-benzyl-β-D-xylofuranosyl)-6-nitrobenzimidazole 4a and 3-*N*-(3,5-di-*O*-benzyl-β-D-xylofuranosyl)-5-nitrobenzimidazole 4b Brown viscous syrup. $[\alpha]^{20}_{D\ mixture}$: -2,4 (c 0,67 CH_2Cl_2). ^{1}HNMR ($CDCl_3$) δ (ppm): 5.88/6.01 (dd, 1H, H_1, $J_{1.2}=2.42/3.27$ Hz), 4,30 (m, 2H, $H_{2,4}$), 3,55 (m, 1H, H_3), 3.95 (m, 2H, $H_{5,5'}$), 4.53/4.75 (m, 6H, CH_2-Ph), 7.13/7.45 (m, 15H, $CH_2\underline{Ph}$), 8.21/8.33 (s,1H, CHN_2), 8.47-7.58 (m, 3H, Ph). ^{13}C NMR ($CDCl_3$): 92.38/91.96 (C_1), 81.79/81.16 (C_2), 83.26 (C_3), 79.14/78.99 (C_4), 68.54/66.31 (C_5), 138.05/137.45/136.88 (C_{ipso}), 74.11/72.92 (CH_2Ph), 128.93/128.59/128.39/128.14 (CH_2-$\underline{Ph}$), 147.85 (NC_{IV}), 146.57/145.55 (CHN_2), 144.15/142.73(CNO_2),132.10/137.47(NC_{ipso}),111.36/108.15/118.80/116.72/120.38/119.72 (CH-$_{Ph}$). IR (ATR) cm^{-1}: 3421 (OH), 2987/2955(C_{arom}). HRMS (ESI) m/z calcd for $C_{13}H_{17}N_3O_6Na$ $[M+Na]^+$498.1636, found 498.1658.

General procedure of cytotoxicity testing

The cell lines were cultivated at 37 °C in a humidified atmosphere containing 5% CO2 supplemented with 10% fetal bovine serum, using Dulbecco's modified Eagle's medium (DMEM) for culture cell lines (Hep-2). A standard solution (1 mg/mL) of each compound was solubilized in 10% DMSO. For each compound to be tested, a serial dilution is carried out with culture medium to obtain several concentrations (500; 250; 125; 62.5; 31.3 and 15.6 µg/mL). After that, cells were incubated (10^5 cells/each well of microplate) in a 96-well plate for 24 h. After addition of the compound tested dissolved in fresh medium, the cells were cultured at 37 °C for 48 h. The absorbance of untreated cells was served as the negative controls (culture medium/DMSO). Doxorubicin was used as positive controls [35, 39].

Cytotoxicity index was calculated based on MTT colorimetric assay to assess the antiproliferative activity [36, 39]. The absorbance was measured at 570 nm on microplate reader.

Acknowledgments The authors thank Dr. Dominique Cailleu and Pr. Serge Pilard (Picardie Jules Verne University) for NMR, MS–MS spectrometry and helpful discussion. Special thanks go also to Pr. Mohamed Massoui (Rabat university, Morocco) for performing the biological tests.

References

1. J.A.R. Salvador, R.M.A. Pinto, S.M. Silvestre, Recent advances of bismuth(III) salts in organic chemistry: application to the synthesis of heterocycles of pharmaceutical interest. Curr. Org. Synth. **6**, 426–470 (2009)
2. S. Vandekerckhove, M. Dhooghe, Quinoline-based antimalarial hybrid compounds. Bioorg. Med. Chem. **23**, 5098–5119 (2015)
3. H. Cho, T. Uehara, T.G. Bernhardt, Beta-lactam antibiotics induce a lethal malfunctioning of the bacterial cell wall synthesis machinery. Cell **159**, 1300–1311 (2014)
4. S. Cacchi, G. Fabrizi, Synthesis and functionalization of indoles through palladium-catalyzed reactions. Chem. Rev. **105**, 2873–2920 (2005)
5. M.B. Deshmukh, S.M. Salunkhe, D.R. Patil, P.V. Anbhule, A novel and efficient one step synthesis of 2-amino-5-cyano-6-hydroxy-4-aryl pyrimidines and their anti-bacterial activity. Eur. J. Med. Chem. **44**, 2651–2654 (2009)
6. J.K. Savjani, A.K. Gajjar, Pharmaceutical importance and synthetic strategies for imidazolidine-2-thione and imidazole-2-thione derivatives. Pak. J. Biol. Sci. **14**, 1076–1089 (2011)
7. A.M. Al-Azzawi, M.S.A. Al-Razzak, Synthesis, characterization and antibacterial screening of new Schiff bases linked to phthalimide. Int. J. Res. Pharm. Chem. **3**, 682–690 (2013)
8. D. Stiz, A. Campos, A.L.T.G. Ruiz, J.E. de Carvalho, R. Corrêa, V. Cechinel-Filho, Antiproliferative effect of synthetic cyclic imides (methylphthalimides, carboxylic acid phthalimides and itaconimides) against human cancer cell lines. Z. Naturforsch. C Biol. Sci. **71**, 423–427 (2016)
9. N. Kushwaha, D. Kaushik, Recent advances and future prospects of phthalimide derivatives. J. Appl. Pharm. Sci. **6**, 159–171 (2016)
10. M.R. da Silva, C.P. Santos, M.J.S. Monte, C.A.D. Sousa, Thermochemical studies of phthalimide and two *N*-alkylsubstituted phthalimides (alkyl = ethyl and *n*-propyl). J. Therm. Anal. Calorim. **83**, 533–539 (2006)

11. J.M. Chapman, G.H. Cocolas, I.H. Hall, Hypolipidemic activity of phthalimide derivatives. 1. *N*-substituted phthalimide derivatives. J. Med. Chem. **22**, 1399–1402 (1979)
12. G. Singh, A. Saroa, S. Girdhar, S. Rani, S. Sahoo, D. Choquesillo-Lazarte, Synthesis, characterization, electronic absorption and antimicrobial studies of *N*-(silatranylpropyl) phthalimide derived from phthalic anhydride. Inorg. Chim. Acta **427**, 232–239 (2015)
13. L.M. Lima, P. Castro, A.L. Machado, C.A. Fraga, C. Lugnier, V.L. de Moraes, E.J. Barreiro, Synthesis and anti-inflammatory activity of phthalimide derivatives, designed as new thalidomide analogues. Bioorg. Med. Chem. **10**, 3067–3073 (2002)
14. S.P. Mahapatra, P. Ghode, D.K. Jain, S.C. Chaturvedi, B.C. Maiti, T.K. Maity, Synthesis and hypoglycemic activity of some phthalimide derivatives. Int. J. Pharm. Sci. Res. **2**, 567–578 (2010)
15. O. Akgul, F. Sultan Kilic, K. Erol, V. Pabuccuoglu, Synthesis and anticonvulsant activity of some *N*-phenyl-2-phtalimidoethanesulfonamide derivatives. Arch. Pharm. **340**, 656–660 (2007)
16. S.H. Kok, R. Gambari, C.H. Chui, M.C.W. Yuen, E. Lin, R.S.M. Wong, F.Y. Lau, G.Y.M. Cheng, W.S. Lam, S.H. Chan, K.H. Lam, C.H. Cheng, P.B.S. Lai, M.W.Y. Yu, F. Cheung, J.C. Tang, A.S.C. Chan, Synthesis and anti-cancer activity of benzothiazole containing phthalimide on human carcinoma cell lines. Bioorg. Med. Chem. **16**, 3626–3631 (2008)
17. N.A. Al-Masoudi, E. Abood, Z.T. Al-Maliki, W.A. Al-Masoudi, C. Pannecouque, Amino acid derivatives. Part 6. Synthesis, in vitro antiviral activity and molecular docking study of new *N*-α-amino acid derivatives conjugated spacer phthalimide backbone. Med. Chem. Res. **25**, 2578–2588 (2016)
18. A. Sharma, V. Luxami, K. Paul, Purine-benzimidazole hybrids: synthesis, single crystal determination and in vitro evaluation of antitumor activities. Eur. J. Med. Chem. **93**, 414–422 (2015)
19. D.M. Badgujar, M.B. Talawar, S.N. Asthana, P.P. Mahulikar, Novel synthesis of biologically active nitro heterocyclic compounds. J. Sci. Ind. Res. **67**, 54–57 (2008)
20. Z. Kazimierczuk, J.A. Upcroft, P. Upcroft, A. Gorska, B. Starosciak, A. Laudy, Synthesis, antiprotozoal and antibacterial activity of nitro-and halogeno-substituted benzimidazole derivatives. Acta Biochim. Pol. **49**, 185–195 (2002)
21. P. Jain, P.K. Sharma, H. Rajak, R.S. Pawar, U.K. Patil, P.K. Singour, Design, synthesis and biological evaluation of some novel benzimidazole derivatives for their potential anticonvulsant activity. Arch. Pharm. Res. **33**, 971–980 (2010)
22. W. Zhu, Y. Da, D. Wu, H. Zheng, L. Zhu, L. Wang, Y. Yan, Z. Chen, Design, synthesis and biological evaluation of new 5-nitro benzimidazole derivatives as AT1 antagonists with anti-hypertension activities. Bioorg. Med. Chem. **22**, 2294–2302 (2014)
23. D. Song, S. Ma, Recent development of benzimidazole-containing antibacterial agents. ChemMedChem **11**, 646–659 (2016)
24. G.T. Le, G. Abbenante, B. Becker, M. Grathwohl, J. Halliday, G. Tometzki, W. Meutermans, Molecular diversity through sugar scaffolds. Drug Discov. Today **8**, 701–709 (2003)
25. M.C. Galan, D. Benito-Alifonso, G.M. Watt, Carbohydrate chemistry in drug discovery. Org. Biomol. Chem. **9**, 3598–3610 (2011)
26. N.M. Javier, A.P. Rauter, Enantioselective synthesis in carbohydrate-based drug discovery: imino sugars, alkaloids and macrolide antibiotics. Curr. Top. Med. Chem. **14**, 1235–1243 (2014)
27. A. Benksim, D. Beaupère, A. Wadouachi, A novel stereospecific synthesis of glycosyl cyanides from 1, 2-*O*-sulfinyl derivatives. Org. Lett. **6**, 3913–3915 (2004)
28. A. Benksim, M. Massoui, D. Beaupère, A. Wadouachi, Efficient *O*-glycosylation of diethyl oxoglutarate via 1, 2-*O*-sulfinyl derivatives. Tetrahedron Lett. **48**, 5087–5089 (2007)
29. F. Roussel, A. Wadouachi, D. Beaupere, *N*-glycosylation of aldoses. synthesis of 1,2-cyclic carbamates via a 1,2-*O*-sulfinyl derivative. Carbohydr. Lett. **3**, 397–404 (2000)
30. B. Lakhrissi, A. Benksim, M. Massoui, E.M. Essassi, V. Lequart, N. Joly, D. Beaupere, A. Wadouachi, P. Martin, Towards the synthesis of new benzimidazolone derivatives with surfactant properties. Carbohydr. Res. **343**, 421–433 (2008)
31. K. Nymann, S. Mylvaganam, J.S. Svendsen, Nucleophilic ring opening of cyclic 1, 2-sulfites with nitrogen nucleophiles. A route to enantiopure benzylic amino alcohols. Acta Chem. Scand. **52**, 1060–1063 (1998)
32. J. Gervay, M.J. Hadd, Anionic additions to glycosyl iodides: highly stereoselective syntheses of β C-, N-, and O-glycosides. J. Org. Chem. **62**, 6961–6967 (1997)
33. A. Megia-Fernandez, J. Morales-Sanfrutos, F. Hernandez-Mateo, F. Santoyo-Gonzalez, Synthetic applications of cyclic sulfites, sulfates and sulfamidates in carbohydrate chemistry. Curr. Org. Chem. **15**, 401–432 (2011)
34. N.M. Xavier, S.D. Lucas, A.P. Rauter, Zeolites as efficient catalysts for key transformations in carbohydrate chemistry. J. Mol. Catal. A Chem. **305**, 84–89 (2009)
35. W.F. Rizo, L.E. Ferreira, V. Colnaghi, J.S. Martins, L.P. Franchi, C.S. Takahashi et al., Cytotoxicity and genotoxicity of coronaridine from tabernaemontana catharinensis A. DC in a human laryngeal epithelial carcinoma cell line (Hep-2). Genet. Mol. Biol. **36**(1), 105–110 (2013)
36. F. Grbović, M.S. Stanković, M. Ćurčić, N. Đorđević, D. Šeklić, M. Topuzović, S. Marković, In vitro cytotoxic activity of *Origanum vulgare* L. on HCT-116 and MDA-MB-231 cell lines. Plants **2**, 371–378 (2013)
37. M.M. Ramla, M.A. Omar, A.M.M. El-Khamry, H.I. El-Diwani, Synthesis and antitumor activity of 1-substituted-2-methyl-5-nitrobenzimidazoles. Bioorg. Med. Chem. **14**, 7324–7332 (2006)
38. M.I. Kharitonova, I.D. Konstantinova, A.I. Miroshnikov, Benzimidazole nucleosides: antiviral and antitumor activities and methods of synthesis. Russ. Chem. Rev. **87**(11), 1111 (2018)
39. M.C. Bufalo, J.M. Candeias, J.M. Sforcin, In vitro cytotoxic effect of Brazilian green propolis on human laryngeal epidermoid carcinoma (HEp-2) cells. Evid. Based Complement. Alternat. Med. **6**, 483–487 (2009)