European Journal of Medicinal Chemistry 45 (2010) 2480–2488

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and modulation properties of imidazo[4,5-*b*]pyridin-7-one and indazole-4,7-dione derivatives towards the *Cryptosporidium parvum* CpABC3 transporter

Waël Zeinyeh [a], Hexue Xia [b], Philippe Lawton [b], Sylvie Radix [a,*], Christelle Marminon [a], Pascal Nebois [a], Nadia Walchshofer [a,*]

[a] Université de Lyon, Université Lyon 1, ISPB-Faculté de Pharmacie, EA 4443 (B2C) « Biomolécules, Cancer et Chimiorésistances», 8, av. Rockefeller, F-69373, Lyon Cedex 08, France
[b] Université de Lyon, Université Lyon 1, ISPB-Faculté de Pharmacie, Laboratoire de Parasitologie, 8, av. Rockefeller, F-69373, Lyon Cedex 08, France

ARTICLE INFO

Article history:
Received 27 October 2009
Received in revised form
11 February 2010
Accepted 13 February 2010
Available online 18 February 2010

Keywords:
ABC transporter
Alkylation
Cryptosporidium parvum
Imidazo[4,5-*b*]pyridinone
Quinone

ABSTRACT

The syntheses of new *N*-polysubstituted imidazo[4,5-*b*]pyridine-7-one (IP, **5** and **8a**–**8f**) and indazole-4,7-dione (ID, **9** and **10**) derivatives are described. The binding affinity of IP and ID towards the recombinant Nucleotide Binding Domain NBD1 of *Cryptosporidium parvum* CpABC3 was evaluated by intrinsic fluorescence quenching. IP induced a moderate quenching of the intrinsic fluorescence of H6-NBD1 whereas IDs **9** and **10** showed a binding affinity comparable to the ATP analogue TNP-ATP. In addition, **8d**, **8e** and **10** were shown to be competitive inhibitors of the ATPase activity, but with low affinity. These compounds could thus act like some flavonoid derivatives, which can partly overlap both the nucleotide-binding site and the adjacent hydrophobic steroid-binding region of mammalian P-glycoproteins.

1. Introduction

Cryptosporidium parvum is an emerging infectious enteric protozoan [1]. It is the causative agent of waterborne intestinal infections and as such, may impair development in early infected children or be life-threatening to immunocompromised patients [2]. Since there is no effective drug treatment for cryptosporidiosis [3], the identification of chemotherapeutic targets could lead to new therapeutic approaches and may be a key for the discovery of really effective drugs. It has been suggested that the intrinsic resistance of *C. parvum* to most of the available anti-parasitic molecules was partly due to natural drug efflux involving ATP-Binding Cassette transporters (ABC proteins), which couple the energy of ATP hydrolysis to the transport of various substances through cell membranes [4]. Three types of ABC proteins have been described in *C. parvum* [5] and one of them, CpABC3, is related to the MDR sub-family to which the mammalian ABC transporter P-glycoprotein belongs [6]. The structural analysis of CpABC3 showed a typical structure of a P-glycoprotein with two Trans-Membrane Domains (TMDs) and two cytosolic Nucleotide-Binding Domains (NBD1 and NBD2), the most conserved feature of the MDR sub-family of ABC transporters [7].

We recently described the overexpression in *Escherichia coli* of an hexahistidine-tagged recombinant protein encompassing the N-terminal NBD1 of *C. parvum* CpABC3 containing a single ATP-binding site [8]. We have shown that this recombinant *C. parvum* NBD is fully functional towards known reference molecules acting on mammalian P-glycoproteins, such as TNP-ATP, quercetin, progesterone. On the other hand, we are involved in a program based on the synthesis of azaheterobicyclic molecules, especially benzimidazolediones [9], imidazopyridinones [10], and purine derivatives [11], designed to target parasitic enzymes that accept purine nucleosides as substrates. Thus, in order to identify scaffolds suitable for preparation of CpABC3 ligands, the binding affinity of such azaheterocycles as imidazo[4,5-*b*]pyridin-7-ones (**IP**) and indazole-4,7-diones (**ID**) towards the recombinant NBD1 of CpABC3 was assessed (Fig. 1).

* Corresponding authors. Tel.: +33 478777253; fax: +33 478777158.
E-mail address: sylvie.radix@univ-lyon1.fr (S. Radix).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.02.033

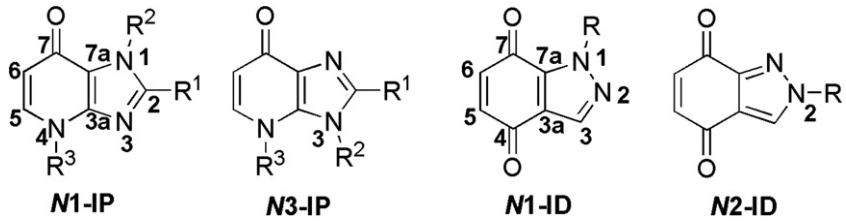


Fig. 1. Structures of *N*-1 and *N*-3-substituted imidazo[4,5-*b*]pyridin-7-one derivatives (*N*1-IP and *N*3-IP) and *N*-1 and *N*-2-substituted indazole-4,7-diones (*N*1-ID and *N*2-ID). Numbering system for NMR data.

2. Results and discussion

2.1. Chemistry

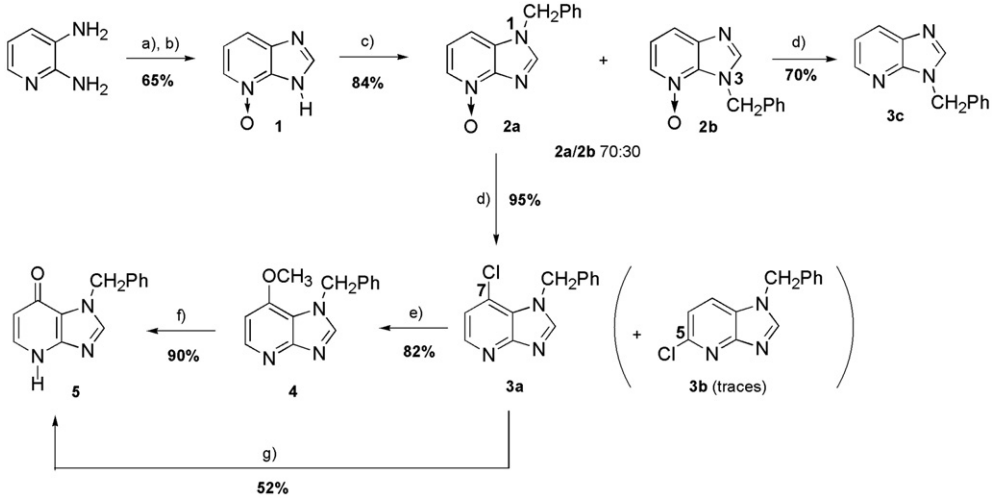
The first step was to prepare imidazo[4,5-*b*]pyridine derivatives (IP) whose synthesis has surprisingly, not been yet described in the literature. The route we have developed to synthesise the main intermediate, 1-benzyl-1,4-dihydroimidazo[4,5-*b*]pyridin-7-one **5**, is illustrated in Scheme 1.

It was prepared in six steps from commercially available pyridine-2,3-diamine with an overall yield of 27%. *N*-benzylation of imidazo[4,5-*b*]pyridine-4-oxide **1** was carried out according to a previously reported method [10]. Classical conditions of nucleophilic substitution, with freshly prepared benzyl iodide [12] as alkylating agent, mainly led to the *N*-1 regioisomer **2a**. Regioisomers **2a** and **2b** were easily separated by column chromatography on silica gel. Treatment of *N*-1 benzylated 4-oxide **2a** with phosphoryl chloride at reflux in chloroform for 24 hours predominantly yielded 7-chloro derivative **3a** [13]. Examination of ^{1}H NMR spectra of the crude reaction mixtures and of the remaining column fractions showed only traces of *N*-1 benzylated 5-chloro regioisomer **3b**. The structure of **3a** was confirmed by the presence of a doublet at lower field (δ 8.37 ppm) for H-5 with a fairly small coupling constant ($J_{5,6} = 5.3$ Hz). The structure of the isomer **3b** was assigned on the basis of the presence of a larger doublet ($J_{6,7} = 8.5$ Hz) for H-7 at 8.07 ppm. Surprisingly, chlorination of the *N*-3 benzylated 4-oxide **2b** under comparable conditions only underwent reduction of the *N*-oxide group to provide 3-benzylimidazo[4,5-*b*]pyridine **3c** with a yield of 70%. Preliminary experiments of hydrolysis of **3a** in acidic conditions (aqueous conc. HCl) only gave a yield of 52% of the desired IP **5** after one week. Unfortunately, a higher yield was not achieved using HCl in MeOH [14]. The mechanism of this latter reaction consists in a nucleophilic aromatic substitution of chloride by methoxide group and a subsequent S_N2 attack of chloride to yield the hydroxypyridine moiety and methyl chloride, which is converted into dimethylether [15]. In an analogous way, we developed a two-step sequence in basic conditions from **3a** to IP **5** *via* 7-methoxylated derivative **4**. The 7-chloroimidazo[4,5-*b*]pyridine **3a** was subjected to aromatic nucleophilic substitution using sodium methoxide to obtain the compound **4** with a very good yield. Subsequently, demethylation was easily performed using sodium ethyl thiolate in DMF as nucleophilic reagent. This convenient two-step procedure from **3a** to **5** provided a better global yield than the direct acidic hydrolysis of **3a**.

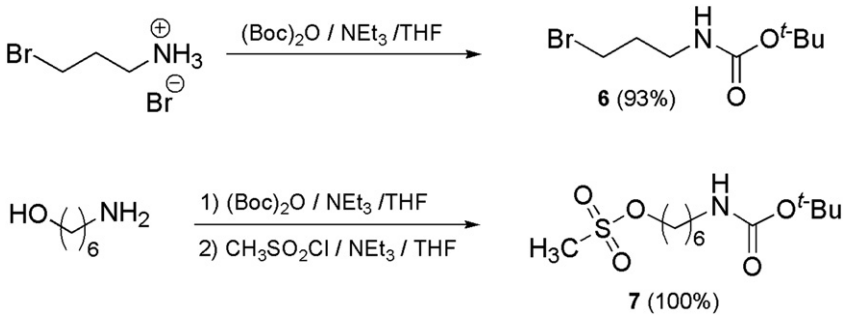
N-4-alkylation of IP 5 was then carried out to obtain poly *N*-substituted imidazo[4,5-*b*]pyridin-7-ones **8**. The alkylating agents **6** [16] and **7** [17] were first synthesised according to literature procedures (Scheme 2).

Reaction of IP **5** with 1.1 to 6 equiv. of various alkylating reagents (MeI, commercially available ω-halo alkyl esters and *N*-Boc protected alkyl amines **6** and **7**) in KOH methanol solution at 90 °C led to a single *N*-4-alkylated regioisomer with moderate to excellent yields (Scheme 3, Table 1).

The regioselectivity of the *N*-4-alkylation was controlled by NMR. 1D NOE difference spectrum of the *O*-methylated compound **4** showed the coupling through-space between CH_3 protons and both H-6 of the imidazopyridine moiety and the phenyl ortho



Scheme 1. Synthesis of 1-benzyl-1,4-dihydroimidazo[4,5-*b*]pyridin-7-one **5**. Reagents and conditions: a) i) $HC(OEt)_3$, reflux, 3h30 ii) 37% HCl, reflux, 1 h (83%); b) *m*-CPBA 90%, AcOH, 50 °C, 16 h (78%); c) i) K_2CO_3, DMF, RT, 1 h ii) $PhCH_2I$, RT, 4 h; d) $POCl_3$, $CHCl_3$, 50 °C, 19 h; e) MeONa, MeOH, 70 °C, 48 h; f) EtSH, NaH 60%, DMF, reflux, 3h30; g) 37% HCl, reflux, one week.



Scheme 2. Synthesis of *N*-Boc-protected alkylamine derivatives **6** and **7**.

protons (Fig. 2A). On the other hand, 1D NOE data registered from **8a** showed interactions of CH_3 protons only with H-5 of the imidazopyridine nucleus (Fig. 2B).

Indazole-4,7-dione derivatives **9** and **10** were then prepared (Fig. 3). The most useful method to obtain indazole-4,7-dione skeleton is the reaction between diazomethane or its derivatives and 1,4-benzoquinone substituted at *C*-2 or/and *C*-3 position(s) [18]. Owing to the hazardous nature of these reagents, we used a previously reported method to synthesise *N*-1- and *N*-2-ID from 7-nitroindazole [19].

ID **10** was synthesised according to the previously reported two-step procedure used to prepare ID **9** (Scheme 4) [19]. The synthesis started with the *N*-alkylation of commercially available 7-nitroindazole using 4-(chloromethyl)-2-methylthiazole as alkylating reagent. The improved alkylation conditions (NaH 1.2eq. in DMF used as a base) led to the highest yield, with a smooth selectivity in favour of the *N*-1-regioisomer **11a** (**11a**: **11b** = 65: 35).

Unambiguous assignment of the structure of both *N*-1 and *N*-2 regioisomers **13a** and **13b** (obtained by *N*-benzylation of 7-nitroindazole) has been previously reported [19]. The comparison of ^{1}H NMR spectra allowed to easily assign the structure of *N*-1 and *N*-2 regioisomers (Fig. 4). Indeed, the H-4 proton signals of *N*-2 products (**11b** and **13b**) were significantly deshielded compared with the same protons in *N*-1 compounds (**11a** and **13a**): $\Delta\delta = +0.11$ or $+0.16$ ppm.

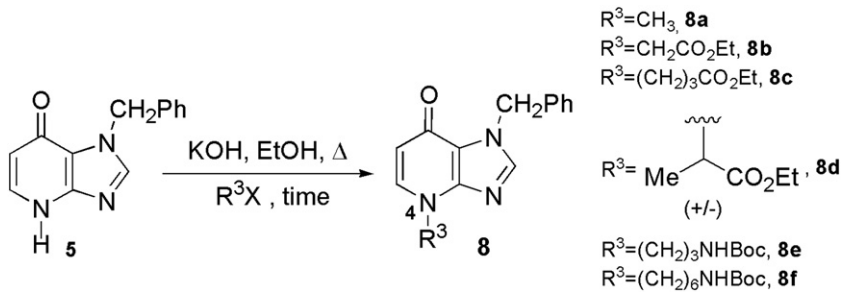
The synthesis was completed *via* reduction of the nitro group of **11a** by catalytic hydrogenation in acidic medium [20] followed by oxidation of the amino derivative **12** with phenyliodine(III) *bis* (trifluoroacetate) (PIFA) [21] to provide quinone **10**.

2.2. Activity tests

The affinity binding of the synthesised molecules **5**, **8a**–**8f**, **9** and **10** was monitored by quenching of the intrinsic fluorescence of the recombinant *C. parvum* H6-NBD1, which showed a characteristic emission peak at 328 nm due to buried Trp residues [8]. 2′-3′-O-(2,4,6-trinitrophenyl) adenosine 5′-triphosphate (TNP-ATP) and progesterone were used as reference molecules. TNP-ATP is a classical competitive inhibitor of ATPase activity [22]. Progesterone is a known steroidal P-glycoprotein modulator [23], and the steroid-interacting hydrophobic region is postulated to be located close to the ATP-binding site of P-glycoproteins [24,25].

The analogues **5** and **8a** did not bind to the recombinant H6-NBD1, indicating the great importance of the intracyclic nitrogen substitution. The results obtained for **8b**–**8f**, **9** and **10** are described in Fig. 5 and Table 2. Compounds **8b**, **8c**, **8d, 8f** induced a moderate quenching of the intrinsic fluorescence of H6-NBD1, similar to this caused by progesterone. Compound **8e** was not significantly different from the control. Conversely, compounds **9** and **10** were much more efficient and were comparable to the ATP analogue TNP-ATP.

The recombinant H6-NBD1 produced a measurable ATPase activity weaker than that of a purified P-glycoprotein, as already reported for a yeast NBD1 [26]. Nevertheless, the ability to inhibit the recombinant H6-NBD1 ATPase activity was evaluated for a set of four molecules, **8d** (IP derivative with an ester group), **8e**, **8f** (IP derivatives with a Boc-amino group), and **10** (ID) (Table 2, Fig. 5B–D). Compound **8f** was not further tested due to its lack of inhibitory activity. Compounds **8d**, **8e** and **10** were competitive inhibitors. However, compound **8e** was as bad an inhibitor as a quencher. The *N*-1-alkylated indazole-4-7-dione **10** caused the best inhibition of ATPase activity, about four times less than TNP-ATP, but twice as much than compound **8d**. It is worthy to note that the compounds **8d**, **8e**, **10** required rather high concentrations to inhibit the ATPase activity, whereas compound **10** showed an intrinsic fluorescence quenching similar to TNP-ATP. It could be hypothesised that the tested compounds could act like some flavonoid derivatives, which can partly overlap both the nucleotide-binding site and an adjacent hydrophobic steroid-binding region of P-glycoprotein [24,25].



Scheme 3. N^4-Alkylation of 1-benzyl-1,4-dihydroimidazo[4,5-*b*]pyridin-7-one **5** in basic conditions.

Table 1
Conditions and results of N^4-alkylation of **5**.

R^3X	Product	KOH	Time	Yield[a]
CH_3I (1.2 eq.)	**8a**	1.1 eq.	5h30	91%
EtO_2CCH_2Br (1.2 eq.)	**8b**	1.1 eq.	5h30	82%
$EtO_2C(CH_2)_3Br$ (1.2 eq.)	**8c**	1.1 eq.	22h30	64%
$MeCH(Cl)CO_2Et$ (+/-) NaI (1.2 eq./1.2 eq.)	**8d**	1.1 eq.	22h30	51%
6 (6 eq.)	**8e**	8 eq.	4 days	60%
7 (6 eq.)	**8f**	8 eq.	4 days	62%

[a] Isolated yields.

3. Conclusion

We report a useful and short access to original imidazo[4,5-*b*]pyridin-7-one derivatives. The binding affinity of these compounds (IP) and indazole-4,7-diones (ID, **9** and **10**) towards the recombinant H6-NBD1 of CpABC3 evidenced that compounds **9-10** were more active than imidazo[4,5-*b*]pyridin-7-ones, but for the latter, the nature of the intracyclic *N*-4 substitution appears critical and will be further explored. Aromatic azaheterocycles IP or ID seem then mandatory for an efficient binding to the H6-NBD1, but with a moderate inhibition of ATPase activity. IP and ID, which could be substituted by different side-chains, should be suitable scaffolds as CpABC3 ligands. Further studies are necessary to determine if these compounds represent a new class of bifunctional derivatives such as flavonoids, which are known to bind simultaneously to adjacent ATP and steroid sites of mammalian P-glycoproteins.

4. Experimental

4.1. Chemistry

4.1.1. General methods and materials

All air- and moisture-sensitive reactions were carried out under an argon atmosphere. All melting points were measured on a Barnstead Electrothermal 9200 apparatus and were uncorrected. Infrared spectra (IR) were recorded using KBr pellets on a Perkin–Elmer FT-IR SPECTRUM ONE spectrometer. ^{1}H spectra were recorded with Bruker ALS300 and Bruker DRX300 Fourier transform spectrometers, using an internal deuterium lock, operating at 300 MHz. ^{13}C NMR spectra were recorded with a Bruker DRX300 Fourier transform spectrometer, using an internal deuterium lock, operating at 75 MHz. All spectra were recorded at 25 °C. Chemical shifts are reported in parts per million (ppm) relative to residual protons or carbons thirteen of deuterated solvent ($\delta = 2.54$ ppm for ^{1}H NMR and $\delta = 40.45$ ppm for ^{13}C NMR for DMSO-d_6 and ($\delta = 7.26$ ppm for ^{1}H NMR and $\delta = 77.36$ ppm for ^{13}C NMR for $CDCl_3$). Carbon multiplicity was determined by DEPT experiments. Low- and high-resolution mass spectroscopy was recorded with a ThermoQuest FINNIGAN MAT 95 XL apparatus operating at 70 eV. Elemental analyses were performed at the Centre de Microanalyse du CNRS at Solaize (France). Product purification by flash column chromatography was performed using Merck Kieselgel 60 Å (40–63 μm). Analytical thin layer chromatography (TLC) was carried out using Merck commercial aluminium sheets coated (0.2 mm layer thickness) with Kieselgel 60 F254, with visualization by ultraviolet. All commercially available chemicals were used as received. Imidazo[4,5-*b*]pyridine-4-oxide **1** [13,27], benzyl iodide [12], *N*-Boc-3-bromopropylamine **6** [16], and *N*-Boc-6-amino-hexan-1-ol methanesulfonate **7** [17] were prepared according to literature procedures.

4.1.2. N-alkylation of 3H-imidazo[4,5-b]pyridine-4-oxide (*1*)

K_2CO_3 (0.370 g, 2.66 mmol) was added under argon to a suspension of compound **1** (0.300 g, 2.22 mmol) in DMF (5 mL). After stirring at room temperature for 1 h, freshly prepared benzyl iodide (0.400 mL, 3.20 mmol) was added. The mixture was stirred for 4 h and concentrated to give dark red oil which was partitioned between CH_2Cl_2 and water. Aqueous phase was extracted by CH_2Cl_2 and the combined organic extracts were dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/methanol, 95:5) to give a mixture of regioisomers **2a** as a white solid (0.295 g, 59%, $R_f = 0.08$) and **2b** as a light yellow solid (0.125 g, 25%, $R_f = 0.22$).

*4.1.2.1. 1-Benzyl-1H-imidazo[4,5-b]pyridine-4-oxide (**2a**).* M.p. 174–176 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 8.64$ (s, 1H, H-2), 8.20 (d, ^3J(H,H) = 6.3 Hz, 1H, H-5), 7.60 (d, ^3J(H,H) = 8.3 Hz, 1H, H-7), 7.40–7.25 (m, 5H, H-phenyl), 7.21 (dd, ^3J(H,H) = 6.3 and 8.3 Hz, 1H, H-6), 5.54 ppm (s, 2H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 147.5$ (C), 146.0 (C), 136.8 (CH, C-2), 134.1 (CH, C-5), 131.2 (C, C-phenyl ipso), 129.7 (2 × CH, C-phenyl), 129.2 (CH, C-phenyl para), 128.4 (2 × CH, C-phenyl), 120.0 (CH, C-7), 110.7 (CH, C-6), 49.4 ppm (CH_2); IR (KBr): $\nu = 3034, 2946, 1578, 1442, 1256, 1216, 1007, 729$ cm^{-1}; MS (70 eV, EI): *m/z* (%): 225 (4) [M^+], 209 (54) [M^+-O], 208 (96) [M^+-OH], 195 (42), 119 (16), 91 (100) [$C_7H_7^+$], 77 (14), 65 (16); HRMS (CI): *m/z*: calcd for $C_{13}H_{11}N_3O + H$: 226.0980; found: 226.0977.

*4.1.2.2. 3-Benzyl-3H-imidazo[4,5-b]pyridine-4-oxide (**2b**).* M.p. 101–103 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 8.39$ (d, ^3J (H,H) = 6.8 Hz, 1H, H-5), 8.37 (s, 1H, H-2), 8.32 (d, ^3J (H,H) = 7.7 Hz, 1H, H-7), 7.51-7.39 (m, 5H, H-phenyl), 7.17 (dd, ^3J (H,H) = 6.8 and 7.7 Hz, 1H, H-6), 5.72 ppm (s, 2H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 160.2$ (CH, C-2), 146.3 (C), 146.1 (C), 133.0 (C, C-phenyl ipso), 129.9 (2 × CH,

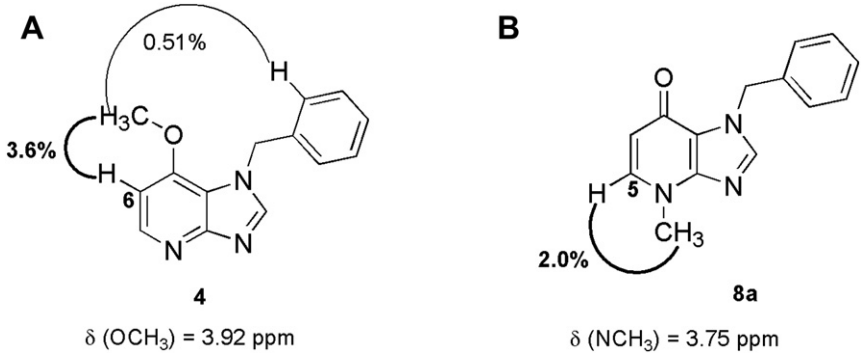
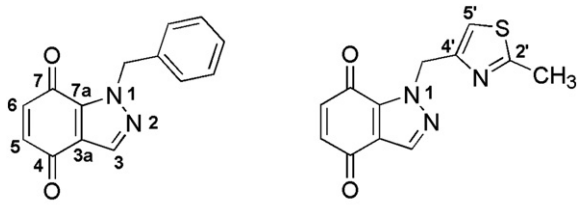


Fig. 2. Establishment of the regioselectivity of *N*-4-methylation of **5** by 1D NOE difference spectroscopy. ^{1}H NMR spectra were registered in DMSO-d_6. A) Irradiation of *O*-CH_3 resonance ($\delta = 3.92$ ppm) in compound **4**. B) Irradiation of *N*-CH_3 resonance ($\delta = 3.75$ ppm) in compound **8a**.



9 **10**

Fig. 3. Structure of *N*-1-alkylated indazole-4-7-diones **9** and **10**. Numbering system for NMR data.

C-phenyl), 129.5 (CH, C-5), 129.4 (CH, C-phenyl para), 128.9 (CH, C-7), 128.6 (2 × CH, C-phenyl), 112.0 (CH, C-6), 80.1 ppm (CH_2); IR (KBr): $\nu = 3088, 2923, 1394, 1297, 1185, 843, 747, 645\ cm^{-1}$; MS (70 eV, ESI): *m*/*z* (%): 226 (100) [MH^+], 210 (13); HRMS (EI): *m*/*z*: calcd for $C_{13}H_{11}N_3O$: 225.0902; found: 225.0903.

*4.1.3. Chlorination of imidazo[4,5-b]pyridine-4-oxide derivatives (**2a**) and (**2b**)*

$POCl_3$ (2.4 mL, 25.8 mmol) and 15 pieces of 4Å molecular sieves were added to a solution of compounds **2a** or **2b** (1.1 g, 4.8 mmol) in chloroform (30 mL) and the mixture was heated at 50 °C for 24 hours. The mixture was cooled to room temperature and quenched with aqueous $NaHCO_3$ (0.6 M, 100 mL). Aqueous phase was extracted by chloroform and the combined organic extracts were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH = 95:5).

*4.1.3.1. 1-Benzyl-7-chloro-1H-imidazo[4,5-b]pyridine (**3a**).* White solid (1.15 g, 95%, $R_f = 0.45$). M.p. 162–164 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 8.78$ (s, 1H, H-2), 8.37 (d, ^3J(H,H) = 5.3 Hz, 1H, H-5), 7.36 (d, ^3J(H,H) = 5.3 Hz, 1H, H-6), 7.34–7.25 (m, 3H, 2 × H-meta, H-para), 7.12 (d, ^3J(H,H) = 7.2 Hz, 2H, 2 × H-ortho), 5.75 ppm (s, 2H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 158.5$ (C, C-3a), 149.6 (CH, C-5), 145.7 (CH, C-2), 138.4 (C, C-7), 129.6 (2 × CH, C-phenyl), 128.4 (CH, C-phenyl para), 127.1 (2 × CH, C-phenyl), 126.6 (C, C-phenyl ipso), 123.3 (C, C-7a), 120.1 (CH, C-6), 50.2 (CH_2); IR (KBr): $\nu = 3073$, 2926, 1604, 1551, 1495, 1362, 1315, 1177, 733 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 245 (13) [(^{37}Cl)M^+], 243 (92) [(^{35}Cl)M^+], 242 (41) [M^+-H], 91 (100), 65 (8%); HRMS (CI): *m*/*z*: calcd for $C_{13}H_{10}^{37}ClN_3$: 244.06410; found: 244.06419.

*4.1.3.2. 1-Benzyl-5-chloro-1H-imidazo[4,5-b]pyridine (**3b**).* Traces; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 8.74$ (s, 1H, H-2), 8.07 (d, ^3J(H,H) = 8.5 Hz, 1H, H-7), 7.38–7.11 (m, 6H, H-6, H-arom), 5.55 ppm (s, 2H, CH_2).

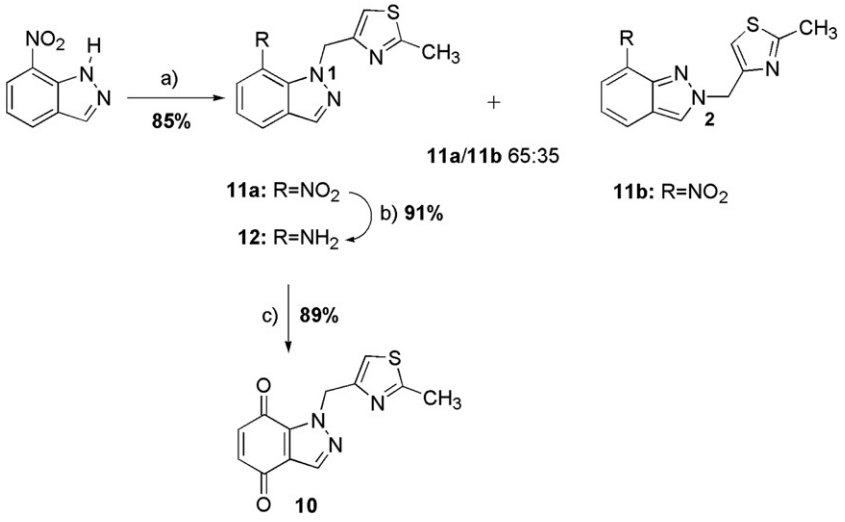
*4.1.3.3. 3-Benzylimidazo[4,5-b]pyridine (**3c**).* Colourless oil (0.70 g, 70%). ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 8.70$ (s, 1H, H-2), 8.41 (d, ^3J(H,H) = 4.7 Hz, 1H, H-5), 7.98 (d, ^3J(H,H) = 8.0 Hz, 1H, H-7), 7.34–7.311 (m, 5H, H-arom), 7.24 (dd, ^3J(H,H) = 4.7 and 8.0 Hz, 1H, H-7), 5.55 ppm (s, 2H, CH_2); MS (70 eV, ESI): *m*/*z* (%): 441 (40) [2 M^+ + Na], 232 (9) [M^+ + H], 210 [MH^+].

*4.1.4. 1-Benzyl-7-methoxy-1H-imidazo[4,5-b]pyridine (**4**)*

Compound **3a** was added to a freshly prepared solution of MeONa (1.25 M) in MeOH (75 mL) under argon and the mixture was refluxed for 48 hours. After cooling down to room temperature, the solvent was evaporated and the residue was partitioned between CH_2Cl_2 and water. Aqueous phase was extracted by CH_2Cl_2. The combined organic extracts were washed once by water, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH = 95:5) to give compound **4** as a white solid (0.90 g, 82%, $R_f = 0.20$). M.p. 169–170 °C (dec.); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 8.50$ (s, 1H, H-2), 8.26 (d, ^3J(H,H) = 5.5 Hz, 1H, H-5), 7.36–7.22 (m, 5H, H-phenyl), 6.87 (d, ^3J(H,H) = 5.5 Hz, 1H, H-6), 5.55 (s, 2H, CH_2), 3.92 ppm (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 157.8$ (C, C-3a), 153.3 (C, C-7), 145.9 (CH, C-5), 145.3 (CH, C-2), 137.8 (C, C-phenyl ipso), 128.6 (2 × CH, C-phenyl meta), 127.6 (CH, C-phenyl para), 127.1 (2 × CH, C-phenyl ortho), 115.6 (C, C-7a), 101.1 (CH, C-6), 55.9 (CH_3), 49.9 ppm (CH_2); IR (KBr): $\nu = 3081$, 3026, 2924, 2848, 1618, 1495, 1455, 1322, 1297, 1184, 1098, 743 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 239 (100) [M^+], 224 (6) [M^+-CH_3], 209 (8), 91 (60), 65 (6); HRMS (CI): *m*/*z*: calcd for $C_{14}H_{13}N_3O$ + H: 240.11370; found: 240,11385.

*4.1.5. Synthesis of 1-benzyl-1,4-dihydroimidazo[4,5-b]pyridin-7-one (**5**)*

*4.1.5.1. Hydrolysis of (**3a**).* Compound **3a** (0.070 g, 0.28 mmol) was dissolved in concentrated hydrochloric acid (37%, 2.7 mL).



Scheme 4. Synthesis of 1-[(2-methylthiazol-4-yl)methyl]-*1H*-indazole-4,7-dione **10**. Reagents and conditions: a) i) NaH, DMF, RT, 5 mn ii) 4-chloromethyl-2-methylthiazole, RT, 15 h; b) H_2 (4 bar), Pd/C, AcOEt, AcOH, RT, 5 h; c) $PhI(OCOCF_3)_2$, MeCN, H_2O, 0 °C then RT, 20 h.

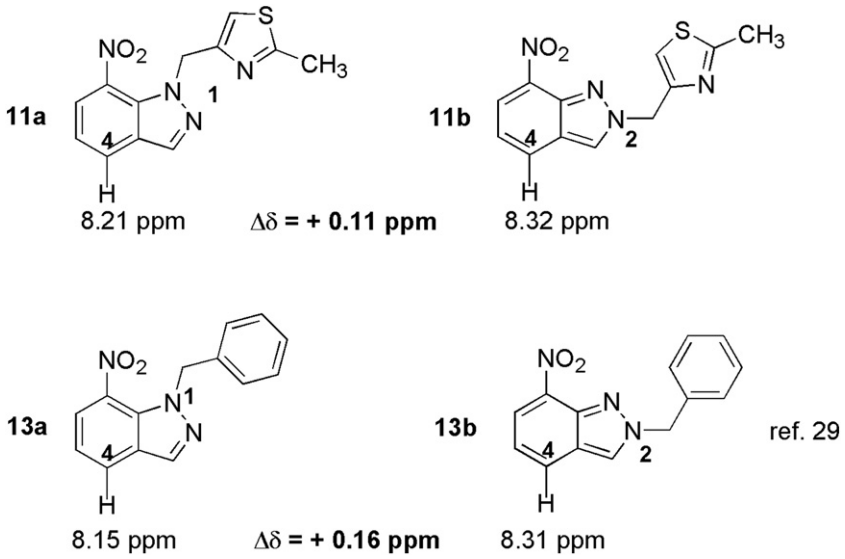


Fig. 4. Assignment of the structure of *N*-1 and *N*-2 regioisomers by comparison of ^{1}H NMR spectra. ^{1}H NMR spectra were recorded at 300 MHz in DMSO-d_6.

The mixture was refluxed for one week. After cooling down to room temperature, the mixture was neutralized adding NaOH pellets to pH 7. Compound **5** partially precipitated after cooling the mixture in an ice bath. After filtration and extraction of filtrate with AcOEt, compound **5** was finally obtained as a light yellow solid (0.033 g, 52%).

*4.1.5.2. Demethylation of (**4**).* NaH (60%, 1.36 g, 34 mmol) was added under argon at 0 °C to a solution of ethanethiol (3.9 mL, 52 mmol) in DMF (35 mL). After 15 mn stirring at 0 °C, this latter solution was added to compound **4** neat (0.82 g, 3.4 mmol) maintained under argon. The mixture was refluxed for 3h30. After cooling down to room temperature, an aqueous solution of AcOH

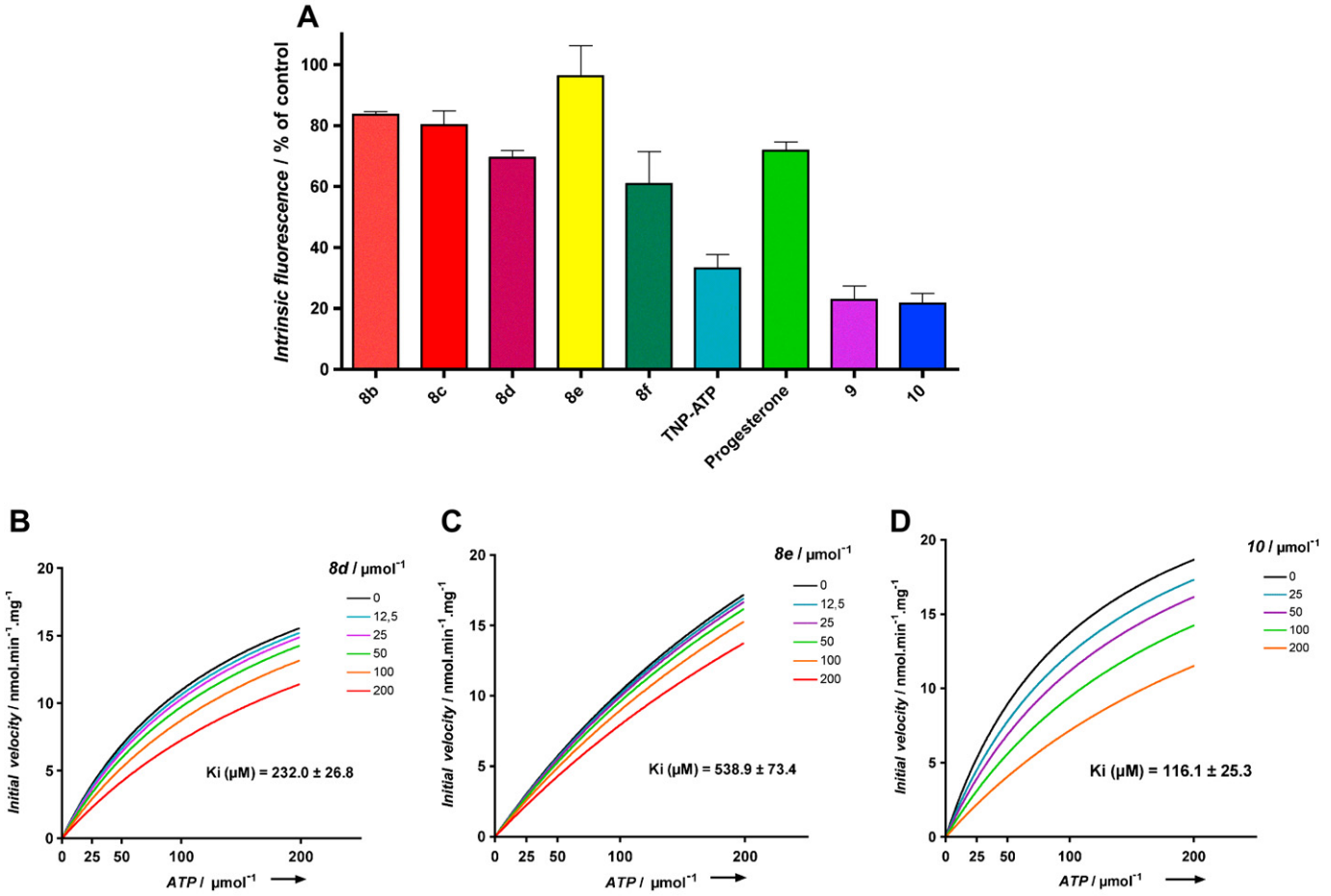


Fig. 5. Quenching of the recombinant H6-NBD1 intrinsic fluorescence by some of the compounds (100 µM) in reference to the ATP analogue TNP-ATP and the steroid modulator progesterone (A) at the same concentration. Competitive inhibition of the ATPase activity by compounds **8d** (B), **8e** (C) and **10** (D).

Table 2
Interactions of some of the compounds with the recombinant H6-NBD1.

Entry	Compound	Intrinsic fluorescence quenching[a] (% of control)	ATPase inhibition Ki (μM)
1	**8b**	85.5 ± 1.8	N.D.[b]
2	**8c**	80.1 ± 8.2	N.D.
3	**8d**	69.3 ± 4,4	232.0 ± 26.8
4	**8e**	96.1 ± 17,4	538.9 ± 73.4
5	**8f**	60.8 ± 18,5	N.I.[c]
6	**9**	22.9 ± 7.8	N.D.
6	**10**	21.6 ± 5.9	116.1 ± 25.3
7	**TNP-ATP**	33.1 ± 7.9	36.6 ± 4.5
8	**Progesterone**	71.7 ± 5.1	N.D.[d]

[a] In fluorescence experiments, the compounds were tested at a concentration of 100 μM. Results from 3 separate experiments.
[b] N.D.: not determined.
[c] N.I.: not inhibitory.
[d] Progesterone is a P-gp modulator but not an inhibitor of the ATPase activity.

was added drop by drop to pH 7. Aqueous phase was extracted by CH_2Cl_2 and the combined organic extracts were dried (Na_2SO_4), filtered and concentrated. The residue was recrystallised in AcOEt/Petroleum ether (PE) to give compound **5** as a white solid (0.690 g, 90%, $R_f = 0.22$ for CH_2Cl_2/MeOH = 95:5). M.p. 236–237 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.32 (bs, 1H, NH or OH), 8.23 (s, 1H, H-2), 7.62 (d, ^3J (H,H) = 7.3 Hz, 1H, H-5), 7.43–7.26 (m, 5H, H-phenyl), 6.00 (d, ^3J (H,H) = 7.3 Hz, 1H, H-6), 5.68 ppm (s, 2H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.6 (C, C-7), 147.6 (C, C-3a), 142.4 (CH, C-2), 139.0 (C, C-phenyl ipso), 135.3 (CH, C-5), 129.4 (2 × CH, C-phenyl meta), 128.5 (3 × CH, 2 × C-phenyl ortho, C-phenyl para), 120.1 (C, C-7a), 112.4 (CH, C-6), 49.5 ppm (CH_2); IR (KBr): ν = 3436, 2962, 2925, 2854, 2525, 1613, 1532, 1517, 1478, 1261, 1217, 1078, 1092, 1026, 803, 731 cm^{-1}; MS (70 eV, EI): *m/z* (%): 225 (100) [M$^+$], 224 (88), 197 (8), 148 (28), 91 (28), 65 (7); HRMS (EI): *m/z*: calcd for $C_{13}H_{11}N_3O$ + H: 226.0980; found: 226.0983.

4.1.6. *N*-alkylation of 1-benzyl-1,4-dihydroimidazo[4,5-b]pyridin-7-one (**5**)

Compound **5** (0.115 g, 0.5 mmol) was added to a solution of KOH (0.55 mmol or 4 mmol, see Table 1) in EtOH (5 mL). R^{3}X (0.6 mmol or 3 mmol, see Table 1) was added and the mixture was refluxed until reaction did not evolve anymore (time reaction indicated in Table 1). After cooling down to room temperature, EtOH was evaporated and the residue was partitioned between CH_2Cl_2 and water. Aqueous phase was thoroughly extracted by CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH = 95:5).

*4.1.6.1. Imidazo[4,5-b]pyridin-7-one (**8a**).* White solid (0.109 g, 91%, R_f = 0.32 for CH_2Cl_2/MeOH = 95:5). M.p. 165–166 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.26 (s, 1H, H-2), 7.66 (d, ^3J (H,H) = 7.5 Hz, 1H, H-5), 7.38–7.22 (m, 5H, H-phenyl), 5.98 (d, ^3J (H,H) = 7.5 Hz, 1H, H-6), 5.67 (s, 2H, CH_2), 3.75 ppm (s, 3H, NCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 169.9 (C, C-7), 147.9 (C, C-3a), 142.1 (CH, C-2), 139.4 (CH, C-5), 138.9 (C, C-phenyl ipso), 129.4 (2 × CH, C-phenyl meta), 128.5 (3 × CH, 2 × C-phenyl ortho, C-phenyl para), 120.1 (C, C-7a), 112.5 (CH, C-6), 49.5 (CH_2), 37.6 ppm (CH_3); IR (KBr): ν = 3080, 2930, 2922, 2852, 1638, 1628, 1573, 1499, 1473, 1456, 1429, 1401, 1301, 1229, 1148, 809, 728, 703 cm^{-1}; MS (70 eV, EI): *m/z* (%): 239 (100) [M$^+$], 224 (8), 162 (30), 133 (5), 91 (26), 65 (5); HRMS (CI): *m/z*: calcd for $C_{14}H_{13}N_3O$ + H: 240.1137; found: 240.1135.

*4.1.6.2. Imidazo[4,5-b]pyridin-7-one (**8b**).* White solid (0.130 g, 82%, R_f = 0.64 for CH_2Cl_2/MeOH = 95:5). M.p. 136–138 °C (Et_2O); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.23 (s, 1H, H-2), 7.67 (d, ^3J (H,H) = 7.5 Hz, 1H, H-5), 7.40–7.24 (m, 5H, H-phenyl), 6.03 (d, 1H, ^3J (H,H) = 7.5 Hz, H-6), 5.65 (s, 2H, NC**H**$_2$Ph), 5.03 (s, 2H, NC**H**$_2$$CO_2$Et), 4.14 (q, 2H, ^3J (H,H) = 7.1 Hz, CO_2CH_2), 1.19 ppm (t, 3H, ^3J (H,H) = 7.1 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.3 (C, COO), 168.9 (C, C-7), 147.6 (C, C-3a), 142.0 (CH, C-2), 139.4 (CH, C-5), 138.7 (C, C-phenyl ipso), 129.5 (2 × CH, C-phenyl meta), 128.64 (CH, C-phenyl para), 128.62 (2 × CH, C-phenyl ortho), 119.7 (C, C-7a), 112.9 (CH, C-6), 62.1 (CH_2, CO_2**C**H_2), 51.0 (CH_2, N**C**H_2CO_2Et), 49.6 (CH_2, N**C**H_2Ph), 14.8 ppm (CH_3); IR (KBr): ν = 3085, 3063, 3052, 2979, 2939, 1735, 1626, 1585, 1474, 1451, 1395, 1374, 1324, 1231, 1200, 1024, 911, 818, 732; MS (70 eV, EI): *m/z* (%): 311 (100) [M$^+$], 282 (10), 238 (32), 234 (17), 224 (10), 206 (7), 91 (23); HRMS (CI): *m/z*: calcd for $C_{17}H_{17}N_3O_3$ + H: 312.1348; found: 312.13461.

*4.1.6.3. Imidazo[4,5-b]pyridin-7-one (**8c**).* Colourless oil (1.110 g, 64%, R_f = 0.52 for CH_2Cl_2/MeOH = 95:5). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.60 (s, 1H, H-2), 7.40–7.26 (m, 6H, H-5, 5 × H-phenyl), 6.23 (d, 1H, ^3J (H,H) = 7.5 Hz, H-6), 5.72 (s, 2H, NC**H**$_2$Ph), 4.22 (t, ^3J (H, H) = 7.1 Hz, 2H, NC**H**$_2$$(CH_2)_2CO_2$Et), 4.09 (t, ^3J (H,H) = 7.2 Hz, 2H, CO_2CH_2), 2.32 (t, ^3J (H,H) = 7.1 Hz, 2H, C**H**$_2CO_2$Et), 2.17 (qn, ^3J (H, H) = 7.1 Hz, 2H, C**H**$_2CH_2CO_2$Et), 1.21 ppm (t, ^3J (H,H) = 7.2 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 172.5 (C, COO), 170.4 (C, C-7), 147.0 (C, C-3a), 140.1 (CH, C-2), 136.8 (CH, C-5), 136.5 (C, C-phenyl ipso), 129.0 (2 × CH, C-phenyl meta), 128.4 (2 × CH, C-phenyl ortho), 128.3 (CH, C-phenyl para), 120.4 (C, C-7a), 113.3 (CH, C-6), 60.7 (CH_2, CO_2**C**H_2), 50.1 (CH_2, N**C**$H_2(CH_2)_2CO_2$Et), 49.8 (CH_2, N**C**H_2Ph), 30.8 (CH_2), 24.9 (CH_2), 14.2 ppm (CH_3); MS (70 eV, ESI): *m/z* (%): 701 (44) [2 M$^+$ + Na], 679 (26) [2 M$^+$ + H], 340 (100) [MH$^+$]; HRMS (CI): *m/z*: calcd for $C_{19}H_{21}N_3O_3$ + H: 340.1661; found: 340.16602.

*4.1.6.4. Imidazo[4,5-b]pyridin-7-one (**8d**).* Colourless oil (0.083 g, 51%, R_f = 0.65 for CH_2Cl_2/MeOH = 95:5). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.58 (s, 1H, H-2), 7.43 (d, ^3J (H,H) = 7.5 Hz, 1H, H-5), 7.39–7.27 (m, 5H, H-phenyl), 6.31 (d, ^3J (H,H) = 7.5 Hz, 1H, H-6), 5.71 (d, ^2J (H,H) = 14.8 Hz, 1H, AB system, NC**H**$_a$H$_b$), 5.67 (d, ^2J (H, H) = 14.8 Hz, 1H, AB system, NCH$_a$**H**$_b$), 5.57 (q, ^3J (H,H) = 7.2 Hz, 1H, NC**H**CH_3), 4.19 (q, ^3J (H,H) = 7.2 Hz, 2H, CO_2CH_2), 1.77 (d, ^3J (H,H) = 7.2 Hz, 3H, C**H**$_3$CH), 1.21 ppm (t, ^3J (H,H) = 7.2 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 172.8 (C, COO), 170.2 (C, C-7), 147.1 (C, C-3a), 139.8 (CH, C-2), 136.4 (C, C-phenyl ipso), 134.1 (CH, C-5), 129.0 (2 × CH, C-phenyl meta), 128.3 (2 × CH, C-phenyl ortho), 128.7 (CH, C-phenyl para), 120.0 (C, C-7a), 113.8 (CH, C-6), 62.2 (CH_2, CO_2**C**H_2), 55.7 (NCH), 50.2 (CH_2, N**C**H_2Ph), 17.0 (CH_3), 14.1 ppm (CH_3, CO_2CH_2**C**H_3); MS (70 eV, ESI): *m/z* (%): 673 (43) [2 M$^+$ + Na], 651 (22) [2 M$^+$ + H], 326 (100) [MH]; HRMS (CI): *m/z*: calcd for $C_{18}H_{19}N_3O_3$ + H: 326.1505; found: 326.15059.

*4.1.6.5. Imidazo[4,5-b]pyridin-7-one (**8e**).* White solid (0.115 g, 60%, R_f = 0.30 for CH_2Cl_2/MeOH = 95:5). M.p. 136–137 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.24 (s, 1H, H-2), 7.71 (d, ^3J (H,H) = 7.5 Hz, 1H, H-5), 7.32-7.24 (m, 5H, H-phenyl), 6.90 (t, ^3J (H,H) = 5.4 Hz, 1H, NH), 5.98 (d, ^3J (H,H) = 7.5 Hz, 1H, H-6), 5.66 (s, 2H, NC**H**$_2$Ph), 4.14 (t, ^3J (H,H) = 6.8 Hz, 2H, NC**H**$_2$$(CH_2)_2$NHBoc), 2.91 (td, ^3J (H,H) = 5.4 and 6.8 Hz, 2H, C**H**$_2$NHBoc), 1.87 (qn, ^3J (H,H) = 6.8 Hz, 2H, C**H**$_2CH_2$NHBoc), 1.36 ppm (s, 9H, $C(CH_3)_3$); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 169.9 (C, C-7), 156.4 (C, NH**C**O^{t}Bu), 147.4 (C, C-3a), 142.0 (CH, C-2), 138.8 (C, C-phenyl ipso), 138.7 (CH, C-5), 129.5 (2 × CH, C-phenyl meta), 128.65 (2 × CH, C-phenyl ortho), 128.6 (CH, C-phenyl para), 120.1 (C, C-7a), 112.6 (CH, C-6), 78.5 (C, **C**$(CH_3)_3$), 49.5 (CH_2, N**C**H_2Ph), 48.4 (CH_2, N**C**$H_2(CH_2)_2$NHBoc), 38.0 (CH_2, **C**H_2NHBoc), 30.5 (CH_2), 29.1 ppm (3 × C, C(**C**$H_3)_3$); IR (KBr): ν = 3368, 2976, 2960, 1697, 1626, 1571, 1496, 1469, 1393, 1366, 1253, 1168, 1079, 818, 726; HRMS (ESI): *m/z*: calcd for $C_{21}H_{26}N_4O_3$ + H: 383.2083; found: 383.20823.

*4.1.6.6. Imidazo[4,5-b]pyridin-7-one (**8f**).* Colourless oil (0.131 g, 62%, $R_f = 0.40$ for CH_2Cl_2/MeOH = 95:5). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.22$ (s, 1H, H-2), 7.69 (d, ^3J (H,H) = 7.5 Hz, 1H, H-5), 7.39–7.24 (m, 5H, H-phenyl), 6.73 (m, 1H, NH), 5.97 (d, ^3J (H,H) = 7.5 Hz, 1H, H-6), 5.66 (s, 2H, NC**H**$_2$Ph), 4.14 (m, 2H, NC**H**$_2$$(CH_2)_5$NHBoc), 2.91-2.82 (m, 4H, 2 × CH_2), 1.74 (m, 2H, CH_2), 1.37-1.23 ppm (m, 13H, C$(CH_3)_3$, 2 × CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.7$ (C, C-7), 156.4 (C, NH**C**O^{t}Bu), 147.4 (C, C-3a), 140.5 (CH, C-2), 137.1 (CH, C-5), 136.9 (C, C-phenyl ipso), 129.3 (2 × CH, C-phenyl meta), 128.8 (2 × CH, C-phenyl ortho), 128.6 (CH, C-phenyl para), 120.7 (C, C-7a), 113.4 (CH, C-6), 79.4 (C, **C**$(CH_3)_3$), 51.1 (CH_2, NCH_2), 50.5 (CH_2, NCH_2), 33.0 (CH_2, **C**H_2NHBoc), 30.2 (CH_2), 30.0 (CH_2), 28.8 (3 × C, C(**C**$H_3)_3$), 26.6 (CH_2), 26.5 ppm (CH_2); HRMS (ESI): *m*/*z*: calcd for $C_{24}H_{32}N_4O_3 + H$: 425.2553; found: 425.25522.

4.1.7. N-alkylation of 7-nitroindazole

To a solution of 7-nitroindazole (0.500 g, 3.06 mmol) in dry DMF (15 mL), was added NaH (60% in mineral oil, 0.147 g, 3.67 mmol). After stirring for 5 min, 4-chloro-2-methylthiazole (0.677 g, 3.67 mmol) was added and stirring was maintained for 15 h. The solution was then poured into ice-water (200 mL) and diluted HCl was added to adjust the pH to about 6. This solution was extracted with AcOEt and the combined organic layers were washed with water and brine, dried over Na_2SO_4 and evaporated under vacuum. The residue was purified by column chromatography (AcOEt/petroleum ether (PE) = 25/75 then 70/30) to give a mixture of regioisomers **11a** as a brown solid (0.252 g, 30%, $R_f = 0.54$ for AcOEt/PE = 15:85) and **11b** as a light yellow solid (0.459 g, 55%, $R_f = 0.14$ for AcOEt/PE = 60:40).

*4.1.7.1. 1-[(2-Methylthiazol-4-yl)methyl]-7-nitro-1H-indazole (**11a**).* M.p. 151 °C; ^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 8.49$ (s, 1H, H-3), 8.29 (d, ^3J (H,H) = 7.7 Hz, 1H, H-6), 8.21 (d, ^3J (H,H) = 7.5 Hz, 1H, H-4), 7.40 (dd, ^3J (H,H) = 7.5 and 7.7 Hz, 1H, H-5), 7.18 (bs, 1H, H-5′), 5.87 (s, 2H, CH_2), 2.50 ppm (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) $\delta = 166.0$ (C, C-2′), 150.8 (C, C-4′), 135.6 (C, C-7a), 135.3 (CH, C-3), 129.9 (C, C-7), 128.9 (C, C-3a), 128.7 (CH, C-4), 124.7 (CH, C-5), 120.4 (CH, C-6), 116.2 (CH, C-5′), 51.9 (CH_2), 18.6 ppm (CH_3); IR (KBr): $\nu = 1524$, 1328, 739 cm^{-1}; Anal. Calcd for $C_{12}H_{10}N_4O_2$: C, 52.54; H, 3.67; N, 20.43; O, 11.67; S, 11.69. Found: C, 52.81; H, 3.68; N, 20.66; O, 11.93; S, 11.44.

*4.1.7.2. 2-[(2-Methylthiazol-4-yl)methyl]-7-nitro-1H-indazole (**11b**).* M.p. 168 °C; ^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 8.90$ (s, 1H, H-3), 8.34 (d, ^3J (H,H) = 7.5 Hz, 1H, H-6), 8.32 (d, ^3J (H,H) = 8.3 Hz, 1H, H-4), 7.61 (bs, 1H, H-5′), 7.29 (dd, ^3J (H,H) = 7.5 and 8.3 Hz, 1H, H-5), 5.86 (s, 2H, CH_2), 2.63 ppm (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) $\delta = 166.4$ (C, C-2′), 149.6 (C, C-4′), 139.6 (C, C-7a), 136.6 (C, C-7), 130.2 (CH, C-3), 127.6 (CH, C-4), 125.2 (C, C-3a), 124.9 (CH, C-5), 119.8 (CH, C-6), 118.3 (CH, C-5′), 52.7 (CH_2), 18.7 ppm (CH_3); IR (KBr): $\nu = 1633$, 1508, 1322, 1291, 1136, 746 cm^{-1}; Anal. Calcd for $C_{12}H_{10}N_4O_2$: C, 52.54; H, 3.67; N, 20.43; O, 11.67; S, 11.69. Found: C, 52.73; H, 3.67; N, 20.86; O, 11.79; S, 11.05.

*4.1.8. 7-Amino-1-[(2-methylthiazol-4-yl)methyl]-7-nitro-1H-indazole (**12**)*

A solution of 7-nitroindazole **11a** (1.210 g, 4.42 mmol) in AcOEt (63 mL) was treated with acetic acid (19 mL) and then added to a suspension of prereduced 10% Pd/C (0.390 g, 32% w/w) in AcOEt (15 mL). The mixture was stirred under 5 bar of H_2 for 5 h, and then filtered through celite. The residue was neutralized with diluted cooled NH_4OH and extracted with AcOEt. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (silica gel neutralized with NEt_3, AcOEt/PE = 30:70) to give compound **12** as a purple oil (0.980 g, 91%, $R_f = 0.51$ for AcOEt/PE = 70:30). ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 8.01$ (s, 1H, H-3), 7.26 (d, ^3J (H,H) = 8.0 Hz, 1H, H-4), 7.24 (s, 1H, H-5′), 7.04 (dd, ^3J (H,H) = 7.3 and 8.0 Hz, 1H, H-5), 6.73 (d, ^3J (H,H) = 7.3 Hz, 1H, H-6), 5.98 (s, 2H, CH_2), 5.11 (bs, 2H, NH_2), 2.71 ppm (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 166.7$ (C, C-2′), 151.8 (C, C-4′), 133.2 (CH, C-3), 132.7 (C, C-3a), 131.8 (C, C-7a), 125.9 (C, C-7), 121.7 (CH, C-5), 116.5 (CH, C-5′), 112.3 (CH, C-6), 111.4 (CH, C-4), 50.2 (CH_2), 18.8 ppm (CH_3); IR (KBr): $\nu = 3390$, 1586, 1273, 735 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 244 (100) [M$^+$], 146 (65), 112 (31), 71 (33); HRMS (CI): *m*/*z*: calcd for $C_{12}H_{12}N_4S + H$: 245.08; found: 245.08640.

*4.1.9. 1-[(2-methylthiazol-4-yl)methyl]-1H-indazole-4,7-dione (**10**)*

A solution of 7-aminoindazole **12** (0.126 g, 0.516 mmol) in $CH_3CN/H_2O = 4.3:1.5$ (mL:mL) was added dropwise to a solution of $PhI(OCOCF_3)_2$ (0.555 g, 1.29 mmol) in $CH_3CN/H_2O = 4.3:1.5$ (mL:mL) cooled at 0 °C. The solution was stirred for 2 h at room temperature. Then, water and AcOEt (50 mL) were added, and aqueous phase was extracted by AcOEt. Organic layer was washed with brine to adjust the pH to about 7, dried (Na_2SO_4), and concentrated. The residue was then purified by flash chromatography on silica gel (AcOEt/PE = 35:65) to give quinone **10** as a red solid (0.119 g, 89%, $R_f = 0.49$ for AcOEt/PE = 60:40). M.p. 128-129 °C; ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 7.98$ (s, 1H, H-3), 7.12 (s, 1H, H-5′), 6.72 (d, ^3J (H,H) = 10.4 Hz, AB system, 2H, H-5 and H-6), 5.85 (s, 2H, CH_2), 2.69 ppm (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 181.2$ (CO), 177.4 (CO), 166.9 (C, C-2′), 149.3 (C, C-4′), 138.5 (CH, C-3), 136.7 (CH, C-5 or C-6), 136.6 (CH, C-6 or C-5), 135.5 (C, C-7a), 121.5 (C, C-3a), 117.0 (CH, C-5′), 50.6 (CH_2), 19.1 ppm (CH_3); IR (KBr): $\nu = 1665$, 1170, 847 cm^{-1}; HRMS (CI): *m*/*z*: calcd for $C_{12}H_9N_3O_2S$: 260.0494; found: 260.04931.

4.2. Activity tests

4.2.1. Production of the recombinant C. parvum H6-NBD1

The recombinant protein was overexpressed in *E. coli* BL21(DE3) pLysS and its medium scale production was carried out under 3.5 L in a glass bioreactor controlled by the BioXpert 1.3 software (Applikon, Systeme C Industrie, St-Paul-Trois-Châteaux, France) for 5 hours. The temperature was set at 37 °C, the pH at 7.0 and the dissolved oxygen (dO_2) at 20%. Three hours after inoculation, induction was performed with 0.1 mM IPTG for 2 h and the bacterial pellet was stored in aliquots at −80 °C. The H6-NBD1 was subsequently affinity-purified as previously described [8] on Ni-NTA columns and dialyzed overnight against 50 mM Tris, pH 7.3 with 100 mM KCl, 0.02% HECAMEG (6-0-[(*N*-heptylcarbamoyl) methyl] α-D-glucopyranoside) and 20% glycerol. The protein was used directly for the ATPase inhibition assay or stored in aliquots at −80 °C.

4.2.2. Fluorescence assays

Fluorescence experiments were performed in triplicates on a Perkin–Elmer LS-3B spectrofluorimeter in a 1 cm-path quartz microcuvette. The recombinant protein was diluted at a concentration of 0.25–0.5 μM in 390 μl of 50 mM Tris pH 7.3, 100 mM KCl, 0.02% HECAMEG. The analogues were added in 10 μl and the mixture was incubated for 30 min at 20 ± 2 °C. Intrinsic fluorescence emission was measured upon excitation at 295 nm and the binding of the analogues was assayed by quenching of the intrinsic fluorescence at 328 nm. Controls were made in the same conditions with the last dialysis buffer to eliminate any interference due to the remaining imidazole. The hydrophobic modulators were dissolved as stock solutions in DMSO and controls included the same solvent concentration, not exceeding 2.5%. Statistics and curve fitting were made with the Prism 4 software program from GraphPad (San Diego, CA).

4.2.3. ATPase inhibition assays

The analogues were serially diluted in various ATP concentrations and their inhibitory activity was measured by incubating

10 min at 37 °C the purified recombinant H6-NBD1 (2.5 μM final) in 50 mM Tris pH 7.3, 100 mM KCl, 4 mM $MgCl_2$ and 0,02% HECAMEG. The reaction products were measured in triplicates by HPLC on a 5 μm reverse phase Hypersil column (Interchim, Montluçon, France) with 1,8% methanol (v/v) in 25 mM $(NH)_4H_2PO_4$ as the mobile phase. The injection volume was 20 μl, the flow rate was 0.5 ml/min and the effluent was monitored at 254 nm. The type of inhibition and Ki were computed by non-linear regression using the GraphPad Prism 4 software program.

References

[1] S. Tzipori, Microbes Infect. 4 (2002) 1045.
[2] R.A. Dillingham, A.A. Lima, R.L. Guerrant, Microbes Infect. 4 (2002) 1059–1066.
[3] H.V. Smith, G.D. Corcoran, Curr Opin. Infect. Dis. 17 (2004) 557–564.
[4] M.E. Perkins, Y.A. Riojas, T.W. Wu, S. Leblancq, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 5734–5739.
[5] M.T. Bonafonte, P.A. Romagnoli, N. Mc Nair, A.P. Shaw, M. Scanlon, G.J. Leitch, J. R. Mead, Exp. Parasitol. 106 (2004) 126–134.
[6] F. Zapata, M.E. Perkins, Y.A. Riojas, T.W. Wu, S.M. Le Blancq, Mol. Biochem. Parasitol. 120 (2002) 157–161.
[7] P. Matsson, J.M. Pederson, U. Norinder, C.A.S. Bergström, P. Artursson, Pharm. Res. 26 (2009) 1816–1831.
[8] P. Lawton, M. Pelandakis, A.-F. Pétavy, N. Walchshofer, Mol. Biochem. Parasitol. 152 (2007) 101–107.
[9] F. Alvarez, A. Ghérardi, P. Nebois, M.-E. Sarciron, A.-F. Pétavy, N. Walchshofer, Bioorg. Med. Chem. Lett. 12 (2002) 977–979.
[10] W. Zeinyeh, J. Pilmé, S. Radix, N. Walchshofer, Tetrahedron Lett. 50 (2009) 1828–1833.
[11] M.E. Million, S. Marrot, M. Imbert, S. Radix, J. Paris, Lett. Org. Chem. 5 (2008) 484–489.
[12] M. Namavari, N. Satyamurthy, M.E. Phelps, J.R. Barrio, Tetrahedron Lett. 31 (1990) 4973–4976.
[13] T. Itoh, K. Ono, T. Sugawara, Y. Mizuno, J. Heterocycl. Chem. 19 (1982) 513–517.
[14] M. Rommel, A. Ernst, U. Koert, Eur. J. Org. Chem. (2007) 4408–4430.
[15] N.D. Heindel, S.A. Fine, J. Org. Chem. 35 (1970) 796–798.
[16] N.S. Georgiades, J. Clardy, Org. Lett. 7 (2005) 4091–4094.
[17] (a) P.G. Mattingly, Synthesis (1990) 366–368;
(b) D.E. Bergbreiter, P.L. Osburn, C. Li, Org. Lett. 4 (2002) 737–740.
[18] (a) G.A. Conway, L.J. Loeffler, I.H. Hall, J. Med. Chem. 26 (1983) 876–884;
(b) G.A. Conway, L.J. Loeffler, J. Heterocycl. Chem. 20 (1983) 1315–1320;
T. Ayoma, T. Nakano, S. Nishigaki, T. Shioiri, Heterocycles 30 (1990) 375–379;
R.A. Tapia, C. Carrasco, S. Ojeda, C. Salas, J.A. Valderrama, A. Morello, Y. Repetto, J. Heterocycl. Chem. 39 (2002) 1093–1096.
[19] C. Marminon, J. Gentili, R. Barret, P. Nebois, Tetrahedron 63 (2007) 735–739.
[20] F.J. Brown, Y.K. Yee, L.A. Cronk, K.C. Hebbel, R.D. Krell, D.W. Snyder, J. Med. Chem. 33 (1990) 1771–1781.
[21] R. Barret, M. Daudon, Tetrahedron Lett. 31 (1990) 4871–4872.
[22] R. Liu, F.J. Sharom, Biochemistry 36 (1997) 2836–2843.
[23] C.P. Yang, D. Cohen, L.M. Greenberger, S.L. Hsu, S.B. Horwitz, J. Biol. Chem. 265 (1990) 10282–10288.
[24] G. Conseil, H. Baubichon-Cortay, G. Dayan, J.-M. Jault, D. Barron, A. Di Pietro, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 9831–9836.
[25] G. Conseil, A. Decottignies, J.M. Jault, G. Comte, D. Barron, A. Goffeau, A. Di Pietro, Biochemistry 39 (2000) 6910–6917.
[26] V. Rai, S. Shukla, S. Jha, S.S. Komath, R. Prasad, Biochemistry 44 (2005) 6650–6661.
[27] I. Antonini, G. Cristalli, P. Franchetti, M. Grifantini, S. Martelli, F. Petrelli, J. Pharm. Sci. 73 (1984) 367–369.