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Original article

Synthesis, evaluation and absolute configuration assignment of novel dihydropyrimidin-2-ones as picomolar sodium iodide symporter inhibitors

Pierre Lacotte, David-Alexandre Buisson, Yves Ambroise*

CEA Saclay, Institut de Biologie et de Technologies de Saclay (iBiTecS), Service de Chimie Bioorganique et de Marquage (SCBM), 91191 Gif-sur-Yvette, France

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ABSTRACT

A small library of dihydropyrimidin-2-ones (DHPMs) was synthesized and evaluated for their potency to block iodide entrapment in rat thyroid cells. Synthesis was achieved using the multicomponent Biginelli reaction. Twelve compounds were tested for the inhibition of sodium iodide symporter (NIS) in a cell-based assay. One newly synthesized derivative exhibited a remarkably strong activity, with a half-maximum inhibitory concentration value (IC_{50}) of 65 pM. Three DHPMs were further resolved from racemates using chiral HPLC and absolute configurations were assigned using circular dichroism spectroscopy. Biological evaluation showed that most of the activity against NIS resides in one enantiomer. This study provides new insights for the development of anti-thyroid drugs, as well as for the synthesis of novel pharmacological tools designed to investigate iodide transport mechanisms at cellular and molecular levels.

1. Introduction

Iodide translocation into thyroid cells is the first and rate-limiting step in the biosynthesis of iodinated hormones T3 and T4 [1]. This process is mediated by the sodium iodide symporter (NIS), a glycoprotein with 13 putative transmembrane domains mainly expressed in the thyroid gland and also in other tissues including salivary glands, gastric mucosa and mammary glands during lactation [2]. NIS was cloned in 1996 [3]. Since then, extensive characterization has unraveled the role of NIS in many thyroid as well as non-thyroid diseases such as cancer (thyroid, breast), thyrotoxicosis and congenital hypothyroidism [4]. Several studies conclude that thyroid gland failure occurs in 5–7% of the population across different countries [5]. Furthermore, the ability of NIS-expressing cells to efficiently take up iodide has provided a basis for extra-thyroid cancer cell destruction by radioiodide after tumor-selective introduction of exogenous NIS [6]. This strategy showed promising results with successful tumor growth inhibition and volume reduction in models of many types of cancers including colon and pancreatic cancers, hepatoma, and melanoma [7]. In cases of accidental exposure of the population to radioactive iodine species, NIS is directly responsible for human contamination, leading to an increased risk of cancer and birth defects [8]. Solutions for body decontamination are still awaited. At a cellular level, the post-translational mechanisms of NIS regulation are still poorly understood and small molecules modulating NIS function are promising tools for investigation of the intracellular signaling pathways and protein/protein interactions in which NIS is involved. Moreover, the development of new drugs capable of disrupting NIS function for the treatment of overactive thyroid may be relevant as it is known that current anti-thyroid drugs such as methimazole, carbimazole and propylthiouracil can cause severe adverse effects [9].

In 2008, a high-throughput screening campaign led to the discovery of dihydropyrimidin-2-ones (DHPMs) as very potent iodide uptake inhibitors [10]. Iodide transport blocker 9 (ITB9, compound **1** herein) was identified as the lead within the DHPM family, with a half maximal inhibitory concentration (IC_{50}) value of 89 nM (Fig. 1) in rat thyroid-derived cells (FRTL5). Further analysis of the effect of **1** on the iodide-induced current in NIS-expressing *Xenopus laevis* oocytes showed that the inhibition was specific and immediate [11]. Additional isotopic flux experiments showed that **1** can trigger a rapid and total iodide discharge from preloaded hNIS-HEK293 cells. Preliminary experiments run in our laboratory showed that compound **1** has no impact on cell viability at concentrations up to 200 μM. Altogether, these results show that the

Abbreviations: CD, circular dichroism; DHPM, dihydropyrimidin-2-one; FRTL5, Fischer rat thyroid 5; IC_{50}, half maximal inhibitory concentration; ITB, iodide transport blocker; MTT, methylthiazolyldiphenyl-tetrazolium bromide; NIS, sodium iodide symporter; SAR, structure–activity relationship.

* Corresponding author. Tel.: +33 1 69 08 24 70; fax: +33 1 69 08 79 91.
E-mail address: yves.ambroise@cea.fr (Y. Ambroise).

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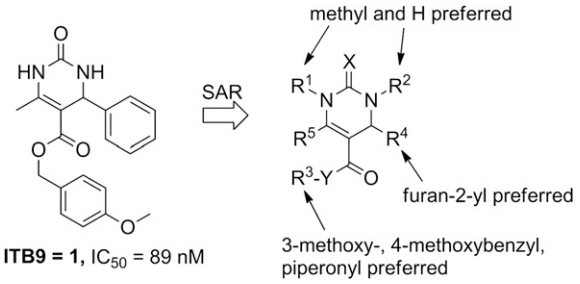

Fig. 1. Structure of ITB9 (compound **1**) and summary of a SAR study from previous work [12].

dihydropyrimidin-2-one core is a promising chemical platform for development of new anti-thyroid drugs.

We recently reported the results of an extensive SAR study of compound **1** using single-point modifications at five key positions on the pyrimidinone ring [12]. The synthesis and evaluation of 115 derivatives provided valuable data and identified a novel potent and non-toxic compound, exhibiting an IC_{50} value of 3.2 nM in FRTL5 (compound **6**, Fig. 2). In this work, it was shown that the furan-2-yl group at C4, methyl and H on *N*1 and *N*3, and methoxybenzyl ester groups at C5 were optimal for potency (Fig. 1). With these data in hand, we decided to synthesize and evaluate a second generation of compounds by combining these optimal modifications. A small library of DHPMs was generated and its evaluation led to a spectacular IC_{50} value improvement from 3.2 nM to 65 pM.

2. Chemistry

The 3,4-dihydropyrimidin-2(1*H*)-ones reported herein were prepared using the three-component ring-forming Biginelli reaction as depicted in Fig. 2A [13]. This reaction involves the condensation of an aldehyde, a β-keto ester and a urea derivative, and is catalyzed with acid. First, we assayed several combinations of Brønsted and Lewis catalysts, solvents and temperature in a model reaction using dimethylurea in order to optimize the conversion rates. The optimal conditions were obtained using 0.1 eq zinc triflate in refluxing acetonitrile. Using these conditions, we combined 4 acetoacetates and 3 urea derivatives while keeping the 2-furaldehyde unchanged to produce a set of 12 individual DHPMs (Fig. 2B). Biginelli condensation using monomethyl urea provided exclusively the *N*1 methylated DHPM (**7**, **10**, **13**, **16**). This was verified by ^{1}H and ^{13}C NMR, and was concordant with previous observations [14]. The 12 DHPMs were obtained after flash chromatography on silica gel with satisfactory yields (30–73%) and excellent chemical purities (>98%). Acetoacetates **2**–**5** were prepared from 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one and diverse R^5OH in the presence of potassium acetate (Fig. 2A) using a slightly modified version of the procedure of Menéndez et al. [15].

3. Biological evaluation

We evaluated the potency of each DHPM (**6**–**17**) by measuring their effect on iodide entrapment in the rat thyroid-derived cell lines FRTL5 [16]. The half maximal inhibitory concentration (IC_{50}) values were measured in at least two independent experiments

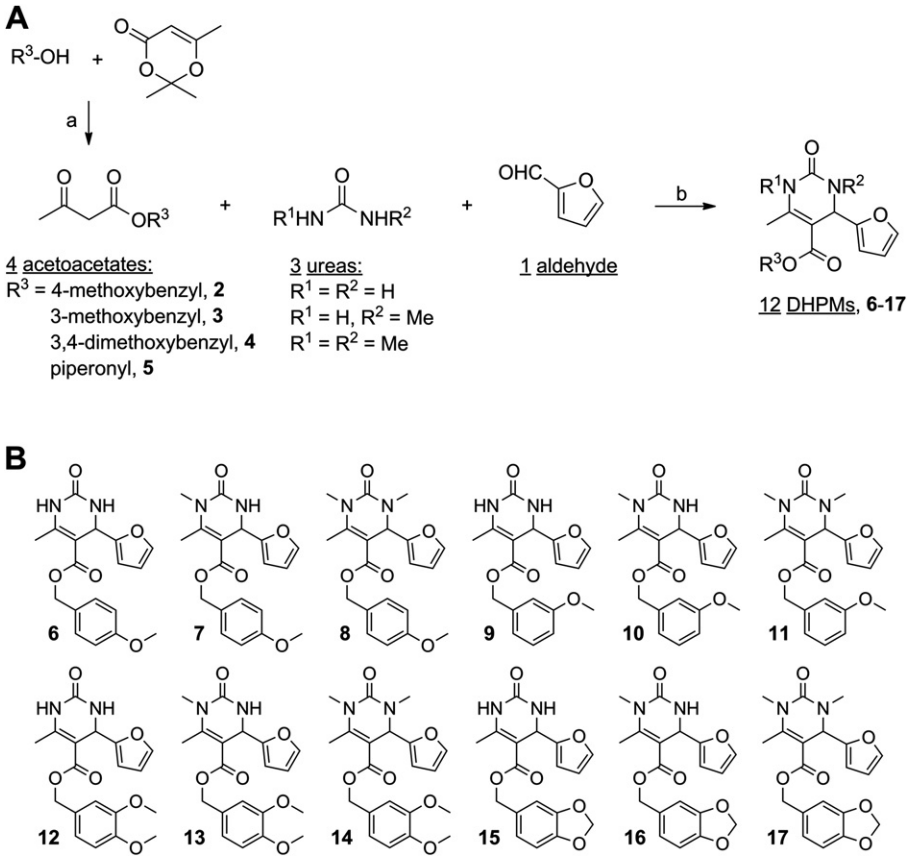

Fig. 2. A: Synthesis of target 3,4-dihydropyrimidinones by the Biginelli reaction. Reagents and conditions: (a) AcOK, microwave irradiation, 120 °C, 30 min, 70–91%; (b) $Zn(OTf)_2$, MeCN, reflux, 2 h, 30–73%. **B**: Structure of the 12 DHPMs.

Table 1
IC_{50} values of the 12 DHPMs (**6**–**17**) for the inhibition of iodide entrapment in FRTL5 cells.[a]

Compound	IC_{50} (nM)
1	89[b]
6	3.2
7	90
8	1.6
9	0.57
10	75
11	0.065
12	400
13	1000
14	65
15	4.0
16	73
17	0.85

[a] IC_{50} values were averaged from at least two independent experiments. $NaClO_4$ and **1** were used as controls. A two-fold standard deviation was deemed acceptable. DHPMs were tested as racemic mixtures.
[b] From Ref. [12].

using the non-radioactive arsenic/cerium titration method developed earlier by our group [17]. Compound **1** was used as the reference compound (IC_{50} = 89 nM) and sodium perchlorate as an assay control (IC_{50} = 0.1 μM).

Table 1 reports the IC_{50} values for the 12 tested DHPMs. As reported earlier, replacing the C4-phenyl ring of **1** by a furan-2-yl group improved potency, as shown by the IC_{50} value of 3.2 nM for compound **6** [12]. When the 4-methoxybenzyl ester group was replaced by a 3-methoxybenzyl ester, the resulting compound **9** showed a great improvement in activity with an IC_{50} value of 0.57 nM. A significant loss of activity for the 3,4-dimethoxy derivative **12** was observed (IC_{50} = 0.4 μM). However, the potency was almost completely recovered with the piperonyl group (**15**, IC_{50} = 4.0 nM). The *N*1 methylation had a negative impact on activity as judged by the IC_{50} values of **7**, **10**, **13** and **16**, which ranged from 73 nM to 1 μM. Very interestingly, the *N*1,*N*3-dimethylated compounds (**8**, **11**, **14**, **17**) exhibited much stronger potencies (IC_{50} = 65 pM–65 nM) than their non-methylated counterparts. This observation was especially true for the 3-methoxybenzyl ester derivative (**11**), which exhibited a remarkable IC_{50} value of 65 pM. This compound is the most potent NIS inhibitor reported to date. On the urea motif, potency improved in the order *N*,*N*-dimethyl > non-methyl > *N*-monomethyl and the ranking of activities for the ester position was 3-methoxybenzyl > piperonyl > 4-methoxybenzyl > 3,4-dimethoxybenzyl. A cell-based MTT assay was performed and none of the compounds tested herein was shown to be toxic to FRTL5 cell lines at 1 μM concentration. In addition, Lipinski's rule of five is respected for DHPMs **6**–**17**. Molecular weights are in the range 342–386 g/mol and ClogP are in the range 2.5–3.7 [18]. Taken together, among the analogs tested herein, compounds **6**, **8**, **9**, **11**, **15** and **17** are the most promising leads for further investigations in animal models.

4. Assignment of the bioactive absolute configuration

The resolution of **1**, **6** and **17** into their respective enantiomers was performed in order to evaluate the impact of stereochemistry on activity. The three compounds were applied to chiral HPLC using analytical Chiralcel OD or Chiralpak AD columns (250 × 4.6 mm). Each pair of enantiomers was obtained with excellent stereochemical purities (>99%). The absolute configuration of each enantiomer was assigned by circular dichroism (CD) spectroscopy. Based on the comparison of experimental CD spectra (Fig. 3) with reference CD spectra of DHPMs with known absolute configuration [19], the enantiomers showing a negative Cotton effect around 280–300 nm were identified as (*R*)-**1**, (*S*)-**6** and (*S*)-**17**. Noteworthily, (*R*)-**1**, (*S*)-**6** and (*S*)-**17** have the same stereochemical orientation at C4 [20]. Biological evaluation of each pair of enantiomers showed that the activity is concentrated in one enantiomer whereas the other does not contribute significantly to the observed activity (Table 2). Therefore, it can be concluded that the mechanism of action of **1**, **6** and **17** for the inhibition of NIS is strongly stereoselective. It probably involves a highly specific interaction between the DHPM and the chiral environment of the recognition site. However, it must be kept in mind that pharmacodynamic processes *in cellulo* may, at least partially, account for the observed difference in activity between two enantiomers. It can also be noted that Pfeiffer's rule is not violated [21], as the eudismic ratios of the two most active compounds (14,000 and 900 for **6** and **17**, resp.) are higher than that of the less active derivative (21 for **1**) [22].

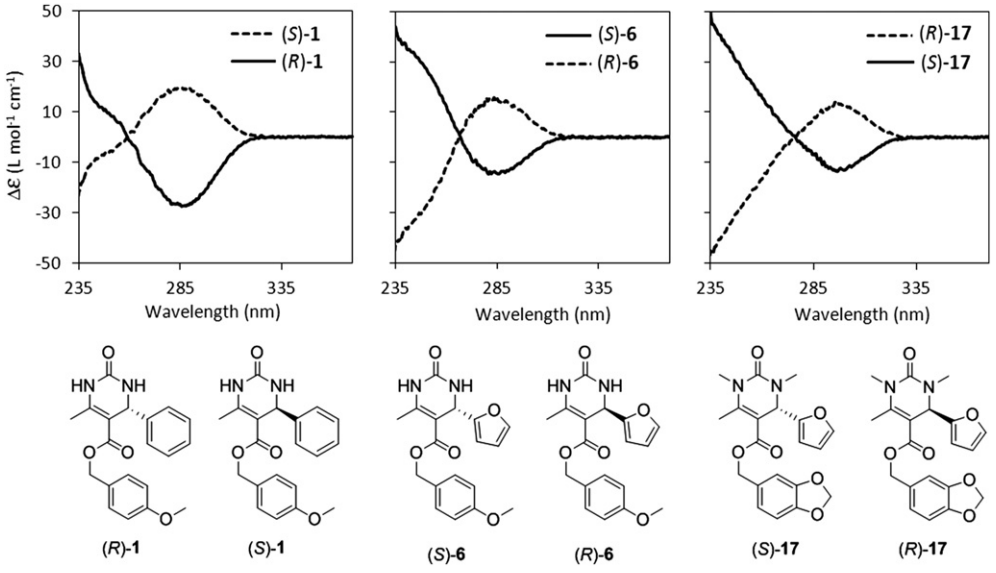

Fig. 3. CD spectra and structure of (*R*)- and (*S*)-**1**, (*R*)- and (*S*)-**6**, (*R*)- and (*S*)-**17**.

Table 2
IC_{50} values of separated enantiomers of **1**, **6** and **17** in the inhibition of iodide entrapment in FRTL5 cells.[a]

Compound	IC_{50} (nM)
(*R*)-**1**	70
(*S*)-**1**	1500
(*R*)-**6**	14,000
(*S*)-**6**	1.0
(*R*)-**17**	630
(*S*)-**17**	0.70

[a] IC_{50} values were averaged from at least two independent experiments. Each compound had a stereochemical purity >99% (chiral HPLC).

5. Conclusion

In summary, a series of dihydropyrimidin-2-ones (**6**–**17**) was synthesized and evaluated as NIS inhibitors in a cellular assay. Among the derivatives, compounds **6**, **8**, **9**, **11**, **15** and **17** are the most potent and **11** exhibited a remarkable IC_{50} value of 65 pM. The inhibition of NIS function by DHPMs was shown to be stereoselective and the absolute configuration of the most active enantiomers was assigned. This study provides important SAR information that will be valuable for the development of compounds with *in vivo* efficacy in the fields of thyroidology and radioprotection.

6. Experimental section

6.1. General methods

Reagents and solvents were from Sigma–Aldrich without further purification. Microwave-assisted reactions were run on a Discover SP system (CEM) equipped with an explorer module. Flash chromatography was performed on a CombiFlash Rf system (Teledyne Isco) using normal phase Redisep (Teledyne Isco) or SNAP (Biotage) cartridges. The HPLC–MS analysis was performed on a system equipped with a binary gradient solvent delivery system (LC-20AB, Shimadzu), a SIL-20A autosampler (Shimadzu) and a photodiode array detector (SPD-20A, Shimadzu, 200–400 nm). This system was coupled to an electrospray ionization Micromass-ZQ spectrometer (Waters) operating in both positive and negative mode. Each compound (8–15 μg) was applied to a 250 × 4.6 mm (5 μm) Zorbax SB-C18 (Agilent) equilibrated with acetonitrile/water = 30/70 (1 mL/min). Samples were eluted by increasing acetonitrile to 45% (10 min), then 85% (25–30 min). ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX 400 spectrometer operating at 400 MHz (^1H) and 100 MHz (^{13}C). The chemical shifts (δ) were expressed in ppm. Melting points (B-540, Büchi) are uncorrected. High-resolution mass spectra (HRMS) were performed on the imagif platform (CNRS – Gif sur Yvette, France), and recorded on a ESI/TOF LCP premier XE mass spectrometer (Waters) using flow injection analysis mode. Circular dichroism spectra were recorded on a Jasco-815 (Jasco) equipped with a Peltier type thermostating accessory (CDF-426S, Jasco). Measurements were carried out at 20 °C using a 1-mm quartz cell in a volume of 300–350 μL. Compounds (200 μg) were dissolved in MeOH (500 μL)/DMSO (26–29 μL). The instrument settings were: bandwidth, 1.0 nm; data pitch, 0.2 nm; speed, 50 nm/min; accumulation, 2; wavelengths, 370–220 nm.

6.2. General procedure (Method A) for the synthesis of intermediate acetoacetates **2**–**5**

2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (1.0 mL, 7.7 mmol) and alcohol (5.9 mmol) were mixed with potassium acetate (241 mg, 2.9 mmol) in a microwave vial. The mixture was microwaved for 20 min at 130 °C and the resulting mixture was chromatographed on silica gel (cHex/EtOAc 100/0–70/30).

6.2.1. 4-Methoxybenzyl 3-oxobutanoate (**2**)

Compound **2** was prepared using Method A and isolated as a yellow liquid (73%). ^1H NMR (400 MHz, $CDCl_3$) δ 2.16 (s, 3H), 3.64 (s, 2H), 3.75 (s, 3H), 5.06 (s, 2H), 6.93 (d, J = 8.0 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.5, 50.0, 55.5, 66.3, 114.2, 128.1, 130.5, 159.6, 167.7, 202.2.

6.2.2. 3-Methoxybenzyl 3-oxobutanoate (**3**)

Compound **3** was prepared using Method A and isolated as a yellow liquid (87%). ^1H NMR (400 MHz, $CDCl_3$) δ 2.24 (s, 3H), 3.49 (s, 2H), 3.80 (s, 3H), 5.14 (s, 2H), 6.84–6.92 (m, 3H), 6.90 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.4, 50.2, 55.5, 67.2, 113.9, 114.2, 120.6, 129.9, 137.0, 160.0, 167.1, 200.5.

6.2.3. 3,4-Dimethoxybenzyl 3-oxobutanoate (**4**)

Compound **4** was prepared using Method A and isolated as a yellow liquid (70%). ^1H NMR (400 MHz, $CDCl_3$) δ 2.24 (s, 3H), 3.48 (s, 2H), 3.76 (s, 3H), 3.77 (s, 3H), 5.19 (s, 2H), 6.78–6.83 (m, 2H), 6.90 (d, J = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.3, 50.3, 56.0, 56.2, 62.8, 111.8, 114.4, 115.9, 124.7, 151.9, 153.7, 167.2, 200.7.

6.2.4. 3,4-(Methylenedioxy)benzyl 3-oxobutanoate (**5**)

Compound **5** was prepared using Method A and isolated as a yellow liquid (91%). ^1H NMR (400 MHz, $CDCl_3$) δ 2.24 (s, 3H), 3.48 (s, 2H), 5.07 (s, 2H), 5.97 (s, 2H), 6.77–6.85 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.5, 50.0, 66.4, 101.5, 108.5, 109.3, 122.6, 129.9, 147.6, 147.7, 167.6, 202.0.

6.3. General procedure (Method B) for the parallel synthesis of DHPMs **6**–**17** by the Biginelli reaction

Acetoacetate **2**–**5** (0.50 mmol), aldehyde (0.60 mmol), urea (0.75 mmol) and $Zn(OTf)_2$ (10 mol%) were dissolved in acetonitrile and refluxed for 2–16 h. The reaction mixture was then allowed to cool to room temperature. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel (cHex/EtOAc 100/0–50/50).

6.3.1. 4-Methoxybenzyl 4-(furan-2-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**6**)

Compound **6** was prepared using Method B and isolated as an ocher powder (40%). mp 158–159 °C. TLC R_f = 0.26 (cHex/EtOAc 1/1). ^1H NMR (400 MHz, DMSO-d_6) δ 2.23 (s, 3H), 3.74 (s, 3H), 4.99 (s, 2H), 5.20 (d, J = 3.2 Hz, 1H), 6.04 (d, J = 2.8 Hz, 1H), 6.35 (dd, J = 2.0, 3.2 Hz, 1H), 6.89 (d, J = 8.4 Hz, 2H), 7.20 (d, J = 8.4 Hz, 2H), 7.55 (s, 1H), 7.76 (s, 1H), 9.29 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 18.2, 48.1, 55.5, 65.2, 96.9, 105.8, 110.8, 114.2, 128.9, 129.9, 142.6, 150.4, 152.8, 156.3, 159.4, 165.5. HPLC t_R = 13.1 min. MS m/z 343 ($[M + H]^+$). HRMS-ESI-TOF m/z calculated 341.1137, found 341.1141 ($[M - H]^-$).

6.3.2. 4-Methoxybenzyl 4-(furan-2-yl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7**)

Compound **7** was prepared using Method B and isolated as a yellow solid (49%), mp 112–113 °C. TLC R_f = 0.28 (cHex/AcOEt 1/1). ^1H NMR (400 MHz, $CDCl_3$) δ 2.49 (s, 3H), 3.15 (s, 3H), 3.76 (s, 3H), 5.03 (s, 1H), 5.04 (s, 1H), 5.41 (d, J = 3.6 Hz, 1H), 5.97 (d, J = 3.2 Hz, 1H), 6.15–6.19 (m, 1H), 6.32 (d, J = 3.2 Hz, 1H), 6.82 (d, J = 8.8 Hz, 2H), 7.16 (d, J = 8.8 Hz, 2H), 7.24 (d, J = 0.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.6, 30.4, 47.6, 55.3, 65.9, 101.3, 105.8, 110.2, 113.9, 128.3, 129.8, 142.3, 151.4, 154.5, 155.0, 159.5, 165.6. HPLC t_R = 16.1 min. MS m/z 357 ($[M + H]^+$). HRMS-ESI-TOF m/z calculated 357.1450, found 357.1464 ($[M + H]^+$).

*6.3.3. 4-Methoxybenzyl 4-(furan-2-yl)-1,3,6-trimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**8**)*

Compound **8** was prepared using Method B and isolated as a yellow oil (46%). TLC R_f = 0.28 (cHex/EtOAc 1/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.48 (s, 3H), 2.96 (s, 3H), 3.20 (s, 3H), 3.78 (s, 3H), 5.02 (d, J = 12.4 Hz, 1H), 5.08 (d, J = 12.0 Hz, 1H), 5.28 (s, 1H), 6.00 (d, J = 2.8 Hz, 1H), 6.22–6.23 (m, 1H), 6.84 (d, J = 8.8 Hz, 2H), 7.18 (d, J = 8.8 Hz, 2H), 7.26 (s, J = 0.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.7, 31.3, 34.9, 54.5, 55.4, 66.0, 100.5, 107.0, 110.2, 114.0, 128.5, 129.9, 142.5, 151.5, 153.3, 154.1, 159.6, 165.6. HPLC t_R = 19.1 min. MS m/z 371 ($[M + H]^+$). HRMS-ESI-TOF m/z calculated 371.1607, found 371.1592 ($[M + H]^+$).

*6.3.4. 3-Methoxybenzyl 4-(furan-2-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**9**)*

Compound **9** was prepared using Method B and isolated as an orange solid (70%). mp 165–166 °C. TLC R_f = 0.35 (cHex/EtOAc 4/6). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.38 (s, 3H), 3.78 (s, 3H), 5.07 (d, J = 12.8 Hz, 1H), 5.13 (d, J = 12.8 Hz, 1H), 5.52 (m, 2H), 6.09 (d, J = 3.2 Hz, 1H), 6.25–6.27 (m, 1H), 6.78–6.85 (s, 3H), 7.21–7.32 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.1, 49.1, 55.4, 66.1, 89.1, 106.4, 110.5, 113.5, 113.7, 120.3, 129.8, 137.8, 142.7, 154.7, 157.1, 159.9, 171.8. HPLC t_R = 13.4 min. MS m/z 343 ($[M + H]^+$). HRMS-ESI-TOF m/z calculated 341.1137, found 341.1140 ($[M - H]^-$).

*6.3.5. 3-Methoxybenzyl 4-(furan-2-yl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**10**)*

Compound **10** was prepared using Method B and isolated as a yellow solid (51%), mp 155–156 °C. TLC R_f = 0.27 (cHex/AcOEt 1/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.51 (s, 3H), 3.18 (s, 3H), 3.74 (s, 3H), 5.06 (d, J = 8.4 Hz, 1H), 5.12 (d, J = 8.4 Hz, 1H), 5.45 (d, J = 2.8 Hz, 1H), 6.01–6.02 (d, J = 2.8 Hz, 1H), 6.07 (d, J = 2.8 Hz, 1H), 6.20–6.22 (m, 1H), 6.77–6.82 (m, 3H), 7.19–7.23 (m, 1H), 7.26 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.7, 30.5, 47.6, 55.3, 66.0, 101.2, 105.9, 110.3, 113.4, 113.7, 120.2, 129.6, 137.8, 142.4, 151.8, 154.5, 154.9, 159.8, 165.5. HPLC t_R = 16.3 min. MS m/z 357 ($[M + H]^+$). HRMS-ESI-TOF m/z calculated 357.1450, found 357.1459 ($[M + H]^+$).

*6.3.6. 3-Methoxybenzyl 4-(furan-2-yl)-1,3,6-trimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**11**)*

Compound **11** was prepared using Method B and isolated as a yellow oil (43%). TLC R_f = 0.31 (cHex/EtOAc 6/4). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (s, 3H), 2.97 (s, 3H), 3.21 (s, 3H), 3.75 (s, 3H), 5.05 (d, J = 12.4 Hz, 1H), 5.13 (d, J = 12.8 Hz, 1H), 5.32 (s, 1H), 6.03 (d, J = 3.2 Hz, 1H), 6.21–6.23 (m, 1H), 6.79–6.82 (m, 3H), 7.20–7.24 (m, 1H), 7.27 (s, J = 0.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.7, 31.3, 34.9, 54.4, 55.3, 66.0, 100.3, 107.0, 110.2, 113.4, 113.7, 120.2, 129.7, 137.9, 142.5, 151.8, 153.3, 154.1, 159.9, 165.4. HPLC t_R = 19.3 min. MS m/z 371 ($[M + H]^+$). HRMS-ESI-TOF m/z calculated 371.1607, found 371.1595 ($[M + H]^+$).

*6.3.7. 3,4-Dimethoxybenzyl 4-(furan-2-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**12**)*

Compound **12** was prepared using Method B and isolated as a yellow solid (73%), mp 72–75 °C. TLC R_f = 0.22 (cHex/AcOEt 1/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.31 (s, 3H), 3.68 (s, 3H), 3.70 (s, 3H), 5.10 (d, J = 12.8 Hz, 1H), 5.15 (d, J = 13.2 Hz, 1H), 5.48 (d, J = 2.8 Hz, 1H), 6.04 (d, J = 3.2 Hz, 1H), 6.17–6.19 (m, 1H), 6.52 (s, 1H), 6.72–6.75 (m, 3H), 7.24 (s, 1H), 8.94 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.5, 48.8, 55.8, 56.0, 61.4, 98.3, 106.1, 110.3, 111.5, 113.6, 115.3, 125.6, 142.4, 148.7, 151.6, 153.5, 154.7, 154.9, 165.4. HPLC t_R = 13.8 min. MS m/z 373 ($[M + H]^+$). HRMS-ESI-TOF m/z calculated 373.1400, found 373.1398 ($[M + H]^+$).

*6.3.8. 3,4-Dimethoxybenzyl 4-(furan-2-yl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**13**)*

Compound **13** was prepared using Method B and isolated as a yellow solid (48%), mp 155–156 °C. TLC R_f = 0.28 (cHex/AcOEt 1/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.51 (s, 3H), 3.18 (s, 3H), 3.69 (s, 3H), 3.72 (s, 3H), 5.12 (d, J = 12.8 Hz, 1H), 5.17 (d, J = 13.2 Hz, 1H), 5.45 (d, J = 3.2 Hz, 1H), 6.00–6.02 (m, 2H), 6.19-6.20 (m, 1H), 6.74–6.79 (m, 3H), 7.25 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.6, 30.5, 47.7, 55.9, 56.0, 61.6, 101.5, 105.9, 110.3, 111.5, 113.7, 115.3, 125.6, 142.4, 151.4, 151.6, 153.5, 154.5, 154.9, 165.6. HPLC t_R = 16.6 min. MS m/z 387 ($[M + H]^+$). HRMS-ESI-TOF m/z calculated 387.1556, found 387.1554 ($[M + H]^+$).

*6.3.9. 3,4-Dimethoxybenzyl 4-(furan-2-yl)-1,3,6-trimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**14**)*

Compound **14** was prepared using Method B and isolated as a white solid (44%), mp 122–124 °C. TLC R_f = 0.28 (cHex/AcOEt 6/4). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (s, 3H), 2.98 (s, 3H), 3.21 (s, 3H), 3.71 (s, 3H), 3.74 (s, 3H), 5.11 (d, J = 13.2 Hz, 1H), 5.19 (d, J = 12.8 Hz, 1H), 5.32 (s, 1H), 6.04 (d, J = 3.2 Hz, 1H), 6.21–6.22 (m, 1H), 6.77–6.78 (m, 3H), 7.26 (s, J = 0.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.7, 30.4, 34.9, 54.5, 55.9, 56.1, 61.6, 100.7, 107.0, 110.2, 111.6, 113.7, 115.4, 125.8, 142.5, 151.5, 151.7, 153.3, 153.6, 154.2, 165.6. HPLC t_R = 19.5 min. MS m/z 401 ($[M + H]^+$). HRMS-ESI-TOF m/z calculated 401.1713, found 401.1719 ($[M + H]^+$).

*6.3.10. Benzo[d][1,3]dioxol-5-ylmethyl 4-(furan-2-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**15**)*

Compound **15** was prepared using Method B and isolated as a white solid (45%), mp 169–171 °C. TLC R_f = 0.33 (cHex/EtOAc 4/6). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.24 (s, 3H), 4.95 (d, J = 12.0 Hz, 1H), 4.99 (d, J = 12.4 Hz, 1H), 5.21 (d, J = 3.2 Hz, 1H), 6.00 (s, 2H), 6.05 (d, J = 3.2 Hz, 1H), 6.34–6.35 (m, 1H), 6.76 (d, J = 8.0 Hz, 1H), 6.81 (s, 1H), 6.86 (d, J = 8.0 Hz, 1H), 7.54 (d, J = 0.8 Hz, 1H), 7.77 (s, 1H), 9.30 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 18.2, 48.1, 65.3, 96.8, 101.4, 105.8, 108.5, 108.8, 110.8, 122.0, 130.7, 142.6, 147.3, 147.7, 150.5, 152.7, 156.3, 165.2. HPLC t_R = 12.7 min. MS m/z 357 ($[M + H]^+$). HRMS-ESI-TOF m/z calculated 355.0930 found 355.0930 ($[M - H]^-$).

*6.3.11. Benzo[d][1,3]dioxol-5-ylmethyl 4-(furan-2-yl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**16**)*

Compound **16** was prepared using Method B and isolated as a yellow solid (30%), mp 141–143 °C. TLC R_f = 0.42 (cHex/AcOEt 4/6). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.49 (s, 3H), 3.10 (s, 3H), 5.00 (s, 2H), 5.21 (d, J = 4.0 Hz, 1H), 6.01 (s, 2H), 6.05 (d, J = 3.2 Hz, 1H), 6.34–6.35 (m, 1H), 6.78 (d, J = 8.0 Hz, 1H), 6.82 (s, 1H), 6.87 (d, J = 8.0 Hz, 1H), 7.54 (d, J = 0.8 Hz, 1H), 7.96 (d, J = 4.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.5, 30.3, 46.9, 65.6, 100.2, 101.5, 105.9, 108.5, 108.9, 110.8, 122.1, 130.5, 142.7, 147.4, 147.7, 152.7, 153.6, 155.9, 165.4. HPLC t_R = 15.7 min. MS m/z 371 ($[M + H]^+$). HRMS-ESI-TOF m/z calculated 371.1243, found 371.1230 ($[M + H]^+$).

*6.3.12. Benzo[d][1,3]dioxol-5-ylmethyl 4-(furan-2-yl)-1,3,6-trimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**17**)*

Compound **17** was prepared using Method B and isolated as a brown oil (70%). TLC R_f = 0.28 (cHex/EtOAc 1/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.51 (s, 3H), 2.99 (s, 3H), 3.23 (s, 3H), 5.00 (d, J = 12.0 Hz, 1H), 5.08 (d, J = 12.4 Hz, 1H), 5.32 (s, 1H), 5.94 (s, 2H), 6.05 (d, J = 3.2 Hz, 1H), 6.25–6.26 (m, 1H), 6.74–6.75 (m, 3H), 7.30 (d, J = 0.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.5, 31.1, 34.7, 54.2, 65.9, 100.2, 101.1, 106.8, 108.1, 108.7, 110.1, 121.8, 130.0, 142.3, 147.4, 147.7, 151.5, 153.1, 153.9, 165.3. HPLC t_R = 18.5min. MS m/z 385 ($[M + H]^+$). HRMS-ESI-TOF m/z calculated 385.1400, found 385.1386 ($[M + H]^+$).

6.4. Chiral resolution

6.4.1. Chiral separation of **1**

Compound **1** (8 batches of 0.48 mg each) was applied to a 250 × 4.6 mm Chiralcel OD column (DAICEL) equilibrated with nHex/EtOH = 92/8. (*S*)-**1** (1.70 mg, t_R = 23.9 min) and (*R*)-**1** (1.85 mg, t_R = 28.3 min) were isolated and further HPLC analysis of separated enantiomers showed >99% stereochemical purity. (*S*)-**1** and (*R*)-**1** did not racemize after 5 months at −20 °C in 20 mM DMSO.

6.4.2. Chiral separation of **6**

Compound **6** (10 batches of 0.36 mg each) was applied to a 250 × 4.6 mm Chiralcel OD column (DAICEL) equilibrated with nHex/EtOH = 90/10. (*S*)-**6** (1.64 mg, t_R = 27.6 min) and (*R*)-**6** (1.65 mg, t_R = 30.4 min) were isolated and further HPLC analysis of separated enantiomers showed >99% stereochemical purity. (*S*)-**6** and (*R*)-**6** did not racemize after 4 months at −20 °C in 20 mM DMSO.

6.4.3. Chiral separation of **17**

Compound **17** (7 batches of 0.60 mg each) was applied to a 250 × 4.6 mm Chiralpak AD column (DAICEL) equilibrated with nHex/EtOH = 70/30. (*R*)-**17** (1.71 mg, t_R = 7.50 min) and (*S*)-**17** (1.71 mg, t_R = 18.3 min) were isolated and further HPLC analysis of separated enantiomers showed >99% stereochemical purity. (*S*)-**17** and (*R*)-**17** did not racemize after 4 months at −20 °C in 20 mM DMSO.

6.5. Biological evaluation of the synthesized compounds

The biological activity of each compound was determined in FRTL5 cells [16], using the non-radioactive arsenic/cerium assay as described elsewhere [17]. Compound potency was expressed as IC_{50}, the concentration of compound necessary to achieve 50% inhibition of iodide uptake. Briefly, to FRTL5 cells at 70–90% confluence was added the compound (200 μM, 10 μM, 0.5 μM, 25 nM, 1.2 nM, 60 pM, 30 pM, and 0.15 pM), followed by NaI (10 μM). After 1-h incubation at 20 ± 1 °C, supernatant was removed and the cells were immediately assayed for iodide content using the modified As/Ce Sandell–Kolthoff reaction. Stock solutions of DHPMs (**1**, **6**–**17**) were in DMSO (20 mM) and $NaClO_4$ was in water at (20 mM). Compounds were tested in columns 4–11 of 96-well polystyrene microplates (Costar 9017, VWR). $NaClO_4$ and **1** were tested in each microplate (in columns 2 and 3) as assay controls. Columns 1 and 12 were for iodide standards. For IC_{50} determination, experimental data were fitted by non-linear regression to the four-parameter sigmoidal Hill equation using an "in-house" application developed in Visual Basic for Excel. The IC_{50} values of all compounds were measured at least twice independently.

6.6. Cell viability

Cell viability was tested according to an MTT-based assay [23]. Briefly, to FRTL5 cells at ~50% confluence was added the compound (1 μM). Cell viability was determined at the 24-h end point before the addition of MTT (1.2 mg/mL). Absorbance at 570 nm was determined after 3-h incubation at 37 °C using a 96-well plate reader (Spectramax plus 384, Molecular Devices). Ouabain was tested as an assay control at eight distinct concentrations (2 μM–1 mM).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.01.043.

References

[1] (a) O. Dohan, A. De la Vieja, V. Paroder, C. Riedel, M. Artani, M. Reed, C.S. Ginter, N. Carrasco, Endocr. Rev. 24 (2003) 48–77;
(b) C. Spitzweg, J.C. Morris, Clin. Endocrinol. 57 (2002) 559–574;
(c) D.H.Y. Shen, R.T. Kloos, E.L. Mazzaferri, S.M. Jhiang, Thyroid 11 (2001) 415–425.
[2] (a) J.Y. Cho, R. Leveille, R. Kao, B. Rousset, A.F. Parlow, W.E. Burak, E.L. Mazzaferri, S.M. Jhiang, J. Clin. Endocrinol. Metab. 85 (2000) 2936–2943;
(b) U.H. Tazebay, I.L. Wapnir, O. Levy, O. Dohan, L.S. Zuckier, Q. Hua Zhao, F.H. Deng, P.S. Amenta, S. Fineberg, R.G. Pestell, N. Carrasco, Nat. Med. 6 (2000) 871–878.
[3] (a) G. Dai, O. Levy, N. Carrasco, Nature 379 (1996) 458–460;
(b) P.A. Smanik, Q. Liu, T.L. Furminger, K. Ryu, S. Xing, E.L. Mazaferi, S.M. Jhiang, Biochem. Biophys. Res. Commun. 226 (1996) 339–345.
[4] (a) G. Riesco-Eizaguirre, P. Santisteban, Eur. J. Endocrinol. 155 (2006) 495–512;
(b) C. Schmutzler, J. Kohrle, Exp. Clin. Endocrinol. Diabetes 106 (Suppl. 3) (1998) S1–S10.
[5] M.P.J. Vanderpump, W.M.G. Tunbridge, in: L.E. Braverman, R.D. Utiger (Eds.), Werner and Ingbar's the Thyroid: A Fundamental and Clinical Text, seventh ed., J.B. Lippincott-Raven, Philadelphia, 1996, pp. 474–482.
[6] (a) T. Kogai, G.A. Brent, Pharmacol. Ther. 135 (2012) 355–370;
(b) M. Hingorani, C. Spitzweg, G. Vassaux, K. Newbold, A. Melcher, H. Pandha, R. Vile, K. Harrington, Curr. Cancer Drug Targets 10 (2010) 242–267.
[7] (a) S.J. Ahn, Y.H. Jeon, Y.J. Lee, Y.L. Lee, S.W. Lee, B.C. Ahn, J.H. Ha, J. Lee, Cancer Gene Ther. 17 (2010) 492–500;
(b) J. Hervé, A. Sa Cunha, B. Liu, Y. Valogne, M. Longuet, R. Boisgard, O. Brégerie, J. Roux, C. Guettier, P. Calès, B. Tavitian, D. Samuel, J. Clerc, C. Bréchot, J. Faivre, Hum. Gene Ther. 19 (2008) 915–926;
(c) G. Riesco-Eizaguirre, A. De la Vieja, I. Rodríguez, S. Miranda, P. Martín-Duque, G. Vassaux, P. Santisteban, J. Clin. Endocrinol. Metab. 96 (2011) E1435–E1443;
(d) R.M. Dwyer, E.R. Bergert, M.K. O'Connor, S.J. Gendler, J.C. Morris, Hum. Gene Ther. 17 (2006) 661–668.
[8] K. Baverstock, D. Williams, Environ. Health Perspect. 114 (2006) 1312–1317.
[9] (a) N. Watanabe, H. Narimatsu, J. Yoshimura Noh, T. Yamaguchi, K. Kobayashi, M. Kami, Y. Kunii, K. Mukasa, K. Ito, K. Ito, J. Clin. Endocrinol. Metab. 97 (2012) E49–E53;
(b) http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm209023.htm;
(c) M. Yang, H. Qu, H.C. Deng, Thyroid 22 (2012) 94–96.
[10] N. Lecat-Guillet, G. Merer, R. Lopez, T. Pourcher, B. Rousseau, Y. Ambroise, ChemBioChem 9 (2008) 889–895.
[11] S. Lindenthal, N. Lecat-Guillet, A. Ondo-Mendez, Y. Ambroise, B. Rousseau, T. Pourcher, J. Endocrinol. 200 (2009) 357–365.
[12] P. Lacotte, C. Puente, Y. Ambroise, ChemMedChem 8 (2013) 104–111.
[13] (a) P. Biginelli, Gazz. Chim. Ital. 23 (1893) 360–416;
(b) C.O. Kappe, Acc. Chem. Res. 33 (2000) 879–888.
[14] O. Kappe, Tetrahedron 49 (1993) 6937–6963.
[15] V. Sridharan, M. Ruiz, J.C. Menéndez, Synthesis 6 (2010) 1053–1057.
[16] F.S. Ambesi-Impiombato, L.A.M. Parks, H.G. Coon, Proc. Natl. Acad. Sci. U.S.A. 77 (1980) 3455–3459.
[17] F. Waltz, L. Pillette, Y. Ambroise, Anal. Biochem. 396 (2010) 91–95.
[18] Calculated on (last accessed 10.10.12), www.molinspiration.com.
[19] W. Krenn, P. Verdino, G. Uray, K. Faber, C.O. Kappe, Chirality 11 (1999) 659–662.
[20] (*R*)-**1**, (*S*)-**6** and (*S*)-**17** are homochirals. There is an inversion of Cahn–Ingold–Prelog priority (CIP) rules between 6/17 and 1 due to the replacement of the phenyl ring by a furan-2-yl.
[21] C.C. Pfeiffer, Science 124 (1956) 29–31.
[22] The eudismic ratio is defined here as: IC_{50} (distomer)/IC_{50} (eutomer). Eutomer is the enantiomer presenting the highest activity. Distomer is the enantiomer presenting the lowest activity.
[23] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.