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Synthesis and Evaluation of 3,4-Dihydropyrimidin-2(1*H*)-ones as Sodium Iodide Symporter Inhibitors

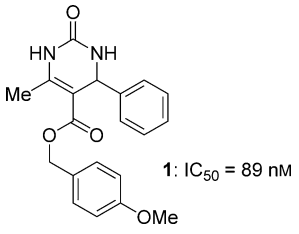
Pierre Lacotte, Celine Puente, and Yves Ambroise*[a]

The sodium iodide symporter (NIS) is responsible for the accumulation of iodide in the thyroid gland. This transport process is involved in numerous thyroid dysfunctions and is the basis for human contamination in the case of exposure to radioactive iodine species. 4-Aryl-3,4-dihydropyrimidin-2(1*H*)-ones were recently discovered by high-throughput screening as the first NIS inhibitors. Described herein are the synthesis and evaluation of 115 derivatives with structural modifications at five key positions on the pyrimidone core. This study provides extensive structure–activity relationships for this new class of inhibitors that will serve as a basis for further development of compounds with in vivo efficacy and adequate pharmacokinetic properties. In addition, the SAR investigation provided a more potent compound, which exhibits an IC_{50} value of 3.2 nM in a rat thyroid cell line (FRTL5).

Introduction

The translocation of iodide from blood into the thyroid gland is an essential step for the biosynthesis of thyroid hormones T3 and T4, which are responsible for many vital mechanisms in vertebrates, such as regulation of metabolism and development of the central nervous system.[1] This transport is mediated by the sodium iodide symporter (NIS), an integral membrane glycoprotein located at the basolateral side of thyrocytes. The molecular characterization of NIS was carried out after cloning the rat and human forms in 1996.[2,3] NIS is essentially expressed in thyroid follicular cells and also in several other tissues including salivary glands, gastric mucosa, and the lactating mammary gland. Other monovalent anions such as ClO_4^-, SCN^-, BF_4^-, PF_6^-, and NO_3^- are transported by NIS.[4,5] They decrease iodide transport in rat thyroid-derived (FRTL5) cells with IC_{50} values of 0.14, 14, 0.75, 0.009, and 250 μM, respectively.[6] Thorough biochemical analysis has clarified the mechanism of iodide uptake and revealed the key role of NIS in many thyroid and extra-thyroid diseases such as cancer (thyroid, breast),[7] autoimmune diseases (Hashimoto's disease and Graves–Basedow disease), toxic nodules, multinodular goiter, and thyroiditis.[8] The prevalence of these thyroid-related disorders is close to 7% in western countries. In the case of nuclear accident, the entrapment of radioactive isotopes of iodide by the thyroid gland is a major source of concern, as it increases the incidence of cancer in the exposed population. A dramatic example of this is the Chernobyl nuclear reactor accident in 1986, after which the World Health Organization predicted that 9000 individuals would die from cancer as a direct result of this disaster. More recent events in Fukushima serve as a reminder of how urgent it is to find solutions to prevent and treat radioactive contamination.[9] One solution is to develop radioprotective small molecules that are capable of blocking radioiodide uptake, or better yet, that enable chemoremediation after contamination has occurred. On the other hand, the ability of the thyroid gland to accumulate radioiodine has long provided the basis for the diagnosis and treatment of thyroid disorders.[10] Today it is proposed to extend this strategy to extra-thyroid tissue for the diagnosis of cancer and the destruction of cancer cells by ^{131}I after targeted NIS gene transfer.[11,12] In this case, compounds that increase radioiodide retention in NIS-expressing cells would be very useful in ensuring a strong and specific toxic effect.[7,13] Small molecules that affect NIS function are unique tools for the study and treatment of many thyroid and non-thyroid dysfunctions.

High-throughput screening has led to the discovery of new potent iodide uptake blockers in human embryonic kidney cells stably expressing the human NIS (hNIS-HEK293).[14] 3,4-Dihydropyrimidin-2(1*H*)-ones were among the most potent inhibitors, and ITB-9 (compound **1**, Figure 1) was selected as the lead in this compound family. The half-maximal inhibitory concentration (IC_{50} value) of **1** was reported to be 0.4 μM in hNIS-



1: IC_{50} = 89 nM

Figure 1. Structure of compound **1**, initially discovered as an inhibitor of NIS function on rat thyroid-derived cells (FRTL5) and hNIS-HEK293.

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HEK293 cells as well as in the rat-thyroid-derived cell line, FRTL5. The potency of compound **1** was later refined using pure resynthesized compound, leading to a more reliable IC_{50} value of 89 nM in FRTL5 cells. Further analysis of the effect of **1** on the iodide-induced current in NIS-expressing oocytes from *X. laevis* showed that the inhibition is rapid (< 5 s) and confirmed a specific inhibitory effect on NIS.[15] More interestingly, it was observed that compound **1** can trigger a rapid efflux of iodide from preloaded hNIS-HEK293 cells, and was not cytotoxic at concentrations up to 200 μM.[14] The discovery of **1** as a powerful iodide uptake inhibitor is particularly attractive, because dihydropyrimidin-2(1*H*)-ones are small versatile structures that are easily synthesized at low cost and on a large scale.

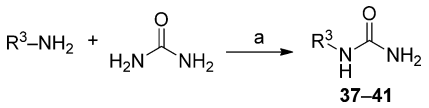
The aim of the study presented herein was to explore 3,4-dihydropyrimidin-2(1*H*)-ones and to develop the first and exhaustive structure–activity relationship of this recently discovered class of compounds in NIS inhibition. We present the synthesis of 115 analogues of **1**, each having a single structural modification. We explored five distinct positions on the pyrimidone core and evaluated the effects of these structural variations on iodide uptake in FRTL5 cells by measuring the IC_{50} values of each compound.

Results and Discussion

Chemistry

Most target 3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) reported herein were prepared according to the general Scheme 1 using the three-component ring-forming Biginelli reaction.[16,17] This reaction involves the condensation of an aldehyde, β-keto ester, and urea with catalytic acid.

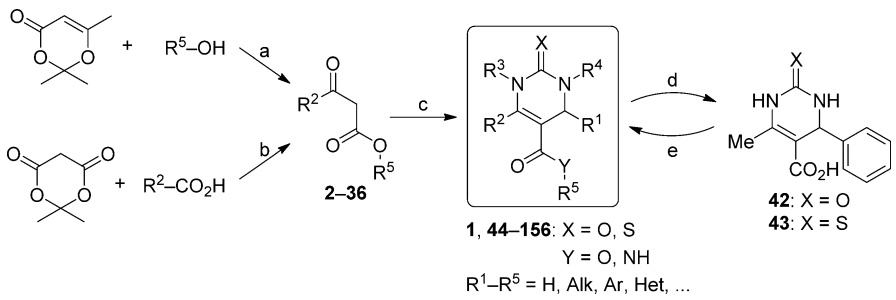
The β-keto esters used to prepare target compounds were obtained from commercial sources or synthesized by published methods. Acetoacetates **2**–**26**, with variation at the ester R^5 position, were prepared from 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one and various R^5OH species in the presence of potassium acetate (Scheme 1).[18] For β-keto esters **27**–**36**, with variations at the R^2 position, 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) was acetylated with various carboxylic acids (R^2CO_2H), and the resulting intermediates were treated with 4-methoxybenzyl alcohol.[19] The monosubstituted ureas **37**–**41** were prepared by reaction of urea with various amines (R^3NH_2) in aqueous hydrochloric acid at reflux (Scheme 2).[20]



Scheme 2. Synthesis of mono-alkylated amines **37**–**41**. *Reagents and conditions:* a) 3 M HCl, H_2O, reflux, 14 h, 38–84%.

Three different Biginelli reaction conditions were used for the final ring-forming reaction: $Yb(OTf)_3$/solvent-free (*Method A*),[21] $Zn(OTf)_2$ in MeCN (*Method B*), or HCl in H_2O/MeOH (*Method C*). The Biginelli reaction with monoalkylureas selectively provided the *N*1-alkyl DHPMs **147**–**153**. This was verified by ^1H and ^{13}C NMR and was in accord with other reports.[22] Some synthesized DHPMs also served as starting materials for the preparation of additional target compounds. The benzyl ester of DHPM **108** provided carboxylic acid **42** under hydrogenolytic conditions (H_2, Pd/C).[23] The 4-methoxyphenyl ester **122** was prepared from **42** under standard coupling conditions (EDC/DMAP). Carboxamide derivative **135** was obtained by coupling **42** with 4-methoxyphenylamine using EDC. The 3,4-dihydropyrimidin-2(1*H*)-thione **155** was prepared in a two-step sequence by treatment of allyl ester **136** with $Pd(PPh_3)_4$ and diethylamine in tetrahydrofuran to provide the intermediate carboxylic acid **43**.[23] This intermediate was subsequently allowed to react with 4-methoxybenzyl alcohol to give DHPM **155**. Finally, oxidation of compound **1** in the presence of $CuCl_2$ and $tBuO_2H$ afforded the 2-hydroxypyrimidine **157** (Scheme 3).[24]

Yields were not optimized. The identities of compounds **1** and **44**–**157** were verified by MS and ^1H, ^{13}C, and ^{19}F NMR (when adequate). The purity of all compounds tested was found to exceed 98% by using a high-performance liquid chromatography (HPLC) system.

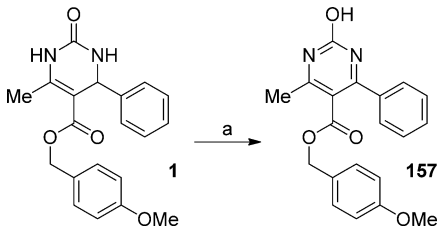


Scheme 1. Synthesis of compounds **1**–**36** and **44**–**156**. *Reagents and conditions:* a) for compounds **2**–**26** (R^2 = methyl): AcOK, μw, 120 °C, 30 min, 26–97%; b) for compounds **27**–**36** (R^5 = 4-methoxybenzyl): DCC, DMAP, CH_2Cl_2, RT, 14 h, then 4-methoxybenzyl alcohol, μw, toluene, 100 °C, 30 min, 50–75% (two steps); c) *Method A:* $Yb(OTf)_3$, solvent free, 100 °C, 45 min — *Method B:* $Zn(OTf)_2$, MeCN, reflux, 2 h — *Method C:* HCl, MeOH, 40 °C, 1–3 days; d) for compound **42** (from **108**): H_2, Pd/C, MeOH, RT, 3.5 h, 79%, for compound **43** (from **136**): $Pd(PPh_3)_4$, DEA, THF, RT, 4 h, 35%; e) for compound **135** (from **42**): 4-methoxybenzyl amine, EDC, DIEA, DMA, μw, 110 °C, 15 min, 59%, for compound **122** (from **42**): 4-methoxyphenol, EDC, DMAP, DMA, μw, 120 °C, 1 h, 37%, for compound **155** (from **43**): 4-methoxybenzyl alcohol, EDC, DMAP, DMA, 80 °C, 5 h, 18%.

Biological evaluation and discussion

The 115 target compounds were evaluated for their ability to inhibit iodide entrapment in FRTL5 cells. The IC_{50} values were averaged from two to four independent experiments. Parent compound **1** was used as the reference compound (IC_{50} = 89 ± 38 nM) and sodium perchlorate as an assay control (IC_{50} = 101 ± 27 nM).[25]

Table 1 presents the IC_{50} values of the first series of com-



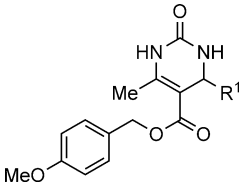
Scheme 3. Synthesis of 2-hydroxypyrimidine analogue **157**. *Reagents and conditions:* a) $CuCl_2$, K_2CO_3, *t*BuOOH, CH_2Cl_2, reflux, 24 h, 54%.

pounds with various R^1 groups (compounds **1** and **44**–**107**). A rapid review of this series shows that increasing the size of the substituent at C4 decreases activity, with IC_{50} values ranging from <100 nM for small groups (furans, thiophenes **93**–**97**) to >20 μM for bulky groups (biaryls **79**, **80**, **102**). The broad range of activity exhibited by DHPMs within this series suggests that the R^1 position is very sensitive to the steric and electronic nature of the substituents. Notably, the presence of a substituent is indispensable for activity, as shown by the total loss of activity of compound **87**, for which the phenyl ring was replaced by a hydrogen atom. Firstly, various substitution patterns on the phenyl group were investigated (compounds **44**–**80**). Electron-withdrawing groups (in **44**–**52**, **56**, and **62**) exhibited very good to moderate activity, with IC_{50} values in the range of 0.075–8.5 μM, compared with electron-donating groups (in **57**–**61** and **64**–**66**) exhibiting only moderate to very low potency (IC_{50}: 5–90 μM). If the phenyl group was *meta*-substituted with Br (**45**), Cl (**47**), F (**50**), or OH (**58**), the DHPMs were more potent (IC_{50}: 0.35, 0.10, 0.075, and 5 μM, respectively) than their *ortho* and *para* counterparts **44**, **46**, **48**, **49**, **51**, **57**, and **59**. In the *meta* series, the potency increases with electron-withdrawing groups as shown by the IC_{50} values, which follow the sequence 3-F > 3-Cl > 3-Br ⩾ 3-OH > 3-OBn. Surprisingly, the 3-CN compound **56** did not fit with the previous observation, with an IC_{50} value of 6.5 μM. Except for the 4-F compound **51** (150 nM), which had an IC_{50} value close to that of **1** (89 nM), every *para*-substituted phenyl derivative exhibited a significant loss of activity, with IC_{50} values ranging from 2.5 to 90 μM (**46**, **48**, **54**–**55**, **59**, **61**–**64**, **66**, **68**, **70**, **71**, **72**, and **74**–**77**). This result was also observed by comparing the activity of 4-biphenyl **80** with 3-biaryl **79** (IC_{50}: >100 and 20 μM, respectively). Multiple substitution of the phenyl ring gave compounds that were significantly less potent, even if the *para* position was left free (**67**, **69** and **73**; IC_{50}: 10, 40, and 25 μM, respectively). Insertion of small spacers between the C4 phenyl group and the DHPM ring (compounds **81**–**86**) caused a significant decrease in potency. The IC_{50} values gradually augmented from 4 μM for a methylene bridge (in **81**) to 75 μM for the oxodimethylene **86**, showing that direct attachment of an aromatic moiety on the 4-DHPM is optimal for activity. Similar to the previous observation, replacing the phenyl ring with alkyl substituents (compounds **88**–**92**) led to significant decreases in potency (IC_{50}: 0.75–8 μM). Based on these results, a variety of aromatic heterocycles were synthesized for further investigation. Satisfyingly, most of the five-membered ring heterocycles displayed improved activities. The furan-2-yl **93** was 28-fold more potent than **1**, with an IC_{50} value of 3.2 nM. The furan-3-yl **94**, thiophen-2-yl **95**, and thiophen-3-yl **96** were also very potent, with activities slightly greater than or similar to that of **1**. When the five-membered ring heterocycle was substituted with a bulky moiety (compounds **101**–**105**), we observed a significant decrease in potency, with IC_{50} values ranging from 13.5 to >100 μM. These results provide new directions for further optimization of the DHPM ring. Compound **100**, with an imidazole ring, led to a dramatic 280-fold decrease in potency ($IC_{50}=25$ μM). This behavior may not be due to protonation of the imidazole ring, as it is only weakly protonated at physiological pH. Six-membered ring heterocycles such as pyridine **106** and quinoline **107** only retained moderate activity (IC_{50}: 2 and 6 μM, respectively).

We next investigated the SAR at the ester position by varying the R^5 group (Table 2). Deletion of the 4-methoxybenzyl group resulted in polar carboxylic acid **42**, with a 56-fold loss of activity. Benzyl ester **108**, lacking the methoxy group, retained most of the activity, with an IC_{50} value of 190 nM. Substitutions at the benzyl ester with small groups such as halogen atoms (**109**–**113**), methyl (**114**), methoxy (**115**, **116**), or methylenedioxy (**119**) had a limited impact on potency, with IC_{50} values ranging from 0.05 to 0.9 μM. Notably, 3-methoxy **116** (0.05 μM) and 3,4-methylenedioxy **119** (0.08 μM) had activities similar to that of the 4-methoxy parent compound **1** (0.089 μM). In contrast, bulky hydrophobic groups on the benzyl ester such as phenyloxy (**117**) or phenyl (**120**, **121**) are not well tolerated, and an increase in IC_{50} values was observed ($IC_{50}=7.5$ to 45 μM). Also, the distance of the 4-methoxyphenyl group from the ester function is critical. A deleterious effect was observed upon deletion of the bridging methylene unit, as determined by the total loss of potency for 4-methoxyphenate **122**. Replacement of the same methylene bridge by a longer ethylene spacer led to compound **123**, with only poor activity ($IC_{50}=20$ μM). Alkyl esters **126**–**129** were also found to be very weak inhibitors. As an illustration, the ethyl ester **126** had an IC_{50} value of >100 μM. Replacement of 4-methoxyphenyl by heterocyclic groups led to esters **130**–**134**, with moderate (**132**) to significant (**134**) loss of activity. Finally, substituting the ester function by amide (compound **135**) resulted in a ~200-fold decrease in activity, probably due to loss of flexibility in the carboxamide bond. Replacement of the *O*-benzyl ester was initially attempted to enhance metabolic stability. However, various DHPMs bearing *O*-alkyl esters (such as SQ 32926 and Monastrol) have been reported to have satisfactory in vivo stability.[26]

We next investigated compounds with a common DHPM core but various R^2 groups at the C6 position (Table 3). Increasing the size of R^2 from methyl (**1**) to ethyl (**137**) had no effect on activity. Other analogues with bulkier groups such as isobutyl (**138**), *n*-pentyl (**139**), cyclohexyl (**140**), phenyl (**141**), and benzyl (**141**) exhibited only minor decreases in activity (IC_{50}: 0.25–0.95 μM). A similar result was obtained with phenoxymethyl (**143**), carboxyamidomethyl (**144**), and heterocyclic rings such as thiophen-2-yl (**145**) and thiophen-2-yl methyl (**146**). These results show that compound potency is not very sensitive to the nature of substituents at C6. Finally, switching

Table 1. Inhibitory activity of target compounds **1** and **44**–**107** against iodide uptake in FRTL5 cells: variations at the R^1 position.



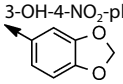
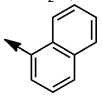
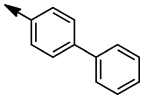
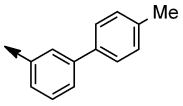
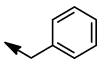
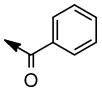
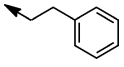
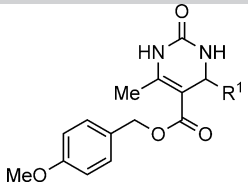
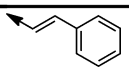
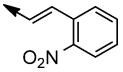
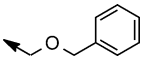
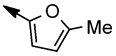
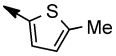
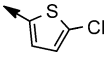
Compd	R^1	IC_{50} [μM][a]
1	Ph	0.089
44	2-Br-phenyl	8.5
45	3-Br-phenyl	0.35
46	4-Br-phenyl	35
47	3-Cl-phenyl	0.10
48	4-Cl-phenyl	13.5
49	2-F-phenyl	0.065
50	3-F-phenyl	0.075
51	4-F-phenyl	0.15
52	2-I-phenyl	3.5
53	3-Cl-4-F-phenyl	20
54	4-CF_3-phenyl	65
55	4-NO_2-phenyl	25
56	3-CN-phenyl	6.5
57	2-OH-phenyl	55
58	3-OH-phenyl	5
59	4-OH-phenyl	13
60	2-OMe-phenyl	20
61	4-OMe-phenyl	50
62	4-OAc-phenyl	2.5
63	4-NHAc-phenyl	30
64	4-$N(Me)_2$-phenyl	25
65	3-OBn-phenyl	70
66	4-OBn-phenyl	90
67	3,5-diOBn-phenyl	10
68	3-OMe-4-OBn-phenyl	20
69	2,5-diOMe-phenyl	40
70	3-OH-4-OMe-phenyl	12.5
71	3-OH-4-NO_2-phenyl	10
72		25
73	2-OH-5-Br-phenyl	25
74	3,4-diOH-phenyl	65
75	2,4,5-triOMe-phenyl	40
76	3,5-diOMe-4-OH-phenyl	20
77	2-NO_2-4,5-diOH-phenyl	85
78		5.5
79		> 100
80		20
81		4
82		6
83		25

Table 1. (Continued)



Compd	R^1	IC_{50} [μM][a]
84		55
85		13.5
86		75
87	H	> 100
88	Et	8
89	*n*Pent	5
90	*c*Pr	0.75
91	*c*Pent	7.5
92	*c*Hex	3
93		0.0032
94		0.02
95		0.09
96		0.05
97		0.04
98		10
99		1
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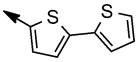
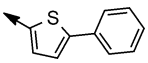
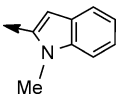
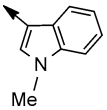
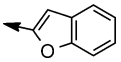
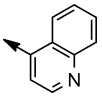
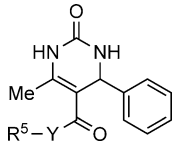
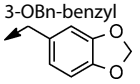
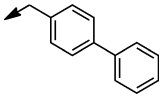
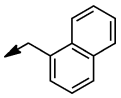
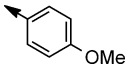
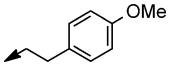
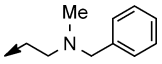
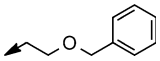
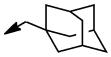
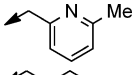
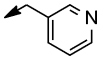
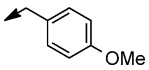
101		25
102		50
103		> 100
104		85
105		13.5
106		2
107		6

[a] Values are the average from two to four independent experiments; a standard deviation of twofold was judged acceptable; $NaClO_4$ and compound **1** were tested as controls in each plate.

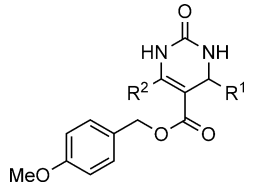
Table 2. Inhibitory activity of target compounds **42** and **108**–**135** against iodide uptake in FRTL5 cells: variations at the Y and R^5 positions.

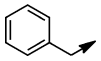
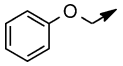
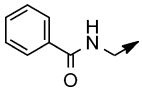


Compd	Y	R^5	IC_{50} [μM][a]
42	O	H	5
108	O	Bn	0.19
109	O	2-Cl-benzyl	0.35
110	O	4-Cl-benzyl	0.3
111	O	4-Br-benzyl	0.4
112	O	2-I-benzyl	0.9
113	O	4-F-benzyl	0.2
114	O	2-Me-benzyl	0.25
115	O	2-OMe-benzyl	0.6
116	O	3-OMe-benzyl	0.05
117	O	3-OPh-benzyl	7.5
118	O	3-OBn-benzyl	1.4
119	O		0.08
120	O		45
121	O		19
122	O		> 100
123	O		20
124	O		2.5
125	O		> 100
126	O	Et	> 100
127	O		2
128	O		35
129	O		> 100
130	O		0.9
131	O		1
132	O		0.4
133	O		5.5
134	O		13.5
135	NH		16.5

[a] Values are the average from two to four independent experiments; a standard deviation of twofold was judged acceptable; $NaClO_4$ and compound **1** were tested as controls in each plate.

Table 3. Inhibitory activity of target compounds **137**–**146** and **156** against iodide uptake in FRTL5 cells: variations at the R^2 and R^1 positions.



Compd	R^2	R^1	IC_{50} [μM][a]
137	Et	Ph	0.07
138	*i*Bu	Ph	0.55
139	*n*Pent	Ph	0.35
140	*c*Hex	Ph	0.85
141	Ph	Ph	0.95
142		Ph	0.25
143		Ph	0.5
144		Ph	1.20
145		Ph	0.55
146		Ph	0.15
156	Ph	Me	20

[a] Values are the average from two to four independent experiments; a standard deviation of twofold was judged acceptable; $NaClO_4$ and compound **1** were tested as controls in each plate.

both R^1 (phenyl) and R^2 (methyl) substituents in **1** resulted in compound **156**, which is equivalent to a double bond migration from C5/C6 to C4/C5 in the DHPM core. This modification had a dramatic impact on activity, with an IC_{50} value of 20 μM.

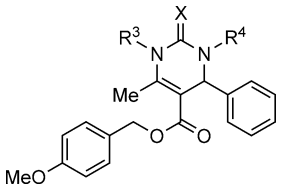
As shown in Table 4, methyl substitution at N1 (**147**) or both N1 and N3 (**154**) resulted in compounds with similar activities (IC_{50}: 0.075 and 0.065 μM, respectively). However, mono-substitution at N1 with bulkier groups (compounds **148**–**153**) caused a more pronounced effect, with IC_{50} values from 0.55 to 10 μM. Replacement of the oxygen atom by sulfur led to compound **155**, with no significant loss of potency (IC_{50}: 0.095 μM). Finally, the fully oxidized analogue **157** (Scheme 3) was not found to be very potent, with an IC_{50} value of 20 μM.

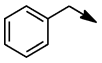
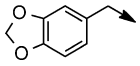
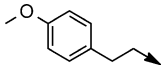
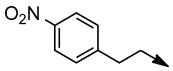
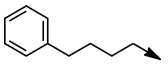
Viability of the FRTL5 cells was also tested with a standard MTT assay in the presence of compounds at 1 μM. None of the DHPMs had an impact on cell growth at this concentration. Higher concentrations were not tested. However, it was reported elsewhere that compound **1** is not toxic at concentrations up to 200 μM.[14]

Conclusions

We have described the synthesis and evaluation of 115 derivatives of dihydropyrimidin-2(1*H*)-one **1** as inhibitors of the sodium iodide symporter in rat-thyroid-derived cell lines. Extensive investigation at five distinct key positions on the

Table 4. Inhibitory activity of target compounds **147–155** against iodide uptake in FRTL5 cells: variations at the R^3, X, and R^4 positions.



Compd	R^3	X	R^4	IC_{50} [μM][a]
147	Me	O	H	0.075
148		O	H	0.55
149		O	H	10
150		O	H	8.7
151		O	H	1.3
152		O	H	1.0
153		O	H	2.0
154	Me	O	Me	0.065
155	H	S	H	0.095

[a] Values are the average from two to four independent experiments; a standard deviation of twofold was judged acceptable; $NaClO_4$ and compound **1** were tested as controls in each plate.

DHPM core led to structure–activity relationships summarized in Figure 2. We found that small five-membered ring heterocycles at the C4 position are among the most potent compounds. The optimal substitution at the carboxy ester is a 3-methoxybenzyl (**116**), 4-methoxybenzyl (**1**), or piperonyl (**119**). Substitutions at C6 and N1 had no or only minimal impact on activity, suggesting that these positions face the solvent, whereas C4 and C5 are significantly involved in NIS binding. This result may aid the design of new fluorescent, radioactive, or photoactivatable probes for the study of NIS function at the molecular level. We identified a new lead compound with improved potency: **93**. This compound exhibited low-nanomolar activity (IC_{50} = 3.2 nM) while showing no impact on cell viability up to 1 μM. It represents the most potent NIS inhibitor reported to date. The physicochemical parameters of **93** were determined and none were found to violate the optimal requirements for druggability (Table 5). The broad SAR investigation

Table 5. Physicochemical properties of compound **93**.

Property	Optimal range	Parameters for **93**[a]
M_r [Da]	< 500	342
Clog *P*	< 5	2.9
H-bond donors	< 5	2
H-bond acceptors	< 10	7
Rotatable bonds	< 5	3
PSA [Å²][b]	< 140	89.8

[a] Calculated with Molinspiration property engine v. 2011.04 (http://www.molinspiration.com). [b] Polar surface area.

reported herein will serve as a base for the development of compounds with adequate pharmacokinetic profiles. Further efforts are necessary to investigate the druggability of the lead(s). First, the metabolic stability of the benzyl ester should be tested, as this function may be hydrolyzed in vivo. Second, the impact of stereochemistry should be determined. Finally, the selectivity of the compounds should be investigated, as DHPMs are known to inhibit other cellular targets including the kinesin spindle protein (HsEG5) and calcium channels.[26]

Experimental Section

General methods: Reagents and solvents were obtained from Sigma–Aldrich and were used without further purification. Microwave-assisted reactions were carried out on a Discover SP system (CEM) operating at 300 W and equipped with an explorer module. Flash chromatography was performed on a CombiFlash R_f system (Teledyne Isco) using normal-phase Redisep (Teledyne Isco) or SNAP (Biotage) cartridges. HPLC–MS analyses were 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). This system was coupled to an electrospray ionization Micromass-ZQ spectrometer (Waters) operating in both positive and negative modes. Each compound (8–15 μg) was applied to a 250×4.6 mm (5 μm) Zorbax SB-C_{18} column (Agilent) equilibrated with MeCN/H_2O 30:70 (1 mL min^{-1}). Samples were eluted by increasing MeCN to 45% (10 min), then 85% (25–30 min). ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on a Bruker Avance DPX 400 spectrometer operating at 400 (^{1}H), 100 (^{13}C), and 160 MHz (^{19}F). Chemical shifts (δ) are expressed in ppm. Melting points (B-540, Büchi) are uncorrected. High-resolution mass spectrometry (HRMS) was performed using the *imagif* platform (CNRS, Gif-sur-Yvette, France), and recorded on an ESI/TOF LCP premier XE mass spectrometer (Waters) using flow injection analysis mode.

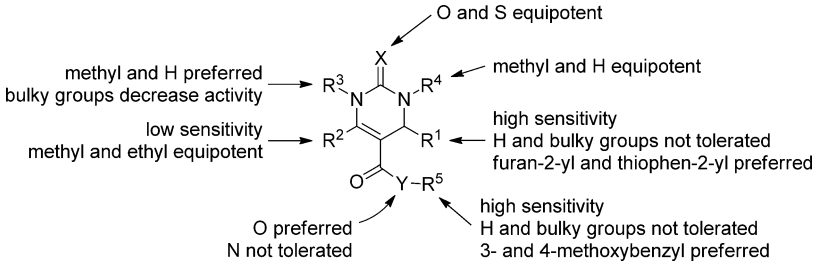


Figure 2. Summary of structure–activity relationships of DHPMs relative to parent compound **1**.

General procedures for the synthesis of compounds 1, 44–121, 123–134, and 136–156 by the Biginelli reaction

Method A: β-Ketoester (0.50 mmol), aldehyde (0.60 mmol), urea (0.75 mmol), and $Yb(OTf)_3$ (5 mol%) were heated at 100 °C for 45 min. The reaction mixture was allowed to cool to room temperature. EtOH (2.5 mL) was added,

and the resulting mixture was left to stand at 0–4 °C for 3 days. In most cases, the precipitate formed was collected by filtration. When the solid did not meet the purity standard of >98% (LC–MS), it was subjected to further chromatography (SiO_2). In a few cases no precipitate formed. Solvents and volatiles were then evaporated under reduced pressure, and the resulting residue was purified by silica gel chromatography to afford the title compound.

Method B: β-Ketoester (0.50 mmol), aldehyde (0.60 mmol), urea (0.75 mmol), and $Zn(OTf)_2$ (10 mol%) were dissolved in MeCN and held at reflux for 2–16 h. The reaction mixture was allowed to cool to room temperature. The solvent was evaporated under reduced pressure, and the residue was subjected to chromatography on silica gel or purified by preparative HPLC to afford the title compound.

Method C: β-Ketoester (1.0 equiv), aldehyde (1.0 equiv), and urea (2.0 equiv) were dissolved in MeOH/HCl_{conc} 1:1 at a final concentration of 1 M (versus aldehyde). The mixture was stirred at 40 °C for 1–3 days. The reaction mixture was allowed to cool to room temperature. The solid formed was collected by filtration and washed with H_2O and/or EtOH to afford the title compound.

Preparation of 4-methoxybenzyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1). Compound **1** was prepared using *Method A* from 4-methoxybenzyl acetoacetate **2** (111 mg), benzaldehyde (51 μL), and urea (45 mg). Isolation by filtration afforded **1** as a white solid (97 mg, 55%): R_f=0.24 (*c*Hex/EtOAc 1:1); mp: 162–164 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=2.25 (s, 3H), 3.74 (s, 3H), 4.95 (s, 2H), 5.13 (s, 1H), 6.86 (d, J=8.8 Hz, 2H), 7.13 (d, J=8.4 Hz, 2H), 7.15–7.20 (m, 2H), 7.24–7.30 (m, 3H), 7.74 (s, 1H), 9.24 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ= 18.3, 54.3, 55.5, 65.1, 99.4, 114.1, 126.7, 127.8, 128.8, 128.9, 130.0, 145.1, 149.4, 152.5, 159.4, 165.6 ppm; HPLC: t_R=14.9 min (>98%); MS-ESI: m/z 353 $[M+H]^+$; HRMS-ESI/TOF (neg.): m/z calcd for $C_{20}H_{19}N_2O_4$: 351.1345 $[M-H]^-$, found: 351.1361.

6-Methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid (42). To a solution of **108** (21.4 mmol) in MeOH (60 mL), under H_2 atmosphere was added Pd/C 10% (0.1 equiv, 2.14 mmol). The mixture was stirred at room temperature for 3.5 h. Excess solvent was removed in vacuo, and the residue was suspended in 0.5 M KOH (100 mL). After vigorous stirring for 3 h at room temperature, the suspension was filtered on Celite. The filtrate was acidified to pH 1–2 with HCl (37%, ~15 mL), and the resulting precipitate was collected by filtration and air dried to afford **42** as a white solid (3.92 g, 79%): R_f=0.35 (*c*Hex/EtOAc 1:1); mp: 232–233 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=2.24 (s, 3H), 5.11 (d, J=3.2 Hz, 1H), 7.24–7.34 (m, 5H), 7.68 (s, 1H), 9.09 (s, 1H), 11.89 ppm (bs, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=18.2, 54.4, 100.3, 126.7, 127.6, 128.8, 145.3, 148.2, 152.8, 167.6 ppm; HPLC: t_R=3.9 min (> 98%); MS-ESI: m/z 233 $[M+H]^+$.

Preparation of 6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid (43). Et_2NH (24.3 mmol) and $Pd(PPh_3)_4$ (0.24 mmol) were added to a solution of **136** (2.4 mmol) in anhydrous THF (5.5 mL) under Ar. The reaction mixture was stirred at room temperature for 4 h. Excess solvent was removed in vacuo. KOH (0.5 M, 20 mL) was added to the residue, and the resulting suspension was filtered on Celite. The filtrate was acidified to pH 1–2 with HCl (37%), and the resulting precipitate was collected by filtration and air dried to afford **43** as a yellow powder (208 mg, 35%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=2.28 (s, 3H), 5.15 (d, J=3.6 Hz, 1H), 7.22–7.37 (m, 5H), 9.60 (s, 1H), 10.26 (s, 1H), 12.25 ppm (bs, 1H); MS-ESI: m/z 249 $[M+H]^+$.

Preparation of 4-methoxybenzyl 4-(furan-2-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (93). Compound **93** was prepared using *Method A* from 4-methoxybenzyl acetoacetate **2** (111 mg), furan-2-carboxaldehyde (50 μL), and urea (45 mg). Isolation by chromatography on silica gel (*c*Hex/EtOAc 100:0→60:40) afforded **93** as an ochre powder (68 mg, 40%): R_f=0.26 (*c*Hex/EtOAc 1:1); mp: 158–159 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): 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 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): 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 ppm; HPLC: t_R=13.1 min (>98%); MS-ESI: m/z 343 $[M+H]^+$; HRMS-ESI-TOF (neg.): m/z calcd for $C_{18}H_{17}N_2O_5$: 341.1137 $[M-H]^-$, found: 341.1141.

Preparation of 4-methoxyphenyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (122). In a 10-mL microwave vial, carboxylic acid **42** (0.42 mmol), 4-methoxyphenol (0.84 mmol), EDCI (0.63 mmol), and DMAP (0.42 mmol) were dissolved in dimethylacetamide (2.0 mL). This reaction mixture was microwaved for 1 h at 120 °C. Excess solvent was removed under reduced pressure. The residue was dissolved in EtOAc (10 mL), washed twice with HCl (1 M, 5 mL) and once with H_2O (5 mL). The dried (Na_2SO_4) organic layer was concentrated in vacuo. The resulting residue was triturated in EtOH, filtered, and dried to afford **122** as a white powder (53 mg, 37%): R_f=0.31 (*c*Hex/EtOAc 1:1); mp: 265–268 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=2.31 (s, 3H), 3.71 (s, 3H), 5.31 (d, J=2.8 Hz, 1H), 6.79 (d, J=9.2 Hz, 2H), 6.89 (d, J= 9.2 Hz, 2H), 7.29–7.39 (m, 5H), 7.89 (s, 1H), 9.45 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=18.5, 54.6, 55.8, 98.4, 114.7, 123.0, 126.8, 127.9, 129.0, 144.2, 145.2, 151.3, 152.0, 157.0, 164.6 ppm; HPLC: t_R=13.7 min (>98%); MS-ESI: m/z 339 $[M+H]^+$.

Preparation of *N*-4-methoxybenzyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxamide (135). In a 10-mL microwave vial, carboxylic acid **42** (0.86 mmol), 4-methoxybenzylamine (0.86 mmol), EDCI (1.29 mmol), and $(iPr)_2NH$ (1.29 mmol) were dissolved in dimethylacetamide (5.0 mL). The reaction mixture was microwaved for 15 min at 110 °C. HCl (1 M, 10 mL) was added, and this solution was extracted with EtOAc (3×20 mL). The organic layers were pooled, dried (Na_2SO_4) and concentrated in vacuo. The resulting residue was purified by chromatography on silica gel (CH_2Cl_2/MeOH 100:0→90:10) to yield **135** as a pale-yellow powder (178 mg, 59%): R_f=0.25 (CH_2Cl_2/MeOH, 95:5); mp: 153–155 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=2.00 (s, 3H), 3.71 (s, 3H), 4.15 (d, J=6.0 Hz, 2H), 5.28 (d, J=2.0 Hz, 1H), 6.76 (d, J=8.8 Hz, 2H), 6.93 (d, J=8.4 Hz, 2H), 7.22–7.33 (m, 5H), 7.49 (s, 1H), 8.06 (t, J=5.8 Hz, 1H), 8.55 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=17.4, 42.0, 55.4, 55.5, 105.4, 113.9, 126.9, 127.7, 128.7, 128.8, 132.1, 137.8, 144.7, 153.1, 158.4, 166.7 ppm; HPLC: t_R=7.7 min (>98%); MS-ESI: m/z 352 $[M+H]^+$.

Preparation of 4-methoxybenzyl 6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (155). A solution of **43** (0.83 mmol), 4-methoxybenzyl alcohol (1.67 mmol), EDCI (1.25 mmol), and DMAP (0.83 mmol) in anhydrous dimethylacetamide (6.0 mL) was heated under Ar at 80 °C for 5 h. In vacuo concentration of the reaction mixture followed by preparative HPLC purification (19×150 mm, 5 μm, XBridge C_{18} in H_2O/MeCN) afforded **155** as a white solid (55 mg,18%): R_f=0.77 (*c*Hex/EtOAc 4:6); mp: 174–175 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=2.29 (s, 3H), 3.74 (s, 3H), 4.95 (d, J=12.4 Hz, 1H), 5.00 (d, J=12.0 *Hz*, 1H), 5.16 (d, J=3.6 Hz, 1H), 6.86 (d, J=8.4 Hz, 2H), 7.13 (d, J=8.8 Hz, 2H), 7.15–7.17 (m, 2H), 7.28–7.32 (m, 3H), 9.66 (s, 1H), 10.37 ppm (s,

1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 17.6, 54.4, 55.5, 65.5, 100.8, 114.1, 126.8, 128.2, 128.5, 129.0, 130.1, 143.7, 146.0, 159.4, 165.4, 174.6 ppm; HPLC: t_R = 18.4 min (> 98%); MS-ESI: *m/z* 369 $[M+H]^+$.

Preparation of 4-methoxybenzyl 2-hydroxy-4-methyl-6-phenylpyrimidine-5-carboxylate (157). A solution of *tert*-butyl hydroperoxide (70% in H_2O, 0.60 mmol) was added slowly (via syringe) to a solution of **1** (0.30 mmol), K_2CO_3 (0.03 mmol), and CuCl (3 mol%) in anhydrous CH_2Cl_2 (1.0 mL). The reaction mixture was held at reflux under Ar for 24 h. The reaction mixture was then treated with saturated $Na_2S_2O_3$ (1.0 mL), followed by saturated NH_4Cl (0.5 mL). The mixture was vigorously stirred for 5 min. After checking for peroxides, the organic layer was concentrated in vacuo. The resulting residue was purified by chromatography on silica gel (*c*Hex/EtOAc 100:0→65:35) to afford **157** as a light-yellow powder (57 mg, 54%): R_f = 0.10 (*c*Hex/EtOAc 4:6); mp: 135–138 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 2.39 (s, 3H), 3.73 (s, 3H), 4.91 (s, 2H), 6.80 (d, *J* = 8.4 Hz, 2H), 6.87 (d, *J* = 8.0 Hz, 2H), 7.42–7.44 (m, 4H), 7.48–7.52 (m, 1H), 12.40 ppm (bs); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 55.5, 67.1, 114.0, 127.1, 128.0, 128.8, 130.6, 130.7, 159.6, 166.3 ppm; HPLC: t_R = 11.9 min (> 98%); MS-ESI: *m/z* 351 $[M+H]^+$.

Biological evaluation

The biological activity of each compound was determined in FRTL5 cells,[27] using the non-radioactive arsenic/cerium assay as described elsewhere.[6] Compound potency was expressed as IC_{50}, the concentration of compound necessary to achieve 50% inhibition of iodide uptake. Briefly, test compound (200 μM, 10 μM, 0.5 μM, 25 nM, 1.2 nM, 60 pM, 30 pM, and 0.15 pM) was added to FRTL5 cells at 70–90% confluence, followed by NaI (10 μM). After incubation for 1 h at 20 ± 1 °C, the supernatant was removed, and the cells were immediately assayed for iodide content using the modified As/Ce Sandell–Kolthoff reaction. Stock solutions of DHPMs were in DMSO (20 mM) and $NaClO_4$ was in H_2O 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 used for iodide standards. For IC_{50} determination, experimental data were fitted by nonlinear regression to the four-parameter sigmoidal Hill equation using an in-house application developed in Visual Basic for Microsoft Excel. The IC_{50} values of all compounds were measured at least twice independently.

Cell viability

Cell viability was tested according to an MTT-based assay.[28] Briefly, compound (1 μM) was added to FRTL5 cells at ~50% confluence. Cell viability was determined at the 24 h end point before addition of MTT (1.2 mg mL^{-1}). Absorbance at λ 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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