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ABSTRACT

The high distribution of CB_2 receptors in immune cells suggests their important role in the control of inflammation. Growing evidence offers this receptor as an attractive therapeutic target: selective CB_2 agonists are able to modulate inflammation without triggering psychotropic effects. In this work, we report a new series of selective CB_2 agonists based on a benzo[*d*]thiazol-2(3*H*)-one scaffold. This drug design project led to the discovery of compound **9**, as a very potent CB_2 agonist (K_i = 13.5 nM) with a good selectivity *versus* CB_1. This compound showed no cytotoxicity, acceptable ADME-Tox parameters and demonstrates the ability to counteract colon inflammatory process *in vivo*.

Keywords:

CB_2 agonist, inflammation, colon inflammatory

INTRODUCTION

Isolated in 1964 by Yechiel Gaoni and Raphael Mechoulam [1], Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the main psychoactive substance found in the cannabis plant (Figure 1) [2]. Δ^9-THC displays a wide range of physiological effects including analgesic, anti-inflammatory and immunosuppressive activities [3-4] but its clinical use is limited because of its abuse potential and psychomimetic side effects. Δ^9-THC acts mainly on the endocannabinoid system, which modulates various physiological functions: motor function, memory, motivation, energy, pain and emotion [5]. More specifically, most of the Δ^9-THC properties are mediated by two G-protein coupled receptors, called cannabinoid receptors, CB_1 and CB_2 [6].

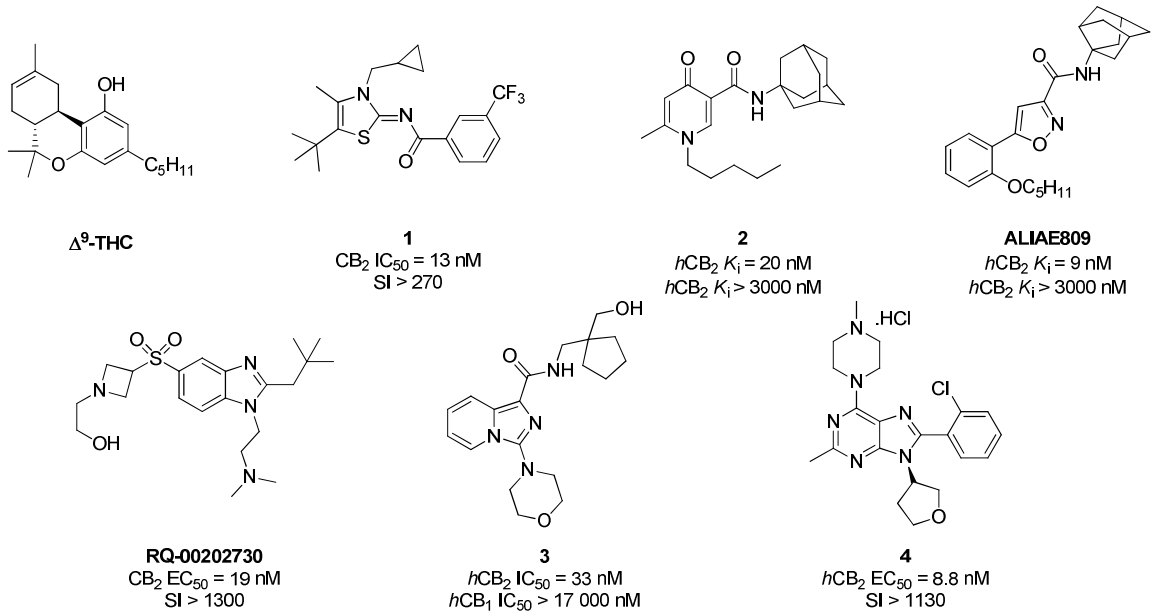


Figure 1. Δ^9-Tetrahydrocannabinol (Δ^9-THC) and selected CB_2 agonists.

Since the last decade, the interest for the therapeutic use of cannabis was reconsidered leading to many research works on the subject [7-11]. Natural and synthetic cannabinoids have beneficial effects on several diseases including asthma, glaucoma and Alzheimer's disease [12]. They exert also antiemetic, anti-inflammatory and analgesic effects [4, 13]. Unfortunately, these therapeutic effects are associated with side effects linked to the CB_1 receptor [14], like memory alteration, dysphoria and sedation [15]. Indeed, CB_1 receptors are mostly located in the brain and thus are responsible for central effects of cannabinoids [16]. However, CB_2 receptors are mainly expressed in peripheral immune cells [17] and their activation mediates immune responses and explains their therapeutic potential [13].

To prevent these adverse effects, several strategies can be envisaged to target CB_2 selectively: the development of (1) selective CB_2 agonists that will not activate CB_1 receptors; (2) endocannabinoid degradation enzyme inhibitors, like FAAH inhibitors, that will enhance the level of endocannabinoids; (3) ligands that do not pass the blood-brain barrier; or (4) ligand vectors like nanoparticles to specifically reach the target.

Recently, a great deal of research have been undertaken in the development of selective CB_2 agonists.

Various molecules have been synthesized with very good biological properties. These selective CB_2 agonists are structurally very different like heterocyclic ylidenes (compound **1**) [18], 4-oxo-1,4-dihydropyridines (compound **2**) [19], isoxazoles (**ALIAE809**) [20], benzoimidazoles (**RQ-00202730**) [21], imidazopyridines (compound **3**) [22] or purines (compound **4**) [23], for example (Figure 1). Despite the structural diversity of these compounds, they are all characterized by lipophilic properties due to the presence of aromatic heterocycles, bulky alkyl or aryl substituents. Some selective CB_2 agonists are currently in clinical trials for the treatment of pain, osteoartitis, atopic dermatitis or systemic scleroderma (Figure 2) [24].

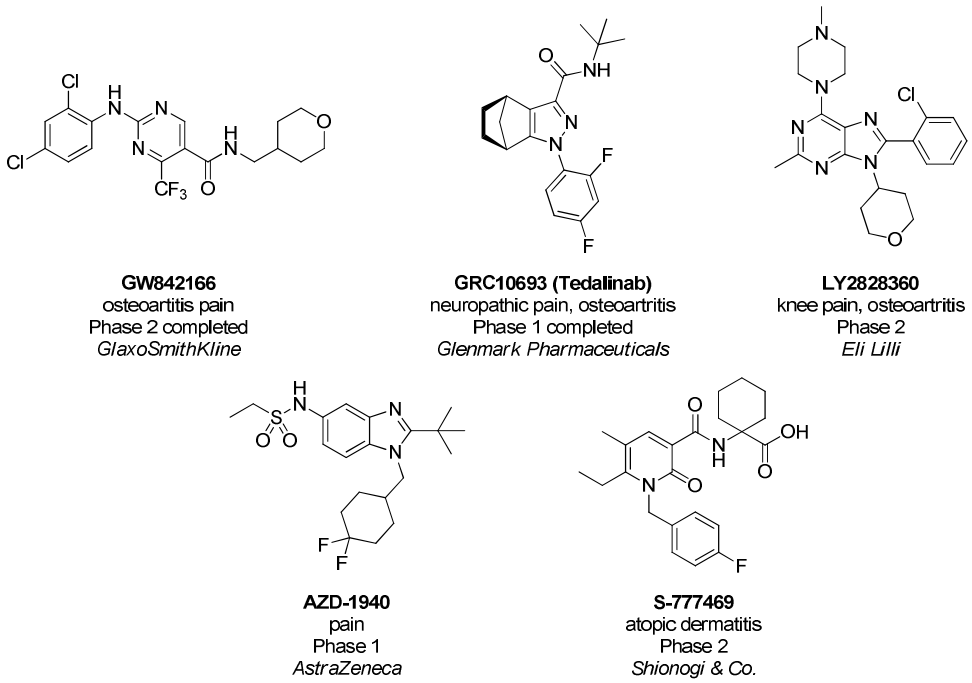


Figure 2. Selected most important CB_2 agonists in clinical development.

Benzothiazolone and benzoxazolone have been qualified as “privileged scaffolds” in drug design [25]. These frameworks have found broad therapeutic applications from analgesic and anti-inflammatory compounds [26-28] to Alzheimer’s disease treatment [29] and anticonvulsant compounds [30]. Nevertheless, only few benzothiazolone derivatives have been reported with anti-inflammatory properties. Among these molecules, we can point tiaramide [31-32] and S-14080 [33] (Figure 3).

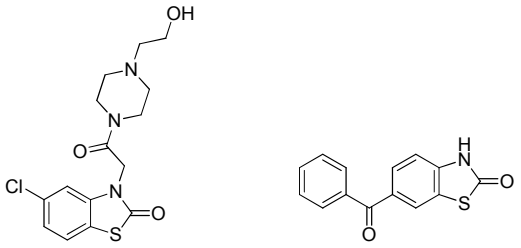


Figure 3. Anti-inflammatory benzothiazolone derivatives

With the ambition to develop selective CB_2 agonists for the treatment of inflammatory bowel diseases, we investigated the synthesis and structure-activity relationship of benzothiazolone

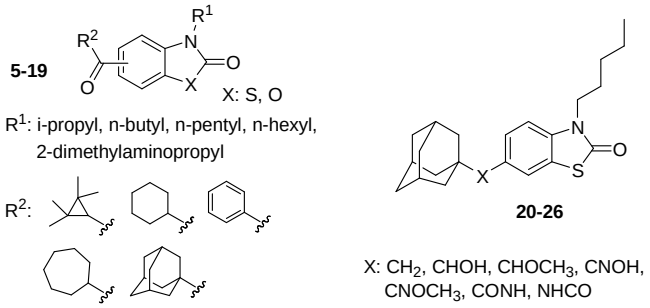


Figure 4: Design and synthesis of benzazolone derivatives **5-26**

22 new compounds (**5-26**) were synthesized and tested for their CB_2 binding affinity. The CB_2 selectivity compared to CB_1, functionality and cytotoxicity were evaluated for the most affine molecules. Finally, ADME properties of our best selective CB_2 agonist (compound **9**) were determinated and *in vivo* anti-inflammatory activity was evaluated on a dextran sulfate sodium (DSS)-induced experimental colitis assay.

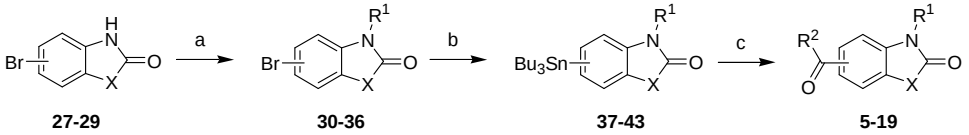
Chemistry

As described previously, compounds **5-19** were synthesized *via* a Stille coupling from the tributylstannyl intermediates **37-43**, which were obtained after *N*-alkylation at the *N*3-position of the corresponding 6-

bromobenzo[*d*]thiazol-2(3*H*)-ones **30-34**, and 5-bromobenzo[*d*]thiazol-2(3*H*)-one **35** and 6-bromobenzo[*d*]xazol-2(3*H*)-one **36** (Scheme 1) [34]. It has already been demonstrated that it is necessary to *N*-alkylate at the *N*3-position before coupling because the NH acid group of the benzo[*d*]thiazol-2(3*H*)-one interacts in the Stille reaction [35]. Indeed, Stille reaction is sensitive to acid media or acid groups.

6-Bromobenzo[*d*]thiazol-2(3*H*)-one **27** [36], 5-bromobenzo[*d*]thiazol-2(3*H*)-one **28** [37] and 6-bromobenzo[*d*]xazol-2(3*H*)-one **29** [36] were prepared according to already described procedures. Compounds **30-36** were obtained by nucleophilic substitution of corresponding haloalcane by the heterocyclic nitrogen atom of the corresponding benzo[*d*]thiazol-2(3*H*)-one or benzo[*d*]xazol-2(3*H*)-one. This reaction was performed in DMF at 80°C in presence of an excess of Cs_2CO_3. The desired compounds **30-36** were obtained with moderate to good yields (38-98%). Then, the tin intermediates **37-43** were prepared by reaction of the previous alkylated compounds (**30-36**) with $Bu_3Sn)_2$ in dry toluene at 80°C in the presence of $Pd(PPh_3)_4$ with low to good yields (9-79%). The obtained stannyl intermediates **37-43** were then refluxed in dry toluene with the corresponding acyl chloride in the presence of $PdCl_2(PPh_3)_2$. The final compounds **5-19** were obtained with low to good yields (6-89%).

Scheme 1. Synthesis of compounds **5-19**.



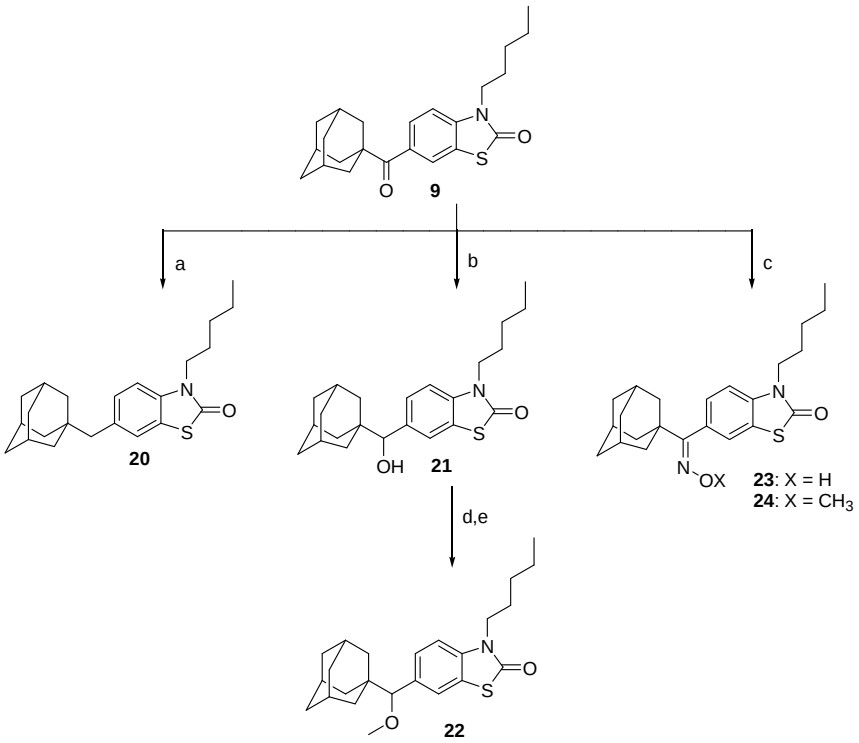
Reagents and conditions: (a): K_2CO_3, R^1Br, DMF, 80°C; (b): $(SnBu_3)_2$, $Pd(PPh_3)_4$, toluene, 80°C; (c): $PdCl_2(PPh_3)_2$, R^2COCl, toluene, reflux.

Compounds	X	R^1	R^2	Position of $(C=O)R^2$
5	S	*n*-pentyl	cyclohexyl	6- position
6	S	*n*-pentyl	phenyl	6- position
7	S	*n*-pentyl	2,2,3,3-tetramethyl cyclopropyl	6- position
8	S	*n*-pentyl	cyclopentyl	6- position
9	S	*n*-pentyl	1-adamantyl	6- position

10	S	*i*-propyl	1-adamantyl	6- position
11	S	*n*-butyl	1-adamantyl	6- position
12	S	*n*-hexyl	1-adamantyl	6- position
13	S	2-dimethyl aminopropyl	1-adamantyl	6- position
14	O	*n*-pentyl	1-adamantyl	6- position
15	O	*n*-pentyl	cyclohexyl	6- position
16	O	*n*-pentyl	2,2,3,3-tetramethyl cyclopropyl	6- position
17	S	*n*-pentyl	1-adamantyl	5- position
18	S	*n*-pentyl	cyclohexyl	5- position
19	S	*n*-pentyl	2,2,3,3-tetramethyl cyclopropyl	5- position

Modification of the ketone function of compound **9** was carried out either starting from compound **9** (Scheme 2) or from benzo[d]thiazol-2(3H)-one (Schemes 3 and 4).

Scheme 2. Synthesis of compounds **20**-**24**.



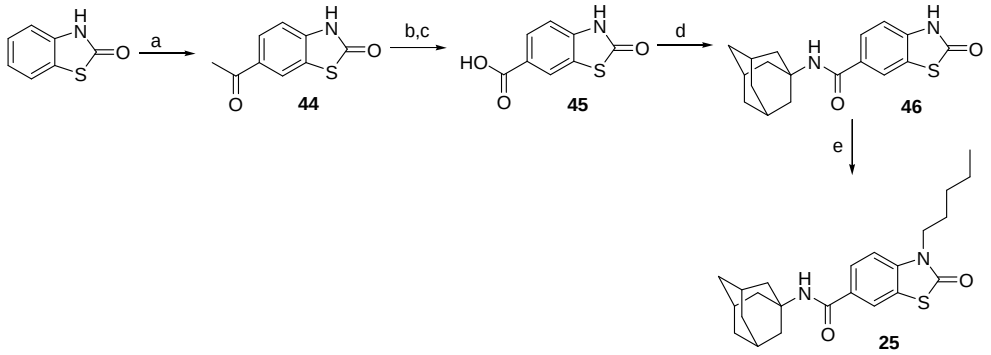
Reagents and conditions: (a): TFA, Et_3SiH, rt; (b): $NaBH_4$, MeOH, rt; (c) XO-NH_3^+ Cl^-, pyridine, MeOH, reflux; (d): NaH, THF, rt; (e) CH_3I.

Starting from compound **9**, compound **20** was obtained by total reduction of the ketone function using

Et_3SiH in TFA with a yield of 16%. Compound **21** was obtained by partial reduction of the ketone function of compound **9** using $NaBH_4$ in methanol with a yield of 26 % [38]. The hydroxyl function of compound **21** was then methylated in THF in presence of NaH and methyl iodide to give compound **22** with a yield of 39%. The hydroxyimine **23** and methoxyimine **24** were synthesized by reaction of compound **9** with hydroxylamine or methylamine hydrochlorides respectively, in refluxing methanol in the presence of pyridine. The desired compounds **23** and **24** were obtained with a yield of 25% and 15%, respectively.

Carboxamide **25** was synthesized starting from benzo[*d*]thiazol-2(3*H*)-one (Scheme 3).

Scheme 3. Synthesis of compound **25**.



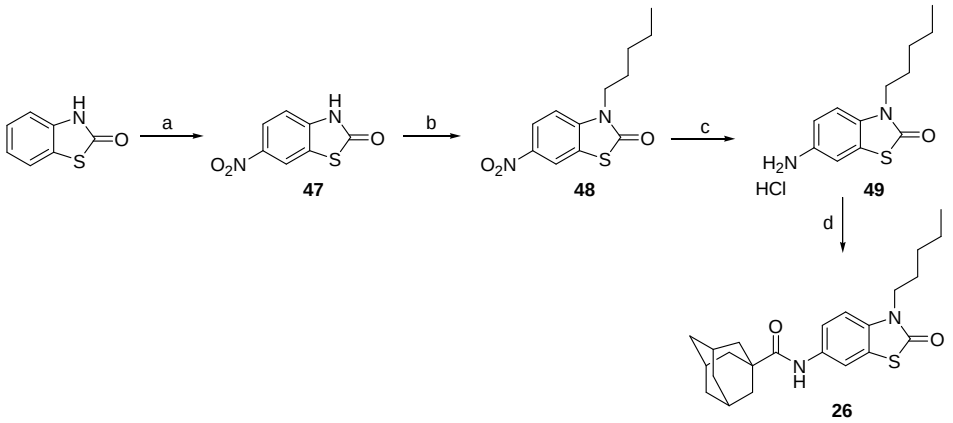
Reagents and conditions: (a): acetylchloride, AlCl3, DMF, 70°C; (b): NaOCl, NaOH, H2O, reflux; (c): HCl; (d): 1-adamantaneammonium chloride, HOBt, HBTU, DIEA, DMF; (e): C5H11Br, K2CO3, DMF, 80°C

First, the benzothiazole was acetylated regioselectively at the *C*6-position by a Friedel-Crafts reaction in presence of aluminium chloride ($AlCl_3$) in DMF at 70°C to give acetylbenzo[*d*]thiazol-2(3*H*)-one **44** with a yield of 48% [38]. The acetyl group was then oxidized through an haloform reaction in a NaOH solution in presence of NaOCl at reflux [39]. The carboxylic acid **45** was obtained with a yield of 50%. The resulted carboxylic acid **45** was coupled to 1-adamantylamine in presence of HBTU, HOBt and DIEA in DMF at room temperature to give carboxamide **46** with 14% yield. Finally, the *N*3-position was alkylated using 1-bromopentane in DMF in presence of K_2CO_3 at 80°C to give the desired

compound **25** with 22%.

Carboxamide **26** was synthesized in 4 steps starting from benzo[*d*]thiazol-2(3*H*)-one (Scheme 4).

Scheme 4. Synthesis of compound **22**.



Reagents and conditions: (a): HNO_3, $(CH_3CO)_2O$, 0°C; (b): $C_5H_{11}Br$, K_2CO_3, DMF, 80°C; (c): H_2, Pd/C, MeOH, rt; (d): adamantylacid chloride, K_2CO_3, H_2O, EtOAc, rt.

Nitration of the benzothiazole with nitric acid in acetic anhydride at 0°C gave mainly 6-nitrobenzo[*d*]thiazol-2(3*H*)-one **47** with 60% yield [40]. Two side products were also obtained: nitration at the position 4 and bisnitration at positions 4 and 6. After *N*-alkylation by 1-bromopentane at the *N*3-position in DMF in presence of an excess of K_2CO_3 at 80°C (compound **48**, yield 69%), the nitro function was reduced by catalytic hydrogenation (H_2, Pd/C, MeOH) to afford to amine **49** with a yield of 57%. Finally, the benzo[*d*]thiazol-2(3*H*)-one **26** was obtained by nucleophilic substitution. This Schotten-Baumann reaction was carried out in a two-phase medium (water/ethylacetate) in presence of adamantane-1-carbonyl chloride and K_2CO_3 to give the final compound **26** with a yield of 69%.

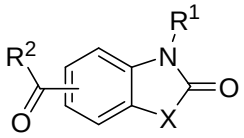
RESULTS AND DISCUSSION

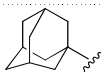
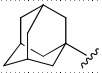
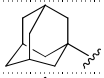
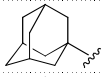
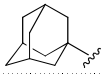
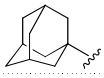
From our previous work on selective CB_2 agonists [41-43], some pharmacophoric elements seem to be essential for activity and selectivity: (a) a long aliphatic chain, which extends toward a hydrophobic

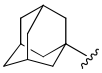
region of the receptor, is optimal for a good CB_2 affinity ; (b) a hydrogen bond acceptor able to interact with Ser285 plays an important role in the molecular recognition and (c) a bulky hydrophobic group fitting with a second hydrophobic pocket, is favorable for a good CB_2 affinity and selectivity.

Following this combination of hydrogen bond and hydrophobic interactions, we assumed that the benzo[*d*]thiazol-2(3*H*)-one scaffold, suitably substituted with an aliphatic chain and a bulky hydrophobic group, could provide the starting point for the identification of new selective CB_2 agonists. Thus, we first synthesized a series of 5 compounds (**5-19**, Table 1) characterized by different substituents at positions 3, 5, 6 of the benzothiazolone or benzoxazolone scaffold.

Table 1. *h*CB_2 affinities[a] of compounds **5-19.**



Compd	X	R^1	R^2	Position of $(C=O)R^2$	*h*CB_2 K_i (nM)
5	S	*n*-pentyl		6	49.5 ± 14.5
6	S	*n*-pentyl		6	> 1000
7	S	*n*-pentyl		6	> 1000
8	S	*n*-pentyl		6	210 ± 36
9	S	*n*-pentyl		6	13.5 ± 1.5
10	S	*i*-propyl		6	280.5 ± 14.5
11	S	*n*-butyl		6	26 ± 7
12	S	*n*-hexyl		6	39 ± 6
13	S	2-dimethyl aminopropyl		6	506 ± 264
14	S	*n*-pentyl		5	525.8 ± 58.6
15	S	*n*-pentyl		5	> 1000

16	S	*n*-pentyl		5	> 1000
17	O	*n*-pentyl		6	> 1000
18	O	*n*-pentyl		6	> 1000
19	O	*n*-pentyl		6	> 1000
WIN-55,212-2		-	-		1.57 ± 0.21
JWH-133		-	-		8 ± 1

[a] The K_i values were obtained from nonlinear analysis of competition curves using [^{3}H]-CP-55,940 as radioligand for *h*CB_2 cannabinoid receptors and are expressed as mean ± SEM of at least four experiments performed in duplicate.

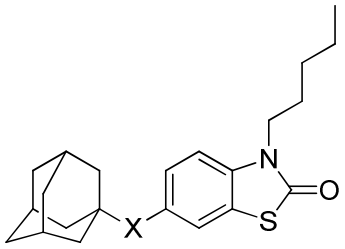
The affinities of the new synthesized compounds together with WIN-55,212-2 and JWH-133, reference compounds, for the human CB_2 receptor (*h*CB_2) were determined by a competitive radioligand displacement assay using [^{3}H]-CP55,940 as radioligand [44]. Membranes from Chinese hamster ovary (CHO) cells expressing *h*CB_2 were used in these experiments. All compounds were first screened at a concentration of 10 µM for their affinity toward the cannabinoid receptor. Inhibition constant (K_i) values were determined for compounds exhibiting a specific displacement superior to 60% for *h*CB_2. As shown in Table 1, among these 9 molecules, 7 displayed good to moderate inhibition constants. No CB_2 affinity was observed for compounds **6** and **7** with a phenyl or a 2,2,3,3-tetramethylcyclopropyl group at position 6 and compound **8** with a cyclopentyl group at the same position showed only moderate CB_2 affinity. These observations suggest that an adamantyl group or a cyclohexyl group are the best substituents at position 6. Regarding the alkyl chains introduced on the heterocyclic nitrogen atom at position 3, the optimal length appears to be chains varying from 4 to 6 carbons. When a shorter (isopropyl, compound **11**) or a dimethylamino functionalized chain (compound **14**) are introduced, a decrease of CB_2 affinity is observed. From these 9 molecules, compound **9** with a *n*-pentyl chain on the heterocyclic nitrogen and an adamantly group at position 6 showed the best affinity for CB_2.

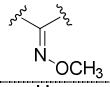
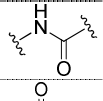
Using this compound **9** as a starting hit, a first phase of structural analysis has been achieved by preserving both substituents and varying (1) the position of the bulky group R^2 from position 6 to

position 5 (compounds **14-16**, Table 1) and (2) the nature of the central scaffold by replacement of the sulfur by an oxygen (compounds **17-19**, Table 1). As show, the shift of the bulky group to the 5 position, as well as the substitution of the benzothiazolone by a benzoxazolone scaffold result in a deep decrease or even a loss of CB_2 affinity.

In order to validate the role of the ketone between the central heterocycle and the adamantyl group in the binding, this function was removed or substituted by other functions. Seven additional compounds were synthesized to this end (Table 2).

Table 2. *h*CB_2 affinities[a] of compounds **20-26**.



Compd	X	*h*CB_2 K_i (nM)
20	CH_2	144 ± 18
21		272 ± 4
22		344 ± 120
23		251 ± 134
24		438 ± 136
25		> 1000
26		> 1000
WIN-55,212-2	-	1.57 ± 0.21
JWH-133	-	8 ± 1

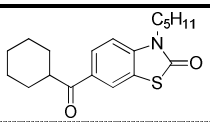
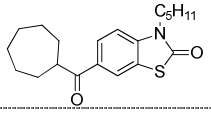
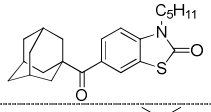
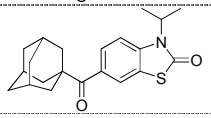
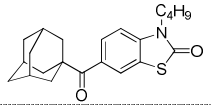
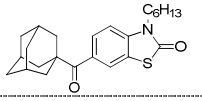
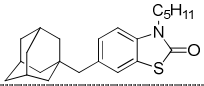
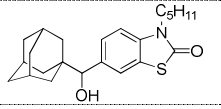
[a] The K_i values were obtained from nonlinear analysis of competition curves using [^{3}H]-CP-55,940 as radioligand for *h*CB_2 cannabinoid receptors and are expressed as mean ± SEM of at least four experiments performed in duplicate.

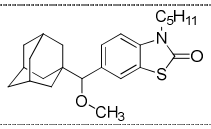
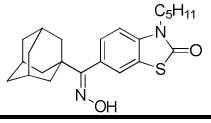
In 4 cases, the replacement of the ketone by an hydroxylmethyl (compound **21**), a methoxy (compound **22**), an hydroxyiminomethyl (compound **23**) or a methoxyiminomethyl (compound **24**) group strongly decreased CB_2 affinity. When the ketone is replaced with amides (compounds **25-26**), the compounds lost totally their CB_2 affinity. Surprisingly, the suppression of the ketone function (compound **20**), that increase the flexibility of the molecule, showed only a slight diminution in CB_2 affinity.

Selectivity analysis

In order to evaluate the selectivity of our new CB_2 ligands, their affinity toward *h*CB_1 receptor was determinated. Only molecules showing a good to moderate affinity for *h*CB_2 (K_i < 400 nM) were tested with the same competitive radioligand displacement assay using the same radioligand. All CB_2 ligands showed no significant affinity for CB_1 (K_i > 590 nM) (Table 3).

Table 3. *h*CB_2 and *h*CB_1 affinities[a] and selectivity index (SI) of identified CB_2 ligands **5**, **8-9** and **10-23**.

Compd	Structure	***h*CB$_2$ K_i (nM)**	***h*CB$_1$ K_i (nM)**	**SI**
5		49.5 ± 14.5	> 1000	> 20
8		210 ± 36	> 1000	> 5
9		13.5 ± 1.5	627 ± 230	46
10		280.5 ± 14.5	> 1000	> 4
11		26 ± 7	780 ± 191	30
12		39 ± 6	903 ± 10	23
20		144 ± 18	593 ± 217	4
21		272 ± 4	> 1000	> 4

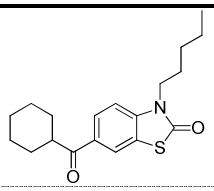
22		344 ± 120	> 1000	> 3
23		251 ± 134	> 1000	> 4
WIN-55,212-2	-	1.57 ± 0.21	19 ± 11	12
JWH-133	-	8 ± 1	> 1000	> 125

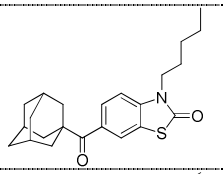
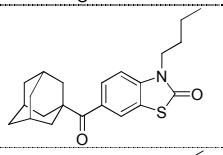
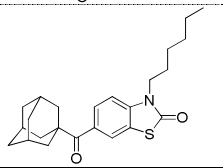
[a] The K_i values were obtained from nonlinear analysis of competition curves using [^{3}H]-CP-55,940 as radioligand for *h*CB_2 and *h*CB_1 cannabinoid receptors and are expressed as mean ± SEM of at least four experiments performed in duplicate.

Functionality analysis

Function activity of most selective benzo[*d*]thiazol-2(3*H*)-ones (SI > 20) with the best *h*CB_2 affinities (K_i < 50 nM) was investigated by using a guanosine-5'-*O*-(3-[^{35}S]-GTPγS) binding assay and *h*CB_2-CHO cells membranes, as previously described [42]. This assay consists in a functional measurement of the interaction between the receptor and the G-protein, which constitutes the first step of the G-protein coupled receptor activation. In this assay, antagonists do not affect [^{35}S]-GTPγS interaction whereas agonists and inverse agonists increase or decrease the binding, respectively. The functional activity of the reference cannabinoid agonist, WIN-55,212-2 (CB_1 and CB_2 agonist), was also determined. Maximum efficacy (E_{max}) and half-maximal effective concentration (EC_{50}) values of the new synthesized compounds and reference are summarized in Table 4. All tested compounds showed a good to moderate efficacy and have been identified as *h*CB_2 agonists (E_{max} > 100%) (Table 4).

Table 4. Functionality and cytotoxicity on HT29 cells[b] of identified selective CB_2 ligands **5**, **9** and **11-12.**

		[^{35}S]-GTPγS(*h*CB_2)		Cytotoxicity
Compd	**Structure**	**EC_{50} (nM)**	**E_{max} (%)**	**(HT29) at 10 µM**
5		41.5 ± 3.6	178 ± 15	67%

9		41.9 ± 5.2	157 ± 3	0%
11		150 ± 18	152 ± 7	0%
12		32.8 ± 8.7	169 ± 8	0%
WIN-55,212-2	-	11.5 ± 3.4	181 ± 12	-

[b] The cytotoxicity values are expressed as the percentage of cellular proliferation inhibition of at least four experiments performed in duplicate.

Cell proliferation assay

Cytotoxicity of our 11 selective CB_2 agonists was determined at 10 µM using a cell proliferation assay on human colorectal adenocarcinoma cells HT29. This test is based on a colorimetric method, which measures the activity of cellular enzymes that reduce the tetrazolium dye (MTS, uncolored) to its insoluble formazan giving a purple color. This assay measures cellular metabolic activity *via* NADPH-dependent cellular oxidoreductase enzymes and reflects, under defined conditions, the number of viable cells. The majority of our selective CB_2 agonists showed no cyctotoxicity. Only compound **5** showed a cytotoxicity on HT29 cells at 10 µM (Table 4).

Anti-inflammatory Effects of 9 in a Murin Model of Acute Colitis

Considering its good affinity for *h*CB_2, selectivity *versus h*CB_1 and agonist property, compound **9** has been selected for the *in vivo* study. Specific Pathogen Free male C57/Bl6 mice received 2.5% dextran sodium sulfate (DSS) in drinking water during 9 days. Concomitantly, they were dosed intraperitoneally with compound **9** in hydroxypropyl β cyclodextrine (150 mM) at the dosage of 10 mg/kg body weight. Control mice were injected with hydroxypropyl β cyclodextrine only. Mice developed progressive weight loss, the first clinical sign of colitis development, starting day 4 after DSS administration

initiation (Figure 4A). At day 9, whereas control mice presented a 79.5 ± 1.8 % of body weight variation from their initial body weight, the body weight change in mice treated with compound **9** was significantly improved (85.9 ± 2.0 %, p = 0.04 respectively, Figure 4B). Another disease indicator measured was colon length/size ratio because DSS typically results in shortening and thickening of the colon, therefore to an increased colon weight/size ratio. We showed that mice treated with **9** had significantly lower colon weight/size ratio (0.044 ± 0.0024, p = 0.004 respectively) compared to control mice (0.050 ± 0.0014, Figure 4C). We then measured colon myeloperoxidase (MPO) activity, reflecting polynuclear neutrophil infiltration (Figure 4D). A 43 % inhibition of MPO activity was quantified in colons of mice treated with **9** compared to control mice (1.3 ± 0.20 *vs* 2.2 ± 0.31, p = 0.04). These data concordantly bring evidence that intraperitoneal administration of **9** inhibit the development of DSS-induced colitis.

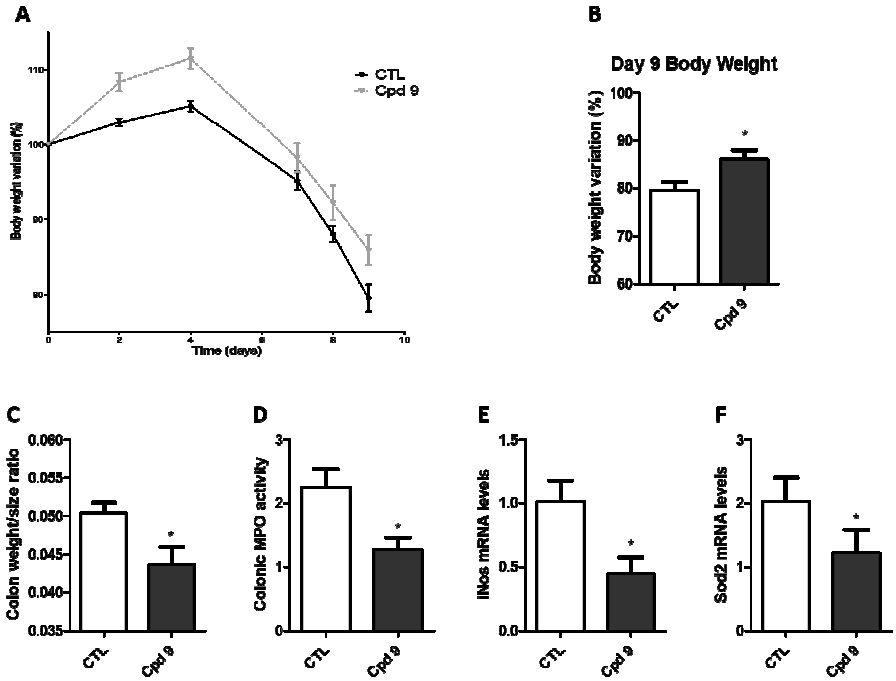


Figure 4. Effects of **9** daily treatment (10 mg/kg, IP) on body weight (A and B), colon weight/size ratio (C) and MPO activity (D) during DSS-induced acute colitis. Quantification by real-time PCR of colon iNos (E) and Sod2 (F) mRNA levels in mice with DDS-induced colitis treated with vehicle and **9**. Values are expressed as a mean ± SEM, n = 10.*p < 0.01, ***p < 0.001.

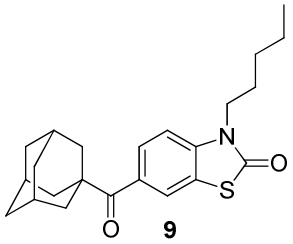
To go further, we quantified by real-time PCR the colonic mRNA levels of several mediators of

inflammation. Administration of **9** resulted in a significant reduction of 2 key enzymes of oxidative stress, *iNos* (0.4 ± 0.1 *vs* 1.0 ± 0.2, p = 0.02, Figure 4E) and *Sod2* (1.2 ± 0.4 *vs* 2.0 ± 0.4, p = 0.02, Figure 4F). Taken together, these different parameters demonstrate the ability of compound **9** to counteract colon inflammatory process.

In vitro ADME-Tox Parameters

In vitro ADME-Tox properties have been performed for compound **9**. ADME profiling comprises solubility, plasma protein binding, Caco-2 permeability and human microsomal stability (Table 5).

Table 5. Determination and evaluation of selected physicochemical and *in vitro* ADME-Tox parameters for compound **9**.



9

Parameters[c]	
MW (g/mol)	383.55
Log P	5.6
tPSA	37.38
***In vitro* ADME-Tox**	
Aqueous solubility (µM)[d]	
Simulated intestinal fluid	107.3
PBS, pH 7.4	1.0
Simulated gastric fluid	183.4
Protein binding (plasma, human)[e]	
% Protein Bound	89%
% Recovery	100%
Caco-2 permeability[f]	
A-B permeability ($\times 10^{-6}$ cm·s^{-1})	1.8
B-A permeability ($\times 10^{-6}$ cm·s^{-1})	1.3
Ratio (B-A)/(A-B)	0.7
Intrinsic clearance (liver microsomes – human)[g]	
% compound remaining after 60 min incubation	43
Half-Life (min)	48
Cl_{int} (µL.min^{-1}.mg^{-1})	145.6

[c] Determined with ChemBioDraw Ultra 12.0; [d] Assessed by shake-flask method (24 h) at RT; [e] Assessed by equilibrium dialysis (4 h) at 37 °C; [f] Compound **9** was incubated (0 and 60 min) at 37 °C with Caco-2 cell line (pH 6.5/7.4); [g] Compound **9** was incubated (0 and 60 min) at 37 °C with human liver microsomes (0.1 mg/mL)

Compound **9** exhibited high *in vitro* metabolic stability with a half-life around 50 min and 43% of compound remaining after 60 min of incubation with human liver microsomes. Caco-2 permeability was moderate and the efflux ratio of 0.7 indicates that compound **9** is not a substrate of an efflux pump. Intestinal and gastric solubility was high (107 and 183 µM, respectively). Nevertheless, no solubility at pH 7.4 was observed. It was found that compound **9** binds strongly to plasma proteins, resulting in 10% being in the free form. ADME profile of compound **9** is acceptable but needs to be improved in the future optimization study to consider oral administration.

CONCLUSION

An original series of selective CB_2 agonists was designed around the benzo[*d*]thiazol-2(3*H*)-one scaffold. 22 new compounds have been synthesized, leading to the discovery of a very potent and selective CB_2 agonist (compound **9**, K_i = 13.5 nM). Pharmacomodulations were carried out and allowed to evidence that the ketone function between the central heterocycle and the bulky aliphatic group plays an important role in the molecular recognition. Position 6 was highlighted to be the optimal position for the hydrophobic group. We have also shown that the replacement of the benzothiazolone by a benzoxazolone resulted in the loss of CB_2 affinity. Compound **9** has shown a strong protective effect in the *in vivo* DSS-induced colitis mouse model, with an improved body weight, a lower colon weight/size ratio, and a decrease of MPO activity. A reduction of 2 key enzymes of oxidative stress (*iNos* and *Sod2*) highlight the ability of these compounds to counteract colon inflammatory process. ADME-Tox profile of compound **9** is acceptable but needs to be improved to consider oral administration. Taking together, these results suggest that benzo[*d*]thiazol-2(3*H*)-one scaffold could open new perspectives for the development of new CB_2 receptor agonists.

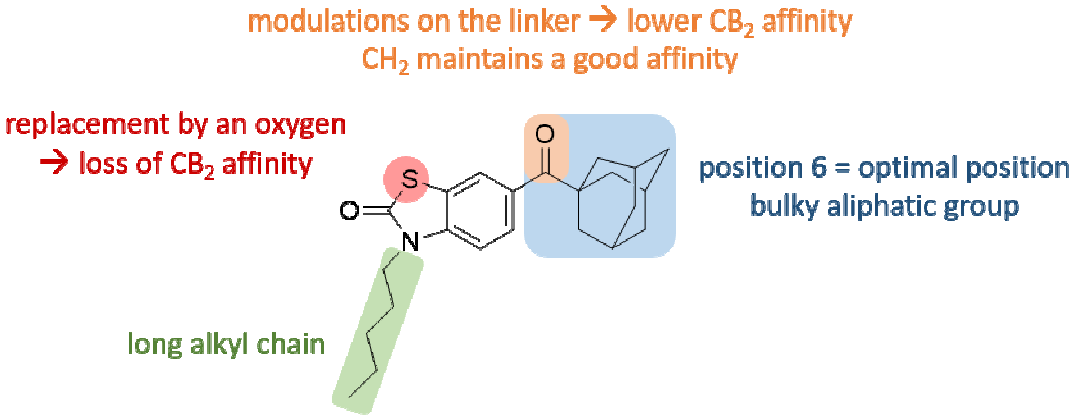


Figure 5. Structure-affinity relationships of benzo[*d*]thiazol-2(3*H*)-one-based selective CB_2 agonists.

EXPERIMENTAL SECTION

Pharmacology. *h*CB_1 and *h*CB_2 membranes of CHO cells were purchased from Perkin Elmer. Fatty acid free bovine serum albumin (BSA) was purchased from Sigma Chemical Co. (St. Louis, MO). WIN-55,212-2 was purchased from RBI (Natick, MA) and JWH-133 from Tocris (Bristol, UK). [^{3}H]-CP-55,940 (101 C_i/mol) was purchased from NEN Life Science (Zaventem, Belgium). Glass fiber filters were purchased from Whatman (Maidstone, UK), while Aqualuma was from PerkinElmer (Schaesberg, TheNetherlands). [^{35}S]-GTPγS (1173 C_i/mmol) was from Amersham (Roosendaal, The Netherlands).

Binding activities. Stock solutions of the compounds were prepared in DMSO and further diluted (100 times) with the binding buffer to the desired concentration. Final DMSO concentrations in the assay were less than 0.1 %. The competitive binding experiments were performed as described earlier [45]. Briefly, [^{3}H]-CP-55,940 (1 nM) as radioligand for the *h*CB_1 and the *h*CB_2 cannabinoid receptor was added to 40 µg of membranes resuspended in 0.5 mL (final volume) binding buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 1 mM EDTA, 0.5% bovine serum albumine, pH 7.4). After 1 h at 30°C, the incubation was stopped and the solutions were rapidly filtered through 0.5% PEI pretreated GF/B glass fiber filters on a M-48T Brandell cell harvester and washed twice with 5 mL of ice-cold binding buffer without serum albumin. The radioactivity on the filters was measured using a Pharmacia Wallac 1410 β-counter using

10 mL of Aqualuma, after 10 s shaking and 3 h resting. Assays were performed at least in triplicate. The non specific binding was determined in the presence of 10 µM HU-210.

[^{35}S]-GTPγS Assays. The binding experiments were performed at 30°C in tubes containing 40 µg protein in 0.5 mL (final volume) binding buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 1 mM EDTA, 100 mM NaCl, 0.1% bovine serum albumin, pH 7.4) supplemented with 20 µM GDP. The assay was initiated by the addition of [^{35}S]-GTPγS (0.05 nM, final concentration). After 1 h, the incubations were terminated by the addition of 5 mL of ice-cold washing buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 1 mM EDTA, 100 mM NaCl). The suspension was immediately filtered through GF/B filters using a 48 well Brandell cell harvester and washed twice with the same ice-cold buffer. The radioactivity on the filters was counted as mentioned above. Assays were performed in triplicate. The non specific binding was measured in the presence of 100 µM Gpp(NH)p. Results were expressed as EC_{50} (nM) and E_{max} (%). Basal constitutive activity of the receptor has been set at a value of 100%; reported E_{max} values above 100% indicated that the compound behaves as an agonist (either partial or full), values fewer than 100% indicated inverse agonist properties.

Cell culture and Cell proliferation assay. Colon cancer cells (HT29) were grown at 37°C in a humidified atmosphere containing 5% CO_2 in DMEM + Glutamax-I (Gibco) supplemented with 10% fetal bovine serum, penicillin (100 IU/mL), and streptomycin (100 µg/mL). In the cell proliferation assay, cells were plated in triplicate on 96-well plates (3000 cells/well) and incubated for 24 h. The cells were then incubated in culture medium that contained a 10 µM concentration of tested compounds, each dissolved in less than 0.1% DMSO. After 72 h, cell growth was estimated by the colorimetric MTS test.

Animals. Six weeks old C57BL6 male mice were purchased from JANVIER Laboratory (Le Genest St. Isle, France). Animals were maintained in specific pathogen free conditions. All animal experiments were approved by local animal care program (Authorization number 00448.01) and were in accordance with European convention on research animal protection.

Induction of acute colitis. Acute colitis was induced with 2.5% (w/v) DSS (molecular mass 35-50 kDa,

TdB consultancy) dissolved in sterile, distilled water ad libitum for 9 days. The DSS solutions were made fresh every 2 days. Body weight was determined regularly. At day 9, mice were euthanized, colons were weighted and sized then stored for molecular analysis.

Colon myeloperoxidase activity measurement. MPO activity was measured to monitor the degree of neutrophil infiltration in the colonic lesions in DSS-induced colitis. Colon specimens were homogenized with an Ultra Turrax T8 (Ika-Werke, Staufen, Germany) in a phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl ammonium and subjected to two sonication and freeze-thaw cycles. The suspensions were centrifuged at 14,000 g for 15 min at 4°C and the supernatants were reacted with 1 mg/ml o-dianisidine hydrochloride and 0.0005% hydrogen peroxide. Optical density of each sample was read at 450nm with a Versamax microplate reader (MDS analytical technologies). One unit of MPO activity was defined as the amount that degraded 1 µmol peroxidase per minute at 25°C. The results were expressed as absorbance per total quantity of proteins determined by the Bradford method.

RNA extraction and Real-Time qPCR. Total RNA was extracted from colonic samples with NucleoSpin RNAII kit (Macherey-Nagel). cDNA was prepared with the High Capacity cDNA Archive kit and RT-qPCR was performed with SyBrGreen (Applied Biosystems). Polymerase RNA II (PolR2A) was used as a reference gene and primer sequences are listed in Supplementary Table 1.

Statistical analysis. Significance was determined using Mann-Whitney U tests (GraphPad prism software, version 6.01).

ADME properties. ADME properties have been determined by Eurofins Panlabs (USA) as described previously [46-49].

Chemistry. Analytical thin-layer chromatography was performed on precoated Kieselgel $60F_{254}$ plates (Merck); the spots were located by UV (254 nm). Silica gel 60 230-400 mesh purchased from Merck was used for column chromatography. Preparative thick-layer chromatography (TLC) was performed using silica gel from Merck, the compounds were eluted from the silica using EtOAc/EtOH (8:2, v/v). All melting points were determined with a Büchi 535 capillary apparatus and remained uncorrected.

Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded at room temperature on a Bruker AC 300 spectrometer. Tetramethylsilane (TMS) was used as an internal standard and $CDCl_3$ or DMSO-d_6 as the solvents. ^{1}H NMR analyses were obtained at 300 MHz (s: singlet, d: doublet, t: triplet, q: quadruplet, quint.: quintuplet, sext.: sextuplet, hept.: heptuplet, dd: double doublet, m: multiplet); whereas ^{13}C NMR analyses were obtained at 75.4 MHz. The chemical shifts (δ) are given in parts per million (ppm) relative to TMS (δ = 0.00). All compounds were analyzed by LC-MS on a HPLC combined with a Surveyor MSQ (Thermo Electron) equipped with an APCI-source. All tested compounds showed purity higher than 96% in APCI$^+$ mode.

6-bromobenzo[*d*]thiazol-2(3*H*)-one **27** [39], 5-bromobenzo[*d*]thiazol-2(3*H*)-one **28** [40], 6-bromobenzo[*d*]xazol-2(3*H*)-one **29** [39] and tetrakis(triphenylphosphine)palladium [50] were prepared according to already described procedures.

General procedure for the preparation of the *N*-alkylated-5 or 6-bromobenzo[*d*]thiazol-2(3*H*)-one and benzo[*d*]xazol-2(3*H*)-one derivatives 30-36. 6-bromobenzo[*d*]thiazol-2(3*H*)-one, 6-bromobenzo[*d*]xazol-2(3*H*)-one or 5-bromobenzo[*d*]thiazol-2(3*H*)-one (10 mmol) was dissolved in 10 mL dry DMF. Cesium carbonate (20 mmol) and the corresponding haloalkane (2-bromopropyl, 1-bromobutyl, 1-bromopentyl, 1-bromohexyl, 2-dimethylaminopropyl chloride, 4-(2-chloroethyl)morpholine) (24 mmol) were added to the solution. The mixture was stirred at 80°C overnight. The solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (50 mL) and washed with water (2 × 50 mL). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure.

6-Bromo-3-pentylbenzo[*d*]thiazol-2(3*H*)-one (30). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 9:1, v/v). A white powder was obtained: yield 65%; mp 47 ± 1°C; ^{1}H NMR (DMSO-d_6) δ 7.95 (d, J = 2.0 Hz, 1H), 7.50 (dd, J = 2.0; 8.6 Hz, 1H), 7.30 (d, J = 8.6 Hz, 1H), 3.90 (t, J = 7.1 Hz, 2H), 1.60 (quint., J = 6.9 Hz, 2H), 1.25 (m, 4H), 0.85 (t, J = 6.9 Hz, 3H); LC-MS (APCI$^+$) *m/z* 300.0 (MH$^+$).

6-Bromo-3-isopropylbenzo[*d*]thiazol-2(3*H*)-one (31). The product was purified by silica gel column chromatography (cyclohexane/EtOAc 8:2, v/v). A beige powder was obtained: yield 56%; mp 67 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.50 (d, J = 2.0 Hz, 1H), 7.36 (dd, J = 2.0; 8.7 Hz, 1H), 7.05 (d, J = 8.7 Hz, 1H), 4.75 (hept., J = 7.1 Hz, 1H), 1.54 (d, J = 7.1 Hz, 6H); LC-MS ($APCI^+$) *m/z* 272.0 (MH^+).

6-Bromo-3-butylbenzo[*d*]thiazol-2(3*H*)-one (32). The product was recristallized in cyclohexane. A white powder was obtained: yield 56%; mp 73 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.56 (d, J = 2.0 Hz, 1H), 7.43 (dd, J = 2.0; 8.6 Hz, 1H), 6.92 (d, J = 8.6 Hz, 1H), 3.93 (t, J = 7.4 Hz, 2H), 1.71 (quint., J = 7.5 Hz, 2H), 1.43 (hex., J = 7.5 Hz, 2H), 0.97 (t, J = 7.4 Hz, 3H); LC-MS ($APCI^+$) *m/z* 327.0 ($MH^+ + CH_3CN$).

6-Bromo-3-hexylbenzo[*d*]thiazol-2(3*H*)-one (33). The product was purified by silica gel column chromatography (cyclohexane/EtOAc 9:1, v/v). A beige powder was obtained: yield 66%; mp 67 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.49 (d, J = 2.0 Hz, 1H), 7.37 (dd, J = 2.0; 8.6 Hz, 1H), 6.88 (d, J = 8.6 Hz, 1H), 3.88 (t, J = 7.5 Hz, 2H), 1.68 (quint., J = 7.1 Hz, 2H), 1.29 (m, 6H), 0.85 (t, J = 6.4 Hz, 3H); LC-MS ($APCI^+$) *m/z* 314.0 (MH^+).

6-Bromo-3-(2-dimethylaminopropyl)benzo[*d*]thiazol-2(3*H*)-one (34). The product was purified by silica gel column chromatography (cyclohexane/EtOAc 9:1, v/v). A yellow oil was obtained: yield 38%; ^{1}H NMR ($CDCl_3$) δ 7.52 (d, J = 2.0 Hz, 1H), 7.39 (dd, J = 2.0; 8.6 Hz, 1H), 7.04 (d, J = 8.6 Hz, 1H), 3.97 (t, J = 7.1 Hz, 2H), 2.32 (t, J = 7.0 Hz, 2H), 2.21 (s, 6H, CH_3), 1.87 (quint., J = 7.0 Hz, 2H); LC-MS ($APCI^+$) *m/z* 315.0 (MH^+).

5-Bromo-3-pentylbenzo[*d*]thiazol-2(3*H*)-one (35). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 9.8:0.2, v/v). A yellow oil was obtained: yield 84%; ^{1}H NMR ($CDCl_3$) δ 7.28 (s, 1H), 7.27 (m, 1H), 7.18 (m, 1H), 3.90 (t, J = 7.4 Hz, 2H), 1.72 (m, 2H), 1.35 (m, 4H), 0.90 (t, J = 6.9 Hz, 3H); LC-MS ($APCI^+$) *m/z* 300.0 (MH^+).

6-Bromo-3-pentylbenzo[*d*]xazol-2(3*H*)-one (36). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 9.9:0.1, v/v). A brown powder was obtained: yield 98%; mp 54 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.38 (d, J = 1.7 Hz, 1H), 7.32 (dd, J = 1.7; 8.3 Hz, 1H), 6.85 (d, J = 8.3

Hz, 1H), 3.80 (t, J = 7.3 Hz, 2H), 1.60 (m, 2H), 1.35 (m, 4H), 0.90 (t, J = 6.6 Hz, 3H); LC-MS ($APCI^+$) *m/z* 284.0 (MH^+).

General procedure for the preparation of the tributylstannyl intermediates 37-43. Under nitrogen atmosphere, compounds **30-36** (3 mmol) and tetrakis(triphenylphosphine)palladium (0.3 mmol) were dissolved in 10 mL dry toluene. Hexa-*n*-butylditin (4.5 mmol) was added to the solution. The reaction was stirred at 70°C for 20h. The solid was filtered and the filtrate evaporated under reduced pressure. The residue was washed with petroleum ether, filtered and the filtrate was evaporated under reduced pressure.

3-Pentyl-6-(tributylstannyl)benzo[*d*]thiazol-2(3*H*)-one (37). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 95:5, v/v). A yellow oil was obtained: yield 45%; ^1H NMR ($CDCl_3$) δ 7.50 (s, 1H), 7.38 (d, J = 7.8 Hz, 1H), 7.00 (d, J = 7.8 Hz, 1H), 3.98 (t, J = 7.5 Hz, 2H), 1.65 (m, 8H), 1.35 (m, 12H), 1.10 (m, 4H), 0.90 (m, 12H) ; LC-MS ($APCI^+$) *m/z* 512.2 (MH^+).

3-Isopropyl-6-(tributylstannyl)benzo[*d*]thiazol-2(3*H*)-one (38). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 99:1, v/v). An orange oil was obtained: yield 9%; ^1H NMR ($CDCl_3$) δ 7.49 (s, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.19 (d, J = 8.0 Hz, 1H), 4.82 (hept., J = 7.1 Hz, 1H), 1.58 (d, J = 7.1 Hz, 6H), 1.56 (m, 6H), 1.35 (m, 12H), 1.08 (m, 9H); LC-MS ($APCI^+$) *m/z* 484.2 (MH^+).

3-Butyl-6-(tributylstannyl)benzo[*d*]thiazol-2(3*H*)-one (39). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 98:2, v/v). A colorless oil was obtained: yield 36%; ^1H NMR ($CDCl_3$) δ 7.48 (s, 1H), 7.38 (d, J = 7.8 Hz, 1H), 7.03 (d, J = 7.8 Hz, 1H), 3.92 (t, J = 7.3 Hz, 2H), 1.65 (m, 8H), 1.37 (m, 2H), 1.35 (m, 12H), 0.89 (m, 12H); LC-MS ($APCI^+$) *m/z* 498.2 (MH^+).

3-Hexyl-6-(tributylstannyl)benzo[*d*]thiazol-2(3*H*)-one (40). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 98:2, v/v). A colorless oil was obtained: yield 25%; ^1H NMR ($CDCl_3$) δ 7.44 (d, J = 0.9 Hz,1H), 7.33 (dd, J = 0.9; 7.8 Hz, 1H), 7.00 (d, J = 7.8 Hz, 1H), 3.87 (t, J = 7.4 Hz, 2H), 1.61 (m, 8H), 1.26 (m, 12H), 1.03 (m, 6H), 0.85 (m, 12H); LC-MS ($APCI^+$) *m/z* 526.2

(MH^+).

3-(2-Dimethylaminopropyl)-6-(tributylstannyl)benzo[*d*]thiazol-2(3*H*)-one (41). The product was purified by silica gel column chromatography (dichloromethane/MeOH 9:1, v/v). A yellow oil was obtained: yield 79%; ^{1}H NMR ($CDCl_3$) δ 7.49 (d, J = 2.0 Hz, 1H), 7.37 (dd, J = 2.0; 8.6 Hz, 1H), 6.88 (d, J = 8.6 Hz, 1H), 3.88 (t, J = 7.5 Hz, 2H), 1.68 (m, 8H), 1.29 (m, 14H), 0.85 (m, 15H); LC-MS ($APCI^+$) *m/z* 526.2 (MH^+).

3-Pentyl-5-(tributylstannyl)benzo[*d*]thiazol-2(3*H*)-one (42). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 9.6:0.4, v/v). A colorless oil was obtained: yield 52%; ^{1}H NMR ($CDCl_3$) δ 7.40 (d, J = 7.5 Hz, 1H), 7.24 (d, J = 7.5 Hz, 1H), 7.12 (s, 1H), 3.97 (t, J = 7.5 Hz, 2H), 1.75 (m, 2H), 1.60 (m, 6H), 1.35 (m, 12H), 1.12 (m, 4H), 0.90 (m, 12H); LC-MS ($APCI^+$) *m/z* 512.2 (MH^+).

3-Pentyl-6-(tributylstannyl)benzo[*d*]xazol-2(3*H*)-one (43). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 96:04, v/v). A yellow oil was obtained: yield 37%; ^{1}H NMR ($CDCl_3$) δ 7.30 (s, 1H), 7.23 (d, J = 7.5 Hz, 1H), 6.92 (d, J = 7.5 Hz, 1H), 3.81 (t, J = 7.4 Hz, 2H), 1.80 (m, 2H), 1.60 (m, 6H), 1.35 (m, 12H), 1.10 (m, 4H), 0.90 (m, 12H); LC-MS ($APCI^+$) *m/z* 496.2 (MH^+).

General procedure for the preparation of compounds 5-19. Under nitrogen atmosphere, a mixture of the corresponding tributyltin intermediate **37-43** (1 mmol), $PdCl_2(PPh_3)_2$ (0.1 mmol) and the corresponding acyl chloride (1-adamantanecarbonyl chloride, benzoyl chloride, acetyl chloride, 2,2,3,3-tetramethylcyclopropanecarbonyl chloride, cyclopentanecarbonyl chloride, cyclohexanecarbonyl chloride, cycloheptanecarbonyl chloride) (2.3 mmol) in dry toluene (4 mL) was stirred at reflux overnight. The reaction mixture was filtered and evaporated under reduced pressure.

6-(Cyclohexanecarbonyl)-3-pentylbenzo[*d*]thiazol-2(3*H*)-one (5). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 98:2, v/v). A yellow oil was obtained: yield 26%; ^{1}H NMR ($CDCl_3$) δ 8.04 (d, J = 1.7 Hz, 1H), 7.93 (dd, J = 1.7; 8.5 Hz, 1H), 7.09 (d, J = 8.5 Hz, 1H),

3.95 (t, J = 7.4 Hz, 2H), 3.20 (m, 1H), 1.80 (m, 6H), 1.30 (m, 10H), 0.90 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 201.8 (^{IV}C), 170.0 (^{IV}C), 140.5 (^{IV}C), 131.4 (^{IV}C), 127.1 (CH), 123.2 (^{IV}C), 123.1 (CH), 110.2 (CH), 45.5 (CH_2), 43.1 (CH), 29.5 ($2CH_2$), 28.8 (CH_2), 27.3 (CH_2), 26.3 (CH_2), 25.9 ($2CH_2$), 22.3 (CH_2), 13.9 (CH_3); LC-MS (ESI) *m/z* 332.1 (MH^+), t_r 5.06 min, λ_{max} 237 nm, purity 96.5%.

6-Benzoyl-3-pentylbenzo[*d*]thiazol-2(3*H*)-one (6). The product was purified by silica gel column chromatography (CH_2Cl_2/MeOH 99:1, v/v). A yellow powder was obtained: yield 89%; mp 65 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 8.13 (d, J = 1.7 Hz, 1H), 7.70 (m, 7H), 3.85 (t, J = 7.3 Hz, 2H), 1.65 (m, 2H), 1.30 (m, 4H), 0.85 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 194.9 (^{IV}C), 170.0 (^{IV}C), 140.5 (^{IV}C), 137.6 (^{IV}C), 132.4 (^{IV}C), 132.4 (CH), 129.8 (2CH), 129.1 (CH), 128.4 (2CH), 125.0 (CH), 122.9 (^{IV}C), 110.0 (CH), 43.2 (CH_2), 28.9 (CH_2), 27.4 (CH_2), 22.3 (CH_2), 13.9 (CH_3); LC-MS (ESI) *m/z* 326.1 (MH^+), t_r 4.64 min, λ_{max} 237 nm, purity 95.6%.

3-Pentyl-6-(2,2,3,3-tetramethylcyclopropanecarbonyl)benzo[*d*]thiazol-2(3*H*)-one (7). The product was purified by silica gel column chromatography (cyclohexane/EtOAc 9:1, v/v). A white powder was obtained: yield 50%; mp 95 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.62 (d, J = 1.8 Hz, 1H), 7.49 (dd, J = 1.8; 8.8 Hz, 1H), 7.12 (d, J = 8.8 Hz, 1H), 3.98 (t, J = 7.3 Hz, 2H), 1.78 (quint., J = 7.3 Hz, 2H), 1.44 (m, 1H), 1.41 (m, 4H), 1.26 (m, 6H), 1.21 (m, 6H), 0.93 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 178.2 (^{IV}C), 169.9 (^{IV}C), 136.7 (^{IV}C), 135.7 (^{IV}C), 125.2 (CH), 123.9 (CH), 121.1 (^{IV}C), 111.0 (CH), 43.2 (CH_2), 35.7 (CH), 31.4 (CH_2), 29.0 (CH_2), 27.5 (2^{IV}C), 23.7 (CH_2), 22.5 ($2CH_3$), 16.7 ($2CH_3$), 14.1 (CH_3); LC-MS ($APCI^+$) *m/z* 346.2 (MH^+), t_r 5.50 min, λ_{max} 235 nm, purity 97.2%.

6-(Cyclopentanecarbonyl)-3-pentylbenzo[*d*]thiazol-2(3*H*)-one (8). The product was purified by silica gel column chromatography (cyclohexane/EtOAc 95:5, v/v). A yellow oil was obtained: yield 6%; ^{1}H NMR ($CDCl_3$) δ 8.08 (d, J = 1.7 Hz, 1H), 7.97 (dd, J = 1.7; 8.5 Hz, 1H), 7.09 (d, J = 8.5 Hz, 1H), 3.97 (t, J = 7.4 Hz, 2H), 3.69 (quint., J = 7.8 Hz, 1H), 1.92 (m, 4H), 1.72 (m, 6H), 1.36 (m, 4H), 0.90 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 201.0 (^{IV}C), 170.1 (^{IV}C), 140.7 (^{IV}C), 132.2 (^{IV}C), 127.4 (CH), 123.4 (CH),

123.2 (^{IV}C), 110.2 (CH), 46.3 (CH), 43.3 (CH_2), 30.2 ($2CH_2$), 29.0 (CH_2), 27.5 (CH_2), 26.4 ($2CH_2$), 22.4 (CH_2), 14.0 (CH_3); LC-MS (APCI$^+$) *m/z* 318.1 (MH^+), t_r 4.60 min, λ_{max} 245 nm, purity 98.7%.

6-(Adamantane-1-carbonyl)-3-pentyl-benzo[*d*]thiazol-2(3*H*)-one (9). The product was purified by silica gel column chromatography (petroleum ether/EtOAc/ammoniac saturated MeOH 94:5.5:0.5, v/v/v). A white powder was obtained: yield 68%; mp 119 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.82 (d, J = 1.3 Hz, 1H), 7.70 (dd, J = 1.3; 8.4 Hz, 1H), 7.05 (d, J = 8.4 Hz, 1H), 3.96 (t, J = 7.3 Hz, 2H), 2.11 (m, 3H), 2.06 (m, 6H), 1.78 (m, 6H), 1.73 (m, 2H), 1.38 (quint., J = 3.7 Hz, 4H), 0.92 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 207.2 (^{IV}C), 184.4 (^{IV}C), 139.1 (^{IV}C), 133.7 (^{IV}C), 126.8 (2CH), 122.8 (^{IV}C), 109.9 (CH), 47.2 (^{IV}C), 43.2 (CH_2), 39.5 ($3CH_2$), 36.7 ($3CH_2$), 29.8 (CH_2), 29.0 (CH_2), 28.3 (3CH), 22.5 (CH_2), 14.1 (CH_3); LC-MS (ESI) *m/z* 384.2 (MH^+), t_r 5.77 min, λ_{max} 237 nm, purity 99.6%.

6-(1-Adamantanecarbonyl)-3-isopropylbenzo[*d*]thiazol-2(3*H*)-one (10). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 98:2, v/v). A white powder was obtained: yield 27%; mp 121 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.77 (d, J = 1.7 Hz, 1H), 7.67 (dd, J = 1.7; 8.6 Hz, 1H), 7.20 (d, J = 8.6 Hz, 1H), 4.82 (hept., J = 7.0 Hz, 1H), 2.09 (m, 3H), 2.04 (m, 6H), 1.90 (m, 6H), 1.57 (d, J = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 207.1 (^{IV}C), 184.1 (^{IV}C), 169.8 (^{IV}C), 138.6 (^{IV}C), 133.2 (CH), 126.5 (CH), 122.7 (^{IV}C), 110.7 (CH), 47.1 (^{IV}C), 40.5 (CH), 39.5 ($3CH_2$), 36.6 ($3CH_2$), 28.3 (3CH), 19.5 ($2CH_3$); LC-MS (ESI) *m/z* 356.1 (MH^+), t_r 4.91 min, λ_{max} 255 nm, purity 97.2%.

6-(1-Adamantanecarbonyl)-3-butylbenzo[*d*]thiazol-2(3*H*)-one (11). The product was purified by preparative TLC (petroleum ether/EtOAc 99:1, v/v). A white powder was obtained: yield 17%; mp 98 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.79 (d, J = 1.7 Hz, 1H), 7.70 (dd, J = 1.7; 8.5 Hz, 1H), 7.04 (d, J = 8.5 Hz, 1H), 3.96 (t, J = 7.4 Hz, 2H), 2.10 (s, 3H), 2.05 (s, 6H), 1.82 (s, 6H), 1.75 (quint., J = 7.6 Hz, 2H), 1.44 (sext., J = 7.5 Hz, 2H), 0.97 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 207.2 (^{IV}C), 170.0 (^{IV}C), 139.1 (^{IV}C), 133.7 (^{IV}C), 126.8 (CH), 122.8 (CH), 122.7 (^{IV}C), 109.8 (CH), 47.1 (^{IV}C), 43.0 (CH_2), 39.5 ($3CH_2$), 36.6 ($3CH_2$), 29.8 (CH_2), 28.3 (3CH), 20.2 (CH_2), 13.8 (CH_3); LC-MS (ESI) *m/z* 370.1 (MH^+), t_r 4.99 min, λ_{max} 245 nm, purity 99.6%.

6-(1-Adamantanecarbonyl)-3-hexylbenzo[*d*]thiazol-2(3*H*)-one (12). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 9:1, v/v). A white powder was obtained: yield 16%; mp 106 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.79 (d, J = 1.5 Hz, 1H), 7.70 (dd, J = 1.5; 8.5 Hz, 1H), 7.04 (d, J = 8.5 Hz, 1H), 3.95 (t, J = 7.4 Hz, 2H), 2.10 (s, 3H), 2.04 (s, 6H), 1.92 (s, 6H), 1.76 (m, 2H), 1.34 (m, 6H), 0.93 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 207.2 (^{IV}C), 170.0 (^{IV}C), 139.0 (^{IV}C), 133.7 (^{IV}C), 126.8 (CH), 122.7 (CH), 122.7 (^{IV}C), 109.8 (CH), 47.1 (^{IV}C), 43.2 (CH_2), 39.5 ($3CH_2$), 36.6 ($3CH_2$), 31.5 (CH_2), 27.9 (3CH), 27.7 (CH_2), 26.5 (CH_2), 22.6 (CH_2), 13.8 (CH_3); LC-MS (ESI) *m/z* 398.2 (MH^+), t_r 5.78 min, λ_{max} 240 nm, purity 99.7%.

6-(1-Adamantanecarbonyl)-3-(2-dimethylaminopropyl)benzo[*d*]thiazol-2(3*H*)-one (13). The product was purified by silica gel column chromatography (dichloromethane/MeOH 9:1, v/v). A white powder was obtained: yield 25%; mp 114 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.79 (d, J = 1.5 Hz, 1H), 7.70 (dd, J = 1.5; 8.5 Hz, 1H), 7.04 (d, J = 8.5 Hz, 1H), 3.95 (t, J = 7.4 Hz, 2H), 2.10 (s, 3H), 2.04 (s, 6H), 1.92 (s, 6H), 1.76 (m, 2H), 1.24 (m, 5H), 0.93 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 207.2 (^{IV}C), 170.0 (^{IV}C), 139.1 (^{IV}C), 133.7 (^{IV}C), 126.8 (CH), 122.8 (CH), 122.7 (^{IV}C), 109.8 (CH), 47.1 (^{IV}C), 43.0 (CH_2), 39.5 ($3CH_2$), 36.6 ($3CH_2$), 29.8 ($2CH_2$), 28.3 (3CH), 20.2 (CH_3), 13.8 (CH_3); LC-MS ($APCI^+$) *m/z* 399.2 (MH^+), t_r 5.78 min, λ_{max} 235 nm, purity 99.7%.

5-(1-Adamantanecarbonyl)-3-pentylbenzo[*d*]thiazol-2(3*H*)-one (14). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 9:1, v/v). A yellow oil was obtained: yield 61%; ^{1}H NMR ($CDCl_3$) δ 7.50 (dd, J = 1.4; 8.1 Hz, 1H), 7.45 (d, J = 8.1 Hz, 1H), 7.30 (s, 1H), 3.95 (t, J = 7.6 Hz, 2H), 2.05 (m, 9H), 1.75 (m, 8H), 1.35 (m, 4H), 0.90 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 208.1 (^{IV}C), 183.2 (^{IV}C), 169.5 (^{IV}C), 137.1 (^{IV}C), 125.8 (^{IV}C), 122.1 (CH), 121.8 (CH), 110.0 (CH), 47.1 (^{IV}C), 43.1 (CH_2), 39.3 ($3CH_2$), 38.9 (CH_2), 36.5 ($3CH_2$), 28.9 (CH_2), 28.0 (3CH), 22.6 (CH_2), 14.0 (CH_3); LC-MS ($APCI^+$) *m/z* 406.2 (M + Na), t_r 5.54 min, λ_{max} 240 nm, purity 98.2%.

5-(Cyclohexanecarbonyl)-3-pentylbenzo[*d*]thiazol-2(3*H*)-one (15). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 9:1, v/v). A yellow oil was obtained: yield 30%;

^{1}H NMR ($CDCl_3$) δ 7.74 (dd, J = 1.3; 8.2 Hz, 1H), 7.66 (d, J = 1.3 Hz, 1H), 7.52 (d, J = 8.2 Hz, 1H), 4.00 (t, J = 7.5 Hz, 2H), 3.25 (m, 1H), 1.90 (m, 4H), 1.75 (m, 2H), 1.40 (m, 10H), 0.90 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 202.8 (^{IV}C), 169.4 (^{IV}C), 137.9 (^{IV}C), 134.7 (^{IV}C), 128.7 (^{IV}C), 123.1 (CH), 122.4 (CH), 110.0 (CH), 45.7 (CH), 43.3 (CH_2), 31.8 (CH_2), 29.6 (CH_2), 29.0 (CH_2), 27.7 (CH_2), 26.8 (CH_2), 26.0 ($2CH_2$), 22.6 (CH_2), 14.2 (CH_3); LC-MS (APCI$^+$) *m/z* 354.2 (M + Na), t_r 4.83 min, λ_{max} 225 nm, purity 98.5%.

3-Pentyl-5-(2,2,3,3-tetramethylcyclopropanecarbonyl)benzo[*d*]thiazol-2(3*H*)-one (16). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 9:1, v/v). A yellow powder was obtained: yield 35%; mp 61 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.55 (d, J = 8.1 Hz, 1H), 7.35 (dd, J = 1.5; 8.1 Hz, 1H), 7.18 (d, J = 1.5 Hz, 1H), 4.05 (t, J = 7.5 Hz, 2H), 1.80 (m, 2H), 1.30 (m, 17H), 0.88 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 201.8 (^{IV}C), 167.8 (^{IV}C), 147.5 (^{IV}C), 135.1 (^{IV}C), 129.2 (CH), 123.0 (^{IV}C), 121.2 (CH), 104.4 (CH), 47.1 (CH_2), 34.0 (CH), 30.5 (CH_2), 30.2 (CH_2), 29.0 (2^{IV}C), 22.3 (CH_2), 21.8 ($2CH_3$), 17.5 ($2CH_3$), 13.8 (CH_3); LC-MS (APCI$^+$) *m/z* 368.2 (M + Na), t_r 4.94 min, λ_{max} 240 nm, purity 98.9%.

6-(1-Adamantanecarbonyl)-3-pentylbenzo[*d*]xazol-2(3*H*)-one (17). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 9:1, v/v). A beige powder was obtained: yield 24%; mp 75 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.60 (s, 1H), 7.55 (d, J = 8.6 Hz, 1H), 6.98 (d, J = 8.6 Hz, 1H), 3.85 (t, J = 7.3 Hz, 2H), 2.10 (m, 9H), 1.75 (m, 8H), 1.35 (m, 4H), 0.90 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 207.1 (^{IV}C), 154.6 (^{IV}C), 142.1 (^{IV}C), 133.4 (^{IV}C), 133.3 (^{IV}C), 124.7 (CH), 110.0 (CH), 107.6 (CH), 47.2 (^{IV}C), 42.7 (CH_2), 39.6 ($3CH_2$), 36.7 ($3CH_2$), 31.7 (CH_2), 28.3 (3CH), 27.9 (CH_2), 22.7 (CH_2), 14.1 (CH_3); LC-MS (APCI$^+$) *m/z* 390.2 (M + Na), t_r 5.33 min, λ_{max} 235 nm, purity 97.2%.

6-(Cyclohexanecarbonyl)-3-pentylbenzo[*d*]xazol-2(3*H*)-one (18). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 9:1, v/v). A beige powder was obtained: yield 21%; mp 65 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.88 (dd, J = 1.4; 8.2 Hz, 1H), 7.81 (d, J = 1.4 Hz, 1H), 7.03 (d, J = 8.2 Hz, 1H), 3.24 (m, 1H), 1.85 (m, 6H), 1.40 (m, 12H), 0.90 (t, J = 6.8 Hz, 3H); ^{13}C NMR

($CDCl_3$) δ 202.0 (^{IV}C), 154.6 (^{IV}C), 142.8 (^{IV}C), 135.2 (^{IV}C), 131.3 (^{IV}C), 125.3 (CH), 110.0 (CH), 107.9 (CH), 45.7 (CH), 42.8 (CH_2), 31.7 (CH_2), 29.7 (CH_2), 28.9 (CH_2), 27.9 (CH_2), 26.7 (CH_2), 26.0 ($2CH_2$), 22.7 (CH_2), 14.1 (CH_3); LC-MS ($APCI^+$) *m/z* 344.2 (M + Si), t_r 4.93 min, λ_{max} 246 nm, purity 97.7%.

3-Pentyl-6-(2,2,3,3-tetramethylcyclopropanecarbonyl)benzo[*d*]xazol-2(3*H*)-one (19). The product was purified by silica gel column chromatography (petroleum ether/EtOAc 9:1, v/v). A yellow oil was obtained: yield 50%; ^1H NMR ($CDCl_3$) δ 7.15 (m, 2H), 6.98 (d, J = 7.1 Hz, 1H), 3.85 (t, J = 7.3 Hz, 2H), 1.80 (m, 2H), 1.30 (m, 17H), 0.90 (m, 3H); LC-MS ($APCI^+$) *m/z* 352.2 (M + Na), t_r 4.73 min, λ_{max} 235 nm, purity 96.4%.

6-(1-Adamantylmethyl)-3-pentylbenzo[*d*]thiazol-2(3*H*)-one (20). Triethylsilane (11 mmol) was added to a solution of compound **9** (5 mmol) in trifluoroacetic acid (15 mL). The reaction mixture was stirred at room temperature overnight. The solution was hydrolyzed with iced water (20 mL) and extracted with dichloromethane (2 × 50 mL). The organic layer was washed with 10% aqueous potassium carbonate (40 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The product was purified by silica gel column chromatography (petroleum ether/EtOAc 98:2, v/v) and recrystallized in methanol. A white powder was obtained: yield 16%; mp 80 ± 1°C; ^1H NMR ($CDCl_3$) δ 7.15 (s, 1H), 7.04 (d, J = 8.3 Hz, 1H), 6.93 (d, J = 8.3 Hz, 1H), 3.94 (t, J = 7.4 Hz, 2H), 2.40 (s, 2H), 1.92 (m, 5H), 1.75 (m, 2H), 1.57 (m, 5H), 1.48 (m, 5H), 1.40 (m, 4H), 0.92 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 170.0 (^{IV}C), 135.4 (^{IV}C), 133.4 (^{IV}C), 128.7 (CH), 124.3 (CH), 122.2 (^{IV}C), 109.8 (CH), 50.9 (^{IV}C), 43.0 (CH_2), 42.5 ($3CH_2$), 37.1 ($3CH_2$), 33.7 (CH_2), 29.1 (CH_2), 28.8 (3CH), 27.5 (CH_2), 22.5 (CH_2), 14.1 (CH_3); LC-MS (ESI) *m/z* 392.2 (M + Na), t_r 5.76 min, λ_{max} 338 nm, purity 97.2%.

6-(1-Adamantylhydroxylmethyl)-3-pentylbenzo[*d*]thiazol-2(3*H*)-one (21). Sodium borohydride (30 mmol) was added to a solution of compound **9** (10 mmol) in methanol (15 mL). The reaction mixture was stirred at room temperature for 5 h. The solvent was evaporated under reduced pressure and the residue was hydrolyzed with water (20 mL). The solution was extracted with dichloromethane (2 × 30 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The product was purified by silica

gel column chromatography (petroleum ether/EtOAc 95:5, v/v) and recrystallized in acetonitrile. A grey powder was obtained: yield 26%; mp 153 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.36 (d, J = 1.4 Hz, 1H), 7.20 (dd, J = 1.4; 8.3 Hz, 1H), 6.95 (d, J = 8.3 Hz, 1H), 4.22 (s, 1H), 2.92 (t, J = 7.6 Hz, 2H), 1.98 (m, 5H), 1.90 (s, 1H), 1.70 (m, 7H), 1.50 (m, 5H), 1.35 (m, 4H), 0.90 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 170.1 (^{IV}C), 136.5 (^{IV}C), 136.3 (^{IV}C), 126.1 (CH), 122.3 (^{IV}C), 122.0 (CH), 109.6 (CH), 82.7 (CH), 43.1 (^{IV}C), 38.2 ($3CH_2$), 37.1 ($3CH_2$), 29.8 (CH_2), 29.1 (CH_2), 28.4 (3CH), 27.5 (CH_2), 22.5 (CH_2), 14.1 (CH_3); LC-MS ($APCI^+$) *m/z* 386.2 (MH^+), t_r 5.18 min, λ_{max} 235 nm, purity 97.8%.

6-(1-Adamantylmethoxylmethyl)-3-pentylbenzo[*d*]thiazol-2(3*H*)-one (22). Sodium hydride (1.67 mmol) was added to a solution of compound **21** (0.39 mmol) in THF (15 mL). Methyl iodide (1.67 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 5 h. The solution was evaporated under reduced pressure and hydrolyzed with water (20 mL). The solution was extracted with dichloromethane (2×30 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The product was purified by silica gel column chromatography (petroleum ether/EtOAc 95:5) and recrystallized in acetonitrile. A yellow powder was obtained: yield 39%; mp 153 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.27 (d, J = 1.4 Hz, 1H), 7.14 (dd, J = 1.4; 8.4 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 3.93 (t, J = 7.3 Hz, 2H), 3.60 (s, 1H), 3.17 (s, 3H), 1.93 (m, 3H), 1.76 (quint., J = 7.3 Hz, 2H), 1.64 (m, 10H), 1.41 (m, 6H), 0.92 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 170.1 (^{IV}C), 136.5 (^{IV}C), 134.0 (^{IV}C), 126.8 (CH), 122.5 (^{IV}C), 122.3 (CH), 109.6 (CH), 92.6 (CH), 57.7 (CH_3), 43.1 (CH), 38.6 ($3CH_2$), 37.5 (CH_2), 37.2 ($3CH_2$), 29.1 (CH_2), 28.5 (3CH), 27.5 (CH_2), 22.5 (CH_2), 14.1 (CH_3); LC-MS ($APCI^+$) *m/z* 399.1 (MH^+), t_r 5.50 min, λ_{max} 235 nm, purity 95.3%.

6-(1-Adamantyl-1-hydroxyiminomethyl)-3-pentylbenzo[*d*]thiazol-2(3*H*)-one (23). Hydroxylamine hydrochloride (20 mmol) and pyridine (25 mmol) were added to a solution of compound **9** (5 mmol) in methanol (20 mL). The reaction mixture was stirred at reflux for 4 h. The solvent was evaporated under reduced pressure. The residue was hydrolyzed with water (10 mL) and the solution was acidified to pH 1 with 1N HCl solution. The solution was extracted with dichloromethane (20 mL), dried over $MgSO_4$

and evaporated under reduced pressure. The product was purified by recrystallisation in absolute ethanol. White crystals were obtained: yield 25%; mp 227 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 10.42 (s, 1H, OH), 7.33 (d, J = 8.2 Hz, 1H), 7.31 (s, 1H), 6.97 (d, J = 8.2 Hz, 1H), 3.93 (t, J = 7.1 Hz, 2H), 1.95 (m, 5H), 1.65 (m, 7H), 1.50 (m, 5H), 1.30 (m, 4H), 0.86 (t, J = 6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 169.9 (^{IV}C), 166.2 (^{IV}C), 137.0 (^{IV}C), 127.8 (^{IV}C), 126.1 (CH), 123.0 (^{IV}C), 122.0 (CH), 110.2 (CH), 43.2 (^{IV}C), 39.9 ($3CH_2$), 39.5 (CH_2), 36.6 ($3CH_2$), 29.1 (CH_2), 28.2 (3CH), 27.5 (CH_2), 22.5 (CH_2), 14.1 (CH_3); LC-MS (ESI) *m/z* 399.2 (MH^+), t_r 5.14 min, λ_{max} 235 nm, purity 95.3%.

6-(1-Adamantyl-1-methoxyiminomethyl)-3-pentylbenzo[*d*]thiazol-2(3*H*)-one (24). Methoxylamine hydrochloride (20 mmol) and pyridine (25 mmol) were added to a solution of compound **9** (5 mmol) in methanol (20 mL). The reaction mixture was stirred at reflux for 4 h. The solvent was evaporated under reduced pressure. The residue was hydrolyzed with water (10 mL) and the solution was acidified to pH 1 with 1N HCl solution. The solution was extracted with dichloromethane (20 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The product was purified by preparative TLC (petroleum ether/EtOAc 95:5, v/v). A white powder was obtained: yield 15%; mp 141 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.09 (d, J = 1.3 Hz, 1H), 7.05 (d, J = 8.3 Hz, 1H), 6.97 (dd, J = 1.3; 8.3 Hz, 1H), 3.94 (t, J = 7.4 Hz, 2H), 3.75 (s, 3H), 2.05 (m, 5H), 1.70 (m, 7H), 1.50 (m, 5H), 1.40 (m, 4H), 0.93 (t, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 169.9 (^{IV}C), 165.0 (^{IV}C), 136.7 (^{IV}C), 128.7 (^{IV}C), 126.0 (CH), 122.7 (^{IV}C), 121.9 (CH), 110.0 (CH), 61.8 (CH_3), 43.1 (^{IV}C), 40.2 ($3CH_2$), 39.2 (CH_2), 36.7 ($3CH_2$), 29.1 (CH_2), 28.3 (3CH), 27.5 (CH_2), 22.5 (CH_2), 14.1 (CH_3); LC-MS (ESI) *m/z* 413.2 (MH^+), t_r 5.59 min, λ_{max} 250 nm, purity 98.4%.

6-Acetylbenzo[*d*]thiazol-2(3*H*)-one (44). Dry DMF (25 mL) was added dropwise to a flask containing aluminium chloride (80 mmol). 2,3-Dihydro-1,3-benzothiazol-2-one (10 mmol) and acetyl chloride (18 mmol) were added dropwise to the mixture. The reaction mixture was stirred at 70°C for 5 h. The solution was hydrolyzed with iced water (10 mL) and the precipitate was filtered off, washed with water until the washing water is neutral and with absolute ethanol. The product was purified by recrystallization in absolute ethanol. A brown powder was obtained: yield 48%; mp 180 ± 1°C; ^{1}H

NMR ($CDCl_3$) δ 12.10 (s,1H, NH), 8.20 (d, J = 1.4 Hz, 1H), 7.90 (dd, J = 1.4; 8.3 Hz, 1H), 7.20 (d, J = 8.3 Hz, 1H), 2.60 (s, 3H); LC-MS ($APCI^+$) *m/z* 194.0 (MH^+).

2-Oxo-2,3-dihydro-1,3-benzothiazole-6-carboxylic acid (45). Sodium hydroxide (155 mmol) was dissolved in water (5 mL). Sodium hypochlorite (100 mL) was added to the solution and compound **44** (15 mmol) was then added dropwise. The reaction mixture was stirred at reflux for 2 h. The solution was hydrolyzed with iced water (40 mL) and acidified to pH 1 with 3N HCl solution. The precipitate was filtered off, washed with water until the washing water is neutral and washed with ethanol. The product was purified by recrystallization in ethanol. A brown powder was obtained: yield 50%; mp > 260°C; ^1H NMR ($CDCl_3$) δ 12.90 (s, 1H, OH), 12.20 (s, 1H, NH), 8.15 (d, J = 1.6 Hz, 1H), 7.88 (dd, J = 1.6; 8.4 Hz, 1H), 7.15 (d, J = 8.4 Hz, 1H); LC-MS ($APCI^+$) *m/z* 196.0 (MH^+).

***N*-(Adamantan-1-yl)-2-oxo-2,3-dihydrobenzo[*d*]thiazole-6-carboxamide (46).** HOBt (0.5 mmol), HBTU (1.5 mmol), adamantanamine hydrochloride (1.1 mmol) and DIEA (2.2 mmol) were added to a solution of compound **45** (1 mmol) in dry DMF (20 mL). The reaction mixture was stirred at room temperature under nitrogen for 18 h. The solvent was evaporated under reduced pressure. The residue was dissolved in 1N HCl (20 mL) and extracted with dichloromethane (20 mL). The organic layer was washed with 5% aqueous sodium bicarbonate (20 mL) and water (20 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The product was purified by recrystallization in methanol. A yellow powder was obtained: yield 14%; mp > 260°C; ^1H NMR ($CDCl_3$) δ 12.10 (s, 1H, NH), 8.15 (s, 1H, NH), 7.75 (d, J = 8.3 Hz, 1H), 7.50 (s, 1H), 7.10 (d, J = 8.3 Hz, 1H), 2.10 (m, 5H), 1.60 (m, 5H), 1.50 (m, 5H); LC-MS ($APCI^+$) *m/z* 329.1 (MH^+).

***N*-(Adamantan-1-yl)-2-oxo-3-pentyl-2,3-dihydrobenzo[*d*]thiazole-6-carboxamide (25).** Compound **46** (0.5 mmol) was dissolved in 1 mL dry DMF. Potassium carbonate (0.75 mmol) and 1-bromopentyl chloride (1.2 mmol) were added to the solution. The mixture was stirred at 80°C overnight. The solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (10 mL) and washed with water (2 × 10 mL). The organic layer was dried over $MgSO_4$ and evaporated under reduced

pressure. The product was purified by preparative TLC (cyclohexane/EtOAc 82:18, v/v). A yellow powder was obtained: yield 22%; mp 145 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 7.82 (d, J = 1.7 Hz, 1H), 7.70 (dd, J = 1.7; 8.4 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 5.80 (s, 1H, NH), 3.96 (t, J = 7.5 Hz, 2H), 2.15 (m, 5H), 1.65 (m, 7H), 1.50 (m, 5H), 1.38 (m, 4H), 0.90 (t, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 176.2 (^{IV}C), 169.7 (^{IV}C), 133.7 (^{IV}C), 133.6 (^{IV}C), 123.3 (CH), 118.7 (^{IV}C), 115.0 (CH), 110.4 (CH), 66.0 (^{IV}C), 42.9 (CH_2), 39.2 ($3CH_2$), 36.4 ($3CH_2$), 28.9 (3CH), 28.1 (CH_2), 27.3 (CH_2), 22.3 (CH_2), 13.9 (CH_3); LC-MS (ESI) *m/z* 399.2 (MH^+), t_r 5.12 min, λ_{max} 260 nm, purity 99.8%.

6-Nitrobenzo[*d*]thiazol-2(3*H*)-one (47). Benzo[*d*]thiazol-2(*3H*)-one (10 mmol) was added to a flask containing anhydride acetic (150 mL) at 0°C. Fuming nitric acid (30 mmol) was added dropwise at 0°C. The precipitate was filtered off, washed with diethyl ether (50 mL) and dried. The product was purified by recrystallization in ethanol/acetonitrile (90:10, v/v). A yellow powder was obtained: yield 60%; mp 242 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 8.37 (d, J = 2.2 Hz, 1H), 8.23 (dd, J = 2.2; 8.7 Hz, 1H), 7.30 (s, 1H, NH), 7.22 (d, J = 8.7 Hz, 1H); LC-MS ($APCI^+$) *m/z* 197.0 (MH^+).

6-Nitro-3-pentylbenzo[*d*]thiazol-2(3*H*)-one (48). Compound **47** (0.5 mmol) was dissolved in 1 mL dry DMF. Potassium carbonate (0.75 mmol) and 1-bromopentyl chloride (1.2 mmol) were added to the solution. The mixture was stirred at 80°C overnight. The solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (10 mL) and washed with water (2 × 10 mL). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The product was purified by silica gel column chromatography (dichloromethane/petroleum ether 50:50, v/v). A yellow powder was obtained: yield 69%; mp 55 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 8.37 (d, J = 2.3 Hz, 1H), 8.25 (dd, J = 2.3; 8.9 Hz, 1H), 7.14 (d, J = 8.9 Hz, 1H), 4.01 (t, J = 7.5 Hz, 2H), 1.75 (m, 2H), 1.40 (m, 4H), 0.90 (t, J = 7.0 Hz, 3H); LC-MS ($APCI^+$) *m/z* 267.1 (MH^+).

6-Amino-3-pentylbenzo[*d*]thiazol-2(3*H*)-one hydrochloride (49). Palladium on carbon (catalytic amount) was added to a solution of compound **48** (4 mmol) in methanol (30 mL). The reaction mixture was stirred at room temperature under hydrogen atmosphere for 4 days. The palladium was filtered over

celite and filtrate was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (30 mL) and saturated HCl in diethyl ether was added to the solution (10 mL). The precipitate was filtered off. The product was purified by recrystallization in acetonitrile/methanol (50:50, v/v). A silver powder was obtained: yield 57%; mp 218 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 10.10 (s, 3H, NH_3^+), 7.63 (d, J = 2.0 Hz, 1H), 7.42 (d, J = 8.6 Hz, 1H), 7.30 (dd, J = 2.0; 8.6 Hz, 1H), 3.93 (t, J = 7.3 Hz, 2H), 1.65 (m, 2H), 1.30 (m, 4H), 0.84 (t, J = 6.9 Hz, 3H); LC-MS ($APCI^+$) *m/z* 237.1 (MH^+).

***N*-(2-Oxo-3-pentyl-2,3-dihydrobenzo[*d*]thiazol-6-yl)adamantane-1-carboxamide (26).** Benzoyl chloride (1.4 mmol) was added to a solution of compound **49** (0.7 mmol) in 5% aqueous potassium carbonate/EtOAc (1:2, v/v). The reaction mixture was stirred at room temperature for 30 min. The organic layer was separated from the aqueous solution and washed with 3N HCl (10 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The product was purified by silica gel column chromatography (dichloromethane). A yellowish powder was obtained: yield 77%; mp 115 ± 1°C; ^{1}H NMR ($CDCl_3$) δ 8.20 (s, 1H, NH), 7.55 (m, 7H), 7.00 (d, J = 8.7 Hz, 1H), 3.90 (t, J = 7.4 Hz, 2H), 1.70 (m, 2H), 1.35 (m, 4H), 0.90 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 170.0 (^{IV}C), 165.6 (^{IV}C), 139.4 (^{IV}C), 131.1 (^{IV}C), 125.4 (CH), 123.1 (^{IV}C), 121.6 (CH), 110.2 (CH), 52.6 (^{IV}C), 43.2 (CH_2), 41.8 ($3CH_2$), 36.5 ($3CH_2$), 29.6 (3CH), 29.0 (CH_2), 27.4 (CH_2), 22.5 (CH_2), 14.1 (CH_3); LC-MS (ESI) *m/z* 341.2 (MH^+), t_r 5.02 min, λ_{max} 238 nm, purity 97.1%.

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SUPPORTING INFORMATION PARAGRAPH

Primers sequences.

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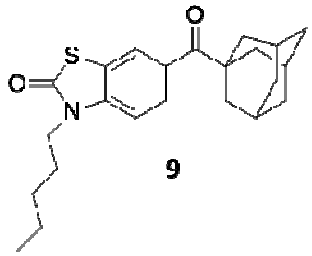
TABLE OF CONTENTS GRAPHIC

Benzo[*d*]thiazol-2(3*H*)-one

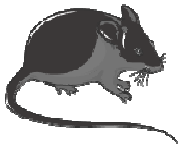


9

Selective CB_2 agonist

K_i (hCB_2) = 13.5 ± 1.5 nM
K_i (hCB_1) = 627 ± 230 nM
Selectivity index = 46
EC_{50} = 41.92 nM, E_{max} = 157%

Murin model of acute colitis



Intraperitoneal administration
Anti-inflammatory effects

GRAPHICAL ABSTRACT

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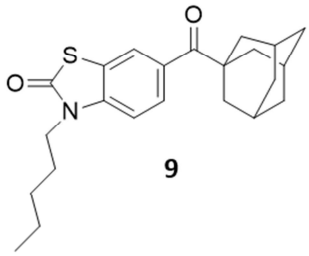

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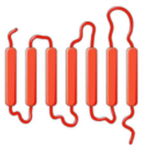

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