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Use of 5-hydroxy-4*H*-benzo[1,4]oxazin-3-ones as β_2-adrenoceptor agonists

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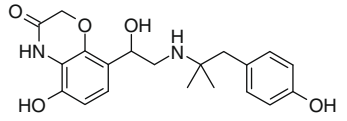
ABSTRACT

Novel β_2-agonists with a 5-hydroxy-4*H*-benzo[1,4]oxazin-3-one moiety as head group are described. Systematic chemical variations at the phenethylamine residue of these compounds lead to the discovery of compound **6m** as potent, full agonist of the β_2-adrenoceptor with a high β_1/β_2-selectivity. Molecular modeling revealed an interaction between the carboxylic acid group of **6m** and a lysine residue (K305) of the β_2-receptor as putative explanation for the high observed selectivity. Further, compound **6m** displayed in a guinea pig in vivo model a complete reversal of acetylcholine induced bronchoconstriction which lasted over the complete study time of 5 h.

β_2-Adrenoceptor agonists have been used as bronchodilating agents for the last decades for the treatment of pulmonary diseases like asthma.[1] The first truly β_2-selective agonists, salbutamol and terbutaline, were discovered some 40 years ago and since then efforts continued to develop drugs with an extended duration of action and a superior safety profile devoid of undesired effects like tachycardia and muscle tremor associated with the activation of β-adrenoceptors outside the lung tissue. This endeavor culminated in the development of the so-called third generation of β_2-agonists suitable for a once a day regimen.[2]

A screening of betamimetic-like structures from our compound collection revealed phenyl ethanolamine **1** as potent β_2-agonist (Fig. 1 and Table 1).[3] Compound **1** contains at its left-hand side a 5-hydroxy-4*H*-benzo[1,4]oxazin-3-one moiety. This heterocycle has been previously used only by our company in the design of β_2-adrenoceptor agonists and offered a more advantageous intellectual property position than other head groups from known β_2-agonists. The right-hand side of compound **1** is described by a phenethylamine residue with two geminal methyl groups at the carbon atom next to the amine, a structure that closely resembles the amine portion of the marketed b.i.d. (twice-daily)[4] β_2-agonist formoterol **2**.

We concluded from the in vitro characteristics of formoterol (compound **2**, Fig. 1 and Table 1) that a high β_1/β_2-selectivity is



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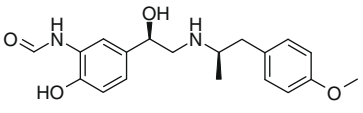

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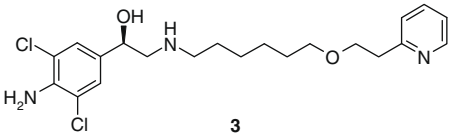

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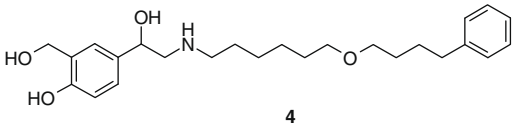

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Figure 1. Structures of the 5-hydroxy-4*H*-benzo[1,4]oxazin-3-one **1**, the investigational drug picumeterol **3** and the b.i.d. LABAs formoterol **2** and salmeterol **4**.

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Table 1
Potency and intrinsic activity at the human β1- and β2-adrenoceptors for formoterol fumarate **2** and the compounds shown in Scheme 1

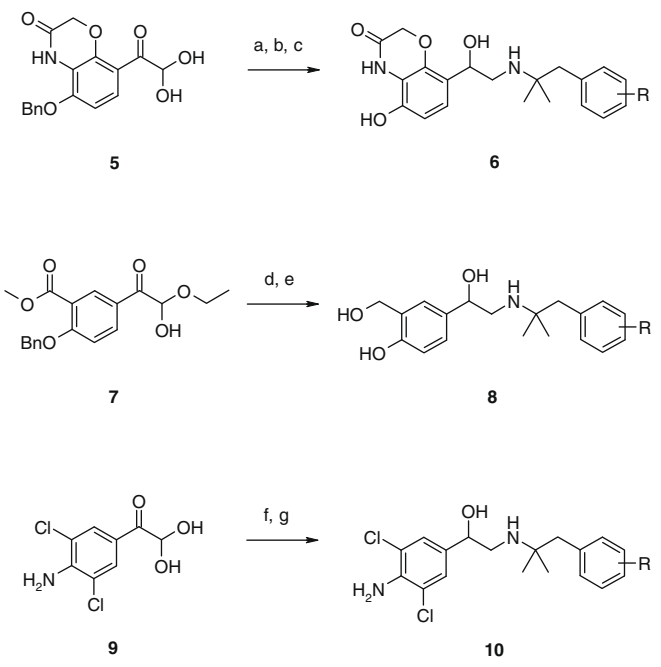
Compounds	R	hβ1 EC_{50} (nM)	hβ1 IA[a] (%)	hβ1 *n*	hβ2 EC_{50} (nM)	hβ2 IA[a] (%)	hβ2 *n*	β1/β2
2		86 ± 23	122 ± 7	2	0.4 ± 0.2	133 ± 48	3	215
1	4-OH	0.9	154	1	0.1 ± 0.1	92 ± 21	2	9
6a	H	0.8	107	1	0.2	139	1	4
6b	2-Me	0.6	92	1	0.1	118	1	6
6c	3-Me	5.0 ± 1.9	73 ± 7	2	0.4 ± 0.0	144 ± 13	2	13
6d	4-Et	5.6	140	1	0.6	170	1	9
6e	4-i-Pr	6.7 ± 4.7	95 ± 16	2	1.5 ± 1.3	108 ± 1	2	4
6f	4-t-Bu	26.5 ± 19.1	131 ± 98	2	13.4 ± 0.9	84 ± 30	2	2
6g	4-Ph	5.0	76	1	1.7	117	1	3
6h	3,5-Di-fluoro	7.2 ± 1.1	66 ± 13	2	0.5 ± 0.2	121 ± 6	2	14
6i	4-CF_3	3.7 ± 0.6	112 ± 77	2	0.4 ± 0.1	125 ± 34	2	9
6j	3-CF_3	3.7 ± 1.4	129 ± 51	2	0.6 ± 0.6	163 ± 42	2	6
6k	4-OCF_3	3.6 ± 0.8	124 ± 35	2	0.7 ± 0.5	132 ± 14	2	5
6l	4-CO_2H	33.0 ± 7.8	75 ± 11	3	2.8 ± 0.1	130 ± 11	2	12
6m	3-CO_2H	253 ± 124	51 ± 4	3	2.9 ± 0.6	116 ± 12	3	87
8a	H	>10,000	19 ± 4	2	70 ± 38	44 ± 6	2	>144
8b	2-Me	294	73	1	20.0	76	1	15
8d	4-Et	>10,000	15	1	170	21	1	>59
8j	3-CF_3	~1869	8	1	131	44	1	14
10d	4-Et	~695	9	1	>10,000	5	1	n.m.
10e	4-*i*-Pr	~1004	9	1	>10,000	2	1	n.m.
10h	3,5-Di-fluoro	~645	7	1	>10,000	1	1	n.m.

[a] Cloned human β1- and β2-adrenoceptors are expressed in CHO-K1 cell lines and the intracellular accumulation of cAMP after addition of various test compounds is measured. The intrinsic activity (IA) is reported as percentage of the maximal effect of isoprenaline (=100%). The standard deviation is given; n.m. = not meaningful.

required to minimize β_1-adrenoceptor mediated cardiovascular effects. A high selectivity was therefore defined as an important optimization goal in the development of a long acting beta2-agonist (LABA) and consequently an improvement of the modest β_1/β_2-selectivity observed for compound **1** was imperative. This study focuses on the introduction of substituents at the right phenyl group of **1** and their influence on the molecule's potency and efficacy towards the β_1- and β_2-adrenoceptor. Mainly lipophilic substituents were selected for this purpose due to reports claiming that the duration of action is increasing with the lipophilicity of the β_2-agonist.[5,6]

During our early optimization phase, analogs of the 5-hydroxy-4*H*-benzo[1,4]oxazin-3-one containing compounds were synthesized with head groups previously used in β_2-agonists. To evaluate the influence of these head groups, the analogs were investigated with respect to their agonistic activity and sub-type selectivity towards the β-adrenoceptors. The outcome of such a comparison with two aryl moieties, notably the 2,6-dichloro-phenylamine and the 2-hydroxymethyl-phenol (saligenin), is also reported in this publication. Both head groups are well established for use in β_2-agonists. The 2,6-dichloro-phenylamine moiety can be found for instance in the investigational β_2-agonist picumeterol **3** whereas the saligenin is present in salmeterol **4** or salbutamol (Fig. 1).

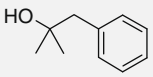
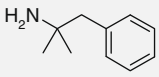
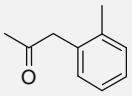
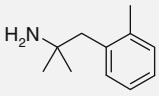
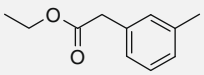
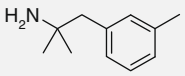
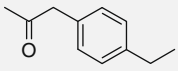
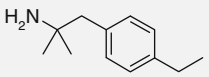
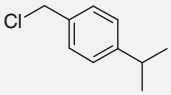
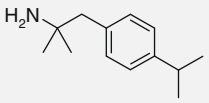
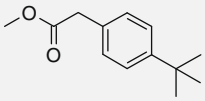
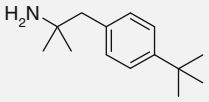
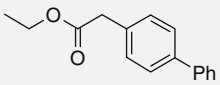
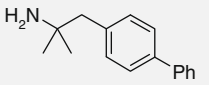
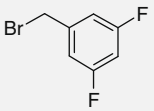
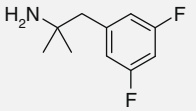
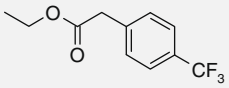
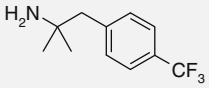
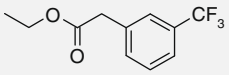
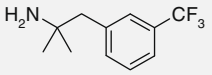
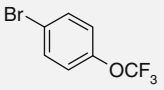
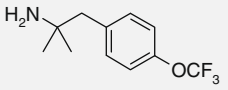
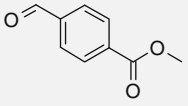
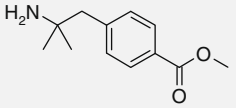
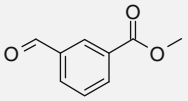
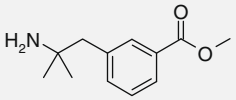
The target compounds described in this study are listed in Table 1 and the general preparative methods used to access the compounds are outlined in Scheme 1.[7] Reaction of the glyoxal hydrate **5**[3] with the phenethylamines **15** in the presence of a reducing agent afforded benzyl protected phenyl ethanolamines. This one-pot reaction comprises a reductive amination and a reduction of the keto functionality. The subsequent removal of the protecting group yielded the 5-hydroxy-4*H*-benzo[1,4]oxazin-3-ones **6**. The saligenines **8** and 2,6-dichloro-phenylamines **10** were obtained in an analogous fashion from the coupling of the hemi-acetal **7**[8] or glyoxal hydrate **9**[9] with the amines **15**. Raney nickel was preferably used as catalyst in the final debenzylation step required for the preparation of the saligenines since palladium on carbon resulted in some cases in the formation of the 2-methyl phenol derivative as side product.



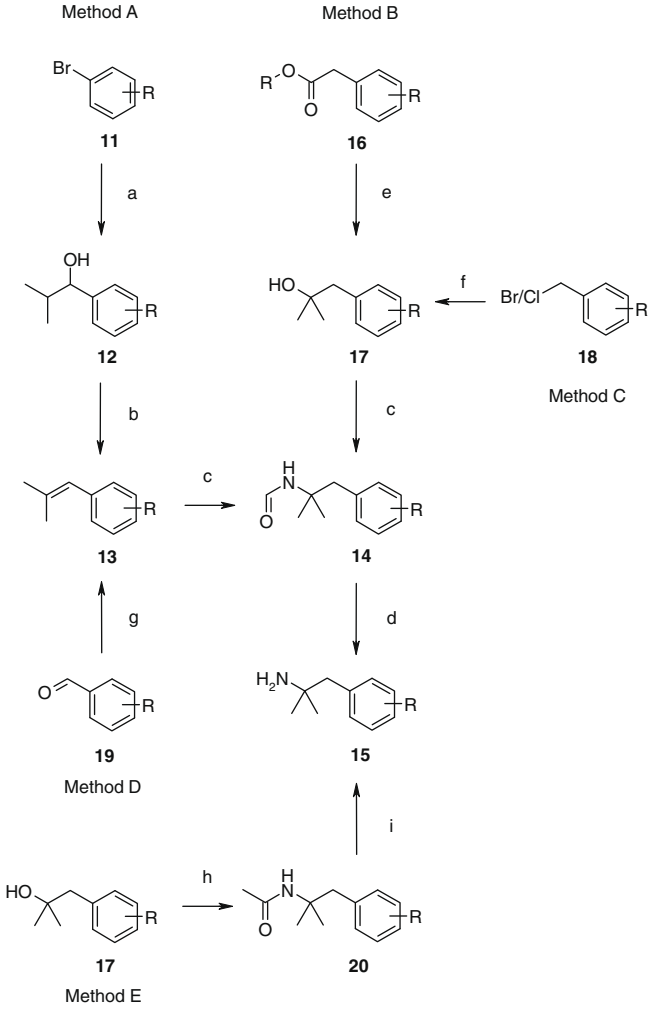
Scheme 1. Reagents and conditions: (a) 1 equiv of amine **15**, THF, 30 min, then $LiBH_4$, 0 °C to rt; (b) Pd/C, H_2, MeOH; (c) only for **6l** and **6m**: aqueous 1 N NaOH, rt; (d) 1 equiv of amine **15**, THF, rt, 30 min, then $LiBH_4$, 0–50 °C; (e) Pd/C, H_2, MeOH or Raney-Ni, H_2, EtOH; (f) amine **15**, EtOH, 50–60 °C; (g) $NaBH_4$, EtOH, rt.

The phenethylamines **15** which were used as substrates in the above mentioned coupling reactions are shown in Table 2 and their synthesis is presented in Scheme 2. A common key step in the preparation of all amines is a Ritter reaction with the (2-methyl-propenyl)-benzenes **13** or the tertiary alcohols **17** as substrate and sodium cyanide or acetonitrile as reagent. Both substrates can be derived by multiple ways. The (2-methyl-propenyl)-benzenes **13** can be either obtained from a nucleophilic attack of phenylmagnesiumbromide to isobutyraldehyde followed by an elimination reaction (Scheme 1, method A) or from benzaldehydes

Table 2
Phenethylamines **15** prepared according to Scheme 2

Educt	Product		Method
		**15a**	E
		**15b**	E[a]
		**15c**	B
		**15d**	E[a]
		**15e**	C
		**15f**	B
		**15g**	B
		**15h**	C
		**15i**	B
		**15j**	B
		**15k**	A
		**15l**	D
		**15m**	D

[a] The 1-phenyl-propan-2-one derivative was converted into the tertiary alcohol **17** through reaction with MeMgBr.



Scheme 2. Reagents and conditions: (a) Mg-Grignard preparation in Et_2O, then isobutyraldehyde; (b) *p*-toluenesulfonic acid monohydrate, toluene, reflux; (c) NaCN, AcOH, concd H_2SO_4; (d) concd HCl, EtOH or MeOH, reflux; for **15k**: potassium hydroxide, ethylene glycol, 140 °C; (e) MeMgBr, THF; (f) Mg-Grignard preparation in Et_2O, then acetone; (g) isopropyl triphenylphosphonium iodide, *n*-BuLi, THF, Et_2O; (h) MeCN, AcOH, concd H_2SO_4; (i) potassium hydroxide, ethylene glycol, reflux.

via a Wittig olefination (Scheme 1, method D). Reaction of phenyl acetic acid esters or acetone with the appropriate Grignard reagents delivers the tertiary alcohols **17** (Scheme 1, methods B and C, respectively).

All examples from this study were tested in cellular functional assays to determine their potency and efficacy (here, intrinsic activity compared to the full agonist isoprenaline) for the human β_1- and β_2-adrenoceptor. In these assays, the intracellular rise of cAMP induced by a β-agonist is quantified in CHO cell lines stably expressing either the human β_1- or β_2-adrenoceptor. Marketed formoterol fumarate was measured for comparison and the results are listed in Table 1.

All 5-hydroxy-4*H*-benzo[1,4]oxazin-3-ones **6a–m** from this study can be classified as full or even super agonists of the β_2-adrenoceptor based on their intrinsic activities (>>100% for super agonists). The examples **6a–d** and **6h–k** with sterically less demanding lipophilic substituents R showed subnanomolar EC_{50} values at this receptor. These compounds were roughly equipotent to formoterol **2** and more potent than their analogs **6e–g** with larger lipophilic groups. Unfortunately, only a modest β_1/β_2-selectivity was observed for all 5-hydroxy-4*H*-benzo[1,4]oxazin-3-ones

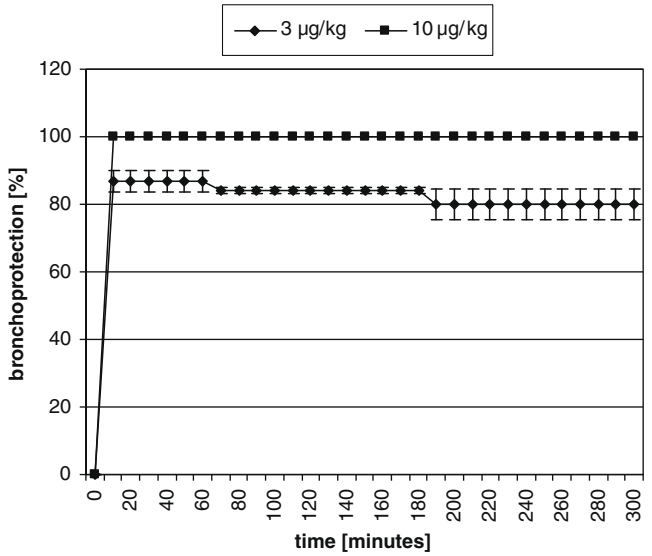


Figure 2. Bronchoprotective effect of example **6m** in the Konzett–Roessler model. Bronchospams were induced in guinea pigs by iv injections of acetylcholine every 10 min. Bronchoprotection is expressed as the percentage of inhibition of the increase in pulmonary resistance induced by acetylcholine ($N = 2$ animals per dose).

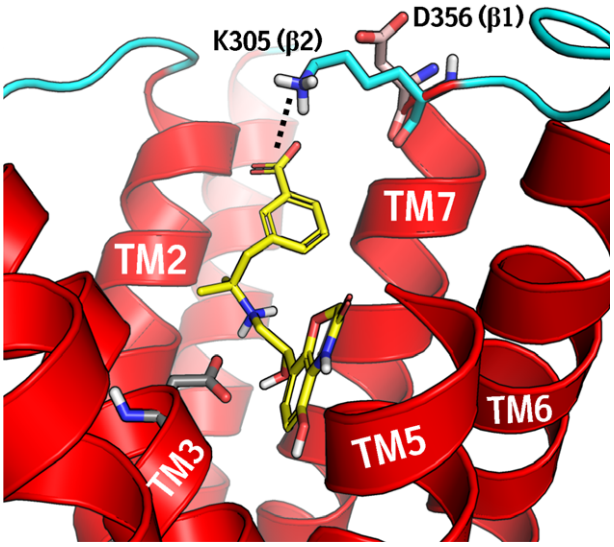


Figure 3. Proposed binding mode of compound **6m** into the β_2-receptor. The carboxylic acid group of the ligand coordinates with a lysine residue (K305, cyan) at the extracellular end of the transmembrane domain (TM) 7 as indicated by the dotted line. In the β_1-receptor, an aspartate residue is found at the corresponding position (D356, pink), unable to form a favorable interaction with the acid. The aspartate residue which is identical in the β_1- and β_2-adrenoceptors is also shown (gray).

with lipophilic groups and it became evident from these data that the position of the substituent at the phenyl ring does not have any substantial influence on the selectivity. EC_{50} values around 3 nM were measured for the carboxylic acid containing compounds **6l–m** at the β_2-receptor. Only the *meta*-substituted example **6m** displayed a high β_1/β_2-selectivity of 87 and a reduced intrinsic activity of 51% at the β_1-adrenoceptor warranting a further investigation of this compound.

For this purpose, compound **6m** was tested in a guinea pig in vivo model[10] of acetylcholine induced bronchoconstriction as previously reported.[11] In this model, the ability of a test compound to reverse the acetylcholine induced bronchoconstriction is determined. Additionally, the heart rate is recorded as an indicator of the systemic availability of the β_2-agonist. Example **6m** showed a dose-dependent bronchodilation in this model which lasted for the applied doses of 3 and 10 µg/kg over the complete study period of 5 h (Fig. 2). A full reversal of the induced bronchospasm was observed at the highest tested dose which was unfortunately accompanied by a significant increase (>10%) in heart rate.[12] Formoterol which was tested as comparator in this model exhibited also a dose-dependent bronchoprotection and exerted a full bronchoprotection of 100% at doses $\geqslant 1$ µg/kg.[12] In contrast to example **6m**, a significant increase in heart rate was only evident at a dose of 10 µg/kg which was 10-fold above the first dose reaching 100% bronchoprotection. Consequently, the profiling of compound **6m** was stopped, since it did show an inferior therapeutic window compared to formoterol.

A receptor model was established based on the recently published structure of the β_2-adrenoceptor co-crystallized with the inverse agonist carazolol with the aim to locate the amino acid residues responsible for the high selectivity of compound **6m**.[13–15] The model revealed an interaction between the carboxylic acid group of the ligand and a lysine residue (K305) within helix7 of the loop area. In the β_1-receptor, an aspartate residue is found at the corresponding position that should reduce the overall binding energy of the ligand through electrostatic repulsion of the two carboxylic acid groups (Fig. 3). This interaction has been previously not reported in the literature and should be useful in the future design of selective β_2-agonists.

β_2-Agonists with a saligenin[16] or a 2,6-dichloro-phenylamine[17] head group and a phenethylamine residue with two geminal methyl groups have been already reported in the literature. However, functional cellular assays were not available for the human β-adrenoceptors at the time of their first publication and an in vitro characterization of such compounds was therefore of interest.

The saligenines **8** displayed a significant lower in vitro potency at the β_2-adrenoceptor compared to the corresponding 5-hydroxy-4*H*-benzo[1,4]oxazin-3-ones. The most potent example, compound **8b**, had an EC_{50} value of 20 nM at the β_2-receptor, which was considered as insufficient to justify a further profiling.

Only a marginal activity was observed in the in vitro assays for the three 2,6-dichloro-phenylamines **10**. These findings are in line with results obtained for other 2,6-dichloro-phenylamines synthesized in our laboratories (data not shown) and consequently the use of this head group was discontinued.

In conclusion, example **6m** was identified from a series of compounds with a 5-hydroxy-4*H*-benzo[1,4]oxazin-3-one head group as potent and selective β_2-agonist. A duration of action over the complete study period of 5 h was demonstrated for this compound in a guinea pig in vivo model. Unfortunately, the tested 5-hydroxy-4*H*-benzo[1,4]oxazin-3-one displayed an inferior therapeutic window in this model compared to the marketed drug formoterol. These results prompted us to focus our optimization efforts on a different part of the phenyl ethanolamine structure and the results will be reported in due course.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.013.

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7. *Synthesis protocol of a representative example* (compound **6c**): A solution of 329 mg (1.00 mmol) 5-benzyloxy-8-(2,2-dihydroxy-acetyl)-4*H*-benzo[1,4]oxazin-3-one and 163 mg (1.00 mmol) 1,1-dimethyl-2-*m*-tolyl-ethylamine in 5 mL THF was stirred for 30 min at ambient temperature and then cooled to 0 °C. Lithiumborohydride (1.5 mL of a 2 M solution in THF) was slowly added and stirring was continued over 30 min at ambient temperature. The solution was diluted with 10 mL dichloromethane and 3 mL water, stirred for additional 1 h and then filtered through a short column of Extrelut®. The column was washed with dichloromethane (3 × 10 mL) and the combined filtrates were evaporated. The residue was dissolved in methanol and hydrogenated in the presence of 100 mg palladium on carbon at ambient temperature and a hydrogen pressure of 50 psi. The catalyst was filtered off, the solvent removed in vacuo and the remainder purified by chromatography (reverse phase, acetonitrile/water with 0.1% trifluoroacetic acid). Yield: 218 mg (45%; trifluoroacetate).
   Analytical data of compound **6c**: Mass spectroscopy: $[M+H]^+$ = 371. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.97 (s, 1H), 9.95 (s, 1H), 8.59 (m, 1H), 8.44 (m, 1H), 7.22 (1H, m), 7.01 (d, 1H, J = 7.6 Hz), 7.00 (m, 2H), 6.97 (d, 1H, J = 8.6 Hz), 6.58 (d, 1H, J = 8.6 Hz), 5.94 (m, 1H), 5.08 (d, 1H, J = 10.0 Hz), 4.62 (d, 1H, J = 14.9), 4.56 (d, 1H, J = 14.9 Hz), 3.16 (m, 1H), 2.91 (m, 3H), 2.30 (s, 3H), 1.19 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 164.2, 157.7 ($^2J_{C,F}$ = 31 Hz), 144.9, 141.2, 137.3, 135.2, 131.2, 128.1, 127.7, 127.6, 120.0, 120.0, 117.2 ($^1J_{C,F}$ = 300 Hz), 115.3, 109.0, 66.9, 63.3, 59.4, 46.7, 42.4, 22.4, 22.3, 20.9.
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