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Potential metabolites of a condensed 2,3-benzodiazepine derivative

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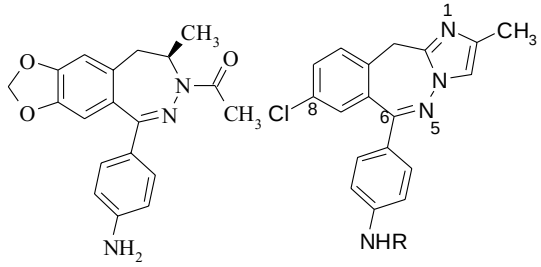
Abstract—Putative metabolites of an AMPA antagonist imidazo-2,3-benzodiazepine (**2**) were synthesized and compared to constituents formed from the parent compound by a rat liver perfusion method. As metabolic transformations, hydroxylation of the 2-methyl group and N-acetylation of the amino functionality in parent compound (**2**) were registered. The hydroxylated analogue **12** of **2** exhibits a weak AMPA antagonist activity.

AMPA (2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid) antagonists possess a significant role among the ionotropic glutamate receptor ligands and have the capacity among others to serve as anticonvulsant and neuroprotective drugs.[1,2]

Talampanel (**1**) (Fig. 1) is a non-competive AMPA antagonist 2,3-benzodiazepine (BDZ) that is now under advanced Phase II clinical investigation.[3–5] Broad structure activity relationship studies have revealed several different structural features related to a BDZ ring system that greatly influences the APMA antagonist activity, consequently, some successful substitutes for the functions of molecule **1** were found.[6] One of the major recognitions was that BDZs with a condensed azole ring, attached to atoms 3,4 of the BDZ skeleton, possess a high AMPA antagonist activity.[7–9] As a representative of this condensed BDZ derivative **2** (GYKI-47261) is exemplified, which showed a broad spectrum anticonvulsant activity against seizures evoked by electroshock and different chemoconvulsive agents; however, its neuroprotective effects in a focal ischemia test and a MPTP-induced Parkinson model were more pronounced.[8] The unique structure of BDZs with an attached azole ring from among the different AMPA antagonists of the



1 **2** (R = H)
3 (R = -$COCH_3$)

Figure 1. Structures of talampanel and a representative of condensed BDZs.

BDZ type was also corroborated by the finding that their corresponding pharmacophore model differed only in one donor site attaching point from that of other BDZ structures and the biological consequences of which are still to be clarified.[10]

Remarkable neuroprotective potency of **2** has paved the way for becoming a potential drug candidate, necessitating the study of its metabolism.[11] A widely accepted method of research on metabolism is to employ radiolabeled compounds in animal experiments. This way the metabolites can be detected in biological samples (blood, excreta, bile, etc.) using selective detection by means of radiochromatography. A drawback of this

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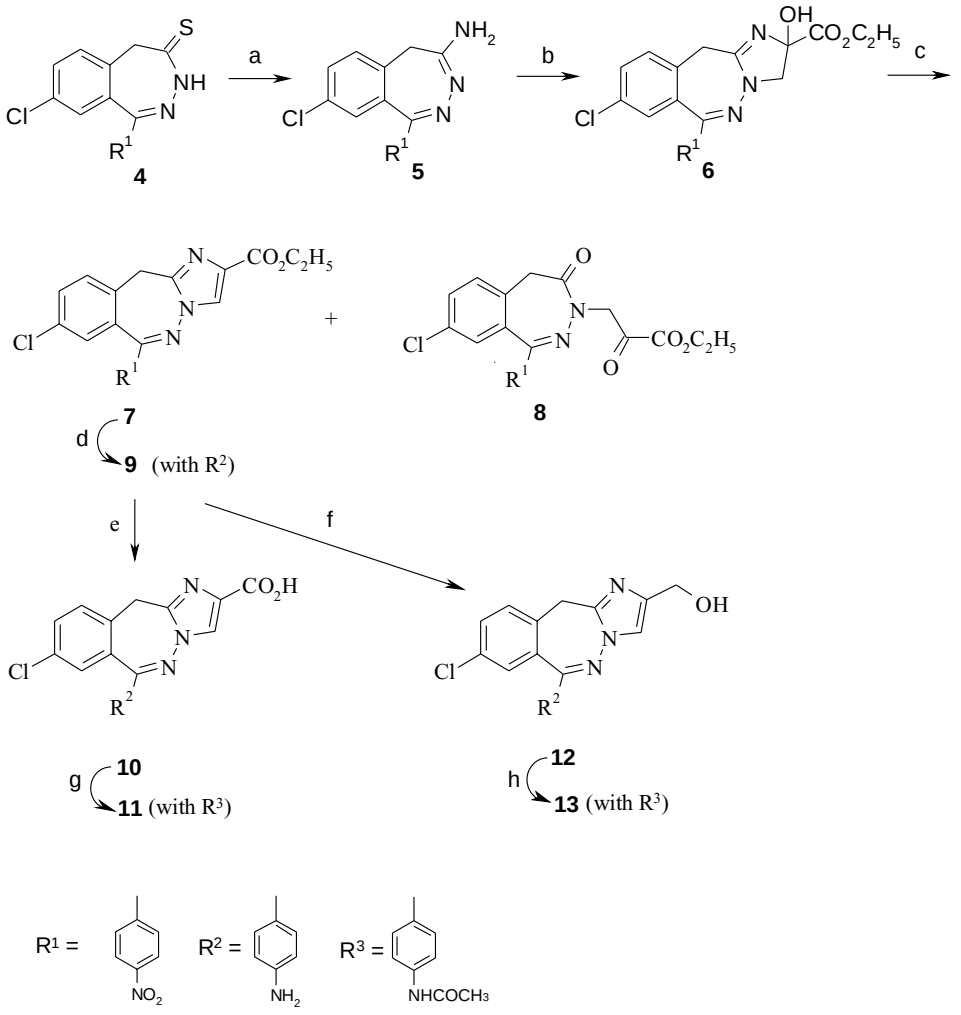
procedure, however, lies in the fact that a tedious sample cleanup procedure is usually required for a structure elucidation. In this study, we used a reverse approach, namely the potential metabolites were synthesized and compared to the constituents formed from the parent compound. An ex vivo technique was applied to obtain samples containing a far less amount of endogenous constituents than the samples of in vivo experiments.[12] Additionally, by this method radiolabeling was dispensed with. Therefore, on the basis of different considerations, several metabolite-like derivatives of **2** have been synthesized and analyzed for their identity with main metabolites of **2.**

As a possible metabolic transformation of **2**, except that of acetylation of the amino function,[13] an oxidation of the 2-methyl group was envisaged, resulting in the formation of derivatives with different oxygenated groups at position 2. Therefore, 2-carboxylic acid and related derivatives of **2** were prepared, according to Scheme 1. Thus, thio-oxo compound **4**[8] was reacted in a Teflon®-coated autoclave with a saturated ammonia–THF solution in the presence of mercury chloride to give an amidine, possessing the tautomeric structure **5** in DMSO solution. Alkylation of **5** with ethyl bromopyruvate resulted in a product which showed no keto-carbonyl group in the IR spectrum and which according to ^{1}H NMR investigations can be characterized by the hemiaminal structure **6**. Latter, when treated with *p*-toluenesulfonic acid, it gave a 2:1 mixture of **7** and **8**, respectively. The formation of hydrolysis product **8** is a proof that alkylation with ethyl bromopyruvate takes place at the ring nitrogen atom. Compound **7** served as the starting material for all the potential metabolite-like analogues of **2**. Transfer hydrogenation gave amino-ester **9** and hydrolysis of the latter resulted in carboxylic acid **10**, whereas a lithium aluminum hydride reduction of **9** gave 2-hydroxymethyl derivative **12**. The necessary N-acetyl derivatives were prepared by simple acetylation of **10** and **12** to provide **11** and **13**, respectively (for physical and spectroscopic data, see Ref. 14).

Liver perfusion was performed, according to standard procedure,[12] and the perfusate was analyzed, as given in Ref. 15.

In Table 1 chromatographic and mass spectrometric characteristics of the synthesized compounds to those



Scheme 1. Reagents and conditions: (a) NH_3/THF/$HgCl_2$, autoclave, 70 °C, 81%; (b) ethyl bromopyruvate, DMF, K_2CO_3, rt, 65%; (c) cat. *p*-TsOH, EtOH/$CHCl_3$, 58% (**7**), 28% (**8**); (d) RaNi, NH_2NH_2monohydrate, CH_2Cl_2/MeOH, 87%; (e) NaOH, EtOH, reflux, 63%; (f) $LiAlH_4$, THF, 59%; (g) Ac_2O, CH_2Cl_2, rt, 79%; (h) AcCl, pyridine, rt, 41%.

Table 1. Comparison of chromatographic and mass spectrometric properties of synthesized standards and metabolites

Compounds	Synthesized standards			Rat liver perfusate		
	Retention time (min)	Molecular mass	Major fragments	Retention time (min)	Molecular mass	Major fragments
2	23.81	323	282 (60%), 217 (40%)	23.84	323	282 (60%), 217 (40%)
3	21.57	365	350 (15%), 338 (15%), 324 (55%), 217 (15%)	21.61	365	350 (15%), 338 (15%), 324 (55%), 217 (15%)
10	8.23	353	335 (100%)	n.d.	n.d.	n.d.
11	8.41	395	377 (100%)	n.d.	n.d.	n.d.
12	13.31	339	321 (100%)	13.42	339	321 (100%)
13	12.08	381	363 (100%)	12.06	381	363 (100%)

n.d.: not detected.

Table 2. Screening results with some new BDZs

Compounds	Behavioural changes (100 mg/kg ip; 200 mg/kg po)	MES test ED_{50}, mg/kg po	Inclined screen test ED_{50}, mg/kg ip	Retinal spreading depression test IC_{50} (μM) or inhibition (%) in 20 μM
1 (referent)	Loss of righting reflex	8.6 (7.0–10.6)	13.4 (11.2–16.0)	1.7
2 (referent)	Loss of righting reflex	24.0 (17.9–32.1)	36.5 (29.4–45.2)	7.3
3[a]	Decrease of SMA,[b] ataxy	56.0	>200[c]	>20[b]
9	Decrease of SMA[b]	>100[c]	>200[c]	10–20
10	Slight decrease of SMA[b]	>100[c]	>200[c]	55%
11		>100[c]	>200[c]	33%
12	Decrease of SMA,[b] ataxia, decrease of muscle tone	25–50	100–200	5.0
13	n.t.[d]	>100[b]	n.t[d]	n.t[c]

[a] See also Ref. 8.
[b] Spontaneous motor activity.
[c] Highest test concentration.
[d] Not tested.

of metabolites present in liver perfusate are compared. It is seen that apart from the parent compound three peaks eluted with retention identical to those of synthetic standards. In addition, these compounds exhibited mass spectra identical to those of standards. Thus, these metabolites were identified as the *N*-acetylated analogue **3** of the parent compound **2, 12** (a hydroxylated analogue of the parent compound **2**), and **13** (*N*-acetylated derivative of **12**). As a consequence, the above findings show that the main metabolic transformations of the compound under study (**2**) are those of N-acetylation, as well as hydroxylation of the side-chain methyl group. A similar metabolic hydroxylation of an analogous methyl-imidazo-BDZ was noticed recently.[9]

In further experiments, the new aminophenyl BDZs (**9**, **10**, **11**, **12**, **13**) were subjected to in vitro screening for inhibition of AMPA (5 μM) evoked spreading depression in isolated retina prepared from young chicken.[16] In addition, these molecules were also tested for anticonvulsant activity using the maximal electroshock seizure model,[17] as well as for muscle relaxant activity, using the inclined screen test in mice.[18] The gross behavioral changes were evaluated in mice according to Irwin[19] the results of which are shown in Table 2.

Data in Table 2 demonstrate the fact that certain compounds possessing an oxygenated side chain on the imidazole moiety show a slight effect however, this fact can be demonstrated in the in vitro retinal spreading depression test only (e.g., **9**–**11**) and could not be observed in vivo in the given test system up to the test concentrations used. The only compound that possesses a weak AMPA antagonist activity in all of the tests was the hydroxymethyl derivative **12**, which was also detected among the metabolites formed from **2** under ex vivo conditions. As a result, a conclusion can be drawn that out of five putative metabolites actually three compounds formed under ex vivo conditions; however, none of them exhibited any strong AMPA antagonist activity.

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14. Physical and spectroscopic data of new compounds. Compound **6**: mp: 188–191 °C, ^{1}H NMR (DMSO-d_6, T = 25 °C) δ 8.32 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 8.5 Hz, 2H), 7.65 (dd, J_1 = 8.3 Hz, J_2 = 1.9 Hz, 1H), 7.60 (d, J = 8.3 Hz, 1H), 7.15 (d, J = 1.9 Hz, 1H), 6.72 (s, 1H, exchangeable), 4.25 (d, J = 10.8 Hz, 1H), 4.10 (q, J = 7.2 Hz, 2H), 3.85 (s, 2H), 3.75 (d, J = 10.8 Hz, 1H), 1.13 (t, J = 7.2 Hz, 3H); ^{13}C NMR δ 170.7 (s), 166.9 (s), 154.3 (s), 147.8 (s), 143.1 (s), 136.1 (s), 133.4 (s), 131.6 (s), 131.3 (d), 130.6 (d), 129.8 (d), 128.4 (d), 123.6 (d), 93.6 (s), 62.7 (t), 61.0 (t), 33.0 (t) 13.9 (q); IR (KBr past.) 1754 cm^{-1}; compound **7**: mp: >260 °C, ^{1}H NMR (DMSO-d_6, T = 25 °C) δ 8.42 (d, J = 8.8 Hz, 2H), 8.23 (s, 1H), 7.96 (d, J = 8.8 Hz, 2H), 7.76 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.22 (d, J = 2.0 Hz, 1H), 4.25 (s, 2H), 4.23 (q, J = 6.9 Hz, 2H), 1.36 (t, J = 6.9 Hz, 3H); compound **8**: mp: 151–153 °C, ^{1}H NMR (DMSO-d_6, T = 27 °C) δ 8.33 (d, J = 8.8 Hz, 2H), 7.83 (d, J = 8.8 Hz, 2H), 7.73 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H), 7.63 (d, J = 8.4 Hz, 1H), 7.23 (d, J = 2.0 Hz, 1H), 5.10 (s, 2H), 4.22 (q, J = 7.2 Hz, 2H), 3.17 (br s, 2H), 1.23 (t, J = 7.2 Hz, 3H), ^{13}C NMR δ 187.9 (s), 167.9 (s), 159.4 (s), 158.2 (s), 148.3 (s), 142.7 (s), 135.5 (s), 132.3 (d), 131.7 (s), 130.6 (d), 130.1 (d), 128.0 (d), 123.6 (d), 62.0 (t), 57.1 (t), 40.3 (t), 13.7 (q); IR (KBr past.) 1734 cm^{-1}, 1668 cm^{-1}; compound **9**: mp: 250–253 °C, ^{1}H NMR (DMSO-d_6, T = 298 K) δ 8.00 (s, 1H), 7.67 (dd, J_1 = 8.3 Hz, J_2 = 1.8 Hz, 1H), 7.58 (d, J = 8.3 Hz, 1H), 7.40 (d, J = 8.5 Hz, 2H), 7.22 (d, J = 1.8 Hz, 1H), 6.63 (d, J = 8.5 Hz, 2H), 5.95 (s, 2H, exchangeable), 4.17 (q J = 7.0 Hz, 2H), 4.05 (br s, 2H), 1.25 (t, J = 7.0 Hz, 3H); compound **10**: mp: >260 °C, EI-MS: *m*/*z*:352, 354 [M]$^{+\cdot}$ (44,17) 308, 310 (100,33), 268 (14), CI-MS: 353, 355 [M+H] (100,42); compound **11**: mp: >260 °C, EI-MS: *m*/*z*: 394, 396 [M]$^{+\cdot}$ (44,15), 350, 352, (100,48), 349, 351 (28,33), CI-MS: 351 [M−CO^2+H]$^+$ (100), 350 [M−CO^2]$^+$ (74); compound **12**: mp: >260 °C, ^{1}H NMR (DMSO-d_6, T = 300K) δ 7.63 (dd, J_1 = 8.2 Hz, J_2 = 2.3 Hz, 1H), 7.56 (d, J = 8.2 Hz, 1H), 7.37 (d, J = 8.4 Hz, 2H), 7.20 (d, J = 2.3 Hz, 1H), 7.20 (s, 1H), 6.64 (d, J = 8.4 Hz, 2H), 5.81 (s, 2H, exchangeable), 4.81 (t, J = 5.5 Hz, 1H, exchangeable), 4.28 (d, J = 5.5 Hz, 2H), 3.84 (br s, 2H); compound **13**: mp: >260 °C, EI-MS: *m*/*z*: 380, 382 [M]$^{+\cdot}$ (100,34), 337 (28), 218 (43).
15. Wistar male rat liver was cannulated into vena portae and washed with Hanks' balanced salt perfusion solution saturated with O_2/CO_2 (95/5) for about 10 min. This solution was spiked with 1 mg/ml of **2** in DMSO to give a final concentration of 10 g/ml. Perfusion was carried out at 37 °C for 2 h. The flow rate was 40 ml/min. Sample cleanup of perfusate solution was done using C18 solid-phase extraction cartridges, as described earlier.[11] Methanol eluate was evaporated to dryness and was reconstituted in a mixture of ethanol/0.05 M ammonium-acetate, pH 8.5 buffer (1/1) and subjected to HPLC/MS analysis. HPLC conditions: Purospher RP18 5 μm 250 × 4.0 mm column with 4 × 4.0 mm guard column, flow rate: 1 ml/min, eluents: (A) acetonitrile/ammonium-acetate buffer pH 8.5 (10/90), (B) acetonitrile/ammonium-acetate buffer pH 8.5 (90/10). Gradient program: 0–2 min: 10% B, 2–4 min: 10–20% B, 4–6 min: 20–30% B, 6–16 min: 30–40% B, 16–27 min: 40–80% B, 27–30 min: 80–10% B, and 30–35 min: 10% B. IT/MS conditions: positive electrospray ionization, flow rate: 0.3 ml/min, sheath gas: 20, spray voltage: 4.5 kV, capillary temperature: 200 °C, capillary voltage: 7. Selected ion monitoring: 0–10 min: *m*/*z* 350–400, 10–12.3 min: *m*/*z* 380–390, 12.3–15.5 min: *m*/*z* 338–345, 15.5–17.6 min: *m*/*z* 380–390, 17.6–20.9 min: *m*/*z* 338–345, 20.9–23.4 min: *m*/*z* 364–369, 23.4–35 min: *m*/*z* 320–330. MS/MS experiment: normalized collision energy: 50.
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