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Short communication

New GABA-modulating 1,2,4-oxadiazole derivatives and their anticonvulsant activity

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Abstract

A series of 3- and 5-aryl-1,2,4-oxadiazole derivatives were prepared and tested for anticonvulsant activity in a variety of models. These 1,2,4-oxadiazoles exhibit considerable activity in both pentylenetetrazole (PTZ) and maximal electroshock seizure (MES) models. Compound **10** was protective in the PTZ model in rats with an oral ED_{50} of 25.5 mg/kg and in the MES model in rats with an oral ED_{50} of 14.6 mg/kg. Neurotoxicity (rotarod) was observed with an ED_{50} of 335 mg/kg. We found several oxadiazoles that acted as selective GABA potentiating compounds with no interaction to the benzodiazepine binding site.

Keywords: 1,2,4-Oxadiazole derivatives; Synthesis; Pentylenetetrazole test; Maximal electroshock seizure test; Anticonvulsant activity

1. Introduction

Despite the development of several new anticonvulsants, the treatment of epilepsy remains still inadequate. About one third of patients do not respond well to currently available treatment, even if multiple drugs with complementary activities are used [1,2]. According to a recent prospective study [3], only 63% of a cohort of 525 patients diagnosed and treated at the Epilepsy unit in Glasgow, Scotland, was seizure free. Furthermore, more than 50% of epilepsy patients experience unwanted side effects of drug treatment [4–6]. Based on this observation, it was our aim to find drugs which are more potent and at the same time better tolerated than existing drugs. However, there is no rational strategy available to find new anticonvulsants which are more potent, i.e. which are especially effective in drug resistant patients [7]. Furthermore, no drug target has yet been identified to treat drug resistant patients. Despite the fact that all new drugs were tested in so-called drug resistant patients using an add on treatment design, the rate of drug resistant patients has not been significantly reduced [8].

We therefore selected an in vivo efficacy approach over a target driven screening approach. The usefulness of this in vivo approach was recently re-enforced [9]. We selected three easy screening models, the MES seizure model, the PTZ seizure model, and the rotarod procedure to get a first hint for efficacy and safety. This procedure is in line with the screening approach at the NIH [10]. Indeed, part of the screening was performed at NIH. Drugs which are active in both, the MES and PTZ model are considered to be broad-spectrum anticonvulsants. So far, most marketed anticonvulsants are not active or only active at high doses in the PTZ model but are instead active in the MES seizure model [10,11]. Prominent members of the drugs active in both models are valproate and the benzodiazepines. These drugs are effective in a wide variety of epilepsy patients with different seizure types. While this characteristic does not predict efficacy in drug resistant patients, it may give a hint towards higher efficacy. Benzodiazepines are

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highly potent anticonvulsants that are commonly used as first line rescue medication to block acute seizure or a status epilepticus [12]. However, due to many side effects including addictive potential and frequent development of tolerance, benzodiazepines have limited utility for long-term treatment.

Therefore our strategy was to find drugs which are effective in both the MES and the PTZ seizure model which are better tolerated than the benzodiazepines.

2. Chemistry

The 3-aryl[1,2,4]oxadiazoles **1**–**9** listed in Table 1 are easily available by direct alkylation of imidazole or triazole with the appropriate 3-aryl-5-chloromethyl[1,2,4]oxadiazoles in acetone by means of potassium carbonate. The 5-chloromethyl intermediates were prepared by reaction of equimolar amounts of *N*-hydroxy benzamidines with chloroacetic acid chloride according to previously described procedure [13]. The 5-aryl-3-chloromethyl[1,2,4]oxadiazoles, intermediates for the synthesis of 5-aryl[1,2,4]oxadiazoles **10**–**15**, listed in Table 1 are available by the reaction of 2-chloro-*N*-hydroxy acetamidines with the appropriate benzoyl chlorides (see Schemes 1 and 2) [14].

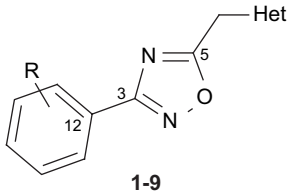
3. Pharmacology and first mechanistical studies

The oxadiazole compounds **1**–**15** showed a remarkable in vivo activity against electrically and chemically induced seizures. Additionally, a sufficient therapeutic index could be achieved by **2** and **10** (see Table 2).

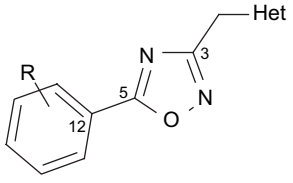
Mechanistical studies on selected compounds revealed some interesting findings. First, all the compounds with in vivo activity potentiated GABA-induced currents in rat cortical neurones. This positive GABA modulation is reminiscent of a benzodiazepine drug. However, no interaction with the benzodiazepine binding site was observed using conventional binding assays. These data indicate that our compounds modulate the GABA receptor via a binding site distinct from the benzodiazepine site (see Table 3). Second, the compounds **1**, **2**, **4** and **7** also acted as potent sodium channel blockers in cortical neurones. Both the GABA potentiation and the sodium channel blocking are mechanisms known to result in anticonvulsant activity. A combination of both mechanisms in one molecule maybe a novel approach to develop a potent anticonvulsant. Among these drugs, **2** is the most interesting candidate. Whether the interaction with a new binding site of the GABA receptor is responsible for the unique pharmacological profile is to be tested in future experiments. Compound **3** is the most effective candidate which does not block sodium channels.

While different modulatory binding sites are described for GABA receptors, further work is needed to identify the binding site for our compounds. Loreclezole, *R*(−) etomidate and some anti-inflammatory agents are compounds with no interaction with the benzodiazepine binding site [16]. There is some similarity between the 1,2,4-oxadiazoles and loreclezole (Fig. 1). The benzene ring and the triazole ring of compound **4** and loreclezole are in a similar position and the oxygen atom or the nitrogen atom in the oxadiazole ring corresponds with the electronegative chloride of the double bond of loreclezole.

Table 1
Physicochemical data and molecular formulas of 3-aryl and 5-aryl[1,2,4]oxadiazoles



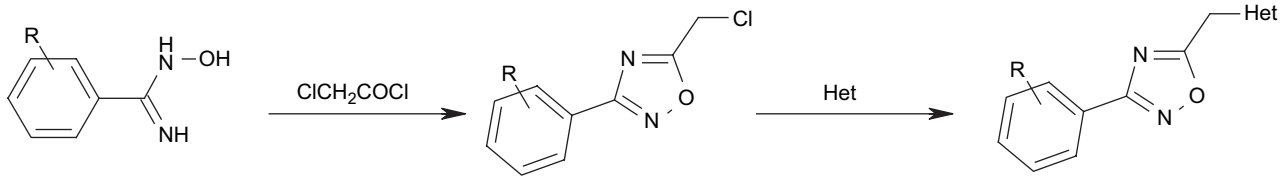
1-9



10-15

Compound	Heterocycle	R	Mp [°C]	Cryst. Solvent	Molecular formula	Anal.[a]
1	1-Imidazolyl	H	79	MeOH–H_2O	$C_{12}H_{10}N_4O$	C, H, N
2	1-Imidazolyl	4-Cl	111	MeOH–H_2O	$C_{12}H_9ClN_4O$	C, H, N, Cl
3	1-[1,2,4]Triazolyl	H	93	CCl_4	$C_{11}H_9N_5O$	C, H, N
4	1-[1,2,4]Triazolyl	4-Cl	98	MeOH–H_2O	$C_{11}H_8ClN_5O$	C, H, N, Cl
5	1-[1,2,4]Triazolyl	2-F	100	MeOH–H_2O	$C_{11}H_8FN_5O$	C, H, N
6	1-[1,2,4]Triazolyl	3-F	83	MeOH–H_2O	$C_{11}H_8FN_5O$	C, H, N
7	1-[1,2,4]Triazolyl	3-CH_3	85	MeOH–H_2O	$C_{12}H_{11}N_5O$	C, H, N
8	1-[1,2,4]Triazolyl	4-CH_3	91	MeOH–H_2O	$C_{12}H_{11}N_5O$	C, H, N
9	5-Methyl-1-[1,2,4]triazolyl	3-F	154	EtOH	$C_{12}H_{10}FN_5O$	C, H, N
10	1-[1,2,4]Triazolyl	H	140	EtOH	$C_{11}H_9N_5O$	C, H, N
11	1-[1,2,4]Triazolyl	2-F	81	MeOH	$C_{11}H_8FN_5O$	C, H, N
12	1-[1,2,4]Triazolyl	3-F	121	EtOH	$C_{11}H_8FN_5O$	C, H, N
13	1-[1,2,4]Triazolyl	4-F	89	EtOH	$C_{11}H_8FN_5O$	C, H, N
14	1-[1,2,4]Triazolyl	3-CH_3	96	EtOH	$C_{12}H_{11}N_5O$	C, H, N
15	1-[1,2,3]Triazolyl	4-Cl	113	EtOH	$C_{11}H_8ClN_5O$	C, H, N, Cl

[a] Detailed results of elemental analyses are given in Section 4.1.



Scheme 1. Synthesis of compound **1–9**.

4. Experimental protocols

4.1. Chemistry

All melting points were determined on a Boetius melting point apparatus PHMK 05. They are uncorrected. The IR spectra were registered on a Perkin–Elmer 1725x spectrometer. All absorption values are expressed in wavenumbers (cm^{-1}). Proton (^1H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on a Bruker ARX 300 NMR spectrometer. Chemical shifts (δ) are in parts per million (ppm) relative to $Si(CH_3)_4$ and coupling constants (J) are in hertz.

4.1.1. General procedure for the synthesis of oxadiazoles

Key intermediates for the synthesis of 3-aryl[1,2,4]oxadiazole derivatives are 3-aryl 5-chloromethyl[1,2,4]oxadiazoles and for the synthesis of 5-aryl[1,2,4]oxadiazole derivatives are 5-aryl 3-chloromethyl[1,2,4]oxadiazoles, respectively. We used synthetic routes similar to those described in Refs. [13,14].

4.1.1.1. Alkylation of imidazole and triazole. To a mixture of 50 mmol chloromethyl-oxadiazole derivative and 50 mmol imidazole or triazole in 150 ml of dried acetone 100 mmol ultra dried potassium carbonate was added. The resulting mixture was heated under reflux for 2 h and then it was allowed to cool to room temperature. The remaining salts were removed by filtration. The filtrate was evaporated to yield brown crystals, which were washed with water for two times and subsequently purified by recrystallization. Solvents for recrystallization are mentioned in Table 1.

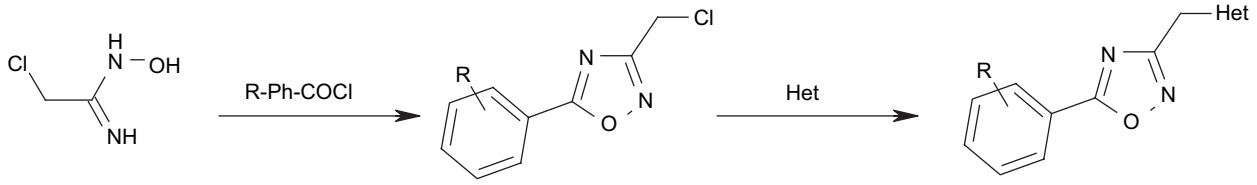
*4.1.1.1.1. 5-Imidazol-1-ylmethyl-3-phenyl[1,2,4]oxadiazole (**1**).* Yield 68%. ^1H NMR (DMSO-d_6): δ 5.75 (s, 2H, CH_2), 6.98 (s, 1H, H-11), 7.27 (s, 1H, H-10), 7.50 (m, 3H, H-14, 15, 16), 7.88 (s, 1H, H-8), 7.98 (d, $^{1,2}J_{HH}$ 8.1 Hz, 2H, H-13, 17). ^{13}C NMR: δ 42.10 (CH_2), 120.72 (C-11), 126.14 (C-12), 127.42 (C-13, 17), 129.27 (C-10), 129.62 (C-14, 16), 132.08 (C-15), 138.55 (C-8), 168.25 (C-3), 176.19 (C-5). Anal. Calc. for $C_{12}H_{10}N_4O$ ($M_r = 226.24$): C, 63.71; H, 4.46; N, 24.76. Found: C, 64.12; H, 4.61; N, 24.94%.

*4.1.1.1.2. 3-(4-Chlorophenyl)-5-imidazol-1-ylmethyl[1,2,4]-oxadiazole (**2**).* Yield 90%. ^1H NMR (DMSO-d_6): δ 5.82 (s, 2H, CH_2), 7.05 (d, 1H, $^{1,2}J_{HH}$ 1.0 Hz, H-11), 7.42(d, 1H, $^{1,2}J_{HH}$ 8.1 Hz, H-10), 7.68 (m, 2H, $^{1,2}J_{HH}$ 8.1 Hz, H-14, 15), 7.88 (s, 1H, H-8), 8.02 (d, 2H, $^{2}J_{HH}$ 8.4 Hz, H-13, 17). ^{13}C NMR: δ 42.10 (CH_2), 120.72 (C-11), 124.99 (C-12), 129.20 (C-13, 17), 129.27 (C-10), 129.82 (C-14, 16), 136.90 (C-15), 138.54 (C-8), 167.25 (C-3), 176.47 (C-5). Anal. Calc. for $C_{12}H_9ClN_4O$ ($M_r = 260.68$): C, 55.29; H, 3.48; N, 21.49. Found: C, 55.24; H, 3.57; N, 21.55%.

*4.1.1.1.3. 3-Phenyl-5-[1,2,4]triazol-1-ylmethyl[1,2,4]oxadiazole (**3**).* Yield 81%. ^1H NMR (DMSO-d_6): δ 5.81 (s, 2H, CH_2), 7.02 (d, 1H, H-11), 7.42 (d, 1H, $^{2,3}J_{HH}$ 7.4 Hz, H-11), 7.66 (d, 2H, $^{1,2}J_{HH}$ 8.2 Hz, H-14, 16), 7.89 (s, 1H, H-9), 8.00 (d, 2H, $^{1,2}J_{HH}$ 8.2 Hz, H-13, 17). ^{13}C NMR: δ 44.74 (CH_2), 126.03 (C-12), 127.42 (C-13, 17), 129.63 (C-14, 16), 132.12 (C-15), 145.86 (C-11), 152.56 (C-9), 167.45 (C-3), 176.47 (C-5). Anal. Calc. for $C_{11}H_9N_5O$ ($M_r = 227.23$): C, 58.15; H, 3.99; N, 30.82. Found: C, 58.09; H, 4.17; N, 30.00%.

*4.1.1.1.4. 3-(4-Chlorophenyl)-5-[1,2,4]triazol-1-ylmethyl[1,2,4]-oxadiazole (**4**).* Yield 89%. ^1H NMR (DMSO-d_6): δ 5.86 (s, 2H, CH_2), 7.45 (d, 2H, $^{1,2}J_{HH}$ 8.4 Hz, H-14, 16), 7.77 (d, $^{1,2}J_{HH}$ 8.4 Hz, 2H, H-13, 17), 7.91 (s, 1H, H-11), 8.63 (s, 1H, H-9). ^{13}C NMR: δ 44.71 (CH_2), 124.88 (C-12), 129.25 (C-13, 17), 129.88 (C-14, 16), 136.96 (C-15), 145.45 (C-11), 152.56 (C-9), 167.53 (C-3), 175.32 (C-5). Anal. Calc. for $C_{11}H_8ClN_5O$ ($M_r = 261.67$): C, 50.49; H, 3.08; N, 26.76. Found: C, 50.28; H, 2.59; N, 26.70%.

*4.1.1.1.5. 3-(2-Fluorophenyl)-5-[1,2,4]triazol-1-ylmethyl[1,2,4]-oxadiazole (**5**).* Yield 76%. ^1H NMR (DMSO-d_6): δ 6.01 (s, 2H, CH_2), 7.32 (m, 2H, H-14, 16), 7.59 (m, 1H, H-15), 7.88 (dd, 1H, H-17), 8.09 (s, 1H, H-9), 8.72 (s, 1H, H-11). ^{13}C NMR: δ 44.66 (CH_2), 114.11 ($^{2}J_{CF}$ 12.38 Hz, C-12), 117.07 ($^{2}J_{CF}$ 20.60 Hz, C-14), 125.49 ($^{3}J_{CF}$ 3.62 Hz, C-15), 130.89 ($^{4}J_{CF}$ 1.81 Hz, C-16), 134.09 ($^{3}J_{CF}$ 8.60 Hz, C-17), 145.86 (C-11), 152.57 (C-9), 158.49 ($^{1}J_{CF}$ 255.92 Hz, C-13), 165.14 ($^{3}J_{CF}$ 5.36 Hz, C-3), 174.65 (C-5). Anal. Calc. for $C_{11}H_8FN_5O$



Scheme 2. Synthesis of compound **10–15**.

Table 2
ED_{50} results of 3-aryl and 5-aryl[1,2,4]oxadiazoles in anticonvulsant and neurotoxicity tests

Compound	ED_{50} PTZ rat p.o. [mg/kg] after (*x*) h[a]	ED_{50} MES rat p.o. [mg/kg] after (*x*) h[a]	ED_{50} NT rat p.o. [mg/kg] after (*x*) h[a]
1	>250.0 (1.0)[b]	5.8 (2.0)	>500 (2.0)[b]
2	46.2 (2.0)[b]	23.2 (1.0)[b]	>500 (1.0)
3	37.0 (2.0)	13.1 (4.0)	77 (2.0)
4	29.5 (2.0)	21.8 (1.0)	<100 (1.0)
5	<50.0 (2.0)	44.3 (2.0)	<250 (2.0)
6	39.2 (1.0)	26.2 (1.0)	128 (1.0)
7	95.9 (0.5)	<30.0 (2.0)[b]	219 (1.0)
8	76.5 (1.0)	>70.0 (2.0)	171 (1.0)
9	35.5 (0.5)	n.d.[c]	n.d.[c]
10	25.5 (1.0)	14.6 (1.0)	335 (1.0)
11	≥50.0 (0.5)[b]	≥30.0 (0.5)[b]	>30 (0.5)[b]
12	73.9 (1.0)	47.2 (0.5)	<250 (0.5)
13	15.2 (0.3)[b]	19.7 (4.0)[b]	<150 (4.0)
14	>50.0 (3.0)	<50.0 (0.5)	>50 (0.5)
15	>100.0 (1.0)	n.d.[c]	n.d.[c]
Diazepam[d]	5.5 (0.5)[d]	62.1 (1.0)[d]	13.3 (0.25)[d]
Valproate[d]	620.0 (0.5)[d]	395.0 (0.5)[d]	859 (0.5)[d]

[a] Time after substance administration in hours.
[b] Data determined by National Institute of Health, Bethesda, USA.
[c] Not determined.
[d] Data taken from Ref. [15] for reference.

($M_r = 245.22$): C, 53.88; H, 3.29; N, 28.56. Found: C, 54.23; H, 3.30; N, 28.75%.

*4.1.1.1.6. 3-(3-Fluorophenyl)-5-[1,2,4]triazol-1-ylmethyl-[1,2,4]oxadiazole (**6**).* Yield 85%. ^{1}H NMR (DMSO-d_6): δ 6.19 (s, 2H, CH_2), 7.61 (m, 1H, H-15), 7.80 (m, 2H, H-13, 15), 7.98 (d, 1H, H-17), 8.18 (s, 1H, H-9), 8.90 (s, 1H, H-11). ^{13}C NMR: δ 44.71 (CH_2), 113.91 ($^2J_{CF}$ 23.70 Hz, C-15), 118.99 ($^2J_{CF}$ 21.06 Hz, H-13), 123.66 ($^4J_{CF}$ 2.87 Hz, H-17), 128.08 ($^3J_{CF}$ 8.45 Hz, C-12), 131.97 ($^3J_{CF}$ 8.38 Hz, C-16), 145.87 (C-11), 152.57 (C-9), 161.03 ($^1J_{CF}$ 245.20 Hz, C-14), 167.44 ($^4J_{CF}$ 3.02 Hz, C-5), 175.35 (C-3). Anal. Calc. for $C_{11}H_8FN_5O$ ($M_r = 245.22$): C, 53.88; H, 3.29; N, 28.56. Found: C, 53.87; H, 3.51; N, 28.37%.

*4.1.1.1.7. 3-(3-Methylphenyl)-5-[1,2,4]triazol-1-ylmethyl-[1,2,4]oxadiazole (**7**).* Yield 81%. ^{1}H NMR (DMSO-d_6): δ 2.42 (s, 3H, CH_3), 6.10 (s, 2H, CH_2), 7.45 (m, 2H, H-15, 16), 7.78 (m, 2H, H-13, 17), 8.17 (s, 1H, H-9), 8.76 (s, 1H, H-11). ^{13}C NMR: δ 21.16 (CH_3), 44.72 (CH_2), 124.60 (C-17), 125.97 (C-12), 127.78 (C-16), 129.53 (C-13), 132.77 (C-15), 139.10 (C-14), 145.84 (C-11), 152.56 (C-9), 168.37 (C-3), 174.93 (C-5). Anal. Calc. for $C_{12}H_{11}N_5O$ ($M_r = 241.25$): C, 59.74; H, 4.6; N, 29.03. Found: C, 59.62; H, 4.29; N, 28.93%.

*4.1.1.1.8. 3-(4-Methylphenyl)-5-[1,2,4]triazol-1-ylmethyl-[1,2,4]oxadiazole (**8**).* Yield 83%. ^{1}H NMR (DMSO-d_6): δ 2.34 (s, 3H, CH_3), 5.99 (s, 2H, CH_2), 7.31 (d, 2H, $^{1,2}J_{HH}$ 7.8 Hz, H-14, 16), 7.81 (d, 2H, $^{1,2}J_{HH}$ 7.8 Hz, H-13, 17), 8.09 (s, 1H, H-9), 8.77 (s, 1H, H-11). ^{13}C NMR: δ 21.41 (CH_3), 44.72 (CH_2), 123.26 (C-12), 127.37 (C-14, 16), 130.20 (C-13, 17), 142.18 (C-15), 145.83 (C-11), 152.54 (C-9), 168.28 (C-5), 174.84 (C-3). Anal. Calc. for $C_{12}H_{11}N_5O$ ($M_r = 241.25$): C, 59.74; H, 4.6; N, 29.03. Found: C, 59.68; H, 4.34; N, 28.92%.

*4.1.1.1.9. 3-(3-Fluorophenyl)-5(5-methyl[1,2,4]triazol-1-yl)-methyl[1,2,4]oxadiazole (**9**).* Yield 76%. ^{1}H NMR (DMSO-d_6): δ 2.50 (s, 3H, CH_3), 6.09 (s, 2H, CH_2), 7.49 (m, 1H, H-4), 7.65 (m, 2H, H-2, 5), 7.82 (d, 1H, H-6), 8.31(s, 1H, H-11). ^{13}C NMR: δ 11.39 (−CH_3), 44.42 (CH_2), 114.35 ($^2J_{CF}$ 24.15 Hz, C-4), 119.47 ($^2J_{CF}$ 21.13 Hz, H-2), 123.85 ($^4J_{CF}$ 3.02 Hz, H-6), 128.19 ($^3J_{CF}$ 9.06 Hz, C-1), 132.27 ($^3J_{CF}$ 8.30 Hz, C-5), 148.82 (C-11), 153.83 (C-10), 164.35 ($^1J_{CF}$ 245.28 Hz, C-3), 167.52 ($^4J_{CF}$ 3.02 Hz, C-7), 174.91 (C-8). Anal. Calc. for $C_{12}H_{10}FN_5O$ ($M_r = 259.24$): C, 55.6; H, 3.89; N, 27.01. Found: C, 55.54; H, 4.02; N, 27.86%.

*4.1.1.1.10. 5-Phenyl-3-[1,2,4]triazol-1-ylmethyl[1,2,4]oxadiazole (**10**).* Yield 86%. ^{1}H NMR (DMSO-d_6): δ 5.93 (s, 2H, CH_2), 7.80 (m, 3H, H-14, 15, 16), 8.25 (m, 3H, H-13, 17, 9), 8.92 (s, 1H, H-11). ^{13}C NMR: δ 44.37 (CH_2), 123.37 (C-12), 128.37 (C-14, 16), 129.96 (C-13, 17), 133.90 (C-15), 145.53 (C-11), 152.30 (C-9), 167.12 (C-3), 176.20 (C-5).

Table 3
Pharmacological data from in vitro experiments of 3-aryl and 5-aryl [1,2,4]oxadiazoles

Compound	Inhibition binding BZR [%]/[μM][a]	Potentiation of the GABA-induced current [%]/[μM][a]	Inhibition of the voltage dependent sodium current [%]/[μM][a]
1	0.6/10	134/100	83/100
			30/10
2	0.2/10	298/100	72/100
		103/10	
3	2.7/10	286/100	8/100
4	−8.0/10	553/100	31/100
		141/10	
5	2.2/10	191/100	n.d.[b]
6	−1.6/10	212/100	12/100
7	−1.1/10	387/100	22/100
		110/10	
10	−3.1/10	210/100	9/100
		131/10	
Diazepam	>99/10 ($K_i = 6.8$ nM)	216/10	n.d.[b]

[a] Administered concentration.
[b] Not determined.

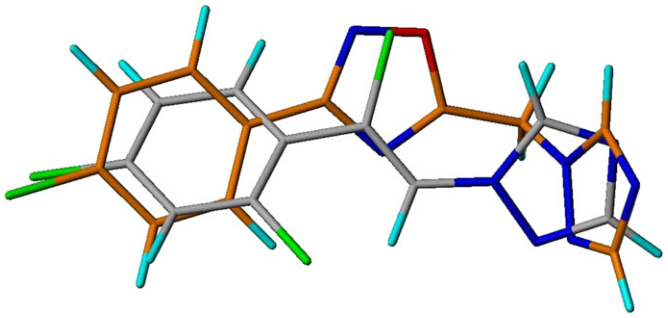


Fig. 1. Superimposition of loreclezole (grey) and compound **4** (orange). Details are given in Section 4.

Anal. Calc. for $C_{11}H_9N_5O$ ($M_r = 227.23$): C, 58.15; H, 3.99; N, 30.82. Found: C, 57.64; H, 4.31; N, 30.01%.

*4.1.1.1.11. 5-(2-Fluorophenyl)-3-[1,2,4]triazol-1-ylmethyl-[1,2,4]oxadiazole (**11**).* Yield 77%. ^{1}H NMR (DMSO-d_6): δ 5.81 (CH_2), 7.39 (m, 2H, H-14, 16), 7.73 (m, 1H, H-15), 8.05 (m, 2H, 13, 9), 8.75 (s, 1H, H-11). ^{13}C NMR: δ 44.35 (CH_2), 111.74 ($^2J_{CF}$ 11.24 Hz, C-12), 117.46 ($^2J_{CF}$ 20.68 Hz, C-16), 125.76 ($^4J_{CF}$ 3.55 Hz, C-14), 131.10 (C-13), 136.09 ($^3J_{CF}$ 7.02 Hz, C-15), 145.55 (C-11), 153.32 (C-9), 158.57 ($^1J_{CF}$ 258.48 Hz, C-17), 166.86 (C-3), 173.16 ($^3J_{CF}$ 4.14 Hz, C-5). Anal. Calc. for $C_{11}H_8FN_5O$ ($M_r = 245.22$): C, 53.88; H, 3.29; N, 28.56. Found: C, 53.97; H, 3.43; N, 28.51%.

*4.1.1.1.12. 5-(3-Fluorophenyl)-3-[1,2,4]triazol-1-ylmethyl-[1,2,4]oxadiazole (**12**).* Yield 80%. ^{1}H NMR (DMSO-d_6): δ 5.84 (CH_2), 7.50 (m, 1H, H-15), 7.63 (m, 1H, H-16), 7.85 (m, 2H, H-13, 17), 8.02 (s, 1H, H-9), 8.67 (s, 1H, H-11). ^{13}C NMR: δ 44.34 (CH_2), 114.77 ($^2J_{CF}$ 24.22 Hz, C-15), 120.70 ($^2J_{CF}$ 21.13 Hz, C-13), 124.53 ($^4J_{CF}$ 2.87 Hz, C-17), 125.21 ($^3J_{CF}$ 8.75 Hz, C-12), 132.23 ($^3J_{CF}$ 8.30 Hz, C-16), 145.54 (C-11), 152.31 (C-9), 160.89 ($^1J_{CF}$ 246.11 Hz, C-14), 167.21 (C-3), 175.11 ($^3J_{CF}$ 3.16 Hz, C-5). Anal. Calc. for $C_{11}H_8FN_5O$ ($M_r = 245.22$): C, 53.88; H, 3.29; N, 28.56. Found: C, 54.10; H, 3.48; N, 28.65%.

*4.1.1.1.13. 5-(4-Fluorophenyl)-3-[1,2,4]triazol-1-ylmethyl-[1,2,4]oxadiazole (**13**).* Yield 63%. ^{1}H NMR (DMSO-d_6): δ 5.68 (s, 2H, CH_2), 7.54 (d, 2H, $^{1,2}J_{HH}$ 8.1 Hz, H-14, 16), 7.96 (s, 1H, H-9), 8.10 (d, 2H, $^{1,2}J_{HH}$ 8.1 Hz, H-13, 17), 8.67 (s, 1H, H-11). ^{13}C NMR: δ 44.34 (CH_2), 117.11 ($^2J_{CF}$ 22.49 Hz, H-14, 16), 120.08 ($^4J_{CF}$ 2.94 Hz, C-12), 131.16 ($^3J_{CF}$ 9.58 Hz, C-13, 17), 145.53 (C-11), 152.30 (C-9), 163.75 ($^1J_{CF}$ 255.39 Hz, C-15), 167.13 (C-3), 175.36 (C-5). Anal. Calc. for $C_{11}H_8FN_5O$ ($M_r = 245.22$): C, 53.88; H, 3.29; N, 28.56. Found: C, 54.02; H, 2.69; N, 28.43%.

*4.1.1.1.14. 5-(3-Methylphenyl)-3-[1,2,4]triazol-1-ylmethyl-[1,2,4]oxadiazole (**14**).* Yield 60%. ^{1}H NMR (DMSO-d_6): δ 2.29 (s, 3H, CH_3), 5.75 (s, 2H, CH_2), 7.28 (d, 2H, $^{4,5}J_{HH}$ 8.2 Hz, H-15, 16), 7.78 (m, 2H, H-13, 17), 8.00 (s, 1H, H-9), 8.71 (s, 1H, H-11). ^{13}C NMR: δ 21.00 (CH_3), 44.41 (CH_2), 123.27 (C-12), 125.35 (C-17), 128.45 (C-16), 129.70 (C-13), 134.42 (C-15), 139.46 (C-14), 145.54 (C-11), 152.30 (C-9), 167.04 (C-3), 176.28 (C-5). Anal. Calc. for $C_{12}H_{11}N_5O$ ($M_r = 241.25$): C, 59.74; H, 4.6; N, 29.03. Found: C, 59.41; H, 4.72; N, 28.38%.

*4.1.1.1.15. 5-(4-Chlorophenyl)-3-[1,2,3]triazol-1-ylmethyl-[1,2,4]oxadiazole (**15**).* Yield 50%. ^{1}H NMR (DMSO-d_6): δ 6.16 (s, 2H, CH_2), 7.88 (d, 2H, $^{1,2}J_{HH}$ 8.3 Hz, H-14, 16), 8.00 (s, 1H, H-10), 8.24 (d, 2H, $^{1,2}J_{HH}$ 8.3 Hz, H-13, 17), 8.48 (s, 1H, H-11). ^{13}C NMR: δ 44.73 (CH_2), 122.20 (C-12), 126.29 (C-13, 17), 130.08 (C-14, 16), 130.12 (C-10), 134.01 (C-11), 138.83 (C-15), 167.11 (C-3), 175.44 (C-5). Anal. Calc. for $C_{11}H_8ClN_5O$ ($M_r = 261.67$): C, 50.49; H, 3.08; N, 26.76. Found: C, 50.61; H, 3.32; N, 26.54%.

4.2. Biology

We used male Wistar rats with a body weight of 130–200 g for all experiments. Animals were allowed to adapt to the laboratory environment for one week before the experiments started. All experiments with drug injection were then carried out within one week to minimize the effect of increasing age on drug susceptibility. Each rat was used for only one experiment.

Rats were kept in groups of five in plastic cages at controlled temperature (22 ± 2 °C) and humidity (about 55 ± 15%) with a 12-h light cycle beginning at 6 a.m. They received standard laboratory rodent chow and tap water ad libitum.

4.2.1. Anticonvulsant tests

We used three different tests for primary characterisation of compounds in rats as described previously [17]: (1) the MES test (maximal electroshock seizure), (2) the PTZ test (pentylenetetrazole induced seizures), and (3) the rotarod test.

For the MES test, male rats were stimulated with ear electrodes by means of a constant-current stimulator (Rodent-shocker type 221, Hugo Sachs Elektronik KG, Germany) with a supra-threshold fixed current sinus wave stimulus (50 Hz, 150 mA, 0.2 s). The maximal tonic extension of the hind limbs was used as endpoint.

For the PTZ test, PTZ (70 mg/kg b.w.) was injected s.c. (0.2 ml/100 g b.w.) at the back of the neck of the rats. After injection the animals were observed for 30 min. The first generalised clonic seizure with loss of righting reflexes was used as the endpoint.

To test the motor coordination of animals a rotating rod was used (diameter 8 cm, 8 rpm). The day before the animals were trained on a rotating rod until they were able to remain on it for 1 min. On the day of the experiment the ability of animals to stay on the rod for 1 min was tested before and after administration of substance or vehicle. Each animal had three trials at each time interval.

4.2.1.1. Calculation of ED_{50}. In the seizure tests an approximation of the time peak effect was achieved by administrating the test substance at different pre-treatment times and determining the percentage of protection. The time at which the highest protection measured was used for dose–response curve and determination of the median effective dose (ED_{50}).

4.2.1.2. Calculation of TD_{50}. In the rotarod test an approximation of the time peak effect was achieved by testing the same animals at different times after administration of the test substance and determining the percentage of disturbance of motor coordination. The time at which the highest neurotoxic effect measured was used for dose–response curve and determination of the median toxic dose (TD_{50}). The ED_{50} or TD_{50} were calculated by probit analysis [18].

4.2.2. Electrophysiological investigations

4.2.2.1. Cell culture. For current recordings, rat cortical neurons were obtained from cortical tissue of 18-day old embryos. Cells were cultivated together with astrocytes on poly-L-lysine coated glass cover slips at a density of 5×10^5 cells/cm^2. The

cells were cultivated in BME supplemented with 10% horse serum, 10% fetal calf serum and glutamine (2 mM). After three days cytosine-1β-D-arabinofuranoside (5 μM) was added in order to inhibit astrocyte propagation and thereby concealment of neurons through large numbers of astrocytes. The cultivation was continued with BME supplemented with 5% horse serum, 5% fetal calf serum and glutamine (2 mM). For the experiments neurons were used between day seven and eight in culture.

4.2.2.2. Patch-clamp recording. The whole-cell variant of the patch-clamp technique was used for the voltage-clamp experiments [19]. The micropipettes were pulled (Sutter Instruments Company, Novato, USA) from borosilicate glass capillaries (Science Products, Hofheim, Germany) and heat polished at the tip (ALA Scientific Instruments, New York, USA). The pipette resistances were between 2 and 5 MΩ. The composition of the internal solution for GABA-induced currents was 140 mM CsCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM *N*-hydroxyethyl-piperazine-*N*′-2-ethanesulfonic acid (HEPES), and 11 mM ethylene glycol-bis(2-aminoethyl ether)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA). The composition of the internal solution for voltage gated sodium currents was 135 mM CsCl, 2 mM $MgCl_2$, 10 mM HEPES, 5 mM D-glucose, and 10 mM 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA). The pH was adjusted to 7.3 with CsCl. The osmolality was adjusted to about 300 mOsmol. The internal solution was prepared in advance and deep-frozen in aliquots of 1 ml. To fill the recording pipettes, the solution was thawed every morning and 2 mM $ATPNa_2$ was added.

Current signals were amplified by an EPC-9 amplifier digitised, stored and analysed using the TIDA system (HEKA Elektronik, Lambrecht, Germany). The data were sampled, digitally filtered (Bessel 10 kHz) and stored on computer disk at a frequency of 2 kHz. For recording, a cover slip was transferred into the recording chamber. For GABA-induced currents the cells were permanently superfused with modified extracellular solutions containing 140 mM NaCl; 5 mM KCl, 2 mM CsCl, 1 mM $MgCl_2$, 10 mM HEPES, 5 mM D-glucose, and 0.003 mM Tetrodotoxine (TTX), pH = 7.3–7.4 (NaOH). The extracellular solution for voltage dependent sodium currents contained 140 mM NaCl; 5.4 mM CsCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 10.8 mM $BaCl_2$, 2 mM $CoCl_2$, 10 mM HEPES, and 5 mM D-glucose and adjusted to pH = 7.3 (NaOH).

4.2.2.3. GABA-induced currents. The cells were clamped at a potential of −80 mV. The concentration of GABA was selected to elicit approximately 20% of the maximal current induced by high concentrations of GABA. This concentration was determined to be 3 μM and elicited a current of -268 ± 35 pA ($n = 16$). The GABA solution or the test compounds (at concentrations between 1 and 100 μM) together with GABA were locally applied onto the clamped cell using a eight channel rapid solution exchanger (DAD8; ALA Scientific Instruments, New York, USA). For the evaluation of the drug effect, maximum current amplitude induced by application of 3 μM GABA was set to 100% and the relative maximum current amplitude induced by the test compound and GABA in relation to the GABA-induced maximum current amplitude in the same cell was calculated.

4.2.2.4. Voltage-dependent sodium currents. The cells were held at −80 mV and superfused for at least 1 min with control or drug solution before initiating the channel opening by depolarising steps with a frequency of 1 Hz for 25 ms in 10 mV increments to various potentials between −80 and +20 mV. Capacitive and leakage currents were subtracted from the active currents. Current traces were evaluated before, during, and after application.

4.2.2.5. Inhibition of specific [^{3}H]-flunitrazepam binding to benzodiazepine binding site. Neuronal membrane fractions from rat forebrain (excluding the cerebellum) were prepared using standard techniques described in Ref. [20]. Membrane fraction of about 150 μl was incubated with 0.5 nmol/l [^{3}H]-flunitrazepam and an appropriate concentration of the test compound for 30 min at 4 °C. Nonspecific binding was determined in the presence of 10 μM diazepam. Binding was terminated by filtration of the incubated membrane fraction using Filtermat A (Pharmacia, Uppsala, Sweden) pre-soaked with 1% polyethylene imine and a micro cell harvester (Skatron, Liver, Norway). Then, the Filtermat A was carefully washed with 0.05 Tris–HCl buffer, pH = 7.7, to eliminate unbound radioactivity. The filters were counted in a scintillation counter (Betaplate 1205, Berthold, Wildbad, Germany) to determine the specific binding of [^{3}H]-flunitrazepam. Compounds were screened at 6–10 concentrations to determine IC_{50} and K_i. In the assay, the dissociation constant of [^{3}H]-flunitrazepam was 1.5 nmol/l, its maximal number of binding sites was 0.38 nmol/l and the specific binding was 90%.

4.3. Molecular modelling

Initial molecular models of loreclezole and compound **4** were built using the molecular sketcher within SYBYL [21]. Geometries were optimized by means of the semiempirical AM1 Hamiltonian in MOPAC [22]. The ‘‘Fit atoms’’ option of SYBYL was used to align the two structures. In detail, the chloro residues in the 4-position of the phenyl substituents, the phenyl rings and the nitrogens of the triazoles were matched. Additionally, the chloro atom of the loreclezole vinyl group was matched with the oxygen of the oxadiazole ring.

5. Conclusion

In summary, the current data indicate that both, oxadiazoles acting as selective GABA potentiating drugs and oxadiazoles which have a dual GABA/sodium channel mechanism were found to be potent anticonvulsants. Selected candidates from both subgroups were better tolerated than benzodiazepines indicating that such drugs maybe developed into interesting new anticonvulsants.

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