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Hypoglycemic effect of several substituted amides and acylhydrazides of succinic acid

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The presence of the succinamic moiety could provide not only less toxicity but also hypoglycemic effect in the light of the previous findings [1–4]. In the course of our investigation of the dicarboxylic acid substituted amides and hydrazides we prepared several succinic acid derivatives [2–5]. As part of a search for novel hypoglycemic agents 20 mentioned compounds were screened for their impact on glucose blood level in normal rats. The most effective hypoglycemic compounds were tested in alloxan-induced diabetic rats and their acute toxicity was studied.

Compounds **1**–**20** were synthesized as outlined in Scheme.

The changes of blood glucose levels in normal and alloxan-induced rats are presented in the Table. The hypoglycemic (**2, 4, 12**–**14, 16, 17, 20**) as well as hyperglycemic (**1, 6, 8, 9, 15**) compound effects were obtained. Arylamides (**2, 4**) and acylhydrazides (**12**–**14, 16, 17, 20**) of succinic acid were found to lower glucose blood level. Every acetylhydrazid of succinic acid tested (**12**–**14**) showed hypoglycemic effect. The most potent blood sugar decreases were observed after administration of compounds **4**, **14** and **17**. The acute toxicity determi-

Scheme

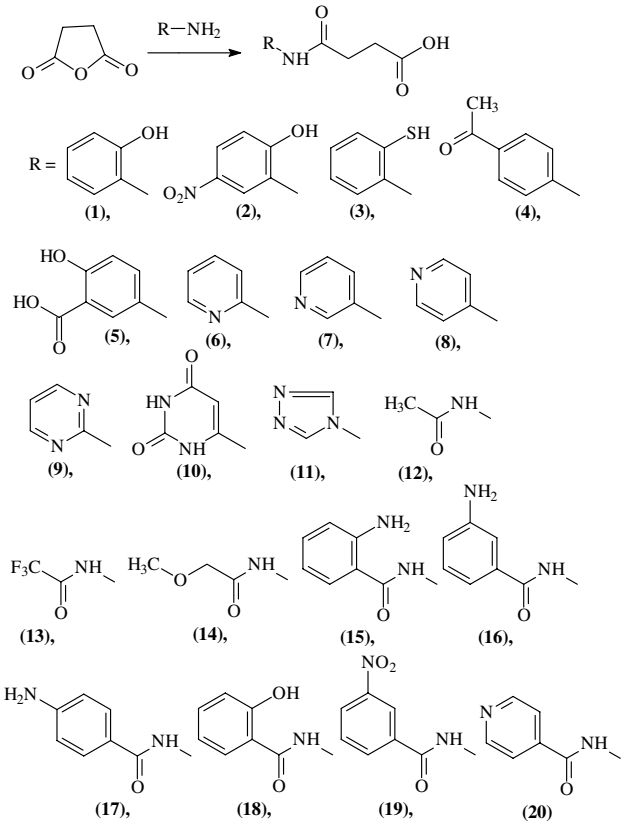

Table: Effect of some succinic acid amides and hydrazides on blood glucose level in normal (1–20) and alloxan-induced diabetic (4, 14, and 17) rats

Compound	n	Dose (mg/kg)	Initial value of blood glucose level (mM)	Changes of blood glucose levels (%)	
				After 3 h	After 5 h
Normal rats					
1	6	50	4.5 ± 0.2	3.1 ± 9.1	7.6 ± 5.6*
Control	6	–	4.7 ± 0.2	−4.2 ± 1.2	−8.9 ± 2.8
2	6	50	5.3 ± 0.1	−7.5 ± 2.2	−17.0 ± 0.6***
Control	6	–	5.4 ± 0.2	−8.5 ± 1.6	−7.8 ± 2.2
3	3	50	5.0 ± 0.1	−12.3 ± 5.4	−11.1 ± 4.3
Control	6	–	5.4 ± 0.2	−8.5 ± 1.6	−7.8 ± 2.2
4	7	50	6.0 ± 0.2	−15.0 ± 3.8	−23.3 ± 3.8***
Control	6	–	5.4 ± 0.2	−8.5 ± 1.6	−7.8 ± 2.2
5	6	50	4.4 ± 0.1	0.9 ± 4.8	0.5 ± 3.6
Control	6	–	4.7 ± 0.2	−4.2 ± 1.2	−8.9 ± 2.8
6	6	50	4.7 ± 0.1	9.4 ± 2.2****	0.6 ± 3.6
Control	6	–	4.7 ± 0.2	−4.2 ± 1.2	−8.9 ± 2.8
7	6	50	4.8 ± 0.1	−8.3 ± 2.3	−12.4 ± 2.0
Control	6	–	4.7 ± 0.2	−4.2 ± 1.2	−8.9 ± 2.8
8	6	5	4.3 ± 0.1	0.9 ± 4.8	30.6 ± 8.4****
Control	6	–	4.1 ± 0.2	−3.7 ± 3.3	−13.5 ± 4.3
9	6	50	4.1 ± 0.3	3.9 ± 3.2	4.0 ± 3.8**
Control	6	–	4.1 ± 0.2	−3.7 ± 3.3	−13.5 ± 4.3
10	6	50	4.8 ± 0.1	−6.4 ± 1.7	−15.4 ± 2.3
Control	6	–	4.7 ± 0.2	−4.2 ± 1.2	−8.9 ± 2.8
11	3	50	5.1 ± 0.1	−9.8 ± 6.3	−19.6 ± 5.0
Control	6	–	5.4 ± 0.2	−8.5 ± 1.6	−7.8 ± 2.2
12	6	50	4.8 ± 0.1	−15.5 ± 2.2***	−4.6 ± 2.6
Control	6	–	4.7 ± 0.2	−4.2 ± 1.2	−8.9 ± 2.8

Table (Continued)

Compound	n	Dose (mg/kg)	Initial value of blood glucose level (mM)	Changes of blood glucose levels (%)	
				After 3 h	After 5 h
13	6	50	4.0 ± 0.1	−12.6 ± 1.9*	−15.9 ± 1.9
Control	6	–	4.1 ± 0.2	−3.7 ± 3.3	−13.5 ± 4.3
14	7	50	5.4 ± 0.1	−11.4 ± 9.0	−22.2 ± 1.8****
Control	6	–	5.4 ± 0.2	−8.5 ± 1.6	−7.8 ± 2.2
15	6	50	4.7 ± 0.1	0.9 ± 4.1	0.7 ± 2.3*
Control	6	–	4.7 ± 0.2	−4.2 ± 1.2	−8.9 ± 2.8
16	6	50	4.4 ± 0.1	−15.1 ± 1.0****	−8.6 ± 1.3
Control	6	–	4.7 ± 0.2	−4.2 ± 1.2	−8.9 ± 2.8
17	6	50	4.0 ± 0.3	−11.0 ± 4.5	−24.7 ± 4.0***
Control	6	–	4.7 ± 0.2	−4.2 ± 1.2	−8.9 ± 2.8
18	6	50	5.3 ± 0.1	−4.7 ± 1.5	−2.1 ± 2.9
Control	6	–	5.4 ± 0.2	−8.5 ± 1.6	−7.8 ± 2.2
19	6	50	5.1 ± 0.1	−4.5 ± 1.2	−6.7 ± 1.6
Control	6	–	5.4 ± 0.2	−8.5 ± 1.6	−7.8 ± 2.2
20	6	50	4.6 ± 0.1	−12.9 ± 5.5	−19.0 ± 3.1*
Control	6	–	4.7 ± 0.2	−4.2 ± 1.2	−8.9 ± 2.8
Gliclazide	6	50	4.3 ± 0.1	−27.3 ± 3.3****	−12.1 ± 3.3
Control	6	–	4.7 ± 0.2	−4.2 ± 1.2	−8.9 ± 2.8
Metformin	6	50	5.0 ± 0.1	4.3 ± 4.1	5.0 ± 0.1****
Control	6	–	4.7 ± 0.2	−4.2 ± 1.2	−8.9 ± 2.8
Alloxan-induced diabetic rats					
4	6	100	9.6 ± 0.8	−35.6 ± 11.7*	−54.3 ± 6.8****
Control	6	–	9.1 ± 1.3	−8.8 ± 1.8	−12.5 ± 2.8
14	6	100	12.5 ± 1.1	−22.4 ± 5.4	−48.8 ± 3.9****
Control	6	–	12.1 ± 0.9	−13.3 ± 1.7	−22.0 ± 2.5
17	5	100	9.5 ± 1.6	−51.6 ± 5.6****	−63.6 ± 5.8****
Control	6	–	9.1 ± 1.3	−8.8 ± 1.8	−12.5 ± 2.8
Gliclazide	6	50	11.9 ± 0.6	−29.8 ± 3.4***	−40.5 ± 3.6****
Control	6	–	11.9 ± 0.7	−13.8 ± 2.4	−20.3 ± 1.6
Metformin	6	50	13.7 ± 1.3	−29.2 ± 2.7****	−49.5 ± 3.8****
Control	6	–	12.1 ± 0.9	−13.3 ± 1.7	−22.0 ± 2.5

* $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$, **** $p < 0.001$ compared with control

nation of these derivatives displayed that they have LD_{50} values higher than 2000 mg/kg after i.p. injection, LD_{50} of corresponding drugs were 980 (903–1063) and 355 (290–420) mg/kg for metformin and glyclazide, respectively.

The compounds **4, 14** and **17** showed significant hypoglycemic effect in alloxan-induced diabetic rats. 4-Aminobenzoylhydrazide of succinic acid (**17**) is the most effective hypoglycemic agent, it has statistically significant more intensive effect on reducing blood glucose level than metformin and glyclazide and less toxicity.

Thus, the pharmacological screening of some succinic acid substituted amides and acylhydrazides displayed 8 hypoglycemic agents. Among the compounds tested, **17** appears to be the most interesting agent because of its low toxicity and hypoglycemic effect in normal rats as well as in rats with induced diabetes.

Experimental

1. Chemistry

Synthetic procedures and spectral data of compounds **1**–**20** were reported previously [2–5].

2. Hypoglycemic activity

2.1. Normal rats

Experiments were performed in normal male and female rats with body weights of 180–220 g. Rats were fasted for 14 h before the treatment. Animals had free access to tap water. Test compounds were administered i.p. as aqueous suspension in 1.0% starch. The control group rats were injected with the same amount of 1.0% starch. Blood samples were collected from the tails of the treated rats at predetermined time points. The blood glucose level was measured by the glucose oxidase/peroxidase method [6], and the effect was expressed as the percentage change of the blood glucose level from the initial value. The total number of animals used in each experiment and the compound doses are given in Table.

2.2. Alloxan-induced diabetic rats

The hypoglycemic effect of the most active compounds (**4, 14, 17**) was determined in alloxan-induced diabetic rats [7]. Male and female rats with body weight of 200–240 g were fasted for 24 h before inducing of diabetes mellitus. The animals were injected i.m. in alloxan (alloxanhydrate) solution at 160 mg/kg and fed to decrease nocturnal mortality due to hypoglycemia. Tree days after alloxan treatment, the rats were injected with insulin (2 U/kg, s.c.) to prevent diabetic hyperglycemic lethality. After one month of alloxan treatment, the stable hyperglycemia was achieved and rats were included in further investigations. The diabetic rats were fasted for 14 h and then were orally administered with solution of **4, 14, 17** (100 mg/kg), metformin (50 mg/kg), gliclazide (50 mg/kg) or placebo (saline) by using a gavage needle. Blood samples were collected and the blood glucose level was measured as described above.

3. Acute toxicity

The compounds **4, 14, 17** were tested for LD_{50} in male and female mice with body weight of 17–25 g after i.p. injection by Prozorovsky [8].

4. Statistical analysis

The statistical significance of the difference between values of compound-treated versus control animals was evaluated using the Student's *t* test.

References

1 Brzozowski, Z.; Gajewski, F.; Kozakiewicz, I.; Dominiak, K.; Wojcikowski, C.: Acta Pol. Pharm. **42**, 244 (1985)
2 Dolzhenko, A.V.; Kolotova, N.V.; Kozminykh, V.O.; Syropyatov, B.Ya.; Kotegov, V.P.; Godina, A.T.: Khim.-Farm. Zh. **36** (2), 6 (2002)
3 Kolotova, N.V.; Kozminykh, V.O.; Dolzhenko, A.V.; Kozminykh, E.N.; Kotegov, V.P.; Godina, A.T.; Syropyatov, B.Ya.; Novoselova G.N.: Khim.-Farm. Zh. **35** (3), 26 (2001)
4 Kolotova, N.V.; Dolzhenko, A.V.; Kozminykh, V.O.; Kotegov, V.P.; Godina, A.T.: Khim.-Farm. Zh. **33** (12), 9 (1999)
5 Kolotova, N.V.; Kozminykh, E.N.; Kolla, V.E.; Syropyatov, B.Ya.; Voronina, E.V.; Kozminykh, V.O.: Khim.-Farm. Zh. **33** (5), 22 (1999)
6 Hettwer, H.; Dahlmann, B.; Marjanovic, S.D.: Cell. Mol. Biol. **23**, 355 (1978)
7 Werner, W.; Rey, H.-G.; Wiclenger, H.: Z. Anal. Chem. **252**, 224 (1970)
8 Prozorovsky, V.B.; Prozorovskaya, M.P.; Demchenko V.M.: Farmakol. Toksikol. **41**(4), 497 (1978)

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New illudane sesquiterpenes from the basidiomycete *Clitocybe rivulosa HKI* 0273

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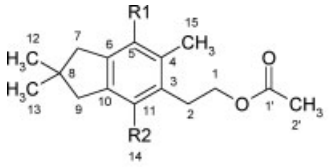
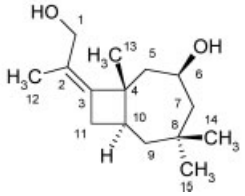
Bicyclic illudanes are frequently occurring fungal sesquiterpenes displaying various biological activities [1–6]. Representatives of this family such as fomajorins [1], radulactone [2], illudone [3], phaliotic acid [4], tsugicoline [5] and 5(2′-hydroyethyl)-2,2,4,6-tetramethyl-1,2-dihydroindane [6] are generated by different genera of basidiomycetes such as *Russula*, *Pholiota*, *Fomitopsis*, *Laurilia*, *Fomes* and *Radulomyces*. Here we report on isolation, structures and biological activities of new metabolites **1, 2** and **3** (Fig. 1) as new illudane sesquiterpenes from cultures of *Clitocybe rivulosa* HKI 0273. Another unusual bicyclic sesquiterpenoid compound (**4**) was co-isolated as a possible shunt metabolite of illudane biosynthesis. The producing strain HKI 0273 from the collection of the Hans-Knöll-Institute was conserved as mycelial culture of *Clitocybe rivulosa* HKI 0273. This was derived from tissue plugs of a fruiting body of *Clitocybe rivulosa* collected from a meadow near Oberbodnitz (Saale-Holzland district, Thuringia, Germany). At the end of the fermentation the whole culture broth (20 l) was extracted three-times under stirring by ethyl acetate (1 : 1). The combined and dried extracts were evaporated to yield 1.5 g oily residue. The material was chromatographed on silica gel 60 (0.063–0.1 mm, elution by $CHCl_3$/MeOH 9 : 1). The obtained fractions were evaporated and rechromatographed on TLC (Merck silica gel aluminium sheets, $CHCl_3$/MeOH; 9 : 1) whereby zones were eluted staining reddish-blueish with 1% vanillin/conc. H_2SO_4. Final purification was achieved by preparative TLC using silica gel aluminium sheets RP_{18} and MeCN/H_2O 83 : 17 with 0.5% TFA as eluent. **1** (15 mg), **2** (12 mg), **3** (5 mg) and **4** (10 mg) were thus obtained as colorless wax in addition to sulcatins A and B [7, 8]. The physico-chemical properties of **1**–**4** are shown in the Experimental part.

Structure elucidation of compounds **1**–**4** was carried out by MS, one- and two-dimensional NMR spectroscopy (^1H, ^{13}C, DEPT 13T, ^1H, ^1H-COSY, HSQC, HMBC, NOESY).





1 (R_1=H, R_2=CH_2OH), **2** (R_1=OH, R_2=CH_2OH), **3** (R_1=H, R_2=OH) and bicyclic metabolite **4** from *Clitocybe rivulosa* HKI 0273