Search for potential hypoglycemic agents among potassium salts of 3-benzyl-8-substituted xanthines

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Nowadays, the prevalence of metabolic syndrome (MS) is a serious problem among the world's population. Metabolic syndrome includes the so-called "deadly" quartet – hypertension, type 2 diabetes mellitus (diabetes mellitus), dyslipidemia and alimentary obesity.

After all, type 2 diabetes is included in the list of pathologies of the metabolic syndrome, it is important to find ways to alleviate the disease. Derivatives of such a heterocyclic system as xanthine are of great interest in this aspect.

The aim of the study was to explore the hypoglycemic activity of newly synthesized water-soluble derivatives of 3-benzyl-8-substituted xanthines.

Materials and methods. We obtained water-soluble potassium 3-benzyl-8-R-xanthin-7-ides, the structure and individuality of which were confirmed by a set of physical-chemical studies.

Results. The hypoglycemic effect of the newly synthesized compounds was assessed by an oral glucose tolerance test. The obtained data were processed using modern statistical *in silico*-methods.

Conclusions. The results of the hypoglycemic activity study among the potassium salts of 3-benzyl-8-substituted xanthine derivatives showed, that some new compounds are in close vicinity to the reference drug's hypoglycemic action.

Key words: xanthines, organic synthesis, hypoglycemic activity.

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Пошук потенційних гіпоглікемічних засобів серед калієвих солей 3-бензил-8-заміщених ксантинів

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Нині поширеність метаболічного синдрому (МС) – актуальна проблема всесвітньої охорони здоров'я. Метаболічний синдром включає в себе так званий «смертельний» квартет: артеріальну гіпертензію, цукровий діабет (ЦД) 2 типу, дисліпідемію та аліментарне ожиріння.

Цукровий діабет 2 типу належить до переліку патологій метаболічного синдрому, й актуальним є пошук засобів, які б давали змогу полегшити перебіг захворювання. В цьому аспекті чималий науковий інтерес викликають похідні такої гетероциклічної системи, як ксантин.

Мета роботи – дослідження гіпоглікемічної активності новосинтезованих водорозчинних похідних 3-бензил-8-заміщених ксантинів.

Матеріали та методи. Одержали водорозчинні калій 3-бензил-8-R-ксантиніди-7, будову та індивідуальність яких підтверджено комплексом фізико-хімічних досліджень.

Результати. Гіпоглікемічну дію новосинтезованих сполук оцінили за допомогою перорального тесту толерантності до глюкози. Результати опрацювали за допомогою сучасних статистичних *in silico*-методів.

Висновки. Результати дослідження гіпоглікемічної активності серед новосинтезованих калієвих солей 3-бензил-8-заміщених ксантинів підтверджують наявність сполук, що за рівнем гіпоглікемічної дії наближаються до референс-препарату.

Ключові слова: ксантини, органічний синтез, гіпоглікемічна активність.

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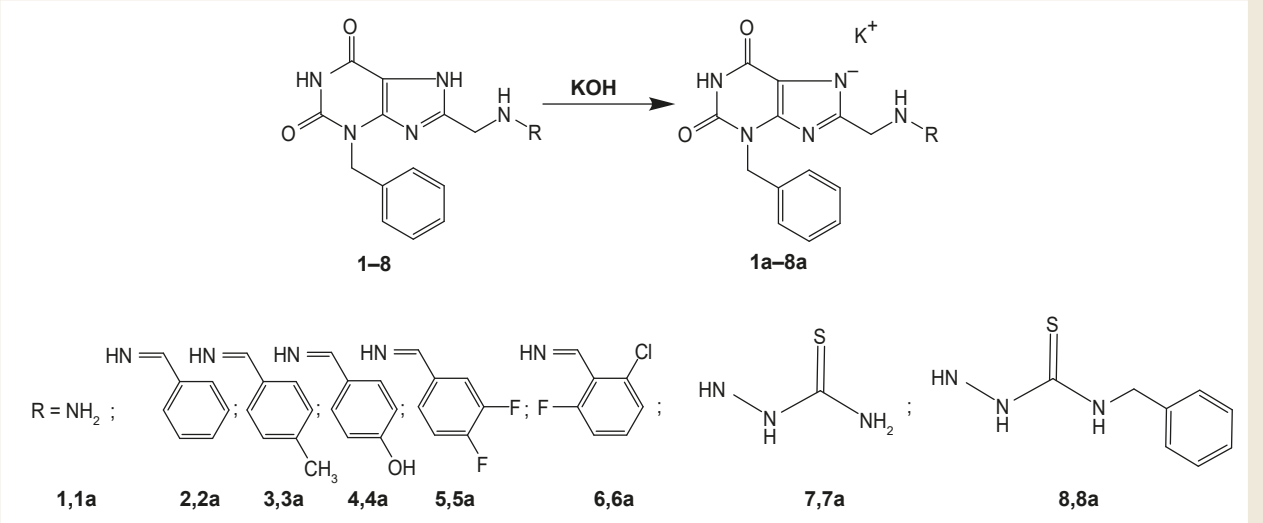

Fig. 1. Synthesis of potassium 3-benzyl-8-R-xanthin-7-ides (1a–8a).

According to the WHO, the prevalence of diabetes has almost quadrupled since 1980 and is now the ninth leading cause of death in the world [1]. Diabetes mellitus type 2 (T2DM) is on the rise worldwide and it's considered to be a global epidemic of our age. The most important goal of treatment of T2DM is the timely prescription of intensive antidiabetic therapy for targeting specific levels of carbohydrate and lipid metabolism in such patients to prevent the progression of vascular complications, which increases mortality [1].

Modern treatment of T2DM involves a set of measures aimed at maintaining good glycemic control, which involves dietary restriction, regular exercise, and finally, taking antidiabetic drugs [2]. The drug should be chosen by considering its effect on metabolic processes, cardiovascular system, and the development of hypoglycemic conditions. According to the clinical guidelines, standard drug treatment is started with metformin, with the subsequent application of sulfonylurea (e. g. Glibenclamide), and DPP-4 and SGLT-2 inhibitors [3,4].

In our previous work [5] we showed the efficacy of 7-substituted 3-benzyl-8-propylxanthin application in the treatment of metabolic syndrome pathologies, which possess high hypoglycemic effect. Thus, we can conclude, that synthesis of new xanthine-based drug candidates with hypoglycemic activity can be very promising.

Aim

The aim of the present study was to explore hypoglycemic activity of new water-soluble derivatives of 3-benzyl-8-substituted xanthines.

Materials and methods

In our previous study [6], we synthesized novel 3-benzyl-8-R-xanthine derivatives. Conversion of the latter into water-soluble potassium salts gave us good objects for pharmacological study.

Potassium 3-benzyl-8-R-xanthin-7-ides (1a–8a). A mixture of 0.003 mol of the original xanthine (1–8) and 0.0036 mol of potassium hydroxide in 10 ml of water was boiled until completely dissolved. The solution was filtered hot, and the filtrate was cooled, acetone was added until product precipitation, which was filtered off, washed with acetone, diethyl ether and dried at 80–85 °C (*Fig. 1*). All synthesized substances were crystalline colorless or yellowish powders, well soluble in water, insoluble in acetone and chloroform.

The melting point was determined by open capillary method on the PTM device (M). Elemental analysis was performed on an Elementar Vario L cube. ^{1}H NMR spectra of the compounds 1–8 revealed protons' signals of the xanthine moiety. Meanwhile, the protons of the aliphatic and aromatic groups resonate with the corresponding shifts [6]. ^{1}H NMR spectra recorded on a Brucker SF-400 spectrometer (solvent DMSO-*d6* or DMSO-*d6* + CDCl4, internal standard – TMS).

The hypoglycemic activity of xanthine derivatives was assessed by an oral glucose tolerance test [7]. This test is performed after 12 hours of fasting and it's started from the first blood sample taken out of incision at the tip of the tail (time -0.5 hours) and test solutions are inserted. After 30 minutes (0 hours), the blood glucose level is measured, then the rats are injected with 20 % glucose solution (2 g/kg body weight) through a rigid gastric tube. Blood samples are collected at 0.5, 1, 2 and 4 hours.

A drop of blood was obtained by creating a puncture at the end of the tail with a scalpel. The basal level of glucose in the blood was measured (-0.5 hours). All subsequent samples were taken through this wound. The total amount of blood used was approximately 30–40 µl.

In experimental studies, 70 Wistar rats about 300 g were used, which underwent the acclimatization for 14 days.

All manipulations with animals were performed out in accordance with the requirements of GLP, the recommendations of "European Union Directive 2010/63 / EU on the protection of animals used for scientific purposes" [8].

Table 1. Hypoglycemic activity of synthesized compounds (**1a–8a**)

	Time, min						AUC, mmol*h/l
	-30	0	30	60	120	240	
1a	4.00 ± 0.07 -11.11 *P < 0.001 **P < 0.01	4.54 ± 0.05 1.92 *P > 0.05 **P > 0.05	9.27 ± 0.10 12.28 *P < 0.001 **P < 0.01	9.00 ± 0.12 0.64 *P > 0.05 **P > 0.05	5.99 ± 0.12 -3.90 *P > 0.05 **P > 0.05	6.61 ± 0.06 1.09 *P > 0.05 **P > 0.05	30.25 ± 0.20 0.58 *P > 0.05 **P > 0.05
2a	4.47 ± 0.07 -0.63 *P > 0.05 **P > 0.05	4.47 ± 0.05 0.32 *P > 0.05 **P > 0.05	8.40 ± 0.14 1.73 *P > 0.05 **P > 0.05	8.96 ± 0.14 0.16 *P > 0.05 **P > 0.05	6.16 ± 0.10 -1.15 *P > 0.05 **P > 0.05	6.23 ± 0.08 -4.80 *P < 0.01 **P < 0.01	29.74 ± 0.20 -1.13 *P > 0.05 **P > 0.05
3a	4.47 ± 0.09 -0.63 *P > 0.05 **P > 0.05	4.31 ± 0.03 -3.21 *P > 0.05 **P < 0.05	8.90 ± 0.12 7.79 *P < 0.01 **P < 0.01	9.36 ± 0.11 4.63 *P < 0.05 **P < 0.05	6.51 ± 0.06 4.59 *P < 0.05 **P < 0.01	6.51 ± 0.06 -0.44 *P > 0.05 **P > 0.05	31.03 ± 0.17 3.17 *P < 0.001 **P < 0.01
4a	4.81 ± 0.08 6.98 *P < 0.05 **P < 0.01	4.43 ± 0.04 -0.64 *P > 0.05 **P > 0.05	8.20 ± 0.13 -0.69 *P > 0.05 **P > 0.05	8.97 ± 0.12 0.32 *P > 0.05 **P > 0.05	5.17 ± 0.06 -16.97 *P < 0.001 **P < 0.001	5.87 ± 0.07 -10.26 *P < 0.001 **P < 0.001	27.88 ± 0.15 -7.32 *P < 0.001 **P < 0.01
5a	4.47 ± 0.06 -0.63 *P > 0.05 **P > 0.05	4.19 ± 0.03 -6.09 *P < 0.05 **P < 0.01	8.37 ± 0.11 1.38 *P > 0.05 **P > 0.05	9.61 ± 0.12 7.51 *P < 0.01 **P < 0.01	6.29 ± 0.07 0.92 *P > 0.05 **P > 0.05	6.56 ± 0.05 0.22 *P > 0.05 **P > 0.05	30.59 ± 0.16 1.72 *P < 0.05 **P < 0.05
6a	4.37 ± 0.08 -2.86 *P > 0.05 **P > 0.05	4.3 ± 0.08 -3.53 *P > 0.05 **P < 0.05	8.01 ± 0.13 -2.94 *P > 0.05 **P > 0.05	9.09 ± 0.10 1.6 *P > 0.05 **P > 0.05	6.41 ± 0.07 2.98 *P > 0.05 **P > 0.05	6.47 ± 0.04 -1.09 *P > 0.05 **P > 0.05	30.16 ± 0.21 0.27 *P > 0.05 **P > 0.05
7a	5.11 ± 0.06 13.65 *P < 0.001 **P < 0.001	4.40 ± 0.07 -1.28 *P > 0.05 **P > 0.05	7.84 ± 0.12 -5.02 *P < 0.05 **P < 0.05	9.5 ± 0.16 6.23 *P < 0.05 **P < 0.05	6.61 ± 0.15 6.19 *P < 0.05 **P < 0.05	7.43 ± 0.05 13.54 *P < 0.001 **P < 0.001	31.88 ± 0.24 5.99 *P < 0.001 **P < 0.01
8a	4.46 ± 0.08 -0.95 *P > 0.05 **P > 0.05	4.63 ± 0.07 3.85 *P > 0.05 **P > 0.05	7.14 ± 0.14 -13.49 *P < 0.001 **P < 0.01	9.71 ± 0.09 8.63 *P < 0.001 **P < 0.01	6.31 ± 0.12 1.38 *P > 0.05 **P > 0.05	6.90 ± 0.04 5.46 *P < 0.001 **P < 0.001	30.66 ± 0.29 1.94 *P > 0.05 **P > 0.05
Control group	4.5 ± 0.07 0 *P = 1 **P > 0.05	4.46 ± 0.08 0 *P = 1 **P > 0.05	8.26 ± 0.10 0 *P = 1 **P > 0.05	8.94 ± 0.10 0 *P = 1 **P > 0.05	6.23 ± 0.08 0 *P = 1 **P > 0.05	6.54 ± 0.04 0 *P = 1 **P > 0.05	30.08 ± 0.15 0 *P = 1 **P > 0.05
Gliben clamide	4.61 ± 0.086 2.54 *P > 0.05 **P > 0.05	4.54 ± 0.037 1.92 *P > 0.05 **P < 0.05	7.40 ± 0.14 -10.38 *P < 0.001 **P < 0.01	5.46 ± 0.15 -38.98 *P < 0.001 **P < 0.01	5.13 ± 0.13 -17.66 *P < 0.001 **P < 0.01	4.33 ± 0.06 -33.84 *P < 0.001 **P < 0.001	23.24 ± 0.33 -22.73 *P < 0.001 **P < 0.01

Mean ± standard deviation of the arithmetic mean (mmol/l), the ratio to the control group in %: ***P:** Student's t-test; ****P:** Mann–Whitney U test.

Rats were obtained from the regular animal chamber of the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine. Care, keeping and feeding of animals was carried out in standard conditions of stable microclimate in the vivarium of the ZSMU. A light-dark regime was maintained at 12:12. Animals had ad libitum access to food (standardized ration feed "Rezon-1" KP-120-1) and water that was processed (acidified (pH 5.8–6.4), chlorinated (6–8 ppm), softened, and filtered (0.02 μm)).

Experimental samples' sizes were calculated by the formula [9] with standard deviation (±1.31 mmol/l) [10]. We expect the effect to be 2 mmol/l [11], where the sample size n = 2; 1.312 (1.96 + 0.842)2/22 = 6.74, i. e., 7 animals per study group.

Randomization and grouping were performed using pseudo-random sequences of the "random" module for the python programming language [12].

The results were processed by modern computer-aided statistical methods using NumPy (BSD License), SciPy (BSD License), pandas (BSD License), pandas-profiling (MIT License) libraries. For data visualization Python matplotlib library (BSD License) was used [13].

The normality hypothesis test of the studied indicators was tested using the Shapiro–Wilk test and the Kolmogorov–Smirnov test. The arithmetic mean (M) and standard error of the mean (±m) were calculated. The statistical significance of intergroup differences according to the obtained data was established using the parametric Student's t-test (*) and the non-parametric Mann–Whitney *U* test (P***).

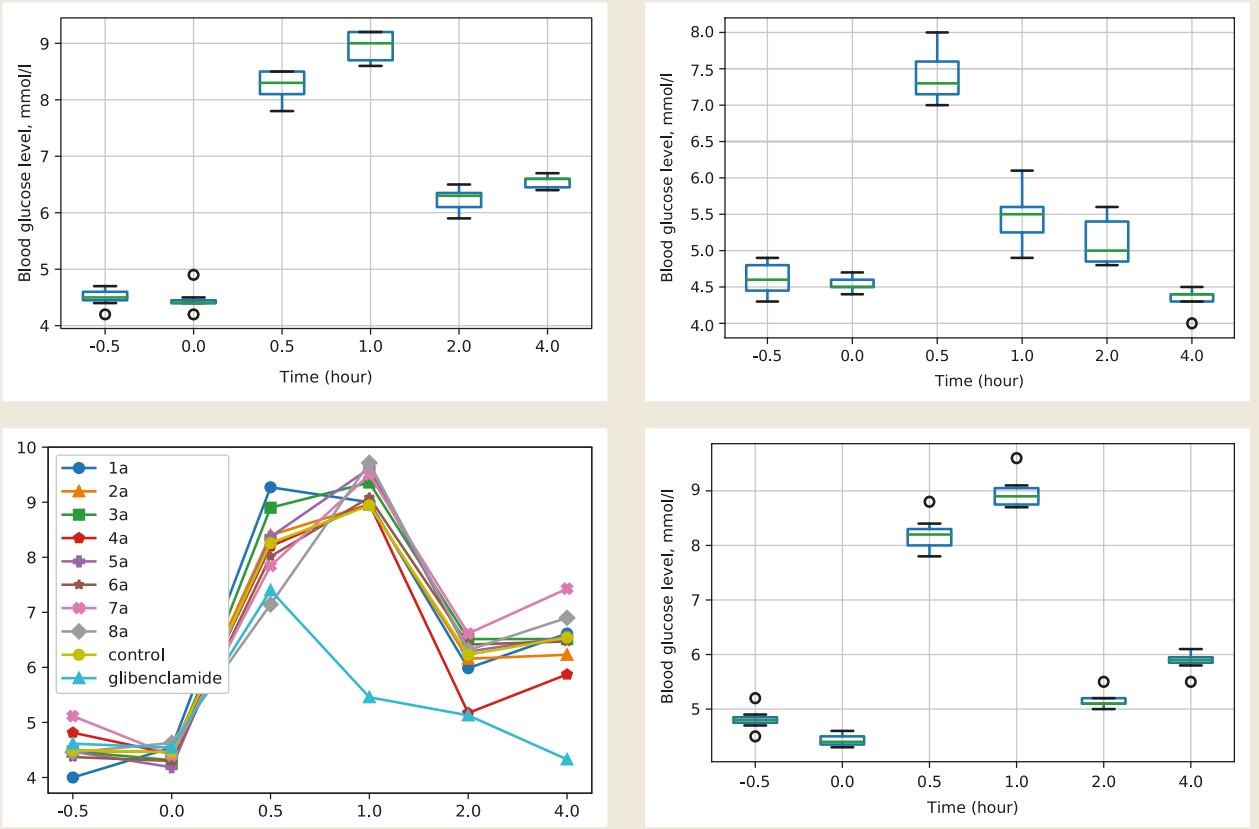

Fig. 2. Changes in glucose levels of experimental animals under OGTT conditions.

The research results were interpreted by applying three difference levels of statistical significance – $P < 0.05$, $P < 0.01$ and $P < 0.001$.

Results

The results of the study of hypoglycemic activity of xanthine derivatives by the oral glucose tolerance test are shown in *Table 1* and *Fig. 2*.

Discussion

After processing and analyzing the results (*Table 1, Fig. 2*), it was found that glibenclamide was most effectively reduced glucose in OGTT conditions (AUC 23.24 ± 0.33), which was 22.73 lower than in the control group (*$P < 0.001$, **$P < 0.01$).

An increase in glucose levels was observed in all groups in 30 min after administration of glucose solution, in compounds **2a, 4a, 5a, 6a** this indicator was equal to the control group (*/**$P > 0.05$), and no statistically significant differences were observed. The presence of the hydrazide group in xanthine moiety (**1a**) led to a significant hyperglycemic action in 1 h after its administration, while the replacement of the hydrazide group by the ylidene hydrazide moiety led to increased hypoglycemic effect in compounds **2a, 3a, 4a, 5a**. The introduction of carbathioamide fragments into xanthine core (**7a, 8a**) led to an increase of glucose level.

In 2 h and 4 h, only compound **4a** approached the standard of glibenclamide by its hypoglycemic activity. Thus, we can conclude, that the presence of a hydroxyl group as a substituent in the benzyl moiety of ylidenhydrazides positively contributes to glucose lowering. It also worth mentioning, that halogens and/or methyl groups do not affect hypoglycemic action.

Conclusions

The results of the hypoglycemic activity study among the potassium salts of 3-benzyl-8-substituted xanthine derivatives showed, that some new compounds were in close vicinity to the reference drug's hypoglycemic action.

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References

[1] WHO (2016, October 21). Global report on diabetes: Executive Summary. https://www.who.int/publications/i/item/who-nmh-nvi-16.3
[2] Humphrey, L. L., Kansagara, D., Qaseem, A., High Value Care Committee of the American College of Physicians, Centor, R., DeLong, D. M., Gantzer, H. E., Horwitch, C. A., Jokela, J. A., Li, J., Lohr, R. H., López, A. M., & McLean, R. M. (2018). World Health Organization Guidelines on Medicines for Diabetes Treatment Intensification: Commentary From the American College of Physicians High Value Care Committee. *Annals of internal medicine*, *169*(6), 398-400. https://doi.org/10.7326/M18-1148
[3] Doyle-Delgado, K., Chamberlain, J. J., Shubrook, J. H., Skolnik, N., & Trujillo, J. (2020). Pharmacologic Approaches to Glycemic Treatment of Type 2 Diabetes: Synopsis of the 2020 American Diabetes Association's Standards of Medical Care in Diabetes Clinical Guideline. *Annals of internal medicine*, *173*(10), 813-821. https://doi.org/10.7326/M20-2470
[4] Inoue, H., Tamaki, Y., Kashihara, Y., Muraki, S., Kakara, M., Hirota, T., & Ieiri, I. (2019). Efficacy of DPP-4 inhibitors, GLP-1 analogues, and SGLT2 inhibitors as add-ons to metformin monotherapy in T2DM patients: a model-based meta-analysis. *British journal of clinical pharmacology*, *85*(2), 393-402. https://doi.org/10.1111/bcp.13807
[5] Mykhalchenko, Y., Aleksandrova, K., Shkoda, O., & Pruglo, Y. (2019). Prospective biological active compounds among 7-substituted of 3-benzyl-8-propylxanthines for treatment of metabolic syndrome pathologies. *Eureka: Health Sciences*, (5), 52-58. https://doi.org/10.21303/2504-5679.2019.00988
[6] Aleksandrova, K. V., Levich, S. V., Mykhal'chenko, Ye. K., & Sinchenko, D. M. (2016). Synthesis and physical-chemical properties of 3-aryl-(aralkyl)-8-hydrazinemethylxanthines and their N-substituted derivatives. *Current issues in pharmacy and medicine: science and practice*, (2), 26-31. https://doi.org/10.14739/2409-2932.2016.2.70906
[7] Kuo, F. Y., Cheng, K. C., Li, Y., & Cheng, J. T. (2021). Oral glucose tolerance test in diabetes, the old method revisited. *World journal of diabetes*, *12*(6), 786-793. https://doi.org/10.4239/wjd.v12.i6.786
[8] European Parliament (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection ofanimals used for scientific pur poses. *Official Journal of the European Communities*, *L276*, 33-79. https://www.legislation.gov.uk/eudr/2010/63/contents
[9] Charan, J., & Kantharia, N. D. (2013). How to calculate sample size in animal studies?. *Journal of pharmacology & pharmacotherapeutics*, *4*(4), 303-306. https://doi.org/10.4103/0976-500X.119726
[10] Wang, Z., Yang, Y., Xiang, X., Zhu, Y., Men, J., & He, M. (2010). [Estimation of the normal range of blood glucose in rats]. *Wei sheng yan jiu = Journal of hygiene research*, *39*(2), 133-142.
[11] Chaimum-aom, N., Chomko, S., & Talubmook, C. (2017). Toxicology and oral glucose tolerance test (OGTT) of Thai medicinal plant used for diabetes controls, Phyllanthus acidus L.(Euphorbiaceae). *Pharmacognosy Journal*, *9*(1), 58-61. https://doi.org/10.5530/pj.2017.1.11
[12] Frassia, M. G. (2018, March 5-7). Enhanced statistical thinking in secondary school with python programming language: a realistic mathematics education approach. *Proceedings of the 12th International Technology, Education and Development Conference* (pp. 3462-3471). http://dx.doi.org/10.21125/inted.2018.0666
[13] Python. *GitHub*. https://github.com/chendaniely/2021-07-13-scipy-pandas