



(51) International Patent Classification:

C07F 9/54 (2006.01) A61P 35/00 (2006.01)
A61P 3/10 (2006.01) A61K 31/66 (2006.01)

(21) International Application Number:

PCT/CZ2015/050016

(22) International Filing Date:

23 December 2015 (23.12.2015)

(25) Filing Language:

Czech

(26) Publication Language:

English

(30) Priority Data:

PV2015-227 31 March 2015 (31.03.2015) CZ

(71) Applicants: **KKCG SE** [CY/CY]; Christodoulides Building, 8 Alasias Street, Limassol, CY-3095 (CY). **SMART BRAIN S.R.O.** [CZ/CZ]; Videnska 1083, 142 20 Praha 4 - Kre (CZ). **MITOTAX S.R.O.** [CZ/CZ]; Sokolovska 47/73, 186 00 Praha 8 - Karlin (CZ). **BIOTECHNOLOGICKY USTAV AV CR, V.V.I.** [CZ/CZ]; Prumyslova 595, Vestec, 252 42 Jesenice u Prahy (CZ).

(72) Inventors: **NEUZIL, Jiri**; Poljanovova 3158, 143 00 Praha 4 (CZ). **WERNER, Lukas**; Chomutovska 1229, 432 01 Kadan (CZ). **STURSA, Jan**; Petyrkova 1948, 148 00 Praha 4 (CZ).

(74) Agent: **HARTVICOVA, Katerina**; INVENTIA s.r.o., Na Belidle 3, 15000 Praha 5 (CZ).

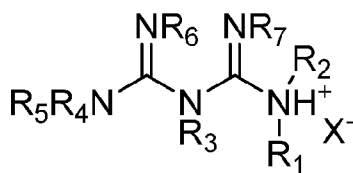
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

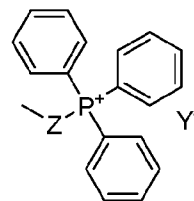
Published:

— with international search report (Art. 21(3))

(54) Title: TRIPHENYLPHOSPHONIUM BIGUANIDE ANALOGUES, THEIR METHOD OF PREPARATION AND USE AS DRUGS



(I)



(II)

(57) Abstract: The present invention provides triphenylphosphonium biguanide analogs of general formula (I), whereas the general formula (I) includes resonance structures and pharmaceutically acceptable salts, protonated form as well as free base, (Formula (I)) where each one of R1, R2, R3, R4, R5, R6, R7 is independently selected from the group comprising H; C1-C6 alkyl; C6-C10 aryl; (C1-C6)alkyl(C6-C10)aryl; -C(=O)-R'; -C(=O)OR'; -C(=O)NR'R''; -C(=S)R'; -C(=S)NR'R''; wherein R' and R'' are independently selected from the group comprising H, C1-C6 alkoxy, C1-C6 alkyl, C6-C10aryl, (C1-C6)alkyl(C6-C10)aryl; whereas C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl can be unsubstituted or substituted by one or more substituents selected independently from the group comprising C1-C4 alkyl, N(H or C1-C4 alkyl)2, wherein alkyls are the same or different, phenyl, benzyl, OH, SH, F, Cl, Br, I, C1-C4alkoxy, C1-C4 acyloxy, C1-C4 mercapto; and substituent of general formula (II) (Formula (I)) wherein Z is as defined in the claims, whereas at least one of R1, R2, R3, R4, R5, R6, R7 is the substituent of general formula (II), X- is a pharmaceutically acceptable anion, preferably an anion of inorganic or organic acid, Y- is a pharmaceutically acceptable anion, preferably an anion of inorganic or organic acid. The invention further provides a method of preparation of these derivatives. These new compounds are suitable in particular for the treatment of diabetes mellitus type 2 and/or pancreatic carcinoma.



Triphenylphosphonium biguanide analogues, their method of preparation and use as drugs

Field of Art

- 5 The invention relates to novel biguanide derivatives with high effectivity against type 2 diabetes mellitus and pancreatic cancer.

Background Art

- 10 Type 2 diabetes mellitus (T2DM) is a metabolic disease affecting a growing number of subjects in industrialised countries. During last decades the number of patients suffering from T2DM increased in an unprecedented manner. It is expected that around 2030 the number of patients suffering from this disease will double. At present, the disease can be considered a civilization epidemic. T2DM is resistant to insulin treatment. The most
15 commonly used drug against this pathology worldwide is metformin, prescribed to tens of millions of patients. Metformin decreases the level of glucose, which is released by glycogenolysis or synthesized by gluconeogenesis in hepatic cells. Metformin is a biguanide drug with low risk of side effects and complications. Oxidative phosphorylation-related processes are affected also by other clinically relevant biguanides such as
20 fenformin, buformin, proguanil and cycloguanil or chlorhexidine.

- Epidemiological and clinical studies clearly show that T2DM is related to neoplastic diseases (Richardson LC, Pollack LA. Nat Clin Pract Oncol. 2005, 2, 48-53), in particular to pancreatic cancer (Bosetti C et al. Ann Oncol 2014 25, 2065-2072. Rahman A. Lancet Oncol 2014
25 15, e420). T2DM is sometimes considered to a "precarcinogenic" stage of pancreatic cancer (Eijgenraam P et al. Br J Cancer 2013, 109, 2924-2932). Pancreatic carcinoma is an extremely hard to treat type of cancer, with basically resection being an option. This is possible only in a limited group of patients, depending on the location and stage of the carcinoma. Pancreatic cancer belongs to neoplastic diseases with the highest number of deaths. One of the complicating factors in
30 pancreatic cancer stems from the fact that up to 90 % of patients is are positive for the oncogene Ras, that causes malignant transformations and considerably complicates therapy.

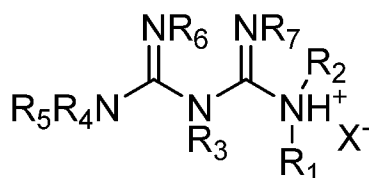
Metformin, the most commonly prescribed drug against T2DM, has diabetes mellitus type 2 patients was found to suppress pancreatic cancer, although the effect is not very high and

the compound is efficient at high, milimolar concentrations (Gong J et al. Front Physiol 2014, 5, 426).

- 5 T2DM as well as pancreatic cancer are very serious conditions that are at present almost untreatable and the incidence of which is constantly increasing. There is a strong need to find novel agents and novel medical approaches against these diseases.

Disclosure of the Invention

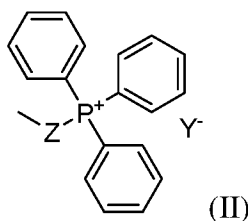
- 10 The present invention provides a new generation of substances derived from the basic structure of alkylated biguanides (buformin, metformin and fenformin) that show 3-4 orders of magnitude higher effect against pancreatic cancer and T2DM compared to metformin, while not causing toxic effects in experimental animals.
- 15 The present invention thus provides triphenylphosphonium biguanide analogues of the general formula I, whereas the general formula I shall be considered to include also resonance (isomeric) structures and pharmaceutically acceptable salts, protonated biguanide forms as well as free bases,



- 20 (I)

- wherein each of R1, R2, R3, R4, R5, R6, R7 is independently selected from the group comprising H; C1-C6 alkyl; C6-C10 aryl; (C1-C6)alkyl(C6-C10)aryl; -C(=O)-R'; -C(=O)OR'; -C(=O)NR'R''; -C(=S)R'; -C(=S)NR'R''; wherein R' and R'' are independently selected from the group comprising H, C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl; whereas C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl can be unsubstituted or substituted by one or more substituents selected independently from the group comprising C1-C4 alkyl, N(H or C1-C4 alkyl)₂, whereas alkyls are the same or different, phenyl, benzyl, OH, SH, F, Cl, Br, I, C1-C4 alkoxy, C1-C4 acyloxy, C1-C4 mercapto; and substituent of general formula II
- 25

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wherein Z is a linear hydrocarbyl chain selected from alkylene, alkenylene or alkynylene, containing 2 to 20 carbon atoms, preferably 4 to 14 carbon atoms, more preferably 8 to 12 carbon atoms, whereas optionally one or more carbon atoms couples in the hydrocarbyl chain may be replaced by one or more 5-membered or 6-membered aromatic rings or heteroaromatic rings containing the heteroatoms O, S and/or N, preferably phenylenes or pyridylenes, and/or one or more carbon atoms in the hydrocarbyl chain may be replaced by one or more heteroatoms selected from O, S, NH, and whereas the hydrocarbyl chain can be unsubstituted or substituted by one or more substituents selected independently from the group comprising C1-C4 alkyl, N(H or C1-C4 alkyl)₂, wherein alkyls are the same or different, phenyl, benzyl, OH, SH, F, Cl, Br, I, C1-C4 alkoxy, C1-C4 acyloxy, C1-C4 mercapto, whereas at least one of R₁, R₂, R₃, R₄, R₅, R₆, R₇ is a substituent of general formula II,

X⁻ is a pharmaceutically acceptable anion, in particular anion of inorganic or organic acid, particularly suitable are Cl⁻, Br⁻, I⁻, sulphate, mesyl, acetate, formate, succinate, Y⁻ is a pharmaceutically acceptable anion, in particular anion of inorganic or organic acid, particularly suitable are Cl⁻, Br⁻, I⁻, sulphate, mesyl, acetate, formate, succinate.

In one preferred embodiment, R₁ is a substituent of general formula II, R₂-R₇ are independently selected from the group comprising H, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl, -C(=O)-R', -C(=O)OR', -C(=O)NR'R'', -C(=S)R', -C(=S)NR'R'', wherein R' and R'' are independently selected from the group comprising H, C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl; whereas C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl can be unsubstituted or substituted by one or more substituents selected independently from the group comprising C1-C4 alkyl, N(H or C1-C4 alkyl)₂, wherein alkyls are the same or different, phenyl, benzyl, OH, SH, F, Cl, Br, I, C1-C4 alkoxy, C1-C4 acyloxy, C1-C4 mercapto.

In another preferred embodiment of the invention, R₁ and R₂ are substituents of general

formula II, R3-R7 are independently selected from the group comprising H, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl, -C(=O)-R', -C(=O)OR', -C(=O)NR'R'', -C(=S)R', -C(=S)NR'R'', wherein R' and R'' are independently selected from the group comprising H, C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl; whereas C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl can be unsubstituted or substituted by one or more substituents selected independently from the group comprising C1-C4 alkyl, N(H or C1-C4 alkyl)₂, wherein alkyls are the same or different, phenyl, benzyl, OH, SH, F, Cl, Br, I, C1-C4 alkoxy, C1-C4 acyloxy, C1-C4 mercapto.

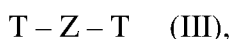
In yet another preferred embodiment of the invention, R1 and R4 are substituents of general formula II, R2, R3, R5-R7 are independently selected from the group comprising H, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl, -C(=O)-R', -C(=O)OR', -C(=O)NR'R'', -C(=S)R', -C(=S)NR'R'', wherein R' and R'' are independently selected from the group comprising H, C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl; whereas C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl can be unsubstituted or substituted by one or more substituents selected independently from the group comprising C1-C4 alkyl, N(H or C1-C4 alkyl)₂, wherein alkyls are the same or different, phenyl, benzyl, OH, SH, F, Cl, Br, I, C1-C4 alkoxy, C1-C4 acyloxy, C1-C4 mercapto.

In another preferred embodiment of the invention, R3 is a substituent of general formula II, R1-R2, R4-R7 are independently selected from the group comprising H, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl, -C(=O)-R', -C(=O)OR', -C(=O)NR'R'', -C(=S)R', -C(=S)NR'R'', wherein R' and R'' are independently selected from the group comprising H, C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl; whereas C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl can be unsubstituted or substituted by one or more substituents selected independently from the group comprising C1-C4 alkyl, N(H or C1-C4 alkyl)₂, wherein alkyls are the same or different, phenyl, benzyl, OH, SH, F, Cl, Br, I, C1-C4 alkoxy, C1-C4 acyloxy, C1-C4 mercapto.

In yet another preferred embodiment of the invention, R1 and R3 are substituents of general formula II, R2, R4-R7 are independently selected from the group comprising H,

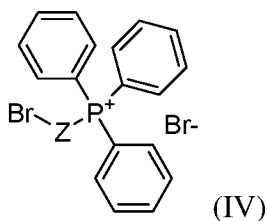
- C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl, -C(=O)-R', -C(=O)OR', -C(=O)NR'R'', -C(=S)R', -C(=S)NR'R'', wherein R' and R'' are independently selected from the group comprising H, C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl; whereas C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl
- 5 can be unsubstituted or substituted by one or more substituents selected independently from the group comprising C1-C4 alkyl, N(H or C1-C4 alkyl)₂, wherein alkyls are the same or different, phenyl, benzyl, OH, SH, F, Cl, Br, I, C1-C4 alkoxy, C1-C4 acyloxy, C1-C4 mercapto.
- 10 Preferably, C1-C6 alkyl is selected from methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl.
- Preferably, C6-C10 aryl is 4-chlorfenyl.
- Preferably, (C1-C6)alkyl(C6-C10)aryl is phenylethyl or 2-(2-acetoxy-4,6-dimethylphenyl)-propyl.
- 15 Preferably, the group R' in the substituent -C(=O)OR' is benzyl.

The present invention further provides a method for preparation of the compounds of general formula I, wherein in the first step, a compound of general formula III



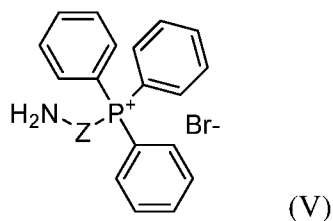
- 20 wherein T is halogen, mesyl, tosyl or other cleavable group and Z has the meaning as defined above,

is subjected to a reaction with triphenylphosphine, preferably in dimethylformamide (DMF), yielding triphenylphosphonium hydrocarbyl derivative of general formula IV



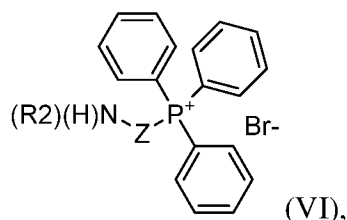
- 25 which is then treated with methanolic ammonia solution, yielding primary aminohydrocarbyl triphenylphosphonium of general formula V

6



or

with methanolic solution of (R₂)NH₂, yielding secondary amino-hydrocarbyl triphenylphosphonium of general formula VI



and the corresponding primary or secondary aminohydrocarbyl triphenylphosphonium, respectively, is subsequently condensed with 2-cyanoguanidine, preferably in DMF, yielding triphenylphosphonium hydrocarbyl biguanide derivative of general formula I.

- 10 The compounds of the present invention were tested for their biological effects and compared with the known compound - metformin (compound having the structure corresponding to formula I, wherein R₁ = R₂ = CH₃). In all cases we have found that the compounds of the present invention killed pancreatic carcinoma cells with effects higher by 3-4 orders of magnitude than those of metformin. This is unprecedented and very
- 15 unexpected. An important finding is that the compounds of the present invention do not show toxic effects on non-malignant cells, hence, they are selective in killing the cells of pancreatic carcinoma. The compounds of the present invention highly effectively inhibit growth of experimental pancreatic carcinoma.
- 20 Antidiabetics activity is characteristic in that these compounds decrease glucose levels in circulating blood. In diabetic patients, glucose levels are increased mainly as a result of glucose release from its storage source, which is glycogen in hepatocytes, by glycogenolysis, and also as a result of gluconeogenesis (formation of glucose). Metformin acts on these processes and thus decreases glucose levels in patients suffering from diabetes mellitus type 2. The compounds of the
- 25 present invention decrease glucose levels in concentrations which are 3 orders of magnitude lower than the effective concentrations of metformin.

Object of the present invention are thus compounds of general formula I for use as medicaments, in particular for use in a method of treatment of diabetes mellitus type 2 and/or pancreatic carcinoma.

5 Object of the present invention is use of compounds of general formula I for preparation of a medicament for the treatment of diabetes mellitus type 2 and/or pancreatic carcinoma.

Further, object of the present invention is a method of treatment of mammals, including human, in which one or more compounds of general formula I is administered to a subject suffering from diabetes mellitus type 2 and/or pancreatic carcinoma.

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Object of the present invention is also a pharmaceutical preparation containing at least one compound of general formula I and at least one pharmaceutical auxiliary substance, such as a carrier, a solvent, a filler, a colorant, a binder, etc.

15 Brief Description of Drawings

Fig.1 Concentration dependence of growth inhibition of PANC-1 cells as a response to studied compounds and metformin using the crystal violet method.

20 Table 1. IC₅₀ values for compounds 7, 8, 9 and 10, and metformin for suppression of growth of pancreatic cell lines (n.d. = not determined).

Fig. 2 IC₅₀ values for compound 7 and metformin for cancer cells PANC-1 and non-malignant BJ fibroblasts.

25 Fig. 3 A – The comparison of the effect of different concentrations of compound 7 on the growth curve of PANC-1 cells acquired by the xCellingence method. B – Concentration and time dependence of growth curve slope.

Fig. 4. IC₅₀ values for compounds 7, 15 and metformin determined from growth curves.

Fig. 5. Annexin test of apoptosis for pancreatic cancer cell lines using compound 7 and metformin.

30 Fig. 6. The comparison of relative level of active caspase-3, a marker of apoptosis detection for control cells, cells treated with compound 7, and cells treated with metformin. Treatment with staurosporin is used as positive control.

Fig. 7. Inhibition of experimental pancreatic cancer with compound 7 compared with the control.

Fig. 8. Concentration dependence of inhibition of cellular respiration via mitochondrial

complex I by compounds 7, 9, 10 and metformin.

Fig. 9. The effect of compound 7 and metformin on mitochondrial membrane potential in a pancreatic cell line PANC-1.

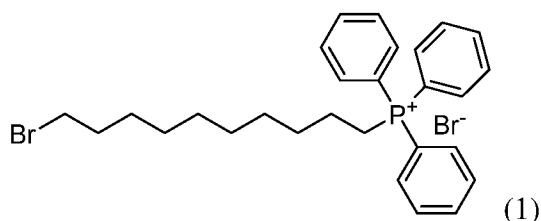
Fig. 10. The effect of compound 7 and metformin on formation of reactive oxygen species.

5 Fig. 11. Suppression of glucose level in HepG2 cells by compound 7 and metformin.

Examples of carrying out the Invention

Example 1

10 1,10-Dibromodecane (5.72 g, 19.063 mmol) and triphenylphosphine (1 g, 3.813 mmol) were dissolved in DMF (5 ml) and the mixture was heated at 90 °C for 12 h. The reaction mixture was cooled to room temperature, dissolved in minimal amount of dichloromethane (5 ml) and added drop-wise to cold (0 °C) diethylether (200 ml). The resulting precipitate was decanted, and concentrate was purified using column chromatography on silicagel (40
15 mL) (chloroform/methanol gradient 0-10% of methanol) to obtain desired (10-bromodecyl) triphenylphosphonium bromide (1.98 g, 92 %) of the formula 1.



1H NMR (401 MHz, Methanol-d₄) δ 8.03 – 7.64 (m, 15H), 3.50 – 3.36 (m, 4H), 1.84 (p, J = 14.4, 6.8 Hz, 2H), 1.79 – 1.63 (m, 2H), 1.63 – 1.52 (m, 2H), 1.50 – 1.22 (m, 10H).

13C NMR (101 MHz, Methanol-d₄) δ 134.87 (d, J = 3.0 Hz), 133.43 (d, J = 10.0 Hz), 130.13 (d, J = 12.5 Hz), 118.62 (d, J = 86.3 Hz), 33.14, 32.54, 30.17 (d, J = 16.1 Hz), 28.93, 28.83, 28.43, 28.33, 27.69, 22.13 (d, J = 4.4 Hz), 21.29 (d, J = 51.0 Hz).

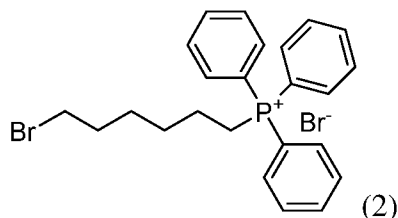
IR (KBr pellet): ν = 3052, 3006, 2988, 2926, 2853, 2792, 1918, 1830, 1617, 1587, 1485,
25 1465, 1438, 1338, 1317, 1251, 1189, 1161, 1113, 996, 750, 723, 691.

HRMS calculated for C₂₈H₃₅BrP: 481.16543 and 483.16338, found: 481.16534 and 483.16318.

Example 2

30 (1-bromohexyl)triphenylphosphonium bromide of formula 2 was prepared using the same

procedure as described in example 1.



^1H NMR (401 MHz, Methanol- d_4) δ 7.96 – 7.74 (m, 15H), 3.52 – 3.39 (m, 4H), 1.83 (p, J = 6.8 Hz, 2H), 1.78 – 1.67 (m, 2H), 1.67 – 1.56 (m, 2H), 1.56 – 1.45 (m, 2H).

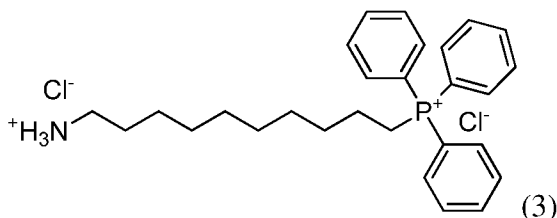
^{13}C NMR (101 MHz, Methanol- d_4) δ 134.88 (d, J = 3.0 Hz), 133.45 (d, J = 10.0 Hz), 130.16 (d, J = 12.6 Hz), 118.56 (d, J = 86.3 Hz), 32.75, 32.18, 29.29 (d, J = 16.4 Hz), 27.00, 22.04 (d, J = 4.3 Hz), 21.28 (d, J = 51.2 Hz).

IR (KBr pellet): ν = 3053, 3040, 3005, 2990, 2938, 2860, 2796, 2212, 2006, 1931, 1821, 1685, 1610, 1587, 1575, 1483, 1459, 1435, 1333, 1252, 1186, 1110, 995, 786, 751, 722, 692.

HRMS calculated for $\text{C}_{24}\text{H}_{27}\text{BrP}$: 425.10283 and 427.10078, found: 425.10279 and 427.10061.

Example 3

(10-bromodecyl)triphenylphosphonium bromide (9.26 g, 16.448 mmol) was dissolved in 7M solution of ammonia in methanol (60 ml) and the reaction mixture was stirred at 50 °C. After 6 h, additional ammonia in methanol (40 ml) was added and the reaction mixture was heated for additional 24 h at 50 °C. The reaction mixture was concentrated under reduced pressure, and crude product was purified using column chromatography on silicagel (200 ml) (chloroform/methanol gradient 0-10% of methanol). The product was acidified with HCl (36%, 5 ml) and filtered through Dowex 1x8 in chloride form (50 g) to obtain desired (10-aminodecyl)triphenylphosphonium chloride hydrochloride (5.135 g, 63%) of the formula 3.



¹H NMR (500 MHz, Methanol-d₄) δ 7.92 – 7.85 (m, 3H), 7.85 – 7.68 (m, 12H), 3.46 – 3.37 (m, 2H), 2.90 (t, J = 7.5 Hz, 2H), 1.72 – 1.60 (m, 4H), 1.56 (p, J = 7.5 Hz, 2H), 1.43 – 1.20 (m, 12H);

¹³C NMR (126 MHz, Methanol-d₄) δ 136.23 (d, J = 3.0 Hz), 134.79 (d, J = 9.9 Hz), 131.51 (d, J = 12.5 Hz), 120.00 (d, J = 86.3 Hz), 40.76, 31.59 (d, J = 16.2 Hz), 30.29 (2C), 30.12, 29.89, 28.53, 27.42, 23.57 (d, J = 4.4 Hz), 22.68 (d, J = 51.1 Hz).

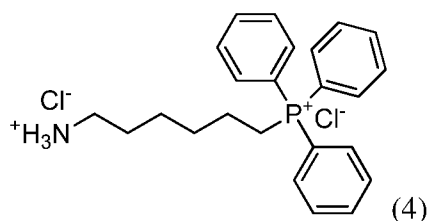
IR (KBr pellet): ν = 3051, 3007, 2927, 2854, 2006, 1825, 1601, 1587, 1485, 1465, 1438, 1402, 1337, 1318, 1189, 1161, 1113, 996, 751, 723, 691.

HRMS calculated for C₂₈H₃₇NP: 418.26581, found: 418.26567.

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Example 4

(6-aminohexyl)triphenylphosphonium chloride hydrochloride of formula 4 was prepared using the same procedure as described in example 3.



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¹H NMR (401 MHz, Methanol-d₄) δ 8.01 – 7.65 (m, 15H), 3.59 – 3.45 (m, 2H), 3.37 (s, 1H, NHD), 2.96 (t, J = 7.5 Hz, deuterium coupling, J = 28.0 Hz, 2H), 1.81 – 1.59 (m, 6H), 1.48 (p, J = 6.8 Hz, 2H).

¹³C NMR (101 MHz, Methanol-d₄) δ 134.86 (d, J = 3.0 Hz), 133.50 (d, J = 10.0 Hz), 130.17 (d, J = 12.6 Hz), 118.56 (d, J = 86.4 Hz), 39.20, 29.59 (d, J = 16.7 Hz), 26.85, 25.32, 22.03 (d, J = 4.2 Hz), 21.35 (d, J = 51.3 Hz).

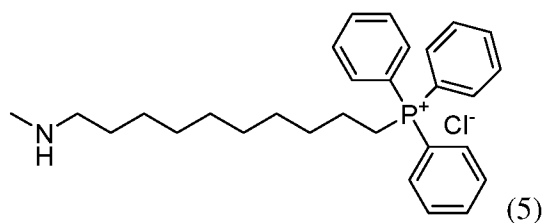
IR (KBr pellet): ν = 3410, 2935, 2864, 2616, 2521, 2008, 1830, 1600, 1586, 1484, 1463, 1437, 1337, 1317, 1187, 1161, 1113, 1026, 996, 940, 749, 723, 690.

Example 5

(10-bromodecyl)triphenylphosphonium bromide (562 mg, 1 mmol) was dissolved in 2M solution of methylamine in methanol (2 ml) and the reaction mixture was stirred at 50 °C for 24 h. The mixture was concentrated under reduced pressure and crude product was purified using column chromatography on silicagel (chloroform/methanol gradient 0-10% of methanol). The resulting (10-((methylamino)decyl)triphenylphosphonium bromide (485

30

mg, 95%) was filtered through Dowex 1x8 in chloride form to obtain the desired (10-((methylamino)decyl)triphenylphosphonium chloride of the formula 5.



5

^1H NMR (401 MHz, Methanol- d_4) δ 8.01 – 7.69 (m, 15H), 3.52 – 3.40 (m, 2H), 3.01 (t, J = 7.7 Hz, 2H), 2.72 (s, 3H), 1.78 – 1.64 (m, 4H), 1.59 (p, J = 6.7 Hz, 2H), 1.46 – 1.24 (m, 10H).

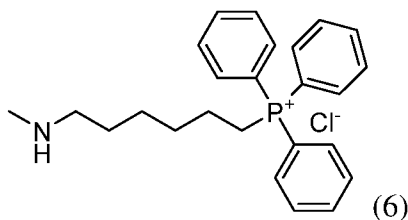
^{13}C NMR (101 MHz, Methanol- d_4) δ 134.85 (d, J = 3.0 Hz), 133.46 (d, J = 10.0 Hz), 130.15 (d, J = 12.5 Hz), 118.63 (d, J = 86.3 Hz), 49.07, 32.29, 30.20 (d, J = 16.1 Hz), 28.87, 28.85, 28.70, 28.49, 28.49, 22.20 (d, J = 4.4 Hz), 21.34 (d, J = 50.9 Hz).

IR (KBr pellet): ν = 3360, 3052, 3008, 2932, 2920, 2852, 2779, 2756, 2729, 2513, 2554, 2410, 2378, 2009, 1934, 1905, 1825, 1778, 1715, 1616, 1586, 1554, 1480, 1469, 1437, 1411, 1318, 1190, 1161, 1113, 1035, 997, 959, 866, 768, 753, 739, 725, 713, 692.

15 HRMS calculated for $\text{C}_{29}\text{H}_{39}\text{NP}$: 432.28146, found: 432.28135.

Example 6

((6-methylamino)hexyl)triphenylphosphonium chloride of the formula 6 was prepared using the same procedure as described in example 5.



20

^1H NMR (401 MHz, Methanol- d_4) δ 8.00 – 7.57 (m, 15H), 3.58 – 3.44 (m, 2H), 3.02 (t, J = 7.6 Hz, 2H), 2.71 (s, 3H), 1.82 – 1.58 (m, 6H), 1.48 (p, J = 6.8 Hz, 2H).

^{13}C NMR (101 MHz, Methanol- d_4) δ 134.87 (d, J = 3.0 Hz), 133.50 (d, J = 10.0 Hz), 130.17 (d, J = 12.6 Hz), 118.55 (d, J = 86.4 Hz), 48.81, 32.29, 29.51 (d, J = 16.7 Hz), 25.44, 25.28, 21.97 (d, J = 4.2 Hz), 21.32 (d, J = 51.3 Hz).

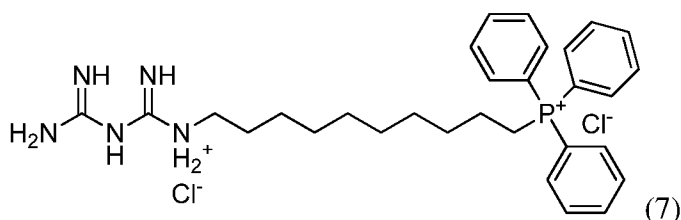
25

IR (KBr pellet): ν = 3409, 3051, 3007, 2935, 2864, 2739, 2417, 2215, 2002, 1924, 1832, 1783, 1614, 1586, 1484, 1462, 1438, 1318, 1189, 1162, 1113, 996, 751, 723, 691.

HRMS calculated for C₂₅H₃₁NP: 376.21886, found: 376.21889.

5 Example 7

(10-aminodecyl) triphenylphosphonium chloride hydrochloride (973 mg, 1.983 mmol) and dicyandiamide (750 mg, 8.922 mmol) was dissolved in DMF (5 ml) and refluxed under constant stirring for 4-8 h at 160 °C. The reaction mixture was concentrated and the crude material purified using column chromatography on silicagel (40 ml) (chloroform/methanol gradient 0-20% of methanol) to obtain the desired biguanide derivative (352 mg, 31 %) of formula 7.



¹H NMR (500 MHz, Methanol-d₄) δ 7.92 – 7.85 (m, 3H), 7.83 – 7.72 (m, 12H), 3.45 – 3.36 (m, 2H), 3.18 (t, J = 7.1 Hz, 2H), 1.70 – 1.60 (m, 2H), 1.58 – 1.48 (m, 4H), 1.37 – 1.22 (m, 12H);

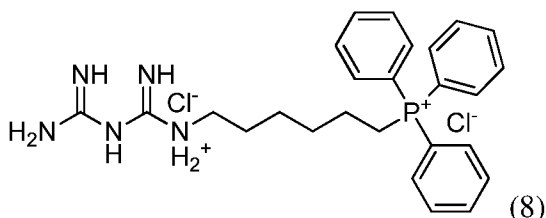
¹³C NMR (126 MHz, Methanol-d₄) δ 159.89, 159.39, 134.85, 133.40 (d, J = 8.8 Hz), 130.01 (d, J = 12.0 Hz), 118.65 (d, J = 86.4 Hz), 41.24, 30.21(d, J = 15.85 Hz), 29.31, 29.09, 28.97(2C), 28.52, 26.47, 22.17, 21.30 (d, J = 50.6 Hz).

IR (KBr pellet): ν = 3162, 2926, 2854, 1635, 1554, 1508, 1486, 1466, 1438, 1399, 1334, 1190, 1163, 1113, 996, 794, 723, 691.

HRMS calculated for C₃₀H₄₁N₅P: 502.30941, found: 502.30942.

Example 8

Biguanide derivative of the formula 8 was prepared using the same procedure as described in example 7.



¹H NMR (401 MHz, Methanol-d₄) δ 8.03 – 7.65 (m, 15H), 3.59 – 3.42 (m, 2H), 3.22 (t, J = 6.9 Hz, 3H), 1.77 – 1.67 (m, 2H), 1.68 – 1.59 (m, 2H), 1.59 – 1.51 (m, 2H), 1.48 – 1.38 (m, 2H).

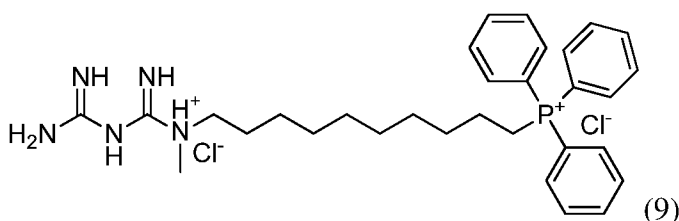
¹³C NMR (101 MHz, Methanol-d₄) δ 159.84, 158.92, 134.87 (d, J = 3.0 Hz), 133.45 (d, J = 10.0 Hz), 130.16 (d, J = 12.6 Hz), 118.60 (d, J = 86.3 Hz), 29.80 (d, J = 16.4 Hz), 25.67 (3C), 22.12 (d, J = 4.3 Hz), 21.26 (d, J = 51.1 Hz).

IR (KBr pellet): ν = 3315, 3180, 3052, 2935, 3861, 2804, 1690, 1631, 1587, 1559, 1503, 1438, 1113, 996, 750, 723, 691.

HRMS calculated for C₂₆H₃₃N₅P: 446.24681, found: 446.24679.

Example 9

(10-((methylamino)decyl)triphenylphosphonium chloride hydrochloride (422 mg, 0.836 mmol) and dicyandiamide (140 mg, 1.665 mmol) was dissolved in DMF (5 ml) and refluxed under constant stirring for 8 h at 160 °C. The reaction mixture was concentrated and the crude material purified using column chromatography on silicagel in chloroform/methanol (gradient 0-10% methanol) to obtain desired biguanide derivative (113 mg, 26 %) of the formula 9.



¹H NMR (400 MHz, Methanol-d₄) δ 8.08 – 7.62 (m, 15H), 3.49 – 3.39 (m, 4H), 3.03 (s, 3H), 1.74 – 1.64 (m, 2H), 1.64 – 1.52 (m, 4H), 1.43 – 1.24 (m, 10H).

¹³C NMR (101 MHz, Methanol-d₄) δ 159.33, 158.85, 134.86 (d, J = 3.0 Hz), 133.43 (d, J = 10.0 Hz), 130.14 (d, J = 12.5 Hz), 118.63 (d, J = 86.3 Hz), 49.95, 34.68, 30.24 (d, J = 16.1 Hz), 29.12, 29.03, 29.01, 28.56, 27.09, 26.23, 22.20 (d, J = 4.4 Hz), 21.28 (d, J = 51.0

Hz).

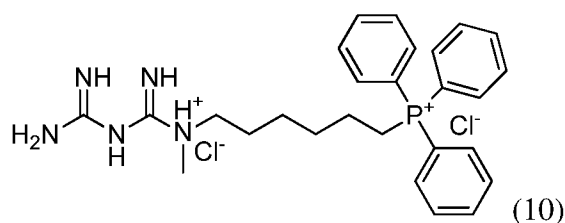
IR (KBr pellet): ν = 3318, 3183, 3057, 2925, 2854, 1629, 1559, 1496, 1465, 1438, 1419, 1365, 1325, 1113, 1048, 996, 752, 723, 692.

HRMS calculated for C₃₁H₄₃N₅P: 516.32506, found: 516.32498.

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Example 10

Biguanide derivative of the formula 10 was prepared using the same procedure as described in example 9.



¹H NMR (400 MHz, Methanol-d₄) δ 8.02 – 7.62 (m, 15H), 3.52 – 3.38 (m, 4H), 3.02 (s, 3H), 1.78 – 1.51 (m, 6H), 1.46 – 1.25 (m, 2H).

13C NMR (101 MHz, Methanol-d₄) δ 159.30, 158.91, 134.88 (d, J = 3.0 Hz), 133.43 (d, J = 10.0 Hz), 130.15 (d, J = 12.6 Hz), 118.58 (d, J = 86.3 Hz), 49.71, 29.95 (d, J = 16.4 Hz), 26.79 (2C), 25.48, 22.14 (d, J = 4.3 Hz), 21.26 (d, J = 51.1 Hz).

IR (KBr pellet): ν = 3311, 3160, 2926, 2853, 1636, 1533, 1507, 1438, 1398, 1337, 1162, 1113, 996, 784, 723, 691.

HRMS calculated for C₂₇H₃₅N₅P: 460.26246, found: 460.26258.

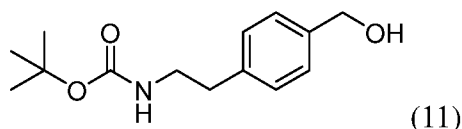
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Example 11

Triethylamine (5mL) and (Boc)₂O (2.160 g, 9.181 mmol) were added to the solution of *p*-aminoethylbenzoic acid hydrochloride (1.000g, 4.959 mmol) in dichloromethane (15 ml). Resulting mixture was stirred for 48 hours at laboratory temperature. Reaction mixture was diluted with diethylether (300 ml) and washed with citric acid (15%, 3 x 50 ml). Organic layer was dried over MgSO₄ and filtered. Filtrate was placed under the argon atmosphere and solution of diisobutylaluminiumhydride (140 ml, 1M v DCM) was added dropwise under the cooling. Reaction was quenched with addition of citric acid (5%, 300 ml) and organic layer was extracted with NaOH (1M, 100 ml) to get rid of residue of citric acid. Organic layer was dried over MgSO₄ and concentrated under vacuum. Crude material

was purified using column chromatography on silicagel (chloroform/methanol/ammonium (100:1:0.1 (500 ml) and 100:3:0.3 (250 ml)) to obtain desired *tert*-butyl (4-(hydroxymethyl)phenethyl)carbamate (355 mg, 26%) of the formula 11 in the form of colorless oil.

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¹H NMR (500 MHz, cdcl₃) δ 7.31 (d, J = 7.8 Hz, 1H), 7.19 (d, J = 7.8 Hz, 1H), 4.67 (d, J = 5.8 Hz, 1H), 4.54 (s, 1H), 3.43 – 3.30 (m, 1H), 2.80 (t, J = 6.9 Hz, 1H), 1.87 (t, J = 5.8 Hz, 1H), 1.44 (s, 9H).

10

¹³C NMR (126 MHz, cdcl₃) δ 155.84 (s), 139.01 (s), 138.42 (s), 128.96 (s), 127.31 (s), 79.23 (s), 65.07 (s), 41.75 (s), 35.84 (s), 28.37 (s).

HRMS calculated for: C₁₄H₂₁NNaO₃: 274.14136, found: 274.14093.

IR (KBr pellet): ν = 3400, 3367, 1684, 1617, 1529, 1512, 1456, 1391, 1367, 1251, 1169, 1055, 811,

15

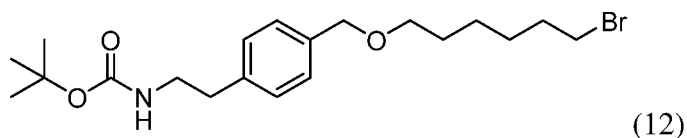
Example 12

Suspension of NaH (47.7 mg, 60%) in THF (3 ml) was slowly added dropwise to the solution of

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tert-butyl (4-(hydroxymethyl)phenethyl)carbamate (200 mg, 0.795 mmol) and 1,6-dibromohexane (1.940 g, 7.952 mmol) in THF (3 ml). Resulting reaction mixture was heated at 63°C and stirred for 16 hours. Reaction was quenched with addition of silicagel (4 g), concentrated and purified using column chromatography on silicagel (chloroform/dichloromethane 1:1) to obtain *tert*-butyl (4-(((6-bromohexyl)oxy)methyl)phenethyl)carbamate (130 mg, 38%) of formula 12 in the form of colorless oil.

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¹H NMR (500 MHz, cdcl₃) δ 7.28 (d, J = 8.0 Hz, 2H), 7.18 (d, J = 7.9 Hz, 2H), 4.55 (s,

¹H), 4.47 (s, 2H), 3.48 (t, J = 6.5 Hz, 2H), 3.41 (t, J = 6.8 Hz, 2H), 3.39 – 3.30 (m, 2H), 2.79 (t, J = 6.8 Hz, 2H), 1.87 (p, J = 6.8 Hz, 2H), 1.64 (p, J = 6.8 Hz, 2H), 1.52 – 1.36 (m, 13H).

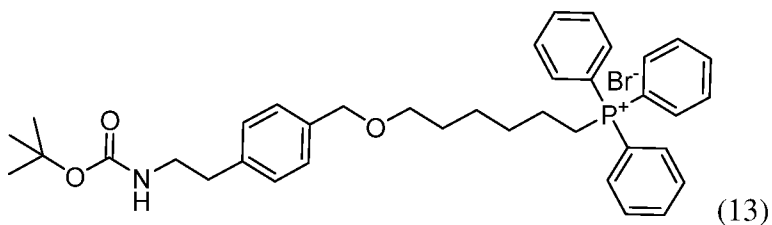
¹³C NMR (126 MHz, cdcl₃) δ 155.81, 138.27, 136.66, 128.78, 127.94, 79.15, 72.67, 70.24, 41.72, 35.85, 33.83, 32.70, 29.59, 28.37, 27.95, 25.38.

HRMS calculated for: C₂₀H₃₂NNaO₃: 436.14578, found: 436.14580.

IR (KBr pellet): ν = 3382, 1713, 1684, 1648, 1457, 1389, 1365, 1252, 1240, 1108, 1095, 647,

10 Example 13

Mixture of *tert*-butyl(4-(((6-bromohexyl)oxy)methyl)phenethyl)carbamate (65 mg, 0.157 mmol) and triphenylphosphine (822 mg, 3.137 mmol) was stirred for 16 hours at temperature of 85°C. Reaction mixture was cooled to laboratory temperature and diluted with mixture of diethyl ether/petrol ether (1:1, 15 ml). Resulting emulsion was left to stand overnight, product was sedimented as oil at the bottom of a flask, and clear solvent was subsequently decanted. Crude product was purified using column chromatography on silicagel (V(SiO₂) = 15 ml) (chloroform/ methanol/ammonium 100:5:0.5) to obtain desired Boc-carbamate (35 mg, 33%) of formula 13 in the form of colorless oil.



¹H NMR (500 MHz, cd₃od) δ 7.96 – 7.86 (m, 3H), 7.85 – 7.70 (m, 12H), 7.24 (d, J = 8.0 Hz, 2H), 7.17 (d, J = 8.0 Hz, 2H), 4.44 (s, 2H), 3.65 (s, 1H), 3.46 (t, J = 6.3 Hz, 2H), 3.44 – 3.37 (m, 2H), 3.23 (t, J = 7.4 Hz, 2H), 2.74 (t, J = 7.2 Hz, 2H), 1.74 – 1.63 (m, 2H), 1.62 – 1.50 (m, 4H), 1.48 – 1.36 (m, 11H).

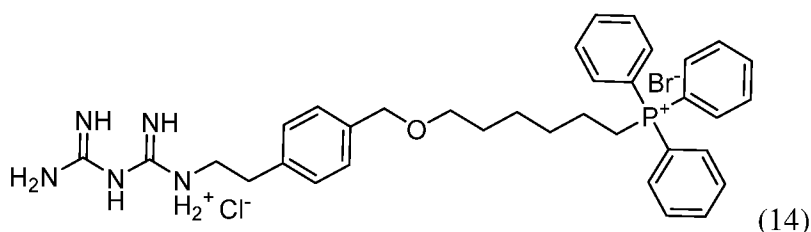
¹³C NMR (126 MHz, cd₃od) δ 156.98, 138.75, 136.29, 134.86 (d, J = 3.0 Hz), 133.40 (d, J = 10.0 Hz), 130.12 (d, J = 12.5 Hz), 128.44, 127.69, 118.57 (d, J = 86.3 Hz), 78.52, 72.32, 69.65, 41.65, 35.52, 29.84 (d, J = 16.2 Hz), 28.86, 27.39, 25.06, 22.01 (d, J = 4.4 Hz), 21.24 (d, J = 51.1 Hz).

HRMS calculated for: C₃₈H₄₇NO₃P: 596.32881, found: 596.32840.

IR (KBr pellet): ν = 1700, 1587, 1512, 1485, 1438, 1364, 1250, 1090, 999, 691

Example 14

HCl (50 μ L, 36%) was added dropwise to the solution of Boc-carbamate of formula 13 (30 mg, 0.0443 mmol) in ethanol (2 mL) and stirred for 2 hour at 60 °C. Completion of the conversion was checked with TLC (chloroform/methanol 80:20, ninhydrine stain detection). Reaction mixture was evaporated to dryness, dissolved in dimethylformamide (0.5 mL) and dicyandiamide (20 mg, 0.2447 mmol) was added in one portion. Reaction mixture was heated for 16 hour at 150°C and then separated using preparative TLC (chloroform/methanol 95:5) to obtain desired biguanid (5 mg, 16 %) of formula 14.

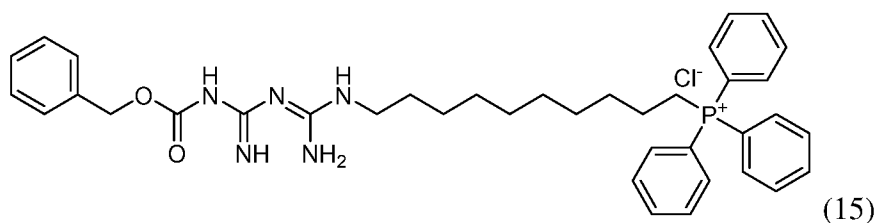


¹H NMR (500 MHz, cd_3od) δ 7.94 – 7.85 (m, 3H), 7.80 – 7.68 (m, 12H), 7.22 (d, J = 8.0 Hz, 2H), 7.16 (d, J = 8.0 Hz, 2H), 4.42 (s, 2H), 3.45 (t, J = 6.3 Hz, 2H), 3.44 – 3.37 (m, 2H), 3.18 (t, J = 7.3 Hz, 2H), 2.71 (t, J = 7.2 Hz, 2H), 1.73 – 1.64 (m, 2H), 1.61 – 1.50 (m, 4H), 1.46 – 1.40 (m, 2H).

HRMS calculated for: $C_{35}H_{43}N_5O^+$: 580.31997, found: 580.32015.

Example 15

Solution of benzylchloroformiate (15 μ L) in dichloromethane (1mL) was added dropwise into the cooled (0-4°C) solution of biguanide of formula 7 (40 mg, 0.0696 mmol) and triethylamine (97 μ L) in dry dichloromethane (1 mL). Reaction mixture was stirred in ice bath for 6 hours and then quenched with addition of methanol (1 mL). Reaction mixture was concentrated and separated using preparative TLC (3 times eluted, chloroform/methanol/ammonium 100:10:1). The sorbent was then divided into four parts, scratched out, pulverized and extracted using methanol (4 x 2 mL). The main fraction of the monosubstituted product of the formula 15 weighted 7 mg.



¹H NMR (500 MHz, cd₃od) δ 7.92 – 7.85 (m, 3H), 7.83 – 7.72 (m, 12H), 7.55 – 7.25 (m, 5H), 5.01 (s, 2H), 3.49 – 3.37 (m, 2H), 2.75 (t, *J* = 7.2 Hz, 2H), 1.72 – 1.59 (m, 2H), 1.59 – 1.50 (m, 4H), 1.35 – 1.20 (m, 12H).

MS calculated for: C₃₈H₄₇N₅O₂P⁺: 636.34619, found: 636.39; calculated for: C₃₈H₄₈N₅O₂P²⁺: 318.67674, found: 318.42.

Example 16

- Compounds **7**, **8**, **9** and **10**, and the known compound metformin were tested for their capacity to suppress growth and viability of human carcinoma cells isolated from patients with pancreatic cancer. The effect on growth was tested using the rather sensitive crystal violet assay (Bonnekoh B et al., (1989) Colorimetric growth assay for epidermal cell cultures by their crystal violet binding capacity. Arch Dermatol Res 281, 487-490).
- Concentration dependence of growth inhibition in cancer line PANC-1 after 24 h incubation in the presence of tested compounds is shown in Figure 1. The growth curves were used for determination of IC₅₀ values, which are shown for individual cancer cell lines in Table 1.
- Table 1: IC₅₀ values for compounds 7, 8, 9 and 10, and metformin for suppression of growth of pancreatic cell lines (n.d. = not determined).

	IC ₅₀ (μM)				
Cell line	Compound 7	Compound 9	Compound 8	Compound 10	metformin
MiaPaCa-2	11.30 ± 1.63	17.31 ± 2.57	179.9 ± 12.8	417.7 ± 45.2	30714 ± 2557
PANC-1	13.66 ± 2.25	57.78 ± 8.66	n.d.	n.d.	34889 ± 4209
PaTu 8902	27.8 ± 5.12	106.9 ± 16.9	n.d.	n.d.	> 50000
BxPc-3	71.23 ± 16.16	218.0 ± 42.8	n.d.	n.d.	20236 ± 2748
ASPC-1	92.45 ± 9.61	194.7 ± 18.3	n.d.	n.d.	22250 ± 2708

Using five different pancreatic cancer cell lines, we show that the IC₅₀ values are for the new compounds up to 4 orders of magnitude lower than for the known compound metformin, which is currently undergoing clinical testing as an efficient drug against

pancreatic cancer. The highest anti-cancer effects were found for compound 7, which was therefore studied in further experiments.

Example 17

5 Effective and safe anti-cancer agents should be selective for malignant cells and, at the same time, non-toxic to normal cells. IC₅₀ values for compound 7 determined by the crystal violet assay is obvious that compound 7 suppresses growth of PANC-1 cells at considerably lower concentration compared to non-tumor line (BJ fibroblasts) (Fig. 2), and should therefore suppress only growth of tumours without deleterious effect on healthy
10 cells, using appropriate doses.

Example 18

Anti-proliferative effect of compound 7 was further tested using the xCelligence method, which enables continuous evaluation of cell growth and viability under *in vitro* conditions
15 (Abassi, Y. A., et al. (2009). Kinetic cell-based morphological screening: prediction of mechanism of compound action and off-target effects. Chemistry & Biology 16(7): 712-723.). Figure 3A depicts growth curves of PANC-1 cells under control conditions and in the presence of compound 7 in the cultivation medium (the arrow shows time of supplementation). Lower slope of the curve indicates that compound 7 suppresses growth
20 and that, at higher concentrations, it even causes cell death, which is suggested by negative slope of the growth curve (Figure 3B).

Example 19

Using the xCelligence system, we further confirmed that compounds 7 and 15 suppress the
25 growth of PANC-1 cancer cells by up to 4 orders of magnitude more efficiently than found for metformin (Figure 4). The IC₅₀ value determined for compound 7 was time-dependent and reached its minimum (>10 μ M) at 72 h of incubation, while it was reached already at 24 h for compound 15. This indicates that compound 15 is metabolized into compound 7, therefore compound 15 is a pro-drug for compound 7. Fasted effect of compound 15 is
30 given by its easier uptake into cells across the plasma membrane on the basis of higher lipophilicity and lower pK_a.

Example 20

This example shows whether the new compounds according to the invention triggered programmed cell death, i.e. apoptosis. For this, the pancreatic cancer cell lines PANC-1 and MiaPaCa-2 were used. Apoptotic cell death was determined using annexin V, which is a protein with affinity for phosphatidylserine that is externalized during apoptosis. Under these conditions, annexin V binds to the phospholipid (Weber T et al (2003) Mitochondria play a central role in apoptosis induced by α -tocopheryl succinate, an agent with anticancer activity. Comparison with receptor-mediated pro-apoptotic signaling. Biochemistry 42, 4277-4291). Since annexin V used for this assay was fluorescently labeled, the extent of apoptotic cell death can be quantified using flow cytometry. Fig. 5 shows that, compared to compound 7 that efficiently kills the cancer cells, metformin is inefficient at the same concentration levels.

Example 21

Results documented in Example 20 were verified by evaluation of caspase-3 activation in PANC-1 cancer cells exposed to compound 7 or metformin. Caspase-3 is a key protein of the apoptotic cascade, whose activation causes cell death. Considerable activation of caspase-3 was observed after 48 h of incubation of the cells in the presence of compound 7. Metformin used at 1,000-fold higher concentration was inefficient (Fig. 6). Staurosporin was used here as a positive control, since it is an efficient apoptosis inducer.

Example 22

We tested anti-cancer effects of compound 7 also under *in vivo* conditions using experimental tumours induced in immunocompromised Balb-c nu/nu mice. Using subcutaneous injection, 2×10^6 PANC-1 or MiaPaCa-2 cells were grafted per animal. The cells were grown under standard conditions. At about 70% influence they were harvested using trypsin, washed in physiological solution and re-suspended so that 100 μ l contained 2×10^6 cells. When the tumour size reached about 5 mm³, treatment was initiated. Compound 7 was applied twice per week at doses some 20- and 40-fold lower than those published for metformin (84 nmol/g for MiaPaCa-2 cell-derived tumours and 126 nmol/g for PANC-1 cell-derived tumours), at which level the known compound suppresses pancreatic tumours (Kisfalvi K et al. Metformin inhibits the growth of human pancreatic cancer xenografts. Pancreas 2013, 42, 781-785). Compound 7 was applied orally using the gavage technique, which allows administration of the agent into the digestive system of an

animal, which allows its uptake into the circulation that then takes the agent to target tissues (cells). The tumours were followed and their volume quantified by ultrasound imaging (Vevo770, VisualSONics, Toronto, ON, Canada), which allows for precise evaluation of selected tissue. Fig. 7 documents high anti-cancer activity of compound 7 that suppresses tumour growth in spite of being applied at lower doses compared to the metformin-treated animals (compared to animals with PANC-1 cell-derived tumours treated with metformin, compound 7 suppressed tumour growth by the factor of 54 (12.17×40); for MiaPaCa-2 cell-derived tumours this factor was 93 ($35/15 \times 40$). An important aspect of these experiments was that compound 7 was not toxic, as documented on the bases of animal behaviour and no weight loss.

Example 23

It is known that metformin also suppresses respiration via mitochondrial complex I, which is one of the mechanisms of the anti-cancer activity of the agent ((Wheaton, W. W., et al. (2014). Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis. *Elife* 3: e02242.). We tested whether and at which concentrations compounds 7, 9 and 10 suppress respiration mediated by complex I. We used here MiaPaCa-2 and PaTu 8902 cells. The effect on complex I was evaluated by assessing respiration using permeabilised cells in the presence of complex I substrates (glutamate and malate) by means of the high-resolution respirometer Oxygraph O2k (Oroboros, Innsbruck, Austria) (Kluckova K et al. Evaluation of respiration of mitochondria in cancer cells exposed to mitochondria-targeted agents. *Methods Mol Biol* 2015, 1265, 181-194). Fig. 8 shows that compound 7 suppresses MiaPaCa-2 cell complex I respiration with IC_{50} of 3 μM and that of PaTu 8902 with IC_{50} of 7.5 μM . Metformin started to inhibit complex I respiration at levels of about 1 mM, i.e. it was again some 3 orders of magnitude less efficient than compound 7. Compare to compound 7, compound 9 suppressed complex I activity with slightly higher IC_{50} , while compound 10 was less efficient by about 1 order of magnitude (Fig. 8). We have noticed analogical differences in efficiency also in monitoring of antiproliferative effect of the prepared compounds (Fig. 1, Table 1). Our results denote the same mechanism of activity for the novel compounds as for metformin, however, the modifications lead to an important efficiency increase.

Example 24

Biguanide analogues induce their activity towards cancer cells by targeting mitochondria. We therefore studied their effect on mitochondrial potential that is a prerequisite for proper mitochondrial function, including efficient import of mitochondrial proteins from the cytoplasm. We followed the effect on mitochondrial potential using the fluorescent probe tetramethylrhodamine methyl ester (TMRM) that loses red fluorescence upon a decrease (dissipation) of mitochondrial potential (Rohlena J et al. Mitochondrially targeted α -tocopheryl succinate is antiangiogenic: Potential benefit against tumor angiogenesis but caution against wound healing. *Antiox Redox Signal* 2011, 15, 2923-2935). The studied PANC-1 cells revealed important loss of mitochondrial potential after 24 h incubation with compound 7, whereas when 5 μ M concentration was used, a complete loss of detectable potential occurred. Metformin used at 200-fold higher concentration showed no effect on mitochondrial potential (Fig. 9).

Example 25

It was tested whether the new compounds also act by means of increased production of reactive oxygen species (ROS). PANC-1 cells were incubated with compound 7 or metformin in the presence of the fluorescent probe MitoSOX that at increased production of ROS increases fluorescence intensity (Yan, B., et al. (2015). Mitochondrially targeted vitamin E succinate efficiently kills breast tumour-initiating cells in a complex II-dependent manner. *BMC Cancer* 15: 401.). Fig. 10 shows concentration and time dependence of ROS production for the tested compounds compared to the control conditions (no added agents). The results reveal that incubation in the presence of compound 7 leads to increased mitochondrial ROS production. This may be one of the mechanisms by which compound 7 affects proliferation of cancer cells. Metformin does not stimulate ROS formation even at levels some 100-fold higher than those used for compound 7.

Example 26

Metformin is the world most frequently subscribed agent against type 2 diabetes mellitus. Since the new compounds according the invention are considerably more efficient against pancreatic cancer compared to metformin, we also tested them for a potential effect against aspects of type 2 diabetes mellitus. One aspect of diabetes is increased level of glucose in circulation due to increased formation of glucose in hepatocytes by the process of

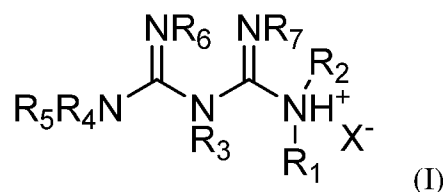
guconeogenesis as well as by release of glucose from glycogen due to glycogenolysis. Therefore we tested the level of glucose using cultured hepatic cell line HepG2 in the presence of metformin and compound 7 using a published protocol (Magni F et al. Determination of serum glucose by isotope dilution mass spectrometry: candidate
5 definitive method. Clin. Chem. 1992, 38, 381-385). Results of this experiment (Fig. 11) show that metformin suppressed glucose level by some 80% at 5 and 10 mM concentrations, while a similar extent of inhibition was found for compound 7 at the concentration of 5 and 10 μ M. These results show that compound 7 is more efficient
10 against pancreatic cancer than found for metformin similarly as against type 2 diabetes mellitus, which is about 3 orders of magnitude in both cases.

Industrial Applicability

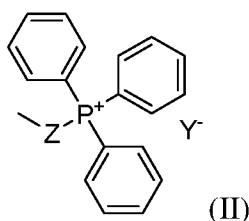
The compounds of the present invention represent a new generation of medicaments for
15 treatment of diabetes mellitus type 2 and pancreatic carcinoma, with high activity and without toxic side effects.

CLAIMS

1. Triphenylphosphonium biguanide analogues of general formula I, whereas the general formula I includes resonance structures and pharmaceutically acceptable salts, protonated
 5 form as well as free base,



where each one of R1, R2, R3, R4, R5, R6, R7 is independently selected from the group comprising H; C1-C6 alkyl; C6-C10 aryl; (C1-C6)alkyl(C6-C10)aryl; -C(=O)-R'; -C(=O)OR'; -C(=O)NR'R''; -C(=S)R'; -C(=S)NR'R''; wherein R' and R'' are
 10 independently selected from the group comprising H, C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl; whereas C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl can be unsubstituted or substituted by one or more substituents selected independently from the group comprising C1-C4 alkyl, N(H or C1-C4 alkyl)₂, wherein alkyls are the same or different, phenyl, benzyl, OH, SH, F, Cl, Br, I, C1-C4
 15 alkoxy, C1-C4 acyloxy, C1-C4 mercapto; and substituent of general formula II



wherein Z is a linear hydrocarbyl chain selected from alkylene, alkenylene or alkynylene, containing 2 to 20 carbon atoms, preferably 4 to 14 carbon atoms, more preferably 8 to 12 carbon atoms, wherein optionally one or more carbon couples in the
 20 hydrocarbyl chain can be replaced by one or more 5-membered or 6-membered aromatic rings or heteroaromatic rings containing O, S and/or N as heteroatoms, preferably phenylenes or pyridylenes, and/or one or more carbon atoms in the hydrocarbyl chain can be replaced by one or more heteroatoms selected from O, S, NH, and wherein the hydrocarbyl chain can be unsubstituted or substituted by one or more
 25 substituents selected independently from the group comprising C1-C4 alkyl, N(H or C1-C4 alkyl)₂, wherein the alkyls are the same or different, phenyl, benzyl, OH, SH, F, Cl, Br, I, C1-C4 alkoxy, C1-C4 acyloxy, C1-C4 mercapto,

whereas at least one of R1, R2, R3, R4, R5, R6, R7 is the substituent of general formula II,

X⁻ is a pharmaceutically acceptable anion, preferably an anion of inorganic or organic acid,

Y⁻ is a pharmaceutically acceptable anion, preferably an anion of inorganic or organic acid.

5

2. The compounds according to claim 1, characterized in that R1 is the substituent of general formula II, R2-R7 are independently selected from the group comprising H; C1-C6 alkyl; C6-C10 aryl; (C1-C6)alkyl(C6-C10)aryl; -C(=O)-R'; -C(=O)OR'; -C(=O)NR'R''; -C(=S)R'; -C(=S)NR'R''; wherein R' and R'' are independently selected from the group comprising H, C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl; wherein C1-C6 alkoxy, C1-C6 alkyl, C6-C10 aryl, (C1-C6)alkyl(C6-C10)aryl can be unsubstituted or substituted by one or more substituents selected independently from the group comprising C1-C4 alkyl, N(H or C1-C4 alkyl)₂, wherein the alkyls are the same or different, phenyl, benzyl, OH, SH, F, Cl, Br, I, C1-C4 alkoxy, C1-C4 acyloxy, C1-C4 mercapto.

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3. The compounds according to claim 1 or 2, characterized in that X⁻ and Y⁻ are independently selected from the group comprising Cl⁻, Br⁻, I⁻, sulphate, mesyl, acetate, formate, succinate.

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4. The compounds according to any one of claims 1 to 3, characterized in that Z is a linear hydrocarbyl chain selected from alkylene, alkenylene or alkynylene comprising 8 to 12 carbon atoms.

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5. The compounds according to any one of claims 1 to 4, characterized in that at least one of R1, R2, R3, R4, R5, R6, R7 is selected from the group comprising methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, 4-chlorophenyl, phenylethyl, 2-(2-acetoxy-4,6-dimethylphenyl)-propyl, substituent -C(=O)OR', wherein the group R' is benzyl.

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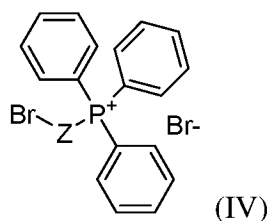
6. A method of preparation of compounds of general formula I according to claim 1, characterized in that:

in the first step, compound of general formula III

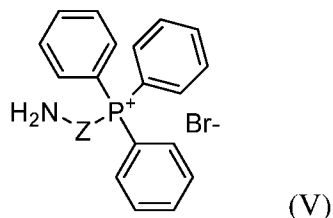
T - Z - T (III),

wherein T is a cleavable group, preferably halogen, mesyl or tosyl, and Z is as defined in claim 1,

is subjected to a reaction with triphenylphosphine, yielding triphenylphosphonium hydrocarbyl derivative of general formula IV

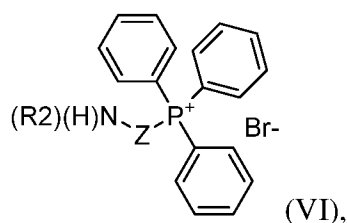


which is then treated with methanolic ammonia solution, yielding primary aminohydrocarbyl triphenylphosphonium of general formula V



or

with methanolic solution of (R2)NH2, yielding secondary amino-hydrocarbyl triphenylphosphonium of general formula VI



and the corresponding primary or secondary aminohydrocarbyl triphenylphosphonium, respectively, is subsequently condensed with 2-cyanoguanidine, yielding triphenylphosphonium hydrocarbyl biguanide derivative of general formula I, wherein Z and R2 are as defined in claim 1.

7. The compounds of general formula I according to any one of claims 1 to 5 for use as medicaments.

8. Use of the compounds of general formula I according to any one of claims 1 to 5 in the manufacture of a medicament for treatment of diabetes mellitus type 2 and/or pancreatic carcinoma.

9. The compounds of general formula I according to any one of claims 1 to 5 for use in a method of treatment of diabetes mellitus type 2 and/or pancreatic carcinoma.
- 5 10. A pharmaceutical composition, characterized in that it contains at least one compound of general formula I according to any one of claims 1 to 5, and at least one pharmaceutical auxiliary substance.

Fig. 1

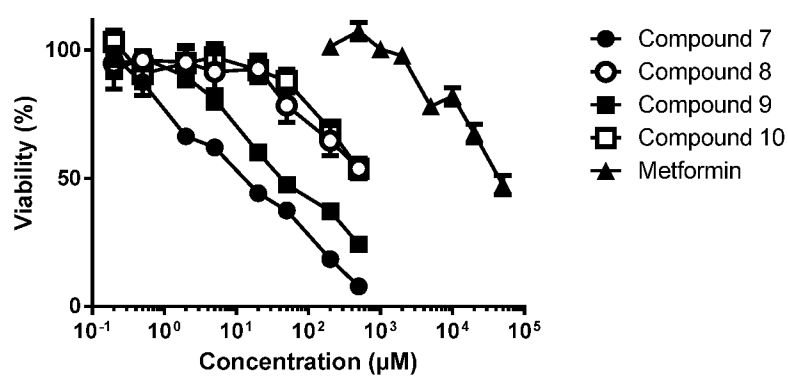
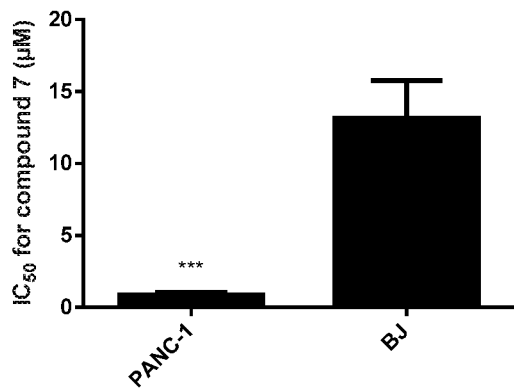


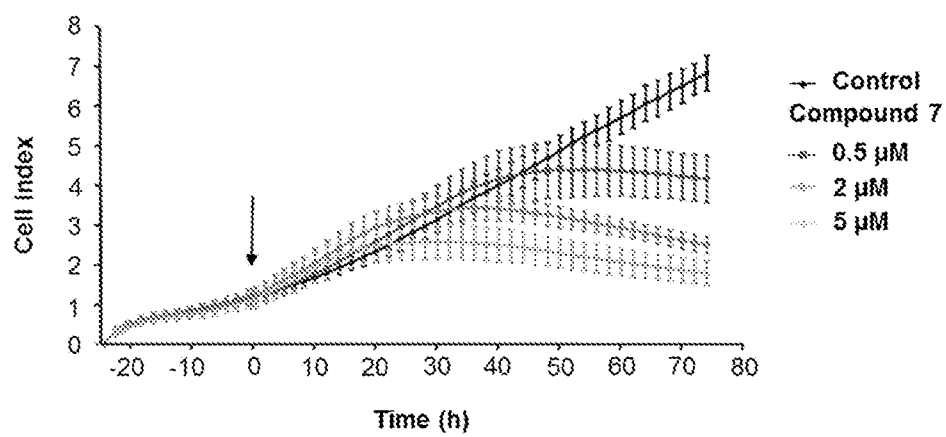
Fig. 2



2 / 8

Fig. 3

A



B

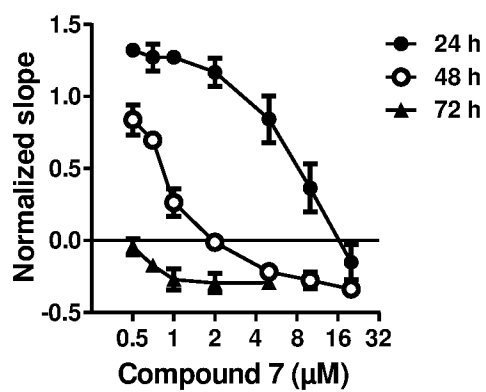


Fig. 4

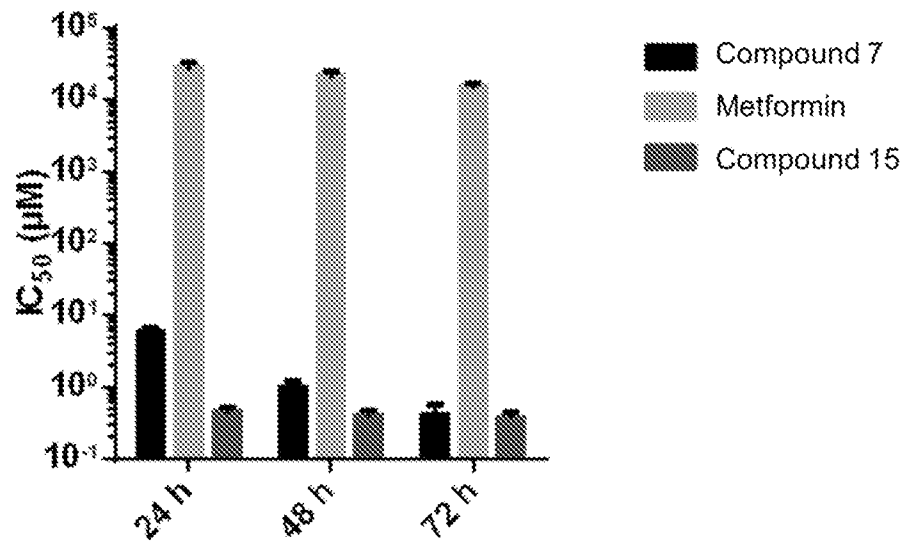


Fig. 5

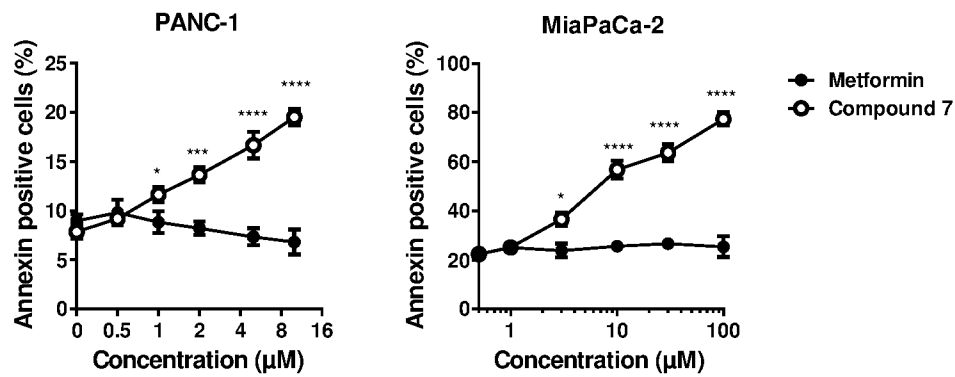


Fig. 6

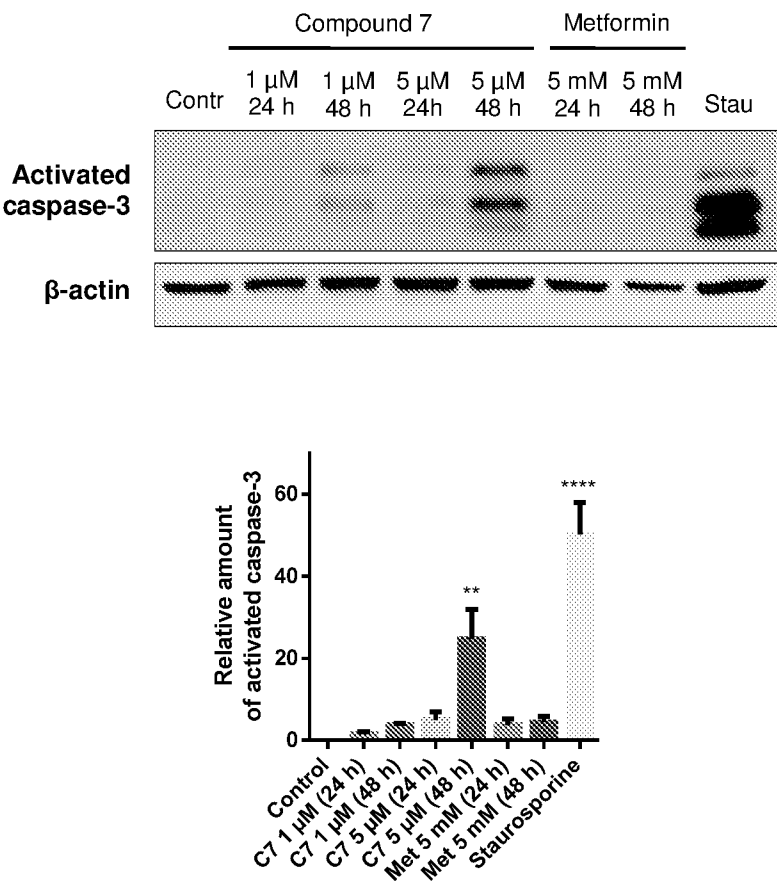


Fig. 7

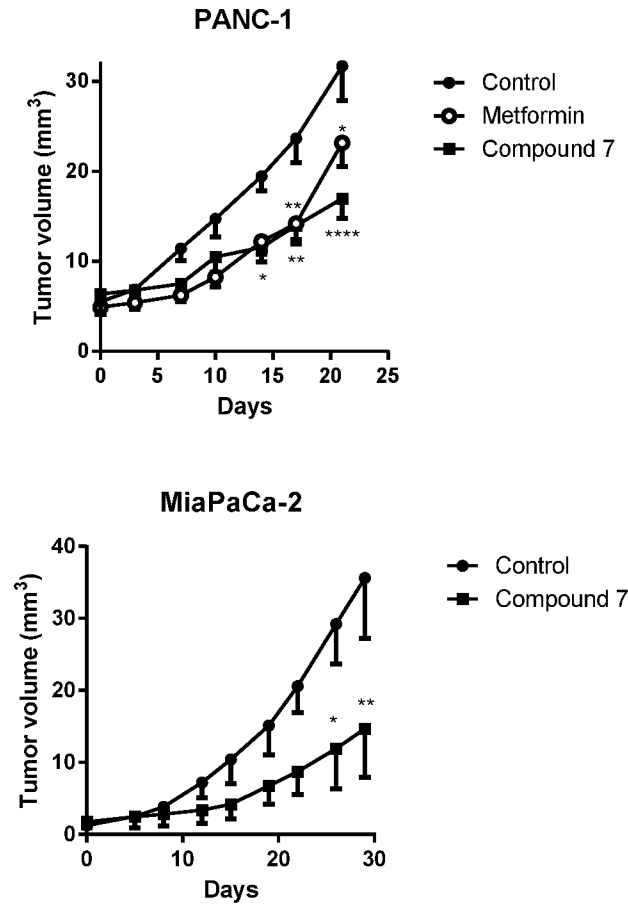


Fig 8

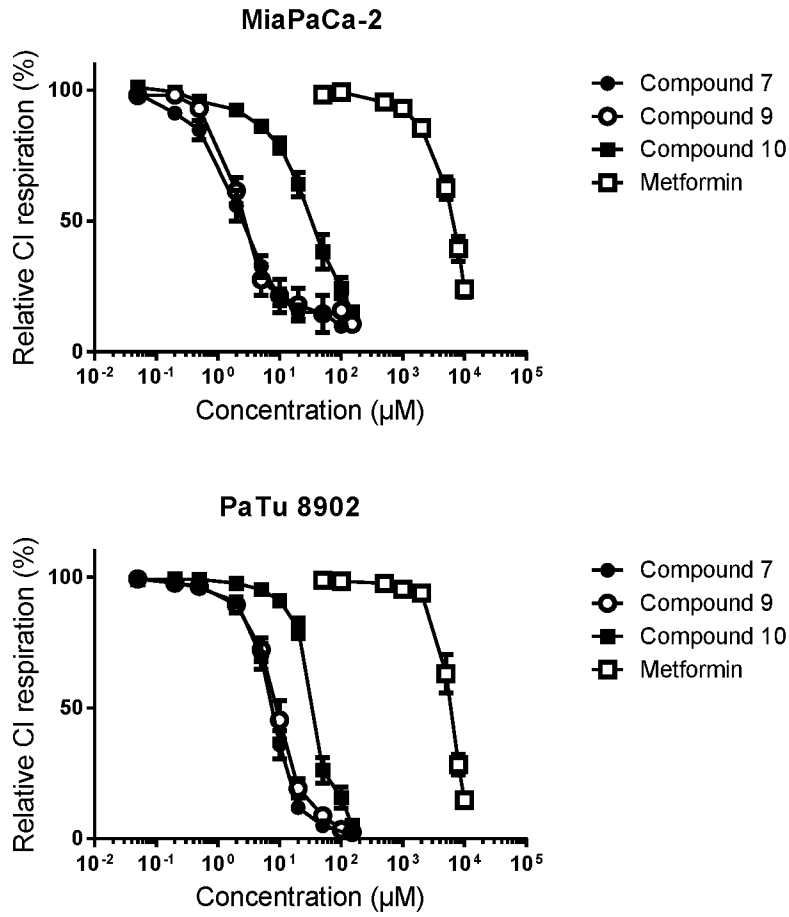


Fig. 9

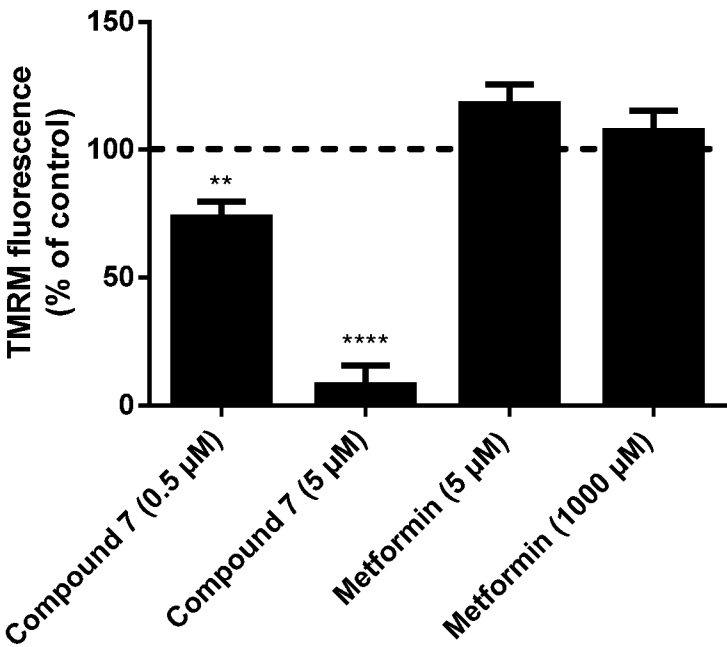


Fig. 10

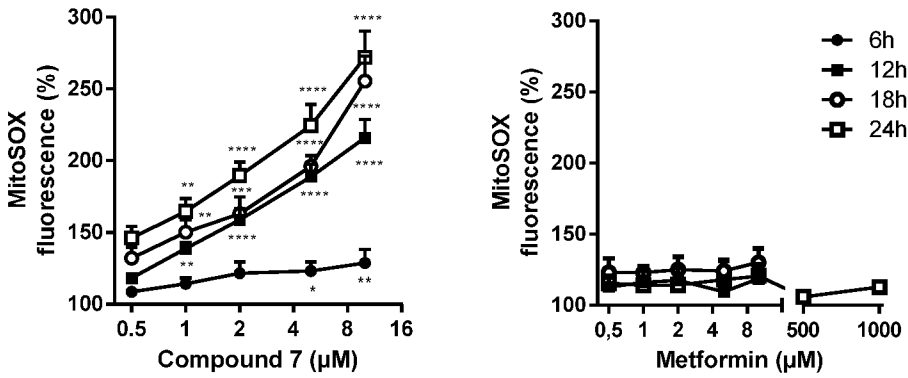
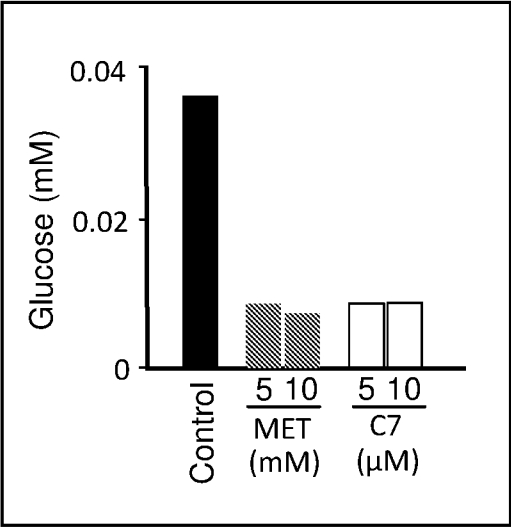


Fig. 11



INTERNATIONAL SEARCH REPORT

International application No
PCT/CZ2015/050016

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07F9/54 A61P3/10 A61P35/00 A61K31/66
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07F A61P A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NEUZIL JIRI ET AL: "Classification of mitocans, anti-cancer drugs acting on mitochondria", MITOCHONDRION, ELSEVIER, AMSTERDAM, NL, vol. 13, no. 3, 28 July 2012 (2012-07-28), pages 199-208, XP028529914, ISSN: 1567-7249, DOI: 10.1016/J.MITO.2012.07.112 pages 200, 203 ----- -/--	1-10



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

26 April 2016

Date of mailing of the international search report

04/05/2016

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Duval, Eric

INTERNATIONAL SEARCH REPORT

International application No
PCT/CZ2015/050016

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LAN-FENG DONG ET AL: "Mitochondrial targeting of -tocopheryl succinate enhances its pro-apoptotic efficacy: A new paradigm for effective cancer therapy", FREE RADICAL BIOLOGY AND MEDICINE, ELSEVIER INC, US, vol. 50, no. 11, 25 February 2011 (2011-02-25), pages 1546-1555, XP028232370, ISSN: 0891-5849, DOI: 10.1016/J.FREERADBIOMED.2011.02.032 [retrieved on 2011-03-12] the whole document	1-10
A	----- WO 2013/103384 A1 (ELCELYX THERAPEUTICS INC [US]; BARON ALAIN D [US]; FINEMAN MARK S [US]) 11 July 2013 (2013-07-11) the whole document	1-10
E	----- WO 2016/025725 A1 (WISCONSIN MED COLLEGE INC [US]) 18 February 2016 (2016-02-18) the whole document	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/CZ2015/050016

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		WO 2013103384 A1	11-07-2013

WO 2016025725	A1	18-02-2016	NONE
