Claims

1. A compound characterized by formula 1 :

Xl Thr Thr Ala Arg X2 (formula 1),

wherein cleavage of the compound into a fragment 1 comprising XI and a fragment 2 comprising X2 generates a detectable signal,

wherein XI comprises or consists of a component Cl and X2 comprises or consists of a component C2, and the detectable signal is generated upon spatial separation of Cl and C2.

2. The compound according to claim 1, wherein the sequence Thr-Thr-Ala-Arg is accessible for a hydrolytic enzyme, in particular a hydrolytic enzyme cleaving the compound into XI-Thr-Thr-Ala-Arg-OH (fragment 1) and NH2-X2 (fragment 2).

3. The compound according to any of claims 1 or 2, wherein one of Cl and C2, in particular C2, is a chromophore having an absorption maximum 1 (AMI) at a wavelength 1, and the compound has an absorption maximum 2 (AM2) at a wavelength 2 that is different from wavelength 1.

4. The compound according to any of claims 1 to 3, wherein Cl and C2 are a pair of a fluorescence donor and a fluorescence acceptor.

5. The compound according to any of claims 1 to 4, wherein the pair of Cl and C2 is selected from the group consisting of 2-aminobenzoic acid (ABZ)/pNA, ABZ/ANB-NH_2, ABZ/DNP, ABZ/EDDNP, ED-AN S/D-ABCYL, TAM/D-ANSYL, ABZ/Tyr(3-$N0_2$), in particular the pair of Cl and C2 is selected from ABZ/pNA and ABZ/ANB-NH_2.

6. An in-vitro method for detecting protease activity in a subject's body fluid, comprising contacting the body fluid with a compound and detecting a signal, wherein the body fluid may comprise a hydrolytic enzyme, in particular a protease, derived from pancreatic cancer cells, and

wherein the compound is characterized by formula 1 :

Xl Thr Thr Ala Arg X2 (formula 1),

wherein cleavage of the compound into a fragment 1 comprising XI and a fragment 2 comprising X2 generates a detectable signal.

7. The in vitro method according to claim 6 for detecting the presence or absence of pancreatic cancer in a subject, wherein the presence of protease activity in the body fluid indicates the presence of pancreatic cancer and the absence of protease activity in the body fluid indicates the absence of pancreatic cancer.

8. The in vitro method according to claim 6 or 7 for the diagnosis of pancreatic cancer.

9. The in vitro method according to any of claims 6 to 8, wherein the body fluid is urine.

10. The method according to any of claims6 to 9, wherein the compound is provided at a concentration of 0.1 10 mg/ml, particularly 0.25 7.5 mg/ml, more particularly 0.5 5 mg/ml, more particularly 0.75 2 mg/ml, even more particularly about 1 mg/ml, in a measurement buffer having neutral or alkaline pH, preferably physiological pH, and the body fluid sample is added to the compound at a ratio of 1 :2 to 1 : 10, particularly 1 :3 to 1 :8, more particularly 1 :4 to 1 :6, even more particularly about 1 :5.

11. The method according to any of claims 6 to 10, wherein detecting the signal comprises measuring absorbance or fluorescence, particularly measuring absorbance intensity at 300 500 nm, more particularly 380 430 nm, preferably for 40 60 min at 25 40°C, in particular at 36 38°C.

12. A kit comprising a compound according to any of claims 1 to 5 and a measurement buffer.

13. Use of the compound characterized by formula 1 :

Xl Thr Thr Ala Arg X2 (formula 1),

wherein cleavage of the compound into a fragment 1 comprising XI and a fragment 2 comprising X2 generates a detectable signal, the methods of any of claims 6 to 11 or the kit comprising said compound and a measurement buffer for the detection of pancreatic cancer, or for monitoring a subject that is suspected of having pancreatic cancer, has an increased risk of developing pancreatic cancer, or has had pancreatic cancer.

~~14. A method of treating pancreatic cancer, the method comprising the steps of:~~
~~a. carrying out the method according to any of claims 7 to 12, and~~
~~b. treating pancreatic cancer in a subject for which protease activity, in particular increased protease activity has been detected in step a.~~

1. A compound characterized by formula 1:

X1-Thr-Thr-Ala-Arg-X2 (formula 1),

wherein cleavage of the compound into a fragment 1 comprising X1 and a fragment 2 comprising X2 generates a detectable signal,
wherein X1 comprises or consists of a component C1 and X2 comprises or consists of a component C2, and the detectable signal is generated upon spatial separation of C1 and C2, by hydrolytic cleaving of the compound,
wherein the detectable signal is optical signal, and
wherein C1 and C2 are a pair of a fluorescence donor and a fluorescence acceptor.

2. The compound according to claim 1, wherein the sequence Thr-Thr-Ala-Arg is accessible for a hydrolytic enzyme, in particular a hydrolytic enzyme cleaving the compound into X1-Thr-Thr-Ala-Arg-OH (fragment 1) and NH_2-X2 (fragment 2).

3. The compound according to any of claims 1 or 2, wherein one of C1 and C2, in particular C2, is a chromophore having an absorption maximum 1 (AM1) at a wavelength 1, and the compound has an absorption maximum 2 (AM2) at a wavelength 2 that is different from wavelength 1.

4. The compound according to any of claims 1 to 3, wherein the pair of C1 and C2 is selected from the group consisting of 2-aminobenzoic acid (ABZ)/pNA, ABZ/ANB-NH_2, ABZ/DNP, ABZ/EDDNP, EDANS/DABCYL, TAM/DANSYL, ABZ/Tyr(3-NO_2), in particular the pair of C1 and C2 is selected from ABZ/pNA and ABZ/ANB-NH_2.

5. An *in vitro* method for detecting protease activity in a subject´s body fluid, comprising contacting the body fluid with a compound and detecting a signal, wherein the body fluid is urine, and wherein the body fluid may comprise a hydrolytic enzyme, in particular a protease, derived from pancreatic cancer cells, and
wherein the compound is characterized by formula 1:

X1-Thr-Thr-Ala-Arg-X2 (formula 1),

wherein cleavage of the compound into a fragment 1 comprising X1 and a fragment 2 comprising X2 generates a detectable signal.

6. The *in vitro* method according to claim 5 for detecting the presence or absence of pancreatic cancer in a subject, wherein the presence of protease activity in the body fluid indicates the presence of pancreatic cancer and the absence of protease activity in the body fluid indicates the absence of pancreatic cancer.

7. The *in vitro* method according to claim 5 or 6 for the diagnosis of pancreatic cancer.

8. The method according to any of claims 5 to 7, wherein the compound is provided at a concentration of 0.1-10 mg/ml, particularly 0.25-7.5 mg/ml, more particularly 0.5-5 mg/ml, more particularly 0.75-2 mg/ml, even more particularly about 1 mg/ml, in a measurement buffer having neutral or alkaline pH, preferably physiological pH, and the body fluid sample is added to the compound at a ratio of 1:2 to 1:10, particularly 1:3 to 1:8, more particularly 1:4 to 1:6, even more particularly about 1:5.

9. The method according to any of claims 5 to 8, wherein detecting the signal comprises measuring absorbance or fluorescence, particularly measuring absorbance intensity at 300-500 nm, more particularly 380-430 nm, preferably for 40-60 min at 25-40°C, in particular at 36-38°C.

10. A kit comprising a compound according to any of claims 1 to 4 and a measurement buffer.

11. Use of the compound characterized by formula 1:

X1-Thr-Thr-Ala-Arg-X2 (formula 1),

wherein cleavage of the compound into a fragment 1 comprising X1 and a fragment 2 comprising X2 generates a detectable signal, the methods of any of claims 5 to 10 or the kit comprising said compound and a measurement buffer for the detection of pancreatic cancer, or for monitoring a subject that is suspected of having pancreatic cancer, has an increased risk of developing pancreatic cancer, or has had pancreatic cancer.

12. Use of the compound characterized by formula 1:

X1-Thr-Thr-Ala-Arg-X2 (formula 1),

wherein cleavage of the compound into a fragment 1 comprising X1 and a fragment 2 comprising X2 generates a detectable signal in a method of treating pancreatic cancer, wherein the method comprises the steps of:

a. carrying out the method according to any of claims 5 to 9, and

b. wherein pancreatic cancer is to be treated in a subject for which protease activity, in particular increased protease activity has been detected in step a.