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New International Patent Application
Gesellschaft für Biotechnologische Forschung mbH (GBF)

Triazolo-epothilones

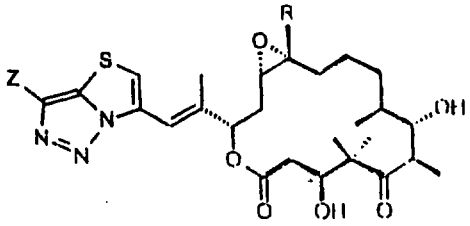
The invention relates to triazolo-thiazole analogues of epothilone A and epothilone B.

Epothilones are macrocyclic lactones having a fungicidal and cytotoxic effect. There is a continuous need for analogues or derivatives having comparable or better activity, which can be used as fungicides or cytostatic agents.

An overview of the chemistry of epothilones is given, for example, by Nicolaou *et al.* in Angew. Chem. Int. Ed. 1998, Vol. 37, 2014-2045.

The problem of the invention is to provide epothilone analogues or derivatives of that kind.

The invention accordingly relates to triazolo-thiazole analogues of epothilone A and epothilone B of formula 4a or 4b:



4a R = H, Z = H, alkyl, aryl, heteroaryl

4b R = CH_3, Z = H, alkyl, aryl, heteroaryl

wherein:

R denotes H, CH_3

Z denotes H, alkyl, aryl, heteroaryl.

The triazolo-thiazole epothilones according to the invention are very effective fungicides and highly potent cytostatic agents having favourable pharmacological properties.

Alkyl denotes straight-chain or branched C_1-C_6alkyl which may be (mono- or poly-)substituted as desired, for example methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl and hexyl. Examples of substituents are C_1-C_6alkoxy, C_1-C_6acyl, hydroxyl and halogen such as bromine, chlorine, fluorine and iodine.

Aryl denotes mononuclear or polynuclear aromatic systems which may be (mono- or poly-)substituted as desired, for example phenyl, o-, m-, p-tolyl, o-, m-, p-xylyl, benzyl, phenethyl and naphthyl. Examples of substituents are C_1-C_6alkyl, C_1-C_6alkoxy, C_1-C_6acyl, hydroxyl and halogen such as bromine, chlorine, fluorine and iodine.

Heteroaryl denotes mononuclear or polynuclear heteroaromatic systems which may be (mono- or poly-)substituted as desired, it being possible for the aromatic nucleus to have one or more hetero atoms selected from N, O and S. Examples of heteroaryl are furyl, pyranyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl and indolyl. Examples of substituents are C_1-C_6alkyl, C_1-C_6alkoxy, C_1-C_6acyl, hydroxyl and halogen such as bromine, chlorine, fluorine and iodine.

The invention relates also to a method of preparing the triazolo-thiazole analogues of epothilone A and epothilone B according to the invention and to fungicidal and pharmaceutical compositions comprising one or more such analogues, and to the use of the analogues and of the fungicidal or pharmaceutical compositions comprising them for combating fungi or for treating diseases that can be treated with cytostatic agents, for example tumour diseases such as cancer or disorders of cell growth.

The fungicidal and pharmaceutical compositions may comprise, besides the actual active ingredient, conventional carriers, diluents or excipients, for example stabilisers such as UV absorbers, anti-oxidants and preservatives.

Figure 1 shows a synthesis route for preparation of the triazolo-thiazole analogues of epothilone A and epothilone B according to the invention.

The preparation of the triazolo-thiazole analogues of epothilone A and epothilone B according to the invention is described hereinbelow, without limitation, with reference to Figure 1. The abbreviation Met denotes metal.

The preparation of C21-modified epothilones, that is to say the aldehydes, ketones and hydrazones of formulae 1, 2 and 3, respectively, is described in the published German Patent Application DE 199 07 588 and in the published International Patent Application WO 2000/050423 of the Applicant. In the method according to the invention, oxidative ring closure is performed on the hydrazone derivatives of formulae 3a and 3b with the aid of metal oxides, preferably NiO_2, $K_3[Fe(CN)_6]$, lead tetraacetate or sodium hypochloride (cf. Houben-Weyl, Vol. E 14b, 4th Edition, 1999).

Synthesis of a triazolo-thiazole analogue of epothilone A (formula 4a, Z = H):

24.6 mg (47.2 µmol) of epothilone-A-21-aldehyde hydrazone (formula 3a) are dissolved in 1.5 ml of absolute dichloromethane. Three aliquots, each of 42.8 mg (472.2 µmol), of nickel peroxide are added at 15-minute intervals, with stirring at room temperature. The mixture is then filtered over Celite, rinsing with dichloromethane. The combined organic phases are concentrated and are dried in a high vacuum. The crude product is purified by means of preparative HPLC (mobile phase: acetonitrile / water 38:62; column: Nucleosil 100 C18 7 µm, 21 x 250 mm). 12.0 mg (49 %) of product were obtained.

The spectroscopic data are identical to epothilone A (cf. DE 4138042 C2) with the exception of:

^{1}H NMR (400 MHz, $CDCl_3$): d = 2.25 (dt, 2a-H), 2.57 (dd, 2b-H), 4.63 (m, 3-H), 5.02 (dd, 3-OH), 1.68 (m, 14a-H), 2.31 (dt, 14b-H), 5.53 (d, 15-H), 6.92 (bs, 17-H), 7.06 (s, 19-H), 7.84 (s, 21-H), 1.08 (s, 22-H), 1.55 (s, 23-H); ^{13}C NMR (100 MHz, $CDCl_3$): d = 73.0 (3-C), 54.7 (4-C), 41.4 (6-C), 71.4 (7-C), 32.0 (14-C), 74.9 (15-C), 145.1 (16-C), 109.2 (17-C), 129.2 (18-C), 115.5 (19-C), 136.4 (20-C), 124.8 (21-C), 15.9 (C-22), 23.3 (23-C), 12.7 (24-.C), 18.1 (27-C); HRMS (DCI): $C_{26}H_{37}N_3O_6S$: [M+NH_4+] calculated 537.2747, found 537.2721.

Synthesis of a triazolo-thiazole analogue of epothilone B (formula 4b, Z = H):

8.1 mg (15.1 µmol) of epothilone-B-21-aldehyde hydrazone (formula 3b) are dissolved in 1 ml of absolute dichloromethane. 13.7 mg (151.4 µmol) of nickel peroxide are added to the solution, whereupon stirring is carried out at room temperature for 15 minutes. The nickel peroxide is filtered off over Celite, washing with dichloromethane. The combined organic phases are concentrated and are dried in a high vacuum. The crude product is purified by means of preparative HPLC (mobile phase: acetonitrile / water 40:60; column: Nucleosil 100 C18 7 µm, 21 x 250 mm), yielding 4.7 mg (58 %) of product.

The spectroscopic data are identical to epothilone B (cf. DE 4138042 C2) with the exception of:

^{1}H NMR (400 MHz, $CDCl_3$): d = 2.25 (dt, 2a-H), 2.59 (dd, 2b-H), 4.69 (m, 3-H), 5.02 (dd, 3-OH), 1.76 (m, 14a-H), 2.31 (dt, 14b-H), 5.53 (d, 15-H), 6.91 (bs, 17-H), 7.06 (s, 19-H), 7.85 (s, 21-H), 1.08 (s, 22-H), 1.56 (s, 23-H); ^{13}C NMR (100 MHz, $CDCl_3$): d = 71.3 (3-C), 54.9 (4-C), 41.0 (6-C), 72.4 (7-C), 33.1 (14-C), 75.2 (15-C), 145.3 (16-C), 108.9 (17-C), 129.2 (18-C), 115.5 (19-C), 136.5 (20-C), 124.9 (21-C), 15.7 (22-C), 23.2 (23-C), 12.0 (24-C), 18.1 (27-C); MS (ESI): $[M+H^+]$ = 534.

The pharmacological activity is shown in the following Table:

Growth test using mammalian cell cultures

Cell line	Origin	Triazolo derivative of epothilone A IC-50 [ng/ml]	epothilone B
L929	Mouse (subcutaneous fat tissue)	10	1.0
K-562	Human (leukaemia)	6	0.7
U-937	Human (lymphoma)	4	0.5

Preparation of 21-*O*-acetyl-epothilone E (5a, R' = CH_3):

2 µl (35.0 µmol) of glacial acetic acid are added to a solution of 3.2 mg (6.2 µmol) of triazolo-epothilone A in 250 µl of dichloromethane and stirring is carried out overnight at room temperature. Water is added to the reaction mixture and extraction with ethyl acetate is carried out three times. The combined organic phases are concentrated and are dried in a high vacuum. 2.8 mg (82 %) of 21-*O*-acetyl-epothilone E were obtained.

The spectroscopic data are identical to epothilone A (cf. DE 4138042 C2) with the exception of:

^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.60 (bs, 17-H), 7.14 (s, 19-H), 5.34 (s, 21-H_2), 2.14 (s, 2'-H_3); ^{13}C NMR (100 MHz, $CDCl_3$): 137.8 (C-16), 119.6 (C-17), 152.5 (C-18), 118.0 (C-19), 163.7 (C-20), 62.5 (C-21), 170.2 (C-1'), 20.9 (C-2'); R_f (CH_2Cl_2/MeOH 95/5): 0.45; HRMS (EI) $C_{28}H_{41}NO_8S$: $[M]^+$ calculated 551.2553, found 551.2519

Preparation of epothilone E-21-*O*-(3'-methoxycarbonyl)propynoic acid ester (5a, R' = CH_3OOC-C_2):

7.0 mg (55.0 µmol) of acetylenedicarboxylic acid monomethyl ester are added to a solution of 5.1 mg (9.8 µmol) of triazolo-epothilone A in 400 µl of dichloromethane. The reaction mixture is stirred overnight at room temperature, water is then added thereto, and extraction with ethyl acetate is carried out three times. The combined organic phases are concentrated and are dried in a high vacuum. After purification by means of PLC (CH_2Cl_2/methanol 95/5) 3.6 mg (59 %) of epothilone E-21-*O*-(3'-methoxycarbonyl)propynoic acid ester were obtained.

The spectroscopic data are identical to epothilone A (cf. DE 4138042 C2) with the exception of:

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.45 (dd, 15-H), 6.60 (17-H), 7.20 (s, 19-H), 5.49 (bs, 21-H_2), 3.85 (s, 5'-H_3); ^{13}C NMR (100 MHz, $CDCl_3$): 76.5 (C-15), 138.2 (C-16), 119.5 (C-17), 153.0 (C-18), 118.8 (C-19), 161.2 (C-20), 64.2 (C-21), 151.1 (C-1'), 152.0 (C-4'), 53.6 (C-5'); R_f (CH_2Cl_2/MeOH 95/5): 0.32; MS (DCI): $[M+NH_4]^+$ = 637.

Photolysis of triazolo-epothilone A to form 21-*O*-methyl-epothilone A (5a, Nu-H = CH_3OH):

9.7 mg (18.7 µmol) of triazolo-epothilone A are dissolved in 1 ml of methanol and, with cooling (ice bath 0°C), are exposed to light for four hours using a mercury vapour lamp (DEMA, HPK-125). The solvent is then reduced and the reaction mixture is separated by means of preparative HPLC (CH_3CN/H_2O 40/60). 2.1 mg (24 %) of 21-methoxy-epothilone A were isolated.

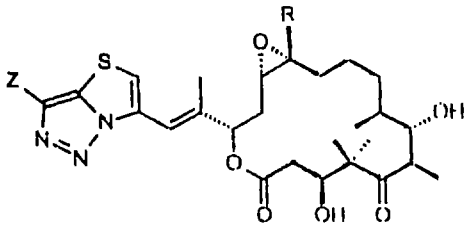
The spectroscopic data are identical to epothilone A (cf. DE 4138042 C2) with the exception of:
^1H NMR (300 MHz, $CDCl_3$): δ = 6.61 (bs, 17-H), 7.13 (s, 19-H), 4.71 (s, 21-H_2), 3.49 (s, 1'-H_3); ^{13}C NMR (75 MHz, $CDCl_3$): 137.5 (C-16), 120.0 (C-17), 152.2 (C-18), 117.3 (C-19), 167.8 (C-20), 71.5 (C-21), 59.1 (C-1'); R_f (CH_2Cl_2/MeOH 95/5): 0.33; HRMS (EI): $C_{27}H_{41}NO_7S$: $[M]^+$ calculated 523.2604, found 523.2609.

1,3-Dipolar cycloaddition of acetylenedicarboxylic acid dimethyl ester and triazolo-epothilone A to form the pyrazole derivative 6a:
1.8 mg (3.5 µmol) of triazolo-epothilone A are dissolved in 200 µl of dichloromethane. Over a period of four hours, three aliquots, each of 4.3 µl (34.7 µmol), of acetylenedicarboxylic acid dimethyl ester are added and stirred at room temperature. The reaction mixture is concentrated slightly and separated by means of PLC (CH_2Cl_2/methanol 95/5). 2.0 mg (87 %) of cycloaddition product were obtained.

The spectroscopic data are identical to epothilone A (cf. DE 4138042 C2) with the exception of:
^1H NMR (400 MHz, $CDCl_3$): δ = 5.49 (dd, 15-H), 6.63 (bs, 17-H), 7.29 (s, 19-H), 3.97 (s, 4'-H_3), 3.95 (s, 6'-H_3); R_f (CH_2Cl_2/MeOH 95/5): 0.17; HRMS (DCI): $C_{32}H_{43}N_3O_{10}S$: $[M+H]^+$ calculated 662.2742, found 662.2778.

Patent claims

1. Triazolo-thiazole analogues of epothilone A and epothilone B of formula 4a or 4b:



4a R = H, Z = H, alkyl, aryl, heteroaryl

4b R = CH_3, Z = H, alkyl, aryl, heteroaryl

wherein:

R denotes H or CH_3

Z denotes H, alkyl, aryl or heteroaryl.

2. Triazolo-thiazole analogues of epothilone A and epothilone B according to claim 1, wherein:

alkyl denotes straight-chain or branched C_1-C_6alkyl, which may be (mono- or poly-)substituted as desired, and/or

aryl denotes mononuclear or polynuclear aromatic systems, which may be (mono- or poly-)substituted as desired, and/or

heteroaryl denotes mononuclear or polynuclear heteroaromatic systems, which may be (mono- or poly-)substituted as desired.

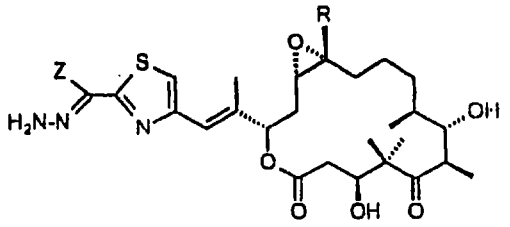
3. Triazolo-thiazole analogues of epothilone A and epothilone B according to claim 2, wherein:

alkyl denotes methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl or hexyl, optionally substituted by C_1-C_6alkoxy, C_1-C_6acyl, hydroxyl or halogen, especially Br, Cl, F or I, and/or

aryl denotes phenyl, o-, m-, p-tolyl, o-, m-, p-xylyl, benzyl, phenethyl or naphthyl, optionally substituted by C_1-C_6alkyl, C_1-C_6alkoxy, C_1-C_6acyl, hydroxyl or halogen, especially Br, Cl, F or I, and/or

heteroaryl denotes furyl, pyranyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl or indolyl, optionally substituted by C_1-C_6alkyl, C_1-C_6alkoxy, C_1-C_6acyl, hydroxyl or halogen, especially Br, Cl, F or I.

4. A method of preparing triazolo-thiazole analogues of epothilone A or epothilone B according to one of the preceding claims, wherein oxidative ring closure is performed on a hydrazone derivative of epothilone A or epothilone B of formula 3a or 3b:



3a R = H, Z = H, alkyl, aryl, heteroaryl

3b R = CH_3, Z = H, alkyl, aryl, heteroaryl

wherein R and Z are as defined hereinbefore, with the aid of metal oxides, $K_3[Fe(CN)_6]$, lead tetraacetate or sodium hypochloride.

5. A method according to claim 4, wherein the metal oxide is NiO_2.

6. A pharmaceutical composition comprising or consisting of one or more triazolo-thiazole analogues of epothilone A or epothilone B according to one of claims 1 to 3 together with a pharmaceutically acceptable carrier, diluent or excipient.

7. Use of a triazolo-thiazole analogue of epothilone A or epothilone B according to one of claims 1 to 3 or of a pharmaceutical composition according to claim 6 in treating tumour diseases and disorders of cell growth.

8. Use according to claim 7, wherein the tumour disease is cancer.

9. A fungicidal composition comprising or consisting of one or more triazolo-thiazole analogues of epothilone A or epothilone B according to one of claims 1 to 3 together with a carrier, diluent or excipient that is acceptable for fungicides.

10. A method of preparing C-21 esters of epothilone A and/or epothilone B, wherein triazolo-epothilone A and/or triazolo-epothilone B (Figure 2, Illustration B) is/are reacted with a carboxylic acid of formula R'COOH (Figure 2, Illustration B) with removal of nitrogen to form a C-21 ester of epothilone A and/or epothilone B and the product(s) of the reaction is/are recovered, wherein R' denotes H, alkyl, alkenyl, alkynyl, aryl or heteroaryl.

11. A method according to claim 10, wherein in the formula R'COOH the radical R' as alkyl, aryl or heteroaryl has one of the meanings according to claim 2.

12. A method according to claim 10, wherein in the formula R'COOH the radical R' denotes C_2-C_8alkenyl or C_2-C_8alkynyl, the multiple bond being in any desired position.

13. A photolytic method wherein triazolo-epothilone A or triazolo-epothilone B is subjected to photolysis in the presence of a nucleophile and the resulting product is recovered (Figure 2, Illustration C).

14. 1,3-Dipolar cycloaddition wherein triazolo-epothilone A or triazolo-epothilone B is subjected to 1,3-dipolar cycloaddition in a manner known *per se* with a double bonded system or a triple bonded system and the heterocyclic cycloaddition product formed is recovered (Figure 2, Illustration D).

15. 1,3-Dipolar cycloaddition according to claim 14, wherein an acetylenecarboxylic acid ester, especially an acetylenedicarboxylic acid diester, is used as triple bonded system.

16. 1,3-Dipolar cycloaddition according to claim 14 or 15, wherein the cycloaddition is carried out in accordance with B. Eistert *et*

al. in Houben-Weyl, Stickstoff-Verbindungen I / Teil 4, Diazoverbindungen, pages 482-894, Thieme-Verlag, Stuttgart 1968.

17. Use of a product of a method or cycloaddition according to one of claims 10 to 16 as a cytotoxic active substance.

18. Use of a product of a method or cycloaddition according to one of claims 10 to 16 in biochemical analysis.

19. Use of the photolytic method according to claim 13 in biochemical analysis, especially for photoaffinity labelling.

20. Product obtainable according to a method or cycloaddition according to one of claims 10 to 16.