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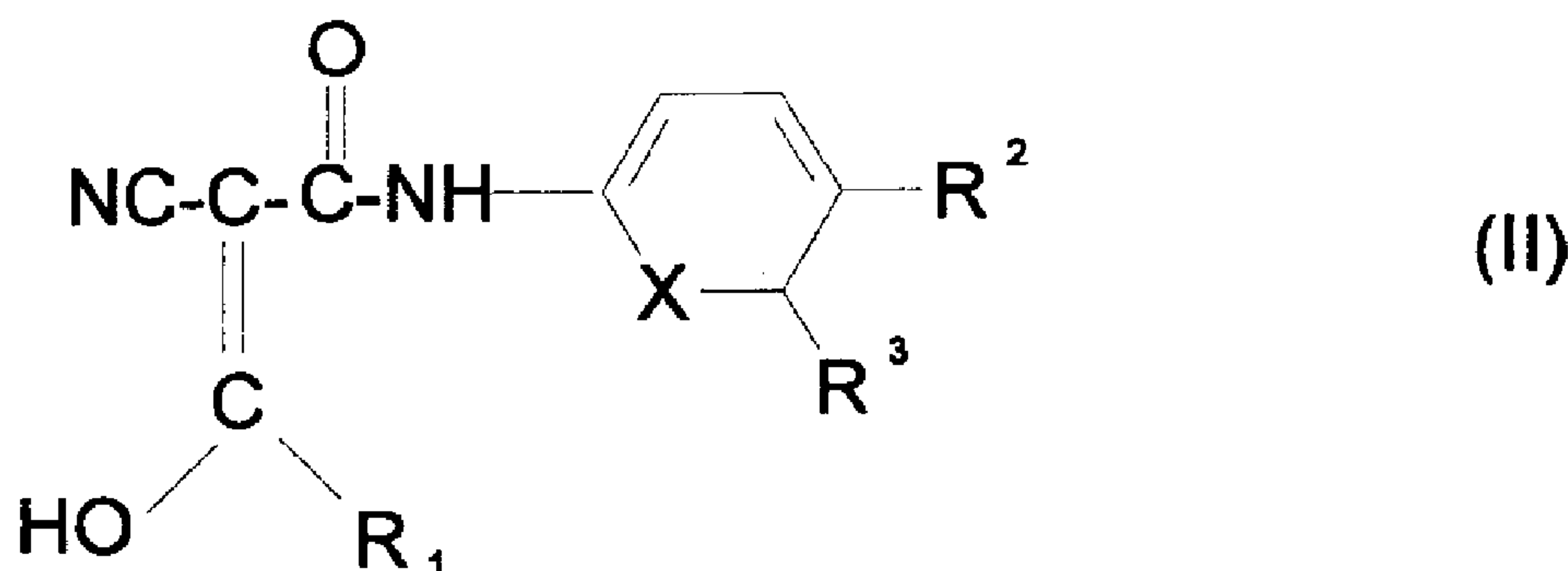
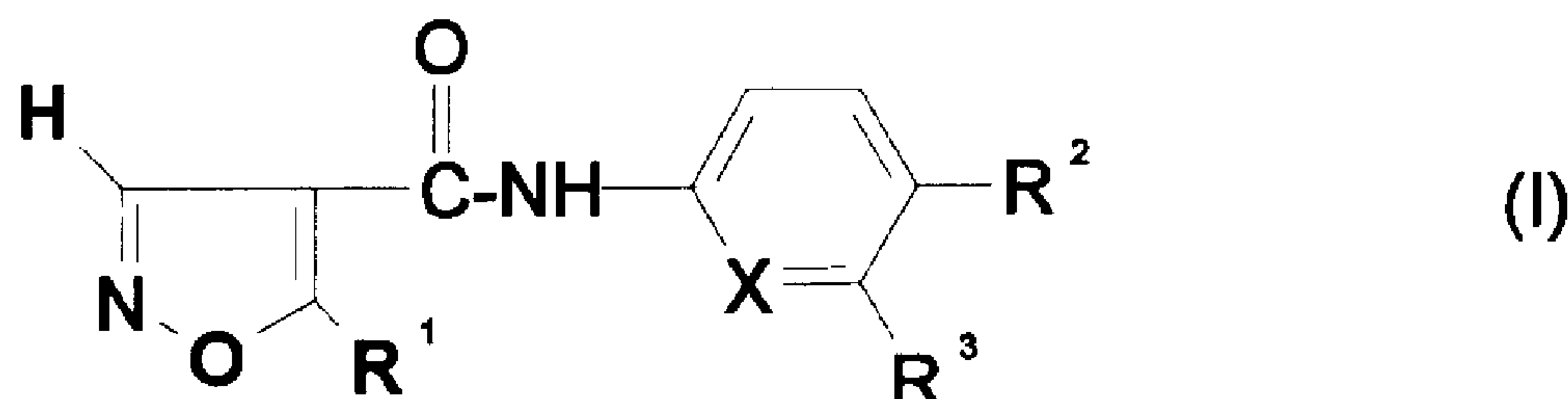
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(54) Titre : UTILISATION DE DERIVES DE L'ISOXAZOLE ET DU CROTONAMIDE POUR LE TRAITEMENT DES TROUBLES CARCINOMATEUX

(54) Title: USE OF ISOXAZOLE AND CROTONAMIDE DERIVATIVES FOR THE TREATMENT OF CARCINOMATOUS DISORDERS



(57) Abrégé/Abstract:

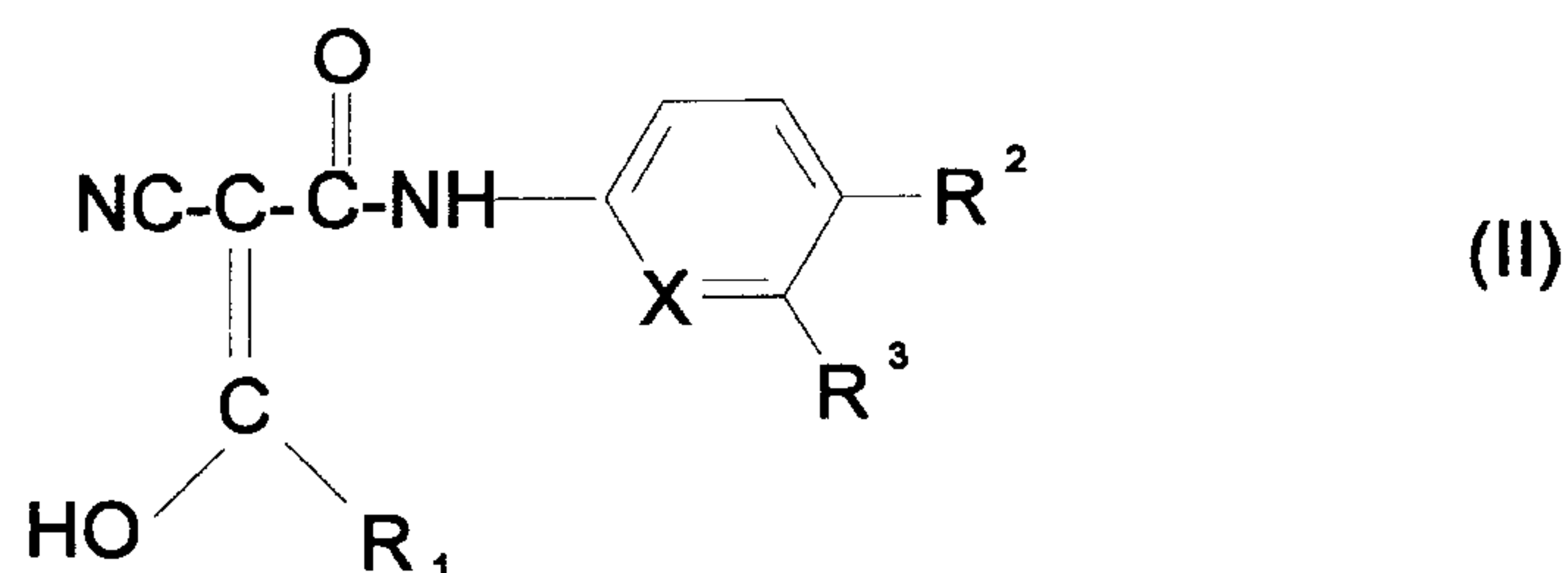
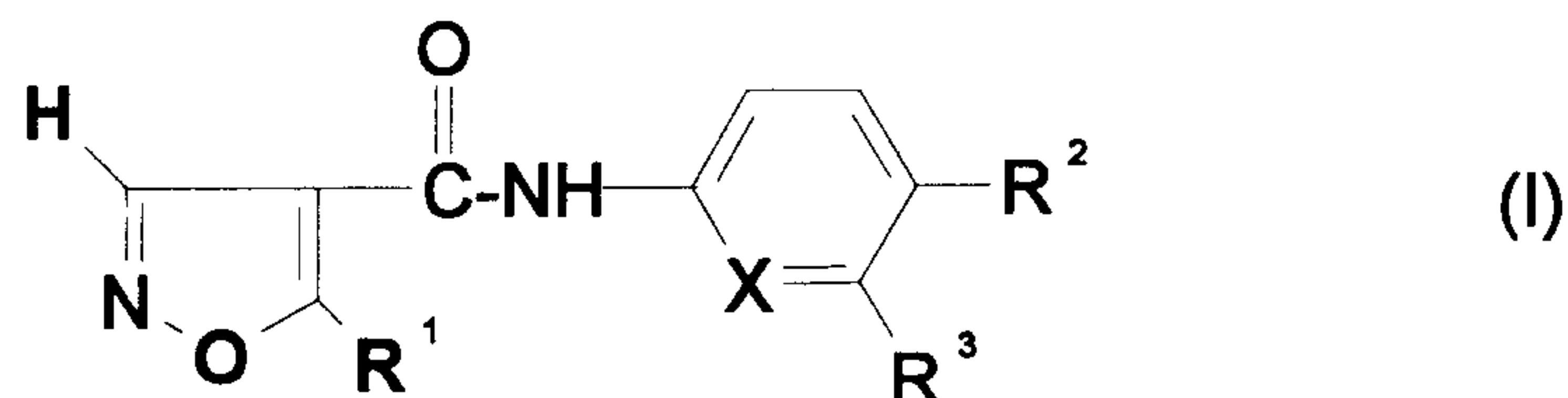
The compound of the formula I or II (see formula I) (see formula II) is suitable for the production of a pharmaceutical for the treatment of carcinomatous disorders, where R¹ is (C₃-C₅)-cycloalkyl, (C₂-C₆)-alkenyl or (C₂-C₆)-alkynyl, R² is CF₃, OCF₃, SCF₃, OH, NO₂, halogen, benzyl, phenyl, CN or O-phenyl, R³ is (C₁-C₄)-alkyl, halogen or a hydrogen atom and X is a -CH group or a nitrogen atom.



Abstract

The use of isoxazole and crotonamide derivatives for the treatment of carcinomatous disorders

The compound of the formula I or II



is suitable for the production of a pharmaceutical for the treatment of carcinomatous disorders, where R^1 is (C_3-C_5) -cycloalkyl, (C_2-C_6) -alkenyl or (C_2-C_6) -alkynyl, R^2 is CF_3 , OCF_3 , SCF_3 , OH, NQ_2 , halogen, benzyl, phenyl, CN or O-phenyl, R^3 is (C_1-C_4) -alkyl, halogen or a hydrogen atom and X is a -CH group or a nitrogen atom.

5 The use of isoxazole and crotonamide derivatives for the treatment of
carcinomatous disorders

Description

10 Chemotherapy is today available for the therapy of advanced hormone
receptor-negative malignant tumors. Beside its limited efficiency, this
form of therapy is characterized by the occurrence of often serious side
effects. The active principle of the chemotherapeutics (inhibition of
proliferation) must be regarded as a cause of the action and the side
15 effects. Since, however, not only tumor cells, but also normal cells are in
division, normal dividing cells in the body of the patient are inhibited from
division just like the actual target cells, the tumor cells. The rapidly
dividing cells of the hair follicles, of the gastrointestinal tract and of the
bone marrow are particularly affected by the undesired side effects of the
antiproliferative therapy.

20

The antiproliferative action of the chemotherapeutics is achieved, for
example, by them intervening in the nucleic acid metabolism of the cell.
Particularly effective antiproliferative substances are the dihydroorotate
dehydrogenase (DHODH) inhibitors. DHODH is a unique enzyme in the
25 de novo synthesis of the pyrimidine nucleotides (Peters et al., 1990,
Biochemical Pharmacology 39: No. 1, 135-144). The enzyme is
concentrated on the outside of the inner mitochondrial membrane.
Inhibition of the enzyme by the active compound DUP-785 (Brequinar)
leads to a depletion of pyrimidine ribo- and deoxyribonukleotides, but not
30 of purine nucleotides (Schwartzmann et al., 1988, Biochem. Pharmacol.
37: 3257-3266). The depletion of dTTP and dCTP is proportional to that
of UTP and CTP and can be prevented by the addition of uridine. The
inhibition of growth exerted on in vitro cell lines by Brequinar can be
abolished by addition of uridine or cytidine, but not by deoxythymidine or
35 deoxycytidine. It can be concluded from this that the inhibition of UMP

synthesis is crucial for the proliferation-inhibiting effect on cell lines in vitro (Peters et al., 1987, Invest. New Drugs, 5: 235-244).

5 In the context of clinical investigations, it was possible to show that Brequinar significantly lowers the plasma uridine values (Peters et al., 1988, Proc. Am Ass. Cancer Res. 29: 350 (Abstract 1392)) (this finding is in agreement with the in vitro observations on various cell lines). In addition, the extent of effects on the in vivo uridine level correlated with the bone marrow and gastrointestinal tract toxicity (the side effects).

10

These clinical observations point to the fact that there is the urgent need to develop cancer therapeutics whose antitumor principle is not based on a general inhibition of proliferation, such as in the case of Brequinar, but on the inhibition of tumor-specific metabolic pathways.

15

It has now been found that the compounds of the formulae I and II only very weakly inhibit human DHODH, but very efficiently block very specific tumor cell lines in their division.

20

Addition of increasing amounts of uridine in the MTT test changes the IC_{50} value of the substances according to the invention on the LoVo cell line only insignificantly, completely in contrast to the massive increase in the IC_{50} value of Brequinar on the same cell line (Example 7).

25

These experimental findings support the assumption that the antiproliferative action of the substances according to the invention is based on a principle of action other than an inhibition of DHODH as in the case of Brequinar.

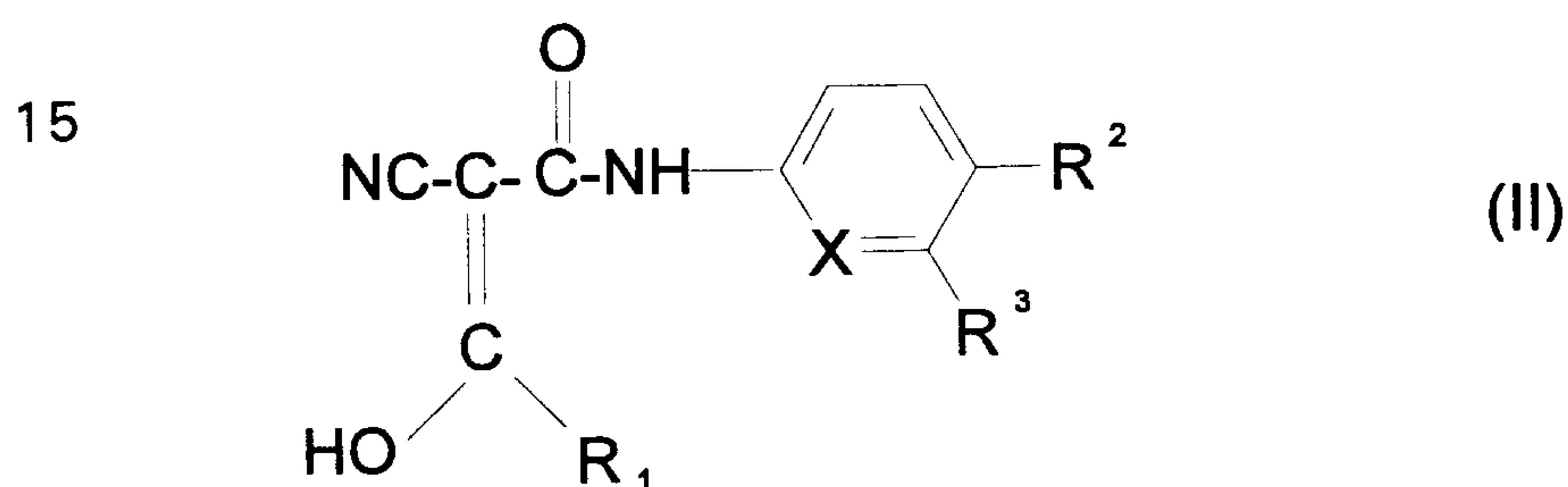
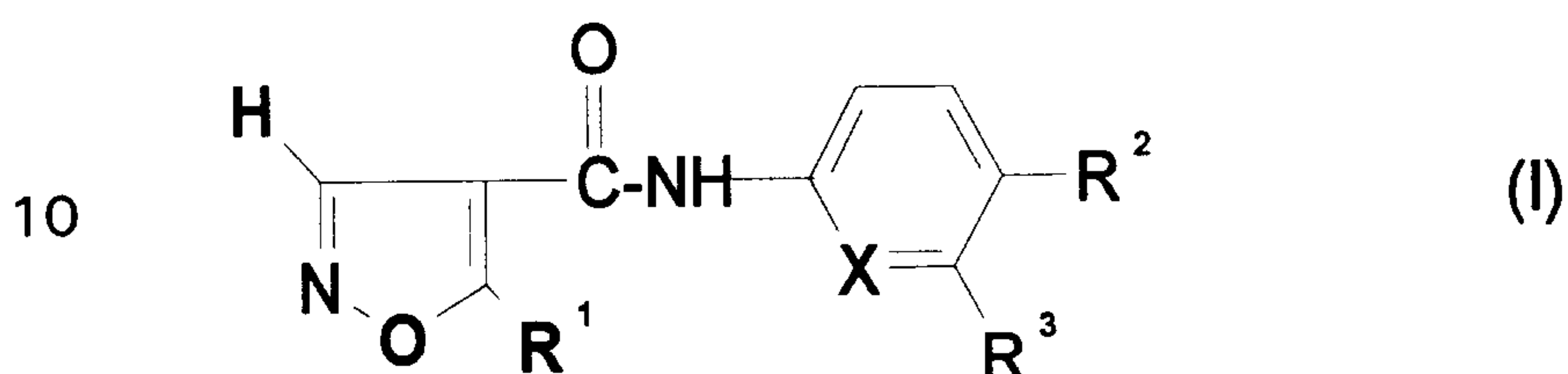
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It was possible to show by means of fluorescence microscopy techniques that those cell lines which strongly overexpress the PDGF receptor and the VEGF receptor are also efficiently prevented from division (Example 6) by very low concentrations of the substances according to the

invention (lower IC_{50}). This observation as a result points to the fact that the substances according to the invention could block specific receptor tyrosine kinases, such as, for example, the PDGF receptor, i.e. advantageously affect the abnormal signal transmission in tumor cells.

5

The invention therefore relates to the use of a compound of the formula I or II



20

and/or an optionally stereoisomeric form of the compound of the formula I or II and/or a physiologically tolerable salt of the compound of the formula II for the production of a pharmaceutical for the treatment of carcinomatous disorders, where

25

R^1 is

- a) (C_3-C_5) -cycloalkyl,
- b) (C_2-C_6) -alkenyl or
- c) (C_2-C_6) -alkynyl,

30

R^2 is

- a) $-CF_3$,
- b) $-O-CF_3$,

- 5 c) -S-CF₃,
d) -OH,
e) -NO₂,
f) halogen,
g) benzyl,
h) phenyl,
i) -CN,
k) -O-phenyl,
l) -O-phenyl, mono- or polysubstituted by
- 10 1) (C₁-C₄)-alkyl,
2) halogen,
3) -O-CF₃ or
4) -O-CH₃,
- 15 R³ is
a) (C₁-C₄)-alkyl,
b) halogen or
c) a hydrogen atom, and
- 20 X is
a) a -CH group or
b) a nitrogen atom.

25 The use is preferred of a compound of the formula I or II and/or an optionally stereoisomeric form of the compound of the formula I or II and/or sodium or lysinium salts of the compound of the formula II, where

R¹ is cyclopropyl, (C₂-C₃)-alkenyl or (C₃-C₅)-alkynyl,
R² is -O-CF₃, -S-CF₃, -O-phenyl, phenyl, -CF₃, -CN or -O-phenyl, mono- or polysubstituted by (C₁-C₄)-alkyl or halogen,

30 R³ is a hydrogen atom or methyl, and
X is a -CH group,

for the production of a pharmaceutical for the treatment of carcinomatous disorders.

The use is particularly preferred of a compound of the formula I or II,
where

R¹ is cyclopropyl, C₃-alkenyl or C₄-alkynyl,

R² is -S-CF₃, CN, 2-methyl-4-chlorophenyl or CF₃,

5 R³ is a hydrogen atom, and

X is a -CH group,

for the production of a pharmaceutical for the treatment of carcinomatous disorders.

10 In particular, the use is preferred of N-(4-trifluoromethylphenyl)-2-cyano-3-hydroxyhexa-2,5-dienecarboxamide, 2-cyano-3-cyclopropyl-3-hydroxyacrylic acid (4-trifluoromethylphenyl)amide, 2-cyano-3-cyclopropyl-3-hydroxyacrylic acid (4-thiotrifluoromethylphenyl)amide, 2-cyano-3-cyclopropyl-3-hydroxyacrylic acid (2-methyl-4-chlorophenyl)amide, N-(4-trifluoromethylphenyl)-2-cyano-3-hydroxy-
15 hept-2-en-6-ynecarboxamide lysine or sodium salt or 2-cyano-3-cyclopropyl-3-hydroxyacrylic acid (4-cyanophenyl)amide lysine or sodium salt.

20 The compounds of the formula I or II are prepared by known processes such as are described in EP 13 376; EP 484 223; EP 538 783; EP 551 230 or US 4 061 767.

The term alkyl, alkenyl or alkynyl is understood as meaning radicals
25 whose carbon chain can be straight-chain or branched. The alkenyl or alkynyl radicals can furthermore also contain two or more double bonds or two or more triple bonds. Cyclic alkyl radicals are, for example, 3- to 5-membered monocyclic systems such as cyclopropyl, cyclobutyl or cyclopentyl. The starting substances for the chemical reactions are
30 known or can be readily prepared by methods known from the literature. The carcinomatous disorders include, for example, leukemia, in particular chronic leukemia of the T- and B-cell type, Hodgkin's or non-Hodgkin's lymphoma, carcinoma, lung cancer, ovarian cancer, lymph node cancer,

sarcoma, Kaposi's sarcoma, meningioma, intestinal cancer, brain tumors, breast cancer, stomach cancer, pancreatic cancer, prostatic cancer or skin cancer.

- 5 The invention also relates to a process for the production of a pharmaceutical for the treatment of carcinomatous disorders, which comprises bringing the compound of the formula I or II and/or a physiologically tolerable salt of the compound of the formula II into a suitable administration form using a pharmaceutically suitable and
- 10 physiologically acceptable excipient and, if appropriate, further suitable active compounds, additives or auxiliaries. The pharmaceuticals according to the invention can be administered orally, topically, rectally, intravenously or alternatively parenterally.
- 15 Suitable solid or liquid pharmaceutical administration forms are, for example, granules, powders, coated tablets, tablets, (micro)capsules, suppositories, syrups, juices, suspensions, emulsions, drops or injectable solutions and also preparations having a protracted release of active compound, in whose preparation customary auxiliaries, such as
- 20 excipients, disintegrants, binders, coating agents, swelling agents, glidants or lubricants, flavorings, sweeteners or solubilizers are used. Frequently used auxiliaries which may be mentioned are, for example, magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, lactoprotein, gelatin, starch, cellulose and its derivatives,
- 25 animal and vegetable oils, polyethylene glycols and solvents, such as, for example, sterile water and mono- or polyhydric alcohols, e.g. glycerol.

Preferably, the pharmaceutical preparations are prepared and administered in dose units, each unit as active constituent containing a

30 certain dose of the compound of the formula I or II and/or physiologically tolerable salts of the compound of the formula II. In the case of solid dose units, such as tablets, capsules or suppositories, this dose can be up to approximately 300 mg, but preferably 10 to 200 mg.

For the treatment of a patient (70 kg), in early phases an intravenous infusion treatment of at most 1200 mg per day and in the later rehabilitation phase an oral administration of 3 times 300 mg per day of the compound of the formula I or II and/or of the corresponding salts of the compound of the formula II are indicated.

Under certain circumstances, however, even higher or lower doses may be appropriate. The dose can be administered both by single administration in the form of an individual dose unit or else of several smaller dose units and by multiple administration of subdivided doses at certain intervals.

Finally, the compounds of the formula I or II and/or their corresponding salts can also be combined together with other suitable active compounds, for example antiuricopathics, platelet aggregation inhibitors, analgesics and steroidal or nonsteroidal antiinflammatories, during the preparation of the abovementioned pharmaceutical administration forms.

Example 1

N-(4-Trifluoromethylphenyl)-2-cyano-3-hydroxyhept-2-en-6-yne-carboxamide sodium salt (compound 1)

- 5 50 g (0.15 mol) of N-(4-trifluoromethylphenyl)-2-cyano-3-hydroxyhept-2-en-6-yne-carboxamide are dissolved in a two-phase system of 50 ml of 5 N sodium hydroxide solution and 500 ml of ethyl acetate, and the organic phase is separated off, washed twice with a little water, dried over sodium sulfate and concentrated. The oily residue is taken up using 500
10 ml of tertiary-butyl methyl ether and stirred at room temperature for 4 hours (h) to complete crystallization, filtered and dried under reduced pressure. For the complete removal of solvent residues, the crystalline product is suspended under reflux for 10 min in 500 ml of toluene, cooled with stirring, filtered off with suction again and dried under reduced
15 pressure. Yield: 41.1 g (77%) of melting point $>244^{\circ}\text{C}$ decomposition (dec.).

$\text{C}_{15}\text{H}_{10}\text{F}_3\text{N}_2\text{O}_2\text{Na}$ (330.24 g/mol):

calculated C: 54.0 H: 3.5 N:8.4 Na: 6.88 (calc. for 1.1% water)

found C: 54.4 H: 3.4 N:8.4 Na: 6.65 water: 1.1%

20

Example 2

2-Cyano-3-cyclopropyl-3-hydroxyacrylic acid (4-cyanophenyl)amide sodium salt (compound 2)

- 25 15 g (0.059 mol) of 2-cyano-3-cyclopropyl-3-hydroxyacrylic acid (4-cyanophenyl)amide are suspended in 120 ml of water and 100 ml of acetone and brought into solution by addition of 60 ml of 1N NaOH. After filtration of traces of undissolved material, the solution is concentrated under reduced pressure in a rotary evaporator to approximately 200 ml,
30 and the product is crystallized overnight at 0°C , filtered off with suction and dried under reduced pressure.

Yield: 13 g, m.p. $>280^{\circ}\text{C}$.

$\text{C}_{14}\text{H}_{10}\text{N}_3\text{O}_2\text{Na}$ (275.24):

calculated C: 60.7 H: 3.7 N:15.2 (calc. for 0.7% water)
found C: 60.8 H: 3.6 N:15.3 water: 0.7%

Example 3

5 N-(4-Trifluoromethylphenyl)-2-cyano-3-hydroxyhept-2-en-6-yne-
carboxamide lysine salt

30 g (0.097 mol) of N-(4-trifluoromethylphenyl)-2-cyano-3-hydroxy-hept-2-
en-6-yne-carboxamide are dissolved in 1 l of water and 25 ml of ethanol
10 together with 17.3 g (0.097 mol) of L-lysine hydrate, filtered and
lyophilized. Adhering residual amounts of ethanol are removed by
repeated freeze drying.

Yield: 44.4 g of mainly amorphous product, m.p. 135-138°C.

¹H-NMR (DMSO-d₆): 1.23-1.77 (m, 6H), 2.3-2.45 (m, 2H), 2.50-2.65 (m,
15 2H), 2.7-2.85 (m, 3H), 3.25 (tb, 1H), 5.7-7.4 (sb, 6H), 7.55 and 7.73
(AA'BB', in each case 2H), 12.35 (s, 1H)

Example 4

20 2-Cyano-3-cyclopropyl-3-hydroxyacrylic acid (4-cyanophenyl)amide lysine
salt

15 g (0.054 mol) of 2-cyano-3-cyclopropyl-3-hydroxyacrylic acid
(4-cyanophenyl)amide are dissolved in 900 ml of water and 10 ml of
ethanol together with 9.6 g (0.054 mol) of L-lysine hydrate, filtered and
25 lyophilized. Adhering residual amounts of ethanol are removed by drying
under reduced pressure.

Yield: 21.8 g of mainly amorphous product, m.p.>100°C (dec.).

¹H-NMR (DMSO-d₆): 0.6-0.82 (m, 4H), 1.27-1.75 (m, 6H), 2.17 (mc, 1H),
2.77 (tb, 2H), 3.28 (tb, 1H), 4.8-7.5 (sb, 6H), 7.63 and 7.7 (AA'BB', in
30 each case 2H), 12.6 (s, 1H)

Example 5

Human DHODH (spleen) enzyme activity is determined according to Williamson et al. (The Journal of Biological Chemistry, 270, (1995), pages 22467-22472). The IC₅₀ value is in each case given in nM.

5

Table 1:

DHODHIC₅₀ in nM

10

Compound 1	292
Compound 2	625
Compound 12	539
Brequinar	4

Example 6

15

Inhibition of the proliferation of tumor cells (MTT test)

20

1 x 10⁴ cells per well are inoculated into a 96-well microtiter plate. After 24 h, the test substances are added at various concentrations. Each group consists of 4 wells, the control is only incubated with medium. After 65 h, 50 µl of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; 2.5 mg/ml in PBS) are added and, after 7 h, the supernatant is removed. The color formed by the living cells is dissolved by addition of 100 µl of dimethyl sulfoxide/well. The extinction is measured at 492 nm for each well with the aid of a Multiscan photometer 340 CC (Flow). The cell lines used are obtainable as follows from the American Type Culture Collection:

25

HUV-EC-C is ATCC CRL 1730; A-172 is ATCC CRL 1620; L 1210 is ATCC CCL 219; LoVo is ATCC CCL 229; C 6 is ATCC CCL 107 and a rat glioblastoma cell line.

30

The mean value is formed from the 4 wells of a group and the IC₅₀ values are calculated from the dose-response curve using the Software 3.0 (Erithacus Software Ltd.). Table 2 shows the results.

Table 2:

MTT Test
IC₅₀ in μM

Cell	Origin (human)	Compound 1	Compound 2	Brequinar
LoVo	Colonic carcinoma	137	392	0.388
HUV-EC-C	Endothelial cell	164	360	9.4
A-172	Glioblastoma	78	169	0.2
L1210	Leukemia (mouse)	9.1	6.1	1.0
C 6	Rat glioblastoma	68	20	9

Example 7

The procedure is as in Example 6; uridine is additionally added to the batches. Table 3 shows the results.

Table 3:

MTT Test on LoVo cells
IC₅₀ in μM

Uridine [μM]	Compound 1	Compound 2	Brequinar
0	168.0	359.2	0.388
1000	277.7	560.0	128.8

The compounds mentioned in Table 4 are prepared as in Examples 1 to 4.

The compounds are tested as described in Example 6.

5

10

15

Com- pound	Structure	A172	C 6	LoVo	HUVE C
1		78	68	137	164
2		169	20	392	360
5		30	5	34	94
6		25	11	24	>400
7		37	11	37	267
8		28	18	13	138
9		193	21	400	>400

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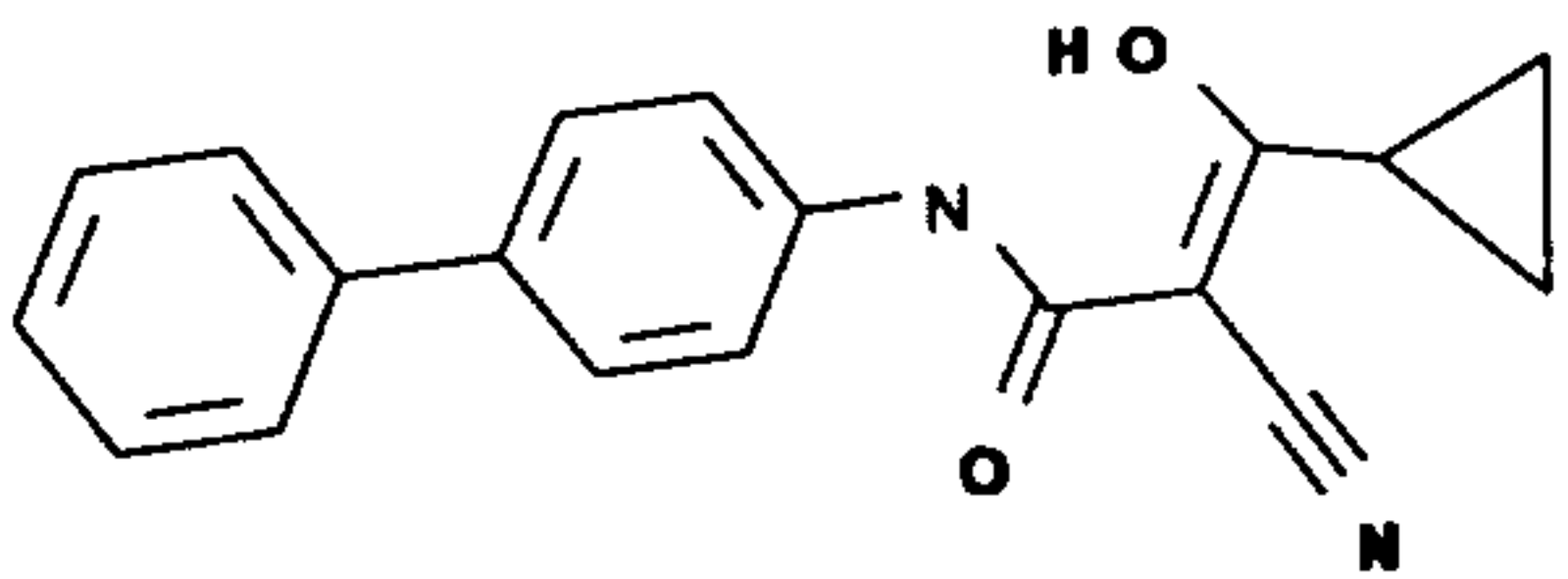
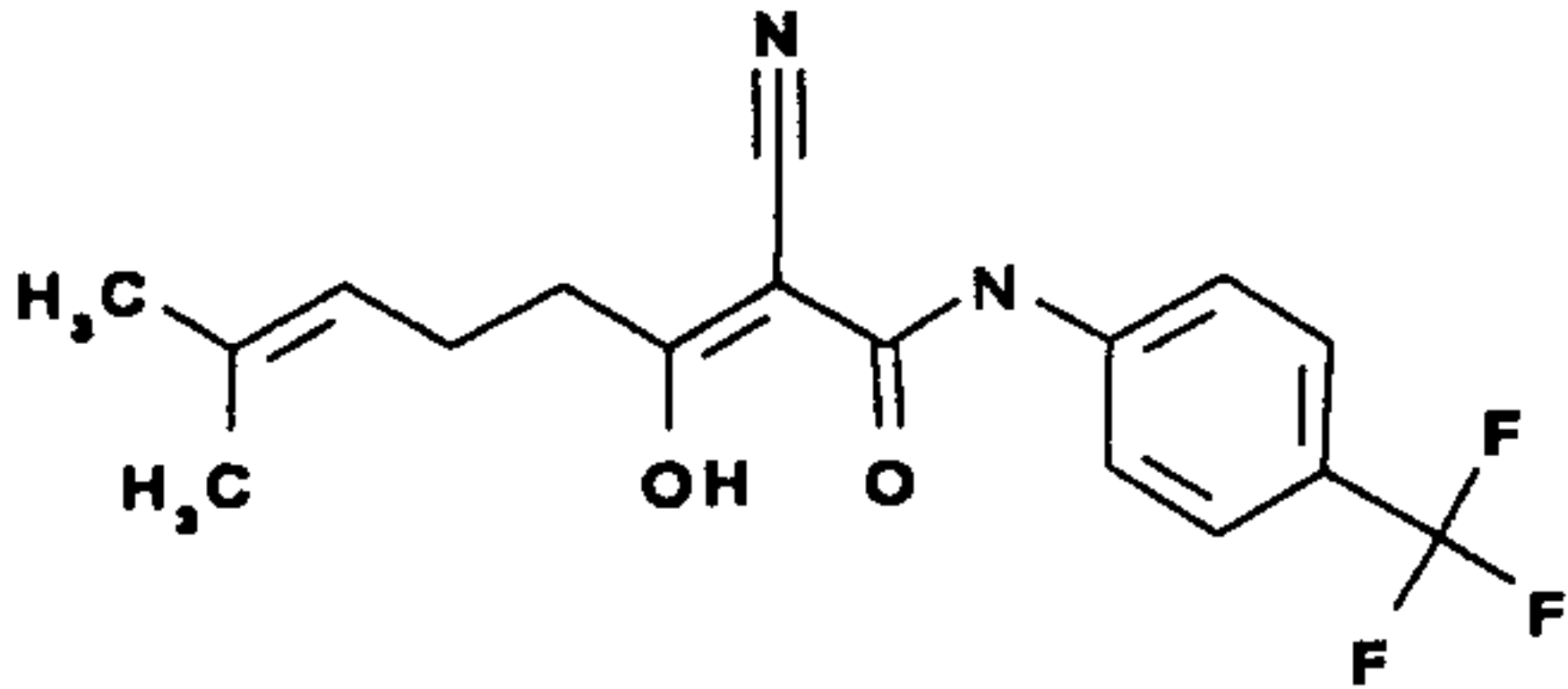
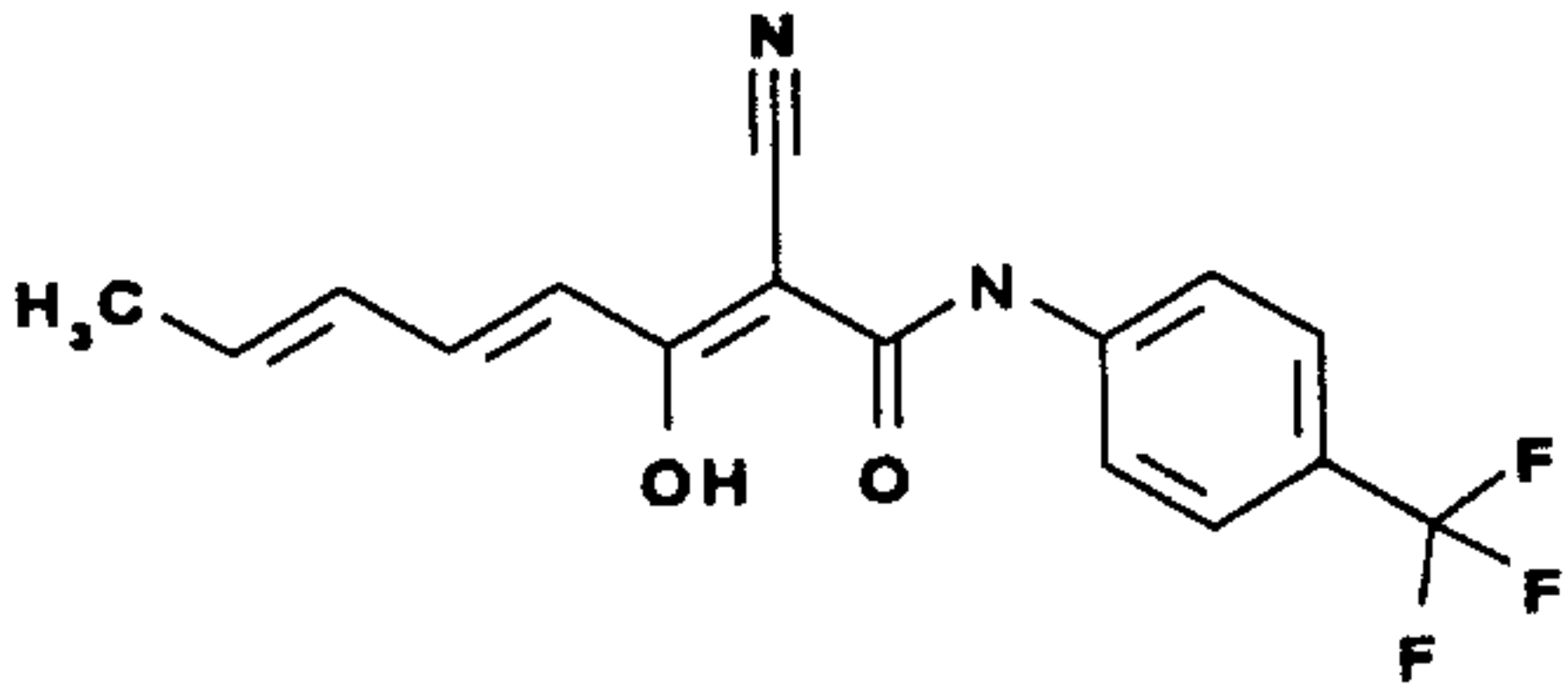
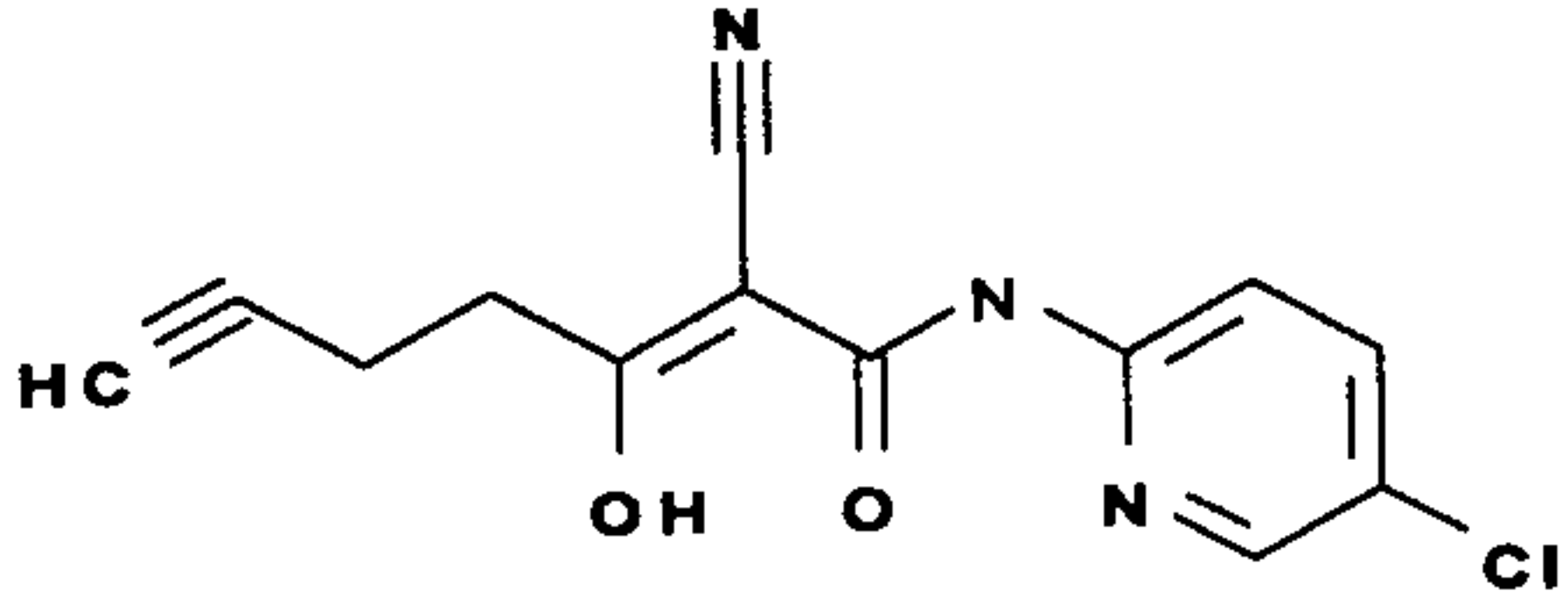
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Com- pound	Structure	A172	C 6	LoVo	HUVE C
10		25	4	21	186
11		55	57	160	187
12		43	6	55	322
13		32	13	15	>400
14		58	7	61	197
15		13	2	21	98
16		17	36	7	299

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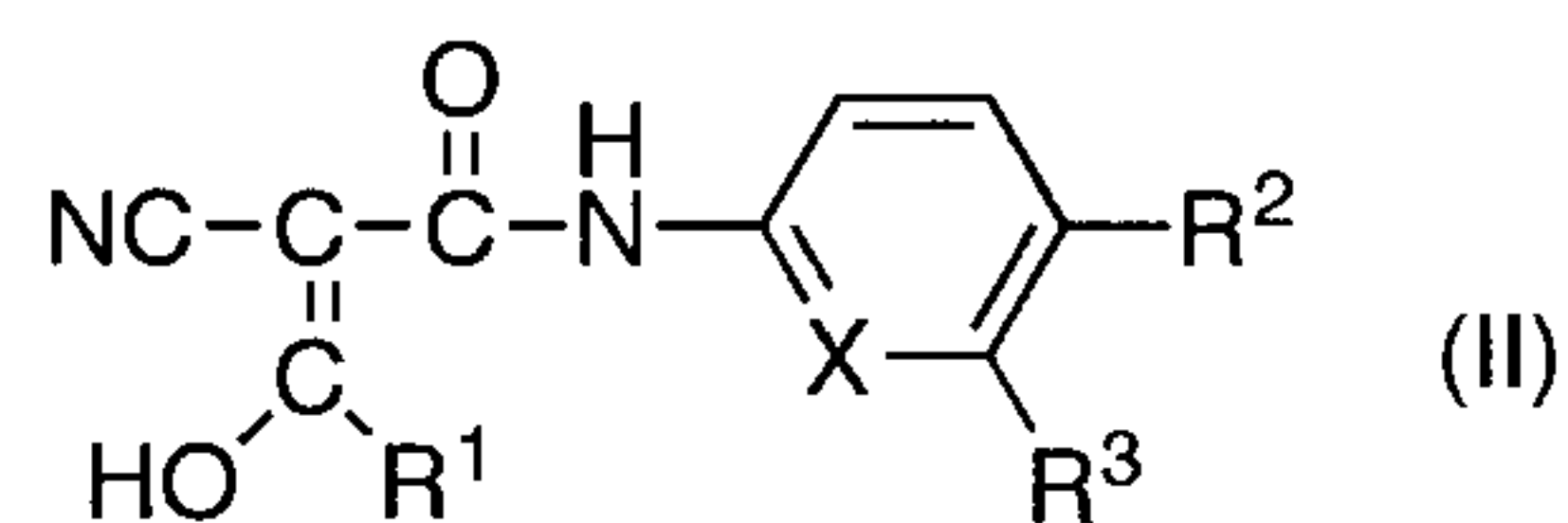
Com- pound	Structure	A172	C 6	LoVo	HUVE C
17		55	186	75	127
18		190	84	110	159
19		118	32	171	135
20		158	182	119	>400
21		63	69	27	339
22		>400	103	149	>400
23		94	7	75	>400

Com-pound	Structure	A172	C 6	LoVo	HUVE C
24		22	34	6	74
25		95	87	306	301
26		200	24	146	378
27		142	126	153	>400

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A use of a sodium or lysinium salt of the compound of the formula II

5



- 10 and/or an optionally stereoisomeric form of the sodium or lysinium salt of the compound of the formula II for the production of a pharmaceutical for the treatment of carcinomatous disorders, where

R¹ is

- a) (C₃-C₅)-cycloalkyl,

15

R² is

- a) -CF₃,
- b) -O-CF₃,
- c) -S-CF₃,
- 20 d) -OH,
- e) -NO₂,
- f) halogen,
- g) benzyl,
- h) phenyl,
- 25 i) -CN,
- k) -O-phenyl or
- l) -O-phenyl, mono- or polysubstituted by
 - 1) (C₁-C₄)-alkyl,
 - 2) halogen,
 - 30 3) -O-CF₃ or

4) -O-CH₃,

R³ is

5

- a) (C₁-C₄)-alkyl,
- b) halogen or
- c) a hydrogen atom, and

X is

10

- a) a -CH group or
- b) a nitrogen atom.

2. The use of the sodium or lysinium salts of the compound of the formula II as claimed in claim 1, where

R¹ is

15

- a) cyclopropyl,

R² is

20

- a) -CF₃
- b) -O-CF₃,
- c) -S-CF₃,
- d) -O-phenyl,
- e) -phenyl,
- f) -CN or
- g) -O-phenyl, mono- or polysubstituted by (C₁-C₄)-alkyl or halogen,

25

R³ is a hydrogen atom or methyl, and

X is a -CH group,

for the production of a pharmaceutical for the treatment of carcinomatous disorders.

30

3. The use of the sodium or lysinium salts of the compound of the formula II as claimed in claim 1 or 2, where

R¹ is cyclopropyl,
R² is -S-CF₃, CN, or CF₃,
R³ is a hydrogen atom, and
5 X is a -CH group,

for the production of a pharmaceutical for the treatment of carcinomatous disorders.

10 4. The use of the compound of the formula II as claimed in any one of claims 1 to 3, wherein the sodium or lysinium salt of 2-cyano-3-cyclopropyl-3-hydroxyacrylic acid (4-trifluoromethylphenyl)amide, 2-cyano-3-cyclopropyl-3-hydroxyacrylic acid (4-thiotrifluoromethyl-phenyl)amide or 2-cyano-3-cyclopropyl-3-hydroxyacrylic acid (4-cyanophenyl)amide is employed.

15

5. The use of the sodium or lysinium salts of the compound of the formula II and/or an optionally stereoisomeric form of the sodium or lysinium salts of the compound of the formula II as claimed in claim 1, for the production of a pharmaceutical for the treatment of leukemia, chronic leukemia of the T-
20 or B-cell type, Hodgkin's or non-Hodgkin's lymphoma, carcinoma, lung cancer, ovarian cancer, lymph node cancer, sarcoma, Kaposi's sarcoma, meningioma, intestinal cancer, brain tumors, breast cancer, stomach cancer, pancreatic cancer, prostatic cancer or skin cancer.

