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(54) **Title:** 4-SUBSTITUTED DERIVATIVES OF PYRAZOLO[3,4-d]PYRIMIDINE AND PYRROLO[2,3-d]PYRIMIDINE AND USES THEREOF

(57) **Abstract:** 4-Substituted derivatives of pyrazolo[3,4-d]pyrimidine and pyrrolo[2,3-d]pyrimidine are described. Compounds are active as antitumoral agents.

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4-SUBSTITUTED DERIVATIVES OF PYRAZOLO[3,4-*d*]PYRIMIDINE AND PYRROLO[2,3-*d*]PYRIMIDINE AND USES THEREOF

* * * *

The present patent concerns 4-substituted derivatives of pyrazolo[3,4-*d*]pyrimidine and pyrrolo[2,3-*d*]pyrimidine. In addition, the patent concerns the use of compounds, herein described, as agents especially, but not limited, for the therapy of tumors, their use for preparing pharmaceutical compositions, methods for the synthesis of compounds and their intermediates.

Adenosine is an endogenous neuro-modulator which acts through the activation of four receptor subtypes termed, A_1, A_{2A}, A_{2B}, e A_3, identified and cloned from several mammalian tissues[1-3].

In spite of the great therapeutic potential, relatively few compounds have been introduced in clinical use [4,5], probably for the poor selectivity of the compounds for a single adenosine receptor subtype.

Nitrogen containing etherocyclic compounds have been characterised as putative A_1 selective antagonist[6]. Among them, pyrazol[3,4-*b*]piridine[8,9] and of pyrazolo[3,4-*d*]pirimidine[10-13] derivatives posses selective A_1 antagonist properties. They have potential therapeutic applications as cognitive enhancers, and anti-dementia agents (e.g. for Alzheimer' disease and cerebrovascular dementia), stimulants, anti-depressants and agents which ameliorate brain functions.[14] A_1AR antagonists have also shown potential therapeutic applications in kidney and cardiac failure'[16].

Ethyl ester derivatives of 4-amino-1-(2-hydroxy-2-phenylethyl)-1*H*-pyrazole [3,4-*b*]piridin-5-carboxylic acid[17] have been previously synthesised. They bind selectively to A_1 receptor and show antagonist activity. In order to improve their activity and search for more potent and A_1 selective antagonists, a pseudoreceptor[18] model was developed. This model theoretically predicts compounds A_1 binding.

The present invention concerns the discovery of a family of 4-substituted derivatives of pyrazolo[3,4-*d*]pyrimidine and pyrrolo[2,3-*d*]pyrimidine which have been designed according to the above described approach. Surprisingly, selected compounds of this series possessed tyrosine kinase inhibitor activity, hence they have potential anti-angiogenic and anti-tumoral properties.

Object of the present invention is a 4-substituted derivatives of pyrazolo[3,4-*d*]pyrimidine and pyrrolo[2,3-*d*]pyrimidine having the following structure:

(I) 

wherein

R_1 = one of the residues **1a** to **1l** of Table 1;

R_2 = $CH_2CHClPh$.

Preferably R_1 is **1h** or **1j** or **1k** as reported in Table 1.

According to the present invention the compounds depicted above may be given as medicament more preferably as anti-tumour agent.

It is another object of the invention a pharmaceutical composition comprising ate least one of the compounds of the invention or a pharmacologically acceptable salt, and suitable excipients and /or diluents. Compounds of the invention or their salts may be administered as pure or as pharmaceutical formulations, i.e. suitable for parenteral, oral, or rectal administrations. Each of said formulations may contain excipients and/or fillers and/or additives and/or binders, coatings and/or suspending agents and/or emulsifying agents, preserving and/or control release agents, suitable for the selected pharmaceutical form.

The invention shall be described with reference to Figure 1 showing histograms of the inhibitory activity of cell proliferation of EGF receptor containing A431 cells (A) and of VEGF containing PAEC cells (B).

Example 1. Synthesis of 4-substituted derivatives of 1-(2-chloro-2-phenylethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine **1a-l** and **2**.

Chemistry

1*H*-Pyrazolo[3,4-*d*]pyrimidines **1a-l** and **6** were synthesized as follows (Scheme). The starting product was the ethyl ester of 5-amino-1-(2-hydroxy-2-phenylethyl)-1*H*-pyrazole carboxylic acid **2**, prepared following (18).

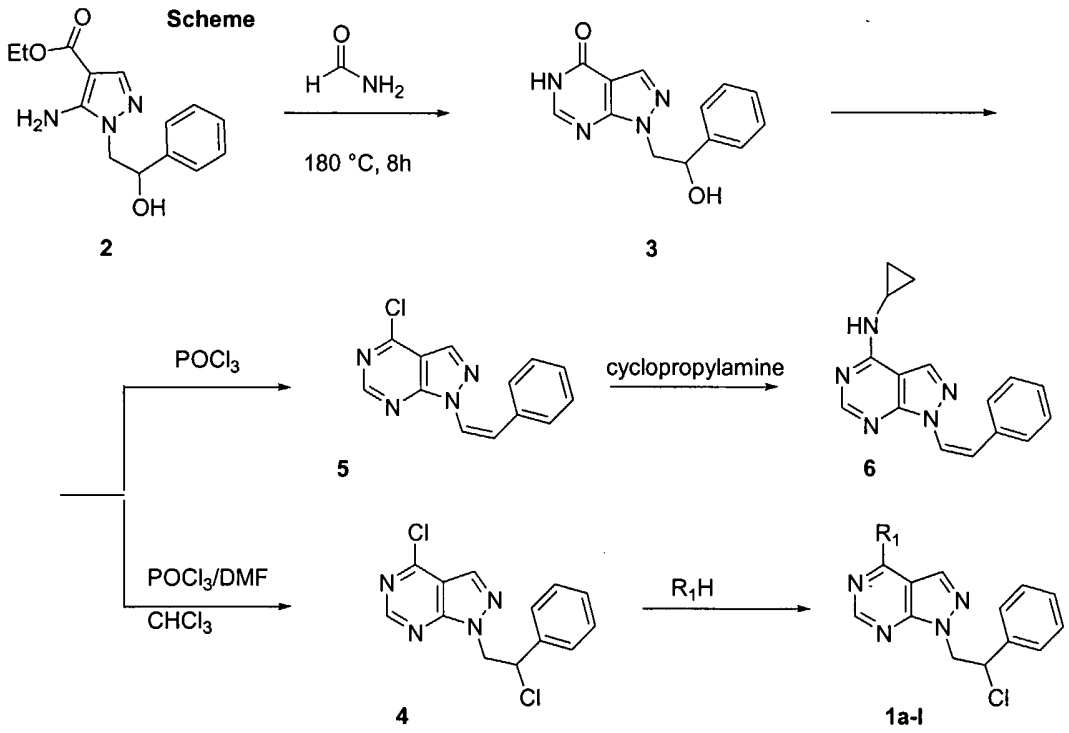
Reaction of **2** with formamide in excess and heating at 200°C for 8 hours afforded the pyrazolo[3,4-d]pyrimidinone **3** which was purified by dissolving the crude in

NaOH 2M, boiling with coal, followed by precipitation with acetic acid (yield 70%, m.p. 271-272°C).

The chlorination with Vilsmeier complex ($POCl_3$/DMF 1:1) in $CHCl_3$ at reflux for 8 hours afforded the dihalogenated derivative **4**, bearing a chlorine atom in position 4 of the pyrimidine ring and on the side chain on the N1 of the pyrazole ring. Product **4** was purified by simply filtration on Florisil® column (yield 70%, m.p. 104-105°C). It is interesting to note that treatment of **3** at reflux with $POCl_3$ afforded the undesired 1-styryl derivative **5** instead.

The reaction scheme is as follows :

Scheme



Regioselective substitution of the C4 chlorine of compounds **4** with an excess of various primary and secondary amines, in toluene at r.t., afforded the desired products **1a-l** in good yield (Table 1).

Table 1. 4-Substituted derivatives of pyrazolo[3,4-*d*]pyrimidine and pyrrolo[2,3-*d*]pyrimidine **1a-l** and **6**.

Compound	R_1	yield %	mp (°C)	Formula
1a	NH cyclopropyl	90	194-195	$C_{16}H_{16}N_5Cl$
1b	NHC_3H_7	88	142-143	$C_{16}H_{18}N_5Cl$
1c	1-pyrrolidinyl	75	161-162	$C_{17}H_{18}N_5Cl$
1d	NHC_4H_9	80	93-94	$C_{17}H_{20}N_5Cl$
1e	$N(CH_2CH_3)_2$	85	147-148	$C_{17}H_{20}N_5Cl$
1f	4-morpholinyl	70	132-133	$C_{17}H_{18}N_5ClO$
1g	$NHCH_2CH_2OC_2H_5$	65	119-120	$C_{17}H_{20}N_5ClO$
1h	1-piperidinyl	72	148-149	$C_{18}H_{20}N_5Cl$
1i	NH cyclohexyl	71	157-158	$C_{19}H_{22}N_5Cl$
1j	1-hexahydroazepinyl	55	134-135	$C_{19}H_{22}N_5Cl$
1k	$NHCH_2C_6H_5$	80	158-160	$C_{20}H_{18}N_5Cl$
1l	$NHCH_2CH_2C_6H_5$	81	102-103	$C_{21}H_{20}N_5Cl$
6	NH cyclopropyl	70	141-142	$C_{16}H_{15}N_5$

It is interesting to point out that the chlorine atom at the side chain of all of the new compounds has never been substituted by the amine group. This finding was proved by the ^{1}H-NMR chemical shifts of the CH_2CH side chain protons, which resonates unchanged at the same position and with the same pattern.

Compound **6** was prepared by reaction of **5** with cyclopropylamine, in toluene at r.t.

Starting materials were purchased from Aldrich-Italia (Milan).

Melting points were determined with a Büchi B 540 apparatus and are uncorrected. IR spectra were recorded in KBr with a Perkin-Elmer 398 spectrophotometer. ^{1}H NMR spectra were recorded in $CDCl_3$ solution on a Varian Gemini 200 (200 MHz) instrument, chemical shifts (deltą are reported in ppm relative to TMS as internal standard; *J* in Hz. ^{1}H patterns are described using the following abbreviations: s = singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, sext = sextet, m = multiplet, br = broad. (Tables 2 and 3).

All compounds were tested for purity by TLC (Merk, Silica gel 60 F_{254}, $CHCl_3$ as eluant)

Analyses for C, H, N were within ± 0.3% of the theoretical value.

Table 2. IR e ^{1}H NMR spectral data of compounds **1a-l**.

compound	IR, cm^{-1} ($CHCl_3$)	^{1}H NMR, δ(ppm) ($CDCl_3$)
1a	3410 (NH)	0.72-0.81 e 0.98-1.09 (2m, 4H, $2CH_2$ cycloprop), 2.88-3.02 (m, 1H, CHNH), 4.71-4.83 and 4.95-5.08 (2dd, 2H, CH_2N), 5.53-5.62 (m, 1H, CHCl), 6.68 (s all, 1H, NH, disappear in D_2O), 7.25-7.40 and 7.42-7.52 (2m, 5HAr), 8.19 (s, 1H, H-3), 8.31 (s, 1H, H-6)
1b	3415 (NH)	1.04 (t, J = 6.9, 3H, CH_3), 1.62-1.85 (m, 2H, $CH_2CH_2CH_3$), 3.58 (q, J = 6.9, 2H, CH_2N prop), 4.68-4.82 e 4.92-5.06 (2dd, 2H, CH_2N), 5.48-5.60 (m, 1H, CHCl), 7.22-7.52 (m, 6H, 5H Ar + H-3), 7.90 (s, 1H, H-6), 8.34 (s all, 1H, NH, decrease in D_2O).
1c		1.95-2.25 (m, 4H, $2CH_2$ pirr), 3.78 (t, 4H, $2CH_2N$ pirr), 4.69-4.79 e 4.95-5.06 (2dd, 2H, CH_2N), 5.53-5.62 (m, 1H, CHCl), 7.22-7.40 e 7.42-7.52 (m, 5H Ar), 7.92 (s, 1H, H-3), 8.36 (s, 1H, H-6).
1d	3415 (NH)	0.98 (t, J = 7.3, 3H, CH_3 but), 1.35-1.55 e 1.56-1.90 (2m, 4H, $2CH_2$ but), 3.61 (q, J = 7.3, 2H, CH_2N but), 4.68-4.82 e 4.92-5.06 (2dd, 2H, CH_2N), 5.48-5.62 (m, 1H, CHCl + s all, 1H, NH, decrease in D_2O), 7.22-7.52 (m, 5H Ar), 7.90 (s, 1H, H-3), 8.34 (s, 1H, H-6).
1e		1.31 (t, J = 7.3, 6H, $2CH_3$ et), 3.74, (q, J = 7.0, 4H, $2CH_2$ et), 4.68-4.82 and 4.85-5.06 (2dd, 2H, CH_2N), 5.53-5.62 (m, 1H, CHCl), 7.22-7.38 e 7.42-7.52 (2m, 5H Ar), 7.92 (s, 1H, H-3), 8.36 (s, 1H, H-6).
1f		3.78-3.88 (m, 4H, $2CH_2N$ morf), 3.90-4.02 (m, 4H, $2CH_2O$), 4.71-4.83 and 4.95-5.06 (2dd, 2H, CH_2N), 5.53-5.62 (m, 1H, CHCl), 7.22-7.40 e 7.42-7.51 (2m, 5H Ar), 7.94 (s, 1H, H-3), 8.37 (s, 1H, H-6).
1g	3340 (NH)	1.24 (t, J = 7, 3H, CH_3), 3.56 (q, J = 7, 2H, OCH_2CH_3), 3.68 (t, J = 6.9, 2H, CH_2O), 3.82 (q, J = 6.9, 2H, CH_2NH), 4.71-4.83 e 4.93-5.06 (2dd, 2H, CH_2N), 5.52-5.60 (m, 1H, CHCl), 5.80 (s all, 1H, NH, disappear in D_2O), 7.26-7.50 (m, 5H Ar), 7.91 (s, 1H, H-3), 8.38 (s, 1H, H-6).
1h		1.62-1.85 (m, 6H, $3CH_2$ pip), 3.93 (t, 4H, $2CH_2N$), 4.68-4.82 e 4.95-5.06 (2dd, 2H, CH_2N), 5.53-5.62 (m, 1H, CHCl), 7.25-7.40 and 7.42-7.52 (2m, 5H Ar), 7.94 (s, 1H, H-3), 8.35 (s, 1H, H-6).
1i	3403 (NH)	1.18-2.20 (m, 10H, $5CH_2$ cicloes), 3.42 (m, 1H, CHNH), 4.05 (s all, 1H, NH, decrease in D_2O), 4.68-4.80 and 4.92-5.06 (2dd, 2H, CH_2N), 5.53-5.60 (m, 1H, CHCl), 7.22-7.50 (m, 5H Ar), 7.85 (s, 1H, H-3), 8.34 (s, 1H, H-6).
1j		1.55-1.75 (m, 4H, $2CH_2$), 1.78-2.00 (m, 4H, $2CH_2$), 3.78-4.10 (m, 4H, $2CH_2N$ hexahydro), 4.68-4.82 and 4.92-5.08 (2dd, 2H, CH_2N), 5.53-5.62 (m, 1H, CHCl), 7.26-7.40 e 7.42-7.52 (2m, 5H Ar), 7.89 (s, 1H, H-3), 8.36 (s, 1H, H-6).

1k	3320 (NH)	4.68-4.84 e 4.92-5.02 (m + dd, 4H, CH_2N + CH_2NH), 4.92-5.06 (dd, 1H, CH_2N), 5.52-5.60 (m, 1H, CHCl), 7.26-7.50 (m, 11H, 10H Ar + NH, decrease in D_2O), 7.85 (s, 1H, H-3), 8.38 (s, 1H, H-6).
1l	3420 (NH)	3.02 (t, J = 6.9, 2H, CH_2Ar), 3.82-3.98 (q, J = 6.9, 2H, CH_2NH feniletil), 4.71-4.84 and 4.94-5.08 (2dd, 2H, CH_2N), 5.52-5.62 (m, 1H, CHCl + 1H, NH, disappear in D_2O), 7.22-7.52 (m, 10H Ar), 7.895 (s, 1H, H-3), 8.40 (s, 1H, H-6).
6	3615 (NH) 1655 (C=C)	0.77-0.87 (m, 2H, CH_2), 1.00-1.11 (m, 2H, CH_2), 2.92-3.06 (m, 1H, CH cyclopr.), 6.35 (br. s, 1H, NH, disappears with D_2O), 7.20-7.58 (m, 6H, 5H Ar + CH=), 8.07 (d, J_{trans} = 14.6, 1H, CH=), 8.30 (s, 1H, H-3), 8.38 (s, 1H, H-6).

Table 3. Elemental analysis of compounds **1a-l** and **6**.

compound		C%	H%	N%
1a	Calc.	61.24	5.14	22.32
	Found	60.91	5.02	22.43
1b	Calc.	60.85	5.74	22.18
	Found	60.80	5.77	22.13
1c	Calc.	62.29	5.53	21.36
	Found	62.10	5.70	21.15
1d	Calc.	61.91	6.11	21.23
	Found	61.60	6.31	21.25
1e	Calc.	61.91	6.11	21.23
	Found	61.36	6.00	21.30
1f	Calc.	59.39	5.28	20.37
	Found	59.19	5.45	20.20
1g	Calc.	59.04	5.83	20.25
	Found	58.81	5.84	20.30
1h	Calc.	63.24	5.90	20.49
	Found	63.00	6.07	20.54
1i	Calc.	64.13	6.23	19.68
	Found	64.16	6.40	19.67
1j	Calc.	64.13	6.23	19.68
	Found	63.86	6.37	19.80
1k	Calc.	66.02	4.99	19.25
	Found	66.00	4.85	19.53
1l	Calc.	66.75	5.33	18.53
	Found	66.51	5.37	18.30
6	Calc.	69.30	5.45	25.25
	Found	69.02	5.50	25.38

Example 2: *1-(2-Hydroxy-2-phenylethyl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one* **3**.

A suspension of **3** (2.75 g, 10 mmol) in formamide (10 g, 333 mmol) was heated at 190°C for 8 h and then poured in H_2O (300 mL). The crude was filtered and purified by dissolving in 2M NaOH (100 mL), boiling with coal, followed by precipitation with glacial acetic acid. The solid was filtered and recrystallized from absolute ethanol to give **3** (1.79 g, 70%) as a white solid: mp 270-271 °C. ^{1}H NMR: □ 4.25-4.55 (m, 2H, CH_2), 5.05-5.15 (m, 1H, CHO), 5.65 (d, 1H, OH, disappears with D_2O), 7.25-7.45 (m, 5H Ar), 8.05 (s, 1H, H-3), 8.10 (s, 1H, H-6), 12.15 (br s, 1H, NH, disappears with D_2O). IR cm^{-1}: 3400 (NH), 3245-2500 (OH), 1740 (C=O).

Example 3: *4-Chloro-1-(2-phenylvinyl)-1H-pyrazolo[3,4-d]pyrimidine* **5**.

$POCl_3$ (14 g, 91 mmol) was added to **4** (2.56 g, 10 mmol), the mixture was refluxed for 12 h and then cooled to room temperature.

The excess of $POCl_3$ was removed by distillation under reduced pressure. H_2O (20 mL) was carefully added to the residue and the suspension was extracted with $CHCl_3$ (3 x 20 mL). The organic solution was washed with H_2O (10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude brown oil was purified by column chromatography (Florisil® 100-200 Mesh), using $CHCl_3$ as the eluant to afford the pure product **5** (1.66 g, 65%) as a white solid: mp 139-140 °C. ^{1}H NMR: □ 7.25-7.61m, 6H, 5H Ar + CH=), 8.04 (d, J_{trans} = 14.6, 1H, CH=), 8.29 (s, 1H, H-3), 8.85 (s, 1H, H-6). IR cm^{-1} 1658 (C=C).

Example 4: *4-Chloro-1-(2-chloro-2-phenylethyl)-1H-pyrazolo[3,4-d]pyrimidine* **4**.

The Vilsmeier complex, previously prepared from $POCl_3$ (6.13 g, 40 mmol) and anhydrous dimethylformamide (DMF) (2.92 g, 40 mmol) was added to a suspension of **3** (2.56 g, 10 mmol) in $CHCl_3$ (20 mL).

The mixture was refluxed for 7 h. The solution was washed with H_2O (2 x 20 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude oil was purified by column chromatography (Florisil®, 100 Mesh), using CH_2Cl_2 as eluant, to afford the pure product **4** (2.2 g, 75%) as a white solid, mp 105-106 °C. ^{1}H NMR□4.78-4.97 and 5.02-5.18 (2dd, 2H, CH_2), 5.50-5.59 (m, 1H, CHCl), 7.25-7.48 (m, 5H Ar), 8.18 (s, 1H, H-3), 8.75 (s, 1H, H-6).

Example 5: *General procedure for 1-(2-chloro-2-phenylethyl)-1H-pyrazolo[3,4-d]pyrimidines 4-amino substituted* **1a-l**.

To a solution of **4** (2.93 g, 10 mmol) in anhydrous toluene (20 mL), the proper amine (40 mmol) was added, and the reaction mixture was stirred at room temperature for 36 h. After it was extracted with H_2O (10mL), the organic phase was dried under reduced pressure; the oil residue crystallized by adding absolute ethanol (10 mL) to give products **1a-l**

1a. White solid, mp 194-195 °C, yield 90%. ^{1}H NMR: □ 0.73-0.87 and 0.98-1.10 (2m, 4H, $2CH_2$), 2.88-3.02 (m, 1H, CH), 4.71-4.83 and 4.95-5.08 (2dd, 2H, CH_2N), 5.53-5.62 (m, 1H, CHCl), 6.68 (br s, 1H, NH, disappears with D_2O), 7.25-7.52 (m, 5H Ar), 8.19 (s, 1H, H-3), 8.32 (s, 1H, H-6). IR cm^{-1}: 3410 (NH).

1b. White solid, mp 142-143 °C, yield 88%. ^{1}H NMR: □ 1.04 (t, J = 7.0, 3H, CH_3), 1.76 (sx, J = 6.0, 2H, CH_2), 3.58 (q, J = 6.0, 2H, CH_2Nprop), 4.68-4.82 and 4.92-5.06 (2dd, 2H, CH_2N), 5.53-5.62 (m, 2H, CHCl), 5.90-6.20 (br s, 1H, NH, disappears with D_2O), 7.25-7.52 (m, 5H Ar), 7.90 (s, 1H, H-3), 8.35 (s, 1H, H-6). IR cm^{-1}: 3415 (NH).

1c. White solid, mp 161-162 °C, yield 75%. ^{1}H NMR: □ 1.95-2.25 (m, 4H, $2CH_2$), 3.78 (t, 4H, $2CH_2$Npyrr), 4.68-4.82 and 4.95-5.06 (2dd, 2H, CH_2N), 5.53-5.62 (m, 1H, CHCl), 7.22-7.52 (m, 5H Ar), 7.92 (s, 1H, H-3), 8.36 (s, 1H, H-6).

1d. White solid, mp 148-149 °C, yield 72%. ^{1}H NMR: □ 1.62-1.85 (m, 6H, $3CH_2$), 3.95-4.07 (m, 4H, $2CH_2$Npip), 4.67-4.82 and 4.92-5.08 (2dd, 2H, CH_2N), 5.53-5.63 (m, 1H, CHCl), 7.25-7.52 (m, 5H Ar), 7.94 (s, 1H, H-3), 8.35 (s, 1H, H-6).

1e. White solid, mp 132-133 °C, yield 70%. ^{1}H NMR: □ 3.79-3.89 and 3.91-4.04 (2m, 8H, $4CH_2$ morph), 4.71-4.83 and 4.94-5.08 (2dd, 2H, CH_2N), 5.53-5.62 (m, 1H, CHCl), 7.24-7.52 (m, 5H Ar), 7.94 (s, 1H, H-3), 8.37 (s, 1H, H-6).

1f. White solid, mp 119-120 °C, yield 65%. ^{1}H NMR: □ 1.24 (t, J = 7.0, 3H, CH_3), 3.56 (q, J = 7.0, 2H, $CH_3\underline{CH_2}O$), 3.68 (t, 2H, CH_2O), 3.83 (q, 2H, $\underline{CH_2}NH$), 4.71-4.83 and 4.95-5.06 (2dd, 2H, CH_2N), 5.52-5.60 (m, 1H, CHCl), 5.80 (br s, 1H, NH, disappears with D_2O), 7.26-7.50 (m, 5H Ar), 7.91 (s, 1H, H-3), 8.39 (s, 1H, H-6). IR cm^{-1}: 3439 (NH).

1g. White solid, mp 157-158 °C, yield 71%. ^{1}H NMR: □ 1.19-1.58, 1.62-1.95 and 2.04-2.20 (3m, 10H, 5CH_2), 3.95-4.15 (m, 1H, CHNH), 4.69-4.81 and 4.91-5.08 (2dd, 2H, CH_2N), 5.51-5.61 (m, 1H, CHCl), 7.12-7.62 (m, 5H Ar), 7.86 (s, 1H, H-3), 8.38 (s, 1H, H-6), 8.79 (br s, 1H, NH, disappears with D_2O). IR cm^{-1}: 3403 (NH).

1h. White solid, mp 134-135 °C, yield 55%. ^{1}H NMR: □1.55-1.78 and 1.80-2.03 (2m, 8H, 4CH_2), 3.75-4.04 (m, 4H, 2CH_2N), 4.68-4.82 and 4.93-5.08 (2dd, 2H, CH_2N), 5.52-5.65 (m, 1H, CHCl), 7.27-7.57 (m, 5H Ar), 7.88 (s, 1H, H-3), 8.36 (s, 1H, H-6).

1i. White solid, mp 102-103 °C, yield 81%. ^{1}H NMR: □ 3.02 (t, J = 7.0, 2H, CH_2), 3.91 (q, J = 7.0, 2H, CH_2NH), 4.70-4.85 and 4.95-5.10 (2dd, 2H, CH_2N), 5.51-5.64 (m, 2H, CHCl + NH, 1H disappears with D_2O), 7.22-7.55 (m, 10H Ar), 7.87 (s, 1H, H-3), 8.40 (s, 1H, H-6). IR cm^{-1}: 3420 (NH).

1j. White solid, mp 161-162 °C, yield 85%. ^{1}H NMR: □ 1.30 (t, J = 7.0, 6H, 2CH_3), 3.74 (q, J = 7.0, 4H, 2CH_2), 4.68-4.83 and 4.85-5.06 (2dd, 2H, CH_2N), 5.52-5.63 (m, 1H, CHCl), 7.15-7.58 (m, 5H Ar), 7.85 (s, 1H, H-3), 8.36 (s, 1H, H-6).

1k. White solid, mp 93-94 °C, yield 80%. ^{1}H NMR: □ 0.98 (t, J = 7.0, 3H, CH_3), 1.35-1.59 (sx, J = 7.0, 2H, CH_2), 1.61-1.79 (quintet, J = 7.0, 2H, CH_2), 3.60 (q, J = 7.0, 2H, CH_2NH), 4.70-4.81 and 4.92-5.08 (2dd, 2H, CH_2N), 5.48-5.63 (m, 2H, CHCl + NH, 1H disappears with D_2O), 7.25-7.55 (m, 5H Ar), 7.90 (s, 1H, H-3), 8.35 (s, 1H, H-6). IR cm^{-1}: 3415 (NH).

1l. White solid, mp 158-160 °C, yield 82%. ^{1}H NMR: □ 4.69-4.80 and 4.93-5.07 (2dd, 2H, CH_2N), 4.82-4.88 (m, 2H, CH_2NH), 5.51-5.59 (m, 2H, CHCl), 7.25-7.51 (m, 10H Ar), 7.85 (s, 1H, H-3), 8.39 (s, 1H, H-6). IR cm^{-1}: 3425 (NH).

N-cyclopropyl-1-(2-styryl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine **6.**

Compound **6** was prepared according to the synthetic sequence described for compounds **1** starting from product **5**. White solid: mp 220-221 °C., yield 85%. ^{1}H NMR: □ 0.77-0.87 (m, 2H, CH_2), 1.00-1.11 (m, 2H, CH_2), 2.92-3.06 (m, 1H, CH cyclopr.), 6.35 (br. s, 1H, NH, disappears with D_2O), 7.20-7.58 (m, 6H, 5H Ar + CH=), 8.07 (d, J_{trans} = 14.6, 1H, CH=), 8.30 (s, 1H, H-3), 8.38 (s, 1H, H-6). IR cm^{-1} 3615 (NH), 1655 (C=C).

Example 6. Tyrosine kinase inhibitors activity

The aim of the experiments herein described was to evaluate compounds, matter of the invention, as inhibitors of tyrosine kinase activity linked to receptors involved in endothelial cell growth such as VEGFFR, FGFR and EGFR. Compounds have been evaluated for their ability to influence the survival, and proliferation of cultered cells over-expressing VEGF, FGF, and EGF receptors.

Methods

Compounds were dissolved in DMSO 0.1M, then diluted in cultured medium. Reference compounds were:

SU5614: {5-cloro-3-[(3,5-dimetil-2-pirroil)metilen]-2-indolinone} selective for VEGF

SU55402: {3-[3-(2-carbossietil)-4-metilpirrol-2-metilidenil]-2-indolinone}selective for FGFR1

AG 1478: [4-(3-Cloroanilino)-6,7-dimetossichinazolina] selective for EGF

Cells

The following cell lines were used: A431 a stable tumor cell line endowed with high density of EGF receptors; PAEC a porcine coronary endothelial cell line transfected with VEGFR2 receptors; Chinese hamster ovarian cells (CHO) transfected with FGFR receptors. Cultured medium was DMEM.

Toxicity

Compounds (10 uM) were added to suspended cells in culture and incubated for 1 hr. To asses cell survival the Trypan Blue exclusion test was performed at the end of incubation.

Proliferation

Cells were seeded in 96 well microtiter plates (2500 cells/well) and incubated for 5hr in 10% FCS. Medium was then changed to 0.1% FCS and incubation prolonged at 37C. After 16 hr stabilisation, reference and experimental compounds were added to the medium at 50 nM. The MTT test was used to monitor cell proliferation at 48 hr. PAEC and CHO growth was stimulated by adding VEGF (20 ng/ml) or FGF(20 ng/ml) during the 48 hr incubation. For the A431 cell lines, EGF was not added since its receptor was constitutively activated.

Results

Most compounds (10 uM) exerted no toxic effects on the cell lines examined (Table 4). Few compounds influenced CHO cell survival at concentration lower than 10 uM.

The inhibitory effects exhibited by the compounds at 50 nM on cell proliferation are reported in Figure 1a, Figure 1b, and Table 5. Results are expressed as %, relative to the reference compound inhibitory effect (100%). Mean standard error was calculated from six data originating from two separate experiments.

Table 4. Toxicity of compounds **1a-l**.

Compound	**PAEC EC_{50}**	**A431 EC_{50}**	**CHO EC_{50}**
1a	ne	ne	Ne
1l	ne	ne	ne
1f	ne	ne	ne
1b	ne	ne	ne
1e	ne	ne	3 μM
1d	ne	ne	6 μM
1g	ne	ne	ne
1i	ne	ne	ne
1c	ne	ne	ne
1h	ne	ne	ne
1j	ne	ne	ne
1k	ne	ne	ne
Reference compound	ne	ne	ne

ne = no effect at 10 μM

EC_{50}=concentration causing death in 50% of the cell population

Table 5. Inhibitory effects on proliferation of CHO cells

Compound	IC_{50} [μM]	Inhibition (%)
SU5402	0.05	100
1a	>5	<30
1l	>5	<30
1f	>5	<30
1b	>5	<30
1e	>5	<30
1d	>5	<30
1g	>5	<30
1i	>5	<30
1c	>5	<30
1h	>5	<30
1j	>5	<30
1k	**0.1**	**50**

Among the compounds examined **1h**, **1j**, e **1k** demonstrated inhibitory activity on the proliferation of the A-431 e PAEC cell lines. In these cells, several compounds exhibited greater inhibitory activity than the reference compound AG 1478. Growth of CHO cells, stimulated by FGF-2, was rather insensitive to the compounds examined, except for compound **1k**, which was the most active also on PAEC.

The inhibitory activity exerted by the compounds on proliferation may be attributed to blockade of receptors, as non-specific toxic effects were not observed.

The study has therefore selected compounds which inhibit cell growth induced by specific kinases. Among them. compound **1k** is potentially active through mechanisms mediated by its specific receptor.

LITERATURE

1. Daly, J. W. *J. Med. Chem.* **1982**, *25*, 197.

2. Müller, C. E.; Scior, T. *Pharm. Acta Helv.* **1993**, *68*, 77.

3. Jacobson, K. A.; Van Galen, P. J. M.; Williams, M. *J. Med. Chem.* **1992**, *35*, 407, and references cited there

4. Erfurth, A.; Schmauss, M. *Fortschr. Neurol. Psychiatr.* **1995**, *63*, 93.

5 Scammels, P. J.; Baker, S. P.; Beauglehole, A. R. *Bioorg. Med. Chem.* **1998**, *6*, 1517.

6. Williams, M. *Med. Res. Rew.* **1989**, *9*, 219.

7. Poulsen, S. A.; Quinn, R. *Bioorg. Med. Chem.* **1998**, 6, 619.

8. Daly, J. W.; Hutchinson, K. D.; Secunda, S. I.; Shi, D.; Padgett, W. L.; Shamin, M.T. *Med. Chem. Res.* **1994**, *4*, 293.

9. Psychoyos, S.; Ford, C. J.; Phillips, M. A. *Biochem. Pharmacol.* **1982**, *31*, 1441.

10. Hamilton, H. W.; Ortwine, D. F.; Worth, D. F.; Bristol, J. A. *J. Med. Chem.* **1987**, *30*, 91.

11. Davies, L. P.; Chow, S. C.; Skerrit, J. H.; Brown, D. J.; Johnston, G. A. R. *Life Sci.* **1984**, *34*, 2117.

12. Poulsen, S. A.; Quinn, J. R. *J. Med. Chem.* **1996**, *39*, 4156.

13. Harden, F. A. Quinn, J. R.; Scammells, P. J. *J. Med. Chem.* **1991**, *34*, 2892.

14. Ralevic,V.; Burnstock, G. *Pharmacol. Rev.* **1998**, *50*, 413.

15. Biagi, G.; Giorgi, I.; Livi, O.; Pacchini, F.; Pacchini, F.; Rum, P.; Scartoni, V.; Costa, B.; Mazzoni, M.R.; Giusti, L. *Farmaco*, **2002**, *57*, 221.

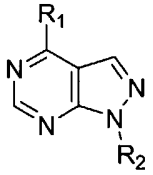
16. Da Settimo , F.; Primofiore, G.; Taliani; S.; Marini, A.M.; La Motta, C.; Novellino, E.; Greco, G.; Lavecchia, A.; Trincavelli, L.; Martini, C. *J. Med. Chem.* **2001**, *44*, 316.

17. Schenone, S.; Bruno, O.; Fossa, P.; Ranise, A.; Menozzi, G.; Mosti, L.; Bondavalli, F.; Martini, C.; Trincavelli, L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2529.

18. Bondavalli, F.; Botta, M.; Bruno, O.; Ciacci, A.; Corelli, F.; Fossa, P.; Lucacchini, A.; Manetti, F.; Martini, C.; Menozzi, G.; Mosti, L.; Ranise, A.; Schenone, S.; Tafi, A.; Trincavelli, L. *J. Med. Chem.* **2002**, *45*, 4875.

CLAIMS

1. 4-substituted derivatives of pyrazolo[3,4-*d*]pyrimidine and pyrrolo[2,3-*d*]pyrimidine having the formula:

(I) 

wherein

R_1 = one of the residues **1a** to **1l** of Table 1;

R_2 = $CH_2CHClPh$.

2. 4-substituted derivatives of pyrazolo[3,4-*d*]pyrimidine and pyrrolo[2,3-*d*]pyrimidine according to Claim 1 wherein R_1 is one of the following residues of Table 1: **1h** or **1j** or **1k**.

3. 4-substituted derivatives of pyrazolo[3,4-*d*]pyrimidine and pyrrolo[2,3-*d*]pyrimidine according to any of previous claims for medical use.

4. 4-substituted derivatives of pyrazolo[3,4-*d*]pyrimidine and pyrrolo[2,3-*d*]pyrimidine according to claims 1 or 2 for antitumoral therapy.

5. Pharmaceutical composition comprising a 4-substituted derivative of pyrazolo[3,4-*d*]pyrimidine and pyrrolo[2,3-*d*]pyrimidine according to claim 1 or 2 or a pharmaceutically acceptable salt thereof and suitable eccipients and/or diluents.

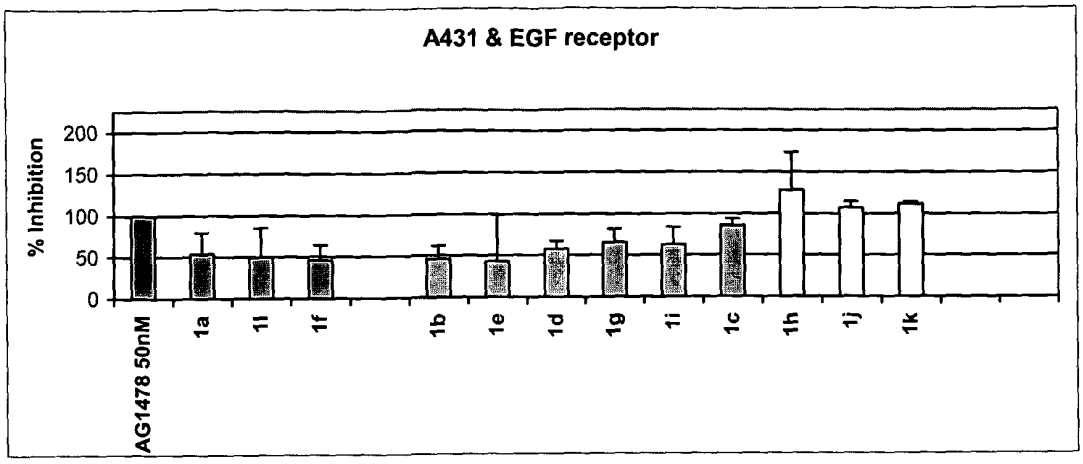

A431 & EGF receptor
% Inhibition
200
150
100
50
0
AG1478 50nM
1a
1l
1f
1b
1e
1d
1g
1i
1c
1h
1j
1k

Fig. 1A

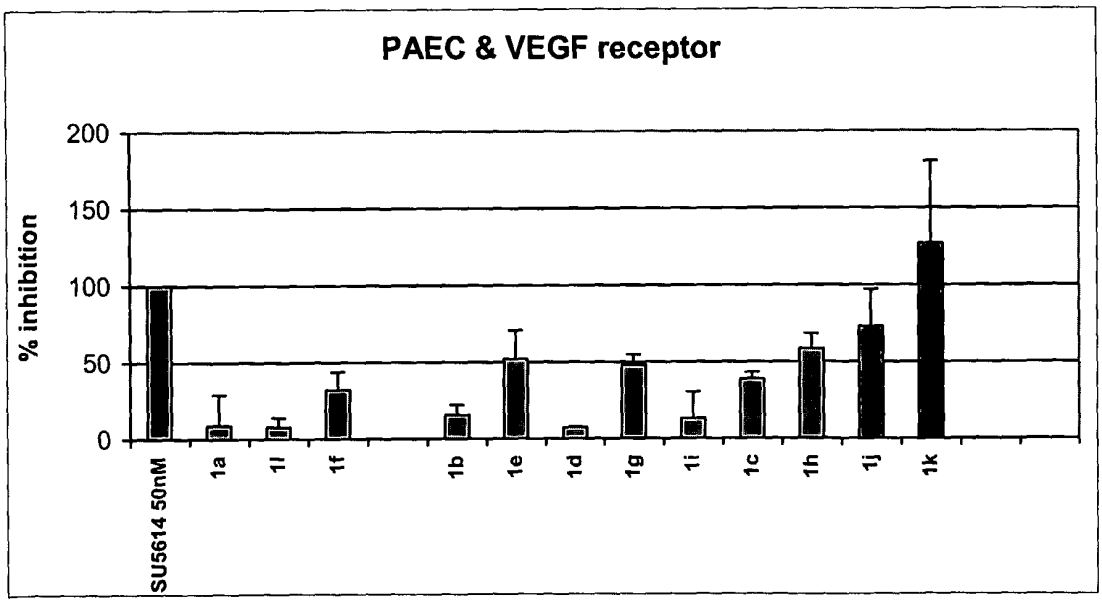

PAEC & VEGF receptor
% inhibition
200
150
100
50
0
SU5614 50nM
1a
1l
1f
1b
1e
1d
1g
1i
1c
1h
1j
1k

Fig. 1B