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POLYHYDROXYLATED 3-(N-PHENYL) CARBAMOYL-2-IMINOCHROMENE DERIVATIVES AS POTENT INHIBITORS OF TYROSINE KINASE $p60^{c\text{-}src}$

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Abstract: Several polyhydroxylated 3-(N-phenyl) carbamoly-2-iminochromene derivatives were synthesized and their inhibitory effects on tyrosine kinase $p60^{c\text{-}src}$ were evaluated. The structure-activity relationship reveals that the positions of the hydroxylation on both the iminochromene ring and the N-phenyl-ring of the carbamoyl group strongly affect the potency of the compounds.

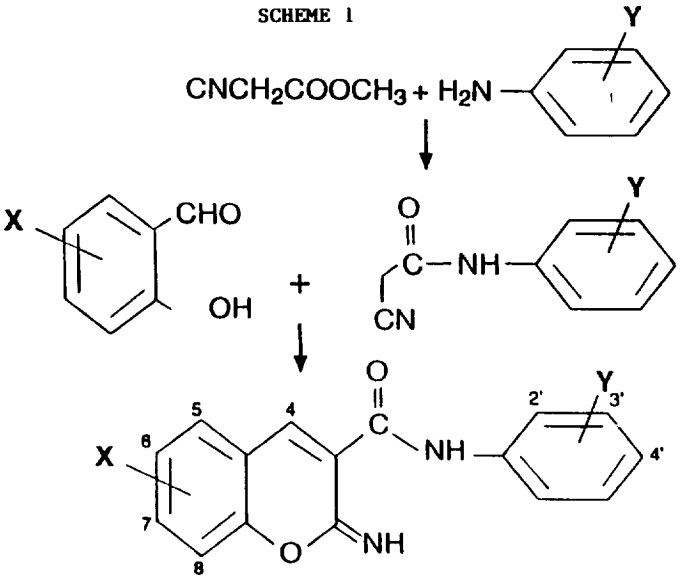
Protein tyrosine kinases (PTK) play an important role in the signal transduction of normal and abnormal cells.[1-3] They include transmembrane receptors for growth factors and intracellular mediators between receptors and target proteins. They mediate cell growth, mitogenic activities and cellular differentiation. Oncogenic kinases contain point mutations, deletions or fusion with other genes. Abnormal expression results in cellular transformation and carcinogenesis.[1-3]

Increasing numbers of PTK inhibitors have recently been introduced as potential anticancer reagents.[4-7] They include isoflavones (genistein),[8] tyrphostins (erbstatin),[9] lavendustin analogues,[10] staurosporine analogues (dianilinophthalmides),[11] dithiobis (indole-alkanoic acid),[12] dihydroxyisoquinolines[13] and others.[4-7]

For the purpose of obtaining highly specific inhibitors, bicyclic compounds as ring-constrained inhibitors of PTK have recently been introduced.[13] They are expected to interact with the flat, cleft-like catalytic cavity of the kinase domain with high specificity. Iminochromenes belong to this type of compound.[13] Several 3-carbamoyl-2-iminochromenes with weak PTK inhibitory activity toward $p56^{lck}$ have been reported.[13] We have synthesized several novel polyhydroxylated 3-(N-phenyl) carbamoyl-2-iminochromenes. We found that several of them are

potent inhibitors of the $p60^{c\text{-}src}$ kinase. The positions of hydroxyl groups on both the iminochromene ring and the (N-phenyl)carbamoyl ring play an important role in determining the potency of these compounds.

SCHEME 1



7: X=6-OH
8: X=7-OH
9: X=8-OH
15: X=7-OCH_3
16: X=6-OCH_3
18: X=7,8-di OH
19: X=5,7-di OH
25: X=-
32: X=8-OCH_3

T: Y=4'-OH
TA: Y=3'-OH
TO: Y=2'-OH
TABA: Y=3'-CH_2OH
TOBA: Y=2'-CH_2OH
ACTA: Y=3'-$COCH_3$
AN: Y=-

Cyanoacetamides were prepared from methyl cyanoacetate and arylamines in ethanol at 70 ℃ for 30 min to 1 h (Scheme. 1). N-phenyl-cyanoacetamides were isolated by cooling and filtration of the reaction mixture.[14] 3-(N-phenyl)carbamoyl-2-iminochromenes were prepared by condensation of various derivatives of 2-hydroxybenzaldehyde with (N-phenyl)cyanoacetamides in ethanol containing piperidine at 40 °C to 60 ℃ for 5 min to 10 min. After cooling, the products were isolated by filtration of the reaction mixture.[13]

Table 1. Structure-Activity Relationship of the 3-(N-phenyl) carbamoyl -2-iminochromenes.

Compound	IC_{50}(μg/ml) $p60^{c\text{-}src}$	$p56^{lck}$	$p56^{lyn}$	$p55^{fyn}$
7T	3.2	>10	i	i
7TA	1.3	i	>10	i
8T	1.2	>10	i	i
8TA	9	i	>10	i
8TO	0.6	i	i	i
9T	1.9	0.62	20	>20
9TA	0.035	0.62	<10	20
9TO	>10	1.2	>20	>20
18AN	>20	>20	>20	>20
18T	3.6	5	>20	>20
18TA	0.225	20	18	>20
18TO	12	20	>20	>20
19T	0.2	2	5	i
19TA	1.25	3.75	5	i
19TO	2.1	i	>>5	i
25T	>20	>10	10	i
25TA	10	i	i	i
25TO	>20	i	i	i
9TABA	2.5	i	20	3
9TOBA	1.8	i	i	i
18TABA	5	>20	20	>20
18TOBA	0.9	5	>20	>20
18ACTA	3	>20	10	20
15T	>10	>10	i	i
16T	12.5	7.5	i	i
32TA	1.3	i	i	i

i = Not Done

Table 1 shows the inhibitory activities (IC_{50}) of several 3-(N-phenyl) carbamoyl-2-iminochromene derivatives on purified tyrosine kinase $p60^{c\text{-}src}$. The parent compound, 3-(N-4'-hydroxyphenyl) carbamoyl-2-iminochromene (25T) inhibits the kinase with an IC_{50} > 20 μg/ml. Mono- or dihydroxylation of the iminochromene ring of the parent compound at positions 5, 6, 7 or 8 leads to an enhancement of the activity (7T (IC_{50} 2.2 μg/ml), 8T (IC_{50} 1.2 μg/ml), 9T (IC_{50} 1.9 μg/ml), 18T (IC_{50} 3.6 μg/ml) and 19T (IC_{50} 0.1 μg/ml). Compound 19T which contains 5,7 dihydroxylation of the iminochromene ring is 200-fold more potent than the parent compound 25T. Substitution of the hydroxyl groups with methoxy groups resulted in the loss of activity (15T is 8-fold less active than 8T and 16T is 4 fold less active than 7T).

The effects of varying the positions of hydroxyl substitution on the N-phenyl ring of the 3-carbamoyl group were studied. Three compounds containing 8-hydroxylation of the iminochromene ring (9T, 9TA, 9TO) were tested. The compound 9TA, which contains

hydroxylation of the N-phenyl ring at 3' position, is 285-fold more potent than the compound 9TO, which contains hydroxylation of the N-phenyl ring at 2'-position. 9TA is 54-fold more potent than the compound 9T, which contains hydroxylation of the N-phenyl ring at the 4'-position. Similar results were observed with the compounds 18T, 18TA and 18TO which contain 7, 8-dihydroxylation of the iminochromene ring but differs in the positions of hydroxyl substitution on the N-phenyl ring: 18T (IC_{50} 3.6 μg/ml), 18TA (IC_{50} 0.225 μg/ml) and 18TO (IC_{50} 12 μg/ml). In contrast, compounds containing hydroxymethylation of the N-phenyl ring at 2'-position (9TOBA, 18TOBA) are much more potent than their hydroxylated parent (9TOBA and 18TOBA are much more potent than 9TO and 18TO, respectively). However, hydroxymethylation of the N-phenyl ring at 3'position does not produce compounds more potent than their parent hydroxylated compounds (9TABA and 18TABA are not more potent than 9TA and 18TA, respectively). Compounds containing hydroxylation of the iminochromene ring at 6-position (7T, 7TA), 7-position (8T, 8TA, 8TO), 5 and 7 positions (19T, 19TA and 19TO) or no hydroxylation (25T, 25TA and 25TO) are less sensitive to the effect of varying the positions of hydroxyl subsititiution on the N-phenyl ring of the carbamoyl group. Compound 18AN which does not contain an hydroxylation group on the N-phenyl-ring is inactive (IC_{50} >20μg/ml). Hydroxylation of the N-phenyl ring at 3' position produces the compound 18TA which is 88 fold more potent than 18AN. Acetoxylation of the 3' position of compound 18TA produced 18ACTA which is 13 fold less potent than 18TA. The most active compound is 9TA (IC_{50} 0.035 μg/ml (120nM)) which is hydroxylated at 3' and 8 positions.

Double reciprocal plots of various concentrations of the substrate [poly [Glu, Tyr] (4:1)] and ATP indicate that 19T is competitive for substrate but noncompetitive for ATP (not shown). Further work is required to fully characterize the mechanism of inhibitory action of these compounds.

Several compounds were also examined for their selectivity against other tyrosine kinases of the $p60^{c\text{-}src}$ family including lck, lyn and fyn. The compounds were reltively weak inhibitors for these kinases. The pattern of structure-activity relationship observed with $p60^{c\text{-}src}$ was not reproduced with these kinases.

$p60^{c\text{-}src}$ kinase may be involved with two major signaling pathways in human breast cancer cells.[15] Its activity is elevated in many tumors.[16-19] Early work demonstrated that some derivatives of 3-(N-alkyl)carbamoyl-2-iminochromene have anti-tumor activities.[20] It will be worthwhile to test if the polyhydroxylated compounds have anti-tumor activities.

In summary, polyhydroxylated 3-(N-phenyl-carbamoyl)-2-imminochromenes represent a novel class of inhibitors of $p60^{c\text{-}src}$. The structure-activity relationship reveals that the positions of the hydroxylation on both the iminochromene ring and the N-phenyl-ring of the carbamoyl group

strongly affect the potency of the compounds. The results presented here and other recent studies[21-23] support the hypothesis of Gazit[9] that it may be possible to prepare potent, specific inhibitors for various tyrosine kinases.

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(24) Purified $p60^{c\text{-}src}$ and *src*-family kinases $p56^{lck}$, $p56^{lyn}$ and $p55^{fyn}$ were obtained from Upstate Biotechnology Inc. Kinase assays were performed as described[14] using poly [Glu,Tyr] (4:1) as a substrate with minor modification. The reaction mixture (40 µl) contains 10mM Hepes (pH 7.4), 10mM MgCl, 3 units of purified tyrosine kinase, 0.1 mg/ml of poly (Glu:Tyr, 4:1), 40 µM ATP, 0.8 µCi [γ-^{32}P]-ATP, with or without the presence of various concentrations of synthesized compounds. Dimethylsulfoxide was the solvent used for

solubilizing the inhibitors. Final concentrations of dimethylsulfoxide in the kinase assays were always less then 0.1% (v/v). The reaction was initiated by the addition of [γ-^{32}P]ATP at room temperature, and terminated after 15 minutes by adding 8 µl of 10mM cold ATP. Aliquots of 25 µl of the reaction mixture were loaded on pieces of Whatman 3MM paper squares (3X3cm). The papers were washed three times for 10 min each in a 5% trichloroacetic acid solution. The papers were dried and counted for acid-precipitable radioactivity (incorporated ^{32}P) in a scintilation counter. The counts obtained without the presence of the compounds in the reaction mixture were used as 100% (control).

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