

## THE DEVELOPMENT OF NOVEL AND SELECTIVE p56<sup>lck</sup> TYROSINE KINASE INHIBITORS<sup>1</sup>

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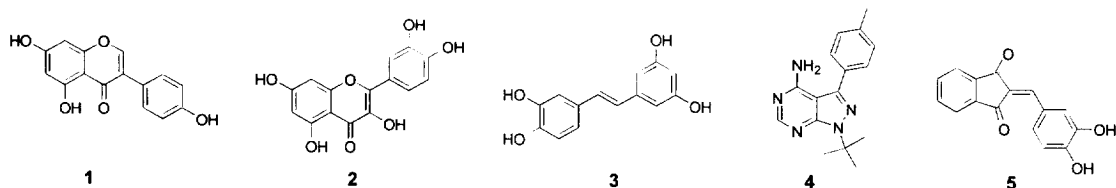
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**Abstract:** Early T-cell receptor mediated signal transduction involves the activation of several tyrosine protein kinases. One of these tyrosine kinases, p56<sup>lck</sup>, is expressed primarily in T-cells and Natural Killer (NK) cells and has been shown to be critical for their proliferative and effector functions. Indandiones have been identified as a potent and selective chemical class that inhibits p56<sup>lck</sup>. © 1998 Elsevier Science Ltd. All rights reserved.

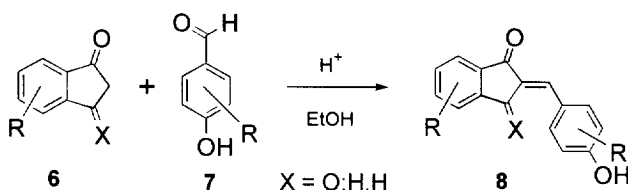
Early T-cell receptor mediated signal transduction involves the activation of several protein tyrosine kinases.<sup>2</sup> One of these tyrosine kinases, a src family tyrosine kinase, p56<sup>lck</sup>,<sup>3–5</sup> is expressed primarily in T-cells and Natural Killer (NK) cells.<sup>6</sup> p56<sup>lck</sup> kinase has been shown to be critical for T-cell proliferative and effector functions.<sup>7</sup> Because p56<sup>lck</sup> kinase is found mainly in T-cells and NK cells, a selective inhibitor has the potential to suppress the immune response with minimal toxic effects.

Many compounds reported to inhibit p56<sup>lck</sup> kinase show similar features.<sup>8–10</sup> Inhibitors of tyrosine kinases have been generally shown to be flat aromatic structures, usually containing electron donor and electron acceptor groups separated by an aromatic spacer thus creating an electronic push-pull situation across the molecule.<sup>11</sup> Often these inhibitors are highly oxygenated species represented by the polyphenols, Genistein, (1)<sup>12,13</sup> Quercetin, (2)<sup>14,15</sup> and Piceatannol (3).<sup>16</sup> Because of the relatively high homology among tyrosine kinases, many of the known inhibitors lack the specificity necessary to avoid effects outside of the immune system, although family-specific tyrosine kinase inhibitors such as compound 4 have been reported.<sup>17</sup> We postulated that binding to a specific tyrosine kinase could be enhanced by the optimization of the stereoelectronic characteristics of the molecule. We based our discovery efforts on this hypothesis and prepared compounds that exhibited flat, conjugated phenolic structures which could be substituted with electron donating and electron withdrawing groups. Compound 5 quickly emerged as an initial lead having an IC<sub>50</sub> of 5 µM against p56<sup>lck</sup>.



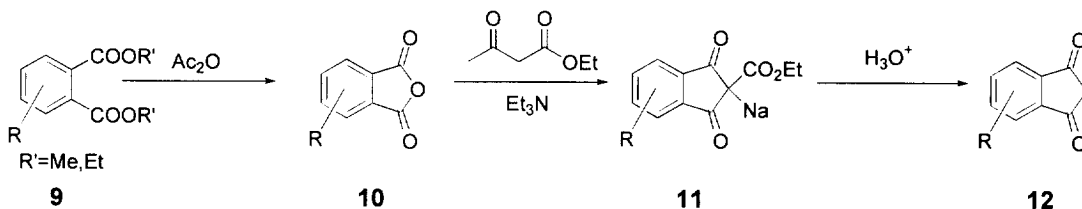
In order to explore the SAR of this chemical series and further elucidate the pharmacophore for p56<sup>lck</sup> activity, we prepared a number of different substituents on both the indanone (X = H,H)/indandione (X = O) portion of the molecule and also the benzylidene portion by condensing the respective indanone or indandione **6** with a variety of benzaldehydes **7** to give the targeted compounds **8** (Scheme 1).<sup>18,19</sup>

#### Scheme 1



A number of substituted indandiones were prepared following previously published procedures usually involving the condensation of substituted phthalic acid esters **9** to give the anhydrides **10** followed by Knoevenagel condensation with ethylacetoacetate. The resultant sodium salt of ester **11** could be decarboxylated to give the indandiones **12**. Alternatively, **11** could be used directly to condense with aldehydes to give the targeted compounds<sup>20</sup> (Scheme 2).

#### Scheme 2

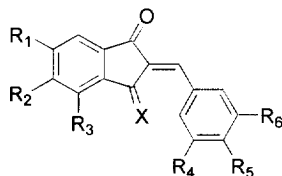


The compounds were assayed *in vitro* for their ability to inhibit p56<sup>lck</sup>.<sup>21</sup> Inhibition of p56<sup>lck</sup> is dependent upon the electronic characteristics of both the indandione system and the benzylidene moiety. Some of our findings are presented in Table 1. Our data have shown that electron-withdrawing groups substituted on the indandione system generally caused a decrease in the inhibition of the enzyme compared to the unsubstituted parent molecule. For instance, compound **17**, with a nitro group substituted at the 5-position of the indandione is significantly less potent than the unsubstituted parent, compound **15**. Halogen substitution of the indandione system provided no significant change in potency when compared to the unsubstituted parent, compound **15** vs.

**16** for example. Electron donating groups have been shown to generally cause an increase in inhibition relative to the unsubstituted parent molecules. Substitution with a methoxy group at the 5-position of the indandione increased potency five-fold when compared to the parent (compound **18** vs. **15**). A 5,6-dimethoxy substitution pattern showed no significant increase in potency compared to the 5-methoxy substitution (compound **27** vs.

**Table 1**

p56<sup>lck</sup> inhibition by substituted indanones/indandiones



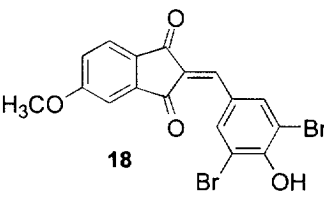
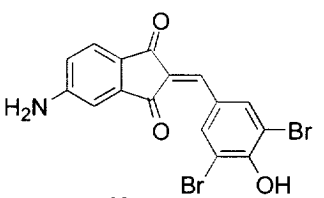
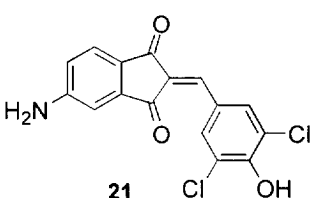
Compd	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	X	IC <sub>50</sub> (μM)
<b>3</b>	H	H	H	H	OH	OH	O	5.0
<b>13</b>	NO <sub>2</sub>	H	H	H	OH	OH	O	>5
<b>14</b>	NH <sub>2</sub>	H	H	H	OH	OH	O	0.60
<b>15</b>	H	H	H	Br	OH	Br	O	0.74
<b>16</b>	Br	H	H	Br	OH	Br	O	1.0
<b>17</b>	NO <sub>2</sub>	H	H	Br	OH	Br	O	>5
<b>18</b>	OMe	H	H	Br	OH	Br	O	0.14
<b>19</b>	NH <sub>2</sub>	H	H	Br	OH	Br	O	0.032
<b>20</b>	NH <sub>2</sub>	H	H	OMe	OH	OMe	O	1.7
<b>21</b>	NH <sub>2</sub>	H	H	Cl	OH	Cl	O	0.10
<b>22</b>	OMe	OMe	H	Cl	OH	Cl	O	0.31
<b>23</b>	NH <sub>2</sub>	H	H	Br	OH	Br	H,H	0.71
<b>24</b>	H	NH <sub>2</sub>	H	Br	OH	Br	H,H	5.0
<b>25</b>	OMe	H	H	H	OH	OH	O	1.5
<b>26</b>	OMe	H	H	Br	OH	OMe	O	0.88
<b>27</b>	OMe	OMe	H	Br	OH	Br	O	0.11
<b>28</b>	Cl	Cl	H	H	OH	OMe	O	5.0
<b>29</b>	Cl	Cl	H	Br	OH	OMe	O	1.7
<b>30</b>	H	H	NO <sub>2</sub>	H	OH	OH	O	5.0
<b>31</b>	H	H	NH <sub>2</sub>	Br	OH	Br	O	0.47
<b>32</b>	H	H	NO <sub>2</sub>	Cl	OH	Cl	O	1.4

**18**). An amino group provided the greatest increase in potency, resulting in a > 20 fold increase in inhibition over the unsubstituted analog (compound **19** vs. **15**). A 4-hydroxy group on the benzylidene portion of the molecule is critical for activity, which compares with the known p56<sup>lck</sup> inhibitors **1**, **2**, and **3**. Indandiones are

significantly more potent than indanones (compounds **23** and **24** vs. **19**). The 3,5-dibromo-4-hydroxy substituted benzylidene is optimal over other substitution patterns prepared including other 3,5-dihalo-4-hydroxy substitutions (compound **19** vs. **21**). Cushman et. al. have also seen increased activity in their p56<sup>lck</sup> inhibitors with this substitution pattern.<sup>22</sup> The most potent compounds in the series were evaluated in other kinase assays to determine their enzyme selectivity. Inhibition studies were run using the related tyrosine kinase c-src<sup>21</sup> and Protein Kinase A<sup>23</sup> (PKA). The results are summarized in Table 2.

**Table 2**

Selectivity data of most potent compounds

	Enzyme Inhibition IC <sub>50</sub> $\mu$ M		
	Lck	c-Src	PKA
 <b>18</b>	0.144	>50	10
 <b>19</b>	0.032	0.46	8
 <b>21</b>	0.102	3.5	5

As Table 2 indicates, our three most potent compounds are selective for p56<sup>lck</sup> kinase with significantly reduced activity towards the related kinase c-src and also PKA. The 5-aminoindandiones **19** and **21** were greater than 50 times less potent in the PKA assay while showing at least 14 times less activity against c-src. The 5-methoxyindandione **18** again showed good selectivity for p56<sup>lck</sup> over PKA. At concentrations above 0.4  $\mu$ M **18** appears to stimulate c-src activity. Compounds **18**, **19**, and **21** were concluded to be ATP competitive, based on

double reciprocal plots of  $1/v_o$  vs.  $1/[S]$  (Lineweaver-Burke plots). These plots generated a series of straight lines intersecting a common point on the y axis ( $1/v_o$ ), and were therefore concluded to be competitive for the ATP substrate.

In summary, we have identified a novel series of indandiones that exhibit potent inhibition of p56<sup>lck</sup> in vitro. Using our initial electron donor/acceptor hypothesis that the optimization of electron donating and electron withdrawing groups substituted on a flat, conjugated phenolic system could enhance the binding of these compounds to a specific tyrosine kinase, we have produced a greater than 1000-fold increase in potency over our earliest leads resulting in compounds that inhibit p56<sup>lck</sup> in the nanomolar range. The indandione series has been shown to be selective for p56<sup>lck</sup> over the related kinase c-src and PKA generally having at least 14 times greater inhibition of p56<sup>lck</sup> over c-src and at least 50 times greater inhibition over PKA. Encouraged by the potency and selectivity of this series, we are continuing to evaluate the series for selectivity against other enzymes, as well as for in vivo activity.

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19. **Representative procedure for indandione condensation: 2-[(3,5-Dibromo-4-hydroxyphenyl)methylene]-1H-1,3(2H)-dione 15:** 1,3-Indandione (0.70 g, 4.79 mmol) and 3,5-Dibromo-4-hydroxybenzaldehyde was dissolved in abs. ethanol (30 ml). The mixture was saturated with gaseous hydrogen chloride and heated to reflux for 18 h. The resulting slurry was cooled to room temperature, filtered, and washed with abs. ethanol to give **15** (1.74 g, 89%) as a light brown solid. <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) δ 8.91 (2H, s), 7.95 (4H, m), 7.73 (1H, s). mp 329–331 °C
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