

## Synthesis and Biological Evaluation of Quinoline Salicylic Acids As P-Selectin Antagonists

Neelu Kaila,\* Kristin Janz, Silvano DeBernardo, Patricia W. Bedard, Raymond T. Camphausen,† Steve Tam, Desirée H. H. Tsao, James C. Keith, Jr., Cheryl Nickerson-Nutter, Adam Shilling, Ruth Young-Sciame, and Qin Wang

Chemical and Screening Sciences, Cardiovascular and Metabolic Disease, Drug Safety and Metabolism, Inflammation, Wyeth Research, 200 Cambridge Park Drive, Cambridge, Massachusetts 02140

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Leukocyte recruitment of sites of inflammation and tissue injury involves leukocyte rolling along the endothelial wall, followed by firm adherence of the leukocyte, and finally transmigration of the leukocyte across cell junctions into the underlying tissue. The initial rolling step is mediated by the interaction of leukocyte glycoproteins containing active moieties such as sialyl Lewis<sup>x</sup> (sLe<sup>x</sup>) with P-selectin expressed on endothelial cells. Consequently, inhibition of this interaction by means of a small molecule P-selectin antagonist is an attractive strategy for the treatment of inflammatory diseases such as arthritis. High-throughput screening of the Wyeth chemical library identified the quinoline salicylic acid class of compounds (**1**) as antagonists of P-selectin, with potency in *in vitro* and cell-based assays far superior to that of sLe<sup>x</sup>. Through iterative medicinal chemistry, we identified analogues with improved P-selectin activity, decreased inhibition of dihydroorotate dehydrogenase, and acceptable CYP profiles. Lead compound **36** was efficacious in the rat AIA model of rheumatoid arthritis.

### Background

Leukocyte emigration from the vasculature into tissue upon inflammatory stimulus, involves a cascade of events. Leukocyte rolling, the initial step, is followed by firm adhesion and activation, transendothelial migration, and penetration into tissue.<sup>1</sup> Over the past 10 years, several studies have collectively suggested that the cell adhesion molecules E- and P-selectin expressed on the endothelium play important and overlapping roles in the initial leukocyte rolling. In addition, it is clear that leukocyte rolling is an important and feasible target for the prevention and treatment of inflammatory diseases.<sup>2</sup> One such disease is rheumatoid arthritis (RA), a systemic immune disorder characterized by articular joint inflammation and destruction. Studies have shown that selectins play an important role in RA. Elevated levels of E- and P-selectin have been observed in inflamed joints of RA patients.<sup>3</sup> Our goal was to identify P-selectin antagonists for the treatment of inflammatory diseases such as RA.

P-selectin is the predominant selectin in leukocyte recruitment and is expressed on the endothelium minutes after stimulation.<sup>4</sup> Its counter receptor on leukocytes is P-selectin glycoprotein ligand 1 (PSGL-1). The carbohydrate epitope (presented by an O-linked glycan) on PSGL-1 that binds the selectins is the tetrasaccharide sialyl Lewis<sup>x</sup> (sLe<sup>x</sup>). There are numerous reports of selectin antagonists in the literature.<sup>5</sup> One popular strategy has been the design of sLe<sup>x</sup> mimetics through the use of X-ray crystallography and molecular modeling. Our efforts on this approach have been reported.<sup>5,6</sup> Our carbohydrate-based sLe<sup>x</sup> mimetic selectin antagonists have shown activity in preclinical models, but the efficacy was considered to be inadequate for development. Several selectin-directed therapies are being evaluated in clinical trials. Two-protein therapeutics have reached phase II clinical trials. A phase II clinical program of YPSGL (rPSGL-Ig) for prevention of delayed graft function in patients undergoing cadaveric kidney transplantation has been

recently initiated. YPSGL is a recombinant fusion of P-selectin glycoprotein ligand-1 (PSGL-1) and human IgG1 Fc.<sup>7</sup> The second is humanized anti-L-selectin monoclonal antibody being developed for trauma.<sup>8</sup> CY 1503, an sLe<sup>x</sup> analogue, was evaluated in phase II/III trials for reperfusion injury.<sup>9</sup> More recently, the completion of a phase II study with the pan-selectin mannose-based antagonist bimasiamose (TBC-1269) was reported. In this study, reduction in airway recruitment of eosinophils was observed after IV administration of bimasiamose.<sup>10</sup> Further evaluation via an inhaled route of administration is reportedly in progress. In general, poor pharmacokinetic properties of carbohydrate-based drugs have been the major challenge in the development of an orally available selectin antagonist, while protein therapeutics have been limited to intravenous administration for acute interventions.

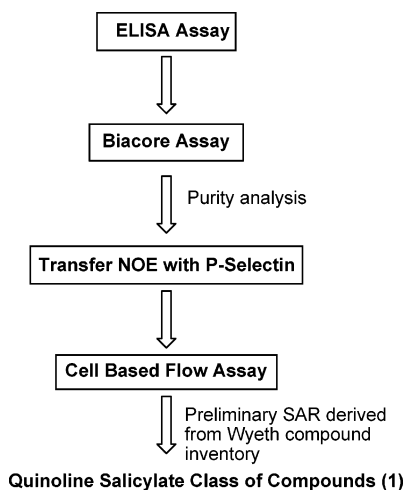
Some of the advanced non-carbohydrate selectin antagonists that have been reported in the literature are imidazole based OC229–648,<sup>11</sup> the substituted thiazepine KF38789,<sup>12</sup> and Eformycine M.<sup>13</sup> These compounds have shown efficacy in some animal models.<sup>14</sup>

In continuation of our search of an orally available selectin antagonist for treatment of inflammatory diseases,<sup>5,6</sup> in 2001 we performed a high throughput screen (HTS) of the Wyeth compound inventory using the P-selectin ELISA assay.<sup>15</sup> The screening hits were further triaged using the testing cascade shown in Table 1. The additional assays were particularly useful in identifying false positives from the ELISA assays.<sup>16</sup> These assays are described in more detail in the following section.

(i) Biacore (surface plasmon resonance) inhibition assay for P-selectin:<sup>16,17</sup> In this assay, the sensitivity of the Biacore instrument allows for the measurement of the weak monomeric selectin interactions in real time under equilibrium flow conditions and, therefore, avoids the pitfalls of avidity in the ELISA assay. In this assay, soluble P-selectin at the  $K_D$  concentration for the interaction is flowed over immobilized PSGL-1 with and without small molecule antagonists. (ii) NMR, transfer NOE (trNOE) experiments of the antagonists in the presence of P-selectin: In transfer NOE experiments, molecules exhibit strong negative trNOEs when bound to the protein and can be

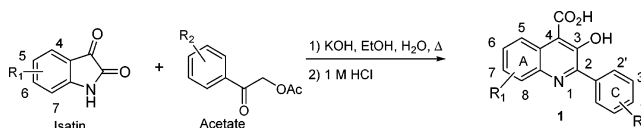
\* Corresponding author. E-mail: nkaila@wyeth.com. Tel.: 617-665-5626. Fax: 617-665-5682.

† Present address: Adnexus Therapeutics, Inc., MA.

**Table 1.** Screening Cascade for HTS Hits from Wyeth Compound Inventory

differentiated from nonbinding molecules with weak positive NOEs.<sup>6</sup> (iii) A rolling cell-based assay:<sup>18</sup> The cell-based in-vitro flow assay measures selectin-mediated rolling of human neutrophils on human umbilical vein endothelial cells (HUVEC) stimulated to express P-selectin in a parallel plate flow chamber. Digital image analysis of rolling and arrested cells is performed to quantitate inhibition.

Only 12% of the ELISA hits were found to be positive in the Biacore assay. This is likely a result of the low affinity of the selectin/ligand interaction and the obligatory washing steps in an ELISA assay. Because the selectins are involved in the attachment and rolling of leukocytes on the vascular surface under the influence of shear forces, it gives them some unusual properties. They exhibit fast-binding kinetics and make few interactions with their counter receptors. These contacts are for the most part electrostatic in nature, thus, the overall affinity is weak. The consequence of the natural ligand binding weakly to the receptor necessitates the use of a multimeric presentation of the receptor or the ligand in the ELISA format. It is necessary to utilize avidity in the ELISA-based assays to ensure sufficient interactions survive wash steps and produce adequate signal to measure differences in inhibition of binding. A drawback to this approach is that multimeric interactions lead to inconsistent complex formation, which in turn leads to larger assay variability than typical biological assays. As a result there are false positives in these nonequilibrium avidity type assays. During the course of our work on the selectins, we have found several examples where activity observed from the E-selectin and P-selectin ELISA assays could not be substantiated by the transferred NOE NMR experiments with the protein. We have reported some of these in the literature.<sup>19</sup> Next, the positives were analyzed in the NMR experiments. Thirty-nine HTS hits were evaluated for their behavior in solution and for their ability to bind P-selectin by trNOE experiments. The trNOE experiment used works very well for weak binders that are in fast exchange,<sup>20</sup> such as sLe<sup>x</sup>, a known tetrasaccharide that binds to all three forms of selectins. sLe<sup>x</sup> was used as a standard, in which binding to P-selectin was confirmed by strong trNOE. Several of the HTS hits that were positive in the Biacore assay were found not to bind P-selectin or bound nonspecifically. Compounds that bind in a nonspecific manner suggest binding to P-selectin with high stoichiometry and were, therefore, not pursued. The false positives from Biacore probably result from small amounts of polyanions picked up during resin purifications.<sup>21</sup> Those hits that displayed

**Scheme 1.** Pfitzinger Reaction

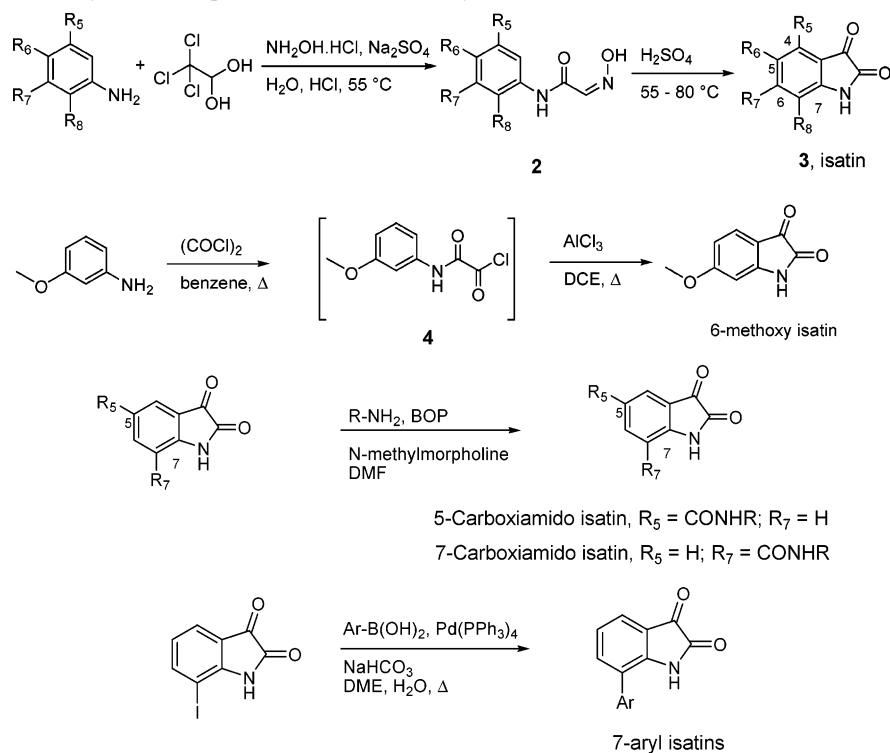
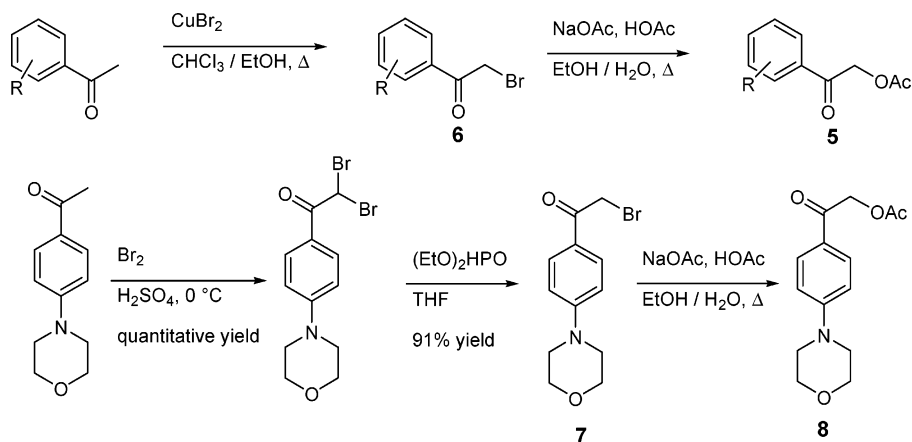
trNOE signals were taken forward to the cell-based assay. In the cell-based assay, a small percentage of these compounds caused cell lysis and others led to neutrophil activation. The hits that were well-tolerated ranked in activity similar to that seen in Biacore. In our final analysis of the compounds from the screening process, the quinoline salicylic acid series (general structure **1**) emerged as the most interesting leads. Herein we report on the optimization of this class of compounds as P-selectin antagonists.

## Chemistry

The target molecules were synthesized using the Pfitzinger reaction (Scheme 1).<sup>22</sup> The majority of the starting isatins for the Pfitzinger reaction were prepared by the Sandmeyer reaction of aniline derivatives with chloral hydrate and hydroxylamine hydrochloride (Scheme 2).<sup>23</sup> The resulting hydroxyiminoacetanilide intermediates **2** were cyclized by heating in concentrated sulfuric acid to give the final isatins **3**. The 4- and 6-substituted isatins were obtained as mixtures when the *meta*-anilines were subjected to the Sandmeyer protocol. These mixtures of isatins were used as such for the Pfitzinger reaction, and the final products were then separated and submitted for testing. Another route to the isatin was by reaction of the aniline with oxalyl chloride to give the oxo acetyl chloride derivative **4**. Upon Friedel–Crafts acylation, intermediate **4** gave the isatin.<sup>24</sup> This method was used to prepare the 6-methoxyisatin but did not work for 7-alkoxyisatins, probably because the alkoxy group of the latter is not able to activate the position (*meta*) at which substitution needs to occur. The 7-alkoxyisatins were prepared using the Sandmeyer reaction. The cyclization of the hydroxyiminoacetanilide intermediate for these isatins worked in only ~7% yield and did not work at all for the 7-phenoxy analogue. The 5- and 7-carboxamidoisatins were prepared by coupling of 5- and 7-carboxyisatin to various amines. Suzuki coupling of aryl boronic acids to 7-iodoisatin gave the 7-arylisatins.<sup>25</sup>

The acetates **5** for the Pfitzinger reaction were synthesized (Scheme 3) in two steps from acetophenones, which were either commercially available or synthesized by known literature procedures. The acetophenones were converted to  $\alpha$ -bromo derivatives **6** with copper(II) bromide. The 4-morpholino acetate could not be prepared using the above method and was synthesized using the procedure of Diwu et al.<sup>26</sup> in which the commercially available 4-morpholino acetophenone was first brominated in the presence of sulfuric acid to afford the dibromo intermediate, then debrominated with diethyl phosphate to give the required monobromide **7** in 90% yield. Acetate displacement of the  $\alpha$ -chloride or bromide gave acetates **5** (a few of the  $\alpha$ -chloro or bromo intermediates were commercially available).

The yields for the Pfitzinger reaction ranged from 5 to 80%. The purification of the final products was not trivial due to the poor solubility of the final quinoline salicylic acids. However, the solubility of the corresponding triethylammonium salts was greatly improved. The products from the Pfitzinger reactions were generally purified either by recrystallization or by chromatography on silica gel as the triethylammonium salts using mixtures of EtOAc–MeCN–MeOH–H<sub>2</sub>O containing 0.5–1% Et<sub>3</sub>N as eluents.

**Scheme 2.** Sandmeyer Isatin Synthesis (Top) and Friedel–Crafts Acylation (Bottom)**Scheme 3**

For further derivatization of the quinoline salicylic acid, a full protection of the salicylic acid functionality was found to be unnecessary. For example, the *N*-acyl derivatives of **9** could be obtained by peracetylation, followed by selective *O*-deprotection (Scheme 4). Also, unprotected bromo quinoline derivative **10** and the 6-iodoquinoline derivative could be successfully used in Suzuki couplings (Scheme 4).

### Results and Discussion

All compounds (Tables 2–7) were evaluated in the Biacore assay. Compared to the natural ligand sLe<sup>x</sup>, which has an IC<sub>50</sub> of 10 mM in this assay, the compounds with IC<sub>50</sub> ranging from 0.1 to 1 mM were considered to be interesting leads.<sup>27</sup>

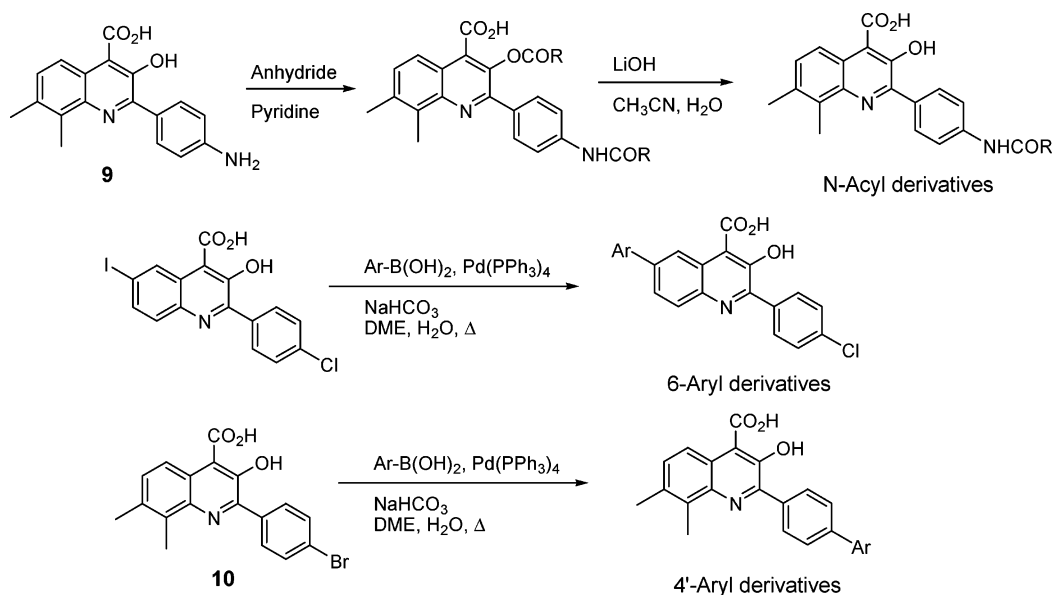
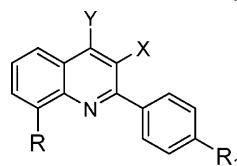
Initial SAR showed that the hydroxyl and carboxyl functionality were both required for activity. Removal of the hydroxyl group or replacement of the hydroxyl group by a methyl ether or methyl group as in **12**, **13**, **16**, and **17** resulted in reduced activity (Table 2). Removal of the carboxyl functionality (**14**) made the compound completely inactive at 6 mM.

Substitution in the A ring was studied next. Shifting the methyl group around the A ring showed that the 8-methyl

analogue was most potent (**22** vs **24**, **26**, and **28**). Other substituents were evaluated only at positions 6, 7, and 8 due to poor activity of **28** and difficulty in synthesizing the 5-substituted analogues. Moving the isopropyl, phenyl, chloro, trifluoromethoxy, trifluoromethyl, and bromo groups around the A ring showed that substituents at the 8 position are most potent, followed by the 6 position (Table 3). Groups at the 7 position had very little effect on activity.

Further analoging was done primarily at the 8 position. A few representative 6-substituted analogues and one 7-substituted analogue were also synthesized (Tables 4 and 5). Hydrophilic groups at the 6 and 8 positions were not desirable for P-selectin activity. Heteroatoms were tolerated if the substituent contained sufficient hydrophobic character (**47**, **49**, **64–70**, **114**, and **115**). Hydrophobic groups at the 6 and 8 position increase P-selectin activity (**22** vs **15** and **26** vs **52**; **60** vs **47** and **74** vs **49**). The most potent compounds resulted from hydrophobic substitution at the 8 position. Some steric bulk was tolerated here (Table 4; **81**, **30**, and **83** vs **85**). Four disubstituted quinoline salicylic acids were also prepared (Table 4). The effect of 6- and

## Scheme 4

**Table 2.** P-Selectin Inhibition; SAR of the Salicylic Acid Functionality

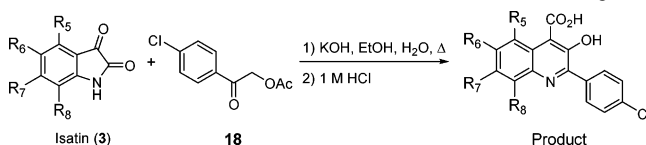
R	Y	X	R <sub>1</sub>	compd <sup>a</sup>	Biacore IC <sub>50</sub> or % inh
H	CO <sub>2</sub> H	OH	H	<b>11</b>	6 mM
H	CO <sub>2</sub> H	OMe	H	<b>12</b>	22% @ 6 mM
H	CO <sub>2</sub> H	H	H	<b>13</b>	22% @ 6 mM
H	H	OH	H	<b>14</b>	NA @ 6 mM
CF <sub>3</sub>	CO <sub>2</sub> H	OH	Cl	<b>15</b>	250 uM
CF <sub>3</sub>	CO <sub>2</sub> H	H	Cl	<b>16</b>	NA @ 500 uM
CF <sub>3</sub>	CO <sub>2</sub> H	Me	Cl	<b>17</b>	NA @ 500 uM

<sup>a</sup> Compounds **11–14** and **16** were obtained from the Wyeth compound inventory.

8-substitution appears to be additive (**93** vs **22** and **26**, **95** vs **22** and **56**, **97** vs **22** and **49**). Insertion of a carboxamido group at the 6 or 8 position resulted in compounds that showed no activity at 500 uM in the Biacore assay (Table 4).

As a further probe of the SAR, we prepared analogues with different substitution patterns on ring C (Tables 6 and 7). Analoging was done using one of our early lead compounds, **91**. Hydrophobic groups at the 4' position showed good activity. Heteroatom substitution was also acceptable (Tables 6 and 7). Polar groups were not tolerated. Moving the trifluoromethoxy and chloro group to the *meta* position had little effect on P-selectin inhibition (**142** and **144**). Introduction of the 4' amide functionality gave compounds that showed no inhibition in the Biacore assay at 1 mM (**148–149**). Similarly, the C-2 biphenyl class of compounds did not show appreciable P-selectin inhibition when tested in the Biacore assay (**132**, and **145–147**).

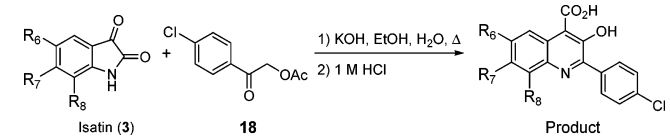
At the conclusion of our SAR study of the quinoline salicylic acids, several potent P-selectin antagonists (e.g., **30**, **36**, **15**, **83**, **87**, **122**, and **124**) had been identified. All of them had small hydrophobic substituents at position 8 and on the C-2 phenyl ring. Figure 1 summarizes the P-selectin activity in the Biacore assay for the quinoline salicylic acid class of compounds.

**Table 3.** P-Selectin Inhibition: Effect of Substitution in the A Ring

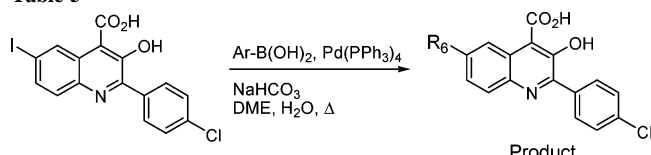
starting isatin	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	product	Biacore IC <sub>50</sub> or % inh
<b>19</b>	H	H	H	H	<b>20</b>	4 mM
<b>21</b>	H	H	H	Me	<b>22</b>	700 uM
<b>23</b>	H	H	Me	H	<b>24</b>	23% @ 1 mM
<b>25</b>	H	Me	H	H	<b>26</b>	15% @ 1 mM
<b>27</b>	Me	H	H	H	<b>28</b>	7% @ 1 mM
<b>29</b>	H	H	H	<i>i</i> -Pr	<b>30</b>	225 uM
<b>31</b>	H	H	<i>i</i> -Pr	H	<b>32</b>	1.5 mM
<b>33</b>	H	<i>i</i> -Pr	H	H	<b>34</b>	55% @ 1 mM
<b>35</b>	H	H	H	Ph	<b>36</b>	200 uM
<b>37</b>	H	H	Ph	H	<b>38</b>	2 mM
	H	Ph	H	H	<b>39</b>	375 uM
<b>40</b>	H	H	H	Cl	<b>41</b>	600 uM
<b>42</b>	H	H	Cl	H	<b>43</b>	14% @ 500 uM
<b>44</b>	H	Cl	H	H	<b>45</b>	38% @ 2 mM
<b>46</b>	H	H	H	OCF <sub>3</sub>	<b>47</b>	550 uM
<b>48</b>	H	OCF <sub>3</sub>	H	H	<b>49</b>	625 uM
<b>50</b>	H	H	H	CF <sub>3</sub>	<b>15</b>	250 uM
<b>51</b>	H	CF <sub>3</sub>	H	H	<b>52</b>	1.6 mM
<b>53</b>	H	H	H	Br	<b>54</b>	625 uM
<b>55</b>	H	Br	H	H	<b>56</b>	1 mM

It is noteworthy that a few close analogues of quinoline salicylic acids, for example, **150**<sup>28</sup> and **151** (Brequinar),<sup>29</sup> were reported to have toxicity issues due to inhibition of dihydro-orotate dehydrogenase (DHOD). DHOD is the fourth enzyme in the *de novo* pyrimidine nucleotide synthesis pathway.<sup>30</sup> Compound **150**, with a high antagonist activity of DHOD, has an LD<sub>50</sub> of 20.5 mg/kg in rats. For this reason, all of our compounds were screened for their human DHOD activity using a colorimetric enzymatic assay. Introduction of a phenyl, *O*-phenyl, or *S*-phenyl group to the 4' position of the quinoline salicylic acid compounds gave rise to potent DHOD inhibitors (Table 8, **11**, **156** vs **152**, **154**, **158**, and **159**). Interestingly, moving a halo, alkyl, or alkoxy group from the 6 to the 8 position resulted in a 10- to 1000-fold loss in DHOD potency (examples: **56** vs **54**; **26** vs **22**; **52** vs **15**; **74** vs **60**; and **49** vs **47**). The activity for 5- or 7-methyl analogues was intermediate relative to the 6- and 8-methyl analogues (**28** or **24** vs **26** and **22**). In general,

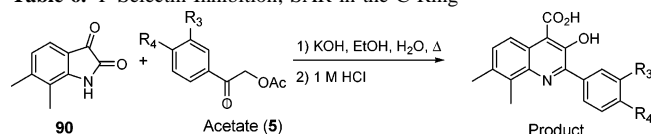


**Table 4.** P-Selectin Inhibition; SAR of the 6, 7 and 8 Positions


starting isatin	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	product	Biacore <sup>a</sup>	starting isatin	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	product	Biacore <sup>a</sup>
<b>57</b>	H	H	CO <sub>2</sub> H	<b>58</b>	NA	<b>50</b>	H	H	CF <sub>3</sub>	<b>15</b>	250 uM
<b>59</b>	H	H	OMe	<b>60</b>	NA	<b>35</b>	H	H	Ph	<b>36</b>	200 uM
<b>61</b>	H	H	OEt	<b>62</b>	26%	<b>86</b>	H	H	3-thiophene	<b>87</b>	175 uM
<b>63</b>	H	H	4-CO <sub>2</sub> H-Ph	<b>64</b>	1.5 mM	<b>53</b>	H	H	Br	<b>54</b>	625 uM
<b>65</b>	H	H	4-CH <sub>2</sub> OH-Ph	<b>66</b>	2 mM	<b>40</b>	H	H	Cl	<b>41</b>	600 uM
<b>67</b>	H	H	4-SO <sub>2</sub> Me-Ph	<b>68</b>	33%	<b>88</b>	H	H	F	<b>89</b>	11%
<b>69</b>	H	H	4-NMe <sub>2</sub> -Ph	<b>70</b>	300 uM	<b>90</b>	H	Me	Me	<b>91</b>	450 uM
<b>71</b>	H	OMe	H	<b>72</b>	NA	<b>92</b>	Me	H	Me	<b>93</b>	500 uM
<b>73</b>	OMe	H	H	<b>74</b>	NA	<b>94</b>	Br	H	Me	<b>95</b>	125 uM
<b>76</b>	OH	H	H	<b>75</b>	1.5 mM	<b>96</b>	OCF <sub>3</sub>	H	Me	<b>97</b>	225 uM
<b>76</b>	NH <sub>2</sub>	H	H	<b>77</b>	3 mM	<b>98</b>	H	H	CONHi-Pr	<b>99</b>	6%
<b>R<sub>6</sub> = NHAc</b>											
<b>78</b>	CO <sub>2</sub> H	H	H	<b>79</b>	6%	<b>100</b>	H	H	CONH <i>t</i> -Bu	<b>101</b>	15%
<b>19</b>	H	H	H	<b>20</b>	4 mM	<b>102</b>	H	H	CONHPh	<b>103</b>	NA
<b>21</b>	H	H	Me	<b>22</b>	700 uM	<b>104</b>	H	H	CONHBn	<b>105</b>	NA
<b>80</b>	H	H	Et	<b>81</b>	525 uM	<b>106</b>	CONHi-Pr	H	H	<b>107</b>	NA
<b>29</b>	H	H	<i>i</i> -Pr	<b>30</b>	225 uM	<b>108</b>	CONH <i>t</i> -Bu	H	H	<b>109</b>	6%
<b>82</b>	H	H	<i>s</i> -Bu	<b>83</b>	75 uM	<b>110</b>	CONHPh	H	H	<b>111</b>	15%
<b>84</b>	H	H	<i>t</i> -Bu	<b>85</b>	23%	<b>112</b>	CONHBn	H	H	<b>113</b>	15%

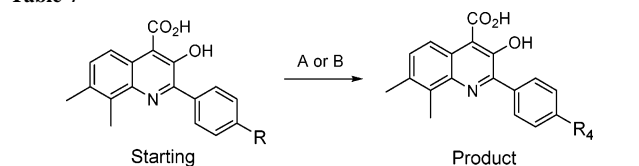
<sup>a</sup> IC<sub>50</sub> or % inhibition at 500 μM.**Table 5**


R <sub>6</sub>	product #	Biacore <sup>a</sup>
4-CO <sub>2</sub> H-Ph	<b>114</b>	27% @ 500 μM
4-OMe-Ph	<b>115</b>	39% @ 250 μM

<sup>a</sup> IC<sub>50</sub> or % inhibition.**Table 6.** P-Selectin Inhibition; SAR in the C-Ring


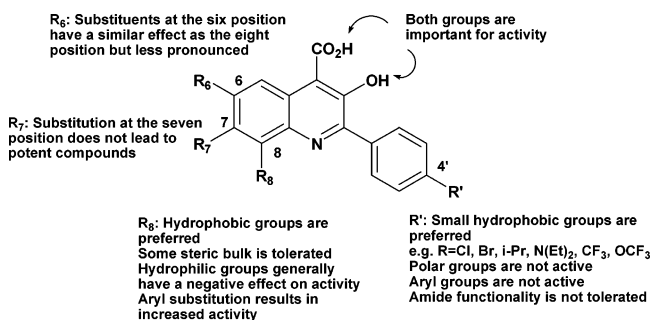
starting acetate	R <sub>3</sub>	R <sub>4</sub>	product	Biacore IC <sub>50</sub> or % inh
<b>116</b>	H	H	<b>117</b>	1.5 mM
<b>118</b>	H	F	<b>119</b>	43% @ 1 mM
<b>18</b>	H	Cl	<b>91</b>	450 uM
<b>120</b>	H	Br	<b>10</b>	400 uM
<b>121</b>	H	<i>i</i> -Pr	<b>122</b>	250 uM
<b>123</b>	H	CF <sub>3</sub>	<b>124</b>	300 uM
<b>125</b>	H	OCF <sub>3</sub>	<b>126</b>	240 uM
<b>127</b>	H	NEt <sub>2</sub>	<b>128</b>	250 uM
<b>129</b>	H	Me	<b>130</b>	1 mM
<b>131</b>	H	Ph	<b>132</b>	6% @ 1 mM
<b>133</b>	H	CO <sub>2</sub> H	<b>134</b>	17% @ 1 mM
<b>R<sub>4</sub> = CO<sub>2</sub>Et</b>				
<b>135</b>	H	OH	<b>136</b>	3.5 mM
<b>137</b>	H	OMe	<b>138</b>	1 mM
<b>139</b>	H	NH <sub>2</sub>	<b>9</b>	17% @ 1 mM
<b>R<sub>4</sub> = NHAc</b>				
<b>8</b>	H	morpholine	<b>140</b>	1 mM
<b>141</b>	OCF <sub>3</sub>	H	<b>142</b>	200 uM
<b>143</b>	Cl	H	<b>144</b>	400 uM

6-aryl analogues showed reduced DHOD inhibition (examples: **160**, **39**, and **114**), whereas 6-halo derivatives increased DHOD potency (for example, **161**, and **56**). Evaluation of a

**Table 7**


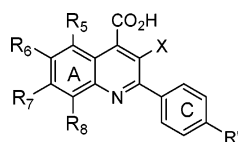
starting rxn cond	R	R <sub>4</sub>	prod #	Biacore IC <sub>50</sub> or % inh
A	Br ( <b>10</b> )	4-Me-Ph	<b>145</b>	49% @ 1 mM
A	Br ( <b>10</b> )	4-(NMe) <sub>2</sub> -Ph	<b>146</b>	NA @ 500 μM
A	Br ( <b>10</b> )	4-OMe-Ph	<b>147</b>	NA @ 500 μM
B	NH <sub>2</sub> ( <b>9</b> )	NHCOCH <sub>3</sub>	<b>148</b>	NA @ 1 mM
B	NH <sub>2</sub> ( <b>9</b> )	NHCOCF <sub>3</sub>	<b>149</b>	NA @ 1 mM

<sup>a</sup> Refer to Scheme 4. Reaction conditions: (A) Ar-B(OH)<sub>2</sub>, Pd(OAc)<sub>2</sub>, K<sub>2</sub>CO<sub>3</sub>; (B) (i) appropriate anhydride, pyridine; (ii) LiOH.

**Figure 1.** Summary of the P-selectin activity in the Biacore assay.

few 6,8-disubstituted compounds further supported the observation that substitution at the 8 position results in loss of DHOD activity. For example, the DHOD activity of compounds **26** and **56** was greatly reduced upon 8-methyl substitution (compounds **93** and **95**). This SAR clearly shows that the structural requirements for P-selectin and DHOD activity are different. The C-8 substituted quinoline salicylic acids seem to have the best combination of P-selectin potency and low DHOD activity.

Compounds with IC<sub>50</sub> ≤ 700 uM in the Biacore assay were further evaluated in our secondary assay, the cell-based Flow

**Table 8.** DHOD Activity for the A and C Ring Analogs

X	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	R'	compd <sup>a</sup>	DHOD		X	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	R'	compd <sup>a</sup>	DHOD	
							IC <sub>50</sub> (μM)									IC <sub>50</sub> (μM)	
NH <sub>2</sub>	H	F	H	H	Ph	<b>150</b>	0.012	OH	H	CF <sub>3</sub>	H	H	H	Cl	<b>52</b>	0.27	
Me	H	F	H	H	Ph	<b>151</b>	0.007	OH	H	OCF <sub>3</sub>	H	H	H	Cl	<b>49</b>	4	
OH	H	H	H	H	H	<b>11</b>	12	OH	H	Me	H	H	H	Cl	<b>26</b>	0.217	
OH	H	H	H	H	Ph	<b>152</b>	0.01	OH	Me	H	H	H	H	Cl	<b>28</b>	51	
OH	H	H	H	H	4-OH-Ph	<b>153</b>	0.006	OH	H	H	Me	H	H	Cl	<b>24</b>	17	
OH	H	H	H	H	OPh	<b>154</b>	0.007	OH	H	H	H	Me	H	Cl	<b>22</b>	212	
OH	H	F	H	H	Ph	<b>155</b>	0.005	OH	H	OMe	H	H	H	Cl	<b>74</b>	43	
OH	H	F	H	H	OH	<b>156</b>	2.1	OH	H	H	OMe	H	H	Cl	<b>72</b>	151	
OH	H	F	H	H	4-OH-Ph	<b>157</b>	0.007	OH	H	H	H	OMe	H	Cl	<b>60</b>	306	
OH	H	F	H	H	OPh	<b>158</b>	0.006	OH	H	H	H	Br	H	Cl	<b>54</b>	132	
OH	H	F	H	H	SPh	<b>159</b>	0.018	OH	H	H	H	OCF <sub>3</sub>	H	Cl	<b>47</b>	130	
OH	H	3-thiophene	H	H	Cl	<b>160</b>	134	OH	H	H	H	CF <sub>3</sub>	H	Cl	<b>15</b>	136	
OH	H	Ph	H	H	Cl	<b>39</b>	230	OH	H	H	H	Cl	H	Cl	<b>41</b>	141	
OH	H	4-CO <sub>2</sub> H-Ph	H	H	Cl	<b>114</b>	135	OH	H	Me	H	Me	H	Cl	<b>93</b>	4.9	
OH	H	F	H	H	Cl	<b>161</b>	0.312	OH	H	Br	H	Me	H	Cl	<b>95</b>	41	
OH	H	Br	H	H	Cl	<b>56</b>	0.211										

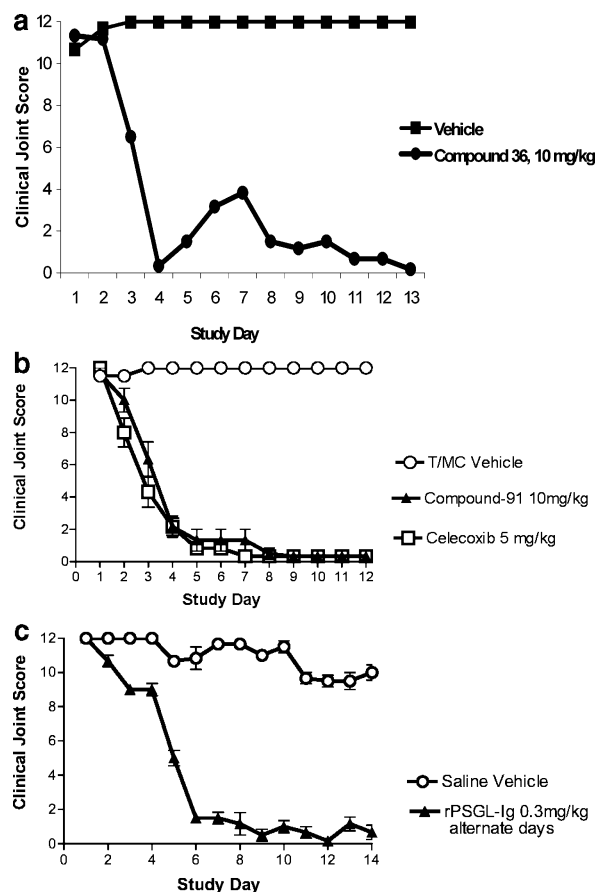
<sup>a</sup> Compounds 150–161 were obtained from the Wyeth compound inventory.

**Table 9.** Cell-Based Flow Activity, Rat PK Parameters (IV at 1 mg/kg) and DHOD Data of Selected Quinoline Salicylic Acids

compd	flow (IC <sub>50</sub> μM)	CL ml/min/kg	AUC min·μM	C <sub>max</sub> μM	DHOD IC <sub>50</sub> μM
<b>10</b>	6	6.2	391	5.5	56
<b>15</b>	13				
<b>22</b>	13				
<b>30</b>	4.7	6.5	402	7	283
<b>36</b>	4.5	9.1	236	9	26
<b>30</b>	10				
<b>41</b>	22				
<b>47</b>	10.5				
<b>49</b>	25				
<b>54</b>	20				
<b>70</b>	20				
<b>81</b>	10				
<b>83</b>	3	13.3	207	6.7	301
<b>91</b>	10	6.2	461	13	72
<b>97</b>	15.5				
<b>122</b>	8				
<b>124</b>	7	4.7	515	9	32
<b>126</b>	15				
<b>128</b>	19				
<b>142</b>	5				
<b>144</b>	5				

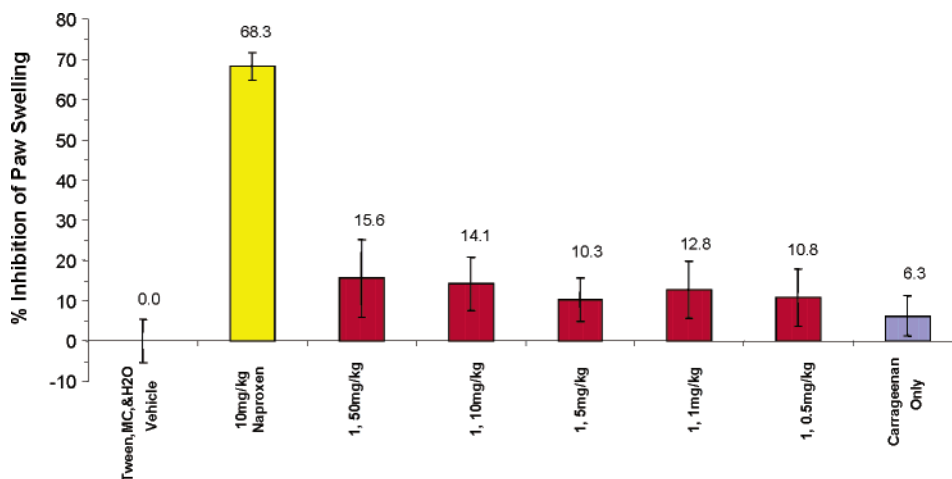
assay. As expected, the Flow IC<sub>50</sub>s were 10- to 500-fold lower than the Biacore IC<sub>50</sub>s (Table 9).<sup>31,18</sup> Selected compounds after this filter were evaluated for IV PK in rats. Several derivatives had low clearance and reasonable exposure (Table 9). All these compounds were poor DHOD inhibitors (2000-fold lower IC<sub>50</sub> compared to compounds **150** and **151**). Among this group, compounds **36** and **91** were found to be most interesting and were taken forward for evaluation in in vivo models.

The rat adjuvant induced arthritis (AIA) model is a well-established model for evaluation of potential drugs for treatment of human RA.<sup>32,33</sup> The role of P-selectin dependent inflammatory cell migration in AIA has been demonstrated.<sup>34</sup> In this model, arthritis was induced by the intradermal injection of Freund's complete adjuvant into the base of the tail of male Lewis rats. As shown in Figure 2, 8 days after adjuvant injection, the joints were almost maximally inflamed. At that time, compounds **36** and **91**, in two separate experiments, were administered to rats by daily oral gavage, and the clinical signs of inflammation



**Figure 2.** (A) P-Selectin antagonist **36** in rat AIA model. Compound **36** showed complete inhibition of the clinical score at a PO dose of 10 mg/kg, qd. (B) P-Selectin antagonist **91** and Celecoxib in rat AIA model. Compound **91** and Celecoxib showed complete inhibition of the clinical score at a PO dose of 10 and 5 mg/kg, qd. (C) Effects of P-selectin antagonist rPSGL-Ig in rat AIA model at an IV dose of 0.3 mg/kg on alternate days.

were measured on a daily basis for 13 days. At a 10 mg/kg dose once daily, both compounds significantly reduced clinical scores (Figure 2) from day 4 of treatment until the end of the



**Figure 3.** P-Selectin antagonist **91** when tested PO at different doses in the CPE model did not show any inhibition in paw swelling.

**Table 10.** Comparison of Activities for Compounds **36** and **91** Across Different Assays

compd #	Biacore IC <sub>50</sub>	flow IC <sub>50</sub>	rat AIA <sup>a</sup>
<b>36</b>	200 $\mu$ M	4.5 $\mu$ M	500 nM
<b>91</b>	450 $\mu$ M	10 $\mu$ M	1800 nM

<sup>a</sup> At PO 10 mg/kg peak plasma concentration.

study ( $p < 0.05$  vs 2% Tween: 0.5% methylcellulose vehicle, student's  $t$  test). These rats showed no signs of compound-related toxicity, and they continued to gain weight during the course of the study (data not shown). Specifically, we did not see toxicity in hematologic parameters, such as declines in white blood cell or erythrocyte number that would be expected with DHOD activity. Lack of toxicity seen with **36** and **91** confirms our finding that with the loss of DHOD activity, toxicity is no longer a concern for our lead analogues. Compound **36** is 2000-fold less active than **86** against DHOD. We have also included the results for Celecoxib treatment along with compound **91**, demonstrating that the activity of compound **91** is similar to this well-known COX-2 inhibitor. In this model, rPSGL-Ig, a recombinant, soluble, and chimeric form of PSGL-1, which is developed as an antagonist to P-selectin, was also tested. Figure 2C shows the effects of rPSGL-Ig 0.3 mg/kg IV dosed on alternate days, beginning on day 12 after intradermal injection of CFA at the ventral aspect of the tail in Lewis rats. A comparison of Biacore IC<sub>50</sub>, Flow IC<sub>50</sub>, and peak plasma concentration in the rat AIA model for compounds **36** and **91** is shown in Table 10. It is interesting to note the paradox of selectin antagonists found in these data. The IC<sub>50</sub> values decreased significantly for compounds **36** and **91** from the primary Biacore assay to the cell-based Flow assay to the in vivo AIA model. There may be two major factors that contribute to this paradox. The first is that in vivo flow rates and selectin densities are difficult to precisely reproduce using in vitro systems. Second, the shear forces, cellular interactions, and variation in expression of receptor ligands seen with cell/cell interactions in vitro and in the more complicated in vivo state may not require the same level of receptor occupancy as is required to block monomeric protein/protein interactions in a

biochemical assay like the Biacore. These correlations, as shown in Table 10, are the basis for pursuing micromolar selectin antagonists. Examples of such correlations can also be seen in the literature with biotherapeutic molecules such as rPSGL-Ig<sup>17,35,36</sup> and small molecules such as TBC1269. These molecules have in vitro and ex vivo IC<sub>50</sub>s typically in the micromolar range but exhibit a markedly increased potency in vivo. TBC1269, which has an IC<sub>50</sub> of 70  $\mu$ M in the P-selectin HL-60 cell assay<sup>37</sup> (sLe<sup>x</sup> has an IC<sub>50</sub> of 3.4 mM in this assay), has shown activity in the kidney allograft rat model<sup>38</sup> and allergic sheep model<sup>39</sup> at a dose of 10 mg/kg IV and 10 mg aerosol, respectively.

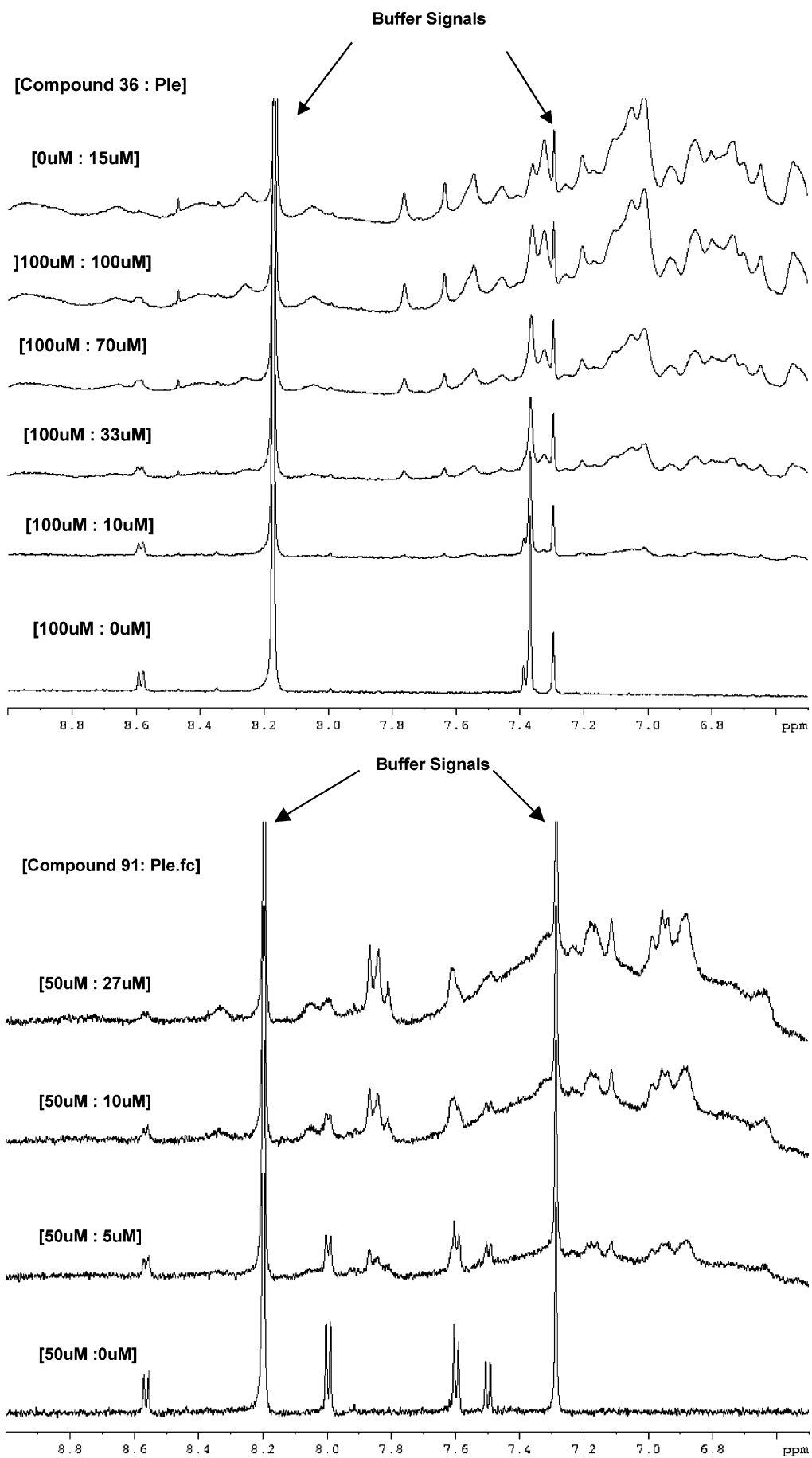
Although designed as P-selectin antagonists, the anti-inflammatory activity seen in AIA could be the result of other pathways. To further evaluate the mechanism of anti-inflammation for these compounds, compound **91** was assessed in the Carrageenan-induced paw edema (CPE) model. Prostaglandins play an important role in the development of edema in this model, and NSAIDs (like naproxen) inhibit the edema in a dose-responsive manner.<sup>40</sup> In addition, glucocorticoids such as Dexamethasone have shown activity in this model. Compound **91** did not show any activity when dosed at 0.5, 1, 5, 10, or 50 mg/kg by the PO route of administration (Figure 3). Thus, the inactivity of compound **91** in this model suggests that NSAID and glucocorticoids pathways are not the mechanism for the anti-inflammatory action of our compound.

Next, compounds **36** and **91** were taken forward for evaluation in NMR studies. On 1D NMR titration of compounds **36** and **91** with P-selectin (Figure 4), gradual line broadening of compounds as P-selectin is added was observed. The titrations were performed at 100 and 50  $\mu$ M compound and protein concentrations (for practical reasons) and, therefore, it was not expected that the binding would reach saturation at the last equimolar point. However, this titration suggests that these compounds titrate well with P-selectin and bind with low stoichiometry.

Compound **36** has an oral bioavailability of 24% in rat and showed a much-improved cytochrome P450 (CYP) profile over that of **91** (Table 11). Therefore, **36** was selected as our lead candidate.

**Table 11.** Data for Inhibition of Human CYP Isozymes for Compounds **36** and **91**

compd #	CYP3A4 IC <sub>50</sub> $\mu$ M	CYP2D6 IC <sub>50</sub> $\mu$ M	CYP2C9 IC <sub>50</sub> $\mu$ M	CYP2C8 IC <sub>50</sub> $\mu$ M	CYP1A2 IC <sub>50</sub> $\mu$ M	CYP2C19 IC <sub>50</sub> $\mu$ M	CYP2A6 IC <sub>50</sub> $\mu$ M
<b>91</b>	7	17	2	9	4	1	20
<b>36</b>	55	151	8.2	4.8	500	146	500



**Figure 4.** (A) 1D NMR titration of compound **36** with P-selectin. (B) 1D NMR titration of compound **91** with P-selectin.



In summary, a new class of P-selectin antagonists, as exemplified by **36**, has been discovered through high-throughput screening. Substitution at the 8 and 4' positions of the quinoline salicylic acid core led to significant improvement of the P-selectin antagonist activity and concomitantly reduced the unwanted side activity toward DHOD and CYP enzymes. The advanced lead compound, **36**, demonstrated acceptable PK properties in rats and exhibited potent in vivo efficacy in the rodent AIA model. These results suggest potential use of these P-selectin antagonists for the treatment of RA in humans.

## Experimental Section

**Chemistry.** Reactions were run using commercially available starting materials and anhydrous solvents without further purification. Proton NMR spectra were recorded at 300 MHz on a Varian Gemini 2000 or on a 400 MHz Bruker AV-400 spectrometer using TMS ( $\delta$  0.0) as a reference. Combustion analyses were obtained using a Perkin-Elmer Series II 2400 CHNS/O analyzer. CHN analyses were carried out by Robertson–Microlit. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were  $\pm 0.4$  of the theoretical values. Low resolution mass spectra were obtained using a Micromass Platform electrospray ionization quadrupole mass spectrometer. High resolution mass spectra were obtained using a Bruker APEXIII Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an actively shielded 7 T superconducting magnet (Magnex Scientific, Ltd., UK) and an external Bruker APOLLO electrospray ionization (ESI) source. Preparative HPLC was run using a Waters reverse-phase prep HPLC with Xterra C18 5  $\mu$ m, 30  $\times$  100 mm column. The flow rate was 40 mL/min and mobile phase A was water, mobile phase B was CH<sub>3</sub>CN, and triethylamine was used as a modifier. Purity in two solvent systems [H<sub>2</sub>O–CH<sub>3</sub>CN (method 1) and H<sub>2</sub>O–MeOH (method 2)] was determined using an Agilent 1100 HPLC instrument, and all compounds analyzed were >95% pure.

**General Procedure for the Synthesis of Bromides.** A solution of appropriately substituted acetophenone (70.67 mmol) in 80 mL of CHCl<sub>3</sub> was added in one portion to a vigorously stirred, refluxing suspension of 31.57 g (141.35 mmol, 2 equiv) of CuBr<sub>2</sub> in 65 mL of ethyl acetate. The reaction was practically complete after refluxing for 1.5 h, as indicated by the conversion of CuBr<sub>2</sub> (black) into CuBr (white), lack of HBr evolution, and TLC (ethyl acetate–cyclohexane, 20:80). The solids were removed by filtration through Celite, washing with ethyl acetate. The residue from evaporation was distributed between ethyl acetate (2  $\times$  350 mL) and semisaturated NaHCO<sub>3</sub> (2  $\times$  200 mL). The organic layers were washed with semisaturated brine (200 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated in vacuo. The crude residue was passed through a quick column, eluting with ethyl acetate–cyclohexane, 20:80, and used as such for the next step. This procedure was used to make the bromides for the following acetates **121**, **139**, and **141**. All other bromides were commercially available.

**General Procedure for the Synthesis of Acetates.** A suspension of appropriately substituted bromoacetophenone (0.21 mol) in 220 mL of ethanol was prepared in a 1 L round-bottomed flask, and a solution of sodium acetate trihydrate (32 g, 0.24 mol) in 110 mL of water and 11 mL of acetic acid was added. The mixture was heated at reflux for 2.5 h, then cooled to room temperature, and refrigerated overnight. In some cases, a solid separated that was collected by filtration and was found to be pure acetate. In other cases, most of the ethanol was removed under reduced pressure, and the resulting oily mixture was distributed between 2  $\times$  300 mL ethyl acetate and 2  $\times$  100 mL of a semisaturated, ice-cold NaHCO<sub>3</sub> solution. The organic extracts were washed in sequence with 100 mL of semisaturated brine, dried with sodium sulfate, and evaporated in vacuo. Crystallization of the residue from ethyl acetate, ethyl acetate–cyclohexane, ether, or ethanol–water afforded the appropriately substituted acetoxyacetophenone as crystals. Acetates **116** and **120** were commercially available.

**2-(4-Fluorophenyl)-2-oxoethyl Acetate (118):** Colorless crystals, yield 57.2%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.13–2.27 (s, 3H), 5.30 (s, 2H), 7.17 (t,  $J$  = 8.59 Hz, 2H), 7.95 (dd,  $J$  = 9.09, 5.31 Hz, 2H).

**2-(4-Chlorophenyl)-2-oxoethyl Acetate (18):** White crystals, yield 83%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.22 (s, 3H), 5.28 (s, 2H), 7.46 (d,  $J$  = 8.6 Hz, 2H), 7.85 (d,  $J$  = 8.6 Hz, 2H).

**2-(4-Isopropylphenyl)-2-oxoethyl Acetate (121):** Colorless crystals, yield 85.6%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  1.27 (d,  $J$  = 6.82 Hz, 6H), 2.23 (s, 3H), 2.85–3.07 (m, 1H), 5.32 (s, 2H), 7.34 (d,  $J$  = 8.08 Hz, 2H), 7.85 (d,  $J$  = 8.59 Hz, 2H).

**2-Oxo-2-(4-(trifluoromethyl)phenyl)ethyl Acetate (123):** Colorless crystals, yield 78.1%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.21–2.27 (s, 3H), 5.17–5.43 (s, 2H), 7.77 (d,  $J$  = 7.33 Hz, 2H), 8.03 (d,  $J$  = 7.33 Hz, 2H).

**2-Oxo-2-(4-(trifluoromethoxy)phenyl)ethyl Acetate (125):** Colorless crystals, yield 89.6%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.24 (s, 3H), 5.31 (s, 2H), 7.29–7.41 (m, 2H), 7.90–8.08 (m, 2H).

**2-(4-(Diethylamino)phenyl)-2-oxoethyl Acetate (127):** Colorless crystals, yield 86.1%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  1.21 (t,  $J$  = 7.07 Hz, 6H), 2.22 (s, 3H), 3.43 (q,  $J$  = 7.07 Hz, 4H), 5.27 (s, 2H), 6.62 (d,  $J$  = 9.09 Hz, 2H), 7.80 (d,  $J$  = 9.35 Hz, 2H).

**2-Oxo-2-*p*-tolylethyl Acetate (129):** Colorless crystals, yield 85.6%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.23 (s, 3H), 2.42 (s, 3H), 5.32 (s, 2H), 7.28 (d,  $J$  = 7.83 Hz, 2H), 7.82 (d,  $J$  = 8.34 Hz, 2H).

**2-(Biphenyl-4-yl)-2-oxoethyl Acetate (131):** Pale tan crystals, yield 88.3%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.25 (s, 3H), 5.37 (s, 2H), 7.41–7.53 (m, 3H), 7.59–7.67 (m, 2H), 7.67–7.77 (m, 2H), 7.92–8.06 (m, 2H).

**Ethyl 4-(2-Acetoxyacetyl)benzoate (133):** Colorless crystals, yield 70.4%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  1.42 (t,  $J$  = 7.07 Hz, 3H), 2.24 (s, 3H), 4.42 (q,  $J$  = 7.16 Hz, 2H), 5.34 (s, 2H), 7.97 (d,  $J$  = 8.84 Hz, 2H), 8.15 (d,  $J$  = 8.59 Hz, 2H).

**2-(4-Hydroxyphenyl)-2-oxoethyl Acetate (135):** Colorless crystals, yield 86%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.40 (s, 3H), 5.42 (s, 2H), 7.04 (d,  $J$  = 9.09 Hz, 2H), 8.00 (d,  $J$  = 9.09 Hz, 2H).

**2-(4-Methoxyphenyl)-2-oxoethyl Acetate (137):** Colorless crystals, yield 86.2%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.41 (s, 3H), 4.06 (s, 3H), 5.48 (s, 2H), 7.14 (d,  $J$  = 9.09 Hz, 2H), 8.08 (d,  $J$  = 9.09 Hz, 2H).

**2-(4-Acetamidophenyl)-2-oxoethyl Acetate (139):** Colorless crystals, yield 75.5%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  2.09 (s, 3H), 2.14 (s, 3H), 5.40 (s, 2H), 7.67–7.77 (m, 2H), 7.81–8.04 (m, 2H), 10.32 (s, 1H).

**2-(4-Morpholinophenyl)-2-oxoethyl Acetate (8):** Pale tan crystals, yield 80%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.23 (s, 3H), 3.26–3.38 (m, 4H), 3.78–3.92 (m, 4H), 5.29 (s, 2H), 6.87 (d,  $J$  = 9.09 Hz, 2H), 7.85 (d,  $J$  = 9.09 Hz, 2H).

**2-Oxo-2-(3-(trifluoromethoxy)phenyl)ethyl Acetate (141):** Pale yellow crystals, yield 73.1%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.23 (s, 3H), 5.31 (s, 2H), 7.41–7.52 (m, 1H), 7.55 (t,  $J$  = 7.96 Hz, 1H), 7.73–7.81 (m, 1H), 7.80–7.90 (m, 1H).

**2-(3-Chlorophenyl)-2-oxoethyl Acetate (143):** Colorless crystals, yield 87%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.23 (m, 3H), 5.30 (s, 2H), 7.44 (t,  $J$  = 7.83 Hz, 1H), 7.55–7.62 (m, 1H), 7.77–7.81 (m, 1H), 7.89 (t,  $J$  = 1.89 Hz, 1H).

**General Procedure for the Synthesis of Indoline-2,3-diones (Isatins).** Chloral hydrate (14.7 g, 88.8 mmol) was added to a 1 L round-bottomed flask containing a suspension of hydroxylamine hydrochloride (18.5 g, 0.266 mol), sodium sulfate (84 g, 0.59 mol), and the appropriate aniline (74.0 mmol) in 500 mL of water and 25 mL of 2 M aqueous hydrochloric acid. The mixture was then heated at 55 °C overnight, with stirring. After cooling to room temperature, the hydroxyiminoacetanilide could usually be collected by filtration. The solid was washed with water and dried under vacuum. To carry out the cyclization, the intermediate was added in small portions, with stirring, to a 250 mL Erlenmeyer flask containing 45 mL of concentrated sulfuric acid, which had been heated to 55 °C. The temperature of the reaction mixture was maintained below 70 °C during this addition. After all the isonitroso had been added, the dark-colored solution was heated at 80 °C for

an additional 10 min and then cooled to room temperature, poured onto 225 mL of crushed ice, and allowed to stand for 30 min. The precipitate was collected by filtration, washing three times with water, and dried under vacuum to yield isatin that was usually of sufficient purity to be used directly in the next step. Occasionally, the first step produced an oil or sludge rather than an isolable solid. In these cases, the hydroxyiminoacetanilide was obtained by ethyl acetate extraction of the cooled reaction mixture; the ethyl acetate solution was washed with brine, dried (MgSO<sub>4</sub>), filtered, and evaporated to give a viscous oil. Cold concentrated sulfuric acid was added to this oil in a 250 mL round-bottomed flask, and the mixture was heated to 80 °C for 30 min (open to the atmosphere). The reaction was then cooled to room temperature and worked up, as described above. The following isatins were commercially available **19**, **25**, **40**, **42**, **44**, **48**, **51**, **55**, **57**, **73**, and **92**.

**7-Methylindoline-2,3-dione (21)**: Orange powder, yield 40%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.19 (s, 3H), 6.99 (t, *J* = 7.6 Hz, 1H), 7.34 (d, *J* = 7.6 Hz, 1H), 7.43 (d, *J* = 7.6 Hz, 1H), 11.09 (s, 1H). MS (electrospray): 162 (M + H)<sup>+</sup>.

**6-Methylindoline-2,3-dione (23) and 4-Methylindoline-2,3-dione (3–5)**: Orange powder, inseparable 1:1 mixture, yield 26%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.35 (s, 1.5H), 2.44 (s, 1.5H), 6.71 (m, 1H), 6.87 (t, 1H), 7.42 (m, 1H), 10.99 (s, 1H). MS (electrospray), 162 (M + H)<sup>+</sup>.

**7-Isopropylindoline-2,3-dione (29)**: Brown powder, yield 46%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.18 (d, *J* = 6.8 Hz, 6H), 3.04 (sep, 1H), 7.06 (t, *J* = 7.7 Hz, 1H), 7.35 (d, *J* = 7.3 Hz, 1H), 7.54 (d, *J* = 7.3 Hz, 1H), 11.09 (s, 1H). MS (electrospray): 188 (M – H)<sup>–</sup>.

**6-Isopropylindoline-2,3-dione (31) and 4-Isopropylindoline-2,3-dione. Hydroxyiminoacetanilide**: Pale golden yellow powder, yield 72%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.19 (d, *J* = 7.1 Hz, 6H), 2.85 (sept, 1H), 6.98 (d, *J* = 7.8 Hz, 1H), 7.24 (t, *J* = 7.8 Hz, 1H), 7.53 (d, *J* = 8.1 Hz, 1H), 7.56 (s, 1H), 7.66 (s, 1H), 10.10 (s, 1H), 12.15 (s, 1H). MS (electrospray): 205 (M – H)<sup>–</sup>. **Isatin**: Dark brown solid, inseparable 8:2 mixture, yield 78%. **6-Isopropylisatin**: <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.20 (d, *J* = 6.8 Hz, 6H), 2.94 (sept, 1H), 6.76 (s, 1H), 6.96 (d, *J* = 7.8 Hz, 1H), 7.43 (d, *J* = 7.8 Hz, 1H), 10.98 (s, 1H). **4-Isopropylisatin**: <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.18 (d, *J* = 7.3 Hz, 6H), 3.69 (sept, 1H), 6.72 (d, *J* = 7.8 Hz, 1H), 7.00 (d, *J* = 7.8 Hz, 1H), 7.50 (t, *J* = 7.8 Hz, 1H), 10.98 (s, 1H). MS (electrospray): 188 (M – H)<sup>–</sup>.

**5-Isopropylindoline-2,3-dione (33)**: Purified by titration with ethyl acetate, yield 71%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.18 (d, *J* = 7.07 Hz, 6H), 2.77–2.95 (m, 1H), 6.84 (d, *J* = 8.08 Hz, 1H), 7.38 (d, *J* = 1.77 Hz, 1H), 7.49 (dd, *J* = 8.08, 2.02 Hz, 1H), 10.94 (s, 1H). MS (electrospray): 188 (M – H)<sup>–</sup>.

**6-Phenylindoline-2,3-dione (37) and 4-Phenylindoline-2,3-dione. Hydroxyiminoacetanilide**: Yellow powder, yield 88%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.39 (m, 1H), 7.48 (m, 1H), 7.62 (dd, *J* = 8.3, 7.3 Hz, 1H), 7.70 (m, 1H), 8.02 (s, 1H), 10.27 (s, 1H), 12.20 (s, 1H). **Isatin**: Dark red powder, inseparable mixture of uncertain isomeric ratio, yield 59%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 6.90 (d, *J* = 7.1 Hz), 7.02 (d, *J* = 7.1 Hz), 7.12 (s), 7.35 (m), 7.50 (m), 7.61 (m), 7.73 (m), 11.13 (s), 11.14 (s). MS (electrospray): 222 (M – H)<sup>–</sup>.

**7-(Trifluoromethoxy)indoline-2,3-dione (46). Hydroxyiminoacetanilide**: Lumpy brown solid, yield 85%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.31 (m, 1H), 7.42 (m, 2H), 7.75 (s, 1H), 7.97 (dd, *J* = 7.8, 1.3 Hz, 1H), 9.71 (s, 1H), 12.39 (s, 1H). **Isatin**: Yield 70%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.15 (t, *J* = 7.8 Hz, 1H), 7.56 (d, *J* = 7.3 Hz, 1H), 7.64 (d, *J* = 8.3 Hz, 1H), 11.71 (s, 1H). MS (electrospray): 230 (M – H)<sup>–</sup>.

**7-(Trifluoromethyl)indoline-2,3-dione (50)**: Yield 61%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.23 (t, *J* = 7.7 Hz, 1H), 7.78 (d, *J* = 7.3 Hz, 1H), 7.85 (d, *J* = 8.1 Hz, 1H), 11.46 (s, 1H). MS (electrospray): 214 (M – H)<sup>–</sup>.

**7-Bromoindoline-2,3-dione (53). Hydroxyiminoacetanilide**: Brown solid, yield 85%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.16 (t, 1H), 7.41 (t, *J* = 7.7 Hz, 1H), 7.69 (m, 2H), 7.91 (d, *J* = 8.1

Hz, 1H), 9.46 (s, 1H), 12.45 (s, 1H). MS (electrospray): 241 (M – H)<sup>–</sup>. **Isatin**: Reddish-brown powder, yield 77%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.02 (t, *J* = 7.8 Hz, 1H), 7.52 (d, *J* = 6.6 Hz, 1H), 7.79 (d, *J* = 8.1 Hz, 1H), 11.32 (s, 1H).

**7-Methoxyindoline-2,3-dione<sup>41</sup> (59)**: <sup>1</sup>H NMR (400 MHz, DMF): δ 4.13 (s, 3H), 7.24–7.33 (m, 1H), 7.34–7.41 (m, 1H), 7.60 (dd, *J* = 8.08, 1.01 Hz, 1H), 11.38 (s, 1H).

**7-Ethoxyindoline-2,3-dione (61)**: Purified by flash chromatography over silica gel (20% ethyl acetate in cyclohexane), yield 9.7%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.33 (t, *J* = 6.95 Hz, 3H), 4.10 (q, *J* = 6.91 Hz, 2H), 6.82–7.01 (m, 1H), 7.02–7.18 (m, 1H), 7.56 (d, *J* = 7.33 Hz, 1H), 10.68 (s, 1H).

**N-(2,3-Dioxindolin-5-yl)acetamide (76)**: Purified by titration with ethyl acetate, yield 50%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.03 (s, 3H), 6.87 (d, *J* = 8.84 Hz, 1H), 7.64 (dd, *J* = 8.34, 2.27 Hz, 1H), 7.80 (d, *J* = 2.27 Hz, 1H), 10.01 (s, 1H), 10.93 (s, 1H).

**2,3-Dioxindoline-5-carboxylic Acid (78)**: Purified by crystallization from ethyl acetate and methanol, yield 45%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.01 (d, *J* = 8.34 Hz, 1H), 7.92 (d, *J* = 1.77 Hz, 1H), 8.15 (dd, *J* = 8.08, 1.77 Hz, 1H), 11.38 (s, 1H).

**7-Ethylindoline-2,3-dione (80)**: Orange-brown powder, yield 27%, recrystallization from ethanol gave orange-red needles. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.14 (t, *J* = 7.5 Hz, 3H), 2.56 (q, *J* = 7.6 Hz, 2H), 7.03 (t, *J* = 7.5 Hz, 1H), 7.35 (d, *J* = 7.3 Hz, 1H), 7.46 (d, *J* = 7.6 Hz, 1H), 11.11 (s, 1H). MS (electrospray): 176 (M + H)<sup>+</sup>.

**7-sec-Butylindoline-2,3-dione (82). Hydroxyiminoacetanilide**: Sticky dark brown oil. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 0.75 (t, *J* = 7.3 Hz, 3H), 1.14 (d, *J* = 6.8 Hz, 3H), 1.51 (m, 2H), 2.86 (m, 1H), 7.24 (m, 4H), 7.68 (s, 1H), 9.57 (s, 1H), 12.16 (s, 1H). MS (electrospray): 219 (M – H)<sup>–</sup>. **Isatin**: Yield 52%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 0.81 (t, *J* = 7.3 Hz, 3H), 1.17 (d, *J* = 6.8 Hz, 3H), 1.55 (m, 2H), 2.83 (m, 1H), 7.06 (t, *J* = 7.6 Hz, 1H), 7.36 (d, *J* = 7.1 Hz, 1H), 7.51 (d, *J* = 7.6 Hz, 1H), 11.09 (s, 1H). MS (electrospray): 202 (M – H)<sup>–</sup>.

**7-tert-Butylindoline-2,3-dione (84)**: Isatin was isolated by ethyl acetate extraction of the solution obtained by pouring the cooled reaction mixture onto ice, yield 55%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.32 (s, 9H), 7.04 (t, 1H), 7.39 (d, *J* = 7.3 Hz, 1H), 7.55 (dd, *J* = 7.8, 1.3 Hz, 1H), 10.76 (s, 1H). MS (electrospray): 204 (M + H)<sup>+</sup>.

**7-Fluoroindoline-2,3-dione (88). Hydroxyiminoacetanilide**: Yield 71%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.20 (m, 2H), 7.29 (m, 1H), 7.74 (s, 1H), 7.86 (m, 1H), 9.81 (s, 1H), 12.30 (s, 1H). **Isatin**: Yield 65%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.08 (ddd, 1H), 7.38 (dt, *J* = 7.5, 0.8 Hz, 1H), 7.54 (ddd, *J* = 10.4, 8.3, 1.0 Hz, 1H), 11.56 (s, 1H). MS (electrospray): 164 (M – H)<sup>–</sup>.

**6,7-Dimethylindoline-2,3-dione<sup>42</sup> (90)**: <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.09 (s, 3H), 2.28 (s, 3H), 6.90 (d, *J* = 7.83 Hz, 1H), 7.25 (d, *J* = 7.58 Hz, 1H), 11.01 (s, 1H).

**7-Methyl-5-(trifluoromethoxy)indoline-2,3-dione (96)**: Purified by titration with ethyl acetate, yield 70%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.23 (s, 3H), 7.35 (s, 1H), 7.50 (s, 1H), 11.26 (s, 1H).

**7-Aryl Indoline-2,3-dione (7-Arylisatins). 7-Iodoindoline-2,3-dione. Hydroxyiminoacetanilide**: Beige solid, yield 83%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 6.99 (t, *J* = 7.7 Hz, 1H), 7.41 (t, 1H), 7.63 (s, 1H), 7.76 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.90 (dd, *J* = 7.8, 1.3 Hz, 1H), 9.38 (s, 1H), 12.42 (s, 1H). MS (electrospray): 289 (M – H)<sup>–</sup>. **Isatin**: Dark red powder, yield 80%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 6.89 (t, *J* = 7.7 Hz, 1H), 7.50 (d, *J* = 7.3 Hz, 1H), 7.95 (d, *J* = 6.8 Hz, 1H), 11.01 (s, 1H). MS (electrospray): 272 (M – H)<sup>–</sup>.

**General Procedure**. To a 1-L three-necked round-bottomed flask fitted with a reflux condenser were added 7-iodoindoline-2,3-dione (2.0 g, 7.33 mmol) and tetrakis[triphenylphosphine]palladium (0.424 g, 0.367 mmol), followed by 225 mL of 1,2-dimethoxyethane. The atmosphere in the reaction vessel was made inert by opening to vacuum, then to a positive pressure of nitrogen (3×). Arylboronic acid (8.06 mmol) and a solution of sodium bicarbonate (1.23 g, 14.7 mmol) in 225 mL of water were added, and the evacuation/



nitrogen procedure was repeated one more time. The reaction mixture was then refluxed until TLC showed complete disappearance of 7-iodoisatin (1–2 h). After cooling to room temperature, the 1,2-dimethoxyethane was removed under reduced pressure. The residue was diluted with 1 M aqueous hydrochloric acid and extracted into ethyl acetate (3×). The organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure to give the crude 7-arylisatin, which could often be used in the next step without purification.

**7-Phenylindoline-2,3-dione (35):** Purified by flash chromatography over silica gel (1% ethyl acetate in dichloromethane), orange needlelike crystals, yield 74%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.18 (t, *J* = 7.6 Hz, 1H), 7.48 (m, 6H), 7.59 (d, *J* = 8.8 Hz, 1H), 10.91 (s, 1H). MS (electrospray): 222 (M – H)<sup>–</sup>.

**4-(2,3-Dioxindolin-7-yl)benzoic Acid (63):** After extraction into ethyl acetate, the organic layer was extracted into 1 M aqueous sodium hydroxide (3×), then acidified with concentrated hydrochloric acid, extracted back into ethyl acetate (3×), and evaporated to give product of sufficient purity to be used directly in the next step: <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.20 (t, *J* = 7.6 Hz, 1H), 7.61 (m, 4H), 7.89 (m, 1H), 8.04 (m, 1H), 8.26 (br s, 1H), 10.97 (s, 1H). MS (electrospray): 266 (M – H)<sup>–</sup>.

**7-(4-(Hydroxymethyl)phenyl)indoline-2,3-dione (65):** <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 4.57 (s, 2H), 7.17 (t, *J* = 7.5 Hz, 1H), 7.44 (s, 4H), 7.59 (m, 2H), 10.90 (s, 1H). MS (electrospray): 252 (M – H)<sup>–</sup>.

**7-(4-(Methylsulfonyl)phenyl)indoline-2,3-dione (67):** Purified by flash chromatography over silica gel (15–100% ethyl acetate in dichloromethane), yield 65%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 3.28 (s, 3H), 7.21 (t, 1H), 7.60 (dt, *J* = 7.4, 1.0 Hz, 1H), 7.64 (dd, *J* = 7.8, 1.3 Hz, 2H), 7.75 (dt, *J* = 8.6, 1.9 Hz, 2H), 8.04 (dt, *J* = 8.6, 1.9 Hz, 2H), 11.03 (s, 1H). MS (electrospray): 300 (M – H)<sup>–</sup>.

**7-(4-(Dimethylamino)phenyl)indoline-2,3-dione (69):** Yield 66%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.96 (s, 6H), 6.83 (d, *J* = 8.6 Hz, 2H), 7.13 (t, *J* = 7.6 Hz, 1H), 7.31 (d, *J* = 8.8 Hz, 2H), 7.44 (d, *J* = 7.1 Hz, 1H), 7.53 (dd, *J* = 7.8, 1.3 Hz, 1H), 10.79 (s, 1H).

**7-(Thien-3-yl)isatin (86):** Purified by flash chromatography over silica gel (3% ethyl acetate in dichloromethane), bright red crystalline material, yield 54%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.15 (t, 1H), 7.36 (dd, *J* = 4.9, 1.4 Hz, 1H), 7.50 (dt, *J* = 7.3, 1.0 Hz, 1H), 7.68 (d, *J* = 1.5 Hz, 1H), 7.71 (m, 2H), 7.75 (dd, *J* = 2.9, 1.4 Hz, 1H), 10.86 (s, 1H). MS (electrospray): 228 (M – H)<sup>–</sup>.

**6-Methoxyindoline-2,3-dione (71):** To a flame-dried 500-mL two-necked round-bottomed flask fitted with a reflux condenser were added, under a nitrogen atmosphere, *m*-anisidine (9.1 mL, 10 g, 81 mmol) and 95 mL anhydrous benzene. Oxalyl chloride (16 mL, 23 g, 0.18 mol) was added dropwise by syringe, evolving HCl gas. The reaction mixture was refluxed for 3 h and then allowed to cool to room temperature. Benzene and excess oxalyl chloride were removed under reduced pressure, and the residue was resuspended in 95 mL of anhydrous 1,2-dichloroethane. The mixture was cooled to 0 °C, aluminum chloride (11.2 g, 83.6 mmol) was added in portions with stirring, then the reaction was allowed to warm to room temperature over the course of the next 3 h, and then heated to reflux until TLC (15% ethyl acetate in dichloromethane) showed complete disappearance of the acid formed when a sample of the reaction mixture was quenched with dilute hydrochloric acid (40 min). After cooling to room temperature, the mixture was poured into crushed ice and then partitioned between 0.1 M aqueous hydrochloric acid and ethyl acetate. The aqueous layer was extracted with additional ethyl acetate (2×), and the combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered, and evaporated. The crude isatin was purified by flash chromatography over silica gel to give pure product as an orange solid (2.23 g, 15% yield): <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 3.88 (s, 3H), 6.40 (d, *J* = 2.3 Hz, 1H), 6.6 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.49 (d, *J* = 8.3 Hz, 1H), 10.97 (s, 1H). MS (electrospray): 176 (M – H)<sup>–</sup>.

**5-Bromo-7-methylindoline-2,3-dione (94):** To a 250-mL two-necked round-bottomed flask fitted with a reflux condenser were added 7-methylisatin (5.46 g, 33.9 mmol) and 55 mL of 1,2-dichloroethane. *N*-Bromosuccinimide (6.03 g, 33.9 mmol) and 2,2'-azobisisobutyronitrile (75 mg, 0.46 mmol) were added, and the mixture was refluxed for 3.5 h. To work up the reaction, the crude reaction mixture was adsorbed onto silica gel and purified by flash chromatography over silica gel (3% ethyl acetate in dichloromethane). This gave an inseparable 55:45 mixture of starting material and 5-bromo-7-methylisatin, a bright orange solid (4.57 g, 25% yield): <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.19 (s, 3H), 7.49 (d, *J* = 2.8 Hz, 1H), 7.65 (dd, *J* = 2.0, 0.8 Hz, 1H), 11.20 (s, 1H). MS (electrospray): 238 (M – H)<sup>–</sup>.

**General Procedure for the Synthesis of 7-Carboxamido Indoline-2,3-dione.** Commercially available 2,3-dioxindoline-7-carboxylic acid (**57**; 1 g, 5.25 mmol) and benzotriazol-1-yloxytri-pyrrolidinophosphonium hexafluorophosphate (pyBOP, 2.99 g, 5.75 mmol) were placed in a flame-dried 50-mL round-bottomed flask fitted with a rubber septum under a nitrogen atmosphere, and 10 mL of anhydrous *N,N*-dimethylformamide was added by syringe. Next, amine (5.8 mmol) and *N*-methylmorpholine (0.63 mL, 0.58 g, 5.8 mmol) were added by syringe. The solution was stirred at room temperature overnight and then worked up by diluting with 50 mL of ethyl acetate and washing with 2 M aqueous hydrochloric acid (3×) and brine (3×). The organic solution was dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure to give the crude amide.

***N*-Isopropyl-2,3-dioxindoline-7-carboxamide (98):** The reaction was carried out in 4:1 DMF/dichloromethane, and the base used was *N,N*-diisopropylethylamine. Purified by flash chromatography over silica gel (2.5% methanol in dichloromethane). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.18 (d, *J* = 6.6 Hz, 6H), 4.11 (m, 1H), 7.14 (t, 1H), 7.64 (d, *J* = 7.3 Hz, 1H), 7.97 (dd, *J* = 8.1, 1.3 Hz, 1H), 8.47 (d, *J* = 7.8 Hz, 1H), 10.56 (s, 1H).

***N*-*tert*-Butyl-2,3-dioxindoline-7-carboxamide (100):** Purified by flash chromatography over silica gel (3–15% ethyl acetate in dichloromethane), yield 17%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.39 (s, 9H), 7.11 (t, 1H), 7.62 (d, *J* = 7.6 Hz, 1H), 7.93 (dd, *J* = 8.1, 1.3 Hz, 1H), 8.01 (s, 1H), 10.58 (s, 1H).

**2,3-Dioxo-*N*-phenylindoline-7-carboxamide (102):** Purified by flash chromatography over silica gel (3% ethyl acetate in dichloromethane), yield 45%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.14 (t, *J* = 7.3 Hz, 1H), 7.21 (t, *J* = 7.6 Hz, 1H), 7.38 (t, 2H), 7.72 (m, 3H), 8.05 (dd, *J* = 7.8, 1.3 Hz, 1H), 10.46 (s, 1H), 10.85 (s, 1H).

***N*-Benzyl-2,3-dioxindoline-7-carboxamide (104):** Purified by flash chromatography over silica gel (5% ethyl acetate in dichloromethane), yield 29%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 4.48 (d, 2H), 7.16 (t, *J* = 7.6 Hz, 1H), 7.26 (m, 1H), 7.34 (d, *J* = 4.3 Hz, 4H), 7.67 (d, *J* = 7.3 Hz, 1H), 8.02 (dd, *J* = 8.0, 1.1 Hz, 1H), 9.27 (t, *J* = 5.8 Hz, 1H), 10.62 (s, 1H).

**General Procedure for the Synthesis of 5-Carboxamido Indoline-2,3-dione.** 2,3-Dioxindoline-5-carboxylic acid (**78**; 1 g, 5.25 mmol) and pyBOP (2.99 g, 5.75 mmol) were placed in a flame-dried 50-mL round-bottomed flask fitted with a rubber septum under a nitrogen atmosphere, and 10 mL of anhydrous *N,N*-dimethylformamide was added by syringe. Next, the appropriate amine (5.8 mmol) and *N*-methylmorpholine (0.63 mL, 0.58 g, 5.8 mmol) were added by syringe. The solution was stirred at room temperature overnight and then worked up by diluting with 50 mL of ethyl acetate and washing with 2 M aqueous hydrochloric acid (3×) and brine (3×). The organic solution was dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure to give the crude amide. These amides were purified by crystallization from ethyl acetate.

***N*-Isopropyl-2,3-dioxindoline-5-carboxamide (106):** Yield 50%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.15 (d, *J* = 6.57 Hz, 6H), 4.00–4.16 (m, 1H), 6.96 (d, *J* = 8.08 Hz, 1H), 8.05 (d, *J* = 1.77 Hz, 1H), 8.10 (dd, *J* = 8.21, 1.89 Hz, 1H), 8.28 (d, *J* = 7.58 Hz, 1H), 11.26 (s, 1H).

***N*-tert-Butyl-2,3-dioxindoline-5-carboxamide (108)**: Yield 42%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.34 (s, 9H), 6.94 (d, *J* = 8.59 Hz, 1H), 7.85 (s, 1H), 8.00–8.10 (m, 2H), 11.22–11.27 (m, 1H).

**2,3-Dioxo-*N*-phenylindoline-5-carboxamide (110)**: Yield 60%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.04 (d, *J* = 8.59 Hz, 1H), 7.10 (t, *J* = 7.33 Hz, 1H), 7.29–7.43 (m, 2H), 7.77 (dd, *J* = 8.59, 1.26 Hz, 2H), 8.15–8.27 (m, 2H), 10.25 (s, 1H), 11.34 (s, 1H).

***N*-Benzyl-2,3-dioxindoline-5-carboxamide (112)**: Yield 59%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 4.46 (d, *J* = 5.81 Hz, 2H), 6.99 (d, *J* = 8.59 Hz, 1H), 7.20–7.28 (m, 1H), 7.29–7.34 (m, 4H), 8.08 (d, *J* = 2.02 Hz, 1H), 8.14 (dd, *J* = 8.34, 1.77 Hz, 1H), 9.09 (t, *J* = 5.81 Hz, 1H), 11.28 (s, 1H).

**General Procedure for the Synthesis of 3-Hydroxyquinoline-4-carboxylic Acids. Pfitzinger Reaction.** In a 50-mL two-necked round-bottomed flask fitted with a reflux condenser, isatin (3.80 mmol) was suspended in 4 mL of 6 M aqueous potassium hydroxide and heated to 100 °C. A solution of the appropriate acetate in 4 mL of warm ethanol was then added by syringe in small portions over the course of 1 h. After the addition had been completed, the reaction mixture was refluxed for an additional 4 h. It was then cooled to room temperature, and ethanol was removed under reduced pressure. The residue was diluted with 20 mL of water, treated with charcoal, and filtered, and the clear solution was acidified to pH 1 with 1 M aqueous hydrochloric acid. The precipitate was collected by filtration, washed with water, and dried under vacuum. The crude product was usually purified by (A) recrystallization or (B) gravity column chromatography over silica gel, eluting with a 70:5:2.5:2.5 ethyl acetate/acetonitrile/methanol/water system to which 0.5% triethylamine had been added; the pure triethylammonium salt was then taken up in 20% acetonitrile in water and acidified with concentrated hydrochloric acid to precipitate the free acid.

**2-(4-Aminophenyl)-3-hydroxy-7,8-dimethylquinoline-4-carboxylic Acid (9)**: Purified by method B, red-orange crystals, yield 82.2%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.42 (s, 3H), 2.69 (s, 3H), 6.79–6.91 (m, 2H), 7.37 (d, *J* = 8.84 Hz, 1H), 7.97–8.08 (m, 2H), 8.44 (d, *J* = 8.84 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>18</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub> (MH<sup>+</sup>), 309.1234; found, 309.1231.

**2-(4-Bromophenyl)-3-hydroxy-7,8-dimethylquinoline-4-carboxylic Acid (10)**: Purified by method B, pale yellow solid, yield 49.5%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.43 (s, 3H), 2.69 (s, 3H), 7.45 (d, *J* = 9.09 Hz, 1H), 7.54–7.64 (m, 2H), 8.14 (d, *J* = 8.59 Hz, 2H), 8.26 (d, *J* = 8.84 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>18</sub>H<sub>14</sub>BrNO<sub>3</sub> (MH<sup>+</sup>), 372.023; found, 372.0231.

**2-(4-Chlorophenyl)-3-hydroxy-8-trifluoromethylquinoline-4-carboxylic Acid (15)**: Recrystallized from chloroform/ethanol, fluffy yellow solid, yield 12%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.63 (d, *J* = 8.6 Hz, 2H), 7.72 (m, 1H), 7.98 (d, *J* = 7.1 Hz, 1H), 8.23 (d, *J* = 8.6 Hz, 2H), 8.95 (d, *J* = 8.3 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>17</sub>H<sub>10</sub>ClF<sub>3</sub>NO<sub>3</sub> (MH<sup>+</sup>), 368.0296; found, 368.0292.

**Triethylammonium 2-(4-Chlorophenyl)-3-hydroxyquinoline-4-carboxylic Acid (20)**: Purified by method B without conversion back to the free acid; yellow solid, yield 53.5%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.17 (t, *J* = 7.33 Hz, 9H), 3.09 (q, *J* = 7.07 Hz, 6H), 7.28–7.36 (m, 1H), 7.35–7.45 (m, 1H), 7.51 (d, *J* = 8.84 Hz, 2H), 7.82 (d, *J* = 8.08 Hz, 1H), 8.17–8.44 (m, 2H), 9.16–9.57 (m, 1H). Anal. (C<sub>22</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>3</sub>·0.25H<sub>2</sub>O) C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>16</sub>H<sub>10</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 300.0422; found, 300.0421.

**2-(4-Chlorophenyl)-3-hydroxy-8-methylquinoline-4-carboxylic Acid (22)**: Purified by method B, fluffy yellow solid, yield 29%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.73 (s, 3H), 7.49 (m, 2H), 7.59 (d, *J* = 8.6 Hz, 2H), 8.15 (d, *J* = 8.6 Hz, 2H), 8.38 (d, *J* = 7.8 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>17</sub>H<sub>13</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 314.0579; found, 314.0576. Anal. (C<sub>17</sub>H<sub>12</sub>ClNO<sub>3</sub>·0.25 H<sub>2</sub>O) C, H, N.

**2-(4-Chlorophenyl)-3-hydroxy-7-methylquinoline-4-carboxylic Acid (24)**: The inseparable mixture of 6-methylisatin and 4-methylisatin was used; the 7-methyl and 5-methylquinoline products could be separated by method B; the 7-methyl isomer was a pumpkin-orange powder, yield 6.0%. <sup>1</sup>H NMR (400 MHz,

DMSO-*d*<sub>6</sub>): δ 2.49 (s, 3H), 7.49 (d, *J* = 8.8 Hz, 1H), 7.60 (d, *J* = 8.3 Hz, 2H), 7.82 (s, 1H), 8.07 (d, *J* = 8.6 Hz, 2H), 8.63 (d, *J* = 9.4 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>17</sub>H<sub>13</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 314.0579; found, 314.0583.

**Triethylammonium 2-(4-Chlorophenyl)-3-hydroxy-6-methylquinoline-4-carboxylic Acid (26)**: The crude product was suspended in the method B solvent system, and triethylamine was added until everything went into solution. Within moments, large crystals precipitated. These were collected by filtration and then converted to the free acid, as described above to give a bright yellow powder, yield 56%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.17 (t, *J* = 7.33 Hz, 9H), 2.51 (s, 3H), 3.09 (q, *J* = 7.07 Hz, 6H), 7.45 (d, *J* = 8.6 Hz, 1H), 7.59 (d, *J* = 8.6 Hz, 2H), 7.91 (d, *J* = 8.6 Hz, 1H), 8.07 (d, *J* = 8.3 Hz, 2H), 8.49 (s, 1H). Anal. C<sub>23</sub>H<sub>27</sub>ClN<sub>2</sub>O<sub>3</sub> C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>17</sub>H<sub>12</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 314.0579; found, 314.0578.

**2-(4-Chlorophenyl)-3-hydroxy-5-methylquinoline-4-carboxylic Acid (28)**: A second purification under method B conditions was required, this time eluting with 70:10:5:5 ethyl acetate/acetonitrile/methanol/water (+0.5% triethylamine); pale yellow powder, yield 3.0%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.67 (s, 3H), 7.41 (d, *J* = 6.6 Hz, 1H), 7.52 (t, *J* = 7.7 Hz, 1H), 7.59 (d, *J* = 8.3 Hz, 2H), 7.87 (d, *J* = 8.1 Hz, 1H), 7.99 (d, *J* = 8.3 Hz, 2H). HRMS (ESI<sup>+</sup>) calcd for C<sub>17</sub>H<sub>13</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 314.0579; found, 314.0575.

**2-(4-Chlorophenyl)-3-hydroxy-8-isopropylquinoline-4-carboxylic Acid (30)**: Recrystallized from chloroform; fine, pale yellow crystals; yield 15%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.33 (d, *J* = 7.1 Hz, 6H), 4.28 (sep, 1H), 7.50 (d, *J* = 6.3 Hz, 1H), 7.59 (m, 3H), 8.14 (d, *J* = 8.6 Hz, 2H), 8.33 (d, *J* = 8.8 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>19</sub>H<sub>17</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 342.0892; found, 342.0887.

**2-(4-Chlorophenyl)-3-hydroxy-7-isopropylquinoline-4-carboxylic Acid (32)**: The inseparable mixture of 6-isopropylisatin and 5-isopropylisatin was used, but the 4-isopropylquinoline could not be isolated. The 7-isopropyl isomer was purified by recrystallization from chloroform/ethanol: orange powder, yield 29%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.31 (d, *J* = 6.8 Hz, 6H), 3.08 (sept, 1H), 7.59 (m, 3H), 7.84 (d, *J* = 2.0 Hz, 1H), 8.09 (dt, *J* = 9.1, 2.5, 2.3 Hz, 2H), 8.67 (d, *J* = 8.6 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>19</sub>H<sub>17</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 342.0892; found, 342.0888.

**2-(4-Chlorophenyl)-3-hydroxy-6-isopropylquinoline-4-carboxylic Acid (34)**: Purified by method B, yellow solid, yield 62.4%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.29 (d, *J* = 7.07 Hz, 6H), 2.94–3.12 (m, 1H), 7.41 (dd, *J* = 8.72, 1.64 Hz, 1H), 7.49–7.62 (m, 2H), 7.85 (d, *J* = 8.59 Hz, 1H), 8.11–8.27 (m, 2H), 8.92 (br s, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>19</sub>H<sub>16</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 342.0892; found, 342.0889.

**2-(4-Chlorophenyl)-3-hydroxy-8-phenylquinoline-4-carboxylic Acid (36)**: Recrystallized from chloroform/ethanol, orange powder, yield 17%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.40 (t, *J* = 7.3 Hz, 1H), 7.50 (m, 4H), 7.63 (dd, *J* = 7.2, 1.4 Hz, 1H), 7.70 (m, 3H), 8.05 (d, *J* = 8.8 Hz, 2H), 8.53 (dd, *J* = 8.5, 1.4 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>22</sub>H<sub>15</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 376.07348; found, 376.07342. Anal. (C<sub>22</sub>H<sub>14</sub>ClNO<sub>3</sub>) C, H, N.

**2-(4-Chlorophenyl)-3-hydroxy-7-phenylquinoline-4-carboxylic Acid (38)**: The inseparable mixture of 6-phenylisatin and 5-phenylisatin was used, but the 4-phenylquinoline could not be isolated. Purified by method B, switching to 70:10:5:5 ethyl acetate/acetonitrile/methanol/water (+0.5% triethylamine) once product began to elute. The pure 7-phenyl isomer was isolated as the 2:1 carboxylate/triethylammonium salt, a fluffy bright yellow solid, yield 8.1%. A portion was converted to the free acid, as described above; bright orange powder. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.42 (t, *J* = 7.3 Hz, 1H), 7.53 (t, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 8.3 Hz, 2H), 7.86 (d, *J* = 7.3 Hz, 2H), 8.00 (dd, *J* = 8.8, 1.8 Hz, 1H), 8.13 (d, *J* = 8.6 Hz, 2H), 8.28 (s, 1H), 8.85 (d, *J* = 8.8 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>22</sub>H<sub>15</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 376.0735; found, 376.0733.

**Triethylammonium 2-(4-Chlorophenyl)-3-hydroxy-6-phenylquinoline-4-carboxylic Acid (39)**: Purified by method B without



conversion back to the free acid; pale yellow solid, yield 50%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.17 (t, *J* = 7.07 Hz, 9H), 3.09 (d, *J* = 6.82 Hz, 6H), 7.30–7.44 (m, 1H), 7.45–7.58 (m, 4H), 7.65 (dd, *J* = 8.59, 2.27 Hz, 1H), 7.76 (dd, *J* = 8.21, 1.14 Hz, 2H), 7.90 (d, *J* = 8.59 Hz, 1H), 8.24–8.44 (m, 2H), 9.78 (d, *J* = 1.77 Hz, 1H). Anal. (C<sub>28</sub>H<sub>29</sub>ClN<sub>2</sub>O<sub>3</sub>·0.9H<sub>2</sub>O) C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>22</sub>H<sub>14</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 376.0735; found, 376.0733.

**8-Chloro-2-(4-chlorophenyl)-3-hydroxyquinoline-4-carboxylic Acid (41):** Recrystallized from ethanol/benzene, pale yellow powder, yield 16%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.57 (dd, *J* = 8.6, 7.6 Hz, 1H), 7.61 (dt, *J* = 9.1, 2.5, 2.3 Hz, 2H), 7.77 (dd, *J* = 7.6, 1.3 Hz, 1H), 8.20 (dt, *J* = 8.9, 2.7, 2.3 Hz, 2H), 8.59 (dd, *J* = 8.6, 1.3 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>16</sub>H<sub>10</sub>Cl<sub>2</sub>NO<sub>3</sub> (MH<sup>+</sup>), 334.0032; found, 334.0027.

**7-Chloro-2-(4-chlorophenyl)-3-hydroxyquinoline-4-carboxylic Acid (43):** Purified by recrystallization from acetonitrile/ethanol/benzene, fluffly bright orange crystals, yield 47%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.59 (dt, *J* = 9.1, 2.5, 2.3 Hz, 2H), 7.65 (dd, *J* = 9.4, 2.3 Hz, 1H), 8.03 (d, *J* = 2.3 Hz, 1H), 8.09 (d, *J* = 8.6 Hz, 2H), 8.73 (d, *J* = 9.4 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>16</sub>H<sub>10</sub>Cl<sub>2</sub>NO<sub>3</sub> (MH<sup>+</sup>), 334.0032; found, 334.0029.

**6-Chloro-2-(4-chlorophenyl)-3-hydroxyquinoline-4-carboxylic Acid (45):** Purified by method B, yellow solid, yield 47%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.54 (dd, *J* = 8.84, 2.27 Hz, 1H), 7.56–7.62 (m, 2H), 7.97 (d, *J* = 8.84 Hz, 1H), 8.09–8.20 (m, 2H), 8.97 (d, *J* = 2.53 Hz, 1H). Anal. C<sub>16</sub>H<sub>9</sub>Cl<sub>2</sub>NO<sub>3</sub>; C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>16</sub>H<sub>9</sub>Cl<sub>2</sub>NO<sub>3</sub> (MH<sup>+</sup>), 334.0032; found, 334.0031.

**2-(4-Chlorophenyl)-3-hydroxy-8-(trifluoromethoxy)quinoline-4-carboxylic Acid (47):** Purified by method B, off-white powder, yield 17%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.63 (m, 4H), 8.15 (d, *J* = 8.6 Hz, 2H), 8.69 (dd, *J* = 8.6, 1.3 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>17</sub>H<sub>10</sub>ClF<sub>3</sub>NO<sub>4</sub> (MH<sup>+</sup>), 384.0245; found, 384.0251. Anal. (C<sub>17</sub>H<sub>9</sub>ClF<sub>3</sub>NO<sub>4</sub>) C, H, N.

**2-(4-Chlorophenyl)-3-hydroxy-6-(trifluoromethoxy)quinoline-4-carboxylic Acid (49):** Purified by method B, yellow solid, yield 60%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.48 (dd, *J* = 9.09, 2.53 Hz, 1H), 7.54–7.63 (m, 2H), 8.07 (d, *J* = 9.09 Hz, 1H), 8.16 (d, *J* = 8.59 Hz, 2H), 8.95 (d, *J* = 1.01 Hz, 1H). Anal. (C<sub>17</sub>H<sub>9</sub>ClF<sub>3</sub>NO<sub>4</sub>·0.2H<sub>2</sub>O) C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>17</sub>H<sub>9</sub>ClF<sub>3</sub>NO<sub>4</sub> (MH<sup>+</sup>), 384.0245; found, 384.0241.

**Triethylammonium 2-(4-Chlorophenyl)-3-hydroxy-6-(trifluoromethyl)quinoline-4-carboxylate (52):** Purified by method B without conversion back to the free acid; yellow solid, yield 48.2%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.17 (t, *J* = 7.33 Hz, 9H), 3.09 (q, *J* = 7.33 Hz, 6H), 7.39–7.68 (m, 2H), 8.01 (d, *J* = 8.59 Hz, 1H), 8.34 (d, *J* = 8.34 Hz, 2H), 8.89 (br s, 1H), 9.89 (s, 1H). Anal. C<sub>23</sub>H<sub>24</sub>ClF<sub>3</sub>N<sub>2</sub>O<sub>3</sub>; C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>17</sub>H<sub>9</sub>ClF<sub>3</sub>NO<sub>3</sub> (MH<sup>+</sup>), 368.0296; found, 368.0295.

**8-Bromo-2-(4-chlorophenyl)-3-hydroxyquinoline-4-carboxylic Acid (54):** Recrystallized from chloroform/ethanol, yellow powder, yield 18%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.49 (t, 1H), 7.62 (d, *J* = 8.6 Hz, 2H), 7.95 (d, *J* = 7.6 Hz, 1H), 8.24 (d, *J* = 8.6 Hz, 2H), 8.65 (d, *J* = 8.6 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>16</sub>H<sub>10</sub>BrClNO<sub>3</sub> (MH<sup>+</sup>), 377.9527; found, 377.9522.

**6-Bromo-2-(4-chlorophenyl)-3-hydroxyquinoline-4-carboxylic Acid (56):** Purified by method B, yellow solid, yield 49%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.52–7.59 (m, 2H), 7.61 (dd, *J* = 8.84, 2.27 Hz, 1H), 7.87 (d, *J* = 9.09 Hz, 1H), 8.12–8.23 (m, 2H), 9.23 (d, *J* = 2.27 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>16</sub>H<sub>9</sub>BrClNO<sub>3</sub> (MH<sup>+</sup>), 377.9527; found, 377.9524.

**2-(4-Chlorophenyl)-3-hydroxyquinoline-4,8-dicarboxylic Acid (58):** Purified by method B, yellow powder, yield 30%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.49 (d, *J* = 8.59 Hz, 2H), 8.58 (dd, *J* = 8.46, 7.45 Hz, 1H), 8.90 (d, *J* = 8.59 Hz, 2H), 9.09 (dd, *J* = 7.33, 1.26 Hz, 1H), 9.88 (dd, *J* = 8.59, 1.26 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>17</sub>H<sub>10</sub>ClNO<sub>5</sub> (MH<sup>+</sup>), 344.0321; found, 344.032.

**2-(4-Chlorophenyl)-3-hydroxy-8-methoxyquinoline-4-carboxylic Acid (60):** Purified by method B, orange yellow crystals, yield 62.2%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 3.96 (s, 3H), 7.07 (dd, *J* = 8.08, 1.01 Hz, 1H), 7.50–7.61 (m, 3H), 7.97–8.12 (m, 3H).

Anal. (C<sub>17</sub>H<sub>12</sub>ClNO<sub>4</sub>) C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>17</sub>H<sub>12</sub>ClNO<sub>4</sub> (MH<sup>+</sup>), 330.05277; found, 330.05193.

**2-(4-Chlorophenyl)-8-ethoxy-3-hydroxyquinoline-4-carboxylic Acid (62):** Purified by method B, orange yellow crystals, yield 77.1%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.44 (t, *J* = 6.95 Hz, 3H), 4.23 (q, *J* = 7.07 Hz, 2H), 6.99–7.06 (m, 1H), 7.42–7.53 (m, 1H), 7.54–7.63 (m, 2H), 8.04–8.13 (m, 2H), 8.20 (d, *J* = 8.08 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>18</sub>H<sub>14</sub>ClNO<sub>4</sub> (MH<sup>+</sup>), 344.0676; found, 344.0684.

**8-(4-Carboxyphenyl)-2-(4-chlorophenyl)-3-hydroxyquinoline-4-carboxylic Acid (64):** Purified by method B (60:10:10:10 ethyl acetate/acetonitrile/methanol/water [+5% *N,N*-diisopropylethylamine]), brownish-orange powder, yield 2.4%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.54 (d, *J* = 8.3 Hz, 2H), 7.68 (m, 2H), 7.82 (d, *J* = 8.1 Hz, 2H), 8.05 (m, 4H), 8.65 (d, *J* = 8.6 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>23</sub>H<sub>15</sub>ClNO<sub>5</sub> (MH<sup>+</sup>), 420.0634; found, 420.0632.

**2-(4-Chlorophenyl)-3-hydroxy-8-(4-hydroxymethylphenyl)quinoline-4-carboxylic Acid (66):** Purified by method B, bright yellow powder, yield 23%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 4.58 (s, 2H), 7.41 (d, *J* = 7.8 Hz, 2H), 7.52 (m, 2H), 7.63 (m, 4H), 8.11 (d, *J* = 8.6 Hz, 2H), 8.82 (d, *J* = 8.3 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>23</sub>H<sub>17</sub>ClNO<sub>4</sub> (MH<sup>+</sup>), 406.0841; found, 406.0847.

**2-(4-Chlorophenyl)-3-hydroxy-8-(4-methanesulfonylphenyl)quinoline-4-carboxylic Acid (68):** Recrystallized from chloroform/ethanol, off-white powder, yield 13%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 3.29 (s, 3H), 7.68 (m, 2H), 7.99 (dd, 4H), 8.06 (m, 6H), 8.74 (dd, *J* = 8.5, 1.4 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>23</sub>H<sub>17</sub>ClNO<sub>5</sub>S (MH<sup>+</sup>), 454.0511; found, 454.0515.

**2-(4-Chlorophenyl)-8-(dimethylaminophenyl)-3-hydroxyquinoline-4-carboxylic Acid (70):** The crude product was taken up in the method B solvent system, and additional triethylamine was added dropwise until all the solid had dissolved. Almost at once, pure product precipitated out of solution as the triethylammonium salt (21% yield). A portion of this was converted back to the free acid, as described above: beige powder. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 3.01 (s, 6H), 6.94 (d, *J* = 7.8 Hz, 2H), 7.54 (m, 3H), 7.63 (m, 3H), 8.11 (d, *J* = 8.6 Hz, 2H), 8.55 (d, *J* = 8.1 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>24</sub>H<sub>20</sub>ClN<sub>2</sub>O<sub>3</sub> (MH<sup>+</sup>), 419.1157; found, 419.1166.

**Triethylammonium 2-(4-Chlorophenyl)-3-hydroxy-7-methoxyquinoline-4-carboxylate (72):** purified by method B without conversion back to the free acid; lyophilization gave a fluffly bright yellow solid, yield 22%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.17 (t, *J* = 7.3 Hz, 9H), 3.10 (m, 6H), 3.85 (s, 3H), 7.10 (dd, *J* = 9.5, 2.9 Hz, 1H), 7.26 (d, *J* = 2.8 Hz, 1H), 7.51 (dt, *J* = 9.1, 2.5, 2.3 Hz, 2H), 8.31 (dt, *J* = 9.1, 2.5, 2.3 Hz, 2H), 8.97 (br s, 1H), 9.35 (d, *J* = 9.4 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>23</sub>H<sub>27</sub>ClN<sub>2</sub>O<sub>4</sub> (MH<sup>+</sup>), 330.05277; found, 330.05255. Anal. (C<sub>23</sub>H<sub>27</sub>ClN<sub>2</sub>O<sub>4</sub>·0.25H<sub>2</sub>O) C, H, N.

**2-(4-Chlorophenyl)-3-hydroxy-6-methoxyquinoline-4-carboxylic Acid (74):** Purified by method B, yellow solid, yield 73.5%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 3.90 (s, 3H), 7.27 (dd, *J* = 9.09, 2.78 Hz, 1H), 7.55–7.63 (m, 2H), 7.94 (d, *J* = 9.09 Hz, 1H), 8.00–8.10 (m, 2H), 8.25 (s, 1H). Anal. (C<sub>17</sub>H<sub>12</sub>ClNO<sub>4</sub>·0.85-(C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>N) C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>17</sub>H<sub>12</sub>ClNO<sub>4</sub> (MH<sup>+</sup>), 330.0528; found, 330.0528.

**6-Amino-2-(4-chlorophenyl)-3-hydroxyquinoline-4-carboxylic Acid (77):** Purified by method B, yellow solid, yield 50%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub> + D<sub>2</sub>O): δ 7.13 (dd, *J* = 9.09, 2.27 Hz, 1H), 7.53–7.63 (m, 2H), 7.82 (d, *J* = 8.84 Hz, 1H), 7.97–8.04 (m, 2H), 8.35 (d, *J* = 2.53 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>16</sub>H<sub>11</sub>ClN<sub>3</sub>O<sub>3</sub> (MH<sup>+</sup>), 315.0531; found, 315.053.

**2-(4-Chlorophenyl)-3-hydroxyquinoline-4,6-dicarboxylic Acid (79):** Purified by method B, yellow solid, yield 50%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.50–7.66 (m, 2H), 7.85–8.07 (m, 2H), 8.10–8.29 (m, 2H), 9.69 (s, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>17</sub>H<sub>10</sub>ClNO<sub>5</sub> (MH<sup>+</sup>), 344.0321; found, 344.0319.

**2-(4-Chlorophenyl)-8-ethyl-3-hydroxyquinoline-4-carboxylic Acid (81):** Purified by method B, off-white powder, yield 26%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.31 (t, *J* = 7.5 Hz, 3H), 3.23 (q, *J* = 7.6 Hz, 2H), 7.46 (d, *J* = 6.3 Hz, 1H), 7.54 (m, 1H), 7.60



(d,  $J = 8.6$  Hz, 2H), 8.15 (d,  $J = 8.6$  Hz, 2H), 8.38 (d,  $J = 8.6$  Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>18</sub>H<sub>15</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 328.0735; found, 328.0740. Anal. (C<sub>18</sub>H<sub>14</sub>ClNO<sub>3</sub>) C, H, N.

**8-sec-Butyl-2-(4-chlorophenyl)-3-hydroxyquinoline-4-carboxylic Acid (83):** Purified by flash chromatography using the method B solvent system, then converted back to the free acid and recrystallized from chloroform; pale yellow solid, yield 5.7%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  0.80 (t,  $J = 7.3$  Hz, 3H), 1.31 (d,  $J = 6.8$  Hz, 3H), 1.74 (m, 2H), 4.11 (m, 1H), 7.47 (d,  $J = 6.3$  Hz, 1H), 7.60 (m, 3H), 8.13 (d,  $J = 8.6$  Hz, 2H), 8.31 (d,  $J = 8.6$  Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>20</sub>H<sub>19</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 356.1048; found, 356.1044.

**8-tert-Butyl-2-(4-chlorophenyl)-3-hydroxyquinoline-4-carboxylic Acid (85):** Purified by flash chromatography using the method B solvent system, then converted back to the free acid and recrystallized from chloroform; fluffly pale yellow crystalline material, yield 2.9%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  1.62 (s, 9H), 7.53 (m, 2H), 7.62 (dt, 2H), 8.11 (dt,  $J = 8.9, 2.5, 2.2$  Hz, 2H), 8.24 (dd,  $J = 6.7, 3.2$  Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>20</sub>H<sub>19</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 356.1048; found, 356.1045.

**2-(4-Chlorophenyl)-3-hydroxy-8-(thien-3-yl)quinoline-4-carboxylic Acid (87):** Recrystallized from chloroform/ethanol, bright yellow crystalline material, yield 20%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  7.57 (dt,  $J = 8.9, 2.7, 2.3$  Hz, 2H), 7.61 (m, 1H), 7.65 (d,  $J = 1.5$  Hz, 1H), 7.71 (dd,  $J = 5.1, 1.3$  Hz, 1H), 7.79 (dd, 1H), 8.03 (dd,  $J = 3.0, 1.3$  Hz, 1H), 8.10 (dt,  $J = 9.1, 2.5, 2.3$  Hz, 2H), 8.51 (dd,  $J = 8.5, 1.4$  Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>20</sub>H<sub>13</sub>ClNO<sub>3</sub>S (MH<sup>+</sup>), 382.02992; found, 382.02935.

**2-(4-Chlorophenyl)-8-fluoro-3-hydroxyquinoline-4-carboxylic Acid (89):** Recrystallized from chloroform/ethanol; pale yellow crystalline material, yield 15%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  7.41 (m, 1H), 7.59 (m, 3H), 8.11 (d,  $J = 8.6$  Hz, 2H), 8.42 (d,  $J = 8.6$  Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>16</sub>H<sub>10</sub>ClFNO<sub>3</sub> (MH<sup>+</sup>), 318.03278; found, 318.03327. Anal. C<sub>16</sub>H<sub>9</sub>ClFNO<sub>3</sub>·0.25H<sub>2</sub>O, C, H, N.

**2-(4-Chlorophenyl)-3-hydroxy-7,8-dimethylquinoline-4-carboxylic Acid (91):** Purified by method B, pale yellow solid, yield 60.4%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  2.43 (s, 1H), 2.69 (s, 3H), 7.45 (d,  $J = 9.09$  Hz, 1H), 7.52–7.67 (m, 2H), 8.09–8.18 (m, 2H), 8.26 (d,  $J = 8.84$  Hz, 1H). Anal. C<sub>18</sub>H<sub>14</sub>ClNO<sub>3</sub> C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>18</sub>H<sub>14</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 328.07334; found, 328.0735.

**2-(4-Chlorophenyl)-3-hydroxy-6,8-dimethylquinoline-4-carboxylic Acid (93):** Purified by method B, yellow solid, yield 50%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  2.46 (s, 3H), 2.69 (s, 3H), 7.32 (s, 1H), 7.50–7.67 (m, 2H), 8.04–8.23 (m, 3H). HRMS (ESI<sup>+</sup>) calcd for C<sub>18</sub>H<sub>14</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 328.07288; found, 328.0735.

**6-Bromo-2-(4-chlorophenyl)-3-hydroxy-8-methylquinoline-4-carboxylic Acid (95):** Purified by flash chromatography over silica gel (70:2.5 ethyl acetate/acetonitrile [+0.5% triethylamine]) and converted back to the free acid as described above, then recrystallized from chloroform/ethanol; fluffly pale yellow solid, yield 41%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  2.71 (s, 3H), 7.58 (m, 3H), 8.20 (d,  $J = 8.6$  Hz, 2H), 8.86 (d,  $J = 2.0$  Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>17</sub>H<sub>12</sub>BrClNO<sub>3</sub> (MH<sup>+</sup>), 391.9684; found, 391.9680.

**2-(4-Chlorophenyl)-3-hydroxy-8-methyl-6-(trifluoromethoxy)quinoline-4-carboxylic Acid (97):** Purified by method B, pale yellow solid, yield 14%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  2.75 (s, 3H), 7.38 (br s, 1H), 7.53–7.61 (m, 2H), 8.25 (d,  $J = 8.59$  Hz, 2H), 8.79 (br s, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>18</sub>H<sub>11</sub>ClF<sub>3</sub>NO<sub>4</sub> (MH<sup>+</sup>), 398.0402; found, 398.0401.

**2-(4-Chlorophenyl)-3-hydroxy-8-isopropylcarbamoylquinoline-4-carboxylic Acid (99):** Purified by method B, pale yellow powder, yield 19%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  1.21 (d,  $J = 6.6$  Hz, 6H), 4.16 (m, 1H), 7.65 (d,  $J = 8.3$  Hz, 2H), 7.70 (t, 1H), 8.08 (d,  $J = 8.3$  Hz, 2H), 8.27 (d,  $J = 6.6$  Hz, 1H), 8.68 (d,  $J = 8.3$  Hz, 1H), 10.27 (d,  $J = 7.3$  Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>20</sub>H<sub>18</sub>ClN<sub>2</sub>O<sub>4</sub> (MH<sup>+</sup>), 385.0950; found, 385.0946.

**8-tert-Butylcarbamoyl-2-(4-chlorophenyl)-3-hydroxyquinoline-4-carboxylic Acid (101):** Recrystallized from acetonitrile/ethanol, pale yellow solid, yield 27%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$

1.40 (s, 9H), 7.63 (d,  $J = 8.6$  Hz, 2H), 7.71 (dd,  $J = 8.6, 7.3$  Hz, 1H), 8.03 (d,  $J = 8.6$  Hz, 2H), 8.33 (d,  $J = 7.3$  Hz, 1H), 8.63 (d,  $J = 9.9$  Hz, 1H), 10.37 (s, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>21</sub>H<sub>20</sub>ClN<sub>2</sub>O<sub>4</sub> (MH<sup>+</sup>), 399.1106; found, 399.1102.

**Triethylammonium 2-(4-Chlorophenyl)-3-hydroxy-8-phenylcarbamoylquinoline-4-carboxylate (103):** Purified by preparative HPLC (water/acetonitrile, triethylamine modifier) and lyophilized, fluffly yellow solid, yield 4.3%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  1.17 (t,  $J = 7.3$  Hz, 9H), 3.10 (m, 6H), 7.10 (t,  $J = 7.5$  Hz, 1H), 7.38 (m, 2H), 7.61 (m, 3H), 7.73 (dd,  $J = 8.7, 1.1$  Hz, 2H), 8.21 (dd,  $J = 7.3, 1.5$  Hz, 1H), 8.25 (dt,  $J = 8.8, 2.5, 2.3$  Hz, 2H), 8.89 (br s, 1H), 9.74 (dd,  $J = 8.6, 1.5$  Hz, 1H), 12.96 (s, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>23</sub>H<sub>16</sub>ClN<sub>2</sub>O<sub>4</sub> (MH<sup>+</sup>), 419.0793; found, 419.0790.

**8-Benzylcarbamoyl-2-(4-chlorophenyl)-3-hydroxyquinoline-4-carboxylic Acid (105):** Recrystallization from ethanol/benzene gave a 2:1 complex with ethanol, bright yellow powder, yield 19%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  1.06 (t,  $J = 7.1$  Hz, 1.5H), 3.44 (q,  $J = 7.2$  Hz, 2H), 4.61 (d,  $J = 5.6$  Hz, 2H), 7.29 (m, 3H), 7.39 (m, 2H), 7.42 (d,  $J = 8.6$  Hz, 2H), 7.72 (dd,  $J = 8.6, 7.3$  Hz, 3H), 7.87 (m, 2H), 8.26 (dd,  $J = 7.3, 1.5$  Hz, 1H), 8.68 (dd,  $J = 8.6, 1.3$  Hz, 1H), 10.48 (t,  $J = 5.8$  Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>24</sub>H<sub>18</sub>ClN<sub>2</sub>O<sub>4</sub> (MH<sup>+</sup>), 433.0950; found, 433.0953.

**2-(4-Chlorophenyl)-3-hydroxy-6-(isopropylcarbamoyl)quinoline-4-carboxylic Acid (107):** Purified by method B, yellow solid, yield 25%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  1.21 (d,  $J = 6.57$  Hz, 6H), 4.06–4.23 (m, 1H), 7.51–7.65 (m, 2H), 7.85–7.95 (m, 1H), 7.96–8.06 (m, 1H), 8.16 (d,  $J = 8.59$  Hz, 2H), 8.41 (d,  $J = 7.33$  Hz, 1H), 9.26 (s, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>20</sub>H<sub>17</sub>ClN<sub>2</sub>O<sub>4</sub> (MH<sup>+</sup>), 385.095; found, 385.0949.

**6-(tert-Butylcarbamoyl)-2-(4-chlorophenyl)-3-hydroxyquinoline-4-carboxylic Acid (109):** Purified by method B, yellow solid, yield 19%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  1.38–1.46 (s, 9H), 7.51–7.63 (m, 2H), 7.78–7.87 (m, 1H), 7.95 (d,  $J = 9.09$  Hz, 2H), 8.13–8.27 (m, 2H), 9.30 (s, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>21</sub>H<sub>19</sub>ClN<sub>2</sub>O<sub>4</sub> (MH<sup>+</sup>), 399.1106; found, 399.1106.

**2-(4-Chlorophenyl)-3-hydroxy-6-(phenylcarbamoyl)quinoline-4-carboxylic Acid (111):** Purified by method B, yellow solid, yield 21%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  7.12 (t,  $J = 7.33$  Hz, 1H), 7.33–7.42 (m, 2H), 7.56–7.62 (m, 2H), 7.82 (d,  $J = 7.58$  Hz, 2H), 7.95–8.02 (m, 1H), 8.04–8.10 (m, 1H), 8.15–8.23 (m, 2H), 9.44 (d,  $J = 1.26$  Hz, 1H), 10.49 (s, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>23</sub>H<sub>15</sub>ClN<sub>2</sub>O<sub>4</sub> (MH<sup>+</sup>), 419.0793; found, 419.079.

**6-(Benzylcarbamoyl)-2-(4-chlorophenyl)-3-hydroxyquinoline-4-carboxylic Acid (113):** Purified by prep HPLC, pale yellow solid, yield 7%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  4.53 (d,  $J = 6.06$  Hz, 2H), 7.21–7.29 (m, 1H), 7.30–7.40 (m, 4H), 7.59 (d,  $J = 8.59$  Hz, 2H), 7.94–8.01 (m, 1H), 8.01–8.06 (m, 1H), 8.15 (d,  $J = 8.34$  Hz, 2H), 9.24 (t,  $J = 5.94$  Hz, 1H), 9.31 (br s, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>24</sub>H<sub>17</sub>ClN<sub>2</sub>O<sub>4</sub> (MH<sup>+</sup>), 433.095; found, 433.0951.

**Triethylammonium 3-Hydroxy-7,8-dimethyl-2-phenylquinoline-4-carboxylic Acid (117):** Purified by method B without conversion back to the free acid, tan powder, yield 71.5%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  1.18 (t,  $J = 7.20$  Hz, 9H), 2.39 (s, 3H), 2.68 (s, 3H), 3.09 (q,  $J = 7.33$  Hz, 6H), 7.25 (d,  $J = 8.84$  Hz, 1H), 7.32–7.57 (m, 3H), 8.15–8.31 (m, 2H), 9.21 (d,  $J = 8.84$  Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>18</sub>H<sub>15</sub>NO<sub>3</sub> (MH<sup>+</sup>), 294.1125; found, 294.1123.

**2-(4-Fluorophenyl)-3-hydroxy-7,8-dimethylquinoline-4-carboxylic Acid (119):** Purified by method B, yellow-orange solid, yield 73.8%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  2.44 (s, 3H), 2.70 (s, 3H), 7.36 (t,  $J = 9.09$  Hz, 2H), 7.44 (d,  $J = 8.84$  Hz, 1H), 8.17 (dd,  $J = 8.97, 5.68$  Hz, 2H), 8.27 (d,  $J = 8.84$  Hz, 1H). Anal. C<sub>18</sub>H<sub>14</sub>FNO<sub>3</sub> C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>18</sub>H<sub>14</sub>FNO<sub>3</sub> (MH<sup>+</sup>), 312.1031; found, 312.1028.

**Triethylammonium 3-Hydroxy-2-(4-isopropylphenyl)-7,8-dimethylquinoline-4-carboxylic Acid (122):** Purified by method B without conversion back to the free acid, yellow brown foam, yield 68.5%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  1.16 (t,  $J = 7.33$  Hz, 9H), 1.27 (d,  $J = 6.82$  Hz, 6H), 2.38 (s, 3H), 2.67 (s, 3H), 2.88–3.01 (m, 1H), 3.07 (m, 6H), 7.21 (d,  $J = 8.84$  Hz, 1H), 7.33 (d,  $J = 8.34$  Hz, 2H), 8.15–8.22 (m, 2H), 9.20 (d,  $J = 8.84$  Hz,

1H). Anal. (C<sub>21</sub>H<sub>21</sub>NO<sub>3</sub>·0.2(C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>N·H<sub>2</sub>O) C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>21</sub>H<sub>21</sub>NO<sub>3</sub> (MH<sup>+</sup>), 336.1594; found, 336.1592.

**Triethylammonium 3-Hydroxy-7,8-dimethyl-2-(4-(trifluoromethyl)phenyl)quinoline-4-carboxylic Acid (124):** Purified by method B, pale yellow solid, yield 13%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.17 (t, *J* = 7.33 Hz, 9H), 2.38 (s, 3H), 2.68 (s, 3H), 3.09 (q, *J* = 7.33 Hz, 6H), 7.26 (d, *J* = 8.84 Hz, 1H), 7.83 (d, *J* = 8.08 Hz, 2H), 8.54 (d, *J* = 8.08 Hz, 2H), 9.22 (d, *J* = 9.09 Hz, 1H). Anal. C<sub>25</sub>H<sub>29</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub> C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>19</sub>H<sub>14</sub>F<sub>3</sub>NO<sub>3</sub> (MH<sup>+</sup>), 362.0999; found, 362.0999.

**Triethylammonium 3-Hydroxy-7,8-dimethyl-2-(4-(trifluoromethoxy)phenyl)quinoline-4-carboxylic Acid (126):** Purified by method B without conversion back to the free acid, yellow-brown foam, yield 71.2%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.16 (t, *J* = 7.20 Hz, 9H), 2.38 (s, 3H), 2.67 (s, 3H), 3.06 (q, *J* = 7.07 Hz, 6H), 7.24 (d, *J* = 8.84 Hz, 1H), 7.45 (d, *J* = 8.08 Hz, 2H), 8.37–8.54 (m, 2H), 9.21 (d, *J* = 8.84 Hz, 1H). Anal. (C<sub>19</sub>H<sub>14</sub>F<sub>3</sub>NO<sub>4</sub>·0.6(C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>N·H<sub>2</sub>O) C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>19</sub>H<sub>14</sub>F<sub>3</sub>NO<sub>4</sub> (MH<sup>+</sup>), 378.0948; found, 378.0945.

**2-(4-(Diethylamino)phenyl)-3-hydroxy-7,8-dimethylquinoline-4-carboxylic Acid (128):** Purified by method B, red crystals, yield 67.9%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.13 (t, *J* = 7.07 Hz, 6H), 2.43 (s, 3H), 2.70 (s, 3H), 3.36–3.73 (m, 4H), 7.42 (d, *J* = 8.59 Hz, 2H), 8.20 (d, *J* = 8.08 Hz, 3H), 8.28 (d, *J* = 8.59 Hz, 1H). Anal. (C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub>·HCO<sub>2</sub>H·H<sub>2</sub>O) C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub> (MH<sup>+</sup>), 365.186; found, 365.1859.

**3-Hydroxy-7,8-dimethyl-2-*p*-tolylquinoline-4-carboxylic Acid (130):** Purified by method B, orange solid, yield 71.3%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.40 (s, 3H), 2.43 (s, 3H), 2.69 (s, 3H), 7.33 (d, *J* = 7.83 Hz, 2H), 7.42 (t, *J* = 8.84 Hz, 1H), 8.01 (d, *J* = 8.34 Hz, 2H), 8.28 (d, *J* = 8.59 Hz, 1H). Anal. C<sub>19</sub>H<sub>17</sub>NO<sub>3</sub> C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>19</sub>H<sub>17</sub>NO<sub>3</sub> (MH<sup>+</sup>), 308.1281; found, 308.128.

**2-(Biphenyl-4-yl)-3-hydroxy-7,8-dimethylquinoline-4-carboxylic Acid (132):** Purified by method B, yellow-tan crystals, yield 72%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.39 (s, 3H), 2.70 (s, 3H), 7.25 (d, *J* = 8.84 Hz, 1H), 7.39 (t, *J* = 7.33 Hz, 1H), 7.51 (t, *J* = 7.58 Hz, 2H), 7.69–7.81 (m, 4H), 8.44 (d, *J* = 8.59 Hz, 2H), 9.22 (d, *J* = 8.84 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>24</sub>H<sub>19</sub>NO<sub>3</sub> (MH<sup>+</sup>), 370.1438; found, 370.1435.

**2-(4-Carboxyphenyl)-3-hydroxy-7,8-dimethylquinoline-4-carboxylic Acid (134):** Purified by method B, pale yellow solid, yield 51.4%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.44 (s, 3H), 2.70 (s, 3H), 7.46 (d, *J* = 8.84 Hz, 1H), 8.03–8.15 (m, 2H), 8.16–8.28 (m, 2H), 8.34 (d, *J* = 8.84 Hz, 1H). Anal. (C<sub>19</sub>H<sub>15</sub>NO<sub>5</sub>·0.6H<sub>2</sub>O) C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>19</sub>H<sub>15</sub>NO<sub>5</sub> (MH<sup>+</sup>), 338.1023; found, 338.1024.

**3-Hydroxy-2-(4-hydroxyphenyl)-7,8-dimethylquinoline-4-carboxylic Acid (136):** Purified by method B, deep yellow crystals, yield 61.1%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.43 (s, 3H), 2.69 (s, 3H), 6.85–6.95 (m, 2H), 7.40 (d, *J* = 9.09 Hz, 1H), 7.99–8.07 (m, 2H), 8.27 (d, *J* = 8.59 Hz, 1H). Anal. C<sub>18</sub>H<sub>15</sub>NO<sub>4</sub> C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>18</sub>H<sub>15</sub>NO<sub>4</sub> (MH<sup>+</sup>), 310.1074; found, 310.1071.

**Triethylammonium 3-Hydroxy-2-(4-methoxyphenyl)-7,8-dimethylquinoline-4-carboxylic Acid (138):** Purified by method B without conversion back to the free acid, tan powder, yield 86.2%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.17 (t, *J* = 7.06 Hz, 9H), 2.37 (s, 3H), 2.67 (s, 3H), 3.09 (m, 6H), 3.83 (s, 3H), 6.96–7.10 (m, 2H), 7.20 (d, *J* = 8.84 Hz, 1H), 8.24–8.43 (m, 2H), 9.19 (d, *J* = 8.59 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>19</sub>H<sub>17</sub>NO<sub>4</sub> (MH<sup>+</sup>), 324.1231; found, 324.1229.

**3-Hydroxy-7,8-dimethyl-2-(4-morpholinophenyl)quinoline-4-carboxylic Acid (140):** Purified by method B, deep red powder, yield 52%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.37 (s, 3H), 2.67 (s, 3H), 3.15–3.24 (m, 4H), 3.72–3.83 (m, 4H), 6.96–7.06 (m, 2H), 7.17 (d, *J* = 8.84 Hz, 1H), 8.27–8.37 (m, 2H), 9.18 (d, *J* = 8.84 Hz, 1H). Anal. (C<sub>22</sub>H<sub>22</sub>N<sub>2</sub>O<sub>4</sub>·0.65(C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>N·CH<sub>3</sub>OH) C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>22</sub>H<sub>22</sub>N<sub>2</sub>O<sub>4</sub> (MH<sup>+</sup>), 379.1653; found, 379.1649.

**3-Hydroxy-7,8-dimethyl-2-(3-(trifluoromethoxy)phenyl)quinoline-4-carboxylic Acid (142):** Purified by method B, dark yellow solid, yield 49%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.44 (s, 3H), 2.70 (s, 3H), 7.37–7.56 (m, 2H), 7.68 (t, *J* = 8.08 Hz, 1H), 8.07 (d, *J* = 1.01 Hz, 1H), 8.17 (dd, *J* = 7.83, 1.52 Hz, 1H), 8.24 (d, *J* = 8.84 Hz, 1H). Anal. C<sub>19</sub>H<sub>14</sub>F<sub>3</sub>NO<sub>4</sub> C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>19</sub>H<sub>14</sub>F<sub>3</sub>NO<sub>4</sub> (MH<sup>+</sup>), 378.09386; found, 378.09477.

**2-(3-Chlorophenyl)-3-hydroxy-7,8-dimethylquinoline-4-carboxylic Acid (144):** Purified by method B, yellow solid, yield 72%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.43 (s, 3H), 2.68 (s, 3H), 7.46 (d, *J* = 8.84 Hz, 1H), 7.54–7.59 (m, 2H), 8.02–8.08 (m, 1H), 8.08–8.13 (m, 1H), 8.24 (d, *J* = 8.59 Hz, 1H). Anal. C<sub>18</sub>H<sub>14</sub>ClNO<sub>3</sub> C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>18</sub>H<sub>14</sub>ClNO<sub>3</sub> (MH<sup>+</sup>), 328.074; found, 328.07411.

**2-(4-Chlorophenyl)-3-methyl-8-(trifluoromethyl)quinoline-4-carboxylic Acid (17):** A suspension of 7-(Trifluoromethyl)indoline-2,3-dione (**3–16**; 0.80 g, 3.7 mmol) in 0.8 mL of ethanol and 3 mL of 10 M aqueous sodium hydroxide in a 50-mL two-necked round-bottomed flask fitted with a condenser was heated to reflux temperature. A solution of 4'-chloropropiophenone (0.82 g, 4.9 mmol) in 5.7 mL of ethanol was added in small portions by syringe over the course of 1 h. After completion of the addition, the mixture was allowed to reflux for an additional hour, then cooled to room temperature and acidified to pH 4 with glacial acetic acid. The resulting mixture was extracted into ethyl acetate (2×), and the organic layer was washed with brine (3×), dried over anhydrous magnesium sulfate, filtered, and evaporated. The residue was purified by preparative HPLC (water/acetonitrile, triethylamine modifier) and then converted back to the free acid as described above, collected by filtration, and dried in air overnight, yielding a free-flowing off-white powder (235 mg, 17% yield). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.46 (s, 3H), 7.61–7.66 (m, 2H), 7.70–7.77 (m, 2H), 7.82 (t, *J* = 7.8 Hz, 1H), 8.10 (d, *J* = 8.6 Hz, 1H), 8.23 (d, *J* = 7.3 Hz, 1H), 14.51 (s, 1H). Anal. C<sub>18</sub>H<sub>11</sub>ClF<sub>3</sub>NO<sub>2</sub> C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>18</sub>H<sub>11</sub>ClF<sub>3</sub>NO<sub>2</sub> (MH<sup>+</sup>), 366.0503; found, 366.0508.

**Triethylammonium 2-(4-Chlorophenyl)-3,6-dihydroxyquinoline-4-carboxylic Acid (75):** A mixture of compound **74** (500 mg, 1.5 mmol) and 11 mL of 48% HBr were refluxed (bath temperature 133 °C) for 4.5 h. The reaction was then cooled and neutralized with 5 M NaOH to a pH ~12. Next, 2 M HCl was added to a pH ~1. The crude precipitate obtained was filtered and purified by prep HPLC to give a brown solid (96 mg, 20%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.17 (t, *J* = 7.20 Hz, 9H), 3.08 (q, *J* = 7.33 Hz, 6H), 6.85 (dd, *J* = 8.84, 2.78 Hz, 1H), 7.38–7.54 (m, 2H), 7.63 (d, *J* = 8.84 Hz, 1H), 8.21–8.35 (m, 2H), 8.68–8.89 (m, 1H), 9.59 (s, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>16</sub>H<sub>10</sub>ClNO<sub>4</sub> (MH<sup>+</sup>), 316.0371; found, 316.037.

**General Procedure for the Synthesis of 6-Aryl Substituted Quinoline Salicylic Acids.** To a 1-L three-necked round-bottomed flask fitted with a reflux condenser were added 2-(4-chlorophenyl)-3-hydroxy-6-iodoquinoline-4-carboxylic acid (3.12 g, 7.33 mmol) and tetrakis(triphenylphosphine)palladium (0.424 g, 0.367 mmol), followed by 225 mL of 1,2-dimethoxyethane. The atmosphere in the reaction vessel was made inert by opening to vacuum and then to a positive pressure of nitrogen (3×). The appropriate arylboronic acid (8.06 mmol) and a solution of sodium bicarbonate (1.23 g, 14.7 mmol) in 225 mL of water were added and the evacuation/nitrogen procedure was repeated one more time. The reaction mixture was then refluxed until TLC showed complete disappearance of starting material (1–2 h). After cooling to room temperature, the 1,2-dimethoxyethane was removed under reduced pressure. The residue was diluted with 1 M aqueous hydrochloric acid and extracted into ethyl acetate (3×). The organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure to give the crude solid residue, which was purified by gravity chromatography (method B) as described in the general procedure for the Pfitzinger reaction.

**6-(4-Carboxyphenyl)-2-(4-chlorophenyl)-3-hydroxyquinoline-4-carboxylic Acid (114):** Yellow solid, yield 15%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.56–7.63 (m, 2H), 7.86–7.95 (m, 3H), 8.06–



8.13 (m, 3H), 8.13–8.22 (m, 2H), 9.23 br s, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>23</sub>H<sub>14</sub>ClNO<sub>5</sub> (MH<sup>+</sup>), 420.0634; found, 420.0632.

**Triethylammonium 2-(4-Chlorophenyl)-3-hydroxy-6-(4-methoxyphenyl)quinoline-4-carboxylic Acid (115):** Pale yellow solid, yield 11%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.16 (t, *J* = 7.07 Hz, 9H), 3.06 (m, 6H), 3.83 (s, 3H), 6.99–7.15 (m, 2H), 7.46–7.56 (m, 2H), 7.62 (dd, *J* = 8.72, 2.15 Hz, 1H), 7.67–7.74 (m, 2H), 7.86 (d, *J* = 8.59 Hz, 1H), 8.26–8.39 (m, 2H), 9.72 (d, *J* = 2.02 Hz, 1H). Anal. (C<sub>23</sub>H<sub>16</sub>ClNO<sub>4</sub>·0.6 (C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>N·H<sub>2</sub>O) C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>23</sub>H<sub>16</sub>ClNO<sub>4</sub> (MH<sup>+</sup>), 406.0841; found, 406.0841.

**General Procedure for the Synthesis of 4'-Aryl Substituted Quinoline Salicylic Acids.** The appropriately substituted boronic acid (0.55 mmol, 1.1 equiv) was added to 346 mg (2.5 mmol, 5 equiv) K<sub>2</sub>CO<sub>3</sub> in 2.5 mL of H<sub>2</sub>O and 2.5 mL of Me<sub>2</sub>CO, and the mixture was stirred 10 min to complete dissolution. Compound **10** (186 mg, 0.5 mmol, 1 equiv) was added next. After its dissolution, the flask was evacuated and flushed with N<sub>2</sub> three times and 20 mg Pd(OAc)<sub>2</sub> was added. The reaction was stirred at room temperature overnight under nitrogen.

The black suspension was distributed between AcOEt (2 × 75 mL) and water (2 × 20 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), evaporated under reduced pressure, and the crude solid residue was purified by gravity chromatography as described in the general procedure for the Pfitzinger reaction.

**Triethylammonium 3-Hydroxy-7,8-dimethyl-2-(4'-methylbiphenyl-4-yl)quinoline-4-carboxylic Acid (145):** Purified by method B without conversion back to the free acid, dark tan solid, yield 63.8%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.17 (t, *J* = 7.20 Hz, 9H), 2.37 (s, 3H), 2.39 (s, 3H), 2.70 (s, 3H), 3.09 (q, *J* = 7.33 Hz, 6H), 7.23 (d, *J* = 8.84 Hz, 1H), 7.31 (d, *J* = 7.83 Hz, 2H), 7.66 (d, *J* = 8.08 Hz, 2H), 7.70–7.80 (m, 2H), 8.33–8.51 (m, 2H), 9.22 (d, *J* = 8.84 Hz, 1H). Anal. (C<sub>25</sub>H<sub>21</sub>NO<sub>3</sub>·0.5(C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>N·H<sub>2</sub>O) C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>25</sub>H<sub>21</sub>NO<sub>3</sub> (MH<sup>+</sup>), 384.1594; found, 384.1595.

**Triethylammonium 2-(4'-(Dimethylamino)biphenyl-4-yl)-3-hydroxy-7,8-dimethylquinoline-4-carboxylic Acid (146):** Purified by method B without conversion back to the free acid, olive green solid, yield 63.8%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.17 (t, *J* = 7.33 Hz, 9H), 2.38 (s, 3H), 2.96 (s, 6H), 3.09 (q, *J* = 7.07 Hz, 6H), 6.84 (d, *J* = 9.09 Hz, 2H), 7.21 (d, *J* = 8.84 Hz, 1H), 7.62 (d, *J* = 8.84 Hz, 2H), 7.69 (d, *J* = 8.59 Hz, 2H), 8.39 (d, *J* = 8.59 Hz, 2H), 9.21 (d, *J* = 8.84 Hz, 1H). Anal. (C<sub>32</sub>H<sub>39</sub>N<sub>3</sub>O<sub>3</sub>·1.6H<sub>2</sub>O) C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>26</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub> (MH<sup>+</sup>), 413.186; found, 413.1861.

**Triethylammonium 3-Hydroxy-2-(4'-methoxybiphenyl-4-yl)-7,8-dimethylquinoline-4-carboxylic Acid (147):** Purified by method B without conversion back to the free acid, yellow orange solid, yield 55%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.17 (t, *J* = 7.2 Hz, 9H), 2.38 (s, 3H), 2.69 (s, 3H), 3.09 (q, *J* = 7.33 Hz, 6H), 3.82 (s, 3H), 7.06 (d, *J* = 8.84 Hz, 2H), 7.22 (d, *J* = 8.84 Hz, 1H), 7.60–7.78 (m, 4H), 8.42 (d, *J* = 8.59 Hz, 2H), 9.22 (d, *J* = 8.59 Hz, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>25</sub>H<sub>21</sub>NO<sub>4</sub> (MH<sup>+</sup>), 400.1544; found, 400.1544.

**General Procedure for the Synthesis of 4'-Acylated Quinoline Salicylic Acids.** To 206 mg (0.668 mMol) of starting quinoline salicylic acid **9** dissolved in 4 mL of dry pyridine under argon was added, while stirring under argon at 0 °C, 0.47 mL (5.06 equiv) of the appropriate acetic anhydride. The solution was stirred in ice water for 1 h and then 16 h at room temperature.

The reaction was quenched with a chip of ice and concentrated under reduced pressure. After distribution of the concentrate between CHCl<sub>3</sub> (2 × 75 mL) and H<sub>2</sub>O (50 mL), followed by drying (Na<sub>2</sub>SO<sub>4</sub>) and evaporation of the organic layers, the residue was purified by gravity chromatography over silica gel, eluting with a 70:5:2.5:2.5 ethyl acetate/acetonitrile/methanol/water system to which 0.5% triethylamine had been added; the pure triethylammonium salt was then taken up in 20% acetonitrile in water and acidified with concentrated hydrochloric acid to precipitate the free acid.

**3-Hydroxy-7,8-dimethyl-2-(4-(2,2,2-trifluoroacetamido)phenyl)quinoline-4-carboxylic Acid (149):** Brown crystals, yield 39.2%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.38 (s, 3H), 2.68 (s, 3H), 7.23 (d, *J* = 8.84 Hz, 1H), 7.77 (d, *J* = 8.84 Hz, 2H), 8.40 (d, *J* = 8.84 Hz, 2H), 9.20 (d, *J* = 8.59 Hz, 1H), 11.38 (s, 1H). HRMS (ESI<sup>+</sup>) calcd for C<sub>20</sub>H<sub>15</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub> (MH<sup>+</sup>), 405.1057; found, 405.1057.

**2-(4-Acetamidophenyl)-3-hydroxy-7,8-dimethylquinoline-4-carboxylic Acid (148):** Yield 66.4% for two steps. The diacetylated product, 2-(4-acetamidophenyl)-3-acetoxy-7,8-dimethylquinoline-4-carboxylic acid, was the major product from acylation of compound **9** with acetic anhydride. A mixture of the diacetylated quinoline salicylic acid (200 mg, 0.51 mmol) and LiOH·H<sub>2</sub>O (75 mg, 1.78 mmol, 3.5 equiv) in 8 mL of MeCN and 8 mL of H<sub>2</sub>O was stirred at room temperature until TLC AcOEt–MeCN–MeOH–H<sub>2</sub>O (60:10:10:10) indicated complete removal of the phenolic acetyl (5 h). After filtration from some turbidity, the solution was diluted with two volumes of water and acidified with 2 N HCl to pH 0. The solid was collected by filtration, washed with water, and dried in vacuo over P<sub>2</sub>O<sub>5</sub> to give 144 mg of **148** as an orange-brown solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.10 (s, 3H), 2.43 (s, 3H), 2.70 (s, 3H), 7.42 (d, *J* = 8.84 Hz, 1H), 7.72 (d, *J* = 8.84 Hz, 2H), 8.00–8.19 (m, 2H), 8.27 (d, *J* = 8.84 Hz, 1H), 10.13 (s, 1H). Anal. (C<sub>20</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>·1.5 H<sub>2</sub>O) C, H, N. HRMS (ESI<sup>+</sup>) calcd for C<sub>20</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub> (MH<sup>+</sup>), 351.134; found, 351.1339.

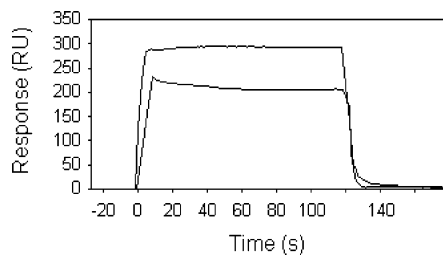
**Transferred NOE (trNOE) Experiment.** The NMR samples were prepared in D<sub>2</sub>O buffer with 20 mM imidazole, pH = 7.4, 150 mM NaCl, 5 mM CaCl<sub>2</sub>, and 0.02% NaN<sub>3</sub>, with either 1 mM or 400 μM small molecule and 40 μM or 25 μM P-selectin-hIgG chimera protein, respectively. The 2D trNOE experiments were performed on a Bruker Avance 600 MHz instrument at 25 °C, with 120–170 ms mixing time. The data were processed with nmrPipe software.<sup>20</sup> Negative control trNOE experiments were also performed either without any added protein or in the presence of IgG instead of P-selectin. Fc, run under the above conditions. The titration of compound **36** was performed with the soluble P-selectin form (PLE).

**Pharmacokinetics in Rats.** The animals used in the study were male adult Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) weighing between 200 and 350 g. The study was performed at Wyeth Research Laboratory (Andover, MA) under the supervision of the Institutional Animal Care and Use Committee. Rats had a jugular vein catheter surgically implanted prior to their arrival at the laboratory. The acclimatization period prior to study was at least one week upon arrival. Each treatment group included three animals. The doses for IV and oral administration were 1 mg/kg formulated in 50:50 DMSO/PEG400 (v/v, 1 mL/kg) and 25 mg/kg formulated aqueous suspension containing 2% Tween and 0.5% methylcellulose, respectively. Blood samples were collected into K<sub>2</sub>EDTA-coated sampling tubes from each rat at 0.083, 0.25, 0.5, 1, 2, 4, 7, and 24 h post dose administration. An equal volume of saline was given to replace the lost blood. Plasma levels of test compounds were determined by LC/MS-MS assay. A log trapezoid noncompartmental analysis was used in pharmacokinetic analysis.

**Cytochrome P450 Inhibition Assay.** Incubations were performed under conditions shown to be linear with respect to time, protein, and substrate concentration based on the cocktail method described previously by Dierks et al.<sup>43</sup> Incubation mixtures consisted of a cocktail of probe substrates at concentrations equal to their approximate *K<sub>m</sub>* values for each of seven human CYP enzymes (see Table 12), 0.5 mg/mL human liver microsomes, 10 mM MgCl<sub>2</sub>, and 100 mM potassium phosphate buffer (pH 7.4) in a total volume of 0.5 mL. Samples were preincubated for 3 min at 37 °C in a shaking water bath. The reactions were initiated by addition of an NADPH regenerating system (final concentrations of 3.5 mM glucose-6-phosphate, 0.4 units/mL of glucose-6-phosphate-dehydrogenase, and 1.3 mM NADP<sup>+</sup>). Incubations were carried out for 20 min and terminated by the addition of 0.50 mL of acetonitrile and 0.5 μM dextrorphan as an internal standard. Samples were centrifuged at 3000 rpm for 10 min at 4 °C to pellet the precipitated protein. The acetonitrile supernatant was evaporated under a stream

**Table 12.** Summary of Probe Substrates and Detected Metabolites (LC-MS/MS method)

human CYP enzyme	substrate (~ $K_m$ concentrated)	detected metabolite
CYP3A4	midazolam (2.5 $\mu$ M)	1'-hydroxymidazolam
CYP2D6	bufuralol (5 $\mu$ M)	1'-hydroxybufuralol
CYP2C9	diclofenac (10 $\mu$ M)	4'-hydroxydiclofenac
CYP1A2	ethoxyresorufin (1 $\mu$ M)	resorufin
CYP2A6	coumarin (2.5 $\mu$ M)	7-hydroxycoumarin
CYP2C8	paclitaxel (10 $\mu$ M)	6 $\alpha$ -hydroxypaclitaxel
CYP2C19	S-mephenytoin (40 $\mu$ M)	4'-hydroxy-S-mephenytoin

**Figure 5.** Representative sensogram showing double reference subtracted signals from P-selectin (P.LE) binding to immobilized PSGL-1 (19ek) in the presence (lower trace) and absence (upper trace) of antagonist. Plot prepared using Scrubber-2 software, Center for Biomolecular Interaction Analysis, David Myszk, University of Utah.

of nitrogen and reconstituted in 20% methanol in water. Metabolites were detected using LC-MS/MS. Approximate  $IC_{50}$  values for the inhibition of CYP3A, CYP2D6, CYP2C9, CYP2A6, CYP2C8, CYP2C19, and CYP1A2 activities were determined by including 0, 0.1, 0.25, 1, 2.5, 10, 25, and 100  $\mu$ M of the P-selectin antagonist in the assay described above.

**Analysis of Results.** Peak area ratios for the metabolites against the internal standard were used to express the metabolite formation. The percent inhibition by the P-selectin antagonist was determined by dividing the peak area ratio obtained in the presence of the antagonist by the corresponding ratio in the absence of the antagonist.  $IC_{50}$  values were determined using percent activity remaining (compared to a control sample with no antagonist added) versus the examined antagonist's concentration (log scale) in WinNonlin version 3.2 Model 103.

## Biology

**Biacore Assay.** Surface plasmon resonance (Biacore) assays were performed on a Biacore 3000 instrument (Biacore, Inc., Piscataway, NJ), at 25 °C at a flow rate of 30  $\mu$ L/minute and consisted of 60-second equilibration, 60  $\mu$ L sample injection (kinject), and 300-second dissociation. Due to the rapid and complete dissociation of P-selectin, the sensogram returned to baseline immediately following the end of each injection (see Figure 5). Previous studies showed that there was little change in the amount of uninhibited binding over a 24-hour period of sample injections. However, because no regeneration step was included, a 1 $\times$  Biacore assay buffer "blank" injection and "uninhibited" 500 nM P.LE solution without small molecule antagonist bracketed every five sample injections. These bracketing controls were used in the analysis (see Table 13 and Biacore analysis section). P.LE is a truncated form of P-selectin containing the lectin and EGF domains.

A purified, monomeric, truncated form of human PSGL-1, "19ek", that contains all the necessary P-selectin binding determinants<sup>44</sup> was biotinylated via amine chemistry (sulfo-NHS-LC-Biotin, Peirce) at a unique C-terminal lysine residue<sup>17</sup> and immobilized on a Biacore SA sensor chip (Biacore, Inc.), using HBS-EP buffer (Biacore, Inc.), with a target of 600–700 RU. The coated chip was re-equilibrated with HBS-P buffer

**Table 13.** Sample Order

sample ID	antagonist concentration ( $\mu$ M)
buffer blank	not applicable
P.LE uninhibited	not applicable
glycyrrhizin	1000
antagonist	250
antagonist	125
antagonist	62.5
antagonist	31.25
buffer blank	not applicable
P.LE uninhibited	not applicable

(Biacore, Inc.) to which 1 mM  $CaCl_2$  and 1 mM  $MgCl_2$  (both from Fisher) was added to ensure sufficient calcium for the calcium-dependent interaction of receptor and ligand. PSI-697 was incubated for 1 h in 1.1 $\times$  Biacore assay buffer. The solution was centrifuged at 2500 g for 7 min through a 96 well 0.2 mM filter plate (Millipore). In validation of this filtration step (data not shown), a structurally varied subset of compounds, which showed apparent solubility in the assay conditions, showed no change in antagonist activity in the Biacore assay when a direct comparison was made between filtered and unfiltered samples. When test compounds showed visible precipitate prior to filtering, this was noted. The only inhibitions reported were those at concentrations free of precipitate.

Glycyrrhizin trisodium salt (TCI) was prepared as a positive control in parallel with our P-selectin antagonist in the same manner. Glycyrrhizin, a demonstrated antagonist of P-selectin (John T. Patton, GlycoTech Corporation, written communication, May 2000), has been shown to inhibit the P-selectin/PSGL-1 interaction with an  $IC_{50}$  of 1 mM in this assay. A soluble recombinant truncated form of human P-selectin, P-LE, comprised of the lectin and EGF domains expressed in CHO cells,<sup>17</sup> was added to each filtered small molecule solution. Final concentrations of reagents were 500 nM P.LE, 31.25 mM to 250  $\mu$ M PSI-697 (or 1 mM glycyrrhizin), 10% DMSO and 1 $\times$  Biacore buffer (100 mM HEPES, 150 mM NaCl, 1 mM  $CaCl_2$ , 1 mM  $MgCl_2$  (all reagents from Fisher), pH 7.4). Ten percent DMSO concentration was needed to ensure solubility of test samples. Test samples were supplied to the Biacore instrument in a 96-well plate.

**Biacore Data Analysis.** The Biacore raw data file was exported as a text file to an Excel spreadsheet where the buffer blanks bracketing the samples are averaged for each Fc and subtracted from the averaged uninhibited P.LE samples and from all the other samples. Then the reference signal from Fc1 (uncoated) is subtracted from its corresponding active (coated) signal for each injection, a process known as double referencing.<sup>45</sup> The percent inhibition of binding is calculated by dividing the reference subtracted inhibited signal by the reference subtracted uninhibited signal, subtracting this value from 1 then multiplying by 100. The replicate percent inhibition values are averaged and expressed as the mean  $\pm$  standard deviation. The interexperiment standard deviation of calculated percent inhibitions in the Biacore assay was  $\pm 5$ . The standard deviation between percent inhibitions calculated for two injections within the same assay was very low, less than 1.

**In Vitro Leukocyte Rolling Assay.** A parallel plate flow chamber (GlycoTech, Rockville, Maryland) was used to measure neutrophil/endothelial cell adhesion events under flow, as previously described,<sup>18</sup> with changes in protocol noted as follows. Confluent monolayers of HUVECs (human umbilical cord endothelial cells) were activated with IL-4 to up regulate P-selectin expression on the cell surface immediately prior to the experiment. Freshly isolated human neutrophils were

incubated for 20 min with or without compound in 4 mL of serum-free cell media containing histamine (to further activate the HUVEC once introduced into the flow chamber). This solution was flowed over the HUVEC monolayer to generate a shear stress of approximately 1 dyn/cm<sup>2</sup>. For each test article, data was collected by digital imaging and videotape on three different monolayers. The number of rolling cells was an average value of 10 fields in each of the three monolayers. Active compounds were titrated to determine an IC<sub>50</sub> concentration. TCI was used as a positive control, inhibiting 50% of rolling flux at 1 mM.

**DHOD Inhibition Assay.** Functional His-tagged recombinant human DHOD was expressed in *E. coli* (PyrD) and purified using Ni-NTA superflow sepharose (Qiagen) column chromatography and a POROS HS-20 cation-exchange column. DHOD inhibition was determined by using absorbance at 590 nm to measure the disappearance of 2,6-dichloroindophenol as a function of time in the presence and absence of small molecule antagonists, adapted from Neihardt et al.<sup>30</sup> to a 96-well format.

**Rat Carrageenan Paw Edema (CPE) Model.** Male Sprague–Dawley rats weighing 190–250 g from Taconic Farms were housed for 1 week prior to experimentation and fed food and water ad libitum. The protocol for the CPE model was adapted from procedures previously described.<sup>29,46</sup> At the beginning of the study, the volume of the left hind footpad of the rat was measured using an Ugo Basile plethysmometer. Immediately following the paw measurement, the animals were dosed with either 0.5, 1, 5, 10, or 50 mg/kg of compound **91** in vehicle (2% Tween 80, 0.5% methylcellulose), 10 mg/kg of naproxen in vehicle, or vehicle only by the PO route of administration (5 mL/kg). Two hours after dosing, the rats were lightly anesthetized and 50 μL of 1% carrageenan in sterile saline was injected subplantar into the left hind footpad. Three hours after the carrageenan injection, the left hind paw volume was again measured using the Ugo Basile plethysmometer. All dosing and measurements were performed in a blinded fashion. Inhibition of paw edema was calculated using the following formula:

$$\text{Percent Inhibition} = \left\{ 1 - \frac{[3 \text{ h paw volume} - 0 \text{ h paw volume (test group)}]}{[3 \text{ h paw volume} - 0 \text{ h paw volume (vehicle group)}]} \right\} \times 100$$

There were 10 rats in each test group.

**Rat Adjuvant-Induced Arthritis.** Arthritis was induced by the intradermal injection of Freund's complete adjuvant into the base of the tail of male Lewis rats, as described previously.<sup>47–51</sup> Compounds **36** and **91** and Celecoxib were administered to rats by daily oral gavage beginning 8 days after adjuvant injection. rPSGL-Ig was administered IV on alternate days. Both erythema (redness) and swelling of the tarsal joints were assessed visually and assigned severity scores of 0–3 for each parameter and limb, with a maximal score per animal being 12.<sup>47–51</sup>

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**Supporting Information Available:** Purity data from HPLC analysis or full combustion data available for all final compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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