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ROCK inhibitors 2. Improving potency, selectivity and solubility through the application of rationally designed solubilizing groups

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ABSTRACT

Solubilizing groups have been frequently appended to kinase inhibitor drug molecules when solubility is insufficient for pharmaceutical development. Such groups are usually located at substitution sites that have minimal impact on target activity. In this report we describe the incorporation of solubilizing groups in a class of Rho kinase (ROCK) inhibitors that not only confer improved solubility, but also enhance target potency and selectivity against a closely related kinase, PKA.

In the period since the introduction of imatinib (**1**) in 2001 as the first explicitly designed therapeutic kinase inhibitor, more than 30 kinase inhibitor drugs have since been approved by the US FDA. Of these kinase-targeted drugs, 19 contain chemical moieties specifically incorporated for the purpose of enhancing solubility, since the parent structure is lacking sufficient solubility for pharmaceutical development (Fig. 1: for a complete illustration of the current FDA-approved kinase inhibitor drugs see Fig. S1 in the Supplementary file). These solubilizing groups are typically attached to a substitution point on the parent molecule that possesses a high degree of SAR tolerance and usually neither enhances nor interferes with target potency. The binding implications of these solubilizing features have been well described and illustrated in a recent review by Wu et al.[1] For example, the X-ray crystal structure of gefitinib (**2**) (PDB: 2ITY) bound to its target, EGFR, shows the solubilizing morpholinopropoxy group to be extended away from the protein surface, not making contact with the protein (Fig. 2). Furthermore, gefitinib (**2**) reportedly inhibits EGFR with IC_{50} = 9 nM, while the analogous 6-methoxy compound has IC_{50} = 23 nM,[2] indicating that the solubilizing group does not significantly contribute to binding. Interestingly, the X-ray structure of imatinib (**1**) bound to its target Abl (PDB: 1IEP) shows the solubilizing methylpiperazine to be in contact with protein backbone residues (Fig. 3). The binding data shows that Bcr-Abl inhibition by imatinib (1: IC_{50} = 38 nM) is approximately 5-fold improved over the unsubstituted (4-methyl) compound (IC_{50} = 200 nM).[3] However, with the exception of the related Bcr-Abl inhibitors nilotinib and ponatinib, the solubilizing appendages of kinase-inhibitor drugs extend into solvent space, not providing any additional value in terms of binding interactions as illustrated by Wu et al.[1]

The Rho-associated kinases, ROCK1 and ROCK2 are highly homologous Ser/Thr kinases, activated by binding of the small GTPase Rho, that act on a variety of substrates, many of which are implicated in smooth muscle contractility. ROCK inhibitors are currently under clinical development for a number of therapeutic applications, most notably for the treatment of glaucoma. These inhibitors have been summarized in a recent review, which also thoroughly covers the ROCK inhibitor literature.[4] We have recently reported our approach to the discovery and design of pyridyl-thiazole and pyridyl-thiophene ROCK inhibitors, exemplified by compounds **20** and **21** shown in Fig 4.[5] X-ray crystallography and molecular modeling facilitated the optimization of this series of molecules from the early lead (**20**) to an optimized molecule (**21**) possessing properties suitable for pharmacological evaluation following oral dosing. During the course of this work key challenges included enhancing potency and selectivity, and improving solubility.

Fig. 5 illustrates the X-ray structure of compound **20** bound to ROCK. Notable interactions include hydrogen bonds between the pyridine nitrogen and Met156 (2.9 Å), the amide carbonyl and the side-chain of catalytic Lys105 (2.8 Å), and the methoxy oxygen and the backbone NH of Phe87 (3.3 Å). In addition, we noted that Asp117, some 12 Å distant from the methoxy group, might offer an additional binding opportunity. Not only could this residue engage in an ionic interaction with a pendant basic moiety, such as piperidine or piperazine, but the analogous residue in PKA, a closely related AGC-family kinase[6] and a key anti-target, is Gln84. The greater steric requirements and neutral nature of this side chain should enhance selectivity for ROCK. To access this region of the target, we prepared 3-substituted phenylacetic acid derivatives bearing extended solubilizing groups and incorporated these into ROCK inhibitors, as illustrated in Schemes 1 and 2.

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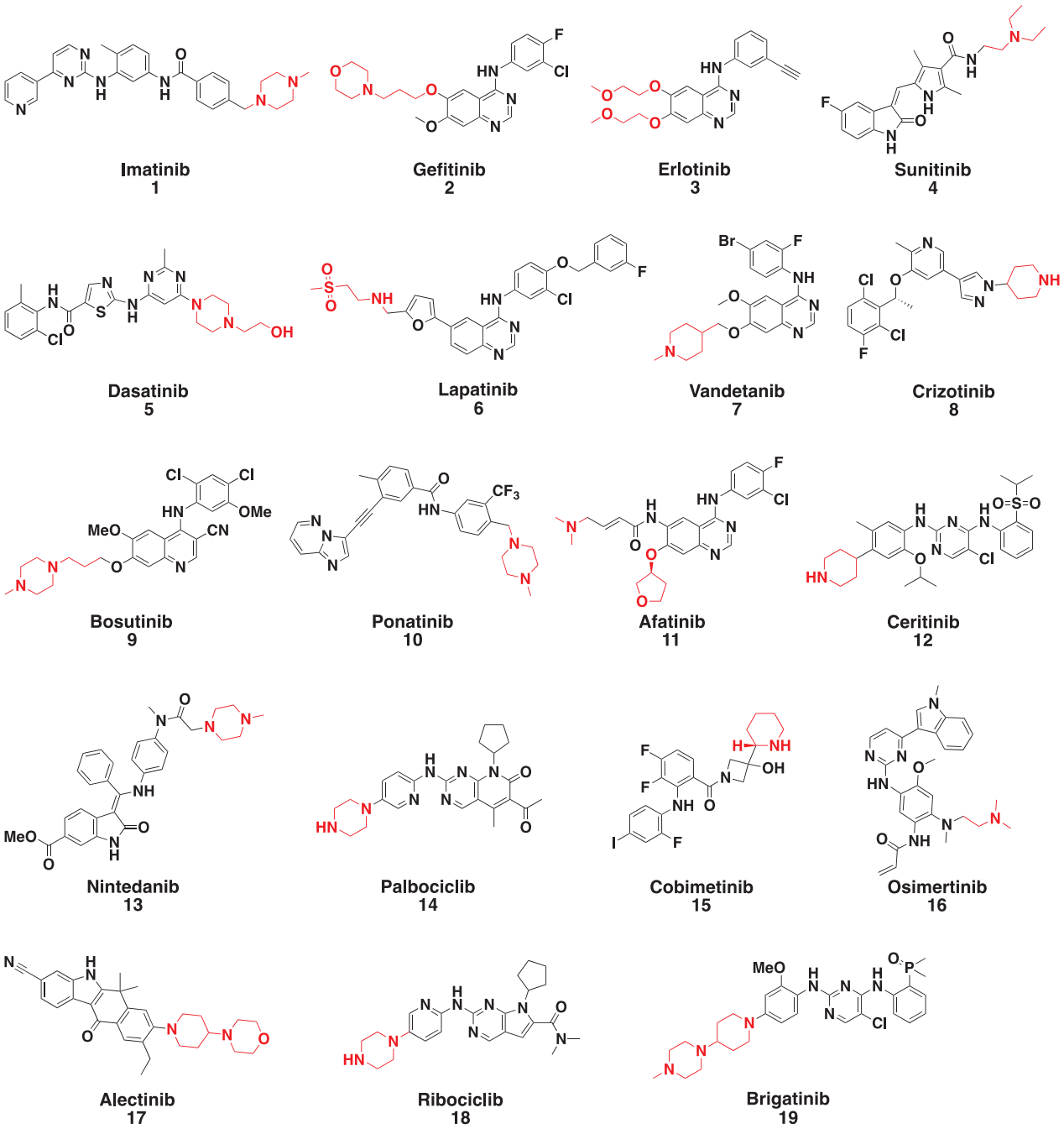

Fig. 1. FDA approved kinase inhibitors containing solubilizing features (highlighted in red).

N-Piperidine, -piperazine and -morpholine compounds **22–29** were prepared as shown in Scheme 1. Methyl 3-hydroxyphenylacetic acid was alkylated with 2-chloro-1-bromoethane, or 3-chloro-1-bromopropane, under basic conditions in acetone at reflux for 24 h. Subsequent base hydrolysis yielded the phenylacetic acids, which were coupled to 4-(pyridin-4-yl)thiazol-2-amine using 1-methanesulfonylbenzotriazole (Ms-Bt),[7] as previously described.[5] Conversion to the final products was accomplished by treatment with the appropriate secondary amine in DMSO at 60–90 °C. Products were purified by preparative HPLC. The preparation of 4-substituted piperidine compounds **30** and **31** is shown in Scheme 2. Methyl 3-hydroxyphenylacetic acid was alkylated with *N*-Boc-4-(3-hydroxypropyl)piperidine under Mitsunobu conditions, the product hydrolyzed and coupled as described above. Boc deprotection using TFA gave compound **30**, while Eschweiler-Clarke methylation gave compound **31**.

Evaluation of the effects of the solubilizing groups is summarized in Table 1.

The introduction of a solubilizing side-chain, attached at the 3-position of the phenylacetamide led to compounds with enhanced potency relative to the parent molecule **20**. With a 2-carbon linker, the piperidine (**22**) and morpholine (**23**) derivatives were *ca*. 5- to 6-fold less potent than **20**, indicating that neither occupancy of the above-