Rapid approach to complex boronic acids

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The compatibility of free boronic acid building blocks in multicomponent reactions to readily create large libraries of diverse and complex small molecules was investigated. Traditionally, boronic acid synthesis is sequential, synthetically demanding, and time-consuming, which leads to high target synthesis times and low coverage of the boronic acid chemical space. We have performed the synthesis of large libraries of boronic acid derivatives based on multiple chemistries and building blocks using acoustic dispensing technology. The synthesis was performed on a nanomole scale with high synthesis success rates. The discovery of a protease inhibitor underscores the usefulness of the approach. Our acoustic dispensing–enabled chemistry paves the way to highly accelerated synthesis and miniaturized reaction scouting, allowing access to unprecedented boronic acid libraries.

INTRODUCTION

Boron is a unique element of great versatility and individuality, although it seems that nature and evolution have generally bypassed it (with the exception of a few natural products, e.g., boromycin) (1). Boron plays an exquisite role in synthetic chemistry, with boronic acids and their esters of paramount importance to all facets of chemical science. Since the introduction of the Pd-catalyzed C=O Suzuki-Miyaura couplings (2) that brought boronate esters into vogue, the boronic acid moiety has become a very important functional group (3). Other highly useful transformations based on boronic acids include the Petasis reaction (4), C═N and C═O coupling (Chan-Lam coupling) (5, 6), Liebeskind-Srogl coupling (7), regioselective deuteriation, or sulfonamide formation (8). Boronic acids as mild electrophiles are also investigated as reversible covalent inhibitors (9, 10), and thousands of different building blocks are now commercially available. As a result, boronic acids are increasingly being seen in approved drugs, e.g., vaborbactam or bortezomib (Fig. 1, A and B) (11, 12).

However, these boron building blocks comprise almost exclusively low–molecular-weight compounds, as the late-stage functionalization of high–molecular-weight boronic acids is synthetically demanding due to their tedious introduction, modest functional group compatibility, regioselectivity issues, and difficulty to parallelize (13, 14). Because of the exquisite differential properties of boronic acids, an easy access to high–molecular-weight elaborated compounds is highly desirable. Isocyanide–based multicomponent reactions (IMCRs) are well established for functional group compatibility that accounts for the immense scaffold diversity that can be generated on the basis of some handful primary IMCRs (15, 16). Furthermore, IMCRs are useful to access a drug-like chemical space and many marketed or experimental drugs (17, 18). Thus, we hypothesized that unprotected boronic acids are compatible with the reaction conditions of IMCR and can be introduced into complex high–molecular-weight compounds of use (19). The use of unprotected boronic acids directly could enable a faster access with limited protecting steps to a large number of boron-based derivatives. In addition, the screening of these compounds (e.g., as covalent inhibitors) could be performed directly

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Fig. 1. Importance of boronic acids, commonly used synthetic methods for the B(OH)2 introduction, and our proposed building block–centered approach. (A) Marketed drugs containing free –B(OH)2 moieties. (B) Common methods for late-stage introduction of the –B(OH)2 moiety. THF-DMF, tetrahydrofuran-dimethylformamide. (C) Building block approach to prepare complex –B(OH)2 moiety containing molecules in large numbers.
without further deprotection (Fig. 1C). To test this hypothesis, we used an acoustic droplet ejection (ADE)–enabled synthesis platform. In ADE, acoustic waves are applied to eject nanoliter droplets from a source plate with building block stock solutions to a destination plate in which the reaction occurs. While ADE is an established dispensing technology in many other scientific areas (e.g., crystallography), it is uncommon in organic synthesis (20). The ADE platform is based on microliter volume chemistry, uses minimal resources, is highly automatable, and is useful to screen many building block combinations in a shorter time frame than other current technologies (21).

RESULTS AND DISCUSSIONS

The mechanism-based functional groups required in IMCRs are carboxylic acids, amines, oxo components, and isocyanides. We synthesized and purchased a number of the first three building block categories. In addition, we also synthesized an unknown isocyanide boronic acid in one example (Fig. 2). We planned to perform four IMCRs and investigate the reaction success rate depending on the reactions, the components, and the substitution pattern (e.g., o-, m-, and p-) in combination with multiple complementary building blocks chosen in a random fashion.

Optimizing the compatibility of boronic acids for MCR is an interesting synthetic challenge, as the C–B bond is well known to react
with common MCR starting materials and intermediates under mild conditions, e.g., primary, secondary amines, and carbonyl compounds (Petasis reaction and others) (22). Moreover, the electrophilic boron could unproductively complex to nucleophilic key functional groups of MCRs and thereby interrupt the reaction progress (23). Here, we used different building blocks (amines, aldehydes, carboxylic acids, and isocyanides) with free boronic acids in different positions to investigate their compatibility with a number of IMCRs (Fig. 2). The stability of the boronic acid moiety in the presence of the isocyanide in one molecule in the absence of such molecules is unknown. To this end, we also synthesized the first free boronic acid–containing isocyanide. We have extensively investigated the compatibility of multiple free boronic acid–containing building blocks in multiple IMCRs, including the classical Ugi four-component reaction (U-4CR) (24),

**Fig. 3. HT synthesis of boronic acids using the building block approach.** (A) Exemplary analytical 384-well plate of the U-4CR scaffold 12 (green, major product formation; yellow, product present; blue, product not present). (B) Statistical analysis of the quality of reactions of the different scaffolds. (C) Structures of some unusual reaction products from different IMCRs.
the Ugi tetrazole (UT-4CR) (25), the Gröbcke-Blackburn-Bienaymé (GBB-3CR) (26–28), and the Ugi-based macrocycles (scaffolds 12 to 20; Fig. 3) (29). In addition, we studied the suitability of the corresponding boronic acid libraries in a secondary Suzuki cross-coupling reaction. We performed the project under extreme resource- and time-saving conditions using the ADE-enabled chemistry platform in a 384-well format.

We accomplished the synthesis of \textit{m}-isocyanophenyl boronic acid by a classical formylation/dehydration procedure (Fig. 2). Because of the presumed instability, we immediately used the isocyanide after preparation. Using the boronic acid building blocks of Fig. 2, we investigated different IMCRs on a nanomole scale using ADE technology. The analytics of the four 384-well format plates were performed using mass spectrometry (MS) as described previously (30), which allowed us to classify the reactions into three groups: major (green color), mediocre (yellow color), or no product formation (blue color). The outcome of the high-throughput (HT) analytics for the different reactions is shown in Fig. 3, and a detailed analysis of the different building blocks is given in the Supplementary Materials.

The rapid collection of information facilitated the ability to predict outcomes from other possible combinations of reagents. For example, it was found that the \textit{ortho} substituted building blocks 4 and 11 reacted less efficiently than the corresponding \textit{meta} or \textit{para} substituted in all MCRs (Fig. 2). This can be rationalized by the neighbor group effect of boronic acid that might hamper formation or reduce reactivity of the key Schiff base. It was also found that boronic acid monoesters 5 and 8 were less reactive than their boronic acid counterparts, probably due to the introduction of ring strain around the boron center, leading to slightly different electronic properties (31).

In addition, it was demonstrated that the U-4CR of the three carboxyphenyl boronic acids 6 to 8 (heat map shown in Fig. 3A) was greatly enhanced when \textit{p}-formaldehyde was used (>60% of the reactions worked; see the Supplementary Materials). Last, it is noteworthy that formylphenyl boronic acids behave well in the GBB-3CR, since more than 50% of the reactions that were performed were successful (see the Supplementary Materials). In general, the use of building blocks without the free \(-\text{B(OH)}_2\) moieties was less successful than those with boronic acids. This could point to a potential catalytic activity of boronic acids in the GBB-3CR as a Brønsted acid as there are many cases of GBB catalysis by Brønsted acids (26–28).

Detailed analysis of the rich data of the complementary building blocks can help to uncover subtle reactivity details.

In our approach, novel substrates could be generated. In the GBB-3CR, compound 21 reacted repeatedly well despite the existence of a hitherto unreported triazolidine-5-thione moiety in this context. Another interesting finding is the good reactivity of building
block 22 in the GBB-3CR, in which the formyl group did not react, as the additional formyl group could theoretically undergo alternative reaction pathways such as condensation and addition reactions. Another pleasant finding is the good reactivity of tetrahydro-β-carboline 23 in the UT-4CR, which is a pharmacophore in multiple natural products and drugs (e.g., harman and tadalafil). Complex medium-sized and macrocycles gave, unexpectedly, very good results (e.g., medium-sized cycle 24). Last, we observed functional group tolerance and selectivity. In the case of U-4CR 25, we used a diamine, which reacted only once, leaving a primary amine behind. The HT synthesis approach displayed here is a treasure trove to uncover interesting unknown reactivities that deserve further investigation and detailed analysis in a narrower compound series.

The scalability from the nanomole to the millimole scale is often problematic. Therefore, we resynthesized multiple examples of each compound series (compounds 12 to 20) on a millimole scale (including...
Modeling studies of 18a in MptpB with Cys 160, Ser 57, and Thr 223 were surface and in the active site of MptpB (the Supplementary Materials). Due to the large number of reactive Ser, Cys, and Thr on 18a, it is the most potent one (Fig. 6). The exact binding mode of 18a in MptpB (the Supplementary Materials). In this assay, we found several hits, the most potent one 18a (Fig. 6). The exact binding mode of 18a is unclear due to the large number of reactive Ser, Cys, and Thr on the surface and in the active site of MptpB (the Supplementary Materials). Modeling studies of 18a in MptpB with Cys 160, Ser 57, and Thr 223 were performed and suggest a covalent adduct to a tetrahedral boron (Fig. 6C and see the Supplementary Materials).

To further underscore the usefulness of our fast, convergent, and highly diverse access of boronic acid libraries, we screened for inhibition of the biological target MptpB, a virulence factor from Mycobacterium tuberculosis (32). MptpB belongs to the notoriously undruggable target class of phosphatases that, despite their overabundance in medicine, suffer from having no approved drug (33). This is generally attributed to the highly positively charged active site of phosphatases requiring negatively charged inhibitors that cannot overcome membrane penetration issues (34). Looking for a potential covalent interaction between the active-site nucleophiles Cys 160, Thr 223, and Ser 57 of MptpB and an electrophilic boronic acid, we screened the library in a colorimetric enzyme assay (see the Supplementary Materials). In this assay, we found several hits, the most potent one 18a (Fig. 6). The exact binding mode of 18a is unclear due to the large number of reactive Ser, Cys, and Thr on the surface and in the active site of MptpB (the Supplementary Materials). Modeling studies of 18a in MptpB with Cys 160, Ser 57, and Thr 223 were performed and suggest a covalent adduct to a tetrahedral boron (Fig. 6C and see the Supplementary Materials).

Classical access to boronic acids by late-stage functionalization of complex molecules suffers from a lack in functional group compatibility and regioselectivity and often requires harsh conditions that are incompatible with molecule stability (35–38). Here, we introduced the concept of boronic acid building blocks combined with the diversity of MCRs as a valid approach for the synthesis of large and unprecedented libraries of boronic acids. Our studies go much beyond a singleton report on the use of a few free boronic acids in the Ugi reaction as we also investigated the GBB-3CR, UT-4CR, and several different IMCR variations more in an unprecedented breadth of building block combinations (35, 36). In other reports, isocyanide-bearing boronic acids are only known in their protected ester form that would need another, often harsh, deprotecting step to yield boronic acids suitable for screening (39, 40). Here, we found that IMCR generally runs under such mild conditions that free boronic acids are widely tolerated. We systematically investigated 10 different boronic acid building blocks with complementary functional groups (primary amine, aldehyde, carboxylic acid, and isocyanide) and combined them with 353 different reactants in four IMCRs. More than 1300 different combinations were investigated in a nanomole miniaturized and automated fashion using ADE technology. HT analytics using MS revealed that the different reactions worked better than satisfactorily in 714 cases (458 giving the main product and 256 cases a satisfactory yield). Many subtle reactivities were uncovered, which in a classical millimole scale reaction, evaluation approach could never have been elucidated in a reasonable time frame. UpScaling of a substantial number of diverse products revealed the synthetic usefulness of the approach. Last, we probed our library to uncover previously unknown boronic acid–based covalent inhibitors for a notoriously undruggable phosphatase target, identifying a micromolar inhibitor. We believe our described building block approach will widen the accessibility of the boronic acid chemical space markedly for applications in synthesis, chemical biology, and drug discovery. This is also true in light of the recently found catalytic enantioselective Ugi reaction (41).
MATERIALS AND METHODS
All the reagents and solvents were purchased from Sigma-Aldrich, AK Scientific, Fluorochem, abcr GmbH, and Acros and were used without further purification. All isocyanides were prepared in-house (see the Supplementary Materials). All microwave irradiation reactions were carried out in a Biotage Initiator microwave synthesizer. Thin-layer chromatography was performed on Milliporte precoated silica gel plates (thickness, 0.20 mm; particle size, 25 μm). Nuclear magnetic resonance spectra were recorded on Bruker Avance 500 spectrometers ($^1$H NMR (nuclear magnetic resonance; 500 MHz), $^{13}$C NMR (126 MHz)]. Chemical shifts for $^1$H NMR were reported as δ values, and coupling constants were in hertz. The following abbreviations were used for spin multiplicities: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; quin, quintet; dd, double of doublets; ddd, double doublet of doublets; and m, multiplet. Chemical shifts for $^{13}$C NMR were reported in parts per million relative to the solvent peak. Flash chromatography was performed on a Reveleris X2 flash chromatography system, using Grace Reveleris Silica flash cartridges (12 g). Mass spectra were measured on a Waters Investigator Supercritical Fluid Chromatograph with a 3100 MS detector (ESI) using a solvent system of methanol and CO$_2$ on a Virdis silica gel column (4.6 × 250 mm, 5-μm particle size) or Virdis 2-ethyl pyridine column (4.6 × 250 mm, 5-μm particle size). High-resolution mass spectra were recorded using an LTQ Orbitrap XL (Thermo Fisher) with phenylboronic acids and cupric acetate. Tetrahedron Lett. 39, 2933–2936 (1998).

REFERENCES AND NOTES
1. D. B. Diaz, A. K. Yudin, The versatility of boron in biological target engagement. Nat. Chem. 9, 731–742 (2017).
2. N. Miyaura, K. Yamada, A. Suzuki, A new stereospecific cross-coupling by the palladium-catalyzed reaction of 1-alkenylboronates with 1-alkenyl or 1-alkynyl halides. Tetrabedron Lett. 20, 3437–3440 (1979).
3. N. Miyaura, A. Suzuki, Palladium-catalyzed cross-coupling reactions of organoboron compounds. Chem. Rev. 95, 2457–2483 (1995).
4. N. A. Petasis, I. Akritopoulos, The boron acid manich reaction: A new method for the synthesis of geometrically pure allamidines. Tetrabedron Lett. 34, 583–586 (1993).
5. D. M. T. Chan, K. L. Monaco, R. P. Wang, M. P. Winters, New N- and O-arylations with phenylboronic acids and cupric acetate. Tetrahedron Lett. 39, 2933–2936 (1998).
6. P. Y. S. Lam, G. C. Clark, S. Saubern, J. Adams, M. P. Winters, D. M. T. Chan, A. Combs, New ary/heteroaryl C-N bond cross-coupling reactions via arylboronic acid/cupric acetate alyation. Tetrahedron Lett. 39, 2941–2944 (1998).
7. L. S. Liebeskind, J. Srogil, Thiol ester–boronic acid coupling. A mechanistically unprecedented and general ketone synthesis. J. Am. Chem. Soc. 122, 11260–11261 (2000).
8. Y. Chen, P. D. R. Murray, A. T. Davies, M. C. Willis, Direct copper-catalyzed three-component synthesis of sulfonamides. J. Am. Chem. Soc. 140, 8781–8787 (2018).
9. M. Lanier, D. C. Cole, Y. Istratiy, M. G. Klein, P. A. Schwartz, R. Tjhen, A. Jennings, M. S. Hixson, Repurposing Suzuki coupling reagents as a directed fragment library targeting serine hydrolases and related enzymes. J. Med. Chem. 60, 5209–5215 (2017).
10. Z. J. Lesnikowski, Recent developments with boron as a platform for novel drug design. Expert Opin. Drug Discovery 11, 569–578 (2016).
11. M. Grof, C. R. Berkers, H. L. Ploegh, H. Ovaa, Crystal structure of the boronic acid-based protease inhibitor bortezomib in complex with the yeast 20S proteasome. Structure 14, 451–456 (2006).
12. R. Smoum, A. Rubinstein, V. Y. Dembitskii, M. Srebniak, Boron containing compounds as protease inhibitors. Chem. Rev. 112, 4156–4220 (2012).
13. C. Li, J. Wang, L. M. Barton, S. Yu, M. Tian, D. S. Peters, M. Kumar, A. W. Yu, K. A. Johnson, A. K. Chatterjee, M. Yan, P. S. Baran, Decarboxylative borylation. Science 356, eaam7355 (2017).
14. M. J. Sharp, W. Cheng, V. Snieckus, Synthetic connections to the aromatic directed arylation. Functionalized arylic boronic acids by ipso borodesilylation. General syntheses of unsymmetrical dibenzenes and m-terphenyls. Tetrahedron Lett. 28, 5093–5096 (1987).
15. A. Domling, I. Ugi, Multicomponent reactions with isocyanides. Angew. Chem. Int. Ed. 39, 3168–3210 (2000).
16. P. Slobbe, E. Ruijter, R. V. A. Orru, Recent applications of multicomponent reactions in medicinal chemistry. MedChemComm. 3, 1189 (2012).
17. A. Domling, W. Wang, K. Wang, Chemistry and biology of multicomponent reactions. Chem. Rev. 112, 3083–3133 (2012).
18. A. Znabat, M. M. Polak, E. Janssen, F. J. J. de Kanter, N. J. Turner, R. V. A. Orru, E. Ruijter, A highly efficient synthesis of telaprevir by strategic use of biocatalysis and multicomponent reactions. Chem. Commun. 46, 7918–7920 (2010).
19. S. C. Solleder, M. A. R. Meier, Sequence control in polymer chemistry through the Passerini three-component reaction. Angew. Chem. Int. Ed. 53, 711–714 (2013).
20. Y. Yin, A. Scalia, L. Leroy, C. M. Cuttitta, G. M. Polizolo, D. L. Ericson, C. G. Roessler, O. Campos, M. Y. Ma, R. Agarwal, R. Jackimowicz, M. Allaire, A. M. Orville, R. M. Sweet, A. S. Soares, Hitting the target: Fragment screening with acoustic in situ co-crystallization of proteins plus fragment libraries on pin-mounted data-collection micromeshes. Acta Crystallogr., Sect. D: Struct. Biol. 70, 1177–1189 (2014).
21. R. Elson, Picoliter: Enabling precise transfer of nanoliter and picoliter volumes. Drug Discov. Today 7, 532–534 (2002).
22. N. R. Candeias, F. Montalbano, P. M. S. D. Cal, P. M. P. Gois, Boronic acids and esters in the Peterson–boron Mannich multicomponent reaction. Chem. Rev. 110, 6160–6193 (2010).

23. J. Tan, A. K. Yudin, Borolated reagents for multicomponent reactions. Drug Discov. Today Technol. 29, 51–60 (2018).

24. I. Ugi, C. Steinbrüchner, Über ein neues Kondensations-Prinzip. Angew. Chem. Int. Ed. 72, 267–268 (1960).

25. C. G. Neochoritis, N. R. Candeias, F. Montalbano, P. M. S. D. Cal, A. K. Yudin, Boronic acids and esters in the Peterson–boron Mannich multicomponent reaction. Chem. Rev. 119, 1970–2042 (2019).

26. K. Groebke, L. Weber, F. Mehlin, Synthesis of imidazo[1,2-a] pyridines. J. Org. Chem. 64, 10725–10729 (2017).

27. C. Blackburn, B. Guan, P. Fleming, K. Shiosaki, S. Tsai, Parallel synthesis of 3-aminoimidazo[1,2-l]pyridines and pyrazines by a new three-component condensation. Tetrahedron Lett. 39, 3635–3638 (1998).

28. H. Bienaymé, K. Bouzid, A new heterocyclic multicomponent reaction for the combinatorial synthesis of fused 3-aminoimidazoles. Angew. Chem. Int. Ed. 37, 2234–2237 (1998).

29. R. Madhavacharya, E. M. M. Abdelrahim, A. Rossetti, A. Twarda-Clapa, B. Musielak, K. Kurpiewska, J. Kalinowska-Tłuścik, A. T. Holak, A. Dömling, Two-step synthesis of complex artificial macrocyclic compounds. Angew. Chem. Int. Ed. 54, 7025–7029 (2015).

30. S. Lin, S. Dikier, W. D. Blinec, R. D. Ferguson, R. S. Shepard, Z. Pong, D. V. Conway, K. Zawatzky, H. Wang, T. Cermak, I. W. Davies, D. A. Dilocco, H. Sheng, C. J. Welch, S. D. Dreher, Mapping the dark space of chemical reactions with extended nanomole synthesis and MALDI-TOF MS. Science 361, eaar6236 (2018).

31. J. M. Gamrat, G. Mancini, S. J. Burke, R. C. Colandrea, N. R. Sadowski, B. C. Figula, J. W. Tomsho, Protection of the benzoazolone moiety: Synthesis and functionalization of aziridinobenzoxaboroles. J. Org. Chem. 83, 6193–6201 (2018).

32. N. Beresford, S. Patel, J. Armstrong, B. Söö, A. P. Fordham-Skelton, L. Tabenero, Mptpb, a virulence factor from Mycobacterium tuberculosis, exhibits triple-specificity phosphate activity. Biochem. J. 406, 13–18 (2007).

33. J. S. Lazo, K. E. McQueeney, E. R. Sharlow, New approaches to difficult drug targets: The phosphate story. SLAS Discov. Adv. Life Sci. R&D. 22, 1071–1083 (2017).

34. R. H. Holf, L. Wu, B. Zhou, Z.-Y. Zhang, A. C. Hengge, Does positive charge at the active site of protein phosphatases cause a change in mechanism? The effect of the conserved arginine on the transition state for phosphoryl transfer in the protein-tyrosine phosphate transferase from Yersinia. J. Am. Chem. Soc. 121, 9514–9522 (1999).

35. S. Guchhait, C. Madaan, B. Thakkar, A highly flexible and efficient Ugi-type multicomponent synthesis of versatile N-fused aminoimidazoles. Synthesis 2009, 3293–3300 (2009).

36. R.-C. Lian, M. H. Lin, P. H. Liao, I. J. Fu, M. J. Wu, Y. C. Wu, F. R. Chang, C. C. Wu, P. S. Pan, Direct synthesis of the arylboronic acid analogues of phenylglycine via microwave-assisted four-component Ugi reaction. Tetrahedron 70, 1800–1804 (2014).

37. N. Sangha, V. Jain, R. Preet, S. Kandekar, A. K. Yudin, u-Boryl isocyanides enable facile preparation of bioactive boropeptides. J. Org. Chem. 85, 8569–8573 (2013).

38. S. J. Kaldas, T. Rogova, G. V. Nenajdenko, A. K. Yudin, Modular synthesis of p53-Mdm2 protein tyrosine phosphate phosphatase. Angew. Chem., Int. Ed. 53, 7296–7302 (2014).

39. M. Gravel, K. A. Thompson, M. Zak, C. Bérubé, D. G. Hall, Universal solid-phase approach for the immobilization, derivatization, and resin-to-resin transfer reactions of boronic acids. J. Org. Chem. 67, 3–15 (2002).

40. A. Zajdlik, Z. Wang, J. L. Hickey, A. Amann, A. D. Schimmer, A. K. Yudin, u-Boryl isocyanides enable facile preparation of bioactive boropeptides. Angew. Chem. Int. Ed. 125, 209–214 (2013).

41. J. Zhang, P. Yu, S. Y. Li, H. Sun, Z. H. Xiang, J. J. Wang, K. N. Houk, A software program for pK(a) prediction and protonation state generation for drug-like molecules. J. Comput. Aided Mol. Des. 21, 681–691 (2007).

42. C. Grundner, D. Perrin, R. Hooft van Huijsduijnen, D. Swinnen, J. Gonzalez, C. L. Gee, T. N. Wells, T. Alber, Structural basis for selective inhibition of Mycobacterium tuberculosis protein tyrosine phosphate phosphatase. Structure 15, 499–509 (2007).

43. G. M. Sastry, M. Adzhigirey, T. Day, R. Annabhimoju, W. Sherman, Protein and ligand preparation: Parameters, protocols, and influence on virtual screening enrichments. J. Comput. Aided Mol. Des. 27, 221–234 (2013).

44. K. Zhu, K. W. Borrelli, J. R. Greenwood, T. Day, R. A. R. Selar, R. Harder, Docking covalent inhibitors: A parameter free approach to pose prediction and scoring. J. Chem. Inf. Model. 54, 1932–1940 (2014).

45. F. Fuller, S. Gul, R. Chatterjee, J. Kern, V. Yachandra, J. Yano, Community Contributed Protocol Exchange (2017). doi:10.1038/protex.2017.017.

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