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Therapeutic Targeting of Myc

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Abstract

Protein-protein interactions between members of the Myc transcription factor network are potential targets of small molecule inhibitors and stabilizers. Diverse screening strategies, including fluorescence resonance energy transfer, fluorescence polarization, two hybrid and protein complementation assays have identified several lead compounds that inhibit Myc-Max dimerization and one compound that stabilizes the Max homodimer. Representative compounds interfere with Myc-induced transcriptional activation, Myc-mediated oncogenic transformation, Myc-driven cellular replication and DNA binding of Myc. For the best characterized compounds, specific binding sites have been determined, and molecular mechanisms of action have been documented. This knowledge of small molecule – protein interaction is currently applied to highly targeted approaches that seek to identify novel compounds with improved potency.

Keywords

Max; Mad; ID proteins; 10058-F4

Introduction

The concept that the abnormal proliferation and metabolism of cancer cells could be exploited pharmacologically is actually quite old and represents the foundation upon which chemotherapeutic approaches have rested for half a century.[1–4] Only recently, however, have we begun to realize its true potential as a result of an improved understanding of the molecular underpinnings responsible for the cancer cell's replicative and survival advantages. This has led to the deliberate design of various inhibitors that attack tumor cells with a much higher degree of specificity and fewer side effects than previously attainable.[5–7] Despite the often stunning successes of this approach in previously refractory diseases, many obvious molecular targets have remained frustratingly immune to attack. In particular, these include transcription factors whose protein-protein or protein-DNA interactions that have been viewed as being intrinsically resistant to small molecules.[8–10] Here we review

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recent progress in therapies designed to target c-Myc (Myc). We weigh the arguments both for and against targeting this ubiquitously expressed and rarely mutated oncoprotein, discuss the various approaches currently being examined and their limitations, and propose how some of the therapeutic bottlenecks might be overcome. Finally, we evaluate the possibility for the use of Myc inhibitors in non-malignant states associated with cellular hyperproliferation.

Arguments against therapeutic targeting of Myc

A priori, there are numerous reasons to view Myc as a challenging therapeutic target. Among the practical and theoretical arguments typically encountered are that:

1. Myc is rarely mutated in cancer despite its high level of expression.[11, 12] Exceptions do occur in Burkitt's and AIDS-related lymphomas where approximately 30% of primary tumors carry amino acid substitutions in Myc.[13–17] The vast majority of these cluster around or directly affect Thr_{58} and affect Myc's half-life by interfering with its ubiquitination-mediated proteasomal degradation.[13, 18] However, the overall paucity of mutations dictates that the design of therapeutics with widespread potential be predicated on different paradigms than have been used so successfully to develop inhibitors of cancer-associated protein kinases, which typically possess gain-of-function mutations that distinguish them from their normal counterparts.[19–22] The development of such agents is guided by the long-established principles of enzyme inhibitor design and will have limited applicability to non-enzymatic proteins such as Myc.[23, 24]
2. Myc expression is a nearly universal property of all proliferating cells and its inhibition might be associated with unacceptable toxicities.
3. Myc inhibitor design will be difficult. The most obvious approaches such as targeting the association between Myc and Max or other essential co-factors such as TRRAP[25] involves the disruption of protein-protein interactions. The surfaces at which these occur tend to be large, flat, relatively featureless and often lack recognizable motifs or clefts such as those found in enzymes. The disruption of protein-protein interactions must also overcome a large free energy of association of the interacting protein moieties.[26–28]

Arguments for therapeutic targeting of Myc

Despite the foregoing objections, there are numerous reasons to believe that the general strategy of inhibiting Myc is both reasonable and attractive and that the potential benefits for pursuing this approach outweigh the concerns and disadvantages. These arguments thus serve to balance the list of disadvantages in the preceding section:

1. Despite the general lack of Myc protein mutations, most tumors are Myc dependent to varying degrees. For example, in a recent survey of over 20 human cancer lines, short hairpin-RNA-mediate depletion of Myc led to a permanent proliferative arrest in every case examined.[29] Consistent with this observation have been the findings obtained with conditional *in vivo* models indicating not only that continuous Myc expression is required to sustain tumor proliferation and viability [30–32] but that, in at least some cases, its re-instatement may actually lead to a seemingly paradoxical apoptotic response.[32] This has not been a universal finding however and certain types of types of tumors appear to lose their dependence on deregulated Myc.[33, 34] However, in none of these cases has their lack of dependence on endogenous Myc been established. Taken together, these studies suggest that at some level, whether it be normally expressed and regulated Myc or the deregulation that accompanies many tumors, Myc inhibition is likely to inhibit tumor progression and/or survival.

The possibility that tumor growth might be further impaired by the pro-apoptotic reestablishment of Myc expression suggests that long-term therapeutic of Myc may, at least in some circumstances, be neither warranted nor even desirable.

2. Myc expression by normal cells might not necessarily limit the use of Myc-based therapies. First, at any given time, most normal cells are quiescent, express little if any Myc, and thus might not be subject to the specific effects of Myc inhibitors. At the whole organism level, this could mean that the associated toxicities of such therapies would mimic those of more standard, non-targeted agents and thus include primarily hematopoietic and gastro-intestinal effects. Consistent with this idea are the findings of Soucek at al. who suppressed endogenous Myc via the tetracycline-regulatable conditional expression of a dominant-negative (DN) Myc protein in transgenic mice that were also engineered to develop K-ras^{G12D}-driven lung adenomas.[35] Although the anticipated pancytopenia, epidermal thinning, and intestinal villus attrition did occur quite quickly upon expression of the DN-Myc, these effects were well-tolerated, unassociated with overt toxicity, and largely reversible even when DN-Myc expression was allowed to persist. Unexpectedly, the mice showed a reduced incidence of adenomas, consistent with the concept that most if not all oncogenic signaling/proliferative pathways are likely to be converge upon Myc and that its inhibition results in nearly universal proliferative arrest.[36, 37]

3. The notion that protein-protein interactions might be refractory to small molecule inhibitors has gradually yielded to experimental evidence to the contrary. Indeed, the finding that single amino acid substitutions in the bHLH-ZIP dimerization domain of Myc could abolish its interaction with Max and abrogate its transcriptional activation function and biological properties [38, 39] speaks directly to this point and provided the initial impetus and rationale for Myc inhibitor discovery by one of our groups.[40] It is also now appreciated that the interaction between the flat surfaces of interacting domains is initiated by a limited number high-affinity interactions, which can sometimes account for the majority of the free energy of binding.[41] This implies that a small molecule capable of recognizing such a site has the potential for exerting a disproportionate effect on protein-protein interaction. Further evidence by analogy for the success of the small molecule approach has now been seen in other areas, including the disruption of TP53-HDM2 interactions by nutlins [10] and the inhibition of Bcl-2, Bcl-X_L and Bcl-w by small molecules BH3 mimetics such as ABT-737.[42,43]

4. Myc-based therapeutics rely upon a novel molecular target. They should therefore be compatible and largely non-cross resistant with many pre-existing chemotherapeutic agents.

Identification of inhibitors of Myc-Max dimeriztion (Myc inhibitors)

There are several *in vitro* and cell-based methods that can be used in screens to identify inhibitors of Myc-Max dimerization. A simple and straightforward *in vitro* technique is based on fluorescence resonance energy transfer (FRET). In this procedure, the basic helix-loop-helix leucine-zipper (bHLHZip) domain of Myc is fused to cyan fluorescent protein (CFP) and the bHLHZip domain of Max is fused to yellow fluorescent protein (YFP). The two proteins are allowed to dimerize, followed by excitation of CFP at the wavelength of 433 nm. Dimerization generates a FRET spectrum characterized by a strong emission signal of YFP at 525 nm and a weaker emission signal of CFP at 475 nm. The ratio of fluorescence intensity at 525 nm over 475 nm is typically 1.7 at complete dimerization of MycCFP with MaxYFP. The intensity ratio is 0.4 for the monomeric state of MycCFP. Inhibitors of Myc-Max dimerization reduce the ratio of fluorescence at 475 nm over that at 525 nm.[44] This *in vitro* technique measures the Myc-Max interaction specifically and directly.

A second *in vitro* technique that has been used in screens for inhibitors of Myc-Max dimerization is based on fluorescence polarization.[45, 46] An oligonucleotide containing several iterations of the Myc-Max consensus binding sequence (E-box) is labeled with a fluorophore (5-carboxyfluorescein, Alexa Fluor 633). This indicator is excited with plane polarized light. The polarization of the emitted light depends on the mobility of the molecule and that in turn is a function of molecular size. Binding of the fluorescent indicator to the Myc-Max dimer increases this size and enhances the fluorescence polarization. Among the advantages of this technique is simplicity; only one of the interacting components needs to be tagged with fluorophore. However, the technique does not discriminate between inhibitors of Myc-Max dimerization and inhibitors of Myc-Max DNA binding and will therefore identify some false positives that have to be eliminated by a different method.

Among the cell-based techniques, the two-hybrid method has been used successfully to isolate inhibitors of Myc-Max dimerization and is described below.[40]

A second *in vivo* technique is the protein complementation assay (PCA). The strategy of the PCA is to mediate the complementation of two β-lactamase fragments by two interacting proteins that are conjugated to the enzyme fragments. Animal cells are devoid of endogenous β-lactamase. Two separate β-lactamase fragments are conjugated to the bHLHLZ domains of Myc and Max.[47, 48] A flexible linker between Myc or Max and lactamase fragments facilitates re-association of the two enzyme fragments when Myc and Max dimerize. We have generated HEK293 cells stably transfected with these chimeric constructs. For the PCA, 1 M CCF2/AM is added to the cultures. CCF2/AM diffuses across the cell membrane and is hydrolyzed by cytoplasmic esterases, generating the β-lactamase substrate CCF2. Complementing fragments of β-lactamase hydrolyze CCF2, generating a coumarin fragment that emits blue fluorescence at 447 nm. The non-hydrolyzed CCF2 emits in the green spectrum at 530 nm. Controls include single transfectants (no lactamase), double transfectants without test compound (active lactamase) and double transfectants with a known Myc-Max inhibitor (reduced lactamase activity). FACS sorting at 447 nm is used for a quantitative determination of inhibitor potency which is reflected by the ratio of nonfluorescing cells to fluorescing cells.[49]

The FRET screening technique has been used in conjunction with two different chemical libraries. One is a peptidometic library generated by solution phase synthesis.[50] The second library, referred to as “credit card” library uses a planar, aromatic core that is further functionalized by the addition of highly diverse motifs. [51] These libraries yielded several inhibitors of Myc-Max dimerization. The lead compounds also interfere with the binding of Myc-Max to DNA in EMSA assays.[44, 51] They are active in reporter assays, inhibiting Myc-mediated but not N-Myc mediated transcriptional activation.[51, 52] For some of these compounds, interference with Myc-Max dimerization was also documented in ELISA assays that measure binding of GFP-coupled Myc to immobilized Max.[44] Oncogenic transformation induced in cultures of primary chicken embryonic cells by Myc is strongly inhibited by most but not all of the identified compounds. The IC_{50} of the Myc-Max inhibitors in all of these assays is between 10 and 50 μM. At higher inhibitor concentrations, cell growth is also negatively affected. The inhibition of oncogenic focus formation extends to Jun-, but not to Src-mediated transformation. Jun and Myc both use leucine zippers to dimerize, and the cross reaction of the inhibitors may reflect structurally similar motifs in Jun and in Myc that interact with the inhibitors. However, there is at present no evidence for this supposition, and more work is required to characterize the interaction between the compounds and their targets. Src-mediated transformation requires Myc function, and inhibitors of Myc are therefore expected to affect the transforming activity of Src.[53] However, under the conditions of the focus assay, the inhibitors never completely block Myc function. The residual Myc activity may be sufficient to support transformation by Src.

Several important questions about these inhibitors of Myc-Max dimerization remain to be answered. It is not known whether these inhibitors bind to Myc or to Max or to both proteins. Binding presumably targets the monomeric form, but the actual binding sites of the inhibitors on Myc or Max have not been determined.

Greater molecular details of the mechanism of inhibitor action have been worked out by Yin *et al.* who utilized a different approach for the identification of candidate compounds.[40] They developed a yeast two-hybrid-based approach in which heterodimerization between the bHLH-ZIP domains of Myc and Max would reconstitute a bipartite but functional Gal4 transcription factor, whose activity could be easily quantified by a β-galactosidase (β-gal) assay. They then screened ca. 10,000 small molecules for those that selectively reduced β-gal activity while having no effect on an identical yeast strain expressing Gal4 fusions of the Id2 and E47 HLH dimerization domains. This latter control allowed for the rapid elimination of compounds with non-specific effects on yeast growth, β-gal enzyme activity, or target gene binding by Gal4. Ultimately, seven compounds were identified whose specificity for Myc-Max inhibition was >90% as judged by their limited inhibition of >30 tested HLH, ZIP, and HLH-ZIP interactions in the same yeast background. A direct effect on Myc-Max association and DNA binding was demonstrated by glutathione-agarose pulldown experiments and electrophoretic mobility shift assays (EMSAs). Similar experiments demonstrated that the compounds both inhibited the formation of new heterodimers and disrupted pre-existing ones. Cells treated with these compounds also showed reduced expression of two different Myc-responsive reporters. Importantly, both normal fibroblasts and those transformed as a result of deregulated Myc expression were growth-inhibited whereas *myc*−/− fibroblasts were not.[54] Transformed fibroblasts also demonstrated markedly reduced tumorigenicity following exposure to the compounds.

The mechanism(s) of Myc compound binding

The above studies, including those of Berg *et al.*,[44] raised several important, and ultimately, related, questions. The first was whether Myc compounds were binding to Myc, Max, or the Myc-Max heterodimer. The second question was whether the compounds were binding to identical or distinct sites. Finally, could Myc compounds be used in combination to provide additive or synergistic effect?

With regard to the first question, the original yeast two-hybrid studies had indirectly implicated the Myc monomer as the site of compound binding since they minimally affected Max homodimerization or Max-Mad member heterodimerization.[40] Monomeric binding was also supported by the finding that the compounds prevented the *de novo* formation of Myc-Max heterodimers *in vitro*. The issue was settled definitely by Wang *et al.*,[37], Follis *et al.*,[55] and Hammoudeh *et al.*[56] who used a combination of fluorescence polarization and circular dichroism (CD) to provide direct physical evidence that the recombinant Myc bHLH-ZIP domain, but not the comparable domain of Max, bound every one of the seven compounds originally identified by Yin *et al*.[40] With regard to the second question, the possibility that Myc compounds were occupying different sites was suggested by their diverse chemical structures. The different patterns of non-specific interactions of these compounds with the numerous other HLH, ZIP, and HLH-ZIP yeast two-hybrid partners used as controls [40] implied this as well. The tools used to establish that all of the compounds were binding to monomeric Myc were also used to localize their exact binding sites via a series of highly purified recombinant Myc bHLH-ZIP deletion and point mutants. Distinct binding sites were identified for three of the original parental compounds: residues 363–381 for 10074-G5, residues 370–407 for 10074-A4, and residues 402–412 for 10058-F4). The first site corresponds to the junction of the basic domain and helix 1, the second site corresponds to the N-terminus of helix 1, and the third site corresponds to the junction of helix 2 and the

ZIP domain. The remaining four compounds bound either to the first site (10050-C10) or the third site (10075-G5, 1009-G9 and 10031-B8). Final confirmation of binding site assignments came from experiments with synthetic peptides whose calculated Kd's for the compounds were in good agreement with those obtained using the longer recombinant bHLH-ZIP proteins (Fig. 1).[37, 55, 56] It will certainly be of interest to determine whether other previously identified Myc inhibitors,[44–46] or those identified in the future, show similar properties. The intrinsically disordered (ID) nature of these three sites suggests that they may be particularly well-adapted as targets for many, if not all, small molecule Myc inhibitors.

As to whether individual compounds could be used more effectively in combination, we observed little more than an additive effect when those with distinct binding site preferences were tested (Wang and Prochownik, unpublished). However, as described in the next section, much different results have been obtained when these compounds were joined via chemical cross-linking to create bi-valent compounds.

Improving Myc compound potency

None of the originally identified Myc compounds bound its cognate site with particularly high affinity. For example the parental compounds 10058-F4 and 10074-G5 showed Kd's of ca. 2.5 μM and ca. 4.4 μM, respectively, as determined by fluorescence polarization.[37, 55] Similarly, binding of the non-fluorescent compound 10074-A4 to its cognate site, which was determined by CD, was calculated to be ca. 21 μM.[56] These values were generally somewhat lower than the empirically determined IC_{50} values in several cell types, which ranged from ca. 20–50 μM.[37, 40] Given that compounds with such low affinities are unlikely to be viable therapeutic candidates, considerable effort has gone into deriving more potent analogs. Three distinct types of approaches have been taken:

1. The "random analog approach". Initially lacking any knowledge of the structure of these compounds in association with their Myc binding sites, a "random analog" screen was conducted to identify more potent structural relatives of the parental compound 10058-F4, which is chemically the simplest of the original seven parental Myc compounds.[40] 10058-F4 contains a six member ethylbenzylidine ring and a five member thioxothiazolidin-4-one, or rhodanine ring. 48 "second generation" analogs containing alterations of only the first ring and 15 analogs with alterations of only the rhodanine ring were screened using a number of independent assays including the aforementioned fluorescence polarization tests, electrophoretic mobility shifts (EMSAs), co-immunoprecipitations from compound-treated cells and cell growth inhibition assays. The overall best of the six-member ring analogs were then combined with the best five-member ring analogs to generate "third generation' compounds with alterations in each ring. In all, a total of 80 chemically distinct 10058-F4 analogs were analyzed.

   Despite some fairly radical departures from the base 10058-F4 structure,[37] a surprisingly large number of analogs retained activity in all assays. However only four analogs had Ka's for recombinant Myc bHLH-ZIP domain that were significantly better than that of 10058-F4, and the best of these was only four-fold better. Moreover, binding affinities did not necessarily correlate with the ability of the compounds to inhibit Myc-Max heterodimerization in intact cells and block proliferation. For example the 6-member ring substituted analog with the best affinity for Myc (28Rh, Ka=1.0 μM) had an IC_{50} in HL60 promyelocytic leukemia cells that was only modestly better than that of 10058-F4 (36 μM vs. 49 μM).[37] In contrast, #764, a five-member ring analog with the best IC_{50} (6.5 μM), actually had a slightly inferior binding affinity relative to that of 10058-F4. The imperfect

correlation between compound binding and growth inhibition probably reflects the differences in biological behaviors among the various analogs, including rates of influx and efflux, metabolism, and intracellular trafficking and non-specific protein binding.

2. Pharmacophore model screens. Subsequent to above-described work, NMR models of representative parental compounds (10058-F4, 10074-G5, and 10074-A4) bound to their cognate synthetic peptides or a short, recombinant segment of the Myc bHLH-ZIP domain were generated.[55, 56] The intrinsically disordered (ID) nature of all three binding sites does not allow these to be represented as actual structures; rather they serve as the best average approximations of the ensemble of dynamic structures most likely to exist at any given time.

The previous studies had yielded a large amount of structure-activity relationships for the active and inactive analogs of 10058-F4.[37, 40] These were next used to generate a molecule-derived pharmacophore model that incorporated these relationships using the GALAHAD program (Genetic Algorithm with Linear Assignment for Hypermolecular Alignment of Datasets) [57, 58] and were further refined using the Tuplets model in SYBYL 8.0 (SYBYL 8.0. www.tripos.com). *In silico* screening of the ZINC database of ca. 5×10^6 compounds [59] initially identified a large number of structurally diverse molecules as determined by their Tanimoto scores of 0.5. This group was ultimately filtered down to a set of 30 compounds with the most desirable ADME properties and then further reduced to nine, which retained their structural diversity and which were viewed as poor substrates for the cytochrome P450 isoform CYP3A4, the major human enzyme responsible for xenobiotic metabolism.[60] Seven of the nine compounds showed activity at high concentrations when tested against pre-formed Myc-Max heterodimers using CD. The four best compounds competing for Max in this assay were generally 2-10-fold better than 10058-F4. They also competed 10058-F4 from its binding site as determined by fluorescence polarization [37, 55] and were able to inhibit DNA binding by Myc-Max heterodimers in an EMSA assay. However, the calculated affinities of the compounds for Myc in these latter two assays were not significantly greater than those for 10058-F4. Finally, when tested in cell proliferation assays, none of the tested compounds were more than two-fold better than 10058-F4. As had been the case with the 10058-F4-derived “random analog” compounds,[37] this likely reflected the more complex nature of the biologically-based assay where inhibition of cell proliferation reflects the balance of many factors other than compound binding, which is the only parameter evaluated in simple, two component-based CD and fluorescence polarization assays.

It seems likely that the failure of the pharmacophore model-based approach to identify molecules with greater biological activity is a measure of both the limited number of compounds that were interrogated as well as imperfections of the model itself. Improving the model by incorporating new and structurally diverse compounds as they become available is likely to increase the rate at which more potent inhibitors are detected. Despite these shortcomings, there have been two dividends of this type of approach, particularly when considered in light of the “random analog” approach. The first has simply been the demonstration that the overall pharmacophore model approach for identifying Myc compounds is viable and reasonably robust. The second has revealed the surprising structural diversity of Myc inhibitors (Fig. 2).

3. Link compounds. The revelation that that many, if not all, Myc compounds bind to monomeric Myc has allowed for a novel approach to identify more potent Myc compounds. This involved the linking together of two small molecules with

different sites to create a bivalent molecule.[61, 62] The idea that such an approach was feasible was supported by the fact that the closely related monomeric bHLH-ZIP domain of v-Myc (and presumably c-Myc as well) is relatively unstructured in solution [63] and assumes a rigid conformation only upon associating with Max.[63, 64] The lack of a well-defined structure of the monomeric Myc bHLH-ZIP domain would be expected to provide sufficient conformational freedom so as to allow it interact readily with even the most rigid bivalent molecule.

To this end, a series of so-called "Link" compounds has recently been generated in which parental Myc inhibitors 10058-F4 and 10074-G5 have been joined at various sites by flexible aliphatic linkers of variable composition and length (Follis *et al.*, in preparation). As expected from the model of the unstructured Myc bHLH-ZIP domain, all Link compounds have demonstrated impressive increases in activity relative to either of the individual monovalent components. In the best case, up to a 5000-fold improvements in binding affinity have been observed, representing >1000-fold better affinity for Myc than the affinity of Max for Myc. It seems likely that there are at least two potential and non-mutually exclusive explanations for these remarkable increases in binding affinities. First, the binding of either moiety to its cognate site on Myc increases the local concentration and the dwell time of the second linked moiety near its binding site. Second, the off-rate of either moiety is countered by virtue of being tethered to its still bound companion moiety. Link compounds provide an immediate solution to the potency problem as high pM-low nM affinities are now readily achieveable. Preliminary results indicate that cellular uptake still remains disproportionately poor but is nevertheless up to 100-fold better than that of even those most potent monovalent compounds thus far discovered. Thus far, Link compounds have been prepared only with parental forms of Myc compounds.[40] Further improvements can be expected when higher affinity monovalent binders are used as Link substrates.

Myc and intrinsic disorder

The finding that the structures of many effective Myc compounds deviated significantly from that of the original parental compound 10058-F4 (Fig. 2), was initially confusing but ultimately understandable in light of the intrinsically disordered (ID) nature of all three Myc binding sites.[56] ID regions have been defined as highly unstructured, dynamic and constantly shifting segments of amino acids that assume an ordered state only upon binding to specific targets, which can themselves be of variable structure. That the binding of ID regions can alter biological function conflicts with prevailing concepts of structure-function paradigms.[65] ID regions are common and appear to be particularly abundant in signaling molecules and transcription factors where they have been proposed to play important roles in regulating their activity.[66, 67] The mapping of Myc compound binding sites to ID regions, together with the frequency with which such sites appear in many transcription factors [66] suggests that the design of small molecule inhibitors of transcription factors will be able to take advantage of ID regions and that the presence of such regions will serve as guides to future drug discovery efforts.

In vivo Myc compound efficacy and metabolism

Thus far, only a single study has assessed the metabolism and/or efficacy of small molecule Myc compounds *in vivo*. Guo *et al.* have studied 10058-F4 in SCID mice bearing xenografts of human PC3 and DU145 prostate cancer cell lines.[68] Although the compound exhibited a high maximally-tolerated dose, it had a short terminal half life of approximately 1 hr and showed extremely rapid breakdown into at least eight metabolites. Highest concentrations of the compound were seen in liver, lung, kidney and fat and peak tumor concentrations were

at least ten-fold lower than those achieved in serum. No significant effects on tumor growth were observed over a two-week period of 5 times/wk i.p treatment. The lack of efficacy was attributed to a combination of rapid metabolism and a failure to reach adequate intratumoral levels. Preliminary studies with the compound 10074-G5 have shown similar rapid metabolism (Liggett *et al.*, in preparation).

The development of more potent Myc compounds whether they be new analogs of monovalent compounds or new Link molecules will require eventual pharmacologic testing that is likely to represent a significant bottleneck to the development of these agents for clinical use. Alternate means of delivery such as encapsulation in liposomes may help to circumvent some of these bottlenecks. Alternatively, direct local delivery, perhaps at a surgical site of excised tumor and perhaps embedded in a bio-degradeable matrix may provide a more protected environment, allow a higher concentration of compound to be attained, and extend compound life-span so as to achieve more pronounced anti-proliferative effects.

The potential for Myc inhibitors in non-neoplastic settings

Interest in Myc inhibition is clearly driven by the potential for its clinical application in the oncologic setting. However, based upon current understanding of Myc action and the known mechanisms of the various Myc inhibitors discussed above, there is reason to believe that these agents might prove equally attractive for the treatment of certain non-neoplastic disease states associated with cellular hyperproliferation. Arterial restenosis and proliferative retinopathy are common conditions that might prove amenable to Myc inhibitors. In each case, effective targeting would involve action of the compounds working in confined spaces and/or over short distances, thus allowing much smaller doses of the inhibitor to be delivered and eliminating or greatly reducing some of the potential side effects and metabolism issues associated with the more systemic delivery approaches.[68]

Balloon angioplasty with the concurrent placement of drug-eluting stents (DES) has emerged as the non-surgical treatment of choice for arterial stenosis.[69] The purpose of DES is to prevent the neointimal hyperplasia and inflammatory response that frequently occurs as a result of local injury induced both by the angioplasty itself and the placement and/or continued presence of the stent. Currently, the most commonly employed drugs in DES included Paclitaxel and sirolimus, with some advantage to the latter having recently been documented.[70] Animal models of restenosis have shown that locally administered Myc antisense oligonucleotides, can lead to improvements in restenosis rates despite achieving only modest reductions in Myc levels.[71, 72] Preliminary results in humans have also shown promise.[73] The results are encouraging enough to consider the far less costly use of small molecule Myc inhibitors that could either replace or be used in conjunction with any of these other approaches and would likely achieve superior levels of Myc inhibition.

Age-related macular degeneration and diabetic retinopathy are the most common causes of blindness in the adult population and are associated with a high degree of endothelial proliferation.[74, 75] Anti VEGF therapies are effective forms of therapy but are expensive, require frequent intravitreal injection, and can be associated with complications such as retinal detachment.[76] The direct delivery of low molecular weight Myc inhibitors would be significantly less expensive. Intravitreal compounds might also show significantly longer half-lives by virtue of being protected from the rapid metabolic breakdown that has thus far been seen with systemically administered Myc inhibitors.

Stabilizers of the Max homodimer

Unlike Myc the Max protein can homodimerize *in vitro* and *in vivo*.[77, 78] Max homodimers are less stable than its heterodimers.[63] The reduced stability of the Max homodimer reflects a packing defect at its protein-protein interface.[64] Max homodimers do not affect transcription except if they are overexpressed with the help of exogenous vectors.[79] Overexpressed Max can then repress transcription and interfere with Myc-induced oncogenesis.[80–82]

The Max-Max homodimers function as the cellular repository for Max which is the essential and universal partner for all other proteins of the Myc network. Because of the relative instability of the Max-Max homodimer, and because Max levels usually exceed those of its partners, Max is readily captured by its heterodimer partners into more stable complexes that function in transcriptional control. Among these partners, Myc is the one that can be highly overexpressed, notably in cancer cells. High levels of Myc require an abundance of Max to become functional. Stabilization of the Max-Max homodimer could therefore affect preferentially such overexpressed Myc and attenuate its oncogenic effects while still allowing life-sustaining functions of other Myc network heterodimers.

Max-Max stabilizers have been identified by virtual ligand screening, using the AutoDock program.[83] Virtual ligand screening has been used to predict inhibitors of protein-protein interactions.[84–87] This type of screening is dependent on structural information for the target molecules. The monomeric form of Myc is only partially structured, and this is probably also true for Max.[63] In contrast, the Myc-Max and Max-Max dimers are highly structured and are therefore suitable targets for *in silico* docking screens.[64] Such screens with the AutoDock suite canvassed about 1700 compounds from the NCI Diversity Set. Potential ligands were identified by their low docking energies and were clustered according to three predicted binding sites: a site between the DNA-binding helices of the Max-Max dimer, the basic and the adjacent neutral HLH region and the intersection of the leucine zipper and the HLH region. The latter cluster yielded ligands that showed specificity for the Max-Max homodimer and did not interact with the Myc-Max heterodimer. One of the compounds identified by virtual ligand screening, NSC13728, is a particularly effective stabilizer of Max-Max as documented by FRET. Stabilization is independent of interaction with DNA. In ELISA and Surface Plasmon Resonance assays, the stabilizer inhibits the binding of MycCFP to immobilized Max. Analytical ultracentrifugation suggests that the stabilizer strongly reduces the Kd of the dimer, confirming stabilization. Compound NSC13728 also strongly interferes with Myc-mediated oncogenic transformation in cell culture while not affecting transformation induced by Jun, Src or PI3K. Myc-induced transcriptional activation of specific target genes is also inhibited. Important questions about the mechanism of action of the Max-Max stabilizer still remain to be answered. Although virtual ligand screening suggests a binding site for the stabilizer, that site has not been experimentally verified. A priori reasoning would suggest that the binding of the stabilizer is to the dimer, not the monomer, a view supported by the structural differences between dimer and monomer.[64]

Conclusion

The inhibitors of Myc-Max and stabilizers of Max-Max constitute a promising beginning. These small molecules can interfere with all Myc functions, with the stimulation of cell replication, with DNA binding, with transcriptional activation and with oncogenic transformation. For some of these compounds, binding sites have been determined and probable mechanisms of action have been established. Promising pharmacophores have been identified. The stage is set for further progress which will concentrate on enhanced potency

and greater mechanistic understanding. The ultimate goal remain compounds that are therapeutically effective in clinical situations that involve gain of function in Myc.

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References

1. Brues AM, Jacobson LO. Comparative therapeutic effects of radioactive and chemical agents in neoplastic diseases of the hemopoietic system. Am J Roentgenol Radium Ther 1947;58:774–782.
2. Farber S, Diamond LK. Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid. N Engl J Med 1948;238:787–793. [PubMed: 18860765]
3. Huggins C. Endocrine Control of Prostatic Cancer. Science 1943;97:541–544. [PubMed: 17773799]
4. Mashburn LT, Wriston JC Jr. Tumor Inhibitory Effect of L-Asparaginase from Escherichia Coli. Arch Biochem Biophys 1964;105:450–452. [PubMed: 14186753]
5. Dhomen N, Marais R. BRAF signaling and targeted therapies in melanoma. Hematol Oncol Clin North Am 2009;23:529–545. ix. [PubMed: 19464601]
6. Druker BJ. Translation of the Philadelphia chromosome into therapy for CML. Blood 2008;112:4808–4817. [PubMed: 19064740]
7. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344:783–792. [PubMed: 11248153]
8. Deng J, Grande F, Neamati N. Small molecule inhibitors of Stat3 signaling pathway. Curr Cancer Drug Targets 2007;7:91–107. [PubMed: 17305481]
9. Diaz-Gonzalez JA, Russell J, Rouzaut A, Gil-Bazo I, Montuenga L. Targeting hypoxia and angiogenesis through HIF-1alpha inhibition. Cancer Biol Ther 2005;4:1055–1062. [PubMed: 16294030]
10. Patel S, Player MR. Small-molecule inhibitors of the p53-HDM2 interaction for the treatment of cancer. Expert Opin Investig Drugs 2008;17:1865–1882.
11. Lee JW, Soung YH, Kim SY, Nam SW, Park WS, Lee JY, et al. Mutational analysis of MYC in common epithelial cancers and acute leukemias. APMIS 2006;114:436–439. [PubMed: 16856965]
12. Nesbit CE, Tersak JM, Prochownik EV. MYC oncogenes and human neoplastic disease. Oncogene 1999;18:3004–3016. [PubMed: 10378696]
13. Bahram F, von der Lehr N, Cetinkaya C, Larsson LG. c-Myc hot spot mutations in lymphomas result in inefficient ubiquitination and decreased proteasome-mediated turnover. Blood 2000;95:2104–2110. [PubMed: 10706881]
14. Bhatia K, Huppi K, Spangler G, Siwarski D, Iyer R, Magrath I. Point mutations in the c-Myc transactivation domain are common in Burkitt's lymphoma and mouse plasmacytomas. Nat Genet 1993;5:56–61. [PubMed: 8220424]
15. Clark HM, Yano T, Otsuki T, Jaffe ES, Shibata D, Raffeld M. Mutations in the coding region of c-MYC in AIDS-associated and other aggressive lymphomas. Cancer Res 1994;54:3383–3386. [PubMed: 8012955]
16. Pulverer BJ, Fisher C, Vousden K, Littlewood T, Evan G, Woodgett JR. Site-specific modulation of c-Myc cotransformation by residues phosphorylated in vivo. Oncogene 1994;9:59–70. [PubMed: 8302604]

17. Yano T, Sander CA, Clark HM, Dolezal MV, Jaffe ES, Raffeld M. Clustered mutations in the second exon of the MYC gene in sporadic Burkitt's lymphoma. Oncogene 1993;8:2741–2748. [PubMed: 8397370]
18. Gregory MA, Hann SR. c-Myc proteolysis by the ubiquitin-proteasome pathway: stabilization of c-Myc in Burkitt's lymphoma cells. Mol Cell Biol 2000;20:2423–2435. [PubMed: 10713166]
19. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer 2009;9:550–562. [PubMed: 19629070]
20. Kantarjian HM, Cortes J, La Rosee P, Hochhaus A. Optimizing therapy for patients with chronic myelogenous leukemia in chronic phase. Cancer 2010;116:1419–1430. [PubMed: 20120030]
21. McCubrey JA, Steelman LS, Abrams SL, Chappell WH, Russo S, Ove R, et al. Emerging Raf inhibitors. Expert Opin Emerg Drugs 2009;14:633–648. [PubMed: 19715444]
22. Vogt PK, Gymnopoulos M, Hart JR. PI 3-kinase and cancer: changing accents. Curr Opin Genet Dev 2009;19:12–17. [PubMed: 19185485]
23. Ross DM, Hughes TP. Cancer treatment with kinase inhibitors: what have we learnt from imatinib? Br J Cancer 2004;90:12–19. [PubMed: 14710199]
24. Thaimattam R, Banerjee R, Miglani R, Iqbal J. Protein kinase inhibitors: structural insights into selectivity. Curr Pharm Des 2007;13:2751–2765. [PubMed: 17897021]
25. Cowling VH, Cole MD. Mechanism of transcriptional activation by the Myc oncoproteins. Semin Cancer Biol 2006;16:242–252. [PubMed: 16935524]
26. Huang Z. Structural chemistry and therapeutic intervention of protein-protein interactions in immune response, human immunodeficiency virus entry, and apoptosis. Pharmacol Ther 2000;86:201–215. [PubMed: 10882809]
27. Jones S, Thornton JM. Principles of protein-protein interactions. Proc Natl Acad Sci U S A 1996;93:13–20. [PubMed: 8552589]
28. Zutshi R, Brickner M, Chmielewski J. Inhibiting the assembly of protein-protein interfaces. Curr Opin Chem Biol 1998;2:62–66. [PubMed: 9667909]
29. Wang H, Mannava S, Grachtchouk V, Zhuang D, Soengas MS, Gudkov AV, et al. c-Myc depletion inhibits proliferation of human tumor cells at various stages of the cell cycle. Oncogene 2008;27:1905–1915. [PubMed: 17906696]
30. Felsher DW. Reversibility of oncogene-induced cancer. Curr Opin Genet Dev 2004;14:37–42. [PubMed: 15108803]
31. Felsher DW, Bishop JM. Reversible tumorigenesis by MYC in hematopoietic lineages. Mol Cell 1999;4:199–207. [PubMed: 10488335]
32. Jain M, Arvanitis C, Chu K, Dewey W, Leonhardt E, Trinh M, et al. Sustained loss of a neoplastic phenotype by brief inactivation of MYC. Science 2002;297:102–104. [PubMed: 12098700]
33. Boxer RB, Jang JW, Sintasath L, Chodosh LA. Lack of sustained regression of c-MYC-induced mammary adenocarcinomas following brief or prolonged MYC inactivation. Cancer Cell 2004;6:577–586. [PubMed: 15607962]
34. Karlsson A, Giuriato S, Tang F, Fung-Weier J, Levan G, Felsher DW. Genomically complex lymphomas undergo sustained tumor regression upon MYC inactivation unless they acquire novel chromosomal translocations. Blood 2003;101:2797–2803. [PubMed: 12517816]
35. Soucek L, Whitfield J, Martins CP, Finch AJ, Murphy DJ, Sodir NM, et al. Modelling Myc inhibition as a cancer therapy. Nature 2008;455:679–683. [PubMed: 18716624]
36. Bazarov AV, Adachi S, Li SF, Mateyak MK, Wei S, Sedivy JM. A modest reduction in c-myc expression has minimal effects on cell growth and apoptosis but dramatically reduces susceptibility to Ras and Raf transformation. Cancer Res 2001;61:1178–1186. [PubMed: 11221849]
37. Wang H, Hammoudeh DI, Follis AV, Reese BE, Lazo JS, Metallo SJ, et al. Improved low molecular weight Myc-Max inhibitors. Mol Cancer Ther 2007;6:2399–2408. [PubMed: 17876039]
38. Bello-Fernandez C, Packham G, Cleveland JL. The ornithine decarboxylase gene is a transcriptional target of c-Myc. Proc Natl Acad Sci U S A 1993;90:7804–7808. [PubMed: 8356088]

39. Smith MJ, Charron-Prochownik DC, Prochownik EV. The leucine zipper of c-Myc is required for full inhibition of erythroleukemia differentiation. Mol Cell Biol 1990;10:5333–5339. [PubMed: 2204813]
40. Yin X, Giap C, Lazo JS, Prochownik EV. Low molecular weight inhibitors of Myc-Max interaction and function. Oncogene 2003;22:6151–6159. [PubMed: 13679853]
41. Clackson T, Wells JA. A hot spot of binding energy in a hormone-receptor interface. Science 1995;267:383–386. [PubMed: 7529940]
42. Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 2005;435:677–681. [PubMed: 15902208]
43. Sasi N, Hwang M, Jaboin J, Csiki I, Lu B. Regulated cell death pathways: new twists in modulation of BCL2 family function. Mol Cancer Ther 2009;8:1421–1429. [PubMed: 19509269]
44. Berg T, Cohen SB, Desharnais J, Sonderegger C, Maslyar DJ, Goldberg J, et al. Small-molecule antagonists of Myc/Max dimerization inhibit Myc-induced transformation of chicken embryo fibroblasts. Proc Natl Acad Sci U S A 2002;99:3830–3835. [PubMed: 11891322]
45. Kiessling A, Wiesinger R, Sperl B, Berg T. Selective inhibition of c-Myc/Max dimerization by a pyrazolo[1,5-a]pyrimidine. ChemMedChem 2007;2:627–630. [PubMed: 17315254]
46. Kiessling A, Sperl B, Hollis A, Eick D, Berg T. Selective inhibition of c-Myc/Max dimerization and DNA binding by small molecules. Chem Biol 2006;13:745–751. [PubMed: 16873022]
47. Galarneau A, Primeau M, Trudeau LE, Michnick SW. Beta-lactamase protein fragment complementation assays as in vivo and in vitro sensors of protein protein interactions. Nat Biotechnol 2002;20:619–622. [PubMed: 12042868]
48. Wehrman T, Kleaveland B, Her JH, Balint RF, Blau HM. Protein-protein interactions monitored in mammalian cells via complementation of beta -lactamase enzyme fragments. Proc Natl Acad Sci U S A 2002;99:3469–3474. [PubMed: 11904411]
49. Lee HK, Brown SJ, Rosen H, Tobias PS. Application of beta-lactamase enzyme complementation to the high-throughput screening of toll-like receptor signaling inhibitors. Mol Pharmacol 2007;72:868–875. [PubMed: 17615244]
50. Boger DL, Lee JK, Goldberg J, Jin Q. Two comparisons of the performance of positional scanning and deletion synthesis for the identification of active constituents in mixture combinatorial libraries. J Org Chem 2000;65:1467–1474. [PubMed: 10814111]
51. Xu Y, Shi J, Yamamoto N, Moss JA, Vogt PK, Janda KD. A credit-card library approach for disrupting protein-protein interactions. Bioorg Med Chem 2006;14:2660–2673. [PubMed: 16384710]
52. Lu X, Vogt PK, Boger DL, Lunec J. Disruption of the MYC transcriptional function by a small-molecule antagonist of MYC/MAX dimerization. Oncol Rep 2008;19:825–830. [PubMed: 18288422]
53. Bowman T, Broome MA, Sinibaldi D, Wharton W, Pledger WJ, Sedivy JM, et al. Stat3-mediated Myc expression is required for Src transformation and PDGF-induced mitogenesis. Proc Natl Acad Sci U S A 2001;98:7319–7324. [PubMed: 11404481]
54. Mateyak MK, Obaya AJ, Adachi S, Sedivy JM. Phenotypes of c-Myc-deficient rat fibroblasts isolated by targeted homologous recombination. Cell Growth Differ 1997;8:1039–1048. [PubMed: 9342182]
55. Follis AV, Hammoudeh DI, Wang H, Prochownik EV, Metallo SJ. Structural rationale for the coupled binding and unfolding of the c-Myc oncoprotein by small molecules. Chem Biol 2008;15:1149–1155. [PubMed: 19022175]
56. Hammoudeh DI, Follis AV, Prochownik EV, Metallo SJ. Multiple independent binding sites for small-molecule inhibitors on the oncoprotein c-Myc. J Am Chem Soc 2009;131:7390–7401. [PubMed: 19432426]
57. Richmond NJ, Abrams CA, Wolohan PR, Abrahamian E, Willett P, Clark RD. GALAHAD: 1. pharmacophore identification by hypermolecular alignment of ligands in 3D. J Comput Aided Mol Des 2006;20:567–587. [PubMed: 17051338]

58. Shepphird JK, Clark RD. A marriage made in torsional space: using GALAHAD models to drive pharmacophore multiplet searches. J Comput Aided Mol Des 2006;20:763–771. [PubMed: 17016746]
59. Irwin JJ, Shoichet BK. ZINC--a free database of commercially available compounds for virtual screening. J Chem Inf Model 2005;45:177–182. [PubMed: 15667143]
60. Wrighton SA, Schuetz EG, Thummel KE, Shen DD, Korzekwa KR, Watkins PB. The human CYP3A subfamily: practical considerations. Drug Metab Rev 2000;32:339–361. [PubMed: 11139133]
61. Erlanson DA, Wells JA, Braisted AC. Tethering: fragment-based drug discovery. Annu Rev Biophys Biomol Struct 2004;33:199–223. [PubMed: 15139811]
62. Liu J, Begley D, Mitchell DD, Verlinde CL, Varani G, Fan E. Multivalent drug design and inhibition of cholera toxin by specific and transient protein-ligand interactions. Chem Biol Drug Des 2008;71:408–419. [PubMed: 18373548]
63. Fieber W, Schneider ML, Matt T, Krautler B, Konrat R, Bister K. Structure, function, and dynamics of the dimerization and DNA-binding domain of oncogenic transcription factor v-Myc. J Mol Biol 2001;307:1395–1410. [PubMed: 11292350]
64. Nair SK, Burley SK. X-ray structures of Myc-Max and Mad-Max recognizing DNA. Molecular bases of regulation by proto-oncogenic transcription factors. Cell 2003;112:193–205. [PubMed: 12553908]
65. Uversky VN, Oldfield CJ, Dunker AK. Showing your ID: intrinsic disorder as an ID for recognition, regulation and cell signaling. J Mol Recognit 2005;18:343–384. [PubMed: 16094605]
66. Miyamoto-Sato E, Fujimori S, Ishizaka M, Hirai N, Masuoka K, Saito R, et al. A comprehensive resource of interacting protein regions for refining human transcription factor networks. PLoS One 2010;5:e9289. [PubMed: 20195357]
67. Sigalov AB. Protein intrinsic disorder and oligomericity in cell signaling. Mol Biosyst 2010;6:451–461. [PubMed: 20174674]
68. Guo J, Parise RA, Joseph E, Egorin MJ, Lazo JS, Prochownik EV, et al. Efficacy, pharmacokinetics, tisssue distribution, and metabolism of the Myc-Max disruptor, 10058-F4 [Z,E]-5-[4-ethylbenzylidine]-2-thioxothiazolidin-4-one, in mice. Cancer Chemother Pharmacol 2009;63:615–625. [PubMed: 18509642]
69. Giordano A. Molecular basis of different outcomes for drug-eluting stents that release sirolimus or tacrolimus. Curr Opin Drug Discov Devel 2010;13:159–168.
70. Zhang F, Dong L, Ge J. Meta-analysis of five randomized clinical trials comparing sirolimus- versus paclitaxel-eluting stents in patients with diabetes mellitus. Am J Cardiol 2010;105:64–68. [PubMed: 20102892]
71. De Feo M, Forte A, Onorati F, Renzulli A, Cipollaro M, Cotrufo M, et al. Rat carotid arteriotomy: c-myc is involved in negative remodelling and apoptosis. J Cardiovasc Med (Hagerstown) 2006;7:61–67. [PubMed: 16645362]
72. Forte A, Galderisi U, De Feo M, Gomez MF, Esposito S, Sante P, et al. c-Myc antisense oligonucleotides preserve smooth muscle differentiation and reduce negative remodelling following rat carotid arteriotomy. J Vasc Res 2005;42:214–225. [PubMed: 15849475]
73. Kipshidze N, Tsapenko M, Iversen P, Burger D. Antisense therapy for restenosis following percutaneous coronary intervention. Expert Opin Biol Ther 2005;5:79–89. [PubMed: 15709911]
74. Andreoli CM, Miller JW. Anti-vascular endothelial growth factor therapy for ocular neovascular disease. Curr Opin Ophthalmol 2007;18:502–508. [PubMed: 18163003]
75. Emerson MV, Lauer AK. Emerging therapies for the treatment of neovascular age-related macular degeneration and diabetic macular edema. BioDrugs 2007;21:245–257. [PubMed: 17628122]
76. Figueroa MS, Contreras I, Noval S. Anti-angiogenic drugs as an adjunctive therapy in the surgical treatment of diabetic retinopathy. Curr Diabetes Rev 2009;5:52–56. [PubMed: 19199899]
77. Blackwood EM, Eisenman RN. Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. Science 1991;251:1211–1217. [PubMed: 2006410]
78. Blackwood EM, Luscher B, Eisenman RN. Myc and Max associate in vivo. Genes Dev 1992;6:71–80. [PubMed: 1730411]

79. Yin X, Grove L, Prochownik EV. Lack of transcriptional repression by max homodimers. Oncogene 1998;16:2629–2637. [PubMed: 9632139]

80. Lindeman GJ, Harris AW, Bath ML, Eisenman RN, Adams JM. Overexpressed max is not oncogenic and attenuates myc-induced lymphoproliferation and lymphomagenesis in transgenic mice. Oncogene 1995;10:1013–1017. [PubMed: 7898919]

81. Cogliati T, Dunn BK, Bar-Ner M, Cultraro CM, Segal S. Transfected wild-type and mutant max regulate cell growth and differentiation of murine erythroleukemia cells. Oncogene 1993;8:1263–1268. [PubMed: 8479748]

82. Kretzner L, Blackwood EM, Eisenman RN. Transcriptional activities of the Myc and Max proteins in mammalian cells. Curr Top Microbiol Immunol 1992;182:435–443. [PubMed: 1490382]

83. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. Journal of Computational Chemistry 1998;19:1639–1662.

84. Brooijmans N, Kuntz ID. Molecular recognition and docking algorithms. Annu Rev Biophys Biomol Struct 2003;32:335–373. [PubMed: 12574069]

85. Li C, Xu L, Wolan DW, Wilson IA, Olson AJ. Virtual screening of human 5-aminoimidazole-4-carboxamide ribonucleotide transformylase against the NCI diversity set by use of AutoDock to identify novel nonfolate inhibitors. J Med Chem 2004;47:6681–6690. [PubMed: 15615517]

86. Dickerson TJ, Beuscher AEt, Rogers CJ, Hixon MS, Yamamoto N, Xu Y, et al. Discovery of acetylcholinesterase peripheral anionic site ligands through computational refinement of a directed library. Biochemistry 2005;44:14845–14853. [PubMed: 16274232]

87. Rogers JP, Beuscher AEt, Flajolet M, McAvoy T, Nairn AC, Olson AJ, et al. Discovery of protein phosphatase 2C inhibitors by virtual screening. J Med Chem 2006;49:1658–1667. [PubMed: 16509582]

88. Mustata G, Follis AV, Hammoudeh DI, Metallo SJ, Wang H, Prochownik EV, et al. Discovery of novel myc-max heterodimer disruptors with a three-dimensional pharmacophore model. J Med Chem 2009;52:1247–1250. [PubMed: 19215087]

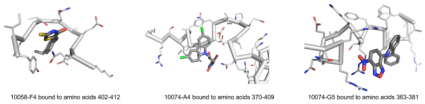

Fig. 1. NMR models of Myc compounds bound to their cognate sites in the Myc monomer

Synthetic peptides encompassing each of the previous determined binding sites were used to obtain NMR models. Because of the intrinsically disordered nature of each binding site, the images shown do not represent actual structures; rather they are composites of the ensembles of structures representing the most likely bound conformation for each site.[56] (Re-printed with permission, Journal of the American Chemical Society)

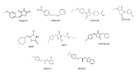

Fig. 2. Examples of the structural diversity of active Myc compounds identified by different methods

All of the compounds shown bind to the same site on Myc, namely residues 402–412.[37, 55, 56] 10058-F4, 10075-G5, 1009-G9, and 10031-B8 were among the original parental Myc compounds identified by Yin *et al.*[40] using the yeast two-hybrid-based approach. 22RH, 474, and 12RH-NCN1 are analogs 10058-F4 that were identified by the "random analog" approach described by Wang *et al.*[37] Relative to 10058-F4, they contain structural modifications of the six member ring only, the five member ring only, and both rings, respectively. 5360134 and 6525237 are molecules from the ZINC library and identified by pharmacophore model screening.[88]