Small-Molecule Inhibitors of Acetyltransferase p300 Identified by High-Throughput Screening Are Potent Anticancer Agents

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Abstract

Acetyltransferase p300 (KAT3B) plays key roles in signaling cascades that support cancer cell survival and sustained proliferation. Thus, p300 represents a potential anticancer therapeutic target. To discover novel anticancer agents that target p300, we conducted a high-throughput screening campaign. A library of 622,079 compounds was assayed for cytotoxicity to the triple-negative breast cancer (TNBC) cell line MDA-MB-231 but not to the human mammary epithelial cells. The resulting compounds were tested in a biochemical assay for inhibiting the enzymatic activity of p300. One compound (L002, NSC764414) displayed an IC50 of 1.98 μmol/L against p300 in vitro, inhibited acetylation of histones and p53, and suppressed STAT3 activation in cell-based assays. L002 could be docked to the active site of the p300 catalytic domain. Biochemical tests of a series of related compounds revealed functional groups that may impact inhibitory potency of L002 against p300. Interestingly, these analogs showed inhibitory activities against the cellular paralog of p300 (CBP), p300/CBP-associated factor, and GCN5, but not to other acetyltransferases (KAT5, KAT6B, and KAT7), histone deacetylases, and histone methyltransferases. Among the NCI-60 panel of cancer cell lines, leukemia and lymphoma cell lines were extremely sensitive to L002, whereas it is toxic to only a limited number of cell lines derived from solid tumors. Notably, breast cancer cell lines, especially those derived from TNBC, were highly susceptible to L002. In vivo, it potently suppressed tumor growth and histone acetylation of MDA-MB-468 xenografts. Thus, these new acetyltransferase inhibitors are potential anticancer therapeutics. Mol Cancer Ther; 12(5): 610–20. ©2013 AACR.
hypoxia-induced cancer cell survival and sustained proliferation. Not only does p300 serve as their coactivator, but STAT3 and NF-κB are also substrates of p300-mediated acetylation (10, 11). Recent studies revealed that p300-mediated acetylation of STAT3 at multiple sites is a prerequisite for its phosphorylation at Tyr705 by Janus-activated kinases (JAK; ref. 12). Interestingly, Marotta and colleagues reported that the IL-6/JAK2/STAT3 pathway is preferentially active in CD44+/CD24− breast cancer stem cells (CSC) and is required for their growth. Inhibition of this pathway by a JAK2 inhibitor is effective in killing CSCs and causing regression of xenografted tumors (13). In addition, CD44 has been shown to undergo nuclear translocation. In the nucleus, CD44 mediates the p300–STAT3 interaction for acetylating STAT3, thus promoting its activation (14). STAT3 can further strengthen oncogenic signaling through activating NF-κB directly or indirectly (14, 15). Aside from acetylation of substrates that are directly involved in transcription, p300 acetylates proteins that impact metabolism (16), autophagy, (17) and motility (18). For example, p300 might contribute to metastasis through acetylation of the chaperone protein Hsp90 (18). These observations provide a strong rationale for targeting p300 as an anticancer therapeutic strategy. Targeting p300 may offer additional advantages. Because multiple growth factor receptors converge to activate STAT3, NF-κB, HIF-1α, and other transcription factors that recruit p300 to their target genes, inhibition of p300 may be more effective than suppression of receptor tyrosine kinases, as inhibition of one kinase often leads to the activation of an alternative pathway that still permits cancer cell survival. Furthermore, chemical inhibition of p300 that possesses intrinsic enzymatic activity is more feasible than blocking transcription factors with small molecules, as discovery of chemical inhibitors of transcription factors has proven extremely challenging.

To identify chemical inhibitors of p300 as potential anticancer agents, we conducted a high-throughput screening (HTS) campaign. The breast cancer cell line, MDA-MB-231, was used in our primary HTS assay coupled with counterscreening against normal human mammary epithelial cells (HMEC) to discover compounds that are only toxic to cancer cells, but benign to normal cells. MDA-MB-231 was derived from a breast cancer subtype known as triple-negative breast cancer (TNBC) that still has no effective treatments, and consequently carries poor prognosis (19, 20). p300 is expressed in MDA-MB-231 and seems to play a critical role in driving its invasive growth (21). Here, we report the identification of L002 and its analogs as a new class of lead compounds that inhibit the activity of p300 and related acetyltransferases with in vivo anticancer effects in a mouse xenograft model.

Materials and Methods

HTS assays

All cancer cell lines were obtained from American Type Culture Collection, and HMECs were purchased from Invitrogen. Although cell lines were not subjected to further genetic authentication, their characteristic morphologies and certain biochemical features were specifically verified. For example, overexpression of EGF receptor in MDA-MB-468 was always observed. HTS assays were adapted to the 1,536-well format. In vitro high-throughput p300 acetyltransferase activity assays were done using a fluorescence assay. Furthermore, details of HTS assays are described in the Supplementary Material and Methods. Radioactivity-based filter-binding (HotSpot) histone acetyltransferase (HAT) assays, in which [3H]-acetyl-CoA and histone H3 were used as substrates, were done by Reaction Biology Corporation.

Cell viability, cell cycle, and colony formation assays

Cell viability and cell-cycle assays were done as described (22). In colony formation assay, cells were seeded in 6-well plates and exposed to various concentrations of L002 for 48 hours. The treated cells were trypsinized and 3,500 cells per well were reseeded. Cells were cultured with a medium containing L002 at the same concentrations as in the initial treatment. The medium with a proper concentration of L002 was changed very 4 days until colonies appeared in about 2 weeks. Colonies were stained with 1% methylene blue. [3H]-thymidine incorporation assays were conducted essentially as described previously (23). Briefly, HCT116 cells were treated for 24 hours with various concentrations of L002. The cells were then pulse labeled for 2 hours with [3H]-thymidine and the incorporation was quantitated and normalized to that of the vehicle dimethyl sulfoxide (DMSO)-treated cells.

Histone isolation and Western blotting

Cells grown in a complete medium including 10% bovine calf serum were exposed to various concentrations of L002 for a specified period of time. One hour before cell harvest, cells were treated with DMSO or 0.2 μmol/L of trichostatin A (TSA). Histones were detected in total cell lysates or as isolated forms using acid extraction method as described previously (24). Conventional SDS-PAGE or acid–urea gel electrophoresis (24) was used to separate histones. Detection antibodies used in this study are described in the Supplementary Materials and Methods.

In silico molecular docking

L002 was docked to a crystal structure of the p300 catalytic domain (25). The details of the docking procedure are described in the Supplementary Materials and Methods.

In vivo efficacy of L002 against TNBC xenograft

MDA-MB-468 cells (5 × 105) in 50 μL of PBS were mixed with 50 μL Matrigel, which was injected into the flank of a female NU/NU mouse (4- to 7-week-old, Charles River). Palpable tumors developed in 2 to 3 weeks. Tumor dimensions were measured with a digital caliper. Tumor volume was calculated with the formula (W2 × L) × 0.5, where W is the width and L the length of a tumor (W < L). When tumor volumes reached approximately 100 mm³, mice
were randomized into 3 treatment cohorts (n = 5): no treatment (group 1), DMSO (group 2), or L002 (group 3) at 0.5 mg in 100 μL DMSO per injection intraperitoneally twice weekly for 3 weeks. Tumor-bearing mice were monitored for 2 additional weeks after treatment termination. Data are shown as means ± SEM. The 2-tailed Student t test was used to compare differences between treatment groups, and the differences were considered statistically significant if P < 0.05. Tumors were removed from euthanized mice at the end point of the animal protocol, and sectioned for hematoxylin and eosin (H&E) staining and immunohistochemical analysis with an antibody against acetylated histone H4 (H4ac, Upstate 06-866, 1:200 dilution). The animal protocol was approved by the University of Florida Institutional Animal Care and Use Committee (Gainesville, Florida).

Results

HTS campaign for identifying p300 inhibitors as new anticancer agents

Our goal was to identify novel anticancer compounds through inhibiting p300. The primary screen identified compounds that are toxic to the TNBC cell line MDA-MB-231 but not to HMECs. These compounds were then tested for their ability to inhibit p300 acetyltransferase activity in an in vitro biochemical assay. Our original hypothesis was that inhibition of p300 might potentiate histone deacetylase (HDAC) inhibitor (HDACi)-mediated cytotoxicity based on our initial observation that shRNA-mediated knockdown of p300 and MDA-MB-231 cells to HDACi-induced cell death (data not shown). Thus, the primary screen was conducted in the presence of 1.5 μmol/L of SAHA (vorinostat), which induced 12% growth inhibition for MDA-MB-231 cells (CC12), to discover HDACi “potentiators.” Each compound in the library was screened as a single point, single dose at 6.2 μmol/L in the MDA-MB-231 cytotoxicity assay with the presence of 1.5 μmol/L of SAHA. The primary screen exhibited a Z’ score of 0.82. To identify nominally active compounds (hits), a standard hit-cutoff algorithm was used based on an average plus 3-fold SD calculation method (26). Thus, a compound was declared a hit if its inhibition exceeded 22.70%. This cut off yielded a total of 6,471 primary hits at a 1.04% hit-rate (Supplementary Fig. S1). From these hits, 6,400 compounds exhibiting the highest inhibition values were selected for secondary screening. Eight compounds were not available for further testing; therefore, 6,392 were included in subsequent experiments. These compounds were retested in the potentiator confirmation assay in the presence of 1.5 μmol/L of SAHA against MDA-MB-231 in triplicate at a single dose (6.2 μmol/L). They were also tested in counterscreens against MDA-MB-231 and HMECs without SAHA. From the set of hits, 4,272 compounds were confirmed to be active against MDA-MB-231 cells, and the vast majority of these compounds exhibited similar inhibitory activities in assays in the presence or absence of SAHA, suggesting that few potentiators could be uncovered in our screen (Supplementary Fig. S2).

Of the 4,272 compounds emerging from the primary/confirmation screens with the MDA-MB-231 cells, 640 compounds were chosen based on their selectivity against MDA-MB-231 cells in comparison with activity versus HMECs for 10-point dose–response assays. The 640 compounds were also tested for inhibiting p300 enzymatic activity in vitro using a fluorescence-based assay (see Materials and Methods section and Supplementary Fig. S3). The CC50 cytotoxicity values of the 640 compounds versus MDA-MB-231 in the presence or absence of SAHA and versus HMEC as well as IC50 of p300 inhibition were determined. Of these 640 compounds, 10 exhibited selectivity against MDA-MB-231 (CC50 ≤ 10 μmol/L vs. MDA-MB-231 and ≥ 10 μmol/L vs. HMEC), and inhibited p300 in vitro with IC50 ≤ 10 μmol/L (Supplementary Fig. S4).

In vitro activity of L002 and its analogs

One compound (L002, ChemBridge ID 6625948) exhibited potent inhibition of the p300 catalytic domain in vitro with an IC50 of 1.98 μmol/L. Notably, at high concentrations, L002 completely suppressed the catalytic activity of p300, whereas anacardic acid, a known HAT inhibitor (27), exerted only approximately 60% inhibition at 52 μmol/L (Fig. 1A). We have identified 7 additional compounds structurally related to L002 (Fig. 1B). All these analogs inhibited p300 with varying potencies (Table 1). Independent biochemical experiments using radioisotope HAT activity assays confirmed the inhibitory effects of these compounds on p300 (Table 1). Among these compounds, L003, L004, and L007 were least potent, compared with L002 (Table 1), whereas other compounds showed less than a 2-fold difference in IC50. Structural comparison of these analogs suggests that a functional (methyl, methoxyl, or bromide) group opposite to the sulfonyl moiety in the phenyl ring is largely unimportant or detrimental to p300 inhibition. Regardless, these results reveal a new chemical scaffold that is not shared among known acetyltransferase inhibitors, which provides a starting point for further optimization to improve the inhibitory potency and specificity of these compounds against p300.

We determined the selectivity of these compounds among various human acetyltransferases. Cellular paralog of p300 (CBP), an acetyltransferase closely related to p300, was inhibited by some of these analogs, and in general, they were less potent to CBP than to p300 (Table 1). L002 also inhibited p300/CBP-associated factor (PCAP) and GCN5, 2 proteins of the GNAT (GCN5-related N-acetyltransferase) family, whereas other compounds were much less effective (Table 1). In contrast, these compounds displayed no inhibition against the MYST family of HATs [KAT5 (Tip60), KAT7 (MYST2)] and KAT6B (MYST4), Table 1).

To further assess target specificity of L002, we tested it against a panel of HDACs and histone methyltransferases (HMT). L002 did not inhibit HDAC1 (class I), HDAC6 (class IIb), and HDAC11 (class IV, Supplementary Fig. S5).
Similarly, L002 did not display inhibitory effects against a panel of 8 diverse HMTs (DOT1, EZH1, G9a, PRMT1, SETD2, SET7-9, SMYD2, and SUV39H2; see Supplementary Fig. S6). Of note, L002 was not flagged as a promiscuous inhibitor in HTS assays. It emerged as a hit only in this HTS campaign among 25 HTS campaigns. Thus, L002 is a specific inhibitor of acetyltransferases.

In silico docking of L002 to the catalytic domain of p300
To assess whether L002 could dock to the active site of p300, we fitted L002 to a crystal structure of the p300 catalytic domain (25). In an energy-minimized model of
the L002–p300 complex, an oxygen atom in the sulfonyl moiety of L002 potentially forms 2 hydrogen bonds with the side-chain of R1410 in helix α3 of the p300 catalytic domain (Fig. 2). R1410 of p300 was shown to form hydrogen bonds with the synthetic bisubstrate p300 inhibitor Lys–CoA in its cocrystal structure with the p300 catalytic domain (25), and was also proposed to form similar hydrogen bonds with the recently identified p300 inhibitor C646 (28). In addition, a possible hydrogen bond might also form between the oxygen linked to the nitrogen atom of the imine bond in L002 and the side-chain of Q1455 from the L1 loop of the p300 catalytic domain (Fig. 2). In general, L002 fits nicely into the acetyl-CoA–binding pocket (Fig. 2B and C), making key contacts with residues R1410, Q1455, W1466, R1462, and K1456.

Figure 2. Docking of L002 to the acetyl-CoA–binding pocket in the p300 catalytic domain. L002 was fitted to the acetyl-CoA–binding pocket of a crystal structure of the p300 catalytic domain. Potential hydrogen bonds between L002 and the indicated residues of p300 are depicted (dotted lines). The residues of interest are labeled in white. A, a cartoon representation of a model of L002–p300 interaction. B, a space-filled representation of the p300 catalytic domain in complex with the inhibitor. C, the L002–p300 complex in a space-filled model. L002 and the p300 catalytic domain are shown in dark and light gray, respectively.

Figure 3. Inhibition of p300-dependent functions in cells. A, MDA-MB-468 cells were untreated (NT, lane 1), treated with DMSO (lane 2), TSA (lane 3), L002 (lane 4), and L002 plus TSA (lane 5). The cells cultured in a complete medium with 10% bovine calf serum were exposed to DMSO or L002 for 7 hours. TSA was added to 0.2 µmol/L at 1 hour before lysing cells for Western blotting. A blot was probed with an antibody against acetylated lysine (top) or acetylated histone H4 (H4ac, middle). The blot was reprobed with an anti-PCNA antibody as a loading control. In a separate gel, an equal amount of the samples was loaded and stained with colloidal blue (bottom). B, HCT116 cells were exposed to DMSO or L002 as indicated for 7 hours. Etoposide was added 1 hour after the addition of L002. Cells were lysed for Western blotting with the indicated antibodies. C, MIA PaCa-2 cells were exposed to the indicated concentrations of L002 or L004 for 6 hours before harvesting cells for Western blotting using the indicated antibodies.
in helices α3, α4, and the L1 loop. L002 could also be docked to the acetyl-CoA–binding pockets of other acetyltransferases with the docking scores of −47.3 (PCAF), −43 (p300), −34.5 (KAT5/Tip60), and −17 (MYST1; the lower the scores, the higher the possible inhibitor–enzyme affinity). These docking scores correlate well with the inhibition data shown in Table 1.

**L002 inhibits p300-mediated acetylation in cell-based assays**

HTS hit compounds were tested for their effects on p300-mediated cellular mechanisms. In TNBC cell line MDA-MB-468, L002 markedly suppressed acetylation of histone H3 (H3ac) and H4 (H4ac; Fig. 3A). Inhibition of histone acetylation at various lysine residues by L002 was also observed in other cancer cell lines including MDA-MB-231 and HCT116 (Supplementary Fig. S7). Together, these cell-based assays provide a validation for the inhibitory effects of L002 on histone acetylation mediated by p300 and other acetyltransferases.

In addition to histones, p300 acetylates numerous other proteins. p53 is specifically acetylated at Lys382 by p300 (3). Acetylation at this site is elevated in response to DNA damage and other cellular stresses (29). To assess whether L002 could inhibit acetylation of p53 at Lys382 (K382ac), HCT116 cells expressing wt p53 were exposed to L002, the genotoxic drug etoposide or a combination thereof. As shown in Fig. 3B, L002 markedly suppressed acetylation of p53 (pS392); however, inhibition of p53 acetylation by L002 seems to compromise p53 protein stability, supporting the notion that acetylation antagonizes ubiquitin-mediated p53 degradation. Nonetheless, L002 did not affect etoposide-induced p21 expression despite attenuated p53 acetylation at Lys382 (Fig. 3B).

To further understand the cellular mechanism of action for L002, we examined the influence of L002 on STAT3 activation, which requires p300-mediated acetylation of STAT3 (12). The pancreatic cancer cell line MIA PaCa-2, in which STAT3 is constitutively active (31), was exposed to varying doses of L002 or L004. As shown in Fig. 3C, the levels of STAT3 phosphorylated at Y705 were reduced in a concentration-dependent manner by both L002 and L004, and the former seemed more potent. Collectively, these lines of evidence provide strong support for our hypothesis that inhibition of p300 is one specific cellular mechanism of action by L002.

**Cytotoxicity of L002 to cancer cell lines**

The differential cytotoxic effects of L002 to HMECs and MDA-MB-231 cells, as determined in the HTS assays, suggest that it selectively kills cancer cells. To further validate cytotoxicity of L002 to cancer cell lines, we tested the sensitivity of a panel of cancer cell lines derived from diverse types of solid cancer to L002. As shown in Table 2 and Fig. 4, these cell lines exhibited varying sensitivities to L002. Notably, all 4 tested TNBC cell lines were highly susceptible to treatment with L002 with CC50 at low micromolar concentrations, whereas cell lines of luminal subtype breast cancer were more resistant. We further examined the impact of L002 treatment on clonal growth.

**Table 2. CC50 values of L002 against HMEC and various cancer cell lines**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tissue/cancer type</th>
<th>CC50 (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMEC</td>
<td>Normal</td>
<td>&gt;15.53a</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>TNBC</td>
<td>1.03</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>TNBC</td>
<td>2.57</td>
</tr>
<tr>
<td>MDA-MB-436</td>
<td>TNBC</td>
<td>2.85</td>
</tr>
<tr>
<td>MDA-MB-435S</td>
<td>TNBC</td>
<td>1.91</td>
</tr>
<tr>
<td>MCF7</td>
<td>Breast cancer (luminal)</td>
<td>21.67</td>
</tr>
<tr>
<td>ZR-75-1</td>
<td>Breast cancer (luminal)</td>
<td>8.91</td>
</tr>
<tr>
<td>SK-BR-3</td>
<td>Breast cancer (luminal)</td>
<td>11.6</td>
</tr>
<tr>
<td>LNCaP</td>
<td>Prostate cancer</td>
<td>3.54</td>
</tr>
<tr>
<td>PC-3</td>
<td>Prostate cancer</td>
<td>4.74</td>
</tr>
<tr>
<td>DU 145</td>
<td>Prostate cancer</td>
<td>3.75</td>
</tr>
<tr>
<td>Mia PaCa-2</td>
<td>Pancreatic cancer</td>
<td>21.7</td>
</tr>
<tr>
<td>HPAC</td>
<td>Pancreatic cancer</td>
<td>&gt;50a</td>
</tr>
<tr>
<td>BxPC3</td>
<td>Pancreatic cancer</td>
<td>18.56</td>
</tr>
<tr>
<td>Panc-1</td>
<td>Pancreatic cancer</td>
<td>8.44</td>
</tr>
<tr>
<td>HCT 116</td>
<td>Colon cancer</td>
<td>7.40</td>
</tr>
<tr>
<td>DLD-1</td>
<td>Colon cancer</td>
<td>8.35</td>
</tr>
<tr>
<td>A549</td>
<td>Non-small cell lung cancer</td>
<td>&gt;50a</td>
</tr>
<tr>
<td>SH-SYSY</td>
<td>Neuroblastoma</td>
<td>0.19</td>
</tr>
</tbody>
</table>

aCell growth inhibition did not surpass 50% at the highest concentration tested.

Although this cell line was isolated from the pleural effusion of a patient with breast cancer and has a gene expression profile consistent with a breast cancer origin, some studies showed that it exhibits properties indicative of a melanoma origin.
of cancer cell lines. As shown in Fig. 4B, in the presence of 1 µmol/L of L002, the clonal growth of MDA-MB-231 cells was completely suppressed, whereas colonies of MCF7 cells were largely unaffected, compared with the control (DMSO-treated cells). For MCF7 cells, a significant number of colonies survived at 5 µmol/L of L002, although no colonies were detected at 10 µmol/L (Fig. 4B). Inhibition of cell growth seemed reversible, as drug washout permitted recovery of treated cells (Fig. 4C). Nonetheless, a significant fraction of MDA-MB-231 cells was already killed within 24 hours of drug exposure (Fig. 4C).

Among these cell lines from solid tumors, SH-SY5Y (neuroblastoma) was most sensitive, whereas A549 (non–small cell lung cancer) and MIA PaCa-2 (pancreatic cancer) were highly resistant to L002 (Table 2). L002 was further tested against the NCI-60 panel of human cancer cell lines. Strikingly, diverse leukemia and lymphoma cell lines were all extremely sensitive to L002 (Supplementary Table S1). Among the solid tumor cell lines, most of the breast cancer cell lines including MDA-MB-231, MCF7 and MDA-MB-435 were sensitive to L002. In contrast, cell lines of non–small cell lung and central nervous system cancer were largely insensitive to L002.

To explore the potential mechanism by which L002 kills cancer cells, we assessed effects of L002 treatment on cell-cycle profiles of MDA-MB-231 cells. As shown in Fig. 5A, compared with treatment with DMSO, exposure of MDA-MB-231 cells to L002 increased cell numbers in the G1 phase of the cell cycle with a concomitant reduction of the percentage of cells in the S-phase. The proportion of cells with sub-G1 DNA content, indicative of cells undergoing apoptosis, was higher in MDA-MB-231 cells exposed to L002 compared with those treated with DMSO. In contrast, anacardic acid had no effects on cell-cycle progression of MDA-MB-231 cells (Fig. 5A). In HCT116 cells, L002 potently inhibited DNA replication, as measured in thymidine incorporation assays (Fig. 5B). Taken together, these results indicate that L002 can induce growth arrest and apoptosis in cancer cells to exert antiproliferative effects.

**In vivo anticaner efficacy of L002**

To test whether L002 could suppress tumor growth in vivo, L002 was administrated intraperitoneally to mice bearing tumor xenografts of the TNBC cell line MDA-MB-468. Figure 6A shows that L002 effectively suppressed tumor growth during systemic treatment and importantly, tumors did not grow back after treatment termination. The twice-weekly dosing regimen was well tolerated, as the change of body weight was within 10% (Fig. 6B). Tumor sections from mice treated with DMSO or L002 were subjected to immunohistochemical staining with an antibody against H4ac. As shown in Fig. 6C, the levels of H4ac were markedly lower in tumors from mice treated with L002 in comparison with those from mice treated with DMSO. Altogether, these data represent the first in vivo proof-of-principle regarding the feasibility of acetyltransferase inhibition for treating solid malignancy.
Discussion

Here, we reported the discovery of a new class of inhibitors of acetyltransferases. These compounds share the same core chemical scaffold and potently inhibited p300 and related acetyltransferases in vitro and in cells. A number of chemical inhibitors of p300 and CBP have been identified. These compounds include natural products curcumin (32), garcinol (33), and anacardic acid (27). These natural products exhibited only moderate inhibitory potency and structure—activity relationship (SAR)-based approaches to improve selectivity and potency are quite challenging due to their complex chemical structures. A HTS of 69,000 compounds identified iso-thiazolones as inhibitors of PCAF and other acetyltransferases (34). These compounds seem to form covalent adducts with their targets and seem to induce irreversible inhibition and toxicity (34), thus limiting their potential applications. A series of analogs of the synthetic bisubstrate HAT inhibitor Lys–CoA have been described. These synthetic compounds are highly potent in inhibiting acetyltransferases (35), but they have relatively large molecular weights and are poorly cell permeable. The most potent p300 inhibitor reported thus far, C646, inhibits p300 at submicromolar potency (28). Inhibition of the enzymatic activity of p300 by C646 has been shown in diverse applications (36, 37). Nonetheless, C646 seems to be inactivated in the presence of serum (38). Furthermore, the requirement of the nitroaromatic moiety for C646 to inhibit p300 (28) might limit its in vivo application due to hepatotoxicity associated with nitroaromatic compounds (39). Thus, novel and potent chemical inhibitors of p300 could broaden the appeal of such compounds for further preclinical and clinical evaluation.

L002 is structurally distinct from any known HAT inhibitors. The 2 ring moieties (quinone imine and methoxyphenyl group) are connected by the sulfonaryl group. The 2 rings are not in the same plane, and this 3-dimensional arrangement seems to fit well in the acetyl-CoA pocket of the p300 catalytic domain (Fig. 2). Initial SAR assessment suggests that the absence of the oxygen atom in the methoxy group enhances the inhibitory potency (Table 1). Furthermore, replacement of the methoxy group with a bromine atom in L002 also markedly reduced the inhibitory potency against p300 (Table 1).

Interestingly, L002 and its analogs displayed differential inhibition to p300/CBP and other acetyltransferases. Most of the 8 analogs showed potent inhibition to p300, whereas only L001 and L002 detectably inhibited PCAF and GCN5 (Table 1). In contrast, these compounds did not inhibit the MYST family of acetyltransferases (Table 1). Furthermore, L002 did not inhibit HDACs and a panel of diverse HMTs (Supplementary Figs. S5 and S6). Although not extensively tested against kinases, L002 did not seem to impact p53 phosphorylation (Fig. 3B). The demonstrated selectivity of these compounds to different classes of acetyltransferases perhaps reflects their structural differences. Although these enzymes have divergent amino acid sequences, their catalytic domains share structural similarity in the central core associated with acetyl-CoA binding, but they also have pronounced differences (25). Structure-guided modifications of these analogs will likely improve selectivity and potency against acetyltransferases.

Targeting p300 might have therapeutic implications in a range of different diseases including heart malfunction (40), diabetes mellitus (41), and HIV infection (42), probably due to the fact that p300 is a pleiotropic protein that is involved in diverse biologic mechanisms ranging from cell survival, proliferation, metabolism, and viral infection. More than 400 protein-binding partners have been described for p300/CBP, making them among the most connected interaction "hubs" in cells (2). p300 and CBP are found in mammals and Drosophila, and their placement in the interaction hubs indicates that they play diverse roles in a multicellular organism, some of which may be essential for cell and organism viability (2). Indeed, homozygous knockout of either p300 or CBP as well as their compound heterozygous knockout causes embryonic lethality in mice (2, 43, 44). These observations indicate...
that pharmacologic inhibition of p300/CBP might cause unintended toxicity. Thus, therapeutic strategies based on targeting p300/CBP might be more applicable against diseases that rely on excessive p300/CBP activity. Our data showed that TNBC cell lines are highly susceptible to L002 (Table 2). In vivo, L002 potently suppressed tumor growth of TNBC cell line MDA-MB-468 xenografts (Fig. 6). This represents the first demonstration of in vivo anticancer efficacy of an acetyltransferase inhibitor using systemic drug administration. Precisely why L002 is more potent in killing TNBC cells requires further investigation; however, the importance of p300 in pathways that sustain CSCs in TNBC provides a plausible explanation. In particular, because of the critical role of p300 in the JAK/STAT (45) and Wnt/β-catenin pathways (46–48) that underpin CSC self-renewal, survival, and proliferation (15), chemical inhibitors of p300 might be more effective against TNBC and other cancers that are still refractory to currently available chemotherapies and targeted therapies.

Notably, among the NCI-60 panel of cancer cell lines, those derived from hematologic malignancies were extremely sensitive to L002 (Supplementary Table S1). This seems to mirror the more pronounced vulnerability of leukemias and lymphomas to agents targeting epigenetic pathways than solid cancers (49). Indeed, HDAC inhibitors SAHA (vorinostat) and romidepsin (ISTODAX) are already in clinical use for treating certain types of lymphoma. Significantly, agents targeting p300 acetyltransferase activity were effective in suppressing acute myelogenous leukemia in an ex vivo treatment approach using a mouse model (36). Because systemic administration of L002 efficiently suppressed tumor growth in vivo (Fig. 6), it is feasible to vigorously test the efficacy of L002 for treating hematologic malignancies using various animal models.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Writing, review, and/or revision of the manuscript: H. Yang, C.E. Pinello, J. Luo, S.C. Jahn, B.K. Law, W.R. Roush, P. Hodder, D. Liao
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Yang, C.E. Pinello, J. Luo, D. Li, Y. Wang, W.R. Roush, P. Hodder, D. Liao
Study supervision: J. Luo, P. Hodder, D. Liao

Acknowledgments
The authors thank Pierre Baillargeon, Lina DeLuca, and Louis Scampavia for compound management and quality control, Katharine Emery (Scripps Florida) for secretarial assistance, Jennifer Xiao for reading the...
article, and the NCI Developmental Therapeutics Program for the NCI-60 cell line testing.

Grant Support
The work was supported by a grant from Bankhead-Coley Cancer Research Program, Florida Department of Health (09BW-05 to D. Liao). H. Yang, D. Li, and Y. Wang received scholarships from the China Scholarship Council.

References

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Received September 21, 2012; revised March 6, 2013; accepted March 6, 2013; published OnlineFirst April 26, 2013.
Correction: Small-Molecule Inhibitors of Acetyltransferase p300 Identified by High-Throughput Screening Are Potent Anticancer Agents

In this article (Mol Cancer Ther; 2013;12:610–20), which appeared in the May 2013 issue of Molecular Cancer Therapeutics (1), the authors regret that there are two errors related to author information to correct: (i) The zip code for the corresponding author is incorrect. The correct zip code is 32610. (ii) Peter Chase, who conducted the high-throughput screen for this project, was inadvertently omitted as an author in this article. Corrected author information is listed below:

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Reference


Published OnlineFirst August 1, 2013.
doi: 10.1158/1535-7163.MCT-13-0441
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Molecular Cancer Therapeutics

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