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Structure-Activity Relationship (SAR) Study of Ethyl 2-Amino-6-(3,5-dimethoxyphenyl)-4-(2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (CXL017) and the Potential of the Lead against Multidrug Resistance in Cancer Treatment

Gopalakrishnan Aridoss, Bo Zhou, David L. Hermanson, Nicholas P. Bleeker, and Chengguo Xing*

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, 308 Harvard Street SE, Minneapolis, Minnesota 55455, United States

Abstract

Multidrug resistance (MDR) against standard therapies poses a serious challenge in cancer treatment, and there is a clinical need for new anticancer agents that would selectively target MDR malignancies. Our previous studies have identified a 4*H*-chromene system, CXL017 (**4**) as an example, that can preferentially kill MDR cancer cells. To further improve its potency, we have performed detailed structure–activity relationship (SAR) studies at the 3, 4, and 6 positions of the 4*H*-chromene system. The results reveal that the 3 and 4 positions prefer rigid and hydrophobic functional groups while the 6 position prefers a meta or para-substituted aryl functional group and the substituent should be small and hydrophilic. We have also identified and characterized nine MDR cancer cells that acquire MDR through different mechanisms and demonstrated the scope of our new lead, **9g**, to selectively target different MDR cancers, which holds promise to help manage MDR in cancer treatment.

Graphical Abstract

INTRODUCTION

Multidrug resistance (MDR) is a general phenomenon among all malignancies. Anticancer agents that can circumvent MDR are highly needed to achieve effective treatment. One

*Corresponding Author Phone: 612-626-5675. Fax: 612-624-0139. xingx009@umn.edu.

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ASSOCIATED CONTENT

Supporting Information

HMBC spectra of **9b**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

major mechanism in acquiring MDR is overexpression of the antiapoptotic Bcl-2 family proteins, which occurs in 60–90% of all types of cancers.¹ Such overexpression is proposed as one general MDR mechanism because many standard cancer therapies introduce their activity via activation of the apoptotic machinery.^{2,3} The other major mechanism is overexpression of ATP-binding cassette (ABC) transporter proteins, such as p-glycoprotein.^{4,5} The overexpressed ABC transporter proteins decrease the concentration of anticancer agents in tumor cells through enhanced drug efflux, leading to MDR.

The Bcl-2 family proteins regulate apoptosis through both the mitochondrial and the endoplasmic reticulum (ER) pathways.^{6,7} The ER pathway communicates with the mitochondrial pathway via control of intracellular Ca^{2+} distribution.⁸ Mechanistically, the antiapoptotic Bcl-2 family proteins on the ER physically interact with inositol triphosphate receptor (IP_3R)^{9–11} or sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA),^{12,13} two key ER-localized Ca^{2+} channel/pump, to regulate Ca^{2+} homeostasis. Studies also suggest that modulating intracellular Ca^{2+} may affect the activity of p-glycoprotein.^{14–16} These lines of evidence suggest that there may be communications among the Bcl-2 family proteins, ER-localized calcium channel/pump, and p-glycoprotein in MDR with intracellular Ca^{2+} as a possible link.

Significant progress has been achieved over the past decades toward the development of anticancer agents that may overcome MDR via inhibition of the antiapoptotic Bcl-2 family proteins or the ABC transporter proteins. For instance, ABT-737 (**1**) and its analogue ABT-263 (**2**) have been developed as inhibitors against Bcl-2 and Bcl-X_L proteins (Figure 1), aiming to reactivate the apoptotic machinery in cancer cells, and candidate **2** has revealed some anticancer effects in recent clinical trials.^{17,18} These drug candidates have also shown potential to treat MDR cancers.^{19,20} Similarly, various small-molecule modulators for ABC transporter proteins have been developed and some of them have demonstrated promise to nullify MDR in different cancer models. In particular, the recent work from Gottesman's group explored and demonstrated the feasibility of selectively targeting p-glycoprotein based MDR cancer cells.^{21,22} One lead candidate, isatin- β -thiosemicarbazone (**3**, Figure 1), reveals increased cytotoxicity, namely, collateral sensitivity, toward p-glycoprotein based MDR cancer cells. Nonetheless, no anticancer candidates have been designed to simultaneously target both MDR mechanisms, which may be necessary, since cancer cells can acquire their MDR through multiple mechanisms simultaneously.

We have recently developed a series of CXL candidates, CXL017 (**4**) as one example, based on HA 14–1 (**5**), a putative Bcl-2 inhibitor (Figure 1).²³ Candidate **5** was selected as our initial lead because of its ability to selectively kill cancer cells with elevated antiapoptotic Bcl-2 protein.²⁴ Candidate **5** also modulates the intracellular Ca^{2+} homeostasis²⁵ and synergizes with various cancer therapies in different cancer models.²⁶ In comparison to **5**, **4** and its analogues have significantly improved stability with some of them having improved potency.^{27–30} Genetically overexpressing antiapoptotic Bcl-2 or Bcl-X_L protein fails to introduce resistance to CXL candidates, although such overexpression confers significant MDR to standard cancer therapies.²⁸ More importantly, five natural MDR cancer cell lines show no resistance to CXL candidates.^{29,30} Some of them actually demonstrate collateral sensitivity to CXL candidates. Results of our mechanistic investigation suggest that CXL

candidates cause ER Ca^{2+} release and induce apoptosis mainly through the ER pathway via antagonizing the antiapoptotic Bcl-2 family proteins and SERCA protein.³¹ Given the potential communications between antiapoptotic Bcl-2 family proteins and p-glycoprotein via ER calcium pump/channel and Ca^{2+} homeostasis, we hypothesize that CXL candidates can effectively target MDR introduced by both mechanisms in cancer treatment. Indeed, our preliminary data show that candidate **4** effectively inhibits the transporting activity of p-glycoprotein in whole cells, and the mechanism of such inhibition is currently under investigation (unpublished results).

Nonetheless, our lead candidate demonstrates relatively weak potency against parental cancer cell lines, limiting their in vivo evaluation. In order to improve the potency of our lead candidates, we have performed additional structural modifications at the 3, 4, and 6 positions of the 4*H*-chromene system. This report summarizes our sharp SAR results at these positions and the identification of several candidates with submicromolar potency. We have also characterized nine MDR cancer cells and evaluated the potential of **9g**, our new lead candidate, to overcome MDR in comparison to several drug candidates that target antiapoptotic Bcl-2 family proteins (**1**), p-glycoprotein (**3**), or SERCA (thapsigargin, **6**).

RESULTS AND DISCUSSION

Chemistry

We prepared the 4*H*-chromene analogues according to the procedures adopted from our previous work with slight modifications (Schemes 1–3).^{29,30}

Results from our previous work suggest that the potency of CXL candidates is very sensitive to the functional groups at the 3 and 4 positions. In this study we further characterized the preferred property of the functional groups at these positions, including size, lipophilicity, and flexibility (Scheme 1, series **7**). Of the two routes to 6-(3',5'-dimethoxyphenyl)coumarin, Suzuki–Miyaura coupling was carried out with 6-bromocoumarin³² instead of 5-bromosalicylaldehyde, as the former route gave higher percentage of conversion along with significantly lower dimer formation derived from the boronic acid and easier purification. To explore the impact of lipophilicity, the diallylic analogue (**7f**) was converted to the dioxirane methyl derivative (**7j**) via standard epoxidation. **7j** can be compared with the dicyclopropylmethyl candidate (**7i**) given their similarity in size, shape, and flexibility. Introduction of an oxadiazole ring system at the 3 position (**7l**) from the corresponding nitrile (**7k**) was achieved via 1,3-dipolar cycloaddition reaction with nitrile oxide generated in situ from 4-methoxyphenylhydroximinoyl chloride³³ in the presence of triethylamine.³⁴

We next introduced fluorine substituted aryls or heterocyclic aromatic systems at the 6 position (series **8**). As outlined in Scheme 2, each candidate was obtained starting from 5-bromosalicylaldehyde through three steps: Suzuki coupling, cyclization to coumarin, and chromene formation. The sequence of these three steps and the conditions of Suzuki coupling reaction vary for different analogues because a universal protocol that would work for all of these analogues could not be found because of different reactivity among the

substrates and ease of purification. The Suzuki reactions with heterocyclic counterparts were achieved through Burke's modification.³⁵

Results from our previous studies also indicate that the meta and para positions of the 6-phenyl group are critical to the potency of CXL candidates: functionalizing each of these positions with a methoxy or a methyl improved their cytotoxicity, while functionalizing both positions led to significant loss of activity. In this study, we prepared a panel of primary, secondary, and tertiary amino candidates at these positions to further define the functional group preference, including size and hydrophilicity (series **9**, Scheme 3). Suzuki coupling of a nitro-substituted arylboronic acid with 5-bromocoumarin followed by Pd/C-catalyzed reduction furnished the desired aminocoumarins, which in turn were converted to the target 4*H*-chromenes with ethanol or propargyl alcohol. It is worth mentioning that the nature of the alcohols used for the synthesis of 4*H*-chromene from 6-arylcoumarin greatly influences the conversion rate, the final product(s), and their yields. Unlike ethanol, where the yields are relatively good and the reaction rate is relatively fast, reaction with propargyl alcohol is very moisture sensitive and slow with low yield. As expected, in situ base catalyzed transesterification occurred when propargyl alcohol was used. Indeed, transesterification is relatively fast that dipropargyl derivative is the major product with minimal or no monopropargyl counterpart. With the support of 2D NMR (see Supporting Information Figures S1 and S2), it was confirmed that the C-4 ester bears the propargyl group in the monopropargyl analogue, consistent with its reaction mechanism.³⁶ For the preparation of secondary and tertiary amino analogues, direct alkylation of the primary amine was not successful in some cases. Under such circumstances, we started with the *N*-Boc-protected arylboronic acid to furnish the *N*-Boc protected 4*H*-chromene, which through conventional protocol led to the desired *N*-alkylated derivatives in moderate to good yield. Interestingly, attempted methylation of *N*-Boc protected amine (**11n**) with excess molar ratio of MeI and NaH led to the cleavage of the 4*H*-chromene to furnish **11r1**,³⁷ whereas the equivalent molar ratio yielded the *N*-methylated derivative as the major product with minor amount of **11r1**. The ring cleavage reaction was not noticed when ethyl iodide was used that it gave solely the desired product. *N,N*-Dimethylated analogues were obtained by further methylation of the corresponding *N*-methylated analogues. The amino analogue (**9c**) was also functionalized to provide compounds **9n** and **9o** by following earlier reports,³⁸ whereas 1*H*-1,2,3,4-tetrazole (**9q**) was conveniently synthesized in one-pot from **9c** by reaction with TMSN₃ and CH(OEt)₃ using the readily available, recyclable, Brønsted acidic ionic liquids [EtNH₃][NO₃].³⁹

In Vitro Cytotoxicity in HL60

The cytotoxic potency of these candidates was first evaluated in HL60 following established procedures,²⁹ given that HL60 parental line is generally less sensitive to CXL candidates compared to HL60/MX2, an MDR resistant cell line developed from HL60.³⁰ The cytotoxicity results of series **7** (Table 1) demonstrate that the functional groups at the 3 and 4 positions are important. There is a 13-fold difference in cytotoxicity between the methyl (**7a**) and the *n*-butyl (**7e**) analogues with the ethyl (**7c**) and *n*-propyl (**7d**) analogues in between. Reduction in flexibility at these positions improves activity. This is reflected by the dipropargyl esters (**7g**) being 3 and 5 times more potent than the diallyl (**7f**) and di-*n*-propyl

(**7d**) candidates, respectively. Similarly, the cyclopropylmethyl analogue (**7i**) is more potent than the *n*-butyl analogue (**7e**). Incorporation of a cyano functional group at the 3 position (**7k**), which is the least flexible and the smallest in size, however, leads to significant loss of activity relative to **7c**. This suggests that the substitution at the 3 position cannot be too small or that the lipophilicity of the functional group is important as well. Converting the cyano group into an oxadiazole (**7l**) results in complete loss of activity, likely due to increased hydrophilicity or size. In comparison to the cyclopropylmethyl candidate, the oxirane methyl substitution (**7j**) is completely inactive, confirming that the 3 and 4 positions favor lipophilic functional groups over hydrophilic functional groups. Overall, the alkoxy groups on the 3 and 4 esters prefer to be lipophilic and rigid with a size of one to three carbons.

Incorporating fluorine on the 6-phenyl ring of the 4*H*-chromene does not show significant improvement over the parent compound (sHA 14–1) except **8f** (Table 2). Among the heterocyclic analogues (Table 3), 4'-pyridinyl shows the greatest improvement over sHA 14–1 and the position of the nitrogen in the pyridine ring is important; the 4'-pyridinyl candidate (**8k**) is 2- and 8-fold more potent than the 3'-pyridinyl (**8j**) and 2'-pyridinyl (**8i**) candidates, respectively. As our previous SAR showed that introduction of a methoxy group at the 3', position significantly improved the potency, we have designed and evaluated analogue **8m**. Disappointingly, incorporation of a 3'-methoxy group in the 4'-pyridinyl candidate causes no change in potency. Given that the 3,4-dipropargyl ester of the 3',5'-dimethoxy analogue (**7g**) is much more potent than the 3,4-diethyl ester counterpart (**7c**), we also prepared the 3,4-dipropargyl ester analogue of **8k**. Disappointingly, instead of improving potency, it suffered a slight loss of activity (data not included).

Our previous results and the results from heterocyclic analogues suggest that the meta and para positions of the 6-phenyl ring prefer hydrophilic functional groups, but the impact of size had not been fully investigated. We therefore introduced varied amino functional groups at these two positions to confirm the preference of hydrophilicity and to explore the optimal size (Table 4). Consistent with our observation in series 7, the 3,4-dipropargyl esters (**9c**, **9g**, **9j**, **9k**, **9m**, and **9u**) are significantly more potent than the propargyl ethyl ester (**9b**, **9f**, **9i**, **9l**, and **9t**) and the diethyl ester (**9a**, **9e**, and **9h**) while an introduction of a cyano functional group leads to a 40-fold loss of activity (**9d**). Generally speaking, primary amino or secondary amino functional groups are tolerated while tertiary amino functional groups (**9k** and **9s**) lead to a 4- to 12-fold loss of activity. With respect to the secondary amino functional group, the smaller methylamino substituent (**9i**, **9j**, and **9r**) is preferred over the larger ethylamino substituent (**9l**, **9m**, and **9u**). The carboxyamido functional group (**9p** vs **9a**) is tolerated, while the chloroacetyl substitution (**9o** vs **9c**) leads to a 4-fold loss of activity. Replacement of the 3'-NH₂ in **9c** by 1*H*-tetrazole (**9q**) does not affect the cytotoxicity, whereas the introduction of 2-hydroxyethyl group (**9n**) improves the activity by 2-fold. These results overall confirmed that hydrophilic functional groups at the meta and para positions of the 6-phenyl are preferred. The size of the substitutions at the para position needs to be small, while the meta position has more tolerance on the size of the functional group. Among these analogues, **9g**, **9j**, and **9n** reveal submicromolar potency.

Characterization of Nine Pairs of Parental and MDR Cancer Cell Lines (Table 5)

In order to evaluate the scope of our CXL candidate against MDR in cancer treatment, we acquired four pairs of AML cell lines (HL60 vs HL60/MX2, HL60/ADR, HL60/DNR, and HL60/DOX), three pairs of ALL cell lines (CCRF-CEM vs CCRF-CEM/C2, CCRF-CEM/VM-1–5, and CCRF-CEM/VLB100), and two pairs of CML cancer lines (K562 vs K562/DOX and K562/HHT300). The MDR cell lines were developed from the parental cell lines through chronic exposure to different cancer therapies, including mitoxantrone (a topoisomerase II inhibitor, MX2), adriamycin (a topoisomerase II inhibitor, ADR, also named as doxorubicin), daunorubicin (a topoisomerase II inhibitor, DNR), doxorubicin (a topoisomerase II inhibitor, DOX), camptothecin (a topoisomerase I inhibitor, C2), homoharringtonine (an antimicrotubule agent, HHT), and vinblastine (an antimicrotubule agent, VLB). Most of these cancer therapies are the first- or second-line therapies against AML, ALL, or CML, substantiating the clinical relevance of these MDR cancer cell line models. These cell lines acquire MDR through different mechanisms. For instance, the apoptotic machinery is impaired in HL60/DOX via the up-regulation of c-inhibitor of apoptosis (c-IAP).⁴⁰ HL60/MX2 confers its resistance against mitoxantrone via down-regulating of topoisomerase II β with no involvement of p-glycoprotein.^{41,42} CCRF-CEM/C2 and CCRF-CEM/VM-1–5 gain resistance via target mutation.^{43–45} HL60/MX2 and CCRF-CEM/C2 also have elevated levels of antiapoptotic Bcl-2 family proteins.²⁹ On the other hand, HL60/ADR, HL60/DNR, K562/DOX, K562/HHT300, and CCRF-CEM/VLB100 mediate their resistance at least partially via the overexpression of p-glycoprotein.^{46,47} These parental and MDR cell lines were evaluated for their sensitivity against a panel of standard therapies in a dose-dependent manner to determine the IC₅₀ values. The ratio of the IC₅₀ against MDR cell line to the IC₅₀ against corresponding parental cell line is summarized in Table 5 with the IC₅₀ for the parental lines presented as well. As expected, the MDR cell lines generally demonstrate cross-resistance to standard cancer therapies (Table 5). Since several of these (HL60/MX2, HL60/ADR, HL60/DNR, HL60/DOX, K562/DOX, and CCRF-CEM/VM-1–5) acquire their resistance through exposure to topoisomerase II inhibitors, they all demonstrate significant cross-resistance to doxorubicin, mitoxantrone, and etoposide, three topoisomerase II inhibitors. Similarly, K562/HHT300 demonstrates significant cross-resistance to vincristine given that its resistance was developed upon exposure to an antimicrotubule agent. On the other hand, CCRF-CEM/C2 reveals no or weak cross-resistance toward the therapies evaluated. This is reasonable, since its resistance was developed upon exposure to camptothecin, a topoisomerase I inhibitor, via topoisomerase I mutation.^{43,44} There are three additional interesting observations. First, HL60/ADR and HL60/DOX both are derived from HL60 upon exposure to the same topoisomerase II inhibitor, doxorubicin. They, however, reveal quite distinct drug sensitivity profiles in that HL60/DOX demonstrates a greater extent of cross-resistance while HL60/ADR even shows collateral sensitivity toward Ara-C. On the basis of reported characterization, they acquire resistance through different mechanisms. Such a distinction may be caused by multiple factors, such as different treatment regimens when HL60 cells were exposed to adriamycin/doxorubicin during the resistance development period. Second, these resistant cell lines reveal either substantial (>1000-fold) or no resistance to vincristine. On the basis of the reported characterization of these cell lines, HL60/DNR, K562/DOX, K562/HHT300, and CCRF-CEM/VLB100 all overexpress p-glycoprotein while HL60/DOX

has not been well characterized for this protein. Since vincristine is an excellent substrate for drug efflux, p-glycoprotein overexpression is probably one major resistant mechanism among these cell lines to vincristine. Third, none of these MDR cell lines reveals >10-fold resistance to Ara-C. Indeed, two of them reveal collateral sensitivity to Ara-C. These data suggest that Ara-C is less likely to suffer cross-resistance derived from the nonantimetabolite cancer therapies evaluated herein. Such an observation is consistent with the reported major mechanism responsible for Ara-C resistance: reduction in the activity of deoxycytidine kinase (dCK), an enzyme in the rate limiting step for Ara-C activation,⁴⁸ which is unlikely to change in these MDR cancer cells. These data overall demonstrate that there are several different mechanisms responsible for MDR among these cancer cells, establishing a system to evaluate the scope of our lead compound (**9g**) against MDR in comparison to several leads that target antiapoptotic Bcl-2 family proteins (**1**), p-glycoprotein (**3**), or SERCA (**6**).

Sensitivity Profiling of the Nine Pairs of Parental and MDR Cancer Cell Lines to **1**, **3**, **6**, and **9g** (Table 6)

1 is selected for evaluation because it is one of the most potent inhibitors against the antiapoptotic Bcl-2 family proteins, although it does not inhibit Mcl-1.¹⁷ **3** is a unique small molecule developed by Gottesman et al. that selectively kills cancer cell lines that overexpress p-glycoprotein.²¹ **6** is a potent inhibitor against SERCA and has been used as a lead for prodrug development against prostate cancer.⁴⁹ **9g** is selected as the lead of our CXL candidates in this multi-MDR cell line screening because of its submicromolar potency, being the most potent candidate among the newly synthesized candidates. These four compounds were evaluated for their cytotoxicity against the parental and MDR cell lines in a dose-dependent manner to determine their IC₅₀ values. The ratio of the IC₅₀ against MDR cell line to the IC₅₀ against the corresponding parental cell line is summarized in Table 6. With respect to **1**, seven of the nine MDR cell lines reveal 2- to 20-fold resistance and two reveal significant collateral sensitivity. These data suggest that targeting the antiapoptotic Bcl-2 family proteins alone, particularly targeting only a subset of the antiapoptotic Bcl-2 family proteins, is not sufficient to overcome MDR. Since c-IAP is downstream of the antiapoptotic Bcl-2 family proteins along the mitochondrial apoptotic pathway, HL60/DOX demonstrates the greatest extent of resistance to **1** (20-fold). Consistent with the results reported by Gottesman et al. in other MDR models, MDR cancer cells that overexpress p-glycoprotein, such as HL60/ADR, HL60/DNR, K562/DOX, K562/HHT300, and CCRF-CEM/VLB100, reveal collateral sensitivity to **3**. CCRF-CEM/C2 also shows decent collateral sensitivity to **3**, although its ability to accumulate camptothecin has been reported to be similar as the parental cells,⁵⁰ suggesting no change in the activity of ABC transporter proteins. HL60/MX2, HL60/DOX, and CCRF-CEM/VM-1–5 fail to show collateral sensitivity to **3** given that they acquire resistance via target mutation/reduction or impairment of apoptotic machinery, independent of p-glycoprotein. Indeed, CCRF-CEM/VM-1–5 reveals a 2-fold resistance to **3**. These results suggest that **3** is unlikely to be effective against p-glycoprotein independent MDR. Six of the nine MDR cell lines confer cross-resistance to **6**, while HL60/MX2 demonstrates a 50-fold collateral sensitivity to **6**. The mechanism of this significant selectivity is under investigation. For **9g**, five of the nine MDR cell lines show collateral sensitivity, and none of them demonstrate any resistance. Since these cells acquire MDR through several mechanisms, our results support the

hypothesis that **9g** targets several key mechanisms involved in MDR and that cancer cells are unlikely to acquire resistance to **9g** relative to **1**, **3**, and **6**.

CONCLUSION

On the basis of the potential of **4** to target MDR, we have performed a detailed SAR study to characterize the optimal functional groups at the 3, 4, and 6 positions of the 4*H*-chromene system in order to improve the potency of the lead candidate. The 3 and 4 positions are very sensitive to the size, flexibility, and lipophilicity of the functional groups with propargyl esters being the best (Tables 1 and 4). Increasing flexibility or hydrophilicity significantly reduces the cytotoxicity of the candidates. Similarly, increasing or significantly decreasing the size of the functional groups also leads to a decrease in cytotoxicity. At the 6 position, incorporation of fluorophenyl or heterocyclic aryl systems has no or a limited improvement on potency (Tables 2 and 3). The functional groups at the meta and para positions on the 6-phenyl position, however, can significantly affect the cytotoxicity of the candidate (Table 4). Typically these positions prefer small and hydrophilic functional groups. These findings culminate in the discovery of **9g**, **9j**, and **9n** with IC₅₀ of 600–800 nM against HL60 cells, which are about 20 times more potent than **4**.

We have also evaluated the scope of our lead candidate, **9g**, against MDR in cancer treatment and compared it with several leads that each target specific mechanisms involved in MDR. We first characterized the sensitivity of nine MDR cancer cell lines, which acquire their drug resistance via different mechanisms (Table 5). These MDR cancer cell lines generally reveal cross-resistance toward standard therapies. Among the three classes of anticancer agents evaluated, topoisomerase II inhibitors suffer the greatest extent of cross-resistance; all nine MDR cancer cell lines are resistant to all three topoisomerase II inhibitors. This is not surprising given that most of these MDR cell lines acquire their resistance upon exposure to topoisomerase inhibitors. Vincristine generally suffers either significant resistance when the cells overexpress p-glycoprotein or no resistance. All MDR cell lines show no or weak resistance to Ara-C, consistent with its distinct mechanisms of action and resistance.

Candidate **1** suffers cross-resistance from seven of the nine MDR cancer cell lines, especially when the resistance is acquired downstream of the mitochondrial Bcl-2 family proteins (HL60/DOX). Candidate **3** suffers cross-resistance from one MDR cell line, which does not overexpress p-glycoprotein. Candidate **6** suffers cross-resistance from six of these cell lines, four of which have been characterized to overexpress p-glycoprotein. Candidate **9g**, on the other hand, suffers no cross-resistance from any of these nine MDR cancer cell lines. Two MDR cell lines reveal collateral sensitivity to candidate **1**, and the mechanism remains to be established. HL60/MX2 cell line shows a 50-fold collateral sensitivity to candidate **6**. Six of these MDR cell lines reveal collateral sensitivity toward candidate **3**, five of which have been characterized to overexpress p-glycoprotein, consistent with the observation from Dr. Gottesman et al.²¹ Five of these MDR cell lines reveal collateral sensitivity toward candidate **9g**, and these five MDR cells have distinct mechanisms, including overexpression of antiapoptotic Bcl-2 family proteins (CCRF-CEM/C2),²⁹ overexpression of p-glycoprotein (HL60/ADR and HL60/DNR), apoptotic defect

downstream of the mitochondrial Bcl-2 family proteins (HL60/DOX), and target mutation (CCRF-CEM/VM-1-5 and CCRF-CEM/C2). Most importantly, each of these nine MDR cancer cell models evaluated herein reveals collateral sensitivity to at least one candidate among **1**, **3**, **6**, and **9g**.

In conclusion, we have performed SAR studies of CXL candidates and defined the optimal functional groups at the 3, 4, and 6 positions of the 4*H*-chromene system, leading to the identification of more potent candidates. One lead compound, **9g**, demonstrates submicromolar potency against all the cancer cell lines evaluated (Table 6), 5- to 35-fold more potent than **3** and can be >10 fold more potent than **1**. **9g** also reveals selective anticancer potential toward MDR cancer cell models that have different resistant mechanisms. **9g** in combination with **1**, **3**, and **6** represents a set of promising leads that may help conquer MDR in cancer treatment.

EXPERIMENTAL SECTION

Chemistry

All commercial reagents and anhydrous solvents were purchased from vendors and were used without further purification or distillation unless otherwise stated. Analytical thin layer chromatography was performed on Whatman silica gel 60 Å with fluorescent indicator (partisil K6F). Compounds were visualized by UV light and/or stained with iodine or potassium permanganate solution followed by heating. Flash column chromatography was performed on Whatman silica gel 60 Å (230–400 mesh). NMR (¹H and ¹³C) spectra were recorded on a Varian 400 MHz or a Bruker 400 MHz spectrometer and calibrated using an internal reference. ESI mode mass spectra were recorded on a Bruker BiotofII mass spectrometer. All the compounds synthesized are racemic mixtures. Purity of the compounds was analyzed by HPLC using 75:25 ACN/H₂O as the mobile phase with a flow rate of 0.9 mL/min on a C18 column. All compounds exhibited greater than 95% purity.

General Procedure for the Synthesis of Salicylaldehyde (10a–c)²⁹

5-Bromosalicylaldehyde (1 mmol), arylboronic acid (1.2 mmol), palladium(II) acetate (0.05 mmol), potassium carbonate (3 mmol), and triphenylphosphine (0.05 mmol) were added to 4 mL of DMF/H₂O (1:1) mixture and degassed well with nitrogen. The mixture was then heated to 60 °C under N₂ atmosphere for 6 h. The crude mixture was diluted with water and extracted with ethyl acetate (25 mL × 3) and washed with brine. The combined organic phase was dried over Na₂SO₄ followed by solvent evaporation, which gave a crude product which was further purified by column chromatography to yield the pure product.

2'-Fluoro-4-hydroxy-[1,1'-biphenyl]-3-carbaldehyde (10a)

Yield: 77%. ¹H NMR (400 MHz, CDCl₃): δ 11.10 (1H, s), 9.98 (1H, s), 7.82–7.72 (2H, m), 7.45 (1H, td, *J* = 7.8, 1.7 Hz), 7.41–7.33 (1H, m), 7.31–7.17 (2H, m), 7.11 (1H, d, *J* = 8.6 Hz).

3'-Fluoro-4-hydroxy-[1,1'-biphenyl]-3-carbaldehyde (10b)

Yield: 76%. ¹H NMR (400 MHz, CDCl₃): δ 11.04 (1H, s), 9.98 (1H, s), 7.80–7.71 (2H, m), 7.42 (1H, td, *J* = 7.9, 6.1 Hz), 7.36–7.30 (1H, m), 7.29–7.21 (1H, m), 7.12–7.01 (2H, m).

4'-Fluoro-4-hydroxy-[1,1'-biphenyl]-3-carbaldehyde (10c)

Yield: 71%. ¹H NMR (400 MHz, CDCl₃): δ 11.00 (1H, s), 9.97 (1H, s), 7.74–7.66 (2H, m), 7.55–7.46 (2H, m), 7.18–7.10 (2H, m), 7.10–7.04 (1H, m).

General Procedure for the Synthesis of Coumarin (11a–c)²⁹

To *N,N*-dimethylacetamide (1.98 mmol) stirred at 0 °C, phosphorus oxychloride (1.98 mmol) was added slowly. The reaction mixture was allowed to stir at 0 °C for 30 min followed by addition of the corresponding salicylaldehyde (0.99 mmol) in dry DCM. The reaction mass was then refluxed (at 60 °C) for about 3 h. Following this, it was cooled to room temperature and saturated NaHCO₃ solution (10 mL) was added to it (solid formation was noticed). The combined mass was heated again at 68–70 °C for another 30 min (the formed solid dissolves and gets into DCM layer), cooled, and acidified (1 N HCl), followed by extraction with methylene chloride. The extracts were combined, dried (anhydrous MgSO₄), and concentrated under reduced pressure to afford a crude product, which upon base treatment (NaOH) followed by recrystallization gave pure compound. In some cases, purification was carried out by flash column chromatography.

6-(2-Fluorophenyl)-2H-chromen-2-one (11a)

Yield: 46%. ¹H NMR (400 MHz, CDCl₃): δ 7.75 (1H, d, *J* = 9.6 Hz), 7.70 (1H, d, *J* = 8.6 Hz), 7.66 (1H, s), 7.48–7.31 (3H, m), 7.24 (1H, t, *J* = 7.2 Hz), 7.18 (1H, dd, *J* = 10.5, 8.5 Hz), 6.46 (1H, d, *J* = 9.6 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 160.58, 159.62 (d, *J*_{C-F} = 247.0 Hz), 153.44, 143.37, 132.46 (d, *J*_{C-F} = 2.2 Hz), 132.19, 130.47 (d, *J*_{C-F} = 3.7 Hz), 129.60 (d, *J*_{C-F} = 8.2 Hz), 128.15 (d, *J*_{C-F} = 3.7 Hz), 127.18 (d, *J*_{C-F} = 13.5 Hz), 124.60 (d, *J*_{C-F} = 3.7 Hz), 118.79, 116.99, 116.97, 116.25 (d, *J*_{C-F} = 23.1 Hz).

6-(3-Fluorophenyl)-2H-chromen-2-one (11b)

Yield: 48%. ¹H NMR (400 MHz, CDCl₃): δ 7.76 (1H, d, *J* = 9.6 Hz), 7.72 (1H, dd, *J* = 8.7, 1.9 Hz), 7.66 (1H, d, *J* = 2.2 Hz), 7.47–7.38 (3H, m), 7.31–7.24 (1H, m), 7.08 (1H, td, *J* = 8.3, 2.5 Hz), 6.48 (1H, d, *J* = 9.6 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 163.19 (d, *J*_{C-F} = 245.5 Hz), 160.47, 153.69, 143.26, 141.57 (d, *J*_{C-F} = 7.5 Hz), 136.47 (d, *J*_{C-F} = 2.2 Hz), 130.57, 130.53 (d, *J*_{C-F} = 8.2 Hz), 126.08, 122.65 (d, *J*_{C-F} = 3.0 Hz), 119.08, 117.42, 117.26, 114.60 (d, *J*_{C-F} = 20.9 Hz), 113.96 (d, *J*_{C-F} = 22.2 Hz).

6-(4-Fluorophenyl)-2H-chromen-2-one (11c)

Yield: 49%. ¹H NMR (400 MHz, CDCl₃): δ 7.75 (1H, d, *J* = 9.4 Hz), 7.69 (1H, d, *J* = 8.5 Hz), 7.62 (1H, d, *J* = 2.1 Hz), 7.58–7.47 (2H, m), 7.38 (1H, dd, *J* = 8.5, 2.1 Hz), 7.15 (2H, t, *J* = 8.3 Hz), 6.46 (1H, d, *J* = 9.4 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 162.64 (d, *J*_{C-F} = 245.5 Hz), 160.55, 153.33, 143.30, 136.80, 135.50 (d, *J*_{C-F} = 3.0 Hz), 130.53, 128.62 (d, *J*_{C-F} = 8.2 Hz), 125.88, 119.02, 117.27, 117.13, 115.89 (d, *J*_{C-F} = 21.6 Hz).

General Procedure for the Synthesis of 6-Aryl-Substituted Coumarin (11d-o)

6-Bromocoumarin (1 mmol), K₂CO₃ (2.5 mmol), and the respective boronic acid (1.2 mmol; all the heterocyclic derivatives were synthesized from their corresponding boronic acid MIDA ester) were taken in toluene/water (10:2) (15 mL) and degassed well with nitrogen. To this, Pd(PPh₃)₄ (0.05 mol %) was added, and the mixture was stirred at 80–90 °C under an atmosphere of nitrogen overnight. It was then acidified using HCl (1 N) on an ice bath (for pyridyl derivatives, HCl wash was not given), extracted with ethyl acetate (25 mL × 3), washed with water, brine, and dried over MgSO₄. Removal of the solvent under reduced pressure gave the crude mass which upon purification by flash column chromatography gave the desired 6-aryl-substituted coumarin.

6-(2,4-Difluorophenyl)-2H-chromen-2-one (11d)

Base: sodium carbonate (4.5 mmol). Solvent: ethanol/water/toluene in 1:2:3 ratio (5 mL). Yield: 48%. ¹H NMR (400 MHz, CDCl₃): δ 7.75 (1H, d, *J* = 9.6 Hz), 7.65 (1H, dd, *J* = 8.8, 1.2 Hz), 7.62 (1H, s), 7.45–7.35 (2H, m), 7.04–6.88 (2H, m), 6.47 (1H, d, *J* = 9.6 Hz).

6-(3,5-Difluorophenyl)-2H-chromen-2-one (11e)

Base: sodium carbonate (4.5 mmol). Solvent: ethanol/water/toluene in 1:2:3 ratio (10 mL). Yield: 62%. ¹H NMR (400 MHz, CDCl₃): δ 7.76 (1H, d, *J* = 9.6 Hz), 7.71 (1H, dd, *J* = 8.6, 2.1 Hz), 7.65 (1H, d, *J* = 2.1 Hz), 7.42 (1H, d, *J* = 8.6 Hz), 7.15–7.05 (2H, m), 6.83 (1H, tt, *J* = 8.8, 2.2 Hz), 6.50 (1H, d, *J* = 9.6 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 163.40 (dd, *J*_{C-F} = 247.8 Hz, 12.7 Hz), 160.30, 154.04, 143.08, 142.63 (t, *J*_{C-F} = 9.3 Hz), 135.43 (t, *J*_{C-F} = 2.7 Hz), 130.42, 126.12, 119.18, 117.62, 117.51, 109.97 (dd, *J*_{C-F} = 18.6 Hz, 7.4 Hz), 103.08 (t, *J*_{C-F} = 25.3 Hz).

6-(Pyridin-2-yl)-2H-chromen-2-one (11f)

Solvent: DMF/IPA in 4:1 ratio (10 mL). Additive: Cu(OAc)₂ (0.5 mmol). Yield: 61%. ¹H NMR (400 MHz, CDCl₃): δ 8.73–8.66 (1H, m), 8.18 (1H, d, *J* = 2.2 Hz), 8.14 (1H, d, *J* = 8.8, 2.2 Hz), 7.84–7.71 (3H, m), 7.42 (1H, d, *J* = 8.8 Hz), 7.28 (1H, ddd, *J* = 7.2, 4.8, 1.3 Hz), 6.46 (1H, d, *J* = 9.6 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 160.50, 155.38, 154.41, 149.78, 143.52, 136.98, 135.76, 130.12, 126.25, 122.51, 120.16, 118.98, 117.12, 116.98.

6-(Pyridin-3-yl)-2H-chromen-2-one (11g)

Solvent: dioxane/water in 5:1 ratio (10 mL). Yield: 50%. ¹H NMR (400 MHz, CDCl₃): δ 8.81 (1H, s), 8.59 (1H, d, *J* = 4.5 Hz), 7.84 (1H, d, *J* = 7.8 Hz), 7.76 (1H, d, *J* = 9.6 Hz), 7.69 (1H, d, *J* = 8.6 Hz), 7.65 (1H, s), 7.44–7.31 (2H, m), 6.44 (1H, d, *J* = 9.6 Hz).

6-(Pyridin-4-yl)-2H-chromen-2-one (11h)

Solvent: dioxane/water in 5:1 ratio (10 mL). Yield: 61%. ¹H NMR (400 MHz, CDCl₃): δ 8.67 (2H, d, *J* = 6.0 Hz), 7.83–7.70 (3H, m), 7.48 (2H, d, *J* = 6.0 Hz), 7.41 (1H, d, *J* = 8.6 Hz), 6.47 (1H, d, *J* = 9.4 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 163.19, 160.23, 154.47, 150.53, 146.53, 143.10, 134.69, 130.40, 126.29, 121.46, 119.32, 117.78.

6-(2-Methoxypyridin-4-yl)-2H-chromen-2-one (11i)

Solvent: dioxane/water in 5:1 ratio (10 mL). Yield: 22%. ^1H NMR (400 MHz, CDCl_3): δ 8.24 (1H, d, $J = 5.5$ Hz), 7.80–7.73 (2H, m), 7.71 (1H, d, $J = 2.2$ Hz), 7.42 (1H, d, $J = 8.6$ Hz), 7.09 (1H, dd, $J = 5.4, 1.1$ Hz), 6.93 (1H, d, $J = 1.1$ Hz), 6.49 (1H, d, $J = 9.6$ Hz), 3.99 (3H, s). ^{13}C NMR (100 MHz, CDCl_3): δ 164.97, 160.28, 154.36, 149.28, 147.58, 143.13, 134.81, 130.38, 126.20, 119.17, 117.61, 117.42, 115.02, 108.44, 53.62.

6-(Pyrimidin-5-yl)-2H-chromen-2-one (11j)

Solvent: dioxane/water in 5:1 ratio (10 mL). Yield: 12%. ^1H NMR (400 MHz, CDCl_3): δ 9.25 (1H, s), 8.97 (2H, s), 7.79 (1H, d, $J = 9.6$ Hz), 7.74 (1H, dd, $J = 8.6, 2.2$ Hz), 7.69 (1H, d, $J = 2.2$ Hz), 7.50 (1H, d, $J = 8.6$ Hz), 6.53 (1H, d, $J = 9.6$ Hz). ^{13}C NMR (100 MHz, CDCl_3): δ 160.01, 157.92, 154.81, 154.43, 142.77, 132.86, 130.82, 130.30, 126.20, 119.60, 118.21, 117.89.

3-(2-Oxo-2H-chromen-6-yl)benzamide (11k)

Solvent: dioxane/water in 5:1 ratio (10 mL). (400 MHz, CDCl_3): δ 8.22 (1H, t, $J = 1.9$ Hz), 8.18–8.07 (3H, m), 8.00 (1H, dd, $J = 8.6, 2.3$ Hz), 7.93–7.84 (2H, m), 7.58 (1H, t, $J = 7.6$ Hz), 7.53 (1H, d, $J = 8.6$ Hz), 7.47 (1H, br s), 6.57 (1H, d, $J = 9.6$ Hz). ^{13}C NMR (100 MHz, CDCl_3): δ 168.08, 160.34, 153.60, 144.72, 139.03, 136.29, 135.50, 130.84, 129.81, 129.51, 127.25, 126.97, 126.15, 119.61, 117.37, 117.15.

6-(3-Nitrophenyl)-2H-chromen-2-one (11l)

Yield: 61%. ^1H NMR (400 MHz, CDCl_3): δ 8.46 (1H, t, $J = 2.0$ Hz), 8.26–8.24 (1H, m), 7.93–7.91 (1H, m), 7.81–7.74 (3H, m), 7.66 (1H, t, $J = 8$ Hz), 7.47 (1H, d, $J = 8.8$ Hz), 6.52 (1H, d, $J = 9.6$ Hz). ^{13}C NMR (100 MHz, CDCl_3): δ 160.40, 154.29, 148.98, 143.18, 141.21, 135.36, 133.03, 130.71, 130.22, 126.49, 122.69, 122.04, 119.49, 117.97, 117.81.

6-(4-Nitrophenyl)-2H-chromen-2-one (11m)

Yield: 58%. ^1H NMR (400 MHz, CDCl_3): δ 8.34 (2H, d, $J = 8.8$ Hz), 7.81–7.73 (5H, m), 7.47 (1H, d, $J = 8.4$ Hz), 6.52 (1H, d, $J = 9.6$ Hz). ^{13}C NMR (100 MHz, CDCl_3): δ 160.37, 154.48, 147.52, 145.83, 143.19, 135.45, 130.89, 127.93, 126.74, 124.48, 119.47, 117.95, 117.79.

tert-Butyl (3-(2-Oxo-2H-chromen-6-yl)phenyl)carbamate (11n)

Yield: 75%. ^1H NMR (400 MHz, CDCl_3): δ 7.78–7.74 (3H, m), 7.68 (1H, d, $J = 2.0$ Hz), 7.39–7.35 (2H, m), 7.23 (2H, dd, $J = 2.2, 8$ Hz), 6.59 (1H, s), 6.46 (1H, d, $J = 9.56$ Hz), 1.54 (9H, s). ^{13}C NMR (100 MHz, CDCl_3): δ 160.87, 153.66, 152.85, 143.64, 140.52, 139.22, 137.72, 130.98, 129.71, 126.30, 121.85, 119.14, 117.86, 117.37, 117.18, 80.94, 28.49 (two coinciding carbon resonances).

tert-Butyl (4-(2-Oxo-2H-chromen-6-yl)phenyl)carbamate (11o)

Yield: 78%. ^1H NMR (400 MHz, CDCl_3): δ 7.75 (1H, d, $J = 9.6$ Hz), 7.71 (1H, dd, $J = 2, 8.4$ Hz), 7.62 (1H, d, $J = 2.0$ Hz), 7.52–7.46 (4H, m), 7.37 (1H, d, $J = 8.4$ Hz), 6.59 (1H, s), 6.45 (1H, d, $J = 9.6$ Hz), 1.54 (9H, s). ^{13}C NMR (100 MHz, CDCl_3): δ 160.89, 153.33, 152.79,

143.65, 138.37, 137.43, 134.09, 130.53, 127.68, 125.69, 119.17, 119.05, 117.37, 117.14, 80.96, 28.48.

General Procedure for Reduction of Nitro to Amine (11p and 11q)

To a stirred suspension of the respective nitrocoumarin **11l** or **11m** (5 mmol) in methanol (20 mL), 10% Pd/C (0.4 g) was added followed by dry ammonium formate (25–30 mmol). The contents were stirred well under nitrogen at room temperature. Upon completion of reaction, the catalyst was removed by filtration through Celite bed and the solvent was evaporated under reduced pressure. The resulting mass was extracted with ethyl acetate, washed with water, and dried (over MgSO₄), and the solvent was removed under reduced pressure. The crude solid was purified by flash chromatography to isolate the desired amino derivative.

6-(3-Aminophenyl)-2H-chromen-2-one (11p)

Yield: 65%. ¹H NMR (400 MHz, CDCl₃): δ 7.76–7.71 (2H, m), 7.63 (1H, d, J = 2.4 Hz), 7.38 (1H, d, J = 8.4 Hz), 7.26–7.23 (1H, m), 6.96 (1H, d, J = 8 Hz), 6.88 (1H, t, J = 1.8 Hz), 6.71 (1H, dd, J = 1.6, 8 Hz), 6.46 (1H, d, J = 9.2 Hz), 3.79 (2H, s). ¹³C NMR (100 MHz, CDCl₃): δ 160.90, 153.51, 147.13, 143.67, 140.71, 138.18, 130.86, 130.10, 126.12, 119.06, 117.54, 117.25, 117.08, 114.64, 113.73.

6-(4-Aminophenyl)-2H-chromen-2-one (11q)

Yield: 72%. ¹H NMR (400 MHz, CDCl₃): δ 7.74 (1H, d, J = 9.6 Hz), 7.68 (1H, dd, J = 2, 8.8 Hz), 7.58 (1H, d, J = 2 Hz), 7.39 (2H, d, J = 8.4 Hz), 7.35 (1H, d, J = 8.4 Hz), 6.77 (2H, d, J = 8.4 Hz), 6.44 (1H, d, J = 9.6 Hz), 3.79 (2H, s). ¹³C NMR (100 MHz, CDCl₃): δ 161.05, 152.92, 146.47, 143.76, 138.01, 130.24, 129.69, 128.08, 125.16, 119.13, 117.24, 116.96, 115.59.

Synthesis of N-Alkyl-Boc-amines (11r-u)

To a stirred solution of the respective amine (1 mmol) and NaH (1.5 mmol) in dry DMF under nitrogen, the desired alkyl iodide (2.5 mmol) was added. The contents were stirred well at room temperature. Upon completion, water was added and the contents were extracted with DCM, washed with water, cold brine, and dried over MgSO₄. The crude mass obtained by evaporation under reduced pressure was purified by flash chromatography to afford the desired N-alkylated amines. When NaH and alkyl iodide were used at 2.5 and 5 equiv, respectively, N-alkylation followed by the lactone ring cleavage occurred leading to the formation of 11r1 as the sole product.

tert-Butyl Methyl(3-(2-oxo-2H-chromen-6-yl)phenyl)-carbamate (11r)

Yield: 72%. ¹H NMR (400 MHz, CDCl₃): δ 7.78–7.72 (2H, m), 7.66 (1H, d, J = 2.0 Hz), 7.48–7.36 (4H, m), 7.28–7.28 (1H, m), 6.47 (1H, d, J = 9.6 Hz), 3.32 (3H, s), 1.49 (9H, s). ¹³C NMR (100 MHz, CDCl₃): δ 160.77, 154.81, 153.65, 144.65, 143.55, 140.07, 137.54, 130.88, 129.34, 126.25, 124.83, 124.38, 124.14, 119.17, 117.42, 117.25, 80.68, 37.52, 28.52.

(cis)-Methyl 3-(3'-((tert-Butoxycarbonyl)(methyl)amino)-4-methoxy-[1,1'-biphenyl]-3-yl)acrylate (11r1)

Yield: 60% (12%). ¹H NMR (400 MHz, CDCl₃): δ 7.81 (1H, d, *J* = 2 Hz), 7.53 (1H, dd, *J* = 2.0, 8.4 Hz), 7.42 (1H, s), 7.35 (2H, d, *J* = 4.8 Hz), 7.22–7.17 (2H, m), 6.94 (1H, d, *J* = 8.8 Hz), 6.03 (1H, d, *J* = 12.4 Hz), 3.86 (3H, s), 3.68 (3H, s), 3.30 (3H, s), 1.47 (9H, s). ¹³C NMR (100 MHz, CDCl₃): δ 166.77, 156.94, 154.94, 144.36, 141.29, 139.19, 132.65, 129.73, 129.06, 128.99, 124.33, 124.11, 124.02, 123.96, 120.05, 110.73, 80.43, 55.79, 51.47, 37.54, 28.52.

tert-Butyl Ethyl-(3-(2-oxo-2H-chromen-6-yl)phenyl)-carbamate (11s)

Yield: 80%. ¹H NMR (400 MHz, CDCl₃): δ 7.76 (1H, d, *J* = 9.6 Hz), 7.71 (1H, dd, *J* = 2.4, 8.8 Hz), 7.65 (1H, d, *J* = 2.0 Hz), 7.42–7.37 (4H, m), 7.23–7.20 (1H, m), 6.45 (1H, d, *J* = 9.6 Hz), 3.72 (2H, q, *J* = 7.2 Hz), 1.46 (9H, s), 1.19 (3H, t, *J* = 7.0 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 160.73, 154.57, 153.61, 148.54, 143.33, 140.16, 137.43, 130.83, 129.44, 126.42, 126.21, 125.83, 124.66, 119.14, 117.37, 117.19, 80.32, 45.19, 28.50, 14.12.

tert-Butyl Methyl-(4-(2-oxo-2H-chromen-6-yl)phenyl)-carbamate (11t)

Yield: 78%. ¹H NMR (400 MHz, CDCl₃): δ 7.76 (1H, d, *J* = 9.6 Hz), 7.73 (1H, dd, *J* = 2, 8.8 Hz), 7.64 (1H, d, *J* = 2.0 Hz), 7.53 (2H, d, *J* = 8.4 Hz), 7.40–7.34 (3H, s), 6.46 (1H, d, *J* = 9.6 Hz), 3.31 (3H, s), 1.49 (9H, s). ¹³C NMR (100 MHz, CDCl₃): δ 160.65, 154.62, 153.35, 143.43, 137.18, 136.15, 130.57, 127.51, 127.10, 125.84, 125.72, 119.03, 117.27, 117.06, 80.60, 37.19, 28.34.

tert-Butyl Ethyl-(4-(2-oxo-2H-chromen-6-yl)phenyl)-carbamate (11u)

Yield: 81%. ¹H NMR (400 MHz, CDCl₃): δ 7.75 (1H, d, *J* = 9.6 Hz), 7.71 (1H, dd, *J* = 2.4, 8.8 Hz), 7.64 (1H, d, *J* = 2.0 Hz), 7.52 (2H, d, *J* = 8.4 Hz), 7.37 (1H, d, *J* = 8.4 Hz), 7.29 (2H, d, *J* = 8.4 Hz), 6.44 (1H, d, *J* = 9.6 Hz), 3.71 (2H, q, *J* = 7.2 Hz), 1.46 (9H, s), 1.18 (3H, t, *J* = 7.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 160.74, 154.52, 153.47, 143.56, 142.37, 137.27, 136.82, 130.68, 127.42, 127.36, 126.00, 119.14, 117.34, 117.13, 80.35, 45.05, 28.46, 14.05.

General Procedure for Deprotection of Boc-amine by TFA (11v-za)

To a stirred solution of the respective Boc-amine (1 mmol) in dry DCM, TFA (5 mmol) was added slowly at low temperature under nitrogen. Stirring was continued for about 6 h, and then the reaction was quenched with 10% bicarbonate solution. The mixture was extracted with DCM, washed with water, brine, and dried over MgSO₄. The crude product obtained after removal of solvent itself was pure enough to use for further reaction.

6-(3-(Methylamino)phenyl)-2H-chromen-2-one (11v)

Yield: 83%. ¹H NMR (400 MHz, CDCl₃): δ 7.75–7.71 (2H, m), 7.64 (1H, d, *J* = 2.0 Hz), 7.36 (1H, d, *J* = 8.4 Hz), 7.27 (1H, t, *J* = 8 Hz), 6.90–6.88 (1H, m), 6.76 (1H, t, *J* = 2 Hz), 6.64 (1H, dd, *J* = 2.0, 8.0 Hz), 6.44 (1H, d, *J* = 9.6 Hz), 3.88 (1H, s), 2.89 (3H, s). ¹³C NMR (100 MHz, CDCl₃): δ 160.92, 153.43, 149.95, 143.71, 140.59, 138.56, 130.93, 129.94, 126.14, 119.01, 117.15, 116.97, 116.21, 112.04, 110.90, 30.85.

6-(3-(Ethylamino)phenyl)-2H-chromen-2-one (11w)

Yield 85%. ¹H NMR (400 MHz, CDCl₃): δ 7.77–7.72 (2H, m), 7.65 (1H, d, *J* = 2.4 Hz), 7.38 (1H, d, *J* = 8.4 Hz), 7.26 (1H, t, *J* = 7.8 Hz), 6.9 (1H, d, *J* = 7.6 Hz), 6.77 (1H, t, *J* = 2 Hz), 6.63 (1H, dd, *J* = 2.2, 8.4 Hz), 6.46 (1H, d, *J* = 9.2 Hz), 3.7 (1H, s), 3.23 (2H, q, *J* = 7.2 Hz), 1.29 (3H, t, *J* = 7.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 160.96, 153.51, 149.11, 143.72, 140.71, 138.66, 130.99, 130.03, 126.18, 119.07, 117.24, 117.07, 116.27, 112.38, 111.34, 38.65, 15.04.

6-(4-(Methylamino)phenyl)-2H-chromen-2-one (11x)

Yield: 85%. ¹H NMR (400 MHz, CDCl₃): δ 7.74 (1H, d, *J* = 9.6 Hz), 7.70 (1H, dd, *J* = 2.4, 8.8 Hz), 7.59 (1H, d, *J* = 2.4 Hz), 7.43 (2H, d, *J* = 8.8 Hz), 7.35 (1H, d, *J* = 8.8 Hz), 6.70 (2H, d, *J* = 8.8 Hz), 6.44 (1H, d, *J* = 9.6 Hz), 3.89 (1H, br s), 2.89 (3H, s). ¹³C NMR (100 MHz, CDCl₃): δ 161.10, 152.79, 149.26, 143.82, 138.18, 130.16, 128.24, 127.98, 124.97, 119.14, 117.22, 116.91, 112.87, 30.81.

6-(4-(Ethylamino)phenyl)-2H-chromen-2-one (11y)

Yield: 89%. ¹H NMR (400 MHz, CDCl₃): δ 7.73 (1H, d, *J* = 9.6 Hz), 7.68 (1H, dd, *J* = 2.4, 8.8 Hz), 7.58 (1H, d, *J* = 2 Hz), 7.41 (2H, d, *J* = 8.4 Hz), 7.33 (1H, t, *J* = 8.4 Hz), 6.68 (2H, d, *J* = 8.8 Hz), 6.44 (1H, d, *J* = 9.6 Hz), 3.72 (1H, br s), 3.21 (2H, q, *J* = 7.2 Hz), 1.29 (3H, t, *J* = 7.6 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 161.09, 152.75, 148.36, 143.82, 138.16, 130.12, 128.12, 127.97, 124.92, 119.12, 117.19, 116.87, 113.15, 38.53, 14.96.

6-(3-(Dimethylamino)phenyl)-2H-chromen-2-one (11z)

Yield: 65%. ¹H NMR (400 MHz, CDCl₃): δ 7.76 (1H, d, *J* = 9.5 Hz), 7.72 (1H, dd, *J* = 2, 8.2 Hz), 7.68 (1H, d, *J* = 2.4 Hz), 7.40 (1H, d, *J* = 8.8 Hz), 7.33 (1H, t, *J* = 8.6 Hz), 6.92–6.88 (2H, m), 6.78 (1H, m), 6.46 (1H, d, *J* = 8.6 Hz), 3.01 (6H, s). ¹³C NMR (100 MHz, CDCl₃): δ 160.82, 153.34, 151.03, 143.59, 140.44, 138.89, 130.99, 129.69, 126.15, 118.91, 117.07, 116.89, 115.48, 111.96, 111.13, 40.64.

6-(4-(Dimethylamino)phenyl)-2H-chromen-2-one (11za)

Yield: 71%. ¹H NMR (400 MHz, CDCl₃): δ 7.74 (1H, d, *J* = 9.6 Hz), 7.7 (1H, dd, *J* = 2, 8.4 Hz), 7.6 (1H, d, *J* = 2.4 Hz), 7.48 (2H, d, *J* = 8.8 Hz), 7.35 (1H, d, *J* = 8.8 Hz), 6.8 (2H, d, *J* = 8.4 Hz), 6.44 (1H, d, *J* = 9.6 Hz), 3.01 (6H, s). ¹³C NMR (100 MHz, CDCl₃): δ 161.09, 152.75, 150.34, 143.83, 138.07, 130.12, 127.74, 127.23, 124.91, 119.14, 117.21, 116.87, 112.88, 40.61.

General Procedure for the Synthesis of Substituted Alkyl-4H-chromene-3-carboxylate Compounds (7a, 7b, 7j-l, 8a-m, and 9a-u)

²⁹ Freshly cut sodium (0.096 mmol) was added to the respective dry alcohol (5 mL), followed by the addition of ethyl cyanoacetate (0.192 mmol). The reaction mixture was stirred at room temperature under an inert atmosphere for 30 min, followed by the addition of a solution of the corresponding coumarin (0.08 mmol) in anhydrous alcohol or DCM (3 mL). The resulting reaction mixture was stirred at room temperature. Upon consumption of the coumarin, the reaction mass was concentrated, diluted with water (30 mL), and extracted

using ethyl acetate (3 × 20 mL). The organics were combined, washed with water, brine, and dried (MgSO₄). Removal of the solvent under reduced pressure afforded the crude mass which upon column purification gave the pure product. Note: Propargyl alcohol used for this reaction must be previously dried with 3 Å molecular sieves. The monopropargyl (R_f = 0.43) and dipropargyl (R_f = 0.41) esters resulting from this reaction could be separated by flash column using the acetone/hexane solvent (3:7) system.

Methyl 2-Amino-6-(3,5-dimethoxyphenyl)-4-(2-methoxy-2-oxoethyl)-4H-chromene-3-carboxylate (7a)

This compound was synthesized by following the same general procedure except that methyl cyanoacetate was used instead of ethyl cyanoacetate. Yield: 35%. ¹H NMR (400 MHz, CDCl₃): δ 7.45 (1H, d, J = 2.20 Hz), 7.41 (1H, dd, J = 2.25, 8.31 Hz), 7.03 (1H, d, J = 8.30 Hz), 6.67 (2H, d, J = 2.35 Hz), 6.46 (1H, t, J = 2.15 Hz), 6.35 (2H, br s), 4.35 (1H, dd, J = 4.70, 7.24 Hz), 3.85 (6H, s), 3.78 (3H, s), 3.59 (3H, s), 2.69 (1H, dd, J = 4.70, 14.80 Hz), 2.62 (1H, dd, J = 7.20, 14.80 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 172.0, 169.4, 161.6, 161.1, 142.5, 137.5, 127.0, 126.5, 125.8, 116.1, 105.2, 105.0, 99.2, 76.5, 55.4, 51.4, 51.0, 43.5, 31.4. MS (ESI, positive) m/z calcd for C₂₂H₂₄NO₇ (M + H): 414.16; found, 414.21. HPLC purity: 100%.

Methyl 2-Amino-6-(3,5-dimethoxyphenyl)-4-(2-ethoxy-2-oxoethyl)-4H-chromene-3-carboxylate (7b)

To a 25 mL flask was added ethyl 2-amino-6-(3,5-dimethoxyphenyl)-4-(2-ethoxy-2-oxoethyl)-4H-chromene-3-carboxylate (15.5 mg, 0.035 mmol) followed by methanol (1 mL). Sodium borohydride (6.61 mg, 0.0175 mmol) was slowly added, and the mixture was stirred at room temperature for 5 h. The resulting mixture was diluted with 25 mL of DCM and washed with saturated NH₄Cl solution. The aqueous phase was extracted with DCM (20 mL × 3), and the combined organic layer was washed with brine and dried over Na₂SO₄. Solvent evaporation gave a crude oil which was further purified by column chromatography to yield the pure product. Yield: 21%. ¹H NMR (400 MHz, CDCl₃): δ 7.44 (1H, d, J = 2.1 Hz), 7.39 (1H, dd, J = 8.3, 2.1 Hz), 7.02 (1H, d, J = 8.3 Hz), 6.66 (2H, d, J = 2.2 Hz), 6.45 (1H, t, J = 2.2 Hz), 6.34 (2H, br s), 4.33 (1H, dd, J = 7.1, 4.8 Hz), 4.02 (2H, q, J = 7.1 Hz), 3.84 (6H, s), 3.77 (3H, s), 2.67 (1H, dd, J = 14.8, 4.8 Hz), 2.61 (1H, dd, J = 14.8, 7.1 Hz), 1.13 (3H, t, J = 7.1 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 171.63, 169.40, 161.55, 161.06, 149.60, 142.50, 137.50, 127.06, 126.46, 125.83, 116.06, 105.19, 99.20, 76.58, 60.21, 55.41, 50.98, 43.70, 31.39, 14.08. MS (ESI, positive) m/z calcd for C₂₃H₂₆NO₇ (M + H): 428.2; found 428.2. HPLC purity: 95%.

Oxiran-2-ylmethyl 2-Amino-6-(3,5-dimethoxyphenyl)-4-(2-(oxiran-2-ylmethoxy)-2-oxoethyl)-4H-chromene-3-carboxylate (7j)

To a stirred solution of 7f (0.17 mmol) in dry DCM under nitrogen at 0 °C was added 0.37 mmol of *m*-CPBA over a period of 5 min. After 1 h, the ice bath was removed and the mixture was left to reach room temperature over 24 h. The reaction mixture was extracted with sodium bicarbonate, washed with brine, dried over Na₂SO₄, filtered, and concentrated. The residue was purified by chromatography to afford the pure compound. Yield: 48%. ¹H NMR (400 MHz, CDCl₃): δ 7.52 (1H, dd, J = 2.25, 8.51 Hz), 7.40 (1H, d, J = 2.15 Hz), 7.29

(1H, d, J = 8.41 Hz), 6.64 (2H, d, J = 2.15 Hz), 6.48 (1H, t, J = 2.15 Hz), 5.76–5.95 (1H, m), 5.23–5.34 (2H, m), 5.19–5.26 (2H, m), 5.12 (2H, dd, J = 4.60, 9.88 Hz), 5.04 (2H, br s), 4.64 (2H, tt, J = 1.20 Hz, J = 5.84 Hz), 4.59 (2H, tt, J = 1.20 Hz, 5.80 Hz), 3.85 (6H, s), 3.29 (1H, dd, J = 9.98, 17.22 Hz), 2.74 (1H, dd, J = 4.50, 17.22 Hz). ^{13}C NMR (100 MHz, CDCl_3): δ 191.4, 171.1, 161.1, 160.0, 154.0, 148.1, 141.9, 139.6, 131.8, 130.6, 128.5, 128.0, 123.6, 119.7, 118.5, 105.5, 99.5, 66.9, 65.7, 55.4, 45.0, 35.8 (two coinciding carbon resonances). MS (ESI, positive) m/z calcd for $\text{C}_{26}\text{H}_{27}\text{NO}_9$ ($M + H$): 498.18; found, 498.16. HPLC purity: 95.6%.

Ethyl 2-(2-Amino-3-cyano-6-(3,5-dimethoxyphenyl)-4H-chromen-4-yl)acetate (7k)

This compound was synthesized by following the same general procedure except that malononitrile was used instead of ethyl cyanoacetate. Yield: 85%. ^1H NMR (400 MHz, CDCl_3): δ 7.43–7.38 (2H, m), 7.00 (1H, d, J = 8.0 Hz), 6.64 (2H, d, J = 2.0 Hz), 6.45 (1H, t, J = 2.0 Hz), 4.73 (2H, s), 4.14–4.09 (3H, m), 3.84 (6H, s), 2.74–2.72 (2H, m), 1.20 (3H, t, J = 6.8 Hz). ^{13}C NMR (100 MHz, CDCl_3): δ 170.84, 161.26, 160.93, 148.97, 142.17, 138.29, 127.23, 126.78, 122.84, 119.76, 116.76, 105.37, 99.45, 60.87, 57.99, 55.56, 43.51, 32.14, 14.19. MS (ESI, positive) m/z calcd for $\text{C}_{22}\text{H}_{22}\text{N}_2\text{O}_5$ ($M + H$): 395.15; found 395.2. HPLC purity: 100%.

Ethyl 2-(2-Amino-6-(3,5-dimethoxyphenyl)-3-(3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-yl)-4H-chromen-4-yl)acetate (7l)

To a solution of 4-methoxyphenylhydroximinoyl chloride (1 mmol), and ethyl 2-(2-amino-3-cyano-6-(3,5-dimethoxyphenyl)-4H-chromen-4-yl)acetate (1 mmol) in benzene was added triethylamine (1 mmol) in benzene slowly. The contents were refluxed for 6 h and concentrated under reduced pressure. This was diluted with water and extracted with ethyl acetate. The organics were combined, washed with water, brine, and dried over MgSO_4 . Removal of the solvent under reduced pressure gave a crude mass which upon column purification furnished pure product. Yield: 46%. ^1H NMR (400 MHz, CDCl_3): δ 8.02 (2H, d, J = 8.8 Hz), 7.53 (1H, d, J = 2.0 Hz), 7.43 (1H, dd, J = 2.0, 8.4 Hz), 7.07 (1H, d, J = 8.4 Hz), 6.98 (2H, d, J = 8.4 Hz), 6.69 (2H, d, J = 2 Hz), 6.59 (2H, br s), 6.47 (1H, t, J = 2 Hz), 4.57 (1H, t, J = 5.4 Hz), 4.02 (2H, q, J = 6.8 Hz), 3.87 (6H, s), 3.85 (3H, s), 2.81 (2H, d, J = 5.6 Hz), 1.12 (3H, t, J = 6.8 Hz). ^{13}C NMR (100 MHz, CDCl_3): δ 175.63, 171.28, 166.96, 161.80, 161.26, 158.43, 149.69, 142.49, 137.95, 129.09, 127.25, 126.93, 124.60, 120.02, 116.45, 114.23, 105.38, 99.44, 72.25, 60.50, 55.57, 55.49, 43.10, 31.48, 14.19. MS (ESI, positive) m/z calcd for $\text{C}_{29}\text{H}_{24}\text{N}_4\text{O}_5$ ($M + H$): 543.20; found 544.1. HPLC purity: 96.9%.

Ethyl 2-Amino-6-bromo-4-(2-ethoxy-2-oxoethyl)-4H-chromene-3-carboxylate (8a)

Yield: 85.0%. ^1H NMR (400 MHz, CDCl_3): δ 7.38 (1H, d, J = 2.3 Hz), 7.28 (1H, dd, J = 8.6, 2.3 Hz), 6.84 (1H, d, J = 8.6 Hz), 6.30 (2H, br s), 4.28–4.17 (3H, m), 4.11 – 3.99 (2H, m), 2.63 (1H, dd, J = 15.2, 4.5 Hz), 2.57 (1H, dd, J = 15.2, 4.5 Hz), 1.31 (3H, t, J = 7.0 Hz), 1.17 (3H, t, J = 7.1 Hz). ^{13}C NMR (100 MHz, CDCl_3): δ 171.39, 168.73, 161.11, 149.03, 131.11, 130.55, 127.77, 117.50, 116.61, 76.23, 60.31, 59.58, 43.32, 31.06, 14.52, 14.08.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(2-fluorophenyl)-4H-chromene-3-carboxylate (8b)

Yield: 18%. ¹H NMR (400 MHz, CDCl₃): δ 7.44 (1H, s), 7.42–7.34 (2H, m), 7.34–7.24 (1H, m), 7.22–7.09 (2H, m), 7.01 (1H, d, *J* = 8.4 Hz), 6.34 (2H, br s), 4.35 (1H, dd, *J* = 6.7, 4.1 Hz), 4.24 (2H, q, *J* = 7.0 Hz), 4.02 (2H, q, *J* = 7.0 Hz), 2.75–2.55 (2H, m), 1.33 (3H, t, *J* = 7.0 Hz), 1.12 (3H, t, *J* = 7.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 171.66, 169.00, 161.40, 159.65 (d, *J*_{C-F} = 247.1 Hz), 149.51, 131.95 (d, *J*_{C-F} = 1.5 Hz), 130.50 (d, *J*_{C-F} = 3.0 Hz), 129.03 (d, *J*_{C-F} = 3.0 Hz), 128.89 (d, *J*_{C-F} = 8.9 Hz), 128.35 (d, *J*_{C-F} = 3.0 Hz), 125.67, 124.34 (d, *J*_{C-F} = 3.8 Hz), 116.20, 115.98, 115.84, 76.77, 60.20, 59.53, 43.66, 31.29, 14.57, 14.01. MS (ESI, positive) *m/z* calcd for C₂₂H₂₃FNO₅ (M + H): 400.2; found 400.2. HPLC purity: 98.9%.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(3-fluorophenyl)-4H-chromene-3-carboxylate (8c)

Yield: 31%. ¹H NMR (400 MHz, CDCl₃): δ 7.60–7.20 (5H, m), 7.10–7.00 (2H, m), 6.37 (2H, br s), 4.42–4.34 (1H, m), 4.32–4.20 (2H, m), 4.10–4.00 (2H, m), 2.77–2.60 (2H, m), 1.36 (3H, t, *J* = 7.2 Hz), 1.12 (3H, t, *J* = 7.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 171.66, 168.96, 163.14 (d, *J*_{C-F} = 244.8 Hz), 161.35, 149.80, 142.52 (d, *J*_{C-F} = 8.2 Hz), 136.13, 130.21 (d, *J*_{C-F} = 8.2 Hz), 127.06, 126.31, 126.08, 122.41 (d, *J*_{C-F} = 3.0 Hz), 116.25, 113.92 (d, *J*_{C-F} = 21.6 Hz), 113.68 (d, *J*_{C-F} = 21.6 Hz), 76.62, 60.22, 59.55, 43.60, 31.29, 14.55, 14.03. MS (ESI, positive) *m/z* calcd for C₂₂H₂₃FNO₅ (M + H): 400.2; found 400.2. HPLC purity: 98.9%.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(4-fluorophenyl)-4H-chromene-3-carboxylate (8d)

Yield: 42%. ¹H NMR (400 MHz, CDCl₃): δ 7.55–7.44 (2H, m), 7.42 (1H, d, *J* = 2.1 Hz), 7.35 (1H, dd, *J* = 8.4, 2.1 Hz), 7.15–7.05 (2H, m), 7.01 (1H, d, *J* = 8.4 Hz), 6.34 (2H, br s), 4.35 (1H, dd, *J* = 6.7, 4.1 Hz), 4.24 (2H, q, *J* = 7.0 Hz), 4.02 (2H, q, *J* = 7.0 Hz), 2.75–2.55 (2H, m), 1.33 (3H, t, *J* = 7.0 Hz), 1.12 (3H, t, *J* = 7.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 171.69, 168.96, 162.33 (d, *J*_{C-F} = 244.1 Hz), 161.40, 149.39, 136.46, 136.41 (d, *J*_{C-F} = 2.2 Hz), 128.35 (d, *J*_{C-F} = 9.0 Hz), 126.92, 126.20, 125.99, 116.15, 115.59 (d, *J*_{C-F} = 21.6 Hz), 76.65, 60.19, 59.53, 43.64, 31.30, 14.55, 14.03. MS (ESI, positive) *m/z* calcd for C₂₂H₂₃FNO₅ (M⁺): 400.2; found 400.2. HPLC purity: 98.5%.

Ethyl 2-Amino-6-(2,4-difluorophenyl)-4-(2-ethoxy-2-oxoethyl)-4H-chromene-3-carboxylate (8e)

Yield: 53%. ¹H NMR (400 MHz, CDCl₃): δ 7.42–7.28 (3H, m), 7.02 (1H, d, *J* = 8.4 Hz), 6.97–6.81 (2H, m), 6.35 (2H, br s), 4.34 (1H, dd, *J* = 7.4, 4.3 Hz), 4.23 (2H, q, *J* = 7.0 Hz), 4.02 (2H, q, *J* = 7.2 Hz), 2.75–2.55 (2H, m), 1.32 (3H, t, *J* = 7.0 Hz), 1.13 (3H, t, *J* = 7.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 171.64, 168.94, 162.15 (dd, *J*_{C-F} = 247.0, 11.9 Hz), 159.60 (dd, *J*_{C-F} = 248.9, 11.5 Hz), 161.37, 149.53, 131.15 (t, *J*_{C-F} = 8.2 Hz), 131.14 (d, *J*_{C-F} = 9.6 Hz), 128.92 (d, *J*_{C-F} = 2.2 Hz), 128.20 (d, *J*_{C-F} = 3.0 Hz), 125.79, 124.45 (dd, *J*_{C-F} = 13.4, 3.8 Hz), 115.93, 111.53 (dd, *J*_{C-F} = 20.8, 3.8 Hz), 104.34 (t, *J*_{C-F} = 25.7 Hz), 76.69, 60.19, 59.54, 43.63, 31.24, 14.54, 14.00. MS (ESI, positive) *m/z* calcd for C₂₂H₂₂F₂NO₅ (M + H): 418.2; found 418.2. HPLC purity: 96.1%.

Ethyl 2-Amino-6-(3,5-difluorophenyl)-4-(2-ethoxy-2-oxoethyl)-4H-chromene-3-carboxylate (8f)

Yield: 69%. ¹H NMR (400 MHz, CDCl₃): δ 7.44 (1H, d, *J* = 2.2 Hz), 7.36 (1H, dd, *J* = 8.4, 2.2 Hz), 7.08–6.99 (3H, m), 6.76 (1H, tt, *J* = 8.9 Hz, 2.3 Hz), 6.34 (2H, br s), 4.35 (1H, dd, *J* = 7.2, 4.3 Hz), 4.24 (2H, q, *J* = 7.1 Hz), 4.03 (2H, q, *J* = 7.1 Hz), 2.69 (1H, dd, *J* = 15.0, 4.3 Hz), 2.63 (1H, dd, *J* = 15.0, 7.2 Hz), 1.33 (3H, t, *J* = 7.1 Hz), 1.13 (3H, t, *J* = 7.1 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 171.62, 168.89, 163.28 (dd, *J*_{C-F} = 247.0, 13.4 Hz), 161.25, 150.21, 143.57 (t, *J*_{C-F} = 9.7 Hz), 135.03 (t, *J*_{C-F} = 2.6 Hz), 127.07, 126.29, 126.25, 116.40, 109.60 (dd, *J*_{C-F} = 18.6, 7.4 Hz), 102.36 (t, *J*_{C-F} = 25.3 Hz), 76.57, 60.25, 59.59, 43.54, 31.23, 14.54, 14.04. MS (ESI, positive) *m/z* calcd for C₂₂H₂₂F₂NO₅ (M + H): 418.2; found 418.2. HPLC purity: 96.3%.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(furan-2-yl)-4H-chromene-3-carboxylate (8g)

This compound was synthesized by Suzuki-Miyaura coupling of 8a with 2-furylboronic acid MIDA ester. (Refer to the general procedure for the synthesis of 6-arylcoumarins.) Yield: 15%. ¹H NMR (400 MHz, CDCl₃): δ 7.55 (1H, d, *J* = 2.1 Hz), 7.49 (1H, dd, *J* = 8.6, 2.1 Hz), 7.43 (1H, dd, *J* = 2.0, 0.7 Hz), 6.97 (1H, d, *J* = 8.6 Hz), 6.56 (1H, dd, *J* = 3.3, 0.7 Hz), 6.45 (1H, dd, *J* = 3.3, 2.0 Hz), 6.31 (2H, br s), 4.31 (1H, dd, *J* = 7.1, 4.4 Hz), 4.23 (2H, q, *J* = 7.0 Hz), 4.03 (2H, q, *J* = 7.2 Hz), 2.68 (1H, dd, *J* = 14.8, 4.4 Hz), 2.61 (1H, dd, *J* = 14.8, 7.1 Hz), 1.33 (3H, t, *J* = 7.0 Hz), 1.14 (3H, t, *J* = 7.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 171.64, 168.99, 161.32, 153.31, 149.18, 141.87, 127.40, 125.91, 123.75, 123.28, 116.12, 111.62, 104.46, 76.60, 60.23, 59.53, 43.54, 31.29, 14.55, 14.03. MS (ESI, positive) *m/z* calcd for C₂₀H₂₂NO₆ (M + H): 372.1; found 372.1. HPLC purity: 98.1%.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(thiophen-2-yl)-4H-chromene-3-carboxylate (8h)

This compound was synthesized by Suzuki-Miyaura coupling of 8a with 2-thiopheneboronic acid MIDA ester. (Refer to the general procedure for the synthesis of 6-arylcoumarins.) Yield: 25%. ¹H NMR (400 MHz, CDCl₃): δ 7.48 (1H, d, *J* = 2.2 Hz), 7.43 (1H, dd, *J* = 8.4, 2.2 Hz), 7.25 (1H, dd, *J* = 5.1, 1.0 Hz), 7.23 (1H, dd, *J* = 3.6, 1.0 Hz), 7.06 (1H, dd, *J* = 5.1, 3.6 Hz), 6.97 (1H, d, *J* = 8.4 Hz), 6.32 (2H, br s), 4.32 (1H, dd, *J* = 7.2, 4.3 Hz), 4.23 (2H, q, *J* = 7.1 Hz), 4.03 (2H, q, *J* = 7.2 Hz), 2.68 (1H, dd, *J* = 14.8, 4.3 Hz), 2.61 (1H, dd, *J* = 14.8, 7.2 Hz), 1.33 (3H, t, *J* = 7.1 Hz), 1.15 (3H, t, *J* = 7.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 171.63, 168.97, 161.32, 149.36, 143.56, 130.83, 128.00, 126.11, 125.81, 125.30, 124.56, 122.81, 116.27, 76.60, 60.27, 59.55, 43.60, 31.25, 14.57, 14.08. MS (ESI, positive) *m/z* calcd for C₂₀H₂₂NO₅S (M + H): 388.1; found 388.1. HPLC purity: 95.6%.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(pyridin-2-yl)-4H-chromene-3-carboxylate (8i)

Yield: 62%. ¹H NMR (400 MHz, CDCl₃): δ 8.69–8.61 (1H, m), 7.90 (1H, d, *J* = 2.0 Hz), 7.85 (1H, dd, *J* = 8.4, 2.0 Hz), 7.75–7.63 (2H, m), 7.22–7.16 (1H, m), 7.04 (1H, d, *J* = 8.4 Hz), 6.36 (2H, br s), 4.37 (1H, dd, *J* = 6.6, 4.8 Hz), 4.23 (2H, q, *J* = 7.1 Hz), 4.01 (2H, q, *J* = 7.1 Hz), 2.70 (1H, dd, *J* = 15.0, 4.8 Hz), 2.65 (1H, dd, *J* = 15.0, 6.6 Hz), 1.32 (3H, t, *J* = 7.1 Hz), 1.12 (3H, t, *J* = 7.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 171.70, 169.03, 161.40, 156.49, 150.71, 149.61, 136.74, 135.65, 127.04, 126.29, 125.91, 121.93, 120.06, 116.15,

76.63, 60.22, 59.53, 43.53, 31.34, 14.58, 14.04. MS (ESI, positive) m/z calcd for $C_{21}H_{23}N_2O_5$ (M + H): 383.2; found 383.2. HPLC purity: 99.7%.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(pyridin-3-yl)-4H-chromene-3-carboxylate (8j)

Yield: 69%. 1H NMR (400 MHz, $CDCl_3$): δ 8.79 (1H, d, J = 1.6 Hz), 8.57 (1H, dd, J = 5.0, 1.6 Hz), 7.81 (1H, dt, J = 7.9, 1.6 Hz), 7.47 (1H, d, J = 2.2 Hz), 7.40 (1H, dd, J = 8.4, 2.2 Hz), 7.34 (1H, dd, J = 7.9, 5.0 Hz), 7.06 (1H, d, J = 8.4 Hz), 6.37 (2H, br s), 4.36 (1H, dd, J = 7.2, 4.3 Hz), 4.29–4.19 (2H, m), 4.02 (2H, q, J = 7.2 Hz), 2.69 (1H, dd, J = 15.0, 4.3 Hz), 2.62 (1H, dd, J = 15.0, 7.2 Hz), 1.33 (3H, t, J = 7.1 Hz), 1.12 (3H, t, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.63, 168.90, 161.31, 150.03, 148.35, 148.00, 135.76, 134.02, 133.99, 127.16, 126.42, 126.39, 123.50, 116.52, 76.57, 60.23, 59.56, 43.58, 31.24, 14.55, 14.05. MS (ESI, positive) m/z calcd for $C_{21}H_{23}N_2O_5$ (M + H): 383.2; found 383.2. HPLC purity: 99.8%.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(pyridin-4-yl)-4H-chromene-3-carboxylate (8k)

Yield: 41%. 1H NMR (400 MHz, $CDCl_3$): δ 8.63 (2H, d, J = 4.7 Hz), 7.55 (1H, d, J = 2.0 Hz), 7.50–7.40 (3H, m), 7.06 (1H, d, J = 8.4 Hz), 6.38 (2H, br s), 4.36 (1H, dd, J = 7.3, 4.4 Hz), 4.23 (2H, q, J = 7.0 Hz), 4.02 (2H, q, J = 7.0 Hz), 2.69 (1H, dd, J = 15.2, 4.4 Hz), 2.61 (1H, dd, J = 15.2, 7.3 Hz), 1.33 (3H, t, J = 7.0 Hz), 1.12 (3H, t, J = 7.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.63, 168.85, 161.20, 150.70, 150.22, 147.33, 134.16, 127.16, 126.47, 126.29, 121.23, 116.59, 76.55, 60.25, 59.61, 43.55, 31.20, 14.54, 14.04. MS (ESI, positive) m/z calcd for $C_{21}H_{23}N_2O_5$ (M + H): 383.2; found 383.2. HPLC purity: 99.9%.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(pyrimidin-3,5-yl)-4H-chromene-3-carboxylate (8l)

Yield: 38%. 1H NMR (400 MHz, $CDCl_3$): δ 9.19 (1H, s), 8.91 (2H, s), 7.50 (1H, d, J = 2.2 Hz), 7.41 (1H, dd, J = 8.4, 2.2 Hz), 7.11 (1H, d, J = 8.4 Hz), 6.36 (2H, br s), 4.37 (1H, dd, J = 7.3, 4.2 Hz), 4.30–4.18 (2H, m), 4.03 (2H, q, J = 7.1 Hz), 2.70 (1H, dd, J = 15.0, 4.2 Hz), 2.63 (1H, dd, J = 15.0, 7.3 Hz), 1.33 (3H, t, J = 7.1 Hz), 1.14 (3H, t, J = 7.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.57, 168.81, 161.13, 157.36, 154.61, 150.72, 133.54, 130.36, 127.11, 127.00, 126.32, 116.98, 76.53, 60.31, 59.66, 43.49, 31.12, 14.56, 14.10. MS (ESI, positive) m/z calcd for $C_{20}H_{22}N_3O_5$ (M + H): 384.2; found 384.2. HPLC purity: 98.4%.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(2-methoxypyridin-4-yl)-4H-chromene-3-carboxylate (8m)

Yield: 44%. 1H NMR (400 MHz, $CDCl_3$): δ 8.18 (1H, d, J = 5.5 Hz), 7.51 (1H, d, J = 2.2 Hz), 7.43 (1H, dd, J = 8.4, 2.2 Hz), 7.07–7.00 (2H, m), 6.88 (1H, d, J = 0.8 Hz), 6.36 (2H, br s), 4.34 (1H, dd, J = 7.2, 4.3 Hz), 4.28–4.18 (2H, m), 4.01 (2H, q, J = 7.1 Hz), 3.97 (3H, s), 2.68 (1H, dd, J = 15.2, 4.3 Hz), 2.61 (1H, dd, J = 15.2, 7.2 Hz), 1.32 (3H, t, J = 7.1 Hz), 1.12 (3H, t, J = 7.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.58, 168.88, 164.89, 161.23, 150.59, 150.15, 147.22, 134.34, 127.10, 126.28, 126.27, 116.41, 114.98, 108.02, 76.55, 60.23, 59.57, 53.49, 43.53, 31.21, 14.55, 14.04. MS (ESI, positive) m/z calcd for $C_{22}H_{25}N_2O_6$ (M + H): 413.2; found 413.2. HPLC purity: 99.8%.

Ethyl 2-Amino-6-(3-aminophenyl)-4-(2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (9a)

Yield: 63%. ¹H NMR (400 MHz, CDCl₃) δ 7.45 (1H, d, *J* = 2.26 Hz), 7.39 (1H, dd, *J* = 2.26, 8.53 Hz), 7.21 (1H, t, *J* = 7.78 Hz), 7.00 (1H, d, *J* = 8.53 Hz), 6.94 (1H, d, *J* = 7.78 Hz), 6.87 (1H, t, *J* = 1.88 Hz), 6.68 (1H, dd, *J* = 2.01, 7.78 Hz), 6.33 (2H, br s), 4.35 (1H, dd, *J* = 4.52, 7.03 Hz), 4.24 (2H, q, *J* = 7.11 Hz), 4.02 (2H, q, *J* = 7.19 Hz), 2.69 (1H, dd, *J* = 4.52, 15.00 Hz), 2.63 (1H, dd, *J* = 7.03, 15.00 Hz), 1.34 (3H, t, *J* = 7.15 Hz), 1.13 (3H, t, *J* = 7.15 Hz). ¹³C NMR (100 MHz, CDCl₃) δ 171.7, 169.1, 161.5, 149.4, 146.7, 141.4, 137.6, 129.7, 126.9, 126.3, 125.7, 117.3, 116.0, 114.0, 113.5, 60.2, 59.5, 43.6, 31.3, 14.6, 14.0 (two coinciding carbon resonances). MS (ESI, positive) *m/z* calcd for C₂₂H₂₄N₂O₅ (M + H): 397.17; found, 397.07. HPLC purity: 95.5%.

Ethyl 2-Amino-6-(3-aminophenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4*H*-chromene-3-carboxylate (9b)

Yield: 29%. ¹H NMR (400 MHz, CDCl₃): δ 7.45 (1H, d, *J* = 2 Hz), 7.38 (1H, dd, *J* = 2.4, 8.4 Hz), 7.19 (1H, t, *J* = 8 Hz), 7.00 (1H, d, *J* = 8.4 Hz), 6.93–6.91 (1H, m), 6.85 (1H, t, *J* = 2 Hz), 6.66–6.64 (1H, m), 6.34 (2H, s), 4.58 (2H, t, *J* = 2.4 Hz), 4.39–4.37 (1H, m), 4.24 (2H, q, *J* = 6.8 Hz), 3.74 (2H, s), 2.76–2.62 (2H, m), 2.28 (1H, t, *J* = 2.4 Hz), 1.33 (3H, t, *J* = 7.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 170.99, 169.07, 161.68, 149.45, 146.85, 141.58, 137.92, 129.78, 127.09, 126.59, 125.58, 117.56, 116.21, 114.09, 113.82, 77.74, 76.76, 74.99, 59.71, 51.86, 43.53, 31.45, 14.72. MS (ESI, positive) *m/z* calcd for C₂₃H₂₂N₂O₅ (M + H): 407.15; found 407.05. HPLC purity: 99.72%.

Prop-2-yn-1-yl 2-Amino-6-(3-aminophenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4*H*-chromene-3-carboxylate (9c)

Yield: 59%. ¹H NMR (400 MHz, CDCl₃): δ 7.45 (1H, d, *J* = 2 Hz), 7.38 (1H, dd, *J* = 2, 8.4 Hz), 7.19 (1H, t, *J* = 8 Hz), 7.01 (1H, d, *J* = 8.4 Hz), 6.92 (1H, d, *J* = 7.6 Hz), 6.85 (1H, t, *J* = 2 Hz), 6.66 (1H, dd, *J* = 1.6, 8 Hz), 6.41 (2H, s), 4.79 (2H, t, *J* = 2.2 Hz), 4.60–4.59 (2H, m), 4.41–4.38 (1H, m), 3.74 (2H, s), 2.78–2.64 (2H, m), 2.47 (1H, t, *J* = 2.0 Hz), 2.29 (1H, t, *J* = 2.6 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 170.81, 167.97, 162.35, 149.26, 146.85, 141.41, 138.02, 129.75, 127.02, 126.63, 125.33, 117.45, 116.18, 114.11, 113.75, 78.83, 77.71, 75.89, 75.04, 74.41, 51.89, 51.21, 43.47, 31.23. MS (ESI, positive) *m/z* calcd for C₂₄H₂₀N₂O₅ (M + H): 417.14; found 417.1. HPLC purity: 99.5%.

Prop-2-yn-1-yl 2-(2-Amino-6-(3-aminophenyl)-3-cyano-4*H*-chromen-4-yl)acetate (9d)

This compound was synthesized by following the same general procedure except that malononitrile was used instead of ethyl cyanoacetate. Yield: 61%. ¹H NMR (400 MHz, CDCl₃): δ 7.41 (1H, dd, *J* = 2, 8.4 Hz), 7.39 (1H, d, *J* = 2 Hz), 7.20 (1H, t, *J* = 7.8 Hz), 7.01 (1H, d, *J* = 8.4 Hz), 6.92 (1H, d, *J* = 8 Hz), 6.83 (1H, s), 6.66 (1H, dd, *J* = 1.8, *J* = 7.8 Hz), 4.67 (2H, d, *J* = 2 Hz), 4.66 (2H, s), 4.16–4.11 (1H, m), 3.76 (2H, s), 2.81–2.77 (2H, m), 2.35 (1H, t, *J* = 2.4 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 170.07, 160.97, 148.71, 146.95, 141.16, 138.76, 129.93, 127.34, 126.72, 122.42, 119.69, 117.54, 116.81, 114.40, 113.76, 77.36, 75.29, 58.05, 52.35, 43.25, 32.17. MS (ESI, positive) *m/z* calcd for C₂₁H₁₇N₃O₃ (M + H): 360.13; found 360.1. HPLC purity: 95%.

Ethyl 2-Amino-6-(4-aminophenyl)-4-(2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (9e)

Yield: 72%. ¹H NMR (400 MHz, CDCl₃): δ 7.39 (1H, d, *J* = 2 Hz), 7.35–7.32 (3H, m), 6.97 (1H, d, *J* = 8.4 Hz), 6.72 (2H, d, *J* = 8.4 Hz), 6.33 (2H, s), 4.35–4.32 (1H, m), 4.23 (2H, q, *J* = 7.2 Hz), 4.01 (2H, q, *J* = 7.2 Hz), 3.73 (2H, s), 2.70–2.58 (2H, m), 1.33 (3H, t, *J* = 7.2 Hz), 1.12 (3H, t, *J* = 7.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 171.91, 169.21, 161.69, 148.81, 145.89, 137.61, 130.75, 127.81, 126.23, 125.83, 125.67, 116.08, 115.49, 60.29, 59.61, 43.85, 31.53, 14.70, 14.18 (two coinciding carbon resonances). MS (ESI, positive) *m/z* calcd for C₂₂H₂₄N₂O₅ (M + H): 397.17; found 397.2. HPLC purity: 99.4%.

Ethyl 2-Amino-6-(4-aminophenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4*H*-chromene-3-carboxylate (9f)

Yield: 22%. ¹H NMR (400 MHz, CDCl₃): δ 7.40 (1H, d, *J* = 2 Hz), 7.35 (3H, d, *J* = 8.4 Hz), 6.99 (1H, d, *J* = 8.4 Hz), 6.73 (2H, d, *J* = 8.4 Hz), 6.31 (2H, s), 4.58 (2H, t, *J* = 2.8 Hz), 4.38–4.35 (1H, m), 4.24 (2H, q, *J* = 7.2 Hz), 3.72 (2H, s), 2.76–2.62 (2H, m), 2.27 (1H, t, *J* = 2.4 Hz), 1.33 (3H, t, *J* = 7.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 171.04, 169.14, 161.78, 148.82, 145.90, 137.80, 130.85, 128.00, 126.30, 125.91, 125.59, 116.22, 115.47, 77.77, 77.37, 74.93, 59.70, 51.86, 43.56, 31.50, 14.75. MS (ESI, positive) *m/z* calcd for C₂₃H₂₂N₂O₅ (M + H): 407.15; found 407.05. HPLC purity: 94.4%.

Prop-2-yn-1-yl 2-Amino-6-(4-aminophenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4*H*-chromene-3-carboxylate (9g)

Yield: 67%. ¹H NMR (400 MHz, CDCl₃): δ 7.41 (1H, d, *J* = 1.8 Hz), 7.35 (3H, d, *J* = 8.4 Hz), 7.00 (1H, d, *J* = 8.4 Hz), 6.73 (2H, d, *J* = 8.4 Hz), 6.39 (2H, s), 4.35–4.79 (2H, t, *J* = 2 Hz), 4.58 (2H, t, *J* = 2.4 Hz), 4.39–4.37 (1H, m), 3.72 (2H, s), 2.77–2.65 (2H, m), 2.47 (1H, t, *J* = 2 Hz), 2.28 (1H, t, *J* = 2.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 170.88, 168.07, 162.47, 148.68, 145.95, 137.97, 130.71, 127.98, 126.27, 125.97, 125.39, 116.23, 115.47, 78.87, 77.77, 76.06, 74.97, 74.37, 51.92, 51.25, 43.53, 31.32. MS (ESI, positive) *m/z* calcd for C₂₄H₂₀N₂O₅ (M + H): 417.14; found 417.2. HPLC purity: 96.9%.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(3-(methylamino)-phenyl)-4*H*-chromene-3-carboxylate (9h)

Yield: 74%. ¹H NMR (400 MHz, CDCl₃): δ 7.46 (1H, d, *J* = 2 Hz), 7.39 (1H, dd, *J* = 2.1, 8.4 Hz), 7.22 (1H, t, *J* = 8 Hz), 7.00 (1H, d, *J* = 8.4 Hz), 6.87 (1H, d, *J* = 7.6 Hz), 6.75 (1H, s), 6.59 (1H, dd, *J* = 2.1, 8 Hz), 6.38 (2H, s), 4.36 (1H, m), 4.24 (2H, q, *J* = 7.0 Hz), 4.03 (2H, q, *J* = 7.2 Hz), 3.74 (1H, br s), 2.88 (3H, s), 2.72–2.60 (2H, m), 1.34 (3H, t, *J* = 7.2 Hz), 1.13 (3H, t, *J* = 7.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 171.83, 169.16, 161.65, 149.78, 149.41, 141.47, 138.14, 129.65, 127.07, 126.47, 125.78, 116.14, 116.01, 111.49, 110.88, 76.74, 60.26, 59.59, 43.79, 31.47, 30.85, 14.67, 14.15. MS (ESI, positive) *m/z* calcd for C₂₃H₂₆N₂O₅ (M + H): 411.18; found 411.2. HPLC purity: 99.2%.

Ethyl 2-Amino-6-(3-(methylamino)phenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4*H*-chromene-3-carboxylate (9i)

Yield: 23%. ¹H NMR (400 MHz, CDCl₃): δ 7.46 (1H, d, *J* = 2 Hz), 7.41 (1H, dd, *J* = 2, 8.4 Hz), 7.22 (1H, t, *J* = 8 Hz), 7.00 (1H, d, *J* = 8.2 Hz), 6.86 (1H, d, *J* = 7.6 Hz), 6.75 (1H, s),

6.58 (1H, dd, $J=1.8, 8$ Hz), 6.34 (2H, s), 4.58 (2H, t, $J=2.5$ Hz), 4.40–4.37 (1H, m), 4.24 (2H, q, $J=7.0$ Hz), 3.81 (1H, s), 2.89 (3H, s), 2.76–2.62 (2H, m), 2.27 (1H, t, $J=2.4$ Hz), 1.33 (3H, t, $J=7.0$ Hz). ^{13}C NMR (100 MHz, CDCl_3): δ 170.99, 169.09, 161.71, 149.78, 149.41, 141.53, 138.36, 129.66, 127.16, 126.70, 125.54, 116.37, 116.17, 111.50, 111.51, 77.73, 76.77, 74.98, 59.71, 51.86, 43.53, 31.47, 30.93, 14.73. MS (ESI, positive) m/z calcd for $\text{C}_{24}\text{H}_{24}\text{N}_2\text{O}_5$ ($M + H$): 421.17; found 421.2. HPLC purity: 97.2%.

Prop-2-yn-1-yl 2-Amino-6-(3-(methylamino)phenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4H-chromene-3-carboxylate (9j)

Yield: 63%. ^1H NMR (400 MHz, CDCl_3): δ 7.47 (1H, d, $J=2$ Hz), 7.40 (1H, dd, $J=2.2, 8.6$ Hz), 7.23 (1H, t, $J=8$ Hz), 7.01 (1H, d, $J=8.4$ Hz), 6.86 (1H, d, $J=7.6$ Hz), 6.74 (1H, s), 6.59 (1H, dd, $J=2, 8.1$ Hz), 6.40 (2H, s), 4.79 (2H, t, $J=2$ Hz), 4.59 (2H, t, $J=2.8$ Hz), 4.39 (1H, m), 3.80 (1H, br s), 2.89 (3H, s), 2.78–2.66 (2H, m), 2.47 (1H, t, $J=2.4$ Hz), 2.28 (1H, t, $J=2.8$ Hz). ^{13}C NMR (100 MHz, CDCl_3): δ 170.82, 168.03, 162.40, 149.77, 149.28, 141.41, 138.52, 129.68, 127.14, 126.77, 125.33, 116.32, 116.18, 111.54, 111.09, 78.85, 77.73, 75.99, 75.01, 74.39, 51.91, 51.25, 43.49, 31.29, 30.91. MS (ESI, positive) m/z calcd for $\text{C}_{25}\text{H}_{22}\text{N}_2\text{O}_5$ ($M + H$): 431.15; found 431.3. HPLC purity: 97.5%.

Prop-2-yn-1-yl 2-Amino-6-(3-(dimethylamino)phenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4H-chromene-3-carboxylate (9k)

Yield: 60%. ^1H NMR (400 MHz, CDCl_3): δ 7.48 (1H, d, $J=2.1$ Hz), 7.43 (1H, dd, $J=2.2, 8.5$ Hz), 7.28 (1H, t, $J=8$ Hz), 7.02 (1H, d, $J=8.4$ Hz), 6.89–6.85 (2H, m), 6.72 (1H, dd, $J=2, 8.0$ Hz), 6.40 (2H, s), 4.79 (2H, t, $J=2$ Hz), 4.59 (2H, t, $J=2.8$ Hz), 4.42–4.39 (1H, m), 3.0 (6H, s), 2.78–2.67 (2H, m), 2.47 (1H, t, $J=2.4$ Hz), 2.27 (1H, t, $J=2.8$ Hz). ^{13}C NMR (100 MHz, CDCl_3): δ 170.84, 168.08, 162.45, 151.08, 149.31, 141.37, 139.00, 129.55, 127.31, 126.95, 125.37, 116.19, 115.77, 111.74, 111.46, 78.86, 77.73, 76.07, 74.99, 74.39, 51.94, 51.29, 43.50, 40.85, 30.32. MS (ESI, positive) m/z calcd for $\text{C}_{26}\text{H}_{24}\text{N}_2\text{O}_5$ ($M + H$): 445.17; found 445.2. HPLC purity: 95.2%.

Ethyl 2-Amino-6-(3-(ethylamino)phenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4H-chromene-3-carboxylate (9l)

Yield: 21%. ^1H NMR (400 MHz, CDCl_3): δ 7.45 (1H, d, $J=2$ Hz), 7.40 (1H, dd, $J=2, 8.4$ Hz), 7.21 (1H, t, $J=7.8$ Hz), 7.00 (1H, d, $J=8.2$ Hz), 6.85 (1H, d, $J=7.7$ Hz), 6.75 (1H, s), 6.58 (1H, dd, $J=2.2, 8$ Hz), 6.32 (2H, s), 4.57 (2H, t, $J=2.8$ Hz), 4.37 (1H, m), 4.23 (2H, q, $J=7.5$ Hz), 3.66 (1H, s), 3.21 (2H, q, $J=7.0$ Hz), 2.77–2.57 (2H, m), 2.26 (1H, t, $J=2.3$ Hz), 1.32 (3H, t, $J=7.2$ Hz), 1.27 (3H, t, $J=7.2$ Hz). ^{13}C NMR (100 MHz, CDCl_3): δ 171.01, 169.12, 161.72, 149.42, 148.91, 141.55, 138.39, 129.71, 127.16, 126.70, 125.55, 116.35, 116.18, 111.81, 111.52, 77.74, 77.36, 74.98, 59.73, 51.86, 43.54, 38.68, 31.48, 15.07, 14.75. MS (ESI, positive) m/z calcd for $\text{C}_{25}\text{H}_{26}\text{N}_2\text{O}_5$ ($M + H$): 435.18; found 435.2. HPLC purity: 98.6%.

Prop-2-yn-1-yl 2-Amino-6-(3-ethylamino)phenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4H-chromene-3-carboxylate (9m)

Yield: 61%. NMR (400 MHz, CDCl₃): δ 7.46 (1H, d, J = 2 Hz), 7.39 (1H, dd, J = 2.2, 8.6 Hz), 7.21 (1H, t, J = 8 Hz), 7.00 (1H, d, J = 8.4 Hz), 6.86 (1H, d, J = 7.6 Hz), 6.74 (1H, s), 6.58 (1H, dd, J = 2, 8 Hz), 6.41 (2H, s), 4.79 (2H, t, J = 2 Hz), 4.59 (2H, t, J = 2.6 Hz), 4.39 (1H, m), 3.65 (1H, br s), 3.21 (2H, q, J = 7.4 Hz), 2.77–2.66 (2H, m), 2.48 (1H, t, J = 2.2 Hz), 2.28 (1H, t, J = 2.4 Hz), 1.28 (3H, t, J = 7.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 170.81, 168.01, 162.39, 149.23, 148.85, 141.37, 138.48, 129.67, 127.08, 126.71, 125.29, 116.25, 116.14, 111.83, 111.44, 78.83, 77.71, 75.93, 75.01, 74.39, 51.89, 51.23, 43.47, 38.60, 31.26, 14.98. MS (ESI, positive) m/z calcd for C₂₆H₂₄N₂O₅ (M + H): 445.17; found 445.2. HPLC purity: 99%.

Prop-2-yn-1-yl 2-Amino-6-(3-((2-hydroxyethyl)amino)- phenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4H-chromene-3- carboxylate (9n)

To the amine 9c (1 mmol) in dry acetonitrile, 2- iodoethanol (1 mmol) was added. The mixture was refluxed at 80 °C overnight. It was concentrated followed by the usual workup, giving the crude mass which upon column purification furnished the monosubstituted product without any disubstitution. Yield: 58%. ¹H NMR (400 MHz, CDCl₃): δ 7.46 (1H, d, J = 2 Hz), 7.40 (1H, dd, J = 2.4, 8.4 Hz), 7.23 (2H, t, J = 8.0 Hz), 7.02 (1H, d, J = 8.4 Hz), 6.90 (1H, d, J = 8 Hz), 6.35 (1H, dd, J = 2.0, 8.0 Hz), 6.41 (2H, br s), 4.79 (2H, t, J = 2 Hz), 4.59 (2H, t, J = 2.4 Hz), 4.41–4.38 (1H, m), 4.13 (1H, br s), 3.88 (2H, t, J = 5.2 Hz), 3.38 (2H, t, J = 5.2 Hz), 2.78–2.66 (2H, m), 2.47 (1H, t, J = 2.4 Hz), 2.29 (1H, t, J = 2.4 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 170.88, 168.07, 162.41, 149.38, 148.63, 141.59, 138.40, 129.84, 127.22, 126.79, 125.43, 116.99, 116.26, 112.35, 111.99, 78.86, 77.77, 76.09, 75.04, 74.44, 61.48, 51.97, 51.30, 46.29, 43.53, 31.30. MS (ESI, positive) m/z calcd for C₂₆H₂₄N₂O₆ (M + H): 461.16; found 461.07. HPLC purity: 95.9%.

Prop-2-yn-1-yl 2-Amino-6-(3-(2-chloroacetamido)phenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4H-chromene-3-carboxy- late (9o)

To an ice cold solution of amine 9c (1 mmol) in dry DCM, chloroacetyl chloride (1.1 mmol) was added slowly. After the addition, the ice bath was removed and the contents stirred at room temperature. Upon completion, the contents were extracted with DCM, washed with bicarbonate, water, brine, and dried over MgSO₄. Removal of the solvent under reduced pressure gave crude amide which upon column purification furnished pure product. Yield: 62%. ¹H NMR (400 MHz, CDCl₃): δ 8.29 (1H, s), 7.71 (1H, s), 7.55–7.47 (2H, m), 7.43–7.33 (3H, m), 7.04 (1H, d, J = 8.4 Hz), 6.41 (2H, s), 4.79 (2H, m), 4.60 (2H, t, J = 2.5 Hz), 4.40 (1H, m), 4.22 (2H, s), 2.78–2.66 (2H, m), 2.47 (1H, t, J = 2.4 Hz), 2.29 (1H, t, J = 2.4 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 170.82, 168.00, 164.00, 162.32, 149.70, 141.45, 137.27, 137.13, 129.69, 127.26, 126.86, 125.67, 123.99, 119.06, 118.82, 116.48, 78.79, 77.77, 75.98, 74.97, 74.44, 51.97, 51.32, 43.45, 43.04, 31.25. MS (ESI, positive) m/z calcd for C₂₆H₂₁ClN₂O₆ (M + H): 493.11; found 493.3. HPLC purity: 95.3%.

Ethyl 2-Amino-6-(3-carbamoylphenyl)-4-(2-ethoxy-2-oxoethyl)-4H-chromene-3-carboxylate (9p)

Yield: 45%. ¹H NMR (400 MHz, CDCl₃): δ 8.00 (1H, t, *J* = 1.5 Hz), 7.74 (1H, dt, *J* = 7.8, 1.5 Hz), 7.68 (1H, dd, *J* = 7.7, 1.5 Hz), 7.55–7.45 (2H, m), 7.42 (1H, dd, *J* = 8.4, 7.2 Hz), 7.03 (1H, d, *J* = 8.4 Hz), 6.31 (2H, br s), 5.89 (2H, br s), 4.35 (1H, dd, *J* = 6.8, 4.5 Hz), 4.23 (2H, q, *J* = 7.1 Hz), 4.02 (2H, q, *J* = 7.1 Hz), 2.69 (1H, dd, *J* = 14.8, 4.5 Hz), 2.63 (1H, dd, *J* = 14.8, 6.8 Hz), 1.33 (3H, t, *J* = 7.1 Hz), 1.12 (3H, t, *J* = 7.1 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 171.72, 169.21, 168.96, 161.36, 149.81, 140.83, 136.32, 133.91, 130.27, 129.06, 127.13, 126.43, 126.11, 125.99, 125.86, 116.29, 76.55, 60.25, 59.56, 43.57, 31.32, 14.55, 14.05. MS (ESI, positive) *m/z* calcd for C₂₃H₂₅N₂O₆ (M + H): 425.2; found 425.2. HPLC purity: 99.2%.

Prop-2-yn-1-yl 6-(3-(1 H-Tetrazol-1-yl)phenyl)-2-amino-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4H-chromene-3-carboxylate (9q)

To the ionic liquid (EAN, 5 mmol) in a screw-capped vial, triethyl orthoformate (1.2 mmol), TMSN₃ (1 mmol), and amine 9c (1 mmol) were added, and the contents were stirred vigorously at room temperature for about 1 h. Upon completion, the compound was extracted in DCM, washed a few times with water, brine, and dried over MgSO₄. The crude product obtained by evaporating the solvent under reduced pressure was purified by column chromatography. Yield: 43%. ¹H NMR (400 MHz, CDCl₃): δ 9.06 (1H, s), 7.87 (1H, s), 7.71–7.64 (3H, m), 7.56 (1H, *J* = 2.0 Hz), 7.47 (1H, dd, *J* = 2, 8.4 Hz), 7.10 (1H, d, *J* = 8.4 Hz), 6.4 (2H, br s), 4.81–4.80 (2H, m), 4.66–4.56 (2H, m), 4.43–4.41 (1H, m), 2.81–2.68 (2H, m), 2.47 (1H, t, *J* = 2.0 Hz), 2.29 (1H, t, *J* = 2.4 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 170.84, 167.93, 162.17, 150.29, 142.91, 140.68, 135.72, 130.77, 128.66, 127.54, 126.92, 126.17, 119.92, 119.88, 116.89, 78.73, 77.36, 75.95, 75.00, 74.51, 52.02, 51.41, 43.45, 31.19 (two coinciding carbon resonances). MS (ESI, positive) *m/z* calcd for C₂₅H₁₉N₅O₅ (M + H): 470.14; found 470. HPLC purity: 99.1%.

Prop-2-yn-1-yl 2-Amino-6-(4-(methylamino)phenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4H-chromene-3-carboxylate (9r)

Yield: 35%. ¹H NMR (400 MHz, CDCl₃): δ 7.41–7.34 (4H, m), 6.98 (1H, d, *J* = 8.4 Hz), 6.66 (2H, d, *J* = 8.4 Hz), 6.39 (2H, br s), 4.78 (2H, t, *J* = 2.4 Hz), 4.59 (2H, t, *J* = 2.8 Hz), 4.39–4.34 (1H, m), 3.8 (1H, br s), 2.87 (3H, s), 2.77–2.65 (2H, m), 2.46 (1H, t, *J* = 2.4 Hz), 2.28 (1H, t, *J* = 2.4 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 170.89, 168.09, 162.50, 148.85, 148.52, 138.13, 129.28, 127.87, 126.11, 125.84, 125.36, 116.20, 112.74, 78.89, 77.78, 76.07, 74.97, 74.36, 51.91, 51.23, 43.54, 31.34, 30.87. MS (ESI, positive) *m/z* calcd for C₂₅H₂₂N₂O₅ (M + H): 431.15; found 431. HPLC purity: 95.5%.

Prop-2-yn-1-yl 2-Amino-6-(4-(dimethylamino)phenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4H-chromene-3-carboxylate (9s)

Yield: 37%. ¹H NMR (400 MHz, CDCl₃): δ 7.45–7.43 (3H, m), 7.37 (1H, dd, *J* = 2.0, 8.4 Hz), 6.98 (1H, d, *J* = 8.4 Hz), 6.78 (1H, d, *J* = 8.8 Hz), 6.42 (2H, s), 4.79 (2H, t, *J* = 2.4 Hz), 4.59 (2H, t, *J* = 2.8 Hz), 4.40–4.38 (2H, m), 2.99 (6H, s), 2.78–2.66 (2H, m), 2.47 (1H, t, *J* = 2.4 Hz), 2.29 (1H, t, *J* = 2.4 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 170.87, 168.06, 162.49,

150.01, 148.48, 137.98, 128.36, 127.63, 126.06, 125.79, 125.34, 116.19, 112.85, 78.89, 77.76, 75.99, 74.97, 74.36, 51.89, 51.19, 43.52, 40.68, 31.31. MS (ESI, positive) m/z calcd for $C_{26}H_{24}N_2O_5$ (M + H): 445.17; found 445.13. HPLC purity: 99.1%.

Ethyl 2-Amino-6-(4-(ethylamino)phenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4H-chromene-3-carboxylate (9t)

Yield: 9%. 1H NMR (400 MHz, $CDCl_3$): δ 7.41–7.34 (4H, m), 6.98 (1H, d, J = 8.4 Hz), 6.65 (2H, d, J = 8.4 Hz), 6.32 (2H, br s), 4.58 (2H, t, J = 2.8 Hz), 4.38–4.35 (1H, m), 4.23 (2H, q, J = 7.0 Hz), 3.66 (1H, br s), 3.19 (2H, q, J = 7.2 Hz), 2.76–2.62 (2H, m), 2.27 (1H, t, J = 2.8 Hz), 1.33 (3H, t, J = 7.2 Hz), 1.27 (3H, t, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.06, 169.15, 161.82, 148.65, 147.93, 137.96, 129.37, 127.92, 126.11, 125.76, 125.55, 116.18, 113.06, 77.79, 77.36, 74.93, 59.69, 51.85, 43.57, 38.65, 31.52, 15.03, 14.75. MS (ESI, positive) m/z calcd for $C_{25}H_{26}N_2O_5$ (M + H): 435.18; found 435.20. HPLC purity: 97.3%.

Prop-2-yn-1-yl 2-Amino-6-(4-(ethylamino)phenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4H-chromene-3-carboxylate (9u)

Yield: 38%. 1H NMR (400 MHz, $CDCl_3$): δ 7.41–7.34 (4H, m), 6.98 (1H, d, J = 8.4 Hz), 6.65 (2H, d, J = 8.8 Hz), 6.38 (2H, br s), 4.78 (2H, t, J = 2.4 Hz), 4.59 (2H, t, J = 2.8 Hz), 4.39–4.37 (1H, m), 3.62 (1H, br s), 3.19 (2H, q, J = 7.2 Hz), 2.77–2.65 (2H, m), 2.46 (1H, t, J = 2.4 Hz), 2.28 (1H, t, J = 2.4 Hz), 1.28 (3H, t, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.87, 168.06, 162.48, 148.48, 147.93, 138.09, 129.17, 127.86, 126.05, 125.78, 125.32, 116.17, 113.03, 78.88, 77.76, 76.01, 74.96, 74.36, 51.89, 51.21, 43.52, 38.58, 31.32, 14.98. MS (ESI, positive) m/z calcd for $C_{26}H_{24}N_2O_5$ (M + H): 445.17; found 417.12. HPLC purity: 98.6%.

Cell Cultures

HL60, HL60/MX2, CCRF-CEM, and CCRF-CEM/C2 were purchased from ATCC. HL60/MX2 was developed from HL60 upon exposure to mitoxantrone, a topoisomerase II inhibitor. CCRF-CEM/C2 was developed from CCRF-CEM upon exposure to camptothecin, a topoisomerase I inhibitor. K562, K562/HHT300, K562/DOX, HL60/ADR, and HL60/DNR cell lines were provided by Dr. Tang.⁴⁷ K562/HHT300 was developed from K562 upon exposure to homoharringtonine, an antimicrotubule agent. K562/DOX was developed from K562 upon exposure to doxorubicin, a topoisomerase II inhibitor. HL60/ADR and HL60/DNR were developed from HL60 upon exposure to adriamycin (which is doxorubicin) and daunorubicin topoisomerase II inhibitors, respectively. HL60 and HL60/DOX cell lines were provided by Dr. Ganapathi.⁴⁰ HL60/DOX was developed from HL60 upon exposure to doxorubicin. CCRF-CEM, CCRF-CEM/VM-1–5, and CCRF-CEM/VLB100 were provided by Dr. Beck.^{45,46} CCRF-CEM/VM-1–5 was developed from CCRF-CEM upon exposure to teniposide, a topoisomerase II inhibitor. CCRF-CEM/VLB100 was developed from CCRF-CEM upon exposure to vinblastine, an antimicrotubule agent. As HL60/ADR and HL60/DOX are from two different sources, they do not behave the same. Similarly even the three parental cell lines for HL60 and the two parental cell lines for CCRF-CEM do not behave the same. We therefore have evaluated all the parental cell lines and the MDR cell lines, using the names as in their original reports. Comparison has been

made only between the MDR cell line with its corresponding parental cell line. All cell lines were grown in RPMI 1640 purchased from ATCC, supplemented with 10% FBS at 37 °C with 5% CO₂ in air atmosphere.

Cytotoxicity Measurement

In vitro cytotoxicity of small molecules was assayed by determining their ability to inhibit the growth of the tumor cells following our established procedures.²⁹

Statistical Analysis

In vitro cytotoxicity assay was performed at least twice with triplicates in each experiment. Data in Tables 1–4 are presented as the mean ± SEM. Data in Tables 5 and 6 are presented as mean values only.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

MDR	multidrug resistance
SAR	structure–activity relationship
ABC	ATP-binding cassette; ER, endoplasmic reticulum
IP₃R	inositol triphosphate receptor
SERCA	sarco/endoplasmic reticulum Ca ²⁺ -ATPase
AML	acute myeloid leukemia
ALL	acute lymphoblastic leukemia
CML	chronic myelogenous leukemia
Ara-C	cytarabine
MX	mitoxantrone
ADR	adriamycin
DNR	daunorubicin
HHT	homoharringtonine
VLB	vinblastine

IAP	inhibitor of apoptosis
dCK	deoxycytidine kinase
GI₅₀	50% growth inhibitory concentration
SEM	standard error of the mean

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Figure 1.

Structures of the representative inhibitors for antiapoptotic Bcl-2 family proteins (**1**, **2**, and **5**), p-glycoprotein (**3**), SERCA (**6**), and one CXL candidate (**4**).

Scheme 1. Synthesis of Series 7^a

^aReagents and conditions: (i) 3,5-dimethoxyphenylboronic acid, Pd(OAc)₂, TPP, DME/H₂O (1:1), K₂CO₃, rt, 24 h; (ii) DMA, POCl₃, DCM, 80 °C, 4 h; NaHCO₃, 60 °C, 1 h; (iii) 3,5-dimethoxyphenylboronic acid, Pd(PPh₃)₄, toluene/H₂O (8:2), K₂CO₃, 80 °C, overnight; (iv) methyl cyanoacetate or ethyl cyanoacetate, R₁OH, NaOR₁, rt, 2 h; (v) NaBH₄, MeOH, rt, 5 h; (vi) ethyl cyanoacetate, allyl alcohol, Na, rt, 2 h; (vii) *m*-CPBA, DCM, 0 °C to rt, 24 h; (viii) malononitrile, EtOH, NaOEt, rt, 3 h; (ix) benzene, NEt₃, reflux, 6 h.

Scheme 2. Synthesis of Series 8^a

^aReagents and conditions: (i) R-B(OH)₂ or R-B(OH)₂ MIDA ester, Pd(OAc)₂ or Pd(PPh₃)₄, suitable base and solvent, 80 °C, overnight; (ii) DMA, POCl₃, DCM, 80 °C, 4 h; NaHCO₃, 60 °C, 1 h; (iii) ethyl cyanoacetate, EtOH, NaOEt, rt, 2 h.

Scheme 3. Synthesis of Series 9^a

^aReagents and conditions: (i) $\text{ArB}(\text{OH})_2$, $\text{Pd}(\text{PPh}_3)_4$, K_2CO_3 , toluene/ H_2O (8:2), 90 °C, overnight (for **11k**, (3-carbamoylphenyl)boronic acid MIDA ester and dioxane-water were used); (ii) ECA, R_1OH , NaOR_1 , rt, 2–12 h; (iii) Pd/C (10%), HCOONH_4 , MeOH, rt, 6 h; (iv) malononitrile, dry propargyl alcohol, Na, rt, 12 h; (v) $\text{ICH}_2\text{CH}_2\text{OH}$, ACN, 80 °C, overnight; (vi) ClCH_2COCl , NEt_3 , dry DCM, rt, 2 h; (vii) ethylammonium nitrate ionic liquid, $\text{CH}(\text{OEt})_3$, TMSN_3 , rt, 1 h; (viii) RI, NaH, Dry DMF, rt, 8 h; (ix) MeI (5 equiv), NaH (2.5 equiv), dry DMF, rt, 2 h; (x) TFA, dry DCM, rt, 6 h; (xi) MeI, NaH, dry DMF, rt, 2 h.

Table 1.

IC₅₀ (μM) of Analogues 7a–l in HL60

compd	R ₁	R ₂	IC ₅₀ ± SEM ^a
7a	Me	Me	2.42 ± 0.77
7b	Et	Me	10.8 ± 0.5
7c ²⁹	Et	Et	10.7 ± 0.5
7d ²⁹	<i>n</i> -Pr	<i>n</i> -Pr	7.6 ± 0.3
7e ²⁹	<i>n</i> -Bu	<i>n</i> -Bu	32 ± 2
7f ²⁹	allyl	allyl	4.7 ± 0.3
7g ²⁹	C3H3	C3H3	1.5 ± 0.1
7h ²⁹	C3H3	Et	4.7 ± 0.2
7i ²⁹	cyclopropylmethyl	cyclopropylmethyl	6.7 ± 0.5
7j	oxirane methyl	oxirane methyl	>80
7k			19.6 ± 3.4
7l			>80

^aResults are given as the mean of at least two independent experiments with triplicates in each experiment.

Table 2.IC₅₀ (μ M) of Fluorine-Containing Analogues 8b–f in HL60

compd	R ₁	R ₂	R ₃	R ₄	IC ₅₀ $\pm$ SEM ^a
sHA 14–1 ²⁹	H	H	H	H	91.0 $\pm$ 0.3
8b	F	H	H	H	73.0 $\pm$ 12.3
8c	H	F	H	H	76.4 $\pm$ 6.2
8d	H	H	F	H	72.1 $\pm$ 8.0
8e	F	H	F	H	76.6 $\pm$ 6.9
8f	H	F	H	F	35.9 $\pm$ 4.3

^aResults are given as the mean of at least two independent experiments with triplicates in each experiment.

Table 3.

IC₅₀ (μM) of Heterocyclic Analogues 8g–m in HL60 **8g-8m**

Compd.	R ₁	IC ₅₀ ± SEN ^a
8g		>80
8h		>80
8i		76.9 ± 4.5
8j		21.5 ± 1.8

8g-8m

Compd.	R ₁	IC ₅₀ ± SEN ^a
8k		10.2 ± 4.6
8l		37.9 ± 3.8
8m		10.2 ± 0.4

^aResults are given as the mean of at least two independent experiments with triplicates in each experiment.

Table 4.

IC₅₀ (μ M) of Analogues 9a–u in HL60

compd	R ₁	R ₂	R ₃	R ₄	IC ₅₀ ± SEM ^a
9a	Et	Et	NH ₂	H	20.2 ± 2.1
9b	C ₃ H ₃	Et	NH ₂	H	3.4 ± 0.3
9c	C ₃ H ₃	C ₃ H ₃	NH ₂	H	1.24 ± 0.27
9d					43.9 ± 4.0
9e	Et	Et	H	NH ₂	22.3 ± 1.3
9f	C ₃ H ₃	Et	H	NH ₂	10.5 ± 0.4
9g	C ₃ H ₃	C ₃ H ₃	H	NH ₂	0.61 ± 0.08
9h	Et	Et	NHMe	H	7.45 ± 0.17
9i	C ₃ H ₃	Et	NHMe	H	7.13 ± 0.29
9j	C ₃ H ₃	C ₃ H ₃	NHMe	H	0.88 ± 0.06
9k	C ₃ H ₃	C ₃ H ₃	NMe ₂	H	12.00 ± 0.71
9l	C ₃ H ₃	Et	NHEt	H	17.2 ± 3.49
9m	C ₃ H ₃	C ₃ H ₃	NHEt	H	1.86 ± 0.45
9n	C ₃ H ₃	C ₃ H ₃	NHCH ₂ CH ₂ OH	H	0.63 ± 0.28
9o	C ₃ H ₃	C ₃ H ₃	NHCOCH ₂ Cl	H	5.3 ± 0.35
9p	Et	Et	CONH ₂	H	5.40 ± 0.07
9q	C ₃ H ₃	C ₃ H ₃	tetrazole	H	1.62 ± 0.15
9r	C ₃ H ₃	C ₃ H ₃	H	NHMe	2.45 ± 0.42
9s	C ₃ H ₃	C ₃ H ₃	H	NMe ₂	2.56 ± 0.26
9t	C ₃ H ₃	Et	H	NHEt	33.0 ± 9.9
9u	C ₃ H ₃	C ₃ H ₃	H	NHEt	7.27 ± 1.23

^aResults are given as the mean of at least two independent experiments with triplicates in each experiment.

Table 5.

Relative Sensitivity of MDR Cancer Cell Lines against Standard Therapies^a

resistant cell line	relative sensitivity with IC ₅₀ for the corresponding parental line in parentheses ^b				
	doxorubicin (μM)	vincristine (nM)	Ara-C (μM)	mitoxantrone (μM)	etoposide (μM)
HL60/MX2	5.3 (0.14)	1 (0.06)	0.4 (4.0)	>20 (0.016)	9.0 (1.6)
HL60/ADR	>15 (0.32)	1 (0.1)	0.1 (5.6)	2.4 (0.97)	>10 (8.1)
HL60/DNR	>50 (0.32)	>1000 (0.1)	1.3 (5.6)	>10 (0.97)	>50 (8.1)
HL60/DOX	>100 (0.04)	>1000 (0.62)	7.5 (0.049)	>20 (3.1)	>200 (0.11)
K562/DOX	>10 (0.44)	>1000 (0.1)	1.5 (14)	5.1 (0.51)	>15 (2.3)
K562/HHT300	>20 (0.44)	>1000 (0.1)	7.0 (14)	>10 (0.51)	>1000 (2.3)
CCRF-CEM/C2	6.7 (0.46)	1 (0.59)	4.1 (0.006)	3.0 (0.036)	2.5 (0.24)
CCRF-CEM/VM-1-5	>15 (0.11)	1 (0.17)	6.5 (0.02)	>30 (0.085)	>50 (0.99)
CCRF-CEM/VLB100	6.1 (0.11)	>1000 (0.17)	1 (0.02)	2.3 (0.085)	8.6 (0.99)

^aRelative sensitivity is defined as the ratio of IC₅₀ in the resistant cell line relative to that in the corresponding parental cell line. When relative sensitivity is >1, MDR cell shows resistance to the therapy. When relative sensitivity is <1, MDR cell shows collateral sensitivity to the therapy.

^bResults are given as the mean of at least two independent experiments with triplicates in each experiment. Because these cell lines were developed from different sources as detailed in the Experimental Section, the parental cell lines of the same cell line do not demonstrate the same sensitivity to the same therapy.

Table 6.Relative sensitivity of MDR cancer cell lines towards 1, 3, 6, and 9^a

resistant cell line	relative sensitivity with IC ₅₀ for the parental line in parentheses ^b			
	1 (μM)	3 (μM)	6 (μM)	9 (μM)
HL60/MX2	1.5 (1.39)	0.86 (19.8)	0.02 (3.0)	1.0 (0.62)
HL60/ADR	4.5 (10.5)	0.47 (30.6)	1.0 (0.078)	0.26 (0.89)
HL60/DNR	4.9 (10.5)	0.64 (30.6)	>50 (0.078)	0.27 (0.89)
HL60/DOX	19.7 (0.76)	1.0 (6.8)	>50 (0.007)	0.60 (0.28)
K562/DOX	2.7 (34.7)	0.47 (26.9)	4.2 (0.17)	1.08 (2.36)
K562/HHT300	2.3 (34.7)	0.43 (26.9)	>10 (0.17)	1.1 (2.36)
CCRF-CEM/C2	0.2 (0.74)	0.58 (7.05)	1.45 (0.27)	0.54 (1.47)
CCRF-CEM/VM-1-5	2.2 (1.17)	2.1 (13.7)	1.0 (0.31)	0.42 (1.82)
CCRF-CEM/VLB100	0.3 (1.17)	0.66 (13.7)	7.7 (0.31)	0.93 (1.82)

^aRelative sensitivity is defined as the ratio of the IC₅₀ in the resistant cell line relative to that in the corresponding parental cell line. When relative sensitivity is >1, MDR cell shows resistance to the therapy. When relative sensitivity is <1, MDR cell shows collateral sensitivity to the therapy.

^bResults are given as the mean of at least two independent experiments with triplicates in each experiment. Because these cell lines were developed from sources as detailed in the Experimental Section, the parental cell lines of the same cell line do not demonstrate the same sensitivity to the same therapy.