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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

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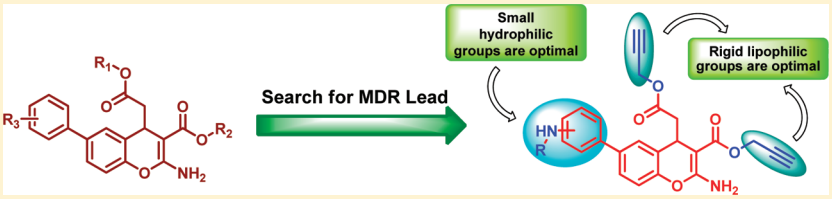
Supporting Information



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.

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 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

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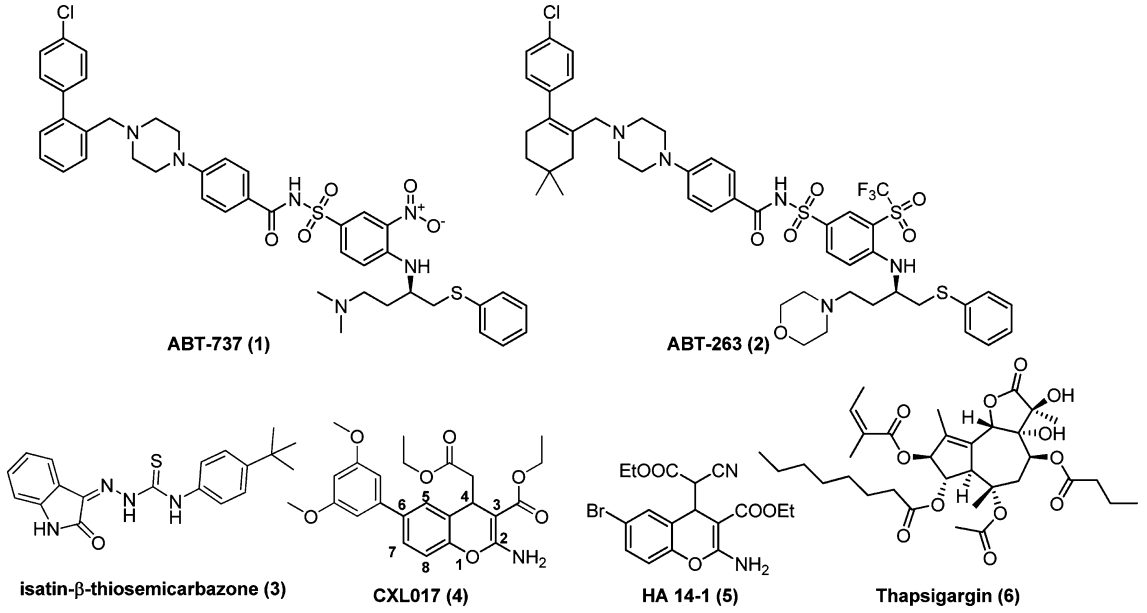
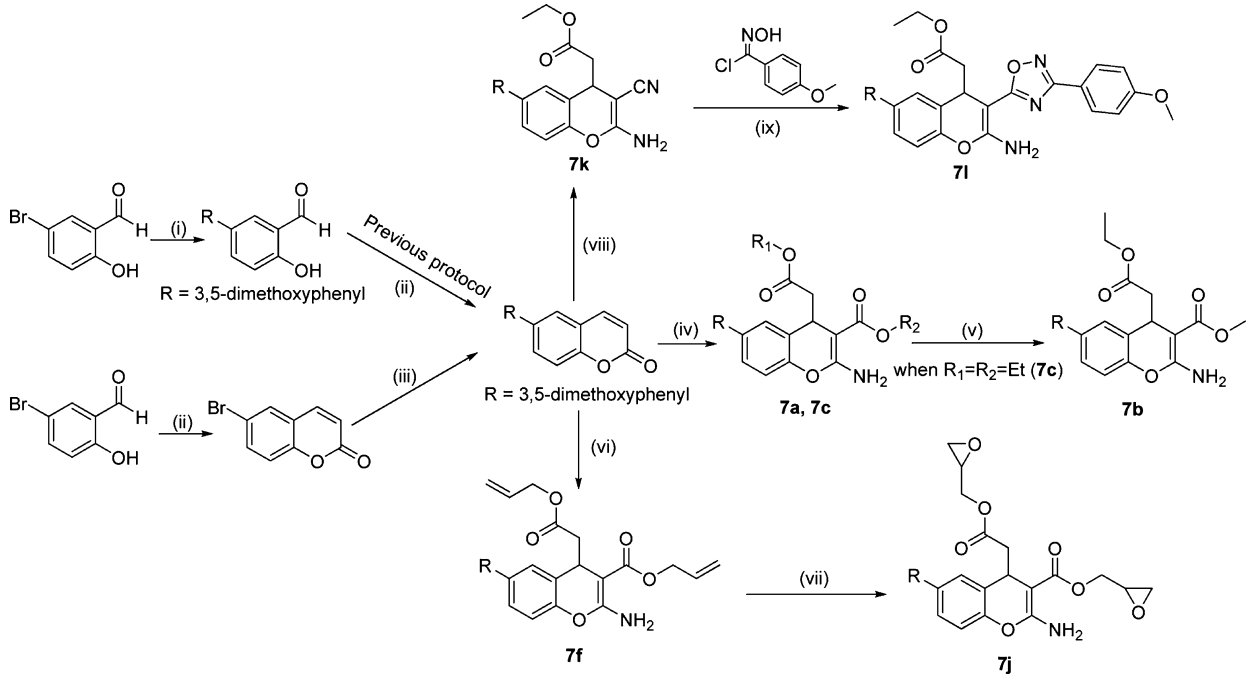


Figure 1. Structures of the representative inhibitors for antiapoptotic Bcl-2 family proteins (**1**, **2**, and **5**), p-glycoprotein (**3**), SERCA (**6**), and one CXL candidate (**4**).

Scheme 1. Synthesis of Series 7[a]



[a]Reagents and conditions: (i) 3,5-dimethoxyphenylboronic acid, $Pd(OAc)_2$, TPP, DME/H_2O (1:1), K_2CO_3, rt, 24 h; (ii) DMA, $POCl_3$, DCM, 80 °C, 4 h; $NaHCO_3$, 60 °C, 1 h; (iii) 3,5-dimethoxyphenylboronic acid, $Pd(PPh_3)_4$, toluene/H_2O (8:2), K_2CO_3, 80 °C, overnight; (iv) methyl cyanoacetate or ethyl cyanoacetate, R_1OH, $NaOR_1$, rt, 2 h; (v) $NaBH_4$, 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, NEt_3, reflux, 6 h.

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+} homeostasis[25] 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

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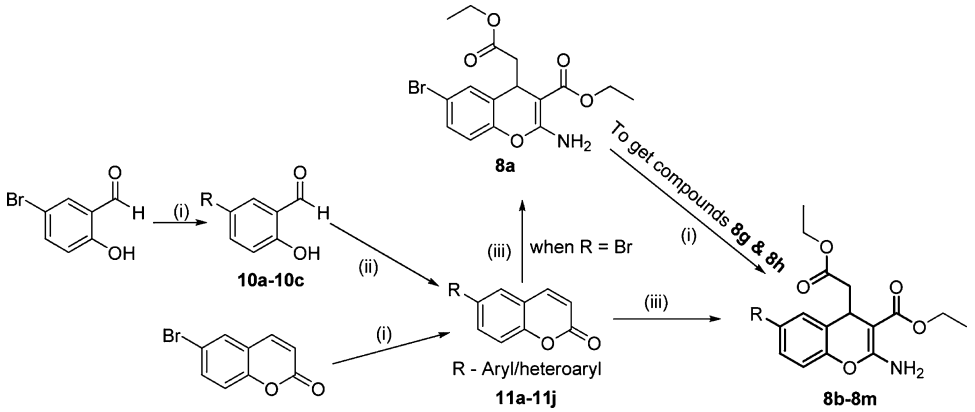
Scheme 2. Synthesis of Series 8[a]



[a]Reagents and conditions: (i) R-B(OH)$_2$ or R-B(OH)$_2$ MIDA ester, Pd(OAc)$_2$ or Pd(PPh$_3$)$_4$, suitable base and solvent, 80 °C, overnight; (ii) DMA, POCl$_3$, DCM, 80 °C, 4 h; NaHCO$_3$, 60 °C, 1 h; (iii) ethyl cyanoacetate, EtOH, NaOEt, rt, 2 h.

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-bromocoumarin[32] 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 chloride[33] 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

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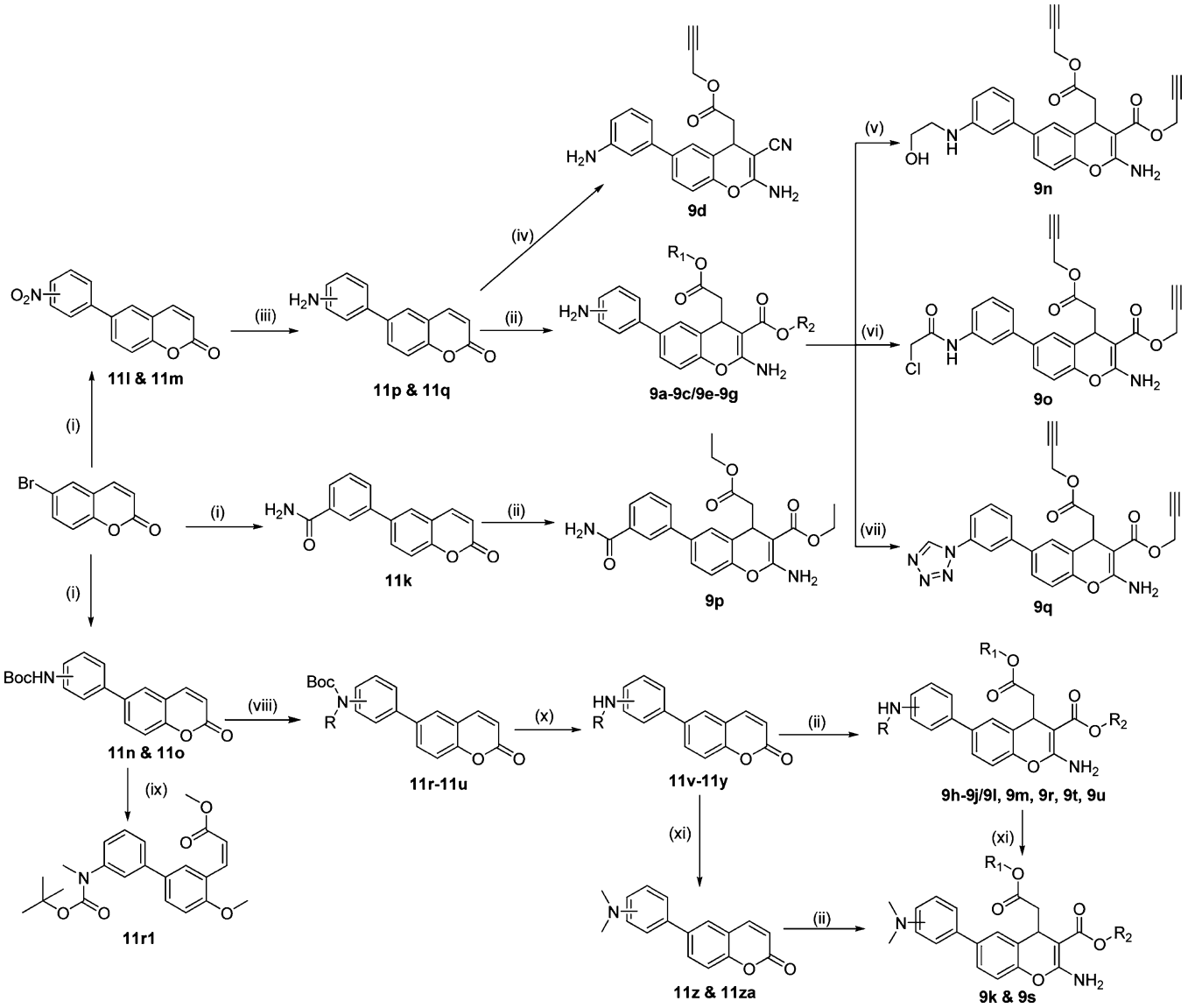
Scheme 3. Synthesis of Series 9[a]



[a]Reagents and conditions: (i) $ArB(OH)_2$, $Pd(PPh_3)_4$, 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) ICH_2CH_2OH, ACN, 80 °C, overnight; (vi) $ClCH_2COCl$, NEt_3, dry DCM, rt, 2 h; (vii) ethylammonium nitrate ionic liquid, $CH(OEt)_3$, $TMSN_3$, 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.

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 $TMSN_3$ and $CH(OEt)_3$ using the readily available, recyclable, Brønsted acidic ionic liquids $[EtNH_3][NO_3]$.[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

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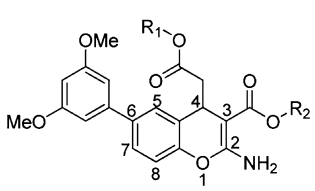
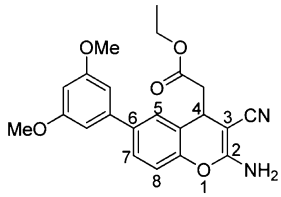
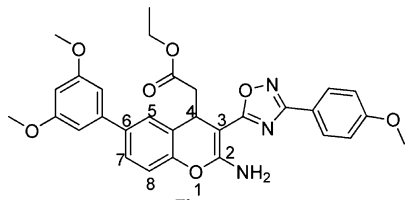
Table 1. IC_{50} (μM) of Analogues 7a–l in HL60



7a-7j



7k



7l

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]	*n*-Pr	*n*-Pr	7.6 ± 0.3
7e[29]	*n*-Bu	*n*-Bu	32 ± 2
7f[29]	allyl	allyl	4.7 ± 0.3
7g[29]	C_3H_3	C_3H_3	1.5 ± 0.1
7h[29]	C_3H_3	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

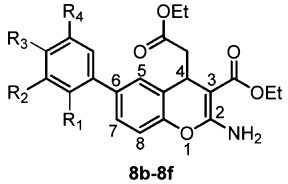
[a]Results are given as the mean of at least two independent experiments with triplicates in each experiment.

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**. Disappointedly, 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′-NH_2 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

Table 2. IC_{50} (μM) of Fluorine-Containing Analogues 8b–f in HL60



8b-8f

compd	R_1	R_2	R_3	R_4	IC_{50} ± SEM[a]
sHA 14-1[29]	H	H	H	H	91.0 ± 0.3
8b	F	H	H	H	73.0 ± 12.3
8c	H	F	H	H	76.4 ± 6.2
8d	H	H	F	H	72.1 ± 8.0
8e	F	H	F	H	76.6 ± 6.9
8f	H	F	H	F	35.9 ± 4.3

[a]Results are given as the mean of at least two independent experiments with triplicates in each experiment.

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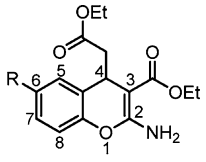
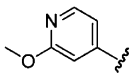
Table 3. IC_{50} (μM) of Heterocyclic Analogues 8g−m in HL60



8g-8m

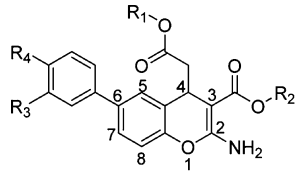
Compd.	R_1	$IC_{50} \pm SEM^a$
8g		>80
8h		>80
8i		76.9 ± 4.5
8j		21.5 ± 1.8
8k		10.2 ± 4.6
8l		37.9 ± 3.8
8m		10.2 ±0.4

[a]Results are given as the mean of at least two independent experiments with triplicates in each experiment.

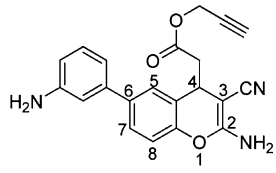
hydrophilic functional groups at the meta and para positions of the 6-phenyl are preferred. The size of the substitutions at the para position needs to be small, while the meta position has more tolerance on the size of the functional group. Among these analogues, **9g**, **9j**, and **9n** reveal submicromolar 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

Table 4. IC_{50} (μM) of Analogues 9a−u in HL60



9a-9c/9e-9u



9d

compd	R_1	R_2	R_3	R_4	$IC_{50} \pm SEM^a$
9a	Et	Et	NH_2	H	20.2 ± 2.1
9b	C_3H_3	Et	NH_2	H	3.4 ± 0.3
9c	C_3H_3	C_3H_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_3H_3	Et	H	NH_2	10.5 ± 0.4
9g	C_3H_3	C_3H_3	H	NH_2	0.61 ± 0.08
9h	Et	Et	NHMe	H	7.45 ± 0.17
9i	C_3H_3	Et	NHMe	H	7.13 ± 0.29
9j	C_3H_3	C_3H_3	NHMe	H	0.88 ± 0.06
9k	C_3H_3	C_3H_3	NMe_2	H	12.00 ± 0.71
9l	C_3H_3	Et	NHEt	H	17.2 ± 3.49
9m	C_3H_3	C_3H_3	NHEt	H	1.86 ± 0.45
9n	C_3H_3	C_3H_3	$NHCH_2CH_2OH$	H	0.63 ± 0.28
9o	C_3H_3	C_3H_3	$NHCOCH_2Cl$	H	5.3 ± 0.35
9p	Et	Et	$CONH_2$	H	5.40 ± 0.07
9q	C_3H_3	C_3H_3	tetrazole	H	1.62 ± 0.15
9r	C_3H_3	C_3H_3	H	NHMe	2.45 ± 0.42
9s	C_3H_3	C_3H_3	H	NMe_2	2.56 ± 0.26
9t	C_3H_3	Et	H	NHEt	33.0 ± 9.9
9u	C_3H_3	C_3H_3	H	NHEt	7.27 ± 1.23

[a]Results are given as the mean of at least two independent experiments with triplicates in each experiment.

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 IIβ 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 IC_{50} values. The ratio of the IC_{50} against MDR cell line to the IC_{50} against corresponding parental cell line is summarized in Table 5 with the IC_{50} 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-

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Table 5. Relative Sensitivity of MDR Cancer Cell Lines against Standard Therapies[a]

	relative sensitivity with IC_{50} for the corresponding parental line in parentheses[b]				
resistant cell line	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)

[a]Relative sensitivity is defined as the ratio of IC_{50} 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. [b]Results 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 9g[a]

	relative sensitivity with IC_{50} for the parental line in parentheses[b]			
resistant cell line	**1** (μM)	**3** (μM)	**6** (μM)	**9g** (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)

[a]Relative sensitivity is defined as the ratio of the IC_{50} 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. [b]Results 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.

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, K560/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

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against the parental and MDR cell lines in a dose-dependent manner to determine their IC_{50} values. The ratio of the IC_{50} against MDR cell line to the IC_{50} 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 IC_{50} 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 ^{13}C) 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/H_2O as the

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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/H_2O (1:1) mixture and degassed well with nitrogen. The mixture was then heated to 60 °C under N_2 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 Na_2SO_4 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%. ^{1}H NMR (400 MHz, $CDCl_3$): 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%. ^{1}H NMR (400 MHz, $CDCl_3$): 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%. ^{1}H NMR (400 MHz, $CDCl_3$): 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 $NaHCO_3$ 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 $MgSO_4$), 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)-2*H*-chromen-2-one (11a). Yield: 46%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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)-2*H*-chromen-2-one (11b). Yield: 48%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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)-2*H*-chromen-2-one (11c). Yield: 49%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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), K_2CO_3 (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, $Pd(PPh_3)_4$ (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 $MgSO_4$. 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)-2*H*-chromen-2-one (11d). Base: sodium carbonate (4.5 mmol). Solvent: ethanol/water/toluene in 1:2:3 ratio (5 mL). Yield: 48%. ^{1}H NMR (400 MHz, $CDCl_3$): 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)-2*H*-chromen-2-one (11e). Base: sodium carbonate (4.5 mmol). Solvent: ethanol/water/toluene in 1:2:3 ratio (10 mL). Yield: 62%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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)-2*H*-chromen-2-one (11f). Solvent: DMF/IPA in 4:1 ratio (10 mL). Additive: $Cu(OAc)_2$ (0.5 mmol). Yield: 61%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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)-2*H*-chromen-2-one (11g). Solvent: dioxane/water in 5:1 ratio (10 mL). Yield: 50%. ^{1}H NMR (400 MHz, $CDCl_3$): 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)-2*H*-chromen-2-one (11h). Solvent: dioxane/water in 5:1 ratio (10 mL). Yield: 61%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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)-2*H*-chromen-2-one (11i). Solvent: dioxane/water in 5:1 ratio (10 mL). Yield: 22%. ^{1}H 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)-2*H*-chromen-2-one (11j). Solvent: dioxane/water in 5:1 ratio (10 mL). Yield: 12%. ^{1}H 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-2*H*-chromen-6-yl)benzamide (11k). Solvent: dioxane/water in 5:1 ratio (10 mL). Yield: 13.7%. ^{1}H NMR (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)-2*H*-chromen-2-one (11l). Yield: 61%. ^{1}H 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

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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)-2*H*-chromen-2-one (11m). Yield: 58%. ^{1}H 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-2*H*-chromen-6-yl)phenyl)carbamate (11n).** Yield: 75%. ^{1}H 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-2*H*-chromen-6-yl)phenyl)carbamate (11o).** Yield: 78%. ^{1}H 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 $MgSO_4$), 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)-2*H*-chromen-2-one (11p). Yield: 65%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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)-2*H*-chromen-2-one (11q). Yield: 72%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $MgSO_4$. 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-2*H*-chromen-6-yl)phenyl)-carbamate (11r).** Yield: 72%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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%). ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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-2*H*-chromen-6-yl)phenyl)-carbamate (11s).** Yield: 80%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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-2*H*-chromen-6-yl)phenyl)-carbamate (11t).** Yield: 78%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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-2*H*-chromen-6-yl)phenyl)-carbamate (11u).** Yield: 81%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $MgSO_4$. The crude product obtained after removal of solvent itself was pure enough to use for further reaction.

6-(3-(Methylamino)phenyl)-2*H*-chromen-2-one (11v). Yield: 83%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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)-2*H*-chromen-2-one (11w). Yield 85%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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)-2*H*-chromen-2-one (11x). Yield: 85%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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)-2*H*-chromen-2-one (11y). Yield: 89%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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)-2*H*-chromen-2-one (11z). Yield: 65%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz,

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$CDCl_3$): 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)-2*H*-chromen-2-one (11za). Yield: 71%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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-4*H*-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 ($MgSO_4$). 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)-4*H*-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%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{22}H_{24}NO_7$ (M + H): 414.16; found, 414.21. HPLC purity: 100%.

Methyl 2-Amino-6-(3,5-dimethoxyphenyl)-4-(2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (7b). To a 25 mL flask was added ethyl 2-amino-6-(3,5-dimethoxyphenyl)-4-(2-ethoxy-2-oxoethyl)-4*H*-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 NH_4Cl solution. The aqueous phase was extracted with DCM (20 mL × 3), and the combined organic layer was washed with brine and dried over Na_2SO_4. Solvent evaporation gave a crude oil which was further purified by column chromatography to yield the pure product. Yield: 21%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{23}H_{26}NO_7$ (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)-4*H*-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 Na_2SO_4, filtered, and concentrated. The residue was purified by chromatography to afford the pure compound. Yield: 48%. ^{1}H NMR (400 MHz, $CDCl_3$): 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 $C_{26}H_{27}NO_9$ (M + H): 498.18; found, 498.16. HPLC purity: 95.6%.

Ethyl 2-(2-Amino-3-cyano-6-(3,5-dimethoxyphenyl)-4*H*-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%. ^{1}H 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 $C_{22}H_{22}N_2O_5$ (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)-4*H*-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)-4*H*-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%. ^{1}H 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 $C_{29}H_{24}N_4O_5$ (M + H): 543.20; found 544.1. HPLC purity: 96.9%.

Ethyl 2-Amino-6-bromo-4-(2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (8a). Yield: 85.0%. ^{1}H 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)-4*H*-chromene-3-carboxylate (8b). Yield: 18%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{22}H_{23}FNO_5$ (M + H): 400.2; found 400.2. HPLC purity: 98.9%.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(3-fluorophenyl)-4*H*-chromene-3-carboxylate (8c). Yield: 31%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{22}H_{23}FNO_5$ (M + H): 400.2; found 400.2. HPLC purity: 98. 9%.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(4-fluorophenyl)-4*H*-chromene-3-carboxylate (8d). Yield: 42%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{22}H_{23}FNO_5$ (M^{+1}): 400.2; found 400.2. HPLC purity: 98.5%.

Ethyl 2-Amino-6-(2,4-difluorophenyl)-4-(2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (8e). Yield: 53%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{22}H_{22}F_2NO_5$ (M + H): 418.2; found 418.2. HPLC purity: 96.1%.

Ethyl 2-Amino-6-(3,5-difluorophenyl)-4-(2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (8f). Yield: 69%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{22}H_{22}F_2NO_5$ (M + H): 418.2; found 418.2. HPLC purity: 96.3%.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(furan-2-yl)-4*H*-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%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{20}H_{22}NO_6$ (M + H): 372.1; found 372.1. HPLC purity: 98.1%.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(thiophen-2-yl)-4*H*-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%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{20}H_{22}NO_5S$ (M + H): 388.1; found 388.1. HPLC purity: 95.6%.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(pyridin-2-yl)-4*H*-chromene-3-carboxylate (8i). Yield: 62%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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)-4*H*-chromene-3-carboxylate (8j). Yield: 69%. ^{1}H 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)-4*H*-chromene-3-carboxylate (8k). Yield: 41%. ^{1}H 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)-4*H*-chromene-3-carboxylate (8l). Yield: 38%. ^{1}H 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)-4*H*-chromene-3-carboxylate (8m). Yield: 44%. ^{1}H 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%. ^{1}H NMR (400 MHz, $CDCl_3$) 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 =

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7.15 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{22}H_{24}N_2O_5$ (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%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{23}H_{22}N_2O_5$ (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%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{24}H_{20}N_2O_5$ (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%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{21}H_{17}N_3O_3$ (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%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{22}H_{24}N_2O_5$ (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%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{23}H_{22}N_2O_5$ (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%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{24}H_{20}N_2O_5$ (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%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{23}H_{26}N_2O_5$ (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%. ^{1}H NMR (400 MHz, $CDCl_3$): 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 $C_{24}H_{24}N_2O_5$ (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)-4*H*-chromene-3-carboxylate (9j). Yield: 63%. ^{1}H 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 $C_{25}H_{22}N_2O_5$ (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)-4*H*-chromene-3-carboxylate (9k). Yield: 60%. ^{1}H 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 $C_{26}H_{24}N_2O_5$ (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)-4*H*-chromene-3-carboxylate (9l). Yield: 21%. ^{1}H 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 $C_{25}H_{26}N_2O_5$ (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)-4*H*-chromene-3-carboxylate (9m).

Yield: 61%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{26}H_{24}N_2O_5$ (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)-4*H*-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%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{26}H_{24}N_2O_6$ (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)-4*H*-chromene-3-carboxylate (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 $MgSO_4$. Removal of the solvent under reduced pressure gave crude amide which upon column purification furnished pure product. Yield: 62%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{26}H_{21}ClN_2O_6$ (M + H): 493.11; found 493.3. HPLC purity: 95.3%.

Ethyl 2-Amino-6-(3-carbamoylphenyl)-4-(2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (9p). Yield: 45%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{23}H_{25}N_2O_6$ (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)-4*H*-chromene-3-carboxylate (9q). To the ionic liquid (EAN, 5 mmol) in a screw-capped vial, triethyl orthoformate (1.2 mmol), $TMSN_3$ (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 $MgSO_4$. The crude product obtained by evaporating the solvent under reduced pressure was purified by column chromatography. Yield: 43%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{25}H_{19}N_5O_5$ (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)-4*H*-chromene-3-carboxylate (9r). Yield: 35%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{25}H_{22}N_2O_5$ (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)-4*H*-chromene-3-carboxylate (9s). Yield: 37%. ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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)-4*H*-chromene-3-carboxylate (9t). Yield: 9%. ^{1}H 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)-4*H*-chromene-3-carboxylate (9u). Yield: 38%. ^{1}H 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% CO_2 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.

■ ASSOCIATED CONTENT

Supporting Information

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

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

MDR, multidrug resistance; SAR, structure−activity relationship; ABC, ATP-binding cassette; ER, endoplasmic reticulum; IP_3R, 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; GI_{50}, 50% growth inhibitory concentration; SEM, standard error of the mean

■ REFERENCES

(1) Reed, J. C.; Pellecchia, M. Apoptosis-based therapies for hematologic malignancies. *Blood* **2005**, *106*, 408−418.
(2) Glenda, G.; Rubin, M.; Gail, W.; Ihor, S.; Ralph, B. Apoptosis and expression of Bcl-2, Bcl-XL, and Bax in renal cell carcinomas. *Cancer Invest.* **2002**, *20*, 324−332.
(3) Sartorious, U. A.; Krammer, P. H. Upregulation of Bcl-2 is involved in the mediation of chemotherapy resistance in human small cell lung cancer cell lines. *Int. J. Cancer* **2002**, *97*, 584−592.
(4) Gottesman, M. M.; Ludwig, J.; Xia, D.; Szakacs, G. Defeating drug resistance in cancer. *Discovery Med* **2006**, *6*, 18−23.
(5) Szakacs, G.; Paterson, J. K.; Ludwig, J. A.; Booth-Genthe, C.; Gottesman, M. M. Targeting multidrug resistance in cancer. *Nat. Rev. Drug Discovery* **2006**, *5*, 219−234.
(6) Youle, R. J.; Strasser, A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 47−59.
(7) Segezdi, E.; Macdonald, D. C.; Ni Chonghaile, T.; Gupta, S.; Samali, A. Bcl-2 family on guard at the ER. *Am. J. Physiol.: Cell Physiol.* **2009**, *296*, C941−953.
(8) Distelhorst, C. W.; Shore, G. C. Bcl-2 and calcium: controversy beneath the surface. *Oncogene* **2004**, *23*, 2875−2880.
(9) Chen, R.; Valencia, I.; Zhong, F.; McColl, K. S.; Roderick, H. L.; Bootman, M. D.; Berridge, M. J.; Conway, S. J.; Holmes, A. B.; Mignery, G. A.; Velez, P.; Distelhorst, C. W. Bcl-2 functionally interacts with inositol 1,4,5-trisphosphate receptors to regulate calcium release from the ER in response to inositol 1,4,5-trisphosphate. *J. Cell Biol.* **2004**, *166*, 193−203.
(10) Hanson, C. J.; Bootman, M. D.; Distelhorst, C. W.; Wojcikiewicz, R. J.; Roderick, H. L. Bcl-2 suppresses Ca^{2+} release through inositol 1,4,5-trisphosphate receptors and inhibits Ca(2+) uptake by mitochondria without affecting ER calcium store content. *Cell Calcium* **2008**, *44*, 324−338.
(11) Li, C.; Wang, X.; Vais, H.; Thompson, C. B.; Foskett, J. K.; White, C. Apoptosis regulation by Bcl-x(L) modulation of mammalian inositol 1,4,5-trisphosphate receptor channel isoform gating. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 12565−12570.
(12) Kuo, T. H.; Kim, H. R.; Zhu, L.; Yu, Y.; Lin, H. M.; Tsang, W. Modulation of endoplasmic reticulum calcium pump by Bcl-2. *Oncogene* **1998**, *17*, 1903−1910.
(13) Dremina, E. S.; Sharov, V. S.; Schöneich, C. Displacement of SERCA from SR lipid caveolae-related domains by Bcl-2: a possible mechanism for SERCA inactivation. *Biochemistry* **2006**, *45*, 175−184.
(14) Grabowski, D. R.; Dubyak, G. R.; Rybicki, L.; Hidaka, H.; Ganapathi, R. Tumor cell resistance to topoisomerase II poisons: role for intracellular free calcium in the sensitization by inhibitors or calcium-calmodulin-dependent enzymes. *Biochem. Pharmacol.* **1998**, *56*, 345−349.
(15) Tombal, B.; Weeraratna, A. T.; Denmeade, S. R.; Isaacs, J. T. Thapsigargin induces a calmodulin/calcineurin-dependent apoptotic cascade responsible for the death of prostatic cancer cells. *Prostate* **2000**, *43*, 303−317.
(16) Morjani, H.; Madoulet, C. Immunosuppressors as multidrug resistance reversal agents. *Methods Mol. Biol.* **2010**, *596*, 433−446.
(17) Oltersdorf, T.; Elmore, S. W.; Shoemaker, A. R.; Armstrong, R. C.; Augeri, D. J.; Belli, B. A.; Bruncko, M.; Deckwerth, T. L.; Dinges, J.; Hajduk, P. J.; Joseph, M. K.; Kitada, S.; Korsmeyer, S. J.; Kunzer, A. R.; Letai, A.; Li, C.; Mitten, M. J.; Nettesheim, D. J.; Ng, S.; Nimmer, P. M.; O'Connor, J. M.; Oleksijew, A.; Petros, A. M.; Reed, J. C.; Shen, W.; Tahir, S. K.; Thompson, C. B.; Tomaselli, K. J.; Wang, B.; Wendt, M. B.; Zhang, H.; Fesik, S. W.; Rosenberg, S. H. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* **2005**, *435*, 677−681.
(18) Roberts, A. W.; Seymour, J. F.; Brown, J. R.; Wierda, W. G.; Kipps, T. J.; Khaw, S. L.; Carney, D. A.; He, S. Z.; Huang, D. C.; Xiong, H.; Cui, Y.; Busman, T. A.; McKeegan, E. M.; Krivoshik, A. P.; Enschede, S. H.; Humerickhouse, R. Substantial susceptibility of chronic lymphocytic leukemia to Bcl2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. *J. Clin. Oncol.* **2012**, *30*, 488−496.
(19) Wong, M.; Tan, N.; Zha, J.; Peale, F. V.; Yue, P.; Fairbrother, W. J.; Belmont, L. D. Navitoclax (ABT-263) reduces Bcl-xL mediated chemo-resistance in ovarian cancer models. *Mol. Cancer Ther.* **2012**, *11*, 1017−1025.
(20) Witham, J.; Valenti, M. R.; De-Haven-Brandon, A. K.; Vidot, S.; Eccles, S. A.; Kaye, S. B.; Richardson, A. The Bcl-2/Bcl-X_L family inhibitor ABT-737 sensitizes ovarian cancer cells to carboplatin. *Clin. Cancer Res.* **2007**, *13*, 7191−7198.
(21) Hall, M. D.; Brimacombe, K. R.; Varonka, M. S.; Pluchino, K. M.; Monda, J. K.; Li, J.; Walsh, M. J.; Boxer, M. B.; Warren, T. H.;

Fales, H. M.; Gottesman, M. M. Synthesis and structure−activity evaluation of isatin-beta-thiosemicarbazones with improved selective activity toward multidrug-resistant cells expressing P-glycoprotein. *J. Med. Chem.* **2011**, *54*, 5878−5889.
(22) Hall, M. D.; Handley, M. D.; Gottesman, M. M. Is resistance useless? Multidrug resistance and collateral sensitivity. *Trends. Pharmacol. Sci.* **2009**, *30*, 546−556.
(23) Wang, J.; Liu, D.; Zhang, Z.; Shan, S.; Han, X.; Srinivasula, S. M.; Croce, C. M.; Alnemri, E. S.; Huang, Z. Structure-based discovery of an organic compound that binds Bcl-2 protein and induces apoptosis of tumor cells. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 7124−7129.
(24) Lickliter, J. D.; Wood, N. J.; Johnson, L.; McHugh, G.; Tan, J.; Wood, F.; Cox, J.; Wickham, N. W. HA14-1 selectively induces apoptosis in Bcl-2-overexpressing leukemia/lymphoma cells, and enhances cytarabine-induced cell death. *Leukemia* **2003**, *17*, 2074−2080.
(25) An, J.; Chen, C.; Huang, Z. Critical upstream signals of cytochrome c release induced by a novel Bcl-2 inhibitor. *J. Biol. Chem.* **2004**, *279*, 19133−19140.
(26) Doshi, J. M.; Xing, C. Antagonists against anti-apoptotic Bcl-2 family proteins for cancer treatment. *Mini-Rev. Org. Chem.* **2008**, *5*, 171−178.
(27) Doshi, J. M.; Tian, D.; Xing, C. Ethyl-2-amino-6-bromo-4-(1-cyano-2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (HA 14-1), a prototype small-molecule antagonist against anti-apoptotic Bcl-2 proteins, decomposes to generate reactive oxygen species (ROS) that induce apoptosis. *Mol. Pharmaceutics* **2007**, *4*, 919−928.
(28) Tian, D.; Das, S.; Doshi, J. M.; Peng, J.; Lin, J.; Xing, C. sHA 14-1, a stable and ROS-free antagonist against anti-apoptotic Bcl-2 proteins, bypasses drug resistances and synergizes cancer therapies in human leukemia cell. *Cancer Lett.* **2008**, *259*, 198−208.
(29) Das, S. G.; Doshi, J. M.; Tian, D.; Addo, S. N.; Srinivasan, B.; Hermanson, D.; Xing, C. Structure activity relationships and molecular mechanisms of sHA 14-1 and its analogs. *J. Med. Chem.* **2009**, *52*, 5937−5949.
(30) Das, S. G.; Srinivasan, B.; Hermanson, D. L.; Bleeker, N.; Doshi, J. M.; Tang, R.; Beck, W. T.; Xing, C. Structure activity relationship and molecular mechanisms of ethyl 2-amino-6-(3,5-dimethoxyphenyl)-4-(2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (CXL017) and its analogs. *J. Med. Chem.* **2011**, *54*, 5937−5948.
(31) Hermanson, D.; Addo, S. N.; Bajer, A. A.; Marchant, J. S.; Das, S. G. K.; Srinivasan, B.; Al-Mousa, F.; Michelangeli, F.; Thomas, D. D.; LeBien, T. W.; Xing, C. Dual mechanisms of sHA 14-1 in inducing cell death through endoplasmic reticulum and mitochondria. *Mol. Pharmacol.* **2009**, *76*, 667−678.
(32) Starcevic, S.; Brozic, P.; Turk, S.; Cesar, J.; Rizner, T. L.; Gobec, S. Synthesis and biological evaluation of (6- and 7-phenyl) coumarin derivatives as selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1. *J. Med. Chem.* **2011**, *54*, 248−261.
(33) Liu, K. C.; Shelton, B. R.; Howe, R. K. A particularly convenient preparation of benzohydroximinoyl chlorides, nitrile oxide precursors. *J. Org. Chem.* **1980**, *45*, 3916−3918.
(34) Kumar, R. R.; Perumal, S.; Menendez, J. C.; Yogeeswari, P.; Sriram, D. Antimycobacterial activity of novel 1,2,4-oxadiazole-pyranopyridine/chromene hybrids generated by chemoselective 1,3-dipolar cycloadditions of nitrile oxides. *Bioorg. Med. Chem.* **2011**, *19*, 3444−3450.
(35) Knapp, D. M.; Gillis, E. P.; Burke, M. D. A general solution for unstable boronic acids: slow-release cross-coupling from air-stable MIDA boronates. *J. Am. Chem. Soc.* **2009**, *131*, 6961−6963.
(36) Curini, M.; Rosati, O.; Marcotullio, M. C.; Montanari, F.; Campagna, V.; Pace, V.; Cravotto, G. Preparation of 2-amino-4*H*-chromene derivatives from coumarins in basic media. *Eur. J. Org. Chem.* **2006**, 746−751.
(37) Ramesh, D.; Srinivasan, M. Studies on ring opening of coumarins. *Curr. Sci.* **1984**, *53*, 369−371.
(38) Bhanu Prasad, B. A.; Gilbertson, S. R. One-pot synthesis of N-heterocyclic carbene ligands from a *N*-(2-iodoethyl)arylamine salts. *Org. Lett.* **2009**, *11*, 3710−3713.
(39) Aridoss, G.; Laali, K. K. Building heterocyclic systems with $RC(OR)_2^+$ carbocations in recyclable Brønsted acidic ionic liquids: facile synthesis of 1-substituted 1*H*-1,2,3,4-tetrazoles, benzazoles and other ring systems with $CH(OEt)_3$ and $EtC(OEt)_3$ in $[EtNH_3][NO_3]$ and $[PMIM(SO_3H)][OTf]$. *Eur. J. Org. Chem.* **2011**, 2827−2835.
(40) Vaziri, S. A.; Grabowski, D. R.; Tabata, M.; Holmes, K. A.; Sterk, J.; Takigawa, N.; Bukowski, R. M.; Ganapathi, M. K.; Ganapathi, R. c-IAP1 is overexpressed in HL-60 cells selected for doxorubicin resistance: effects on etoposide-induced apoptosis. *Anticancer Res.* **2003**, *23*, 3657−3661.
(41) Harker, W. G.; Slade, D. L.; Drake, F. H.; Parr, R. L. Mitoxantrone resistance in HL-60 leukemia cells: reduced nuclear topoisomerase II catalytic activity and drug-induced DNA cleavage in association with reduced expression of the topoisomerase II beta isoform. *Biochemistry* **1991**, *30*, 9953−9961.
(42) Harker, W. G.; Slade, D. L.; Dalton, W. S.; Meltzer, P. S.; Trent, J. M. Multidrug resistance in mitoxantrone-selected HL-60 leukemia cells in the absence of P-glycoprotein overexpression. *Cancer Res.* **1989**, *49*, 4542−4549.
(43) Fujimori, A.; Harker, W. G.; Kohlhagen, G.; Hoki, Y.; Pommier, Y. Mutation at the catalytic site of topoisomerase I in CEM/C2, a human leukemia cell line resistant to camptothecin. *Cancer Res.* **1995**, *55*, 1339−1346.
(44) Fujimori, A.; Hoki, Y.; Popescu, N. C.; Pommier, Y. Silencing and selective methylation of the normal topoisomerase I gene in camptothecin-resistant CEM/C2 human leukemia cells. *Oncol. Res.* **1996**, *8*, 295−301.
(45) Chen, M.; Beck, W. T. DNA topoisomerase II expression, stability, and phosphorylation in two VM-26-resistant human leukemic CEM sublines. *Oncol. Res.* **1995**, *7*, 103−111.
(46) Beck, W. T.; Mueller, T. J.; Tanzer, L. R. Altered surface membrane glycoproteins in vinca alkaloid-resistant human leukemic lymphoblasts. *Cancer Res.* **1979**, *39*, 2070−2076.
(47) Tang, R.; Cohen, S.; Perrot, J. Y.; Faussat, A. M.; Zuany-Amorim, C.; Marjanovic, Z.; Morjani, H.; Fava, F.; Corre, E.; Legrand, O.; Marie, J. P. P-gp activity is a critical resistance factor against AVE9633 and DM4 cytotoxicity in leukaemia cell lines, but not a major mechanism of chemoresistance in cells from acute myeloid leukaemia patients. *BMC Cancer* **2009**, *9*, 199.
(48) Cai, J.; Damaraju, V. L.; Groulx, N.; Mowles, D.; Peng, Y.; Robins, M. J.; Cass, C. E.; Gros, P. Two distinct molecular mechanisms underlying cytarabine resistance in human leukemic cells. *Cancer Res.* **2008**, *68*, 2349−2357.
(49) Denmeade, S. R.; Jakobsen, C. M.; Janssen, S.; Khan, S. R.; Garrett, E. S.; Lilja, H.; Christensen, S. B.; Isaacs, J. T. Prostate-specific antigen-activated thapsigargin prodrug as targeted therapy for prostate cancer. *J. Natl. Cancer Inst.* **2003**, *95*, 990−1000.
(50) Kapoor, R.; Slade, D. L.; Fujimori, A.; Pommier, Y.; Harker, W. G. Altered topoisomerase I expression in two subclones of human CEM leukemia selected for resistance to camptothecin. *Oncol. Res.* **1995**, *7*, 83−95.