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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 Analogues

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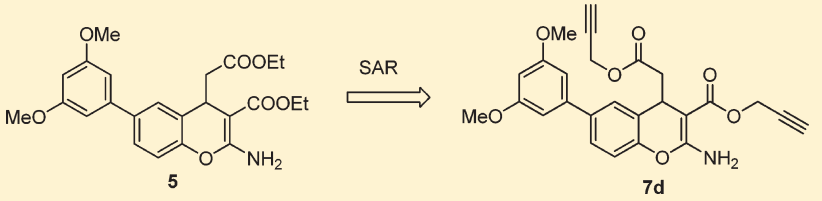
Supporting Information

ABSTRACT:



Multidrug resistance (MDR) in cancer is a phenomenon in which administration of a single chemotherapeutic agent causes cross-resistance of cancer cells to a variety of therapies even with different mechanisms of action. Development of MDR against standard therapies is a major challenge in the treatment of cancer. Previously we have demonstrated a unique ability of CXL017 (**5**) to selectively target MDR cancer cells and synergize with mitoxantrone (MX) in HL60/MX2 MDR cells. Here we expand its scope and demonstrate that **5** can synergize with both vincristine and paclitaxel in three different MDR cell lines (HL60/DNR, K562/HHT300, and CCRF-CEM/VLB100). We also demonstrate that **5** has potent cytotoxicity in the NCI-60 panel of cell lines with an average IC_{50} of 1.04 μM. In addition, **5** has a unique mechanism of action in comparison with standard agents in the NCI database based on COMPARE analysis. Further structure−activity relationship study led to the development of a more potent analogue, compound **7d**, with an IC_{50} of 640 nM in HL60/MX2. Additionally, one enantiomer of **5** is 13-fold more active than the less active enantiomer. Taken together, our study has led to the discovery of a series of analogues that selectively target drug-resistant cancer cells with the potential for the treatment of drug-resistant cancers.

INTRODUCTION

Cancer is a disease that has been known to humans for centuries. Although there has been a substantial improvement in the survival rate and patient care, finding a complete cure for it has been a challenging task. One primary cause of treatment failure is the emergence of resistance in cancer. For example, acute myeloid leukemia (AML) patients have a remission rate of 60−80% but only ∼20% of these patients survive more than 5 years.[1−3] The remaining patients relapse with residual diseases that are typically resistant to standard chemotherapies. Therefore, there is an unmet clinical need for new therapies targeting drug-resistant malignancies.

Cancer cells utilize multiple mechanisms for development of drug resistance, such as alteration in drug transport, protein targets, or cellular repair mechanisms.[4,5] One of the major mechanisms is the alteration of the apoptotic pathway through overexpression of the antiapoptotic Bcl-2 family proteins.[6] Bcl-2 family proteins are the key regulators of apoptosis, a programmed cell death mechanism.[7] Antiapoptotic members, such as Bcl-2, Bcl-X_L, and Mcl-1, and proapoptotic members, including Bax, Bak, and Bad, antagonize each other to maintain the balance between cell survival and death.[8] Overexpression of the antiapoptotic Bcl-2 family proteins in cancer cells disrupts this balance and prevents cell death.[9] Indeed, over 60−90% of all cancers reveal increased expression of the antiapoptotic Bcl-2 family proteins.[10,11] Therefore, the antiapoptotic Bcl-2 family proteins are promising targets for the development of new therapies to treat drug-resistant malignancies.[12]

Over the years, tremendous progress has been made in designing inhibitors for the antiapoptotic Bcl-2 family proteins.[13] Many of these inhibitors are currently in clinical trials for the treatment of various cancers.[14] Our research has focused on a putative inhibitor of the antiapoptotic Bcl-2 family proteins

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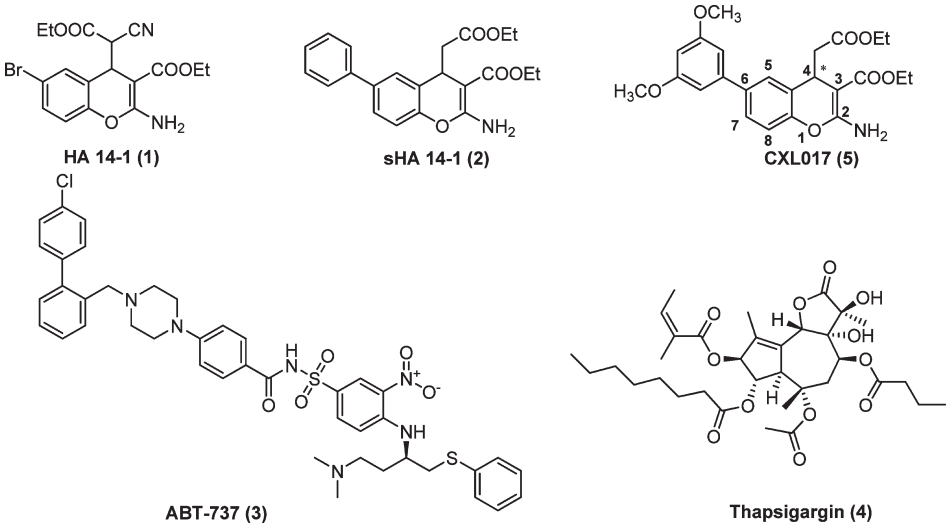

Figure 1. Structures of compounds. The asterisk (*) represents chiral centers.

Table 1. Cross-Resistance (Ratio of IC_{50} in Resistant Cell Lines Relative to That in Parent Cell Lines) of MDR Cancer Cell Lines for Standard Therapies and 5[a]

	cross-resistance					
resistant cell line	doxorubicin	vincristine	Ara-C	paclitaxel	MX	**5**
HL60/DNR	80 ± 21	>1000	1.3 ± 0.5	>1000	7.5 ± 0.6	0.77 ± 0.15
K562/HHT300	32 ± 11	>1000	2.2 ± 0.1	91 ± 15	11 ± 0.5	0.93 ± 0.15
CCRF-CEM/VLB100	6.1 ± 2.1	>1000	0.88 ± 0.16	266 ± 16	2.0 ± 0.5	1.32 ± 0.02

[a] Results are the mean of two independent experiments in triplicate in each experiment.

known as HA 14-1 (**1**) (Figure 1). **1** was selected for study because it overcomes drug resistance in cancer cells that overexpress antiapoptotic Bcl-2 proteins and demonstrates synergism with a variety of standard cancer therapies.[15,16] However, **1** has a short half-life and therefore is not an appropriate drug candidate.[17] On the basis of the decomposition pathway of **1**, we have successfully developed a stable analogue, namely, sHA 14-1 (**2**) (Figure 1).[18]

One remarkable feature of **2** was that cancer cells genetically overexpressing the antiapoptotic Bcl-2 family proteins reveal no resistance to **2**,[18] despite the fact that such overexpression conferred significant resistance against standard cancer therapies. In addition, **2** functions quite differently from ABT-737 (**3**) (Figure 1), a well-known inhibitor against Bcl-2 and Bcl-X_L proteins, in that **2** induces programmed cell death mainly through the endoplasmic reticulum (ER) pathway by inducing ER stress and ER Ca^{2+} release.[19] **2** also inhibits the ATPase activity of sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), while its anticancer profile differs from that of thapsigargin (**4**) (Figure 1) as well, a standard SERCA inhibitor.[19] These data, overall, suggest that **2** represents a novel scaffold that may be used to develop therapies against drug-resistant cancers.

On the basis of the interesting anticancer profile of **2**, we performed a structure−activity relationship (SAR) study that led to the discovery of CXL017 (**5**) (Figure 1), which exhibits cytotoxicity comparable to that of **3**.[20] Compound **5** demonstrated low micromolar cytotoxicity over 11 different cancer cell lines, including both solid and hematological malignancies.[20] Of interest, **5** demonstrated selective cytotoxicity toward two drug-resistant cancer cell lines, HL60/MX2 and CCRF-CEM/CT, which are cross-resistant to standard cancer therapies. In this report, we provide additional evidence toward the unique ability of **5** to target drug resistant cancers and discuss an extended SAR study of **5** to understand the role of various functional groups on its cytotoxicity and selectivity toward drug resistant cancers.

RESULTS AND DISCUSSION

Cytotoxicity of 5 in Multidrug Resistant Cancer Cells. Previously, we have shown that multidrug resistant (MDR) cancer cells like HL60/MX2 and CCRF-CEM/CT show no cross-resistance to **5**.[20] Instead **5** demonstrates increased cytotoxicity toward such MDR cancer cells relative to the parental counterparts. To evaluate the scope of **5** in targeting drug-resistant cancers, we acquired several additional drug resistant cancer cell lines, such as HL60/DNR, K562/HHT300, CCRF-CEM/VLB100, selected for resistance toward daunorubicin (DNR), homoharringtonine (HHT), and vinblastine (VLB), respectively. We first evaluated these cell lines for their potential cross-resistance to standard therapies. Our results (Table 1) show that these drug-resistant cell lines reveal cross-resistance to all standard therapies tested. Evaluation of compound **5** in these cell lines again revealed that none of these resistant cell lines show cross-resistance to **5** (Table 1) and that one cell line (HL60/DNR) may be collaterally sensitive to **5**. These results further support the unique ability of **5** to target drug-resistant malignancies.

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Table 2. CI Values of Vincristine and Paclitaxel with 5 in MDR (HL60/DNR, K562/HHT300, and CCRF-CEM/VLB100) Cancer Cell Lines[a]

	combination index (CI)		
treatment	HL60/DNR	K562/HHT300	CCRF-CEM/VLB100
vincristine + **5**	0.61	0.56	0.55
paclitaxel + **5**	0.77	0.53	0.53

[a] The MDR cell lines were treated with single drugs vincristine, paclitaxel, and **5** or in combination in a fixed ratio for 48 h. The fixed ratios used for vincristine with **5** are 1:2.5, 1:5, and 1:1 and those for paclitaxel with **5** are 1:8, 1:8, and 1:1 for HL60/DNR, K562/HHT300 and CCRF-CEM/VLB100, respectively. The CI (combination index) was calculated by the combination index equation of Chou and Talalay. CI < 1, CI = 1, and CI > 1 indicate synergism, additive effect, and antagonism, respectively.

Synergism of 5 with Standard Therapies. **5** has been shown to synergize with mitoxantrone in HL60/MX2 resistant cells.[20] In this study, we tested the potential synergism of **5** in combination with vincristine or paclitaxel in the three new MDR cell lines. The combination index (CI) of these drugs was calculated using the Chou and Talalay method.[21] Our results show that **5** synergizes with these clinically used drugs with a CI of ~0.5−0.8 in all three MDR cell lines (Table 2), suggesting that **5** can be used in combination with standard therapies to treat drug-resistant cancer cells.

Cytotoxicity of 5 in the NCI-60 Cell Line Panel. The NCI-60 human tumor cell line anticancer drug screen was developed in the late 1980s and has been used for a variety of purposes such as identifying potential anticancer drug candidates, determining the molecular target of a compound, gene, and chemosensitization profiling.[22] We used the NCI-60 cell line screen to evaluate the activity profile of **5**. Notably, **5** revealed decent cytotoxicity across the entire NCI cell panel with a mean GI_{50} of 1.04 μM (Table 3). Very interestingly, the natural MDR cell line HCT-15 and in vitro drug selected MDR cell line NCI/ADR-RES are both sensitive to treatment by **5** with GI_{50} values of 0.55 and 0.49 μM, respectively (Table 3). Further analysis of the NCI data suggests that **5** was generally more effective against leukemia, colon cancer, melanoma, and breast cancer cells but shows decreased activity in renal, ovarian and non-small-cell lung cancer cells. These results suggest that **5** may be useful for the treatment of a variety of different cancers including naturally occurring MDR cancers.

COMPARE Analysis of 5. To explore the potential similarity of **5** to other compounds tested in the NCI-60 cell line screen, we used the COMPARE algorithm provided by NCI to measure the similarity of **5** (the seed compound) in responses to the 60 cell lines of an entire database of compounds. It then ranks all the compounds in the entire database in their order of similarity toward the seed compound, in which a compound with a rank 1 signifies the highest similarity in the possible mechanism of action to the seed compound. Using this approach, we compared the mean GI_{50} profile ("fingerprint") of **5** to the standard agents. The standard agent database includes 171 compounds ranging from cancer treatment new drug applications and investigational new drug applications to compounds with a particularly high interest at the NCI. The similarity pattern compared to that of the seed is expressed using a Pearson's correlation coefficient (PCC). A PCC of >0.5 is generally considered significant based on previous reports.[23,24]

Table 3. Cytotoxicity of 5 in μM across a panel of 60 cancer cell lines from NCI

panel/cell lines	GI_{50} (μM)	panel/cell lines	GI_{50} (μM)
leukemia		melanoma	
HL-60(TB)	0.35	UACC-62	0.55
K-562	0.39	M14	0.55
SR	0.45	LOX IMVI	0.72
CCRF-CEM	0.81	SK-MEL-5	0.89
MOLT-4	0.80	SK-MEL-2	1.4
RPMI-8226	1.9	SK-MEL-28	2.6
non-small-cell lung cancer		UACC-257	12
NCI-H460	0.54	ovarian cancer	
NCI-H522	0.62	OVCAR-3	0.32
HOP-62	0.89	NCI/ADR-RES	0.49
A549/ATCC	1.2	IGROV1	0.93
EKVX	1.3	SK-OV-3	1.4
HOP-92	1.4	OVCAR-8	3.8
NCI-H23	2.0	OVCAR-5	4.1
NCI-H322M	4.0	OVCAR-4	5.2
NCI-H226	4.4	renal cancer	
colon cancer		A498	0.36
HT29	0.39	CAKI-1	0.71
HCT-116	0.42	UO-31	1.4
SW-620	0.49	ACHN	1.5
KM12	0.50	786−0	2.3
HCT-15	0.55	RXF 393	2.5
COLO 205	1.2	SN12C	2.8
HCC-2998	2.5	TK-10	3.4
CNS cancer		prostate cancer	
SF-295	0.48	PC-3	0.83
SF-539	0.49	DU-145	1.5
U251	0.63	breast cancer	
SF-268	1.1	HS 578T	0.32
SNB-75	1.3	MCF7	0.45
SNB-19	1.9	MDA-MB-468	0.67
melanoma		BT-549	0.77
MDA-MB-435	0.27	MDA-MB-231/ATCC	1.5
MALME-3M	0.39	T-47D	3.7

Our COMPARE analysis results show that compound **5** is unique in its mechanism of action with little correlation to "standard" agents; specifically none of the 171 compounds tested, which includes some clinically used agents, show a PCC of >0.5 to **5** in the COMPARE analysis (Table 4). These data suggest that **5** is a scaffold with a unique mechanism of action, potentially accounting for its novel ability to selectively eliminate drug-resistant cancers.

Structure−Activity Relationship (SAR) of 5. *Rationale.* Previous SAR studies of **5** mainly focused on its cytotoxicity. Here we further explore the SAR of **5** to understand the importance of various functional groups on **5** toward both its cytotoxicity and selectivity. Since **5** has two methoxy groups on the 3′ and 5′ positions on the phenyl ring relative to **2**, resulting in 10-fold improvement in cytotoxicity, we designed molecules **6a**−**e** (Table 5) to investigate the importance of the number of methoxy groups and their positions on the phenyl ring. Previous SAR studies have also shown that the phenyl ring plays an

important role in the activity of **2**; therefore, we evaluated compounds **6f**−**k** (Table 6) to determine the effect of the phenyl substitution on selectivity toward MDR cells. Compounds **6l**−**p** (Table 7) were chosen to study the effect of electron donating groups (EDGs) on the phenyl ring, while compounds **6q** and **6r** were designed to determine the effect of electron withdrawing groups (EWGs). Previous SAR study results also show that substitution at the 4′ position on the phenyl ring decreases activity. Here we have expanded our SAR to include fused and extended ring system analogues (**6s**−**v**) (Table 7) to further understand the impact of sterics on the activity and selectivity of **5**. We also designed molecules (**7a**−**f**) (Table 8) with various alkyl and alkynyl groups on the ester at the third and fourth positions to evaluate the effect of modulating chain length and flexibility on the ester functionality. To explore the importance of ester functionality at the third and fourth position on the chromene ring, analogues **8a**−**f** (Table 9) were designed. Finally, the role of the amino functionality at the second position of the chromene ring was explored with the help of compounds **7g**, **8g**, and **8h**. These compounds were evaluated in HL60 and HL60/MX2 (the drug-resistant cell line) to evaluate their cytotoxicity and selectivity.

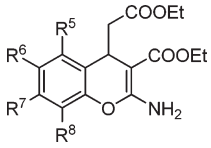
Table 4. List of the Top Compounds with Growth Inhibitory Patterns Similar to That of 5 (NSC No. S753690)[a]

rank	PCC	NSC no.	name	mechanism	high concn
1	0.50	S35212	trimetrexate	antimetabolite/antifolate	−6.0
2	0.49	S163501	acivicin	antimetabolite	−4.0
3	0.48	S126771	dichloroallyl lawsone	antimetabolite	−3.6
4	0.48	S153858	maytansine	antimitotic agent	−3.6
5	0.48	S332598	rhizoxin	antimitotic agent	−4.3
14	0.40	S49842	vinblastine	antimitotic agent	−5.6
16	0.39	S619003	vincristine	antimitotic agent	−5.0
21	0.38	S19893	5-fluorouracil	antimetabolite	−4.0
22	0.38	S740	methotrexate	antimetabolite/antifolate	−3.6
32	0.36	S125973	paclitaxel	antimitotic agent	−6.0

[a] Seed NSC: S753690 (**5**). EXP ID: AVGDATA. High concn: −4.0. Database: Standard Agents. Seed level: GI_{50}. High concn: −4.0.

Chemistry. Compounds **6f**−**k** and **8a**−**f** were synthesized previously with their synthesis and characterization reported before.[20] The general structures of the new series of 4*H*-chromene derivatives, designed based on our lead compound **5**, are depicted in Scheme 1 with compounds **6a**−**e**,**l**−**u** synthesized from their corresponding boronic acids with overall yields of ∼70%. Compound **6v** was synthesized via a nucleophilic substitution reaction between **14** and **15** as outlined in Scheme 2 to give intermediate **16**, followed by a Michael addition reaction sequence to give **6v** in 61% yield.[25] Compounds **7a**−**f** with various alkyl or alkynyl groups on the ester functionality were synthesized by treating 3,5-dimethoxyphenylcoumarin (**17**) with ethyl cyanoacetate in the presence of various sodium alkoxides generated in situ as outlined in Scheme 3 with overall yields ranging from 45% to 60%. Compound **7g** was synthesized from 3,5-dimethoxyphenylsalicylaldehyde (**18**) using the method described in Scheme 4.[26] Briefly, **18** was converted to the ester

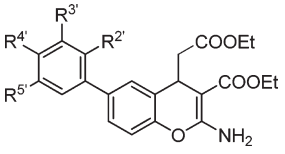
Table 6. IC_{50} (μM) of Analogues 6f−k in HL60 and HL60/MX2



					IC_{50} ± SEM[a]		
compd	R^5	R^6	R^7	R^8	HL60	HL60/MX2	selectivity HL60/(HL60/MX2)
6f	H	H	H	H	202 ± 10	99.1 ± 2.3	2.04
6g	H	Br	H	H	74.4 ± 5.6	35.4 ± 0.7	2.10
6h	H	*n*-Pr	H	H	79.2 ± 4.2	46.7 ± 1.9	1.70
6i	Ph	H	H	H	56.8 ± 2.0	38.7 ± 1.2	1.47
6j	H	H	Ph	H	84.5 ± 4.2	34.3 ± 5	2.47
6k	H	H	H	Ph	87.3 ± 6.5	44.4 ± 3.4	1.97

[a] Results are given as the mean of three independent experiments in triplicate in each experiment.

Table 5. IC_{50} (μM) of Analogues 2 and 6a−e in HL60 and HL60/MX2



					IC_{50} ± SEM[a]		
compd	$R^{2'}$	$R^{3'}$	$R^{4'}$	$R^{5'}$	HL60	HL60/MX2	selectivity HL60/(HL60/MX2)
5	H	OMe	H	OMe	10.7 ± 0.5	2.43 ± 0.18	4.41
2	H	H	H	H	91.0 ± 0.3	23.2 ± 1.5	3.93
6a	H	OMe	H	H	38.7 ± 1.2	9.7 ± 0.5	4.02
6b	H	OMe	OMe	OMe	33.3 ± 0.8	28.1 ± 1.0	1.19
6c	H	H	OMe	H	66.6 ± 0.3	15.1 ± 0.6	4.41
6d	OMe	H	H	H	68.4 ± 1.3	16.0 ± 0.3	4.28
6e	H	OMe	OMe	H	54.6 ± 7.2	14.5 ± 0.4	3.77

[a] Results are given as the mean of three independent experiments in triplicate in each experiment.

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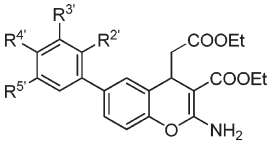
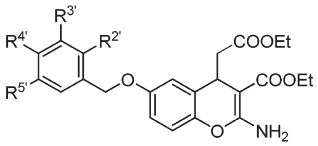
Table 7. IC_{50} (μM) of Analogues 6l−v in HL60 and HL60/MX2



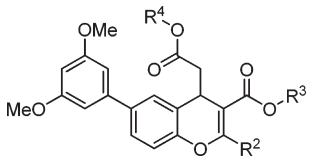


6v

Cmpd.	$R^{2'}$	$R^{3'}$	$R^{4'}$	$R^{5'}$	HL60 $IC_{50} \pm SEM^a$	HL60/MX2 $IC_{50} \pm SEM^a$	Selectivity HL60/(HL60/MX2)
6l	H	Me	H	H	12.2 ± 0.6	4.3 ± 0.05	2.82
6m	H	Me	H	Me	7.29 ± 0.4	2.46 ± 0.2	2.97
6n	H	OH	H	OMe	84.5 ± 8.9	11.9 ± 1.6	7.10
6o	H	OH	H	OH	> 400	67.5 ± 5.7	5.93
6p	H	H	*t*-BuPh	H	48.3 ± 0.5	24.4 ± 0.4	1.98
6q	H	CF_3	H	CF_3	264.5 ± 5.7	81.3 ± 0.5	3.26
6r	H	NO_2	H	H	39.2 ± 4.7	13.4 ± 0.76	2.92
6s			H	H	39.5 ± 2.8	15.1 ± 1.9	2.61
6t	H			H	57.7 ± 1.9	18.2 ± 1.7	3.17
6u	H			H	73.1 ± 1.5	19.2 ± 0.5	3.82
6v	H	OMe	H	OMe	32.6 ± 13	11.5 ± 0.4	2.82

[a] Results are given as the mean of three independent experiments in triplicate in each experiment.

Table 8. IC_{50} (μM) of Analogues 7a−g in HL60 and HL60/MX2



				$IC_{50} \pm SEM^a$		
compd	R^2	R^3	R^4	HL60	HL60/MX2	selectivity HL60/(HL60/MX2)
7a	NH_2	*n*-Pr	*n*-Pr	7.6 ± 0.3	3.9 ± 0.3	1.97
7b	NH_2	*n*-Bu	*n*-Bu	32 ± 1.7	14.1 ± 0.5	2
7c	NH_2	allyl	allyl	4.7 ± 0.3	2.5 ± 0.1	1.62
7d	NH_2	C_3H_3	C_3H_3	1.5 ± 0.1	0.64 ± 0.01	2.38
7e	NH_2	Et	C_3H_3	4.7 ± 0.2	1.87 ± 0.06	2.54
7f	NH_2	cyclopropylmethyl	cyclopropylmethyl	6.7 ± 0.5	3.55 ± 0.45	1.87
7g	Me	Et	Et	63.6 ± 7.4	9.55 ± 0.90	6.66

[a] Results are given as the mean of three independent experiments in triplicate in each experiment.

intermediate **19** using the Wittig reaction with 72% yield. Intermediate **19** then underwent an oxa-Michael/Michael addition sequence with ethyl buta-2,3-dieonate (**20**) to give compound **7g** in 82% yield.

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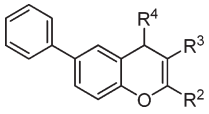
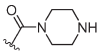
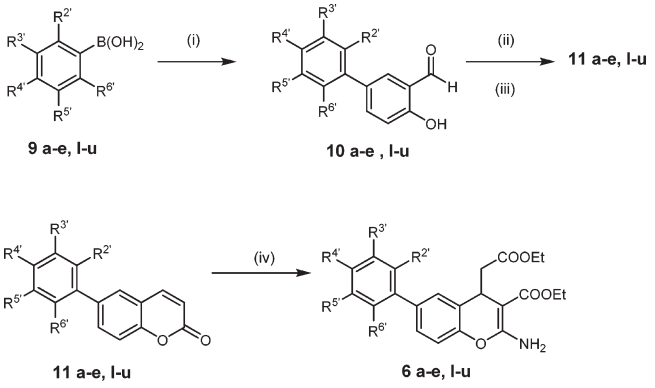
Table 9. IC_{50} (μM) of Analogues 8a−h in HL60 and HL60/MX2



Cmpd.	R^2	R^3	R^4	HL60 $IC_{50} \pm SEM^a$	HL60/MX2 $IC_{50} \pm SEM^a$	Selectivity HL60/(HL60/MX2)
8a	NH_2	CN	OEt	76.1 ± 1.1	26.5 ± 5.7	2.87
8b	NH_2	CO_2i-Pr	CO_2i-Pr	74.3 ± 6.5	22.1 ± 1.5	3.37
8c	NH_2	CO_2Et		24.9 ± 2.5	38.8 ± 1.04	0.64
8d	NH_2	CO_2Et		57 ± 6	23.4 ± 3.2	2.44
8e	NH_2	CO_2Et		> 400	> 400	1.00
8f	NH_2	CO_2Et		60.0 ±2.7	31.1 ± 1.9	1.93
8g	NH(COMe)	CH_2CO_2Et	CO_2Et	79.6 ± 5	44.9 ± 3.8	1.77
8h	$N(COMe)_2$	CH_2CO_2Et	CO_2Et	58.9 ± 4.4	45.8 ± 2.2	1.29

[a] Results are given as the mean of three independent experiments in triplicate in each experiment.

Scheme 1[a]



[a] Reagents and condition: (i) 5-bromosalicylaldehyde, $Pd(OAc)_2$, K_2CO_3, DME/H_2O (1:1), room temp; (ii) DMA, $POCl_3$ 60 °C, 4−8 h; (iii) $NaHCO_3$, 60 °C, 0.5 h; (iv) ethyl cyanoacetate, EtOH, NaOEt, room temp.

In Vitro Cytotoxicity in HL60 and HL60/MX2 Cells. The in vitro cytotoxicity results of **2**, **5**, **6a**−**e** (Table 5) revealed that the number of methoxy groups on the phenyl ring played an important role in the activity. For example, compound **2** with no methoxy group shows a significant loss in activity in comparison with analogues **6a** (one methoxy at 3′ position) and **5** (two methoxy groups at 3′ and 5′ positions). Interestingly, further increasing the number of methoxy groups (**6b**) led to decreased activity in the HL60/MX2 cells. A possible reason for this observation could be the change in sterics of the ring on addition of a third methoxy group at the 4′ position. This observation was further supported by the data from compounds **6c**−**e** (Table 5). Compounds **6c** and **6d** that have methoxy groups at 4′ and 2′ positions of the phenyl ring, respectively, showed decreased activity in comparison to **6a** that has a methoxy group at the 3′ position. In addition, **6e** with methoxy groups at 3′ and 4′ positions was less active than **5**. Taken together, these data suggest that the number and relative position of the methoxy groups play an important role in the activity of **5**.

We showed previously that removal of the phenyl group from the sixth position of the chromene ring leads to a decrease in activity in JURKAT cells.[20] A similar trend was observed when these analogues (**6f**−**h**) (Table 6) were tested in HL60 and HL60/MX2 cells. In addition, moving the phenyl ring to positions 5, 7, and 8 (**6i**−**k**) on the chromene core also led to a decrease in activity (Table 6), suggesting that the 6-phenyl is probably optimal for activity.

When analogues with EDG (**6l**−**p**) (Table 7) were tested, we found that small lipophilic groups like methyl (**6l**, **6m**) led to an improvement in activity while bulky lipophilic groups, such as *tert*-butyl in **6p**, led to a decrease in activity possibly due to unfavorable steric interactions as observed for **6b**. Interestingly, replacing the lipophilic groups with hydrophilic groups like hydroxyl (**6n**, **6o**) also led to a decrease in activity, suggesting that small lipophilic functional groups were optimal for activity. We also tested the effect of EWG on the phenyl ring (**6q** and **6r**) and found that substituting electron withdrawing groups led to a decrease in activity.

As observed in an earlier SAR study,[20] steric bulkiness at the 4′ position on the phenyl ring led to a decrease in activity. To further explore the importance of the 4′ position, we introduced rigidity and flexibility in the scaffold with fused and extended ring system analogues (**6s**−**v**) (Table 7). All of these analogues show

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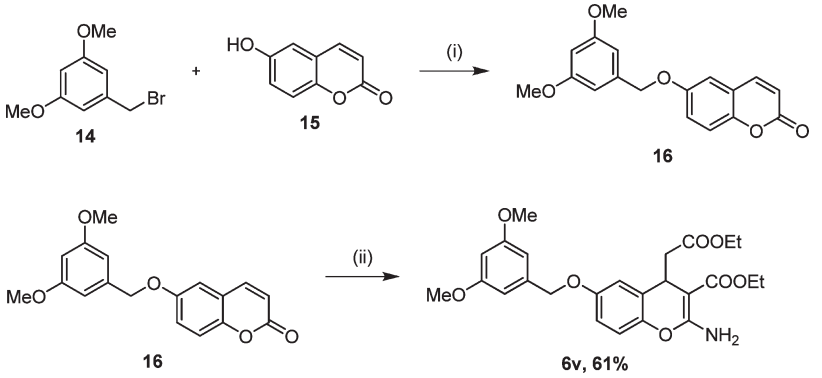
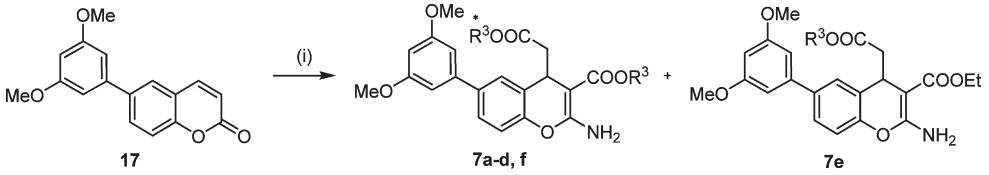
Scheme 2[a]



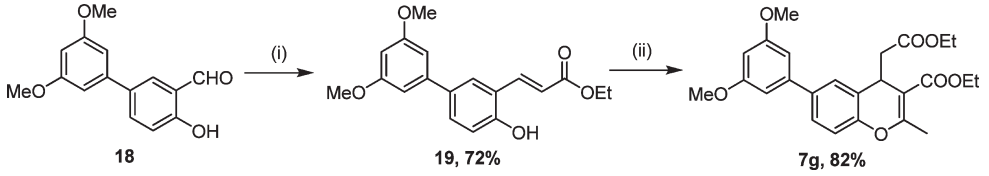
[a] Reagents and condition: (i) NaH, DMF, room temp; (ii) ethyl cyanoacetate, EtOH, NaOEt, room temp.

Scheme 3[a]



[a] Reagents and condition: (i) ethyl cyanoacetate, R^3OH, $NaOR^3$, room temp.

Scheme 4[a]



[a] Reagents and condition: (i) $Ph_3P{=}CHCO_2Et$, toluene, 80 °C; (ii) $H_2C{=}C{=}CHCO_2Et$, K_2CO_3 (10 mol %), DMSO, 120 °C.

a decrease in activity in comparison to **5**, further supporting that increased sterics lead to decreased activity.

Previously, we showed that changes on the ester did not significantly affect the activity in JURKAT cells.[20] In this study, we evaluated the ester functionality by designing analogues with various alkyl and alkynyl substitutions at the ester position (**7a**−**f**) (Table 8). Interestingly, replacing the ethyl ester with an *n*-propyl (**7a**) slightly improves activity. However, a further increase in chain length to the *n*-butyl ester (**7b**) led to a significant decrease in activity. Interestingly, introducing unsaturation in the propyl group led to a further improvement in activity (**7c** and **7d**). Analogue **7d** with a propargyl group on the ester is the most active compound with an IC_{50} of 1.53 μM in HL60 cells and an IC_{50} of 0.64 μM in HL60/MX2 cells, about 4 to 7-fold more active than **5**.

Other positions on the chromene core, including the second, third, and fourth positions, were also explored. The amino group at the second position was evaluated by acetylation (**8g** and **8h**) (Table 9) or replacement by a methyl (**7g**) (Table 8), which resulted in a loss in activity suggesting that the amino group was optimal for activity. Replacing the ester with CN or amide led to a slight decrease in activity (Table 9), suggesting that the ester functionality may be optimal for activity.

Selectivity toward MDR Resistant HL60/MX2 Cells. The selectivity of these analogues toward MDR resistant cell lines was determined based on the ratio of their IC_{50} in HL60 cells to that in HL60/MX2 cells. We used the Bonferroni multiple comparison test of statistical analysis to classify the compounds into three categories based on their selectivity ratio and statistical analysis. Compounds with selectivity ratios between 0.5 and 2.0 had $p > 0.05$, indicating that they are likely to be nonselective. Analogues with a selectivity ratio of 2−4 had $p < 0.01$ and were considered to have medium selectivity. Analogues with a selectivity ratio of >4 had $p < 0.001$ and were considered to have significant selectivity.

On the basis of such criteria, 14 analogues demonstrate no selectivity, 18 analogues demonstrate medium selectivity, while 7 analogues (**5**, **6a**, **6c**, **6d**, **6n**, **6o**, and **7g**) demonstrate significant selectivity with **6n**, **6o**, and **7g** being the most selective. Our results show that removing a phenyl group (**6f**−**h**) from **5** led to

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Table 10. IC_{50} (μM) of 5 and Its Enantiomers in MDR Cell Lines[a] and Selectivity

cell line	5	(+)-enantiomer	(−)-enantiomer
HL60, IC_{50}	10.7 ± 0.5	82.6 ± 1.4	6.1 ± 0.1
HL60/MX2, IC_{50}	2.4 ± 0.2	21.8 ± 1.8	1.9 ± 0.1
HL60/(HL60/MX2) selectivity	4.4	3.8	3.2

[a] Results are given as the mean of two independent experiments in triplicate in each experiment.

a decrease in selectivity (Table 6). However, removing the methoxy groups (**6a**) or replacing them with other electron donating groups such as a methyl (**6m**) does not change the selectivity (Tables 5 and 7). In addition, replacing an EDG with an EWG such as NO_2 or CF_3 does not cause significant change in selectivity. These data overall suggest that electronic effects are unlikely to play an essential role in the selectivity of **5** toward drug-resistant cells. Interestingly, converting the methoxy to a hydroxyl group, compounds **6n** and **6o** (Table 7), led to an improvement in selectivity, suggesting that the hydrophilic nature of a hydroxyl group might play a role in the improved selectivity. Sterically hindered molecules like (**6s**−**v**) do not show much change in selectivity in comparison to **5**, indicating that sterics do not play a major role in the selectivity of **5**. Converting the amino group at the second position to a methyl group (**7g**) (Table 8) improves the selectivity, suggesting that introducing lipophilic groups at the second position might lead to more selective candidate.

The results of our current SAR studies also suggest that there is no correlation between the selectivity and cytotoxicity of these compounds in cancer cells. Nonetheless, both selectivity and cytotoxicity of **5** are very sensitive to functional modifications. For example, the methoxy groups on **5** play an important role in its activity and selectivity (**5** vs **6n**). Similarly, replacement of the amino group from **5** with a methyl (compound **7g**) led to a slight decrease in cytotoxicity but improvement in selectivity.

Chiral Separation and Cytotoxicity of Stereoisomers of 5. As shown in Figure 1, compound **5** has one chiral center at position 4 on the chromene core. Our study so far has only evaluated the racemate of **5**. We wished to determine whether the optically pure isomers of compound **5** would present a different in vitro activity profile. For this purpose, we used a chiral HPLC column to separate the two enantiomers. The optical purity of the enantiomers was determined using polarimetry. The optical rotation for one enantiomer was +0.107 (*c* 1.0, MeOH) and that for the other enantiomer was −0.102 (*c* 1.0, MeOH). The two enantiomers were evaluated for their cytotoxicity and selectivity in HL60 and HL60/MX2 cancer cells. The results show that (−)-**5** is about 13-fold more cytotoxic than the (+)-**5** while there is no significant change with respect to selectivity (Table 10).

CONCLUSION

Given that the ability to selectively target MDR cancer cells is one crucial factor for future cancer therapy development, we have identified compound **5** as a potential lead. In this report, we demonstrate that a variety of MDR cells show no cross-resistance to **5**. **5** synergizes with several standard anticancer agents in MDR cells, including vincristine, paclitaxel, and mitoxantrone. Additionally, we have shown that **5** is effective across the panel of NCI-60 cell lines and seems to have a unique mechanism of action based on its low correlation with any known agents. With these results, further SAR studies to make more potent and selective analogues of **5** led to the discovery of compound **7d**, which is 4-fold more potent than **5**. We were also able to improve the selectivity of **5** with compounds **6m** and **7g** with a selectivity of 7.1 and 6.7, respectively, toward HL60/MX2 MDR cells relative to the parent HL60 cells. Finally, we have established that the (−)-enantiomer for compound **5** is about 13 times more active than the (+)-enantiomer; however, there is no difference in their selectivity toward MDR cancer cells. The significant difference in cytotoxicity and lack of difference in selectivity of the enantiomers suggest that **5** and its analogues have distinct cellular targets responsible for cytotoxicity and selectivity, which are currently under investigation. In conclusion, we have demonstrated that compound **5** reveals a unique mechanism of action to selectively kill MDR cancer cell lines, which merits further investigation for its potential as a drug candidate for the treatment of MDR cancers.

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 potassium permanganate solution followed by heating. Flash column chromatography was performed on Whatman silica gel 60 Å (230−400 mesh). NMR (^{1}H, ^{13}C) spectra were recorded on a Varian 300/400 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 compounds synthesized are racemic mixtures and are more than 95% pure, analyzed using HPLC. Optical rotation was measured using a Rudolph Research Autopol III polarimeter at 589 nm Na D-line.

General Procedure for the Synthesis of Salicylaldehyde (10a−u). 5-Bromosalicylaldehyde (1 g, 4.97 mmol), K_2CO_3 (2.061 g, 14.91 mmol), boronic acid (0.9954 g, 5.47 mmol), triphenylphosphine (TPP) (1 mol %), and $Pd(OAc)_2$ (1 mol %) were taken in DME/water (1:1) (12 mL). The mixture was stirred at room temperature under an atmosphere of nitrogen for 24 h. The reaction mixture was acidified using HCl (1 N) on an ice bath, followed by extraction with ethyl acetate. The extracts were combined, dried ($MgSO_4$), and the solvent was removed under vacuum. The crude solid was purified by flash chromatography to isolate the desired salicylaldehyde.

4-Hydroxy-3′-methoxy-[1,1′-biphenyl]-3-carbaldehyde (10a). Yield: 71%. ^{1}H NMR ($CDCl_3$): δ 11.01 (1H, s), 9.98 (1H, s), 7.76−7.78 (2H, m), 7.37 (1H, t, J = 4.0 Hz), 7.13 (1H, d, J = 7.6 Hz), 7.06−7.08 (2H, m), 6.90 (1H, dd, J = 2.0 Hz, 8.4 Hz), 3.87 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.66, 161.08, 160.12, 140.83, 135.77, 133.17, 131.92, 130.03, 120.68, 119.08, 118.11, 112.60, 112.55, 55.35.

4-Hydroxy-4′-methoxy-[1,1′-biphenyl]-3-carbaldehyde (10c). Yield: 76%. ^{1}H NMR ($CDCl_3$): δ 10.96 (1H, s), 9.97 (1H, s), 7.70−7.74 (2H, m), 7.47−7.49 (2H, m), 7.05 (1H, d, J = 8.4 Hz), 6.98−7.07 (2H, m), 3.86 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.70, 160.54, 159.20, 135.45, 133.07, 131.93, 131.34, 127.66, 120.70, 118.05, 114.41, 55.39.

4-Hydroxy-2′-methoxy-[1,1′-biphenyl]-3-carbaldehyde (10d). Yield: 65%. ^{1}H NMR ($CDCl_3$): δ 11.02 (1H, s), 9.93 (1H, s), 7.70−7.73 (2H, m), 7.29−7.36 (2H, m), 7.01−7.06 (3H, m), 3.83 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.78, 160.63, 156.36, 138.43, 134.47, 130.38, 128.94, 128.67, 121.01, 120.34, 117.24, 111.24, 111.07, 55.55.

4-Hydroxy-3′,4′-dimethoxy-[1,1′-biphenyl]-3-carbaldehyde (10e). Yield: 78%. ^{1}H NMR ($CDCl_3$): δ 10.97 (1H, s), 9.97 (1H, s), 7.70−7.73 (2H, m), 7.04−7.10 (3H, m), 6.95 (1H, d, J = 8.4 Hz), 3.96 (3H, s), 3.93 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.67, 160.64, 149.35, 148.72, 135.56, 133.26, 132.40, 131.46, 120.67, 118.96, 118.06, 111.62, 109.96, 56.02.

4-Hydroxy-3′-methyl-[1,1′-biphenyl]-3-carbaldehyde (10l). Yield: 67%. ^{1}H NMR ($CDCl_3$): δ 10.99 (1H, s), 9.98 (1H, s), 7.76 (2H, m), 7.35 (3H, m), 7.18 (1H, m), 7.07 (1H, d, J = 8.4 Hz), 2.43 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.69, 160.92, 139.32, 138.66, 135.79, 133.46, 131.86, 128.90, 128.15, 127.39, 123.70, 120.71, 118.06, 21.54.

4-Hydroxy-3′,5′-dimethyl-[1,1′-biphenyl]-3-carbaldehyde (10m). Yield: 53%. ^{1}H NMR ($CDCl_3$): δ 10.99 (1H, s), 9.99 (1H, s), 7.77 (2H, m), 7.18 (2H, s), 7.07 (1H, d, J = 8.4 Hz), 7.02 (1H, s), 2.40 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.72, 160.86, 139.31, 138.56, 135.83, 133.57, 131.85, 129.03, 124.52, 120.68, 117.98, 21.39.

4-Hydroxy-3′,5′-bis(trifluoromethyl)biphenyl-3-carbaldehyde (10q). Yield: 60%. ^{1}H NMR ($CDCl_3$): δ 11.13 (1H, s), 10.03 (1H, s), 7.98 (2H, s), 7.87 (1H, s), 7.82−7.79 (2H, m), 7.16 (1H, d, J = 8.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.28, 162.09, 141.46, 135.47, 132.59, 132.26, 132.14, 130.18, 126.64, 126.6, 124.6, 121.88, 121.05, 120.96, 120.86, 118.96.

4-Hydroxy-3′-nitro-[1,1′-biphenyl]-3-carbaldehyde (10r). Yield: 51%. ^{1}H NMR ($CDCl_3$): δ 11.11 (1H, s), 10.02 (1H, s), 8.434−8.425 (1H, m), 8.23−8.20 (1H, m), 7.90−7.88 (1H, m), 7.84−7.80 (2H, m), 7.64 (1H, m) 7.15 (1H, d, J = 8.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.34, 162.16, 147.08, 140.64, 135.64, 132.35, 130.73, 127.32, 127.19, 124.38, 124.22, 120.84, 118.83.

5-(Benzo[*d*][1,3]dioxol-5-yl)-2-hydroxybenzaldehyde (10u). Yield: 58%. ^{1}H NMR ($CDCl_3$): δ 10.96 (1H, s), 9.95 (1H, s), 6.67 (2H, m), 7.01 (3H, m), 6.88 (1H, d, J = 8.0 Hz), 6.01 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.64, 160.70, 148.33, 147.17, 135.54, 133.73, 133.14, 131.53, 120.65, 120.12, 118.08, 108.73, 107.19, 101.28.

General Procedure for the Synthesis of Coumarin (11a−u). 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). The reaction mass was then heated at 68−70 °C for 3 h. Following this, the reaction mass was cooled to room temperature and saturated $NaHCO_3$ solution (10 mL) was added to it. The reaction mass was heated at 68−70 °C for another 30 min, 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 residue, which upon column chromatography afforded the desired coumarin.

6-(3-Methoxyphenyl)-2*H*-chromen-2-one (11a). Yield: 79%. ^{1}H NMR ($CDCl_3$): δ 7.75 (2H, m), 7.66 (1H, d, J = 2.4 Hz), 7.36−7.41 (2H, m), 7.14−7.17 (1H, m), 7.09 (1H, t, J = 2.0 Hz), 6.92−6.95 (1H, m), 6.46 (1H, d, J = 9.2 Hz), 3.88 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.68, 160.11, 153.51, 143.45, 140.89, 137.69, 130.78, 130.07, 126.11, 119.51, 119.01, 117.25, 117.08, 113.01, 112.98, 55.57.

6-(2-Methoxyphenyl)-2*H*-chromen-2-one (11d). Yield: 82%. ^{1}H NMR ($CDCl_3$): δ 7.74 (1H, d, J = 9.6 Hz), 7.70 (1H, dd, J = 2.4 Hz, 8.4 Hz), 7.63 (1H, d, J = 2.0 Hz), 7.34−7.38 (2H, m), 7.31 (1H, dd, J = 2.0 Hz, 7.6 Hz), 6.99−7.07 (2H, m), 6.44 (1H, d, J = 9.6 Hz), 3.83 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.97, 156.34, 153.07, 143.73, 135.02, 133.36, 130.69, 129.28, 128.73, 128.54, 121.02, 118.52, 116.61, 116.46, 111.27, 55.56.

6-(3,4-Dimethoxyphenyl)-2*H*-chromen-2-one (11e). Yield: 75%. ^{1}H NMR ($CDCl_3$): δ 7.76 (1H, d, J = 9.6 Hz), 7.71 (1H, dd, J = 2.0 Hz, 8.4 Hz), 7.61 (1H, d, J = 2.4 Hz), 7.38 (1H, d, J = 8.4 Hz), 7.13 (1H, dd, J = 2.0 Hz, 8.4 Hz), 7.07 (1H, d, J = 2.0 Hz), 6.96 (1H, d, J = 8.4 Hz), 6.46 (1H, d, J = 9.6 Hz), 3.97 (3H, s), 3.93 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.73, 153.13, 149.38, 149.04, 143.45, 137.72, 132.36, 132.36, 130.52, 125.62, 119.45, 119.01, 117.21, 117.04, 111.61, 110.30, 56.04.

6-(*m*-Tolyl)-2*H*-chromen-2-one (11l). Yield: 84%. ^{1}H NMR ($CDCl_3$): δ 7.75 (2H, m), 7.66 (1H, d, J = 2.0 Hz), 7.34−7.40 (4H, m), 7.21 (1H, d, J = 6.4 Hz), 6.46 (1H, d, J = 9.4 Hz), 2.44 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.74, 153.38, 143.50, 139.38, 138.70, 137.97, 130.79, 128.93, 128.54, 127.84, 126.04, 124.15, 119.00, 117.21, 117.01, 21.53.

6-(3,5-Dimethylphenyl)-2*H*-chromen-2-one (11m). Yield: 79%. ^{1}H NMR ($CDCl_3$): δ 7.76 (1H, d, J = 9.4 Hz), 7.72 (1H, d, J = 2.0 Hz), 7.65 (1H, d, J = 2.4 Hz), 7.19 (2H, s), 6.46 (1H, d, J = 9.2 Hz), 2.40 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.79, 153.33, 143.53, 139.38, 138.61, 138.09, 130.82, 129.42, 126.02, 124.97, 118.96, 117.13, 116.96, 21.39.

6-(3,5-Bis(trifluoromethyl)phenyl)-2*H*-chromen-2-one (11q). Yield: 65%. ^{1}H NMR ($CDCl_3$): δ 8.01 (2H, s), 7.89 (1H, s), 7.81−7.76 (2H, m), 7.73 (1H, d, J = 2.4 Hz), 7.49 (1H, d, J = 8.8 Hz), 6.53 (1H, d, J = 9.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.08, 154.33, 142.86, 141.53, 134.71, 132.64, 132.30, 130.57, 127.14, 127.10, 126.45, 124.55, 121.47, 121.40, 119.42, 117.94, 117.82.

6-(3-Nitrophenyl)-2*H*-chromen-2-one (11r). Yield: 72%. ^{1}H NMR ($CDCl_3$): δ 8.46 (1H,s), 8.27−8.24 (1H, m), 7.93−7.91 (1H, m), 7.81−7.78 (2H,m), 7.737 (1H, d, J = 2 Hz), 7.66 (1H,t, J = 8 Hz), 7.48 (1H, d, J = 8.4 Hz), 6.53 (1H, d, J = 9.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.23, 154.15, 142.99, 141.07, 135.21, 132.86, 130.55, 130.06, 126.33, 122.55, 121.88, 119.35, 117.82, 117.68.

6-(Benzo[*d*][1,3]dioxol-5-yl)-2*H*-chromen-2-one (11u). Yield: 72%. ^{1}H NMR ($CDCl_3$): δ 7.72 (1H, d, J = 8.8 Hz), 7.66 (1H, dd, J = 2.4 Hz, 8.8 Hz), 7.58 (1H, d, J = 2.0 Hz), 7.37 (1H, d, J = 8.4 Hz), 7.03 (2H, m), 6.90 (1H, m), 6.47 (1H, d, J = 8.4 Hz), 6.02 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.72, 153.18, 148.38, 147.52, 143.44, 137.59, 133.70, 130.51, 125.69, 120.67, 119.01, 117.07, 108.77, 107.53, 101.35.

General Procedure for the Synthesis of Substituted Ethyl 4*H*-Chromene-3-carboxylate Compounds (6a−v, 7a−g). Freshly cut sodium (0.096 mmol) was added to anhydrous ethanol (2 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 ethanol (1 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 methylene chloride (3 × 20 mL). The organics were combined, dried ($MgSO_4$), and the solvent was removed under vacuum to afford an oil. This crude oil was subjected to column chromatography to afford the pure product.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(3-methoxyphenyl)-4*H*-chromene-3-carboxylate (6a). Yield: 74%. ^{1}H NMR ($CDCl_3$): δ 7.46 (1H, d, J = 2.4 Hz), 7.40 (1H, dd, J = 2.0 Hz, 8.4 Hz), 7.32 (1H, t, J = 8.0 Hz), 7.11 (1H, d, J = 8.0 Hz), 7.06 (1H, t, J = 2.0 Hz), 7.00 (1H, d, J = 8.4 Hz), 6.87 (1H, dd, J = 2.0 Hz, 8.0 Hz), 6.34 (2H, bs), 4.35 (1H, m), 4.23 (2H, q, J = 7.2 Hz), 4.02 (2H, q, J = 7.2 Hz), 3.85 (3H, s), 2.59−2.71 (2H, m), 1.33 (3H, t, J = 7.2 Hz), 1.13 (3H, t, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.73, 169.04, 161.49, 159.96, 149.54, 141.81, 137.32, 129.78, 127.07, 126.42, 125.89, 119.37, 116.10, 112.60, 112.59, 60.20, 59.54, 55.28, 43.67, 31.38, 14.59, 14.07. MS (ESI, positive) m/z 434.36. Calcd for $C_{23}H_{25}NO_6Na$: 434.17.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(4-methoxyphenyl)-4*H*-chromene-3-carboxylate (6c). Yield: 79%. ^{1}H NMR ($CDCl_3$): δ 7.44−7.47 (2H, m), 7.42 (1H, d, J = 2.0 Hz), 7.35 (1H, dd, J = 2.4 Hz, 8.4 Hz), 6.99 (1H, d, J = 8.4 Hz), 6.95 (1H, d, J = 8.8 Hz), 6.32 (2H, bs), 4.32−4.35 (1H, m), 4.23 (2H, q, J = 7.2 Hz), 4.02 (2H, q, J = 7.2 Hz), 3.84 (3H, s), 2.58−2.70 (2H, m), 1.33 (3H, t, J = 7.2 Hz), 1.12 (3H, t, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.76,

169.06, 161.52, 159.05, 149.00, 137.12, 132.88, 127.85, 126.54, 125.92, 125.84, 116.05, 114.21, 60.19, 59.51, 55.34, 43.70, 31.38, 14.58, 14.07. MS (ESI, positive) m/z 434.35. Calcd for $C_{23}H_{25}NO_6Na$: 434.17.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(2-methoxyphenyl)-4*H*-chromene-3-carboxylate (6d). Yield: %. ^{1}H NMR ($CDCl_3$): δ 7.40 (1H, d, J = 2.0 Hz), 7.36 (1H, dd, J = 2.4 Hz, 8.4 Hz), 7.26−7.32 (2H, m), 6.96−7.03 (3H, m), 6.35 (2H, bs), 4.32−4.35 (1H, m), 4.23 (2H, q, J = 7.2 Hz), 4.02 (2H, q, J = 7.2 Hz), 3.83 (3H, s), 2.58−2.69 (2H, m), 1.33 (3H, t, J = 7.2 Hz), 1.12 (3H, t, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.75, 169.13, 161.56, 156.37, 148.93, 134.71, 130.68, 129.76, 129.48, 128.90, 128.60, 125.08, 120.84, 115.32, 111.21, 60.13, 59.49, 55.53, 43.75, 31.34, 14.59, 14.04. MS (ESI, positive) m/z 434.36. Calcd for $C_{23}H_{25}NO_6Na$: 434.17.

Ethyl 2-Amino-6-(3,4-dimethoxyphenyl)-4-(2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (6e). Yield: 71%. ^{1}H NMR ($CDCl_3$): δ 7.42 (1H, d, J = 2.0 Hz), 7.36 (1H, dd, J = 2.0 Hz, 8.4 Hz), 7.07 (1H, dd, J = 2.0 Hz, 8.0 Hz), 7.04 (1H, d, J = 2.0 Hz), 7.00 (1H, d, J = 8.4 Hz), 6.92 (1H, d, J = 8.0 Hz), 6.34 (2H, bs), 4.35 (1H, m), 4.23 (2H, q, J = 7.2 Hz), 4.02 (2H, q, J = 7.2 Hz), 3.94 (3H, s), 3.92 (3H, s), 2.58−2.67 (2H, m), 1.33 (3H, t, J = 7.2 Hz), 1.13 (3H, t, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.76, 169.05, 161.50, 149.15, 149.11, 148.54, 137.33, 133.36, 126.67, 126.06, 125.88, 119.12, 116.06, 111.48, 110.17, 60.18, 59.53, 55.98, 55.93, 43.72, 31.37, 14.58, 14.09. MS (ESI, positive) m/z 464.68. Calcd for $C_{24}H_{27}NO_7Na$: 464.18.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(*m*-tolyl)-4*H*-chromene-3-carboxylate (6l). Yield: 69%. ^{1}H NMR ($CDCl_3$): δ 7.46 (1H, d, J = 2.4 Hz), 7.40 (1H, dd, J = 2.4 Hz, 8.4 Hz), 7.28−7.34 (3H, m), 7.15 (1H, d, J = 6.4 Hz), 7.01 (1H, d, J = 8.4 Hz), 6.33 (2H, bs), 4.35 (1H, m), 4.23 (2H, q, J = 6.8 Hz), 4.02 (2H, q, J = 7.2 Hz), 2.63 (2H, m), 2.41 (3H, m), 1.33 (3H, t, J = 6.8 Hz), 1.13 (3H, t, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.74, 169.07, 161.50, 149.38, 140.27, 138.35, 137.60, 128.68, 127.91, 127.66, 127.01, 126.37, 125.84, 123.95, 116.05, 60.20, 59.53, 43.68, 31.38, 21.52, 14.58, 14.06. MS (ESI, positive) m/z 418.28. Calcd for $C_{23}H_{25}NO_5Na$: 418.17. HPLC purity: 93%

Ethyl 2-Amino-6-(3,5-dimethylphenyl)-4-(2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (6m). Yield: 61%. ^{1}H NMR ($CDCl_3$): δ 7.45 (1H, d, J = 2.0 Hz), 7.39 (1H, dd, J = 2.4 Hz, 8.4 Hz), 7.14 (2H, d, J = 2.0 Hz), 7.00 (1H, d, J = 8.4 Hz), 6.97 (1H, s), 6.32 (2H, bs), 4.35 (1H, m), 4.23 (2H, q, J = 7.2 Hz), 4.02 (2H, q, J = 7.2 Hz), 2.66 (2H, m), 2.37 (6H, m), 1.33 (3H, t, J = 7.2 Hz), 1.13 (3H, t, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.74, 169.08, 161.51, 149.32, 104.28, 138.27, 137.71, 128.80, 126.98, 126.36, 125.77, 124.79, 115.98, 60.20, 59.52, 43.68, 31.38, 24.74, 21.39, 14.57, 14.06. MS (ESI, positive) m/z 432.38. Calcd for $C_{24}H_{27}NO_5Na$: 432.19. HPLC purity: 92.2%

Ethyl 2-Amino-6-(3,5-bis(trifluoromethyl)phenyl)-4-(2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (6q). Yield: 55%. ^{1}H NMR ($CDCl_3$): δ 7.95 (2H, s), 7.83 (1H, s), 7.51 (1H, d, J = 2.0 Hz), 7.45 (1H, dd, J = 2.4 Hz, 8.4 Hz), 7.11 (1H, d, J = 8.4 Hz), 6.4 (2H, bs), 4.40−4.37 (1H, m), 4.27 (2H, q, J = 7.2 Hz), 4.07 (2H, q, J = 7.2 Hz), 2.74−2.6 (2H, m), 1.35 (3H, t, J = 7.2 Hz), 1.17 (3H, t, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.56, 168.87, 161.16, 150.58, 142.39, 134.36, 132.31, 131.98, 127.34, 126.72, 121.95, 120.79, 116.76, 60.34, 59.66, 43.56, 31.22, 14.54, 14.06. MS (ESI, positive) m/z 517.27. Calcd for $C_{24}H_{21}F_6NO_5$.: 517.13.

Ethyl 2-Amino-4-(2-ethoxy-2-oxoethyl)-6-(3-nitrophenyl)-4*H*-chromene-3-carboxylate (6r). Yield: 45%. ^{1}H NMR ($CDCl_3$): δ 8.39−8.38 (1H, m), 8.19−8.16 (1H, m), 7.87−7.85 (1H, m), 7.6 (1H, t, J = 8.0 Hz), 7.53 (1H, d, J = 2.4 Hz), 7.47 (1H, dd, J = 2.0 Hz, 8.4 Hz), 7.1 (1H, d, J = 8.4 Hz), 6.4 (2H, bs), 4.39−4.36 (1H, m), 4.27 (2H, q, J = 6.8 Hz), 4.07 (2H, q, J = 7.2 Hz), 2.73−2.61 (2H, m), 1.35 (3H, t, J = 7.2 Hz), 1.17 (3H, t, J = 6.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.65, 168.89, 161.24, 150.34, 148.72, 141.96, 134.84, 132.69, 129.75, 127.27, 126.54, 121.92, 121.61, 116.62, 60.31, 59.63, 43.56, 31.24, 14.56, 14.08. MS (ESI, positive) m/z 426.4. Calcd for $C_{25}H_{29}NO_8Na$: 426.14.

Ethyl 2-Amino-6-(benzo[*d*][1,3]dioxol-5-yl)-4-(2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (6u). Yield: 52%. ^{1}H NMR ($CDCl_3$): δ 7.26−7.48 (2H, m), 6.93−7.01 (2H, m), 6.80−6.89 (2H, m), 5.98 (2H, s), 4.06−4.18 (5H, m), 2.89−3.13 (2H, m), 1.14−1.22 (6H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.71, 166.04, 165.17, 152.80, 148.09, 146.77, 134.81, 134.10, 127.62, 127.18, 124.82, 120.10, 117.00, 108.58, 107.29, 101.12, 63.00, 61.24, 41.79, 36.31, 34.99, 14.00, 13.79. MS (ESI, positive) m/z 448.42. Calcd for $C_{23}H_{23}NO_7Na$: 448.15.

Ethyl 2-Amino-6-((3,5-dimethoxybenzyl)oxy)-4-(2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (6v). Yield: 61%. ^{1}H NMR ($CDCl_3$): δ 6.84−6.88 (2H, m), 6.78 (1H, dd, J = 3.2 Hz, 8.8 Hz), 6.56 (2H, d, J = 2.4 Hz), 6.40 (1H, t, J = 2.0 Hz), 6.29 (2H, bs), 4.93 (2H, s), 4.19−4.26 (3H, m), 4.01 (2H, q, J = 7.2 Hz), 3.79 (6H, s), 2.54−2.65 (2H, m), 1.31 (3H, t, J = 7.2 Hz), 1.16 (3H, t, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.76, 169.06, 161.73, 160.99, 155.22, 144.18, 139.28, 126.53, 116.53, 114.45, 113.78, 105.16, 99.90, 70.40, 60.18, 59.45, 55.35, 43.58, 31.54, 14.58, 14.10. MS (ESI, positive) m/z 494.27. Calcd for $C_{25}H_{29}NO_8Na$: 494.19. HPLC purity: 85%

Propyl 2-Amino-6-(3,5-dimethoxyphenyl)-4-(2-oxo-2-propoxyethyl)-4*H*-chromene-3-carboxylate (7a). Yield: 58%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (d, J = 2.26 Hz, 1H), 7.40 (dd, J = 2.13, 8.41 Hz, 1H), 7.02 (d, J = 8.28 Hz, 1H), 6.67 (d, J = 2.26 Hz, 2H), 6.46 (t, J = 2.26 Hz, 1H), 6.34 (br s, 2H), 4.37 (dd, J = 4.02, 7.78 Hz, 1H), 4.15 (t, J = 6.65 Hz, 2H), 3.94 (dt, J = 2.38, 6.71 Hz, 2H), 3.85 (s, 6H), 2.58−2.75 (m, 2H), 1.75 (sxt, J = 7.08 Hz, 2H), 1.53 (qd, J = 7.19, 14.31 Hz, 2H), 1.03 (t, J = 7.40 Hz, 3H), 0.81 (t, J = 7.40 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.74, 169.10, 161.43, 161.05, 149.56, 142.53, 137.44, 127.05, 126.45, 125.79, 116.08, 105.19, 99.21, 65.89, 65.25, 55.40, 43.67, 31.42, 29.69, 22.31, 21.84, 10.66, 10.28. MS (ESI, positive) m/z calcd for $C_{26}H_{31}NO_7$ (M + H), 470.22; found, 470.20.

Butyl 2-Amino-4-(2-butoxy-2-oxoethyl)-6-(3,5-dimethoxyphenyl)-4*H*-chromene-3-carboxylate (7b). Yield: 63%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (d, J = 2.26 Hz, 1H), 7.40 (dd, J = 2.26, 8.28 Hz, 1H), 7.02 (d, J = 8.28 Hz, 1H), 6.68 (d, J = 2.26 Hz, 2H), 6.46 (t, J = 2.26 Hz, 1H), 6.34 (br s, 2H), 4.35 (dd, J = 4.02, 7.53 Hz, 1H), 4.19 (t, J = 6.15 Hz, 2H), 3.98 (t, J = 7.53 Hz, 2H), 3.85 (s, 6H), 2.56−2.74 (m, 2H), 1.65−1.76 (m, 2H), 1.41−1.55 (m, 4H), 1.24 (qd, J = 7.38, 15.00 Hz, 2H), 0.98 (t, J = 7.40 Hz, 3H), 0.84 (t, J = 7.40 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.74, 169.10, 161.41, 161.04, 149.53, 142.50, 137.42, 127.05, 126.43, 125.76, 116.06, 105.19, 99.17, 64.18, 63.45, 55.38, 43.66, 31.39, 31.01, 30.53, 19.33, 19.03, 13.78, 13.62. MS (ESI, positive) m/z calcd for $C_{28}H_{35}NO_7$ (M + H), 498.25; found, 498.21.

Allyl 4-(2-(Allyloxy)-2-oxoethyl)-2-amino-6-(3,5-dimethoxyphenyl)-4*H*-chromene-3-carboxylate (7c). Yield: 47%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (d, J = 2.01 Hz, 1H), 7.40 (dd, J = 2.13, 8.41 Hz, 1H), 7.02 (d, J = 8.53 Hz, 1H), 6.67 (d, J = 2.26 Hz, 2H), 6.46 (t, J = 2.13 Hz, 1H), 6.38 (br s, 2H), 5.95−6.08 (m, 1H), 5.73−5.85 (m, 1H), 5.39 (d, J = 17.32 Hz, 1H), 5.07−5.28 (m, 3H), 4.70 (d, J = 5.27 Hz, 2H), 4.48 (d, J = 5.77 Hz, 2H), 4.41 (dd, J = 4.27, 7.28 Hz, 1H), 3.85 (s, 6H), 2.62−2.79 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.23, 168.54, 161.75, 161.05, 149.53, 142.44, 137.54, 133.00, 132.02, 127.06, 126.52, 125.66, 118.18, 117.18, 116.11, 105.21, 99.21, 65.01, 64.25, 55.39, 43.58, 31.35. MS (ESI, positive) m/z calcd for $C_{26}H_{27}NO_7$ (M + H), 466.19; found, 466.21.

Prop-2-yn-1-yl 2-Amino-6-(3,5-dimethoxyphenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4*H*-chromene-3-carboxylate (7d). Yield: 43%. ^{1}H NMR ($CDCl_3$): δ 7.46 (1H, d, J = 2.0 Hz), 7.40 (1H, dd, J = 2.0 Hz, 8.4 Hz), 7.02 (1H, d, J = 8.4 Hz), 6.66 (2H, d, J = 2.0 Hz), 6.44 (1H, t, J = 2.0 Hz), 6.39 (2H, br s), 4.78−4.79 (2H, m), 4.54−4.64 (2H, m), 4.39 (1H, dd, J = 4.8 Hz, 8.4 Hz), 3.84 (6H, s), 2.66−2.77 (2H, m), 2.47 (3H, t, J = 2.4 Hz), 2.31 (3H, t, J = 2.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.64, 167.88, 162.20, 161.05, 149.45, 142.40, 137.78, 127.09, 126.70, 125.39, 116.19, 105.31, 99.24, 78.68,

75.86, 74.89, 74.28, 55.43, 51.81, 51.16, 43.33, 31.12. MS (ESI, positive) m/z 484.46. Calcd for $C_{26}H_{23}NO_7Na$: 484.15.

Ethyl 2-Amino-6-(3,5-dimethoxyphenyl)-4-(2-oxo-2-(prop-2-yn-1-yloxy)ethyl)-4*H*-chromene-3-carboxylate (7e). Yield: 22%. ^{1}H NMR ($CDCl_3$): δ 7.46 (1H, d, J = 2.4 Hz), 7.39 (1H, dd, J = 2.4 Hz, 8.4 Hz), 7.01 (1H, d, J = 8.8 Hz), 6.67 (2H, d, J = 2.0 Hz), 6.44 (1H, t, J = 2.0 Hz), 6.32 (2H, br s), 4.53−4.62 (2H, m), 4.38 (1H, q, J = 7.2 Hz), 4.23 (2H, dd, J = 4.8 Hz, 8.4 Hz), 3.84 (6H, s), 2.62−2.75 (2H, m), 2.30 (3H, t, J = 2.4 Hz), 1.33 (3H, t, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.80, 168.92, 161.50, 161.03, 149.56, 142.49, 137.58, 127.09, 126.61, 125.57, 116.16, 105.32, 99.18, 77.58, 74.87, 59.60, 55.43, 51.73, 43.35, 31.29, 14.59. MS (ESI, positive) m/z 474.15. Calcd for $C_{25}H_{25}NO_7Na$: 474.16. HPLC purity: 88%

Cyclopropylmethyl 2-Amino-4-(2-(cyclopropylmethoxy)-2-oxoethyl)-6-(3,5-dimethoxyphenyl)-4*H*-chromene-3-carboxylate (7f). Yield: 46%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 (d, J = 2.26 Hz, 1H), 7.40 (dd, J = 2.26, 8.28 Hz, 1H), 7.02 (d, J = 8.53 Hz, 1H), 6.67 (d, J = 2.26 Hz, 2H), 6.45 (t, J = 2.26 Hz, 1H), 6.00−6.42 (br s, 2H), 4.40 (dd, J = 4.27, 7.28 Hz, 1H), 3.98−4.08 (m, 2H), 3.85 (s, 6H), 3.80 (dd, J = 1.25, 7.28 Hz, 2H), 2.65−2.79 (m, 2H), 1.27 (s, 2H), 0.56−0.62 (m, 2H), 0.42−0.48 (m, 2H), 0.31−0.37 (m, 2H), 0.13−0.18 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.8, 168.1, 160.4, 160.0, 148.6, 141.5, 136.4, 126.1, 125.4, 124.8, 115.1, 104.2, 98.2, 76.3, 76.0, 75.7, 68.0, 67.1, 54.4, 42.5, 30.4, 28.7, 9.1, 8.7, 2.2, 2.1, 2.1, 2.1. MS (ESI, positive) m/z calcd for $C_{28}H_{31}NO_7$ (M + H), 494.22; found, 494.40. HPLC purity: 90%

Ethyl 6-(3,5-Dimethoxyphenyl)-4-(2-ethoxy-2-oxoethyl)-2-methyl-4*H*-chromene-3-carboxylate (7g). Yield: 82%. ^{1}H NMR ($CDCl_3$): δ 7.43 (1H, d, J = 2.0 Hz), 7.40 (1H, dd, J = 2.4 Hz, 8.4 Hz), 7.03 (1H, d, J = 8.4 Hz), 6.66 (2H, d, J = 2.4 Hz), 6.44 (1H, t, J = 2.0 Hz), 4.24 (1H, m), 4.05 (2H, q, J = 7.2 Hz), 4.03 (2H, q, J = 7.2 Hz), 3.83 (6H, s), 2.68 (1H, dd, J = 4.4 Hz, 14.8 Hz), 2.56 (1H, dd, J = 8.0 Hz, 15.2 Hz), 2.45 (3H, s), 1.34 (3H, t, J = 6.8 Hz), 2.45 (3H, s), 1.13 (3H, t, J = 6.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.32, 166.90, 162.52, 161.07, 150.30, 142.57, 137.47, 126.87, 126.59, 124.49, 116.27, 105.14, 104.72, 99.18, 60.36, 60.35, 55.40, 43.59, 32.4.

6-((3,5-Dimethoxybenzyl)oxy)-2*H*-chromen-2-one (11v). Yield: 56%. ^{1}H NMR ($CDCl_3$): δ 7.63 (1H, d, J = 8.4 Hz), 7.26 (1H, d, J = 8.4 Hz), 7.16−7.19 (1H, m), 6.97 (1H, d, J = 2.4 Hz), 6.57 (1H, d, J = 2.4 Hz), 6.40−6.43 (2H, m), 5.04 (2H, s), 3.80 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.12, 160.92, 155.10, 148.63, 143.16, 138.69, 120.17, 119.19, 117.94, 117.11, 111.40, 105.16, 99.88, 70.63, 55.38.

(*E*)-Ethyl 3-(4-Hydroxy-3′,5′-dimethoxy-[1,1′-biphenyl]-3-yl)acrylate (19). Yield: 72%. ^{1}H NMR ($CDCl_3$): δ 8.06 (1H, d, J = 16 Hz), 7.67 (1H, d, J = 2.4 Hz), 7.45 (1H, dd, J = 2.4 Hz, 8.4 Hz), 6.91 (1H, d, J = 8.4 Hz), 6.73 (1H, d, J = 16 Hz), 6.67 (2H, d, J = 2 Hz), 6.50 (1H, s), 6.44 (1H, t, J = 2.0 Hz), 4.30 (2H, q, J = 6.8 Hz), 3.85 (6H, s), 1.36 (3H, t, J = 5.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.23, 161.11, 154.90, 142.44, 140.30, 134.01, 130.11, 127.84, 121.88, 119.04, 116.75, 105.05, 99.00, 60.74, 55.45, 14.33.

Determination of Purity and Chiral Separation. The purity of the final compounds were determined using 85:15 (ACN/H_2O) as the mobile phase with a flow rate of 1.0 mL/min on a C18 column. The chiral separation was done using a RegisPack chiral column (Regis Technologies). The enantiomers of **5** were separated using 70:30 (hexane/IPA) with a flow rate of 1.5 mL/min. The retention time for the (+)-enantiomer was 3.8 min, and that for the (−)-enantiomer was 5.0 min.

Cell Cultures. CCL-240 (HL-60) and CRL-2258 (HL-60/mitoxantrone (MX) resistant) cells were purchased from ATCC. K562, K562/HHT300 and HL60/DNR cell lines were developed by one of us (R.T.). CCRF-CEM and CCRF-CEM-VLB100 were developed and described by one of us (W.T.B.). The HL-60 cell line was grown in IMDM Glutamax medium supplemented with 20% FBS. All other cell lines were grown in RPMI 1640 purchased from ATCC supplemented with 10% FBS. All cell lines were incubated at 37 °C with 5% CO_2 in air atmosphere.

Cell Viability Measurement. The in vitro cytotoxicity of these small molecules was assayed by determining their ability to inhibit the growth of the tumor cells. In brief, the tumor cells were plated in a 96-well plate (a density of 1×10^4 cells/well). The cells were treated with a series of dilutions of the test compounds of varied concentrations with 1% DMSO in the final cell medium (cells treated with medium containing 1% DMSO served as a control). After a 48 h treatment, the relative cell viability in each well was determined by using CellTiter-Blue cell viability assay kit. The IC_{50} of each candidate was determined by fitting the relative viability of the cells to the drug concentration by using a dose−response model in the Prism program from GraphPad Software, Inc. (San Diego, CA).

Synergism Assay. Synergistic interactions of **5** with vincristine were examined by using median dose−effect analysis as described by Chou and Talalay.[21] Briefly, tumor cells were treated with serial dilutions of each agent individually and in combination simultaneously at a fixed dose ratio for 48 h. The fixed ratios used for vincristine with **5** are 1:2.5, 1:5, and 1:1 and those for paclitaxel with **5** are 1:8, 1:8, and 1:1 for HL60/DNR, K562/HHT300, and CCRF-CEM/VLB100, respectively. The cytotoxic effects of the treatment were measured by evaluating the cell viability using the cell viability assay and the long-term survival assay. Fractional effect was calculated as fraction of cells killed by the individual agent or the combination, in treated versus untreated cells. Median dose effect analysis was performed using CompuSyn program from ComboSyn, Inc. (Paramus, NJ). The software computes combination index (CI) values based on the following equation: $CI = (D)_1/(Dx)_1 + (D)_2/(Dx)_2 + (D)_1(D)_2/(Dx)_1(Dx)_2$, where $(D)_1$ and $(D)_2$ are the doses of drug 1 and drug 2 that have x effect when used in combination and $(Dx)_1$ and$(Dx)_2$ are the doses of drugs 1 and 2 that have the same x effect when used alone. The CI values indicate synergism (<1), additivity (1), or antagonism (>1). CIs of 0.1−0.3, 0.3−0.7, and 0.7−0.85 are considered to indicate strong synergism, synergism, and moderate synergism, respectively.

Statistical Analysis. The in vitro cytotoxicity assay was performed at least twice with triplicates in each experiment. Data are presented as the mean ± SD, and comparisons were made using Student's t test. The synergism assay was performed as a single replicate with triplicates in each experiment. We performed the Bonferroni multiple comparison analysis for determining significant selectivity ratios in comparison to no selectivity with a ratio of 1. A probability of 0.05 or less was considered statistically significant.

■ ASSOCIATED CONTENT

Supporting Information. Purity results of final compounds and chiral HPLC spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

MDR, multidrug resistance; AML, acute myeloid leukemia; PCC, Pearson's correlation coefficient; SAR, structure−activity relationship; EDG, electron donating group; EWG, electron withdrawing group; Ara-C, cytarabine; MX, mitoxantrone; DNR, daunorubicin; HHT, homoharringtonine; VLB, vinblastine; ER, endoplasmic reticulum; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase; CI, combination index; GI_{50}, 50% growth inhibitory concentration

■ REFERENCES

(1) Kuendgen, A.; Germing, U. Emerging treatment strategies for acute myeloid leukemia (AML) in the elderly. *Cancer Treat. Rev.* **2009**, *35*, 97–120.

(2) Lowenberg, B.; Downing, J. R.; Burnett, A. Acute myeloid leukemia. *N. Engl. J. Med.* **1999**, *341*, 1051–1062.

(3) Fanning, S. R.; Sekeres, M. A.; Theil, K. *Accute Myelogenous Leukemia*; The Cleveland Clinic Foundation: Cleveland, OH, 2009; http://www.clevelandclinicmeded.com/medicalpubs/diseasemanagement/hematology-oncology/acute-myelogenous-leukemia/.

(4) Lage, H. An overview of cancer multidrug resistance: a still unsolved problem. *Cell. Mol. Life Sci.* **2008**, *65*, 3145–3167.

(5) Mellor, H. R.; Callaghan, R. Resistance to chemotherapy in cancer: a complex and integrated cellular response. *Pharmacology* **2008**, *81*, 275–300.

(6) Campos, L.; Rouault, J. P.; Sabido, O.; Oriol, P.; Roubi, N.; Vasselon, C.; Archimbaud, E.; Magaud, J. P.; Guyotat, D. High expression of bcl-2 protein in acute myeloid leukemia cells is associated with poor response to chemotherapy. *Blood* **1993**, *81*, 3091–3096.

(7) Elmore, S. Apoptosis: a review of programmed cell death. *Toxicol. Pathol.* **2007**, *35*, 495–516.

(8) 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.

(9) Reed, J. C.; Miyashita, T.; Takayama, S.; Wang, H. G.; Sato, T.; Krajewski, S.; Aime-Sempe, C.; Bodrug, S.; Kitada, S.; Hanada, M. BCL-2 family proteins: regulators of cell death involved in the pathogenesis of cancer and resistance to therapy. *J. Cell. Biochem.* **1996**, *60*, 23–32.

(10) Reed, J. C. Bcl-2 family proteins. *Oncogene* **1998**, *17*, 3225–3236.

(11) Adams, J. M.; Cory, S. The Bcl-2 protein family: arbiters of cell survival. *Science* **1998**, *281*, 1322–1326.

(12) Kang, M. H.; Reynolds, C. P. Bcl-2 inhibitors: targeting mitochondrial apoptotic pathways in cancer therapy. *Clin. Cancer Res.* **2009**, *15*, 1126–1132.

(13) Leber, B.; Geng, F.; Kale, J.; Andrews, D. W. Drugs targeting Bcl-2 family members as an emerging strategy in cancer. *Expert Rev. Mol. Med.* **2010**, *12*, No. e28.

(14) Kang, M. H.; Reynolds, C. P. Bcl-2 inhibitors: targeting mitochondrial apoptotic pathways in cancer therapy. *Clin. Cancer Res.* **2009**, *15*, 1126–1132.

(15) 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.

(16) Skommer, J.; Wlodkowic, D.; Matto, M.; Eray, M.; Pelkonen, J. HA14-1, a small molecule Bcl-2 antagonist, induces apoptosis and modulates action of selected anticancer drugs in follicular lymphoma B cells. *Leuk. Res.* **2006**, *30*, 322–331.

(17) 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 antiapoptotic Bcl-2 proteins, decomposes to generate reactive oxygen species that induce apoptosis. *Mol. Pharmaceutics* **2007**, *4*, 919–928.

(18) Tian, D.; Das, S. G.; 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.

(19) Hermanson, D.; Addo, S. N.; Bajer, A. A.; Marchant, J. S.; Das, S. G.; 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.

(20) Das, S. G.; Doshi, J. M.; Tian, D.; Addo, S. N.; Srinivasan, B.; Hermanson, D. L.; Xing, C. Structure−activity relationship and molecular mechanisms of ethyl 2-amino-4-(2-ethoxy-2-oxoethyl)-6-phenyl-4*H*-chromene-3-carboxylate (sha 14-1) and its analogues. *J. Med. Chem.* **2009**, *52*, 5937–5949.

(21) Chou, T. C.; Talalay, P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv. Enzyme Regul.* **1984**, *22*, 27–55.

(22) Shoemaker, R. H. The NCI60 human tumour cell line anticancer drug screen. *Nat. Rev. Cancer* **2006**, *6*, 813–823.

(23) Naasani, I.; Seimiya, H.; Yamori, T.; Tsuruo, T. FJ5002: a potent telomerase inhibitor identified by exploiting the disease-oriented screening program with COMPARE analysis. *Cancer Res.* **1999**, *59*, 4004–4011.

(24) Yamori, T.; Matsunaga, A.; Sato, S.; Yamazaki, K.; Komi, A.; Ishizu, K.; Mita, I.; Edatsugi, H.; Matsuba, Y.; Takezawa, K.; Nakanishi, O.; Kohno, H.; Nakajima, Y.; Komatsu, H.; Andoh, T.; Tsuruo, T. Potent antitumor activity of MS-247, a novel DNA minor groove binder, evaluated by an in vitro and in vivo human cancer cell line panel. *Cancer Res.* **1999**, *59*, 4042–4049.

(25) Richardson, T. I.; Dodge, J. A.; Durst, G. L.; Pfeifer, L. A.; Shah, J.; Wang, Y.; Durbin, J. D.; Krishnan, V.; Norman, B. H. Benzopyrans as selective estrogen receptor beta agonists (SERBAs). Part 3: synthesis of cyclopentanone and cyclohexanone intermediates for C-ring modification. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4824–4828.

(26) Dai, L. Z.; Shi, Y. L.; Zhao, G. L.; Shi, M. A facile synthetic route to 2*H*-chromenes: reconsideration of the mechanism of the DBU-catalyzed reaction between salicylic aldehydes and ethyl 2-methylbuta-2,3-dienoate. *Chemistry* **2007**, *13*, 3701–3706.