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A rapid synthesis of lavendustin-mimetic small molecules by click fragment assembly

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ABSTRACT

Lavendustin-mimetic small molecules modifying the linker $-CH_2-NH-$ with an 1,2,3-triazole ring have been synthesized via a click chemistry. Two pharmacophoric fragments of lavendustin were varied to investigate chemical space and the auxophoric $-CH_2-NH-$ was altered to an 1,2,3-triazole for rapid click conjugation. The small molecules were evaluated against HCT116 colon cancer and CCRF-CEM leukemia cell lines. Among 28 analogues, 3-phenylpropyl ester **26b** inhibited CCRF-CEM leukemia cell growth with GI_{50} value of 0.9 μM.

Due to the growing interest in small molecules to help understand biological processes, the demand for the generation of small-molecule libraries inspired by bioactive natural products and their metabolites has increased significantly. In this context, a rapid access to natural product-like small molecules has become crucial for systematically dissecting the complex protein functions and for discovering drug leads. Chemistry tailored to produce molecules rapidly and reliably by connecting small units is click chemistry,[1] which is a chemical philosophy introduced by Barry K. Sharpless. The Cu(I)-catalyzed Huisgen [3+2] cycloaddition,[2] the representative click chemistry, has been used in various applications such as drug discovery and chemical biology due to high chemical yield, high selectivity, wide scope, atom economy, and simple purification. Especially, fragment-based rapid assembly using click chemistry and in situ screening are emerging as a versatile tool to identify novel inhibitors quickly against a number of biological targets.[3] The resulting 1,4-disubstituted 1,2,3-triazoles are similar to amide bonds in terms of distance and planarity, and often used as a scaffold in the discovery of enzyme inhibitors. Recently, potential pharmaceuticals based on 1,2,3-triazoles have been developed exponentially.

Lavendustin A (**1**) (Fig. 1), a metabolite of *Streptomyces griseolavendus*, was first isolated by Onoda in 1989.[4] This natural metabolite and its synthetic partial structure **3** were reported to inhibit protein-tyrosine kinase (PTK) in cell-free extract.[4] However, due to their high polarity and poor cell permeability, they significantly lost their cellular activity in intact cell line.[4] The further research on improving cell penetration and antiproliferative activity led to the discovery of novel structures including compound **4** and its derivatives,[5] which were found to inhibit not only the nonreceptor PTK Syk and the receptor PTK EGFR but also tubulin polymerization.

Lavendustin C (**3**) consists of two fragments, salicylic acid and hydroxybenzene, which are responsible for biological activity and also found in lavendustin A (**1**) and B (**2**). However, such polar fragments are also considered to decrease cell permeability. Therefore, when devising a rapid route to lavendustin-mimetic small molecules, the followings should be considered: (1) the modification of pharmacophoric fragments to improve cell permeability; (2) the change of the auxophoric $-CH_2-NH-$ with a linkage to facilitate the assembly of fragments. In this study, we synthesized a series of lavendustin-mimetic small molecules replacing the $-CH_2-NH-$ with an 1,2,3-triazole ring. The salicylic acid fragment and the hydroxybenzene fragment were varied structurally to explore subtle chemical space and linked with a rigid lipophilic triazole (Fig. 2). This bioisosteric triazole replacement would increase hydrophobicity and make these molecules suitable for rapid parallel synthesis. Particularly, click chemistry would allow the substituents of the 1,2,3-triazole ring to be easily varied through the use of different azides or alkynes. Similar bioisosteric triazole replacements are often found in the literatures. Recently, Genazzani and co-workers modified the –CH=CH– of natural product resveratrol

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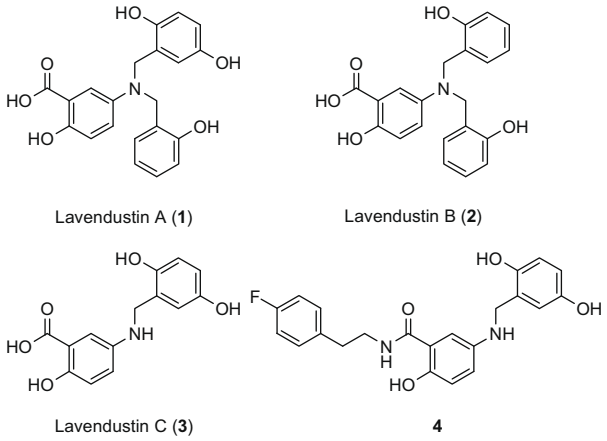


Figure 1. Structures of lavendustin analogues.

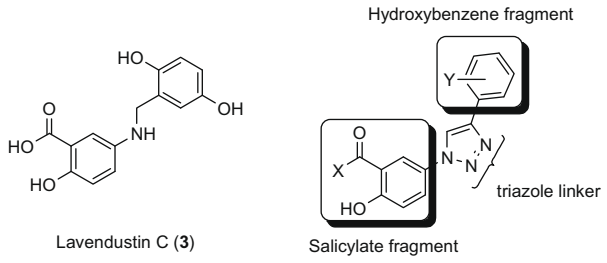
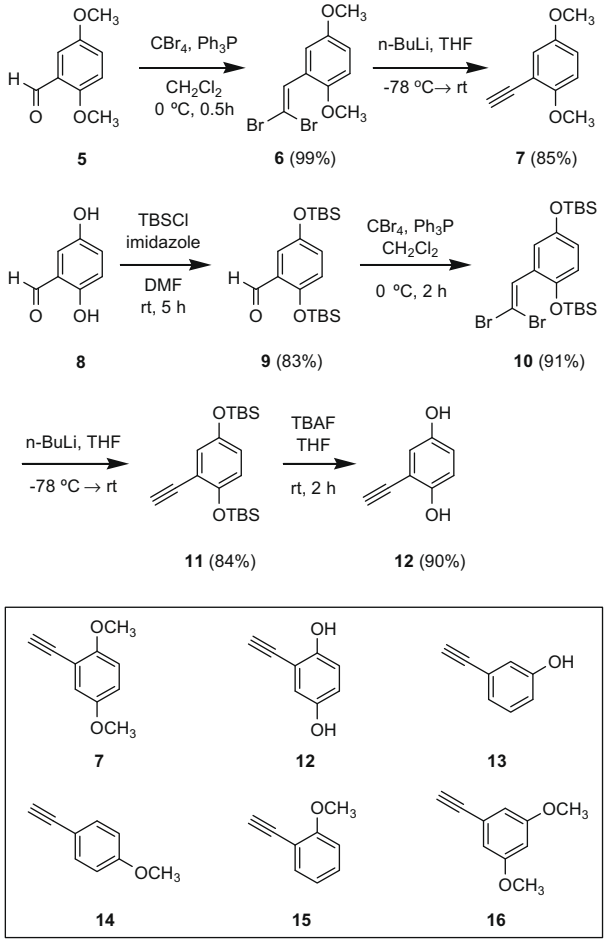


Figure 2. Fragment-based click approach for lavendustin-mimetic small molecule synthesis.



Scheme 1. Structure and synthesis of alkyne building blocks.

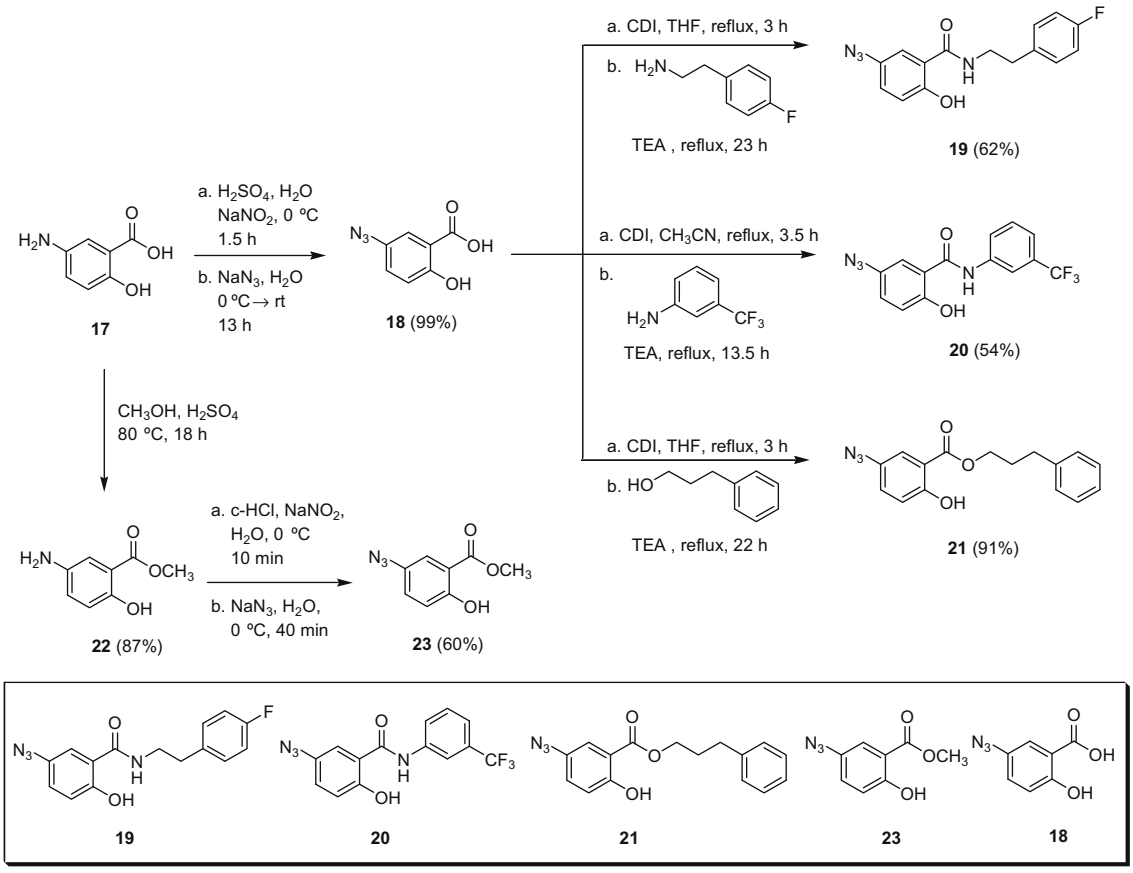
with 1,2,3-triazole and identified a potent antiproliferative agent showing low nanomolar IC_{50}.[3b]

We used six alkynes and five azides for the rapid assembly of salicylate fragments and hydroxybenzene fragments of lavendustin-mimetic small molecules. Alkyne **7** and **12** were readily prepared under Corey–Fuchs conditions[6] from the aldehyde **5** and **8**, respectively (Scheme 1). Commercially available alkynes **13**–**16** were also used to investigate the role of –OH in the hydroxybenzene fragment of lavendustin C (**3**). Five different azide building blocks bearing salicylamide or alkylsalicylate were synthesized as lipophilic surrogates of the salicylate fragment (Scheme 2). Synthesis of the azide **23** commenced from commercially available 5-aminosalicylic acid (**17**). After esterification, a treatment of the diazonium salt, formed by the addition of aqueous solution of $NaNO_2$ to a HCl solution of **22**, with NaN_3 led to an azide **23**. However, the same condition did not provide azide **18**. After changing HCl to H_2SO_4,[7] we were able to obtain azide **18** in 99% yield, which was coupled with amines and alcohols in the presence of 1, 1′-carbonyldiimidazole.

With alkyne and azide building blocks in hand, we performed a small screening of catalytic conditions for azide **18**, **23** and alkyne **13**, **16** to find the optimal conditions for click reaction (Table 1). In this simulation, azide **23** bearing methylester afforded the corresponding triazoles in almost quantitative yields. However, azide **18** bearing salicylic acid did not react with alkynes even in the presence of 20 mol % of catalyst. Interestingly, when we used 20 mol % of catalyst, we observed the line broadening effect in ^1H NMR of the crude reaction mixture, presumably caused by the bidentate complex formation of Cu and salicylic acid.[8] Therefore, the salicylic acid analogues **28a**–**28f** were synthesized from hydrolysis of the corresponding methylsalicylate analogues **27a**–**27f** after click reaction of azide **23** and alkyne **7**, **12**–**16** (Scheme 3).

Library synthesis was well proceeded in 24 conical tubes. After four days, the resulting triazoles were precipitated, separated by centrifugation, and washed with H_2O and ether. The conical tube reactions really facilitated work-up processes such as solid separation by centrifugation and solvent removal by GeneVac™ without sample transfer. All precipitated compounds were submitted to HPLC and ^1H NMR analysis to verify their purity and authenticity. Indeed, 23 out of 24 were confirmed as the expected product. Only **25b** was not yielded under standard conditions (Table 2). To ensure the complete removal of a trace of Cu, which is a potentially bioactive metal, all crude products were further purified through a short pad of silica gel before bioassay. Hydrolysis of methyl ester analogues **27a**–**27f** provided the salicylic acid analogues **28a** and **28c**–**28f** except **28b**. We eventually synthesized the 28-membered lavendustin-mimetic small molecules with minimum side products, as judged by HPLC and ^1H NMR characterizations. The yields of the synthesized compounds are shown in Table 2. The yields of the salicylic acid analogues **28a**–**28f** are for two steps; click reaction and hydrolysis.

The 28-membered library was next screened for antiproliferative activity against HCT116 colon cancer and CCRF-CEM leukemia cell lines.[9] For easy comparison, the potency was expressed as % inhibition at 10 μM concentration of the reaction products (Table 2). GI_{50} values for selected compounds are listed in Table 3 compared to the anticancer drug, doxorubicin, as a positive control. Among the 28 small molecules, two ester (**26b** and **27b**) analogues and seven salicylamide (**24a**, **24c**, **24d**, **24f**, **25a**, **25e**, and

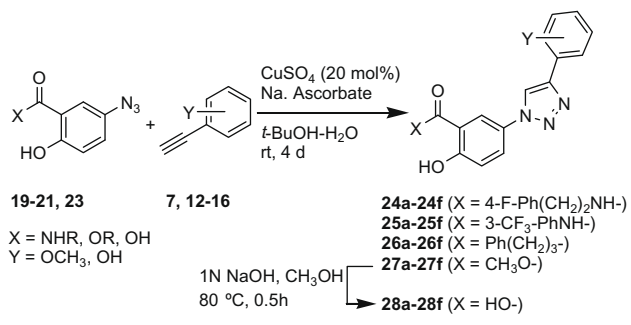


Scheme 2. Structure and synthesis of azide building blocks.

Table 1
Screening of loadings of catalyst and reducing agent

Entries	Azide	Alkyne	$CuSO_4$ (mol %)	Na ascorbate (mol %)	Yield (%)
1	**23**	**13**	2	10	99
2	**23**	**16**	2	10	77
3	**23**	**13**	20	100	99
4	**23**	**16**	20	100	99
5	**18**	**13**	2	10	0[a]
6	**18**	**16**	2	10	0[a]
7	**18**	**13**	20	100	0[b]
8	**18**	**16**	20	100	0[b]

[a] No reaction.
[b] No reaction, the line broadening in ^1H NMR was observed.



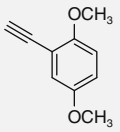
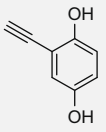
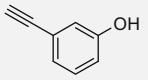
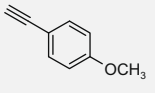
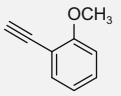
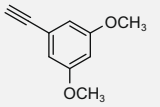
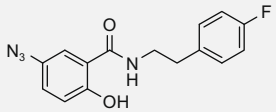
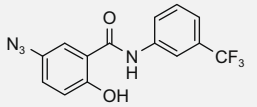
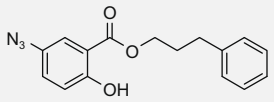
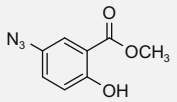
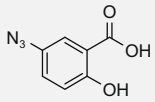
Scheme 3. Click assembly for lavendustin-mimetic small molecules.

25f) analogues showed moderate potency with GI_{50} values of 0.9–23 μM. Particularly, 3-phenylpropyl ester **26b**[10] was the best among a series of compounds against CCRF-CEM leukemia cell lines and exhibited the GI_{50} value of 0.9 μM. Interestingly, 4-fluorophenylethyl amide **24b**, a rigid triazole analogue of **4**, was less active (15% inhibition of CCRF-CEM and 18% inhibition of HCT116 at 10 μM) than **4**, which showed GI_{50} values of 0.29 μM (CCRF-CEM) and 9.2 μM (HCT116).[11]

Previously, it was reported that lavendustin C potently inhibited EGF-R tyrosine kinase in A431 cell-free extract with 0.012 μg/mL IC_{50}.[4] However, lack of cellular activity was observed in the HaCaT keratinocyte proliferation assay (IC_{50} >100 μM).[12] It was attributed to insufficient cell penetration caused by the polar functional groups, in particular the carboxylic moiety.[12] Not surprisingly, all the salicylic acid analogues **28a**, **28c**–**28f** exhibited no cellular activity presumably due to the high polarity and poor cell permeability of salicylic acid. This result is consistent with the cell-based assay of natural lavendustins[4] and supports the library design that the salicylic acid fragment should be replaced by a lipophilic surrogate.

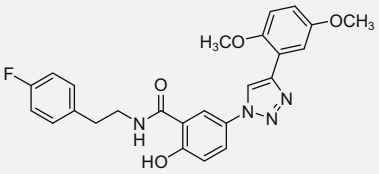
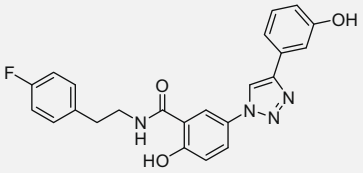
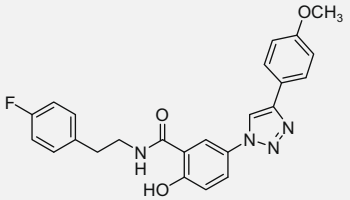
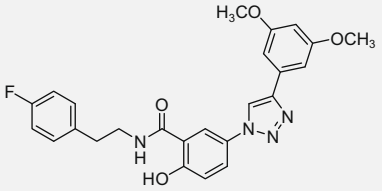
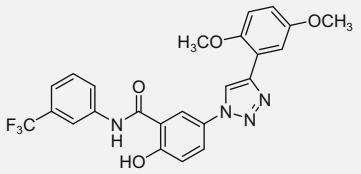
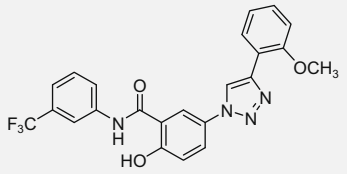
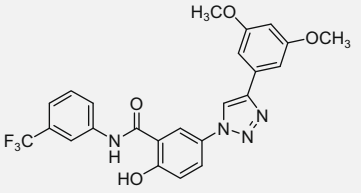
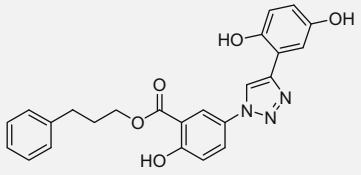
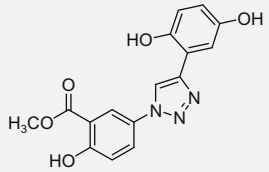
In conclusion, we have developed synthetic protocol for the rapid assembly of 28-membered lavendustin-mimetic small molecules using 'click chemistry': Cu(I)-catalyzed 1,3-dipolar cycloaddition between alkynes and azides, which afforded triazoles in good yields after minimum work-up processes. Further silica gel filtration completely removed a trace of Cu and afforded pure compounds suitable for biological evaluation against cancer cell lines. In antiproliferative screening, **26b** showed cytotoxic activity on the CCRF-CEM leukemia cell line with GI_{50} value of 0.9 μM. This compound could be a good candidate for further design of cell-permeable antiproliferative agents.

Table 2
Chemical yields and cell growth inhibition of lavendustin-mimetic small molecules synthesized by 'click chemistry' (**28a**–**28f** yields for two steps)

		 7	 12	 13	 14	 15	 16
 **19**	Yield (%) % Inhibition (HCT116)[a] % Inhibition (CCRF-CEM)[a]	**24a**, 70 44 35	**24b**, 41 18 15	**24c**, 57 0 35	**24d**, 80 62 21	**24e**, 60 23 11	**24f**, 75 43 35
 **20**	Yield (%) % Inhibition (HCT116)[a] % Inhibition (CCRF-CEM)[a]	**25a**, 29 46 15	**25b**, 0 — —	**25c**, 99 25 13	**25d**, 21 9 12	**25e**, 24 61 65	**25f**, 45 62 73
 **21**	Yield (%) % Inhibition (HCT116)[a] % Inhibition (CCRF-CEM)[a]	**26a**, 76 13 5	**26b**, 34 39 81	**26c**, 72 11 9	**26d**, 81 12 5	**26e**, 69 7 4	**26f**, 76 19 8
 **23**	Yield (%) % Inhibition (HCT116)[a] % Inhibition (CCRF-CEM)[a]	**27a**, 84 6 3	**27b**, 29 15 52	**27c**, 77 6 5	**27d**, 85 6 16	**27e**, 74 8 6	**27f**, 94 3 3
 **18**	Yield (%) % Inhibition (HCT116)[a] % Inhibition (CCRF-CEM)[a]	**28a**, 74[b] 0 9	**28b**, 0[b] — —	**28c**, 82[b] 5 3	**28d**, 64[b] 8 9	**28e**, 78[b] 4 9	**28f**, 76[b] 2 9

[a] Inhibition at 10 μM.
[b] Yields for two steps; click reaction and hydrolysis.

Table 3
GI_{50} of selected lavendustin-mimetic small molecules

Compounds	GI_{50} (μM)	
	HCT116	CCRF-CEM
24a 	13.57 ± 0.96	22.87 ± 0.67
24c 	>100	22.78 ± 0.04
24d 	12.50 ± 0.77	>100
24f 	16.10 ± 1.54	>100
25a 	12.90 ± 0.79	>100
25e 	7.92 ± 0.67	10.45 ± 0.37
25f 	4.37 ± 0.39	6.41 ± 0.50
26b 	20.40 ± 2.79	0.93 ± 0.03
27b 	>100	13.90 ± 1.66
Doxorubicin	0.40 ± 0.21	0.02 ± 0.003

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.014.

References and notes

1. Kolb, H. C.; Finn, M. G.; Sharpless, B. K. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.
2. For recent reviews on the Huisgen 1,3-dipolar cycloaddition reaction see: Meldal, M.; Tornøe, C. W. *Chem. Rev.* **2008**, *108*, 2952.
3. (a) Kalesh, K. A.; Liu, K.; Yao, S. Q. *Org. Biomol. Chem.* **2009**, *7*, 5129; (b) Pagliai, F.; Pirali, T.; Grosso, E. D.; Brisco, R. D.; Tron, G. C.; Sorba, G.; Genazzani, A. A. *J. Med. Chem.* **2006**, *49*, 467; (c) Srinivasan, R.; Uttamchandani, M.; Yao, S. Q. *Org. Lett.* **2006**, *8*, 713.
4. Onoda, T.; Iinuma, H.; Sasaki, Y.; Hamada, M.; Isshiki, K.; Naganawa, H.; Takeuchi, T.; Tatsuta, M.; Umezawa, K. *J. Nat. Prod.* **1989**, *52*, 1252.
5. Mu, F.; Coffing, S. L.; Riese, D. J. I.; Geahlen, R. L.; Verdier-Pinard, P.; Hamel, E.; Johnson, J.; Cushman, M. *J. Med. Chem.* **2001**, *44*, 441.
6. Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* **1972**, *13*, 3769.
7. Xiong, Y.; Bernardi, D.; Bratton, S.; Ward, M. D.; Battaglia, E.; Finel, M.; Drake, R. R.; Radominska-Pandya, A. *Biochemistry* **2006**, *45*, 2322.
8. Hanic, F.; Michalov, J. *Acta Crystallogr.* **1960**, *13*, 299.
9. Cytotoxic activity of the synthesized compounds against human cancer cell lines was investigated using the MTT assay. HCT116 (Human colorectal carcinoma:KCLB) were supplied from the KCLB/ATCC and grown in RPMI 1640/DMEM (Gibco BRL) supplemented with 10% (V/V) heat inactivated Fetal Bovine Serum (FBS) and maintained at 37 °C in a humidified atmosphere with 5% CO_2. The cells (5×10^4 cells/ml) were seeded into 96-well plate. Various concentrations of samples was added to each well in duplicate, then incubated at 37 °C with 5% CO_2 for two days such that time cells are in the exponential phase of growth at the time of sample addition. Add 15 μL of the Dye Solution (Promrga, Cell Titer 96) to each well. Incubate the plate at 37 °C for up to 4 h in a humidified, 5% CO_2 atmosphere. After incubation, add 100 μL of the Solubilization Solution/Stop Mix (Promrga, Cell Titer 96) to each well. Allow the plate to stand overnight in a sealed container with a humidified atmosphere at room temperature to completely solubilize the formazan crystals. The optical density was measured using a microplate reader (Versamax, Molecular Devices) with a 570 nm wavelength and the anticancer effective concentration was expressed as a GI_{50}.
10. Yield: 34%. TLC: R_f 0.27 (EtOAc/Hex = 2:3). ^{1}H NMR (400 MHz, acetone-d_6): δ 10.96 (br s, 1H), 9.66 (br s, 1H), 8.92 (s, 1H), 8.35 (d, 1H, J = 3.2 Hz), 8.10 (dd, 1H, J = 8.8 Hz, 3.2 Hz), 7.92 (br s, 1H), 7.39 (d, 1H, J = 3.2 Hz), 7.29–7.26 (m, 4H), 7.23 (d, 2H, J = 8.8 Hz), 7.13 (m, 1H), 6.86 (d, 1H, J = 8.8 Hz), 6.77 (dd, 1H, J = 8.8 Hz, 3.2 Hz), 4.49 (t, 2H, J = 6.8 Hz), 2.85 (t, 2H, J = 7.2 Hz), 2.19 (m, 2H). ^{13}C NMR (100 MHz, acetone-d_6): δ 205.9, 170.1, 162.5, 151.2, 149.3, 147.5, 142.2, 130.1, 129.3, 129.2, 126.8, 123.1, 120.6, 119.7, 118.2, 117.6, 116.2, 113.9, 113.3, 66.4, 32.8, 30.8. LRMS (FAB) m/z (rel int): (pos) 432 ([M+H]$^+$, 9). HRMS m/z calcd $C_{24}H_{22}N_3O_5$ 432.1559; found 432.1565.
11. Mu, F.; Hamel, E.; Lee, D. J.; Pryor, D. E.; Cushman, M. *J. Med. Chem.* **2003**, *46*, 1670.
12. Nussbaumer, P.; Winiski, A. P.; Cammisuli, S.; Hiestand, P.; Weckbecker, G.; Stutz, A. *J. Med. Chem.* **1994**, *37*, 4079.