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Amino Derivatives of Indole As Potent Inhibitors of Isoprenylcysteine Carboxyl Methyltransferase

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The enzyme isoprenylcysteine carboxyl methyltransferase (Icmt) plays an important role in the post-translational modification of proteins that are involved in the regulation of cell growth. The indole acetamide cysmethynil is by far the most potent and widely investigated Icmt inhibitor, but it has modest antiproliferative activity and may have pharmacokinetic limitations due to its lipophilic character. We report here that cysmethynil can be structurally modified to give analogues that are as potent in inhibiting Icmt but with significantly greater antiproliferative activity. Key modifications were the replacement of the acetamide side chain by tertiary amino groups, the *n*-octyl side chain by isoprenyl and the 5-*m*-tolyl ring by fluorine. Moreover, these analogues have lower lipophilicities that could lead to improved pharmacokinetic profiles.

1. Introduction

Proteins that terminate with a C-terminal CaaX motif (where C is cysteine, a represents an aliphatic amino acid and X denotes any amino acid) regulate a number of pathways that are important for oncogenesis. These proteins undergo a series of post-translational modifications at their C-termini that are critical to their subcellular localization, stability, and functional activity.[1,2] The sequence of reactions begins with the transfer of a 15-carbon farnesyl or 20-carbon geranylgeranyl residue from the corresponding isoprenoid diphosphate to the thiol of the cysteine residue in the CaaX motif.[3] This is followed by the endoproteolytic removal of the C-terminal aaX tripeptide by a prenyl-CaaX specific protease termed Ras converting enzyme 1 (Rce1[a])[4,5] and carboxyl methylation of the newly exposed C-terminal S-prenylated cysteine by isoprenylcysteine carboxyl methyltransferase (Icmt).[6−8] As a result of these modifications, hydrophilic cytosolic proteins are rendered hydrophobic and primed for interaction with cellular membranes and other proteins.[2,9]

The most widely studied example of CaaX proteins is the Ras family of regulatory proteins. Ras is an important molecular switch for a variety of signaling pathways that regulate normal cell growth and malignant transformation.[10] Activating mutations in Ras genes are implicated in the pathogenesis of a large number of solid tumors and hematological malignancies.[11] Many cancers may also contain alterations in signaling pathways that lie upstream of Ras, and the resulting hyperactivity of Ras is thought to contribute to tumorigenesis.[12−14] The membrane targeting and transforming abilities of Ras proteins are dependent on the processing of their terminal CaaX motif by prenylation and postprenylation events.[15,16] For this reason, the possibility of blocking Ras-induced oncogenic transformation by inhibiting the enzymes involved in the post-translational processing of the CaaX motif has been explored for its therapeutic potential.

Protein farnesyltransferase (FTase), the enzyme that catalyzes the prenylation of the cysteine residue, was an early target of several drug discovery programs.[17,18] FTase inhibitors had significant activity in mouse models, but clinical trials in patients with Ras-driven tumors failed to demonstrate convincing activity.[19] This was attributed to the likely occurrence of an alternative prenylation (geranylgeranylation) pathway when FTase activity was limiting.[20,21] For this reason, attention has shifted to the postprenylation enzymes Rce1 and Icmt as potential therapeutic targets in oncogenesis. Of the two enzymes, Icmt is generally viewed as the more druggable target as mammalian genomes encode only one member of the Icmt class of methyltransferases and Icmt lacks homology to other protein methyltransferases.[8] Targeted inactivation of the Icmt gene in mammalian cells led to the mislocation of K-Ras and the inability of K-Ras and B-Raf to promote transformation of fibroblasts.[22] In mice, inactivation of Icmt inhibited the progression of K-Ras-induced myeloproliferative disease and lung cancer[23] as compared to the acceleration of Ras-induced myeloproliferative disease when Rce-1 was inhibited.[24]

Several small molecule inhibitors of Icmt have been reported in the literature,[25−32] but most of these agents are either not sufficiently potent as inhibitors[25−29] or have limited specificity.[30,31] The most promising Icmt inhibitor identified to date is the indole acetamide cysmethynil (2-(1-octyl-5-*m*-tolyl-1*H*-indol-3-yl) acetamide, Figure 1), which was discovered through the screening of a diverse library of over 70 subfamilies with different structural scaffolds.[32] Cancer cells exposed to cysmethynil showed mislocalization of Ras and impaired growth factor signaling.[32] Cysmethynil also blocked anchorage independent growth of human colon cancer cells that was reversed by overexpression of Icmt.[32] Cell cycle arrest

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[a]Abbreviations: Rce1: Ras converting enzyme; Icmt: isoprenylcysteine carboxyl methyltransferase; FTase: farnesyltransferase ; Adomet: *S*-adenosylmethionine ; CMC: critical micelle concentration.

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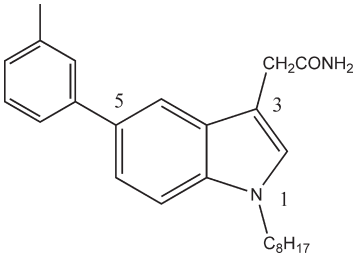


Figure 1. Structure of cysmethynil. Modifications investigated were at positions 1 (*n*-octyl), 3 (acetamide), and 5 (*m*-tolyl).

and induction of autophagy were found to contribute to the cell death that accompanied the inhibition of Icmt by cysmethynil.[33] While cysmethynil is the most promising inhibitor reported to date, we have noted its poor water solubility and strong binding to plasma proteins. A quick assessment of its compliance to drug-like filters like the Lipinski's "Rule of Five"[34] and other criteria[35] shows that it exceeds the lipophilic threshold for drug-likeness (ClogP of cysmethynil is 7) and just complies with the cutoff value[35] for rotatable bonds. Less lipophilic cysmethynil analogues will have a better solubility−lipophilicity balance, which may translate to an improved pharmacokinetic profile and bioavailability.[36] On the other hand, an excessive reduction in lipophilicity may result in compounds that are not able to gain access to the membrane bound Icmt and thus fail to bring about adequate inhibition. In an effort to reconcile these conflicting requirements, we have synthesized cysmethynil analogues with a 10^4 fold variation in lipophilicities and evaluated them for Icmt inhibitory activities as well as antiproliferative activity on breast cancer MDA-MB-231 cells. Our results showed that cysmethynil can be structurally modified to give analogues that are significantly more potent than cysmethynil and yet possess lower lipophilicities that could lead to improved bioavailability.

2. Chemistry

The synthesized compounds were classified as series 1−6 based on modifications made to positions 1, 3, and 5 of cysmethynil (Table 1). Except for series 6, where compounds were modified at two or more positions, only one position was systematically altered in the other series. Briefly, series 1 comprised compounds that were modified at position 5, while in series 2, changes were made to the *n*-octyl side chain at position 1. The acetamide side chain at position 3 was replaced by tertiary amides in series 3, tertiary amines in series 4, and related homologues and isosteric groups in series 5.

Scheme 1 gives the reaction sequence for series 1 (except **1**−**7**, **1**−**8**) and series 2. Starting with 5-bromo-1*H*-indole-3-carbaldehyde, the aldehydic group was converted to the nitrile **1** in a stepwise process that involved in situ reduction to an alcohol by sodium borohydride, followed by displacement of the alcoholic OH by a nitrile anion.[37] Nitrile **1** was hydrolyzed under basic conditions to give the carboxamide **2**, which was then reacted by Suzuki coupling with a suitably substituted phenylboronic acid. In the final step, *N*-alkylation of the ring nitrogen of the indole acetamide was achieved by reaction with an alkyl halide in the presence of sodium hydride. In the case of **1**−**7** and **1**−**8**, the same reactions were carried out on indole-3-carbaldehyde or 5-fluoro-1*H*-indole-3-carbaldehyde but without the Suzuki coupling reaction.

The tertiary amides of series 3 were synthesized by *N*-alkylation of the indole nitrogen of 2-(5-bromo-1*H*-indol-3-yl)acetic acid to give the octyl analogue **4**,[38] followed by conversion of the carboxylic acid to an acid chloride and reaction with a secondary or heterocyclic amine (Scheme 2). Suzuki coupling was then initiated at position 5 to introduce the *m*-tolyl ring.

Scheme 3 outlines the reaction sequences for the synthesis of the tertiary amines of series 4. Starting from 5-bromoindole, *N*-alkylation and Suzuki coupling were carried out to give **3**−**1**, the cysmethynil analogue without the acetamide side chain. **3**−**1** was reacted with a secondary amine and aqueous formaldehyde in a Mannich reaction to give the amines of series 4.

Series 5 comprised of various analogues of cysmethynil in which the acetamide side chain was replaced by its homologues (**5**−**1**, **5**−**2**) and isosteric groups (methyl/ethyl esters **5**−**3**, **5**−**4**, retroamide **5**−**5**, and sulphonamide **5**−**6**). The esters were prepared from 2-(5-bromo-1*H*-indol-3-yl) acetic acid. The latter was functionalized at position 1, converted to the ester in an acid catalyzed reaction, and then subjected to Suzuki coupling (Scheme 4).

Scheme 5 outlines the synthesis of the retroamide **5**−**5**, sulphonamide **5**−**6**, and the short chain homologue **5**−**1**. Starting from 5-bromo-1*H*-indole-3-carbaldehyde, sequential *N*-alkylation and Sukuzi coupling gave **9**. The aldehydic group of **9** was converted to the oxime **10** (mixture of *E* and *Z*) with hydroxylamine[39] and reacted with sodium borohydride to give the primary amine analogue **4**−**1**, which was then acetylated to give the retroamide **5**−**5** or sulphonylated to give the sulphonamide **5**−**6**. The short chain homologue of cysmethynil (**5**−**1**) was prepared by oxidation[40] of the aldehydic group of **9** to give the carboxylic acid **11**, which was converted in situ to the acid chloride and then reacted with gaseous ammonia to give **5**−**1**.

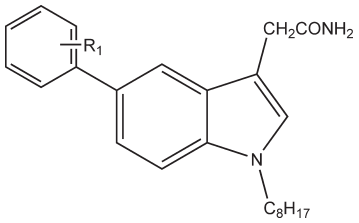
The long chain homologue of cysmethynil (**5**−**2**) was obtained by converting 2-(5-bromo-1*H*-indol-3-yl) acetic acid to its methyl ester **12**, followed by hydride reduction to the corresponding alcohol **13**,[41] displacement of the alcohol OH with the nitrile anion,[41] and hydrolysis of the nitrile **14** to the carboxamide **15**. The 5-*m*-tolyl substituent was introduced by Suzuki coupling, followed by *N*-alkylation to give **5**−**2** (Scheme 6)

Series 6 was synthesized following the earlier reported procedures. The amines **6**−**1**, **6**−**2**, **6**−**4** to **6**−**7**, **6**−**9**, and **6**−**10** were prepared by the Mannich reaction of functionalized indoles. The reaction pathways are given in Scheme 7. The acetamide **6**−**8** was synthesized in the same way as **1**−**8**, starting with 5-fluoro-1*H*-indole-3-carbaldehyde but with 1-chloro-3-methylbut-2-ene as alkylating agent. The synthesis of the tertiary amides **6**−**11** and **6**−**12** were carried out following the reaction sequences described in Scheme 2. **6**−**3** was synthesized from 5-bromo-1*H*-indole by alkylation with 1-chloro-4-methylbut-2-ene, followed by Suzuki coupling.

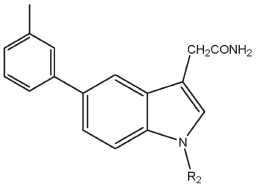
3. Results

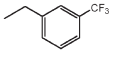
3.1. Icmt Inhibitory Activity. Icmt inhibitory activity was determined[32] and expressed in terms of IC_{50} (concentration required to reduce by 50%, the rate at which the tritiated methyl group of the methyl donor *S*-adenosylmethionine (AdoMet) was transferred to the substrate biotin-farnesyl-L-cysteine, BFC). Briefly, the assay involved incubation of enzyme and test compound for a period of time, followed by addition of the substrate cocktail of AdoMet and BFC. The concentrations of AdoMet, BFC, and test compounds ranged from 0.1 to 100 μM, hence under all conditions exceeded the

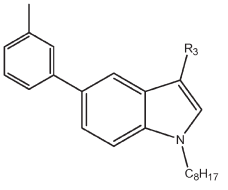
Table 1. IC_{50} Values for Icmt Inhibition, Antiproliferative Activities on MDA-MB-231 Cells and SlogP Values of Test Compounds[a]



Compound Series 1	R_1	SlogP[1]	IC_{50} (μM) Icmt inhibition [2]	IC_{50} (μM) Antiproliferative activity [2]
1-1 (Cysmethynil)	*m*-CH_3	6.3	1.5 ±0.2	21.8 ± 0.8
1-2	*o*-CH_3	6.3	1.0 ±0.2	21.2 ± 1.1
1-3	*p*-CH_3	6.3	1.0 ±0.2	ND [3]
1-4	*m*-OCH_3	6.0	1.9 ±0.3	22.4 ±1.2
1-5	*m*-OC_2H_5	6.4	1.3 ±0.2	18.7 ±1.0
1-6	H	6.0	1.8 ±0.3	26.0 ±1.5
1-7	No 5-phenyl [4]	4.3	6.5 ±2.6	50 ±4
1-8	5-F [4]	4.4	7.0 ±3.4	70 ±20



Compound Series 2	R_2	SlogP[1]	IC_{50} (μM) Icmt inhibition [2]	IC_{50} (μM) Antiproliferative activity [2]
2-1	H	3.2	33 ±10	> 100
2-2		6.3	2.5 ±0.3	17.2 ±2.3
2-3	Isoprenyl	4.9	7.7 ±2.0	28.5 ±5.3
2-4	Geranyl	6.6	1.1 ±0.2	10.6 ±0.7



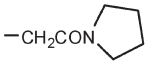
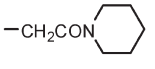
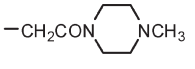
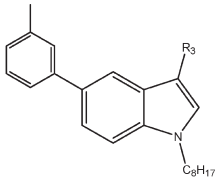
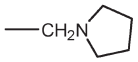
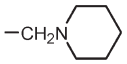
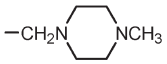
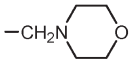
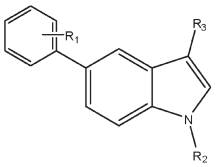
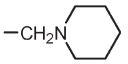
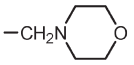
Compound Series 3,4 and 5	R_3	SlogP[1]	IC_{50} (μM) Icmt inhibition [2]	IC_{50} (μM) Antiproliferative activity [2]
3-1	H	7.2	> 100	> 100
3-2	-$CH_2CON(CH_3)_2$	6.9	1.5 ± 0.3	31 ±3
3-3	-$CH_2CON(C_2H_5)_2$	7.7	1.4 ±0.8	27.0 ±6.7
3-4		7.4	1.2 ±0.3	15.6 ± 2.7
3-5		7.8	1.8 ± 0.8	28.9 ±5.2
3-6		5.1	1.7 ±0.4	19.7 ±0.9
4-1	-CH_2NH_2	6.3	0.7 ±0.1	2.9 ± 0.2
4-2	-$CH_2N(CH_3)_2$	6.2	1.3 ± 0.5	6.8 ±0.5
4-3	-$CH_2N(C_2H_5)_2$	6.9	0.7 ±0.1	3.6 ±0.1

Table 1. Continued



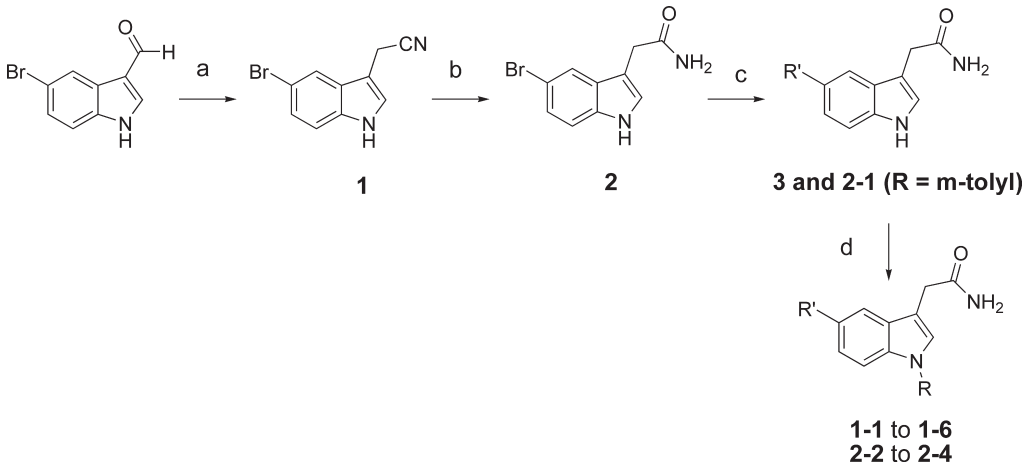
Compound Series 3,4 and 5	R_3	SlogP[1]	IC_{50} (μM)	
			Icmt inhibition [2]	Antiproliferative activity [2]
4-4	$-CH_2N(i\text{-}C_3H_7)_2$	7.7	1.7 ±0.1	20.1 ± 5.2
4-5	$-CH_2N(n\text{-}C_3H_7)_2$	7.7	0.8 ± 0.2	13.0 ±0.7
4-6	$-CH_2NCH_3(i\text{-}C_3H_7)$	6.9	0.9 ±0.1	5.3 ±0.2
4-7		6.7	0.5 ±0.1	5.1 ± 1.2
4-8		7.1	0.7 ± 0.1	4.9 ±0.2
4-9		4.4	0.9 ±0.1	6.1 ±0.2
4-10		5.9	2.7 ±0.9	24.5 ±2.3
5-1	$-CONH_2$	6.3	30 ±29	>100
5-2	$-CH_2CH_2$ CONH2	6.7	1.2 ±0.3	20.7 ± 0.8
5-3	$-CH_2COOCH_3$	7.0	76 ± 45	>100
5-4	$-CH_2COOC_2H_5$	7.3	>100	>100
5-5	$-CH_2NHCOCH_3$	7.1	1.8 ±0.4	30 ±3
5-6	$-CH_2NHSO_2CH_3$	6.6	1.2 ±0.2	17.5 ± 0.9



Compound Series 6	R_1	R_2	R_3	SlogP[1]	IC_{50} (μM)	
					Icmt inhibition [2]	Antiproliferative Activity [2]
6-1	*o*-CH_3	n-C_8H_{17}	$-CH_2N(C_2H_5)_2$	6.9	0.6 ±0.2	5.5 ± 0.3
6-2	*p*-CH_3	n-C_8H_{17}	$-CH_2N(C_2H_5)_2$	6.9	0.6 ±0.2	3.4 ±0.1
6-3	*m*-CH_3	isoprenyl	H	5.8	>100	>100
6-4	*m*-CH_3	isoprenyl	$-CH_2N(C_2H_5)_2$	5.5	2.4 ±0.3	3.8 ± 0.3
6-5	*m*-CH_3	isoprenyl		5.7	2.1 ±0.7	3.9 ± 0.4
6-6	*m*-CH_3	isoprenyl		4.5	2.0 ±0.8	34 ± 3
6-7	No 5- phenyl	isoprenyl	$-CH_2N(C_2H_5)_2$	3.6	67 ±28	74 ± 10
6-8	5- F	isoprenyl	$-CH_2CONH_2$	3.0	69 ±56	>100
6-9	5-F	n-C_8H_{17}	$-CH_2N(C_2H_5)_2$	5.1	4.1 ±1.1	7.2 ± 0.4
6-10	5-F	isoprenyl	$-CH_2N(C_2H_5)_2$	3.7	35 ±6	32 ± 2
6-11	*m*-OCH_3	n-C_8H_{17}	$-CH_2CON(C_2H_5)_2$	7.4	0.8 ±0.5	52 ±13
6-12	*m*-OC_2H_5	n-C_8H_{17}	$-CH_2CON(C_2H_5)_2$	7.7	1.0 ±0.7	26.8 ±7.8

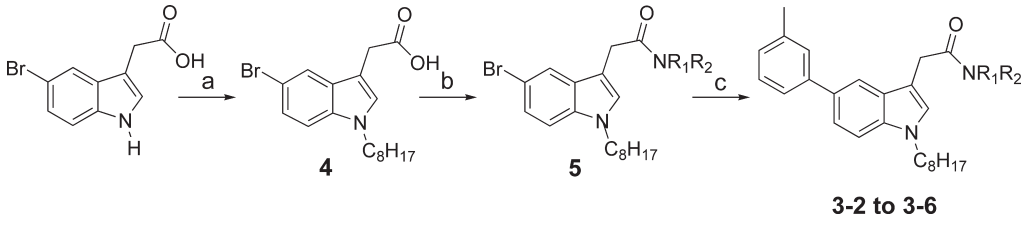
[a] [1]SlogP is the logarithm of the octanol/water partition coefficient of the test compound in its protonated state. If the compound has no basic groups and does not exist in a protonated state, SlogP will be calculated from the existing state of the compound. SlogP values were determined from geometry minimized structures using the software MOE (2008.1001) (Chemical Computing Group, Montreal, Canada). [2]Mean and SD of 3 or more determinations. [3]ND = Not determined because compound was insoluble at higher concentrations. [4]5-Phenyl is absent in **1**−**7** and **1**−**8** and replaced by H and F respectively.

Scheme 1[a]



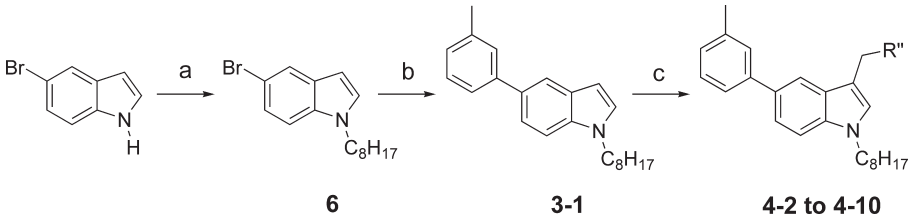
[a]Reagents and conditions: (a) (i) $NaBH_4$, NH_2CHO-MeOH, rt, (ii) KCN, 100 °C, 2.5 h; (b) KOH, *t*-BuOH, reflux, 3 h; (c) substituted phenylboronic acid, $Pd(PPh_3)_4$, $NaHCO_3$, EtOH/toluene, reflux; (d) alkyl/arylalkyl halide, NaH, DMF, rt → 53−58°C, 3−6 h.

Scheme 2[a]



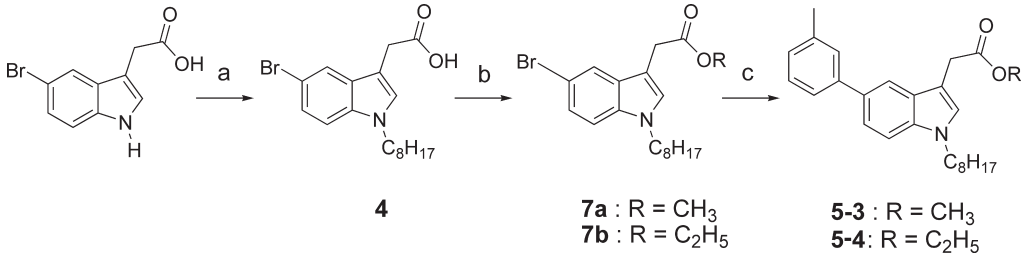
[a]Reagents and conditions: (a) $C_8H_{17}Br$, NaH, THF, 0 °C → rt, 4 h; (b) (i) $SOCl_2$, benzene, reflux, 4 h; (ii) 2° amine/heterocyclic amine, THF; (c) *m*-tolylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, EtOH/DME, reflux, 5 h.

Scheme 3[a]



[a]Reagents and conditions: (a) 1-bromooctane, NaH, DMSO, rt, 3 h; (b) substituted phenylboronic acid, $Pd(PPh_3)_4$, EtOH/DME, Na_2CO_3, reflux, 5 h; (c) aq HCHO, 2° amine (R″H), $ZnCl_2$, EtOH, rt, 10 h.

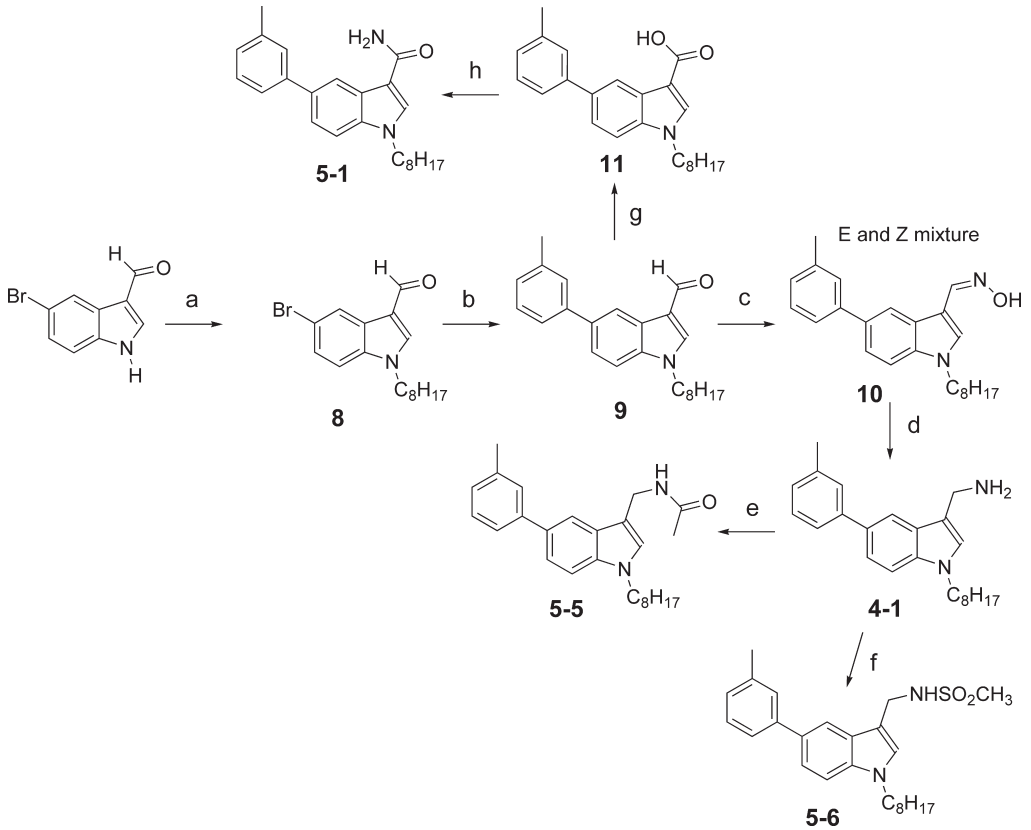
Scheme 4[a]



[a]Reagents and conditions: (a) $C_8H_{17}Br$, NaH, THF, 0 °C → rt, 4 h; (b) R-OH, cat. H_2SO_4, reflux, 1−1.5 h; (c) *m*-tolylboronic acid, $Pd(PPh_3)_4$, EtOH/DME, Na_2CO_3, reflux, 5 h.

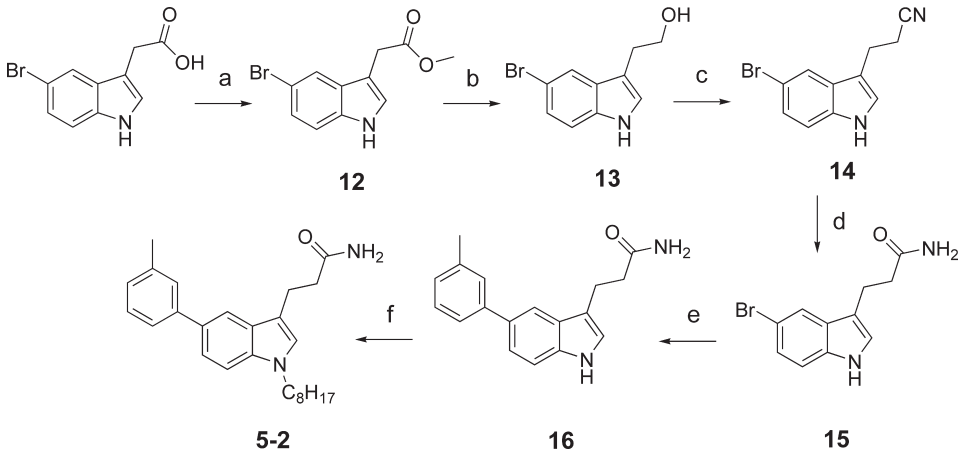
enzyme concentration which was estimated to be in the pM range. Under these conditions, a side-by-side comparison of the IC_{50} values of test compounds for the purpose of rank-ordering of potencies is considered valid. IC_{50} values are given in Table 1, which also tabulates the lipophilicity descriptor SlogP[42] of the compounds. SlogP is the logarithm of the octanol/water partition coefficient of the compound in its protonated state, and it is used in preference to ClogP[43] in

Scheme 5[a]



[a]Reagents and conditions: (a) $C_8H_{17}Br$, NaH, DMSO, rt, 3 h, DMF; (b) *m*-tolylboronic acid, $Pd(PPh_3)_4$, EtOH/DME, Na_2CO_3, reflux, 5 h; (c) $NH_2OH \cdot HCl$, Py, EtOH; (d) $NaBH_4$, $NiCl_2 \cdot 6H_2O$, MeOH; (e) CH_3COCl, Et_3N, THF, rt, 1 h; (f) CH_3SO_2Cl, Et_3N, THF, rt, 1 h; (g) $KMnO_4$, acetone, rt, 5 h; (h) (i) $SOCl_2$, benzene, reflux, 4 h, (ii) NH_3, THF, rt, 30 min.

Scheme 6[a]



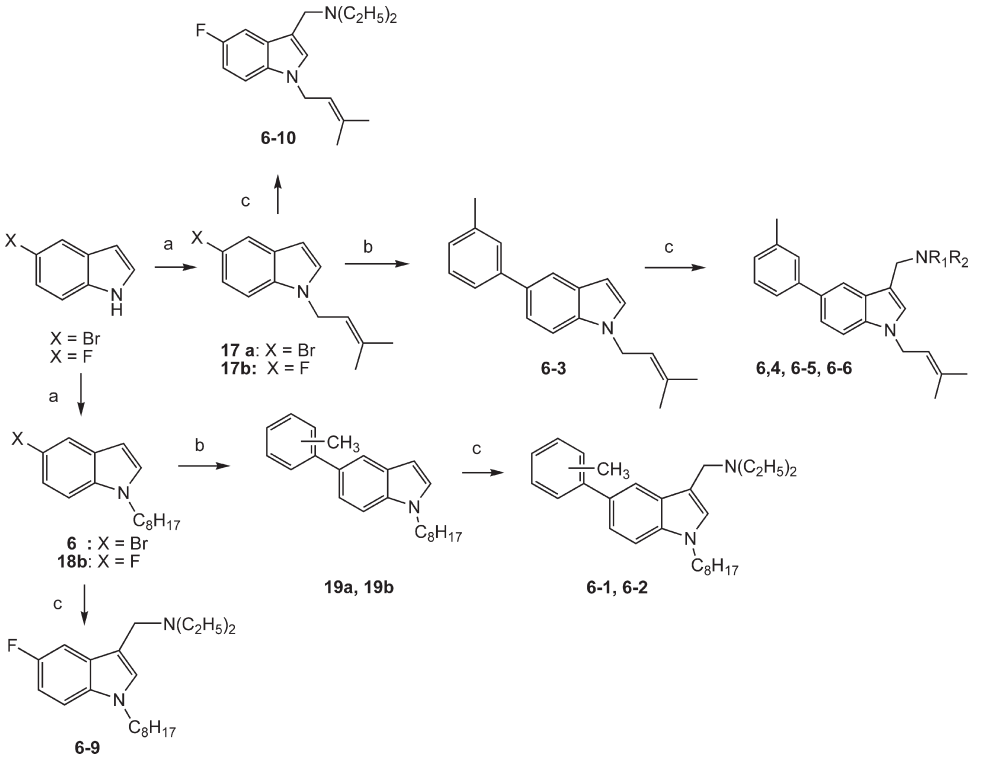
[a] Reagents and conditions: (a) MeOH, H_2SO_4, reflux, 1−1.5 h; (b) $LiAlH_4$, THF, rt, 30 min; (c) (i) MsCl, TEA, CH_2Cl_2, 0 °C, 30 min, (ii) KCN, DMSO, 100 °C; (d) KOH, *t*-BuOH, reflux, 1 h; (e) *m*-tolylboronic acid, $Pd(PPh_3)_4$, $NaHCO_3$, EtOH/toluene, reflux, 1 h; (f) bromooctane, NaH, DMF, rt → 53−58°C, 4 h.

this report as most of the test compounds have basic functionalities which are protonated at physiological conditions.

Among the series investigated, the compounds in series 1 are notable in having the narrowest variation in Icmt inhibitory activity. Changes in the substitution on the 5-phenyl ring (**1**−**1** to **1**−**6**) had minimal effects on activity while a larger but still modest 7-fold loss in activity was observed when the 5-phenyl ring was removed altogether (**1**−**7**) or replaced with 5-fluoro (**1**−**8**). The implication is that the substitution at position 5 makes a relatively small contribution to overall activity and a range of structural modifications are permitted at this position.

Unlike position 5, changes at position 1 caused marked changes to Icmt inhibitory activity, as seen from the sharp 20-fold decline in inhibition when position 1 was not substituted (**2**−**1**). Interestingly, the inhibitory activities of the series 2 compounds were closely aligned to their lipophilic character as assessed from SlogP values. Replacing *n*-octyl of

Scheme 7[a]



[a]Reagents and conditions: (a) 1-chloro-3-methylbut-2-ene or 1-bromooctane, NaH, DMSO, rt, 3 h; (b) *o*-, *m*-, or *p*-tolylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, EtOH/DME, reflux, 5 h; (c) aq HCHO, 2° amine (R_1R_2NH or diethylamine), $ZnCl_2$, EtOH, rt, 3 h.

cysmethynil with side chains that are of comparable or higher lipophilicities (*m*-trifluoromethylbenzyl **2**−**2**, geranyl **2**−**4**) had minimal effects on inhibitory activity, but introducing a less lipophilic group like isoprenyl (**2**−**3**) caused a larger drop in activity. A possible explanation may be that as Icmt is found in the hydrophobic environment of the endoplasmic reticulum, only inhibitors with sufficient lipophilicities are able to gain access to the enzyme. Clearly, the *n*-octyl side chain of cysmethynil played a key role in imparting the desired level of lipophilicity.

The role of the acetamide moiety at position 3 was investigated in series 3, 4, and 5. Its removal gave compound **3**−**1**, which had no measurable Icmt inhibitory activity. The loss of activity was far greater than that observed for **1**−**7** (removal of 5-*m*-tolyl from position 5) or **2**−**1** (removal of *n*-octyl from position 1), indicating that the contribution of the acetamide exceeded that of the other functionalities. The poor activity of **3**−**1** was clearly not due to a loss in lipophilicity as the SlogP of **3**−**1** was almost an order of magnitude greater than that of cysmethynil. When the acetamide side chain was modified to give the tertiary amides of series 3 and the amines of series 4, we found that all the tertiary amides (**3**−**2** to **3**−**6**) were comparable to cysmethynil in terms of Icmt inhibition. On the other hand, most of the amino analogues (except **4**−**2**, **4**−**4**, **4**−**10**) had submicromolar IC_{50} values and were 2−3 times more potent than cysmethynil. These potent analogues included the primary amine **4**−**1**, tertiary amines with alkyl substituents (*N*,*N*-diethyl **4**−**3**, *N*,*N*-di(*n*-propyl) **4**−**5**, *N*-methyl-*N*-isopropyl **4**−**6**), and those that had basic nitrogen atoms in heterocyclic rings (pyrrolidine **4**−**7**, piperidine **4**−**8**, *N*-methylpiperazine **4**−**9**). Interestingly, the least active amino analogue was the *N*-morpholino **4**−**10**, which was coincidentally less basic (pK_a ca. 8) than the other heterocyclic amines. These findings raised the question as to the possible contribution of the amide or amino side chain to Icmt inhibition. A hydrogen (H) bond donor role is unlikely as the tertiary amides of series 3 cannot function in this capacity but were still as potent as cysmethynil. A H bond acceptor role is more probable as the electron pairs on the carbonyl oxygen of the amides (series 3) and the basic amino side chain (series 4) can function in this capacity. For the amines which are largely protonated at physiological pH, this would necessitate deprotonation to the free base, an event that may occur in the hydrophobic membrane environment of the enzyme.

In series 5, the acetamide side chain is replaced by groups that are its isosteres or homologues. The short chain homologue **5**−**1** was significantly less inhibitory than the longer chain homologue **5**−**2**, which was as active as cysmethynil. The marked difference in activities may imply the need to maintain a minimum distance between the acetamide and the indole ring. Of the isosteric groups, the esters (**5**−**4**, **5**−**5**) fared poorly but the retroamide (**5**−**5**) and sulphonamide (**5**−**6**) were as active as cysmethynil.

Unlike the compounds in series 1−5, those in series 6 were modified at two or more positions (1, 3, or 5). These modifications included *n*-octyl or isoprenyl at position 1, an amine or amide side chain at position 3, and substituted phenyl or fluoro at position 5. In spite of these multiple modifications, structure−activity trends are generally similar to those observed earlier where changes are limited to a single position. For instance, the inactivity of the isoprenyl analogue **6**−**3**, like that of its *n*-octyl analogue **3**−**1**, emphasized the importance of retaining a side chain at position 3. The preference for an amino group at this position is again seen from the improved activities of the amino analogues

6−4, **6−5**, and **6−6** (they have isoprenyl in place of *n*-octyl at position 1) as compared to the isoprenyl analogue of cysmethynil **2−3**. In the case of the amino analogues (**6−1**, **6−2**) and tertiary amides (**6−11**, **6−12**), which have substituents other than *m*-tolyl at position 5, they were as active as their corresponding analogues which retained the *m*-tolyl ring. This is in keeping with the view that position 5 exerts a limited influence on activity.

There is however a notable difference in SAR among compounds that have concurrent changes at positions 1 and 5. This is observed among the isoprenyl analogues (**6−7**, **6−10**) that do not have a *m*-tolyl group at position 5: they were largely inactive as inhibitors despite the potency-enhancing amino side chain at position 3. In fact, for compounds without the 5-phenyl ring, a lipophilic side chain at position 1 plays a determining role as seen from the 5-fluoro analogues **6−9** (*n*-octyl, amine) and **6−10** (isoprenyl, amine) as well as **1−8** (*n*-octyl, amide) and **6−8** (isoprenyl, amide). In both instances, more potent inhibition was found for the *n*-octyl analogues **6−9** (> **6−10**) and **1−8** (> **6−8**). There appears to be a need to maintain a certain lipophilicity threshold for activity, and in the absence of the 5-phenyl ring, the *n*-octyl side chain fulfills this role better than the shorter and less lipophilic isoprenyl side chain.

3.2. Effect on Cell Growth and Proliferation. The compounds were evaluated for their effects on the viability of MDA-MB-231 human breast cancer cells by the colorimetric tetrazolium assay. Inhibitory IC_{50} values were determined after incubation for 72 h. As seen from Table 1, cell viability IC_{50} values are generally an order of magnitude higher than those for Icmt inhibition. They are spread over a narrow 30-fold concentration range, unlike the wider 100-fold variation observed for Icmt IC_{50} values. The two inhibitory activities are significantly correlated (Spearman's $\rho = 0.707$, $p = 0.001$, two-tail), which means that strong inhibitors of Icmt are also those that have potent effects on cell viability. Moreover, structure−activity trends for the two activities share many similarities. Accordingly, we found that of the three functionalities (5-*m*-tolyl, *n*-octyl, acetamide) in cysmethynil, removal of the *m*-tolyl ring (**1−7**) had the least effect on antiproliferative activity. Likewise, the antiproliferative activity of the series 3 tertiary amides (IC_{50} 16−31 μM) were comparable to cysmethynil (IC_{50} 22 μM), while the series 4 amines were significantly more potent (IC_{50} 3−13 μM). The correlation with Icmt inhibitory activity is also observed for the series 5 and 6 compounds. There are, however, exceptions. For instance, **6−11** had weak antiproliferative activity (IC_{50} 50 μM) in spite of strong inhibition of Icmt (IC_{50} 0.8 μM), and **2−4**, **6−4**, and **6−5** had better than expected antiproliferative activities compared to their Icmt inhibitory activities. The isoprenyl analogues (**6−4**, **6−5**) were notable in having antiproliferative activities that were comparable to the most potent amines (**4−3**, **4−8**) in series 4.

4. Discussion

The present study has provided useful insight into the structure−activity relationship of cysmethynil as an Icmt inhibitor. Of the three functionalities attached to the indole ring of cysmethynil, the substituent at position 5 is seen to have the least influence on Icmt inhibitory activity as only incremental changes were observed when modifications were made here. This may in fact work to advantage as functionalities that can moderate physicochemical properties while having minimal adverse effects on activity may be introduced at this position. Compound **6−9** is a case in point where introducing a fluoro atom at position 5 caused a desired fall in lipophilicity without significant loss of Icmt inhibitory activity.

In the case of position 1, its main contribution is related to the size and lipophilicity of the attached side chain. This is clearly demonstrated in series 2, where the correlation between lipophilicity (SlogP) arising from changes at position 1 and inhibitory IC_{50} values is striking. While the geranyl side chain (present in **2−4**) is a viable alternative to *n*-octyl (present in cysmethynil **1−1**), it is associated with a further increase in lipophilicity. Interestingly, we found that analogues with the shorter isoprenyl side chain (**6−4**, **6−5**) retained good activities (including antiproliferative activity), which would imply that a less lipophilic side chain is permissible but only if appropriate functionalities like the potency-enhancing amino group is present at position 3. It is also apparent that analogues with an isoprenyl side chain must have a 5-phenyl substituent if good activity is to be retained, possibly to compensate for the loss of the lipophilic *n*-octyl side chain. This is seen from the activities of **6−4** (> **6−7**) and **1−8** (> **6−8**).

The critical role of position 3 in influencing Icmt inhibition and cell viability is an important finding of the present investigation. The absence of a side chain at this position caused dramatic losses in both activities. On the other hand, introducing an amino functionality improved activities in most instances. The potency-enhancing effect of the amino side chain raised the question as to whether interaction with the enzyme involved the protonated or nonprotonated state of the amine. The amino analogues would undoubtedly be protonated at physiological pH because of their basic character. Of note is that Icmt is a membrane bound enzyme that acts on a lipidated cysteine residue, which is likely, at least, to be a component of the active site located in a hydrophobic environment. Thus it is not improbable that the protonated amino analogue loses a proton in the membrane environment to give the nonprotonated specie which then interacts with the enzyme, while the more polar features of the compound are accommodated in the hydrophilic AdoMet binding site.

Notwithstanding the hydrophobic environment in which Icmt is found, several compounds that are less lipophilic than cysmethynil demonstrated comparable Icmt inhibitory activities. They are the amino analogues **4−9**, **6−4**, **6−5**, and **6−9**, which are 10−100 times less lipophilic than cysmethylnil based on SlogP values yet comparable to it in terms of Icmt inhibition and more potent as antiproliferative agents. The reduced lipophilicities of these compounds are due to the dibasic *N*-methylpiperazine ring in **4−9**, the isoprenyl side chain in **6−4**, **6−5**, and 5-fluoro in **6−9**. There is, however, a threshold level of lipophilicity beyond which activity is compromised, and this is apparent from the need to couple the isoprenyl side chain with 5-phenyl (**6−4**, **6−5**) and 5-fluoro with *n*-octyl (**6−9**) in the target compounds.

There is a concern that the amphiphilic nature of the test compounds (lipophilic side chain at position 1 coupled with a polar amide or amino side chain at position 3) will lead to nonspecific detergent-like effects that may confound the inhibition of Icmt which is a polytopic membrane protein. Hence, the critical micelle concentrations (CMC) of the parent compound (cysmethynil, **1−1**) and a potent analogue **4−3** were determined under identical buffer conditions used in the Icmt assay and found to be 28 and 12 μM, respectively. As these concentrations are several-fold higher than their Icmt inhibitory IC_{50} (**1−1**, 1.5 μM; **4−3**, 0.7 μM), nonspecific

inhibition involving micelle formation is unlikely to interfere with the Icmt inhibitory activities of these compounds.

We found the K_i of cysmethynil (**1**−**1**) and **4**−**3** to be 3.4 and 4.5 μM, respectively (unpublished results), which are somewhat higher than the IC_{50} values (**1**−**1**, 1.5 μM; **4**−**3**, 0.7 μM) reported in Table 1. The inhibition of Icmt by cysmethynil has been shown to be strongly time-dependent, with more than 10-fold variation in IC_{50} depending on whether the value was determined using initial velocity measurements or those on final enzyme−inhibitor complex.[44] In this instance, K_i determinations were performed under early time conditions. Given the complexity of the interaction of the indole-based compounds with Icmt, kinetics analyses of the interaction of **4**−**3** and other promising analogues with Icmt warrants further investigation.

Many of the compounds with strong Icmt inhibitory activity have pronounced effects on cell proliferation. The functional consequences of Icmt inhibition remain to be fully elucidated. We have shown in an earlier report that cysmethynil arrested the cell cycle of prostate cancer cells at the G1 phase at concentrations close to its antiproliferative IC_{50} and induced autophagic cell death at higher concentrations.[33] These effects were not observed with **3**−**1**, which had negligible Icmt inhibition.[33] On the other hand, the more potent Icmt inhibitors **4**−**3** and **6**−**4** demonstrated similar effects as cysmethynil but at significantly lower concentrations.[45]

Taken together, the available evidence on the biological consequences of Icmt inhibition strongly supports the potential of the enzyme as a novel therapeutic target for oncogenesis. Hence there is a compelling rationale for developing specific Icmt inhibitors as pharmacological tools to elucidate the mechanism of action of the enzyme and the functional consequences of its inhibition and, more importantly, as potential therapeutic agents for cancer. We have identified several compounds that are at least equivalent to cysmethynil in terms of Icmt inhibition but have significantly greater antiproliferative activity on MDA-MB-231 cells. The gains in potency were achieved in many instances without an increase in lipophilicity, and this may translate to an improved solubility−lipophilicity balance, leading to a more desirable pharmacokinetic profile that may include a reduction in plasma protein binding and improved bioavailability. Hence, the findings of the present investigation may be usefully employed to guide future synthetic efforts aimed at deriving clinically useful potent inhibitors of Icmt.

5. Experimental Section

5.1. General Details. Reagents (synthetic grade or better) were obtained from commercial suppliers (Sigma-Aldrich Chemical Co. Inc., Singapore; Alfa Aesar, MA) and used without further purification. ^{1}H (300 MHz) and ^{13}C (75 MHz) spectra were measured on a Bruker Spectrospin 300 Ultrashield spectrometer magnetic resonance spectrometer. Chemical shifts were reported in ppm and referenced to TMS or residual deuterated solvents ($CHCl_3$ δ_H 7.26 ppm, DMSO-d_6 δ_H 2.50 ppm; CD_3OD δ_H 3.31 ppm) for ^{1}H spectra and residual $CHCl_3$ (δ_C 77.16 ppm) and DMSO-d_6 (δ_C 39.52 ppm) for proton decoupled ^{13}C NMR spectra. Coupling constants (J) were reported in Hertz (Hz). Reactions were routinely monitored by thin layer chromatography (TLC) on precoated plates (Silica Gel 60 F254, Merck) with ultraviolet light as visualizing agent. Column chromatography was carried out with Silica Gel 60 (0.04−0.063 mm). Nominal mass spectra were captured on an LCQ Finnigan MAT equipped with an atmospheric pressure chemical ionization (APCI) probe and m/z values for the molecular ion were reported. High resolution mass was determined using a Finnigan Mat 95/XL-T spectrometer equipped with an electron spray ionization probe (ESI). Spectroscopic data of final compounds (series 1−6) are given in Supporting Information. Purity of final compounds was verified by reverse phase HPLC on two different solvent systems (isocratic mode) and found to be ≥95%, except for **1**−**8**, **3**−**6**, **4**−**10**, **6**−**8**, and **6**−**10** (92−93% purity). Details are given in Supporting Information. Compounds **4**−**6**, **6**−**4**, **6**−**5**, **6**−**6**, and **6**−**9** were analyzed by combustion analysis (C and H) on a Perkin-Elmer PRE-2400 elemental analyzer and had C and H values that fall within ±5% of theoretical values.

5.2. Synthesis of Series 1 and 2. 5.2.1. 2-(5-Bromo-1*H*-indol-3-yl) Acetonitrile (1). The method of Yamada et al.[37] was followed. Briefly, 5-bromo-1*H*-indole-3-carbaldehyde (4.5 mmol, 1 equiv) in formamide-methanol (NH_2CHO−MeOH) was reacted with sodium borohydride ($NaBH_4$; 13.5 mmol, 3 equiv). Potassium cyanide (KCN; 45 mmol, 10 equiv) was added and the mixture refluxed (100 °C, 2.5 h). Yield: 81%. ^{1}H NMR (300 MHz, CD_3OD): δ 3.73 (s, 2 H), 7.19−7.33 (m, 3 H), 7.69 (s, 1 H). MS (APCI): m/z 236.1 $[M + H]^+$. 2-(5-Fluoro-1*H*-indol-3-yl)acetonitrile, the intermediate for **1**−**8**, was prepared in a similar manner starting from 5-fluoro-1*H*-indole-3-carbaldehyde (52% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.66 (s, 2H), 6.90 (dt, J_1 = 9 Hz, J_2 = 2.1 Hz, 1 H), 7.08 (s, 1 H), 7.13−7.22 (m, 2 H), 8.4 (1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 102.8, 104.2, 111.0, 112.4, 118.3, 124.8, 126.2, 132.8, 156.3.

5.2.2. 2-(5-Bromo-1*H*-indol-3-yl)acetamide (2). Nitrile **1** (3.5 mmol, 1 equiv) was refluxed in tertiary butanol (*t*-BuOH;10 mL) containing finely powdered 85% KOH (28 mmol, 8 equiv) for 3 h. The reaction mixture was cooled to room temperature, diluted with water, and acidified (1 M HCl) The resulting suspension was filtered under reduced pressure, and the residue was washed with water and dried in vacuo. The product was isolated as an off-white/light-brown solid. Yield: 89%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.44 (s, 2 H), 7.15−7.38 (m, 4 H), 7.73 (s, 1 H), 11.07 (s, 1 H). MS (APCI): m/z 254.1 $[M + H]^+$. 2-(5-Fluoro-1*H*-indol-3-yl) acetamide (intermediate for **1**−**8** and **6**−**8**) was obtained from the corresponding nitrile under similar reaction conditions in 61% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.69 (s, 2 H), 5.29 (s, 2 H), 6.98 (dt, J_1 = 9 Hz, J_2 = 2.4 Hz, 1 H), 7.21 (s, 1 H), 7.25−7.33 (m, 2 H), 8.34 (s, 1 H).

5.2.3. General Procedure for Preparation of 2-(5-(Substituted Phenyl)-1*H*-indole-3-yl) Acetamides (3). To a suspension of bromoindole **2** (2 mmol, 1 equiv) in anhydrous toluene (40 mL) in an ice bath was added $Pd(PPh_3)_4$ (5.7 mol %). The bright-yellow suspension was stirred for 0.5 h, after which was added (in one portion) the substituted phenylboronic acid (3 mmol, 1.5 equiv) in absolute EtOH (10 mL), followed immediately by a saturated $NaHCO_3$ solution (25 mL). After refluxing for 1−6 h, the biphasic mixture was cooled to room temperature and poured into a solution of brine. The organic phase was separated, and the aqueous phase was extracted with ethyl acetate (EtOAc). The organic extracts were combined, dried (anhydrous Na_2SO_4), the drying agent was removed by filtration, and the filtrate concentrated under reduced pressure to give the crude product **3**, which was used without further purification for the *N*-alkylation reaction.

5.2.4. General Procedure for Preparation of 2-(1-Substituted-5-(phenyl/substituted phenyl)-1*H*-indol-3-yl)acetamides (1−1 to 1−6, 2−2 to 2−4). To a stirred suspension of sodium hydride (NaH, 60% dispersion in mineral oil; 3 mmol, 1.5 equiv) in anhydrous dimethylformamide (DMF; 5 mL) in ice bath was added dropwise a solution of **3** (2 mmol, 1 equiv) in anhydrous DMF (10 mL) over 10 min. After stirring at room temperature for 1.5 h, the alkyl or arylalkyl halide (6 mmol, 3 equiv) was added dropwise over 5 min. The reaction mixture was heated in an oil bath (53−58 °C, 3−6 h), cooled to room temperature, and poured into ice water. The suspension was stirred (10 min) and extracted with diethyl ether (Et_2O). The organic extracts were combined, washed with brine, and dried (anhydrous Na_2SO_4).

The drying agent was removed by filtration and the filtrate concentrated in vacuo to give the crude product, which was purified by flash silica gel column chromatography (EtOAc/CH_2Cl_2) to give **1**−**1** to **1**−**6**, and **2**−**2** to **2**−**4**. Compounds **1**−**7** and **1**−**8** were obtained by the same method starting from 1*H*-indol-3-ylacetamide and 2-(5-fluoro-1*H*-indol-3-yl)acetamide, respectively.

5.3. Synthesis of Series 3. 5.3.1. 2-(5-Bromo-1-octyl-1*H*-indol-3-yl)acetic Acid (4). The method of Roy et al.[38] was followed. Briefly, 2-(5-bromo-1*H*-indol-3-yl)acetic acid (28 mmol, 1 equiv) was reacted with 1-bromooctane (83 mmol, 3 equiv) in the presence of NaH (60% dispersion in mineral oil, 138 mmol, 5 equiv) in tetrahydrofuran (THF; 100 mL) at 0 °C. **4** was obtained in 64% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71 (s, 1 H), 7.26 (d, J = 2.1 Hz, 1 H), 7.16 (d, J = 8.7 Hz, 1H), 7.07 (s, 1 H), 4.03 (t, J = 7.2 Hz, 2 H), 3.73 (s, 2 H), 1.79 (t, J = 9.9 Hz, 2H), 1.28−1.24 (m, 10 H), 0.86 (t, J = 6.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.3, 134.8, 129.2, 128.0, 124.5, 121.5, 112.5, 110.9, 105.6, 46.5, 31.7, 30.7, 30.1, 29.1, 29.0, 26.9, 22.5, 14.0.

5.3.2. General Procedure for the Synthesis of 2-(5-Bromo-1-octyl-1*H*-indol-3-yl)-*N*-substituted Acetamides (5). A mixture of **4** (1 mmol, 1 equiv) and thionyl chloride ($SOCl_2$; 2 mL, 27 equiv) were refluxed for 4 h in dry benzene (5 mL), after which excess $SOCl_2$ and benzene were removed by distillation in vacuo. The crude acid chloride was dissolved in dry THF (4 mL) and added dropwise to a stirred solution of the amine in dry THF at 0−5 °C. The reaction mixture was stirred at 0−5 °C for 1 h, after which THF was removed in vacuo and the residue extracted with CH_2Cl_2 and dried (anhydrous Na_2SO_4). Removal of the solvent under reduced pressure gave the desired amide, which was purified by column chromatography with EtOAc/hexane as eluting solvents. Yields and spectroscopic data of the amides are given in Supporting Information.

5.3.3. General Procedure for the Synthesis of the Tertiary Amides (3−2 to 3−6). The method of Li et al.[46] was followed with some modifications. Briefly, a solution of the acetamide **5** (1 mmol, 1 equiv) in 4 mL of dimethoxyethane (DME) was added $Pd(PPh_3)_4$ (0.05 mmol, 0.05 equiv), followed by stirring under argon for 15 min. A solution of *m*-tolylboronic acid (1 mmol, 1 equiv) in 1.5 mL of EtOH was added, stirred for 15 min, after which 2 M Na_2CO_3 (4 mL) was added and the mixture was refluxed (5 h) under argon. On cooling, the organic solvent was removed and the resulting suspension was extracted with CH_2Cl_2 and dried (anhydrous Na_2SO_4). The residue obtained on removal of the solvent was purified by column chromatography on silica gel with EtOAc/hexane as eluting solvents.

5.4. Synthesis of Series 4. 5.4.1. 5-Bromo-1-octyl-1*H*-indole (6). The method of Na et al.[47] was followed. Briefly, 5-bromo-1*H*-indole (5 mmol, 1 equiv) was reacted with NaH (60% in mineral oil dispersion, 6 mmol, 1.2 equiv) in anhydrous dimethylsulfoxide (DMSO) at room temperature. After 1 h of stirring at the same temperature, 1-bromooctane was added and the mixture stirred for 3 h. Distilled water was added to stop the reaction and mixture was worked up in the usual manner. Yield: 92%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.82 (t, J = 6.9 Hz, 3 H), 1.20−1.24 (m, 10 H), 1.76 (t, J = 6.9 Hz, 2 H), 4.03 (t, J = 7.2 Hz, 2 H), 6.37 (d, J = 3 Hz, 1 H), 7.04 (d, J = 3 Hz, 1H), 7.17 (s, 1 H), 7.21 (d, J = 1.2 Hz, 1 H), 7.69 (d, J = 1.5 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 22.5, 26.9, 29.1(2C), 30.1, 31.7, 46.5, 100.4, 110.7, 112.4, 123.3, 124.0, 128.8, 130.1, 134.6.

5.4.2. 1-Octyl-5-*m*-tolyl-1*H*-indole (3−1). Indole **6** was reacted with *m*-tolylboronic acid as described in Section 5.3.3. to give **3**−**1**. Yield: 85%.

5.4.3. General Procedure for the Synthesis of *N*-Substituted (1-Octyl-5-*m*-tolyl-1*H*-indol-3-yl) methyl) ethanamine (4−2 to 4−10). The amine (1 mmol, 1 equiv), zinc chloride ($ZnCl_2$; 1.5 mmol, 1.5 equiv), formaldehyde (HCHO; 1 mmol, 36% aq, 1 equiv), indole **3**−**1** (1 mmol, 1equiv), and EtOH (3 mL) were stirred together in a round-bottom flask for 10 h at room temperature. Distilled water was added to the mixture, which was made alkaline by the addition of 4 M NaOH. The mixture was extracted with EtOAc, the solvent was removed under reduced pressure, and the residue purified by column chromatography with CH_2Cl_2/MeOH or EtOAc/hexane as eluting solvents. Yields and spectroscopic data of **4**−**2** to **4**−**10** are given in Supporting Information.

5.5. Synthesis of 5−3 and 5−4. 5.5.1. General Method for the Preparation of Alkyl Esters of 2-(5-Bromo-1-octyl-1*H*-indol-3-yl) Acetic Acid (7a, 7b). To a solution of acid **4** (4 mmol, 1 equiv) in 20 mL of the required alcohol (MeOH or EtoH) was added 10 drops of concentrated H_2SO_4. The mixture was heated under reflux for 1−1.5 h, cooled to room temperature, and evaporated in vacuo. The residue was treated with a saturated solution of $NaHCO_3$ (50 mL), extracted with EtOAc, and dried (anhydrous Na_2SO_4). Removal of the solvent under reduced pressure gave the crude product, which was purified by column chromatography with CH_2Cl_2 as eluting solvent. Yields and spectroscopic data of **7a** and **7b** are given in Supporting Information.

5.5.2. General Method for the Preparation of Alkyl Esters of 2-(1-Octyl-5-*m*-tolyl-1*H*-indol-3-yl) Acetic Acid (5−3, 5−4). The esters **7a**, **7b** were reacted with *m*-tolylboronic acid as described in Section 5.3.3. Yields and spectroscopic details of **5**−**3** and **5**−**4** are given in Supporting Information.

5.6. Synthesis of 4−1, 5−1, 5−5, 5−6. 5.6.1. 5-Bromo-1-octyl-1*H*-indole-3-carbaldehyde (8). 5-Bromo-1*H*-indole-3-carbaldehye was reacted with 1-bromooctane and sodium hydride as described in Section 5.4.1. Yield: 85%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.91 (s, 1 H), 8.43 (s, 1 H), 7.66 (s, 1 H), 7.36 (d, J = 7.5 Hz, 1 H), 7.20 (d, J = 8.7, 1 H), 4.11 (t, J = 7.2 Hz, 2 H), 1.85 (t, J = 6.3 Hz, 2 H),1.30−1.24 (m,10 H), 0.86 (t, J = 6 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.1, 138.7, 135.8, 126.8, 126.8, 124.7, 117.3, 116.4, 111.4, 47.4, 32.7, 31.6, 29.6, 29.0, 26.7, 22.5, 14.0.

5.6.2. 1-Octyl-5-*m*-tolyl-1*H*-indole-3-carbaldehyde (9). Aldehyde **8** was reacted with *m*-tolylboronic acid as described in Section 5.3.3. Yield: 62%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.98 (s, 1 H), 8.53 (s, 1 H), 7.66 (s, 1 H), 7.57−7.46 (m, 2 H), 7.39−7.29 (m, 2 H), 7.13 (d, J = 7.2 H, 1 H), 4.1 (t, J = 8.7 Hz, 2 H), 2.42 (s, 3 H), 1.86 (t, 2 H), 1.29−1.24 (m, 10 H), 0.86 (t, J = 6.6 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.4, 141.4, 138.9, 138.2, 136.6, 136.4, 128.5, 128.2, 127.6, 125.9, 124.5, 123.5, 120.4, 118.1, 110.3, 47.3, 31.7, 29.7, 29.0 (2C), 26.8, 22.5, 21.5, 14.0.

5.6.3. (*E* and *Z*)-1-Octyl-5-*m*-tolyl-1*H*-indole-3-carbaldehyde Oxime (10). The method of Jansen et al.[39] was followed. Briefly, **9** (1.03 mmol, 1 equiv) was refluxed (2 h) with hydroxylamine hydrochloride ($NH_2OH\cdot HCl$; 2.57 mmol, 2.5 equiv) and pyridine (2.67 mmol, 2.6 equiv) in EtOH (20 mL). The mixture was acidified with 10% HCl and extracted with Et_2O, and the ethereal layer was washed successively with 10% HCl and water and dried (anhydrous Na_2SO_4). Removal of solvent in vacuo gave a residue that was triturated with petroleum ether and filtered to give **10** in 74% yield. It was used without further purification for the next step.

5.6.4. 1-Octyl-5-*m*-tolyl-1*H*-indol-3-yl) Methanamine (4−1). To a stirred solution of nickel chloride ($NiC1_2\cdot 6H_2O$; 0.76 mmol, 1 equiv) in MeOH (12 mL) was added **10** (0.8 mmol, 1 equiv) and $NaBH_4$ (4.2 mmol, 5.5 equiv) in one portion. After 5 min of stirring, the black precipitate was removed by vacuum filtration and the filtrate concentrated in vacuo to approximately $^1/_3$ of its original volume. It was poured into aqueous ammonia solution (15% v/v), extracted with EtOAc, and dried (anhydrous Na_2SO_4). Removal of solvent gave a viscous, dark-colored oil which was purified by flash silica gel column chromatography with CH_2Cl_2/MeOH to give **4**−**1**. Yield: 54%.

5.6.5. *N*-[(1-Octyl-5-*m*-tolyl-1*H*-indol-3-yl)methyl]acetamide (5−5). Acetyl chloride (0.0.628 mmol, 1 equiv) was added to a solution of amine **4**−**1** (0.63 mmol, 1 equiv) and triethylamine (0.94 mmol, 1.5 equiv) in THF (4 mL) at 0 °C. The reaction mixture was stirred at room temperature (27 °C) for 1 h, after

which the precipitate was removed by vacuum filtration, the filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography with CH_2Cl_2 as eluting solvent. Yield: 65%.

5.6.6. *N*-((1-Octyl-5-*m*-tolyl-1*H*-indol-3-yl)methyl)methanesulfonamide (5−6). Methanesulfonyl chloride (0.434 mmol, 1 equiv) was added to a solution of amine **4**−**1** (0.434 mmol, 1 equiv) and triethylamine (0.651 mmol, 1.5 equiv) in THF (4 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. The precipitate was removed by filtration, the filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography with CH_2Cl_2 as eluting solvent. The sulfonamide **5**−**6** was obtained in 61% yield.

5.6.7. 1-Octyl-5-*m*-tolyl-1*H*-indole-3-carboxylic Acid (11). Compound **9** (0.573 mmol, 1 equiv) was dissolved in acetone (10 mL) and treated with an aqueous solution of potassium permanganate ($KMnO_4$, 5 mL, 1.5 mmol).[40] The reaction mixture was stirred at room temperature for 5 h, treated with 10% H_2O_2 until the pink color of $KMnO_4$ was not observed, and then filtered. The solvent was removed under reduced pressure, and the resulting residue was filtered again (if necessary), acidified with 2 M HCl, and extracted with Et_2O. The organic solvent was removed in vacuo, and the residue was purified by column chromatography with hexane/EtOAc as eluting solvents. Yield: 62%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.84 (s, 1 H), 7.92 (s, 1 H), 7.54−7.48 (m, 2 H), 7.41−7.29 (m, 3 H), 7.14 (d, J = 6.6 Hz, 1 H), 4.12 (t, J = 6.6 Hz, 2 H), 2.44 (s, 3 H), 1.87 (t, J = 6.6 Hz, 3 H), 1.37−1.25 (m, 10 H), 0.86 (t, J = 6.6 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3, 142.3, 141.9, 138.4, 138.2, 136.1, 136.0, 135.8, 128.5, 128.3, 127.4, 124.6, 120.3, 110.3, 106.3, 47.2, 31.7, 31.1, 29.8, 29.1, 22.6, 21.5, 21.5, 14.0.

5.6.8. 1-Octyl-5-*m*-tolyl-1*H*-indole-3-carboxamide (5−1). A mixture of **11** (0.4 mmol, 1 equiv) and $SOCl_2$ (1 mL, 13 equiv) in dry benzene (5 mL) was refluxed for 4 h. Excess $SOCl_2$ and benzene were removed by distillation under reduced pressure, and the residue containing the acid chloride was dried in vacuo. It was dissolved in dry THF (4 mL), and ammonia gas was bubbled into the stirred solution for 30 min. THF was removed under vacuum, the residue was extracted with CH_2Cl_2, dried (anhydrous Na_2SO_4), and the solvent removed under reduced pressure to give carboxamide **5**−**1**. Yield: 74%.

5.7. Synthesis of 5−2. 5.7.1. Methyl-2-(5-bromo-1*H*-indol-3-yl)acetate (12). The method of Bascop et al.[41] was followed. Briefly, 2-(5-bromo-1*H*-indol-3-yl)acetic acid (4 mmol) in 20 mL of MeOH acidified with ca. 10 drops concentrated H_2SO_4 was refluxed (1−1.5 h), cooled to room temperature, and evaporated in vacuo. The acidic residue was neutralized with saturated $NaHCO_3$ (50 mL) and extracted with EtOAc. Removal of solvent gave a residue that was purified by flash silica gel column chromatography (CH_2Cl_2) to give **12**. Yield: 89%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.66 (s, 3 H), 3.68 (s, 2 H), 7.18−7.21 (m, 4 H). MS (APCI), m/z 269.2 $[M + H]^+$.

5.7.2. 2-(5-Bromo-1*H*-indol-3-yl)ethanol (13). The method of Bascop et al.[41] was followed. Ester **12** (4 mmol, 1 equiv) in anhydrous THF (50 mL) was reacted with lithium aluminum hydride ($LiAlH_4$; 16 mmol, 4 equiv) at 0 °C. Excess $LiAlH_4$ was destroyed with a saturated solution of Na_2SO_4 at 0 °C and the reaction mixture was worked up in the usual way. The alcohol **13** was obtained in 83% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.97 (t, J = 18 Hz, 2 H), 3.86−3.93 (m, 2 H), 4.09 (t, J = 21 Hz,1 H), 7.09 (d, J = 3 Hz, 1 H), 7.22 (d, J = 9 Hz, 1 H), 7.75 (s, 1 H), 8.10 (s, 1 H). MS (APCI): m/z 241.2 $[M + H]^+$.

5.7.3. 2-(5-Bromo-1*H*-indol-3-yl)propanenitrile (14). The method of Bascop et al.[41] was followed. Briefly, methanesulphonyl chloride (MsCl, 0.55 mL, 2 equiv) was added to a stirred solution of **13** (3.2 mmol, 1 equiv), triethylamine (0.9 mL, 2 equiv), and anhydrous CH_2Cl_2 (20 mL) at 0 °C. The reaction mixture was stirred for 30 min, 0 °C under N_2, after which it was treated with 2 M NaOH and extracted with CH_2Cl_2. The combined organic layer was washed with water, dried (anhydrous Na_2SO_4), and evaporated to dryness. The residue was dissolved in anhydrous DMSO and reacted with KCN (3.25 mmol, 3 equiv) by heating at 100 °C for 1 h. The mixture was diluted with a water−ice mixture, extracted with $CHCl_3$, and the combined organic layer was washed with water, dried (anhydrous Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by column chromatography with $CHCl_3$ as eluting solvent to give **14** in 75% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.78 (t, J = 15 Hz, 2 H), 3.58 (t, J = 12 Hz, 2 H), 7.14−7.17 (m, 2 H), 7.20 (d, J = 27 Hz, 1 H), 7.68 (s, 1 H), 11.01 (s, 1 H). MS (APCI): m/z 261.3 $[M + H]^+$.

5.7.4. 2-(5-Bromo-1*H*-indol-3-yl)propanamide (15). Amide **15** was prepared from the nitrile **14** by the method described in Section 5.2.2. Yield: 82%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.44 (t, J = 15 Hz, 2 H), 2.96 (t, J = 15 Hz, 2 H), 7.09−7.58 (m, 4 H). MS (APCI): m/z 268.1 $[M + H]^+$.

5.7.5. 3-(5-*m*-Tolyl-1*H*-indol-3-yl)propanamide (16). 16 was prepared from the **15** by the method described in Section 5.2.3. Yield: 50%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.38 (s, 3 H), 2.49 (t, J = 18 Hz, 2 H), 3.02 (t, J = 15 Hz, 2 H), 7.12−7.61 (m, 8 H). MS (APCI), m/z 279.4 $[M + H]^+$.

5.7.6. 3-(1-Octyl-5-*m*-tolyl-1*H*-indol-3-yl)propanamide (5−2). 5−2 was prepared from **16** by the method described in Section 5.2.4. **5**−**2** was obtained in 77% yield.

5.8. Synthesis of Series 6. 5.8.1. General Method for the Synthesis of 5-Halo-1-(3-methylbut-2-enyl)-1*H*-indole (17a, 17b). 5-Bromo-1*H*-indole or 5-fluoro-1*H*-indole was reacted with 1-chloro-3-methylbut-ene by the method described in Section 5.4.1. to give the 5-bromo (**17a**) or 5-fluoro (**17b**) analogue. Yields and spectroscopic details of **17a** and **17b** are given in Supporting Information.

5.8.2. 1-(3-Methylbut-2-enyl)-5-*m*-tolyl-1*H*-indole (6−3). 17a was reacted with *m*-tolylboronic acid as described in Section 5.3.3. **6**−**3** was obtained in 75% yield.

5.8.3. General Procedure for the Synthesis of *N*-Substituted (1-(3-Methylbut-2-enyl)-5-*m*-tolyl-1*H*-indol-3-yl)methyl)ethanamines (6−4, 6−5, 6−6). Indole **6**−**3** was reacted with the amine in the presence of $ZnCl_2$ and aqueous HCHO as described in Section 5.4.3. Yields and spectroscopic details of **6**−**4**, **6**−**5**, and **6**−**6** are given in Supporting Information.

5.8.4. *N*-Ethyl-*N*-((5-fluoro-1-(3-methylbut-2-enyl)-1*H*-indol-3-yl)methyl)ethanamine (6−10). 17b was reacted with diethylamine in the presence of $ZnCl_2$ and aqueous HCHO as described in Section 5.4.3. **6**−**10** was obtained in 77% yield.

5.8.5. 5-Fluoro-1-octyl-1*H*-indole (18b). 5-Fluoro-1*H*-indole was reacted with 1-bromooctane and NaH by the method described in Section 5.4.1. Yield: 92%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27−7.19 (m, 2 H), 7.10 (d, J = 3 Hz, 1 H), 6.93 (dt, J_1 = 8.4 Hz, J_2 = 2.1 Hz, 1H), 6.41 (d, J = 2.7 Hz, 1 H), 4.06 (t, J = 6.9 Hz, 2 H), 1.79 (t, J = 6.6 Hz, 2 H), 1.28−1.24 (m, 10 H), 0.86 (t, J = 6 Hz, 3 H). ^{1}H NMR (300 MHz, $CDCl_3$): δ 156.1, 132.6, 129.3, 109.8, 109.4, 105.6, 105.3, 100.7, 46.6, 31.7, 30.2, 29.1, 26.9, 22.6, 14.0.

5.8.6. *N*-Ethyl-*N*-[(5-fluoro-1-octyl-1*H*-indol-3-yl)methyl]-ethanamine (6−9). 18b was reacted with diethylamine in the presence of $ZnCl_2$ and aqueous HCHO by the method described in Section 5.4.3. to give **6**−**9** in 73% yield.

5.8.7. General Method for the Synthesis of 1-Octyl-5-tolyl-1*H*-indoles (19a, 19b). Compound **6** was reacted with *o*-tolylboronic acid or *p*-tolylboronic acid by the method described in Section 5.3.3. Yields and spectroscopic details of **19a** and **19b** are given in Supporting Information.

5.8.8. General Method for the Synthesis of *N*-Ethyl-*N*-((1-octyl-5-tolyl-1*H*-indol-3-yl) methyl)ethanamines 6−1 and 6−2. 19a or **19b** was reacted with diethylamine in the presence of $ZnCl_2$ and aqueous HCHO by the method described in Section 5.4.3. to give **6**−**1** or **6**−**2**. Yields and spectroscopic details of **6**−**1** and **6**−**2** are given in Supporting Information.

5.8.9. *N*-Ethyl-*N*-((1-(3-methylbut-2-enyl)-1*H*-indol-3-yl) methyl) Ethanamine (6−7). Indole was reacted with diethylamine,

$ZnCl_2$, HCHO, and ethanol as described in Section 5.4.3. to give *N*-[(1*H*-Indol-3-yl)methyl]-*N*-ethylethanamine in 64% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.07 (t, J = 6.0 Hz, 6 H), 2.55 (q, J = 6.0 Hz, 4 H), 3.77 (s, 2 H), 6.95 (s, 1 H), 7.08- 7.16 (m, 2 H), 7.23 (d, J = 6.9 Hz, 1 H), 7.67 (d, J = 7.8 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.5, 46.4, 50.0, 111.2, 112.1, 119.0, 119.2, 121.6, 124.2, 128.1, 136.1. The resulting amine was reacted with 1-chloro-3-methylbut-ene by the method described in Section 5.4.1. to give **6**−**7** in 62% yield.

5.8.10. 2-(5-Fluoro-1-(3-methylbut-2-enyl)-1*H*-indol-3-yl)acetamide (6−8). 2-(5-Fluoro-1*H*-indol-3-yl) acetamide (Section 5.2.1.) was reacted with 1-chloro-3-methylbut-2-ene by the method described in Section 5.4.1. **6**−**8** was obtained in 67% yield.

5.8.11. *N*,*N*-Diethyl-2-(5-(3-methoxyphenyl)-1-octyl-1*H*-indol-3-yl)acetamide (6−11). Acetamide **5** was reacted with *m*-methoxyphenylboronic acid as described in Section 5.3.3. **6**−**11** was obtained in 80% yield.

5.8.12. 2-(5-(3-Ethoxyphenyl)-1-octyl-1*H*-indol-3-yl)-*N*,*N*-diethylacetamide (6−12). Acetamide **5** was reacted with *m*-ethoxyphenylboronic acid as described in Section 5.3.3. **6**−**12** was obtained in 74% yield.

5.9. Materials for Biological Assay. Sf9 (*Spodoptera frugiperda* ovarian) membranes containing recombinant Icmt were prepared as described previously.[48] The human breast cancer cell line, MDA-MB-231, was obtained from American Type Culture Collection (ATCC, Rockville, MD). Streptavidin sepharose beads were purchased from Amersham Biosciences (Piscataway, NJ), *S*-adenosylmethionine *p*-toluene sulfonate from Sigma-Aldrich (Singapore), and [^{3}H] *S*-adenosylmethionine (AdoMet) from Perkin-Elmer (Waltham, MA). Biotin *S*-farnesylcysteine (BFC) was synthesized by the Duke Small Molecule Synthesis Facility,[49] and its purity was assessed by mass spectrometry and NMR. CellTiter 96 AQueous One Solution was obtained from Promega (Madison, WI). Suppliers for other reagents were: 10% fetal bovine serum (Hyclone Laboratories, Logan, UT), penicillin and streptomycin (Gibco, Invitrogen, Carlsbad, CA), and DMEM (Sigma Aldrich, Singapore).

5.10. Inhibition of Icmt Activity. The method described by Winter-Vann et al.[32] was followed with some modifications. Briefly, the standard reaction mixture contained recombinant Icmt (1.0 μg of Sf9 membrane protein) in a final total volume of 50 μL buffer A (100 mM Hepes buffer pH 7.4, 5 mM $MgCl_2$, and 100 μM EDTA). The test compound (dissolved in DMSO, final concentration of DMSO in mixture was 2% v/v) was added and the mixture was incubated at 37 °C for 20 min. After this time, the substrate mixture, which comprised 5 μM BFC and 2.5 μM [^{3}H] AdoMet (15 Ci/mmol) in buffer A, was added to initiate the reaction, which was carried out at 37 °C for 20 min. The final concentrations of BFC and [^{3}H] AdoMet in the mixture were 1 and 0.5 μM, respectively. The reaction was terminated by the addition of 10% Tween 20 (5 μL) and streptavidin beads (10 μL of packed beads) in 500 μL of buffer B, which consisted of 20 mM NaH_2PO_4 (pH 7.4) and 150 mM NaCl. The interaction between biotin and streptavidin beads was allowed to proceed overnight at 4 °C with gentle agitation. The beads were harvested by centrifugation (1000*g*, 5 min) on a tabletop microcentrifuge and washed 3× with 500 μL buffer B. The beads were then suspended in 500 μL of the same buffer and transferred to scintillation vials (each with 4.5 mL scintillation fluid) for the determination of radioactivity. Radioactivity was measured on the LS6500 multipurpose scintillation counter (Beckmann Coulter Inc., Fullerton, CA) and analyzed by the LS6000 data capture/network software version 2.11 (Beckman Coulter).

5.11. Cell Viability Assay. MDA-MB-231 human breast cancer cells were grown in DMEM supplemented with 10% fetal bovine serum, 50 U/mL penicillin, and 50 μg/mL streptomycin at 37 °C, 5% CO_2. Cells were subcultured at 80−90% confluency and used within 10−25 passages for the assays. For the assay, cells were seeded at 2400 cells per well in DMEM containing 5% FBS in 96-well plates for 24 h. Aliquots of test compounds (various concentrations, prepared in DMSO stock solutions) were then added to each well (final concentration of DMSO maintained at 0.5% v/v per well) and incubated for 72 h. Control wells contained cells and DMSO at the same concentration as those in test wells. At the end of the incubation period, 20 μL of CellTiter 96 AQueous One Solution was added to each well and the plates were incubated in the dark for 2 h at 37 °C before absorbance readings at 490 nm were taken. Background absorbance readings from blank wells that contained only media and DMSO were subtracted from each well. Each condition was determined in triplicate. Cell viability was expressed as % of absorbance at 490 nm (treated cells) over that of control (untreated) cells. The concentration of test compound required to reduce cell growth by 50% (IC_{50}) was determined from the sigmoidal curve obtained by plotting % cell viability versus concentration with GraphPad Prism (Version 4.0, GraphPad Software, San Diego, CA). IC_{50} values were the mean of at least two separate determinations.

5.12. Measurement of CMC of Cysmethynil (1−1) and Compound 4−3. Measurements were carried out by a reported method[50] with modifications. At least seven different concentrations of **1**−**1** or **4**−**3** were prepared in the same buffer solution used for determining Icmt inhibitory activity. Surface tension measurements were made at room temperature (25 °C) on a torsion balance (White Electrical Instrument Co. Ltd., UK) equipped with a du Nouy platinum ring. The instrument was calibrated before each measurement and at least three determinations were made for each concentration. CMC was determined from the plot of surface tension versus concentration.

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Supporting Information Available: Characterization and purity data of synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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