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Synthesis and antiproliferative activity of benzyl and phenethyl analogs of makaluvamines

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Abstract—Analogs of marine alkaloid, makaluvamine, bearing substituted benzyl and substituted phenethyl side chains have been synthesized and their antiproliferative activities have been evaluated. 4-Methyl, 4-chloro, and 4-fluoro substituted benzyl analogs possessed pronounced antiproliferative effects on the breast cancer cell line, MCF-7 at IC_{50} values of 2.3 μM, 1.8 μM, and 2.8 μM, respectively. 4-Methyl, 4-chloro, and 3,4-methylenedioxy derivatives showed the best activity against MCF-7 among the phenethyl analogs with IC_{50} values of 2.3 μM, 2.8 μM, and 2.4 μM, respectively. In general, methoxy substitutions resulted in slight loss in activity in both benzyl and phenethyl series. Benzyl, 4-fluorobenzyl, 3,4-dimethoxyphenethyl, and 3,4-methylenedioxyphenethyl analogs were tested by NCI in their 60 cell lines in vitro human cancer cell screen. All four compounds showed excellent inhibition against several tested cancer cell lines. Benzyl and 4-fluorobenzyl analogs were relatively more active than 3,4-dimethoxy phenethyl and 3,4-methylenedioxy phenethyl analogs. In NCI assays, the best $\mathrm{Log}\,GI_{50}$ values were shown by the fluorobenzyl analog against the renal cancer cell line RXF-393 (<−8.0 M) and dimethoxy phenethyl analog against the CNS cancer cell line, SF-268 (<−8.0 M). The best $\mathrm{Log}\,LC_{50}$ value was shown by the fluorobenzyl analog against the breast cancer cell line MCF-7 (−6.01 M).

1. Introduction

For the past quarter of a century, global marine sources have proven to be a rich source of a vast array of new medicinally valuable compounds.[1] These natural products exist as secondary metabolites in marine invertebrates such as sponges, bryazoa, tunicates, and ascidians. As a result of the potential for drug discovery, marine natural products have attracted scientists from different disciplines, such as organic chemistry, bioorganic chemistry, pharmacology, and biology. About a dozen of marine alkaloids are currently in various phases of human clinical trials for treatment of different types of cancers.[2] The largest number of bioactive marine alkaloids with novel structures have been isolated from marine sponges.[3] Sponges produce a plethora of chemical compounds with widely varying carbon skeletons. Most bioactive compounds from sponges have exhibited a variety of activities such as anti-inflammatory, antitumor, immunosuppressive, neurosuppressive, antiviral, antimalarial, and antibiotic activities.[3] While a number of these alkaloids have been isolated in quantities sufficient to ascertain their biological profile, many with unique structures are available only in minute quantities, precluding their thorough biological evaluations. Laboratory synthesis of these alkaloids and their analogs is the only practical solution to this problem.

Marine sponges of the genera *Latrunculia*, *Batzella*, *Prianos*, and *Zyzzya* are a rich source of alkaloids bearing a pyrrolo[4,3,2-*de*]quinoline skeleton.[4,5] This series of alkaloids comprise of about 60 metabolites' including discorhabdins,[6] epinardins,[7] batzellines'[8] isobatzellines,[8] makaluvamines[9–14], and veiutamine.[15] Pyrrolo[4, 3, 2-*de*]quinoline alkaloids have shown a variety of biological activities such as inhibition of topoisomerase I[9] and II,[14] cytotoxicity against different tumor cell lines,[14,16] antifungal[9] and antimicrobial activities.[17] Pyrrolo[4, 3, 2-*de*]quinoline alkaloids have recently received increasing attention as a source of new anticancer drugs.[18–23] Their unique fused ring skeletons' carrying interesting biological properties' have made them targets for several synthetic and biological studies. There has been a rapid growth of interest in the synthesis and biological evaluation of this class of compounds and their analogs. Several reviews have been published on the chemistry and

Keywords: Marine; Alkaloid; Makaluvamine; Analogs; Benzyl; Phenethyl; Cytotoxicity; Antiproliferative; Antitumor.
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bioactivity of this class of compounds.[4,24,25]. Our interest is focused on makaluvamines which belong to this family of alkaloids. Makaluvamines A–P are a group of 16 marine alkaloids isolated mainly from four species of marine sponges, namely the Fijian sponge *Zyzzya* cf. *marsailis*,[14] Indonesian sponge *Histodermella* sp.,[9] Pohnpeian sponge *Zyzzya fuliginosa*,[13], and Jamaican sponge *Smenospongia aurea*.[12] A few examples of makaluvamine alkaloids are given in Figure 1.

Makaluvamines exhibited in vitro cytotoxicity against human colon tumor cell line, HCT-116. The cytotoxicities of makaluvamines against Xrs-6, a Chinese hamster ovary (CHO) cell line sensitive to agents that cause double stranded breaks,[14] paralleled the data obtained with HCT-116. Makaluvamines are topoisomerase II inhibitors.[14] According to a recent literature report, makaluvamines produce their anticancer activity by direct DNA damage under reductive activation conditions as well.[26]

As a part of our work on analogs of marine natural products with potential pharmacological value, we have been interested in studying the anticancer activity of makaluvamine analogs. Many pyrroloiminoquinone alkaloids with proven anticancer activities were found to have substitutions at the 7-position of the pyrroloiminoquinone ring. We have explored some simple substitutions with increased steric bulk, hydrophobicity, and hydrophilicity at the 7- position of the pyrroloiminoquinone ring and examined their anticancer activity.[27] These derivatives were tested against two human breast cancer cell lines, MCF-7 and MDA-MB-468, and one human colon tumor cell line, HCT-116. All of the makaluvamine analogs showed cytotoxicity against the tested cell lines with IC_{50} values ranging from 0.56 μM to 11 μM. Two analogs (benzyl analog, **4**, and phenethyl analog, **5**) showed excellent activity against MCF-7, MDA-MB-468, and HCT-116 (Fig. 2).[27] Compounds **4** and **5** exhibited topoisomerase II inhibition comparable to two clinically used topoisomerase II targeting drugs, m-AMSA and etoposide.[27]

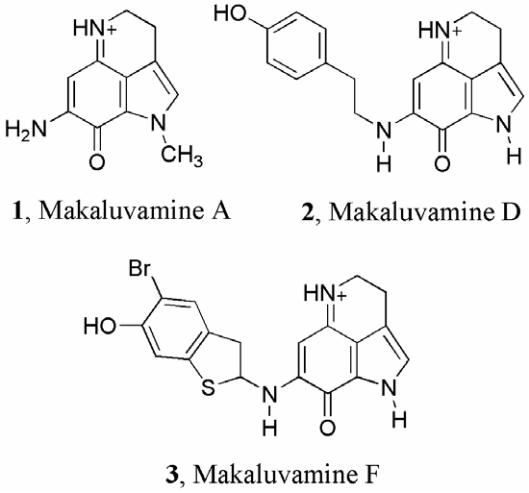
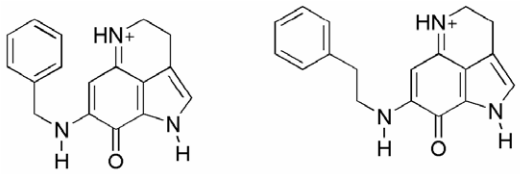


Figure 1. A few examples of naturally occurring makaluvamines.



Cell line	Compound **4**	Compound **5**
HCT-116	1.3 ± 0.2 μM	3.9 ± 1.2 μM
MCF-7	1.0 ± 0.4 μM	1.7 ± 0.5 μM
MDA-MB-468	0.3 ± 0.18 μM	0.3 ± 0.27 μM

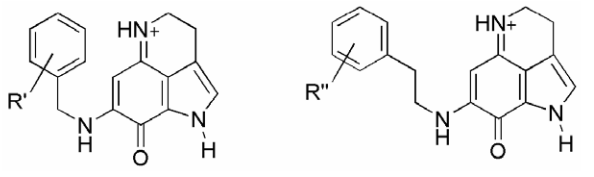
Figure 2. Benzyl and phenethyl analogs of makaluvamine and their IC_{50} values against breast cancer cell lines MCF-7 and MDA-MB-468 and human colon tumor cell line HCT-116.

2. Results and discussion

We have explored structure–activity relationship studies on these two lead compounds **4** and **5** with the objective of optimizing their activity. As a first step, we decided to examine the effect of electron releasing and electron withdrawing polar substituents on the phenyl rings present in these two compounds. Proposed target structures are given in Figure 3. The choice of substituents was limited by the commercial availability of benzyl and phenethyl amines required for the synthesis of these target compounds.

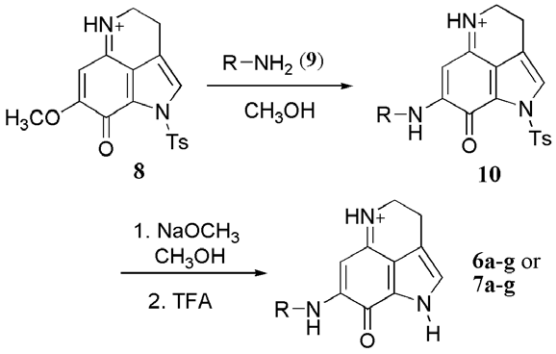
2.1. Chemistry

Synthesis of proposed target compounds is outlined in Scheme 1. All target compounds were synthesized in two steps starting from the known tricyclic pyrroloiminoquinone compound, **8**. Synthesis of compound **8** has been reported in the literature by several groups.[28] We prepared this compound following the 4,6,7-trimethoxyindole approach described previously.[29] Treatment of compound **8** with different amine derivatives (**9**) in anhydrous methanol at room temperature provided the aminated compounds **10**. Intermediate prod-



	R' =		R'' =
6a	4-CH_3	**7a**	4-CH_3
6b	4-OCH_3	**7b**	4-OCH_3
6c	3,4-di-OCH_3	**7c**	3,4-di-OCH_3
6d	3,4,5-tri-OCH_3	**7d**	3,4-di-Cl
6e	3,4-OCH_2O	**7e**	3,4-OCH_2O
6f	4-Cl	**7f**	4-Cl
6g	4-F	**7g**	4-F

Figure 3. Proposed target structures.



Scheme 1. Synthesis of makaluvamine analogs.

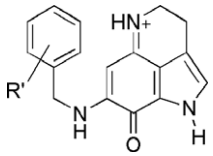
ucts **10** were subjected to detosylation reaction without further purification and characterization. Removal of tosyl protecting group from the compound **10** was accomplished by treatment with NaOMe in MeOH to obtain the final products **6a**–**g** or **7a**–**g** in 48–62 % yield. All final products were completely characterized.

2.2. Biology

Compounds **6a**–**g** and **7a**–**g** were evaluated for their cytotoxicities against breast cancer cell line MCF-7 at UAB. The dose of the compound that inhibits 50% cell proliferation (IC_{50}) was calculated using the data generated from 2 to 4 independent tetrazolium-based (XTT) cytotoxicity assays (R&D Systems Inc., Minneapolis, MN) performed in triplicate and were combined for an average ± standard deviation. Two known topoisomerase II targeting drugs, m-AMSA and etoposide, were included in our experiment for comparison.

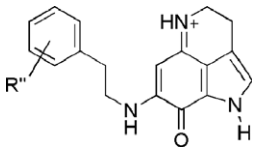
Cytotoxic activities of benzyl analogs against MCF-7 are given in Table 1 and cytotoxic activities of phenethyl analogs against MCF-7 are given in Table 2. All of the synthesized benzyl analogs were found to be more active than the control drug, etoposide. All benzyl analogs except one compound (**6d**) were more active than m-AMSA. All of the phenethyl analogs were found to be more active than both of the control drugs, etoposide and m-AMSA. However, the newly synthesized analogs

Table 1. Activity of benzyl analogs of makaluvamines against MCF-7



Compound	R=	IC_{50} (μM)
6a	4-CH_3	2.3 ± 0.8
6b	4-OCH_3	3.6 ± 1.2
6c	3,4-Di-OCH_3	6.6 ± 0.8
6d	3,4,5-Tri-OCH_3	34.7 ± 5.9
6e	3,4-OCH_2O	5.2 ± 0.9
6f	4-Cl	1.8 ± 0.7
6g	4-F	2.8 ± 0.3
Etoposide		35.6 ± 3.4
m-AMSA		21.7 ± 2.5

Table 2. Activity of phenethyl analogs of makaluvamines against MCF-7



Compound	R″=	IC_{50} (μM)
7a	4-CH_3	2.3 ± 0.6
7b	4-OCH_3	3.4 ± 1.1
7c	3,4-Di-OCH_3	13.7 ± 1.8
7d	3,4-Di-Cl	4.6 ± 0.2
7e	3,4-OCH_2O	2.4 ± 0.4
7f	4-Cl	2.8 ± 0.2
7g	4-F	5.1 ± 0.3

were not more active than the lead compounds **4** or **5**. In the case of benzyl analogs several of the derivatives (compounds **6a**, **6f**, and **6g**) showed comparable activity to the parent compound **4**. Substitution of monomethoxy, dimethoxy, trimethoxy or methylene dioxy groups resulted in decrease in activity as compared to compound **4**. As the number of methoxy groups increased from 1 to 3 a regular decrease in activity was observed (**6b**, 3.6 μM, **6c**, 6.6 μM, and **6d**, 34.7 μM). 3,4-Methylenedioxy substitution exhibited a similar decrease in activity (**6e**, 5.2 μM) as compared to compound **4**. The trimethoxy substituted benzyl derivative was the least active of all benzyl analogs (**6d**, 34.7 μM). Substitution of halogens (4-Cl and 4-F) on the ring did not have a major impact on the activity of benzyl analog **4** (**6f**, 1.8 μM and **6g**, 2.8 μM).

Similar trends in activity as observed in the case of benzyl analogs were observed in the case of phenethyl analogs as well (Table 2). Methyl substitution did not change the activity much (**7a**, 2.3 μM) as compared to the parent compound **5**. Methoxy and dimethoxy substitution did show a decrease in activity (**7b**, 3.4 μM, **7c**, 13.7 μM). However, methylene dioxy substitution did not have a major influence on the activity (**7e**, 2.4 μM). 4-Chloro substitution did not change the activity much (**7f**, 2.8 μM). But, a 3,4-dichloro substitution (**7d**, 4.6 μM) and 4-fluoro substitution (**7e**, 5.1 μM) showed a slight reduction in activity.

Two benzyl analogs (**4**, **6g**) and two phenethyl analogs (**7c**, **7e**) were selected by the Developmental Therapeutic Program of NCI (National Cancer Institute, Bethesda, MD, USA) to undergo in vitro testing against a panel of approximately 60 human tumor cell lines. These compounds were tested against cell lines derived from nine tumor types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast. The compounds were tested at five concentrations at 10-fold dilution.[30,31] The antitumor activity of the tested compounds is expressed as Log GI_{50} values which is log of molar concentration of the compound that inhibits 50% net cell growth and Log LC_{50} values which is log of molar concentration leading to 50% net cell death. The Log GI_{50} and Log LC_{50} values of compounds **4**, **6g**, **7c**, and **7e** are summarized in Table 3.

Table 3. In vitro inhibition[a] of cancer cell lines by compounds **4**, **6g**, **7c**, and **7e**

Compound no.	**4**		**7c**		**7e**		**6g**	
Panel/cell line	A	B	A	B	A	B	A	B
Leukemia								
CCRF-CEM	−6.05	>−4.00	−4.97	>−4.00	−5.53	>−4.00	−5.85	−4.12
HL-60(TB)	−6.49	−5.14	−5.50	>−4.00	−5.72	−5.00	−5.81	−5.07
K-562	−5.63	>−4.00	−4.77	>−4.00	−5.46	>−4.00	−5.97	>−4.00
MOLT-4	−6.50	N.T.[d]	−5.31	>−4.00	−5.70	>−4.00	−6.10	N.T.[d]
RPMI-8226	−5.87	−4.21	N.T.[d]	N.T.[d]	N.T.[d]	N.T.[d]	−6.25	−4.28
SR	−5.76	>−4.00	−5.24	>−4.00	−5.60	>−4.00	−5.92	−5.04
Non-small cell lung cancer								
A549/ATCC	−5.67	−4.60	−5.57	−4.22	N.T.[d]	N.T.[d]	−5.84	−5.25
EKVX	−6.57	−5.05	−5.51	−4.43	−5.71	−4.56	−6.34	−5.23
HOP-62	−5.43	−4.28	−5.29	−4.32	−5.60	−4.38	−5.42	−4.38
HOP-92	−6.51	−4.47	−4.99	>−4.00	−5.26	>−4.00	−5.77	−4.72
NCI-H226	−5.26	−4.37	−4.98	−4.26	−5.36	−4.20	−5.63	−4.89
NCI-H23	−5.83	>−4.00	−5.70	−4.70	−5.77	−5.00	−6.28	>−4.00
NCI-H322M	−6.20	−4.54	−5.55	−4.31	−5.55	−4.44	−5.93	−5.02
NCI-H460	−5.82	−4.36	−5.85	−4.40	−6.00	−4.92	−6.09	−4.71
NCI-H522	−5.77	−5.01	−5.65	−4.38	−5.58	−4.43	−5.70	−5.03
Colon cancer								
COLO 205	−6.42	−5.24	−5.86	−5.13	−5.99	−5.03	−6.44	−5.36
HCC-2998	−5.58	−4.32	−5.63	−4.61	−5.81	−4.87	−5.57	−4.39
HCT-116	−6.51	>−4.00	−5.62	−4.32	−5.62	−4.50	−6.15	−4.24
HCT-15	−5.86	−5.16	−5.58	−4.40	−5.73	−4.46	−5.83	−5.23
HT29	−6.31	−4.48	−5.60	−4.42	−5.68	−4.76	−6.22	−4.71
KM12	−5.69	>−4.00	−5.65	−4.00	−5.64	−4.09	−5.69	>−4.00
SW-620	−5.97	−4.59	−5.51	−4.55	−5.46	−4.48	−5.74	−4.61
CNS cancer								
SF-268	−5.55	−4.25	<−8.00	>−4.00	−5.44	>−4.00	−5.78	−4.36
SF-295	−5.95	−4.64	−5.73	−4.65	−5.73	−5.01	−5.97	−5.14
SF-539	−5.72	−5.01	−5.48	−4.45	−5.66	−4.63	−5.76	−5.14
SNB-19	−5.71	−5.10	−5.49	−4.44	−5.48	−4.41	−5.75	−5.12
SNB-75	−4.78	−4.15	−5.28	−4.29	−5.46	−4.37	−5.28	−4.21
U251	−5.57	−4.62	−5.46	−4.44	−5.52	−4.42	−5.70	−5.01
Melanoma								
LOX IMVI	−5.74	−4.84	−5.63	−4.58	−5.78	−4.64	−5.80	−5.02
MALME-3M	−6.52	−5.41	−6.60	−5.44	−6.47	−5.40	−6.68	−5.65
M14	−5.89	−5.17	−5.65	−4.58	−5.85	−5.12	−5.84	−5.21
SK-MEL-2	−5.28	−4.26	−5.14	>−4.00	−5.23	−4.26	−5.34	−4.31
SK-MEL-28	−5.13	−4.27	−4.99	−4.07	−5.31	−4.32	−5.24	−4.35
SK-MEL-5	−6.34	−5.38	−5.87	−5.29	−6.02	−5.33	−6.49	−5.44
UACC-257	−6.50	−5.29	−5.51	>−4.00	−5.53	>−4.00	−6.72	−5.45
UACC-62	−5.74	−5.11	5.66	−4.88	−5.64	−5.00	−5.81	−5.20
Ovarian cancer								
IGROV1	−6.10	−5.02	−5.37	−4.13	−5.60	−4.32	−5.85	−5.00
OVCAR-3	−5.59	−4.25	−5.79	−4.59	−5.70	−4.57	−5.65	−4.23
OVCAR-4	−6.21	−5.31	−5.93	−5.21	−6.01	−5.25	−5.96	−5.30
OVCAR-5	−5.57	−4.59	−5.29	−4.27	−5.57	>−4.00	−5.63	−4.93
OVCAR-8	−5.68	−4.08	−5.75	−5.12	−5.79	−5.17	−5.70	−4.74
SK-OV-3	−5.15	−4.28	−4.84	>−4.00	−5.01	>−4.00	−5.50	−4.41
Renal cancer								
786-0	−5.49	>−4.00	−5.26	−4.09	−5.40	−4.27	−5.46	−4.27
A498	−4.78	−4.26	−5.14	−4.33	−5.22	−4.32	−5.51	−4.55
ACHN	−5.55	−4.42	−5.38	−4.35	−5.49	−4.06	−5.69	−4.77
CAKI-1	−6.53	−4.49	−5.57	−4.50	−5.75	−4.93	−6.48	−4.45
RXF 393	−5.68	−4.38	N.T.[d]	N.T.[d]	N.T.[d]	N.T.[d]	<−8.00	−5.29
SN12C	−5.72	−5.02	−5.51	−4.47	−5.57	−4.60	−5.71	−5.05
TK-10	−5.33	−4.24	−5.29	>−4.00	−5.35	>−4.00	−5.50	−4.34
UO-31	−5.58	>−4.00	−5.15	>−4.00	−5.23	−4.09	−5.59	−4.27
Prostate cancer								
PC-3	−5.81	>−4.00	−5.12	>−4.00	−5.23	>−4.00	−5.80	−4.27
DU-145	−5.75	−4.60	−5.55	−4.45	−5.87	−5.29	−5.97	−5.32

Table 3 (*continued*)

Compound no.	**4**		**7c**		**7e**		**6g**	
Panel/cell line	A	B	A	B	A	B	A	B
Breast cancer								
MCF7	−7.22	−5.98	−6.58	−5.34	−5.92	−5.25	−6.80	−6.01
NCI/ADR-RES	−5.46	>−4.00	−4.91	>−4.00	−4.75	>−4.00	−5.51	>−4.00
MDA-MB-231/ATCC	−6.36	>−4.00	−5.49	−4.03	−5.63	>−4.00	−6.14	−4.32
HS 578T	−5.28	>−4.00	−5.30	>−4.00	−5.54	>−4.00	−4.36	>−4.00
MDA-MB-435	−5.69	−5.07	−5.69	−5.20	−5.72	−5.21	−5.76	−5.09
BT-549	−5.34	−4.40	−5.34	−4.38	−5.51	−4.43	−5.50	−4.57
T-47D	−6.28	>−4.00	−5.60	>−4.00	−5.53	>−4.00	−6.20	N.T.[d]

Response parameters, $A = \mathrm{Log}GI_{50}$[b] in Molar units and $B = \mathrm{Log}LC_{50}$[c] in Molar units.
[a] Data obtained from the NCI's in vitro disease-oriented human tumor cell screen.
[b] $\mathrm{Log}GI_{50}$ is the log of molar concentration causing 50% growth inhibition of tumor cells.
[c] $\mathrm{Log}LC_{50}$ is the log of the molar concentration leading to 50% net cell death.
[d] Not tested.

All four compounds tested by the Developmental Therapeutic Program of NCI exhibited high potency against several tested cancer cell lines with $\mathrm{Log}GI_{50}$ values ranging from <-8.00 M to -4.36 M and $\mathrm{Log}LC_{50}$ values ranging from -6.01 M to >-4.0 M. The best $\mathrm{Log}GI_{50}$ values were shown by the fluorobenzyl analog, **6g**, against the renal cancer cell line RXF-393 (<-8.0 M) and the dimethoxy phenethyl analog, **7c**, against CNS cancer cell line SF-268 (<-8.00 M). The best $\mathrm{Log}LC_{50}$ value was shown by **6g** against the breast cancer cell line, MCF-7 (-6.01 M).

Overall, $\mathrm{Log}GI_{50}$ and $\mathrm{Log}LC_{50}$ values indicate that the two benzyl analogs, **4** and **6g**, are more active than the two phenethyl analogs, **7c** and **7e**. The benzyl analog, **4**, exhibited $\mathrm{Log}GI_{50}$ values of $\leqslant -6.0$ against leukemia cell lines CCRF-CEM, HL-60 (TB), and MOLT-4; non-small cell lung cancer cell lines, EKVX, HOP-92, and NCI-H332M; colon cancer cell lines COLO205, HCT-116, and HT-29; melanoma cell lines, MALME-3M, SK-MEL-5, and UACC-257; ovarian cancer cell lines, IGROV1 and OVCAR-4; renal cancer cell line CAKI-1, and breast cancer cell lines MCF-7, MDA-MB-231/ATCC, and T47-D. The benzyl analog, **4**, exhibited $\mathrm{Log}LC_{50}$ values of $\leqslant -5.0$ M against several cell lines. This includes leukemia cell line HL-60 (TB); non-small cell lung cancer cell lines EKVX and NCI-H522; colon cancer cell lines COLO205 and HCT-15; CNS cancer cell lines SF-539 and SNB-19; melanoma cell lines MALME-3M, M-14, SK-MEL-5, UACC-257, and UACC-62; ovarian cancer cell lines IGROV1 and OVCAR-4; renal cancer cell line SN12C, and breast cancer cell lines MCF-7 and MDA-MB-435.

Fluorobenzyl analog, **6g**, was also equally potent exhibiting $\mathrm{Log}GI_{50}$ values of $\leqslant -6.0$ against several cancer cell lines. This includes leukemia cell lines MOLT-4 and RPMI-8226; non-small lung cancer cell lines EKVX, NCI-H23, and NCI-H460; colon cancer cell lines COLO205, HCT-116, and HT-29; melanoma cell lines MALME-3M, SK-MEL-5, and UACC-257; renal cancer CAKI-1 and RXF-393 and breast cancer cell lines MCF-7, MDA-MB-231/ATCC, and T-47D. The fluorobenzyl analog, **6g**, exhibited $\mathrm{Log}LC_{50}$ values of $\leqslant -5.0$ M against several cell lines. This includes the leukemia cell lines HL-60 (TB) and SR; non-small lung cancer cell lines A549/ATCC, EKVX, NCI-H322M, and NCI-H522; colon cancer cell lines COLO205 and HCT-15; CNS cancer cell lines SF-295, SF-539, SNB-19, and U251; melanoma cell lines LOX IMVI, MALME-3M, M14, SK-MEL-5, UACC-257, and UACC-62; ovarian cancer cell lines IGROV1 and OVCAR-4; renal cancer cell lines RXF-393 and SN12C; prostate cancer cell line DU-145; and breast cancer cell lines MCF-7, MDA-MB-435.

The best $\mathrm{Log}GI_{50}$ value of the dimethoxy phenethyl analog, **7c**, was against the CNS cancer cell line SF-268 (<-8.0 M). It also exhibited $\mathrm{Log}GI_{50}$ values of $\leqslant -6.0$ M against the melanoma cell line MALME-3M and breast cancer cell line MCF-7. It showed $\mathrm{Log}LC_{50}$ values of $\leqslant -5.0$ M against colon cancer cell line COLO205, melanoma cell lines MALME-3M and SK-MEL-5, ovarian cancer cell lines OVCAR-4 and OVCAR-8, and breast cancer cell lines, MCF-7 and MDA-MB-435.

The best $\mathrm{Log}GI_{50}$ of methylene dioxy phenethyl analog, **7e** was against melanoma cell line MALME-3M (-6.47 M). It also exhibited $\mathrm{Log}GI_{50}$ values of $\leqslant -6.0$ M against non-small lung cancer cell line NCI-H460, melanoma cell line SK-MEL-5, and ovarian cancer cell line OVCAR-4. It had $\mathrm{Log}LC_{50}$ values of $\leqslant -5.0$ M against leukemia cell line HL-60(TB), non-small lung cancer cell line NCI-H23, colon cancer cell line COLO205, CNS cancer SF-295, melanoma cell lines MALME-3M, M14, SK-MEL-5, and UACC-62; ovarian cancer cell lines OVCAR-4 and OVCAR-8; prostate cancer cell line DU-145, and breast cancer cell lines MCF-7 and MDA-MB-435.

Interestingly, all four analogs **4**, **6g**, **7c**, and **7e** also exhibited activity against adriamycin-resistant breast cancer cell line NCI/ADR-RES with $\mathrm{Log}GI_{50}$ values -5.46, -5.51, -4.91, and -4.75, respectively.

3. Conclusions

Analogs of marine alkaloid, makaluvamine, bearing substituted benzyl and phenethyl side chains have been

synthesized and their antiproliferative activities have been evaluated at UAB against breast cancer cell line MCF-7. 4-Methyl, 4-chloro, and 4-fluoro substituted benzyl analogs possessed a pronounced antiproliferative effect on the breast cancer cell line MCF-7, at IC_{50} values of 2.3, 1.8, and 2.8 μM, respectively, as compared to 35.6 μM for etoposide and 21.7 μM for m-AMSA. 4-Methyl, 4-chloro, and 3,4-methylenedioxy derivatives were the best among the phenethyl analogs exhibiting IC_{50} values of 2.3 μM, 2.8 μM, and 2.4 μM, respectively. In general methoxy substitution resulted in a slight loss in activity in both benzyl and phenethyl series. Benzyl, 4-fluorobenzyl, 3,4-dimethoxyphenethyl, and 3,4-methylenedioxyphenethyl analogs were tested by NCI in their 60 cell lines in vitro human cancer cell screen. All four compounds showed excellent inhibition against several tested cell lines. Benzyl and 4-fluorobenzyl analogs were more active than 3,4-dimethoxy and 3,4-methylenedioxy phenethyl analogs. In NCI assays, the best $Log GI_{50}$ values were shown by the fluorobenzyl analog against the renal cancer cell line RXF-393 (<−8.0 M) and 3,4-dimethoxyphenethyl analog against CNS cancer cell line SF-268 (<−8.0 M). The best $Log LC_{50}$ value was shown by the fluorobenzyl analog against the breast cancer cell line MCF-7 (−6.01 M). All four analogs also exhibited activity against adriamycin-resistant breast cancer cell line NCI/ADR-RES.

4. Experimental

4.1. General methods for synthesis

Solvent evaporations were carried out in vacuo with rotary evaporator. Thin layer chromatography (TLC) was performed on silica gel plates with fluorescent indicator (Whatmann, silica gel, UV254, 25 μm plates). Spots were visualized by UV light (254 and 365 nm). Purification by column and flash chromatography was carried out using 'BAKER' silica gel (40 μm) in the solvent systems indicated. The amount (weight) of silica gel for column chromatography was in the range of 50–100 times the amount (weight) of the crude compounds being separated. Proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Brucker DPX-300 spectrometer using TMS as internal standard. The values of chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. Mass spectra were recorded on Micromass Platform LCC instrument. Anhydrous solvents used for reactions were purchased in Sure-Seal™ bottles from Aldrich Chemical Company. Other reagents were purchased from Aldrich, Lancaster or Fisher chemical companies and used as received.

4.2. General procedure for amination of the pyrroloiminoquinone derivative 8

To a solution of pyrroloiminoquinone derivative **8** (1 equiv) in anhydrous MeOH, a solution of amine **9** (1.2 equiv) in anhydrous MeOH was added dropwise over a period of 10–15 min. The resulting solution was stirred at RT for 16–36 h. TLC analysis (MeOH/$CHCl_3$, 1/9) revealed that the reaction was complete. The reaction mixture was cooled to 0 °C and quenched by adding trifluoroacetic acid (2.2 equiv). The reaction mixture was allowed to attain room temperature at which it was stirred for 30–45 min. The solvent was removed under reduced pressure and the residue was passed through a pad of silica gel with MeOH/$CHCl_3$ (1/15) as eluent to furnish the crude product **10**. The crude product **10** as such was subjected to detosylation without further purification.

4.3. General procedure for detosylation

To a solution of *N*-tosyl makaluvamine derivatives **10** (1 equiv) in anhydrous MeOH, sodium methoxide (20 equiv) was added and stirred at room temperature for 4–6 h. TLC analysis (MeOH/$CHCl_3$, 1/9) revealed that the deprotection was complete. The resulting solution was quenched at 0 °C with trifluoroacetic acid (30 equiv) and stirred further at RT for 30 min. The solvent was completely removed under reduced pressure and the residue obtained was co-evaporated three times with $CHCl_3$ to remove the traces of TFA. The crude compound thus obtained was purified by chromatography over a column of Si gel (20 × 2 cm) using MeOH/$CHCl_3$ (1/10) as eluent to afford the pure detosylated makaluvamine analogs **4**, **5**, **6a**–**g** or **7a**–**g** (30–51% overall yield for two steps starting from the compound **8**).

4.3.1. 7-(Benzylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1*H*)-one (4). Following the typical procedure, pyrroloiminoquinone **8** (80 mg, 0.17 mmol) and benzyl amine (**6c**) (22.0 mg, 0.20 mmol) in anhydrous MeOH (15 mL) were stirred at room temperature for 20 h to furnish 48 mg (0.09 mmol) of tosyl compound which was detosylated using NaOMe (97 mg, 1.80 mmol) in MeOH (8 mL) to furnish compound **4** (28 mg, 42%). ^{1}H NMR (CD_3OD) δ 2.92 (t, 2H, J = 7.8 Hz), 3.80 (t, 2H, J = 7.8 Hz), 4.58 (s, 2H), 5.38 (s, 1H), 7.13 (s, 1H), and 7.20–7.45 (m, 5H); ^{13}C NMR (CD_3OD) δ 19.4, 44.2, 48.0, 86.3, 120.2, 123.7, 125.7, 127.1, 128.3 (2C), 128.9, 130.0 (2C), 137.2, 155.2, 159.9, and 168.8; MS (ES^+) *m*/*z* 278 (M^+); HRMS (EI at 70 eV) *m*/*z*. Found: 277.1218; calcd for $C_{17}H_{15}N_3O$: 277.1215 (M^+−H).

4.3.2. 1,3,4,8-tetrahydro-7-(phenethylamino)pyrrolo[4,3,2-de]-quinolin-8(1*H*)-one (5). Following the typical procedure, pyrroloiminoquinone **8** (80 mg, 0.17 mmol) and phenethyl amine (24.5 mg, 0.20 mmol) in anhydrous MeOH (15 mL) were stirred at room temperature for 20 h to furnish 47 mg (0.08 mmol) of tosyl compound which was detosylated using NaOMe (91 mg, 1.68 mmol) in MeOH (8 mL) to furnish compound **5** (27 mg, 39%). ^{1}H NMR (CD_3OD) δ 2.89–3.05 (m, 4H), 3.60 (t, 2H, J = 7.2 Hz), 3.83 (t, 2H, J = 7.5 Hz), 5.41 (s, 1H), 7.14 (s, 1H), and 7.20–7.40 (m, 5H); ^{13}C NMR (CD_3OD) δ 19.5, 35.1, 44.1, 46.2, 85.2, 120.2, 123.9, 125.5, 127.2, 127.9, 129.8 (2C), 129.9 (2C), 139.4, 155.0, 159.7, and 168.5; MS (ES^+) *m*/*z* 292 (M^+). HRMS (EI at 70 eV) *m*/*z*. Found: 291.1371; calcd for $C_{18}H_{17}N_3O$: 291.1371 (M^+−H).

4.3.3. 7-(4-Methylbenzylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1*H*)-one (6a). Following the typical procedure, pyrroloiminoquinone **8** (80 mg, 0.17 mmol)

and 4-methylbenzyl amine (25 mg, 0.20 mmol) in anhydrous MeOH (15 mL) were stirred at room temperature for 15 h to furnish 53 mg (0.095 mmol) of tosyl compound which was detosylated using NaOMe (103 mg, 1.9 mmol) in MeOH (8 mL) to furnish compound **6a** (32 mg, 46%). ^{1}H NMR (CD_3OD) δ 2.29 (s, 3H), 2.91 (t, 2H, J = 7.6 Hz), 3.79 (t, 2H, J = 7.6 Hz), 4.51 (s, 2H), 5.37 (s, 1H), 7.12 (s, 1H), 7.13–7.24 (m, 4H); ^{13}C NMR (CD_3OD) δ 19.4, 21.1, 44.2, 47.8, 86.3, 120.2, 123.7, 125.6, 127.1, 128.4 (2C), 130.6 (2C), 134.1, 138.9, 155.1, 159.8, and 168.8; MS (ES^+) *m*/*z* 292 (M^+); HRMS (EI at 70 eV) *m*/*z*. Found: 291.1379; calcd for $C_{18}H_{17}N_3O$: 291.1372 (M^+-H).

4.3.4. 7-(4-Methoxybenzylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1*H*)-one (6b). Following the typical procedure, pyrroloiminoquinone **8** (80 mg, 0.17 mmol) and 4-methoxybenzyl amine (28 mg, 0.20 mmol) in anhydrous MeOH (15 mL) were stirred at room temperature for 16 h to furnish 48 mg (0.083 mmol) of tosyl compound which was detosylated using NaOMe (90 mg, 1.67 mmol) in MeOH (8 mL) to furnish the compound **6b** (31 mg, 43%). ^{1}H NMR (CD_3OD) δ 2.92 (t, 2H, J = 7.6 Hz), 3.75 (s, 3H), 3.80 (t, 2H, J = 8.0 Hz), 4.49 (s, 2H), 5.40 (s, 1H), 6.89 (d, 2H, J = 6.8 Hz), 7.12 (s, 1H), 7.23 (d, 2H, J = 6.8 Hz); ^{13}C NMR (CD_3OD) δ 19.4, 44.1, 47.5, 55.7, 86.2, 115.3 (2C), 120.2, 123.7, 125.6, 127.1, 129.0, 129.8 (2C), 155.0, 159.8, 160.9, and 168.8; MS (ES^+) *m*/*z* 308 (M^+); HRMS (EI at 70 eV) *m*/*z*. Found: 307.1317; calcd for $C_{18}H_{17}N_3O_2$: 307.1321 (M^+-H).

4.3.5. 7-(3,4-Dimethoxybenzylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1*H*)-one (6c). Following the typical procedure, pyrroloiminoquinone **8** (80 mg, 0.17 mmol) and veratryl amine (34 mg, 0.20 mmol) in anhydrous MeOH (15 mL) were stirred at room temperature for 20 h to furnish 46 mg (0.076 mmol) of tosyl compound which was detosylated using NaOMe (82 mg, 1.52 mmol) in MeOH (8 mL) to furnish compound **6c** (31 mg, 40%). ^{1}H NMR (CD_3OD) δ 2.93 (t, 2H, J = 7.8 Hz), 3.80 (s, 3H), 3.81 (s, 3H), 3.75–3.85 (m, 2H), 4.51 (s, 2H), 5.42 (s, 1H), 6.75–6.95 (m, 3H), 7.14 (s, 1H); ^{13}C NMR (CD_3OD) δ 19.5, 44.2, 47.9, 56.5 (2C), 86.3, 112.3, 113.1, 120.2, 121.0, 123.7, 125.6, 127.1, 129.8, 150.3, 150.9, 155.1, 159.9, and 168.8; MS (ES^+) *m*/*z* 338 (M^+); HRMS (EI at 70 eV) *m*/*z*. Found: 337.1437; calcd for $C_{19}H_{19}N_3O_3$: 337.1426 (M^+-H).

4.3.6. 7-(3,4,5-Trimethoxybenzylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1*H*)-one (6d). Following the typical procedure, pyrroloiminoquinone **8** (80 mg, 0.17 mmol) and 3,4,5-trimethoxybenzyl amine (40 mg, 0.20 mmol) in anhydrous MeOH (15 mL) were stirred at room temperature for 16 h to furnish 41 mg (0.064 mmol) of tosyl compound which was detosylated using NaOMe (70 mg, 1.29 mmol) in MeOH (8 mL) to furnish compound **6d** (28 mg, 34%). ^{1}H NMR (CD_3OD) δ 2.93 (t, 2H, J = 7.5 Hz), 3.72 (s, 3H), 3.81 (s, 6H), 3.82 (t, 2H, J = 7.5 Hz), 4.51 (s, 2H), 5.41 (s, 1H), 6.64 (s, 2H), 7.14 (s, 1H); ^{13}C NMR (CD_3OD) δ 19.4, 44.2, 48.6, 56.6 (2C), 61.0, 86.4, 105.6 (2C), 120.2, 123.7, 125.7, 127.1, 133.3, 138.6, 155.0 (2C), 155.1, 159.9, and 168.8; MS (ES^+) *m*/*z* 368 (M^+); HRMS (EI at 70 eV) *m*/*z*. Found: 367.1534; calcd for $C_{20}H_{21}N_3O_4$: 367.1532 (M^+-H).

4.3.7. 7-(3,4-Methylenedioxybenzylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1*H*)-one (6e). Following the typical procedure, pyrroloiminoquinone **8** (80 mg, 0.17 mmol) and piperonyl amine (31 mg, 0.20 mmol) in anhydrous MeOH (15 mL) were stirred at room temperature for 16 h to furnish 46 mg (0.08 mmol) of tosyl compound which was detosylated using NaOMe (84 mg, 1.56 mmol) in MeOH (8 mL) to furnish compound **6e** (31 mg (42%). ^{1}H NMR (CD_3OD) δ 2.93 (t, 2H, J = 7.5 Hz), 3.82 (t, 2H, J = 7.5 Hz), 4.48 (s, 2H), 5.42 (s, 2H), 5.92 (s, 2H), 6.75–6.83 (m, 3H), 7.14 (s, 1H); ^{13}C NMR (CD_3OD) δ 19.4, 44.2, 47.8, 86.3, 102.6, 108.8, 109.4, 120.2, 122.0, 123.7, 125.7, 127.1, 130.9, 148.8, 149.7, 155.0, 159.9, and 168.8; MS (ES^+) *m*/*z* 322 (M^+); HRMS (EI at 70 eV) *m*/*z*. Found: 321.1122; calcd for $C_{18}H_{15}N_3O_3$: 321.1113 (M^+-H).

4.3.8. 7-(4-Chlorobenzylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1*H*)-one (6f). Following the typical procedure, pyrroloiminoquinone **8** (80 mg, 0.17 mmol) and 4-chlorobenzyl amine (29 mg, 0.20 mmol) in anhydrous MeOH (15 mL) were stirred at room temperature for 36 h to furnish 42 mg (0.072 mmol) of tosyl compound which was detosylated using NaOMe (78 mg, 1.45 mmol) in MeOH (8 mL) to furnish compound **6f** (23 mg, 32%). ^{1}H NMR (CD_3OD) δ 2.93 (t, 2H, J = 7.5 Hz), 3.82 (t, 2H, J = 7.5 Hz), 4.57 (s, 2H), 5.35 (s, 1H), 7.14 (s, 1H), and 7.21–7.42 (m, 4H); ^{13}C NMR (CD_3OD) δ 19.4, 44.2, 47.2, 86.5, 120.2, 123.6, 125.5, 127.1, 129.9 (2C), 130.0 (2C), 134.7, 136.1, 155.1, 160.0, and 168.8; MS (ES^+) *m*/*z* 312 (M^+); HRMS (EI at 70 eV) *m*/*z*. Found: 311.0826; calcd for $C_{17}H_{14}ClN_3O$: 311.0825 (M^+-H).

4.3.9. 7-(4-Fluorobenzylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1*H*)-one (6g). Following the typical procedure, pyrroloiminoquinone **8** (80 mg, 0.17 mmol) and 4-fluorobenzyl amine (26 mg, 0.20 mmol) in anhydrous MeOH (15 mL) were stirred at room temperature for 36 h to furnish 44 mg (0.078 mmol) of tosyl compound which was detosylated using NaOMe (84 mg, 1.56 mmol) in MeOH (8 mL) to furnish compound **6g** (22 mg, (31%). ^{1}H NMR (CD_3OD) δ 2.94 (t, 2H, J = 7.7 Hz), 3.82 (t, 2H, J = 7.7 Hz), 4.57 (s, 2H), 5.38 (s, 1H), 7.01–7.19 (m, 3H), and 7.29–7.40 (m, 2H); ^{13}C NMR (CD_3OD) δ 19.4, 44.2, 47.2, 86.3, 116.5 and 116.8 (C–F coupling), 120.2, 123.6, 125.7, 127.1, 130.3, and 130.4 (C–F coupling), 133.3, 155.1, and 160.0 (C–F coupling), 165.4, 168.8, 173.2; MS (ES^+) *m*/*z* 296 (M^+); HRMS (EI at 70 eV) *m*/*z*. Found: 295.1114; calcd for $C_{17}H_{14}FN_3O$: 295.1121 (M^+-H).

4.3.10. 7-(4-Methylphenethylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1*H*)-one (7a). Following the typical procedure, pyrroloiminoquinone **8** (80 mg, 0.17 mmol) and 4-methylphenethyl amine (27.6 mg, 0.20 mmol) in anhydrous MeOH (15 mL) were stirred at room temperature for 15 h to furnish 49 mg (0.10 mmol) of tosyl compound which was detosylated using NaOMe (112 mg, 2.07 mmol) in MeOH (8 mL)

to furnish compound **7a** (29 mg, 41%). ^{1}H NMR (CD_3OD) δ 2.28 (s, 3H), 2.85–3.00 (m, 4H), 3.56 (t, 2H, J = 7.2 Hz), 3.83 (t, 2H, J = 7.2 Hz), 5.39 (s, 1H), 7.05–7.15 (m, 4H), 7.13 (s, 1H); ^{13}C NMR (CD_3OD) δ 19.5 and 21.0, 34.7, 44.1, 46.3, 85.2, 120.2, 123.9, 125.5, 127.2, 129.7 (2C), 130.3 (2C), 136.2, 137.5, 155.0, 159.7, 168.5; MS (ES^+) m/z 306 (M^+); HRMS (EI at 70 eV) m/z. Found: 305.1518; calcd for $C_{19}H_{19}N_3O$: 305.1528 (M^+-H).

4.3.11. 7-(4-Methoxyphenethylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1*H*)-one (7b). Following the typical procedure, pyrroloiminoquinone **8** (80 mg, 0.17 mmol) and 4-methoxyphenethyl amine (31 mg, 0.20 mmol) in anhydrous MeOH (15 mL) were stirred at room temperature for 16 h to furnish 59 mg (0.10 mmol) of tosyl compound which was detosylated using NaOMe (108 mg, 2.0 mmol) in MeOH (8 mL) to furnish compound **7b** (38 mg, 51%). ^{1}H NMR (CD_3OD) δ 2.81–3.00 (m, 4H), 3.54 (t, 2H, J = 7.5 Hz), 3.73 (s, 3H), 3.82 (t, 2H, J = 7.2 Hz), 5.37 (s, 1H), 6.83 (d, 2H, J = 8.1 Hz), 7.13 (s, 1H), 7.15 (d, 2H, J = 8.1 Hz); ^{13}C NMR (CD_3OD) δ 19.5, 34.3, 44.1, 46.4, 55.6, 85.2, 115.1 (2C), 120.2, 123.9, 125.4, 127.2, 130.8 (2C), 131.2, 155.0, 159.6, 160.1, and 168.5; MS (ES^+) m/z 322 (M^+); HRMS (EI at 70 eV) m/z. Found: 321.1465; calcd for $C_{19}H_{19}N_3O_2$: 321.1477 (M^+-H).

4.3.12. 7-(3,4-Dimethoxyphenethylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1*H*)-one (7c). Following the typical procedure, pyrroloiminoquinone **8** (80 mg, 0.17 mmol) and 3,4-dimethoxyphenethyl amine (37 mg, 0.20 mmol) in anhydrous MeOH (15 mL) were stirred at room temperature for 16 h to furnish 51 mg (0.082 mmol) of tosyl compound which was detosylated using NaOMe (89 mg, 1.65 mmol) in MeOH (8 mL) to furnish compound **7c** (41 mg, 52%). ^{1}H NMR (CD_3OD) δ 2.85–3.00 (m, 4H), 3.57 (t, 2H, J = 7.2 Hz), 3.77 (s, 3H), 3.78 (s, 3H), 3.82 (t, 2H, J = 7.5 Hz), 5.36 (s, 1H), 6.72–6.91 (m, 3H), 7.14 (s, 1H); ^{13}C NMR (CD_3OD) δ 19.5, 34.7, 44.1, 46.3, 56.3, 56.4, 85.2, 113.2, 113.7, 120.2, 122.2, 123.9, 125.4, 127.2, 132.1, 149.4, 150.6, 155.0, 159.6, and 168.5; MS (ES^+) m/z 352 (M^+); HRMS (EI at 70 eV) m/z. Found: 351.1571; calcd for $C_{20}H_{21}N_3O_3$: 351.1583 (M^+-H).

4.3.13. 7-(3,4-Dichlorophenethylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1*H*)-one (7d). Following the typical procedure, pyrroloiminoquinone **8** (80 mg, 0.17 mmol) and 3,4-dichlorophenethyl amine (39 mg, 0.20 mmol) in anhydrous MeOH (15 mL) were stirred at room temperature for 36 h to furnish 46 mg (0.073 mmol) of tosyl compound, which was detosylated using NaOMe (79 mg, 1.46 mmol) in MeOH (8 mL) to furnish compound **7d** (24 mg, 30%). ^{1}H NMR (CD_3OD) δ 2.89–3.02 (m, 4H), 3.60 (t, 2H, J = 7.0 Hz), 3.85 (t, 2H, J = 7.5 Hz), 5.40 (s, 1H), 7.15 (s, 1H), 7.19 (dd, 1H, J_1 = 2.2 Hz, J_2 = 7.9 Hz), and 7.41–7.48 (m, 2H). ^{13}C NMR (CD_3OD) δ 19.5, 34.1, 44.2, 45.5, 85.4, 120.2, 123.8, 125.5, 127.2, 130.0, 131.8 (2C), 132.0, 133.4, 140.4, 155.1, 159.8, and 168.5. MS (ES^+) m/z 360 (M^+). HRMS (EI at 70 eV) m/z. Found: 359.0583; calcd for $C_{18}H_{15}Cl_2N_3O$: 359.0592 (M^+-H).

4.3.14. 7-(3,4-Methylenedioxyphenethylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1*H*)-one (7e). Following the typical procedure, pyrroloiminoquinone **8** (80 mg, 0.17 mmol) was stirred in anhydrous MeOH (8 mL) and a solution of 3,4-methylenedioxyphenethylamine hydrochloride (41 mg, 0.20 mmol) and triethylamine (0.03 mL, 0.25 mmol) in anhydrous MeOH (7 mL) were added to it dropwise. The resulting reaction mixture was stirred at room temperature for 24 h to furnish 57 mg (0.094 mmol) of tosyl compound which was detosylated using NaOMe (102 mg, 1.89 mmol) in MeOH (8 mL) to furnish compound **7e** (34 mg, 45%).^{1}H NMR (CD_3OD) δ 2.88 (t, 2H, J = 7.8 Hz), 2.94 (t, 2H, J = 7.5 Hz), 3.55 (t, 2H, J = 7.2 Hz), 3.84 (t, 2H, J = 7.8 Hz), 5.40 (s, 1H), 5.89 (s, 2H), 6.60–6.80 (m, 3H), 7.14 (s, 1H); ^{13}C NMR (CD_3OD) δ 19.5, 34.8, 44.1, 46.3, 85.2, 102.3, 109.3, 110.0, 120.2, 123.0, 123.9, 125.4, 127.2, 133.0, 147.9, 149.3, 155.0, 159.7, and 168.5; MS (ES^+) m/z 336 (M^+); HRMS (EI at 70 eV) m/z. Found: 335.1276; calcd for $C_{19}H_{17}N_3O_3$: 335.1270 (M^+-H).

4.3.15. 7-(4-Chlorophenethylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1*H*)-one (7f). Following the typical procedure, pyrroloiminoquinone **8** (80 mg, 0.17 mmol) and *p*-chlorophenethyl amine (32 mg, 0.20 mmol) in anhydrous MeOH (15 mL) were stirred at room temperature for 24 h to furnish 48 mg (0.081 mmol) of tosyl compound which was detosylated using NaOMe (88 mg, 1.62 mmol) in MeOH (8 mL) to furnish compound **7f** (28 mg, 37%). ^{1}H NMR (CD_3OD) δ 2.82–3.00 (m, 4H), 3.50–3.69 (m, 2H), 3.85 (t, 2H, J = 7.5 Hz), 5.38 (s, 1H), 7.15 (s, 1H), 7.16–7.33 (m, 4H); ^{13}C NMR (CD_3OD) δ 19.5, 34.4, 44.2, 45.9, 85.3, 120.2, 123.8, 125.5, 127.2, 129.8 (2C), 131.5 (2C), 133.8, 138.2, 155.1, 159.8, and 168.5; MS (ES^+) m/z 326 (M^+); HRMS (EI at 70 eV) m/z. Found: 325.0974; calcd for $C_{18}H_{16}ClN_3O$: 325.0982 (M^+-H).

4.3.16. 7-(4-Fluorophenethylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1*H*)-one (7g). Following the typical procedure, pyrroloiminoquinone **8** (80 mg, 0.17 mmol) and 4-fluorophenethyl amine (28 mg, 0.20 mmol) in anhydrous MeOH (15 mL) were stirred at room temperature for 36 h to furnish 53 mg (0.092 mmol) of tosyl compound which was detosylated using NaOMe (99 mg, 1.84 mmol) in MeOH (8 mL) to furnish compound **7g** (26 mg, (36%). ^{1}H NMR (CD_3OD) δ 2.89–3.01 (m, 4H), 3.58 (t, 2H, J = 6.9 Hz), 3.84 (t, 2H, J = 7.5 Hz), 5.39 (s, 1H), 6.95–7.06 (m, 2H), 7.15 (s, 1H), and 7.20–7.34 (m, 2H); ^{13}C NMR (CD_3OD) δ 19.5, 34.2, 44.2, 46.1, 85.2, 116.2, and 116.5 (C–F coupling), 120.2, 123.9, 125.5, 127.2, 131.6 and 131.7 (C–F coupling), 135.4, 155.1, and 159.8 (C–F coupling), 161.7, 164.9, and 168.5; MS (ES^+) m/z 310 (M^+); HRMS (EI at 70 eV) m/z. Found: 309.1270; calcd for $C_{18}H_{16}FN_3O$: 309.1277 (M^+-H).

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