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The Synthesis and Biological Evaluation of Alkyl and Benzyl Naphthyridinium Analogs of Eupolauridine as Potential Antimicrobial and Cytotoxic Agents

Shahriyar Taghavi-Moghadam,[1] Cecil D. Kwong,[1] John A. Secrist III,[1*] Shabana I. Khan,[2] and Alice M. Clark[2]

[1]Organic Chemistry Department, Southern Research Institute, 2000 Ninth Avenue South, P.O. Box 55305, Birmingham, AL 35255 and [2]National Center for Natural Products Research, and Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677

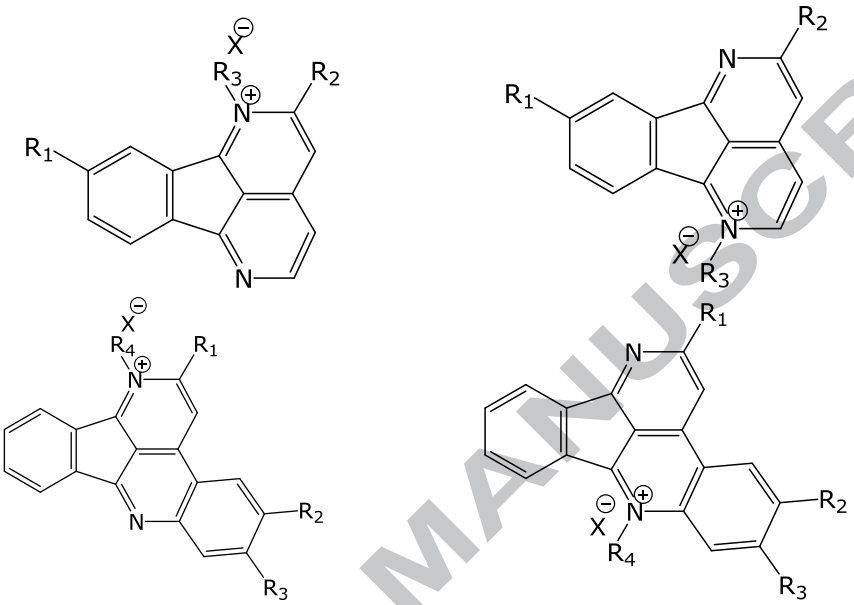
*Corresponding author: secrist@southernresearch.org

Abstract

Eupolauridine, an indenonaphthyridine alkaloid, has been previously reported by us to exhibit antifungal activity. This study describes the synthesis of new alkyl and benzyl naphthyridinium/pyridinium analogs of the eupolauridine as potential antifungal agents. A majority of the analogs exhibited antifungal activity against opportunistic pathogens such as Candida albicans and Cryptococcus neoformans. Several of them were also effective against bacteria (S. aureus, MRS, Pseudomnas and Mycobacterium) and the malaria parasite (Plasmodium falciparum) to variable extents. A number of analogs were also cytotoxic to human cancer cell lines.

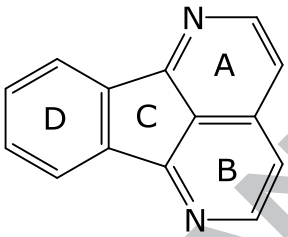
Graphical Abstract

A series of alkyl and benzyl naphthyridinium analogs of the natural product eupolauridine were prepared and evaluated for their antifungal, antibacterial and antimalarial activities. Several compounds showed antifungal, antibacterial and antimalarial activity to varying degrees. Some of them were cytotoxic to human cancer cell lines.



1. Introduction

Eupolauridine is an indenonaphthyridine alkaloid that has been shown to have *in vitro* efficacy against fungal pathogens namely *Candida albicans*, *Cryptococcus neoformans*, and Aspergillus species.[1-4] This natural product, isolated from *Eupomatia laurina* and from *Cleistopholis patens* trees found in North Queensland and West Africa, respectively,[5,6] has also shown *in vivo* efficacy in a murine model of systemic candidiasis.[5] It is nontoxic towards mammalian cell lines and to mice,[4,6] shows no activity against mammalian DNA polymerase α.[7] It inhibits the catalytic activity of *Candida* topoisomerase I (TopoI), completely inhibiting the DNA relaxation activity of purified TopoI at 50 μg/mL.[4] Additionally, it has also shown promising anti-toxoplasma activity in human fibroblast host cells.[8]



Eupolauridine

Our interest in developing new eupolauridine-based antifungal and antibacterial agents was inspired by these biological activities. Since there was little information related to its mechanism of action and no SAR data was available for this compound, we pursued a systematic SAR approach towards our goal of enhancing its biological activity. We have prepared and evaluated a variety of substituents on the A, B. and D rings. Herein we present our results on a series of alkyl and benzyl naphthyridinium derivatives, compounds that should enhance aqueous solubility while maintain the main structural features of eupolauridine. In this paper, we report the synthesis of a series of eupolauridine analogs in which one of the naphthyridine/pyridine rings has been N-alkylated with various alkyl and benzyl groups, producing the corresponding eupolauridinium compounds. The synthesis of *N*-aminoeupolauridine is also reported.

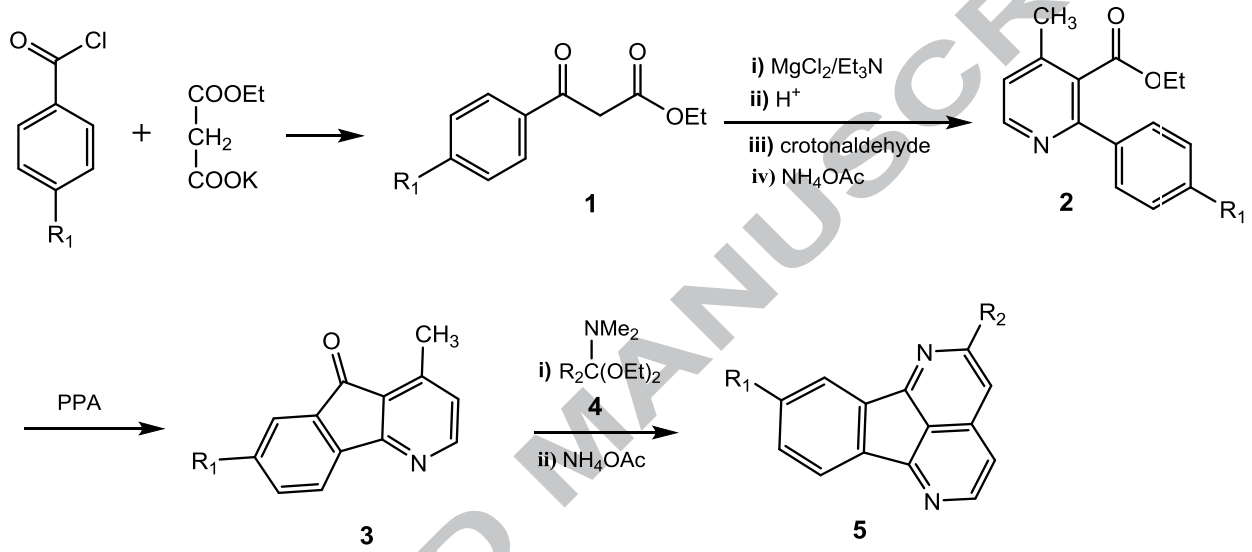
All the newly synthesized analogs were screened for antifungal, antibacterial, and antimalarial activities against a panel of representative pathogenic organisms. They were also screened for anti-cellproliferative activity against four human solid tumor cell lines. DNA topoisomerases are the enzymes that play important role in DNA replication by regulating the topological state of DNA. These enzymes are considered as targets of a number of anti-infective and anticancer agents, and we wished to determine if a topoisomerase played any role in the mechanism of action of eupolauridine derivatives. To further explore if the addition of alkyl and benzyl naphthyridinium moieties affected the TopoI inhibitory activity of the parent compound, newly synthesized analogs were also screened for inhibition of catalytic activity of both fungal and human TopoI.

2. Results and Discussion

2.1. Chemistry

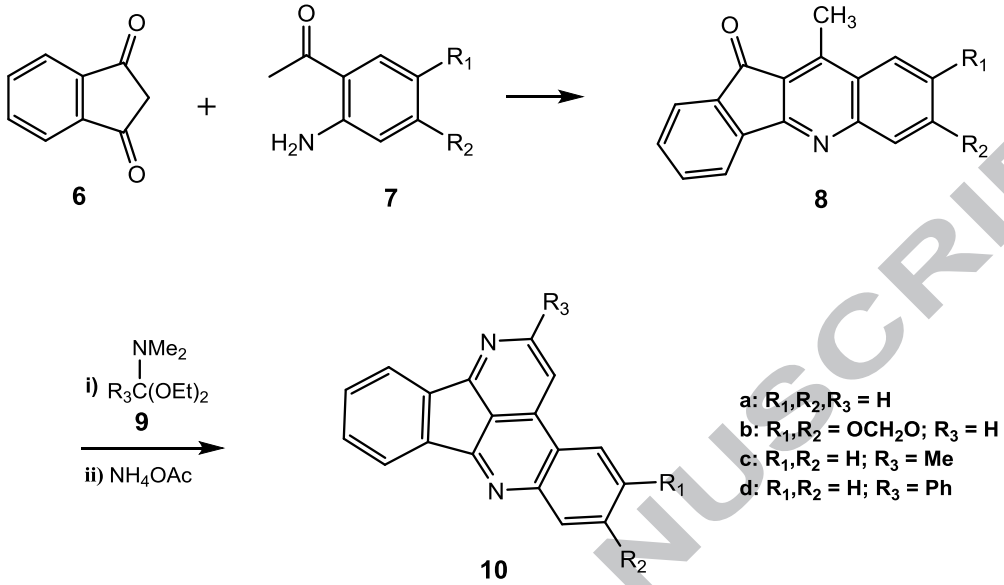
The various alkyl- and benzyl- eupolauridinium targets described in this paper were synthesized from eupolauridine (**5a**)[9] or from other eupolauridine analogs from the literature or prepared in our laboratories including A- and D-ring substituted analogs **5b-f**[10] and benzoannulated analogs **10a-d,**[11] whose syntheses are shown in Schemes 1 and 2.

Scheme 1



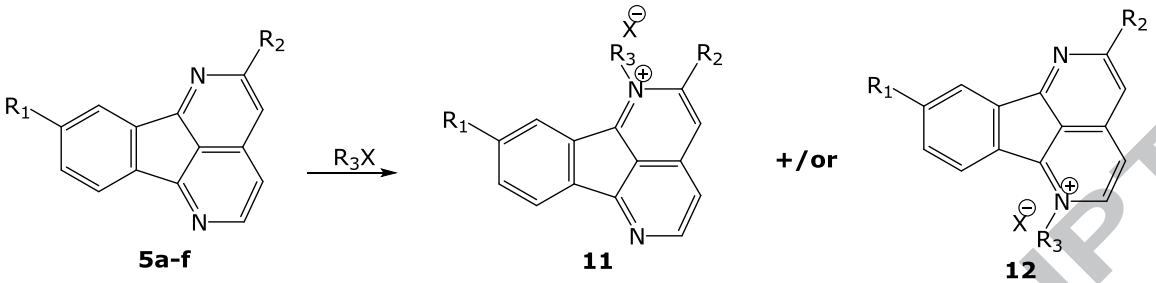
a: R_1 = R_2 = H (eupolauridine)
b: R_1 = F; R_2 = H
c: R_1 = OMe; R_2 = H
d: R_1 = Me; R_2 = H
e: R_1 = H; R_2 = Me
f: R_1 = H; R_2 = Ph

Scheme 2



The initial targets within this series were analogs of the known compound, 1-*N*-methyleupolauridinium iodide **11a**[12] but with different sized groups replacing methyl, including 1-*N*-ethyl and 1-*N*-benzyl eupolauridinium analogs **11b** and **11c**, the corresponding 2-methyl and 2-phenyleupolauridine-based analogs **12p** and **12r**, and benzoeupolauridinium analogs **13a**, **14f**, and **13i**. As shown in Schemes 3 and 4, these compounds were prepared by treating eupolauridines **5a, 5e,** and **5f** and benzoeupolauridines **10a, 10b,** and **10d** with the appropriate alkyl or benzyl halides. After good antifungal and antibacterial activities were seen in the antifungal and antibacterial assays of these analogs (see biological section for details), our study was expanded to determine if substituent groups on the benzyls or on the D-ring could further enhance these activities. Schemes 3 and 4 also show these eupolauridinium targets, which were prepared by reacting **5a-f** and benzoannulated analogs **10a-d** with the appropriate alkyl or benzyl halides or tosylate. We also prepared *N*-aminoeupolauridine **11w**, which was obtained in 15% overall yield by treating eupolauridine with O-mesitylenesulfonyl-hydroxylamine,[13-16] as is also shown in Scheme 3.

Scheme 3



Precursor	Product	R_1	R_2	R_3	X
5a	**11a**	H	H	CH_3	I
5a	**11b**	H	H	CH_3CH_2	I
5a	**11c**	H	H	$C_6H_5CH_2$	Br
5a	**11d**	H	H	p-F-$C_6H_4CH_2$	Br
5a	**11e**	H	H	m-Cl-$C_6H_4CH_2$	Br
5a	**11f**	H	H	p-Br-$C_6H_4CH_2$	Br
5a	**11g**	H	H	o,p-F_2-$C_6H_4CH_2$	Br
5a	**11h**	H	H	p-NO_2-$C_6H_4CH_2$	Br
5a	**11i**	H	H	p-CH_3-$C_6H_4CH_2$	Br
5a	**11j**	H	H	p-CF_3-$C_6H_4CH_2$	Br
5a	**11k**	H	H	3,5-$(OCH_3)_2$-$C_6H_4CH_2$	Br
5a	**11l**	H	H	$C_{10}H_7CH_2$(naphth-2-yl)	Br
5d	**11m, 12m***	CH_3	H	$C_6H_5CH_2$	Br
5c	**11n, 12n***	OCH_3	H	$C_6H_5CH_2$	Br
5b	**11o, 12o***	F	H	$C_6H_5CH_2$	Br
5e	**12p**	H	CH_3	CH_3	I
5e	**12q**	H	CH_3	$C_6H_5CH_2$	Br
5f	**12r**	H	C_6H_5	CH_3	I
5f	**12s**	H	C_6H_5	$C_6H_5CH_2$	Br
5a	**11t**	H	H	$CH_3CH_2OCOCH_2$	Br
5a	**11u**	H	H	$C_6H_5COCH_2$	Br
5a	**11v**	H	H	H_2NCOCH_2	Br
5a	**11w**	H	H	H_2N	Mes**

*) Mixture of both N(1)- and N(6)-isomers (**11** and **12**, respectively) in a ~1:1 ratio.

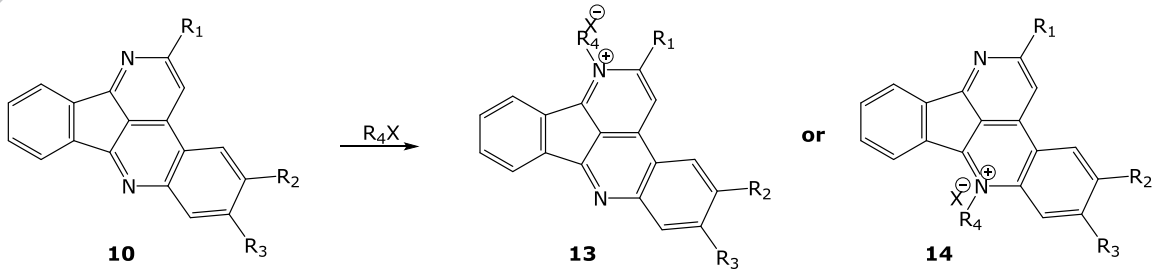
**) Mesitylene sulfonate salt. Prepared by the reaction of eupolauridine with freshly prepared O-mesitylenesulfonylhydroxylamine. See experimental section for details.

Table 1:
Antimicrobial activity of alkyl and benzyl naphthyridinium analogs of eupolauridine (IC_{50} and MIC values are in µg/ml)

	C.albicans		*C.neoformans*		*S. aureus*	MRS	P. auruginosa	A. fumigatus	M.intracellulare
	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	IC_{50}	IC_{50}	MIC	MIC
eupo	5.25	12.5	4.8	25			5	2	
11a	NA		10	50					
11b	35	50	13.33	25	40				
11c	4.17	6.25	0.43	1.56					
11d	9.33	12.5	0.87	3.12					
11e	3	6.25	0.18	0.78					50
11f	2.5	6.25	0.3	0.78			40		50
11g	5.33	6.25	0.58	1.56					
11h	NA		1.19	6.25					50
11i	3.17	6.25	0.33	0.78		35			50
11j	6.5	12.5	0.57	3.12	30		27		50
11k	20	25	0.92	3.12		9			50
11l	0.67	0.78	0.3	0.39	20	22	30		50
11m	6.17	12.5	0.48	1.56					50
11n	16.67	25	0.7	1.56					50
11o	1.5	3.12	0.52	0.78					50
12p	NA		8	50					
12q	13.33	25	0.82	1.56					
12r	0.32	0.39	0.4	0.78	20	20	25		
12s	1.5	3.12	0.95	3.12					
11t	NA	NA	NA	NA					
11u	NA	NA	6.18	25					
11v	NA	NA	30	50					
11w	NA	NA	19.67	50					
13a	23.3	25	2	6.25		20			
13b	16.67	25	6	12.5			50		
13c	NA	NA	2.13	12.5	5	35	4		10
13d	35	NA	1.93	6.25			50		
13e	25	50	1.83	3.12		25			
14f	0.2	0.39	0.23	0.78	4.5	5	4		50
14g	0.04	0.098	0.17	0.39	2	2	2		10
13h	NA		NA						
13i	16.1	25	0.37	1.56					50
13j	5.17	12.5	0.57	3.12					

Amphotericin B was used as control drug which showed an MIC of 0.156 µg/mL for *C.albicans*, 0.078 µg/mL for *C. neoformans* and 1.25 µg/mL for *A. fumigatus*. IC_{50} is the concentration that causes a growth inhibition of 50%. MIC (Minimum Inhibitory Concentration) is the concentration that inhibits 80% growth. NA = not active at the highest concentration tested, 50 µg/mL.

Scheme 4



Precursor	Product	R_1	R_2, R_2	R_4	X
10a	**13a**	H	H	CH_3	I
10a	**13b**	H	H	CH_3CH_2	I
10a	**13c**	H	H	$C_6H_5CH_2$	Br
10a	**13d**	H	H	CH_3	TsO*
10c	**13e**	CH_3	H	CH_3	I
10d	**14f**	C_6H_5	H	CH_3	I
10d	**14g**	C_6H_5	H	CH_3CH_2	Br
10a	**13h**	H	H	$CH_3CH_2OCOCH_2$	Br
10b	**13i**	H	-OCH_2O-	CH_3	I
10b	**13j**	H	-OCH_2O-	$C_6H_5CH_2$	Br

*) *p*-Toluenesulfonate salt.

2.2. Biological Evaluation

Out of a total of 33 alkyl and benzyl naphthyridinium analogs of eupolauridine, 12 analogs (**11c, 11e-g, 11i, 11l, 11o, 12r-s, 14f-g and 13j**) exhibited antifungal activity toward *Candida albicans* with IC_{50} values of <5 µg/ml (Table 1) and they were more potent than eupolauridine (IC_{50} 5.25 µg/mL) except 11c, 11g and 13j. Among them four analogs (**11l, 12r, 14f and 14g**) showed the most potent activity with IC_{50} values of 0.67, 0.32, 0.2 and 0.04 µg/mL and MIC values in the range of 0.098 – 0.78 µg/mL, respectively. The remaining analogs were either less active than eupolauridine or inactive towards *C. albicans*. Though we did not perform any detailed solubility studies, the quaternary salts did have improved solubility properties, as expected.

The alkyl and benzyl naphthyridinium analogs were also active against *Cryptococcus neoformans* except **11t** and **13h**. Interestingly, nineteen of them (**11c-g, 11i-o, 12q-s, 14f-g, and 13i-j**) were very potent with IC_{50} values < 1 µg/ml and MIC values in the range of 0.39 – 3.12 µg/mL in comparison to eupolauridine (IC_{50} 4.8 and MIC 25 µg/mL). Four others (**11h, 13a, 13c-e**) were also more effective (IC_{50} values 1-2 µg/mL) than eupolauridine.

Several analogs (**11a, 11h, 12p, 11u-w,** and **13c**) were selective for Cryptococcus with no activity towards Candida. The analog **14g** was more active towards Candida (IC_{50} 0.04 µg/mL, MIC 0.098 µg/mL) than Cryptococcus (IC_{50} 0.17 µg/mL, MIC 0.39 µg/mL). This compound was the most potent antifungal of the series with an over 100 fold increase in activity for Candida and a 30 fold increase in activity for Cryptococcus compared to the parent compound eupolauridine.

The activity data shows that most of the active analogs belong to the benzyleupolauridinium bromide series, with the exception of the nitro-analog **11h**. Methyl- and ethyleupolauridinium analogs (**11a and 11b**) were not active against

Candida but moderately active against Cryptococcus. From the diazafluoranthenium salts only **14f-g** demonstrated antifungal activities towards both organisms.

Although eupolauridine showed activity against *Aspergillus fumigatus* (MIC 2 µg/mL) none of the synthesized analogs were effective against this fungal pathogen. None of the analogs showed any significant antibacterial activities (data not shown) except **13c**, **14f-g** (IC_{50} 2-5 µg/mL).

Although eupolauridine did not exhibit any antimalarial activity (towards *Plasmodium falciparum),* benzyleupolauridinium bromide and its derivatives (Table 2) showed significant antimalarial activity. The IC_{50} values of **11d-g**, and **11I-k** were <20 ng/mL indicating a potent antimalarial activity. Among methyl-, ethyl-, and methoxy-benzyleupolauridinium salts only **11l** showed good antimalarial activity (IC_{50} 19.5 ng/mL). Phenyleupolauridinium salts were also not very active against *P. falciparum.* From the diazafluorenthenium salts, only dimethylbenzo analog **13e** exhibited antimalarial activity with an IC_{50} of 20 ng/mL.

The alkyl- and benzyl naphthyridinium analogs of eupolauridine reported in this study were also tested for their cytotoxic properties against a panel of four human cancer cell lines and one noncancerous mammalian kidney cell line in terms of their anti-cell proliferative effects (Table 3). The compounds were not cytotoxic to mammalian kidney (VERO) cells up to a concentration of 10 µg/ml except **11j** and **14g**, which showed mild cytotoxicities (IC_{50} 6.4 and 7.8 µg/ml). These concentrations are much higher than the IC_{50} values of growth inhibition of Candida, Cryptococcus and Plasmodium indicating a high selectivity index for antifungal and antimalarial activities.

Although eupolauridine was not cytotoxic to any of the cell lines, most of the analogs have shown cytotoxicity (anti-cell proliferative activity) towards four cancer cell lines included in this study, as shown in Table 3, indicating a possibility of anticancer potential of these compounds. Based on these results it is clear that addition of alkyl- and benzyl naphthyridinium moieties to eupolauridine resulted in molecules that were cytotoxic to cancer cells. This could be explained in terms of topoisomeraseII-related mechanism of action as described in an earlier study[4]. The involvement of other mechanisms of course may well be important. Further evaluation of their cytotoxicities and selectivity in animal models would be a logical next step for selecting potential analogs that could serve as lead compounds.

Table 2: Antimalarial activity of alkyl and benzyl naphthyridinium analogs of eupolauridine

	P. falciparum
	IC_{50} ng/ml
eupo	NA
11a	60
11b	47.5
11c	22.5
11d	17

11e	4.2
11f	13
11g	15
11h	35
11i	9.5
11j	8.5
11k	11
11l	19.5
11m	62
11n	32
11o	33
12p	110
12q	95
12r	460
12s	40
11t	500
11u	170
11v	90
11w	3200
13a	33
13b	33
13c	41.5
13d	38.5
13e	20
14f	NA
14g	NA
13h	560
13i	28
13j	9.4

Chloroquine was used as the control drug which showed IC_{50} <20 ng/mL. NA = not active at the highest concentration tested, 4.76 µg/mL.

Table 3: Cytotoxicity of naphthyridinium analogs

	cytotoxicity (IC_{50} µg/ml)				
	SK-MEL	KB	BT-549	SK-OV-3	VERO
eupo	NA	NA	NA	>10	>10
11a	NA	NA	NA	>10	NA
11b	NA	NA	NA	NA	NA
11c	5.4	NA	NA	1.5	NA
11d	6	>10	7.5	1.5	NA
11e	1.15	0.68	1.6	0.055	NA
11f	0.65	0.18	1.15	0.095	NA
11g	6.2	NA	NA	2.8	NA
11h	4.9	1.8	4	0.62	NA
11i	1.9	6.8	NA	<1.1	NA

11j	0.78	0.54	0.79	0.08	6.4
11k	2	7	>10	<1.1	NA
11l	<1.1	6	>10	<1.1	NA
11m	1.4	0.52	1.8	0.58	NA
11n	1.5	0.2	1.1	0.15	NA
11o	5.6	1.3	2.5	0.53	NA
12p	NA	NA	NA	NA	NA
12q	4.2	>10	3.5	1.7	NA
12r	9	7	6.2	<1.1	NA
12s	0.7	1	1.2	0.25	NA
11t	NA	NA	NA	7.6	NA
11u	NA	>10	>10	6	NA
11v	6.5	NA	NA	>10	>10
11w	>10	NA	NA	NA	NA
13a	NA	6.5	7	1.3	NA
13b	NA	6.8	>10	1.3	NA
13c	1.8	2.9	6	1.2	NA
13d	>10	4.4	7.5	1.3	NA
13e	>10	3.5	5.4	4.5	NA
14f	3.5	>10	7.5	4.5	NA
14g	1.3	6.5	8	4	7.8
13h	>10	NA	NA	5.9	NA
13i	5	4.2	1.2	1.2	NA
13j	0.55	1	1.3	0.6	NA
Doxorubicin	1.11	1.59	1.35	1.3	>10

NA = not active (cytotoxic) at the highest concentration tested, 10 µg/mL.

Eupolauridine was initially found to inhibit the DNA relaxation activity of DNA topoisomerase I and was more selective for fungal TopoI[4]. We also observed inhibition of both human and candida TopoI enzymes in our study as shown in Table 4 with a little bit higher selectivity towards the fungal enzyme in comparison to the human counterpart. Based on this observation and to establish an SAR, we screened the synthesized analogs for inhibition of TopoI isolated from *Candida albicans*[4] and compared their activity with human TopoI (commercially available). All the analogs have retained the activity of topoisomeraseI inhibition except **11o** and **11v** (Table 4). The most active compounds were **13a-e, 14f-g** and **13i-j**, with IC_{50}s of < 1 µg/ml. Interestingly, only one compound (**12r**) was much more selective for Candida TopoI inhibition over human TopoI with a selectivity index of 2.92 as compared to eupolauridine with a selectivity of 1.55 (Table 4). Most of the compounds inhibited both human and fungal topoisomerase I to similar extents. Inhibition of topoisomerase I might be responsible for the antifungal activities of these compounds but certainly involvement of other mechanisms seems likely. Compound **11o** demonstrated no topoisomerase inhibition but showed antifungal activity; similarly, compounds with no or mild antifungal activity have shown mild to moderate inhibition of topoisomerase I. Hence a true correlation between the enzyme inhibition and antifungal properties of these analogs could not be seen and this observation is in support of previous study indicating that inhibition of catalytic activity of DNA TopoI does not seem to be the principal mode of antifungal action of eupolauridine[4] which seems to be

true for the alkyl and benzyl naphthyridinium analogs synthesized in this study. Although, topoisomerase inhibition could be one of the possible contributing factors towards their antifungal activities. We have therefore selected one compound, alkyl naphthyridiniuim analog **14f**, for further mechanistic studies, which will be reported in a separate publication.[16]

Table 4: Topoisomerase I inhibition

	Topol IC_{50} (µg/ml)		Topol
	human	candida	Selectivity Index
eupo	6.5	4.2	1.55
11a	10	10	1
11b	10	15	0.67
11c	19	38	0.5
11d	40	50	0.8
11e	21	22	0.95
11f	21	22	0.95
11g	32	45	0.71
11h	18	21	0.86
11i	11	11	1
11j	30	31	0.97
11k	12.5	15	0.83
11l	21	30	0.7
11m	15	17.5	0.86
11n	5.75	5	1.15
11o	NA	NA	
12p	5	4.5	1.11
12q	11	12	0.92
12r	35	12	2.92
12s	4.3	4.4	0.98
11t	75	90	0.83
11u	50	55	0.91
11v	NA	NA	
11w	7.5	15	0.5
13a	1.5	1.8	0.83
13b	0.7	0.7	1.00
13c	0.55	0.57	0.96
13d	0.7	0.7	1
13e	0.5	0.7	0.71
14f	0.375	0.475	0.79
14g	<6	<6	
13h	2.3	2.4	0.96
13i	0.425	0.45	0.94
13j	0.52	0.52	1

NA = not active at the highest concentration tested, 10 µg/mL.

3. Conclusion

As one phase of our systematic SAR studies on modified eupolauridine analogs, we prepared a series of alkyl and benzyl naphthyridinium analogs and evaluated their biological activities. We found a number of analogs that were significantly more potent than the parent eupolauridine while also having enhanced solubilities. Compounds **14f** and **14g** were the most effective antifungal agents against both Candida and Cryptococcus, while several compounds (**13i**, **13j**, **12q**, and several of the **11** series) were more effective against Crypotococcus than Candida. Though some antimalarial activity was seen, it was relatively modest, though improved from the the phenyleupolauridinium salts. The alkyl and benzyl naphthrydinium compounds were in some cases cytotoxic to cancer cells. Evaluation of the topoisomerase I inhibitory activity of the various analogs showed some inhibition, but it appears likely that one or more other mechanisms of action are involved. We have selected **14f** and studied its mechanism of action in more detail, and those findings will be reported.[16] These results and our other SAR studies on eupolauridine derivatives modified in other ways in the A, B. and D rings demonstrate that it is possible to maintain or improve antifungal activity while incorporating modifications at a variety of sites on the eupolauridine ring system.

4. Experimental Section

4.1. General Methods

Melting points are uncorrected and were determined in open capillary tubes with a Mel-Temp melting point apparatus. ^{1}HNMR spectra were recorded on a Nicolet NT-300 NB spectrometer operating at 300.635 MHz (1H). Chemical shifts are expressed in parts per million downfield from tetramethylsilane. Spectral assignments are supported by proton decoupling. UV absorption spectra were determined on a Perkin-Elmer lambda 9 spectrometer by dissolving each compound in methanol. Numbers in parentheses are extinction coefficients (lg(ε)), [ ] = shoulder. Microanalyses were performed by the Molecular Spectroscopy Section of Southern Research Institute. Mass spectra were recorded on a Varian/MAT 311A double-focusing mass spectrometer in the fast atom bombardment (FAB) mode. HPLC analyses were carried out on a Hewlett-Packard 1100 liquid chromatograph with a Bondapak C18 column (3.9 mm x 30 cm) or Phenomenex Sphereclone 5µ ODS (1) column (250 X 4.6 mm) and diode-array detector for UV monitoring (200-350 nm). All chromatographic separations were carried out by flash chromatography using 230-400 mesh silica gel from E. Merck. TLC was carried out on Merck precoated silica gel (60 F254) sheets or on Schleicher & Schuell cellulose (F 1440/LS 254) sheets with UV detection at 254 nm and 366 nm. Preparative TLC was carried out on silica gel (60 F254, 2 mm) plates with appropriate solvents.

4.2. Synthesis

4.2.1. 1-N-Methyleupolauridinium iodide[13] (11a) (1-*N*-Methyl-indeno[1,2,3-*ij*]naphthyridinium iodide)

To a solution of eupolauridine[1,13] (100 mg, 0.49 mmol) in acetone (5 ml, anhydrous) was added methyl iodide (1 ml, 16.06 mmol), and the mixture was heated at reflux for 24 h. After cooling to room temperature, the resulting orange precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (131 mg, 77%) that was chromatographically pure. m.p: 260-265 °C dec. (lit.[13]: 267-269 °C dec.). TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.65. UV (MeOH), λ_{max}[nm] (lgε): 386 (3.92), 368 (3.89), [347 (3.54)], [310 (4.04)], 296 (4.23), 289 (4.23), [240 (4.45)], 224 (4.61). FAB-MS (m/e): 219 $(M)^+$. ^{1}H-NMR (DMSO-d_6): δ 4.66 (s, 3H, CH_3), 7.71 (t, J=7.9 Hz, 1H, Ph), 7.80 (t, J=7.4 Hz, 1H, Ph), 7.91 (d, J=5.9 Hz, 1H, Py), 8.10 (d, J=7.0 Hz, 1H, Ph), 8.29 (d, J=7.7 Hz, 1H, Ph), 8.33 (d, J=6.8 Hz, 1H, Py), 8.81 (d, J=6.8 Hz, 1H, Py), 8.99 (d, J=5.9 Hz, 1H, Py).

4.2.2. 1-N-Ethyleupolauridinium iodide (11b) (1-*N*-Ethyl-indeno[1,2,3-*ij*][2,7]naphthyridinium iodide)

To a solution of eupolauridine (100 mg, 0.49 mmol) in acetone (5 ml, anhydrous) was added ethyl iodide (1 ml, 12.50 mmol), and the mixture was heated at reflux for 24 h. After cooling to room temperature, the resulting orange precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (62 mg, 35%) as chromatographically pure material. A second fraction of the product (79 mg, 45%) was isolated by additional quantity of ethyl iodide (1 ml, 12,50 mmol) to the filtrate and further heating at reflux for 48 hours. m.p: 225 °C dec. (beginning of dec.); 246-250 °C (completely dec.). TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.71. UV (MeOH), λ_{max}[nm] (lgε): 387 (3.91), 368 (3.89), [349 (3.61)], [310 (4.06)], 296 (4.23), 289 (4.22), [240 (4.47)], 225 (4.62). FAB-MS (m/e): 233 $(M)^+$. ^{1}H-NMR (DMSO-d_6): δ 1.65 (t, J=7.3 Hz, 3H, CH_3), 4.99 (q, J=7.3 Hz, 2H, CH_2), 7.68-7.73 (dt, J=1.1, 7.7 Hz, 1H, Ph), 7.79 (t, J=7.4 Hz, 1H, Ph), 7.91 (d, J=5.9 Hz, 1H, Py), 8.10 (d, J=7.3 Hz, 1H, Ph), 8.28 (d, J=7.5 Hz, 1H, Ph), 8.39 (d, J=6.8 Hz, 1H, Py), 8.87 (d, J=6.8 Hz, 1H, Py), 8.99 (d, J=6.2 Hz, 1H, Py). Anal. Calcd for $C_{16}H_{13}IN_2$: C, 53.35; H, 3.64; N, 7.78. Found: C, 53.35; H, 3.71; N, 7.76.

4.2.3. 1-N-Benzyleupolauridinium bromide (11c) (1-*N*-Benzylindeno[1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of eupolauridine (100 mg, 0.49 mmol) in acetone (5 ml, anhydrous) was added benzyl bromide (1 ml, 8.41 mmol), and the mixture was heated at reflux for 48 h. After cooling to room temperature, the resulting yellow precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (178 mg, 97%) as chromatographically pure material. m.p: 244-246 °C (dec.). TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.75. UV (MeOH), λ_{max}[nm] (lgε): 389 (3.91), 370 (3.89), [352 (3.62)], [310 (4.10)], 297 (4.24), [288 (4.22)], [242 (4.49)], 228 (4.53). FAB-MS (m/e): 295 $(M)^+$. ^{1}H-NMR (DMSO-d_6): δ 6.30 (s, 2H, CH_2), 7.42-7.49 (m, 5H, Ph), 7.58-7.64 (dt, J=71.3, 7.7 Hz, 1H, Ph), 7.75 (t, J=7.7 Hz, 1H, Ph), 7.95 (d, J=6.1 Hz, 1H, Py), 8.08 (d, J=6.8 Hz, 1H, Ph), 8.18 (d, J=7.7 Hz, 1H, Ph), 8.43 (d, J=6.8 Hz, 1H, Py), 8.82 (d, J=7.0 Hz, 1H, Py), 9.01 (d, J=5.93 Hz, 1H, Py). Anal. Calcd for $C_{21}H_{15}BrN_2$ x 0.5 H_2O : C, 65.64; H, 4.20; N, 7.29. Found: C, 65.42; H, 4.01; N, 7.79.

4.2.4. 1-N-(4-Fluoro)benzyleupolauridinium bromide (11d) (1-*N*-(4-Fluoro)benzylindeno[1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of eupolauridine (50 mg, 0.24 mmol) in acetone (5 ml, anhydrous) was added a solution of 4-fluorobenzyl bromide (0.47, 2.5 mmol) in acetone (2 ml, anhydrous), and the mixture was heated at reflux for 72 h. After cooling to room temperature, the resulting yellow precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (62 mg, 62%) as chromatographically pure material. m.p: 180-184 °C (dec.). TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.75. FAB-MS (m/e): 313 $(M)^+$. ^{1}H-NMR (DMSO-d_6): δ 6.27 (s, 2H, CH_2), 7.27-7.32 (m, 2H, Ph), 7.49-7.57 (m, 2H, Ph), 7.62 (t, J=7.7 Hz, 1H, Ph), 7.75 (t, J=7.6 Hz, 1H, Ph), 7.93 (d, J=6.0 Hz, 1H, Py), 8.08 (d, J=7.3 Hz, 1H, Ph), 8.22 (d, J=7.7 Hz, 1H, Ph), 8.40 (d, J=6.9 Hz, 1H, Py), 8.76 (d, J=6.9 Hz, 1H, Py), 9.00 (d, J=6.0 Hz, 1H, Py).

4.2.5. 1-N-(3-Chloro)benzyleupolauridinium bromide (11e) (1-*N*-(3-Chloro)benzylindeno[1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of eupolauridine (50 mg, 0.24 mmol) in acetone (5 ml, anhydrous) was added a solution of 3-chlorobenzyl bromide (0.51 g, 2.5 mmol) in acetone (2 ml, anhydrous), and the mixture was heated at reflux for 72 h. After cooling to room temperature, the resulting yellow precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (73 mg, 73%) as chromatographically pure material. m.p: 218-222 °C. TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.78. FAB-MS (m/e): 329, 331 $(M)^+$. ^{1}H-NMR (DMSO-d_6): δ (6.29 (s, 2H, CH_2), 7.36 (d, J=6.8 Hz, 1H, Ph), 7.43-7.50 (m, 2H, Ph), 7.58-7.63 (m, 2H, Ph), 7.75 (t, J=7.6 Hz, 1H, Ph), 7.94 (d, J=6.0 Hz, 1H, Py), 8.07 (d, J=7.3 Hz, 1H, Ph), 8.16 (d, J=7.7 Hz, 1H, Ph), 8.42 (d, J=6.9 Hz, 1H, Py), 8.78 (d, J=6.9 Hz, 1H, Py), 9.00 (d, J=6.0 Hz, 1H, Py).

4.2.6. 1-N-(4-Bromo)benzyleupolauridinium bromide (11f) (1-*N*-(4-Bromo)benzylindeno[1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of eupolauridine (50 mg, 0.24 mmol) in acetone (5 ml, anhydrous) was added a solution of 4-bromobenzyl bromide (0.62 g, 2.5 mmol) in acetone (2 ml, anhydrous), and the mixture was heated at reflux for 72 h. After cooling to room temperature, the resulting yellow precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (82 mg, 74%) as chromatographically pure material. m.p: >190 °C (beginning of dec.); 215-220 °C (completely dec.). TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.78. FAB-MS (m/e): 373, 375 $(M)^+$. ^{1}H-NMR (DMSO-d_6): δ 6.26 (s, 2H, CH_2), 7.38 (d, J=8.4 Hz, 2H, Ph), 7.58-7.65 (m, 3H, Ph), 7.75 (t, J=7.5 Hz, 1H, Ph), 7.93 (d, J=6.1 Hz, 1H, Py), 8.07 (d, J=7.3 Hz, 1H, Ph), 8.16 (d, J=7.7 Hz, 1H, Ph), 8.40 (d, J=6.9 Hz, 1H, Py), 8.77 (d, J=6.9 Hz, 1H, Py), 9.00 (d, J=6.0 Hz, 1H, Py).

4.2.7. 1-N-(2,4-Difluoro)benzyleupolauridinium bromide (11g) (1-*N*-(2,4-Difluoro)benzylindeno[1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of eupolauridine (50 mg, 0.24 mmol) in acetone (5 ml, anhydrous) was added a solution of 2,4-difluorobenzyl bromide (0.52 g, 2.5 mmol) in acetone (2 ml,

anhydrous), and the mixture was heated at reflux for 72 h. After cooling to room temperature, the resulting yellow precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (60 mg, 60%) as chromatographically pure material. m.p: 192-196 °C (dec.). TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): FAB-MS (m/e): 331 $(M)^+$. R_f: 0.75. ^{1}H-NMR (DMSO-d_6): δ 6.28 (s, 2H, CH_2), 7.16-7.22 (m, 1H, Ph), 7.43-7.52 (m, 1H, Ph), 7.58-7.67 (m, 2H, Ph), 7.78 (t, J=7.6 Hz, 1H, Ph), 7.92 (d, J=6.0 Hz, 1H, Py), 8.09 (d, J=7.2 Hz, 1H, Ph), 8.33 (d, J=7.7 Hz, 1H, Ph), 8.37 (d, J=6.8 Hz, 1H, Py), 8.69 (d, J=6.9 Hz, 1H, Py), 9.00 (d, J=6.0 Hz, 1H, Py).

4.2.8. 1-N-(4-Nitro)benzyleupolauridinium bromide (11h) (1-*N*-(4-Nitro)benzylindeno [1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of eupolauridine (50 mg, 0.24 mmol) in acetone (5 ml, anhydrous) was added a solution of 4-nitrobenzyl bromide (0.54, 2.5 mmol) in acetone (2 ml, anhydrous), and the mixture was heated at reflux for 72 h. After cooling to room temperature, the resulting yellow precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (68 mg, 66%) as chromatographically pure material. m.p: 223-226 °C. TLC, cellulose, (4%-Na-citrate: MeOH, 1:1): R_f: 0.74. FAB-MS (m/e): 340 $(M)^+$. ^{1}H-NMR (DMSO-d_6): δ 6.46 (s, 2H, CH_2), 7.57 (t, J=7.7 Hz, 1H, Ph), 7.67-7.76 (m, 3H, Ph), 7.95 (d, J=6.1 Hz, 1H, Py), 8.05-8.08 (m, 2H, Ph), 8.26 (d, J=8.7 Hz, 2H, Ph), 8.45 (d, J=6.9 Hz, 1H, Py), 8.83 (d, J=6.9 Hz, 1H, Py), 9.02 (d, J=6.0 Hz, 1H, Py).

4.2.9. 1-N-(4-Methyl)benzyleupolauridinium bromide (11i) (1-*N*-(4-Methyl)benzyl-indeno[1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of eupolauridine (50 mg, 0.24 mmol) in acetone (5 ml, anhydrous) was added a solution of 4-methylbenzyl bromide (0.46, 2.5 mmol) in acetone (2 ml, anhydrous), and the mixture was heated at reflux for 72 h. After cooling to room temperature, the resulting yellow precipitate was collected, washed with acetone and ether, and dried under high vacuum to give product (33 mg, 35%) containing ~20 % starting material, according to ^{1}H-NMR. The obtained mixture was screened without purification. m.p: (of the mixture) >184 °C (beginning of dec.), 200-204 °C (completely dec.). TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.78 (product), 0.39 (starting material, eupolauridine). FAB-MS (m/e): 355 $(M)^+$. ^{1}H-NMR (DMSO-d_6): δ (target compound only) 2.29 (s, 3H, CH_3), 6.23 (s, 2H, CH_2), 7.23 (d, J=8.0 Hz, 2H, Ph), 7.30 (d, J=8.1 Hz, 2H, Ph), 7.60 (t, J=8.1 Hz, 1H, Ph), 7.74 (t, J=7.7 Hz, 1H, Ph), 7.91 (d, J=6.0 Hz, 1H, Py), 8.07 (d, J=7.3 Hz, 1H, Ph), 8.20 (d, J=7.7 Hz, 1H, Ph), 8.39 (d, J=6.9 Hz, 1H, Py), 8.76 (d, J=6.8 Hz, 1H, Py), 8.99 (d, J=5.9 Hz, 1H, Py).

4.2.10. 1-N-(4-Trifluoromethyl)benzyleupolauridinium bromide (11j) (1-*N*-(4-Trifluoromethyl)-benzylindeno[1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of eupolauridine (50 mg, 0.24 mmol) in acetone (5 ml, anhydrous) was added a solution of 4-trifluorobenzyl bromide (0.60 g, 2.5 mmol) in acetone (2 ml, anhydrous), and the mixture was heated at reflux for 72 h. After cooling to room temperature, the resulting yellow precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (51 mg, 47%) as chromatographically pure material. m.p:202-206 °C (dec.). TLC, cellulose, (4%-Na-

citrate:MeOH, 1:1):R_f: 0.83. FAB-MS (m/e): 363 $(M)^+$. ^{1}H-NMR (DMSO-d_6): δ 6.41 (s, 2H, CH_2), 7.59 (t, J=7.7 Hz, 1H, Ph), 7.63 (d, J=8.2 Hz, 2H, Ph), 7.74 (t, J=7.6 Hz, 1H, Ph), 7.80 (d, J=6.1 Hz, 2H, Ph), 7.95 (d, J=6.0 Hz, 1H, Py), 8.07 (d, J=7.3 Hz, 1H, Ph), 8.12 (d, J=7.7 Hz, 1H, Ph), 8.43 (d, J=6.8 Hz, 1H, Py), 8.82 (d, J=6.8 Hz, 1H, Py), 9.01 (d, J=6.0 Hz, 1H, Py).

4.2.11. 1-N-(3,5-Dimethoxy)benzyleupolauridinium bromide (11k) (1-*N*-(3,5-Dimethoxy)-benzylindeno[1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of eupolauridine (50 mg, 0.24 mmol) in acetone (5 ml, anhydrous) was added a solution of 3,5-dimethoxyenzyl bromide (0.54, 2.5 mmol) in acetone (2 ml, anhydrous), and the mixture was heated at reflux for 72 h. After cooling to room temperature, the resulting yellow precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the pure product (24 mg, 23%) as chromatographically pure material. m.p: 200-205 °C. TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.78. FAB-MS (m/e): 355 $(M)^+$. ^{1}H-NMR (DMSO-d_6): δ 3.72 (s, 6H, $(OCH_3)_2$), 6.18 (s, 2H, CH_2), 6.55-6.58 (m, 3H, Ph), 7.63 (t, J=7.5 Hz, 1H, Ph), 7.75 (t, J=7.6 Hz, 1H, Ph), 7.92 (d, J=5.9 Hz, 1H, Py), 8.07 (d, J=7.3 Hz, 1H, Ph), 8.20 (d, J=7.6 Hz, 1H, Ph), 8.39 (d, J=6.8 Hz, 1H, Py), 8.76 (d, J=6.7 Hz, 1H, Py), 8.99 (d, J=5.9 Hz, 1H, Py).

4.2.12. 1-N-(Naphth-2-yl)methyleupolauridinium bromide (11l) (1-*N*-(Naphth-2-yl)-methylindeno[1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of eupolauridine (50 mg, 0.24 mmol) in acetone (5 ml, anhydrous) was added 2-(bromomethyl)naphthalene (0.54 g, 2.45 mmol), and the mixture was heated at reflux for 48 h. After cooling to room temperature, the resulting orange precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (66 mg, 63%) as chromatographically pure material. m.p: 230-232 °C (dec.). TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.76. FAB-MS (m/e): 345 $(M)^+$. ^{1}H-NMR (DMSO-d_6): δ 6.42 (s, 2H, CH_2), 7.55-7.60 (m, 4H, Ph), 7.72-7.77 (m, 1H, Ph), 7.83-7.98 (m, 4H, 3 Ph, 1 Py), 8.03-8.12 (m, 3H, Ph), 8.37 (d, J=6.8 Hz, 1H, Py), 8.79 (d, J=6.8 Hz, 1H, Py), 8.99 (d, J=6.2 Hz, 1H, Py).

4.2.13. 1-N-Benzyl-8(9)-methyleupolauridinium bromide (11m, 12m) (1-*N*-Benzyl-8(9)-methylindeno[1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of 8-methyl eupolauridine (50 mg, 0.23 mmol) in acetone (5 ml, anhydrous) was added benzyl bromide (0.5 ml, 4.20 mmol), and the mixture was heated at reflux for 24 h. After cooling to room temperature, the resulting orange precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the pure product (75 mg, 84%) as a mixture of both 8-and 9-methyl –isomers in a tentative ratio of ~ 1:1 according to ^{1}H-NMR. The obtained mixture was screened without purification. m.p. (mixture) : 234-238 °C (dec.). TLC, cellulose, (4%-Na-citrate:MeOH, 1:1):R_f: 0.83. FAB-MS (m/e): 309 $(M)^+$. ^{1}H-NMR (DMSO-d_6): (The ^{1}H-NMR-spectrum shows both the 1-N-benzyl-8-methyl - (the assignment with the index a) and 1-N-benzyl-9-methyl-eupolauridinium bromide isomers (designated a and b, respectively) in tentative ratio of 1:1. The reported coupling constant values are approximated. Because of signal overlapping, an exact calculation of the coupling constants is not possible) δ 2.41 (s, 3H,

CH_3(a)), 2.47 (s, 3H, CH_3(b)), 6.26 (s, 2H, CH_2(a)), 6.29 (s, 2H, CH_2(b)), 7.39-7.45 (m, 11H, 6 Ph(a), 5 Ph(b)), 7.54 (d, J=8.4 Hz, 1H, Ph(b)), 7.88-7.96 (m, 4H, 1 Py(a), 2 Ph(b), 1 Py(b)), 8.05 (d, J=8.1 Hz, 1H, Ph(a)), 8.11 (br s, 1H, Ph(a)), 8.36 (d, J=5.5 Hz, 1H, Py(b)), 8.38 (d, J=5.5 Hz, 1H, Py(a)), 8.74 (d, J=7.0 Hz, 1H, Py(a)), 8.76 (d, J=7.0 Hz, 1H, Py(b)), 8.96 (d, J=6.2 Hz, 1H, Py(a)), 8.99 (d, J=5.9 Hz, 1H, Py(b)). Anal. Calcd for $C_{22}H_{17}BrN_2$: C, 67.88; H, 4.40; N, 7.20. Found: C, 67.71; H, 4.24; N, 7.06.

4.2.14. 1-N-Benzyl-8(9)-methoxyeupolauridinium bromide (11n, 12n) (1-*N*-Benzyl-8(9)-methoxyindeno[1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of 8-methoxy-eupolauridine (50 mg, 0.21 mmol) in acetone (5 ml, anhydrous) was added benzyl bromide (0.5 ml, 4.20 mmol), and the mixture was heated at reflux for 24 h. After cooling to room temperature, the resulting red precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (74 mg, 86%) as a mixture of both 8-and 9-methoxy –isomers in a tentative ratio of ~ 3:2 according to ^{1}H-NMR. The obtained mixture was screened without purification. m.p (mixture): 216-220 °C (dec.). TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.78, FAB-MS (m/e): 325 $(M)^+$. ^{1}H-NMR (DMSO-d_6): (The ^{1}H-NMR-spectrum of **11n** shows the 1-N-benzyl-8-methoxy and 1-N-benzyl-9-methoxy-eupolauridinium bromide isomers (designated a and b, respectively) in tentative ratio of 3:2. The reported coupling constant values are approximated. Because of signal overlapping, an exact calculation of the coupling constants is not possible). δ 3.86 (s, 3H, OCH_3(b)), 3.98 (s, 3H, OCH_3(a)), 6.20 (s, 2H, CH_2(a)), 6.31 (s, 2H, CH_2(b)), 7.05-7.08(dd, J=2.5, 8.8 Hz, 1H, Ph(a)), 7.21 (d, J=8.4 Hz, 1H, Ph(b)), 7.38-7.38 (m, 10H, 5 Ph(a), 5 Ph(b)), 7.59-7.62 (m, 2H, 1 Ph(a), 1 Ph(b)), 7.83 (d, J=5.9 Hz, 1H, Ph(b)), 7.91 (d, J=5.9 Hz, 1H, Ph(a)), 7.96 (d, J=8.4 Hz, 1H, Py(b)), 8.06 (d, J=8.6 Hz, 1H, Py(a)), 8.25 (d, J=6.8 Hz, 1H, Py(a)), 8.41 (d, J=7.0 Hz, 1H, Py(b)), 8.67 (d, J=6.8 Hz, 1H, Py(a)), 8.82 (d, J=6.8 Hz, 1H, Py(b)), 8.91 (d, J=5.5 Hz, 1H, Py(b)), 8.99 (d, J=5.9 Hz, 1H, Py(a)).

4.2.15. 1-N-Benzyl-8(9)-fluoroeupolauridinium bromide (11o) (1-*N*-Benzyl-8(9)-fluoroindeno[1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of 8-fluoro eupolauridine (50 mg, 0.23 mmol) in acetone (5 ml, anhydrous) was added benzyl bromide (0.5 ml, 4.20 mmol), and the mixture was heated at reflux for 24 h. After cooling to room temperature, the resulting orange precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (45 mg, 51%) as a mixture of both 8-and 9-fluoro –isomers in a tentative ratio of ~ 1:1 according to ^{1}H-NMR. A second fraction (21 mg, 23 %) was isolated by heating the filtrate at reflux for another 24 hours. The obtained mixture was screened without purification. m.p (mixture): 194-200 °C (dec.). TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.83. FAB-MS (m/e): 313 $(M)^+$. ^{1}H-NMR (DMSO-d_6): (The ^{1}H-NMR-spectrum showed both the 1-N-benzyl-8-fluoro and 1-N-benzyl-9-fluoro-eupolauridinium bromide isomers (**11o** and **12o**, designated a and b, respectively) in tentative ratio of 1:1. The reported coupling constant values are approximated. Because of signal overlapping an exact calculation of the coupling constants is not possible). δ 6.25 (s, 2H, CH_2(a)), 6.28 (s, 2H, CH_2(b)), 7.38-7.50 (m, 11H, 6 Ph(a), 5 Ph(b)), 7.56-7.62 (m, 1H, Ph(b)), 7.91-8.00 (m, 3H, 1 Py(a), 1 Ph(a), 1 Py(b)), 8.05-8.21 (m, 3H, 1 Ph(a), 2 Ph(b)), 8.36 (d,

J=6.8 Hz, 1H, Py(a)), 8.42 (d, J=6.4 Hz, 1H, Py(b)), 8.72-8.77 (m, 2H, Py(a), Py(b)), 8.98 (d, J=5.9 Hz, 1H, Py(b)), 9.02 (d, J=6.2 Hz, 1H, Py(a)).

4.2.16. 1,5-Dimethyleupolauridinium iodide (12p) (1,5-Dimethyl-indeno[1,2,3-*ij*][2,7] naphthyridinium iodide)

To a solution of 2-methyleupolauridine (100 mg, 0.46 mmol) in acetone (7 ml, anhydrous) was added methyl iodide (1 ml, 16.06 mmol), and the mixture was heated at reflux for 24 h. After cooling to room temperature the resulting orange precipitate was collected, washed with acetone and ether, and dried under high vacuum to give product (148 mg, 90%) that was chromatographically pure. m.p: 259-264 °C dec. TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.70. FAB-MS (m/e): 233 $(M)^+$. UV (MeOH), λ_{max}[nm] (lgε): 387 (3.88), 368 (3.87), [350 (3.60)], [313 (4.05)], [300 (4.19)], 287 (4.25), [240 (4.53)], 229 (4.60). ^{1}H-NMR (DMSO-d_6) δ 2.80 (br s, 3H, C(5)-CH_3), 4.64 (br s, 3H, N(1)CH_3), 7.66-7.82 (m, 2H, Ph & 1H, Py), 8.06 (d, J=7.9 Hz, 1H, Ph), 8.22-8.25 (m, 1H, Ph & 1H, Py), 8.74 (d, J=6.6 Hz, 1H, Py). Anal. Calcd for $C_{16}H_{13}IN_2$: C, 53.35; H, 3.64; N, 7.78. Found: C, 53.19; H, 3.87; N, 7.67.

4.2.17. 1-N-Benzyl-5-methyleupolauridinium bromide (12q) (1-*N*-Benzyl-5-methyl indeno[1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of 2-methyleupolauridine (50 mg, 0.23 mmol) in acetone (5 ml, anhydrous) was added benzyl bromide (0.5 ml, 4.20 mmol), and the mixture was heated at reflux for 48 h. After cooling to room temperature, the resulting yellow precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (66 mg, 74%) as chromatographically pure material. m.p: 218-222 °C. TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.83. FAB-MS (m/e): 309 $(M)^+$. ^{1}H-NMR (DMSO-d_6): δ 2.81 (s, 3H, CH_3), 6.28 (br s, 2H, CH_2), 7.38-7.47 (m, 5H, Ph), 7.57-7.62 (dt, J=1.1, 7.7 Hz, 1H, Ph), 7.73 (t, J=7.5 Hz, 1H, Ph), 7.79 (br s, 1H, Py), 8.05 (d, J=6.8 Hz, 1H, Ph), 8.16 (d, J=7.7 Hz, 1H, Ph), 8.32 (d, J=6.8 Hz, 1H, Py), 8.77 (d, J=7.0 Hz, 1H, Py).

4.2.18. 1-N-Methyl-5-phenyleupolauridinium iodide (12r) (1-N-Methyl-5-phenylindeno [1,2,3-ij][2,7]naphthyridinium iodide)

To a solution of 2-phenyleupolauridine (100 mg, 0.46 mmol) in acetone (10 ml, anhydrous) was added methyl iodide (1 ml, 16.06 mmol), and the mixture was heated at reflux for 24 h. After cooling to room temperature, the resulting orange precipitate was collected, washed with acetone (2x3 ml) and ether (3x10 ml), and dried under high vacuum to give the product (126 mg, 84%) as a chromatographically pure material. m.p: 246-250 °C dec. TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.50. FAB-MS (m/e): 295 $(M)^+$. UV (MeOH), λ_{max}[nm] (lgε), 439 (3.22), 392 (3.91), 372 (3.93), [352 (3.95)], 324 (4.51), 299 (4.38), [225 (4.49)]. ^{1}H-NMR (DMSO-d_6): δ 4.62 (br s, 3H, CH_3), 7.63-7.65 (m, 3H, Ph), 7.71-7.76 (m, 1H, Ph), 7.79-7.82 (m, 1H, Ph), 8.19-8.21 (d, J=7.0 Hz, 1H, Ph), 8.27-8.36 (m, 3H, Ph, 1H, Py), 8.47 (s, 1H, Ph), 8.76 (d, J=6.8 Hz, 1H, Py). Anal. Calcd for $C_{21}H_{15}IN_2$ x 0.2 H_2O: C, 59.23; H, 3.64; N, 6.58. Found: C, 59.02; H, 3.83; N, 6.63.

4.2.19. 1-N-Benzyl-5-phenyleupolauridinium bromide (12s) (1-*N*-Benzyl-5-phenylindeno [1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of 2-phenyleupolauridine (50 mg, 0.18 mmol) in acetone (5 ml, anhydrous) was added benzyl bromide (0.5 ml, 4.20 mmol), and the mixture was heated at reflux for 48 h. After cooling to room temperature, the resulting yellow precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (67 mg, 83%) as chromatographically pure material. m.p: 232-236 °C. TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.62. FAB-MS (m/e): 371 $(M)^+$. UV (MeOH), λ_{max}[nm] (lgε): 442 (3.30), 394 (3.92), 374 (3.96), [356 (3.99)], 325 (4.51), [302 (4.38)], 276 (4.24), [250 (4.42)], 237 (4.47). ^{1}H-NMR (DMSO-d_6): δ 6.30 (s, 2H, CH_2), 7.41-7.46 (m, 5H, Ph), 7.61-7.66 (m, 4H, Ph), 7.78 (t, J=7.7 Hz, 1H, Ph), 8.18 (d, J=7.3 Hz, 2H, Ph), 8.34-8.37 (m, 3H, 2 Ph, Py), 8.52 (br s, 1H, Py), 8.77 (d, J=7.0 Hz, 1H, Py). Anal. Calcd for $C_{27}H_{19}BrN_2$: C, 71.85; H, 4.24; N, 6.21. Found: C, 71.59; H, 4.54; N, 6.12.

4.2.20. 1-N-Ethylacetatoeupolauridinium bromide (11t) (1-*N*-Ethylacetatoindeno[1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of eupolauridine (100 mg, 0.49 mmol) in acetone (5 ml, anhydrous) was added ethyl bromoacetate (1 ml, 9.02 mmol), and the mixture was stirred at room temperature for 1 week.

The resulting yellow precipitate was collected, washed with acetone and ether, and dried under high vacuum to chromatographically pure product (110 mg, 61%) as material. In another experiment, the reaction mixture was heated at reflux for 72 h. Using the same workup procedure mentioned above, the chromatographically pure product (73 mg, 40%) was again isolated. m.p: 207-209 °C (dec.). TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.58. FAB-MS (m/e): 292 $(M)^+$. UV (MeOH), λ_{max}[nm] (lgε): 391 (3.90), 372 (3.88), [353 (3.64)], [311 (4.09)], 298 (4.22), [288 (4.20)], [256 (4.08)], [242 (4.38)], 226 (4.43). ^{1}H-NMR (DMSO-d_6): δ 1.27 (t, J=7.0 Hz, 3H, CH_3), 4.29 (q, J=7.0 Hz, 2H, CH_2), 6.15 (br s, 2H, CH_2), 7.65-7.70 (m, 1H, Ph), 7.77 (t, J=7.7 Hz, 1H, Ph), 7.96 (d, J=5.9 Hz, 1H, Py), 8.04 (d, J=7.5 Hz, 1H, Ph), 8.08 (d, J=6.8 Hz, 1H, Ph), 8.48 (d, J=6.6 Hz, 1H, Py), 8.86 (d, J=6.8 Hz, 1H, Py), 9.03 (d, J=6.2 Hz, 1H, Py). Anal. Calcd for $C_{18}H_{15}BrN_2O_2$ x 0.4 H_2O: C, 57.13; H, 4.21; N, 7.40. Found: C, 57.04; H, 4.13; N, 7.42.

4.2.21. 1-N-Phenacyleupolauridinium bromide (11u) (1-*N*-Phenacylindeno[1,2,3-*ij*][2,7] naphthyridinium bromide)

To a solution of eupolauridine (50 mg, 0.24 mmol) in acetone (5 ml, anhydrous) was added a solution of 2-bromoacetophenone (0.48 g, 2.40 mmol) in acetone (1 ml, anhydrous), and the mixture was heated at reflux overnight. After cooling to room temperature, the resulting precipitate was collected, washed with acetone, ether, and dried under high vacuum to give the pure product (32 mg, 32%) as a yellow solid. A second fraction of the product (26 mg, 26%) was isolated by heating the filtrate at reflux for another 48 h. m.p: 248-252 °C. TLC, cellulose, (4%-Na-citrate: MeOH, 1:1): R_f: 0.74. FAB-MS (m/e): 323 $(M)^+$. UV (MeOH), λ_{max}[nm] (lgε): 388 (3.87), 367 (3.86), [348 (3.61)], [320 (4.05)], [299 (4.25)], 288 (4.27), 236 (4.53), 227 (4.55). ^{1}H-NMR (DMSO-d_6): δ 6.95 (s, 2H, CH_2), 7.53-7.59 (m, 1H, Ph), 7.70-7.77 (m, 3H, Ph), 7.85 (d, J=7.5 Hz, 2H, Ph), 7.98 (d, J=5.9 Hz, 1H, Py), 8.09 (d, J=7.5 Hz, 1H, Ph), 8.19 (d, J=7.3 Hz, 2H,

Ph), 8.47 (d, J=6.8 Hz, 1H, Py), 8.78 (d, J=6.8 Hz, 1H, Py), 9.06 (d, J=5.9 Hz, 1H, Py). Anal. Calcd for $C_{22}H_{15}BrN_2O$: C, 65.52; H, 3.75; N, 6.95. Found: C, 65.34; H, 3.73; N, 7.06.

4.2.22. 1-N-Acetamidoeupolauridinium bromide (11v) (1-*N*-Acetamidoindeno[1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of eupolauridine (100 mg, 0.48 mmol) in acetone (10 ml, anhydrous) was added a solution of bromoacetamide (0.70, 5.00 mmol) in acetone (6 ml, anhydrous), and the mixture was heated at reflux for 48 h. After cooling to room temperature, the resulting yellow precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (25 mg, 15%) as chromatographycally pure material. A second fraction (26 mg, 16%) was isolated by heating of the filtrate at reflux for another 48 h. m.p: 260 °C (beginning of dec.), 274-280 °C (completely dec.). TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.68. FAB-MS (m/e): 262 $(M)^+$. ^{1}H-NMR (DMSO-d_6): δ 5.80 (s, 2H, CH_2), 7.67-7.80 (m, 2H, Ph), 7.93-7.99 (m, 3H, 2 Ph, 1 NH), 8.08 (d, J=7.2 Hz, 1H, Py),8.22 (br s, 1H, NH), 8.42 (d, J=6.7 Hz, 1H, Py), 8.81 (d, J=6.7 Hz, 1H, Py), 9.02 (d, J=5.9 Hz, 1H, Py).

4.2.23. 1-N-Aminoeupolauridinium mesitylene sulfonate (11w) (1-*N*-Amino-1,6-diazafluoranthenium mesitylene sulfonate, 1-*N*-Aminoindeno[1,2,3-*ij*][2,7]naphthyridinium mesitylene sulfonate)

Freshly prepared *O*-mesitylenesulfonylhydroxylamine[14-17] (300 mg, 1.39 mmol) was added to a solution of eupolauridine (100 mg, 0.49 mmol) in methanol (15 ml), and the mixture was stirred at room temperature for 30 min. The green reaction mixture was added dropwise to ether (50 ml), and the resulting precipitate was collected, washed with ether (2x 10 ml), dried under high vacuum to give the product (88 mg, 15%) as a green solid. This material was submitted without any further purification for screening. FAB-MS (m/e): 220 $(M+H)^+$. ^{1}H-NMR (DMSO-d_6): δ 2.15 (s, 3H, CH_3 (O-mesitylene)), 2.47 (s, 6H, CH_3 (O-mesitylene)), 6.71 (s, 2H, Ph (O-mesitylene)), 7.70-7.76 (m, 2H, 2-Ph), 7.90 (d, J=5.9 Hz, 1H, Py), 8.09 (d, J=6.8 Hz, 1H, Ph), 8.29 (d, J=7.03 Hz, 1H, Py), 8.32-8.35 (dd, J=1.1, 7.0 Hz, 1H, Ph), 8.64 (d, J=6.8 Hz, 1H, Py), 8.75 (br s, 2H, NH_2), 8.93 (d, J=5.9 Hz, 1H, Py).

4.2.24. 6-Methyl-2,3-benzo-1,6-diazafluoranthenium iodide (13a) (1-*N*-Methyl-benz[c] indeno[1,2,3-*ij*][2,7]naphthyridinium iodide)

To a solution of 2,3-benzo-1,6-diazafluoranthene (300 mg, 1.18 mmol) in acetone (30 ml, anhydrous) was added methyl iodide (3 ml, 48.18 mmol), and the mixture was heated at reflux for 48 h. After cooling to room temperature, the resulting orange precipitate was collected, washed with acetone and ether, and dried under high vacuum to give product (403 mg, 86%) that was chromatographically pure. m.p: 256-260 °C dec. TLC, cellulose, (4%-Na-citrat:MeOH, 1:1): R_f: 0.38. FAB-MS (m/e): 269 $(M)^+$. UV (MeOH), λ_{max}[nm] (lgε): 399 (3.77), 382 (3.75), 305 (4.66), 241 (4.52). ^{1}H-NMR (DMSO-d_6): δ 4.72 (s, 3H, CH_3), 7.76-7.88 (m, 2H, Ph), 7.94-7.99 (dt, J=1.3, 8.1 Hz, 1H, Ph), 8.08-8.14 (dt, J=1.3, 7.3 Hz, 1H, Ph), 8.25 (d, J=7.5 Hz, 1H, Ph), 8.35 (d, J=8.4 Hz, 2H, Ph), 8.89-8.92 (dd, J=1.3, 7.9 Hz, 1H, Ph), 9.05 (d, J=6.8 Hz, 1H, Py), 9.10 (d, J=7.0

Hz, 1H, Py). Anal. Calcd for $C_{19}H_{13}IN_2$: C, 57.60; H, 3.31; N, 7.07. Found: C, 57.14; H, 3.36; N, 6.97.

4.2.25. 6-Ethyl-2,3-benzo-1,6-diazafluoranthenium iodide (13b) (1-Ethylbenz[*c*] indeno[1,2,3-*ij*][2,7]naphthyridinium iodide)

To a solution of 2,3-benzo-1,6-diazafluoranthene (100 mg, 0.39 mmol) in acetone (10 ml, anhydrous) was added ethyl iodide (1 ml, 48.18 mmol), and the mixture was heated at reflux for 72 h. After cooling to room temperature, the resulting red-orange precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (76 mg, 47%) as chromatographically pure material. m.p: >240 °C (beginning of dec), 250-252 °C (completely dec.). TLC, cellulose, (4%-Na-citrat:MeOH, 1:1): R_f: 0.55. FAB-MS (m/e): 283 $(M)^+$. UV (MeOH), λ_{max}[nm] (lgε): 401 (3.76), 383 (3.74), [350 (3.74)], 307 (4.66), 242 (4.51). ^{1}H-NMR (DMSO-d_6): δ 1.69 (t, J=7.2 Hz, 3H, CH_3) 5.06 (q, J=7.0 Hz, 2H, CH_2), 7.76-7.88 (m, 2H, Ph), 7.93-7.98 (dt, J=1.1, 8.1 Hz, 1H, Ph), 8.08-8.13 (dt, J=1.3, 7.3 Hz, 1H, Ph), 8.24-8.26 (dd, J=1.3, 7.5 Hz, 1H, Ph), 8.34 (d, J=7.9 Hz, 2H, Ph), 8.92 (d, J=7.0 Hz, 1H, Ph), 9.10 (d, J=6.6 Hz, 1H, Py), 9.16 (d, J=6.4 Hz, 1H, Py). Anal. Calcd for $C_{20}H_{15}IN_2$ x 0.5 H_2O: C, 57.30; H, 3.85; N, 6.68. Found: C, 57.67; H, 3.61; N, 6.99.

4.2.26. 6-N-Benzyl-2,3-benzo-1,6-diazafluoranthenium bromide (13c) (1-*N*-Benzyl-benz[*c*]indeno[1,2,3-*ij*][2,7]naphthyridinium bromide)

To a solution of 2,3-benzo-1,6-diazafluoranthene (100 mg, 0.39 mmol) in acetone (10 ml, anhydrous) was added benzyl bromide (1 ml, 8.41 mmol), and the mixture was heated at reflux for 48 h. After cooling to room temperature, the resulting yellow precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (128 mg, 77%) as chromatographically pure material. m.p: 248-252 °C. TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.63. FAB-MS (m/e): 345 $(M)^+$. UV (MeOH), λ_{max}[nm] (lgε): 403 (3.77), 385 (3.75), [350 (3.77)], 307 (4.66), 243 (4.52). ^{1}H-NMR (DMSO-d_6): δ 6.39 (s, 2H, CH_2), 7.39-7.45 (m, 5H, Ph), 7.64-7.70 (dt,J=0.9, 7.7 Hz, 1H, Ph), 7.77-7.82 (dt, J=0.7, 7.7 Hz, 1H, Ph), 7.94-8.00 (dt, J=1.3, 8.4 Hz, 1H, Ph), 8.09-8.12 (dd, J=1.3, 8.1 Hz, 1H, Ph), 8.16 (d, J=7.7 Hz, 1H, Ph), 8.22 (d, J=7.3 Hz, 1H, Ph), 8.34-8.37 (dd, J=1.1, 8.1 Hz, 1H, Ph), 8.92-8.95 (dd, J=1.3, 8.1 Hz, 1H, Ph), 9.17 (s, 2H, Py). Anal. Calcd for $C_{25}H_{17}BrN_2$ x 0.3 H_2O: C, 69.71; H, 4.12; N, 6.50. Found: C, 69.77; H, 4.08; N, 6.69.

4.2.27. 6-Methyl-2,3-benzo-1,6-diazafluoranthenium p-toluenesulfonate (13d) (1-Methyl-benz[*c*]indeno[1,2,3-*ij*][2,7]naphthyridinium *p*-toluenesulfonate)

To a solution of 2,3-benzo-1,6-diazafluoranthene (100 mg, 0.39 mmol) in acetone (10 ml, anhydrous) was added methyl *p*-toluenesulfonate (1 ml, 6.63 mmol), and the mixture was heated at reflux for 48 h. After cooling to room temperature, the resulting yellow precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the crude product (120 mg), contaminated with 13% (by ^{1}H-NMR) starting material. The crude product was suspended in acetone (10 ml, anhydrous), treated with methyl p-toluensulfonate (1 ml, 6.63 mmol) and heated for an additional 72 hours at reflux. The pure product (69 mg, 40%) was isolated as yellow solid. m.p: 238-242 °C. TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.43. FAB-MS (m/e): 269 $(M)^+$. UV

(MeOH), λ_{max}[nm] (lgε): 400 (3.77), 382 (3.75), [350 (3.71)], 305 (4.66), 241 (4.51). ^{1}H-NMR (DMSO-d_6): δ 2.28 (s, 3H, CH_3), 4.72 (s, 3H, CH_3), 7.11 (d, J=7.9, 2H, Ph), 7.46 (d, J=8.1, 2H, Ph), 7.60-7.88 (m, 2H, Ph), 7.93-7.99 (dt, J=1.3, 8.1 Hz, 1H, Ph), 8.08-8.14 (dt, J=1.3, 7.3 Hz, 1H, Ph), , 8.24-8.26 (d, J=7.0 HZ, 1H, Ph), 8.34-8.37 (d, J=8.1 HZ, 2H, Ph), 8.89-8.92 (dd, J=0.9, 9.0 Hz, 1H, Ph), 9.05 (d, J=6.8 Hz 1H, Py), 9.09 (d, J=6.8 Hz 1H, Py). Anal. Calcd for $C_{26}H_{20}N_2O_3S$ x 0.2 H_2O: C, 70.60; H, 4.60; N, 6.33. Found: C, 70.47; H, 4.60; N, 6.72.

4.2.28. 1,2-Dimethyl-benzo[e]-1,6-diazafluoranthenium iodide (13e) (1,2-Dimethyl benz [*f*]indeno[1,2,3-*ij*][2,7]naphthyridinium iodide)

To a solution of 2-methyl-4,5-benzo[e]-1,6-diazafluoranthene (100 mg, 0.37 mmol) in acetone (25 ml, anhydrous) was added methyl iodide (1 ml, 16.06 mmol), and the mixture was heated at reflux for 3 days. The resulting yellow precipitate was filtered from the hot mixture, washed with acetone, chloroform, and ether, and dried under high vacuum to give the chromatographically pure product (60 mg, 40%). m.p: 238-244 °C dec. TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.42. FAB-MS (m/e): 283 $(M)^+$. UV (MeOH), λ_{max}[nm] (lgε): 403 (3.82), 384 (3.81), [356 (3.72)], [322 (4.34)], 303 (4.64), 237 (4.50). ^{1}H-NMR (DMSO-d_6): δ 3.03 (s, 3H, CH_3), 4.57 (s, 3H, CH_3), 7.75-7.86 (m, 2H, Ph), 7.92-7.97 (dt, J=1.2, 7.3 Hz, 1H, Ph), 8.06-8.11 (dt, J=1.3, 8.3 Hz, 1H, Ph), 8.23-8.25 (dd, J=0.8, 7.3 Hz, 1H, Ph), 8.31 –8.34 (dd, J=0.8, 7.3 Hz, 1H, Ph), 8.48 (d, J=7.8 Hz, 1H, Py), 8.78-8.81 (dd, J=1.1, 9.1 Hz, 1H, Py), 9.05 (br s, 1H, Py). Anal. Calcd for $C_{20}H_{15}IN_2$ x 0.4 HI: C, 52.06; H, 3.36; N, 6.07. Found: C, 52.03; H, 3.51; N, 5.95.

4.2.29. 1-N-Methyl-5-phenyl-2,3-benzo-1,6-diazafluoranthenium iodide (14f) (8-*N*-Methyl-2-phenyl-benz[c]indeno[1,2,3-*ij*][2,7]naphthyridinium iodide)

To a solution of 2-phenyl-2,3-benzo-1,6-diazafluoranthene (100 mg, 0.30 mmol) in chloroform (10 ml, anhydrous) was added methyl iodide (1 ml, 16.06 mmol), and the mixture was heated at reflux overnight. After cooling to room temperature, the resulting red precipitate was collected, washed with acetone and ether, and dried under high vacuum to give the product (14 mg, 10%) as a chromatographically pure material. A second fraction (50 mg, 35%) of the product was obtained by adding methyl iodide (1 ml, 16.06 mmol) to the filtrate and further heating at reflux for 48 hours. m.p: 232-236 °C dec. TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.28. FAB-MS (m/e): 345 $(M)^+$. UV (MeOH), λ_{max}[nm] (lgε): 450 (3.47), 402 (3.99), [380 (4.10)], [363 (4.16)], 324 (4.57), [310 (4.41)], 274 (4.52), 236 (4.61). ^{1}H-NMR (DMSO-d_6): δ 4.82 (s, 3H, N(8)-CH_3), 7.63-7.68 (m, 3H, Ph), 7.73-7.78 (dt, J=1.1, 7.9 Hz, 1H, Ph), 7.88 (t, J=7.4 Hz, 1H, Ph), 8.17-8.30 (m, 3H, Ph), , 8.49-8.52 (m, 2H, Ph), 8.60 (d, J=7.9, 1H, Ph), 8.78 (d, J=8.5, 1H, Ph), 9.13 (s, 1H, Py), 9.29 (dd, J=1.3, 7.9 Hz, 1H, Ph). Anal. Calcd for $C_{25}H_{17}IN_2$ x 0.8 H_2O: C, 61.69; H, 3.85; N, 5.76. Found: C, 61.72; H, 3.62; N, 5.53.

4.2.30. 1-N-Ethyl-5-phenyl-2,3-benzo-1,6-diazafluoranthenium iodide (14g) (8-*N*-Ethyl-2-phenylbenz[*c*]indeno[1,2,3-*ij*][2,7]naphthyridinium iodide)

To a solution of 2-phenyl-2,3-benzo-1,6-diazafluoranthene (50 mg, 0.15 mmol) in dioxane (10 ml, anhydrous) was added ethyl iodide (0.5 ml, 6.25 mmol), and the mixture was heated at reflux 48h. After cooling to room temperature, the resulting red precipitate was collected, washed with dioxane and ether, and dried under high vacuum to give the

product (11 mg, 15%) as chromatographically pure material. A second fraction (11 mg, 15%) of the product was isolated by adding of an excess of ethyl iodide (0.5 ml, 6.25 mmol) to the filtrate and further heating at reflux for 48 hours. m.p: >220 °C dec. (beginning of dec.), 230-235 °C (completely dec.). TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.28, FAB-MS (m/e): 359 $(M)^+$. ^{1}H-NMR (DMSO-d_6): δ 1.77 (t, J=7.3 Hz, 3H, CH_3), 5.34 (q, J=7.3 Hz, 2H, CH_2), 7.62-7.71 (m, 3H, Ph), 7.75-7.80 (m, 1H, Ph), 7.90 (t, J=7.5 Hz, 1H, Ph), 8.17-8.31 (m, 3H, Ph), 8.46 (d, J=7.7, 1H, Ph), 8.52-8.55 (m, 2H, Ph), 8.82 (d, J=8.6, 1H, Ph), 9.13 (s, 1H, Py), 9.29 (dd, J=1.5, 8.1 Hz, 1H, Ph).

4.2.31. 1-N-Ethoxycarbonylmethyl-4,5-Benzo-1,6-diazafluoranthenium bromide (13h) (1-*N*- Ethoxycarbonylmethylbenz[*c*]indeno[1,2,3-*ij*][2,7]naphthyridine)

To a solution of 2,3-benzo-1,6-diazafluoranthene (100 mg, 0.39 mmol) in acetone (12 ml, anhydrous) was added ethyl bromoacetate (1 ml, 9.02 mmol) and the mixture was heated at reflux overnight. The resulting yellow precipitate was collected, washed with acetone and ether and dried under high vacuum to give the product (56 mg, 33%) as chromatographically pure material. A second fraction (29 mg, 17%) was isolated by adding excess ethyl bromoacetate (1 ml, 9.02 mmol) to the filtrate and heating the mixture for 48 hours. m.p: 216-218 °C (dec.). TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.69. UV (MeOH), λ_{max}[nm] (lgε): 405 (3.78), 385 (3.76), [352 (3.78)], 309 (4.68), 243 (4.51). FAB-MS (m/e): 341 $(M)^+$. ^{1}H-NMR (DMSO-d_6): δ 1.27 (t, J=7.0 Hz, 3H, CH_3), 4.29 (q, J=7.0 Hz, 2H, CH_2), 6.18 (s, 2H, CH_2), 7.73-7.78 (m, 1H, Ph), 7.82-7.87 (m, 1H, Ph), 7.95-8.00 (m, 1H, Ph), 8.05 (d, J=7.7 Hz, 1H, Ph), 8.11-8.16 (m, 1H, Ph), 8.24 (d, J=7.3 Hz, 1H, Ph), 8.35 (d, J=8.1 Hz, 1H, Ph), 8.89 (d, J=7.0 Hz, 1H, Ph), 9.09 (d, J=7.0 Hz, 1H, Py), 9.18 (d, J=6.8 Hz, 1H, Py). Anal. Calcd for $C_{22}H_{17}BrN_2O_2$ x 0.1 H_2O: C, 62.45; H, 4.10; N, 6.62. Found: C, 62.34; H, 4.08; N, 6.69.

4.2.32. 1-N-Methyl-5,6-dioxomethylenebenz[c]indeno[1,2,3-ij][2,7]naphthyridinium bromide (13i)

To a solution of 5,6-dioxomethylenebenz[*c*]indeno[1,2,3-*ij*][2,7]naphthyridine (100 mg, 0.34 mmol) in chloroform (35 ml, anhydrous) was added methyl iodide (1.0 ml, 16.06 mmol), and the mixture was heated at reflux for 7 days. After cooling to room temperature, the resulting precipitate was collected, washed with chloroform, ether and dried under high vacuum to give the product (85 mg, 58%), along with 10% unreacted starting material as a impurity. This material was recrystallized from methanol (200 ml) and dimethyl formamide (50 ml) to give the product (31 mg, 21%) as a red solid with a purity of 96 %, according to ^{1}H-NMR. m.p: 300-304 °C. TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.22. FAB-MS (m/e): 313 $(M)^+$. ^{1}H-NMR (DMSO-d_6): δ 4.67 (br s, 3H, CH_3), 6.41 (br s, 2H, CH_2), 7.70-7.78 (m, 2H, Ph), 7.81 (s, 1H, Ph), 8.11 (d, J=7.0 Hz, 1H, Ph), 8.29 (d, J=3.5 Hz, 1H, Ph), 8.41 (s, 1H, Ph), 8.90-8.98 (m, 2H, Py).

4.2.33. 1-N-Benzyl-5,6-dioxomethylenebenz[c]indeno[1,2,3-ij][2,7]naphthyridinium bromide (13j)

To a solution of 5,6-dioxomethylenebenz[*c*]indeno[1,2,3-*ij*][2,7]naphthyridine (50 mg, 0.17 mmol) in chloroform (10 ml, anhydrous) was added benzyl bromide (0.5 ml, 4.20 mmol), and the mixture was heated at reflux for 48 h. After cooling to room temperature,

the resulting precipitate was collected, washed with chloroform and ether, and dried under high vacuum to give the pure product (35 mg, 44%) as an orange solid. A second fraction of the product (27 mg, 34%) was isolated by heating the filtrate at reflux for another 48 h. m.p: 280-284 °C. TLC, cellulose, (4%-Na-citrate:MeOH, 1:1): R_f: 0.43. FAB-MS (m/e): 389 $(M)^+$. UV (MeOH), λ_{max}[nm] (lgε): [411 (3.54)], [390 (3.75)], 336 (4.74), [296 (4.14)], 276 (4.28), 251 (4.37), [226 (4.46)]. ^{1}H-NMR (DMSO-d_6): δ 6.33 (s, 2H, CH_2), 6.43 (s, 2H, CH_2), 7.37-7.46 (m, 5H, Ph), 7.61 (t, J=7.7 Hz, 1H, Ph), 7.75 (t, J=7.5 Hz, 1H, Ph), 7.82 (s, 1H, Ph), 8.11 (d, J=7.7 Hz, 2H, Ph), 8.45 (br s, 1H, Ph), 9.01 (d, J=6.8 Hz, 1H, Py), 9.06 (d, J=6.6 Hz, 1H, Py). Anal. Calcd for $C_{26}H_{17}BrN_2O_2$ x 0.7 HBr: C, 59.37; H, 3.39; N, 5.33. Found: C, 59.39; H, 3.58; N, 5.45.

4.3. Biology Methods

4.3.1. Antimicrobial Activity

All compounds were assessed for growth inhibitory activity against a panel of microorganisms including *Candida albicans* B311 (Ca), *Cryptococcus neoformans* (ATCC 52657, Cn), *Mycobacterium intracellulare* (ATCC 23068, Mi), *Aspergillus fumigatus* (ATCC 26934, Afu), *Pseudomonas aeruginosa* (ATCC 15442, Pa), *Staphylococcus aureus* (ATCC 6538, Sa) and methicillin-resistant *Staphylococcus aureus* (MRS). Growth inhibition was determined in 96-well microtiter plates by incubating the organism with diluted samples and assessing growth compared to controls.[17] Ca was cultured in Sabouraud's dextrose broth, while mycophil broth was used to culture Cn and the Aspergillus species. Trypticase soy broth was used for Pa and Eugon for Sa. Mi was cultured in Middlebrook media with OADC supplement. Prior to the assay, the appropriate media were inoculated with microorganisms and allowed to incubate for 24 (Ca, Pa, Sa, MRS) or 72 (Cn, Mi) hours. Yeasts were counted on a hemocytometer and diluted in broth to 10,000 (Ca) or 200,000 (Cn) CFUs/mL. Pa, Sa and MRS were diluted 1:50 in broth while Mi was diluted 1:30. Inocula were prepared from the *Aspergillus* species on the day of the assay by flooding a slant with a mat of fungus growth and gently scraping with a pipet tip to release spores. On the day of the assay, inoculum was added to diluted sample in the wells of a 96 well microtiter plate such that the greatest final concentration tested was 50 μg/mL (the highest DMSO concentration was 2.5%). The plates were incubated at 37°C (Ca, Mi, Pa, Sa, MRS) or at 30°C (Cn, Afl, Afu). Following incubation, the growth is determined as absorbance at 630 nm measured with an EL-340 plate reader (Bio-Tek Instruments, Inc.).The inhibitory activity of the sample was expressed as the Minimum Inhibitory Concentration (MIC), the lowest concentration tested which inhibited 80% of growth compared to controls. IC_{50} values (the concentration which inhibited 50% of growth) were also determined from graphs of concentration versus % growth compared to the controls.[18]

4.3.2. Cytotoxicity

Compounds were assessed for inhibition of growth of human cancer cell lines: SK-MEL, KB, BT-549 and SK-OV-3. Additionally, a cell line derived from monkey kidney fibroblasts, VERO, was also used to represent non-cancerous cells. Cells were cultured in RPMI-1640 media supplemented with 10% bovine serum and 60 μg/mL

amikacin and incubated at 37°C and 5% CO_2. Cells were plated in 96-well tissue culture-treated microtiter plates at 25,000 cells/well and allowed to adhere for 24 hours. Samples, diluted in RPMI, were added to the wells such that highest final concentration was 10 μg/mL. Following 48 hours of incubation with samples, growth was assessed by measuring uptake of the supravital dye, neutral red.[19]

4.3.3. Antimalarial Activity

Compounds were tested for antimalarial activity *in vitro* against a chloroquine sensitive strain of *Plasmodium falciparum* (D6). Parasites were cultured in A+ human red blood cells suspended in RPMI 1640 supplemented with 10% normal type A+ human serum, 25 mM HEPES, 27 mM bicarbonate and 60 ug/mL amikacin at 37°C and under a gas mixture of 90% N_2, 5% O_2 and 5% CO_2. Susceptibility assays were conducted in a manner similar to Makler et al. (1993)[20] in which infected red blood cells were incubated for 48 hours with samples in wells of a microtiter plate. The highest concentration of samples tested was 4.8 μg/mL. Following incubation, the growth of parasite was assessed with the Malstat™ reagent (Flow Inc., Portland, OR) followed by reduction of nitro blue tetrazolium dye. Absorbance of the reduced dye was measured at 630 nm. The growth assay is based on the specificity of 3-acetylpyridine adenine dinucleotide (Malstat™reagent) as a substrate of the parasite lactate dehydrogenase.[20]

4.3.4. Inhibition of TopoisomeraseI

The catalytic activity of TopoI was determined in terms of the conversion of supercoiled DNA to relaxed DNA as described earlier.[4] The assay for inhibition was performed using the TopoI drug kit (TopoGen, Inc.) according to the procedure provided with the kit.The IC_{50} values were calculated rom the dose response curves of %inhibition versus tested concentration.

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