Novel 11-Substituted Ellipticines as Potent Anticancer Agents with Divergent Activity against Cancer Cells

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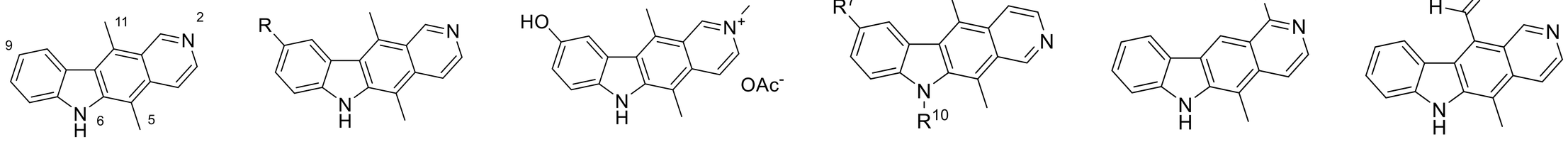
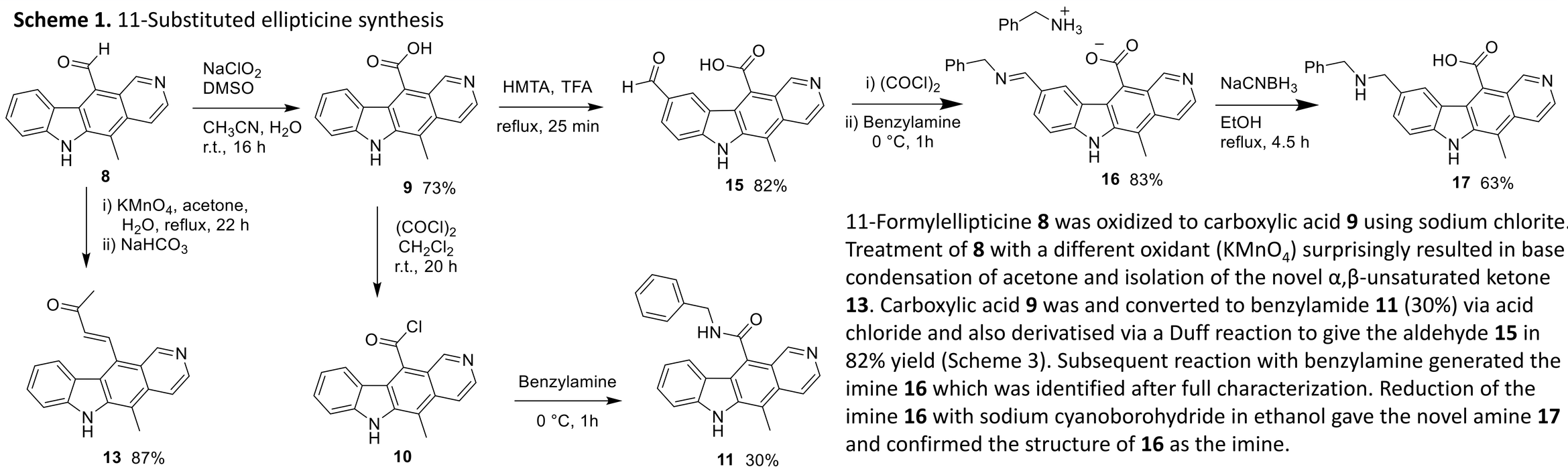
Ellipticine **1** (5,11-dimethyl-6*H*-pyrido [4,3-*b*]carbazole, Figure 1) was isolated in 1959 from a small tropical evergreen tree (Ochrosia elliptica) by Goodwin *et al.* [1]. Since its isolation, the planar tetracyclic structure of ellipticine (and 9-methoxyellipticine **2**) has been the focus of extensive chemical and pharmacological research [2]. Celiptium **3** and 9-hydroxyellipticine **4** both progressed to phase II clinical trials though were subsequently discontinued [3-6]. Recent work within our group has expanded on the ellipticines to isoellipticines (**5** and **6**) which are identified with potent cellular and *in vivo* activity with substitution dependent cellular effect [7-10].

Figure 1. Structures of Ellipticine **1** and related anticancer agents.



The 11-position of ellipticine has received little attention despite evidence that it may be key to bioactivity [12]. Removal of the 11-methyl group to form olivicines maintains potency for DNA topoisomerase inhibition (**7**, R = CH_3, Figure 1) with olivacine, S16020 (**7**, R = carboxamide) progressing to clinical trials [13-15]. Despite synthesis of 11-formyl ellipticine **8** (Figure 1) almost 30 years ago, only four reported compounds exist with carbonyl at 11-position [16-17]. We therefore set out to develop novel 11-substituted ellipticines and evaluate their effect on topoisomerase II and cell growth in the National Cancer Institute's 60 cell line screen [18].

Scheme 1. 11-Substituted ellipticine synthesis



11-Formylellipticine **8** was oxidized to carboxylic acid **9** using sodium chlorite. Treatment of **8** with a different oxidant ($KMnO_4$) surprisingly resulted in base condensation of acetone and isolation of the novel α,β-unsaturated ketone **13**. Carboxylic acid **9** was and converted to benzylamide **11** (30%) via acid chloride and also derivatised via a Duff reaction to give the aldehyde **15** in 82% yield (Scheme 3). Subsequent reaction with benzylamine generated the imine **16** which was identified after full characterization. Reduction of the imine **16** with sodium cyanoborohydride in ethanol gave the novel amine **17** and confirmed the structure of **16** as the imine.

Topoisomerase II has key functions in the change of topological structure of DNA and hence cell replication which can be evaluated using a decatenation assay. As expected, the planar ellipticine **1** and the simple 9-substituted ellipticines (**2**,**4**,**19**) all displayed excellent inhibition of topoisomerase II at 100 μM (see SI). On assessment of the 11-substituted ellipticines, the majority were inactive against topoisomerase II but compounds **13** α,β-unsaturated ketone and **16** 9-substituted imine showed the most promise and are new discovery templates. National Cancer Institute (NCI) evaluation of 11-substituted ellipticines identifies significant effects on the growth of the 60-cell line panel with mean growth values ranging from 18% to 106%. The Mean Growth percent is a reference tool whereby screening at 10 μM concentration is used to filter active anticancer compounds. Of the six compounds tested, two (11-substituted amide **11** and conjugated ketone **13**) achieved Mean Growth percentages of <25% and fulfilled the requirements for progression to the five-dose assay (Table 1).

	11-Substituent	9-Substituent	Topo II Inhibition[a]	NSC No	NCI Mean Growth %
8	CHO	H	–		Not tested
9	COOH	H	–	762124	99.92
11	$CONHCH_2Ph$	H	–	762144	21.22
13	CH=CH-C(O)Me	H	+	762123	17.83
15	COOH	CHO	–	762141	95.56
16	$COO^- {}^+NH_3CH_2Ph$	$CH=NCH_2Ph$	+	762142	106.19
17	COOH	CH_2NHCH_2Ph	–	762143	101.72

Table 1. 11-Substituted ellipticine topo II inhibition and effect on NCI (National Cancer Institute) 60 cancer cell mean growth (one dose 10μM)

a.R^1 = C-9 substituent; R^2 = C11 substituent (+) Inhibition observed at 100 μM; (–) no activity observed at 100 μM.

Cell Line	Cancer Subtype	11		13	
		GI50	LC50	GI50	LC50
HOP-62	Lung	2.15	>100	1.77	26.0
SW-620	Colon	2.86	>100	1.65	44.0
SNB-75	CNS	2.05	>100	2.65	34.8
OVCAR-3	Ovarian	2.33	<10	2.53	28.0
OVCAR-4	Ovarian	1.71	6.19	2.88	41.5
786-0	Renal	2.79	72.0	2.79	29.8
A498	Renal	50.5	>100	0.386	7.48
UO-31	Renal	2.73	>100	1.25	33.8
MCF7	Breast	2.71	>100	1.74	52.3
MDA-MB-231	Breast	2.43	>100	1.74	41.3
HS578T	Breast	2.61	>100	1.96	48.4

Table 2. Selected GI50 and LC50 of the NCI 60 cell line panel **11** and **13** (data reported in μM values; GI50: Growth Inhibition 50%; LC50: Lethal conc. 50%).

Evaluation at of **11** and **13** at five dose confirmed the potency and their specific effects on cells seen in the one dose screen. Benzylamide **11** exerts a broad range of activity from cytostatic to cytotoxic at dose ranges from 1 to 100 μM (Table 2; Figure 3, note divergence from mean GI50 in horizontal bars). Growth is significantly restricted against HOP62, SNB75, OVCAR-3, OVCAR-4 and 786-0 but there is selective cytotoxicity with no evident effect on cell growth of some cancers, in particular melanoma: Leukaemia (HL-60), Lung (EKVX), CNS (SF-295, SNB-19), Melanoma (MALME-3M, M14, SK-MEL-2, SK MEL-28, UACC-257), Ovarian (OVCAR-5, NCI/ADR-RES), Breast (T-47D). Ketone **13** exerts a far more cytotoxic effect across all cell lines with consistent cell death evident at 10 μM (Table 2, LC50 column; Figure 3, note lack of divergence) and is exceptionally potent against the growth of A498 renal cancer (386 nM). It is assumed that the Michael acceptor moiety is involved in alkylation of essential cellular machinery.

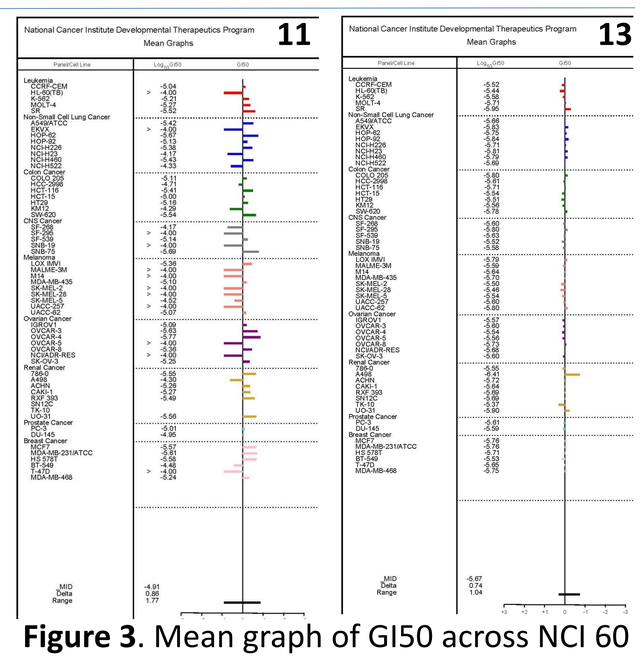


Figure 3. Mean graph of GI50 across NCI 60 panel (horizontal bars represent divergence)

In summary, although limited topo II inhibition was identified it is evident that both benzylamide **11** and unsaturated ketone **13** are highly potent and affect cell growth by different mechanisms with broad cytotoxicity seen for compound **13** but some selectivity of cellular response seen for compound **11**. Benzylamide **11** has the potential for use in ovarian cancers given its exceptional toxicity against the OVCAR-3 and OVCAR-4 phenotypes. COMPARE analysis identified a potential target for compound **11** in Aurora kinase due to correlation of 0.6 with the known inhibitor SCH1473759 [18]. This will be the focus of a new panel of ellipticine 11-amides.

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