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# Novel 6,7-diphenyl-2,3,8,8a-tetrahydro-1H-indolizin-5-one Analogues as Cytotoxic Agents†

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**Abstract**—A series of 6,7-diphenyl-2,3,8,8a-tetrahydro-1H-indolizin-5-one analogues were synthesized and evaluated for cytotoxic activity against eight human cancer cell lines. Compounds **18**, **21**, **28**, **29**, **30** and **31** showed cytotoxic activity with $GI_{50}$ values in the range of 2.1–8.1 μM concentration. Among these, compounds **21** and **28** exhibited good pharmacokinetic properties. These compounds were further evaluated for their in vivo efficacy in modified hollow fibre assay (HFA).


Antimitotic agents are one of the major classes of cytotoxic drugs for cancer treatment and have attracted attention recently due to the clinical success of Taxol.[1–4] Antimitotic agents arrest the cell division by interfering with the normal microtubule polymerization/depolymerization process. Although antimitotic drugs such as Vincristine and Paclitaxel gained wide clinical use for the treatment of various cancers, they suffer from many side effects, difficulty in dosing schedule and lack of efficacy against various multidrug resistant cancer cell lines. Therefore, there is a need to discover novel antitumor agents with fewer side effects, improved pharmacokinetic properties and better efficacy with novel mechanism of actions.

In 1987, Petit et al.,[5] reported the isolation of Combretastatin A-1 (**CA-1**) and B-1 (**CB-1**) from the South African willow tree *Combretum caffrum* (Combretaceae). Both natural products were shown to be significant cancer cell growth inhibitors and antimitotic agents. Later it was found that monophenol derivative of **CA-1**, Combretastatin A-4 (**CA-4**) is much more potent in its phosphate prodrug.[6] Current phase-II clinical trials and the uncovering of its very promising anti-angiogenesis effects[7,8] combined with its strong anti-tubulin activity attracted our attention and prompted us to search for similar novel alkaloids of plant origin with a novel ring structure as part of our ongoing cancer research programme.[9,10]

Our search resulted in identification of an interesting compound Septicine (**32**, Fig. 1), reported in the literature, from the Indian medicinal plant *Tylophora asthamatica*.[11] The prominent difference between Septicine and Combretastatins (**CA-1**, **CB-1** and **CA-4**) is that Septicine has a novel additional indolizidine ring which is absent in Combretastatins. In this paper we describe the synthesis, cytotoxic activity and structure activity relationship of Septicine (**32**) and its analogues.

Literature search revealed that Septicine has been synthesized earlier by different routes.[12–18] We employed the method reported by Bhakuni et al.[17,18] with a few modifications to synthesize analogues as shown in Scheme 1. Appropriately substituted 3-oxo-3-phenyl-propionicacid methyl esters (**1**) were hydrolyzed to corresponding 3-oxo-3-phenyl-propionicacids (**2**), these were condensed with 1-pyrroline **3**[19] to give 1-phenyl-2-pyrrolidin-2-yl-ethanones (**4**). Reaction of **4** with different substituted phenylacetylchlorides (**5**) in the presence of triethylamine yielded 1-phenyl-2-(1-phenylace-

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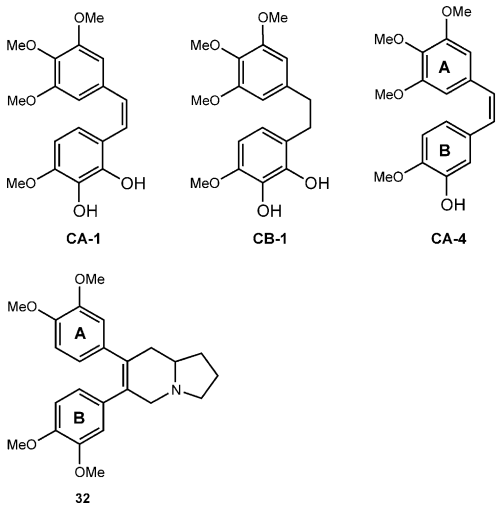
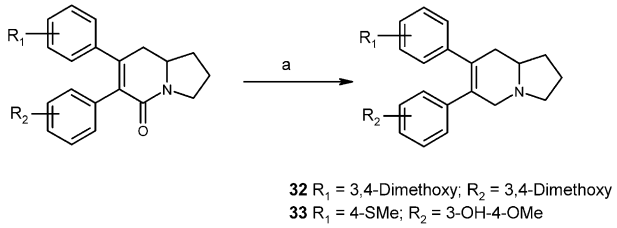



**Figure 1.** Structures of Combretastatin A-1(**CA-1**), Combretastatin B-1(**CB-1**), Combretastatin A-4 (**CA-4**) and Septicine (**32**).

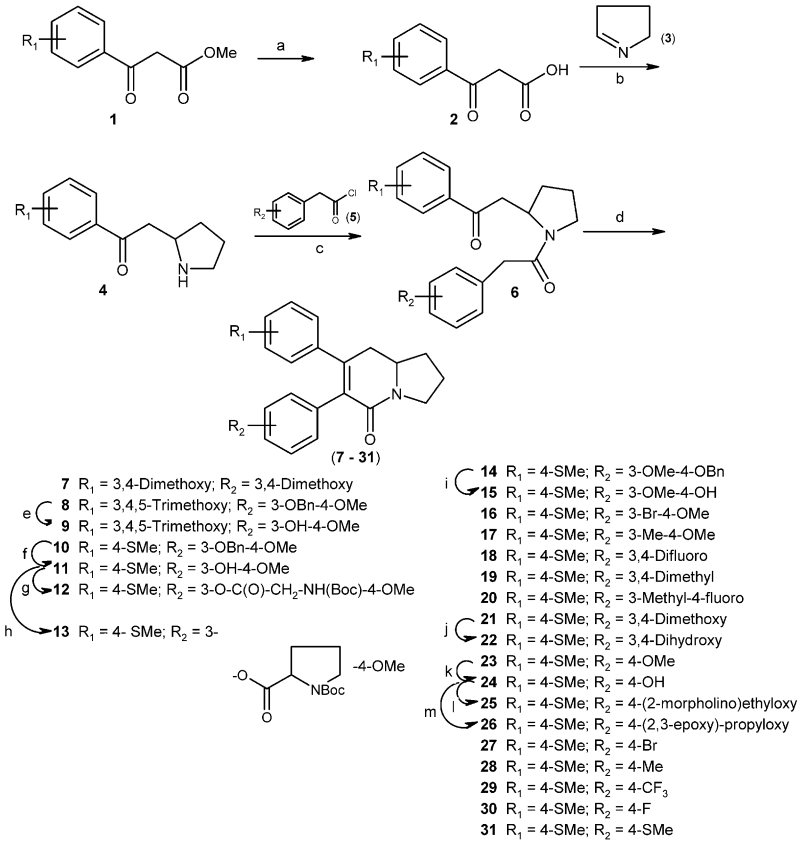
tylpyrrolidin-2-yl-)-ethanones (**6**). These 1-phenyl-2-(1-phenylacetylpyrrolidin-2-yl-)-ethanones (**6**) were cyclized in alcoholic potassium hydroxide to obtain 6,7-diphenyl-2,3,8,8a-tetrahydro-1H-indolizin-5-ones (**7**–**31**) in good yields.




**Scheme 2.** (a) $BH_3$:DMS, THF, rt, 6–12 h, 40–45%.

Reduction of lactum was achieved by using $BH_3$:DMS in THF as shown in Scheme 2.

Cytotoxic activities of the synthesized compounds were tested against Breast (MCF/ADR), CNS (U251), Colon (SW620), Lung (H-522), Melanoma (M14), Ovarian (SKOV3), Prostate (DU145) and Renal (A498) human cancer cell lines. The results are shown in Table 1. For comparison purposes, the average cytotoxic activities of compounds in all cell lines were calculated and the results of this computation are included in Table 1. We started our SAR from septicine (**32**) expecting a minimum activity due to structural similarity with Combretastatin. But to our surprise the compound did not show cytotoxic activity. Then we screened compound **7**, which is a precursor for **32**, and **9** which has similar methoxy/hydroxylation pattern of **CA-4**, against a panel




**Scheme 1.** (a) 2–5% aq KOH, rt, 24 h, 65–70%; (b) phosphate buffer (pH 7.0), rt, 60 h, 55–60%; (c) $Et_3N$, $CH_2Cl_2$, rt, 2–4 h, 48–55%; (d) 1–4% ethanolic KOH, reflux, 2–4 h, 75–80%; (e) concd HCl/MeOH, reflux, 10 h, 85%; (f) concd HCl/MeOH, reflux, 10 h, 87%; (g) *N*,*N*′-Carbonyldiimidazole (1 equiv), *N*-Boc-glycine (1 equiv), $CH_2Cl_2$, rt, 12 h, 62%; (h) *N*,*N*′-carbonyldimidazole (1 equiv), *N*-Boc-(L)-proline (1 equiv), $CH_2Cl_2$, rt, 12 h, 58%; (i) concd HCl/MeOH, reflux, 12 h, 85%; (j) 47% aq HBr, reflux, 2 h, 40%; (k) 47% aq HBr, reflux, 2 h, 85%; (l) Chloroethylmorpholine.HCl (1.5 equiv), $K_2CO_3$ (2 equiv), acetone, reflux, 12 h, 75%; (m) epichlorohydrin (1.5 equiv), NaH (2 equiv), benzene, reflux, 12 h, 87%.

**Table 1.** In vitro cytotoxic activities of indolizidinone derivatives

| Compd | $GI_{50}$ values in μM | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Breast MCF/ADR | CNS U 251 | Colon SW620 | Lung H-522 | Melanoma M14 | Ovarian SKOV3 | Prostate DU145 | Renal A498 | Average $GI_{50}$ (μM) |
| **7** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **9** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **11** | 5.0 | 5.0 | 20.0 | 6.0 | nt | >100 | 6.0 | nt | 23.6 |
| **12** | 6.0 | 6.0 | 20.0 | 7.0 | nt | 20.0 | 10.0 | nt | 11.5 |
| **13** | 4.0 | 2.0 | 10.0 | 5.0 | nt | 10.0 | 5.0 | nt | 6.0 |
| **15** | 8.0 | 8.0 | 9.0 | 7.0 | 9.0 | 8.0 | 8.0 | 5.0 | 7.75 |
| **16** | 6.0 | 0.8 | 5.0 | 0.7 | 3.0 | 3.0 | 0.9 | 0.6 | 2.5 |
| **17** | 0.4 | 0.09 | 0.5 | 0.1 | 0.2 | 15.0 | 0.9 | 0.4 | 2.1 |
| **18** | 0.8 | 15.0 | 0.2 | 0.2 | 0.3 | 0.1 | 0.5 | 0.1 | 2.1 |
| **19** | 0.8 | 10.0 | 0.7 | 0.3 | 0.8 | 0.3 | 0.5 | 0.3 | 1.7 |
| **20** | 0.5 | 0.2 | 0.6 | 0.5 | 0.8 | 6.0 | 0.4 | 0.4 | 1.1 |
| **21** | 0.6 | 0.3 | 3.0 | 2.0 | 5.0 | 9.0 | 1.5 | 8.0 | 3.6 |
| **22** | 80.0 | 30.0 | 50.0 | 60.0 | nt | 80.0 | 60.0 | nt | 60 |
| **23** | 5.0 | 2.0 | 3.0 | 0.8 | 4.0 | 3.0 | 0.9 | 0.8 | 2.4 |
| **24** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **25** | 9.0 | 9.0 | 9.0 | 7.0 | 20.0 | 9.0 | 20.0 | 9.0 | 11.5 |
| **26** | 10.0 | 0.08 | 0.09 | 0.03 | 0.09 | 10.0 | 0.5 | 0.5 | 2.6 |
| **27** | 8.0 | 7.0 | 7.0 | 7.0 | 9.0 | 9.0 | 9.0 | 7.0 | 7.8 |
| **28** | 0.6 | 0.4 | 3.0 | 0.9 | 6.0 | 9.0 | 0.6 | 4.0 | 3.0 |
| **29** | 0.8 | 0.8 | 0.8 | 0.8 | 12.0 | 15.0 | 0.7 | 20.0 | 6.3 |
| **30** | 0.8 | 0.09 | 0.5 | 0.25 | 0.6 | 30.0 | 0.5 | 0.1 | 4.1 |
| **31** | 8.0 | 5.0 | 7.0 | 15.0 | 5.0 | 2.0 | 8.0 | 15.0 | 8.1 |
| **32** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **33** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **CA-4** | 0.09 | 0.006 | 0.005 | 0.007 | 0.0001 | <0.0001 | nt | 0.61 | 0.103 |

$GI_{50}$ μM—Concentration that produces 50% inhibition.
nt, Not tested.

of eight human cancer cell lines. Compounds **7** and **9** were also found inactive. A very slight improvement in activity with an average $GI_{50}$ of 23.6 μM concentration was seen when all the methoxy groups in ring A of compound **9** were replaced with 4-thiomethyl substituent (**11**). However the corresponding reduced compound (**33**) did not show any activity. This proves the importance of the 4-thiomethyl substituent in ring A and the presence of lactum carbonyl in central indolizidine ring for the activity. Further improvement in activity was observed for ester derivatives **12** and **13** with 11.5 and 6.0 average $GI_{50}$ values, respectively. Compound **15**, a positional isomer of **11**, has shown better activity (7.75 μM). Compound **21**,[21] where the hydroxy group of compound **11** was replaced with methoxy, exhibited potent activity in various cell lines ranging from 0.3–9.0 μM. It is significant to note that activity at less than 1 μM concentration in many of the tested cell lines was achieved for the first time in this series with compounds, where one of the methoxy group in ring B of compound **21** was replaced with bromo (**16**) and methyl (**17**) substituents. Comparison of cytotoxic activities of compounds **11**, **15**, **16**, **17**, **21** and compounds **18**, **19**, **20** revealed that methoxy substituents in ring B seem to be not very important for these compounds for their cytotoxicity. Compounds **18**, **19** and **20** where both methoxy groups in ring B of compound **21** were replaced by fluoro and/or methyl substituents resulted in marked increase in antiproliferative activities. However, compound **22**, a dihydroxy derivative was found to be inactive.

At this juncture, we shifted our focus to know whether both the substituents in ring B are required for the biological activity or one is sufficient. To test this, compound **23** was synthesized with 4-methoxy substituent in ring B. The compound displayed similar activity to that of its counterpart **21**. Compounds with 4-bromo (**27**), 4-methyl (**28**[22]), 4-trifluoromethyl (**29**), 4-fluoro (**30**) and 4-thiomethyl (**31**) substituents in ring B showed better activity in the increasing order of $SCH_3 < Br < F < CF_3 < CH_3$. Compound **25**, where morpholinoethyl group was introduced in the place of methyl group of **23** by aiming to improve the solubility and in vitro potency has shown less activity, whereas **26** with (2,3-epoxy)-propyl substituent has shown excellent activity with nanomolar $GI_{50}$ values in three out of eight cell lines. Combretastatin A-4 when tested for its cytotoxic activity in the above human cancer cell lines showed 100-fold potency in comparison to compounds **21** and **28**.

The pharmacokinetic properties in mice, for selected indolizidinone analogues (**18**, **21**, **28**, **29**, **30**, **31**) were studied at 200 mg/kg ip dose. The results are summarized in Table 2. The pharmacokinetic profiles for compounds

**Table 2.** Pharmacokinetic parameters in mice

| Parameters | **18** | **21** | **28** | **29** | **30** | **31** |
|---|---|---|---|---|---|---|
| Dose mg/kg, ip | 100 | 200 | 200 | 200 | 200 | 200 |
| AUC $_{(0\text{-}t)}$ μM. h. | 6.92 | 102.37 | 38.54 | 10.72 | 3.88 | 2.50 |
| $C_{max}$ μM | 3.61 | 22.99 | 8.02 | 3.79 | 0.99 | 0.86 |
| $T_{max}$ h | 0.29 | 0.25 | 0.25 | 0.25 | 1.50 | 1.00 |
| $t_{1/2\beta(2,4,6,8\ h)}$ h | # | 7.29 | 6.24 | 2.66 | 2.59 | # |

# Not possible to calculate because of detection limits of the analytical method and limited data points available in the elimination phase.
Each value is mean ± S.D. of $n = 3$–4 observations.

**21** and **28** are encouraging and are characterized by reasonable $C_{max}$ and $t_{1/2}$ values. On the basis of trends we observed in vitro followed by pharmacokinetic profiles, we chose to examine the preliminary in vivo efficacy of compounds **21** and **28** in modified hollow fiber assay (HFA). Each compound was tested at two different doses 200 and 400 mg/kg intraperitoneally in QDx4 schedule by following the procedure described by Melinda et. al.[20] The actively growing different types of cancer cells in hollow fibre capsules were implanted in SAM (Swiss Albino Mice) aseptically under light ether anesthesia in to the subcutaneous (sc) and intraperitonial (ip) compartments. The anticancer activity in this model was assessed based on percent net growth in both the compartments. Compounds **21** and **28** were tested against all the eight cell lines. To simplify evaluation, a points system has been adopted which allows rapid viewing of the activity of a given compound by using HFA criteria, that is a %T/C of 50 or less in 10 of the 32 possible test concentrations (eight cell lines × two sites × two compound doses). For this a value of two is assigned for each compound dose which results in a 50% or greater reduction in a viable cell mass. The ip and sc samples were scored separately.

Generally, compounds with a total score of $\geq 20$ in IP + SC are referred for xenograft testing. However, compounds **21** and **28** showed poor activity by scoring IP + SC 4/32 and 8/32, respectively. The reason for this may be the physical properties of the compounds, particularly solubility made it difficult to dose in this particular schedule. Plans are on to make some more compounds to strike a balance between solubility and in vitro potency followed by in vivo efficacy.

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21. Note 1. Compound **21**: mp 150–151 °C; IR: 2926.4, 1631.2, 1514.1 $cm^{-1}$; $^1$HNMR ($CDCl_3$) δ 1.5–2.0 (2H, m), 2.1 (1H, m), 2.3 (1H, m), 2.45 (3H, s), 2.7–2.85 (2H, m), 3.5–4.0 (3H, m), 3.65 (3H, s), 3.85 (3H, s), 6.6–6.7 (3H, m), 6.9–7.1 (4H, dd, $J = 8.4$ Hz); EIMS *m*/*z* 395 ($M^+$, 100%).
22. Note 2. Compound **28**: mp 105–106 °C; IR: 1640.6, 1434.8, 1363.0 $cm^{-1}$; $^1$HNMR ($CDCl_3$) δ 1.6–2.0 (2H,m), 2.1(1H, m), 2.2 (1H, m), 2.45 (3H, s), 2.5 (3H, s), 2.8 (2H, m), 3.7 (2H, m), 4.0 (1H, m), 7.0 (8H, m); EIMS *m*/*z* 349 ($M^+$, 100%).