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Microwave expedited synthesis of 5-aminocamptothecin analogs: Inhibitors of hypoxia inducible factor HIF-1α

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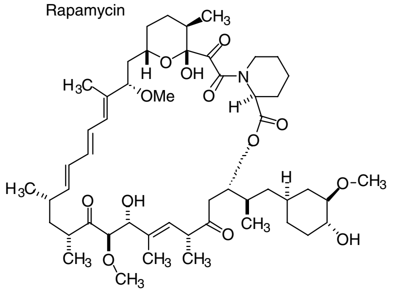
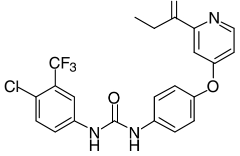
Abstract—A series of 5-aminosubstituted camptothecin analogs were prepared from the corresponding 5-hydroxycamptothecin using microwave irradiation. The analogs were assayed for ability to inhibit the action of hypoxia inducible factors (HIF-1α and HIF-2α). The 5-fluoroethyl analog showed potent inhibitory activity and is now the focus of ongoing pathway analysis and potential as an antiproliferative agent.

Hypoxia is a common characteristic of many solid tumors, and can confer considerable resistance to conventional chemotherapy, contributing to tumor progression in over 70% of human cancers, for example, prostate cancer.[1] It is now recognized that progression to hypoxic state is influenced and regulated by a class of hypoxia inducible factors, exemplified by the heterodimeric transcription factor, HIF-1α.[2] Given the significance of the hypoxic state, inhibitors of these factors could hold promise in the treatment of solid tumors and a variety of agents have been uncovered which directly or indirectly modulate the myriad pathways linked to HIF homeostasis.[3] For example, the immunosuppressant rapamycin has been shown to down regulate HIF-1α production by inhibiting the so-called PI3K/Akt/mTOR signaling pathway, regulating protein synthesis through phosphorylation.[4] The alkylating agent PX-478 causes reduction in HIF-1α levels presumably through a redox-sensing mechanism and has a similar inhibitory effect on VEGF production.[5] The agent nexavar also regulates HIF through its actions as a multi-kinase inhibitor, acting on downstream targets of HIF including VEGFR, B-RAF, C-RAF, and PDGFR.[6] We became interested in HIF inhibitors following reports that camptothecin analogs including topotecan are able to inhibit HIF-1α translation through a topoisomerase I-dependent mechanism.[7] This finding is significant as topotecan is clinically approved for the treatment of solid (ovarian) tumors, thus the possibility exists for rational drug design to improve HIF modulation.

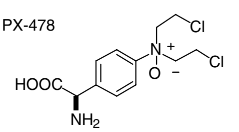
Rapamycin



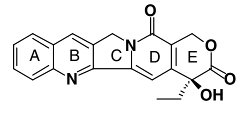
Nexavar



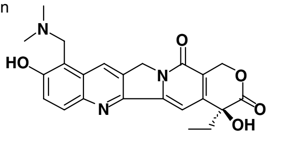
PX-478



Camptothecin



Topotecan



The parent drug campothecin is an alkaloid isolated from *Camptotheca acuminata* and showed significant activity in early L1210 leukemia screens.[8] Though toxic side effects and water insolubility limited precluded its

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Keywords: Camptothecin; Anticancer; Hypoxia; HIF; Microwave.

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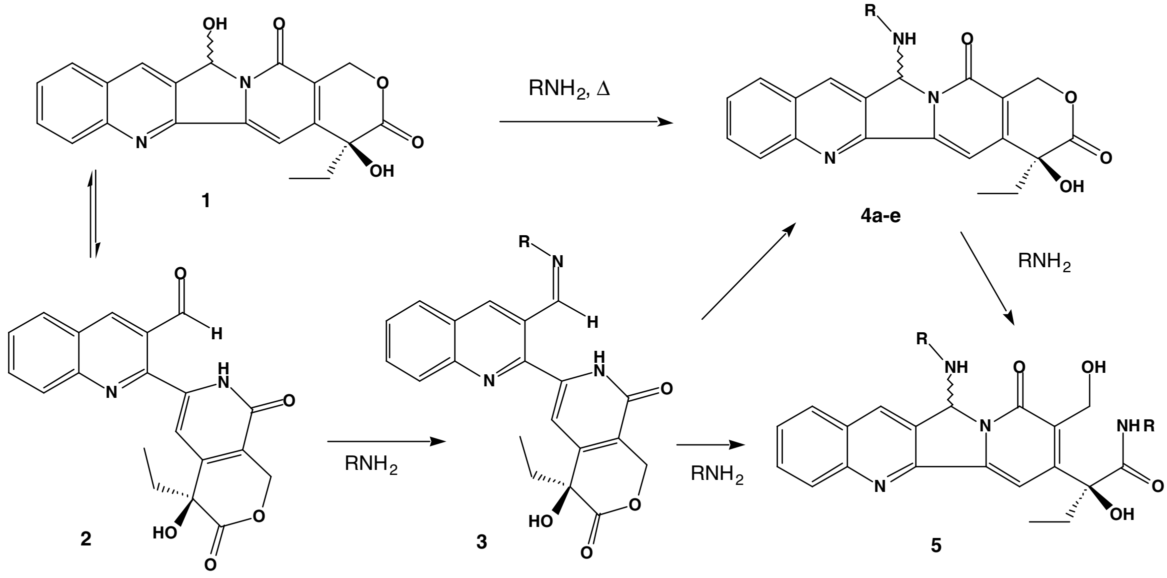
clinical development, subsequent research identified and validated topoisomerase I as its biological target. Second generation agents have been developed which address these problems.[9] The camptothecins possess a pentacyclic ring structure, designated A–E rings, and though modification of the D, E rings usually results in complete loss of activity, various substituents have been introduced in the A ring (topotecan), B ring (irnotecan), and C ring (20*S*-5-ethylidenecamptothecin) with either enhanced or comparable activity to the parent molecule.[9] Substitution at the C5 position forms the basis of our investigation, following reports of facile introduction of 5-amino substituents via displacement of the 5-hydroxy group, which can be installed directly from camptothecin ($FeCl_3/H_2SO_4$/EtOH then HCl, EtOH).[10] 5-OH-Camptothecin **1** exists in equilibrium with its hemi-aminal form **2**, thus facile nucleophilic substitution is possible (Scheme 1). In order to achieve efficient conversion to desired ring closed products **4**, addition to form imine **3** must be achieved under conditions that prevent double addition viz. **5**, thus an expeditious procedure is desirable. Conventional conditions involve thermolysis for 6–24 h in the presence of excess amine and were examined for a range of substrates (Table 1). In each case, though products could be recovered, varying quantities of double addition products **5** were observed, complicating purification and diminishing yields.[11] Based on promising results using microwave irradiation,[12] direct conversion to **4** was surveyed using a range of conditions and a commercial (CEM Navigator™) oven. In each case (Table 1) efficient conversion was achieved within minutes, eliminating the production of double addition products. Workup involved filtering off product through a silica gel plug or in exceptional circumstances using preparative TLC, giving direct access to a family of 5-aminosubstituted analogs.[13] Based on prior expectations, all of the analogs produced were freely soluble in buffer solutions, in polar organic solvents, and in DMSO/DMF aqueous admixes, allowing for facile biological analysis.



Scheme 1. Microwave expedited route to 5-aminocamptothecin analogs.

Table 1. Comparative methods for synthesis of 5-aminocamptothecins

Entry	Amine	Conditions	**4** (%)
1	$PhCH_2NH_2$	12 h reflux, MeOH, Et_3N	48
2	$(4\text{-MeO})PhCH_2NH_2$	12 h reflux, MeOH, Et_3N	43
3	$(4\text{-F})PhCH_2NH_2$	12 h reflux, MeOH, Et_3N	23
4	$(3\text{-F})PhCH_2NH_2$	12 h reflux, MeOH, Et_3N	29
5	$CH_3CH_2CH_2NH_2$	12 h reflux, MeOH, Et_3N	33
6	$FCH_2CF_2NH_2$	12 h reflux, MeOH, Et_3N	27
7	$(4\text{-MeO})PhCH_2NH_2$	MW, 5 min, 150 °C, MeOH, Et_3N	67
8	$(4\text{-MeO})PhCH_2NH_2$	MW, 10 min, 150 °C, MeOH, Et_3N	88
9	$(4\text{-MeO})PhCH_2NH_2$	MW, 15 min, 150 °C, MeOH, Et_3N	73
10	$(4\text{-MeO})PhCH_2NH_2$	MW, 10 min, 100 °C, MeOH, Et_3N	65
11	$(4\text{-MeO})PhCH_2NH_2$	MW, 10 min, 200 °C, MeOH, Et_3N	79
12	$(4\text{-F})PhCH_2NH_2$	MW, 10 min, 150 °C, MeOH, Et_3N	81
13	$(3\text{-F})PhCH_2NH_2$	MW, 10 min, 150 °C, MeOH, Et_3N	77
14	$CH_3CH_2CH_2NH_2$	MW, 10 min, 150 °C, MeOH, Et_3N	83
15	$FCH_2CF_2NH_2$	MW, 10 min, 150 °C, MeOH, Et_3N	80

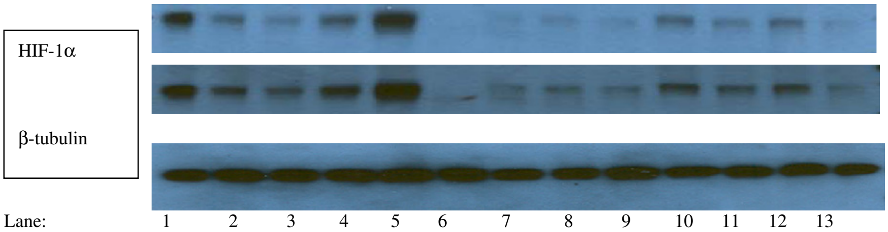


Figure 1. Impact on HIF-1α production in c4-2 cells under influence of camptothecins. Lanes 1, FBS; 2, FBS + 20 nM rapamycin; 3, FBS + 10 nM nexavar; 4, CSS; 5, CSS + 10 nMDHT; 6, pTV cell line (HIF-2α expressing negative control derived from 786-O cells); 7, FBS + 10 μM camptothecin; 8, FBS + 1 μM topotecan; 9, FBS + 10 μM topotecan; 10, FBS + 10 μM **4** (R = *n*-Pr); 11, FBS + 1 μM **4** (R = *n*-Pr); 12, FBS + 1 μM **4** (R = FCH_2CH_2); 13, FBS + 10 μM **4** (R = FCH_2CH_2). (FBS, fetal bovine serum; CSS, charcoal stripped serum; and DHT, dihydrotestosterone.)

Table 2. Relative Inhibition of HIF-1α production by 5-aminocamptothecins[a]

Entry	Compound	Concentration	Inhibition (SD)
1	Rapamycin	20 nM	35% (0.10)
2	Nexavar	10 nM	54% (0.05)
3	Camptothecin	10 μM	69% (0.09)
4	Topotecan	10 μM	69% (0.08)
5	5-*p*-Methoxybenzylaminocamptothecin	10 μM	9% (0.15)
6	5-*p*-Fluorobenzylaminocamptothecin	10 μM	40% (0.04)
7	5-*m*-Fluorobenzylaminocamptothecin	10 μM	0% (0.16)
8	5-Propylaminocamptothecin	10 μM	76% (0.22)
9	5-β-Fluoroethylaminocamptothecin	10 μM	88% (0.06)

[a] c4-2 and 786-O cells were grown for 48 h. then treated with compounds /controls for 24 h in a humidified environment. Cells were then washed with PBS and then lysed with 2% SDS containing 1% protease inhibitor cocktail. Protein content of lysates was determined using Bradford assay. For both HIF-1α and HIF-2α analyses, approximately 13 μg of protein was loaded per lane. The proteins were resolved on SDS–PAGE and transferred to polyvinylidene difluoride membrane (Millipore). Blots were probed for either HIF-1α or HIF-2α using monoclonal anti-HIF-1α or polyclonal anti-HIF2α (Novus Biological Inc.). Control blots were probed for β-tubulin using monoclonal anti-β-tubulin. Blots were quantitated using Bio-Rad gel dock software. Densities of HIF-1α/HIF-2α and β-tubulin bands were evaluated, background densities were also determined and subtracted from appropriate band densities.

The agents, plus control elements camptothecin, rapamycin, nexavar, and topotecan, were assayed for ability to inhibit production of HIF-1α and HIF-2α. HIF expression can be monitored using a variety of cell lines under simulated hypoxic conditions. However, we were keen to employ cells which express HIF under normoxic conditions and selected cell lines c4-2 and 786-O. c4-2 cells are an androgen independent line derived from LNCaP human prostate cancer cells which are xenografted into castrated mice and known to express HIF-1α.[14] The 786-O line is a vhl deficient derivative of renal cell carcinoma and is known to express HIF-2α.[15] Following exposure, HIF levels and those of control proteins (tubulin) were assessed by Western blot (Fig. 1, Table 2). Clearly rapamycin and nexavar have a pronounced impact on HIF-1α production, but within the family of camptothecins the new derivatives had comparable inhibitory activity, and the fluoroethyl analog was superior. Though inhibition of HIF-1α was marked, none of the agents had any discernable impact on HIF-2α production (data not shown), suggesting that a specific signaling event is governed through interaction with the camptothecins.

In conclusion, water soluble 5-aminocoamptothecin analogs have been prepared in three steps from commercially available camptothecin. The 5-fluoroethyl camptothecin analog shows potent HIF-1α inhibitory activity. Thorough investigation of the biological pathway involved will reveal the potential for these agents to be adapted to frontline chemotherapeutics against hypoxic tumors. In this regard the expeditious and efficient microwave mediated route to generate this and related 5-substituted analogs will help accelerate the development of candidate libraries.[16]

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13. Specimen experimental procedure (**4**; R = 4-MeOPhCH_2): 5-hydroxycamptothecin (8.9 mg, 24.5 μmol), 4-methoxybenzylamine (4.8 μl, 36 μmol), and Et_3N (34 μl) were dissolved in anhydrous MeOH (0.5 ml) in a 10 ml CEM microwave thermolysis vial and sealed with an Intelli-Vent® vial cap. The vessel was placed in a CEM Navigator oven and thermolyzed for 10 min (150 °C, 200 psi, 105 W). The solution was diluted with a mixture of 95% EtOAc/5% MeOH (5 ml) and then washed with brine (2× 1 ml) and dried (Na_2SO_4). The solution was filtered then loaded onto a PTLC plate (Merck, silica gel 0.5 mm) and eluted (50:45:5 CH_2Cl_2/hexane/MeOH eluent), to give the product **4** (R = *p*MeOPhCH_2) 10.4 mg, 88% as a colorless solid (*m*/*z*): 484 (M+); ^{1}H NMR (300 MHz, $CDCl_3$), δ 8.70 (s, 1 H), 8.30 (d, $J = 11$ Hz, 1H), 8.05 (d, $J = 11$ Hz, 1H), 7.95 (t, $J = 7$ Hz, 1H), 7.75 (t, $J = 7$ Hz, 1H), 7.55 (s, 1H), 7.35–6.90 (m, 4H), 6.70 (s, 0.5H), 6.60 (s, 0.5H), 5.80–6.30 (br s, 2H), 5.60 (d, $J = 16$ Hz, 0.5H), 5.50 (d, $J = 16$ Hz, 0.5H), 5.20 (d, $J = 16$ Hz, 0.5H), 5.10 (d, $J = 16$ Hz, 0.5H), 3.90 (s, 3H), 3.27 (m, 2H), 1.70–.190 (m, 2H), 1.10–0.95 (m, 3H).
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