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Synthesis and therapeutic evaluation of pyridyl based novel mTOR inhibitors

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ABSTRACT

A series of novel cyanopyridyl based molecules (**1–14**) were designed, synthesized and probed for inhibition of mammalian target of rapamycin (mTOR) activity. Compound **14** was found to be a potent inhibitor of mTOR activity as assessed by enzyme-linked immunoassays and Western blot analysis. Most importantly, systemic application (intraperitoneal; ip) of compound **14** significantly suppressed macroscopic and histological abnormalities associated with chemically-induced murine colitis.

Mammalian target of rapamycin (mTOR) is a protein kinase that regulates cell proliferation and protein synthesis in response to mitogens and nutrients.[1] It is well established that mTOR plays a crucial role in tumorogenesis.[1] More recently, a growing body of evidence causally links increased mTOR activity to heightened inflammatory responses. Indeed, lipopolysaccharide (LPS) stimulation of macrophages leads to the phosphorylation and activation of p70S6K1 as well as that of 4EBP1/PHAS-1;[2] both proteins are bonafide targets of mTOR. Moreover, the mTOR pathway regulates the production of nitric oxide[3] and activates STAT1-dependent transcription in macrophages in response to LPS.[4] Interestingly, a recent study showed that rapamycin, a mTOR inhibitor, blunts leukocyte adhesion and extravasation in the gut mucosa leading to suppression of experimental chronic colitis.[5] In another study, treatment with everolimus (another mTOR inhibitor) reduced the number of T-cells in lamina propria and blocked lymphocytic IFN-γ release thereby ameliorating established murine colitis.[6] These findings suggest that mTOR inhibitors may be useful for treatment of ulcerative colitis (UC).

In the present study we report the synthesis, discovery and structure–activity-relationship (SAR) of novel small molecule inhibitors targeting mTOR. In our quest for finding potential therapeutics we choose pyridine as a basic scaffold. The pyridine scaffold is a very common structural motif that can be found in many natural products and in several pharmacologically interesting compounds. Therefore, the synthesis of pyridine derivatives, with the objective of developing new drugs, is an active area of research. Recently, several reports have emerged which show the therapeutic qualities of cyanopyridine derivatives. For example, 2-cyanopyridylureas derivatives have been claimed for their properties in treating hyper-proliferative and angiogenesis disorders.[7] Furthermore, 3-cyano-2,6-dihydropyridine has been reported as potent inhibitor of dihydrouracil dehydrogenase and its coadministration with 1-ethoxymethyl-5-fluorouracil enhances the antitumor effect.[8] Separately, 3,5-dicyanopyridines derivatives have been described as intermediates in the synthesis of pyrido[2,3-*d*]pyrimidines as antihistaminic agents,[9] pyridothieno- and pyridodithienotriazines endowed with antihistaminic and cytotoxic activity[10] and acyclo-3-deazapyrimidine S-nucleosides that are active toward HIV.[11]

Based on the above rationale, we designed and synthesized cyanopyridyl based molecules **1–14** (Tables 1 and 2) as shown in Schemes 1 and 2. The commercially available 5-amino-2-cyanopyridine or 2-amino-5-cyanopyridine was treated with various acid chlorides or anhydrides using chloroform as solvent and triethylamine (TEA) as base at 0–25 °C for 2–3 h to get target molecules **1–14**. 5-Amino-2-cyanopyridine was treated with β-chloroacetylchloride in presence of TEA to synthesize 3-chloro-*N*-(6-cyanopyridin-3-yl)propanamide. The latter underwent simul-

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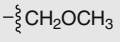
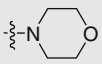
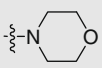
Table 1

Compds	X_1	X_2	R	% mTOR inhibition* at 10 μM
1	N	C		12
2	N	C		31
3	N	C		11
4	N	C		NA
5	N	C		17
7	N	C		<15
8	N	C		<15
9	C	N		NA
10	C	N		NA
12	C	N		NA
13	N	C		NA
14	N	C		70

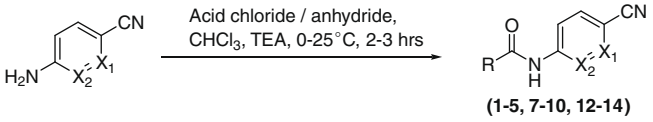
* FCS-induced phosphorylation of p70S6K1 in H460 cells was used as a read out in ELISA assays to ascertain the mTOR inhibitory activity of various compounds.

Table 2

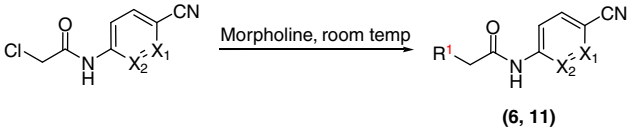
Compds	X_1	X_2	R^1	% mTOR inhibition* at 10 μM
6	N	C		<15
11	C	N		NA

* FCS-induced phosphorylation of p70S6K1 in H460 cells was used as a read out in ELISA assays to ascertain the mTOR inhibitory activity of various compounds.

taneous one pot β-chloro elimination and resulted in *N*-(6-cyanopyridin-3-yl)acrylamide (compound **1**). 5-Amino-2-cyanopyridine was treated separately with chloroacetyl chloride, fluoroacetic anhydride, trifluoroacetic anhydride, methoxyacetyl chloride to obtain compounds **2**, **3**, **4** and **5**, respectively. Analogous to the synthesis of compound **2**, 5-amino-2-cyanopyridine was treated separately with cyclopantanecarbonyl chloride, thiophene-2-carbonyl chloride, 2,2-dichloroacetyl chloride and 2-chloropropanoyl chloride to obtain compounds **7**, **8**, **13** and **14**, respectively. Similarly, 2-amino-5-cyanopyridine was treated separately with chloroacetyl chloride, 2-chloropropanoyl chloride and 2,2-dichloroacetyl chloride to obtain compounds **9**, **10** and **12**, respectively. Compound



Scheme 1. Synthesis of target molecules 1–5, 7–10 and 12–14.



Scheme 2. Synthesis of target molecules 6 and11.

2 and **9** on treatment with morpholine at room temperature afforded compounds **6** and **11**, respectively. All newly synthesized compounds (**1**–**14**) were characterized by nuclear magnetic resonance (NMR) and mass spectrometry (MS). The purity of synthesized compounds was assessed by high performance liquid chromatography (hplc).

In the cyanopyridyl series *N*-(4-cyanophenyl)acrylamide (**1**) showed only weak mTOR inhibitory activity. Indeed, in enzyme-linked immunoassays (ELISA) compound **1** inhibited fetal calf serum (FCS)-induced phosphorylation of p70S6K1 (a bonafide target of mTOR) in H460 human non-small cell lung cancer cells by only 12% even at 10 μM (Table 1). A chloroacetyl substitution of the amine yielded 2-chloro-*N*-(6-cyanopyridin-3-yl)acetamide (**2**) with increased mTOR inhibitory activity (31% inhibition at 10 μM; Table 1). Given these findings, we hypothesized that different carboxamide substitutions would lead to further increases in the mTOR inhibitory activity of compound **2**. Accordingly, as a first step, we tried a 2-fluorocarboxamide (**3**) substitution. Interestingly, this led to a marked decrease in mTOR inhibitory activity (11% inhibition at 10 μM; Table 1). We next tried a 3,3,3-trifluoropropanamide (**4**) substitution. Of note, this led to a complete loss of mTOR inhibitory activity (Table 1). Replacements of the chloro with other electron-donating, -neutral and -withdrawing groups (**5, 6**, **7**, and **8**) did not result in any improvement in mTOR inhibitory activity (Tables 1 and 2). Based on these observations, we surmised that a 2-chloro substitution was necessary for marked mTOR inhibitory activity. We next sought to dissect the contribution of pyridyl nitrogen to the observed mTOR inhibitory activity. Accordingly, we synthesized a series of compounds (**9**, **10**, **11** and **12**). Interestingly, none of these compounds exhibited mTOR inhibitory activity (Tables 1 and 2) clearly demonstrating the importance of pyridyl nitrogen to the observed mTOR inhibition. Given that 2-acetamide framework was 'inactive', we went back to the 3-acetamide scaffold. We tried a 2,2 dichloro (**13**) substitution on the 3-pyridylacetamide scaffold. Surprisingly, this compound did not exhibit any mTOR inhibitory activity (Table 1). We postulated that multiple electron withdrawing groups on the acetamide might compromise mTOR inhibition. To investigate this hypothesis, we substituted one chlorine with a methyl group (**14**). In line with our reasoning, compound **14** exhibited significant mTOR inhibitory activity (70% inhibition at 10 μM; Table 1).[12] Thus, **14** turned out to be the most potent mTOR inhibitor in this series. Western blot analysis was utilized to confirm and further characterize the mTOR inhibitory activity of **14**. The results of these latter experiments revealed that **14** inhibited serum-induced mTOR activity in H460 human non-small cell lung cancer cells as well as HCT-116 human colon carcinoma cells (Fig. 1).

Given that increased mTOR activation can lead to inflammatory complications,[3,4] we investigated the effects of **14** in inflammation assays. As reported elsewhere,[12] **14** did not inhibit in vitro or

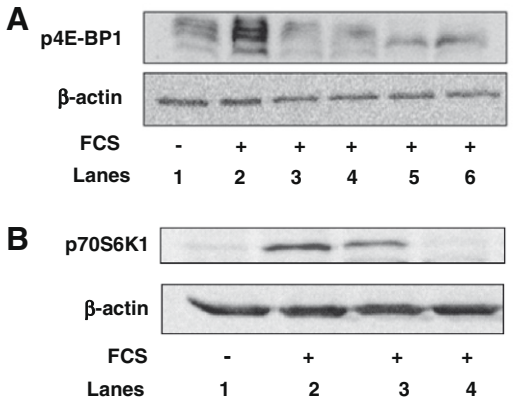


Figure 1. Compound **14** inhibits mTOR activity.[12] (A) Western blots of FCS-stimulated H460 cells treated with **14**, LY2942002 (positive control), rapamycin (positive control) or DMSO. Blots presented are representative of $n = 2$ experiments. (Lanes 1 and 2: pre-treatment with 0.5% DMSO; lane 3: pre-treatment with 3 μM **14**; lane 4: pre-treatment with 10 μM **14**; lane 5: pre-treatment with 30 μM LY294002 and lane 6: pre-treatment with 1 μM rapamycin.) (B) Western blots of FCS-stimulated HCT-116 cells treated with **14**, LY2942002, or DMSO. Blots presented are representative of $n = 2$ experiments. (Lanes 1 and 2: pre-treatment with 0.5% DMSO; lane 3: pre-treatment with 30 μM **14** and lane 4: pre-treatment with 30 μM LY294002.).

in vivo lipopolysaccharide (LPS)-induced tumor necrosis factor-a (TNF-α production. Given that everolimus, a mTOR inhibitor, inhibits interferon-γ (IFN-γ production,[6] we next investigated the effect of **14** on the induced production of IFN-γ. **14** inhibited concanavalin A (ConA)-induced IFN-γ production in a dose dependent manner (Fig. 2).

The observations that rapamycin[5] and evorilimus[6] (both mTOR inhibitors) are efficacious in animal models of colitis, combined with the findings that blocking IFN-γ production elicits a therapeutic effect in experimental colitis,[13] led us to hypothesize that **14** (that inhibits mTOR activation as well as IFN-γ production) would be efficacious in a murine model of colitis. In preliminary experiments we ascertained the plasma stability and pharmacokinetic profile of **14** administered via oral, intraperitoneal (ip) and intravenous (iv) route.

These studies revealed that **14** was stable in mouse, rat and human plasma (data not shown). Furthermore, it was observed that

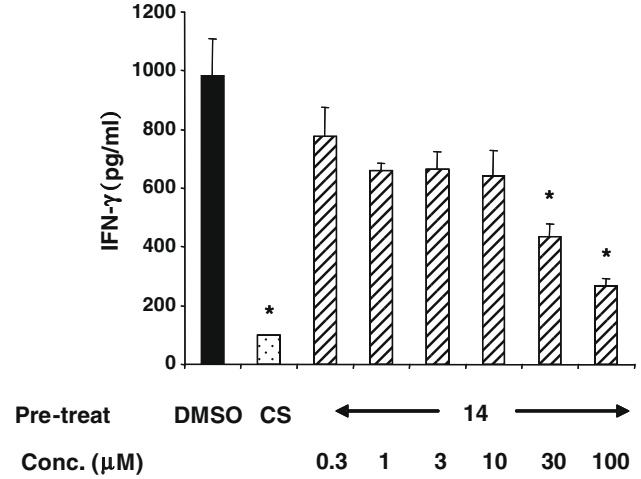


Figure 2. Compound **14** inhibits Con-A-induced IFN-γ production from human peripheral blood mononuclear cells in a dose-dependent manner.[12] CS indicates cyclosporine A. Results presented are representative of $n = 3$ separate experiments. * indicates $p < 0.05$ compared to DMSO control.

an ip dose of 100 mg/kg of **14** results in a maximal concentration (C_{max}) of 180 μM in the plasma of mice (Fig. 3). A rough extrapolation of these findings suggests that a dose of 15 mg/kg **14** (ip) would result in a C_{max} of ~30 μM (concentration at which mTOR inhibition is observed in HCT116 colon cancer cells; Fig. 1B). Accordingly, the effect of **14** on experimental colitis was studied at a dose of 15 mg/kg (ip) (described below).

In the dextran sulfate sodium (DSS)-model of colitis,[12] (i) **14** significantly inhibited DSS-induced weight loss, improved rectal bleeding index,[12] decreased disease activity index and reversed DSS-induced shortening of the colon (Fig. 4); (ii) **14** distinctly attenuated DSS-induced edema, prominently diminished the leukocyte infiltration in the colonic mucosa and resulted in protection against DSS-induced crypt damage[12] and (iii) **14** blocked DSS-in-

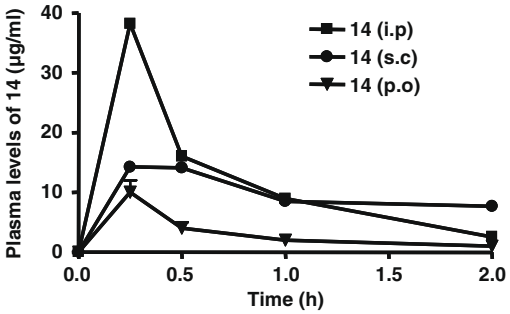


Figure 3. Pharmacokinetic profile of compound **14**. Compound **14** was administered orally (po; $n = 4$), intraperitoneally (ip; $n = 2$), or subcutaneously (s.c; $n = 2$) at dose of 100 mg/kg to mice. Plasma levels of **14** were determined by HPLC method. All values are expressed as mean ± S.E.M.

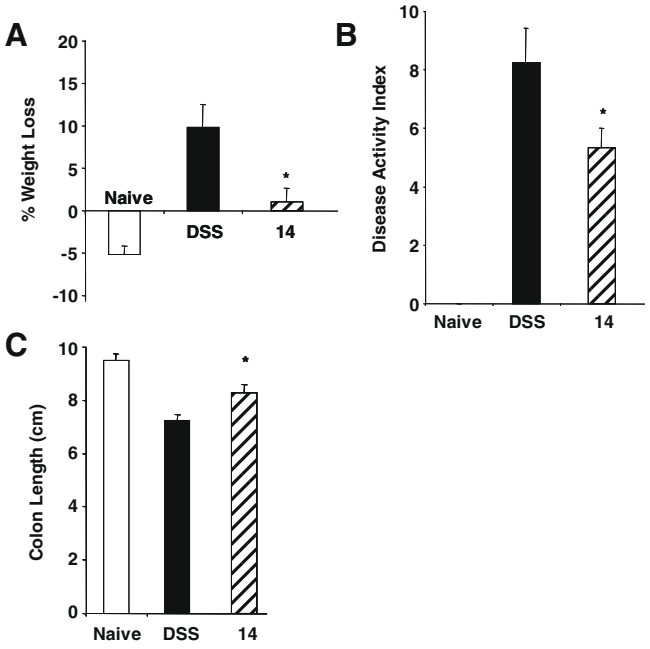


Figure 4. Compound **14** significantly inhibits DSS-induced colitis.[12] Various groups of mice received DSS daily with some groups receiving daily injections of 15 mg/kg **14** or 0.5% carboxymethyl cellulose sodium (CMC). (A) The percentage weight loss during the study. (B) Disease activity index and (C) the longitudinal length of the colon. All values are averages of 6 mice. Results presented are representative of $n = 3$ separate experiments. * indicates $p < 0.05$ compared to DSS-treated, CMC administered control group. (Legend: Na indicates mice were given regular drinking water from day 0 to day 10. DSS indicates mice were given DSS in drinking water from day 0 to day 10 and administered 0.5% CMC daily from day 0 to day 10, **14** indicates mice were given DSS in drinking water from day 0 to day 10 and administered 15 mg/kg **14**, ip, daily from day 0 to day 10).

duced activation of mTOR.[12] Collectively, these results provide direct evidence that **14**, a novel mTOR inhibitor suppresses DSS-induced colitis by inhibiting T cell function, and is potential therapeutic for colitis.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.055.

References and notes

1. Guertin, D. A.; Sabatini, D. M. *Cancer Cell* **2007**, *12*, 9.
2. Dos Santos, S.; Delattre, A. I.; De Longueville, F.; Bult, H.; Raes, M. *Ann. N.Y. Acad. Sci.* **2007**, *1096*, 70.
3. Weinstein, S. L.; Finn, A. J.; Dave, S. H.; Meng, F.; Lowell, C. A.; Sanghera, J. S.; DeFranco, A. L. *J. Leukoc. Biol.* **2000**, *67*, 405.
4. Kristof, A. S.; Marks-Konczalik, J.; Billings, E.; Moss, J. *J. Biol. Chem.* **2003**, *278*, 33637.
5. Farkas, S.; Hornung, M.; Sattler, C.; Guba, M.; Steinbauer, M.; Anthuber, M.; Herfarth, H.; Schlitt, H. J.; Geissler, E. K. *Int. J. Colorectal. Dis.* **2006**, *21*, 747.
6. Matsuda, C.; Ito, T.; Song, J.; Mizushima, T.; Tamagawa, H.; Kai, Y.; Hamanaka, Y.; Inoue, M.; Nishida, T.; Matsuda, H.; Sawa, Y. *Clin. Exp. Immunol.* **2007**, *148*, 348.
7. Scott, W. J.; Dumas, J.; Boyer, S.; Lee, W.; Chen, Y.; Phillips, B.; Verma, S.; Chen, J; Chen, Z.; Fan, J.; Raudenbush, B.; Redman, A.; Yi, L.; Zhu, Q.; Adnane, L. US Patent Application 20040235829, 2004.
8. Tatsumi, K.; Yamauchi, T.; Kiyono, K.; Kishi, K.; Yanagihara, Y.; Imaoka, T.; Kawaguchi, T.; Kubo, M. *J. Biochem.* **1993**, *114*, 912.
9. Quintela, J. M.; Peinador, C.; Botana, L.; Estevez, M.; Riguera, R. *Bioorg. Med. Chem.* **1997**, *5*, 1543.
10. Quintela, J. M.; Peinador, C.; Veiga, C.; Gonzalez, L.; Botana, L. M.; Alfonso, A.; Riguera, R. *Bioorg. Med. Chem.* **1998**, *6*, 1911.
11. Liu, M. C.; Luo, M. Z.; Mozdziesz, D. E.; Lin, T. S.; Dutschman, G. E.; Gullen, E. A.; Cheng, Y. C.; Sartorelli, A. C. *Nucleosides Nucleotides Nucleic Acids* **2000**, *19*, 603.
12. Bhonde, M. R.; Gupte, R. D.; Dadarkar, S. D.; Jadhav, M. G.; Tannu, A. A.; Bhatt, P.; Bhatia, D. R.; Desai, N. K.; Deore, V.; Yewalkar, N.; Vishwakarma, R. A.; Sharma, S.; Kumar, S.; Dagia, N. M. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2008**, *295*, G1237.
13. Fiorucci, S.; Antonelli, E.; Distrutti, E.; Del Soldato, P.; Flower, R. J.; Paul-Clark, M. J.; Morelli, A.; Perretti, M.; Ignarro, L. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15770.