Bioorganic & Medicinal Chemistry Letters 21 (2011) 2389–2393

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and optimisation of orally active TLR7 agonists for the treatment of hepatitis C virus infection

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ARTICLE INFO

Article history:
Received 12 January 2011
Revised 16 February 2011
Accepted 20 February 2011
Available online 16 March 2011

Keywords:
TLR7 agonist
HCV
Interferon inducer
Aldehyde oxidase

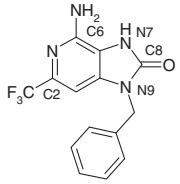
ABSTRACT

The synthesis and structure–activity relationships of a series of novel interferon inducers are described. Pharmacokinetic studies and efficacy assessment of a series of 8-oxo-3-deazapurine analogues led to the identification of compound **33**, a potent and selective agonist of the TLR7 receptor with an excellent in vivo efficacy profile in a mouse model.

Hepatitis C (HCV) is a global health problem currently affecting more than 190 million people worldwide and has been recognized as one of the leading causes of liver impairment such as cirrhosis and hepatocellular carcinoma in humans.[1] The current standard of care for HCV is a combination of pegylated interferon-α (IFN) and ribavirin.[2] Current therapy with IFN is associated with significant side effects including fatigue, headache, myalgia, fever and nausea. Ribavirin also causes haemolytic anemia in 10–20% of patients. Furthermore, the administration of IFN by intramuscular or subcutaneous injection causes pain and irritation at the site of injection. Therefore, the development of an oral IFN inducer, which enhances the release of endogenous IFN by oral administration, is very much desired.

Toll-like receptors (TLRs) are a family of pathogen recognition receptors that mediate the innate immune response.[3] To date, there have been 11 different TLRs genes identified in humans[4] and their stimulation shown to cause the release of multiple cytokines, including type 1 and type 2 interferons. Among these, TLR7 is of particular interest because small molecule ligands for this receptor have been identified, including the guanosine analogue isatoribine,[5] the mixed TLR7/8 agonists Imiquimod[6] and Resiquimod,[7] and the 8-oxo adenine SM-360320[8] (Fig. 1).

As part of our research efforts to identify new therapeutic agents for the treatment of HCV, we recently reported the discovery of the deazapurine lead candidate compound **1** as the prototype compound from our TLR7 agonist programme.[9]



1
EC_{50} 269nM(10)
Sol. 1 μg/mL

Compound **1** was shown to be highly selective against a panel of other TLRs and has an exciting profile with a 1–50 mg *qd* dose predicted to induce the same level of INFα2a required to achieve clin-

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doi:10.1016/j.bmcl.2011.02.092

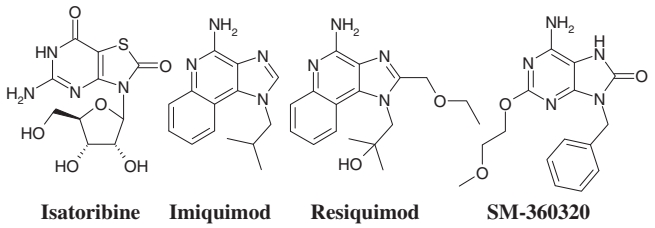
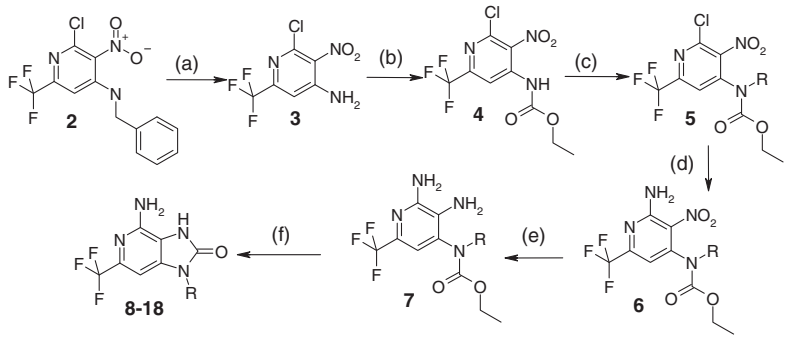


Figure 1. Structure of known TLR7 agonists.

ical efficacy in HCV patients. However, due to its low solubility, the formulation of **1** for oral administration was complicated. This Letter describes our attempts to identify a back-up compound with improved potency and solubility compared to compound **1**.

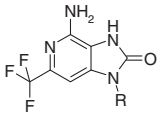
Our strategy to improve solubility was based primarily on driving the lipophilicity down by adding polar substituents on the N-9 position. Test compounds were conveniently prepared by the general methodology described in Scheme 1.

The synthesis of intermediate **2** was described in our previous publication.[11] Compound **2** was debenzylated in concentrated



Scheme 1. Reagents and conditions: (a) concd H_2SO_4, rt, 100%; (b) EtO_2CCl, NEt_3, Me–THF, rt, 80%; (c) R-X, K_2CO_3, NaI, acetone, rt, 65–81%; (d) concd NH_3, THF, rt, 88%; (e) Pd/C, H_2, EtOH, 40psi, 40 °C, 100%; (f) AcOH, 80 °C, 71%.

Table 1
Potency and selected data for N-9 analogues



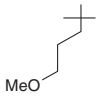
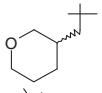
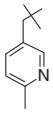
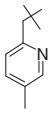
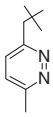
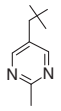
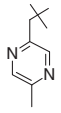
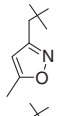
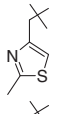
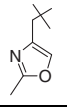
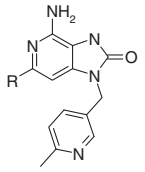
Compound	R	TLR7 EC_{50}[a] (nM)	log *D* (*c* log *P*)	Solubility (μg/mL)	HLM (RLM) (μL/min/kg)	Rat cytosol $T_{1/2}$ (min)
1		269	3.3 (3.3)	1	<7 (<8.5)	ND
8		833	1.9 (1.9)	23	<8 (<11)	ND
9		1440	2.0 (1.8)	37	<7 (<8.5)	ND
10		50	2.3 (2.3)	22	<7 (<8.5)	46
11		95	2.2 (2.3)	3	<8 (<8.5)	83
12		328	2.3 (2.3)	3	<8 (27)	ND
13		411	1.1 (1.1)	21	<8 (<8.5)	ND

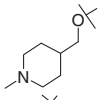
Table 1 (*continued*)

Compound	R	TLR7 EC_{50}[a] (nM)	log *D* (*c* log *P*)	Solubility (μg/mL)	HLM (RLM) (μL/min/kg)	Rat cytosol $T_{1/2}$ (min)
14		366	1.6 (1.4)	152	<8 (<8.5)	ND
15		352	1.8 (1.4)	ND	<8 (<8.5)	ND
16		103	2.1 (1.6)	4	<8 (<9)	Stable (>1000)
17		79	2.3 (2.2)	8	<7 (<10)	Stable (>1000)
18		193	1.7 (1.3)	35	<8 (<10)	Stable (>1000)

HLM = human liver microsomes; RLM = rat liver microsomes.
[a] Values are means of at least two experiments; the TLR7 agonist potency assay is described in Ref. 12.

Table 2
Structure–activity relationships of selected C-2 analogues



	R	TLR7 EC_{50}[a] (nM)	log *D* (*c* log *P*)	Solubility (μg/mL)	HLM RLM (μL/min/kg)	Rat cytosol $T_{1/2}$ (min)	A1 (A2a) IC_{50} (nM)
26		102	1.6 (1.4)	66	9 (<8.5)	46	970 (840)
27		87	2.1 (1.7)	19	<7 (<8.5)	Stable (>1000)	1600 (1500)
28		51	1.6 (1.7)	123	<7 (<8.5)	ND	1900 (2300)
29		633	2.5 (1.8)	45	<8 (<8.5)	ND	2800 (3400)
30		>4000	0.2 (2.8)	ND	<8 (<8.5)	ND	ND (ND)
31		>4000	1.8 (2.3)	ND	<8 (<8.5)	ND	ND (ND)
32		474	1.8 (1.9)	ND	<8 (<8.5)	ND	ND (ND)
33		99	2.1 (2.3)	17	<8 (<8.5)	Stable (>1000)	Not active (not active)

HLM = human liver microsomes; RLM = rat liver microsomes.
[a] Values are means of at least two experiments; the TLR7 agonist potency assay is described in Ref. 12.

sulphuric acid at room temperature with quantitative yield. Intermediate **3** was then converted into the corresponding carbamate **4** by reaction with ethyl chloroformate. Reaction of **4** with a range of electrophiles under basic conditions provided intermediates **5** generally in good yield. Chloride displacement with concentrated ammonia, followed by nitro reduction under pressurised hydrogen provided intermediate **7**, which was then cyclised under acid conditions to provide target compounds **8–18** (Table 1).

Table 3
Rat pharmacokinetics (IV 1 mg/kg, po 0.2 mg/kg)

Compound	**10**	**33**
Cl (mL/min/kg)	25	36
PPB (%)	83	41
C_{max} (nM)	132	116
$T_{1/2}$ (h)	0.5	0.4
F (%)	78	17
F_{abs} (%)	100	40

Cl = Clearance; PPB = rat Plasma protein binding; F = bioavailability; F_{abs} = fraction of compound absorbed.

Numerous N-9 analogues were synthesised and representative examples are summarised in Table 1. Initial attempts to incorporate alkoxy side chains such as **8** or cyclic ethers such as **9** in the N-9 position resulted in a loss in potency. A range of aromatic substitutions were then explored to look for a more polar expression of the benzyl moiety of compound **1**. First, a small number of N-9 substituted pyridine analogues were synthesised (compounds **10–12**) and screened, allowing the identification of compound **10** as more potent and more soluble than **1**. Attempts to introduce a second heteroatom into the pyridine ring (compounds 13–**15**) to improve solubility resulted in a loss of potency. Compound **10** was selected to be progressed into a rat pharmacokinetic study (Table 3). In vivo, compound **10** was completely absorbed when administered as a suspension but the $T_{1/2}$ of this compound was quite short by virtue of metabolism of the pyridine ring by aldehyde oxidase, which represented the major metabolic route in rat. This was confirmed by screening the compound in either isolated cytosol or hepatocytes, in the presence or the absence of Raloxifene, an aldehyde oxidase inhibitor. Scaling from rat to human gave a prediction in human of liver blood flow clearance for **10**. It was decided to look for alternative polar N-9 substituents with the goal of avoiding aldehyde oxidase recognition. Aldehyde oxidase generally oxidises the sp^2 carbon adjacent to the aromatic nitrogen atom of heterocyclic systems, so a very simple strategy was to block the C–H adjacent to this pyridine N aromatic atom.[13]

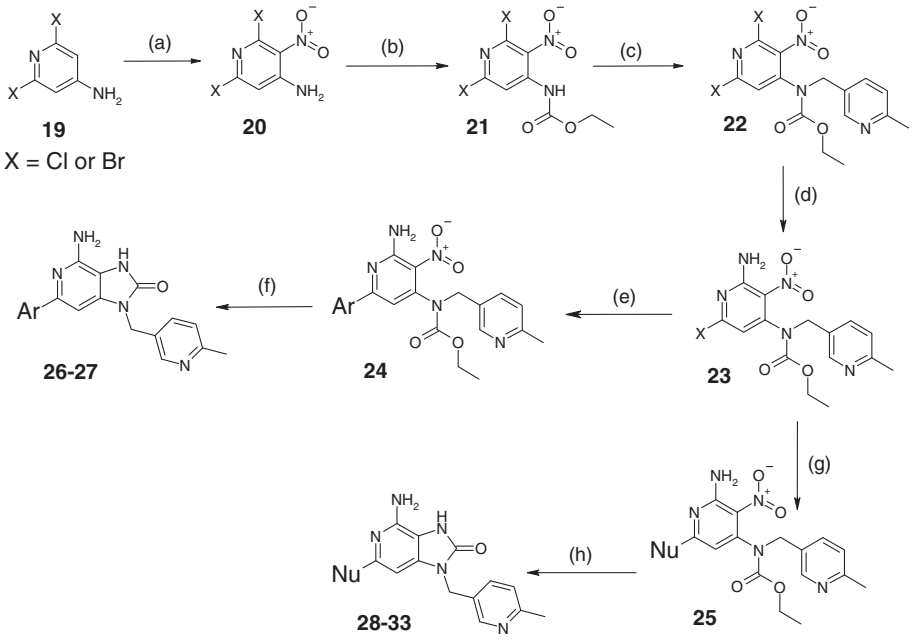
A small set of five-membered heterocycles were then designed and characterised with this strategy in mind. Both isoxazole **16** and oxazole **17** analogues showed promising potency and were stable in rat cytosol (Table 1), however, the improvement in solubility was quite modest. The oxazole analogue **18** offered a 35-fold improvement in solubility, but when dosed in vivo **18** was 4-fold less potent than **1** and was not pursued.[14]

To further improve solubility and potency, we then turned our attention to the C-2 position. Our strategy was to keep the potency and solubility enhancing pyridine substituent at the N-9 position identified in compound **10** and explore a range of more polar C-2 substituents.

Test compounds were prepared by the general methodology described in Scheme 2.

The nitration of the commercially available 2,6-dichloro or 2,6-dibromo-4-amino-pyridine **19** in concentrated sulphuric acid provided intermediate **20**, which was then converted to the corresponding carbamate **21** with ethyl chloroformate. Reaction of intermediate **21** with the favoured N-9 substituent 5-chloromethyl-2-methyl-pyridine provided intermediate **22**, which was then converted to its corresponding amino analogue **23** with concentrated ammonia. When X was bromide, a range of heterocyclic systems were introduced via Negishi coupling reaction.[15] The resulting intermediate **24** was reduced and cyclised to provide target compounds **26 and 27**. When X was chloride, a direct chloride displacement was performed with a range of nucleophiles. The resulting intermediate **25** was reduced and cyclised to provide target compounds **28–33**. Numerous C-2 analogues were synthesised and key examples are summarised in Table 2.

The oxazole analogue **26** retained good potency with improved solubility over compound **1**. In rat cytosol, the compound was not stable, by virtue once again of metabolism of the pyridine ring by aldehyde oxidase. When tested against a broad panel of enzymes, receptors and ion channels, compound **26** inhibited both adenosine receptors A1 and A2a (Table 2). The subsequent five-membered heterocycles synthesised such as the methyl oxazole analogue **27**, N-2 triazole analogue **28** and N-1 pyrazole analogue had similar adenosine receptor polypharmacology issues and were



Scheme 2. Reagents and conditions: (a) concd H_2SO_4, concd HNO_3, 0 °C to rt, 70%; (b) EtO_2CCl, NEt_3, Me–THF, rt, 79%; (c) 5-chloromethyl-2-methyl-pyridine, K_2CO_3, NaI, acetone, rt, 61%; (d) concd NH_3, THF, rt, 88%; (e), X = Br, Ar-ZnCl, $PdCl_2(PPh_3)_2$, microwave, 60 °C, 15 min, 86%; (f) (i) Pd/C, H_2, EtOH, 40psi, 40 °C; (ii) AcOH, 80 °C, 40%; (g) X = Cl, nucleophile, K_2CO_3, MeCN, 65%; (h) (i) Pd/C, H_2, EtOH, 40psi, 40 °C; (ii) AcOH, 80 °C, 40%.

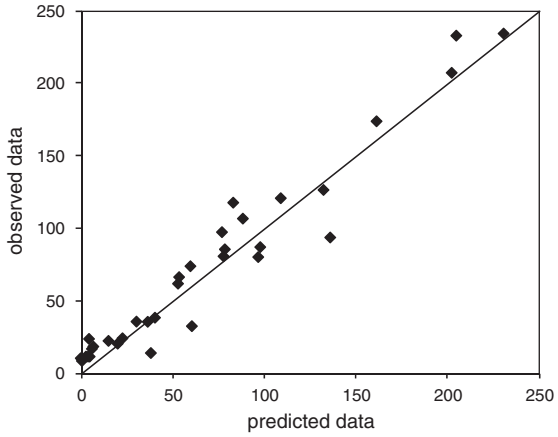


Figure 2. Observed versus predicted data from mouse IFN model fit. The observed data represent the observed IFN levels in the study and predicted data represent the predicted values from the model. All units are in pg/ml.

not pursued. However, it is interesting to note that introducing a methyl substituent to the oxazole ring was enough to confer to analogue **27** its stability in rat liver cytosol. Introducing a basic centre at the C-2 position resulted in a loss of potency as illustrated by analogue **30** and aliphatic ether side chains exemplified by analogue **31** were not tolerated either. The tetrahydrofuran analogue **32** had promising potency, prompting us to explore further cyclic ether rings. The tetrahydropyran **33** had good potency, reasonable solubility, no chirality and it shows no activity when tested against TLRs 2, 3, 4, 5, 8 and 9 at 20 μM, and highly selective when tested against a broad panel of enzymes, receptors and ion channels. The compound was selected for further profiling. Interestingly, like **27**, compound **33** was stable in rat liver cytosol and was not a substrate for aldehyde oxidase. Turning off oxidation by aldehyde oxidase by adding bulky substituents remotely to the vulnerable ring has not been reported in the literature before.[13] This mitigation strategy could be applicable for other drug discovery programmes with aldehyde oxidase mediated oxidation issues.

Following an intravenous administration to the rat, compound **33** demonstrated a mean elimination half-life of 0.4 h due to a blood clearance of 36 ml/min/kg and a volume of distribution of 1.3 L/kg. When dosed orally as a suspension, compound **33** exhibited an oral bioavailability of 17% with the amount absorbed being estimated as 40% (Table 3).

Human pharmacokinetic predictions have been made using allometric scaling from the rat. Scaling to man from the rat gave a predicted blood clearance of 16 ml/min/kg, with a volume of distribution (V_{ss}) of 2.1 L/kg and a half-life of 1.5 h. A dose prediction has been made to man using the murine IFNα model and the scaled pharmacokinetic parameters from the rat.

A PK/PD model was constructed based on the observed clinical efficacy of IFNα2a in HCV patients and the measured IFNα2a induced by **33** in the mouse. The relationship between **33** pharmacokinetics and IFN levels was modelled in mouse using an indirect response model with **33** stimulating the production of IFN.[16] Figure 2 shows the observed IFN levels versus those predicted using the model. The data points are dispersed around the line of identity indicating that the model described accurately the observed IFN data.

The EC_{50} (468 nM, coefficient of variation = 21%, corresponding to the production of ~30 pg/ml/h of IFN) from this model was coupled with a viral dynamic model[17] and a physiologically based pharmacokinetic model[18] to estimate potential efficacious doses in human. Compound **33** was predicted to have comparable efficacy to exogenous IFN when administered to man at doses less than 30 mg once daily.

In summary, a number of TLR7 agonists for the treatment of HCV were synthesized. Evaluation of the potency and pharmacokinetic properties of these compounds led to the identification of compound **33**, a back-up candidate to compound **1** with similar potency and improved solubility to achieve suitable oral absorption for toxicology and human clinical studies.

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Further reading

10. The potency discrepancy for compound **1** between this article and Ref. 9 is due to the use of different assay formats. In this article, a co-culture assay in a 384-well plate format was used compared to a 96-well plate format used in Ref. 9.