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SAR studies of C2 ethers of 2*H*-pyrano[2,3-*d*]pyrimidine-2,4,7(1*H*,3*H*)-triones as nicotinic acid receptor (NAR) agonist

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ABSTRACT

Based on in house screening lead compound **1** for the NAR project, SAR studies have been focused on the modification of the C2 ethers of the pyrimidinedione core structure. In this effort, an unpredictable SAR trend was overcome in the alkyl ether and arylalkyl ether series to identify compound **24** with improved in vitro activity compared to nicotinic acid. More consistent and predictable SAR was achieved in the propargyl ether series. Lead compound **41** was identified with good in vitro and in vivo activity in rat, and much improved rat PK profile.

Heart Disease is the number one cause of death in the USA. High levels of cholesterol, has long been known to be a risk factor for heart attack and stroke.[1–3] LDL-C was well documented as being an independent risk factor for increased cardiovascular risk.[4] HDL-C was recently thought as independent factor for reduction of CVD mortality and emerges as new therapeutic target.[5–7] Niacin as a nicotinic acid receptor agonist was proved to be safe and efficacious which has been used as a lipid lowering drug for over 30 years[8,9] and helped to decrease VLDL-C, LDL-C, triglyceride, and increases HDL-C.[10–13] It can be used alone or in combination with other lipid lowering agents to promote coronary artery disease (CAD) regression, and reduces cardiovascular disease (CVD) mortality. However, the patients needed to take gram amounts of the drug multiple times per day with food due to poor human PK profile (low exposure and short half life). High percentage of patients had intense flushing of the face and upper body and severe itching, some of them quitted the therapy after initial treatment due to this reason.[14] GI side effects including nausea, gastrointestinal discomfort and diarrhea were observed in some patients as well.[15,16] Some might display hepatotoxicity and hyperuricemia and gout.[15,16] Although extended release formulation of NA (niaspan) is available by prescription to alleviate the flushing side effect,[16,17] this comes with compromised efficacy and can not totally eliminate side effects such as potential hepatotoxicity.[18–20] Due to these considerations, the discovery of new NAR agonist without or minimized side effect liability is highly desirable.

Nicotinic acid receptor (NAR), which enables rational drug discovery for NAR agonist with improved profile, was identified in the beginning of 21st century.[21–23] NAR is a G-protein coupled receptor which includes the high affinity agonist known as HM74A (GPR109A) and the low affinity agonist known as HM74 (GPR109B) with 95% homology. Studies suggested that activation of HM74A by nicotinic acid could result in downstream effect to reduce intracellular triglyceride (TG) and free fatty acids (FFA) secretion which eventually lead to the increase of HDL level.[24–28] Thus HM74A as a potential novel therapeutic target has attracted much attention from the pharmaceutical industry. Recently, we started our effort in the NAR agonist area and have identified two pyrimidinedione derived leads (Fig. 1, 1 and 2) with moderate HM74A agonist activity from in house screening. The goal of our NAR project was to identify a NAR agonist that, compared to NA, had (1) decreased flushing liability; (2) similar or improved

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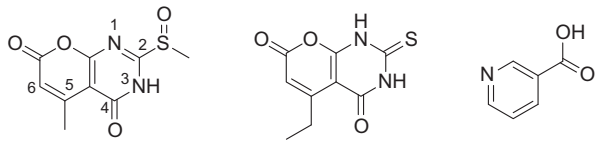


Figure 1. Structures of NAR screening leads and nicotinic acid.

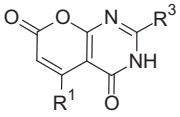
potency and in vivo efficacy; (3) similar or improved pharmacokinetic profile. SAR studies following lead **2** and C2 carbon series have been reported.[29,30] Herein we report our SAR study effort on the C2 substitution modifications of the screening lead **1** focusing on the ether side chain to improve PK/PD profile.

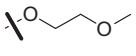
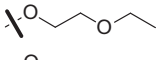
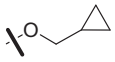
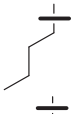
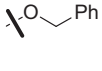
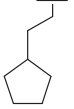
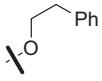
We initially prepared a series of alkylethers at C2 positions and the SAR is summarized in Table 1. When we introduced the methoxy group, the compound showed good binding activity (**3**, 125 nM). However, the SAR of the alkylethers seemed rather narrow and did not follow a rational trend. When an ethyl group was introduced (Table 1 and **4**), the activity decreased significantly. Interestingly cyclopropyl methyl group (**5**) brought back activity. To mimic the electronic effect of a cyclopropyl group, a phenyl group (**6**) was introduced and it gave good activity. Again, one carbon elongation of the linker between phenyl group and the core (**7**) resulted in much reduced activity. Introduction of heteroatoms such as oxygen within the ether side chain helped to maintain agonist activity, but SAR did not follow a predictable trend either (Table 1 and **8** vs **9**). We also modified C5 substitutions to see whether this would help to improve the activity profile. However, longer (**10**) and bulkier chain (**11, 12**) at C5 seemed not helpful to improve the activity.

We next looked into the SAR of substituted benzyl ether series based on compound **6** to see whether we could improve the in vitro activity.[31] As summarized in Table 2, SAR in this series was a little more consistent. Ortho-substitution showed better activity than the meta- and para-substituted compounds with meta- and para-compounds having similar activity (Table 2, **13**–**15** and **16**–**18**). Both electron withdrawing and donating groups gave comparable activity (Table 2, **13** and **16**). Biarylalkyl ethers were tolerated, and the ortho-substitution gave better activity (Table 2, **19** and **20**). Heterobiaryl (**21**) was acceptable as well. Since all these modifications did not lead to better binding activity than NA (100 nM), we next introduced heteroaryl alkyl ethers. Pyridazine substitution (**22**) gave better activity than the pyridine (**23**). Isooxazole derivative (**24**) gave the best activity. SAR was very sensitive to substitution modifications (**25**) and simple relocation of the isooxazole heteroatom position resulted in much reduced activity (**26**).

Encouraged by the improved in vitro activity of compound **24**, and based on SAR information from the alkyl and arylalkyl ether series, we felt that the properties especially electronic nature of the ether side chain might play important role in affecting the activity profile. We thought that an alkyne group could be used to mimic the electronic effect of the aryl group and at the same time to mimic the size of simple alkyl groups. Based on this hypothesis, we prepared a series of alkynylalkyl ethers. It turned out that the propargyl ether series had the most consistent SAR as summarized in Table 3. With a simple propargyl ether, the compound showed moderate in vitro activity (Table 3 and **27**). Introduction of substitutions on the propargyl group resulted in improved activity (**28–31**). The activity increased with the increased size of the substitutions. However, when the size was getting too big such as cyclohexanyl group (Table 3 and **32**), the activity started to drop. Polar groups such as hydroxyl was tolerated in this series as well, and again it showed consistent improvement of activity with the increased length of the spacer between the alkyne and hydroxyl group (Table 3 and **33**–**35**) with the three carbon spacer most active (**35**, 26 nM). Encouraged by these results, we then fixed the C2 substitution as ethyl substituted propargyl ether and modified C5 substitutions. As summarized in Table 3, aryl substitution (**36**), longer chain and bulky substitution (**37**, **38**) were tolerated and resulted in compounds with good activity. Cyclopropyl and cyclobutyl alkyl substitutions at C5 provided compounds with very good in vitro activity (**39–41**). This may be resulted from the subtle electronic effect brought by these groups. To determine the importance of the propargyl group, compounds **42–44** were prepared. The location of the alkyne was important for activity. When the ether oxygen atom was linked by two carbons to the alkyne, decreased

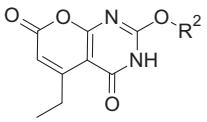
Table 1
SAR summary of C2 alkyl ethers[a]

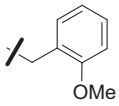
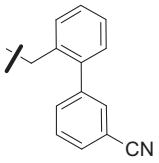
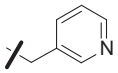
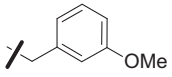
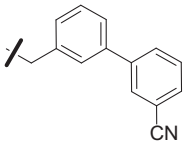
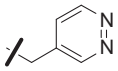
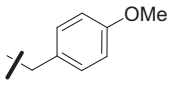
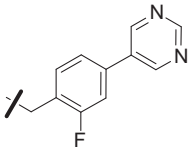
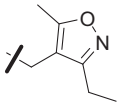
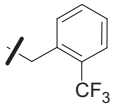
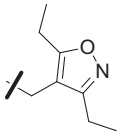
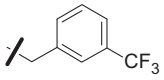
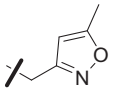
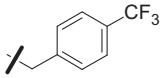


Compound number	R^1	R^3	Ec_{50}	Compound number	R^1	R^3	Ec_{50}
3			125	**8**			138
4			10000	**9**			2060
5			285	**10**			417
6			190	**11**			319
7			10000	**12**			260

[a] Data is in nM for human HM74A receptor and represents an average of multiple determinations (3 or more).

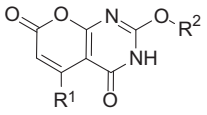
Table 2
SAR Summary of C2 aryl–alkyl ethers[a]



Compound number	R^2	Ec_{50}	Compound number	R^1	Ec_{50}	Compound number	R^1	Ec_{50}
13		113	**19**		238	**22**		738
14		860	**20**		723	**23**		93
15		1061	**21**		291	**24**		63
16		133				**25**		588
17		290				**26**		10000
18		270						

[a] Data is in nM for human HM74A receptor and represents an average of multiple determinations (3 or more).

Table 3
SAR summary of C2 propargyl ethers[a]



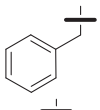
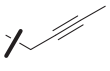
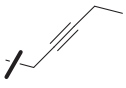
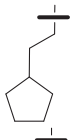
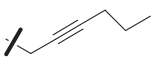
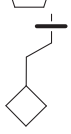
Compound number	R^1	R^2	Ec_{50}	Compound number	R^1	R^2	Ec_{50}
27			911	**36**			137
28			425	**37**			66
29			96	**38**			172
30			53	**39**			22

Table 3 (*continued*)

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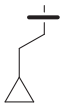
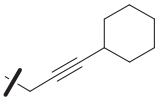
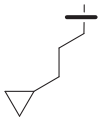
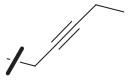
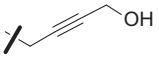
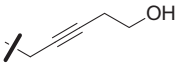
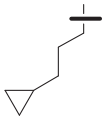
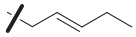
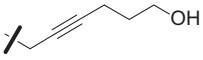
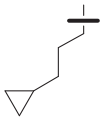
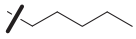
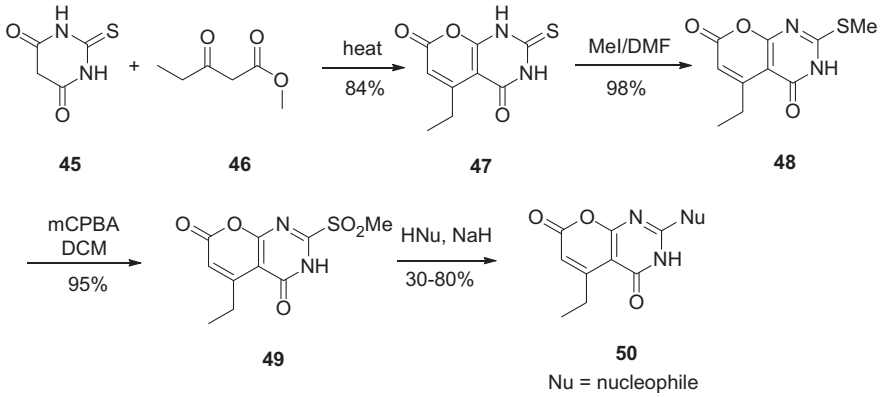
31			34	**40**			90
32			177	**41**			67
33			312	**42**			424
34			179	**43**			>1000
35			26	**44**			>1000

[a] Data is in nM for human HM74A receptor and represents an average of multiple determinations (3 or more).

activity was observed (Table 3 and **42**). The alkyne group was crucial for activity, when the alkyne was reduced to an alkene (**43**) or simple alkyl group (**44**), these compounds lost activity dramatically. With improved in vitro activity, compounds **39** and **41** were selected for further profiling in in vivo studies. Compound **39** had a binding activity of 28 nM in rats, however, it did not show robust TG decrease (2.6% reduction at 0.3 mpk at 6 h time point) in rats. On the other hand, compound **41** had 83 nM binding activity in rat and showed very good in vivo activity in rat TG assay (34% reduction at 0.3 mpk at 6 h time point). This compound had very good PK profile in rat (42.5 μM h AUC at 10 mpk oral dosing in rapid rat studies,[32] C_{max} = 29.2 μM, and T_{max} = 4 h). On the other hand, the rat PK of compound **39** was moderate with much shorter T_{max} (21.8 μM h AUC at 10 mpk oral dosing, C_{max} = 15.7 μM, and T_{max} = 1 h) which might explain the lack of in vivo activity of this compound at 6 h time point.

The synthesis of the C2 substituted compounds is summarized in Scheme 1. Thiobarbituric acid (**45**) was first condensed with the ketoester (**46**) as a neat mixture at 165 °C to give thiopyrimidinedione **47** after washing the crude with water. Methylation of compound **47** was achieved using MeI in DMF without any base and the product (**48**) was crashed out with DMF and water. The methyl thioether (**48**) was oxidized with m-CPBA in dicloromethane (DCM) and provided key intermediate sulfone **49** after solvent removal and washing with ethyl acetate and hexane mixture. Different nucleophiles were introduced at this stage in the presence of base in a mixture of THF and DMF to give the final compounds **50**. The overall synthesis was straight forward and required no column chromatography of the intermediates. Considering the difficulties to handle these compounds which had very poor solubility in organic solvents, this process offered the opportunity to carry out the SAR studies quickly and provided key compounds in sufficient quantity.

In summary, based on in house screening lead compound **1** for the NAR project, SAR studies have been focused on the modification of the C2 ethers. In this effort, we overcame an unpredictable SAR trend in the alkyl ether and arylalkyl ether series to identify compound **24** with improved in vitro activity compared to nicotinic acid. More consistent and predictable SAR was achieved in the propargyl ether series. Lead compound **41** was identified with good in vitro and in vivo activity in rat, and much improved rat PK profile.



Scheme 1. The synthetic route of C2 ethers

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