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Synthesis and biological activity of heteroaryl 3-(1,1-dioxo-2*H*-(1,2,4)-benzothiadizin-3-yl)-4-hydroxy-2(1*H*)-quinolinone derivatives as hepatitis C virus NS5B polymerase inhibitors

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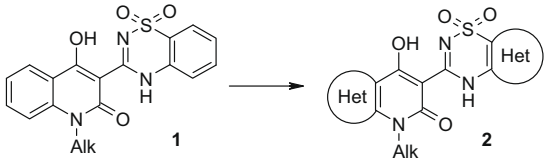
ABSTRACT

Modification of the benzo rings of 3-(1,1-dioxo-2*H*-(1,2,4)-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinones into heteroaromatic systems was investigated to enhance physicochemical properties and potency profile of this class of inhibitors. The synthesis and biological activity of the derived compounds is discussed.

Currently, Hepatitis C Virus (HCV) infects about 3% of the world population and is the leading cause of chronic liver disease and liver transplants worldwide.[1] The most effective treatment thus far consists of PEG-interferon-α (1.5 μg/kg weekly) in combination with ribavirin (800–1200 mg/day), but the rate of success of this treatment in patients affected with genotypes 1a and 1b, which account for 70% of the infected population, is <50%.[2] Considerable side effects are also associated with this therapy, often resulting in discontinuation of treatment.

HCV is a positive, single-stranded RNA virus of the Flaviviridae family, with a ~9600 nucleotide genome. The genome encodes a single polyprotein of approximately 3010 amino acids, which is then processed by the host cell and viral proteases into mature structural (C, E1, E2) and non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A, NS5B).[3] The NS5B is an RNA dependent RNA polymerase (RdRp) essential for viral genome replication which has been well characterized in vitro.[4,5] The availability of a cell based HCV replicon system,[6,7] in addition to a robust biochemical enzymatic assay,[8] has provided the scientific community with the tools required for the discovery of inhibitors of HCV polymerase.[9,10] Among those, the 1-alkyl-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-1-quinolin-2-ones (**1**) were discovered and developed in our laboratories through high-throughput screening and have been the subject of intense structure–activity investigations in several laboratories.[11–15]



In an effort to enhance the physicochemical properties and potency profile of this inhibitor class, substitution on the two benzo rings was undertaken and extensive SAR discussions have been reported elsewhere.[12,13] In addition to these efforts, the modification of the benzo rings into heteroaromatic systems has the potential of improving physical properties, obtaining different metabolic degradation profiles, or improving potency in the presence of serum proteins. The synthesis and preliminary evaluation of such modified derivatives **2** is discussed herein.

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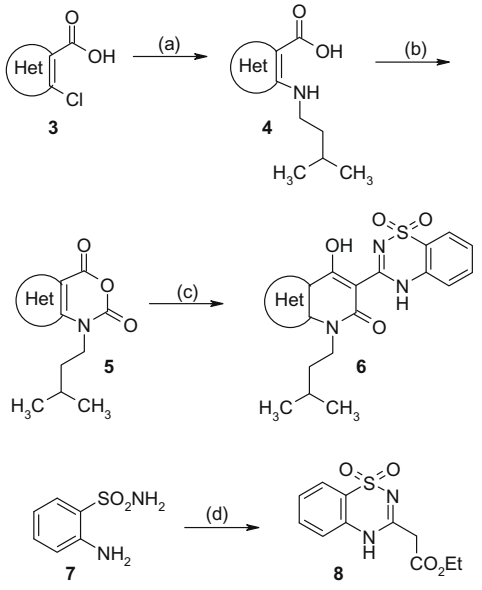
The requisite heteroaryloxazine-2,4-dione starting materials **5** were either commercially available or prepared from the appropriate *o*-amino acid derivatives **4** by reaction with phosgene or a phosgene equivalent. The heteroaryloxazines were then converted to the desired derivatives **6** by condensation with (1,1-dioxo-1,4-dihydrobenzo-[1,2,4]-thiadiazin-3-yl)-acetic acid ethyl ester **8** (Scheme 1).[12]

Synthesis of several of the heteroaryl analogs of **8** by the procedure shown in Scheme 1 proved to be challenging, perhaps due to the reduced nucleophilicity of the corresponding hetero-anilines. Therefore, we pursued an alternate synthetic route utilizing different coupling partners in order to prepare the desired heteroaryl derivatives. Specifically, we had previously observed that 3-cyanoquinolinones **10** were efficient coupling partners with 2-aminobenzenesulfonamides in the presence of trimethylaluminum, followed by base promoted cyclization of the intermediate substituted amidine to afford the thiadiazine system **11** (Scheme 2).[13]

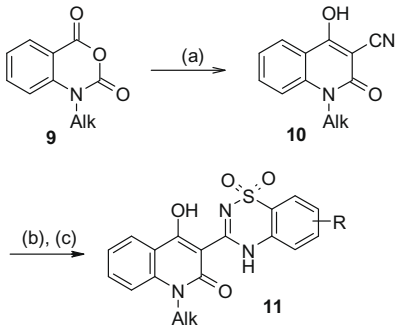
Although this route was ineffective for very poor nucleophilic *o*-aminoheteroaryl-sulfonamides, the conversion of **12** to the unsubstituted amidine in the presence of ammonium chloride and trimethylaluminum, followed by treatment with a 2-chloroheteroarylsulfonylchloride provided a practical route. Using this novel procedure, the target heteroaryl compounds **14** were prepared (Scheme 3).

Pyrido derivative **14a** could be further modified by partial reduction,[16] followed by alkylation or acylation and further transformations to afford the partially saturated compounds **15** (Scheme 4).

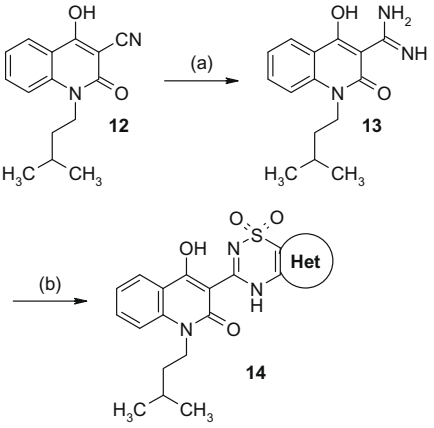
The most active heteroarylquinolinone left-hand side derivatives were also combined with benzothiadiazines possessing 7-oxyacetamide or 7-oxypropionamide substituents, which had been observed previously to improve cellular potency in the presence of serum proteins in the parent benzo-fused system.[13] Coupling of the appropriate heteroaryloxazine-2,4-dione (**5**, R^1 = *i*-propyl) with 7-methoxy-thiadiazin-3-yl-acetate **16a**[17] in the presence of NaH, followed by demethylation and alkylation of the free hydroxyl group, afforded compounds **21**. The propensity of the cyclopropyl moiety to undergo ring opening precluded the use of the harshly acid conditions required for the dealkylation step. In these cases



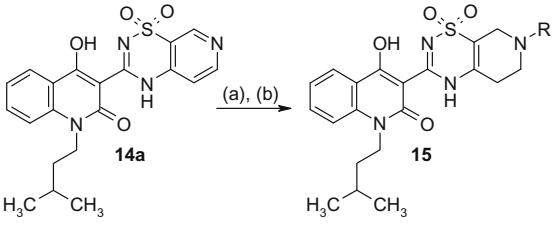
Scheme 1. Reagents and conditions: (a) (1) K_2CO_3, H_2O; (2) 3-methyl-butylamine, Cu(0), DMF, reflux; (b) Na_2CO_3, H_2O, $COCl_2$ (20% in toluene); (c) (1) **8**, NaH, THF, reflux; (2) CH_3CO_2H, reflux; (d) (1) $ClCOCH_2CO_2Et$, pyridine, CH_2Cl_2; (2) 10% aqueous Na_2CO_3.



Scheme 2. Reagents: (a) NaH, CH_3OCOCH_2CN, DMF; (b) 2-amino-*benzene*sulfonamide, Me_3Al, 1,4-dioxane; (c) sodium hydroxide/reflux.



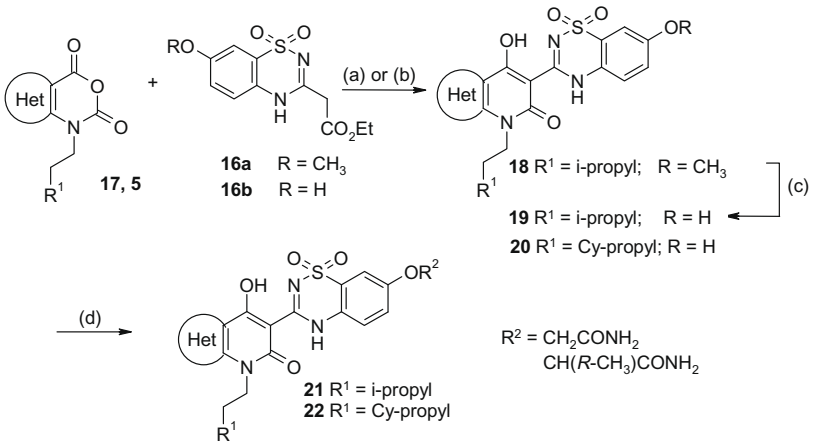
Scheme 3. Reagents: (a) NH_4Cl, Me_3Al, 1,4-dioxane, reflux; (b) 2-chloro-heteroarylsulfonylchloride, NaH, THF or DMF, reflux.



Scheme 4. Reagents: (a) H_2, PtO_2, CH_3CO_2H; (b) RCOCl, Et_3N, CH_2Cl_2 or RBr, K_2CO_3, DMF.

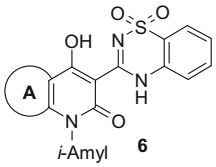
7-hydroxy derivative **16b** was used as the coupling partner for isatoic abhydride **17** (R^1 = cyclopropyl), and coupling was accomplished by the use of DBU, followed by acid promoted cyclization. Alkylation of the free hydroxyl group then provided the derivatives **22** (Scheme 5).

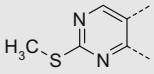
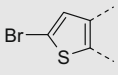
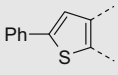
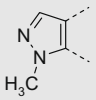
Replacement of the benzo portion of the quinolinone ring system with heteroaryl groups did influence the potency of the resulting inhibitors (Table 1). The 1,8-naphthiridinone system **6b** and the two thieno derivatives **6d** and **6e** displayed activity similar to the parent benzo derivative **6a**, while other replacements were not well tolerated, resulting in compounds with decreased inhibitory activity against the polymerase. Although the human serum albumin (HSA) binding of the pyrazole derivatives **6h** and **6i**, measured



Scheme 5. Reagents: (a) (1) NaH, THF, reflux; (2) CH_3CO_2H, THF, reflux; (b) (1) DBU, DMF; (2) CH_3CO_2H; (c) 48% aqueous HBr, CH_3CO_2H, reflux; (d) $ClCH_2CONH_2$ or TsOCH[(*S*)-CH_3]$CONH_2$, K_2CO_3, DMF.

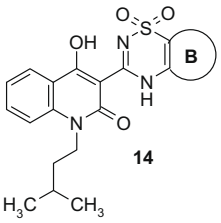
Table 1
Heterocyclic pyrimidinediones

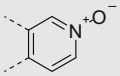
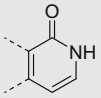
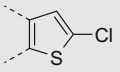
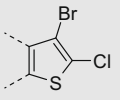
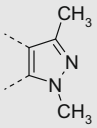


Compd	A	RdRp[a] IC_{50} (nM)	Replicon[a] IC_{50} (nM)	HSA (% bound)
6a		32	417	>99.9
6b		212	1359	>99.9
6c		>30,000	–	–
6d		48	942	>99.9
6e		132	1164	>99.9
6f		1168	–	>99.9
6g		>30000	–	–
6h		3337	–	97.0
6i		4936	–	97.6

[a] Genotype **1b**; – = not tested.

Table 2
Heterocyclic thiadiazines



Compd	B	RdRp IC_{50} (nM)	Replicon IC_{50} (nM)	HSA (% bound)
14a		20	226	99.0
14b		31	743	98.7
14c		51	1681	–
14d		18	2377	97.7
14e		16	13,679	–
14f		20	2887	99.5
14g		57	>20,000	–
14h		3016	–	–
14i		200	>20,000	98.5

chromatographically as the percentage bound to a ChromTech™ HSA column,[18] was significantly reduced, these analogs were unfortunately much less active than the benzo analogs against the polymerase.

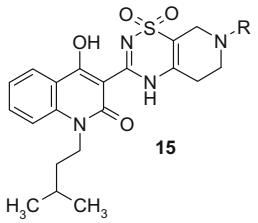
Pyridines (**14a**–**e**), thiophenes (**14f**–**h**) and pyrazole (**14i**) were investigated as replacements for the benzo ring of the thiadiazine system (Table 2). These derivatives all displayed decreased plasma protein binding, with the pyridine derivatives **14a** and **14b** being equipotent to the parent compound. Pyridine *N*-oxide **14d** and the more polar pyridinone derivative **14e**, were not as active in the replicon system, despite their outstanding enzyme inhibition, presumably due to lower cell-membrane permeability (data not shown).

In the attempt to reduce protein binding even further while maintaining enzymatic and cellular potency, pyridine derivative **14a** was reduced to tetrahydroderivative **15a** and the free nitrogen was then alkylated or acylated with a series of functionalized chains (Table 3). Indeed, with the exception of **15i**, compounds **15a**–**k** had measurable plasma protein binding of <99%, however these derivatives all displayed lower potency than the parent benzo system, particularly in the replicon assay. This may be the result of the loss of an apparent edge-to-face π-interaction observed in the X-ray co-crystal structures of NS5B with benzothiadiazine inhibitors between Phe193 and the benzo portion of the thiadiazine ring system.[12,19]

We had previously observed that the presence of an oxyacetamide or oxypropionamide substituent at the benzothiadiazine C-7 position greatly increased the potency in the replicon system in the presence of serum proteins.[13] We therefore combined the best heteroaryl quinolinone derivatives with benzothiadiazines possessing 7-oxy groups to explore the possibility of increasing potency even further. Thienopyridinones **21b**–**d** and 1,8-naphthiridinone systems **21e,f** and **22a,b** were all extremely potent compounds in the enzyme assay and maintained good levels of potency in the replicon system while reducing plasma protein binding (Table 4).

In this Letter, we describe the development of a new series of inhibitors based on the novel scaffold of the heteroaromatic derivatives of **1**. Although this class of inhibitors offers no significant improvement in intrinsic NS5B inhibitory activity when compared to the parent benzo system, the lower plasma protein binding observed with several analogs combined with the good inhibitory properties may result in an improved anti-viral response in vivo. Overall, these compounds represent an alternative series that shows good potential to offer different physical properties within this general class of NS5B inhibitors.

Table 3



15

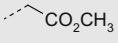
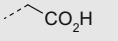
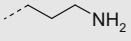
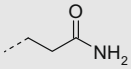
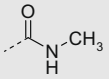
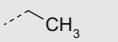
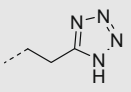
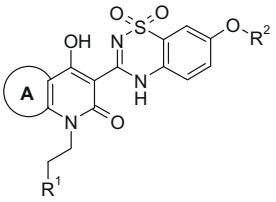
Compd	R	RdRp IC_{50} (nM)	Replicon IC_{50} (nM)	HSA (% bound)
15a	H	604	12,219	98.2
15b		1527	–	98.0
15c		879	–	97.6
15d		132	4320	–
15e		276	>20,000	–
15f		728	–	98.3
15g		288	>20,000	98.0
15h		774	–	98.2
15i		975	–	99.4
15j		679	40,000	97.9
15k		289	>30,000	98.7

Table 4



Compd	A	R^1	R^2	RdRp IC_{50} (nM)	Replicon IC_{50} (nM)	HSA (% bound)
21a			CH_2CONH_2	6	15	99.2
19a			H	16	1186	99.0
21b			CH_2CONH_2	<5	126	98.3
21c			$CH(R\text{-}CH_3)CONH_2$	<5	114	98.3
19b		*i*-Pr	H	25	471	99.1
21d			CH_2CONH_2	<5	238	98.7
19c			H	16	473	99.0
21e			CH_2CONH_2	<5	52	98.4
21f			$CH(R\text{-}CH_3)CONH_2$	<5	47	98.3
20		*c*-Pr	H	12	542	98.8
22a			CH_2CONH_2	<5	51	97.8
22b			$CH(R\text{-}CH_3)CONH_2$	<5	ND	97.8

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