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Non-nucleoside inhibitors of the hepatitis C virus NS5B RNA-dependant RNA polymerase: 2-Aryl-3-heteroaryl-1,3-thiazolidin-4-one derivatives

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Abstract

Hepatitis C virus (HCV) NS5B RNA polymerase is crucial for replicating the HCV RNA genome and is an attractive target for developing anti-HCV drugs. A novel series of 2,3-diaryl-1,3-thiazolidin-4-one derivatives were evaluated for their ability to inhibit HCV NS5B. Of this series, compounds **4c, 5b, 5c** and **6** emerged as more potent, displaying over 95% inhibition of NS5B RNA polymerase activity *in vitro*. The two most active compounds **4c** and **5c** exhibited an IC_{50} of 31.9 μM and 32.2 μM, respectively against HCV NS5B.

Hepatitis C virus (HCV) is a blood-borne pathogen belonging to the *Flaviviridae*[1] family of viruses, which also includes the West Nile, Yellow Fever, and Dengue viruses. HCV infection is one of the most significant cause for liver cirrhosis and hepatocellular carcinoma[2] leading to liver failure and as such is a growing medical problem that affects an estimated 170 million individuals worldwide.[3] HCV is a positive strand RNA virus, and its genome comprises of 9600 base pairs that encode several structural and nonstructural proteins.[4] Non-structural protein 5B (NS5B), encodes the viral RNA dependent RNA polymerase (RdRp), which plays a pivotal role in replicating the HCV RNA genome.[5] By analogy to AIDS, most small molecule inhibitor approaches to HCV have centered on the inhibition of essential viral targets, especially the NS3-4A protease (analogous to HIV protease) and the NS5B RdRp (analogous to HIV RT), although other targets are also being followed.[6] More interestingly, there is no functional counter part of this enzyme in mammalian cells thus making it an ideal drug target.[7] Several classes of potent NS5B inhibitors have been reported in the past couple of years[8] e.g. nucleoside NS5B inhibitors NM283[9] and R-1626,[10] and non-nucleoside inhibitors HCV-796[11] and wedelolactone[12] (Fig. 1) among others. However, despite a proliferation of pharmaceutical and academic research in the past decade, no specific antiviral agents are available for the treatment of HCV. Therefore, development of anti-HCV drugs remains an enormous unmet medical need for adequate therapeutic options.

4-Thiazolidinone scaffold has been gaining prominence in recent years, due to the fact that its derivatives are known to possess wide spectrum of activities such as antibacterial,[13,14] antifungal,[15,16] anticonvulsant,[17,18] antiCOX-1,[19] antituberculosis,[20–22]

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antihistaminic[23] and anticancer.[24] The persuasive antiviral activity of 4-thiazolidinone scaffold has been enlightened by several studies.

These include the inhibition of HIV-1 RT by 2,3-diaryl-1,3-thiazolidin-4-ones.[25–27] More recently, the inhibitory potency of 4-thiazolidinone ring system against HCV NS5B polymerase has been reported by Kaushik-Basu et al.[28]

In this study, we have investigated the therapeutic potential of the 4-thiazolidinone scaffold against HCV NS5B, utilizing a series of 2,3-diaryl-1,3-thiazolidin-4-one derivatives synthesized by our group. The synthesis of all compounds reported in Table 1 except compounds **4c, 4p, 7** and **8** have been described previously.[26] Our investigations have focused on building the structure–activity relationship (SAR) around 2- and 3-positions of the 4-thiazolidinone template in contrast to the recently reported 4-oxo-2-thionothiazolidines, which carry arylsulfonamido and arylidene substituents at 3- and 5-positions, respectively.[28] Here we report the identification of a new series of 4-thiazolidinone derivatives as promising inhibitors of HCV NS5B polymerase. These seminal findings should assist in the development of novel 4-thiazolidinone compounds harboring potent anti-NS5B activity.

The target compounds in this study (**4a–4f, 4q, 5a–5c** and **6**) were prepared by the multi-component DCC mediated reaction protocol[29] earlier reported from this laboratory as shown in Scheme 1. In this protocol *N*,*N*-Dicyclohexylcarbodiimide (DCC) is used as a dehydrating agent to accelerate the intramolecular cyclization resulting in faster reaction and improved yields. The reactions were performed by reacting theappropriate heteroaryl amines (**1**), substituted benzaldehydes (**2**) and mercapto acids (**3**) in the presence of DCC at room temperature. After completion of the reaction ranging around 1.0 hr, the desired products were obtained in excellent yields and purity as confirmed by spectral data analysis. Compounds **7, 4g–4p** and **4r–4s** were synthesized by using the toluene reflux protocol[26] in the presence of 4Å molecular sieve and p-toluene sulphonic acid (PTSA). Reaction time for these compounds varied from 18–24 hours and yielded the desired products in moderate yields and purity. Sulfoxide (**8**) was synthesized by using Oxone® (2 equivalents) in methanol:water (1:1) at room temperature stirring for 30 minutes. The spectral data including the elemental analysis of this compound reported in supplemental information correlates with the expected structure. Physical data for all 4-thiazolidinone derivatives are given in Table 1.

To investigate the influence of the 4-thiazolidinone compounds on the RdRp activity of NS5B, we employed the *N*-terminal His-tagged HCV NS5BCΔ21 (genotype 1b), lacking the *C*-terminal 21-amino acid membrane-spanning domain. Purification of NS5BCΔ21 and determination of its RdRp activity was carried out in accordance with previously described procedures.[12,28] Primarily, the anti-NS5B activity was evaluated for all compounds (**4a–4s, 5a–5c** and **6–8**) and their results are summarized in Table 2. The compounds showed varied pattern of inhibition of HCV NS5B RdRp ranging from moderate to good at 0.25 mM compound concentration. Importantly, compounds **4c, 5b, 5c** and **6** exhibited 95% or higher inhibition of NS5B at this concentration, thus revealing somewhat higher potency than compound **9**.[28] Further, compounds **5b, 5c** and **6** exhibited relatively poor anti-HIV-1 RT activity, in contrast to the inhibition pattern seen with HCV NS5B. More-over, examination of the inhibitory activity of the thiazolidin-4-one derivatives against SARS Co-V RdRp (nsp12) and Klenow polymerase as described previously,[12,28] yielded ≤50% inhibition at 0.5 mM concentration of these compounds (data not shown), thus suggesting their specificity for HCV NS5B.

It may be inferred from the biological activity data reported in Table 2 that the anti-HCV NS5B activity is sturdily dependent on the nature of the substituent at C-2, N-3 and C-5 of the 4-thiazolidinone scaffold. In particular, a high activity level was observed for compounds

possessing a halophenyl and 4-dimethylaminophenyl group at C-2, substituted/unsubstituted pyridine-2-yl, pyridine-3-ylmethyl, substituted pyrimidin-2-yl and furan-2-ylmethyl at N-3 and unsubstituted or methyl substitution at C-5. In fact, the compounds with the best combination of high potency were unsubstituted pyridine-2-yl, pyridine-3-ylmethyl or furan-2-ylmethyl substituted at N-3 of 4-thiazolidinone scaffold, derivatives such as **4c, 5b, 5c** and **6**. The effect of 2,6-dihalosubstituent on the phenyl ring at C-2 was apparent in compound **4i**. This compound was more active than the corresponding 2,6-dichloro (**4g**) and 2-chloro-6-fluoro substituted (**4h**) compounds. Furthermore the favourable effect of 2,6-dibromo was confirmed by the finding that 2,6-dichloro derivative (**4g**) possessed intermediate activity between 2,6-dibromo and 2-chloro-6-fluoro analogues. But the 2,6-difluoro substituted compounds (**4o**) possessed almost same activity as 2,6-dichloro substituted compound (**4m**) in case of 4,6-diphenylpyrimidin-2-yl derivatives.

The introduction of pyrimidin-2-yl substituent at the N-3 atom of the thiazolidinone ring moderated their anti-NS5B activity. Considering the effect of the substituents on the pyrimidine ring at N-3, introduction of 4-phenyl-6-trifluoromethyl pyrimidin-2-yl moiety (**4l**) resulted in moderate activity. Introduction of the 4-methyl-6-phenyl pyrimidin-2-yl moiety (**4j–4k**) led to substantial decrease in their ability to inhibit NS5B. Compounds **4m, 4n** and **4o** with the 4,6-diphenylpyrimidin-2-yl derivatives, compounds **4g** and **4i** with the 4-methyl-6-trifluoromethyl pyrimidin-2-yl derivatives exhibited near equal potency.

However, introduction of the quinolin-2-yl (**4p**), thiophen-2-ylmethyl (**4r**) and 5-ethyl-[1,3,4]-thiadiazol-2-yl (**4s**) at N-3 position also resulted in a substantial decrease in their activity compared to the pyridin-2yl, pyridine-3-ylmethyl and furan-2-ylmethyl containing compounds.

Compounds **4c, 4e, 5b, 5c, 6** and **7**, thus emerged as the most potent compounds of this series with percentage inhibition in the range of 85 to 98%. We therefore evaluated the IC_{50} values of these compounds by monitoring the total incorporation of the radiolabeled UTP on poly rA/U_{12} as a function of inhibitor concentration. Compounds **4c, 4e, 5b, 5c, 6** and **7** yielded IC_{50} value ranging from 31.9–79.4 μM (Table 2). The most potent compound **4c** and **5c** exhibited an IC_{50} value of 31.9 and 32.2 μM whereas the compound **4e, 5b, 6** and **7** exhibited modest IC_{50} values (41.8–79.4 μM).

Earlier reports had focused mostly on exploring the substitution at C-2 and N-3 position of the 4-thiazolidinone scaffold.[28] As a first step towards exploring the importance of thiazolidinone scaffold per se, we have evaluated compounds having methyl substitution at C-5, ring expansion metathiazanone and sulfoxide modification. It is apparent from the data presented in Table 2 that changes in the basic thiazolidinone moiety either by introducing a methyl group at C-5 (**5a–5c**), ring expansion (**6**) or spiro (**7**) has favorable effect on the anti-HCV NS5B activity as opposed to the introduction of sulfoxide (**8**) moiety, which decreased the activity.

In conclusion, previously synthesized novel 2,3-diaryl-1,3-thiazolidin-4-one derivatives were evaluated against HCV NS5B polymerase. Compounds **4c, 4e, 5b, 5c, 6** and **7** of this series showed promise as anti-HCV NS5B agents and exhibited over 85% inhibition. Compounds **4c** and **5c** were the most potent of this group with IC_{50} values of 31.9 and 32.2 μM, respectively. All other derivatives also exhibited greater than 60% NS5B RdRp inhibition. Taken together our data indicate that changes at C-2, N-3 and C-5 position of 4-thiazolidinone scaffold with appropriate substitution may provide compounds with improved potency. Thus 4-thiazolidinone skeleton holds promise for further activity optimization studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References and notes

1. Choo, Q-L.; Han, J.; Weiner, AJ.; Overby, LR.; Bradley, DW.; Kuo, G.; Houghton, M. Hepatitis C virus is a distant relative of the flaviviruses and pestiviruses. Shikata, T.; Purcell, RH.; Uchida, T., editors. Amsterdam, The Netherlands: Viral hepatitis C, D and E Elsevier Science Publishers B.V.; 1991. p. 47-52. (b) Choo Q-L, Richman KH, Han JH, Berger K, Lee C, Dong C, Gallegos C, Coit D, Medina-Selby A, Barr PJ, Weiner AJ, Bradley DW, Kuo G, Houghton M. Proc. Natl. Acad. Sci. USA 1991;88:2451–2455. [PubMed: 1848704]
2. McHutchison JG. Am. J. Manag. Care 2004;10:S21–S29. [PubMed: 15084064]
3. Cohen J. Science 1999;285:26. [PubMed: 10428695]
4. Reed KE, Rice CM. Curr. Top. Microbiol. Immunol 2000;242:55–84. [PubMed: 10592656]
5. Beaulieu PL, Montse L-B. Curr. Med. Chem. Anti-Infective Agents 2002;1:163–176.
6. (a) Gordon CP, Keller PA. J. Med. Chem 2005;48:1–20. [PubMed: 15633995] (b) De Francesco R, Migliaccio G. Nature 2005;436:953–960. [PubMed: 16107835]
7. (a) Wu JZ, Hong Z. Curr. Drug Targets–Infect. Dis 2003;3:207. (b) LaPlante S, Jakalian A, Aubry N, Bousquet Y, Ferland J-M, Gillard J, Lefebvre S, Poirier M, Tsantrizos YS, Kukolj G, Beaulieu PL. Angew. Chem., Int. Ed 2004;43:4306. (c) Tan S-L, He Y, Huang Y, Gale M Jr. Curr. Opin. Pharmacol 2004;4:465. [PubMed: 15351350] (d) Beaulieu PL, Tsantrizos YS. Curr. Opin. Investig. Drugs 2004;5:838. (e) Gordon CP, Keller PA. J. Med. Chem 2005;48:1. [PubMed: 15633995] (f) Condon SM, LaPorte MG, Herbertz T. Curr. Med. Chem.–Anti-Infective Agents 2005;4:99. (g) De Francesco R, Migliaccio G. Nature 2005;436:953. [PubMed: 16107835]
8. (a) Beaulieu PL, Bousquet Y, Gauthier J, Gillard J, Marquis M, McKercher G, Pellerin C, Valois S, Kukolj G. J. Med. Chem 2004;47:6884–6892. [PubMed: 15615537] (b) Harper S, Avolio S, Pacini B, Di Filippo M, Altamura S, Tomei L, Paonessa G, Di Marco S, Carfi A, Giuliano C, Padron J, Bonelli F, Migliaccio G, De Francesco R, Laufer R, Rowley M, Narjes F. J. Med. Chem 2005;48:4547–4557. [PubMed: 15999993] (c) Tedesco R, Shaw AN, Bambal R, Chai D, Concha NO, Darcy MG, Dhanak D, Fitch DM, Gates A, Gerhardt WG, Halegoua DL, Han C, Hofmann GA, Johnston VK, Kaura AC, Liu N, Keenan RM, Lin-Goerke J, Sarisky RT, Wiggall KJ, Zimmerman MN, Duffy KJ. J. Med. Chem 2006;49:971–983. [PubMed: 16451063] (d) Hirashima S, Suzuki T, Ishida T, Noji S, Yata S, Ando I, Komatsu M, Ikeda S, Hashimoto H. J. Med. Chem 2006;49:4721–4736. [PubMed: 16854079]
9. Afdhal, N.; Rodriguez-Torres, M.; Lawitz, E.; Godofsky, E.; Chao, G.; Fielman, B.; Knox, S.; Broen, N. 40th EASL, Enhanced antiviral efficacy for Valopicitabine (NM283) plus Peg-interferon in hepatitis C patients with HCV genotype-1 infection: results of a phase IIa multicenter trial; April 13–17, 2005; Paris, France.
10. Roberts, S.; Cooksley, G.; Shaw, D.; Berns, HK.; Brandl, MT.; Fettner, SH.; Hill, G.; Ipe, D.; Klumpp, K.; Mannino, M.; O'Mara, E.; Tu, Y.; Washington, CB. Abstract 731, 41st EASL, Interim results of a multiple ascending dose study of R1626, a novel nucleoside analog targeting HCV polymerase in chronic HCV patients; Vienna, Austria.
11. Chandra, C.; Raible, D.; Harper, D.; Speth, J.; Villano, S.; Bichier, G. DDW 2006, Antiviral activity of the non-nucleoside polymerase inhibitor, HCV-796, in patients with chronic hepatitis C virus: preliminary results from a randomized, double-blind, placebo-controlled, ascending multiple dose study; May 20–25, 2006; Los Angeles, CA.
12. Kaushik-Basu N, Bopda-Waffo A, Talele TT, Basu A, Costa PR, Da Silva AJ, Sarafianos SG, Noël F. Nucleic Acids Res 2008;36(5):1482–1496. [PubMed: 18203743]
13. Anders CJ, Bronson JJ, D'Andrea SV, Deshpande SM, Falk PJ, Grant-Young KA, Harte WE, Ho H, Misco PF, Robertson JG, Stock D, Sun Y, Walsh AW. Bioorg. Med. Chem. Lett 2000;10:715–717. [PubMed: 10782671]
14. Kucukguzel SG, Oruc EE, Rollas S, Sahin F, Ozbek A. Eur. J. Med. Chem 2002;37:197–206. [PubMed: 11900864]
15. Karali N, Ilhan E, Gu¨rsoy A, Kiraz M. Farmaco 1998;53:346–349.

16. Fahmy HTY. Boll. Chim. Farm 2001;140:422–427. [PubMed: 11822232]
17. Ergenc N, Capan G. Farmaco 1994;49:133–135. [PubMed: 8003182]
18. Capan G, Ergenc N, Ekinci AC, Vidin A. Farmaco 1996;51:729–732. [PubMed: 9035380]
19. Look GC, Schullek JR, Homes CP, Chinn JP, Gordon EM, Gallop MA. Bioorg. Med. Chem. Lett 1996;6:707–712.
20. Bukowski L, Janowiec M, Zwolska-Kwiek Z, Andrezejczyk Z. Pharmazie 1998;53:373–376. [PubMed: 9675766]
21. Ulusoy N. Arzneim.-Forsch.-Drug Res 2002;52:565–571. [PubMed: 12189781]
22. Babaoglu K, Page MA, Jones VC, McNeil MR, Dong C, Naismith JH, Lee RE. Bioorg. Med. Chem. Lett 2003;13:3227–3230. [PubMed: 12951098]
23. Diurno MV, Mazzoni O, Calignano PE, Giordano F, Bolognese A. J. Med. Chem 1992;35:2910–2912. [PubMed: 1353796]
24. Bhatt JJ, Shah BR, Shah HP, Trivedi PB, Undavia NK, Desai NC. Indian J. Chem 1994;33B:189–192.
25. Barreca ML, Balzarini J, Chimirri A, De Clerc E, Luca LD, Holtje MH, Holtje M, Monforte AM, Monforte P, Pannecouque C, Rao A, Zappala M. J. Med. Chem 2002;45:5410–5413. [PubMed: 12431069]
26. (a) Rawal RK, Prabhakar YS, Katti SB, De Clercq E. Bioorg. Med. Chem 2005;13:6771–6776. [PubMed: 16198576] (b) Rawal RK, RajaSolomon V, Prabhakar YS, Katti SB, De Clercq E. Comb. Chem. High Throughput Screening 2005;8:439–443. (c) Rawal RK, Tripathi R, Katti SB, Pannecouque C, De Clercq E. Bioorg. Med. Chem 2007;15:1725–1731. [PubMed: 17178227] (d) Rawal RK, Tripathi R, Katti SB, Pannecouque C, De Clercq E. Bioorg. Med. Chem 2007;15:3134–3142. [PubMed: 17349793] (e) Rawal RK, Tripathi R, Katti SB, Pannecouque C, De Clercq E. Med. Chem 2007;3:355–363. [PubMed: 17627572]
27. Barreca ML, Chimirri A, Luca LD, Monforte AM, Monforte P, Rao A, Zappala M, Balzarini J, De Clercq E, Pannecouque C, Witvrouw M. Bioorg. Med. Chem. Lett 2001;11:1793–1796. [PubMed: 11425562]
28. Kaushik-Basu N, Bopda-Waffo A, Talele TT, Basu A, Chen Y, Guniz Kucukguzel S. Frontiers in Bioscience 2008;13:3857–3868. [PubMed: 18508480]
29. Srivastava T, Haq W, Katti SB. Tetrahedron 2002;58:7619–7624.

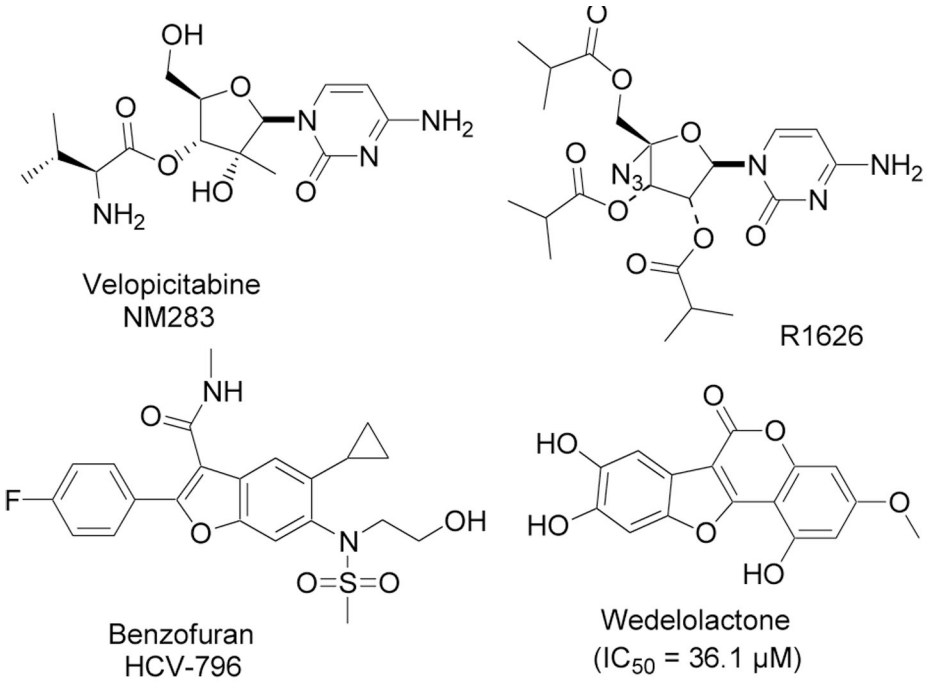
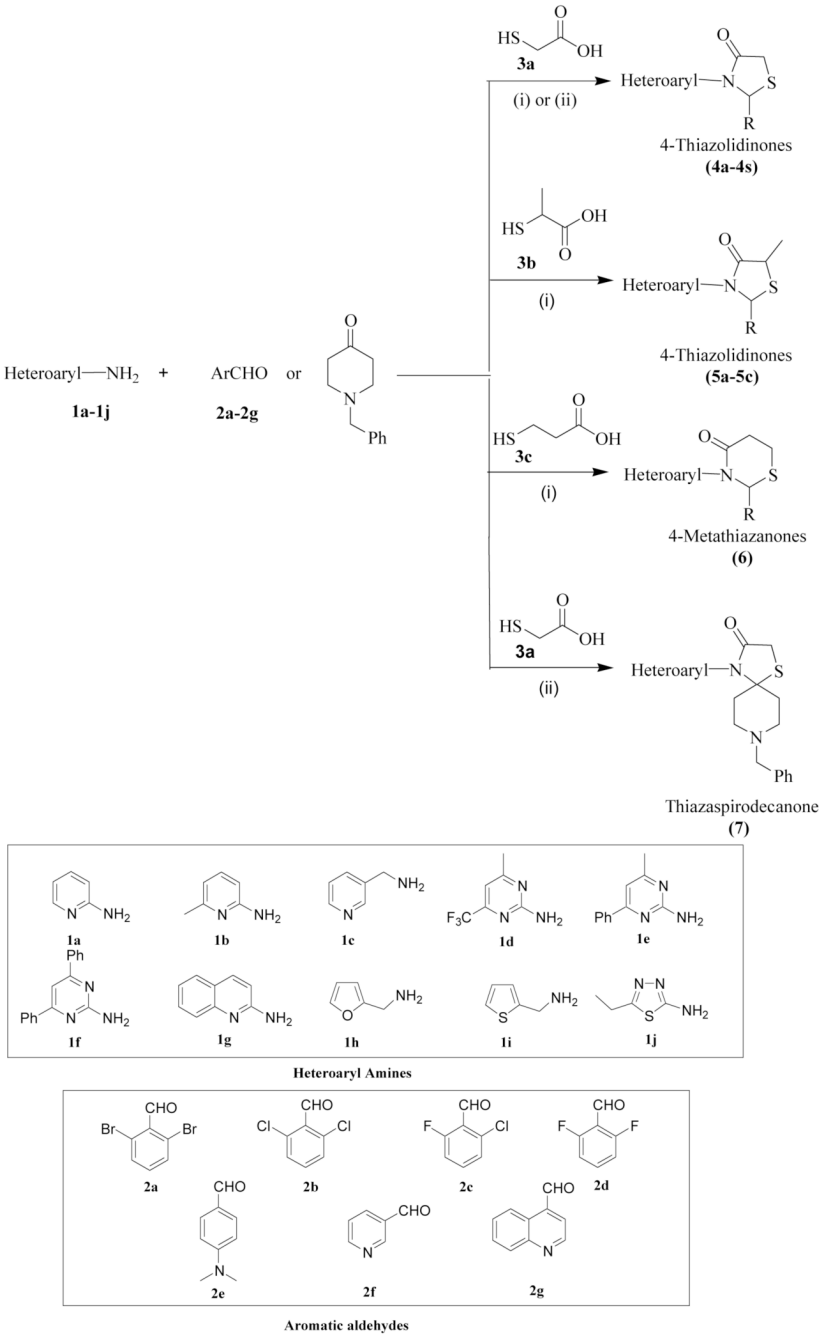


Figure 1.
NS5B RNA polymerase inhibitors.

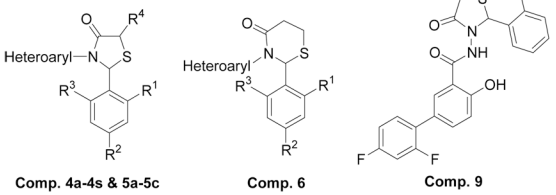


Scheme 1.

Synthesis of Compounds **4a–4s, 5a–5c, 6** and **7**. (i) DCC, THF, at RT (ii) Toluene, 4 Å MS at 120 °C.

Table 1

Physical data of 2,3-diaryl-1,3-thiazolidin-4-one derivatives.



Comp.	Heteroaryl	R^1	R^2	R^3	R^4	MWt	% yield	m.p. °C
4a	Pyridin-2-yl		Quinolin-4-yl		H	307	75	-
4b	Pyridin-2-yl	H	NMe2	H	H	299	68	-
4c	Pyridin-2-yl	H	F	H	H	274	78	-
4d	6-methyl-pyridin-2-yl		Pyridin-3-yl		H	271	55	104–106
4e	6-methyl-pyridin-2-yl	H	NMe2	H	H	313	72	133–135
4f	Pyridin-3-ylmethyl	Cl	H	Cl	H	339	92	-
4g	4-methyl-6-trifluoromethyl-pyrimidin-2-yl	Cl	H	Cl	H	408	50	111–115
4h	4-methyl-6-trifluoromethyl-pyrimidin-2-yl	Cl	H	F	H	392	47	92–94
4i	4-methyl-6-trifluoromethyl-pyrimidin-2-yl	Br	H	Br	H	497	36	133–137
4j	4-methyl-6-phenyl-pyrimidin-2-yl	Cl	H	Cl	H	416	48	168–170
4k	4-methyl-6-phenyl-pyrimidin-2-yl	Cl	H	F	H	400	41	138–140
4l	4-phenyl-6-trifluoromethyl-pyrimidin-2-yl	Cl	H	Cl	H	470	46	206–208
4m	4,6-diphenyl-pyrimidin-2-yl	Cl	H	Cl	H	478	38	206–208
4n	4,6-diphenyl-pyrimidin-2-yl	Cl	H	F	H	462	30	176–178
4o	4,6-diphenyl-pyrimidin-2-yl	F	H	F	H	445	28	192–194
4p	Quinolin-2-yl	Cl	H	F	H	358	56	144–146
4q	Furan-2-ylmethyl	Cl	H	Cl	H	328	92	96–98
4r	Thiophen-2-ylmethyl	Br	H	Br	H	433	64	-
4s	5-ethyl-[1,3,4]-thiadiazol-2-yl	F	H	F	H	327	44	110–114
5a	Pyridin-2-yl	Cl	H	Cl	CH_3	339	80	85–89
5b	Pyridin-3-ylmethyl	Cl	H	Cl	CH_3	353	90	-
5c	Furan-2-ylmethyl	Cl	H	Cl	CH_3	342	88	-
6	Furan-2-ylmethyl	Cl	H	Cl	H	342	90	-
7	Pyridin-2-yl		1-benzyl-piperidinyl		H	339	50	-
8[a]	Furan-2-ylmethyl	Cl	H	Cl	H	344	78	155–158
9	2',4'-Difluoro-4-hydroxy-biphenyl-3-carboxylic acid [2-(2-fluoro-phenyl)-4-oxo-thiazolidin-3-yl]-amide					-	-	-

[a] sulfoxide.

Table 2

Anti-NS5B RdRp activity of compounds (**4a–4s, 5a–5c** and **6–9**).

Comp.	Anti-NS5B Activity % Inhibition[a]	IC_{50} μM
4a	62.2±4.3	-
4b	76.4±3.6	-
4c[b]	98±0.9	31.9±1.2
4d	64.2±4.1	-
4e	84.7±4.2	79.4±1.1
4f	75.6±2.1	-
4g	72.1±1.6	-
4h	63.6±2.5	-
4i	76.3±2.5	-
4j	63.6±2.1	-
4k	65.5±2.3	-
4l	66.4±2.9	-
4m	73.1±3.1	-
4n	70.6±3.8	-
4o	73.3±2.1	-
4p[b]	60.0±2.6	-
4q	72.3±1.6	-
4r	62.3±3.5	-
4s	62.9±1.0	-
5a	73.0±3.6	-
5b	95.6±0.8	41.8±1.1
5c	97.8±0.8	32.2±1.1
6	94.2±2.8	52.6±1.2
7[b]	85.6±4.9	71.9±1.1
8[b]	68.7±5.4	-
9	92.4±2.5	48
wedelolactone	-	36.1

[a]Percentage inhibition was determined at 0.25 mM concentration of the indicated compound and represents an average of at least three independent measurements. NS5B RdRp activity in the absence of the inhibitor was taken as 100 percent after subtraction of residual background activity. The concentration of DMSO in all reactions was kept constant at 10%. The IC_{50} values of the compounds **4c, 4e, 5b, 5c, 6** and **7** were determined from dose–response curves using 8–12 concentrations of each compound in duplicate, in two independent experiments. Curves were fitted to data points using nonlinear regression analysis and IC_{50} values were interpolated from the resulting curves using GraphPad Prism 5.0 software. Wedelolactone (IC_{50}=36.1 μM) was included as an internal reference standard.

[b]compounds 4c, 4p, 7 & 8 are new.