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Parallel synthesis of 5-cyano-6-aryl-2-thiouracil derivatives as inhibitors for hepatitis C viral NS5B RNA-dependent RNA polymerase

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Abstract

From random screening of our compound libraries, we identified a hit compound with an IC_{50} of 27 μM against hepatitis C viral NS5B RNA-dependent RNA polymerase. By using a parallel synthetic strategy, a series of its derivatives were synthesized. From their anti-HCV activity screening, compounds with single digital 3.8 micromolar activity were obtained.

Keywords: HCV; NS5B; 5-Cyano-6-aryl-2-thiouracil derivatives; Inhibitors

1. Introduction

Chronic viral infection caused by hepatitis C virus (HCV) has been recognized as one of the leading causes of liver impairment such as cirrhosis and hepatocellular carcinoma. It is estimated that 3% of the world population or about 170 million people are infected with hepatitis C virus [1]. The recommended standard of care treatment, the pegylated interferon α in the combination with ribavirin, provides a sustained response in about 50% of the treated patients, but side effects could be severe [2]. The need of a more efficacious and

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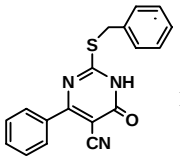
better tolerated treatment has spurred intense research efforts in pharmaceutical industry to develop novel anti-HCV agents.

HCV is a 9.6 kb positive strand RNA virus of the flaviviridae, genus *Hepacivirus*. It contains a single open reading frame coding for a ~3000 amino acid polyprotein, which is further processed into various structural and non-structural viral proteins by host and viral proteases. The NS5B RNA-dependent RNA polymerase (RdRp) is the central enzyme that is responsible for replication of the viral genome, and has since become a target of choice for the screening and design of small molecular inhibitors, which in principle, should interfere with viral replication [3]. Various institutions and pharmaceutical companies have reported structurally diverse non-nucleoside small molecular inhibitors of NS5B polymerase [4].



IC_{50} = 27μM

1

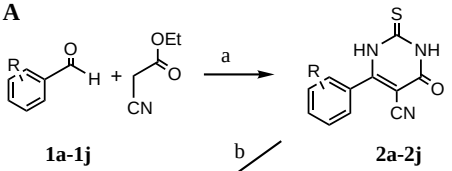
Our efforts towards identifying an HCV NS5B RdRp inhibitor started with a high throughput screening of compound libraries using an NS5B RdRp directed RNA synthesis assay [5]. The effort culminated in identification of a 5-cyano-6-aryl-2-thiouracil compound **1** that had an IC_{50} (inhibitor concentration for 50% inhibition) of 27 μM. Although its potency was not impressive, compound **1** had no apparent structural liability and seemed to possess a pharmacophore of uracil, the nucleobase of uridine that is utilized by an RNA polymerase. In addition, this class of molecule has been previously reported to possess antiviral activity against polio, coxsackie, and semliki forest viruses [6]. A recent patent application also revealed that 5-cyano-6-aryl-2-thiouracils are active against HCV NS3 helicase [7]. In this communication, we report the synthesis of various thiouracil derivatives with different substituents across the ring skeleton and their inhibitory activity against HCV NS5B RdRp.

2. Results and discussion

Based on the structure of compound **1**, we made the chemical modifications on aryl group at C-6 of the ring, alkylation on the S atom, and substitutions on the N of the ring through the parallel synthesis strategy. We like to find out the structure–activity relation of these modifications. The efficient synthetic strategy is summarized in Scheme 1. The thioxopyrimidine heterocyclic cores were constructed by a cyclo-condensation of equivalent molar quantities of 10 substituted aromatic aldehydes **1a**–**1j**, thiourea, and ethyl cyanoacetate in the parallel fashion. After neutralization with acetic acid, the desired 5-cyano-4-oxo-6-aryl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine derivatives **2a**–**2j** were obtained as solids.

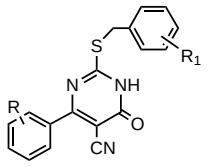
Benzylation of compounds **2a**–**2j** with different substituted benzyl bromides in ethanol in the presence of K_2CO_3 gave 6-aryl-5-cyano-2-benzylthio-4-oxopyrimidines **3aa**–**3je** in high yields. During these reactions, in some cases, the S and N disubstituted pyrimidine derivatives such as compound **3a** were also isolated from the reaction mixture as minor products.

A

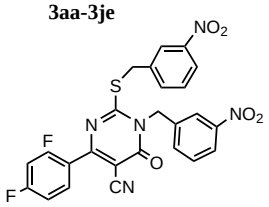


1a-1j **2a-2j**

a R=2, 4-difluoro
b R=2,4-dichloro
c R=4-bromo
d R=3-methyl
e R=3-trifluoromethyl
f R=2-methyl
g R=3,5-difluoro
h R=2-trifluoromethyl
i R=3,5-dichloro
j R=4-isopropyl

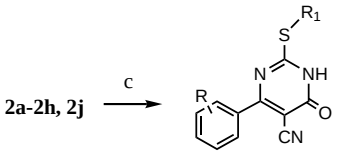


3aa-3je



3a

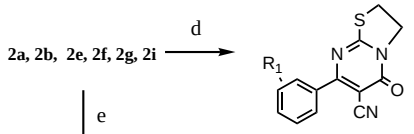
3aa R=2, 4-difluoro, R_1=3,5-dimethoxyl
3ab R=2,4-difluoro, R_1=3-Me
3ac R=2,4-difluoro, R_1=2-Br
3ad R=2,4-difluoro, R_1=3-NO_2
3ba R = 2,4-dichloro, R_1=3,5-dimethoxyl
3bb R = 2,4-dichloro, R_1=3-Me
3bc R = 2,4-dichloro, R_1=4-OCF_3
3ca R=4-Br, R_1=3,5-dimethoxyl
3cb R=4-Br, R_1=3-Me
3cc R=4-Br, R_1=2-Br
3cd R=4-Br, R_1=3-NO_2
3ce R=4-Br, R_1=2-CF_3
3cf R=4-Br, R_1=4-OCF_3
3da R=3-Me, R_1=3,5-dimethoxyl
3db R=3-Me, R_1=3-Me
3dc R=3-Me, R_1=3-NO_2
3dd R=3-Me, R_1=4-OCF_3
3ea R=3-CF_3, R_1=3,5-dimethoxyl
3eb R=3-CF_3, R_1=3-Me
3ec R=3-CF_3, R_1=3-NO_2
3fa R=2-Me, R_1=3,5-dimethoxyl
3fb R=2-Me, R_1=3-Me
3fc R=2-Me, R_1=3-Br
3fd R=2-Me, R_1=3-NO_2
3ga R=3,5-difluoro, R_1=3,5-dimethoxyl
3gb R=3,5-difluoro, R_1=3-Me
3ha R=2-CF_3, R_1=3,5-dimethoxyl
3hb R=2-CF_3, R_1=3-Me
3hc R=2-CF_3, R_1=2-Br
3hd R=2-CF_3, R_1=4-OCF_3
3ia R=3,5-dichloro, R_1=3,5-dimethoxyl
3ib R=3,5-dichloro, R_1=3-Me
3ja R=4-isopropyl, R_1=3,5-dimethoxyl
3jb R=4-isopropyl, R_1=3-Me
3jc R=4-isopropyl, R_1=2-Br
3jd R=4-isopropyl, R_1=3-NO2
3je R=4-isopropyl, R_1=4-OCF_3



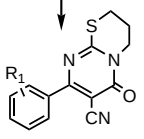
2a-2h, 2j **4aa-4ha, 4ja-4jc**

4aa R=2, 4-difluoro, R_1=$(CH_2)_4CH_3$
4ba R = 2,4-dichloro, R_1=$(CH_2)_4CH_3$
4bb R = 2,4-dichloro, R_1=$(CH_2)_3$Ph
4ca R=4-Br, R_1=$(CH_2)_4CH_3$
4cb R=4-Br, R_1=CH_2CH_2OCOPh
4cc R=4-Br, R_1=(tetrahydro-2H-pyran)methene
4da R=3-Me, R_1=$(CH_2)_4CH_3$
4db R=3-Me, R_1=(tetrahydro-2H-pyran)methene
4ea R=3-CF_3, R_1=$(CH_2)_4CH_3$
4eb R=3-CF_3, R_1=$(CH_2)_3$Ph
4fa R=2-Me, R_1=$(CH_2)_4CH_3$
4ga R=3,5-difluoro, R_1=$(CH_2)_3$Ph
4gb R=3,5-difluoro, R_1=(tetrahydro-2H-pyran)methene
4ha R=2-CF_3, R_1=$(CH_2)_4CH_3$
4ja R=4-isopropyl, R_1=$(CH_2)_4CH_3$
4jb R=4-isopropyl, R_1=$(CH_2)_3$Ph
4jc R=R=4-isopropyl, R_1=(tetrahydro-2H-pyran)methene

B

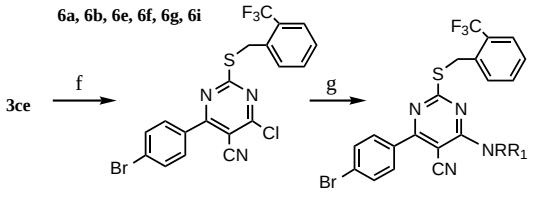


2a, 2b, 2e, 2f, 2g, 2i **5b, 5e, 5f, 5g, 5i**



6a, 6b, 6e, 6f, 6g, 6i

a R_1=2,4-difluoro
b R_1=2,4-dichloro
e R_1=3-methyl
f R_1=3-trifluoromethyl
g R_1=2-methyl
i R_1=4-bromo



3ce **7** **7a-7i**

7a R=2-(3-methoxylphenyl)-ethyl, R_1=H
7b R=cyclbutyl, R_1=H
7c R=2-(phenyl)-ethyl, R_1=H
7d R=2-(ethyl)-heptanyl, R_1=H
7e R=4-(methylamino) piperidine, R_1=Me
7f R=2-(3,4-dimethoxylphenyl)-ethyl, R_1=H
7g R=(2-pyrimidy)-piperazine, R_1=H
7h R=2-(1-methylpyrrolidine)-ethyl
7i R=CH_2CF_3, R_1=H
7j R=2-(3, 4-benzodioxole)-ethyl, R_1=H

Alkylation of compounds **2a**–**2h**, and **2j** with various alkyl bromides in DMF in the presence of triethylamine provided the 6-aryl-5-cyano-2-alkylthio-4-oxopyrimidines **4aa**–**4ha**, **4ja**–**4jc**. Treatment of compounds **2a**, **2b**, **2e**, **2f**, **2g**, and **2i** with 1,2-dibromoethylene or 1,3-dibromopropane in DMF in the presence of triethylamine afforded the five-membered ring bicyclic thiazolo[3,2-*a*]pyrimidines **5b**, **5e**, **5f**, **5g**, **5i**, and the six-membered ring bicyclic pyrimido[2,1-*b*]thiazines **6a**, **6b**, **6e**, **6f**, **6g**, and **6i**. We tried to get the seven-membered ring bicyclic derivatives from the reactions with 1,4-dibromobutane, however, no desired compound was isolated from the reaction mixture even under strong reaction conditions (higher temperature and longer reaction time in DMF or DMSO).

Compound **3ce** was halogenated from the reaction with phosphoryl chloride to yield 6-(4-bromophenyl)-4-chloro-2-(2-trifluoromethyl-benzylthio)-pyrimidine-5-carbonitrile (**7**). This highly activated intermediate was then reacted with different amines to yield 6-(4-bromophenyl)-4-alkylamino-2-(2-trifluoromethyl-benzylthio)-pyridine- 5-carbonitriles **7a**–**7j**.

Each compound was purified on preparative TLC in a parallel fashion. The identity of the compounds described above was confirmed by ESMS (data not shown) and ^{1}H NMR spectra. They normally had more than 90% purity judged by HPLC and LCMS.

All the compounds were evaluated for inhibitory activity against HCV NS5B RdRp using a publishing procedure [5]. In the assay, a compound was first dissolved in DMSO, and then transferred into the assay buffer. Every compound was tested in duplicate. IC_{50} determined for each compound generally carried with a standard deviation <10%.

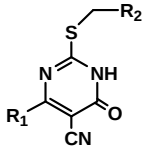
A systematic study of importance of the substitution on the aryl ring and benzyl ring for compounds **3aa**–**3je** is listed in Table 1. The inhibitory activities of 2-alkylthio-6-aryl-3,4-dihydro-6-oxopyrimidine-5-carbonitrile derivatives **4aa**–**4jc** are listed in Table 2. Some of these compounds showed single digital micromolar activity including compounds **3ce** (7.1 μM), **3cf** (8.7 μM), **3dd** (8.6 μM), **3hd** (9.2 μM), **3jc** (3.8 μM), and **3je** (9.9 μM) against hepatitis C viral NS5B RNA-dependent RNA polymerase. The best compound **3jc** was further assayed for its ability to inhibit hepatitis C viral subgenome replication assay in Huh-7 cells. Its activity was weak with an EC_{50} of 32 μM.

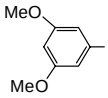
The *para*-substitution on both C-6 aryl group and the S-benzyl group improved the inhibitory activity two-fold over the lead compound. However, a combination of *para*-substitution on C-6 aryl group and a *ortho*-substitution on *S*-benzyl group led to a five-fold increase over the lead compound activity. Non-benzyl alkyl substitution on sulfur did not improve the activity very much.

Five-membered ring bicyclic heterocycles **5b**, **5e**, **5f**, **5g**, and **5f**, six-membered ring bicyclic heterocycles **6a**, **6b**, **6e**, **6f**, **6g**, and **6i**, 4-alkylamino-6-(4-bromophenyl)-2-(2-trifluoromethyl-benzylthio)-pyrimidine-5-carbonitrile analogues **7a**–**7j**, and the S and N disubstituted pyrimidine derivative **3a** did not show any inhibitory activity ($IC_{50} > 100$ μM). All of these compounds have the substitution at N of the ring, indicating free NH of the ring is necessary for the activity.

Scheme 1. Reagents and conditions: (a) **1a**–**1j**, thiourea, K_2CO_3, ethanol, refluxing; (b) **2a**–**2j**, substituted benzyl bromides, K_2CO_3, DMF, room temperature, 10 h; (c) **2a**–**2h**, **2j**, alkyl bromides, triethylamine, DMF, 80 °C, 10 h; (d) **2b**, **2e**, **2f**, **2g**, and **2i**, $BrCH_2CH_2Br$, triethylamine, DMF, 80 °C, 10 h; (e) **2a**, **2b**, **2e**, **2f**, **2g**, and **2i**, $BrCH_2CH_2CH_2Br$, triethylamine, DMF, 80 °C, 10 h; (f) **3ce**, $POCl_3$, 70 °C, 100%; (g) **7**, alkyl amines, triethylamine, CH_3CN, room temperature, 10 h.

Table 1
SAR of the substitution on the aryl and benzyl groups of compound **1**



Compound	R_1	R_2	IC_{50} (μM)
3aa			19
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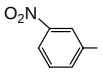
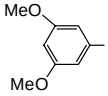
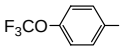
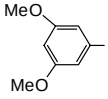
3ac			13
3ad			32
3ba			34
3bb			33
3bc			11
3ca			13
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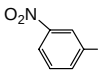
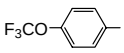
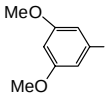
3cc			10
3cd			14
3ce			7.1
3cf			8.7
3da			30

Table 1 (*continued*)

Compound	R_1	R_2	IC_{50} (μM)
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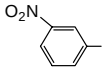
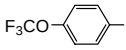
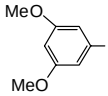
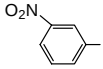
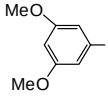
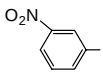
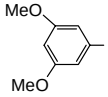
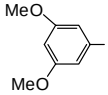
3dc			20
3dd			8.6
3ea			11
3eb			14
3ec			13
3fa			25
3fb			14
3fc			32
3fd			37
3ga			15
3gb			27
3ha			12
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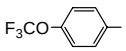
3hd			9.2

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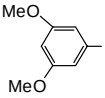
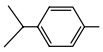
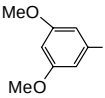
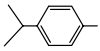
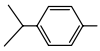
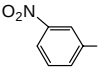
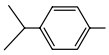
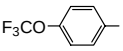
Table 1 (*continued*)

Compound	R_1	R_2	IC_{50} (μM)
3ia			34
3ib			29
3ja			25
3jb			12
3jc			3.8
3jd			10
3je			9.9

All the compounds described here were tested for cellular activity using a cell-based HCV subgenomic replicon assay. They did not show any toxicity up to 250 μM.

3. Conclusions

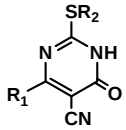
From the screening of our compound libraries using an NS5B-directly RNA synthesis assay, we identified a hit compound **1** with an IC_{50} of 27 μM. By using a parallel synthetic strategy, we were able to synthesize a series of 5-cyano-6-aryl-2-thiouracil derivatives for anti-HCV agent screening. Modification of the hit has resulted in a series of inhibitor with improved potency. The SAR studies suggest that the free NH of the uracil ring seems to be necessary for the inhibitory activity, the *para*-substitution on both C-6 aryl group and the *S*-benzyl group slightly improved the activity, and a combination of the *para*-substitution on the aryl group and an *ortho*-substitution on the sulfur atom increase the activity. Based on these results, further optimization, mechanism of action, and structural studies are in progress to elucidate their mode of action and improve their potency against HCV NS5B RdRp.

4. Experimental

4.1. General

^{1}H spectra were obtained using a Varian Gemini 300 NMR. The proton NMR spectra were recorded at 300 MHz. All reagents and chemicals were obtained from Aldrich Chem-

Table 2
SAR at the substitution of aryl group and the non-beznyl alkyl group on sulfur atom of the core structure



Compound	R_1	R_2	IC_{50} (μM)
4aa		$CH_3(CH_2)_4$	12
4ba		$CH_3(CH_2)_4$	17
4bb		$Ph(CH_2)_3$	12
4ca		$CH_3(CH_2)_4$	11
4cb		$PhCO_2(CH_2)_2$	30
4cc			50
4da		$CH_3(CH_2)_4$	18
4db			>100
4ea		$CH_3(CH_2)_4$	12
4eb		$PhCO_2(CH_2)_2$	13
4fa		$CH_3(CH_2)_4$	18
4ga		$Ph(CH_2)_3$	>100
4gb			>100
4ha		$CH_3(CH_2)_4$	20
4ja		$CH_3(CH_2)_4$	12
4jb		$Ph(CH_2)_3$	>100
4jc			17

ical (USA) and were used as received unless otherwise noted. All the reactions were performed in a Reacto-Station RS 6000 synthesizer. Each compound was purified on a preparative TLC. The purity of the compounds was determined by LCMS (waters Micromass ZQ).

4.2. Spectral data: ^1H NMR data

3aa (CD_3OD): δ 7.72 (m, 1H), 7.20 (m, 2H), 6.54 (s, 2H), 6.37 (s, 1H), 4.23 (s, 2H), 3.68 (s, 6H).

3ab ^{1}H NMR (CD_3OD): δ 7.53 (q, 1H), 7.21 (s, 1H), 7.17–7.01 (m, 5H), 4.33 (s, 2H), 2.29 (s, 3H).

3ac ^{1}H NMR (CD_3OD): δ 7.56 (m, 2H), 7.23 (t, 1H, $J = 7.2$ Hz), 7.13 (m, 3H), 4.51 (s, 2H).

3ad ^{1}H NMR (CD_3OD): δ 8.31 (s, 1H), 8.09 (d, 1H, $J = 8.4$ Hz), 7.88 (d, 1H, $J = 8.4$ Hz), 7.52 (m, 2H), 7.10 (m, 2H), 4.53 (s, 2H).

3a ^{1}H NMR (CD_3OD): δ 8.27 (s, 1H), 8.19 (m, 1H), 8.12 (s, 1H), 8.06 (d, 1H, $J = 8.1$ Hz), 7.80 (d, 1H, $J = 8.1$ Hz), 7.8 (m, 2H), 7.48 (t, 1H, $J = 8.1$ Hz), 7.37 (m, 1H), 7.01 (m, 2H), 4.46 (d, 2H, $J = 8.4$ Hz), 4.28 (d, 2H, $J = 8.4$ Hz).

3ba ^{1}H NMR (CD_3OD): δ 7.69 (s, 1H), 7.53 (s, 1H), 7.52 (s, 1H), 6.51 (s, 2H), 6.36 (s, 1H), 4.39 (s, 2H), 3.68 (s, 6H).

3bb ^{1}H NMR (CD_3OD): δ 7.59 (d, 1H, $J = 2.1$ Hz), 7.45 (dd, 1H, $J = 8.7$, 2.1 Hz), 7.37 (d, 1H, $J = 8.7$ Hz), 7.20 (s, 1H), 7.16 (s, 1H), 7.15 (d, 1H, $J = 6.6$ Hz), 7.02 (d, 1H, $J = 6.6$ Hz), 4.36 (s, 2H), 2.27 (s, 3H).

3bc ^{1}H NMR (CD_3OD): δ 7.59 (s, 1H), 7.52 (d, 2H, $J = 8.7$ Hz), 7.38 (q, 2H, $J = 8.4$ Hz), 7.16 (d, 2H, $J = 8.7$ Hz); 4.38 (s, 2H).

3ca ^{1}H NMR (DMSO-d_6): δ 7.74 (d, 2H, $J = 8.7$ Hz), 7.68 (d, 2H, $J = 8.7$ Hz), 6.56 (s, 2H), 6.32 (s, 1H), 4.24 (s, 2H), 3.64 (s, 6H).

3cb ^{1}H NMR (DMSO-d_6): δ 7.79 (d, 2H, $J = 8.4$ Hz), 7.73 (d, 2H, $J = 8.4$ Hz), 7.18 (m, 2H), 7.16, (s, 1H), 7.04 (m, 1H), 4.35 (s, 2H), 2.22 (s, 3H).

3cc ^{1}H NMR (DMSO-d_6): δ 7.86 (d, 2H, $J = 6.9$ Hz), 7.79 (d, 2H, $J = 6.9$ Hz), 7.64 (d, 1H, $J = 6.9$ Hz), 7.53 (d, 1H, $J = 6.9$ Hz), 7.32 (t, 1H, $J = 6.9$ Hz), 7.23 (t, 1H, $J = 6.9$ Hz), 4.60 (s, 2H).

3cd ^{1}H NMR (CD_3OD): δ 8.33 (s, 1H), 8.09 (d, 2H, $J = 8.1$ Hz), 7.86 (d, 2H, $J = 8.1$ Hz), 7.73 (d, 2H, $J = 8.7$ Hz), 7.64 (d, 2H, $J = 8.7$ Hz), 7.52 (t, 1H, $J = 8.1$ Hz), 4.51 (s, 2H).

3ce ^{1}H NMR (DMSO-d_6): δ 7.78 (m, 6H), 7.64 (t, 1H, $J = 7.5$ Hz), 7.52 (t, 1H, $J = 7.5$ Hz), 4.68 (s, 2H).

3cf ^{1}H NMR (CD_3OD): δ 7.71 (d, 2H, $J = 8.4$ Hz), 7.63 (d, 2H, $J = 8.4$ Hz), 7.53 (d, 1H, $J = 8.2$ Hz), 7.17 (d, 2H, $J = 8.2$ Hz), 4.41 (s, 2H).

3da ^{1}H NMR (CD_3OD): δ 7.78 (s, 1H), 7.76 (d, 1H), 7.41 (m, 2H), 6.59 (s, 2H), 6.36 (s, 1H), 4.47 (s, 2H), 3.64 (s, 6H), 2.43 (s, 3H).

3db ^{1}H NMR (CD_3OD): δ 7.61 (s, 2H), 7.33 (m, 2H), 7.17 (m, 3H), 7.01 (d, 1H, $J = 6.0$ Hz), 4.40 (s, 2H), 2.39 (s, 3H), 2.27 (s, 3H).

3dc ^{1}H NMR (DMSO-d_6): δ 8.27 (s, 1H), 8.06 (d, 1H, $J = 8.1$ Hz), 7.87 (d, 1H, $J = 8.1$ Hz), 7.58 (t, 1H, $J = 8.1$ Hz), 7.49 (d, 1H), 7.48 (s, 1H), 7.31 (t, 1H, $J = 8.1$ Hz), 7.24 (t, 1H, $J = 8.1$ Hz), 4.38 (s, 2H), 2.33 (s, 3H).

3dd ^{1}H NMR ($CDCl_3$): δ 7.47 (d, 1H), 7.44 (s, 1H), 7.11 (m, 4H), 6.89 (d, 2H, $J = 8.1$ Hz), 4.12 (s, 2H), 2.15 (s, 3H).

3ea ^{1}H NMR (CD_3OD): δ 8.30 (s, 1H), 8.25 (d, 1H, $J = 6.9$ Hz), 7.90 (d, 1H, $J = 6.9$ Hz), 7.77 (t, 1H, $J = 6.9$ Hz), 6.57 (s, 2H), 6.36 (s, 1H), 4.49 (s, 2H), 3.65 (s, 6H).

3eb ^{1}H NMR (CD_3OD): δ 8.10 (s, 1H), 8.07 (d, 1H, $J = 8.8$ Hz), 7.80 (d, 1H, $J = 7.8$ Hz), 7.68 (t, 1H, $J = 8.8$ Hz), 7.23 (s, 1H), 7.19 (d, 1H, $J = 8.8$ Hz), 7.14 (t, 1H, $J = 8.8$ Hz), 7.02 (d, 1H, $J = 8.8$ Hz), 4.35 (s, 2H), 2.28 (s, 3H).

3ec ^{1}H NMR (DMSO-d_6): δ 8.27 (s, 1H), 8.05 (t, 1H, $J = 9.0$ Hz), 7.99 (s, 1H), 7.86 (d, 1H, $J = 7.5$ Hz), 7.83 (d, 1H, $J = 7.5$ Hz), 7.69 (t, 1H, $J = 9.0$ Hz), 7.57 (t, 1H, $J = 9.0$ Hz), 4.39 (s, 2H).

3fa ^{1}H NMR (CD_3OD): δ 7.20 (m, 4H), 6.56 (s, 2H), 6.34 (s, 1H), 4.40 (s, 2H), 3.66 (s, 6H), 2.32 (s, 3H).

3fb ^{1}H NMR (CD_3OD): δ 7.38–7.11 (m, 7H), 7.02 (d, 1H, $J = 6.6$ Hz), 4.33 (s, 2H), 2.27 (s, 3H), 2.28 (s, 3H).

3fc ^{1}H NMR (CD_3OD): δ 7.40–7.11 (m, 7H), 7.02 (d, 1H, $J = 6.6$ Hz), 4.38 (s, 2H), 2.27 (s, 3H).

3fd ^{1}H NMR ($CDCl_3$): δ 8.20 (s, 1H), 8.02 (t, 1H), 7.82 (d, 1H, $J = 7.5$ Hz), 7.60 (t, 1H), 7.20 (m, 4H), 4.20 (s, 2H), 2.20 (s, 3H).

3ga ^{1}H NMR (CD_3OD): δ 7.59 (d, 1H), 7.57 (d, 1H), 7.22 (m, 1H), 6.58 (s, 2H), 6.37 (s, 1H), 4.24 (s, 2H), 3.69 (s, 6H).

3gb ^{1}H NMR (CD_3OD): δ 7.41 (m, 2H), 7.23 (s, 1H), 7.17 (d, 1H, $J = 7.5$ Hz), 7.15 (t, 1H, $J = 7.5$ Hz), 7.06 (m, 1H), 7.02 (d, 1H, $J = 7.5$ Hz), 4.34 (s, 2H), 2.29 (s, 3H).

3ha ^{1}H NMR (CD_3OD): δ 7.91 (d, 1H, $J = 7.2$ Hz), 7.77 (t, 2H, $J = 7.2$ Hz), 7.62 (d, 1H, $J = 7.2$ Hz), 6.47 (s, 2H) 6.34 (s, 1H), 4.37 (s, 2H), 3.64 (s, 6H).

3hb ^{1}H NMR (CD_3OD): δ 7.81 (d, 1H, $J = 7.5$ Hz), 7.68 (q, 2H, $J = 7.5$ Hz), 7.47 (d, 1H, $J = 7.5$ Hz), 7.19 (s, 1H), 7.14 (m, 2H), 7.01 (d, 1H, $J = 6.0$ Hz), 4.31 (s, 2H), 2.28 (s, 3H).

3hc ^{1}H NMR (CD_3OD): δ 7.82 (d, 1H, $J = 8.4$ Hz), 7.72 (t, 2H, $J = 8.4$ Hz), 7.66 (d, 1H, $J = 8.4$ Hz), 7.55 (d, 1H, $J = 8.4$ Hz), 7.53 (d, 1H, $J = 8.4$ Hz), 7.47 (d, 1H, $J = 8.4$ Hz), 7.21 (t, 1H, $J = 8.4$ Hz), 7.12 (t, 1H, $J = 8.4$ Hz), 4.49 (s, 2H).

3hd ^{1}H NMR ($CDCl_3$): δ 7.67 (d, 1H, $J = 7.8$ Hz), 7.45 (t, 1H, $J = 7.8$ Hz), 7.33 (t, 1H, $J = 7.8$ Hz), 7.16 (d, 2H, $J = 8.4$ Hz), 7.09 (d, 2H, $J = 7.8$ Hz), 6.93 (d, 2H, $J = 8.4$ Hz), 4.15 (s, 2H).

3ia ^{1}H NMR (CD_3OD): δ 7.77 (s, 2H), 7.58 (s, 1H), 6.60 (s, 2H), 6.33 (s, 1H), 3.77 (s, 2H), 3.72 (s, 6H).

3ib ^{1}H NMR (CD_3OD): δ 7.75 (d, 2H, $J = 2.1$ Hz), 7.57 (t, 1H, $J = 2.1$ Hz), 7.24 (s, 1H), 7.18 (s, 1H), 7.04 (s, 1H), 7.02 (s, 1H), 4.33 (s, 2H), 2.29 (s, 3H).

3ja ^{1}H NMR (DMSO-d_6): δ 7.90 (d, 2H, $J = 9.1$ Hz), 7.43, (d, 2H, $J = 9.1$ Hz), 6.57 (s, 2H), 6.34 (s, 1H), 4.43 (s, 2H), 4.36 (s, 1H, NH), 3.55 (s, 6H), 2.96 (m, 1H), 1.27 (d, 6H, $J = 6.9$ Hz).

3jb ^{1}H NMR (CD_3OD): δ 7.75 (s, 1H), 7.74 (s, 1H), 7.57 (t, 1H, $J = 2.1$ Hz), 7.24 (s, 1H), 7.18 (s, 1H), 7.14 (d, 1H), 7.02 (d, 1H), 4.33 (s, 2H), 2.29 (s, 3H).

3jc ^{1}H NMR (DMSO-d_6): δ 7.84 (d, 2H, $J = 7.8$ Hz), 7.38 (d, 1H, $J = 7.8$ Hz), 7.30 (d, 1H, $J = 7.8$ Hz), 7.13 (d, 2H, $J = 7.8$ Hz), 6.96 (t, 2H, $J = 7.8$ Hz), 4.36 (s, 2H), 2.85 (m, 1H), 1.29 (d, 6H, $J = 6.6$ Hz).

3jd ^{1}H NMR (CD_3OD): δ 8.34 (s, 1H), 8.12 (d, 1H, $J = 9.3$ Hz), 7.88 (d, 2H, $J = 8.4$ Hz), 7.84 (d, 1H, $J = 9.3$ Hz), 7.54 (t, 1H, $J = 9.3$ Hz), 7.40 (d, 2H, $J = 8.4$ Hz), 4.65 (s, 2H), 3.02 (m, 1H), 1.30 (d, 6H, $J = 6.6$ Hz).

3je ^{1}H NMR ($CDCl_3$): δ 7.61 (d, 2H, $J = 8.1$ Hz), 7.04 (m, 4H), 6.83 (d, 4H, $J = 8.1$ Hz), 4.03 (s, 2H), 2.80 (m, 1H), 1.13 (d, 6H, $J = 6.6$ Hz).

4aa ^{1}H NMR (CD_3OD): δ 7.58 (q, 1H, $J = 8.4$ Hz), 7.08 (t, 2H, $J = 8.4$ Hz), 3.09 (t, 2H, $J = 7.6$ Hz), 1.70 (m, 2H), 1.36 (m, 4H), 0.90 (t, 3H, $J = 7.6$ Hz).

4ba ^{1}H NMR (CD_3OD): δ 7.59 (s, 1H), 7.43 (m, 2H), 3.09 (t, 2H, $J = 7.6$ Hz), 1.70 (m, 2H), 1.36 (m, 4H), 0.90 (t, 3H, $J = 7.6$ Hz).

4bb ^{1}H NMR (CD_3OD): δ 7.59 (s, 1H), 7.43 (q, 2H, $J = 7.6$ Hz), 7.21 (m, 5H), 3.09 (t, 2H, $J = 7.6$ Hz), 2.70 (t, 2H, $J = 7.6$ Hz), 2.01 (m, 2H).

4ca ^{1}H NMR (CD_3OD): δ 7.79 (d, 1H, $J = 7.5$ Hz), 7.63 (t, 2H, $J = 7.5$ Hz), 3.09 (t, 2H, $J = 7.2$ Hz), 1.75 (m, 2H), 1.40 (m, 4H), 0.90 (t, 3H, $J = 7.2$ Hz).

4cb ^{1}H NMR (CD_3OD): δ 7.74 (d, 2H, $J = 6.6$ Hz), 7.65 (d, 2H, $J = 6.6$ Hz), 7.34 (m, 3H), 7.12 (m, 2H), 4.10 (t, 2H, $J = 7.4$ Hz), 3.40 (d, 1H, $J = 7.4$ Hz).

4cc ^{1}H NMR (CD_3OD): δ 7.74 (d, 1H, $J = 6.6$ Hz), 7.65 (d, 1H, $J = 6.6$ Hz), 3.95 (dd, 1H, $J = 11.1$, 2.7 Hz), 3.58 (m, 1H), 3.44 (m, 1H), 3.35 (dd, 1H), 3.08 (q, 1H, $J = 13.5$ Hz), 1.82 (m, 2H), 1.52 (m, 3H), 1.31 (m, 1H).

4da ^{1}H NMR (CD_3OD): δ 7.62 (s, 1H), 7.59 (d, 1H, $J = 7.2$ Hz), 7.31 (m, 2H), 3.09 (t, 2H, $J = 7.2$ Hz), 1.75 (m, 2H), 1.40 (m, 4H), 0.90 (t, 3H, $J = 7.2$ Hz).

4db ^{1}H NMR (DMSO-d_6): δ 7.70 (d, 1H, $J = 5.7$ Hz), 7.67 (s, 1H), 7.42 (t, 1H, $J = 5.7$ Hz), 7.40 (d, 1H, $J = 5.7$ Hz), 4.42 (s, 1H, NH), 3.93 (dd, 1H), 3.56 (m, 1H), 3.44 (m, 1H), 3.35 (dd, 1H, $J = 14.1$, 4.8 Hz), 3.17 (q, 1H, 13.5), 2.19 (s, 3H), 1.82 (m, 2H), 1.52 (m, 3H), 1.35 (m, 1H).

4ea ^{1}H NMR (CD_3OD): δ 8.11 (s, 1H), 8.10 (d, 1H), 7.80 (d, 1H, $J = 7.2$ Hz), 7.68 (t, 1H, $J = 7.2$ Hz), (m, 2H), 3.13 (t, 2H, $J = 7.5$ Hz), 1.75 (m, 2H), 1.40 (m, 4H), 0.91 (t, 3H, $J = 7.2$ Hz).

4eb ^{1}H NMR ($CDCl_3$): δ 8.15 (s, 1H), 7.95 (m, 2H), 7.89 (m, 2H), 7.60 (m, 3H), 7.38 (m, 2H), 4.61 (t, 2H, $J = 6.0$ Hz), 3.65 (t, 2H, $J = 6.0$ Hz).

4fa ^{1}H NMR (CD_3OD): δ 7.26 (m, 4H), 3.07 (t, 2H, $J = 7.5$ Hz), 2.27 (s, 3H), 1.75 (m, 2H), 1.40 (m, 4H), 0.91 (t, 3H, $J = 7.2$ Hz).

4ga ^{1}H NMR (CD_3OD): δ 7.21 (m, 8H), 4.04 (t, 2H, $J = 6.3$ Hz), 2.72 (t, 2H, $J = 6.3$ Hz), 1.92 (m, 2H).

4gb ^{1}H NMR (CD_3OD): δ 7.50 (m, 2H), 7.10 (m, 1H), 3.95 (dd, 1H, $J = 11.1$, 2.7 Hz), 3.56 (m, 1H), 3.45 (m, 1H), 3.35 (dd, 1H), 3.12 (q, 1H, $J = 13.5$ Hz), 1.82 (m, 2H), 1.52 (m, 3H), 1.35 (m, 1H).

4ha ^{1}H NMR (CD_3OD): δ 7.80 (d, 1H, $J = 7.5$ Hz), 7.69 (t, 1H, $J = 7.5$ Hz), 7.64 (t, 1H, $J = 7.5$ Hz), 7.46 (d, 1H, $J = 7.5$ Hz), 3.09 (t, 2H, $J = 7.2$ Hz), 1.70 (m, 2H), 1.36 (m, 4H), 0.90 (t, 3H, $J = 7.2$ Hz).

4ja ^{1}H NMR (CD_3OD): δ 7.76 (d, 2H, $J = 8.1$ Hz), 7.33 (d, 2H, $J = 8.1$ Hz), 3.11 (t, 2H, $J = 7.2$ Hz), 2.98 (m, 1H), 1.71 (m, 2H), 1.40 (m, 4H), 1.30 (d, 6H, $J = 6.9$ Hz), 0.91 (t, 3H, $J = 7.2$ Hz).

4jb ^{1}H NMR (CD_3OD): δ 7.77 (d, 2H, $J = 8.4$ Hz), 7.34 (d, 2H, $J = 8.4$ Hz), 7.18 (m, 5H), 3.14 (d, 1H, $J = 7.2$ Hz), 2.98 (m, 1H), 2.75 (t, 2H, $J = 7.2$ Hz), 2.03 (m, 2H).

4jc ^{1}H NMR (CD_3OD): δ 7.76 (d, 2H, $J = 8.4$ Hz), 7.34 (d, 2H, $J = 8.4$ Hz), 3.94 (dd, 1H, $J = 10.5$, 2.7 Hz), 3.67 (m, 1H), 3.44 (m, 1H), 3.33 (dd, 1H, $J = 13.8$, 4.5 Hz), 3.13 (q, 1H, $J = 7.5$ Hz), 2.99 (m, 1H), 1.83 (m, 2H), 1.58 (m, 3H), 1.35 (m, 1H), 1.30 (d, 6H, $J = 6.9$ Hz).

5b ^{1}H NMR (CD_3OD): δ 7.67 (s, 1H), 7.51 (s, 1H), 7.50 (s, 1H), 4.61 (t, 2H, $J = 7.8$ Hz), 3.68 (t, 2H, $J = 7.8$ Hz).

5e ^{1}H NMR ($CDCl_3$): δ 7.75 (s, 1H), 7.73 (t, 1H, $J = 4.8$ Hz), 7.40 (d, 2H, $J = 4.8$ Hz), 4.57 (t, 2H, $J = 7.8$ Hz), 3.65 (t, 2H, $J = 7.8$ Hz).

5f ^{1}H NMR (CD_3OD): δ 8.23 (s, 1H), 8.22 (d, 1H, $J = 6.6$ Hz), 7.89 (d, 1H, $J = 6.6$ Hz), 7.75 (t, 1H, $J = 6.6$ Hz), 4.59 (t, 2H, $J = 7.8$ Hz), 3.67 (t, 2H, $J = 7.8$ Hz).

5g ^{1}H NMR (CD_3OD): δ 7.25 (m, 4H), 4.60 (t, 2H, $J = 7.8$ Hz), 3.60 (t, 2H, $J = 7.8$ Hz), 2.30 (s, 3H).

5i ^{1}H NMR ($CDCl_3$): δ 7.96 (d, 2H, $J = 8.1$ Hz), 7.61 (d, 2H, $J = 8.1$ Hz), 4.60 (t, 2H, $J = 7.8$ Hz), 3.60 (t, 2H, $J = 7.8$ Hz).

6a ^{1}H NMR (CD_3OD): δ 7.67 (m, 1H), 7.18 (m, 2H), 4.15 (m, 2H), 3.30 (m, 2H), 2.34 (m, 2H).

6b ^{1}H NMR (CD_3OD): δ 7.65 (s, 1H), 7.48 (s, 1H), 7.47 (s, 1H), 4.17 (t, 2H, $J = 7.8$ Hz), 3.30 (t, 2H), 2.35 (m, 2H).

6e ^{1}H NMR ($CDCl_3$): δ 7.88 (s, 1H), 7.86 (t, 1H, $J = 8.1$ Hz), 7.69 (d, 2H, $J = 8.1$ Hz), 4.13 (t, 2H, $J = 7.8$ Hz), 3.30 (t, 2H), 2.33 (m, 2H).

6f ^{1}H NMR (CD_3OD): δ 8.213 (s, 1H), 8.22 (d, 1H, $J = 7.8$ Hz), 7.89 (d, 1H, $J = 7.8$ Hz), 7.73 (t, 1H, $J = 7.8$ Hz), 4.16 (t, 2H), 3.30 (t, 2H), 2.34 (m, 2H).

6g ^{1}H NMR ($CDCl_3$): δ 7.25 (m, 4H), 4.60 (d, 2H, $J = 7.8$ Hz), 3.30 (m, 2H), 2.31 (m, 2H), 2.30 (s, 3H).

6i ^{1}H NMR (CD_3OD): δ 7.65 (s, 1H), 7.49 (m, 2H, $J = 6.6$ Hz), 4.18 (m, 4H), 2.35 (d, 2H).

7a ^{1}H NMR (DMSO-d_6): δ 8.31 (t, 1H, NH), 7.74 (m, 6H), 7.63 (t, 1H, $J = 7.8$ Hz), 7.51 (d, 1H, $J = 7.8$ Hz), 7.15 (t, 1H, $J = 7.8$ Hz), 6.74 (d, 1H, $J = 8.1$ Hz), 6.72 (s, 1H), 6.70 (d, 1H, $J = 8.1$ Hz), 4.62 (s, 2H), 3.64 (s, 3H), 3.63 (m, 2H), 2.79 (t, 2H, $J = 7.5$ Hz).

7b ^{1}H NMR ($CDCl_3$): δ 7.81 (d, 2H, $J = 8.4$ Hz), 7.69 (d, 1H, $J = 7.5$ Hz), 7.67 (d, 1H, $J = 7.5$ Hz), 7.61 (d, 2H, $J = 8.4$ Hz), 7.48 (t, 1H, $J = 7.5$ Hz), 7.37 (t, 1H, $J = 7.2$ Hz), 5.75 (t, 1H, NH), 4.65 (s, 2H), 4.58 (m, 1H), 2.35 (m, 2H), 1.95 (m, 2H), 1.78 (m, 2H).

7c ^{1}H NMR ($CDCl_3$): δ 7.81 (d, 2H, $J = 8.4$ Hz), 7.67 (m, 4H), 7.49 (t, 1H, $J = 7.2$ Hz), 7.36 (t, 1H, $J = 7.2$ Hz), 7.28 (m, 3H), 7.16 (d, 2H, $J = 7.8$ Hz), 5.72 (t, 1H, NH), 4.67 (s, 2H), 3.75 (q, 2H), 2.87 (t, 2H, $J = 8.2$ Hz).

7d ^{1}H NMR ($CDCl_3$): δ 7.84 (d, 2H, $J = 8.7$ Hz), 7.69 (d, 1H, $J = 7.2$ Hz), 7.66 (d, 1H, $J = 7.2$ Hz), 7.62 (d, 2H, $J = 8.7$ Hz), 7.48 (t, 1H, $J = 7.2$ Hz), 7.37 (t, 1H, $J = 7.2$ Hz), 5.64 (t, 1H, NH), 4.65 (s, 2H), 3.46 (t, 2H, $J = 6.3$ Hz), 1.34 (m, 1H), 1.27 (m, 8H), 0.88 (t, 6H, $J = 6.0$ Hz).

7e ^{1}H NMR ($CDCl_3$): δ 7.73 (d, 2H, $J = 8.2$ Hz), 7.67 (d, 1H, $J = 8.2$ Hz), 7.64 (d, 1H, $J = 8.15$ Hz), 7.60 (d, 2H, $J = 8.2$ Hz), 7.49 (t, 1H, $J = 8.2$ Hz), 7.39 (t, 1H, $J = 8.2$ Hz), 4.61 (s, 2H), 4.58 (m, 1H), 3.18 (s, 3H), 2.95 (m, 2H), 2.31 (s, 3H), 2.00 (m, 3H), 1.80 (m, 3H), 1.10 (m, 1H).

7f ^{1}H NMR ($CDCl_3$): δ 7.82 (d, 2H, $J = 8.4$ Hz), 7.68 (t, 2H, $J = 9.0$ Hz), 7.65 (d, 2H, $J = 8.4$ Hz), 7.49 (t, 1H, $J = 9.0$ Hz), 7.37 (t, 1H, $J = 9.0$ Hz), 6.82 (d, 1H, $J = 8.1$ Hz), 6.71 (d, 1H, $J = 8.1$ Hz), 6.70 (s, 1H), 5.73 (t, 1H, NH), 4.67 (s, 2H), 3.86 (s, 3H), 3.84 (s, 3H), 3.77 (m, 2H), 2.83 (t, 2H, $J = 6.9$ Hz).

7g ^{1}H NMR ($CDCl_3$): δ 8.21 (m, 1H), 7.74 (d, 2H, $J = 8.7$ Hz), 7.68 (m, 1H), 7.65 (d, 2H, $J = 8.7$ Hz), 7.55 (d, 1H), $J = 8.4$ Hz), 7.53 (t, 1H, $J = 8.4$ Hz), 7.37 (t, 1H, $J = 8.4$ Hz), 6.69 (m, 1H), 6.63 (d, 1H, $J = 8.4$ Hz), 4.64 (s, 2H), 4.06 (t, 4H, $J = 4.5$ Hz), 3.68 (t, 4H, $J = 4.5$ Hz).

7h ^{1}H NMR ($CDCl_3$): δ 9.5 (s, 1H, NH), 7.81 (d, 2H, $J = 8.7$ Hz), 7.67 (d, 1H, $J = 7.4$ Hz), 7.65 (d, 1H, $J = 7.4$ Hz), 7.58 (d, 2H, $J = 8.7$ Hz), 7.48 (t, 1H, $J = 7.4$ Hz), 7.46 (t, 1H, $J = 7.4$ Hz); 4.65 (s, 2H), 3.80 (m, 1H), 3.50 (td, 1H), 3.20 (m, 1H), 2.80 (m, 1H), 2.60 (m, 1H), 2.38 (s, 3H), 2.02 (dt, 2H), 1.99 (m, 2H), 1.82 (m, 2H).

7i ^{1}H NMR ($CDCl_3$): δ 7.85 (d, 2H, $J = 8.7$ Hz), 7.70 (d, 1H, $J = 7.5$ Hz), 7.69 (d, 1H, $J = 7.5$ Hz), 7.65 (d, 2H, $J = 8.7$ Hz), 7.50 (t, 1H, $J = 7.5$ Hz), 7.38 (t, 1H, $J = 7.5$ Hz), 5.99 (t, 1H, NH), 4.65 (s, 2H), 4.20 (m, 2H).

7j ^{1}H NMR ($CDCl_3$): δ 7.84 (d, 2H, $J = 8.4$ Hz), 7.63 (d, 1H, $J = 7.5$ Hz), 7.61 (d, 2H, $J = 7.5$ Hz), 7.60 (t, 2H, $J = 8.4$ Hz), 7.50 (t, 1H, $J = 7.5$ Hz); 7.39 (t, 1H, $J = 7.5$ Hz), 6.74 (d, 1H, $J = 8.1$ Hz), 6.62 (d, 1H, $J = 8.1$ Hz), 6.61 (s, 1H), 5.94 (s, 2H), 5.72 (t, 1H, NH), 4.66 (s, 2H), 3.70 (m, 2H), 2.78 (t, 2H, $J = 6.6$ Hz).

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