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Synthesis and Charecterization of Daclatasvir acid analogues

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ABSTRACT

Daclatasvir is hepatitis-c virus NS5A inhibitor. It stops HCV viral replication and protein translation by directly inhibiting HCV protein NS5A. It was discovered by scientists of Bristol- Myers Squibb. In the present study, we efficiently synthesized a series of Three novel acid substituted Daclatasvir analogues (7a-7c) as potential prodrug.

INTRODUCTION:

Daclatasvir is hepatitis-c virus NS5A inhibitor. It stops HCV viral replication and protein translation by directly inhibiting HCV protein NS5A. It was discovered by scientists of Bristol- Myers Squibb. Molecular docking had already been used successfully in the search for active substances against the corona viruses SARS-CoV-2, The results are also supported by the fact that both SARS-CoV-2 and the hepatitis C virus are a virus of the same type, also-called single-stranded RNA virus. Several drugs approved for the treatment of hepatitis C viral infection have been identified as potential candidates against COVID-19 caused by the SARS-CoV-2 corona virus, according to a study based on extensive calculations using supercomputer simulations. Researchers from Johannes Gutenberg University Mainz (JGU) in Germany. Using the powerful MOGON II supercomputer, the researchers made more than 30 billion single calculations within two months. They found that compounds from the four hepatitis C drugs simeprevir, paritaprevir, grazoprevir, and velpatasvir have a high affinity to bind SARS-CoV-2 very strongly and may therefore be able to prevent infection. Direct-acting antivirals (DAAs) changed the entire landscape of hepatitis C (HCV) treatment. There has been considerable interest with DAAs such as sofosbuvir, daclatasvir, and velpatasvir as new kids in COVID-19 therapeutics. DAAs interfere with replication by targeting specific nonstructural proteins of the HCV.

Several in vitro and clinical trials started in China during the last month with the first approved drug, Favilavir, by the National Medical Products Administration of China is announced in (18 February 2020) in Zhejiang province. Different directly acting antiviral drugs are approved against other viruses, by the Food and Drugs Administration (FDA), such as Sofosbuvir, Ribavirin against RdRp of Hepatitis C Virus (HCV). These drugs are nucleotides derivative competing with physiological nucleotide for RdRp active site. Additionally, a huge number of attempts to develop anti-RdRp compounds are under clinical testing against different viruses. The half-maximal Effective Concentration (EC50) for Ribavirin against COVID-19 is 109.5 μM, while its half-maximum Inhibition Concentration (IC50) against Dengue virus is 8 μM. Sofosbuvir show 4 μM against the Zika virus. Remdesivir shows EC90 of 1.76 μM against COVID-19 in vitro. We focus here in the present study on nucleotide inhibitors due to its strong evidence of inhibiting emerging viral RdRps. We build the COVID-19 RdRp model using homology modeling after sequence comparison to the available structures in the protein data bank. Molecular docking is then performed to test some direct-acting antiviral (DAA) drugs against COVID-19 RdRp (Sofosbuvir, Ribavirin, Remdisvir, IDX-184). Additionally, the native nucleotides GTP and UTP, from which IDX-184 and Sofosbuvir are derived, are also tested against COVID-19 RdRp model. The results are promising and suggest possible inhibition for the currently available therapeutics against the newly emerged coronavirus.

The world health organisation (WHO) 2014 guidelines for the screening, care and treatment persons with hepatitis C infection state that worldwide more than 185 million people are infected with hepatitis C virus (HCV). An estimated one third of these who

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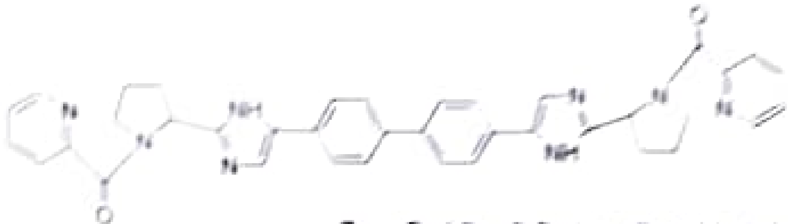
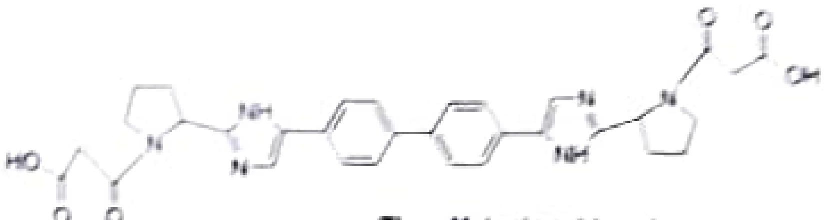
become chronically infected (or) hepatocellular carcinoma. Nearly 350000 to 500000 die each year (HCV affected people). HCV is member of the flaviviridae, with seven major genotypes and major number of sub types. Hepatitis C virus is a serious global medical problem. Action of HCV on proteins can be divided into two groups those are structural proteins (C, E1 And E2) and non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B).

Daclatasvir belongs to a class of new directly acting antivirals that inhibit non-structural proteins NS5A. It has been tested in combination regiments with pegylated interferon and ribavirin as well as with other direct-acting antiviral agents including asunaprevir and sofosbuvir. Daclatasvir stops HCV viral RNA replication and protein translation by directly inhibiting HCV protein NS5A. Daclatasvir reaches steady state in human subjects after about 4 days of once-daily 60 mg oral administration.

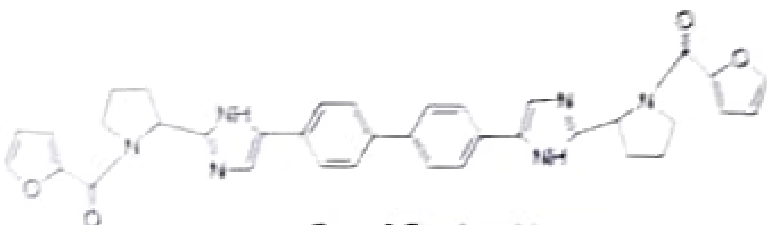
Bristol-Meyers Squibb discovered the drug, and got approval from the United States Food and Drug Administration (FDA) in 2014 for the treatment of genotype 1b chronic HCV. The European medicines agency approved in august 2014 in combination with other drugs for use across genotype for the treatment of chronic hepatitis C virus in adults. In the present study synthesize novel and Daclatasvir analogues.



7a Pyridine 2-Carboxylic acid analogue



7b Malonic acid analogue



7c 2-Furoic acid analogue

RESULTS AND DISCUSSION

With an objective of developing a viable and efficient method would give high yields and easy handling procedure under aerobic conditions. The target symmetrical structure compounds were synthesized from biphenyl (1) on Friedel-Craft acylation with two equivalent chloro acetyl chloride in presence of anhydrous $AlCl_3$ in CH_2Cl_2 converted to di substituted chloro acetyl biphenyl (2). This was coupled with N-BOC proline in presence of ACN and K2CO3 to form (3). The compound 3 treated with ammonium acetate ($CH_3COO^-NH_4^+$) in toluene refluxing for 3hours to form (4). After the deprotection of N-BOC group with Conc. HCl in IPA to give (5). The resulting amine coupled with Carboxylic acids (6a-6c) gave (7a-7c) Carboxylic acid analogues. All the target compounds were new chemical entities.

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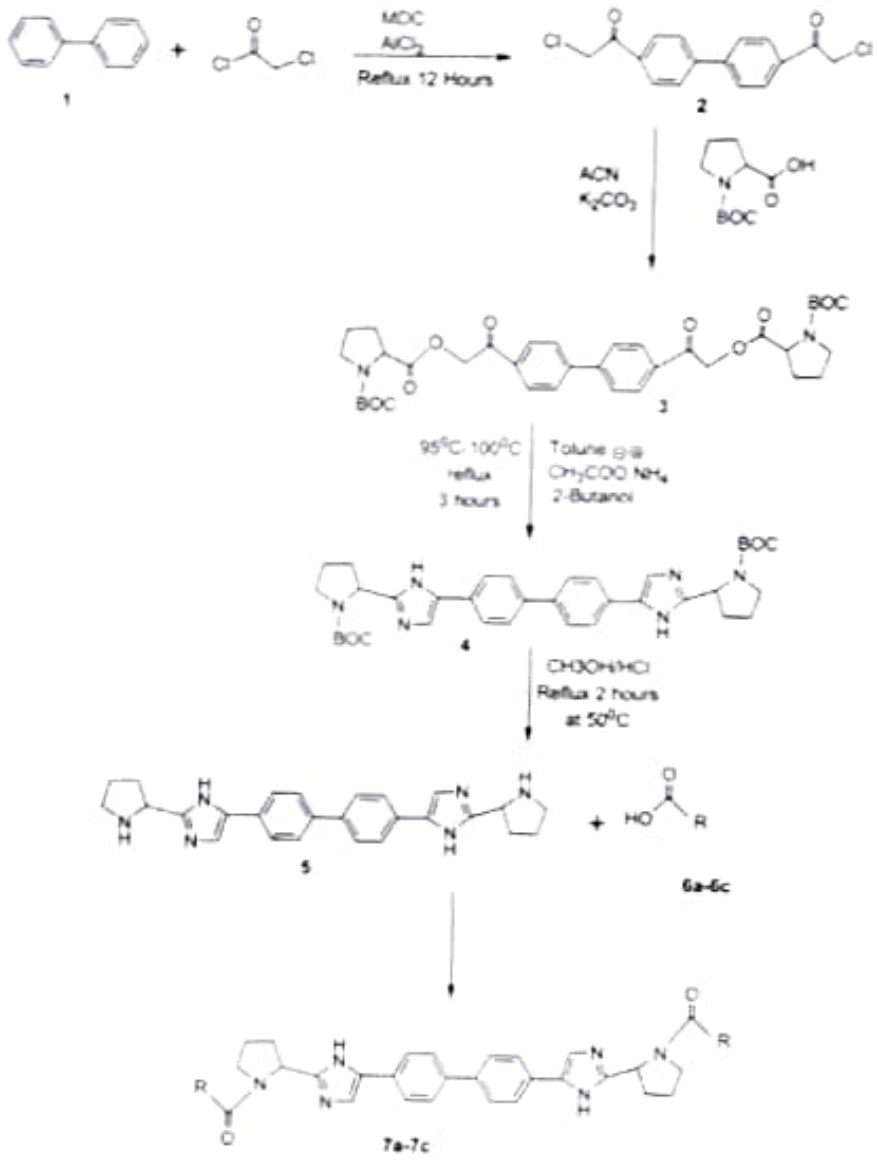
Scheme:



EXPERIMENTAL SECTION

General: All starting materials and other reagents were purchased from commercial suppliers and were used without further purification. Nuclear Magnetic Resonance (NMR) spectra were recorded on bruker instrument operating at 500 MHz and 1H NMR spectra are obtained with TMS as internal standard in DMSO solvent. 13C NMR spectra are also obtained in DMSO instrument operating at 125 MHz IR and Mass spectra were recorded. The reactions were assayed by thin-layer chromatography (TLC) and terminated as judged by the consumption of starting material. When peak multiplicities are reported.

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Step-I:

Anhydrous AlCl3 (**1.33 g**, 10 mmol) and chloro acetyl chloride (1.12g, 10 mmol) suspended in 5 ml of CH2Cl2. And then added the biphenyl (771 **mg**, 5 **mmol**). The contents refluxed for 12 hours. The reaction was monitored by TLC. The reaction mixture was extracted with 6 ml of water 10 ml of acetone added and separate the layers. The organic layer was dried results the solid. The crude was analyzed by NMR, Mass and FTIR.

1H NMR (500 MHz, in DMSO)δ: 7.92 (d, 4H), 7.59 (d, 4H), 4.64(s, 4H)

13 C NMR (500MHz, in DMSO)δ: 129.1, 127.3, 136.3, 141.0, 188.8, 53.8

IR (neat) λ max: 1718 cm^{-1}

MS: m/z = 306.02 [M + H]$^+$, Molecular Weight: 307.17

Step-II:

N-BOC proline (1.07 g, 5 mmol) and K2CO3 (691 mg, 5 mmol) were added to a suspension of 1,1'-(Biphenyl-4,4'-diyl) bis (2-Chloroethanone) **2** (768 mg, 2.5 mmol) in acetonitrile (10 ml). The solution was stirred for 30 min and reflux 18 hours at room temperature. The reaction was monitored by TLC. 25 ml of toluene was added and separate the layers. Organic layer concentrated under reduced pressure. The crude solid was dried and analysed by NMR, Mass and FTIR spectra.

1H NMR (500 MHz, in DMSO) δ: 7.92 (d, H), 7.59 (d, 4H), 5.34 (s, 4H), 4.1 (t, 2H), 3.35 (t, 4H), 1.94 (m, 4H), 1.59 (m, 4H), 1.40 (s, 18H). **13 C NMR (500MHz, in DMSO) δ:** 129.1, 127.3, 136.3, 141.0, 196.5, 73.5, 172.0, 59.7, 22.7, 44.1, 20.1, 159.3, 70.9, 28.7 **IR (neat) λ max: MS:** m/z = 664.30 [M + H]$^+$, Molecular Weight: 664.74

Step-III:

(2S)-2, 2-2, 2'-(Biphenyl-4,4'-diyl)bis(2-oxoethane-2,1-diyl)1-tert-butyl dipyrrolidine-1,2-dicarboxylate **3** (1.32 g, 2 mmol) dissolved in 9 ml of toluene and added ammonium acetate (3.36 g, 43.6 mmol). The contents were stirred and refluxing for 3 hours at 95-100°C. The reaction was monitored by TLC. The combined EtOAc extracts were concentrated under reduced pressure. Separate the solid and dried. The crude was analysed by NMR, Mass and FTIR spectra.

1H NMR (500 MHz, in DMSO) δ: 7.54 (d, 8H), 7.09s, 2H), 13.4 (bs, 2H), 4.63 (t, 2H), 3.35 (t, 4H), 1.88 (m, 4H), 1.59 (m, 4H), 1.40 (s, 18H). **13 C NMR (500MHz, in DMSO) δ:** 127.5, 127.9, 136.6, 135.4, 122, 149, 56.7, 29.4, 20.3, 44.3, 159.3, 70.9, and 28.7. **IR (neat) λ max: MS:** m/z = 624.34 [M + H]$^+$, Molecular Weight: 624.77

Step-IV:

(2S,2'S)-tert-Butyl 2,2'-(5,5'-(biphenyl-4,4'-diyl)bis(1H-imidazole-5,2-diyl))dipyrrolidine-1-carboxylate **4** (1 g, 1.6 mmol) suspended in 2.6 ml of iso propyl alcohol and added 1.68 ml of Conc HCl. The solution refluxed for 3 hours at 50°C. The reaction was monitored by TLC. And add 10 ml of iso propyl alcohol at 50°C cooled to 0-5°C. Filter the solid and dried. The crude was analysed by NMR, Mass and FTIR spectra.

1H NMR (500 MHz, in DMSO) δ: 7.54 (d, 8H), 7.0 (s, 2H), 13.4 (s, 2H), 4.03 (t, 2H), 1.88 (m, 4H), 1.59 (m, 4H), 2.75 (m, 4H), 2.0 (bs, 2H). **13 C NMR (500MHz, in DMSO) δ:** 127.5, 127.9, 136.6, 135.4, 122, 149, 59.4, 32.2, 23.1, 44.5, **IR (neat) λ max: MS:** m/z = 424.24 [M + H]$^+$, Molecular Weight: 424.54

Step-V:

1-hydroxy benzotrizole hydrate (382 mg, 2.5 mmol) in 10 ml of MDC. Added different Caboxylic acid (2 mmol) and EDC HCl (460 mg, 2.4 mmol) stirred the contents for 1 **hour** at room temperature and cooled to 0-5°C. Add Di amine compound **5** (424 mg, 1 mmol) and Di iso propyl ethyl amine (516 mg, 4 mmol) mixture stands at 0-5°C for 30 min. Allowed to raise the temp to room temp , stirred for 12 hours at room temp. The reaction was monitored by TLC. Add ten times water and separate the organic layer washed with aqueous NaOH and water. Concentrated the organic layer results the solid.

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CORRESPONDING AUTHOR ASSOCIATED CONTENT

NMR, IR and Mass spectral data for compounds and supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.06.006.

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The authors declare no competing financial interest.

Author Contribution:

These authors contributed equally to the work described in this paper.

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