Bioorganic & Medicinal Chemistry Letters 23 (2013) 6467–6473

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification of a series of 1,3,4-trisubstituted pyrazoles as novel hepatitis C virus entry inhibitors

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ARTICLE INFO

Article history:
Received 10 June 2013
Revised 20 August 2013
Accepted 12 September 2013
Available online 21 September 2013

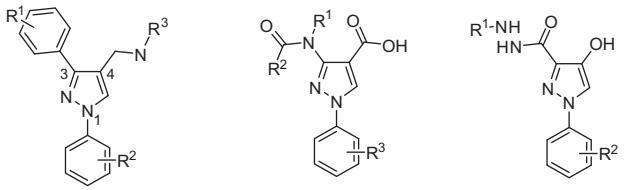
Keywords:
Hepatitis C virus
Phenotypic high-throughput screening
Infectious cell culture system
Pyrazole
Entry inhibitor

ABSTRACT

In this report we describe the identification of novel pyrazole analogs as potent hepatitis C virus (HCV) entry inhibitor. The pyrazoles were identified by our phenotypic high-throughput screening using infectious HCV. A series of pyrazole derivatives was synthesized and evaluated for inhibitory activity against HCV in the infectious cell culture system. Through evaluation of selected compounds we observed that the pyrazoles did not interfere with HCV RNA replication but with viral entry as shown by experiments with HCV replicons and HCV pseudo particles, respectively.

Hepatitis C virus (HCV) is a global health concern, affecting more than 3% of the world's population by causing chronic liver disease.[1] Viral genome replication is prone to high error rates leading to a large diversity of HCV genotypes and subtypes[2] with differences in susceptibility to current treatment and outcome of disease. To date, the standard of care (SOC), a combination of pegylated interferon-alpha (PEG-IFNα) and ribavirin (RBV), results frequently in unsatisfactory sustained virologic response rates (SVR) of only 45–70% for patients infected with HCV genotype (gt) 1 and about 70–80% for those infected with gt 2 or 3.[3,4] Unfortunately, this treatment is associated with side effects responsible for low adherence to therapy.[5–8] More recently, two direct-acting antiviral agents (DAAs) were introduced to the clinics increasing the SVR rates in HCV gt1 infected patients, but are unfortunately accompanied by severe side effects.[9] This emerging clinical data encouraged us to develop a high-throughput screening (HTS) assay to identify novel antiviral targets. By devising screening strategies using infectious HCV expressing a fluorescent marker protein, we screened phenotypically the entire viral life cycle.[10,11] Identified inhibitors were triaged in HCV replicon and HCV pseudo particle (HCVpp) systems to monitor viral RNA replication and viral entry, respectively. The antiviral activity of hit compounds was determined by 10-point dose response curve (DRC) analysis. Briefly, naïve Huh-7 target cells which were plated in 384-well microplates, incubated with serially diluted compounds, and inoculated with cell culture derived HCV (HCVcc) at a multiplicity of infection (MOI) 1. At 72 h post-infection live cells were analyzed and the half maximal effective concentration (EC_{50}) to inhibit viral replication was determined. In parallel, to rule out that the observed antiviral effects were due to toxicity induced by compounds, the cytotoxic concentration (CC_{50}) was calculated by automatically counting cells of each individual well treated with antivirals.

In our phenotypic target-free HTS campaign for the discovery of novel anti-HCV chemical entities we identified among others



1 2 3

Figure 1. Structure of 1,3,4-trisubstituted pyrazoles exhibiting anti-HCV activity (**1**) and HCV RNA replication inhibitor (**2** and **3**).

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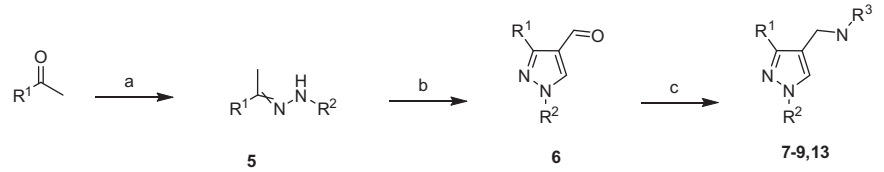
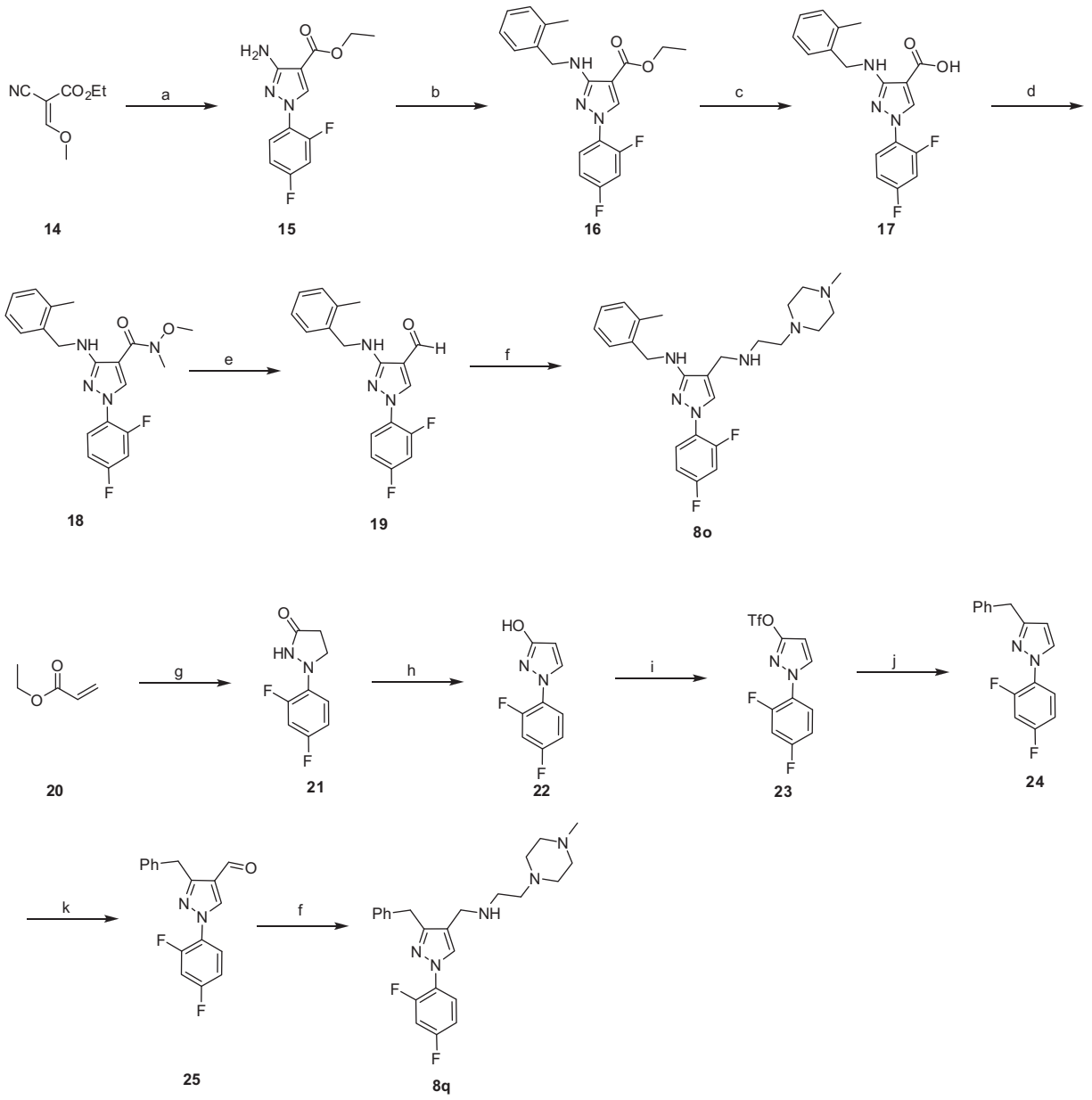
http://dx.doi.org/10.1016/j.bmcl.2013.09.039

active compounds containing a 1,3,4-trisubstituted pyrazole core (Fig. 1 and **1**). Pyrazoles **1** were counter-screened in the HCV replicon system to filter out viral replication inhibitor. The pyrazole core is well-known structure with broad biological spectrum in drug discovery. Two pyrazole structures (**2**[12,13] and **3**[14]) have been previously reported to inhibit HCV RNA replication. However, pyrazoles **1** do not interfere with HCV RNA replication, indicating a novel mechanism of action (MoA), which led us to pursue extensive medicinal chemistry work to improve anti-HCV potency and also to characterize the MoA of pyrazoles. Here, we describe the identification of a series of pyrazole derivatives by conducting structure-activity relationship (SAR) studies with the pyrazole core



Scheme 1. Reagents and conditions: (a) R^2NHNH_2, NaOAc, EtOH, microwave, 130 °C, 1 h; (b) $POCl_3$, DMF, 80 °C, 3 h; (c) R^3NH, $NaBH(OAc)_3$, DCM, rt.



Scheme 2. Reagents and conditions: (a) 2,4-difluorophenyl hydrazine, NaOAc, AcOH, H_2O, microwave, 130 °C, 30 min, 86%; (b) 2-MeBnBr, NaH, DMF, rt, 4 h, 69%; (c) LiOH, H_2O/THF (1:3, v/v), rt, overnight, 77%; (d) *N*,*O*-dimethylhydroxyamine hydrochloride, EDC·HCl, HOBt, DMF, rt, overnight, 93%; (e) LAH, THF, −40 to 0 °C, 5 h, 86%; (f) 2-(4-methylpiperazin-1-yl)ethanamine, $NaBH(OAc)_3$, DCM, rt; (g) 2,4-difluorophenyl hydrazine, NaOAc, AcOH, H_2O, microwave, 130 °C, 30 min, 58%; (h) $FeCl_3$, air, DMF, 80 °C, 6 h, 96%; (i) Tf_2O, TEA, DCM, rt, 98%; (j) BnZnBr, $Pd(OAc)_2$, X-Phos, THF, 50 °C, overnight, 94%; (k) $POCl_3$, DMF, 80 °C, 3 h, 70%.