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Short communication

Discovery of the 2-phenyl-4,5,6,7-Tetrahydro-1*H*-indole as a novel anti-hepatitis C virus targeting scaffold

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A B S T R A C T

Although all-oral direct-acting antiviral (DAA) therapy for hepatitis C virus (HCV) treatment is now a reality, today's HCV drugs are expensive, and more affordable drugs are still urgently needed. In this work, we report the identification of the 2-phenyl-4,5,6,7-Tetrahydro-1*H*-indole chemical scaffold that inhibits cellular replication of HCV genotype 1b and 2a subgenomic replicons. The anti-HCV genotype 1b and 2a profiling and effects on cell viability of a selected representative set of derivatives as well as their chemical synthesis are described herein. The most potent compound **39** displayed EC_{50} values of 7.9 and 2.6 μM in genotype 1b and 2a, respectively. Biochemical assays showed that derivative **39** had no effect on HCV NS5B polymerase, NS3 helicase, IRES mediated translation and selected host factors. Thus, future work will involve both the chemical optimization and target identification of 2-phenyl-4,5,6,7-Tetrahydro-1*H*-indoles as new anti-HCV agents.

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1. Introduction

Hepatitis C virus (HCV) infection represents a global health problem that has an associated high risk for serious liver diseases. On the basis of annual World Health Organization (WHO) reports, more than 130-150 million people are infected and more than 350,000–500,000 individuals die from HCV-related liver pathologies each year [1]. To date, at least eleven HCV genotypes (gt) have been identified. These genotypes can be divided into multiple subtypes. The global distribution of HCV genotypes varies depending on the particular geographical area. HCV gt 1 is the most common in North and South America, Europe and Australia [2]. HCV gt 2 is widespread in America and Europe, while gt 3 is common in Central Asia and Middle East. Finally, HCV gt 4 and gt 5 are found almost exclusively in Africa, and HCV gt 6 is endemic in East and Southeast Asia [2]. Gt 1 and gt 4 are the hardest to treat and are associated with a particularly aggressive form of the disease.

HCV was discovered in 1989, and until recently all treatments included some combination of pegylated interferon-α (pegIFN-α) and ribavirin (RBV), both of which cause debilitating side effects often worse than HCV symptoms. PEG-IFN/RBV treatment alone has been moderately successful and is genotype-dependent as only 40–50% of gt 1 and gt 4 patients have achieved a sustained virological response (SVR) indicative of a cure [3]. This treatment regimen remained the standard-of-care (SOC) until 2011 for gt 1, and until 2014 for the other genotypes. Over the past 20 years, a combination of developments of new models and tools have been able to reveal the different steps of the HCV life cycle and tremendous drug discovery efforts have allowed the development of direct-acting antivirals (DAAs) that specifically target HCV proteins. Since 2011, the new SOC for patients infected with gt 1 is based on a combination of pegIFN-α and RBV with the first-

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generation HCV protease inhibitors telaprevir or boceprevir (Fig. 1). Although the cure rates have improved (SVR = 60–80%), the new SOC provides only limited clinical benefit against HCV gt 2–6 and has resulted in some serious side effects in clinical trials [4,5]. Consequently, two new HCV DAAs, simeprevir and sofosbuvir (Fig. 1), have been approved in December 2013 in the United States and in the first half of 2014 in Europe [6–8]. Simeprevir is a second-generation protease inhibitor that is endowed with a broader genotypic coverage (gt 1, 2 and 4). Its combination with pegIFN-α and RBV has shown improved SVR and a better tolerance profile [6]. Sofosbuvir, the first nucleotide inhibitor of NS5B polymerase approved by FDA, has paved the way for all-oral IFN-free therapies, two of which were approved in 2014: Viekira Pak (ombitasvir, paritaprevir, ritonavir and dasabuvir), and Harvoni (ledipasvir and sofosbuvir) (Fig. 1) [9–11]. Viekira Pak and Harvoni are both approved only for adult HCV patients with gt1 infection; they have displayed >90% SVR and are also effective against other genotype in clinical trials.

There are currently many similar HCV DAAs in development, and most target the NS3 protease, NS5B polymerase and NS5A protein. They are undergoing late stages of clinical development and are close to approval. An up-to-date status of the clinical trials along with comprehensive overviews of the continued and discontinued HCV-specific DAAs have been recently described [12].

The main drawback is that the newly approved drugs and/or regimens are very expensive, thus restricting access for most HCV-infected patients to the new anti-HCV therapies. Another serious medical issue is the high mutation rate of HCV coupled with the rapid emergence of drug resistance to the DAAs [13–15]. These observations serve to encourage continuing research in the field of HCV drug discovery that will lead to the identification of new antiviral agents effective against HCV.

Thus, it is within this context that we herein report the discovery of a new chemical class of anti-HCV compounds that have a 2-phenyl-4,5,6,7-Tetrahydro-1*H*-indole core.

2. Results and discussion

2.1. Cell-based screening of EDASA compounds: hit identification

Compounds **1**–**33** (Fig. 2), representative chemotypes of the EDASA Scientific public compound library (http://www.edasascientific.com/page/catalogue), have been screened for their possible anti-HCV activity using HCV replicons based on the two most widely studied HCV genotypes (gt 1b and gt 2a) (Table 1). All compounds, except **24**, are racemates.

Gt 1b was studied for many years because it is one of the most resistant to pegIFN-α/RBV therapy, and the gt 1b (con1) strain was used in the first subgenomic HCV replicons [16]. Gt 2a exhibits a greater sensitivity than gt 1 to pegIFN-α/RBV treatment, but it was the first to be replicated in a robust cell culture model [17]. Taking this into consideration for our discovery campaign, we decided to screen the compounds against both the HCV genotypes.

The compounds were evaluated against Huh7/Rep-Feo1b and Huh7.5-FGR-JC1-Rluc2A cells, which carry the autonomously replicating HCV RNA of gt 1b and 2a in the firefly and Renilla luciferase reporters, respectively [18]. During initial screening, the 33 EDASA Scientific compounds were assayed at 50 μM against both the HCV replicons in reporter assays. The compounds that inhibited HCV replication by > 50% in the primary assays were then further evaluated in concentration-response assays. The ability of each compound to inhibit activity in gt 1b and 2a replicons, and

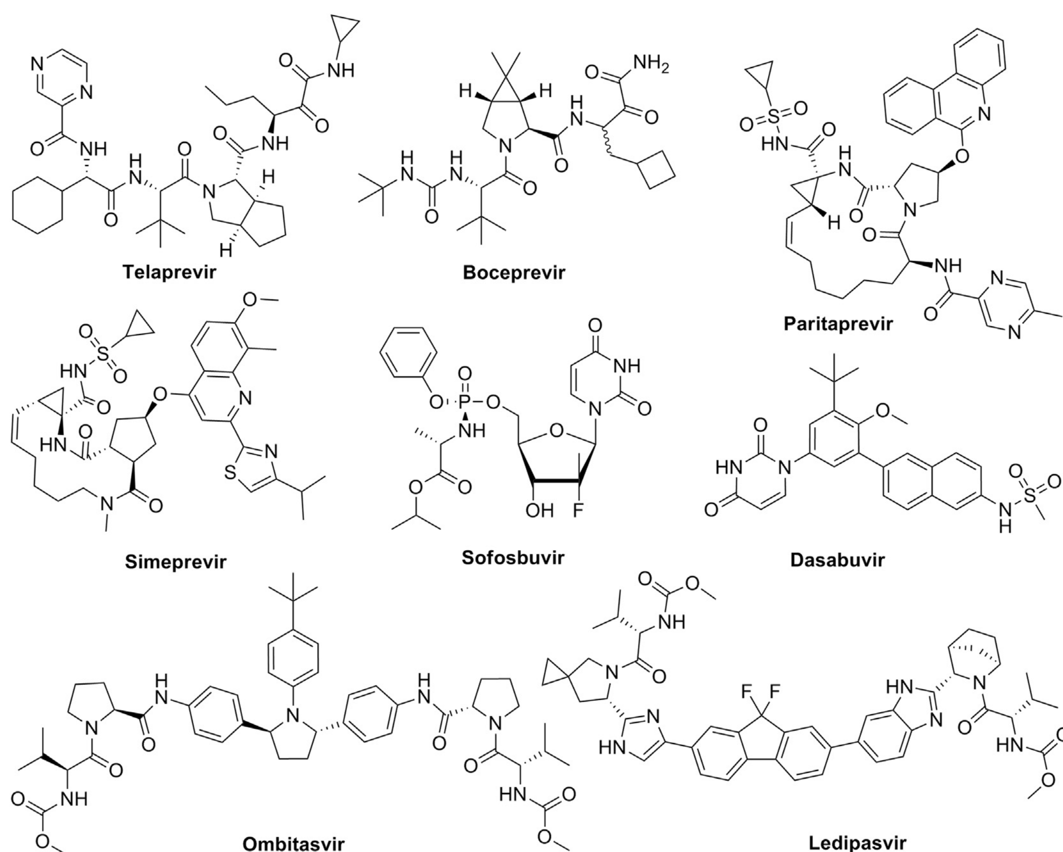


Fig. 1. DAAs – FDA approved drugs for the treatment of Hepatitis C.

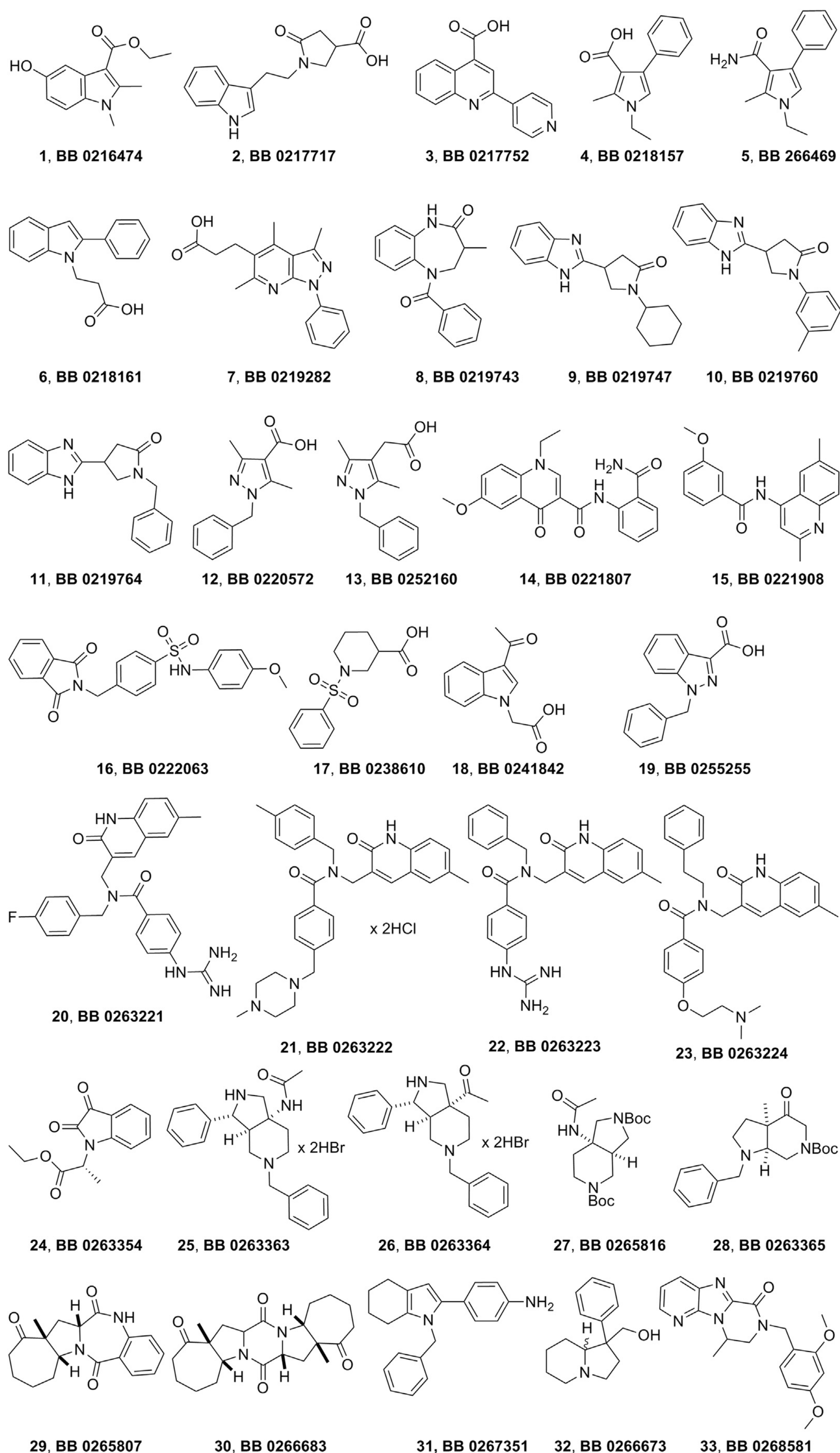


Fig. 2. Chemical structures and internal EDASA Scientific codes of the first set of compounds that underwent biological evaluation.

Table 1
Anti-HCV activities and cytotoxicity of the first 33 EDASA Scientific compounds evaluated on gt 1b and 2a.

Cpd	CC_{50}[a] (μM)	Huh7/Rep-Feo1b			Huh7.5-FGR-JC1-Rluc2A		
		Inhibition,[b] %	EC_{50}[c] (μM)	SI[d]	Inhibition,[b] %	EC_{50}[c] (μM)	SI[d]
1	>200	19 ± 6	ND	ND	80 ± 5	11.1 ± 0.9	>18
2	>200	22 ± 9	ND	ND	51 ± 8	48.5 ± 3.9	>4
3	>200	NI	ND	ND	49 ± 6	ND	ND
4	>200	NI	ND	ND	36 ± 10	ND	ND
5	>200	49 ± 12	ND	ND	66 ± 7	23.6 ± 4.0	>8
6	ND	26 ± 1	ND	ND	54 ± 4	ND	ND
7	>200	NI	ND	ND	78 ± 3	11.7 ± 0.9	>17
8	>200	21 ± 2	ND	ND	77 ± 7	14.1 ± 1.9	>14
9	>200	19 ± 10	ND	ND	72 ± 3	15.6 ± 3.7	>13
10	>200	21 ± 2	ND	ND	77 ± 7	17.3 ± 3.2	>12
11	>200	NI	ND	ND	67 ± 5	21.1 ± 4.4	>9
12	>200	19 ± 8	ND	ND	NI	ND	ND
13	>200	NI	ND	ND	14 ± 8	ND	ND
14	85.6 ± 5.9	17 ± 3	ND	ND	65 ± 5	20.6 ± 2.9	4
15	<25	88 ± 2	ND	ND	99 ± 1	ND	ND
16	>200	39 ± 4	ND	ND	60 ± 6	22.5 ± 3.8	>9
17	>200	NI	ND	ND	62 ± 1	28.1 ± 4.8	>7
18	>200	NI	ND	ND	55 ± 9	ND	ND
19	>200	NI	ND	ND	46 ± 8	ND	ND
20	>200	54 ± 8	ND	ND	29 ± 8	ND	ND
21	<25	92 ± 1	ND	ND	99 ± 1	ND	ND
22	>200	NI	ND	ND	44 ± 9	ND	ND
23	>200	45 ± 6	ND	ND	61 ± 9	24.8 ± 4.5	>8
24	>200	19 ± 9	ND	ND	85 ± 9	7.3 ± 0.5	>27
25	>200	NI	ND	ND	73 ± 3	4.9 ± 0.4	>41
26	>200	NI	ND	ND	76 ± 7	17.4 ± 0.8	>11
27	>200	NI	ND	ND	43 ± 10	ND	ND
28	114.7 ± 14.6	73 ± 9	24.3 ± 1.2	5	98 ± 2	6.0 ± 1.0	>19
29	>200	NI	ND	ND	NI	ND	ND
30	>200	NI	ND	ND	28 ± 4	ND	ND
31	109.9 ± 2.9	66 ± 9	12.4 ± 1.0	9	88 ± 8	8.7 ± 1.9	13
32	>200	50 ± 4	ND	ND	25 ± 5	ND	ND
33	>200	45 ± 2	ND	ND	48 ± 11	ND	ND

[a] CC_{50} values were determined in Huh7.5 parental cells by the MTS assay. CC_{50} = is the concentration required to reduce the bioreduction of MTS (3-(4,5- dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) into formazan by 50%. The reported value represents the means ± SD of data derived from three independent experiments.
[b] Anti-HCV activity of the compounds were carried out at 50 μM in preliminary screening.
[c] The inhibition data from 8 to 12 quarter log dilutions were used to generate the dose response curves. EC_{50} = the effective concentration required to inhibit virus induced cytopathic effect by 50%. The reported values represent the means ± SD of data derived from three independent experiments.
[d] SI: selectivity index ratio of CC_{50} to EC_{50}. ND: not determined. NI: no inhibition.

their effect on cell viability are shown in Table 1. The selectivity index (SI) was calculated as well to estimate the therapeutic potential of the compounds in this system. Only two compounds (**28** and **31**) were found to be active against gt 1b (displaying EC_{50} values of 24.3 and 12.4 μM, respectively), although they showed poor SI (<10). In contrast, a total of 16 compounds were active against gt 2a, with associated EC_{50} values in the range from 4.9 to 28.1 μM and moderate to good SI. Compound **25** was the most potent among all the tested compounds and showed EC_{50} value of 4.9 μM with a SI > 41. Interestingly, the only two compounds found to be active on gt 1b replicons (**28** and **31**) were also active against gt 2a replicons and exhibited EC_{50} values of 6.0 and 8.7 μM, respectively, with SI values > 10.

Overall, compound **31**, having a 2-phenyl-4,5,6,7-Tetrahydro-1*H*-indole scaffold, emerged as a hit compound, displaying low cytotoxicity (CC_{50} = 109.9 μM) and promising anti-HCV activity in replicon reporter cells of both the genotype 1b (EC_{50} = 12.4 μM) and 2a (EC_{50} = 8.7 μM). Following on from this, we had eleven more analogues of **31** available at EDASA Scientific that we decided to further evaluate for their anti-HCV activities (**34**–**44**, Fig. 3).

2.2. Synthesis of derivatives 31, 34–44

Recently, we have developed a two-step one-pot synthetic methodology, which leads to 4,5,6,7-Tetrahydro-1*H*-indoles with a wide range of substituents, including chiral moieties, both at C-2 and at the *N*-1 positions [19]. This synthetic sequence was successfully applied to achieve derivatives **31**, **34**–**44** (Scheme 1).

This one-pot Sonogashira cross-coupling/5-endo-dig cyclization procedure was used as a flexible and versatile synthetic approach. Thus, the trans-stereoselective and highly regioselective nucleophilic epoxide ring opening of **45** with different amines was followed by a subsequent one-pot Pd-catalyzed arylation/cyclization. This short sequence allowed the variation of substituents both at the nitrogen atom and at the C-2 position of the pyrrole ring, along with a judicial design and a fast preparation of the most promising tetrahydroindole derivatives. Furthermore, it utilized mild conditions and inexpensive catalysts, being highly tolerant to a range of functional groups and readily scalable to provide sufficient amounts of tetrahydroindoles on gram scales in a good to excellent yields to effectively assemble the tetrahydroindole compound array for further screening. The full report on the synthetic sequence as well as compound characterization is presented in Supporting Information.

2.3. Cell-based assays of compounds 34–44

The anti-HCV activities of the new analogues of **31** (**34**–**44**) are shown in Table 2. The cell-based assays revealed that, out of the eleven compounds tested, eight derivatives in gt 1b and ten

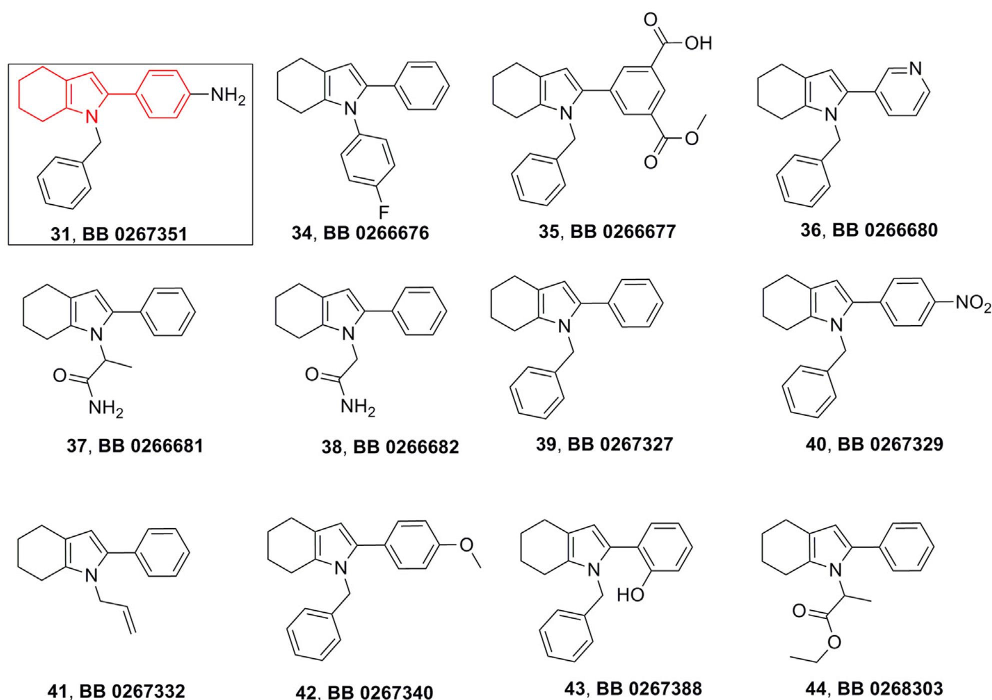


Fig. 3. Structures of EDASA analogues of 2-phenyl-4,5,6,7-Tetrahydro-1*H*-indole **31**.

compounds in gt 2a showed >60% inhibition during preliminary screening.

All these compounds except one were then evaluated for their EC_{50} and SI values; in fact, derivative **36** exerted its HCV replication inhibition at toxic concentration ($CC_{50} < 25$ μM) and thus it was not submitted to EC_{50} evaluation.

Taking into account the obtained biological data (Table 2), some preliminary SAR can be proposed for this new class of anti-HCV agents.

Derivatives **39**, **40** and **42**, all having a *N*-benzyl substitution at the tetrahydroindole core, showed the higher anti-HCV activities with SI values ranging from 10 to 13 for gt 1b, and from 27 to 32 for gt 2a. Among them, compound **39** was found to be the most potent in both gts displaying EC_{50} values of 7.9 μM (1b) and 2.6 μM (2a). Compared to **39**, derivative **34**, having a *para*-fluorophenyl group at the nitrogen atom, showed a nearly 3.7 and 4.7 fold reduction in anti-HCV activity for gt 1b and 2a replicon reporter assays, respectively. Furthermore, when the *N*-benzyl substituent of the tetrahydroindole nucleus was replaced with non-aromatic groups, the anti-HCV activity on gt 1b was completely lost (**37**, **38**, and **41**) or a non selective antiviral effect (i.e. low SI value) was obtained (**44**); the analysis on gt 2a provided similar conclusions with the exception of derivative **37** which turned out to be active.

When analyzing the biological data for the whole subset of *N*-benzyl derivatives (i.e. compounds **31**, **35**, **39**, **40**, **42** and **43**), the key role of the aryl substituent at the C-2 position became evident. An unsubstitued phenyl (**39**) as well as a *para*-substituted phenyl (i.e. **31**: NH_2, **40**: NO_2 and **42**: OCH_3) were both well tolerated; conversely, the presence of either *meta*-disubstituents (**35**) or *ortho*-OH (**43**) substituent led to compounds endowed with high toxicity. Moreover, the replacement of the phenyl (**39**) with a 3-pyridinyl ring (**36**) was also responsible for the increased cytoxicity ($CC_{50} = 80.8$ μM vs $CC_{50} < 25$ μM, respectively).

In order to further validate the anti-HCV activity of these compounds, hit **39** was selected and tested as a representative candidate against a reporter free cell culture system. To achieve this, we treated MH-14 cells carrying stably replicating HCV sub genomic replicon gt 1b with compound **39** and the HCV RNA was quantitated using standard quantitative RT-PCR methods. Notably, **39** inhibited the HCV replication in a dose-dependent manner and exhibited EC_{50} value of 3.13 μM (Fig. 4), which was quite similar to the value obtained in the replicon reporter cells (i.e. $EC_{50} = 7.9$ μM).

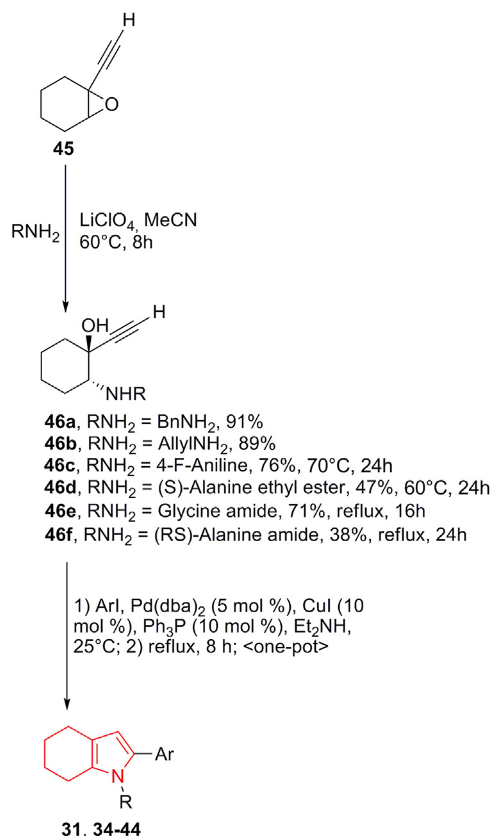
Overall, the results clearly indicated that promising anti-HCV activity coupled with no apparent cytotoxic effects were obtained when the 2-phenyl-4,5,6,7-Tetrahydro-1*H*-indole scaffold was properly functionalized.

2.4. Molecular target investigation

Next, we carried out target investigation for the most active tetrahydro-1*H*-indoles (i.e., **31**, **34**, **39**, **40** and **42**). Towards this end, we tested the compounds for their ability to inhibit the activity of two HCV viral proteins, i.e. NS5B polymerase and NS3 helicase. These two targets were chosen as first choice because indole derivatives have been reported in literature as both HCV NS5B polymerase and NS3 helicase inhibitors [20,21].

We utilized a standard primer-dependent elongation assay to test whether the compounds possessed anti-NS5B RNA-dependent RNA polymerase (RdRp) activity [22,23]. The compounds were investigated at 50 μM concentration in the preliminary assay. The results clearly revealed that none of the compounds was inhibitory to NS5B RdRp activity (data not shown), thus ruling out the possibility of possessing anti-HCV activity by targeting this protein.

The five compounds were also tested in HCV NS3 helicase assays as described previously [24]. None of the compounds inhibited the ability of the NS3 helicase to unwind a DNA substrate even at concentrations as high as 500 μM (data not shown). However, high concentrations of compound **31** inhibited the ability of NS3 helicase to cleave ATP in the presence of RNA. About 420 μM of **31** inhibited HCV helicase catalyzed ATP hydrolysis by 50% (see Fig. S1 Supporting Information).



Scheme 1. Synthesis of 2-aryl-4,5,6,7–1H-tetrahydroindoles. The explicit structures of compounds **31** and **34**–**44** are reported in Fig. 3.

Apart from targeting HCV proteins, small molecules known to interfere with HCV Internal Ribosome Entry Site (IRES)-mediated translation have been documented [25,26]. We therefore investigated if the observed anti-HCV activity of the 2-phenyl-tetrahydro-1*H*-indole scaffold could be due to the down-regulation of HCV IRES-mediated translation. Using compound **39** as representative, our results displayed that this compound had no effect on HCV IRES mediated translation (data not shown).

We also tested the possibility that compound **39** could function as a potential activator or suppressor of host-factor's that facilitate HCV replication. Towards this end, we carried-out cell based assays in which reporter plasmids of cyclooxygenase-2, heme oxygenase-1, interferon-stimulated response element or anti-oxidant response element were transfected, and the ability of derivative **39** to modulate the activation or suppression of the corresponding host-factors at three varying compound concentrations (5, 10 and 25 μM) were investigated. Our results revealed that **39** had no effect in these reporter mediated assays, thus ruling out the specified host factors as targets of the 2-phenyl-4,5,6,7-Tetrahydro-1*H*-indole core.

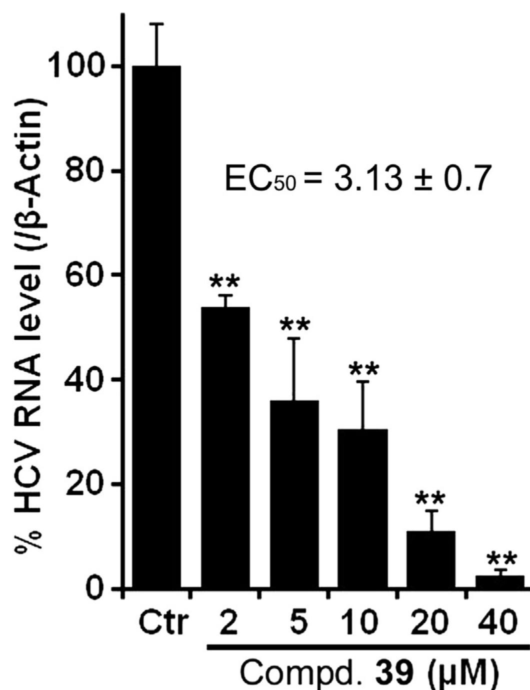


Fig. 4. Dose-dependent response of compound **39** assayed in MH-14 cells. **p < 0.01.

3. Conclusion

Overall, these results highlight the identification of 2-phenyl-4,5,6,7-Tetrahydro-1*H*-indole scaffold as a new anti-HCV chemotype.

Table 2
Anti-HCV activities and cytotoxicity of analogues of **31** evaluated on gt 1b and 2a.

Cpd	CC_{50} [a] (μM)	Huh7/Rep-Feo1b			Huh7.5-FGR-JC1-Rluc2A		
		Inhibition,[b] %	EC_{50} [c] (μM)	SI[d]	Inhibition,[b] %	EC_{50} [c] (μM)	SI[d]
34	>200	71 ± 6	29.2 ± 1.2	>7	66 ± 10	12.3 ± 1.0	>16
35	45.6 ± 6.1	81 ± 3	35.8 ± 3.4	>1	96 ± 3	9.9 ± 1.6	>5
36	<25	92 ± 1	ND	ND	99 ± 1	ND	ND
37	155.6 ± 11	50 ± 8	ND	ND	83 ± 3	15.4 ± 3.9	>10
38	>200	37 ± 18	ND	ND	48 ± 11	ND	ND
39	80.8 ± 3.1	95 ± 4	7.9 ± 0.5	10	99 ± 1	2.6 ± 0.4	32
40	>200	75 ± 7	15.0 ± 1.3	13	98 ± 2	7.3 ± 1.4	27
41	118.8 ± 2.8	35 ± 6	ND	ND	69 ± 2	32.1 ± 4.1	4
42	137.4 ± 1.0	99 ± 1	11.8 ± 0.6	12	96 ± 2	4.9 ± 0.3	28
43	48.9 ± 1.7	96 ± 2	9.2 ± 0.6	5	99 ± 1	6.5 ± 0.6	8
44	84.3 ± 1.3	74 ± 4	13.2 ± 1.4	6	95 ± 3	13.7 ± 2.1	6

[a] CC_{50} values were determined in Huh7.5 parental cells by the MTS assay. CC_{50} = is the concentration required to reduce the bioreduction of MTS (3-(4,5- dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) into formazan by 50%. The reported value represents the means ± SD of data derived from three independent experiments.
[b] Anti-HCV activity of the compounds were carried out at 50 μM in preliminary screening.
[c] The inhibition data from 8 to 12 quarter log dilutions were used to generate the dose response curves. EC_{50} = the effective concentration required to inhibit virus induced cytopathic effect by 50%. The reported values represent the means ± SD of data derived from three independent experiments.
[d] SI: selectivity index ratio of CC_{50} to EC_{50}. ND: not determined.

Preliminary SAR highlighted the key role of both the substituents on the 2-phenyl ring and the *N*-1 benzyl moiety in modulating cytotoxicity and activity, respectively, with derivatives **39**, **40** and **42** being the best hits within this first series of 2-phenyl-4,5,6,7-Tetrahydro-1*H*-indoles.

While the present study has revealed a novel chemotype worthy of further investigation, the exact mechanism by which these derivatives inhibit HCV replication remains to be clarified.

4. Experimental section

4.1. Cell culture

Huh7/Rep-Feo1b and Huh7.5-FGR-JC1-Rluc2A replicon reporter cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, 5% antibiotic and 0.5 mg/mL G418. Huh 7.5 cells were grown similarly as above without G418. All cells were cultured at 37 °C in 5% humidified CO_2.

4.2. NS5B RdRp assay

Recombinant HCV NS5B bearing hexa-histidine tag at N-terminal was expressed in *Escherichia coli* and purified as previously described [23,27]. The anti-NS5B RdRp activity of the compounds was evaluated by using a primer-dependent elongation assay as reported earlier [28]. In brief, the reaction buffer containing 20 mM Tris–HCl (pH 7.0), 100 mM Na-glutamate, 100 mM NaCl, 0.01% BSA, 0.01% Tween-20, 0.1 mM DTT, 5% glycerol, 20 U/mL of RNasin, 20 μM UTP, 1 μCi [α-^{32}P]UTP, 0.25 μM polyrA/U_{12}, 100 ng NS5BCΔ21 was incubated with compounds and the polymerase reaction was started by addition of 1 mM $MnCl_2$ in a final volume of 20 μl. The reactions were incubated at 30 °C for 60 min, and then stopped by adding 5% trichloroacetic acid containing 0.5 mM sodium pyrophosphate, filtered through GF-B filters, and successively washed with water and ethanol. The amount of radiolabeled RNA was quantified using liquid scintillation counter. The activity of NS5B in the presence of an equal amount of DMSO was set at 100% and that in the presence of the compounds was determined relative to this control.

4.3. Huh7/Rep-Feo1b, Huh7.5-FGR-JC1-Rluc2A reporter system and cellular viability assay

The anti-HCV activity of compounds was measured using the Huh7/Rep-Feo1b and Huh7.5-FGR-JC1-Rluc2A replicon reporter cells as described earlier [29,30]. In short, approximately 1×10^4 cells were plated in 96 well plates and treated with compounds or DMSO for 48 h. The concentration of DMSO in cell culture was kept constant at 1.0%. The luciferase activities were measured by following the manufacturer's protocol (Promega Inc, USA). The activity of the compounds was evaluated as the comparative levels of the luciferase signals in compound-treated cells versus DMSO-treated controls. The cellular cytotoxicity assays were conducted in 96 well plate format using parental Huh7.5 cells. Briefly, cells treated at 6-8 doses of compounds for 48 h were evaluated employing the CellTiter 96® AQueous One Solution Cell Proliferation kit (Promega Inc, USA). The luciferase activities of the cells treated with an equal amount of DMSO served as control.

4.4. Target identification reporter assays

The effect of compound **39** on HCV IRES mediated translation was studied using a dual luciferase reporter construct (pClneo-Rluc-IRES-Fluc) in which Rluc was translated in a cap-dependent manner and Fluc was translated via HCV IRES-mediated initiation, as described previously [29]. Transfections were carried our using LipoD293 reagent in Huh7.5 cells. Sixteen h post-transfection, the cells were treated with compound or DMSO and Luciferase activity assay was performed using Dual-Glo Luciferase Assay Kit.

For investigation host-factors as potential targets, hepatoma cells carrying HCV subgenomic replicons (MH-14) were transfected with 300 ng of gene specific reporter plasmid pCOX-2-FLuc [31–34], pHO-1-Luc [35], pISRE-Luc [36], or p3xARE-Luc [37]. Sixteen h post-transfection, cells were treated with compound **39** or DMSO (control) for 48 h and luciferase activities were measured as described above. Transfection efficiencies were normalized by Renilla luciferase expression.

4.5. RT-PCR

Total RNA was isolated using an RNeasy mini kit (Qiagen) and quantified using NanoDrop (ND1000, NanoDrop Technologies). Approximately 500 ng of RNA was reverse transcribed using M-MLV reverse transcriptase (Life Technologies) and either oligo dT_{18} or HCV specific primers in a final volume of 20 μl. Approximately 50 ng of synthesized cDNA's were used for PCR applications using gene specific primers and Power SYBR green PCR master mix (Applied Biosystems) in a final volume of 25 μl. The PCR was performed on Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument. The forward and reverse primer sequence for *β*-Actin was 5′- AGCGAGCATCCCCCAAAGTT-3′ and 5′-GGGCACGAAGGCTCATCATT-3′, respectively. The HCV primer sequence was 5′-CGGGAGAGCCATAGTGG-3′ for forward and 5′-AGTACCACAAGGCCTTTCG-3′ for the reverse primer.

4.6. NS3 helicase assay

4.6.1. Chemicals and reagents

Truncated C-terminally His-tagged NS3 protein lacking the N-terminal protease (NS3h) from the con1 strain of genotype 1b [Genbank accession AB114136], was expressed and purified as previously described [38,39].

4.6.2. Molecular beacon based helicase assays

Molecular beacon-based NS3 helicase assays were performed as described by Hanson et al. [49] Reactions contained 25 mM MOPS pH 6.5, 1.25 mM $MgCl_2$, 5% DMSO, 5 μg/ml BSA, 0.01% (v/v) Tween20, 0.05 mM DTT, 5 nM florescent DNA substrate, 12.5 nM NS3h, and 1 mM ATP.

4.6.3. ATP hydrolysis (ATPase) assays

A modified malachite green-based assay was used to measure helicase-catalyzed ATP hydrolysis (Sweeney et al., 2013). The colorimetric reagent was prepared fresh by mixing 3 volumes of 0.045% (w/v) malachite green, with 1 volume 4.2% ammonium molybdate in 4 N HCl, and 0.05 volumes of 20% Tween 20. Reactions (30 μL) were initiated by adding ATP, incubated for 15 min at 37 °C, and terminated by adding 200 μL of the malachite green reagent, followed by 30 μL of 35% sodium citrate. The color was allowed to develop for 30 min and an absorbance at 630 nm was observed.

HCV Helicase-catalyzed ATP hydrolysis in the absence of RNA was monitored in reactions containing 50 nM HCV NS3h, 25 mM MOPS pH 6.5, 1.25 mM $MgCl_2$, 1 mM ATP, 33 μg/ml BSA, 0.07% (v/v) Tween 20, 0.3 mM DTT, and 10% v/v DMSO. Reactions in the presence of polyU RNA were performed with 4 nM HCV NS3h in the same buffer with 1 μM PolyU (Sigma, expressed and nucleotide concentration) was added to each reaction.

To determine the compound concentration, it was necessary to

reduce helicase-catalyzed ATP hydrolysis by 50% (IC_{50}). Reactions were performed in duplicate through a two-fold dilution series so that final compound concentrations ranged from 0.5 mM to 0.78 μM. Data obtained from all reactions within the linear range of the colorimetric assay as determined with a phosphate standard curve were normalized to controls lacking an inhibitor (100%) and controls lacking an enzyme (0%), and fitted to a normalized dose response equation with a variable Hill slope using GraphPad Prism (v. 6). Reactions were performed in duplicate and each titration conformed to the above concentration response equation. Average IC_{50} values ± standard deviations were reported. In another set of controls, 100 μM of inorganic phosphate was titrated with each compound, followed by the addition of a malachite green reagent. None of the compounds affected the absorbance of the colorimetric reaction products in these controls.

Acknowledgments

We thank Drs. Naoya Sakamoto and Hengli Tang for providing the Huh7/Rep-Feo1b and Huh7.5-FGR-JC1-Rluc2A replicon reporter cells. Plasmids pCIneo-Rluc-IRES-Fluc, pHO-1-Luc and p3xARE-Luc, were generously shared by Drs. Naoya Sakamoto, Anupam Agarwal, and Dr. Being-Sun Wung, respectively. We acknowledge and appreciate grant support from the New Jersey Health Foundation to Neerja Kaushik-Basu and the Russian Foundation for Basic Research (RFBR), Russia (Projects No. 14-03-31685, 14-03-31709, 14-03-01114).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.04.022.

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Supporting Information

Discovery of the 2-Phenyl-4,5,6,7-Tetrahydro-1*H*-indole as a Novel Anti-Hepatitis C Virus Targeting Scaffold.

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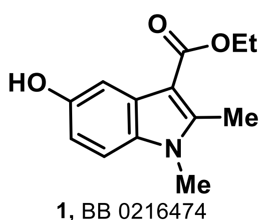
Compounds 1-33 have been procured from the EDASA Scientific public available compound repertory (http://www.edasascientific.com/page/catalogue). A report of their characterization via **^{1}H NMR**, **^{13}C NMR** and **m.p.** can be found on pages S2 to S11.

The synthetic procedure and compound characterization of compounds **34-44** is reported on pages S12 to S22.

Ethyl 1,2-dimethyl-5-hydroxy-indole-3-carboxylate (1)[1]



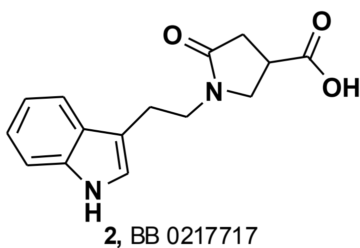
1, BB 0216474

m.p. = 208 – 209 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.35 (t, J = 7.1 Hz, 3H), 2.66 (s, 3H), 3.63 (s, 3H), 4.26 (q, J = 7.1 Hz, 2H), 6.68 (dd, J = 8.7, 2.4 Hz, 1H), 7.26 (d, J = 8.7 Hz, 1H), 7.38 (d, J = 2.2 Hz, 1H), 8.94 (s, 1H).

^{13}C NMR: (DMSO-d_6, 100 MHz): δ = 11.7, 14.5, 29.6, 58.6, 101.9, 105.5, 110.3, 111.3, 127.1, 130.7, 145.3, 152.6, 165.2.

1-[2-(1H-Indol-3-yl)ethyl]-5-oxopyrrolidine-3-carboxylic acid (2)



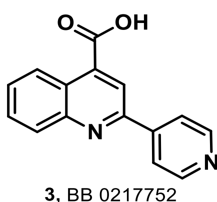
2, BB 0217717

m.p. = 217 – 220 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.40 - 2.55 (m, 2H), 2.87 (t, J = 7.5 Hz, 2H), 3.17 (tdd, J = 9.1, 7.3, 6.0 Hz, 1H), 3.47 (dd, J = 8.9, 7.8 Hz, 2H), 3.50-3.61 (m, 2H), 6.99 (ddd, J = 8.0, 7.1, 1.0 Hz, 1H), 7.08 (td, J = 7.5, 1.1 Hz, 1H), 7.16 (d, J = 2.3 Hz, 1H), 7.35 (d, J = 8.1 Hz, 1H), 7.55 (d, J = 7.8 Hz, 1H), 10.83 (s, 1H), 12.60 (br. s, 1H).

^{13}C NMR: (DMSO-d_6, 100 MHz): δ = 22.9, 33.7, 35.5, 42.5, 49.7, 111.3, 111.4, 118.2, 118.3, 121.0, 122.7, 127.1, 136.3, 171.8, 174.6.

2-Pyridin-4-ylquinoline-4-carboxylic acid (3)[2]



3, BB 0217752

m.p. = 310 – 312 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.77 (t, J = 7.6 Hz, 1H), 7.90 (t, J = 7.6 Hz, 1H), 8.22 (d, J=8.6 Hz, 1H), 8.26 (d, J = 5.1 Hz, 1H), 8.55 (s, 1H), 8.68 (d, J = 8.4 Hz, 1H), 8.79 (d, J = 5.0 Hz, 2H).

^{13}C NMR: (DMSO-d_6, 100 MHz): δ = 119.1, 121.3 (2C), 124.1, 125.5, 128.7, 130.0, 130.6, 138.3, 144.8, 148.3, 150.5 (2C), 153.6, 167.5.

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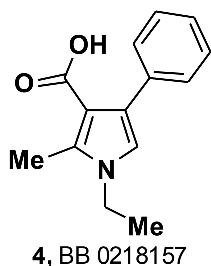
[2] ASTRAZENECA AB Patents: WO2009/82346 A1,2009; WO 2009/082346 A1

1-Ethyl-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid (4)

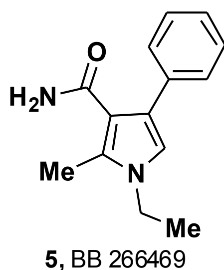


m.p. = 195 °C (decomp.).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.29 (t, J = 7.3 Hz, 3H), 2.47 (s, 3H), 3.90 (q, J = 7.2 Hz, 2H), 6.78 (s, 1H), 7.08-7.22 (m, 1H), 7.24-7.34 (m, 4H), 10.26-12.42 (br. s, 1H).

^{13}C NMR: (DMSO-d_6, 100 MHz): δ = 11.0, 16.0, 40.9, 110.1, 119.6, 124.9, 125.5, 127.4 (2C), 128.8 (2C), 135.1, 136.2, 166.6.

1-Ethyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxamide (5)

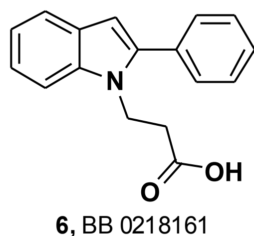


Viscous oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.40 (t, J = 7.3 Hz, 3H), 2.56 (s, 3H), 3.90 (q, J = 7.3 Hz, 2H), 5.30 (br. s., 1H), 5.50 (br. s, 1H), 6.54 (s, 1H), 7.29-7.33 (m, 1H), 7.34-7.44 (m, 4H).

^{13}C NMR: ($CDCl_3$, 100 MHz): δ = 11.1, 16.2, 41.4, 112.9, 118.5, 123.6, 127.0, 128.7 (2C), 129.5 (2C), 134.6, 135.4, 168.4.

3-(2-Phenyl-1H-indol-1-yl)propanoic acid (6)

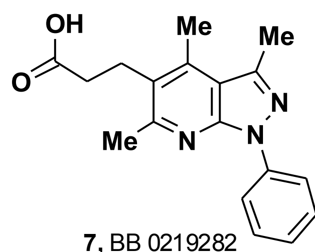


m.p. = 137 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.58 (t, J = 7.6 Hz, 2H), 4.45 (t, J = 7.6 Hz, 2H), 6.54 (s, 1H), 7.09 (t, J = 7.5 Hz, 1H), 7.20 (td, J = 8.1, 0.9 Hz, 1H), 7.43-7.49 (m, 1H), 7.49 - 7.61 (m, 6H), 12.40 (br. s., 1H).

^{13}C NMR: (DMSO-d_6, 100 MHz): δ = 34.2, 39.5, 102.3, 110.5, 119.8, 120.3, 121.7, 127.8, 128.2, 128.8 (2C), 129.1 (2C), 132.4, 137.1, 140.7, 172.1.[3]

3-(3,4,6-Trimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl)propanoic acid (7)



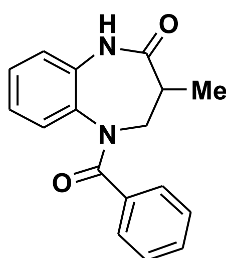
m.p. > 250 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.41 (t, J = 8.1 Hz, 2H), 2.61 (s, 3H), 2.62 (s, 3H), 2.66 (s, 3H), 2.97 (t, J = 8.1 Hz, 2H), 7.25 (t, J =7.3 Hz, 1H), 7.50 (t, J =7.9 Hz, 2H), 8.28 (d, J =7.9 Hz, 2H), 12.30 (br. s., 1H).

^{13}C NMR: (DMSO-d_6, 100 MHz): δ = 14.8, 15.5, 23.6, 23.8, 33.4, 115.0, 119.6 (2C), 124.8, 126.7, 129.0 (2C), 139.5, 140.9, 142.4, 148.8, 157.5, 173.8.

[3] One aliphatic signal is overlapping with the center of DMSO-d_6 septet.

5-Benzoyl-3-methyl-1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-one (8)[4]



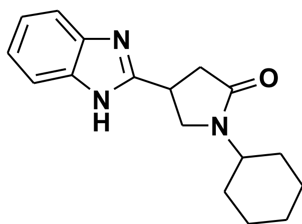
8, BB 0219743

m.p. = 171 – 173 °C.

^{1}H NMR (CDCl$_3$, 400 MHz): δ = 1.24 (d, *J* = 6.5 Hz, 3H), 2.89-3.01 (m, 1H), 3.87 (dd, *J* = 11.3, 5.6 Hz, 1H), 4.50 (t, *J* = 12.9 Hz, 1H), 6.74 (d, *J* = 7.7 Hz, 1H), 6.86 (t, *J* = 6.9 Hz, 1H), 7.10-7.27 (m, 7H), 8.96 (br. s, 1H).

^{13}C NMR: (CDCl$_3$, 75 MHz): δ = 12.9, 35.0, 56.8, 122.7, 126.1, 128.0 (2C), 128.4 (2C), 128.5, 130.38, 130.31, 135.0, 135.2, 135.4, 171.2, 176.0.

4-(1H-Benzimidazol-2-yl)-1-cyclohexylpyrrolidin-2-one (9)



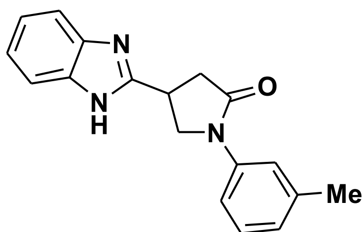
9, BB 0219747

m.p. = 235 °C.

^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ = 1.00-1.14 (m, 1H), 1.20-1.50 (m, 4H), 1.52-1.65 (m, 3H), 1.74 (t, *J* = 11.6 Hz, 2H), 2.73 (d, *J* = 8.2 Hz, 2H), 3.58-3.67 (m, 1H), 3.71-3.87 (m, 3H), 5.48 (br. s., 1H), 7.13 (dd, *J* = 5.9, 3.1 Hz, 2H), 7.50 (tq, *J* = 3.2, 3.1 Hz, 2H).

^{13}C NMR: (DMSO-*d*$_6$, 100 MHz): δ = 25.0, 25.1, 25.2, 29.6, 29.8, 31.4, 36.5, 46.9, 50.1, 114.7 (2C), 121.4 (2C), 138.8, 155.6, 171.4.

4-(1*H*-Benzimidazol-2-yl)-1-(3-methylphenyl)pyrrolidin-2-one (10)



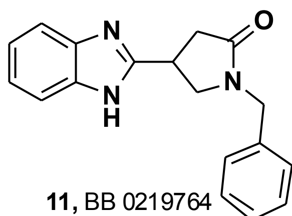
10, BB 0219760

m.p. = 175 – 178 °C.

^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ = 2.31 (s, 3H), 3.02 (dd, *J* = 7.9, 6.4 Hz, 2H), 4.00 (ddt, *J* = 7.8, 7.6, 7.5 Hz, 1H), 4.16-4.33 (m, 2H), 6.96 (d, *J* = 7.5 Hz, 1H), 7.16 (dd, *J* = 5.9, 3.1 Hz, 2H), 7.26 (t, *J* = 8.1 Hz, 1H), 7.47-7.59 (m, 4H), 12.45 (br. s., 1H).

^{13}C NMR: (DMSO-*d*$_6$, 100 MHz): δ = 21.2, 30.7, 37.6, 52.3, 116.7, 120.1, 121.6, 124.8, 128.6, 138.0, 139.3, 155.1, 172.0.

4-(1H-Benzimidazol-2-yl)-1-benzylpyrrolidin-2-one (11)



11, BB 0219764

m.p. = 150 – 153 °C.

^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ = 2.84 (d, *J*=8.3 Hz, 2H), 3.57 (dd, *J*=9.6, 6.5 Hz, 1H), 3.70 (t, *J*=8.9 Hz, 1H), 3.88 (ddd, *J*= 16.6, 8.3, 6.9 Hz, 1H), 4.45 (s, 2H), 7.10-7.18 (m, 2H), 7.23-7.29 (m, 3H), 7.29-7.35 (m, 2H), 7.46-7.55 (m, 2H), 12.36 (br. s., 1H)

^{13}C NMR: (DMSO-*d*$_6$, 100 MHz): δ = 31.0, 35.7, 45.4, 50.7, 121.5, 127.3, 127.7 (2C), 128.6 (2C), 136.75, 155.3, 172.3.

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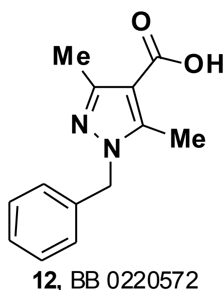
[4] R. Janciene, A. Vektariene, G. Mikulskiene, T. Javorskis, G. Vektaris, A. Klimaviciusa. *ARKIVOC*, **2013**, iv, 1-19.

1-Benzyl-3,5-dimethyl-1H-pyrazole-4-carboxylic acid (12)



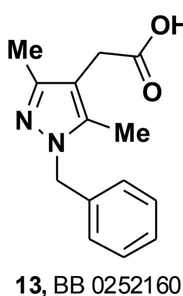
12, BB 0220572

m.p. = 143 – 145 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.30 (s, 3H), 2.42 (s, 3H), 5.27 (s, 2H), 7.12 (d, J = 7.0 Hz, 2H), 7.24-7.30 (m, 1H), 7.30-7.36 (m, 2H).

^{13}C NMR: (DMSO-d_6, 100 MHz): δ = 10.9, 14.1, 51.8, 109.6, 127.0 (2C), 127.5, 128.7 (2C), 136.9, 143.7, 149.4, 165.3.

(1-Benzyl-3,5-dimethyl-1H-pyrazol-4-yl)acetic acid (13)



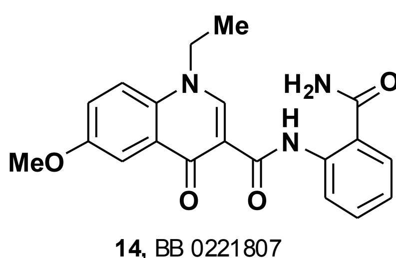
13, BB 0252160

m.p. = 123 – 125°C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.06 (s, 3H), 2.09 (s, 3H), 3.27 (s, 2H), 5.19 (s, 2H), 7.10 (d, J = 7.1 Hz, 2H), 7.22-7.28 (m, 1H), 7.29-7.35 (m, 2H), 12.18 (br. s., 1H).

^{13}C NMR: (DMSO-d_6, 100 MHz): δ = 9.3, 11.7, 29.2, 51.8, 109.9, 126.9 (2C), 127.3, 128.5 (2C), 136.8, 138.0, 145.4, 172.8.

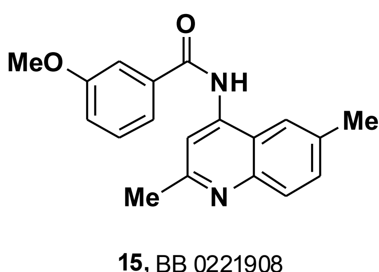
N-(2-Carbamoylphenyl)-1-ethyl-6-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxamide (14)



14, BB 0221807

^{1}H NMR (CD_3OD, 400 MHz): δ = 1.56 (t, J = 7.3 Hz, 3H), 2.17 (s, 3H), 3.98 (s, 3H), 4.54 (q, J = 7.2 Hz, 2H), 7.23 (td, J = 7.6, 1.0 Hz, 1H), 7.48-7.54 (m, 2H), 7.64 (dd, J =7.6, 1.2 Hz, 1H), 7.87 (d, J = 9.3 Hz, 1H), 7.98 (d, J = 2.8 Hz, 1H), 8.35 (dd, J = 8.1, 1.0 Hz, 1H), 8.92 (s, 1H).

***N*-(2,6-Dimethylquinolin-4-yl)-3-methoxybenzamide (15)**



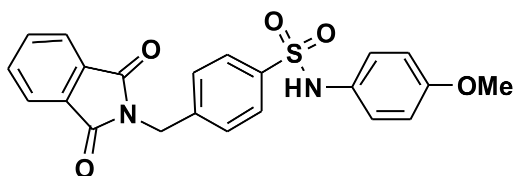
15, BB 0221908

m.p. = 177 – 178 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.50 (s, 3H), 2.64 (s, 3H), 3.87 (s, 3H), 7.22 (dd, J = 8.2, 2.4 Hz, 1H), 7.50 (t, J = 7.9 Hz, 1H), 7.57 (dd, J = 8.7, 1.6 Hz, 1H), 7.63 (s, 1H), 7.68 (d, J = 7.6 Hz, 1H), 7.75 (s, 1H), 7.85 (d, J =8.6 Hz, 1H), 7.94 (s, 1H), 10.51 (s, 1H).

^{13}C NMR: (DMSO-d_6, 100 MHz): δ = 21.3, 25.0, 55.4, 113.2, 115.9, 117.9, 120.3, 121.1, 121.8, 128.4, 129.7, 131.5, 134.5, 135.7, 141.3, 147.0, 157.8, 159.3, 166.2.

4-[(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)methyl]-*N*-(4-methoxyphenyl)benzene sulfonamide (16)



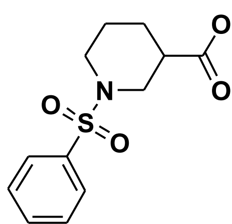
16, BB 0222063

m.p. = 225 – 230 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 3.65 (s, 3H), 4.82 (s, 2H), 6.78 (dt, *J* = 9.1, 2.9 Hz, 2H), 6.98 (dt, *J* = 9.1, 2.9 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.82-7.91 (m, 4H), 9.95 (s, 1H).

^{13}C NMR: (DMSO-d_6, 100 MHz): δ = 40.4, 55.1, 114.3 (2C), 123.2 (2C), 123.3 (2C), 127.0 (2C), 127.8 (2C), 130.1, 131.6, 134.6 (2C), 138.6, 141.5, 156.5, 167.7.

1-(Phenylsulfonyl)piperidine-3-carboxylic acid (17)



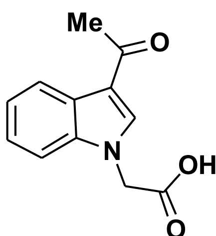
17, BB 0238610

m.p. = 126 – 128 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.26-1.41 (m, 1H), 1.42-1.55 (m, 1H), 1.65-1.74 (m, 1H), 1.75-1.83 (m, 1H), 2.39 (td, *J* = 10.9, 2.4 Hz, 1H), 2.45-2.56 (m, 2H), 3.29-3.37 (m, 1H), 3.48-3.59 (m, 1H), 7.61-7.69 (m, 2H), 7.69-7.79 (m, 3H), 12.39 (br. s., 1H).

^{13}C NMR: (DMSO-d_6, 100 MHz): δ = 23.4, 25.6, 40.1, 46.1, 47.5, 127.4 (2C), 129.5 (2C), 133.2, 135.4, 173.8.

(3-Acetyl-1H-indol-1-yl)acetic acid (18)



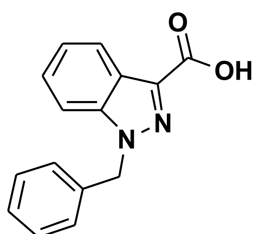
18, BB 0241842

m.p. = 200 – 220 °C (dec.).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.45 (s, 3H), 5.13 (s, 2H), 7.17-7.30 (m, 2H), 7.47-7.53 (m, 1H), 8.16-8.24 (m, 1H), 8.33 (s, 1H), 13.19 (br. s., 1H).

^{13}C NMR: (DMSO-d_6, 100 MHz): δ = 27.3, 47.6, 110.7, 116.3, 121.5, 122.1, 123.0, 125.6, 137.3, 138.2, 169.8, 192.4.

1-Benzyl-1H-indazole-3-carboxylic acid (19)



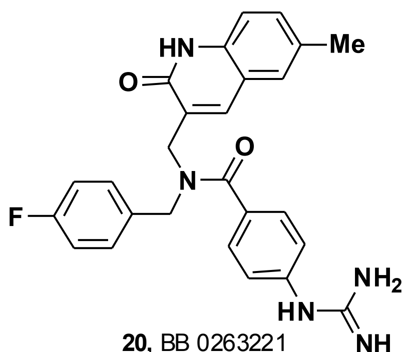
19, BB 0255255

m.p. = 202 – 203 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 5.89 (s, 2H), 7.04 (d, *J* = 7.2 Hz, 2H), 7.13 (t, *J* = 7.5 Hz, 1H), 7.16-7.22 (m, 1H), 7.22-7.31 (m, 2H), 7.36 (d, *J* = 7.0 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.71 (d, *J* = 7.9 Hz, 1H), 12.91 (br. s., 1H).

^{13}C NMR: (DMSO-d_6, 100 MHz): δ = 46.9, 110.5, 111.3, 120.7, 122.4, 124.9, 125.6, 126.3 (2C), 127.0, 128.5 (2C), 138.7, 139.0, 163.0.

N-(4-Fluorobenzyl)-4-guanidino-N-((6-methyl-2-oxo-1,2-dihydroquinolin-3-yl)methyl)benzamide (20)

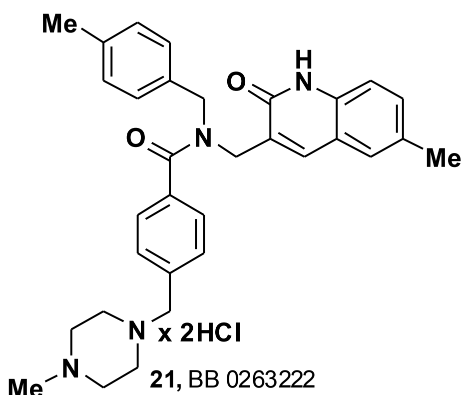


20, BB 0263221

m.p. > 230 °C (dec.).

^{1}H NMR: (DMSO, 400 MHz) δ = 2.36 (s, 3H), 4.34 (br. s, 2H), 4.66 (br. s, 2H), 7.10-7.19 (m, 4H), 7.20-7.25 (m, 1H), 7.26-7.38 (m, 3H), 7.45-7.56 (m, 3H), 7.67 (br. s, 1H), 8.40 (br. s, 1H). Guanidine protons are overlapped with water.

N-((6-Methyl-2-oxo-1,2-dihydroquinolin-3-yl)methyl)-N-(4-methylbenzyl)-4-((4-methylpiperazin-1-yl)methyl)benzamide dihydrochloride (21)



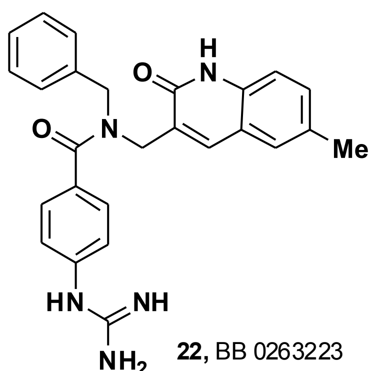
21, BB 0263222

m.p. = 199 – 201 °C.

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 2.35 (s, 3H), 2.38 (s, 3H), 2.42 (s, 3H), 2.55 (br. s, 8H), 3.50 (br. s, 2H), 4.49 (br. s, 1H), 4.59-4.76 (m, 2H), 4.81 (br. s, 1H),7.15 (br. s, 3H), 7.21-7.41 (m, 7H), 7.45 (d, J = 7.2 Hz, 2H), 12.11 (br. s, 1H).

^{13}C NMR: ($CDCl_3$, 100 MHz) δ = 21.1, 21.2, 29.8, 45.6, 48.1, 52.4, 54.9 (2C), 62.4 (2C), 115.7, 119.7, 120.0, 126.7, 127.1, 127.2, 128.5, 129.2 (2C), 129.5, 129.6, 131.8, 132.0, 132.3, 132.6, 134.0, 134.1, 135.1, 137.4, 140.0, 163.0, 172.9.

N-Benzyl-4-guanidino-N-((6-methyl-2-oxo-1,2-dihydroquinolin-3-yl)methyl)benzamide (22)

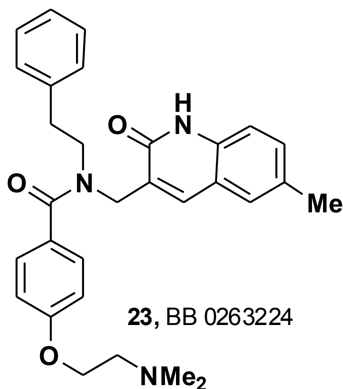


22, BB 0263223

m.p. > 200 °C (dec.).

^{1}H NMR: (DMSO, 400 MHz) δ = 2.36 (s, 3H), 4.21-4.45 (m, 2H), 4.69 (br. s, 2H), 7.11-7.25 (m, 4H), 7.25-7.40 (m, 5H), 7.44-7.61 (m, 3H), 7.61-8.05 (m, 4H), 8.40 (br. s, 1H), 11.80 (br. s, 1H).

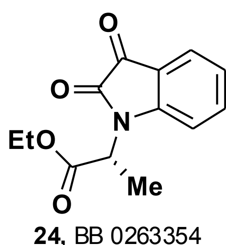
4-(2-(Dimethylamino)ethoxy)-*N*-((6-methyl-2-oxo-1,2-dihydroquinolin-3-yl)methyl)-*N*-phenethylbenzamide (23)



m.p. = 233 – 235 °C.

^{1}H NMR: (DMSO, 400 MHz) δ = 2.19 (br. s, 6H), 2.33 (br. s, 3H), 2.54-2.73 (m, 2H), 2.73-3.06 (m, 2H), 3.44-3.73 (m, 2H), 4.05 (br. s, 2H), 4.22 (br. s, 1H), 4.54 (br. s, 1H), 6.77-7.08 (m, 3H), 7.11-7.45 (m, 8H), 7.53 (br. s, 1H), 7.68 (br. s, 1H), 11.83 (br. s, 1H).

(*R*)-Ethyl 2-(2,3-dioxoindolin-1-yl)propanoate (24)[5]



Orange solid

m.p. = 58 °C.

$[\alpha]_D^{23}$ = + 18 (c = 1, CH_2Cl_2).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.22 (t, J = 7.2 Hz, 3H), 1.69 (d, J = 7.5 Hz, 3H), 4.23 (q, J = 7.2 Hz, 2H), 5.16 (q, J = 7.5 Hz, 1H), 6.85 (d, J = 7.7 Hz, 1H), 7.15 (td, J = 7.7, 0.7 Hz, 1H), 7.57 (td, J = 7.7, 1.4 Hz, 1H), 7.65 (ddd, J = 7.7, 1.4, 0.7 Hz, 1H).

^{13}C NMR: ($CDCl_3$, 100 MHz): δ = 14.1, 14.3, 49.2, 62.2, 111.5, 117.9, 123.9, 125.6, 138.2, 149.5, 157.7, 169.4, 182.7.

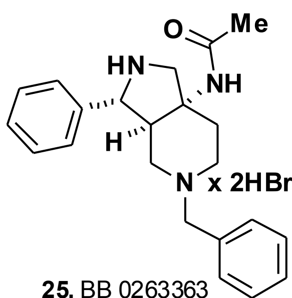
IR ν_{max} (KBr): 3467, 2993, 1739 (CO), 1608, 1468, 1367, 1309, 1246, 1113, 750, 476 cm^{-1}.

m/z (I_{rel}, %): 247 [M+], 174 [M-CO_2Et], 146 [M-CH_3CHCO_2Et], 128 (0.8), 117 (6), 91 (12), 77 (26), 51 (9).

Anal. Calcd for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.66. **Found**: C, 63.20; H, 5.43; N, 5.81.

[5] a) Kurkin, A. V.; Bernovskaya, A. A.; Yurovskaya, M. A. *Tetrahedron: Asymmetry* **2009**, *20*, 1500 – 1505; b) Kurkin, A. V.; Bernovskaya, A. A.; Yurovskaya, M. A. *Tetrahedron: Asymmetry* **2010**, 21, 2100 – 2107; c) Kurkin, A. V.; Bernovskaya, A. A.; Yurovskaya, M. A. *Chem. Heterocycl. Compd.* **2011**, *46*, 1208 – 1214.

N-((3*RS*,3a*SR*,7a*RS*)-5-Benzyl-3-phenyloctahydro-1H-pyrrolo[3,4-c]pyridin-7a-yl)acetamide dihydrobromide (25)



m.p. = 240 – 242°C.

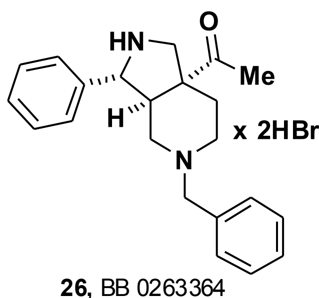
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.96 (s, 3H), 2.19-2.33 (m, 1H), 2.42-2.55 (m, 1H), 2.76 (d, *J* = 13.7 Hz, 1H), 3.13-3.28 (m, 2H), 3.33-3.48 (m, 1H), 3.50-3.63 (m., 2H), 3.63-3.73 (m, 1H), 4.30-4.51 (m, 2H), 5.32-5.45 (m, 1H), 7.37-7.43 (m, 3H), 7.43-7.50 (m, 3H), 7.56-7.71 (m, 4H), 8.55 (s, 1H), 9.24 (br. s., 1H), 10.22 (br. s., 2H).

^{13}C NMR: (DMSO-d_6, 100 MHz): δ = 23.0, 27.4, 44.0, 44.4, 47.3, 52.8, 54.6, 59.2, 59.5, 128.6 (2C), 128.7 (2C), 129.0 (2C), 129.6, 129.8, 131.5, 132.4, 170.5.

IR ν_{max} (KBr): 2920, 2872, 2856, 2702, 2623, 2590, 1699, 1529, 1464, 1414, 1377, 748, 696 cm^{-1}.

HRMS (ESI) for $C_{22}H_{28}N_3O$ $[M + H]^+$ calcd 350.2227, found 350.2224.

1-((3*RS*,3a*SR*,7a*RS*)-5-Benzyl-3-phenyloctahydro-1H-pyrrolo[3,4-c]pyridin-7a-yl)ethanone dihydrobromide (26)



m.p. = 298 – 300 °C (dec.).

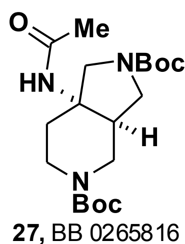
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.39 (s + m, 3 + 1H), 2.52-2.61 (m, 1H), 2.64-2.95 (m, 2H), 3.01-3.29 (m, 2H), 3.36-3.57 (m, 2H), 3.80 (d, *J* = 12.1 Hz, 1H), 4.09-4.57 (m, 2H), 5.47 (d, *J* = 10.5 Hz, 1H), 7.31-7.40 (m, 3H), 7.41-7.50 (m, 3H), 7.52-7.67 (m, 2H), 7.68-7.83 (m, 2H), 9.26 (br. s., 1H), 10.31 (br. s., 2H).

^{13}C NMR: (DMSO-d_6, 100 MHz): δ = 26.1, 26.6, 43.0, 45.6, 48.4, 50.4, 51.7, 59.0, 61.2, 128.6 (2C), 128.8 (2C), 128.9 (2C), 129.5, 129.7, 131.5 (2C), 132.1, 206.2.

IR ν_{max} (KBr): 3543, 3469, 3234, 2931, 2914, 1624, 1549, 1462, 1377, 756, 704 cm^{-1}.

HR–MS (ESI) for $C_{22}H_{27}N_2O$ $[M + H]^+$ calcd 335.2118, found 335.2117.

(3a*RS*,7a*SR*)-di-tert-Butyl 7a-acetamidotetrahydro-1H-pyrrolo[3,4-c]pyridine-2,5(3H,6H)-dicarboxylate (27)



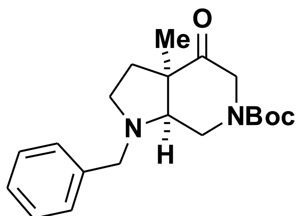
m.p. = 105 – 110 °C.

Mixture of rotamers.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.43 (s, 18H), 1.63-1.83 (m, 1H), 1.96 (s, 3H), 2.12 (d, *J* = 13.7 Hz, 1H), 2.24-2.49 (m, 0.5H), 2.51-2.75 (m, 1.5H), 2.98-3.19 (m, 2H), 3.27 (d, *J* = 13.9 Hz, 1H), 3.42-3.78 (m, 4H), 6.23 (s, 1H).

^{13}**C NMR: (CDCl$_3$, 100 MHz): δ =** (23.68, 23.72), (28.3, 28.4) (3C), 29.0, 40.2 (br., 2C), (45.6, 46.1), (54.7, 55.3), (57.0, 57.5), (79.68, 79.72), (80.03, 80.05), (154.7, 154.8, 155.0), 170.7.

(3a*RS*,7a*SR*)-tert-Butyl 1-benzyl-3a-methyl-4-oxohexahydro-1H-pyrrolo[2,3-c]pyridine-6(2H)-carboxylate (28)



28, BB 0263365

m.p. = 72 – 74 °C.

Mixture of two rotamers.

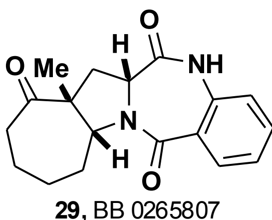
^{1}**H NMR: (CDCl$_3$, 400 MHz) δ =** 1.24 (br. s., 3H), 1.45-1.50 (br. s + m, 10H), 2.17-2.36 (m, 2H), 2.59 (br. s., 1H), 2.88 (d, *J* =18.3 Hz, 1H), 3.23 (d, *J* = 12.3 Hz, 0.5H), 3.39 (d, *J* = 12.5 Hz, 0.5H), 3.50 (d, *J* = 14.1 Hz, 1H), 3.72 (d, *J* = 12.8 Hz, 0.5H), 3.91-4.08 (m, 2H), 4.14 (d, *J* = 12.5 Hz, 0.5H), 4.24 (d, *J* = 18.6 Hz, 1H), 7.18 - 7.41 (m, 5H).

^{13}**C NMR (400 MHz, CDCl$_3$) δ =** (23.2, 23.5), 28.5 (3C), 33.8, (41.8, 43.9), (50.9, 51.1), (52.2, 53.2), 52.9, 57.7, (69.5, 69.8), 80.5, 127.1, 128.2 (2C), (128.7, 128.9) (2C), (138.6, 138.8), 154.5, 210.5.

IR ν_{max} (KBr): 2912, 2870, 1705, 1462, 1456, 1377, 1169, 1140, 758, 744, 731, 102 cm^{-1}.

HRMS (ESI) calcd for $C_{20}H_{29}N_2O_3$ $[M+H]^+$ 345.2173, found 345.2171.

(6a*RS*,11a*RS*,12a*RS*)-3,4-Benzo-11a-methyl-6a,7,8,9,10,11a,12,12a-octahydrocyclohepta[4,5]pyrrolo[1,2-*a*][1,4]diazepine-1,5,11(2*H*)-trione (29)



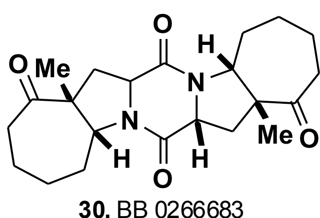
29, BB 0265807

m.p. > 220 °C (dec.).

^{1}**H NMR: (CDCl$_3$, 400 MHz) δ =** 1.21-1.31 (m, 1H), 1.28 (s, 3H), 1.31-1.53 (m, 2H), 1.59-1.76 (m, 1H), 1.80-1.92 (m, 1H), 1.93-2.06 (m, 3H), 2.38-2.50 (m, 1H), 2.71 (td, *J* = 12.8, 1.8 Hz, 1H), 3.24 (dd, *J* = 14.1, 9.1 Hz, 1H), 4.12 (t, *J* = 8.6 Hz, 1H), 4.41 (d, *J* = 11.1 Hz, 1H), 7.12 (d, *J* = 8.1 Hz, 1H), 7.27 (t, *J* = 7.3 Hz, 1H), 7.51 (td, *J* = 8.0, 1.3 Hz, 1H), 8.00 (dd, *J* = 7.8, 1.1 Hz, 1H), 9.70 (s, 1H).

^{13}**C NMR (400 MHz, CDCl$_3$) δ =** 24.6, 27.7, 28.0, 31.9, 33.6, 41.1, 55.0, 57.0, 65.9, 121.4, 125.3, 126.0, 131.2, 132.9, 135.4, 165.8, 171.1, 213.6.

Diketopiperazine (30)



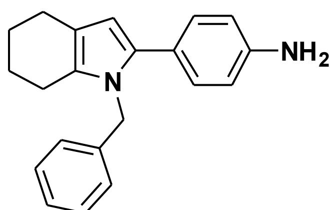
30, BB 0266683

^{1}**H NMR: (CDCl$_3$, 400 MHz) δ =** 1.01 (dd, *J* = 24.7, 12.0 Hz, 2H), 1.34 (s, 6H), 1.36-1.50 (m, 2H), 1.51-1.67 (m, 2H), 1.86 (d, *J* = 11.7 Hz, 2H), 1.96 (d, *J* = 9.8 Hz, 2H), 2.12 (dd, *J* = 13.6, 6.7 Hz, 4H), 2.39-2.52 (m, 4H), 2.68 (t, *J* = 11.0 Hz, 2H), 3.91 (d, *J* = 10.8 Hz,

2H), 4.23 (dd, J = 10.9, 7.1 Hz, 2H).

^{13}C NMR (400 MHz, $CDCl_3$) δ = 23.6, 27.6, 27.7, 32.3, 32.4, 40.8, 58.0, 59.1, 64.6, 167.7, 212.5.

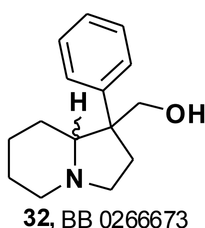
4-(1-Benzyl-4,5,6,7-tetrahydro-1*H*-indol-2-yl)aniline (31)[6]



The full synthetic procedure for THI **31** and analogs **34**-**44** as well as for intermediate aminopropargylic alcohols **46a-e** will be reported below. Though these compounds and synthetic procedures are already thoroughly described in our previous article[6] they will be given in this SI for convenience.

Copies of the NMR spectra (^{1}H and ^{13}C) are provided below only for unknown compounds: aminopropargylic alcohols **46d,e** and 4,5,6,7-tetrahydro-1H-indoles **37** and **38**. For others see reference.[6]

(1-Phenyloctahydroindolizin-1-yl)methanol (32)

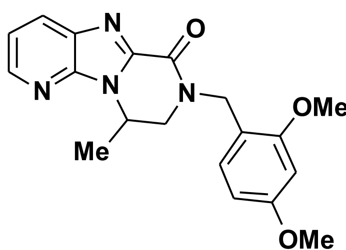


brown oil

Mixture of racemic diastereoisomers (~ 2:1)

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 1.20-1.73 (m, 6H), 1.85-2.02 (m, 2H), 2.11 (ddd, *J* = 12.8, 10.2, 2.5 Hz, 1H), 2.26-2.34 (m, 1H), 2.41-2.73 (m, 2H), 3.06 (dt, *J* = 12.6, 2.0 Hz, 0.4H), 3.16 (d, *J* = 11.0 Hz, 0.6H), 3.20-3.29 (m, 1H), 3.67 (dd, *J* = 10.0, 1.3 Hz, 0.5H), 3.75 (d, *J* = 10.4 Hz, 0.5H), 3.87 (d, *J* = 10.4 Hz, 0.5H), 4.06 (d, *J*=10.0 Hz, 0.5H), 7.19 - 7.37 (m, 5 H).

7-(2,4-Dimethoxybenzyl)-9-methyl-7H,9H-pyrido[3',2':4,5]imidazo[1,2-a]pyrazine-6,8-dione (33)[7]



m.p. = 174 – 176 ºC.

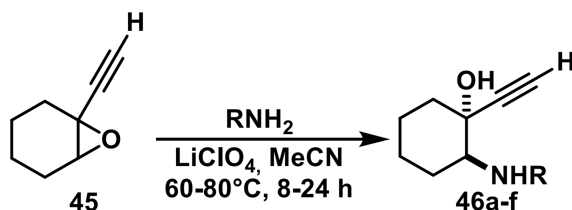
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.02 (d, *J* = 7.1 Hz, 3H), 3.78 (s, 3H), 3.81 (s, 3H), 5.10-5.13 (m, 1H), 5.31-5.33 (m, 1H), 5.52 (q, *J* = 7.0 Hz, 1H), 6.41-6.43 (m, 2H), 7.20 (d, *J* = 8.8 Hz, 1H), 7.42 (dd, *J* = 8.2, 4.6 Hz, 1H), 8.28 (dd, *J* = 8.2, 1.2 Hz, 1H), 8.59 (dd, *J* = 4.6, 1.2, 1H).

^{13}C NMR: ($CDCl_3$, 100 MHz): δ = 20.7, 39.6, 54.2, 55.4, 55.5, 98.6, 104.1, 115.9, 118.6, 120.7, 130.5, 130.6, 136.1, 145.8, 147.8, 155.3, 158.5, 160.6, 169.0.

[6] I. A. Andreev, D. S. Belov, A. V. Kurkin, M. A. Yurovskaya, *Eur. J. Org. Chem*. **2013**, 649 − 652.

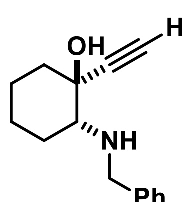
[7] Bukhryakov, K. V.; Kurkin, A. V.; Yurovskaya, M. A. *Chemistry of Heterocyclic Compounds*, **2012**, *48*, 773 – 784.

General procedure for lithium perchlorate mediated epoxide opening with various amines.



To a vigorously stirred solution of epoxide **45** (1 equiv) and amine (1.5 to 3 equiv), alanine ethyl ester[▿] (2 equiv), glycine amide (2 equiv) or alanine amide[·] (2 equiv) in acetonitrile (1 M solution of epoxide) lithium perchlorate (1.5 equiv) was added in one portion. The reaction mixture was stirred at 50-80°C until the full consumption of the starting epoxide (TLC control, typically 8-24 h). The overheating is strictly undesirable and leads to the decrease in yields. The reaction mixture was cooled to an ambient temperature and poured into 2 volumes of water followed by the extraction with 2 to 3 times (half of the reaction mixture volume each time) of dichloromethane. The combined organic extracts were dried over an anhydrous sodium sulfate and concentrated under reduced pressure on a rotary evaporator. The residue was purified by flash chromatography (eluting with petroleum ether (PE) – EtOAc (EA) in proportions varying from 10:1 to 1:1 in the case of **46a-d** or with CH_2Cl_2 – MeOH in proportions varying from 30:1 to 15:1 in the case of **46e,f**) to afford amino propargylic alcohols **46a-f** as bright to dark yellow/orange oils (**46a-d**) or white solids (**46e,f**).

(1*RS*,2*SR*)-2-(Benzylamino)-1-ethynylcyclohexanol (46a)[6]



Compound **46a** was synthesized according to the general procedure from epoxide **45** (20.00 g, 163.7 mmol) and benzylamine (35.09 g, 327.4 mmol, 2 equiv) at 60°C and isolated in the amount of 34.17 g (91%) as a bright-yellow oil. R_f =0.20 (petroleum ether – EtOAc, 3:1).

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 1.18-1.52 (m, 4H), 1.55-1.82 (m, 3H), 2.09-2.21 (m, 2H), 2.38 (dd, *J* = 11.3, 3.8 Hz, 1H), 2.45 (s, 1H), 3.71 (d, *J* = 13.0 Hz, 1H), 4.01 (d, *J*= 13.0 Hz, 1H), 4.33 (br. s., 1H), 7.24-7.29 (m, 1H), 7.31-7.38 (m, 4H).

^{13}C NMR: ($CDCl_3$, 100 MHz) δ = 23.1, 25.1, 28.7, 37.8, 50.8, 64.7, 71.8, 74.1, 85.2, 127.2, 128.2 (2C), 128.5 (2C), 140.3.

IR ν_{max} (KBr): 3465 (br), 3296, 2935, 2858, 1452, 1369, 1095, 1072, 1032, 741, 700 cm^{-1};

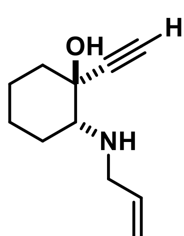
▿ Ethyl ester of L-alanine was preliminary obtained in a free base form from the corresponding hydrochloride by the CH_2Cl_2 extraction from K_2CO_3 solution in 73% yield.

· The free base of glycine and alanine amide was obtained by the treatment of a vigorously stirred 1M suspension of hydrochloride in *i*PrOH with 1 equiv of solid NaOH followed by the filtration (typically after 10-12 h) of the precipitated NaCl and subsequent evaporation of the filtrate in 93% and 98% yield respectively.

m/z (I_{rel}, %): 229 (MH^+, 2), 138 (9), 132 (11), 120 (9), 92 (12), 91 (100), 65 (26), 53 (18), 53 (18), 41 (14), 39 (18).

Anal. Calcd for $C_{15}H_{19}NO$: C, 78.56; H, 8.35; N, 6.11. **Found:** C, 78.47; H, 8.17; N, 6.00.

(1*RS*,2*SR*)-2-(Prop-2-en-1-ylamino)-1-ethynylcyclohexanol (46b)[6]



Compound **46b** was synthesized according to the general procedure from epoxide **45** (5.00 g, 40.9 mmol) and allylamine (9.2 ml, 122.8 mmol, 3 equiv) at 50°C and isolated in the amount of 6.53 g (89%) as non-viscous orange oil after the flash chromatography with PE/EA = 10:1. R_f = 0.18 (petroleum ether – EtOAc, 3**:**1).

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 1.06 (br. s., 1H), 1.19-1.33 (m, 2H), 1.48 (td, *J* = 12.6, 4.0Hz, 1H), 1.54-1.82 (m, 3H), 2.02-2.17 (m, 2H), 2.32 (dd, *J* = 10.9, 3.6Hz, 1H), 2.46 (s, 1H), 3.18 (dd, *J* = 13.9, 5.9Hz, 1H), 3.47 (dd, *J* = 13.9, 5.9, Hz, 1H), 4.32 (br. s., 1H), 5.10 (d, *J* = 10.2 Hz, 1H), 5.20 (dd, *J* = 17.1, 1.6Hz, 1H), 5.90 (dddd, *J* = 17.1, 11.1, 5.9, 1.6Hz, 1H).

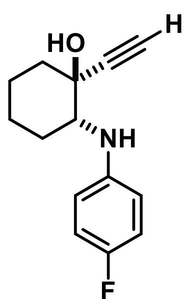
^{13}C NMR: ($CDCl_3$, 100 MHz) δ = 23.2, 25.2, 28.9, 37.9, 49.5, 64.7, 71.8, 74.0, 85.3, 116.0, 137.3.

m/z ($I_{rel.}$, %): 179 (0.7, MH^+), 68 (31), 65 (28), 56 (25), 55 (25), 54 (26), 53 (54), 41 (100), 39 (46), 32 (34).

IR ν_{max} (KBr): 3464 (br.),3306 (br.), 3079w, 2934s, 2860m, 1642w, 1448m, 1369m, 1074m, 921m, 850m, 776m, 648m cm^{-1}.

Anal. Calcd for $C_{17}H_{21}NO$: C, 79.96; H, 8.29; N, 5.49. **Found:** C, 80.01; H, 8.01; N, 5.50.

(1*RS*,2*SR*)-1-Ethynyl-2-[(4-fluorophenyl)amino]cyclohexanol (46c)[6]



Compound **46c** was synthesized according to the general procedure from epoxide **45** (2.00 g, 16.4 mmol) and 4-fluoroaniline (3.64 g, 32.7 mmol, 2 equiv), stirring the reaction mixture at 70°C for 24 h, and isolated in the amount of 2.89 g (76%) as a brown solid with **m.p.** = 78 – 80°C. R_f = 0.42 (petroleum ether – EtOAc, 3**:**1); R_f = 0.13 (petroleum ether – EtOAc, 10**:**1).

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 1.24-1.42 (m, 2H), 1.56-1.70 (m, 2H), 1.70-1.82 (m, 2H), 1.97-2.04 (m, 1H), 2.19-2.25 (m, 1H), 2.60 (s, 1H), 2.74 (dd, *J* = 11.1, 3.4 Hz, 1H), 3.46 (s, 1H), 3.51 (br. s., 1H), 6.69 (dd, *J* = 8.9, 4.4 Hz, 2H), 6.69 (t, *J* = 8.9, 2H).

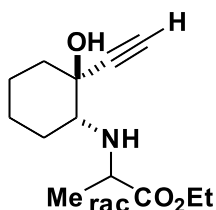
^{13}C NMR: ($CDCl_3$, 100 MHz) δ = 23.3, 25.1, 30.1, 38.1, 62.9, 72.5, 75.1, 84.4, 115.9 (d, *J* = 15.4 Hz, 2C), 116.1 (2C), 143.5, 156.6 (d, *J* = 236.4 Hz).

m/z (I_{rel}, %): 233 (46), 150 (78), 137 (60), 136 (65), 124 (100), 122 (49), 111 (45), 95 (47).

IR ν_{max} (KBr): 3510 (w), 3408 (w), 3298, 2937, 2862, 1512, 1219, 1063, 823, 656 cm^{-1}.

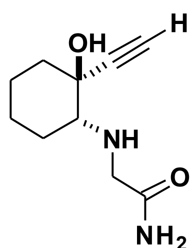
Anal. Calcd for $C_{14}H_{16}FNO$: C, 72.08; H, 6.91; N, 6.00. **Found:** C, 72.25; H, 6.80; N, 5.83.

Ethyl 2-{[(1*RS*,2*SR*)-2-ethynyl-2-hydroxycyclohexyl]amino}propanoate (46d)[6]



Compound **46d** was synthesized according to the general procedure from epoxide **45** (4.99 g, 40.8 mmol) and ethyl L-alaninate (9.57 g, 81.7 mmol, 2 equiv, free base form) stirring at 65°C for 24 h and isolated as a ~1:1 mixture of two diastereomers as dark-yellow oil (4.57 g, 47%; MH^+ = 239, $\mathbf{I_{rel}}$= 2%). R_f = 0.22÷0.34 (mixture of diastereomers, petroleum ether – EtOAc, 3**:**1). The increase of the quantity of either amino acid ester or lithium perchlorate doesn't improve the yield of **2f**. Obtained diastereomeric mixture was subjected directly to the cyclization step without separation.

2-((1*RS*,2*SR*)-2-Ethynyl-2-hydroxycyclohexylamino)acetamide (46e)



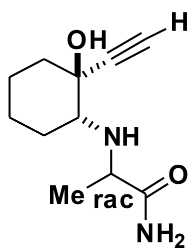
Compound **46e** was synthesized according to the general procedure from epoxide **45** (500 mg, 4.1 mmol) and glycine amide (606 mg, 8.2 mmol, 2equiv, free base form), stirring the reaction mixture at a reflux temperature for 16 h (the complete consumption of the starting epoxide occurred). The reaction mixture was poured into water and washed twice with dichloromethane prior to the saturation with an appropriate cooling with a solid potassium carbonate to achieve a 50% aqueous solution approx. The solids were filtered off and washed with EtOAc. The filtrate was extracted with EtOAc, dried over an anhydrous sodium sulfate and concentrated under reduced pressure on a rotary evaporator to afford 824 mg of a crude amino propargylic alcohol. Flash-chromatography of the residue by dichloromethane – methanol, 15**:**1 affords 571 mg (71%) of a yellow oil which slowly crystallizes into light-yellow (or beige) solid with **m.p.** = 134 – 136 °C. R_f = 0.22 (CH_2Cl_2 – MeOH, 15**:**1; $KMnO_4$ visualization – white spot).

On a ten times bigger quantities (5.12 g of **45** and 6.2 g of glycine amide free base) instead of chromatographic purification to prevent prolonged separations (the title compound absorbs decently on SiO_2) crystallization techniques were applied. Almost completely evaporated EtOAc extract was treated with a minimal amount of CH_2Cl_2. The resulting precipitate was filtered off and washed with Et_2O with rubbing to afford 3.59 g (~ 44%) of an off-white solid. The filtrate was evaporated to dryness, treated with hot benzene and decanted from orange insoluble oil. The extract was evaporated to dryness and treated with rubbing with ether. The resulting light-yellow solid was filtered off, washed with ether and dried on air to provide the second less pure portion in the amount of 2.03 g (~ 25%). The total yield was 5.62 g (68%).

^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 1.05-1.21 (m, 2H), 1.33-1.48 (m, 2H), 1.51-1.66 (m, 2H), 1.75-1.89 (m, 2H), 1.93 (br. s., 1H), 2.16 (dd, J = 10.0, 3.2 Hz, 1H), 3.02 (d, J = 16.6 Hz, 1H), 3.21 (d, J = 16.6 Hz, 1H), 3.27 (s, 1H), 5.59 (s, 1H), 7.03 (s, 1H), 7.53 (s, 1H).

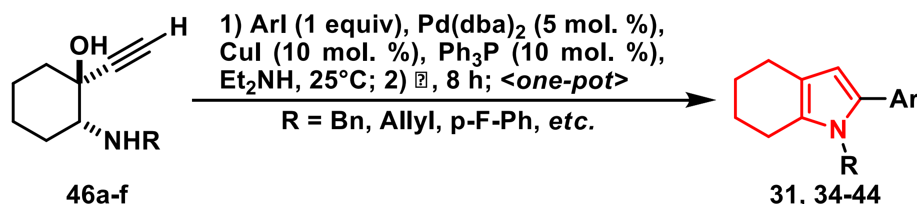
^{13}C NMR: (DMSO-d_6, 100 MHz) δ = 22.9, 24.1, 29.1, 39.1, 49.9, 65.1, 71.4, 75.8, 86.0, 174.3.

2-((1*RS*,2*SR*)-2-Ethynyl-2-hydroxycyclohexylamino)propanamide (46f)



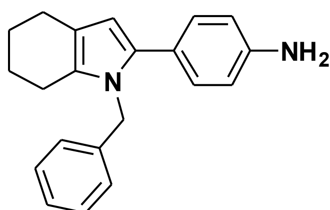
Compound **46f** was synthesized according to the general procedure from epoxide **45** (4.00 g, 32.7 mmol) and alanine amide (5.77 g, 65.5 mmol, 2equiv, free base form) stirring the reaction mixture at a reflux temperature for 24 h (the complete consumption of the starting epoxide occurred). The reaction mixture was poured into 100 ml of water and saturated with an appropriate cooling with a solid potassium carbonate to achieve a 50% aqueous solution approx. The solids were filtered off and washed with EtOAc. The filtrate was extracted with EtOAc, dried over an anhydrous sodium sulfate and concentrated under reduced pressure on a rotary evaporator to afford ~ 7 g of a crude amino propargylic alcohol. Flash-chromatography of the residue by dichloromethane – methanol, 30**:**1 affords 4.00 g (58%) of yellow oily crystals of **46f** as a ~1:1 mixture of two diastereomers. R_f = 0.19 (CH_2Cl_2 – MeOH, 30**:**1; $KMnO_4$ visualization – white spot). Rubbing of the residue in Et_2O and subsequent filtration affords 2.63 g (38%) as a fluffy white solid with **m.p.** = 121 – 123 °C. Obtained diastereomeric mixture was subjected directly to the cyclization step without separation.

General procedure for the synthesis of 4,5,6,7-tetrahydro-1*H*-indoles: *one-pot* tandem Sonogashira coupling/5-endo-dig metal-catalyzed cyclization, employing aminopropargylic alcohols 2a-f as a starting material.[6]



1 equiv (typically **1.00 g** unless otherwise stated) of amino propargylic alcohol **46a-e**, 1 equiv of aryl iodide and 0.1 equiv of triphenylphosphine are placed in a 50 ml oven-dried Schlenk flask equipped with a magnetic stirring bar and a water condenser fitted with an oil bubbler. The reaction vessel is charged with 20 equiv of diethyl amine (**commonly 9 ml**) and after the complete dissolution of the starting material a strong nitrogen flush is introduced for a period of 2-3 minutes. The pressure of inert gas is decreased and 0.05 equiv of Pd(dba)$_2$ followed by 0.1 equiv of CuI are added. The vessel is flushed with a strong stream of nitrogen once again (1 min), the pressure of inert gas is decreased and the reaction mixture is stirred under a slow stream of nitrogen at an ambient temperature overnight (10 to 20 hours). Then reaction mixture is refluxed under a slow stream of nitrogen for 8-12 h (TLC control is possible, generally applying petroleum ether – EtOAc, 3**:**1). The reaction mixture is cooled to an ambient temperature and poured into 50 ml of saturated NH_4Cl solution. The resulting mixture is extracted 3-4 times with 50 ml portions of CH_2Cl_2. Combined organic extracts are dried over anhydrous Na_2SO_4 and concentrated under reduced pressure on a rotary evaporator. The resulting crude mixture is subjected to the flash chromatography, generally (unless otherwise noted) eluting with petroleum ether – EtOAc, 100**:**1 to 50**:**1 to obtain an analytically pure compound.

4-(1-Benzyl-4,5,6,7-tetrahydro-1*H*-indol-2-yl)aniline (31)[6]



The crude reaction mixture was flash chromatographied with petroleum ether – EtOAc, 4**:**1 to afford 1.01 g (77%) of **31** as a deep orange thick oil. R_f = 0.27 (petroleum ether – EtOAc, 3**:**1).

^{1}H NMR: (CDCl$_3$, 400 MHz) δ = 1.73-1.88 (m, 4H), 2.35-2.49 (m, 2H), 2.56-2.69 (m, 2H), 3.64 (s, 2H), 5.07 (s, 2H), 6.06 (s, 1H), 6.62 (d, *J*= 8.3 Hz, 2H), 7.00 (d, *J*= 7.2 Hz, 2H), 7.13 (d, *J*= 8.5 Hz, 2H), 7.22-7.29 (m, 1H), 7.29-7.36 (m, 2H).

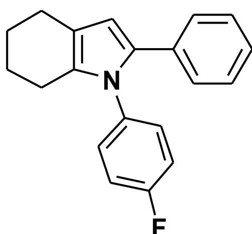
^{13}C NMR: (CDCl$_3$, 100 MHz) δ =22.4, 23.2, 23.5, 23.8, 47.3, 106.4, 115.0 (2C), 117.5, 124.2, 125.9 (2C), 126.9, 128.7 (2C), 130.0 (2C), 134.0, 138.0, 139.6, 145.3.

m/z (I_{rel}, %):303 (13), 302 (57, MH^+), 212 (17), 211 (100), 120 (17), 92 (18), 91 (78), 65 (36), 41 (11), 39 (15).

IR ν_{max} (KBr): 3459m (br.), 3363s (br.), 3217w, 3026m, 2926s (br.), 2847s (br.), 1953w, 1887w, 1620s, 1534s, 1482s, 1443s, 1385s, 1285s, 1177s, 833s, 784s, 738scm^{-1}.

Anal. Calcd for $C_{21}H_{22}N_2$: C, 83.40; H, 7.33; N, 9.26. **Found**: C, 83.78; H, 7.16; N, 9.04.

1-(4-Fluorophenyl)-2-phenyl-4,5,6,7-tetrahydro-1*H*-indole (34)[6, 8]



Crude reaction mixture is flash chromatographied with petroleum ether – EtOAc, 10**:**1 to afford 1.15 g (92%) of **34** as a light-brown solid with **m.p.** = 129 – 131 °C, **lit. m.p.[8]** = 129 – 130 °C. R_f = 0.60 (petroleum ether – EtOAc, 3**:**1).

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 1.77-1.90 (m, 4H), 2.39-2.48 (m, 2H), 2.59-2.70 (m, 2H), 6.27 (s, 1H), 7.00-7.22 (m, 9H).

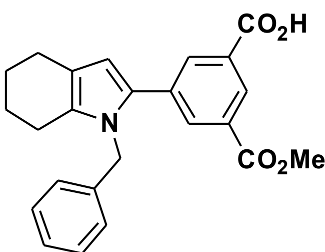
^{13}C NMR: ($CDCl_3$, 100 MHz) δ = 23.2, 23.3, 23.5, 23.7, 108.8, 115.9 (d, *J*= 23.0 Hz, 2C), 118.6, 125.9, 128.0 (2C), 128.1 (2C), 129.6 (d, *J*= 8.4 Hz, 2C), 131.2, 133.3, 133.3, 135.3, 161.4 (d, *J*= 246.9 Hz, C).

m/z (I_{rel}, %): 292 (23), 291 (100, MH^+), 290 (18), 264 (12), 263 (56), 262 (35), 95 (22), 77 (16), 75 (15), 39 (11).

IR ν_{max} (KBr): 3057 (w), 2926 (s), 2851 (m), 1896 (w), 1651 (w), 1601 (m), 1506 (s), 1441 (m), 1387 (m), 1287 (w), 1217 (s), 1138 (w), 1090 (m), 974 (w), 845 (s), 820 (m), 802 (m), 758 (s), 698 (s), 577 (m) cm^{-1}.

Anal. Calcd for $C_{20}H_{18}FN$: C, 82.45; H, 6.23; N, 4.81; F, 6.52. **Found**: C, 82.32; H, 6.03; N, 4.90.

3-(Methoxycarbonyl)-5-(1-benzyl-4,5,6,7-tetrahydro-1*H*-indol-2-yl)benzoic acid (35)[6]



Flash chromatography with CH_2Cl_2 – MeOH = 20**:**1 affords 1.33 g (78%) of **35** as dark orange foam (*sample is of **non**-analytical purity*). R_f = 0.59 ($CHCl_3$ – MeOH, 7**:**1). To obtain the sample of the analytical purity in addition to flash chromatography, compound was subjected to column chromatography (eluting firstly with CH_2Cl_2 and then with CH_2Cl_2 – MeOH = 20**:**1). Thus obtained dark yellow foam was dissolved in 1 ml of diethyl ether followed by 1 ml of petroleum ether yielding gum which was rubbed. The resulting solid was filtered off, washed with small portions of petroleum ether and dried on air to afford 120 mg (7%) of **35** as a pistachio-green solid of analytical purity with **m.p.** = 161 – 163 °C.

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[8] K. Nagarajan, P. K. Talwalker, R. K. Shah, S. R. Mehta, G. V. Nayak, *Ind. J. Chem., Section B: Org. Chem. Incl. Med. Chem.* **1985**, 24, 98 − 111.

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 1.73-1.90 (m, 4H), 2.48 (t, *J*= 5.6 Hz, 2H), 2.61 (t, *J*= 5.5 Hz, 2H), 3.88 (s, 3H), 5.12 (s, 2H), 6.26 (s, 1H), 6.92 (d, *J*= 7.2 Hz, 2H), 7.20-7.35 (m, 3H),8.18 (t, *J*=1.6 Hz, 1H), 8.56 (t, *J*=1.5 Hz, 1H).

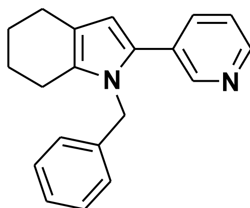
^{13}C NMR: ($CDCl_3$, 100 MHz) δ = 22.4, 23.2, 23.4, 23.8, 47.6, 52.5, 109.3, 118.7, 125.8 (2C), 127.3, 128.9 (2C), 130.1, 131.0, 131.4, 131.5, 132.3, 133.8, 134.0, 134.8, 166.2, 171.1.

m/z (I_{rel}, %): 390 (5), 389 (22, MH^+),298 (13), 239 (3), 194 (7), 92 (11), 91 (100), 65 (14), 59 (4), 77 (4).

IR ν_{max} (KBr): 2928m, 2844m, 2626m (br.), 1724s, 1696s, 1603m, 1501w, 1436m, 1395w, 1323m, 1265s, 1139w, 1077w, 998w, 917w, 757m, 727w, 697w cm^{-1}.

Anal. Calcd for $C_{24}H_{23}NO_4$: C, 74.02; H, 5.95; N, 3.60; O, 16.43. **Found**: C, 73.80; H, 5.99; N, 3.40.

1-Benzyl-2-pyridin-3-yl-4,5,6,7-tetrahydro-1*H*-indole (36)[6]



The crude reaction mixture was flash chromatographied with petroleum ether – EtOAc, 3:1 to afford 0.98 g (78%) of **36** as a deep orange thick oil. R_f = 0.75 (petroleum ether – EtOAc, 1:1).

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 1.72-1.86 (m, 4H), 2.38-2.49 (m, 2H), 2.55-2.65 (m, 2H), 3.88 (s, 3H), 5.07 (s, 2H), 6.18 (s, 1H), 6.93 (d, *J*= 7.5 Hz, 2H), 7.18 (dd, *J*= 7.5, 4.8 Hz, 1H),7.21-7.27 (m, 1H), 7.30 (t, *J*= 7.6 Hz, 2H), 7.53 (d, *J*= 7.8 Hz, 1H), 8.44 (br. s., 1H), 8.59 (br. s., 1H).

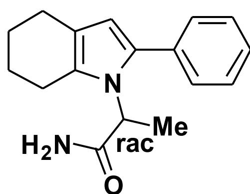
^{13}C NMR: ($CDCl_3$, 100 MHz) δ = 22.3, 23.2, 23.4, 23.7, 47.4, 108.8, 118.6, 123.3, 125.7 (2C), 127.3, 128.9 (2C), 129.8, 129.9, 131.2, 135.3, 138.8, 147.6, 149.4.

m/z (I_{rel}, %): 288 (31, MH^+),197 (17), 195 (24), 92 (23), 91 (100), 77 (17), 65 (54), 51 (23), 39 (26), 32 (32).

IR ν_{max} (KBr): 3028w, 2930s, 2849m, 1594w, 1564m, 1496m, 1452m, 1380m, 1301m, 1022m, 793m, 726s cm^{-1}.

Anal. Calcd for $C_{20}H_{20}N_2$: C, 83.3; H, 6.99; N, 9.71. **Found**: C, 83.32; H, 6.82; N, 9.95.

2-(2-Phenyl-4,5,6,7-tetrahydro-1H-indol-1-yl)propanamide (37)

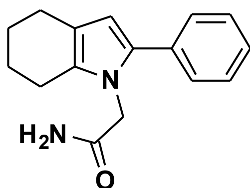


Compound **37** was obtained according to the general procedure as a beige solid with **m.p.** = 164 – 166 °C in a racemic form in the amount of 835 mg (65%) after two successive flash chromatographic separations eluting with CH_2Cl_2 – MeOH, 100:1. R_f = 0.27 (CH_2Cl_2 – MeOH, 100:1).

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 1.66 (d, *J* = 7.3 Hz, 3H), 1.69-1.77 (m, 2H), 1.77-1.86 (m, 1H), 1.87-1.98 (m, 1H), 2.50-2.70 (m, 4H), 4.88 (q, *J* = 7.3 Hz, 1H), 5.27 (br. s, 1H), 5.67 (br. s, 1H), 6.04 (s, 1H), 7.28-7.35 (m, 3H), 7.35-7.43 (m, 2H).

^{13}C NMR: ($CDCl_3$, 100 MHz) δ = 16.7, 23.2, 23.5, 23.7, 24.1, 54.4, 109.0, 119.9, 127.3, 128.8 (2C), 129.0 (2C), 129.5, 133.3, 134.7, 174.8.

2-(2-Phenyl-4,5,6,7-tetrahydro-1H-indol-1-yl)acetamide (38)

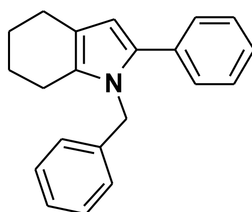


The starting amino propargylic alcohol **46d** (522 mg, 2.7 mmol) was insoluble in diethyl amine. Thus, after the addition of the catalyst the reaction mixture was heated to reflux with a heatgun for 5 min to afford a turbid solution and immediately cooled to an ambient temperature by the means of external bath (containing cold water) which led to an orange transparent solution. TLC control showed the complete consumption of the starting material (R_f = 0.22 (CH_2Cl_2 – MeOH, 15**:**1); $KMnO_4$ visualization – white spot) and presumably the appearance of the arylated amino propargylic alcohol with R_f = 0.42 (CH_2Cl_2 – MeOH, 15**:**1). The reaction mixture was then refluxed for 10 h, cooled to r.t. and worked up as usual. Flash chromatography with CH_2Cl_2 – MeOH, 50**:**1 afforded 515 mg (76%) of **38** as a tan solid with **m.p.** = 190 – 192 °C. R_f = 0.23 (CH_2Cl_2 – MeOH, 50**:**1); R_f = 0.16 (CH_2Cl_2 – MeOH, 100**:**1).

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 1.72-1.82 (m, 2H), 1.83-1.93 (m, 2H), 2.54 (t, *J* = 5.9 Hz, 4H), 4.47 (s, 2H), 5.45 (br. s, 1H), 6.06 (br. s, 1H), 6.10 (s, 1H), 7.26-7.34 (m, 3H), 7.35-7.41 (m, 2H).

^{13}C NMR: ($CDCl_3$, 100 MHz) δ = 22.1, 23.1, 23.3, 23.6, 47.7, 109.1, 119.4, 127.2, 128.5 (2C), 128.9 (2C), 130.0, 132.8, 133.8, 172.4.

1-Benzyl-2-phenyl-4,5,6,7-tetrahydro-1*H*-indole (39)[6, 9]



Flash chromatography affords **39** as a yellow solid with **m.p.** = 83 – 84°C, **lit. m.p.**[9] = 72 – 73°C in the amount of 0.97 g (75%). R_f = 0.86 (petroleum ether – EtOAc, 3**:**1).

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 1.74-1.87 (m, 4H), 2.42 (t, *J*= 5.5 Hz, 2H), 2.62 (t, *J*= 5.5 Hz, 2H), 5.11 (s, 2H), 6.14 (s, 1H), 6.99 (d, *J*= 7.4 Hz, 2H), 7.21-7.37 (m, 8H).

^{13}C NMR: ($CDCl_3$, 100 MHz) δ = 22.4, 23.2, 23.5, 23.8, 47.5, 107.6, 118.1, 125.9 (2C), 126.6, 127.0, 128.5 (2C), 128.7 (2C), 128.8 (2C), 130.0, 133.8, 133.9, 139.4.

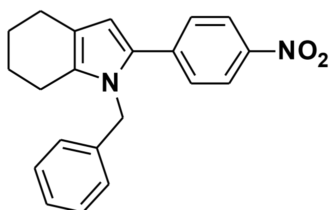
m/z (I_{rel}, %): 288 (19), 287 (82, MH^+), 259 (10), 241 (27), 240 (100), 213 (13), 197 (10), 196 (66), 194 (12), 91 (100), 77 (11), 65 (25), 39 (9).

IR ν_{max} (KBr): 3062w, 3029w, 2928s, 2849s, 1604m, 1443m, 1356m, 1299m, 793w, 761s, 723m, 698s cm^{-1}.

[9] M. A. Volodina, E. A. Pronina, V. G. Mishina, A. P. Terentev, *J. Gen. Chem. USSR* **1963**, 33, 3223.

Anal. Calcd for $C_{21}H_{21}N$: C, 87.76; H, 7.36; N, 4.87. **Found**: C, 87.71; H, 7.21; N, 4.89.

1-Benzyl-2-(4-nitrophenyl)-4,5,6,7-tetrahydro-1*H*-indole (40)[6]



Flash chromatography affords 1.34 g (93%) of **40** as a bright yellow crystals with **m.p.** = 115 – 117°C. R_f = 0.77 (petroleum ether – EtOAc, 3**:**1). To increase the dissolution rate of the starting aryl iodide after addition of the catalyst, 1-iodo-4-nitrobenzene is preliminary grinded into an amorphous mass.

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 1.74-1.87 (m, 4H), 2.42-2.50 (m, 2H), 2.59-2.65 (m, 2H), 5.15 (s, 2H), 6.99 (d, *J*=7.2 Hz, 2H), 7.26-7.38 (m, 3H),7.40 (d, *J*=9.0 Hz, 2H), 8.14 (d, *J* = 9.0 Hz, 2H).

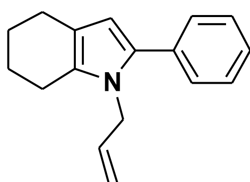
^{13}C NMR: ($CDCl_3$, 100 MHz) δ = 22.4, 23.1, 23.3, 23.6, 47.8, 110.7, 119.5, 124.2 (2C), 125.6 (2C), 127.5, 127.6 (2C), 129.1 (2C), 131.6, 133.4, 138.4, 140.1, 145.6.

m/z (I_{rel}, %): 333 (12), 332 (54, MH^+),195 (17), 194 (13), 92 (16), 91 (100), 65 (18).

IR ν_{max} (KBr): 2927, 1593, 1508, 1335, 856, 729 cm^{-1}.

Anal. Calcd for $C_{21}H_{20}N_2O_2$: C, 75.88; H, 6.06; N, 8.43. **Found**: C, 75.77; H, 6.08; N, 8.14.

2-Phenyl-1-prop-2-en-1-yl-4,5,6,7-tetrahydro-1*H*-indole (41)[6]



Flash chromatography affords **41** in the amount of 0.99 g (75%). The sample contains approx. 20 mol% (according to **^{1}H NMR** analysis) of 2H tetrahydroindole, which is inseparable by chromatographic methods. $Pd(OAc)_2$ catalyzed cyclization of the intermediate arylated tetrahydroindole[10] brings out 0.81 g (87%) of **41** as a yellow solid with **m.p.** = 64 – 65°C. R_f = 0.82 (petroleum ether – EtOAc, 3**:**1).

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 1.73-1.82 (m, 2H), 1.82-1.91 (m, 2H), 2.51-2.61 (m, 4H), 4.43 (ddd, *J* = 4.0, 2.2, 1.8 Hz, 2H), 4.94 (dq, *J* = 17.1, 1.6 Hz, 1H), 5.18 (dq, *J* = 10.4, 1.6 Hz, 1H), 5.86-5.99 (m, 1H), 6.06 (s, 1H), 7.22-7.30 (m, 1H), 7.31-7.43 (m, 4H).

^{13}C NMR: ($CDCl_3$, 100 MHz) δ = 22.3, 23.2, 23.5, 23.9, 46.4, 107.4, 116.1, 117.7, 126.5, 128.4 (2C), 128.6 (2C), 129.9, 133.4, 134.0, 135.2.

m/z (I_{rel}, %): 287 (82, MH^+), 237 (77), 236 (22), 209 (35), 208 (39), 196 (26), 194 (28), 115 (18), 77 (25), 41 (100), 39 (70).

IR ν_{max} (KBr): 3086w, 2912m, 2833m, 1648w, 1601m, 1389m, 1301m, 931m, 793m, 756s, 696s, 596w, 547w, 488w cm^{-1}.

Anal. Calcd for $C_{17}H_{19}N$: C, 86.03; H, 8.07; N, 5.90. **Found**: C, 85.91; H, 8.10; N, 5.94.

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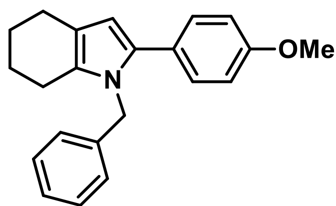
[10] I. A. Andreev, I. O. Ryzhkov, A. V. Kurkin, M. A. Yurovskaya, *Chem. Heterocycl. Compd.* **2012**, *48*, 715 − 719.

1-Benzyl-2-(4-methoxyphenyl)-4,5,6,7-tetrahydro-1*H*-indole (42)[6]



An orange oil with R_f = 0.60 (petroleum ether – EtOAc, 3**:**1) obtained by flash chromatography contained a small amount of the corresponding aryl iodide. The flask with the substance is placed in an oil bath with the internal temperature of 110-120°C for 2-3 hours and aryl iodide is distilled off on a high-vac system. The residue crystallizes to afford 1.17 g (85%) of **42** as an orange solid with **m.p.** = 94 – 96°C.

^{1}H NMR: (CDCl$_3$, 400 MHz) δ = 1.72-1.87 (m, 4H), 2.37-2.46 (m, 2H), 2.58-2.66 (m, 2H), 3.80 (s, 3H), 5.06 (s, 2H), 6.07 (s, 1H), 6.82-6.88 (m, 2H), 6.98 (d, *J*= 7.0 Hz, 2H), 7.21-7.27 (m, 3H), 7.29-7.36 (m, 2H).

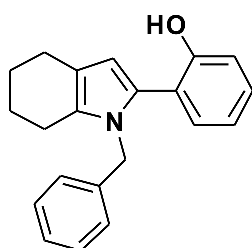
^{13}C NMR: (CDCl$_3$, 100 MHz) δ = 22.4, 23.3, 23.5, 23.9, 47.3, 55.4, 106.9, 113.9 (2C), 117.7, 125.9 (2C), 126.5, 127.0, 128.8 (2C), 129.2, 130.1 (2C), 133.5, 139.5, 158.6.

m/z (I_{rel}, %):318 (13), 317 (54, MH^+), 227 (11), 226 (62), 183 (11), 115 (8), 92 (9), 91 (100), 77 (8), 65 (22).

IR ν_{max} (KBr): 3027w, 2925s, 2851s, 1613w, 1531s, 1483s, 1450s, 1378m, 1283s, 1244s, 1174s, 1105m, 1028s, 838s, 785s, 736s, 694m, 648w, 597m, 555m cm^{-1}.

Anal. Calcd for $C_{22}H_{23}NO$: C, 83.24; H, 7.30; N, 4.41; O, 5.04. **Found**: C, 83.28; H, 7.09; N, 4.21.

2-(1-Benzyl-4,5,6,7-tetrahydro-1*H*-indol-2-yl)phenol (43)[6]



2-Iodophenol was insoluble in diethyl amine. Thus, after the addition of the catalyst the reaction mixture was heated to reflux with a heatgun and immediately cooled to an ambient temperature by the means of external bath (containing cold water) to afford a bright orange solution. Typical protocol employed afterwards lead to 0.86 g of an orange oil, which was contaminated with the corresponding aryl iodide. Flask containing substance was placed in an oil bath with the internal temperature of 80 – 90°C for 2-3 hours and aryl iodide was distilled off on a high-vac system. The residue was subjected once again to flash chromatography (eluting with petroleum ether – EtOAc, 10**:**1) to afford 0.53 g (40%) of **43** as a dark orange oil. R_f = 0.93 (petroleum ether – EtOAc, 3**:**1).

^{1}H NMR: (CDCl$_3$, 400 MHz) δ = 1.74-1.87 (m, 4H), 2.41-2.49 (m, 2H), 2.56-2.64 (m, 2H), 4.95 (s, 2H), 6.01 (s, 1H), 6.14 (s, 1H), 6.83 (dd, *J*= 7.4, 1.2 Hz, 1H), 6.84-6.89 (m, 2H), 6.95-7.00 (m, 1H), 7.08 (dd, *J*= 7.6, 1.6 Hz, 1H), 7.18-7.31 (m, 4H).

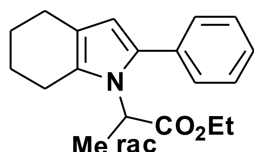
^{13}C NMR: (CDCl$_3$, 100 MHz) δ = 22.5, 23.2, 23.4, 23.7, 47.3, 108.1, 115.3, 118.4, 119.5, 120.0, 125.6, 126.0 (2C), 127.1, 128.7 (2C), 129.5, 130.7 (2C), 138.8, 154.3.

m/z (I_{rel}, %): 303 (27, MH^+), 212 (63), 115 (15), 92 (14), 91 (100), 89 (11), 77 (16), 65 (41), 41 (13), 39 (19).

IR ν_{max} (KBr): 3445s, 3269m (br.), 3061m, 2940s, 2847m, 1670m, 1603m, 1452s, 1391m, 1344m, 1283m, 1215m, 1180s, 1026m, 933w, 799w, 754s, 696mcm^{-1}.

Anal. Calcd for $C_{21}H_{21}NO$: C, 83.13; H, 6.98; N, 4.62; O, 5.27. **Found**: C, 82.99; H, 6.75; N, 4.72.

Ethyl (2*RS*)-2-(2-phenyl-4,5,6,7-tetrahydro-1*H*-indol-1-yl)propanoate (44)[6]



Compound **44** was obtained according to the general procedure as a dark orange non viscous oil in a racemic form in the amount of 1.03 g (83%). R_f = 0.84 (petroleum ether – EtOAc, 3**:**1).

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 1.30 (t, *J*= 7.0 Hz, 3H), 1.60 (d, *J*= 7.2 Hz, 3H), 1.73-1.88 (m, 3H), 1.88-1.98 (m, 1H), 2.44-2.54 (m, 1H), 2.57-2.69 (m, 3H), 4.25 (q, *J*= 7.0 Hz, 2H), 5.00 (q, *J*= 7.2 Hz, 1H), 6.04 (s, 1H), 7.30-7.37 (m, 1H), 7.38-7.46 (m, 4H).

^{13}C NMR: ($CDCl_3$, 100 MHz) δ = 14.3, 17.8, 23.3, 23.6, 23.7 (2C), 53.3, 61.6, 108.1, 118.7, 127.0, 128.5 (2C), 129.1, 129.4 (2C), 133.9, 134.2, 171.8.

m/z(I_{rel}, %): 297 (31, MH^+), 224 (32), 197 (16), 196 (100), 194 (12), 115 (13), 91 (16), 77 (18), 41 (10), 29 (88).

IR ν_{max} (KBr): 3062w, 2932s, 2849m, 1738s, 1603w, 1520w, 1443m, 1374m, 1309w, 1221s, 1075w, 1030w, 792w, 764m, 701mcm^{-1}.

Anal. Calcd for $C_{19}H_{23}NO_2$: C, 76.74; H, 7.80; N, 4.71; O, 10.76. **Found**: C, 76.85; H, 7.59; N, 4.75.

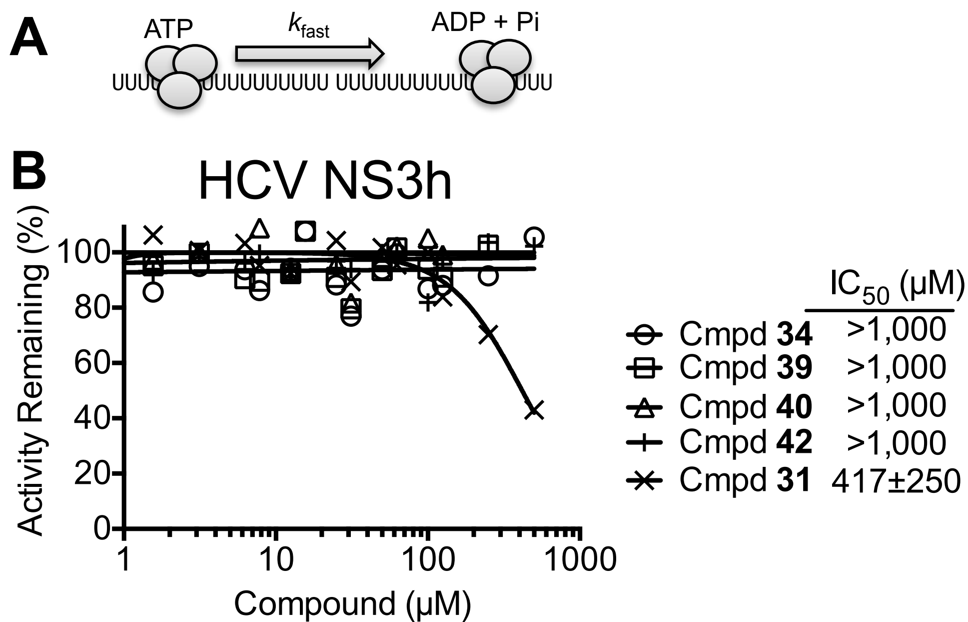


Fig. S1. Effect of compounds on RNA-stimulated helicase-catalyzed ATP hydrolysis (A) Compounds were added to assays monitoring helicase catalyzed ATP hydrolysis in the presence of RNA (B) Activity remaining in reactions catalyzed by the HCV genotype 1b (con1) NS3h in the presence of various concentrations of each compound.