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DNA-Encoded Libraries: Hydrazide as a Pluripotent Precursor for On-DNA Synthesis of Various Azole Derivatives

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Abstract: DNA-encoded combinatorial chemical library (DEL) technology, an approach that combines the power of genetics and chemistry, has emerged as an invaluable tool in drug discovery. Skeletal diversity plays a fundamental importance in DEL applications, and relies heavily on novel DNA-compatible chemical reactions. We report herein a phylogenic chemical transformation strategy using DNA-conjugated benzoyl hydrazine as a common versatile precursor in azole chemical expansion of DELs. DNA-compatible reactions deriving from the common benzoyl hydrazine precursor showed excellent functional group tolerance with exceptional efficiency in the synthesis of various azoles, including oxadiazoles, thiadiazoles, and triazoles, under mild reaction conditions. The phylogenic chemical transformation strategy provides DELs a facile way to expand into various unique chemical spaces with privileged scaffolds and pharmacophores.

Introduction

In 1992, an elegant approach using DNA barcoding technology, a.k.a DNA-encoded combinatorial chemical libraries (DELs), was first described by Lerner and Brenner,[1] in which genetics and chemistry are linked to track and playback the complete transformation processes of chemical molecules. The unique feature of DELs has gained wide attention in both the pharmaceutical industry and academia.[2] DELs make use of the combination of molecular engineering, combinatorial synthesis, deep-sequencing and advanced informatics, including machine learning, to enable the creation and screening of enormously diverse new chemical entities at an unprecedented level in a short period of time. The numerous number of structurally diverse molecules in DELs endows the technology with great potential for hit identification of drug targets with exquisite specificity through library interrogation.[3] DELs consisting of large numbers (millions to billions) can be readily constructed using a strategy called "split and pool",[4] and screened against a broad spectrum of validated protein targets via affinity-based selection. Serval drug candidates have since been discovered and entered into clinical studies, including receptor interacting protein 1 (RIP1) inhibitor GSK3145095 for pancreatic cancer,[5] RIP1 kinase inhibitor GSK2982772 for autoimmune diseases,[6] soluble epoxide hydrolase (sEH) inhibitor GSK2256294 for diabetes,[7] and selective sphingosine-1-phosphate (S1P) receptor agonist GSK18427099 for multiple sclerosis, etc.[8]

As an on-going effort to improve the diversity and quality of DELs, a number of labs, including ours, have been working to develop novel DNA-compatible reactions that generate spatial and regioselective diversities efficiently.[9] However, most traditional reaction conditions in conventional organic chemistry are not compatible with the presence of DNA.[10] Chemical transformations in the presence of DNA require aqueous media and extremely low concentrations in comparison with conventional chemical syntheses (e.g. 0.1–1 mM *vs.* 0.1—1 M, respectively). In addition, DNA-compatible reactions generally must be carried out under mild reaction conditions to ensure the stability and integrity of DNA tags. The incorporation of privileged scaffolds and pharmacophores into DELs would be greatly facilitated by the ability to carry out the required synthetic reactions in the presence of DNA. We describe here a phylogenic approach, in which a family of on-DNA chemical transformations for a diverse range of azole compounds can be carried out using a common precursor molecule.

Substituted azole derivatives with five- or six-membered heterocyclic rings have been frequently used as the fundamental pharmacophores in new drug design due to their highly desirable biochemical properties.[11] Among all the possible azole derivatives, 1,3,4-oxadiazole, 1,3,4-thiadiazole-2-amine and 1,2,4-triazole derivatives have been widely exploited for various applications.[12] Many drugs containing these azole moieties are approved for clinical use, including raltegravir and maraviroc, two anti-HIV agents;[13] nesapidil, an antihypertensive agent;[14] and acetazolamide, an anti-glaucoma and mild cardiac

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edema agent.[15] The important pharmaceutical potential of azole derivatives has led to continuing efforts to development methods for on-DNA synthesis of azole heterocyclic compounds. For example, DNA-compatible synthesis of thiazole,[16] oxazole,[17] tetrazole,[18] imidazole,[19] 1,3,4-oxadiazole[19] and 1,2,4-oxadiazole[20] have been reported in recent years. Among the synthetic transformations of azoles in conventional organic chemistry, benzoyl hydrazine has been used as a versatile functionality with a wide range of substrates to form the corresponding intermediates which can subsequently be transformed into azole derivatives under mild reaction conditions. Compared to conventional organic reactions, DEL chemical reactions usually occur at much lower, e.g. micromolar, substrate concentrations. Moreover, the DNA tag makes the separation of end-products from substrates easy and fast. These properties allow a DEL reaction to be driven kinetically,[21] thus similar organic reactions can be readily clustered around a common precursor. We chose DNA-conjugated benzoyl hydrazine as a common precursor to synthesize various on-DNA azole compounds. Here, as shown in Figure 1, we report a phylogenic approach through condensation cyclization of DNA-conjugated benzoyl hydrazine with different cyclization agents to construct azole-focused DELs. Using five-membered azole ring derivatives including 1,3,4-oxadiazole, 1,3,4-thiadiazole-2-amine, and 1,2,4-triazole as examples, we demonstrated that highly efficient on-DNA syntheses from the same precursor can be achieved.

Results and Discussion

In order to synthesize the starting materials **HP-ArHDs** (Figure 1), the synthetic route was designed as shown in Scheme S1 (see Support Information). First, using (hetero)aryl acid derivatives **1a**–**1j**, the corresponding Boc-protected (hetero)aryl hydrazide derivatives **4a**–**4j** were synthesized using a conventional three-steps reaction (Scheme S1). The DNA-conjugated benzoyl hydrazine with Boc-protection, **HP-ArHBs**, was next prepared by acylation of the DNA headpiece compound, **HP-NH$_2$** (Figure S1), with **4a**–**4j** (Scheme S2). To remove the Boc-protection group of **HP-ArHBs**, different buffer systems were screened with the Na_2HPO_4–NaH_2PO_4 buffer (pH = 6.0, 200 mM) showing the highest conversion yield (Table S1). Under the optimized reaction conditions, the de-Boc product DNA-conjugated benzoyl hydrazine **HP-ArHDs** was obtained in excellent yields at 60 °C in 12 hours (Scheme S3). As expected, all the DNA-conjugated (hetero)aryl hydrazide derivatives, **HP-ArHD-1**–**HP-ArHD-10**, could be obtained in high yields following the same synthetic route (Scheme S3).

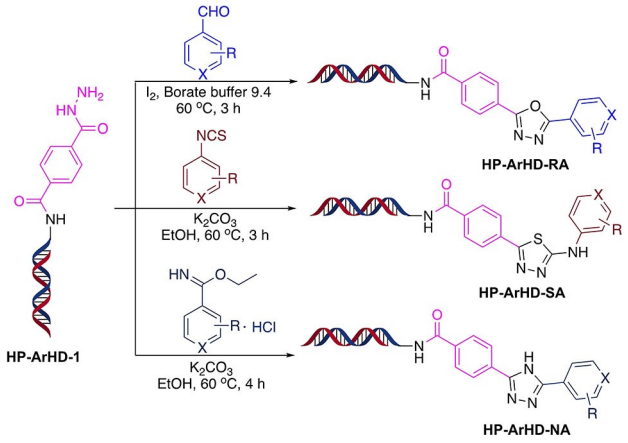

Figure 1. Synthesis of on-DNA azole derivatives.

With **HP-ArHDs** in hand, we investigated synthetic reactions of 1,3,4-oxadiazole derivatives in the presence of DNA. Conventional syntheses for 1,3,4-oxadiazole derivatives were reported using various oxidizing agents such as chloramine T,[22] ceric ammonium nitrate (CAN),[23] bis-(trifluoroacetoxy) iodobenzene,[24] trichloroisocyanuric acid (TCCA),[25] N-chlorosuccinimide,[26] $Cu(OTf)_2$,[27] and oxidized iodine.[28] Using **HP-ArHD-1** and benzaldehyde as model substrates, oxidizing agents suitable for on-DNA synthesis of 1,3,4-oxadiazole derivatives were extensively screened and validated under various reaction conditions (Table S2). For all the examined oxidizing agents including iodine, chloramine T, CAN, TCCA, Dess–Martin, and IBX, only iodine showed a low but encouraging 23% yield in DMSO at 25 °C (Table S2). Subsequently, iodine was chosen as the oxidizing agent for further optimization including buffer, solvent, temperature, and stoichiometry, etc. (Table S2). It was found that under a condition, in which 80 equiv. of benzaldehyde (in 10 μL DMSO) were mixed with 80 equiv. of I_2 (in 10 μL DMSO), 1 equiv. of **HP-ArHD-1** (in 10 μL ddH_2O), and 5 μL 250 mM borate buffer (pH = 9.4) at 60 °C for 3 h, the reaction proceeded smoothly and reached a nearly complete conversion (96% yield). In order to gauge the scope of substrates, various substituted aldehyde derivatives were next tested. As shown in Figure 2a, the aromatic derivatives of benzaldehyde with functional groups both electron-rich (**RA2-RA4**) and electron-deficient (**RA5-RA7**) at *para*-substituted positions of the aromatic ring afforded the corresponding products in medium to excellent yields (69%-98%). Notably, the benzaldehyde derivatives with fluorine- (**RA8**), chlorine- (**RA9**), bromine- (**RA10**), and iodine-substitution (**RA11**) afforded the desired products with excellent yields of 96%, 92%, 94%, and 92%, respectively. Gratifyingly, reactive functional groups such as ester (**RA12**), nitrile (**RA13**) and acid (**RA14**) were all well-tolerated in the reaction (65%-94%). Moreover, the *meta*-substituted (**RA15-RA17**) and sterically hindered *ortho*-substituted benzaldehyde derivatives (**RA18-RA20**) with electron-rich groups (–CH_3, –OCH_3) and electron-deficient (–Cl) afforded the corresponding products in excellent yields (89%–95%). The dual-substituted benzaldehyde derivatives (**RA21-RA22**) also showed excellent conversions of 89% and 94%, respectively. In addition, oxygen-, nitrogen- and sulfur-containing heteroaryl aldehyde derivatives (**RA23-RA30**) were shown to react efficiently with **HP-ArHD-1** in good to excellent yields (67%–97%). It was noted the naphthalene analog, 1-naphthaldehyde, reacted with **HP-ArHD-1** in an excellent yield (82%). To our delight, the aliphatic aldehyde derivatives (**RA32-RA34**) also gave the desired products with excellent yields (80%–92%).

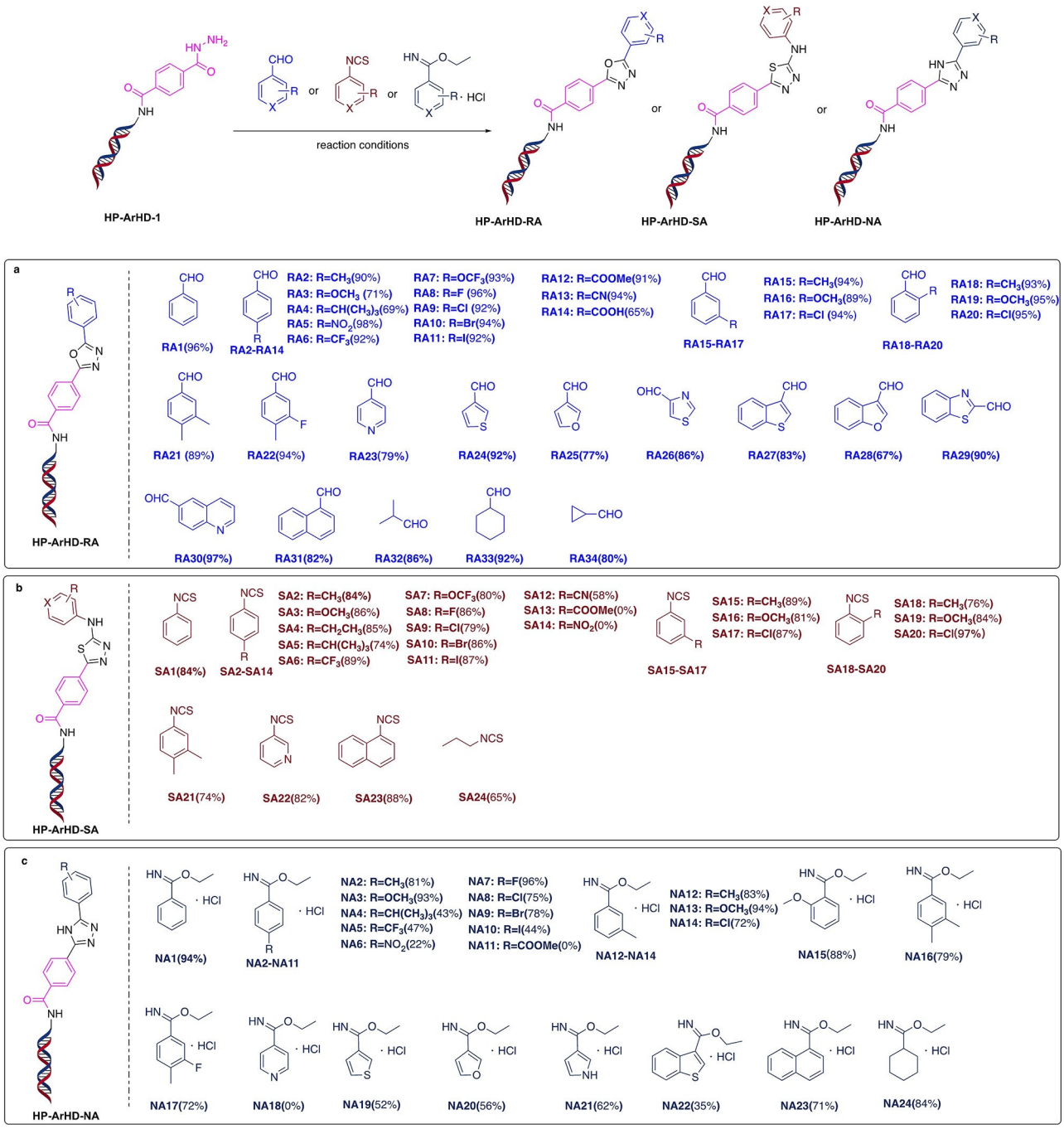

Figure 2. Examples of building blocks used in phylogenic chemical transformation strategy for the synthesis of on- DNA azole derivatives derived from **HP-ArHD-1**. (a) Reaction conditions: 1 equiv. of **HP-ArHD-1** (10 μL, 1 mM in water), 80 equiv. of **RA1** (10 μL, 100 mM in DMSO), 80 equiv. of I_2 (10 μL, 80 mM in DMSO), 5 μL borate buffer (pH = 9.4, 250 mM), 60 °C, 3 h; (b) Reaction conditions: 1 equiv. of **HP-ArHD-1** (10 μL, 1 mM in water), 200 equiv. of **SA** (10 μL, 200 mM in EtOH), 800 equiv. of K_2CO_3 (10 μL, 800 mM in water), 60 °C, 3 h; (c) Reaction conditions: 1 equiv. of **HP-ArHD-1** (10 μL, 1 mM in water), 200 equiv. of **NA** (10 μL, 200 mM in EtOH), 1000 equiv. of K_2CO_3 (10 μL, 1000 mM in water), 60 °C, 3 h. Conversion of **HP-ArHD-1** was determined by LC-MS.

Under the same reaction conditions, the remaining DNA-conjugated (hetero) aryl hydrazide derivatives **HP-ArHD-2**–**HP-ArHD-10** (Scheme S3) were investigated in the syntheses of on-DNA 1,3,4-oxadiazoles derivatives (Scheme S4). As shown in Scheme S4, compared to **HP-ArHD-1**, all the alternative aryl-hydrazides showed reduced reactivities (19%–76%), of which the indole derivative (**HP-ArHD-10**) failed to react with benzaldehyde to afford the desired product.

1,3,4-thiadiazol-2-amine derivatives are another important family of azoles that have demonstrated versatile pharmacological activities. Various synthetic routes for 1,3,4-thiadiazol-2-amine derivatives were reported including reactions between hydrazide and isothiocynate,[29] acid hydrazides and

dithiocarbamates,[30] hydrazination,[11c] and using Vilsmeier and Lawesson's reagents,[31] etc. Taking into account the tolerability, integrity and water-solubility of the DNA barcode in the reaction, the reactant pair between **HP-ArHD-1** and isothiocyanatobenzene (**SA1**) was chosen for the on-DNA synthesis of 1,3,4-thiadiazol-2-amine derivatives. The desired product **HP-ArHD-SA1** was obtained in 60% yield using EtOH (volume ratio: 33%) as the co-solvent (Table S3) suggesting that introduction of isocyanates was a feasible route to synthesize on-DNA 1,3,4-thiadiazol-2-amine derivatives. To improve the conversion yield, reaction conditions were examined and optimized. The reaction with 200 equiv. of isothiocyanatobenzene (**SA1**), 800 equiv. of K_2CO_3, and EtOH (volume ratio: 33%) at 60 °C for 3 h proceeded smoothly with an excellent conversion (84%) (Table S3). In order to explore the substrate scope for the synthesis of on-DNA 1,3,4-thiadiazol-2-amine derivatives, multiple aryl isothiocyanates with various substituents were next tested. As shown in Figure 2b, the derivatives bearing either electron-donating group (**SA2-SA5, SA21**) or electron-withdrawing group (**SA6-SA7**) reacted readily with **HP-ArHD-1** in good to excellent yields (74%–89%). In addition, fluorine-, chlorine-, bromine-, or iodine-substituted isothiocyanatobenzene derivatives at *para*-positions (**SA8-SA11**) afforded the desired products in the yields of 86%, 79%, 86%, and 87%, respectively. However, methyl 4-isothiocyanatobenzoate (**SA13**) and 1-isothiocyanato-4-nitrobenzene (**SA14**) failed to react with **HP-ArHD-1** suggesting that a reactive group like ester (**SA13**) or the strong electron-withdrawing group like nitro (**SA14**) in the *para*-position of the benzene ring was not tolerated under the reaction conditions. It was noted that the *meta*-substituted (**SA15-SA17**) and *ortho*-substituted aryl isocyanates (**SA18-SA20**), despite possible steric hindrance, afforded final products in good to excellent yields (76%-97%). **HP-ArHD-1** was also shown to react readily with the heterocyclic 3-isothiocyanatopyridine (**SA22)** in 82% yield, and the naphthalene analog, 1-isothiocyanatonaphthalene (**SA23**), 88% yield. Furthermore, the aliphatic 1-isothiocyanatopropane (**SA24**) could also afford the desired product with a moderate 65% yield.

Similarly, under the same reaction conditions of **HP-ArHD-1**, the remaining DNA-conjugated (hetero)aryl hydrazide derivatives (**HP-ArHD-2** – **HP-ArHD-10**) were next examined in the syntheses of on-DNA 1,3,4-thiadiazol-2-amine derivatives. As shown in Scheme S5, both electron-donating (**HP-ArHD-2**, **HP-ArHD-4**) and electron-withdrawing (**HP-ArHD-3, HP-ArHD-5, HP-ArHD-6**) substitutions on the aromatic ring of **HP-ArHDs** afforded satisfactory conversions (58%–83%). Aryl-hydrazide at the *meta*-position on the aromatic ring (**HP-ArHD-7)** gave a moderate 58% yield. Moreover, the heterocycle bearing pyridine (**HP-ArHD-8)** and indole (**HP-ArHD-10)** afforded the desired products in yields of 40% and 79%, respectively. Notably, the naphthalene nucleus (**HP-ArHD-9)** gave an impressive 72% yield.

Encouraged by the successful application of phylogenic chemical transformation strategy for on-DNA syntheses of 1,3,4-oxadiazole and 1,3,4-thiadiazol-2-amine derivatives, we next exploited the on-DNA synthesis of 1, 2, 4-triazole derivatives. Many studies reported previously demonstrated diverse synthetic organic approaches for 1, 2, 4-triazole derivatives, which includes microwave irradiation of acid hydrazide and S-methyl isothioamide hydroiodide on the surface of silica gel;[32] condensation of nitrile and hydrazide under microwave irradiation at 150 °C;[33] reacting aldehydes with hydrazonoyl hydrochlorides *via* 1,3-dipolar cycloaddition;[34] and dimerization of amidine hydrochloride in the presence of $Cu(OAc)_2$.[35] These conventional methods required high reaction temperature and strong base, which are likely not to be tolerated with DNA oligonucleotides. In order to synthesize on-DNA 1,2,4-triazole derivatives, a new synthetic route taking advantage of the findings that the acylamidrazone obtained by condensation of imino ether and acylhydrazine could serve as an intermediate in triazole cyclization[36] was designed and exploited. **HP-ArHD-1** and benzimidate hydrochloride (**NA1**) were chosen as model substrates in the reaction. In the presence of 1000 equiv. of K_2CO_3 at 60 °C for 2 h, to our satisfaction, **HP-ArHD-1** reacted with benzimidate hydrochloride (**NA1**) affording the desired product **HP-ArHD-NA1** at a 65% yield (Table S4). Subsequently, reaction conditions including base, solvent, temperature, time, and stoichiometry were examined and optimized (Table S4). Results showed that in the presence of 1000 equiv. of K_2CO_3 with EtOH (%volume: 33%) as a co-solvent, 200 equiv. of benzimidate hydrochloride could react readily with **HP-ArHD-1** at 60 °C and afforded the final product with an excellent 94% yield in 4 h. In order to investigate the substrate scope of this reaction, dozens of imidate hydrochloride derivatives were synthesized from nitrile using the facile and efficient Pinner reaction (Scheme S6).[37] As shown in Figure 2c, these substituted imidate hydrochloride derivatives were examined with **HP-ArHD-1** under the optimized reaction conditions. Electron-donating substituents (**NA2-NA4, NA16-NA17**) appeared to afford higher yields (43%–94%) compared to electron-withdrawing substituents (**NA5-NA6**), which had low to moderate yields (22%–75%). The fluorine-, bromine-, chlorine- and iodine-substituted benzimidate hydrochloride derivatives (**NA7-NA10**) afforded the desired products in yields of 96%, 75%, 78%, and 44%, respectively. No product formation was observed with an ester derivative (**NA15)** that contains a sensitive functional group and ethyl isonicotinimidate hydrochloride (**NA18**) that contains a heterocyclic pyridine. Nevertheless, heterocycles bearing thiophene (**NA19**), furan (**NA20**), pyrrole (**NA21**), or benzothiophene (**NA22**) afforded the corresponding products with moderate yields (35%–62%).

Significantly, the aliphatic imidate hydrochloride (**NA24**) could also react with **HP-ArHD-1** in an excellent 84% yield.

We also tested the above established reaction conditions with all the remaining DNA-conjugated (hetero) aryl-hydrazide derivatives (**HP-ArHD-2**–**HP-ArHD-10)**, and showed moderate to excellent conversion (41%–94% yield) (Scheme S7).

To further validate the chemical transformation of on-DNA 1,3,4-oxadiazoles, 1,3,4-thiadiazol-2-amines, and 1,2,4-triazole derivatives, the standard samples of 4-(5-phenyl-1,3,4-oxadiazol-2-yl)benzoic acid (**RR1**), 4-(5-(phenylamino)-1,3,4-thiadiazol-2-yl)benzoic acid (**SS1**), and 4-(5-phenyl-4*H*-1,2,4-triazol-3-yl) benzoic acid (**NN1**) were individually synthesized using conventional organic synthetic method (Scheme S8) and confirmed by

LC–MS and NMR (see Support Information). The authentic **HP-ArHD-RA1**, **HP-ArHD-SA1** and **HP-ArHD-NA1** were then resynthesized by reacting **RR1**, **SS1** and **NN1**, respectively, with **HP-NH_2** *via* simple amide coupling (Scheme S9). The resynthesized authentic samples of **HP-ArHD-RA1**, **HP-ArHD-SA1** and **HP-ArHD-NA1** were co-injected with those synthesized by on-DNA reactions using UPLC-MS. As shown in Figure S2, S3 and S4, the products from DNA-compatible reactions had identical retention times and molecule weights corresponding to the resynthesized compounds.

To ensure the compatibility of this chemistry with DEL synthesis, it was important to show that these reaction conditions do not damage the DNA-based barcodes.[16b,38] Any effect of these reactions on the integrity of oligo DNA barcodes was next examined by carrying out the enzymatic ligation reactions of a 50-mer oligo DNA primer with on-DNA 1,3,4-oxadiazoles (**HP-ArHD-RA1**), 1,3,4-thiadiazol-2-amine **(HP-ArHD-SA1)**, and 1,2,4-triazole derivatives (**HP-ArHD-NA1)** individually (Figure 3). Results were analyzed by agarose gel electrophoreses (Figure S5) and subjected to deep-sequencing after PCR amplification (Figure S6). As shown in Figure S5 and S6, the ligation reactions proceeded successfully to afford the desired 58-bp products of **HP-ArHD-RA-L**, **HP-ArHD-SA-L**, and **HP-ArHD-NA-L**, respectively. These reaction conditions did not interfere with the DNA headpiece's cohesive ends.

Conclusion

The scope of synthetic transformations that can be used for construction of a DEL is constrained by the requirement that the appended DNA barcode is not modified. Indeed, this constraint has led to a set of studies where classical organic reactions are modified to work under mild conditions in water.[39] Over time, more and more classical organic chemistry has been applied to reactions compatible with the presence of DNA. The results reported herein are another example of this trend. We developed a phylogenic chemical transformation strategy to carry out on-DNA syntheses that cover broad substrate scopes and diverse reaction conditions under conventional organic synthesis. We demonstrated that three chemical families consisting of important pharmacophores, 1,3,4-oxadiazoles, 1,3,4-thiadiazol-2-amines, and 1,2,4-triazoles, could be readily derived from a common DNA-conjugated benzoyl hydrazine (**HP-ArHD**) and excessive amounts of aldehydes, isocyanates, and imidate hydrochlorides, respectively. Moreover the same pluripotent precursor **HP-ArHD** could be extended into the on-DNA syntheses of [1,2,4]triazolo[4, 3-*b*]pyridazines, 4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepines, as well as six-membered heterocyclic rings such as 1,2,4-triazines, etc. Assuming a three-cycle DEL library construction, with the current three azole families, an azole-focused DEL library containing 10^6 unique azole structures (cycle1 × cycle2 × cycle3 = 350 × 10 × 300 building blocks = 1.05×10^6) could be envisioned and assembled readily. It is foreseeable that the described phylogenic chemical transformation strategy could be applied to various different pharmacophore structures, and expand the DELs approach to cover broader and deeper chemical space.

Experimental Section

General procedure for the synthesis of DNA-conjugated (hetero) aryl Boc-protected hydrazide derivatives (HP-ArHBs): To a solution of DNA headpiece (HP-NH_2, 300 mol) in 600 μL borate buffer (pH = 9.4, 250 mM) was added a mixture of O-(7-Azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HATU, 60 μL, 200 mM in N,N-Dimethylaniline (DMA)), 4 (60 μL, 200 mM in DMA) and N,N-Diisopropylethylamine (DIPEA, 60 μL, 200 mM in DMA). The resultant mixture was vortexed and stood at 25 °C for 12 h. Aqueous NaCl (78 μL, 5.0 M) and cold EtOH (2.34 mL) were sequentially added and the resultant mixture was stored at −80 °C for 30 min. The mixture was centrifuged at 4 °C for 30 min at 12000 rpm before the resultant supernatant was removed. The pellet was dissolved in deionized water (300 μL), which was used in the next reaction without further purification.

General procedure for the synthesis of DNA-conjugated (hetero) aryl hydrazide derivatives (HP-ArHDs): To the above solution of **HP-ArHBs** (300 μL, 300 nmol, 1 mM in water) was added 300 μL Na_2HPO_4-NaH_2PO_4 buffer (pH = 6.0, 200 mM), and the resultant mixture was vortexed and stood at 60 °C for 12 h. Aqueous NaCl (60 μL, 5.0 M) and cold EtOH (1.8 mL) were sequentially added and the resultant mixture was stored at −80 °C for 30 min. The mixture was centrifuged at 4 °C for 30 min at 12000 rpm before the resultant supernatant was removed. The pellet was dissolved in deionized water (300 μL), which was used in the next reaction without further purification.

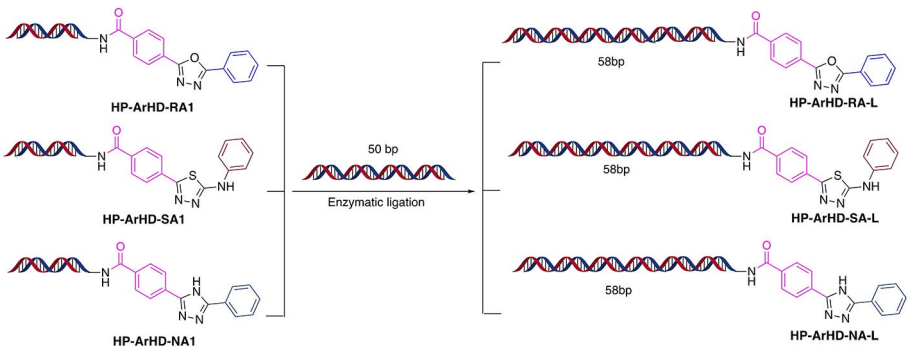

Figure 3. Enzymatic ligation of HP-ArHD-RA1, HP-ArHD-SA1 and HP-ArHD-NA1.

General procedure for the synthesis of on-DNA 1,3,4-oxadiazole derivatives: To the mixture of **HP-ArHD-1** (10 μL, 10 nmol, 1 mM in water) and 5μL borate buffer (pH = 9.4, 250 mM) was added 80 equiv. of aldehydes (10 μL, 80 mM in DMSO). The mixture was vortexed and stood at 60 °C for 1 h. After the reaction, added 80 equiv. of iodine (10 μL, 80 mM in DMSO) and heated the reaction mixture at 60 °C for another 2 h. Aqueous NaCl (10% by volume, 5.0 M) and cold EtOH (2.5 times by volume, EtOH stored at −20 °C) were sequentially added and the resultant mixture was stored at −80 °C for 30 min. The sample was centrifuged for around 30 minutes at 4 °C in a microcentrifuge at 12000 rpm. The above supernatant was removed and the pellet (precipitate) was dissolved in deionized water for LC-MS detection.

General procedure for the synthesis of on-DNA 1,3,4-thiadiazol-2-amine derivatives: To the mixture of **HP-ArHD-1** (10 μL, 10 nmol, 1 mM in water) and 200 equiv. of isocyanates (10 μL, 200 mM in EtOH) was added 800 equiv. of K_2CO_3 (10 μL, 800 mM in water). The mixture was vortexed and stood at 60 °C for 3 h. After the reaction, aqueous NaCl (10% by volume, 5.0 M) and cold EtOH (2.5 times by volume, EtOH stored at −20 °C) were sequentially added and the resultant mixture was stored at −80 °C for 30 min. The sample was centrifuged for around 30 minutes at 4 °C in a microcentrifuge at 12000 rpm. The above supernatant was removed and the pellet (precipitate) was dissolved in deionized water for LC-MS detection.

General procedure for the synthesis of on-DNA 1,2,4-triazole derivatives: To the mixture of **HP-ArHD-1** (10 μL, 10 nmol, 1 mM in water) and 1000 equiv. of K_2CO_3 (10 μL, 1000 mM in water) was added 200 equiv. of imidates hydrochloride (10 μL, 200 mM in EtOH). The mixture was vortexed and stood at 60 °C for 4 h. After the reaction, aqueous NaCl (10% by volume, 5.0 M) and cold EtOH (2.5 times by volume, EtOH stored at −20 °C) were sequentially added and the resultant mixture was stored at −80 °C for 30 min. The sample was centrifuged for around 30 minutes at 4 °C in a microcentrifuge at 12000 rpm. The above supernatant was removed and the pellet (precipitate) was dissolved in deionized water for LC–MS detection.

Co-injection experiment: Samples were dissolved in an appropriate amount of distilled and deionized water (ddH_2O) and injected or co-injected into a reverse-phase chromatography column (Xbridge Oligonucleotide BEH C18 column, 1.7 μm, 2.1 × 50 mm). The elution was carried out as followings: 5–30% solvent B over 30 min, 0.4 mL/min, λ = 260 nm; solvent A: 0.75% v/v hexafluoroisopropanol/ 0.038% v/v triethylamine in methanol/water = 5/95; solvent B: 0.75% v/v hexafluoroisopropanol/ 0.038% v/v triethylamine in methanol/water = 90/10. The effluents were analyzed by a Xevo G2-XS Q-TOF with electrospray ionization source.

Enzymatic ligation experiment: **HP-ArHD-RA1** or **HP-ArHD-SA1 or HP-ArHD-NA1** (5 nmol) was dissolved in 5 μL ddH_2O. To the tube was added the **Primer** (LGC Biosearch Technologies, 1 mM in ddH_2O, 6 μL, Primer-F and Primer-R were freshly annealed at 95 °C for 5 min, then stored at 4 °C for 15 min), Ligation buffer (Thermo Scientific, 10 X, 3 μL), ddH_2O (15.7 μL), T4 ligase (30 U/μL, 0.3 μL). The tube was mixed, eddied and centrifuged, stood at r.t., overnight. For quality control of tag ligations, every sample was analyzed via gel electrophoresis on 4% agarose gel. The amplified DNA samples were subjected to deep-sequencing.

Reporting summary: Further information is available in the support information.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: azole · DNA-compatible reactions · DNA-encoded libraries · hydrazide

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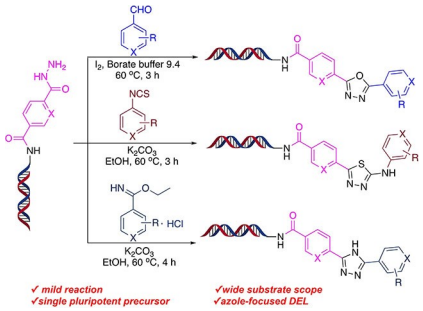
FULL PAPER

The phylogenic chemical transformation approach to synthesize on-DNA 1,3,4-oxadiazole, 1,3,4-thiadiazol-2-amine and 1,2,4-triazole derivatives using DNA-conjugated benzoyl hydrazine as pluripotent precursor are reported here, which shows great potential in facilitating rapid construction of azole-focused DELs.



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