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Extended Multicomponent Reactions with Indole Aldehydes: Access to Unprecedented Polyheterocyclic Scaffolds, Ligands of the Aryl Hydrocarbon Receptor

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Abstract: The participation of reactants undergoing a polarity inversion along a multicomponent reaction, allows the continuation of the transformation with productive domino processes. Thus, indole aldehydes in Groebke-Blackburn-Bienaymé reactions lead to an initial adduct which spontaneously triggers a series of events leading to the discovery of novel reaction pathways together with a direct access to a variety of linked, fused and bridged polyheterocyclic scaffolds. Indole 3- and 4-carbaldehydes with suitable isocyanides and aminoazines afford fused adducts through oxidative Pictet-Spengler processes, whereas indole 2-carbaldehyde yields linked indolocarbazoles under mild conditions, and a bridged macrocycle at high temperature. These novel structures are potent activators of the human aryl hydrocarbon receptor signaling pathway.

Multicomponent reactions (MCRs) are transformations involving three or more reactants that yield a unified adduct in a single step. MCRs are among the strategies of choice to achieve high diversity and complexity levels, offering significant exploratory potential in a simple experimental framework. Accordingly, they have an impressive impact on modern organic synthesis, medicinal chemistry, materials science, etc.[1–3]
Another way to rapidly reach molecular complexity deals with domino processes, where an initial reaction triggers a series of consecutive transformations to generate intermediates that interact with other functionalities present in the substrate.[4,5] Domino reactions are, however, highly dependent on the specific arrangement of the substrates, which usually need to be previously prepared through multistep synthesis. Therefore, these reactions are challenging to generalize, and their use is somewhat restricted. Incidentally, MCRs can be described as *intermolecular* domino reactions featuring simple starting materials.

Here we present the concept of *extended* MCRs, as an alternative approach to tackle the aforesaid limitations of classic domino reactions. Extended MCRs involve reactants that trigger an ensuing cascade of inter/intramolecular transformations due to a polarity inversion, following the initial MCR. Relevant reports in the literature include related processes where this concept is implicit.[6–9]

We particularly focused on electrophilic indolealdehydes that render nucleophilic moieties after the MCR and promote subsequent reactions within the adduct, or with other species present in the medium (Figure 1). This situation is found in several MCRs involving aromatic aldehydes and remains unexplored. Indoles are common scaffolds in drugs and bioactive compounds. Furthermore, they are influential motifs in the design of domino processes [10–12] and indole carbaldehydes have already displayed a rich reactivity in this endeavor.[13–15] Indoles are also present in a variety of MCRs. However, they often appear as mere substituents of the reactive groups on the inputs.[16,17]

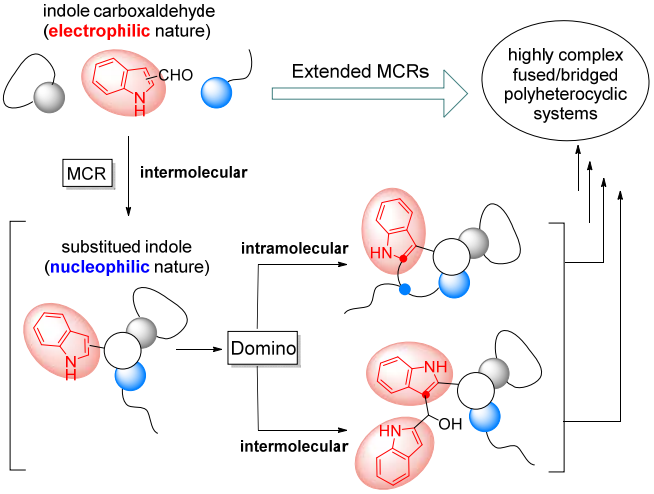
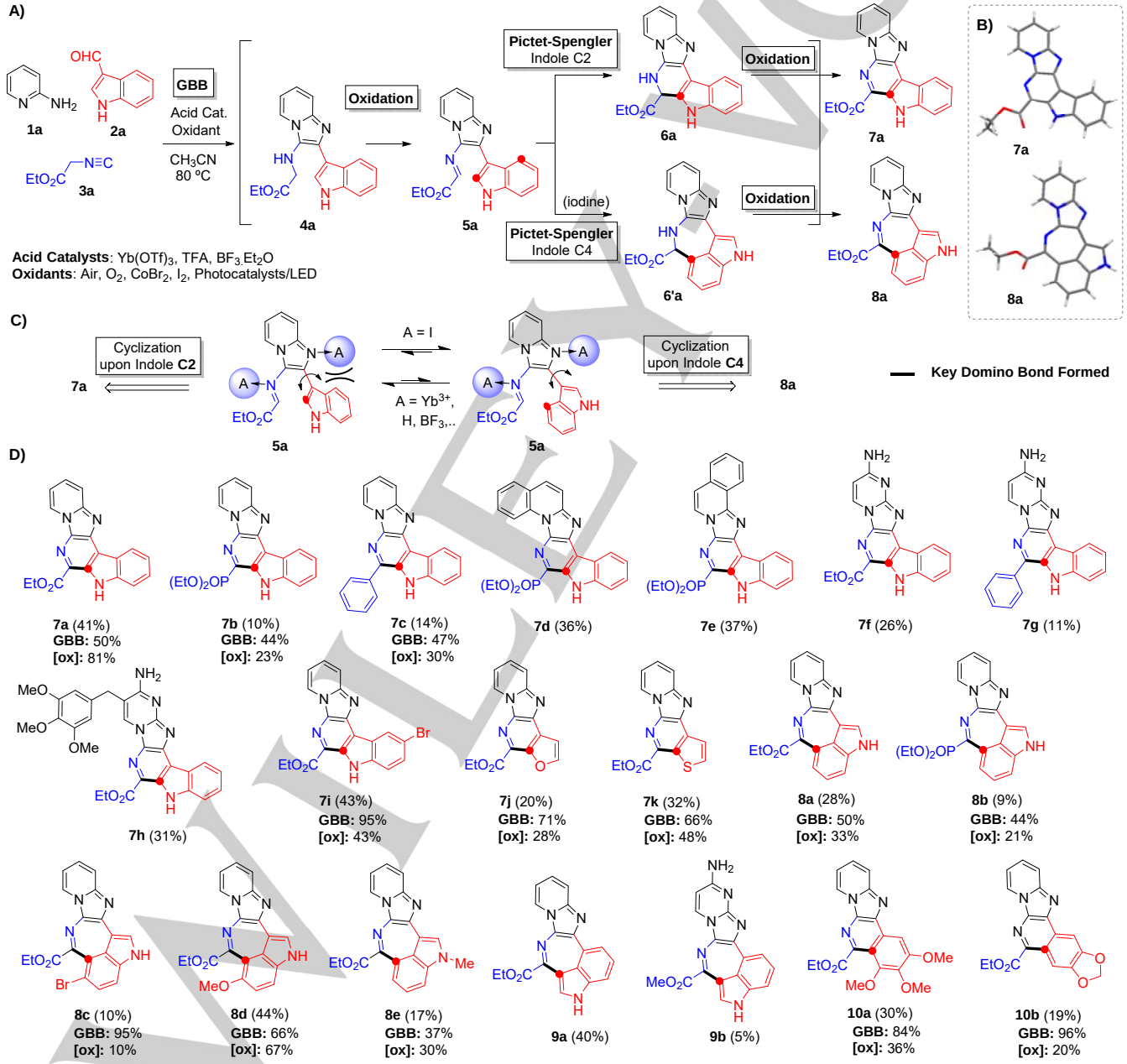


Figure 1. The concept of extended MCRs with indolealdehydes.

We focused on the Groebke-Blackburn-Bienaymé MCR (GBB): the interaction of α-aminoazines, aldehydes and isocyanides to yield imidazoazines, a privileged scaffold in medicinal chemistry.[18–20]

Thus, we performed a GBB with α-aminopyridine **1a**, indole-3-carbaldehyde **2a** and ethyl isocyanoacetate **3a** in open air and observed the formation of the fused adduct **7a** (25%, Scheme 1A). Likely, the initial GBB adduct **4a** was oxidized to the corresponding imine **5a** by atmospheric O_2, which underwent an acid-catalyzed Pictet-Spengler (PS) cyclization upon the indole C2 position to give the dihydropyridine **6a**, that subsequently oxidized to the final product **7a** (structure confirmed by X-Ray crystallography, Scheme 1B). The imine generation can be related to the auto-oxidation of glycine derivatives under analogous conditions.[21] Moreover, the detection of the imine **5a** and the intermediate dihydropyridine **6a** supports the mechanistic hypothesis [See Supporting Information (SI)]. The reaction was repeated in the presence of several acid/oxidant combinations in one-pot and stepwise protocols. The GBB went smoothly with $Yb(OTf)_3$ as the catalyst. In terms of the external oxidant, the performance of $CoBr_2$ particularly stood out from $FeCl_3$, CuI, IBX and blue-LED-promoted photocatalytic oxidations using Eosin Y, Rose Bengal and $Ru(bpy)_3^{2+}$.[22–24] Although the final product is directly achievable from the **1a, 2a** and **3a** mixture, higher yields (41%) and cleaner crudes were obtained when the GBB adduct **4a** was isolated in a prior step, possibly due to the sensitivity of isocyanides in the presence of particular oxidizing agents.



Scheme 1. Extended oxidative GBBs featuring indole-3(4)-aldehydes: A) Reaction conditions and mechanism. B) X-ray and microED structures of compounds **7a** and **8a**. C) Hypothesis for the regioselective PS. D) Scope and variations. For sequential processes, the yield of each step is reported.

Remarkably, the use of iodine brought a dramatic modification in the connectivity pattern of the domino process, as it promoted an unconventional cyclization upon the indole C4, in the presence of the free C2 position, yielding adduct **8a** (28%). Compound **8a** features a complex fused seven-membered ring and, not being able to obtain suitable crystals, its structure was secured by microcrystal Electron Diffraction[25] (Scheme 1B). This cyclization mode is unprecedented in unbiased indole systems, as PS and other electrophilic processes prefer position C2,[26] whereas reactions on position C4 only occur under enzymatic catalysis.[27] Presumably, the coordination with one imidazole N atom in the imine **5a** favors the later cyclization by increasing the steric bulk around C2. This hypothesis was further supported as a standard PS reaction between 4-chlorobenzaldehyde and tryptamine in the presence of iodine[28] yields common C2 products (Scheme 1C and SI).

Next, we studied the scope of this extended MCR. Regarding the isocyanide input, only those capable of yielding conjugated imines (**5**) undergo the domino process. PhosMIC and benzyl isocyanide proceeded in a similar manner to yield adducts **7b** (10%), **8b** (9%) and **7c** (14%). TOSMIC did not yield the expected GBB adduct, likely due to its low nucleophilicity. In agreement with this hypothesis, 4-fluorophenetyl-, 4-methoxyphenyl- and cyclohexyl isocyanide did not evolve beyond their respective GBB adducts (Scheme 1D and SI).
With respect to aminoazines, 1-aminoisoquinoline and 2-aminoquinoline afforded the expected compounds **7d** (36%) and **7e** (37%) respectively in the presence of $CoBr_2$ (Scheme 1D). These adducts display interesting S- and C-shape topologies. 2,4-Diaminopyrimidine yielded the adducts **7f** (26%) and **7g** (11%) in a regioselective manner, following the protocol developed by the group.[29] The antibiotic trimethoprim suitably reacted likewise to give adduct **7h** (31%).[30] Interestingly, 2,4-diaminopyrimidine adducts react smoothly in air, not requiring other oxidants, probably due to their higher electron density.
As for the scope of the aldehyde component, activated (hetero)aromatic aldehydes should react, facilitating the PS step. Accordingly, 5-bromoindole-3-carbaldehyde yielded the expected adducts **7i** (43%) and **8c** (10%) in the presence of $CoBr_2$ and I_2, respectively. 5-Methoxy-3-carbaldehyde and 1-methyl indole 3-carbaldehyde provided adducts **8d** (44%) and **8e** (17%) in the presence of I_2. Furan 3- and thiophene 3-carbaldehyde gave the corresponding adducts **7j** (20%) and **7k** (32%) respectively. The incorporation of indole 4-carboxaldehyde afforded adducts **9a** (40%) and **9b** (5%) through PS cyclization upon indole C3 position, featuring an alternative connectivity of the fused seven-membered ring (Scheme 1D). Electron-rich benzaldehydes, such as 3,4,5-trimethoxy benzaldehyde and piperonal, provided the expected adducts **10a** (30%) and **10b** (19%). Consistently, pyridine aldehydes only yielded GBB adducts (See SI). In general, the processes lead quite cleanly to the oxidized compounds **7-10** with relatively few side reactions (imine oxidation, isocyanide hydrolysis) given the mild conditions used.

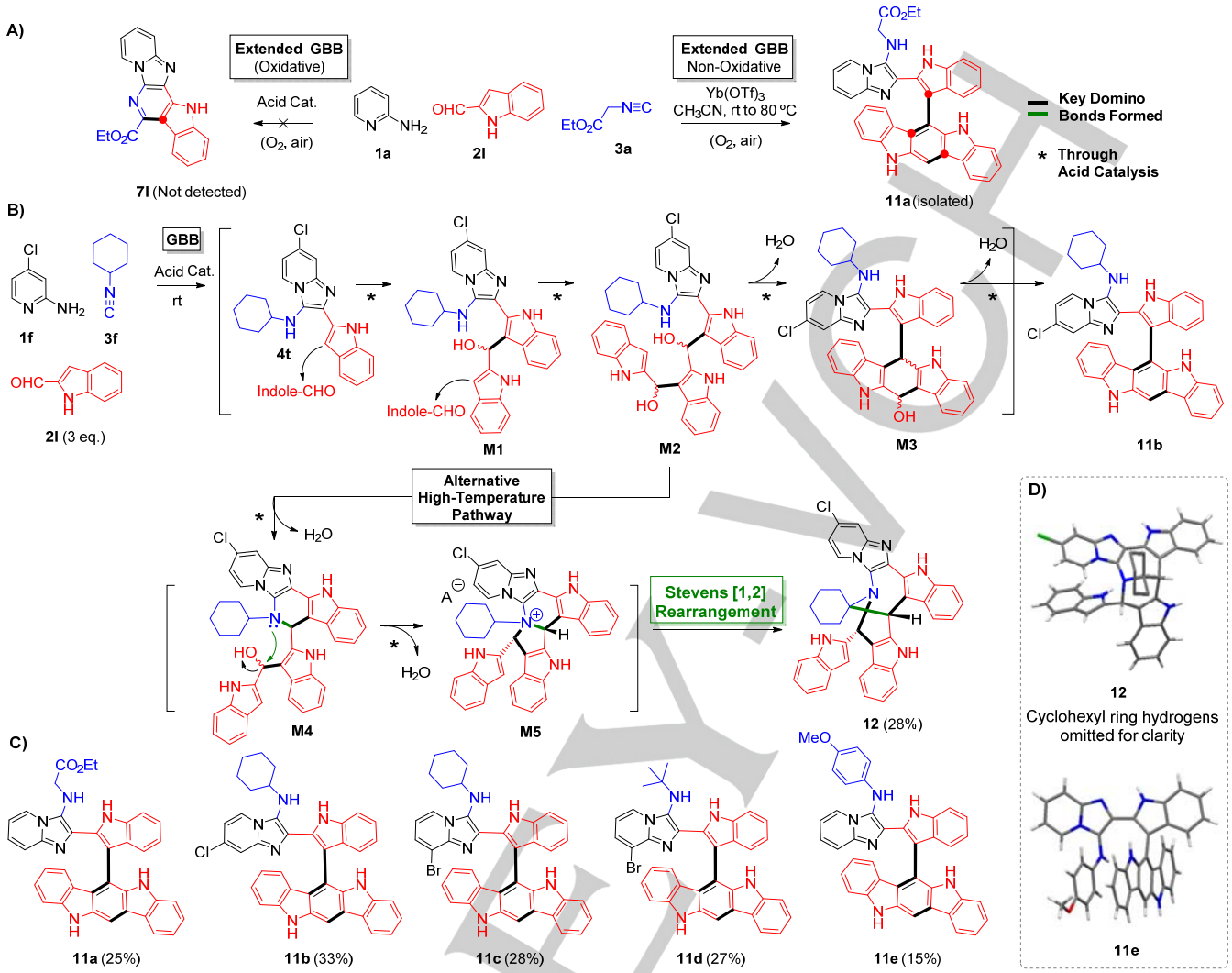
Next, we reacted equimolar quantities of indole 2-carbaldehyde **2l**, 2-aminopyridine **1a**, and ethyl isocyanoacetate **3a** in the presence of $Yb(OTf)_3$, to presumably obtain the adduct **7l**, following the expected oxidative domino process (Scheme 2A). Yet surprisingly, indolocarbazole **11a** was isolated instead, when performing the reaction with three equivalents of the aldehyde input, the unoptimized yield rose to 25%. As the new domino process should, in principle, proceed with any isocyanide, we switched to cyclohexyl isocyanide **3f** and repeated the reaction with the modified stoichiometry, to yield adduct **11b** (33%) in an unprecedented ABC_3 domino fashion (Scheme 2A and SI). Similar reactions involving 5-bromo-1-aminopyridine, *tert*-butyl isocyanide, and 4-methoxyphenyl isocyanide also led to the corresponding adducts **11c** (28%), **11d** (17%) and **11e** (15%) (Scheme 2C). The latter's structure was secured by X-Ray crystallography (Scheme 2D). These results displayed the usefulness of the new intermolecular domino as a remarkably convenient one-pot access to the valuable indolocarbazole scaffold.[31,32]

Astonishingly, when we promoted the reaction with cyclohexyl isocyanide by microwave irradiation (90 minutes at 110 °C), we isolated small amounts of compound **11b** (9%), whereas the major component was its complex isomer **12** (28%), whose structure was elucidated by X-Ray crystallography (Scheme 2D). Compound **12** features a spiroazabicyclo[4.3.1]decane core fused with one benzimidazole, and two indole rings. We detected traces of compound **12** at 80 ºC but not at rt. Moreover, the two isomers **11b** and **12** did not interconvert under acidic and thermal conditions.

The generation of indolocarbazoles **11** and the bicyclic adduct **12** may be explained through a unified mechanism (Scheme 2B). The proposed reaction mechanism involves the formation of the GBB adduct **4t** featuring a highly nucleophilic 2-subsituted indole moiety that attacks a second aldehyde unit to form the intermediate alcohol **M1**. A similar nucleophilic addition takes place between this intermediate and a third aldehyde unit to generate diol **M2**, which cyclizes to afford alcohol **M3**. This intermediate undergoes a dehydration/aromatization step to give the indolocarbazole **11b** (Scheme 2B). At high temperature, however, **M2** may evolve through the generation of intermediate **M4** resulting from the hydroxyl displacement by the amino group and similarly, the quaternary ammonium salt **M5** would arise. This intermediate may undergo a [1,2] Stevens rearrangement[33] justifying the connectivity found in compound **12**. Although several alternative Stevens rearrangements can, in principle, take place, the illustrated pathway presumably leads to the most stable adduct likely involving a reasonably favored intermediate (See SI for a discussion).

Overall, the extended MCRs reach remarkable bond-forming indexes and structural diversity/complexity levels in one-pot operations with modest overall yields, which, however, can be specifically optimized attending to their particular input combinations. For representative examples, see the tuning of conditions leading to compounds **7a** and **7f** in SI.

Having an exclusive access to a variety of novel heteroaromatic scaffolds, we intended to determine their bioactivity profile. We focused on the aryl hydrocarbon receptor (AHR), as indolocarbazoles and related structures are listed among its ligands.[34,35] Upon binding of a ligand in the cytoplasm, AHR translocates to the nucleus and binds to xenobiotic-responsive elements (XRE) in the enhancer of target genes, e.g. encoding cytochrome P450 (CYP) 1A1, to induce their expression.



Scheme 2. Extended non-oxidative GBBs with indole 2-carbaldehyde: A) Domino adducts with ethyl isocyanoacetate. B) Unified mechanistic hypothesis. C) Scope of indolocarbazoles. D) X-Ray structures of compounds **11e** and **12**.

AHR regulates multiple physiological and pathophysiological processes, including xenobiotic metabolism, immune responses, inflammation, and carcinogenesis.[34–36] Recent studies, particularly assessing AHR's function in the context of skin and gut diseases, have unmasked the Janus-faced nature of AHR.[37,38] Depending on cell-type, tissue, microenvironment, and presence of ligand(s), either activation or inhibition of AHR may be beneficial. The AHR binding pocket is still not well described, and although computational studies have shed some light, the generation of potent AHR ligands still relies mostly on appropriate synthesis and screening.[39,40] As shown in Figure 2A, exposure to compounds **8a**, **11b-e** and **12** (but not **7a**) resulted in a dose-dependent induction of CYP1A enzyme activity in AHR-proficient but not AHR-knockdown HaCaT keratinocytes. At the highest test concentration, **8a** interfered with CYP1A enzyme activity, suggesting that this compound is metabolized by CYP1A isoforms and thus competing with the deethylation of 7-ethoxyresorufin. All compounds, except **7a**, increased the CYP1A1 and CYP1B1 copy numbers in HaCaT cells in an AHR-dependent manner (Figure 2B). Aside **7a** and **8a**, all compounds stimulated XRE-driven reporter gene activity in hepatoma cells (Figure 2C).

Taken together, these results strongly indicate that extended MCRs enable the production of a variety of AHR-activating compounds. The tested compounds are almost as potent as the positive control (6-formylindolo[3,2-*b*]carbazole, FICZ). The described modular access to these scaffolds will facilitate further studies to prove their bonding to the AHR and to assess their pharmacokinetic and safety profiles, facilitating a drug discovery program.

To conclude, we have described several domino-prolonged GBBs with indole carbaldehydes that directly yield unprecedented polyheteroaromatic systems. We believe that the concept of extended MCRs may have a positive impact in the exploration of the chemical and reactivity spaces, taking into account that similar polarity changes could take place in many MCRs.

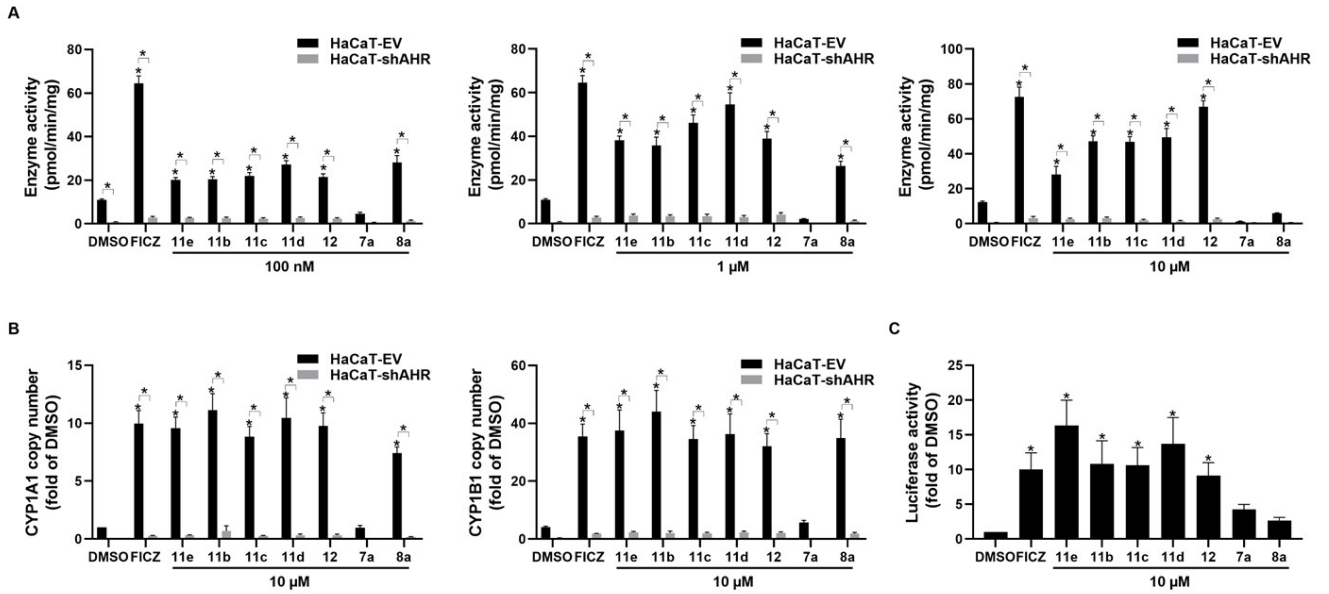


Figure 2. Effect of test compounds on AHR-driven gene expression and CYP1A enzyme activity. A) AHR-proficient HaCaT-EV and AHR-knockdown HaCaT-shAHR keratinocytes were treated with 0.1% DMSO, 100 nM of the AHR agonist FICZ and increasing concentrations of the compounds (100 nM, 1 µM, 10 µM). After 6 h, CYP1A-mediated deethylation of 7-ethoxyresorufin was determined. B) HaCaT-EV and HaCaT-shAHR cells were treated for 6 h with 0.1% DMSO, 100 nM FICZ, and 10 µM of each test compound. After 6 h, total RNA was isolated, reverse transcribed, and copy numbers of CYP1A1 and CYP1B1 were determined by quantitative real-time PCR. C) XRE-HepG2 cells were treated with 0.1% DMSO, 100 nM FICZ, and 10 µM of each compound. After 24 h, AHR/XRE-dependent luciferase activity was determined. * $p \leq 0.05$.

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Keywords: Domino reactions • Multicomponent reactions • Nitrogen heterocycles • Receptors• Synthetic methods

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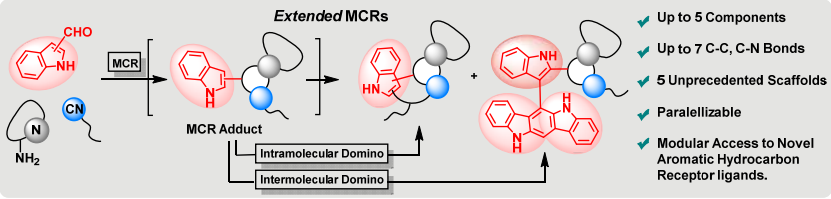

Extend your MCR! By engaging indolealdehydes in an isocyanide Multicomponent Reaction, the polarity inversion of the indole moiety along the transformation triggers an extension of the domino process, leading to a variety of unprecedented fused, linked and bridged scaffolds. The processes are parallelizable and the adducts display remarkable bioactivity as potent ligands of the aryl hydrocarbon receptor.

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