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COMMUNICATION

Relay Tricyclic Pd(II)/Ag(I) Catalysis: Design of a Four-Component Reaction Driven by Nitrene-Transfer on Isocyanide Yields Inhibitors of EGFR

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One-pot synthesis of pyrazolo[1,5-c]quinazolines from four easily available precursors is presented through a one-pot tricyclic Pd(II)/Ag(I) relay catalysis. The bimetallic relay cascade forges five new chemical bonds by concatenating six discrete chemical steps. The relay catalysis enables four-component assembly of pyrazolo[1,5-c]quinazolines that selectively inhibit EGFR, exhibit apoptosis through the ROS-induced mitochondrial-mediated pathway, and arrest the cell cycle at G1 phase.

One of the important objectives of synthetic organic chemistry is to design rapid, efficient and sustainable strategies to access valuable molecular constructs. To this end, chemists have designed tandem or cascade reactions that generate complex molecular frameworks in one-pot without the need for any isolation of an intermediate.[1] Recently, there is an upsurge in the field of organic synthesis for the development of catalytic multistep processes, whereby two or more distinct chemical transformations are promoted by more than one catalysts in a single flask. Classified as cooperative-,[2] synergistic-,[3] relay-[4] or sequential-catalysis[5] depending on the precise experimental details offers the potential for the advancement in the synthesis by removing dependency over iterative processes.[6] Of these, orthogonal relay catalyses of transition-metals, where all metal catalysts are present at the outset, are somewhat difficult to design owing to the redox incompatibilities among various metals and a challenging time-resolution of such methods (Fig. 1). Besides, among various methods reported so far, the second transition metal is often employed as a redox[7] or photoredox[8] partner in the majority of these reports, which doesn't expand the overall range of transformations. In 2011, Lautens reported relay catalysis of Rh-catalyzed alkyne arylation and Pd-catalyzed N-arylation steps for the synthesis of dihydroquinolines [Eq.(1)].[9,10] Tu employed Co(II)/Ag(I) relay catalysis in 2015 for isocyanide insertion/cycloaddition cascade to access a new pyrrolo[2,3-b]indole heterocycle [Eq.(2)].[11] Ji reported Rh(II)/Pd(0) dual catalysis for selective construction of cyclic quarternary carbon center [Eq.(3)][12] and Ramasastry developed trimetallic relay catalysis for the synthesis of β-carbolines in 2016 [Eq.(4)].[13] In 2017, Wang successfully applied Rh(III)/Ag(I) relay catalysis in the total synthesis of aristolactam BII[14] [Eq.(5)] and Xu

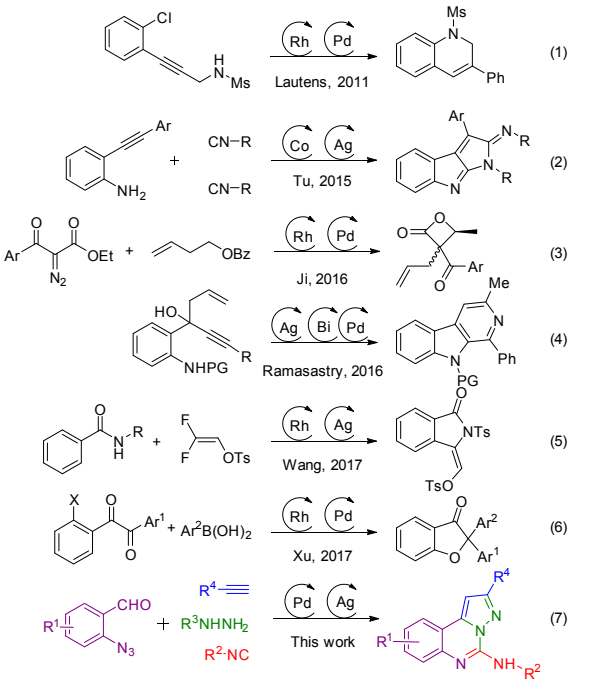

Fig 1. Various strategies reported for relay catalysis promoted by two or more transition metal catalysts.

a.
b.
c.
d.
e.
f.
g.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

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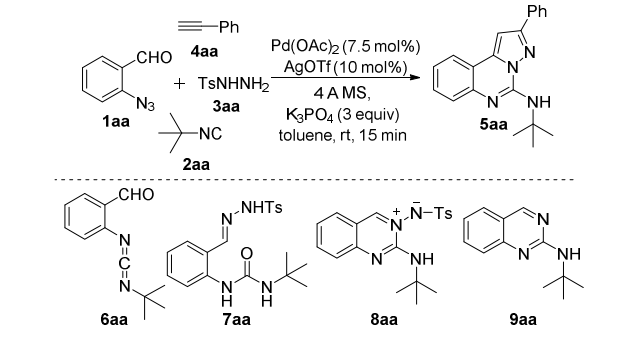
reported rhodium/palladium relay catalysis for enantioselective synthesis of gem-diaryl benzofuran-3(2*H*)-ones [Eq.(6)].[15] In spite of their utility, all methodologies of bimetallic relay catalysis relied on the requirement of prefabricated substrates synthesized by convergent multistep routes. However, the cutting-edge application of multicatalytic relay catalysis lies in promoting multicomponent reactions, since it imitates a biological processe where several enzymes coordinate to generate complex molecular frameworks from multiple, simple and easily available starting materials.[16] To our surprise, multicomponent relay catalysis of two or more transition metals received scant synthetic attention.[11] As a consequence, the scope of the bimetallic relay catalysts is limited, and its true potential for promoting the multicomponent reaction has not been explored to date.

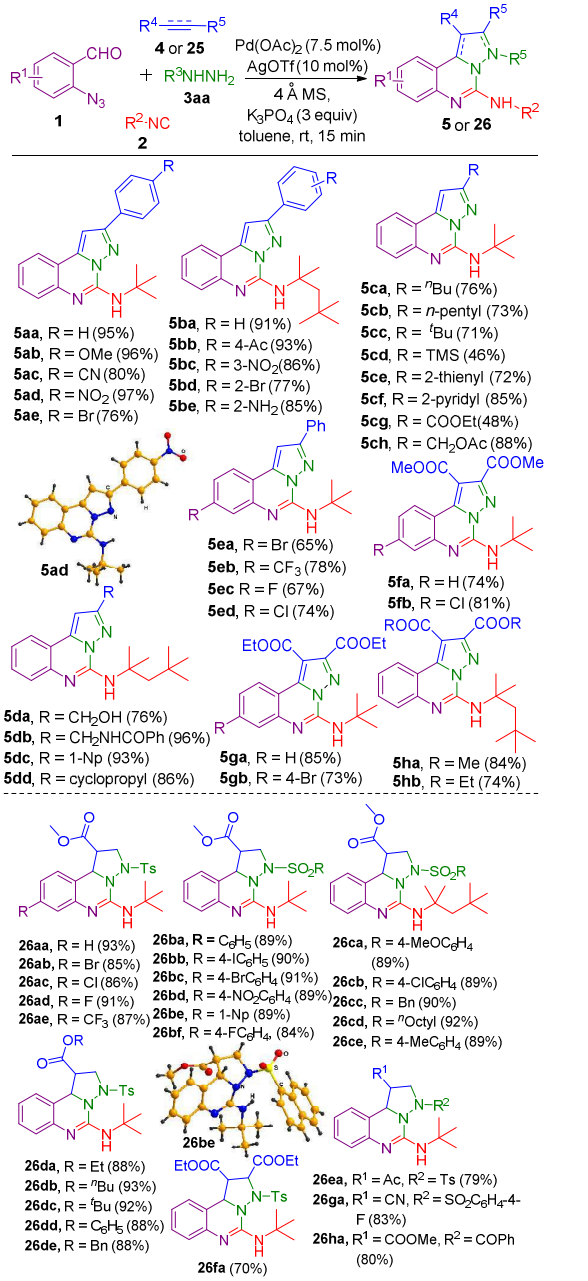
We now report a four component reaction (4-CR) employing four versatile privileged synthons: 2-azidobenzaldehyde, isocyanide, sulfonyl hydrazides and alkynes promoted by two different transition metal catalyst in a time-resolved manner, thus leading to an efficient preparation of pharmacologically active pyrazolo[1,5-*c*]quinazolines.[17] The corresponding focused compound collection of pyrazolo[1,5-c]quinazolines exhibited selective cytotoxicity on cancer cells, inhibited the Epidermal Growth Factor Receptor (EGFR), increased the intracellular Reactive Oxygen Species (ROS) levels and altered the mitochondrial membrane potential ($\Delta\psi m$) leading to apoptosis, cell cycle arrest and cancer cell deaths.

Upon treatment of $Pd(OAc)_2$ (7.5 mol%) and AgOTf (10 mol%) with a mixture of 2-azidobenzaldehyde **1aa**, *tert*-butyl isocyanide **2aa**, tosyl hydrazide **3aa**, phenylacetylene **4aa** and K_3PO_4 in toluene, a four-component adduct, pyrazolo[1,5-c]quinazoline **5aa**, was formed (Scheme 1). The structure of **5** was established by X-ray crystallography[18] and involved the formation of five new chemical bonds in a single operation. Addition of 4 Å molecular sieves was essential to suppress a major side product, urea **7aa** (Scheme 1).[19] Both silver and copper salts worked with equal efficiency in this transformation. Other Pd-sources, bases, and solvents were also screened (for details see section S2 of the Supporting Information), but these turned out to be inferior to the optimal condition. The 4-CR worked well at room temperature, and any further increase in the temperature to 60 °C led to the formation of **9aa**[18] as the major product.



Scheme 1. Reaction condition for four-component reaction.

With an assortment of precursors at hand, a broad diversity of pyrazolo[1,5-c]quinazolines **5** could be constructed from alkynes (Scheme 2, refer Supporting Information for details). The scope of 4-CR was further extended for electron-deficient alkenes, such as acrylates and acrylonitrile, to generate tetrahydropyrazolo[1,5-c]quinazolines **26**[18] of four-point diversity exhibiting excellent atom economy. The investigation underscores high tolerance of the 4-CR reaction to steric and electronic effect. In all cases, the reaction proceeds with high regiochemical fidelity. Interestingly, the formation of a single diastereomer was observed in 4-CR with alkenes.



Scheme 2. Substrate Scope of 4-CR.

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The successful isolation of reaction intermediates, carbodiimide **6aa,** and azomethine imine **8aa**[18] (Fig. 2), provided meaningful insights into the mechanism of the 4-CR. **6aa** was readily generated from 2-azidobenzaldehyde **1aa** and *tert*-butyl isocyanide **2aa** was converted to **8aa** upon reacting with the tosyl hydrazide **3aa** (refer Section S5.2 of Supporting Information for details). Interestingly, both steps required $Pd(OAc)_2$ for catalysis. Conversion of **8aa** to pyrazolo[1,5-*c*]quinazoline **5aa** was catalyzed by silver. Although, these results lend strong support in favor of path A (Fig. 2, *via* **6aa**); an alternate mechanism via **10aa** can also be proposed (Path B). To probe whether the formation of **8aa** could proceed through both path A and path B, or whether one of these pathways was dominating, kinetic studies of 4CR were carried out by monitoring the reaction progress with ^{1}H NMR spectroscopy (section S5.3). The study unequivocally ruled out path B for the formation of **8aa**. Moreover, the sluggish rate of reaction of the competing concurrent catalytic processes, such as path B, ensured the time-resolution of the relay catalytic process. The regiochemical outcome of 4-CR and H/D scrambling experiment (Section S5.4) revealed that a possible route for the formation of **5aa** is base-mediated direct alkynylation of azomethine imine **8aa**. Further, no deuterium incorporation in **5aa** was observed, when the deuterated substrate (ethynyl-*d*)-benzene was reacted with **8aa** (Section S5.5). This observation supports the formation of acetylide during the reaction. Based on experimental studies and literature precedence,[20] it is evident that the 4-CR is mediated by three independent catalytic cycles of Pd- and Ag-metals (refer section S5.6 for details).

To explore if the diversity-rich compound collection generated by the bimetallic orthogonal relay catalysis translates into the biological activity, pyrazolo[1,5-c]quinazolines **5** and **26** were screened in the MTT based cell viability assay (section S6.2) on cancer cell lines of the lung (A549), colon (HCT-116) and human glioblastoma (U-87 MG) (Table S7). Of these, **5dd**, **5fb**, **26ea** and **26ga** exhibited an excellent cytotoxic effect in all three cancer cells lines. Pleasingly, these compounds also presented negligible toxicity to Human Peripheral Blood Mononuclear Cells (HPBMC) implicating a pattern of selectivity to cancer cells (Section S6.3). Owing to their structural resemblance to the quinazoline scaffold of gefitinib and erlotinib, the investigational compounds **5dd**, **5fb**, **26ea** and **26ga** were subsequently scrutinized for inhibition of ATP dependent phosphorylation of EGFR[21] (Table S8). To our delight, **5fb** was found to be the most potent even with respect to the standard drug, erlotinib, while **5dd** and **26ga** showed comparable activity in the nanomolar range. This observation was further supported by molecular docking studies, which revealed that **26ea** and **26ga** bind to the ATP binding site of EGFR, and exhibit hydrogen bonding interactions with MET769, carboxylate group of **5fb**, and carboxylate group of THR830 and THR766 (Fig. 3 and Section S7).

A further biological investigation revealed that the investigational compounds **5dd**, **5fb**, **26ea** and **26ga** increased the ROS level (section S6.5) and altered the mitochondrial membrane potential ($\Delta\psi$m) (section S6.6) of pretreated lung cancer cell lines A549. Both these biochemical events play a key role in inducing the mitochondrial apoptosis and lead to lung cancer cell deaths through the ROS-induced mitochondrial-mediated pathway that involves the release of the cytochrome c from the mitochondria into the cytosol. Anticancer drugs are known to delay the progression of cell cycle and inhibit the cell proliferation.[22] Analysis of the cell cycle of control and A549 cell lines treated with **5fb** and **26ga** by flow cytometry showed a substantial rise in the G1 phase of cells and the overall drop in the percentage of cycling cells in S phase and G2-M phase (Fig. 4). The G1 phase arrest by investigational compounds may be attributed to the fact that EGFR is a mitogen that helps in cell cycle progression from G1 to G2/M. The CDK4/6 gets inhibited and causes the cell cycle arrest at the G1 phase.[23]

Conclusions

In summary, we have developed a novel four-component reaction of 2-azidobenzaldehydes, isocyanides, sulfonyl hydrazides and alkynes/alkenes promoted by orthogonal relay catalysis of Pd(II)/Ag(I). The 4-CR produced a diverse and complex heterocycle, pyrazolo[1,5-*c*]quinazolines with the formation of five new chemical bonds in a single operation. The 4-CR exhibited a broad substrate scope with excellent

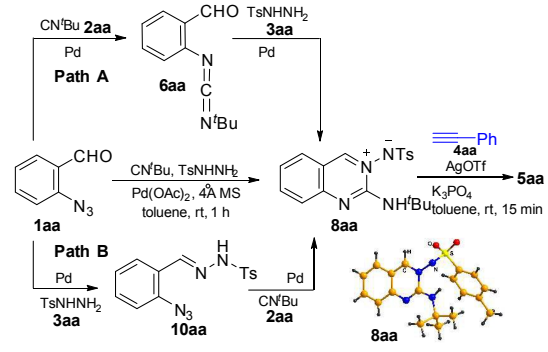

Fig. 2: Control experiments for scrutinizing plausible intermediates.

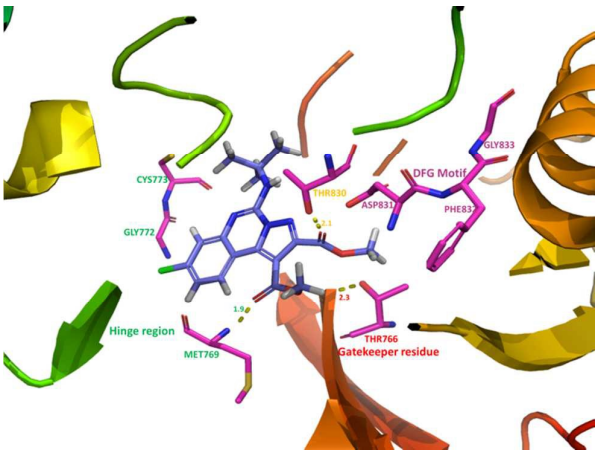

Fig. 3: Binding pattern representation of **5fb** (blue color).

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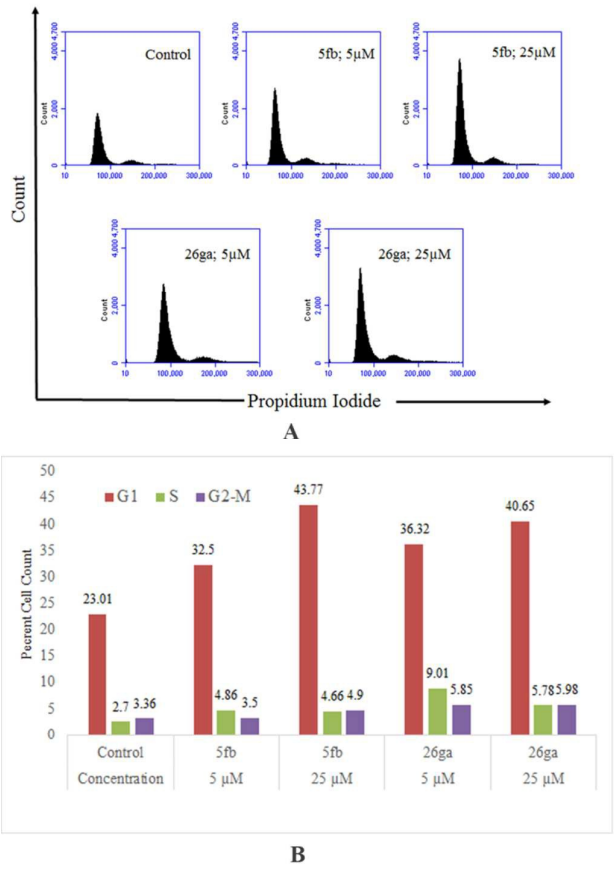

Fig 4. (A) Cell cycle analysis using flow cytometry of A549 cancer cell line treated with **5fb** and **26ga**. (B) The bar graph represents the percent cell count (DNA) at various stages of cell cycle.

regio-and stereocontrol. A focused compound collection of pyrazolo[1,5-c]quinazolines exhibited potent and selective cytotoxicity on cancer cell lines. Of these, **5fb** was found two-times potent inhibitor of EGFR than an existing drug, erlotinib. Pyrazolo[1,5-c]quinazolines led to the mitochondrial apoptosis through the ROS-induced mitochondrial-mediated pathway that involves the release of the cytochrome c from the mitochondria into the cytosol leading to the cytotoxic activity on lung cancer cells (A549) and the cell cycle arrest in G1 phase. The work described herein would pave way for the development of MCR driven design and assembly of bioactive heterocycles.

Conflicts of interest

There are no conflicts to declare.

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