nature portfolio

Peer Review File

Transforming an Azaarene into the Spine of Fused Bicyclics via Cycloaddition-Induced Scaffold Hopping of 5-Hydroxypyrazoles

Corresponding Author: Professor An-Xin Wu

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Wu and their colleagues have disclosed for the first time that skeletal remodeling of the pyrazole ring achieves its ring upgrading, a mode of reaction that appears to be a new form of molecular editing. This work achieves its scaffold hopping based on the cleavage of pyrazole's C=N bond, rather than from the previously reported N-N bond cleavage, and the reaction mechanism was also confirmed by HRMS. To my opinion, this work is interesting and novel. The protocol applied to a wide range of substrates provides good yields. Additionally, the reaction features operation simplicity and easily available substrates, which offers a great advantage in synthetic chemistry. The authors further demonstrated the practicality of the method by the pre-modification transformation of the active molecules. Therefore, this work is highly recommended for publication in Nature Communications with minor revisions:
1.In Table 1, the reaction equation has 2.0 eq. of oxidant and c = 0.1, which is inconsistent with entries and notes, and it is not clear whether there is a clerical error or other considerations here, the authors should make a check.

2. From the results, it appears that the generation of the target product 4a relies firstly on the formation of a diene by a chemo-regioselective reaction of 1a and 2a. The 1a and 2a's amino groups are also capable of forming imine-type diene, and the product obtained based on this seems to have a smaller spatial site resistance. Has the author detected or isolated such regioisomers?

3. From the point of view of the reaction mechanism, could 2a be extended to other types of aromatic amines, for example, aniline, 3-aminoindazole, etc., to afford the corresponding product?

4.In Figure 5d, since there is no substituent at the C-3 position in substrate 3a to act as a hindering group, intermediate D should be easily oxidatively arylated to produce a 5/6/5 tricyclic by-product, can it be detected and isolated?

5. What is the reason for the different reaction conditions observed in Figure 3 for 6m-6p?

Reviewer #2

(Remarks to the Author)
The manuscript communicated in the Nature Communications by Wu and co-workers disclosed the chemistry entitled "From an Azaarene to the Spine of Fused Bicyclics: Cycloaddition-Induced Scaffold Hopping of 5-Hydroxypyrazoles". Although carefully carried out, the work entailed in this manuscript requires some major revisions which are delineated below:
1. In the abstract part, the authors have mentioned about the participation of pyrrole core in their developed protocol but no pyrrole core is found in the MS for the 'scaffold hopping', so this statement needs justification.
2. In this protocol, an equivalent amount of molecular iodine in presence of DMSO solvent is necessary to carry out the Kornblum oxidation of methyl ketones, whereas literature discloses(i. Org. Lett. 2017, 19, 17, 4584–4587; ii. Org. Lett. 2020, 22, 18, 7103–7107) the necessity of catalytic amount of iodine in presence of DMSO is sufficient to perform the role, so in this case illustration is sought for regarding the role of equivalent amount of iodine to forward the reaction .
3. The authors must check the feasibility of the proposed protocol using the aliphatic methyl ketones.

4. The authors are advised to widen the merit of their protocol further and hence attempt should be made to evaluate and report the reactivity pattern of several other heterocyclic enamines other than the amino-pyrazoles and amino-isooxazoles.
5. The HRMS spectra of the reaction intermediates (1ab-D, A, B, C and D) and product 4a are too difficult to understand due to their low resolution and smaller scale size. Authors must provide the clear spectra containing the entire m/z scale.
6. In 1H-NMR spectra, an unidentified peak is present for compound 4u (δ 10.68), 4v (δ 9.91) and 4x (δ 10.55). Authors are suggested to justify this anomaly.
Recommendation: Major revision

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Wu and their colleagues have disclosed for the first time that skeletal remodeling of the pyrazole ring achieves its ring upgrading, a mode of reaction that appears to be a new form of molecular editing. This work achieves its scaffold hopping based on the cleavage of pyrazole's C=N bond, rather than from the previously reported N-N bond cleavage, and the reaction mechanism was also confirmed by HRMS.
The authors have carefully revised and supplemented all the comments from the two reviewers. The authors' workload has been substantial, and the variety of substrates has become quite rich. However, the results for aliphatic methyl ketones as substrates are not very satisfactory. Nonetheless, each reaction possesses its own universal applicability. As a reviewer, I believe that the paper has been significantly improved through revisions and that its quality has greatly enhanced. The paper exhibits high originality and is suitable for publication in the high-level journal Nature Communications.

Reviewer #2

(Remarks to the Author)
The authors have thoroughly addressed all the questions in the revised manuscript. The authors further expanded the substrate scope as suggested. Therefore, this work is highly recommended for publication in Nature Communications.

Response to Manuscript ID: NCOMMS-24-38779

Title: From an Azaarene to the Spine of Fused Bicyclics: Cycloaddition-Induced Scaffold Hopping of 5-Hydroxypyrazoles

Dear Editor,

We are very grateful for the careful evaluation of our manuscript by the reviewers and the editorial office for constructive suggestions. We have thoroughly revised the manuscript following the reviewer's and the editor's comments. Manuscript files with and without highlighted changes have been uploaded as requested. Please find below the point-by-point response to these comments. The reviewer's comments are in **black**, and our responses are in **blue**.

Reviewer's comments:

Reviewer #1 (Remarks to the Author):

Wu and their colleagues have disclosed for the first time that skeletal remodeling of the pyrazole ring achieves its ring upgrading, a mode of reaction that appears to be a new form of molecular editing. This work achieves its scaffold hopping based on the cleavage of pyrazole's C=N bond, rather than from the previously reported N-N bond cleavage, and the reaction mechanism was also confirmed by HRMS. To my opinion, this work is interesting and novel. The protocol applied to a wide range of substrates provides good yields. Additionally, the reaction features operation simplicity and easily available substrates, which offers a great advantage in synthetic chemistry. The authors further demonstrated the practicality of the method by the pre-modification transformation of the active molecules. Therefore, this work is highly recommended for publication in Nature Communications with minor revisions:

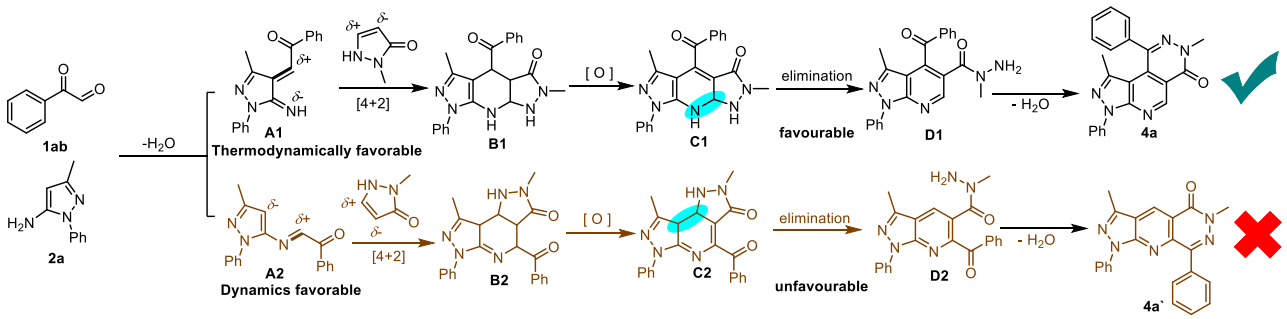
1. In Table 1, the reaction equation has 2.0 eq. of oxidant and c = 0.1, which is inconsistent with entries and notes, and it is not clear whether there is a clerical error or other considerations here, the authors should make a check.

***Response*:** We thank the reviewers for their careful checking, which was a mistake due to our negligence. We have corrected this mistake accordingly.

2. From the results, it appears that the generation of the target product **4a** relies firstly on the formation of a diene by a chemo-regioselective reaction of **1a** and **2a**. The **1a** and **2a**'s amino groups are also capable of forming imine-type diene, and the product obtained based on this seems to have a smaller spatial site resistance. Has the author detected or isolated such regioisomers?

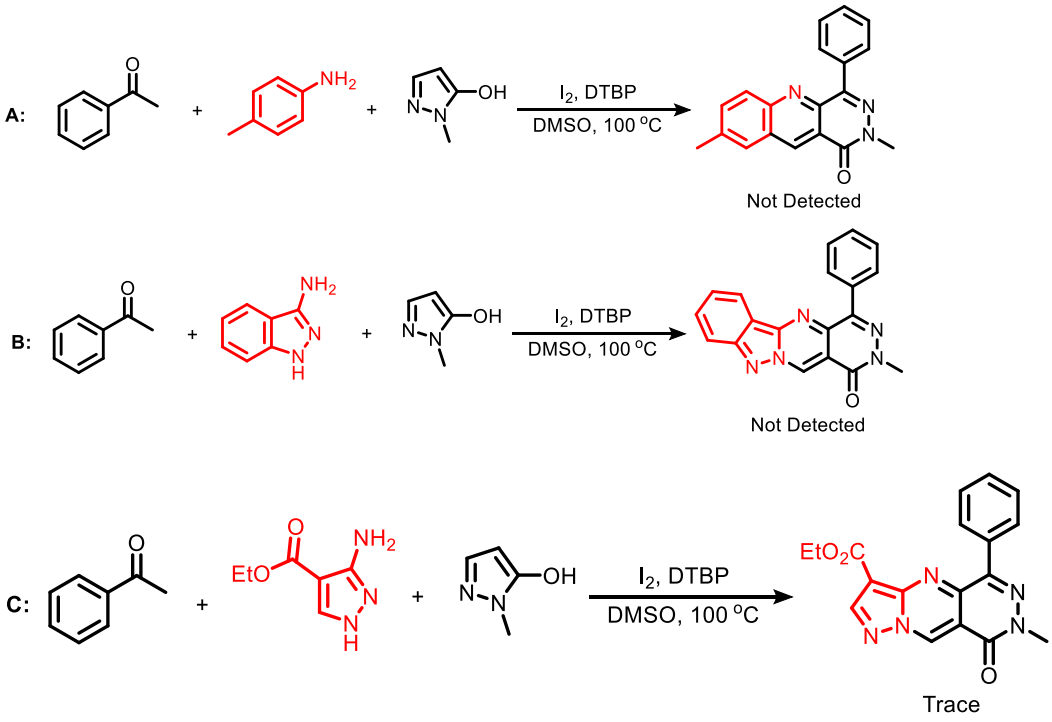
***Response*:** According to the literature (*J. Org. Chem.*, **2014**, *79*, 5258-5268; *Org. Chem. Front.*, **2018**, *5*, 765-768; *Org. Chem. Front.*, **2023**, *10*, 4122-4130), both the C-4 and N-5 positions of 5-aminopyrazole do have the property to undergo a condensation reaction with the aldehyde group to produce the diene. In fact, when we first attempted this reaction, it was once thought to be the **4a`** structure with a much smaller spatial site resistance by NMR spectroscopy and HRMS analysis, until *X*-ray single-crystal diffraction confirmed that **4a** was its true structure. In the process of expanding nearly a hundred products, we did not find the generation of isomers. This reaction has good chemical regioselectivity to generate a single target structure. We believe that the reasons for this high degree of selectivity are manifold; the intermediates **A1** produced by the reaction of **1ab** and **2a** are thermodynamically stable (irreversible), whereas the generation of **A2** is kinetically favorable (reversible); the **C1** and **C2** intermediates produced by the subsequent [4+2] cycloaddition and oxidation of each, **C1** being an aminal structure (*Org. Chem. Front.*, **2021**, *8*, 4508-4513, *Org.*

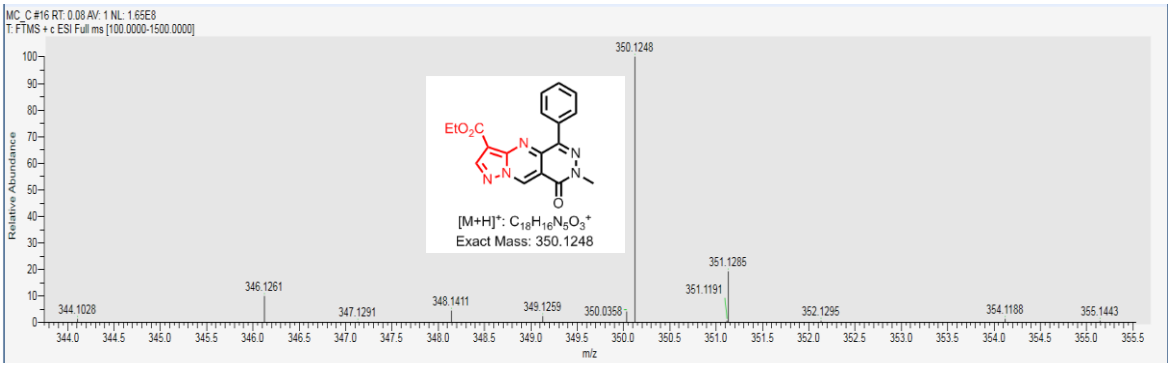
Lett., **2020**, *22*, 8210-8214), elimination of the cleaved C-N bond is much easier and more rapid; whereas the **C2** intermediate does not have the structural advantage of elimination; thus a single product, **4a**, was finally obtained.



3. From the point of view of the reaction mechanism, could **2a** be extended to other types of aromatic amines, for example, aniline, 3-aminoindazole, etc., to afford the corresponding product?

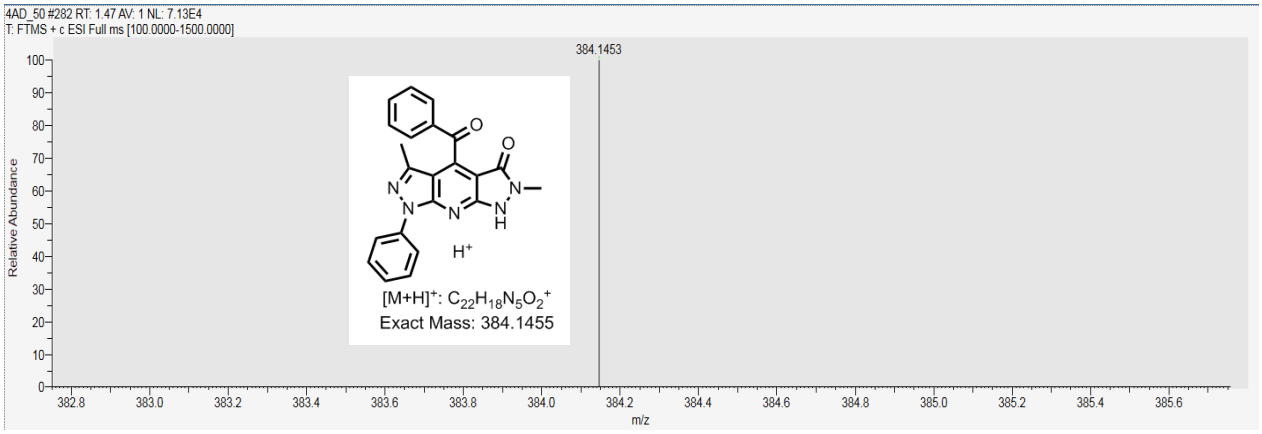
***Response*:** We used *p*-toluidine, 3-aminoindazole, or 2-amino-4-ethylpyrazole instead of **2a** as reaction substrates under standard reaction conditions. Unfortunately, thin-layer chromatography analysis and high-resolution mass spectrometry testing of the reaction solutions revealed that the generation of the target products was not detected for reactions A and B. And reaction C was able to detect the generation of the target products in trace amounts, but was difficult to obtain by separation. From the perspective of the value of synthesis, we consider this result to be of limited significance.





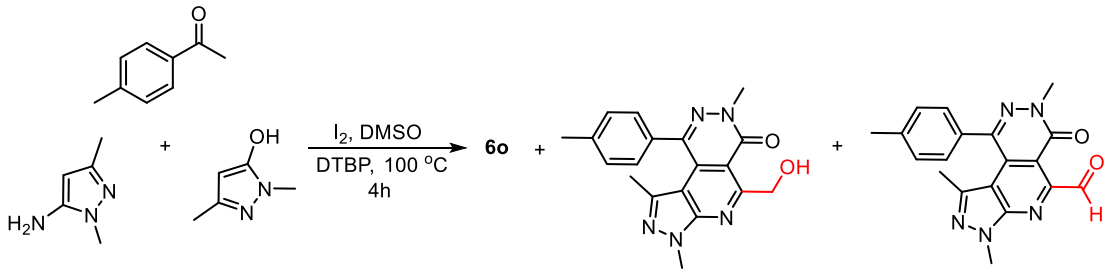
4. In Figure 5d, since there is no substituent at the C-3 position in substrate **3a** to act as a hindering group, intermediate **D** should be easily oxidatively arylated to produce a 5/6/5 tricyclic by-product, can it be detected and isolated?

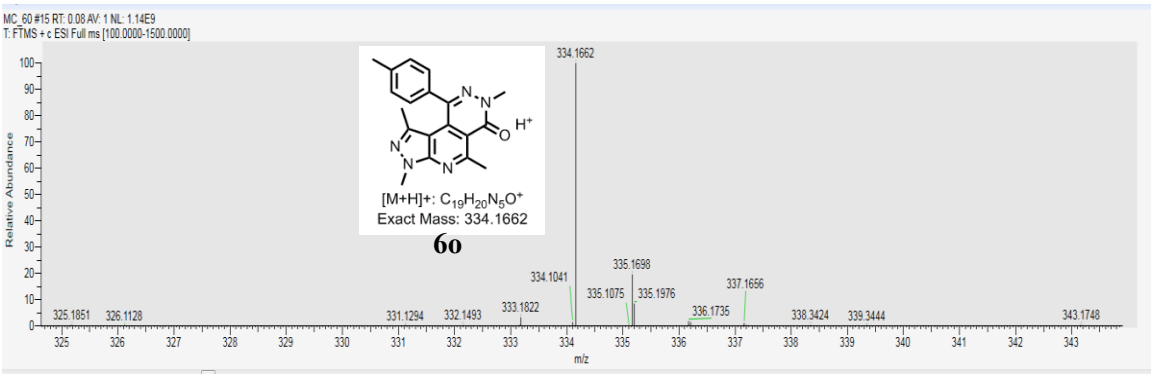
***Response*:** Under standard reaction conditions, we used high-resolution mass spectrometry to detect the reaction solution, and in addition to clearly detecting the target product **4a**; A small amount of by-products **4a-by** formed by oxidative aromatization were also detected, but due to their low concentration, they were difficult to obtain by isolation. Moreover, this type of 5/6/5 tricyclic by-product was not detected or isolated in other examples of our substrate expansion process.

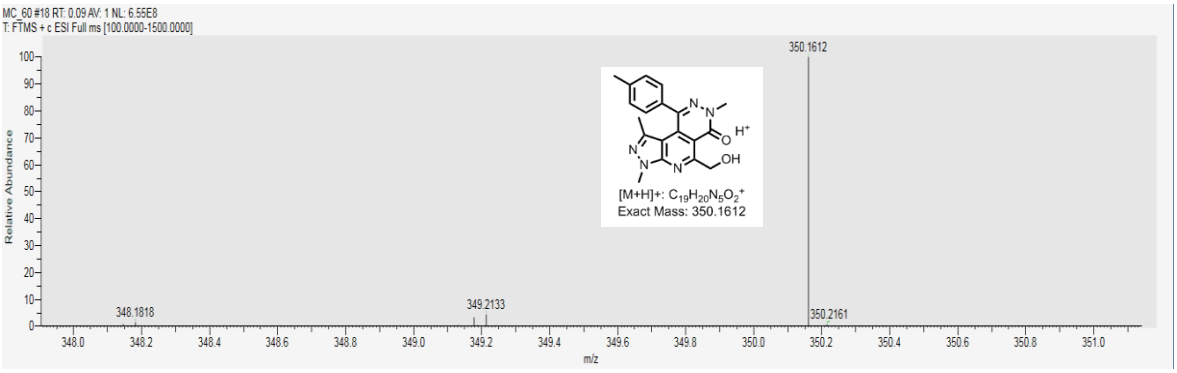


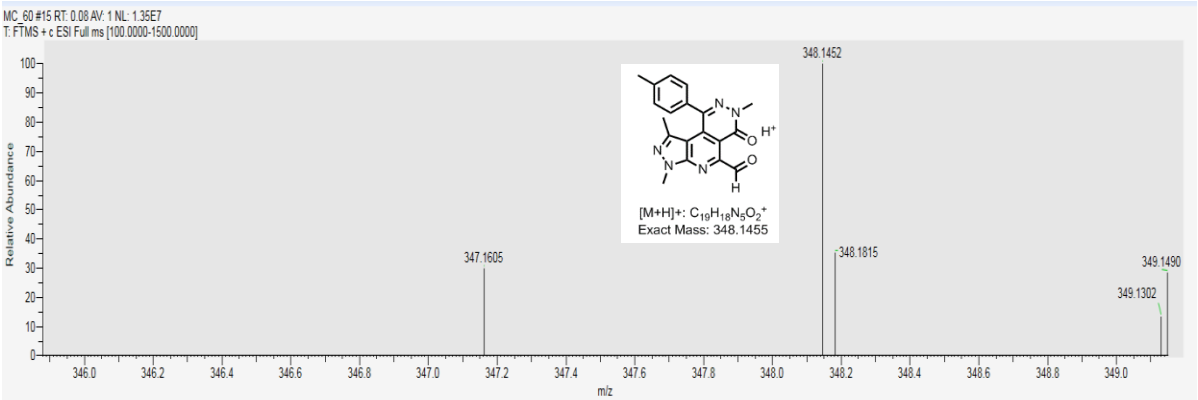
5. What is the reason for the different reaction conditions observed in Figure 3 for **6m-6p**?

***Response*:** According to the reports in the literature (*Org. Lett.*, **2021**, *23*, 9000-9005; *Org. Chem. Front.*, **2022**, *9*, 1403-1409; *RSC Adv.*, **2017**, *7*, 44132-44135), the *ortho*-methyl group of the pyridine ring can be oxidized under I_2 or other oxidizing agents to generate aldehyde groups. Taking product **6o** as an example, under the conditions of I_2 and DTBP, the methyl group of the product will be oxidized, generating by-products bearing corresponding primary alcohol or aldehyde group, resulting in therefore a decrease in the yield of **6o**. We confirmed this conclusion by examining the reaction solution by high-resolution mass spectrometry. Consequently, we recommend using only iodine in the synthesis of **6m-6p** (**6y-7b**) and shortening the reaction time to 2 hours.









Reviewer #2 (Remarks to the Author):

The manuscript communicated in the Nature Communications by Wu and co-workers disclosed the chemistry entitled “From an Azaarene to the Spine of Fused Bicyclics: Cycloaddition-Induced Scaffold Hopping of 5-Hydroxypyrazoles”. Although carefully carried out, the work entailed in this

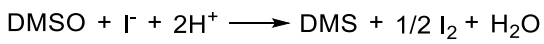
manuscript requires some major revisions which are delineated below:

1. In the abstract part, the authors have mentioned about the participation of pyrrole core in their developed protocol but no **pyrrole core** is found in the MS for the 'scaffold hopping', so this statement needs justification.

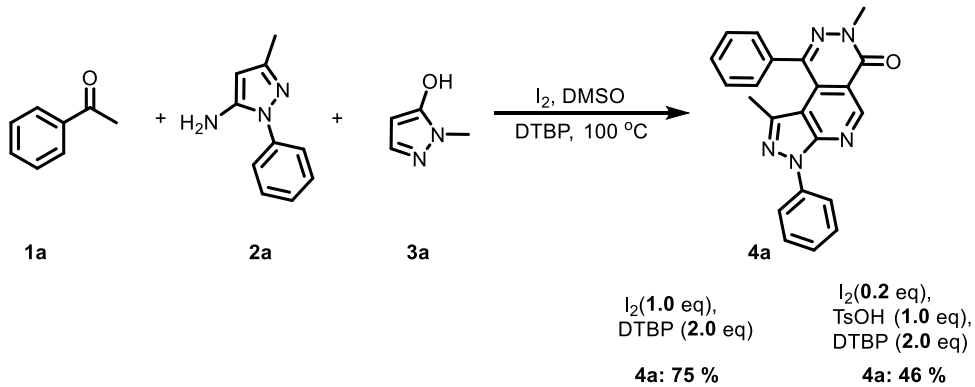
***Response*:** We thank the reviewers for their careful checking. We are so sorry for this mistake; we have corrected the "pyrrole core" into "pyrazole core".

2. In this protocol, an equivalent amount of molecular iodine in presence of DMSO solvent is necessary to carry out the Kornblum oxidation of methyl ketones, whereas literature discloses(i. Org. Lett. 2017, 19, 17, 4584–4587; ii. Org. Lett. 2020, 22, 18, 7103–7107) the necessity of catalytic amount of iodine in presence of DMSO is sufficient to perform the role, so in this case illustration is sought for regarding the role of equivalent amount of iodine to forward the reaction.

***Response*:** Based on our experimental results and our understanding of the I_2-DMSO system, we think that the use of catalytic amounts of iodine to achieve DMSO-enabled catalytic cycling could be realized in some cases (include the paper you cited and please also see: J. Org. Chem. 2022, 87, 15101−15113). We found that higher reaction temperatures and the addition of extra Brönsted acid to the system will facilitate this catalytic cycle. However, in this paper, we did not use acid as additives. As a result, this catalytic cycle is not efficient enough. We believe that the role of acids in this can be explained by the following reaction equation.

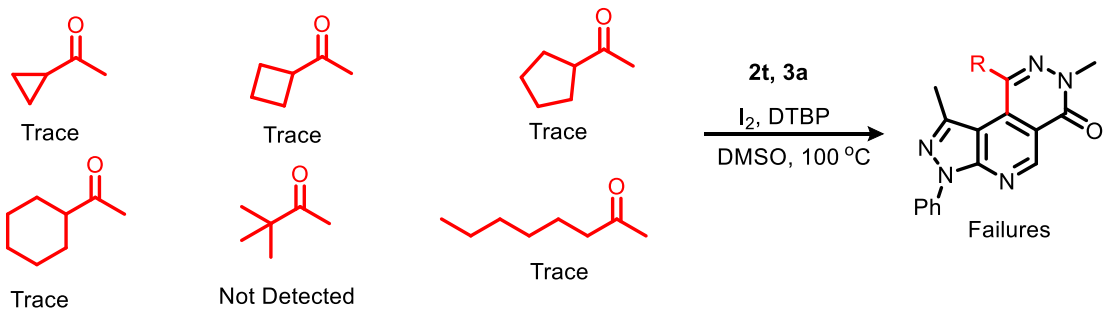


Furthermore, based on our screening of the reaction conditions in this paper, we have found that high yields of the product could not be obtained using catalytic amounts of iodine alone, and that the reaction could not be carried out efficiently with the addition of the extra acid (standard condition: 75%; 0.2eq I_2, 1.0eq TsOH and 2.0eq DTBP: 46%). Based on these experimental results, we chose 1.0 equivalent of iodine as the optimal one. We believe that higher concentrations of iodine are favourable for the oxidation of intermediate **C** to **D**, accelerating the ring opening process of 5-hydroxypyrazole. The iodine is consumed in the first step of methyl ketone substitution by iodine, however, in the presence of DMSO as well as DTBP, iodine could be regenerated and participates the oxidation reaction in the downstream (from **C** to **D**, in Figure 5-d). However, we did not choose to add more than one equivalent of iodine. Indeed, using 2.0 equivalents of iodine resulted in a lower yield of the desired product (please see SI: Screening of the reaction conditions). We believe that the overdose of iodine has a negative effect on the reaction and substrate compatibility due to side reactions, such as iodine substitution on electron-rich aromatic rings (Org. Lett. 2016, 18, 2507−2510), will afford unwanted by-products.

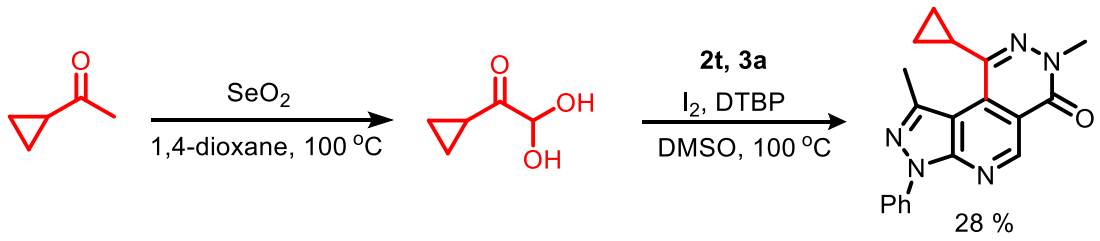


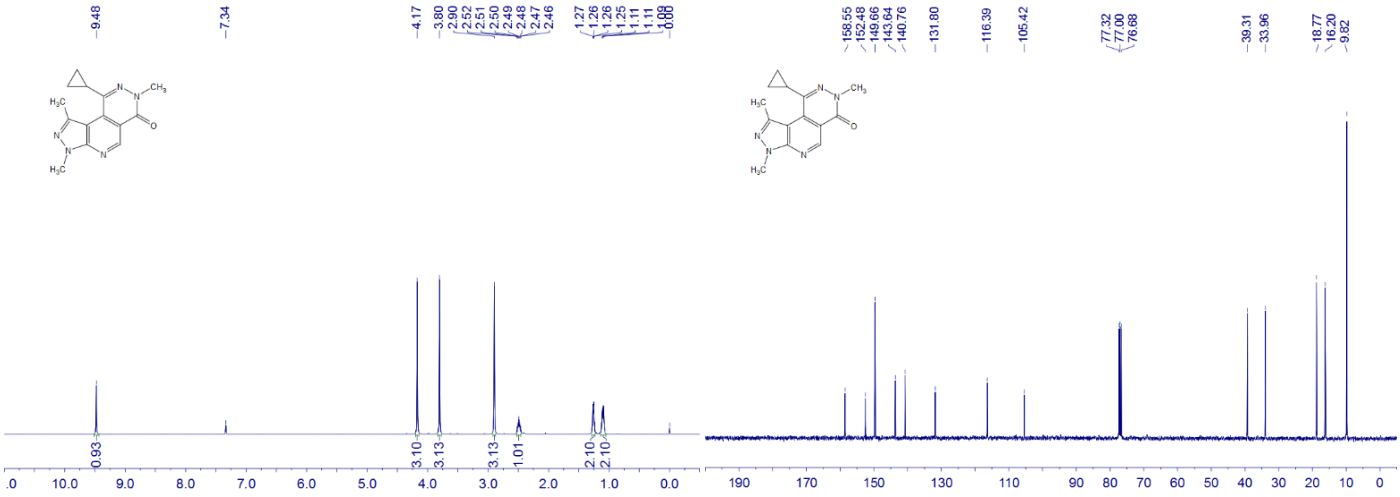
3. The authors must check the feasibility of the proposed protocol using the aliphatic methyl ketones.

***Response*:** We tried the feasibility of six alkyl ketones under standard conditions and found that none of the products could be obtained successfully. As far as we know from our previous research experience, the final product could not be obtained because the alkyl ketones ($RCOCH_3$) are not easily to be transformed into corresponding alkylglyoxal (RCOCHO) under I_2-DMSO conditions. In comparison to the aryl ring, the aliphatic chain substituents are not sufficiently electron-rich. The nucleophilic substitution process (iodination process), the first step of the domino process, is less likely to occur.



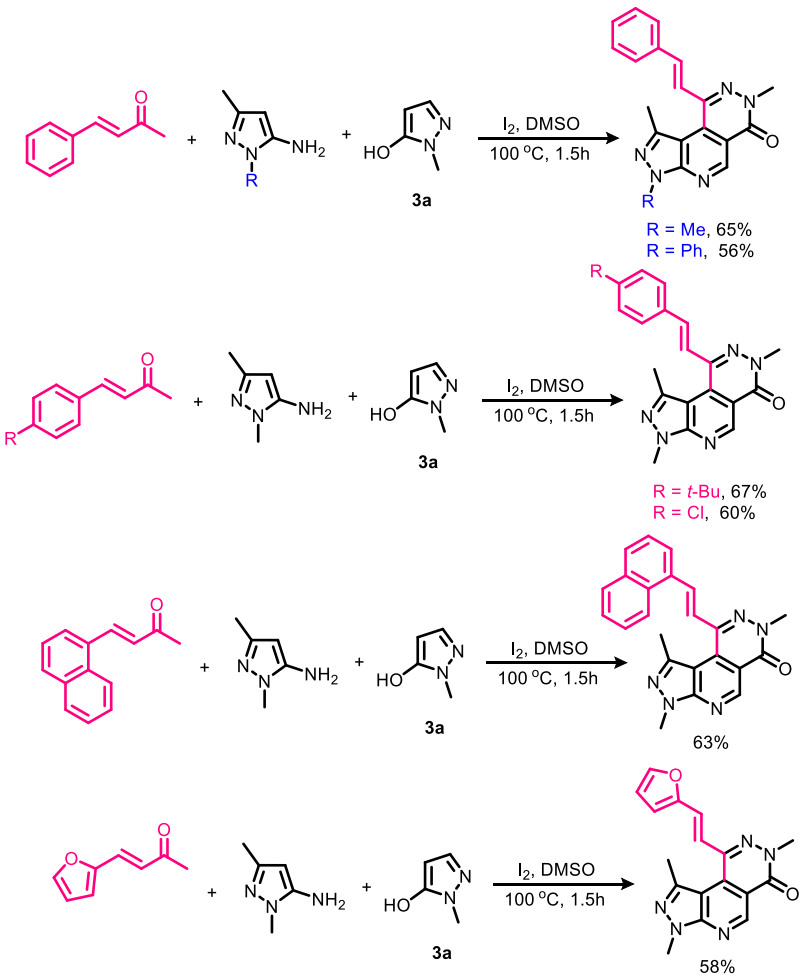
We subsequently oxidized cyclopropyl methyl ketone to afford cyclopropylglyoxal using SeO2. Then used 1-cyclopropyl-2,2-dihydroxyethan-1-one as the starting material to carry out our standard reaction. We only obtain the target product in 28% yield. This result demonstrates that our ring-upgrading reaction can occur when using an aliphatic glyoxal directly as a substrate, but resulting in low yield under our standard conditions. It is promising to improve the yield through further screening the reaction conditions. However, we would like to concentrate in this article on reporting the transformation from methyl ketone as a substrate.

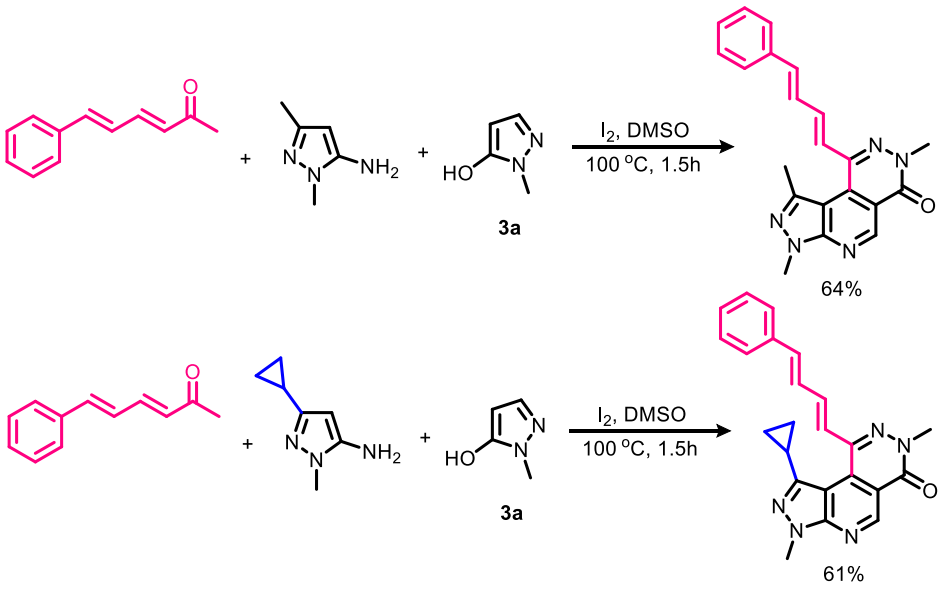




To further demonstrate the diversity of substrates, a variety of α, β-unsaturated methyl ketone was

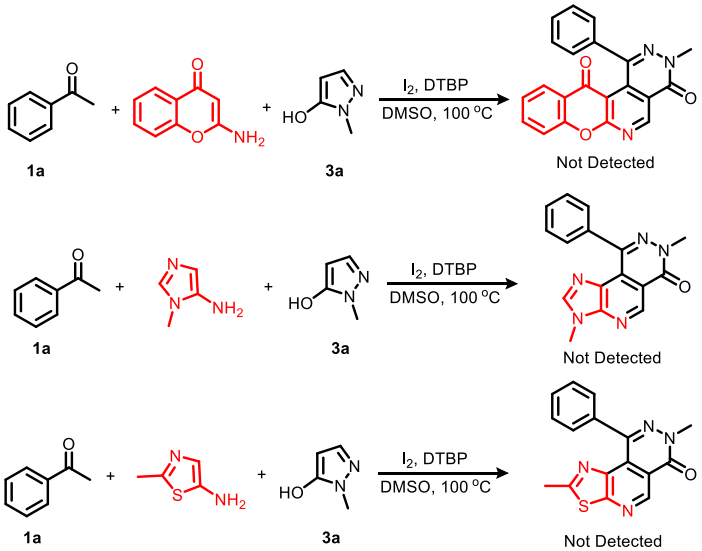
examined. Using reaction condition b, it was found that α, β-unsaturated methyl ketone bearing various substitution such as halogenated aryl group, naphthyl substituent, and heterocyclics yielded the target compounds in moderate yields (6 examples). Encouragingly, we also tried 3,5-diene methyl ketone as a substrate and found that the structure of conjugated olefins is also compatible in the reaction. We believe that the double bonds in the above examples can all be converted to saturated carbon chains through hydrogenation reactions. In this way, we can synthesize some examples of pyrazolopyridopyridazin-6-one skeleton bearing aliphatic substitutions which are limited in our methodology. We added the eight examples from this section to the main article and supporting information after completing the structural characterization.

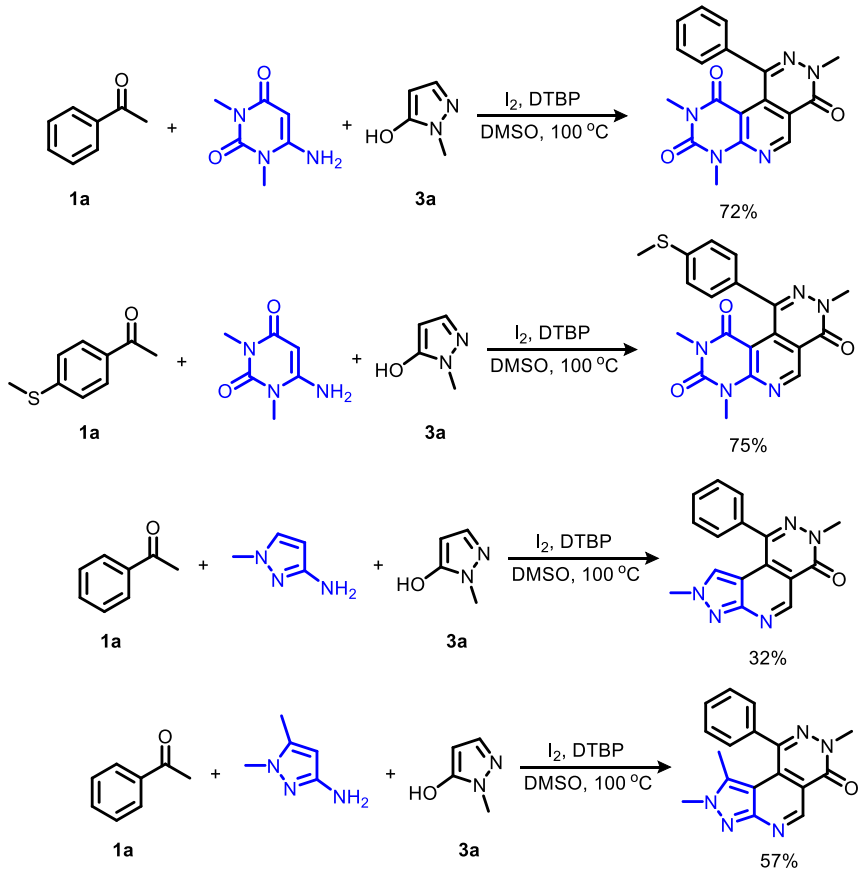




4. The authors are advised to widen the merit of their protocol further and hence attempt should be made to evaluate and report the reactivity pattern of several other heterocyclic enamines other than the amino-pyrazoles and amino-isooxazoles.

***Response*:** Under standard reaction conditions, we tried many enamine substrates instead of 5-aminopyrazole, and found that 3-amino-1-methylpyrazole and 6-aminouracil yielded the corresponding target products. We believe it is valuable to incorporate uracil skeletons in the final product. We thank the reviewers for your suggestions. Four target compounds were synthesized, which were added to the main article and supporting information after a full structural characterization was completed.



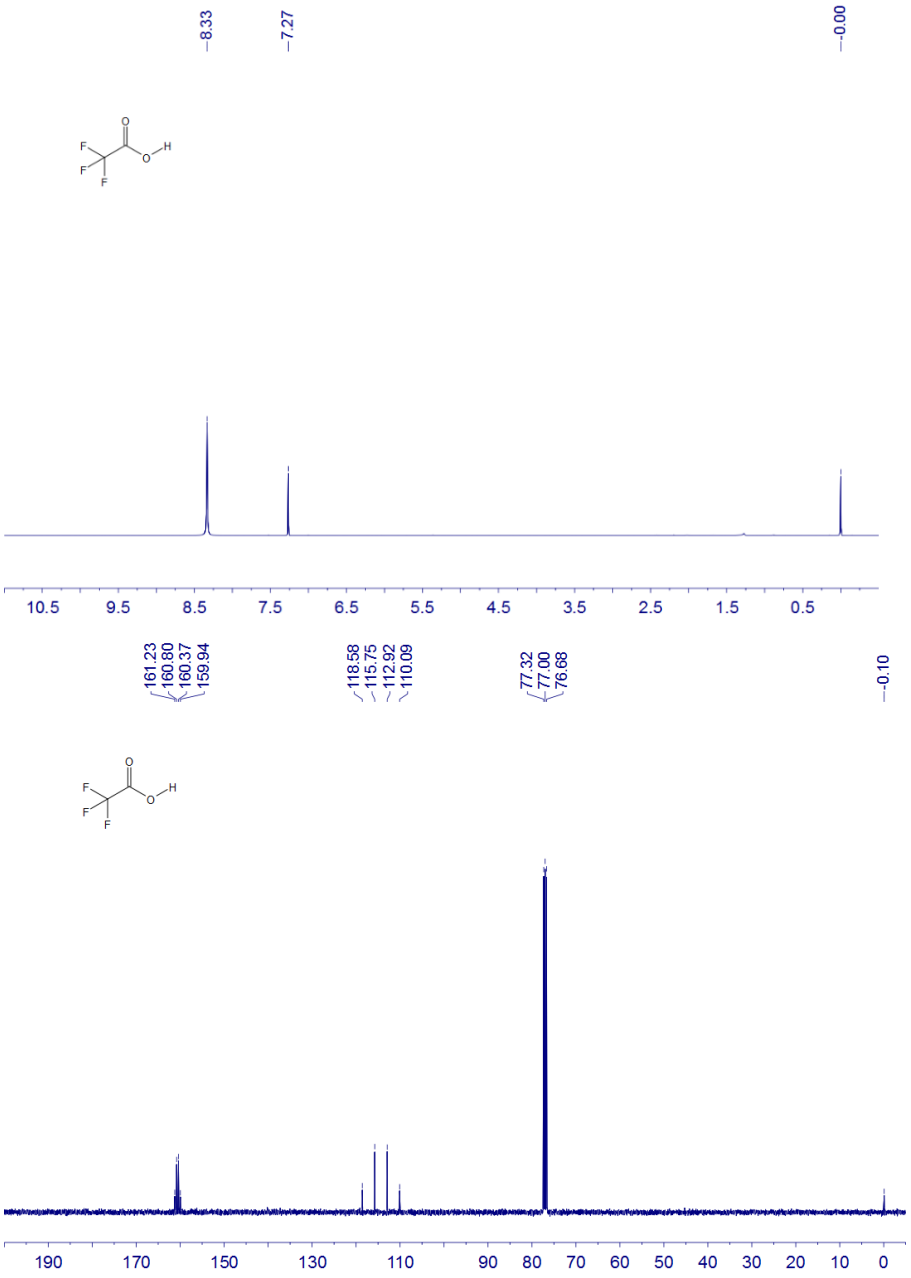


5. The HRMS spectra of the reaction intermediates (1ab-D, A, B, C and D) and product 4a are too difficult to understand due to their low resolution and smaller scale size. Authors must provide the clear spectra containing the entire m/z scale.

***Response*:** We thank the reviewers for checking carefully, this was a messy image due to our negligence and we have re-uploaded the usable image.

6. In 1H-NMR spectra, an unidentified peak is present for compound 4u (δ 10.68), 4v (δ 9.91) and 4x (δ 10.55). Authors are suggested to justify this anomaly.

***Response*:** Thank you reviewers for your careful reading of the manuscript and your questions are very specialized. NMR characterization is unavailable due to the fact that some of the compounds are very difficult to dissolve in solvents such as $CDCl_3$ and CD_3SOCD_3. According to the literature (*Molecules*, **2023**, *28*, 1557), for compounds that are difficult to dissolve, if a few drops of CF_3CO_2D are added as a co-solvency reagent to $CDCl_3$, the products can be dissolved and characterized without any problem; and the single peaks with high chemical shifts in the ^1H NMR spectra of **4u**, **4v**, and **4x** are the result of CF_3CO_2D, and similarly their ^{13}C NMR spectra will have two quadruple peaks present. We provide the NMR spectra of CF_3CO_2D in $CDCl_3$, in which the chemical shift (8.33) of the ^1H NMR spectrum of CF_3CO_2H is related to the trace water content of the solution, with higher chemical shifts at lower water content and vice versa.



Sincerely,

Prof. An-Xin Wu

Prof. Jia-Chen Xiang