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ARTICLE

NHC Catalysed direct addition of HMF to diazo compounds: Synthesis of Acyl Hydrazones with antitumor activity

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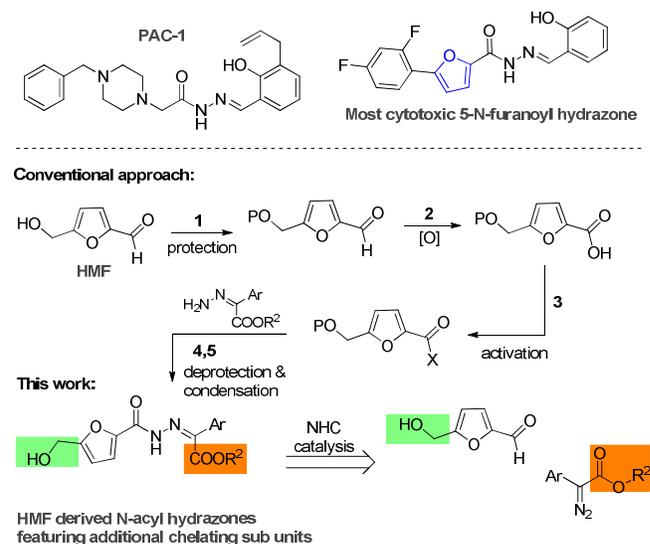
NHC *umpolung* catalysis between 5-hydroxymethyl furfural (HMF) derivatives and diazo compounds overcomes the usual multistep synthesis of acylhydrazones and gives direct access to an unexplored family of HMF-based acylhydrazones displaying promising anti-tumor activity. A preliminary screening of hydroxymethyl's protection groups allowed the identification of the *tert*-butyldimethylsilyl group as essential for the desired biological activity. Compound **25** was found to be very active against MCF-7 breast cell line (with an IC₅₀s of 3.60 μM) and displayed a ten-fold selectivity relatively to CaCo-2 cell lines model.

Introduction

Hydrazones are an important scaffold for medicinal chemistry present in a wide range of molecules with antimicrobial, anti-inflammatory, antiviral and antitumor properties.¹ Among these, acylhydrazones are particular interesting because they often display high cytotoxic activity.²⁻¹¹ For instance, PAC-1 is a potent cytotoxic acylhydrazone that was recently approved for clinical trials (Scheme 1). This molecule is thought to induce apoptosis *via* the activation of procaspase-3 interfering in the zinc-mediated inhibition pathway.^{12,13} Recognizing the importance of these molecules to effectively chelate metal ions, the furan moiety was included in the structure of several acylhydrazones to improve their antitumor activity. This strategy lead to the synthesis of several 5-aryl-*N*-furanoyl hydrazones with promising cytotoxic activities against human promyelocytic leukemic cells (15 μM (IC₅₀) for HL-60, scheme 1).¹⁴ Based on this rational, we conceived that further manipulation of this scaffold to include additional chelating sub units, could render *N*-furanoyl hydrazones with improved biological activity (Scheme 1).

5-Hydroxymethyl furfural (HMF) is a very important synthetic motif which has recently gathered considerable attention for its unique structure and biorenewable origins.¹⁵⁻¹⁹ As shown in Scheme 1, HMF exhibits a substituted furan ring ideally suited to build structurally diverse *N*-furanoyl hydrazones. Despite this, HMF synthetic manipulation is often troublesome due to a limited chemical and thermal stability.^{17,20} For instance, in acidic conditions, HMF suffers hydrolysis and formic acid extrusion to yield levulinic acid, while in basic conditions, it readily affords 5-hydroxymethyl furanoic acid and 2,5-dihydroxymethyl furan *via* a Cannizzaro reaction.²¹⁻²² Therefore, traditional methods to prepare hydrazones became very problematic when using HMF and require a multistep sequence comprising the protection of the alcohol, oxidation of the aldehyde, activation of the carboxylic acid and deprotection of the alcohol (scheme 1). Hence we conceive that an alternative strategy to circumvent this problem would be the

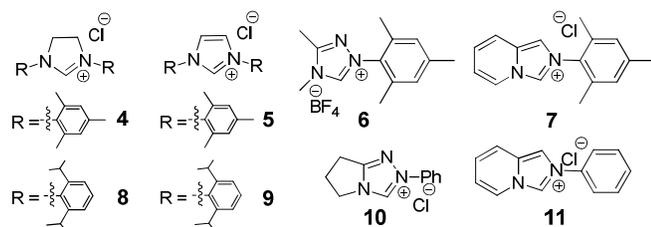
direct addition of the aldehyde to a diazo compound catalysed by *N*-heterocyclic carbenes (NHC).²³ Based on our recent experience on the use of NHCs as organocatalysts²³⁻²⁵ and in the synthesis and manipulation of HMF^{15,16,21}, we initiated a study to establish if NHCs could be effectively used to *umpolung* the reactivity of HMF's aldehyde and to catalyse the direct synthesis of *N*-furanoyl hydrazones with improved cytotoxic activity against model cancer cell lines.



Scheme 1. PAC-1 structure; *N*-acyl hydrazones with antitumoral activity; Conventional *versus* the application of NHC organocatalysis in the synthesis of novel HMF-based *N*-acyl hydrazones with potential antitumor activity.

Results and discussion

We initiated this study by testing the NHC catalysed direct addition of HMF **1** to diazo compound **2**. Based on our previous study on the addition of aldehydes to diazo compounds, we identified NHCs generated from imidazolidinium **4**, imidazolium **5** and triazolium **6** as the most promising catalysts for this transformation (scheme 2).²³ Therefore HMF **1** was slowly added to a pre-stirred solution of 20 mol% of each of the NHC precursors, 40 mol% of DBU and diazo compound **2**. Differently from our previous study, NHCs generated *in situ* from **5** and **6** performed poorly when using HMF as the starting material, affording the desired *N*-acyl hydrazone in only 11 and <5% yield respectively. Differently and to our delight, when using imidazolidinium **4** as the NHC precursor, the desired compound **3** was obtained in 70% isolated yield after 16 hours at room temperature (Table 1, entry 1).



Scheme 2. NHC precursors employed in this work

Table 1. Optimization of reaction conditions

Entry	NHC precursor	Base	Solvent	Yield (%)
1 ^a	4	DBU	CH ₂ Cl ₂	70
2 ^a	5	DBU	CH ₂ Cl ₂	11
3 ^a	6	DBU	CH ₂ Cl ₂	6
4 ^a	7	DBU	CH ₂ Cl ₂	<5
5 ^a	8	DBU	CH ₂ Cl ₂	8
6 ^a	9	DBU	CH ₂ Cl ₂	5
7 ^a	10	DBU	CH ₂ Cl ₂	<5
8 ^a	11	DBU	CH ₂ Cl ₂	<5
9 ^a	4	NaH	CH ₂ Cl ₂	<5
10 ^a	4	NaO ^t Bu	CH ₂ Cl ₂	<5
11 ^a	4	TEA	CH ₂ Cl ₂	<5
12 ^a	4	DIPEA	CH ₂ Cl ₂	<5
13 ^a	4	DBU	THF	41
14 ^a	4	DBU	CH ₃ CN	40
15 ^a	4	DBU	MeOH	<5
16 ^a	4	DBU	Toluene	n.r.
17 ^b	4	DBU (20)	CH ₂ Cl ₂	49
18 ^c	4 (10)	DBU (20)	CH ₂ Cl ₂	38
19 ^d	4	DBU	CH ₂ Cl ₂	80

[a] HMF **1** (1.5 eq), diazo **2** (1 eq.), NHC precursor (20 mol%), base (40 mol%), aldehyde slow addition (0.2 ml solvent in 30 minutes); [b] identical to a) except base (20 mol%); [c] identical to a) except NHC precursor (10 mol%), base (20 mol%); [d] identical to a) except aldehyde slow addition (0.5 ml solvent in 2 hours); N.R. - no reaction; DBU - 1,8-Diazabicyclo[5.4.0]undec-7-ene; TEA - triethyl amine; DIPEA - diisopropyl ethyl amine.

This compound was shown to be a unique organocatalyst for this amidation reaction as all other NHCs tested afforded only

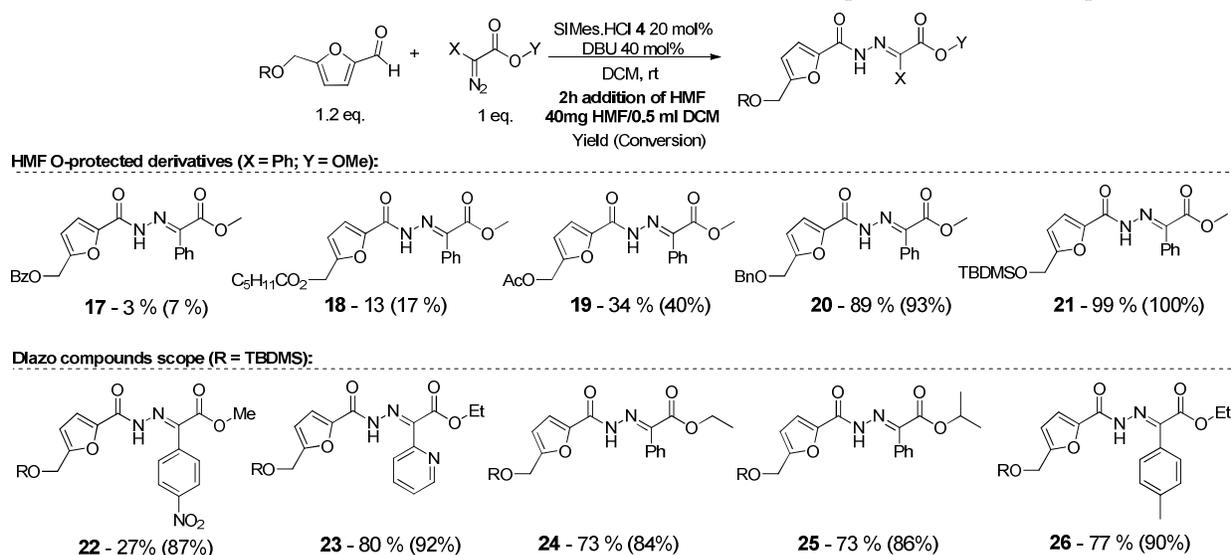
traces of compound **3** (Table 1, entries 4-8). Once established the NHC generated *in situ* from imidazolidinium **4** as the most effective organocatalyst precursor to perform the addition of HMF to diazo compound **2**, we addressed the optimization of the reaction conditions. As shown in table 1, when using bases like NaH, NaO^tBu, triethylamine and diisopropylethyl amine (Table 1, entries 9-12) the yield of hydrazone **3** decreased considerably. The reaction was also less efficient when performed in solvents such as THF and acetonitrile (Table 1, entries 13-14) and was completely inhibited in methanol and toluene (Table 1, entries 15-16). Similarly reducing the amount of based or organocatalyst to 20 mol% and 10 mol% respectively resulted in lower yields of *N*-acyl hydrazone (Table 1, entry 17 and 18). Finally, by increasing the aldehyde addition time, the isolated yield of the compound **3** was improved to 80% (Table 1, entry 19). Interestingly, the analysis of the reaction crude mixture by HPLC (see SI) did not indicate the formation of the expected competitive Cannizzaro products (2,5-dihydroxymethyl furan or 5-hydroxymethyl furoic acid) neither the products resulting from the NHC catalysed self-condensation of HMF. After establishing the optimised reaction conditions and aiming at the synthesis of structurally different *N*-(5-hydroxymethyl furfuryl) hydrazones for biological evaluation, we tested the addition of HMF to several diazo compounds.

Table 2. Diazo compound scope with HMF as nucleophile

Entry	Product	Conv. (%)	Yield (%)
1	3	95	80 ^b
2	12	77	68 ^c
3	13	31	50 ^b
4	14	60	42 ^b
5	15	72	64 ^b
6 ^a	16	73	53 ^d

[a] 1 eq. of furan-2,5-dicarbaldehyde and 2 eq. of diazo compound **2**; [b] isomer ratio = 1:9; [c] isolated as a single isomer and characterized in DMSO- d_6 ; [d] isomer ratio = 4:8

conditions and tested against the same cancer cell lines. As shown in table 3, compounds **22** and **23** were shown to be inactive while compound **24** inhibit the proliferation of this



Scheme 3. Substrate scope of the NHC-catalysed formation of N-acyl hydrazones from HMF O-derivatives

As shown in Table 2, using diazo compounds featuring different aromatic and ester substituents afforded the expected *N*-(5-hydroxymethyl furfuryl) hydrazones in moderate to good yields (42-80%) as a mixture of geometric isomers E/Z (for more details consult SI). Furan-2,5-dicarbaldehyde was also shown to be a viable substrate for this transformation, yielding the symmetrical *N*-acyl hydrazone **16** in 53 % yield (Table 2, entry 6). Once achieved the synthesis of HMF derived *N*-acyl hydrazones **3**, **12-16**, we envisioned that further modification of the hydrazone structure could be obtained by modifying the hydroxyl group at the HMF. Therefore, we tested the direct addition of O-protected HMF derivatives to diazo compounds under the optimized reaction conditions. As shown in scheme 3, the ester derivatives displayed lower reactivity than HMF itself delivering hydrazones **17-19** in up to 34 % yield (scheme 3, top). Very differently, both benzyl and TBDMS ethers afforded the expected products in 89 and 99% isolated yields respectively (scheme 3). With compounds **3**, **12-16**, **20** and **21** in hands we performed the evaluation of their anti-proliferative properties against HT-29, MCF-7 and NCI-H460 cancer cell lines at concentrations within the range 0-15 μ M. Very disappointingly, *N*-(5-hydroxymethyl furfuryl) hydrazones **3**, **12-16** didn't exhibit any relevant biological activity, similarly HMF benzyl ether derivative **20** was also unable to prevent the proliferation of any of the cancer cell lines at these concentrations. Differently, the HMF TBDMS ether **21** proved to be active against MCF-7 cancer cell line with an IC_{50} of 13.32 μ M. These results indicate that the presence of the TBDMS group on the molecule structure is important for the biological activity probably due to an increase of the compound lipophilicity. This is in line with previous observations for other scaffolds and biological activities.²⁶ Therefore, compounds **22-26** were readily prepared under our optimized reaction

cancer cell models in the range of 8.59-13.32 μ MIC₅₀s. Very gratifyingly, these results were further improved with compounds **25** and **26** that displayed IC_{50} s in the interval of 3.60-6.37 and 3.50-7.29 μ M, respectively. Furthermore compound **25** and **26** also displayed the lower toxicities against normal cells as demonstrated by the highest IC_{50} s (40.12 and 27.90 μ M respectively) in the assay with CaCo-2 cell lines model.

Table 3. Determined IC_{50} of the compounds in HT-29, MCF-7, NCI-H460 and CaCo-2 models after 24 hours incubation. Between brackets is presented the selectivity index defined as IC_{50} (CaCo-2) / IC_{50} (HT-29 or MCF-7 or NCI-H460).

$IC_{50} \mu$ M	HT-29	MCF-7	NCI-H460	CaCo-2
3	>15	>15	>15	-
12	>15	>15	>15	-
13	>15	>15	>15	-
14	>15	>15	>15	-
15	>15	>15	>15	-
16	>15	>15	>15	-
20	>15	>15	>15	-
21	>15	13.32(1.44)	>15	19.14
22	>15	>15	>15	-
23	>15	>15	>15	-
24	8.59(1.82)	13.32(1.17)	10.71(1.53)	15.62
25	6.37(6.30)	3.60 (11.14)	5.06 (6.73)	40.12
26	7.29(3.82)	3.50 (7.97)	6.95 (4.01)	27.90

Conclusions

The HMF scaffold offers the possibility to access a diverse and unexplored family of acylhydrazones with potential antitumor activity. However, the utilization of HMF for the synthesis of higher value molecules such as fine chemicals or drugs is a challenging area of research due to its peculiar chemistry and thermal and/or chemical stability. This motivated the discovery

of a new method to directly access acylhydrazones from the HMF scaffold overcoming the common multistep synthesis. Herein, it was demonstrated that NHCs are able to selectively catalyse the nucleophilic addition of HMF and its O-protected derivatives to aryl diazoacetates under mildly basic conditions at room temperature affording the desired acylhydrazones, while avoiding the usual degradation pathways of HMF. From our preliminary biological evaluation studies compounds **25** and **26** were identified as active cytotoxic agents (displayed IC₅₀s in the interval of 3.60-6.37 and 3.50-7.29 μM, respectively) indicating that t-butyltrimethylsilyl group is essential for the desired biological activity. Additionally, compound **25** displayed an increased ten-fold selectivity relatively to CaCo-2 cell lines model displaying an IC₅₀ of 40.12 μM.

Experimental

Synthesis of HMF-based N-acylhydrazones: NHC precursor **4** (20 mol %) and DBU (40 mol %) were dissolved in freshly dry DCM (0.5 mL) inside a round bottom flask under argon. The mixture was stirred for 10 minutes at room temperature. Then a solution of diazo compound (0.24 mmol in 0.5 mL of DCM) was added. Immediately after, HMF (1.2 eq., 40 mg in 0.5 mL of DCM) (or protected HMF) was slowly added over two hours. The mixture was left reacting until most of diazo compound was consumed, usually 16 hours. The final product, N-Acylhydrazone, was isolated by flash chromatography (eluent: Hexane/Ethyl acetate).

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Notes and references

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Electronic Supplementary Information (ESI) available: General information, experimental procedures, data and copies of ¹H and ¹³C NMR spectra for compounds **3**, **12-26** is available in supporting information. See DOI: 10.1039/b000000x/

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