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A new Heck reaction modification using ketone Mannich bases as enone precursors: Parallel synthesis of anti-leishmanial chalcones

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Abstract—A new Heck-type reaction for the synthesis of chalcones has been established using Mannich bases as enone precursors. The novel reaction proceeds rapidly in air atmosphere under ligandless conditions and can be adapted for library synthesis in a parallel reactor station. Screening of the synthesized chalcones revealed *N*-{4-[(1*E*)-3-oxo-3-(3-pyridinyl)-1-propenyl]phenyl}benzamide **(3f)** to be a potent anti-leishmanial agent.

Kala azar or visceral leishmaniasis (VL) is caused by infection with *Leishmania donovani* parasites that are transmitted by the bite of sandflies. Typical symptoms of this disease include hypergammaglobulinemia, anemia, weight loss, hepatosplenomegaly, fever, and immunosuppression. Without early diagnosis and proper treatment VL is fatal. Due to the epidemiological situation and therapeutic options available to populations in endemic countries affected by this disease, the WHO has designated leishmaniasis a 'neglected and emerging disease'. All existing medical treatments against leishmaniasis have major drawbacks (resistance against antimony drugs, side effects of pentamidine and amphotericin B, reproductive toxicity of miltefosine) and there is an urgent need for additional effective medicines.[1,2] Chalcones (=1,3-diarylpropenones) of natural or synthetic origin have repeatedly been mentioned as anti-leishmanial agents.[3–6] It has been shown that licochalcone A, a chalcone from the roots of *Glycyrrhizae inflata*,[7] alters the ultrastructure of mitochondria in *Leishmania* parasites[8] and inhibits the leishmanial fumarate reductase.[9] Oxygenated chalcones exhibited in vivo anti-leishmanial activity in a hamster model.[10] Detailed structure–activity relationships (SAR) of anti-leishmanial chalcones have been published by several groups.[11–14] Chalcones have also been found to show anti-plasmodial activity in vitro.[15] The majority of the chalcones used in these studies were either extracted from natural sources or prepared by a classical aldol condensation reaction employing an aromatic aldehyde and an appropriate aromatic ketone. For a systematic exploitation of the published SAR and the generation of chalcone libraries additional synthetic procedures are desirable. These methods should be broadly applicable, start with inexpensive educts and allow the rapid generation of congeners in a parallel fashion. For example, a variety of substituted haloarenes are commercially available that could serve as educts for carbon–carbon-cross coupling reactions.

For a synthesis strategy involving haloarenes, the palladium catalyzed Heck reaction[16–18] using aryl vinyl ketones as reactants for the construction of the enone substructure is an obvious option. However, a survey of the literature revealed that examples for such reactions are extremely rare,[19–21] presumably because of the low stability of aryl vinyl ketones under the conditions typically used for Heck reactions. We therefore considered the use of suitable stable precursors liberating the aryl vinyl ketones in the course of the reaction. Although ketone Mannich bases have been applied as enone substitutes for the reaction with various nucleophiles,[22] they have to our best knowledge not been employed in transition-metal catalyzed carbon–carbon coupling reactions. Since it has been reported that the

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Table 1. Modification of catalyst in synthesis of chalcone **3a** according to Scheme 1

Catalyst	Yield[a] (%)
2.5 mol% $Pd(OAc)_2$	28
5 mol% $Pd(OAc)_2$	44 (72)
5 mol% $Pd(OAc)_2$/5 mol % triphenylphosphine	37
10 mol% $Pd(OAc)_2$	43
5 mol% $[Ph_3P]_2PdCl_2$	21
5 mol% $Pd[Ph_3P]_4$	13
5 mol% $PdCl_2dppf_2 \times CH_2Cl_2$	37

[a] Yield after work-up and crystallization from ethanol. Yield in brackets determined by HPLC (area% method).

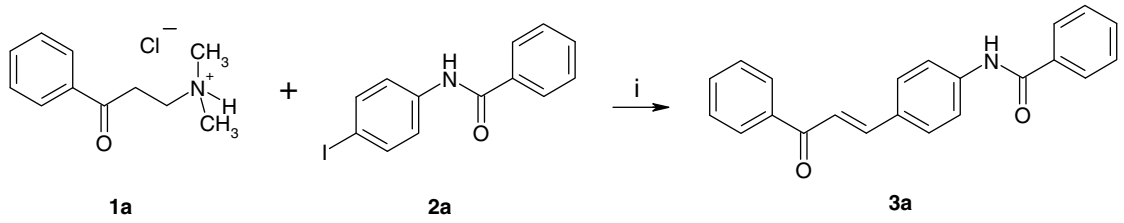
toxicity of anti-leishmanial chalcones against the human host cells is decreased by a large substituent in position 4,[14] we chose for synthetic studies the preparation of 4-benzoylaminochalcone **3a** from *N*,*N*-dimethyl-3-oxo-3-phenylpropan-1-aminium chloride **1a** and *N*-(4-iodophenyl)benzamide **2a** as a model reaction (Scheme 1). The reactions were performed in 20 mL standard vials of a parallel synthesis reactor to establish a method useful for a rapid generation of structure analogues. Initial attempts were carried out in DMF under air atmosphere in the presence of triethylamine as base. Of the four palladium catalysts employed, 5 mol% palladium(II)acetate gave the best result, presumably because of its air stability. The addition of triphenylphosphine as ligand or the modification of catalyst loading (2.5 or 10 mol%) failed to increase the yield of isolated product.

The described method was then implemented for the synthesis of a small exemplary chalcone library starting from ketone Mannich bases **1**with aryl iodides **2** (Scheme 2/Tables 2 and 3). In all cases, the reaction proceeded rapidly and was completed within less than 30 min. Although HPLC analyses of the raw reaction mixtures before work-up indicated product yields between 63% and 72%, the isolated yields of the pure products appear as moderate. The HPLC traces showed the presence of side products in low concentrations, among them the corresponding *Z*-diastereomers. Because of similar chromatographic behaviour of main and side products, the purification by column chromatography was difficult. Therefore, crystallization from ethanol was applied as standard work-up procedure to yield the desired (*E*)-chalcones. Since the crystallization caused some loss of material, the yields indicated in Table 2 still bear some optimization potential.[23]

The reaction was also applicable for the reaction of aromatic ketone Mannich bases with bromoarenes, albeit the reactions furnished lower yields (data not shown). Chloroarenes showed reactivity only when activated by an additional strongly electron-withdrawing substituent, for example, a nitro group (data not shown). This graduated reactivity of haloarenes allowed the synthesis of the chalcone **3c**, because the chloro substituent of the corresponding educt ketone Mannich base remained unaffected during the reaction.

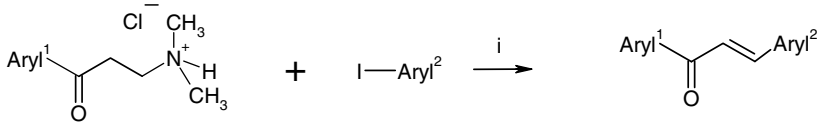
The small exemplary chalcone library generated by the novel parallel synthesis approach was screened for anti-leishmanial activity. Depending on the stage of the life cycle, *Leishmania* exist in two forms: extracellular promastigotes residing in the insect vectors and intracellular amastigotes that multiply in human host macrophages. The anti-leishmanial activity of the chalcones reported here was screened using a fluorescent viability microplate assay with *L. donovani* axenic amastigotes.[29] In an initial screening, the inhibition of the amastigote growth by a 15 μM chalcone concentration was assessed (refer to Table 2). For chalcones showing more than 90% inhibition the GI_{50} (concentration for 50% growth inhibition) was also determined. In addition, the general cytotoxicity of the active chalcones was determined using an alamarBlue viability assay on the human macrophage cell line THP-1 (Table 3).[30]

The initial results revealed that four compounds (**3a**, **3d**, **3e**, **3f**) with the *p*-benzoylamino-substituent at the 1-aryl-ring and the *p*-acetyl derivative **3i** exhibited strong



1a **2a** **3a**

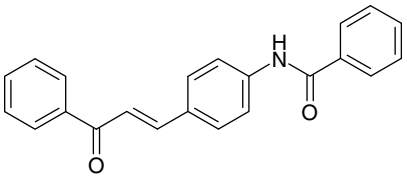
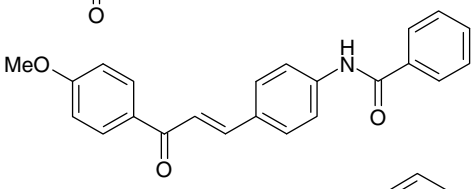
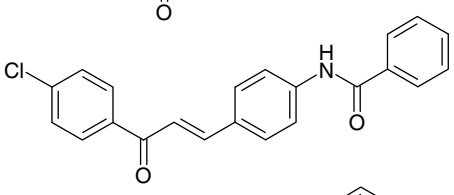
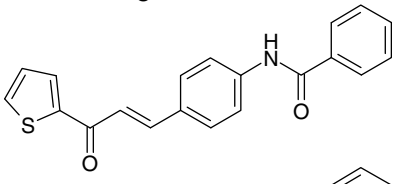
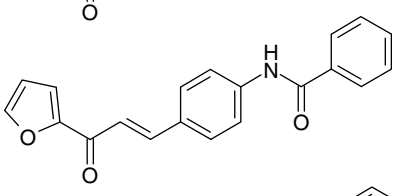
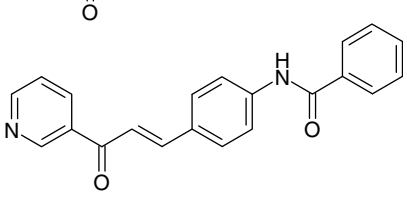
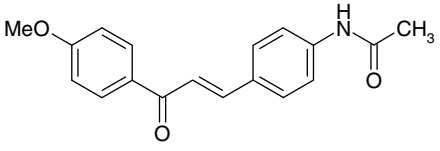
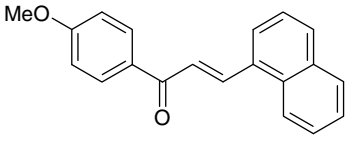
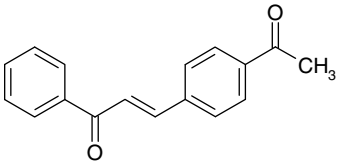
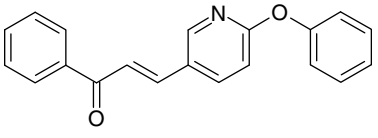
Scheme 1. Modification of reaction conditions for synthesis of chalcone **3a**. Reagents and conditions: Palladium catalyst, DMF, triethylamine, 140 °C, parallel reactor station, 30 min. For catalysts and yields refer to Table 1.



1 **2** **3**

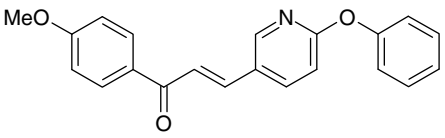
Scheme 2. General procedure for palladium-catalyzed synthesis of chalcones **3** from Mannich bases **1** and iodoarenes **2**. For residues $Aryl^1$ and $Aryl^2$ refer to Table 2. Reagents and conditions: 5 mol% $Pd(OAc)_2$, DMF, triethylamine, 140 °C, parallel reactor station, 30 min.

Table 2. Synthesized chalcones **3a**–**k**[a]

Chalcone	Structure	Yield[b]	Inhibition of axenic *L. donovani* amastigotes at 15 μM[c] (% ± s.e.)
3a		44 (72)	91.6 ± 1.2
3b		29 (73)	2.1 ± 5.1
3c		37 (66)	59.8 ± 2.6
3d		65	95.3 ± 0.73
3e		40 (72)	92.4 ± 0.89
3f		43 (67)	98.7 ± 0.34
3g		41	54.7 ± 6.7
3h[d]		50	51.0 ± 2.0
3i		31	93.6 ± 0.71
3j		24	63.7 ± 1.9

(*continued on next page*)

Table 2 (*continued*)

Chalcone	Structure	Yield[b]	Inhibition of axenic *L. donovani* amastigotes at 15 μM[c] (% ±s.e.)
3k		45	42.0 ± 4.8
DMSO[e]		—	0.0 ± 2.0
Amphotericin B[f]		—	99.0 ± 0.33

[a] Compounds **3a**–**b**,[24] **3d**–**e**,[25] **3g**,[26] **3h**,[12,15] and **3i**[27] have been described before. For melting points of **3c**, **3f**, **3j**, **3k** refer to note.[28]
[b] Yields after work-up and a single crystallization from ethanol. Values in brackets represent yields determined by HPLC from raw reaction mixtures (area% method).
[c] Mean of two experiments (triplicates ± standard error).
[d] Compound **3h** has been reported to show an $IC_{50} > 200$ μM in an assay measuring the viability of *L. donovani* amastigotes using a tetrazolium dye-based reagents.[12]
[e] 1% DMSO in medium was used as negative control.
[f] Amphotericin B used as positive control.

Table 3. Biological activities of selected chalcones

Chalcone	GI_{50} on axenic *L. donovani* amastigotes[a] (μM ± s.e.)	% Killing of THP-1 macrophages at 1 μM[b]
3a	7.6 ± 0.30	37
3d	5.5 ± 0.30	6
3e	6.0 ± 0.0	0
3f	2.5 ± 0.44	0
3i	5.6 ± 0.25	45.7 ± 6.3

[a] Mean of duplicate experiments (**3f**: triplicate) ± standard error.
[b] Determined as single experiments (**3i**: triplicate ± standard error).

anti-leishmanial activity at 15 μM. The following dose–response studies distinguished the pyridinyl derivative **3f**,[31] which showed a GI_{50} of 2.5 μM and proved to be at least twice as potent as the other chalcones tested. While **3f** showed no toxicity at 1 μM in initial studies on the human macrophage cell line THP-1, the chalcones **3a** and **3i** caused a definite cell killing. In conclusion, we have established a novel method for the rapid preparation of chalcones from Mannich Bases and haloarenes. The procedure is suitable for chalcone library generation in a parallel reactor station device. Among the chalcones prepared by the new method, **3f** proved to be a compound with interesting anti-leishmanial properties. This structure will be the basic scaffold for a focused chalcone library we intend to prepare in the search of novel effective antiparasitic agents.

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References and notes

1. Mishra, J.; Saxena, A.; Singh, S. *Curr. Med. Chem.* **2007**, *14*, 1153.
2. Pechan, P.; Jaffe, C. L. *Dosis* **2006**, *22*, 158.
3. Kayser, O.; Kiderlen, A. *Phytother. Res.* **2001**, *15*, 148.
4. Lunardi, F.; Guzela, M.; Rodrigues, A.; Corrêa, R.; Eger-Mangrich, I.; Steindel, M.; Grisard, E.; Assreuy, J.; Calixto, J.; Santos, A. *Antimicrob. Agents Chemother.* **2003**, *47*, 1449.
5. Nowakowska, Z. *Eur. J. Med. Chem.* **2007**, *42*, 125.
6. Torres-Santos, E.; Moreira, D.; Kaplan, M.; Meirelles, M.; Rossi-Bergmann, B. *Antimicrob. Agents Chemother.* **1999**, *43*, 1234.
7. Nielsen, S. F.; Chen, M.; Theander, T. G.; Kharazmi, A.; Christensen, S. B. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 449.
8. Zhai, L.; Blom, J.; Chen, M.; Christensen, S. B.; Kharazmi, A. *Antimicrob. Agents Chemother.* **1995**, *39*, 2742.
9. Chen, M.; Zhai, L.; Christensen, S. B.; Theander, T. G.; Kharazmi, A. *Antimicrob. Agents Chemother.* **2001**, *45*, 2023.
10. Zhai, L.; Chen, M.; Blom, J.; Theander, T.; Christensen, S.; Kharazmi, A. *J. Antimicrob. Chemother.* **1999**, *43*, 793.
11. Boeck, P.; Bandeira Falcao, C.; Leal, P.; Yunes, R.; Filho, V.; Torres-Santos, E.; Rossi-Bergmann, B. *Bioorg. Med. Chem.* **2006**, *14*, 1538.
12. Gutteridge, C.; Vo, J.; Tillett, C.; Vigilante, J.; Dettmer, J.; Patterson, S.; Werbovetz, K.; Capers, J.; Nichols, D.; Bhattacharjee, A.; Gerena, L. *Med. Chem.* **2007**, *3*, 115.
13. Liu, M.; Wilairat, P.; Croft, S.; Tan, A. L.-C.; Go, M.-L. *Bioorg. Med. Chem.* **2003**, *11*, 2729.
14. Nielsen, S.; Christensen, S.; Cruciani, G.; Kharazmi, A.; Liljefors, T. *J. Med. Chem.* **1998**, *41*, 4819.
15. Gutteridge, C. E.; Nichols, D. A.; Curtis, S. M.; Thota, D. S.; Vo, J. V.; Gerena, L.; Montip, G.; Ashe, C. O.; Diaz, D. S.; Ditusa, C.; Smith, K. S.; Bhattacharjee, A. K. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5682.
16. Alonso, F.; Beletskaya, I.; Yus, M. *Tetrahedron* **2005**, *61*, 11771.
17. Heck, R. F.; Nolley, J. P. *J. Org. Chem.* **1972**, *37*, 2320.
18. Knowles, J.; Whiting, A. *Org. Biomol. Chem.* **2007**, *5*, 31.

19. Barabanov, I. I.; Fedenok, L.; Polyakov, N. E.; Shvartzberg, M. S. *Russ. Chem. Bull., Int. Ed.* **2001**, *9*, 1663.
20. Bianco, A.; Cavarischia, C.; Farina, A.; Guiso, M.; Marra, C. *Tetrahedron Lett.* **2003**, *44*, 9107.
21. Bianco, A.; Cavarischia, C.; Guiso, M. *Eur. J. Org. Chem.* **2004**, 2894.
22. Tramontini, M.; Angiolini, L. *Tetrahedron* **1990**, *46*, 1791.
23. Synthesis of chalcones by Heck-type reaction, general procedure: 1 mmol iodoarene, 1.1 mmol ketone Mannich base hydrochloride, 11.25 mg (0.05 mmol) palladium(II)acetate and 2 mL triethylamine are suspended in 5 mL DMF and stirred (20 mL vials, carousel 12 place reactor station, Radley Discovery Technologies, UK) for 30 min at 140 °C vessel reactor block temperature. After filtration, silica gel (1.5 g) is added to the filtrate. After evaporation of the solvent the remaining silica gel/reaction product mixture is added onto a silica gel pad in a glass frit and then eluted with ethylacetate (200 mL). After evaporation of the solvent the remaining solid is purified by crystallization from ethanol.
24. Pfeiffer, P. *Liebigs Ann. Chem.* **1925**, *441*, 228.
25. Rtishchev, N. I.; Nosova, G. I.; Solovskaya, N. A.; Luk'ysahina, V. A.; Galaktionova, E. F.; Kudryavtsev, V. V. *Russ. J. Gen. Chem.* **2001**, *71*, 1272.
26. Edwards, M.; Stemerick, D.; Sunkara, P. *J. Med. Chem.* **1990**, *33*, 1948.
27. Meier, H.; Aust, H.; Ickenroth, D.; Kolshorn, H. *J. Prakt. Chem. (Weinheim, Germany)* **1999**, *341*, 529.
28. Melting points of new compounds: **3c**, 198 °C; **3f**, 192 °C; **3j**, 111 °C; **3k**, 134 °C.
29. Screening was carried out using an assay similar to one reported for leishmanial promastigotes (Mikus, D.; Steverding, D. *Parasitol. Internat*. **2000**, *48*, 265). Compounds to be assayed were diluted to twice the final concentration in complete amastigote medium, containing 1% DMSO, and were aliquoted in triplicate (125 μl/well) into 96-well flat-bottom plates. Amastigotes (5.0×10^5 cells/ml; 125 μl/well) were added to each well and incubated for 24 h at 37 °C in a 5% CO_2 incubator. The alamarBlue viability indicator was added (25 μl/well) and the plates incubated for an additional 24 h at which time the fluorescence (λ_{ex} = 544 nm; λ_{em} = 590 nm) was measured in a microplate reader. Complete medium both with and without DMSO was used as negative controls (0% inhibition of amastigote growth). Amphotericin B was included as a positive control on each plate and gave >90% inhibition of parasite growth at 1 μM. Standardization and optimization of the assay will be described elsewhere (Shimony and Jaffe, in preparation).
30. For the toxicity assay, compounds (2 μM) were diluted in the complete medium containing 1% DMSO and aliquoted in triplicate (125 μl/well) into 96-well plates. THP-1 macrophages in complete RPMI-1640 were added (8×10^5 cells/ml, 125 μl/well) and incubated for 48 h (37 °C, 5% CO_2). AlamarBlue (25 μl) was added, the plates incubated for an additional 3 h and the fluorescence read. Medium both with and without DMSO was used as negative controls (0% inhibition).
31. Analytical data of compound **3f**: ^{1}H NMR 7.54–7.58 (m, 2H, ArH), 7.60–7.64 (m, 2H, ArH), 7.79 (d, 1H, J = 15.7, (C═O)CH═C), 7.97–7.99 (m, 2H, ArH), 7.92 (d, 5H, ArH and (C═O)C═CH), 8.47 (dt, 1H, J = 7.9/2.2/1.8, ArH), 8.84 (dd, 1H, J = 4.8/1.7 Hz, ArH), 9.34 (dd, 1H, J = 2.2/0.7 Hz, ArH), 10.49 (s, 1H, NH); ^{13}C NMR 120.1 (2×), 120.2, 123.9, 127.7 (2×), 128.4 (2×), 129.9 (2×), 131.8, 135.8, 144.5, 149.6, 153.2 (tert. arom. C), 129.6, 133.0, 134.8, 141.7, 165.8, 188.2 (quat. arom. C); Anal. ($C_{21}H_{16}N_2O_2$) calcd. C 76.81, H 4.91, N 8.53; found C 76.77, H 4.99, N 8.45.