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Solution Phase Combinatorial Synthesis and Screening of Mini Libraries of Arylchalcones for Antibacterial Activity

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Abstract

The solution-phase combinatorial synthesis of aryl chalcones was studied by synthesizing a 6x4 array mini library. The mini libraries of chalcones were synthesized by condensation of 4-substituted acetophenones and various aryl/heteroaryl carbaldehydes. All the synthesized mini libraries were screened for antibacterial activity using serial dilution method. The mini-libraries **3{1a,2a–d}, 3{1b,2a–d}, 3{1a–f,2a}** and **3{1a–f,2c}** were found to be most active of the synthesized mini libraries. The common elements present in the identified pool of interest were used to individually synthesize four compounds which were individually subjected to antibacterial activity evaluation. The compound 3-(4-chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one exhibited significant activity that was better than that of the other three compounds synthesized. The anti-bacterial activity of the identified hit was also found to be better than all mini-libraries, indicating utility of combinatorial synthesis and the assumption there in for lead identification.

Keywords

Combinatorial • Chalcones • Antibacterial • Leads

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Introduction

Combinatorial chemistry has emerged as a powerful tool for the discovery and optimization of new leads in the pharmaceutical industry. Because of their ability to mimic the structure of peptides and their ability to reverse binding to proteins, functionalized heterocycles are interesting scaffolds for the preparation of diversity-oriented compound libraries for medicinal and pharmaceutical applications [1–4]. So far, several chalcone containing biologically active compounds have been synthesized [5–7]. However, there are few reports on solution phase combinatorial synthesis and combinatorial antibacterial screening, despite the simplicity, time and material saving involved in the process. Aryl/heteroaryl substituted aldehydes and 4-substituted acetophenones, which are versatile reagents in heterocyclic synthesis, were used as components of mini-library. The synthetic availability and interesting structural features of chalcones, a ubiquitous structural unit in many biologically active compounds, prompted us to carry out the solution phase combinatorial synthesis of 1-(4-substituted phenyl)-3-(aryl)prop-2-en-1-one derivatives for combinatorial screening leading to identification of structures which could be novel leads for antibacterial activity optimization.

Results and Discussion

Parallel Solution-Phase Synthesis of 1-(4-substituted phenyl)-3-(aryl)prop-2-en-1-one mini-libraries

We studied parallel solution-phase coupling of the aldehydes with ketones. For efficient synthesis of larger combinatorial libraries, we were especially interested in identifying a solvent/catalyst that would allow the conjugation process with a minimized load of byproducts. During initial studies, we found that solvents yielding proton were very useful than basic solvents. We also found sodium hydroxide to be an appropriate catalyst, providing stringent conditions to the reaction for complete conversion and easy recovery.

The reaction of ketones **1(a–f)** with aldehydes **2(a–d)** in methanol gave the corresponding combinatorial mini-libraries. Assignments of the structures of combinatorial libraries were done on basis of the data obtained by IR analysis. The absorption bands of α,β-unsaturated carbonyl group were observed at 1720 cm^{-1}. The various substituent groups on aldehydes and ketones used for the synthesis of respective mini-libraries exhibited bands at 745–755(C-Cl); 780–785(C-Br); 1050–1055(C-O); 1350–1375 N-$(CH_3)_2$; 1610–1620(NO_2); 2800–2900(CH); 3200–3350(NH).

Analysis of Mini libraries by GC-MS

Gas Chromatography- Mass Spectrometry (GC-MS) was used to establish that a sizable fraction of the expected compounds are produced in the above synthesis of ethanol soluble mini libraries. Experiments were designed to determine if the combination of a 3-(4-methoxyphenyl)prop-2-en-1-one core with a set of ketone results in the formation of a mixture in which all of the expected compounds are present above a certain concentration threshold. To investigate the mass-spectrometric behavior of these molecules, several pure test mixtures of three compounds were analyzed. Taken together, the molecular ion peaks obtained from these measurements are a set of data directly correlated to the diversity of a given molecular library. Since fragmentation was not a factor, we were able to compare the molecular ion peaks in the mass spectra with the molecular weights expected for each mini-library, and conclude which compounds had been formed and which had not. Result of the GC-MS analysis of a mini-library is compiled in Fig. 1. This indicated that all components might be present in sufficient amount.

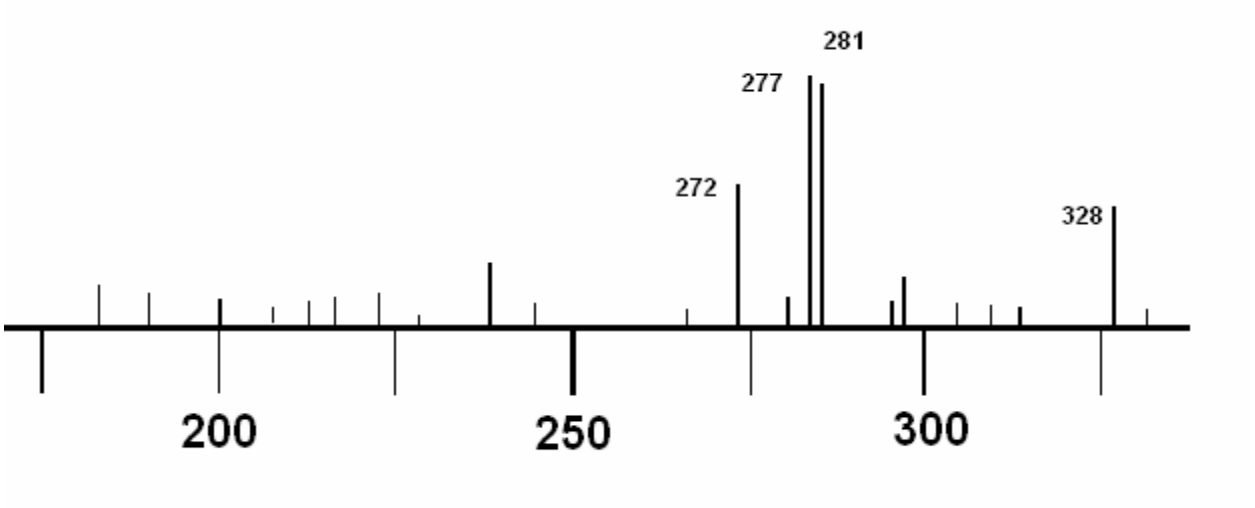


Fig. 1. Results of GC-MS analysis of Library **3{1a–f,2a}**

Combinatorial screening of the mixtures for Antibacterial activity.

The combinatorial mini-library mixtures were subjected to antibacterial activity evaluation against four different bacteria strains *S. aureus, B. subtilis, E. coli, P. aeruginosa*. 2D deconvolution steps deconvoluted activity pools of interest. In the family pool **3{1a,2a–d}, 3{1b,2a–d}, 3{1a–f,2a}** and **3{1a–f,2c}** stood out as most active mini-libraries.

The common elements present in the identified pool of interest were used to individually synthesize four compounds which were individually subjected to antibacterial activity evaluation. The compound **3{1a,2a}** exhibited significant activity that was better than that of the other three compounds synthesized. Activity of this lead molecule was also better than that of any of the mini-combinatorial libraries (Table 1).

Thus, synthesis of mixture mini-libraries proved to be a valuable time and material saving tool in the discovery of new leads for drug discovery projects. Specifically, preparation and screening of mixtures of 1-(4-substituted phenyl)-3-(aryl)prop-2-en-1-one derivatives has lead to the discovery of many potent compounds across a variety of receptor targets.

Tab. 1. Antibacterial activity data of lead molecules

Comp. Code	MIC (μg/ml)			
	S. aureus	*B. subtilis*	*E. Coli*	*P. aeruginosa*
3{1a,2a}	31	63	16	16
3{1a,2c}	125	125	250	250
3{1b,2a}	125	63	250	250
3{1b,2c}	16	63	31	63

The lead **3{1a,2a}** identified from the mini-libraries by deconvolutional analysis after individual synthesis exhibited maximum activity. The lead molecule was an element of the most active mini-libraries which were synthesized. The results obtained have potential to tempt for further evaluation and modification by the tools of combinatorial and computational chemistry for identification of potent and less toxic molecules.

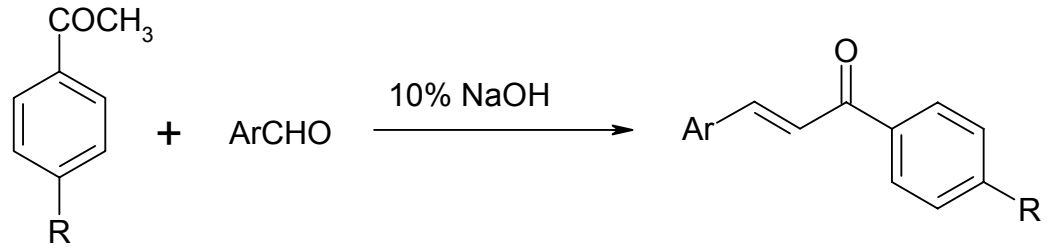
Experimental

The NMR spectra were obtained on a 400 MHz Varian NMR for ^{1}H, using DMSO-d_6 and TMS as the solvent and internal standard respectively. GC-MS spectra were recorded on a Shimadzu GC-2010, MS-2P20100 spectrophotometer; IR spectra, on a Jasco 4100 FTIR spectrophotometer.

Combinatorial Synthesis of 1-(4-substituted phenyl)-3-(aryl)prop-2-en-1-one. 3{1a-f,2a-d} **[8–10]**.

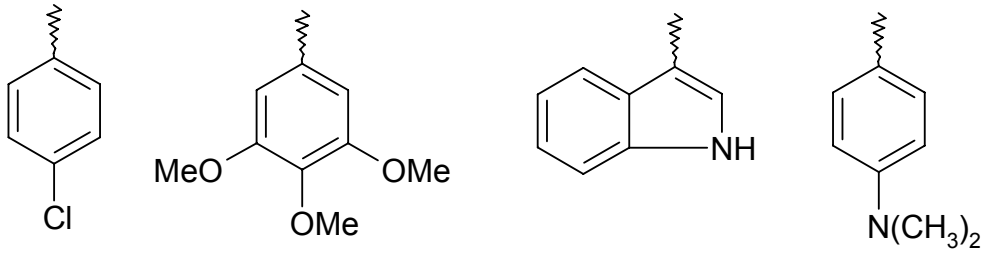
Combinatorial synthesis of mini-library of 1-(4-substituted phenyl)-3-(aryl)prop-2-en-1-one derivatives was done using the reported procedure of formation of chalcones. Mini-libraries of 6x4 array were synthesized by reacting aromatic aldehydes **2{a–d}** in stoichiometric amount with the selected ketones **1{a–f}** in methanol. Vice-a-versa the second set was synthesized by condensation of aldehydes with ketones in stoichiometric amounts in methanol. The reaction mixture was stirred for 1hr in presence of 10% sodium hydroxide and the mixture was kept in cold water for 24 hrs. Spectral data of a representative mini-library

3{1a–f,2a} is given: IR (KBr) 745–755(C-Cl); 780–785(C-Br); 1050–1055(C-O); 1350–1375 N-$(CH_3)_2$; 1610–1620(NO_2); 2800–2900(CH); 3200–3350(NH). ^{1}H NMR (DMSO) 2.10 (s, 6H, N$(CH_3)_2$), 2.50 (s, 3H, CH_3), 3.20 (s, 3H, OCH_3), 5.34–5.45 (2d, 2H, -CH=CH-), 7.0–8.5 (Br, Ar).



1{a–f} **2{a–d}** **3{1a–f,2a–d}**

R= OCH_3, Cl, CH_3, Br, NO_2, N$(CH_3)_2$.



2a **2b** **2c** **2d**

Fig. 1. Synthesis of compounds

Biological Screening

All the synthesized mini libraries were screened for antibacterial activity [11], against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* by cup plate agar diffusion method. Ciprofloxacin was used as standard drug for antibacterial activity. The activity was measured by serial dilution method to determine the Minimum Inhibitory Concentrations of each mini-library. In each plate, four cavities of 6 mm diameter were made with a sterile-borer. Test solutions at the concentrations 1000, 750, 500, 250, 125, 63, 31 and 16 µg/ml were added to the respective cavity

aseptically and labeled accordingly. Standard was added in concentration of 100µg/ml. The plates were kept undisturbed for at least 2 hrs at room temperature to allow diffusion of the solution properly in the nutrient agar medium. After incubation of the plates at 37 ± 1°C for 24 hours. The minimum inhibitory concentration (MIC) values of libraries are given in (Table 2).

Tab. 2. Antimicrobial screening data of mini-libraries.

Library Code	MIC (µg/ml)			
	S. aureus	*B. subtilis*	*E. coli*	*P. areuginosa*
3{1a,2a–d}	63	125	31	31
3{1b,2a–d}	63	31	63	125
3{1c,2a–d}	500	750	500	250
3{1d,2a–d}	250	1000	1000	750
3{1e,2a–d}	500	1000	1000	750
3{1f,2a–d}	1000	250	500	250
3{1a–f,2a}	63	31	63	63
3{1a–f,2b}	250	125	750	1000
3{1a–f,2c}	125	63	31	125
3{1a–f,2d}	250	750	250	250
Cipro.	16	31	31	16

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