Molecular Diversity, 2 (1996) 171–174
ESCOM

MOLDIV 055

Xanthines as a scaffold for molecular diversity

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Received 6 December 1996
Accepted 6 February 1997

Keywords: Xanthines; Combinatorial chemistry; Diversity; Small molecules

Summary

Xanthines represent a new, versatile scaffold for combinatorial chemistry. A five-step solid-phase synthesis of xanthine derivatives is described which includes alkylations, a nucleophilic displacement reaction at a heterocycle and a ring closure reaction by condensation of a nitroso function with an activated methylene group. The selected reaction sequence allows the production of a highly diverse small-molecule combinatorial compound library.

Introduction

Combinatorial compound libraries represent a powerful method to identify new pharmaceutical lead compounds for a given biological target [1]. One of the challenges in generating new kinds of libraries is the development of scaffolds that allow the introduction of a maximum of diversity. Recent investigations showed the successful synthesis on a solid support of several small molecules that are suitable as scaffolds for the generation of molecular diversity [2]. The application of solid-phase technology to the preparation of such new scaffolds for combinatorial chemistry requires the transfer of solution-phase chemistry to conditions that are feasible on the solid support. The reactions should proceed in resin-swelling solvents and the reaction temperatures should be kept at reasonable values.

Xanthines represent a new structurally rigid scaffold for combinatorial chemistry. Certain naturally occurring xanthines (e.g. caffeine, theophylline and theobromine) are well known to exert prominent physiological effects at very low doses, and they have been used as pharmaceuticals, narcotics or stimulants for many centuries [3]. Today, 8-substituted xanthines are studied extensively for clinical application [4], as they inhibit many pharmacological and physiological effects of adenosine [5]. In order to use the xanthine scaffold for combinatorial chemistry, we have developed a method of solid-phase synthesis of highly substitutable xanthines for the generation of new compound libraries.

Results and Discussion

The ring closure reaction of 1,3-dialkyl-6-alkylamino-5-nitrosouracils **1** proceeds easily, giving the corresponding xanthine derivatives **2**, as long as N1 is blocked by a substituent (Scheme 1) [6].

The application of solid-phase technology to the generation of combinatorial compound libraries necessitates the adaptation of this reaction to solid-phase conditions. As a first step, we coupled bromoacetic acid to the free amino function of a solid support **3**, which is modified by the Rink amide linker [7]. We used the Rink linker because of its stability towards a wide variety of reaction conditions. 1-Alkyl-4-chlorouracils **5** were then attached to the solid phase by alkylation of the nitrogen at the uracil system to form intermediates **6**. The chlorine of the resin-bound uracils **6** can easily be replaced by primary benzylamines to form 4-benzylaminouracils **7**.

The intermediates **7**, bound to the solid support, were treated with isopentyl nitrite in the presence of a small amount of acetic acid. The nitroso compounds formed first immediately undergo ring closure to form the xanthine derivatives **8**. This conversion could be followed by UV spectroscopy, since the absorption band at 260–265 nm, characteristic for the uracil-type compounds **7**, disap-

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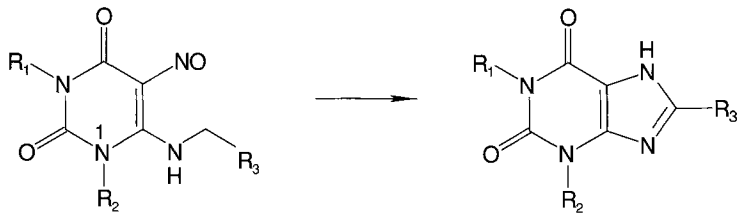


1 2

Scheme 1. Formation of xanthine derivatives **2** by ring closure of 1,3-dialkyl-6-alkylamino-5-nitrosouracils **1**. Note that N1 is blocked.

peared and was shifted to 306–310 nm, characteristic for xanthines **8**. The progress of all reactions was judged by cleavage of a small amount of the reaction product from the solid phase, followed by analysis by UV spectroscopy and comparison of HPLC characteristics of the product with those of product prepared in solution (if available). Alkylation of these compounds proceeded with excess alkylating agent in the presence of triethylamine to give

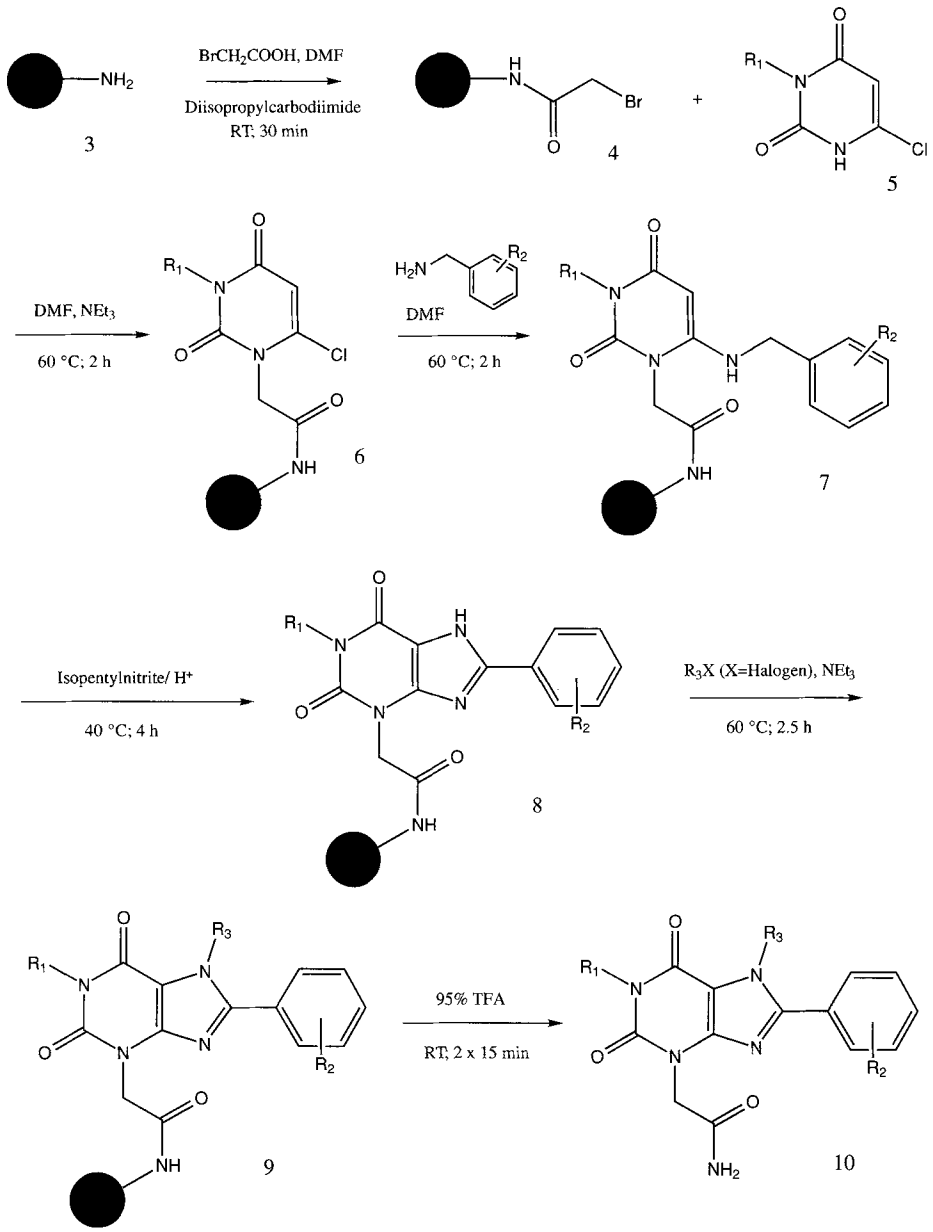
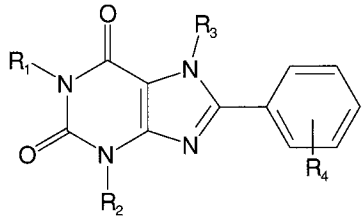
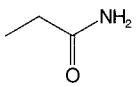
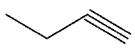
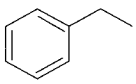
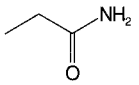
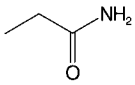
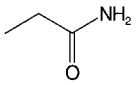
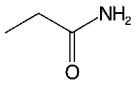
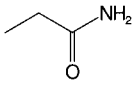
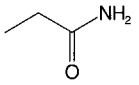
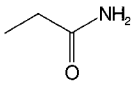
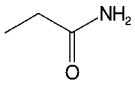
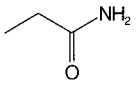
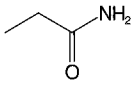
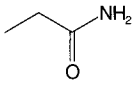
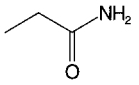
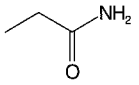
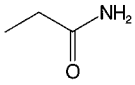


Fig. 1. Solid-phase synthesis of fully substituted xanthines.

TABLE 1
ANALYTICAL DATA OF SYNTHESIZED XANTHINE DERIVATIVES



No.	R_1	R_2	R_3	R_4	Yield[a] (mg)	Retention time (min) (see text)	MW (g/mol) MS (MH^+)[b]
10a	CH_3			2'-OCH_3	1.94 (10%)	10.87	367.37 368.1
10b				4'-CF_3	3.63 (14%)	13.06	500.44 502.2
10c	CH_3		H	4'-F	1.80 (19%)	8.75	317.28 320.4
10d	CH_3			4'-F	3.55 (32%)	6.57	374.33 377.2
10e	CH_3		H	4'-CH_3	2.34 (25%)	9.45	313.32[c] 314.5
10f	CH_3			4'-CH_3	3.56 (32%)	7.46	370.37[c] 371.4
10g	$CH_2CH_2CH_3$		H	4'-F	2.42 (23%)	10.60	345.33 344.1
10h	$CH_2CH_2CH_3$			4'-F	3.10 (26%)	8.20	402.38 403.6
10i	$CH_2CH_2CH_3$		H	4'-CH_3	1.31 (13%)	11.23	341.37 342.4
10j	$CH_2CH_2CH_3$			4'-CH_3	1.52 (11%)	8.98	398.42 400.5

[a] The yield after RP-HPLC purification (based on a resin loading of 0.23 mmol/g) reflects the recovery of pure product. Analysis by RP-HPLC of the crude products (after cleavage from the resin) showed purities indicated by peak areas ranging from 86–98% ($\lambda = 300$ nm) of 65–89% ($\lambda = 220$ nm).

[b] Mass spectrometric data were obtained on a MALDI system (Linear Scientific 1700, Reno, CA, U.S.A.).

[c] For NMR data see text.

the fully substituted xanthines **9**. The final products **10** were cleaved from the solid support by the action of trifluoroacetic acid. The cleavage procedure was as follows. The resin was treated twice with 95% trifluoroacetic acid and washed with 95% trifluoroacetic acid, dichloromethane and trifluoroethanol. The combined solvents were removed over potassium hydroxide in an evacuated desiccator, after which the residues were lyophilized twice from dilute acetic acid and directly purified by HPLC.

Analytical and preparative HPLC was performed on a Jasco system (Tokyo, Japan). Analytical runs were carried out on a Waters Symmetry C_{18} column (3.9 × 150 mm) using a linear solvent gradient from 5% to 100% solvent B in 20 min (solvent A = 0.1% TFA in water; solvent B = 0.1% TFA in acetonitrile/water, 7:3). Preparative runs were carried out on a Waters Symmetry C_{18} column (19 × 150 mm) with a linear solvent gradient from 25% to 60% solvent B in 22 min.

We investigated the applicability of this method by first synthesizing two individual xanthines (**10a** and **10b**). The potential of the synthetic scheme to produce combinatorial compound libraries was then determined by the synthesis of a group of eight related xanthines (**10c–10j**). The original amount of resin was continuously divided into smaller portions along with the progressing reaction sequence (see Fig. 1). In this way, eight individual xanthines were obtained by cleavage from the eight different resin portions. The analytical data of these compounds are summarized in Table 1. The structure of two compounds (**10e** and **10f**) was verified by NMR spectroscopy. NMR spectra were recorded on a Bruker 300 MHz instrument. NMR data of compound **10e**: ^{1}H NMR (DMSO-d_6, ppm): 7.53 d (2H), 7.36 d (2H) ArH; 4.58 s (2H) CH_2CO; 3.30 s (3H) N-CH_3; 2.38 s (3H) p-CH_3. NMR data of compound **10f**: ^{1}H NMR (DMSO-d_6, ppm): 7.67 d (2H) CO-NH_2; 7.53 d (2H), 7.36 d (2H) ArH; 7.28 d (2H) CO-NH_2; 4.94 s (2H) CH_2CO; 4.57 s (2H) CH_2CO; 3.28 s (3H) N-CH_3; 2.38 s (3H) p-CH_3.

Conclusions

We have demonstrated the successful transfer of solution-phase reactions to a solid-phase synthesis of xanthine derivatives. The applicability of this synthetic strategy for the generation of molecular diversity was tested with the preparation of a small xanthine library consisting of eight individual entities. The method can easily be extended to generate libraries with a large number of compounds. A library containing 90 individual compounds was prepared in this way and is currently being tested in various biological assays.

Acknowledgements

We thank Dr. R. Andreatta, Dr. A. Marzinzik and Mr. H. Müller, Ciba-Geigy AG, Basel, Switzerland for carrying out the mass spectrometric and NMR analyses. G.H. was supported by the DF fund.

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