Automated Liquid−Liquid Extraction Workstation for Library Synthesis and Its Use in the Parallel and Chromatography-Free Synthesis of 2-Alkyl-3-alkyl-4-(3H)-quinazolinones

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An automated liquid−liquid extraction workstation has been developed. This module processes up to 96 samples in an automated and parallel mode avoiding the time-consuming and intensive sample manipulation during the workup process. To validate the workstation, a highly automated and chromatography-free synthesis of differentially substituted quinazolin-4(*3H*)-ones with two diversity points has been carried out using isatoic anhydride as starting material.

Introduction

The preparation of combinatorial libraries of heterocyclic compounds either by solid- or solution-phase synthesis is of great interest for the acceleration of lead discovery in pharmaceutical research. The increased demands for small but diverse libraries in the drug discovery process within the past few years has led to a shift in attention away from the classical solid-phase approach toward the solution-phase synthesis. While the generation of a library by solid-phase organic synthesis is associated with lengthy optimization processes, the classical solution-phase parallel synthesis starts to deliver compounds far more rapidly and is thus also very effective in the production of small libraries.

In this respect, the value of automated laboratory equipment to enhance output of research synthesis in drug discovery is usually no longer questioned,[1] and several excellent approaches to automated synthesis have been reported in the past few years.[2] To this aim, several different companies, including Lilly, have devoted specific programs to develop modular workstations able to automate different processes involved in the synthesis of compounds. The workup based on the liquid−liquid extraction is one of the most widely used methods for product isolation in solution-phase synthesis; however, this operation is an intensive manual procedure requiring chemists to perform several well-known and time-consuming actions.

A number of methods or equipment have been developed to increase the level of automation of this tedious task; the ALLEXis workstation from Mettler Toledo is among the most popular.[3] In addition to the ALLEXis and during the preparation of this manuscript, Takeda also reported a very nice parallel liquid−liquid extraction unit based on the Tecan Genesis liquid handler.[4]

In our continuous efforts to develop systems to automate solution-phase chemistry fully adapted to our needs for the production of libraries, we developed an in-house-designed automated liquid−liquid extraction module.[5] We submit this paper with the aim of offering the scientific community the details of an alternative module that has operated in our department in an open-access mode since 2003, also arranged around a Tecan Genesis worktable but using a different process and equipment to perform the workup protocols. Additionally, we report the use of this workstation in a highly automated, highly unattended, and chromatography-free three-step solution-phase approach to 2-carbon-substituted quinazolinones using isatoic anhydride as starting material and primary amines and carboxylic acids as building blocks to introduce the diversity points.

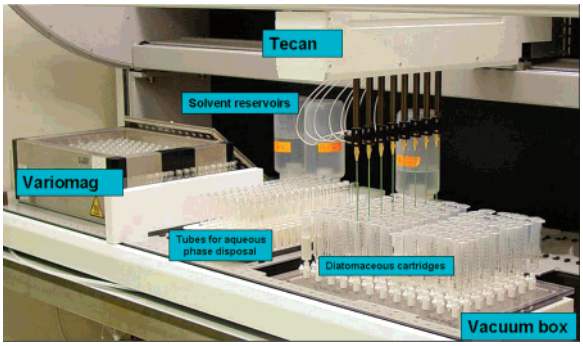

Figure 1. Automated liquid−liquid extraction module.

Results and Discussion

Automated Liquid−Liquid Extraction Workstation. The module described in this paper is integrated by an automated liquid dispenser (Tecan) to manipulate several solvents, a magnetic stirrer block (Variomag) to mix different layers, and a homemade vacuum box able to dry the samples through a filtration process with diatomaceous earth cartridges. All this equipment is arranged around a modified Tecan working table (Figure 1) and controlled by a customizable and easy-to-use software interface (Figure 2), both fully designed and built at Lilly.

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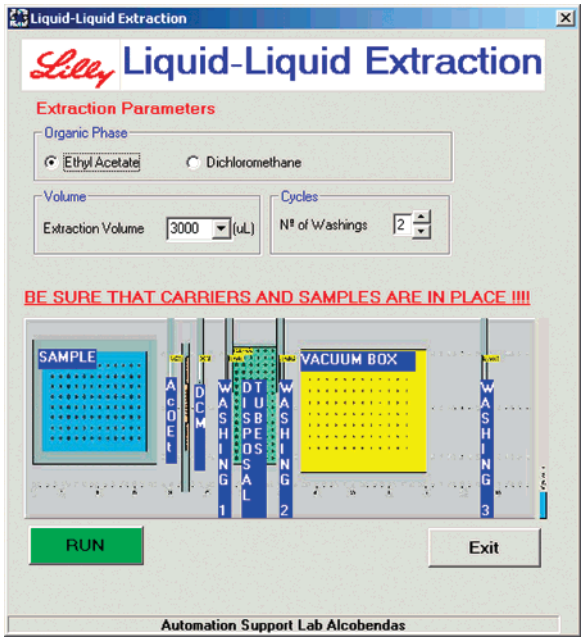


Figure 2. Screen shot of the software interface.

In a typical experiment, the module can perform up to 96 samples that are placed dry in 16 × 100 mm tubes within the Variomag equipment. The liquid−liquid extraction process is outlined in Figure 3.

The first step is the addition of organic solvent to dissolve the samples using the stirring power of the Variomag. The module was validated for solvents with lower and higher densities than water (dichloromethane and ethyl acetate). In a second step, the appropriate aqueous solution (e.g., acid, base, brine, etc.) is added, maintaining strong agitation to achieve an intimate mixing of the two phases. As was previously mentioned, the software is easily customizable, and this version performs every washing step using the same volume of organic and aqueous solution (extraction volume in Figure 2). In the next step, the aqueous solutions are taken by the Tecan and transferred to other tubes placed in a different part of the working table (tubes for aqueous phase disposal in Figure 1). Although the Tecan Genesis has a built-in liquid detector that performs this task very well with conductive liquids, it failed in our hands when organic solvents were used. Consequently, there is no real feedback of the boundary between the organic and aqueous phases sensed by the instrument. For this reason, we developed two different protocols depending on the density of the organic solvent used to carry out the extraction.

Dichloromethane as Organic Phase. The system takes into account the volume added for each phase and the inner diameter of the tube to calculate the height of the boundary from the bottom of the tube. Tecan tips are dipped to that height to take the aqueous phase. To ensure that the whole product is recovered, the aqueous layer is not completely removed.

Ethyl Acetate as Organic Phase. Tecan tips are moved down to the bottom of the tube and aspirate slightly less volume of aqueous solution than the amount added to wash the samples. This aqueous washing step could be repeated as many times as necessary by just configuring the software accordingly (no. of washings in Figure 2) and adding to the Tecan worktable as many different aqueous washing reservoirs as needed.

Finally, the organic layer is automatically transferred to cartridges of diatomaceous earth placed in the homemade vacuum box, and the final dry samples are collected into new 16 × 100 mm tubes placed inside the box.

When our automated liquid−liquid extraction workstation is compared with Takeda's, although both use the Tecan Genesis as liquid handler, our system offers the advantage of doing more than one washing (basic, acid, etc.) on the same organic solution because the module first removes the aqueous phase, maintaining in the same vial the organic phase until all the aqueous washings have been done and the samples are ready to be dried by transferring and passing them through the diatomaceous cartridges.

Synthesis of a Small Library of Quinazolinones. The quinazoline skeleton[6] is frequently encountered as an important component of pharmacologically active compounds associated with biological activities including anticonvulsant,[7] antibacterial,[8] antihypertensive,[9] antitumor,[10] antihistaminic,[11] antiinflamatory,[12] and antidiabetic agents.[13] While several previous reports have described solid-phase synthesis,[14]

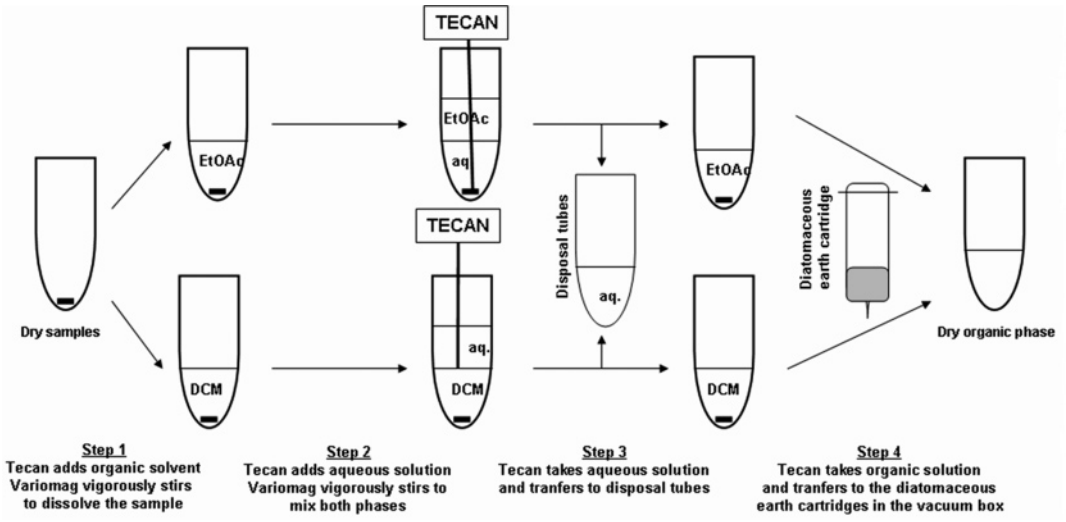
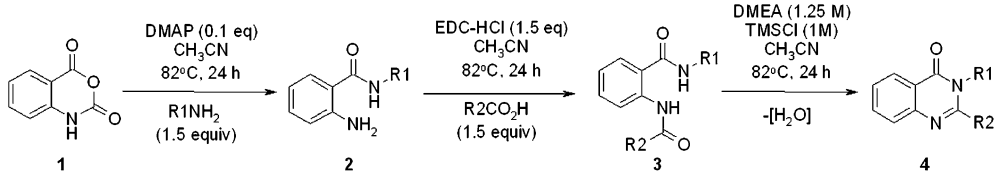


Figure 3. Stepwise procedure for liquid−liquid extraction.

Scheme 1. Synthesis of Quinazolin-4(*3H*)-ones



combination of solid-phase/solution-phase syntheses,[15] or more extensively, solution-phase synthesis[16] of the related 2-carbon-substituted quinazolinone, our goal was to develop a general, chromatography-free, and highly automated solution-based methodology that would enable us to generate quinazolinone libraries with up to three possible diversity points using carboxylic acids and amines as reagents and allow us to validate the removal of acidic and basic impurities during the automated workup process.

The solution-phase synthesis of quinazolin-4(*3H*)-ones is outlined in Scheme 1. The reaction of isatoic anhydride with different amines ($R1NH_2$) in acetonitrile at 82 °C for 24 h and in the presence of a catalytic amount of DMAP gave the corresponding amino amides **2**. After evaporation of the solvent, workup of the reaction mixture was carried out in the automated liquid−liquid extraction module using ethyl acetate as organic solvent and 10% HCl and water as washing aqueous solutions. After parallel removal of the solvent, these samples were dissolved in acetonitrile and treated with EDC-HCl and the corresponding carboxylic acids ($R2CO_2H$) at 82 °C for 24 h. A similar automated workup protocol was employed in this transformation including three washing steps to remove the excess of reagents (10% HCl and saturated $NaHCO_3$ solutions to eliminate the excess of EDC-HCl and carboxylic acids, respectively, with a final washing with water). The last synthetic step to obtain the quinazolinone-based targets involved the cyclodehydration of diamides **3**. This transformation was easily carried out using the protocol already reported by O'Mahony.[15] Dry crude materials containing the diamide compounds **3** were taken up in a solution of dimethyl ethyl amine (DMEA) and TMSCl in acetonitrile and stirred at 82 °C for 24 h in a capped reaction vessel. When the solvent was evaporated, a final, third automated liquid−liquid extraction was done (ethyl acetate as organic solvent and three different aqueous solutions: 10% HCl, saturated $NaHCO_3$, and water, successively) to remove all the undesired impurities.

Table 1 shows the building blocks employed to synthesize the 9 library members used to validate this methodology and the new automated workup module. The final quinazolinones **4** were obtained in moderate to good overall yields and very high purities following the synthetic approach outlined in Scheme 1. All the library compounds were characterized using LC-MS and ^1H NMR.

In summary, we have reported a useful highly automated and highly unattended synthesis of quinazolinones with two diversity points.[17] This three-step chromatography-free process gives the desired final products in very high purities and involves the use of a liquid−liquid extraction module that allows the scientist to perform this time-consuming task in an automated fashion by selecting the organic and aqueous washing solvents, the volume of each solvent, and the number of washing/extraction steps needed to eliminate impurities of the final product. This methodology has been successfully used for the fully automated construction of a highly diverse quinazolinone library, and the workup module is set up and handled very easily and has been applied to other different chemistry.

Table 1. Yields and Purities of Quinazolinones **4** Obtained Following the Synthetic Route Outlined in Scheme 1

c	$R1NH_2$	$R2CO_2H$	overall yield[a] (%)	purity[b] (%)
4a	benzyl amine	phenylacetic acid	33	90
4b	benzyl amine	benzoic acid	37	90
4c	benzyl amine	propionic acid	39	>95
4d	butyl amine	phenylacetic acid	56	>95
4e	butyl amine	benzoic acid	22	91
4f	butyl amine	propionic acid	48	>95
4g	propargyl amine	phenylacetic acid	26	>95
4h	propargyl amine	benzoic acid	12	95
4i	propargyl amine	propionic acid	48	>95

[a] Isolated crude yields after overall synthesis. [b] Average purity determined by HPLC(DAD)/MS analysis of the crude material at 214 nm in two orthogonal methods (low and high pH mobile phases).

Experimental Section

Chromatographic analysis was carried out on an Agilent HP1100 liquid chromatography system. Eluents for acidic conditions, A (0.05% TFA in water) and B (0.05% TFA in acetonitrile), were used in a gradient (0.5 min at 10% B, 10−95% of B in 4.5 min, 2 min at 95% B). The flow rate was 1 mL/min, and the analytical HPLC column used was a 4.6 × 50 mm, 5 μm Kromasil C-18 column. Eluents for basic conditions, A (ammonium bicarbonate 10 mM in water) and B (acetonitrile), were used in a gradient (0.5 min at 10% B, 10−95% of B in 4.5 min, 2 min at 95% B). The flow rate was 1 mL/min, and the analytical HPLC column used was a 4.6 × 50 mm, 5 μm XTerra MS-C18 column. ^1H NMR spectra were acquired on a Bruker 500 MHz spectrometer equipped with a flow injection selective inverse 3 mm probe head. The proton chemical shifts were referenced to the residual DMSO signal at 2.5 ppm.

General Experimental Procedure for 4. A mixture of isatoic anhydride (1 mmol), the corresponding amine (1.5 mmol), and DMAP (0.1 mmol) in acetonitrile (3 mL) was heated at 82 °C for 24 h. After evaporation of the solvent using a homemade nitrogen evaporator, the workup of the reaction mixture was carried out in the automated liquid−liquid extraction module. Ethyl acetate (4 mL) was employed as organic solvent, and a 10% solution of HCl (4 mL) and water (4 mL) were used as washing aqueous solutions. Cartridges of diatomaceous earth were used to dry the organic layers before evaporation of the samples to give amino amides **2**. Those samples were dissolved in acetonitrile (3

mL), treated with EDC-HCl (1.5 mmol) and the corresponding carboxylic acid (1.5 mmol), and heated at 82 °C for 24 h. Removal of the solvent, automated workup (EtOAc to dissolve the samples and 10% HCl, saturated solution of $NaHCO_3$, and water successively to remove the excess of reagents), and final evaporation of the organic solvent after drying of the organic layer with diatomaceous earth yielded the diamides **3**. Diamides **3** were taken up in a solution of dimethyl ethyl amine (DMEA) (1.25 M) and TMSCl (1 M) in acetonitrile (3 mL) and heated at 82 °C for 24 h. Then, the solvent was evaporated, and a third automated workup was done with EtOAc, a 10% solution of HCl, a saturated solution of $NaHCO_3$, and water. Samples were passed through cartridges of diatomaceous earth, followed by evaporation of the organic solvent. to achieve the final quinazolinones **4**.

2,3-Dibenzyl-3H-quinazolin-4-one (4a).[18] ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.23 (d, J = 7.8 Hz, 1H), 7.93 (t, J = 7.6 Hz, 1H), 7.75 (d, J = 7.8 Hz, 1H), 7.58 (t, J = 7.7 Hz, 1H), 7.35−7.17 (m, 10H), 5.30 (s, 2H), 4.13 (s, 2H). ESI-MS: m/z 327 (M + H).

3-Benzyl-2-phenyl-3H-quinazolin-4-one (4b).[16d] ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.23 (d, J = 7.8 Hz, 1H), 7.88 (t, J = 7.8 Hz, 1H), 7.71 (d, J = 7.8 Hz, 1H), 7.59 (t, J = 7.8 Hz, 1H) 7.52−7.38 (m, 5H), 7.24−7.16 (m, 3H), 6.91 (d, J = 7.3 Hz, 2H), 5.20 (s, 2H). ESI-MS: m/z 313 (M + H).

3-Benzyl-2-ethyl-3H-quinazolin-4-one (4c).[16d] ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.17 (d, J = 8.3 Hz, 1H), 7.84 (t, J = 7.8 Hz, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 7.35 (t, J = 7.5 Hz, 2H), 7.29−7.25 (m, 1H), 7.18 (d, J = 7.8 Hz, 2H), 5.40 (s, 2H), 2.77 (q, J = 7.3 Hz, 2H), 1.2 (t, J = 7.8 Hz, 3H). ESI-MS: m/z 265 (M + H).

2-Benzyl-3-butyl-3H-quinazolin-4-one (4d).[18] ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.12 (d, J = 8.5 Hz, 1H), 7.82 (t, J = 7.3 Hz, 1H), 7.65 (d, J = 7.3 Hz, 1H), 7.51 (t, J = 7.3 Hz, 1H), 7.39−7.23 (m, 5H), 4.29 (s, 2H), 3.93 (t, J = 7.3 Hz, 2H), 1.40−1.25 (m, 4H), 0.82 (t, J = 7.3 Hz, 3H). ESI-MS: m/z 293 (M + H).

3-Butyl-2-phenyl-3H-quinazolin-4-one (4e).[19] ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.20 (d, J = 8.5 Hz, 1H), 7.86 (t, J = 7.3 Hz, 1H), 7.69 (d, J = 7.3 Hz, 1H), 7.64−7.59 (m, 6H), 3.89 (t, J = 7.5 Hz, 2H), 1.48 (m, 2H), 1.07 (m, 2H), 0.67 (t, J = 7.3 Hz, 3H). ESI-MS: m/z 279 (M + H).

3-Butyl-2-ethyl-3H-quinazolin-4-one (4f).[20] ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.10 (d, J = 8.5 Hz, 1H), 7.73 (t, J = 7.3 Hz, 1H), 7.59 (d, J = 7.3 Hz, 1H), 7.41 (t, J = 7.3 Hz, 1H), 4.06 (t, J = 7.5 Hz, 2H), 2.85 (q, J = 4.8 Hz, 2H) 1.62 (m, 2H), 1.38 (m, 2H), 1.30 (t, J = 7.5 Hz, 3H), 0.67 (t, J = 7.3 Hz, 3H). ESI-MS: m/z 231 (M + H).

2-Benzyl-3-prop-2-ynyl-3H-quinazolin-4-one (4g).[21] ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.14 (d, J = 8.5 Hz, 1H), 7.83 (t, J = 7.3 Hz, 1H), 7.62 (d, J = 7.3 Hz, 1H), 7.54 (t, J = 7.3 Hz, 1H), 7.39−7.23 (m, 5H), 4.86 (d, J = 1.6 Hz, 2H), 4.36 (s, 2H), 3.30 (t, J = 1.6 Hz, 1H, overlapping with water signal). ESI-MS: m/z 275 (M + H).

2-Phenyl-3-prop-2-ynyl-3H-quinazolin-4-one (4h).[21] ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.22 (d, J = 8.5 Hz, 1H), 7.88 (t, J = 7.3 Hz, 1H), 7.73−7.71 (m 1H), 7.63−7.55 (m, 1H), 7.39−7.23 (m, 5H), 4.62 (d, J = 1.6 Hz, 2H), 3.30 (t, J = 1.6 Hz, 1H, overlapping with water signal). ESI-MS: m/z 261 (M + H).

2-Ethyl-3-prop-2-ynyl-3H-quinazolin-4-one (4i).[22] ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.13 (d, J = 7.8 Hz, 1H), 7.82 (t, J = 7.3 Hz, 1H), 7.64 (d, J = 7.8 Hz, 1H), 7.52 (t, J = 7.3 Hz, 1H), 4.95 (d, J = 1.6 Hz, 2H), 3.34 (t, J = 1.6 Hz, 1H), 3.01 (q, J = 7.3 Hz, 2H), 1.33 (t, J = 6.8 Hz, 3H). ESI-MS: m/z 213 (M + H).

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