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Parallel iterative solution-phase synthesis of 5-amino-1-aryl-[1,2,4]triazolo[1,5-*a*]pyridine-7-carboxylic acid amide derivatives

Bernd Brodbeck, Bernd Püllmann, Sébastien Schmitt and Matthias Nettekoven*

F. Hoffmann-LaRoche Ltd, Pharmaceutical Research Basel, Discovery Chemistry, Lead Generation, CH-4070 Basel, Switzerland

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Abstract—The parallel iterative solution-phase synthesis of 5-amino-1-aryl-[1,2,4]triazolo[1,5-*a*]pyridine-7-carboxylic acid amide derivatives is described. The key intermediate 2,6-bis-aminopyridine-4-carboxylic acid methyl ester was synthesised in a two step procedure in 64% overall yield and elaborated to a variety of triazolopyridine-5-carboxylic acid methyl ester by selective pyridine-*N*-amination, condensation of the adduct with a wide selection of aldehydes and subsequent cyclisation and oxidation. The desired esters were obtained in yields up to 70%. The final transformation to the amide derivatives was accomplished by application of carefully optimised reaction conditions thus giving access to a library of total 500 triazolopyridine amide derivatives. Iterative synthetic cycles (12–48 library members each) allowing for maximal flexibility in chemistry and maximal efficiency in in vitro biological activity optimisation guided by molecular modelling efforts constitute a synergistic procedure for rapid lead optimisation.

Combinatorial chemistry has matured during recent years.[1] Initially, concepts for the synthesis of large numbers of compounds in a split and mix fashion on solid phase as well as the synthesis of compounds in mixtures attracted much interest in academia and especially in pharmaceutical research.[2] In many pharmaceutical companies a conceptual shift could be observed over the last years away from the solid-phase synthesis of large numbers of non-purified compounds in mixtures to the parallel solution-phase synthesis of discrete single compounds of high purity.[3] Libraries with smaller numbers of especially designed compounds with a desired structural pattern and physicochemical properties provided a unalterable balance between the ability to synthesise large numbers of compounds and the wish to maximise the knowledge about certain biological test systems, thus shortening the timelines in medicinal chemistry projects. The purification techniques as well as the post-synthesis handling of compound arrays was streamlined as well as analytical techniques have developed along with this combinatorial chemistry revolution.[4]

In the course of a medicinal chemistry program triazolopyridine derivatives have attracted considerable attention.[5] Especially 5-amino-1-aryl-[1,2,4]triazolo[1,5-*a*]pyridine-7-carboxylic acid amide derivatives **1** promised to be of high value in the lead optimisation phase. The chosen retrosynthetic approach towards those compounds commences with the cleavage of the amide functionality in triazolopyridine derivative **1** to trail back to ester **2** which can be further converted to 2,6-bis-aminopyridine-4-carboxylic acid methyl ester **3** an *N*-aminating source and an appropriate C-1 unit like aldehydes or esters (Scheme 1).

Literature research for the key building block 2,6-bis aminopyridine-4-carboxylic acid methyl ester **3** revealed only one source of information dating back to 1915 where the integrity of the compounds was confirmed by melting point and elemental analysis.[6] Following this protocol, and characterising the intermediates accordingly, we reacted 2,6-dichloro citracinic acid **4** with ammonia under copper catalysis, under pressure (20 bar) and at elevated temperature (180°C). Filtration of the copper, subsequent treatment with base and precipitation upon addition of acid yielded the free acid in 83%. Subsequently, the esterification under acidic conditions in methanol yielded the methyl ester **3** in 77% after extraction with ethyl acetate. Satisfactorily, the two-step procedure described in the original literature

Keywords: triazolo[1,5-*a*]pyridine derivatives; combinatorial chemistry; iterative solution-phase chemistry; lead optimisation; *N*-amination.

* Corresponding author. Fax: +41-61-6886459; e-mail: matthias.nettekoven@roche.com

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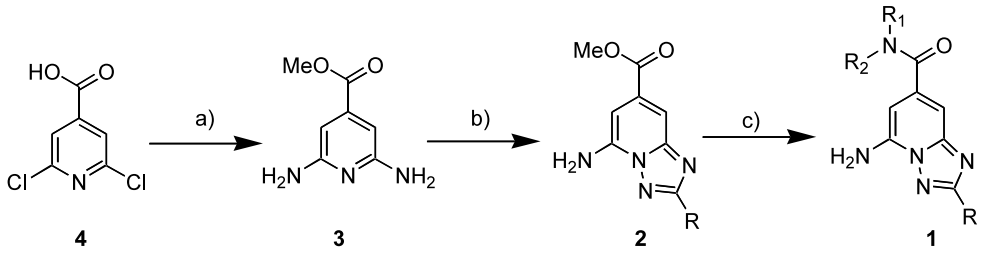


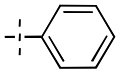
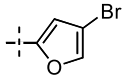
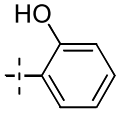
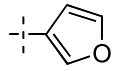
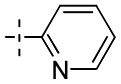
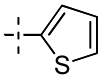
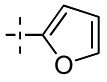
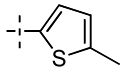
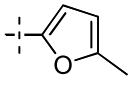
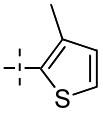
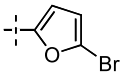
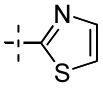
Scheme 1. Synthetic access to 5-amino-1-aryl-[1,2,4]triazolo[1,5-*a*]pyridine-7-carboxylic acid amide derivatives **1**. *Reagents and conditions*: (a) (i) NH_3, Cu, 83%, (ii) HCl (g), MeOH, 77%; (b) (i) *O*-mesitylenesulfonylhydroxylamine, (ii) RCHO, (iii) KOH/MeOH, O_2; (c) methylaluminoxane, R_1R_2NH, dioxane, 90°C.

from 1915 could be reproduced with 64% overall yield.[7] The adduct resulting from *N*-amination of the key intermediate **3** with *O*-mesitylenesulfonylhydroxylamine[8] was used directly in one pot without further purification for the condensation with an aldehyde. At elevated temperature the ring-closure took place upon addition of potassium hydroxide in methanol followed by the final aromatisation initialised by opening of the reaction vessel to ambient air to yield triazolopyridine ester **2**.

In total 21 substituted benzaldehydes, furfurals, pyridine carboxaldehydes and thiophene carboxaldehydes were utilised to affect the generation of 1-aryl-substituted triazolopyridine methyl esters **2** in satisfactory yields up to 51% for the three-step/one-pot reaction sequence.[9] The protocol worked reliably for all selected aldehydes, however, the isolated yields of **2** were dependant on steric and electronic features of the aldehydes which determined their reactivity. A representative selection of the synthesised triazolopyridine derivatives **2a**–**l** is depicted in Table 1.

The conversion of the ester functionality in the triazolopyridine derivatives **2** to form the desired amide derivatives **1** was considered to be straightforward. A direct conversion from the ester to the amide promised to be more economic then a two-step procedure via the liberated acid and subsequent coupling with amines and therefore highly desirable. However, the main obstacle to overcome proofed to be the generality of the reaction conditions. The amines to be employed in the final synthetic array were chosen according to diversity considerations and desired physicochemical properties predictions of the end products.[10] Therefore, a diverse set of amines was selected apart from anilines as they were considered to cause adverse side reactions thus competing with the amino group in 5 position of the triazolopyridine **2**. The generality of reaction conditions was investigated by choosing a training set of triazolopyridines **2j**/**2l** and a set of three different amines (i.e. benzylamine, morpholine and diethylamine). The conditions checked dealt mainly with aluminium and magnesium mediated amidations[11] and a wide combination of amounts of amine, solvents and temperatures was screened. The most promising protocol for a clean transformation of esters **2** to amides **1** employed conditions were methylaluminoxane pre-mixed with amines was reacted with the respective esters in dioxane at

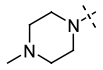
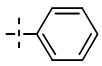
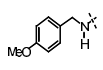
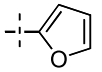
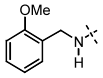
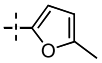
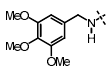
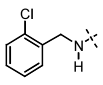
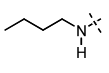
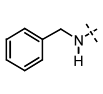
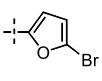
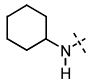
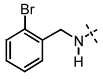
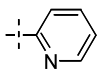
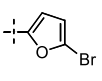
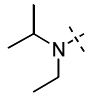
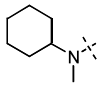
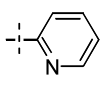
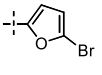
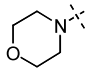
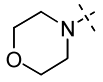
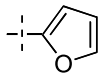
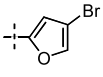
Table 1. Representative examples of triazolopyridine derivatives **2a**–**l**[a]

No	R	Yield (%)	No	R	Yield (%)
2a		50	**2g**		24
2b		18	**2h**		51
2c		32	**2i**		23
2d		43	**2j**		49
2e		50	**2k**		43
2f		27	**2l**		51

[a] Compounds characterised by ^{1}H NMR, MS and microanalysis.

90°C for a prolonged period of time (48–96 h).[12] Acidification, evaporation, dissolution in DMSO and parallel filtration yielded a solution which was directly subjected to preparative HPLC purification on reversed-phase material eluting with an acetonitrile/water gradient. Evaporation of the product fractions yielded the desired triazolopyridine carboxamides **1** in yields up to 70%. The purity was checked by HPLC and the structural integrity was corroborated by MS and ^{1}H NMR in non-deuterated DMSO.[13] A total of 500 compounds was synthesised with the protocol outlined in parallel iterative solution-phase chemistry cycles (synthesis of compound arrays with 12–48 members) allowing for maximal flexibility in the design of

Table 2. Representative triazolopyridine carboxamide derivatives **1**

No	NR_1R_2	R	Yield [%] (purity [%])[a] MH^+ found	No	NR_1R_2	R	Yield [%] (purity[%])[a] MH^+ found	No	NR_1R_2	R	Yield[%] (purity[%])[a] MH^+ found
3a			53 (87) 337.2	**3i**			32 (94) 364.2	**3q**			15 (100) 314.2
3b			29 (100) 374.4	**3j**			25 (100) 314.3	**3r**			35 (95) 440.4
3c			21 (100) 394.2	**3k**			21 (100) 362.2	**3s**			51(100) 330.3
3d			22 (100) 360.3	**3l**			54 (100) 378.3	**3t**			32 (100) 356.3
3e			18 (100) 423.2	**3m**			70 (100) 390.1	**3u**			25 (91) 344.3
3f			19 (100) 351.1	**3n**			22 (100) 454.4	**3v**			34 (100) 344.2
3g			32 (100) 314.2	**3o**			43 (89) 446.2	**3w**			56 (100) 315.3
3h			32 (97) 412.2	**3p**			53 (99) 360.3	**3x**			35 (100) 365.2

[a] Purity was determined by analytical HPLC–MS at 230 nm.

the next synthetic array reacting accordingly to in vitro biology results.[14] Representative examples are shown in Table 2.

The protocol chosen for the above mentioned transformation proofed to work reliably for all sorts of primary/secondary aliphatic amines, cycloalkylamines, various benzylamines and phenethylamines thus affording in the majority of the experiments performed the desired triazolopyridine derivatives **1**. However, the yield of the final product **1** was mainly influenced by steric and electronic features of the selected amines. With the final triazolopyridine derivatives **1** in hand it was possible to rapidly explore the biological diversity of those compounds thereby establishing a preliminary SAR being efficiently supported and guided by molecular modeling efforts.[10]

In conclusion, we devised a new versatile synthetic route towards 5-amino-1-aryl-[1,2,4]triazolo[1,5-*a*]pyridine-7-carboxylic acid amide derivatives **1** commencing from 2,6-dichlorocitrazinic acid **4**. A two step synthesis previously described in a literature reference from 1915 led smoothly in 64% overall yield to the desired key intermediate 2,6-bis-aminopyridine-4-carboxylic acid methyl ester **3**. The elaboration of a variety of triazolopyridine-5-carboxylic acid methyl esters **2** by *N*-amination of the pyridine nitrogen, followed by condensation of the adduct with a wide selection of aldehydes and subsequent cyclisation and oxidation led in yields up to 70% to the precursors **2** for the final coupling. The careful evaluation of reaction conditions proofed to be key to the success of transformation of the methyl ester functionality in **2** to the desired final amide derivatives **1**. A library of total 500 5-amino-1-aryl-[1,2,4]triazolo[1,5-*a*]pyridine-7-carboxylic acid amide derivatives **1** was synthesised in parallel iterative cycles in a combinatorial fashion taking advantage of solution-phase chemistry thus allowing for maximal flexibility in chemistry and maximal efficiency in in vitro biological activity optimisation. Chemistry efforts towards novel triazolopyridine derivatives with improved biological in vitro activities and pharmacological profiles are currently undertaken and will be reported in full in due course.

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7. *2,6-Diaminoisonicotinic acid methyl ester* ***4***: A mixture of 20 g (0.1 mol) 2,6-dichloroisonicotinic acid and 2 g (30 mmol) copper powder in 300 ml aqueous ammonia (~30%) was heated for 12 h to 180°C in an autoclave (20 bar). After cooling to room temperature the copper was filtered of and the filtrate was treated with 1N HCl to pH 5. The precipitate was filtered and purified by repeated dissolving in aqueous ammonia (25%) and subsequent precipitation with 1N HCl. Filtration and drying in HV yielded 13.2 g (83%) 2,6-diaminoisonicotinic acid as a brown solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.23 (s, br, 1H, COOH), 6.10 (s, 2H, Ar-H), 5.64 (s, br, 4H, NH_2). MS m/e (%): 153 (MH^+, 100). A suspension of 11 g (70 mmol) 2,6-diaminoisonicotinic acid in 270 ml methanol was treated at 0°C for 2 h with gaseous HCl. The mixture was concentrated, the residue was dissolved in water and saturated $NaHCO_3$ was added to pH 8. Exhaustive extraction with ethylacetate, drying of the combined organic phases with $MgSO_4$ and removal of the volatiles yielded 9.3 g (77%) 2,6-diaminoisonicotinic acid methyl ester **4** as yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.11 (s, 2H, Ar-H), 5.69 (s, 4H, NH_2), 3.77 (s, 3H, CH_3). MS m/e (%): 167 (MH^+, 100). Anal. calcd for $C_7H_9N_3O_2$: C, 50.30; H, 5.43; N, 25.14. Found: C, 50.27; H, 5.26; N, 24.11.
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9. *General procedure for the synthesis of* ***2***: To a solution of 1 g (5.98 mmol) 2,6-diaminoisonicotinic acid methyl ester **4** in 50 ml dioxane at room temperature was added 1.41 g (6.58 mmol, 1.1 equiv.) *O*-mesitylenesulfonylhydroxylamine and, after 2 h, 1.3 equiv. of the respective aldehyde was added and the mixture was stirred for 3 h at 100°C. After the addition of 6 ml 1N KOH in MeOH the mixture was stirred at room temperature for 12 h and concentrated. The residue was taken up in 50 ml water followed by extraction with dichloromethane, drying of the combined organic layers with $MgSO_4$, and removal of the volatile components. The residue was purified by column chromatography on silica eluting with a gradient of dichloromethane:ethylacetate to afford the title compound.
   Compound **2e**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.38 (s, 3H, 8-H, NH_2), 7.08 (s, 1H, furanyl (3-H)), 6.73 (s, 1H, 6-H)), 6.34 (s, 1H, furanyl (4-H)), 3.89 (s, 3H, OCH_3), 2.40 (s, 3H, furanyl (CH_3)). MS m/e (%): 272.2 (MH^+, 100). Anal. calcd for $C_{13}H_{12}N_4O_3$: C, 57.35; H, 4.44; N, 20.58. Found: C, 57.46; H, 4.61; N, 20.08%.
   Compound **2l**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.09 (d, J=2.8 Hz, 1H, thiazolyl (5-H)), 8.01 (d, J=2.8 Hz, 1H, thiazolyl (4-H)), 7.51 (s, br, 2H, NH_2), 7.50 (s, 1H, 8-H), 6.81 (s, 1H, 6-H), 3.91 (s, 3H, OCH_3). MS m/e (%): 275.3 (MH^+, 100). Anal. calcd for $C_{11}H_9N_5O_2S$: C, 47.99; H, 3.30; N, 25.44; S, 11.65. Found: C, 48.27; H, 3.51; N, 24.49; S, 10.69%.
10. For a more detailed description of the procedure that led to the amine starting material selection for the respective iterative cycle, see: Schneider, G.; Nettekoven, M. *J. Comb. Chem.* **2003**, in press.
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12. *General procedure for the synthesis of* ***1***: To a solution of 0.44 mmol amine in 0.5 ml dioxane was added 0.5 ml methylaluminoxane (10% in toluene) (use of trimethylaluminium instead of methylauminoxane gave comparable results) and the mixture was stirred for 1 h at room temperature. 0.11 mmol [1,2,4]triazolo[1,5-*a*]pyridine-7-carboxylic acid methyl ester in 1 ml dioxane was added and the mixture was heated to 90°C for 72 h. After addition of 0.4 ml 1N HCl the mixture was evaporated to dryness and the residue was taken up in 1.5 ml DMSO, filtered, and the title compound was isolated by reversed-phase HPLC eluting with a water/acetonitrile gradient.
13. The NMR samples were processed with a stop-flow method and the Bruker-efficient sample transfer (BEST) procedure.
   Compound **3j**: ^{1}H NMR (500 MHz, DMSO): δ 7.24 (s, 2H, NH_2), 7.03 (d, J=3 Hz, 1H, furanyl (3-H)), 6.79 (s, 1H, 8-H), 6.32 (d, J=3 Hz, 1H, furanyl (4-H)), 6.09 (s, 1H, 6-H), 2.39 (s, 3H, CH_3), 1.15 (m, 3H, NCH_2CH_3), 1.08 (m, 3H, NCH_2CH_3), signal for NCH_2 under DMSO signal.
   Compound **3s**: ^{1}H NMR (500 MHz, DMSO): δ 8.60 (t, J=5.6 Hz, 1H, NH), 7.63 (d, J=3.6 Hz, 1H, thiophenyl 3-H), 7.32 (s, 1H, 8-H), 7.15 (s, br, 2H, NH_2), 6.93 (d, J=3.6 Hz, 1H, thiophenyl 4-H), 6.63 (s, 1H, 6-H), 3.27 (m, 2H, NCH_2), 1.54 (m, 2H, $CH_2CH_2CH_3$), 1.35 (m, 2H, CH_2CH_3), 0.96 (t, J=7.7 Hz, 2H, CH_3).
   Compound **3w**: ^{1}H NMR (500 MHz, DMSO): δ 8.07 (d, J=2.8 Hz, 1H, thiazolyl 5-H), 7.98 (d, J=2.8 Hz, 1H, thiazolyl 4-H), 7.34 (s, br, 2H, NH_2), 7.10 (s, 1H, 8-H), 6.33 (s, 1H, 6-H), 3.49 (t, J=7.2 Hz, 2H, NCH_2), 3.43 (t, J=7.2 Hz, 2H, NCH_2), 1.89 (m, 2H, CH_2), 1.84 (m, 2H, CH_2).
14. For a more detailed description of workflow procedures and automation technology utilised for this synthetic array, see: Nettekoven, M.; Thomas, A. W. *Curr. Med. Chem.* **2002**, *9*, 2179–2190.