A Short and Unequivocal Synthesis of 5-Aminotetrazolo[1,5-*a*]-quinazoline as a Tricyclic Analogue of 4-(3-Bromoanilino)-6,7-dimethoxyquinazoline (PD 153035)

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The discovery of 4-(3-bromoanilino)-6,7-dimethoxyquinazoline (PD 153035) as an extremely potent inhibitor of the tyrosine kinase activity of the epidermal growth factor receptor led to the preparation of several fused tricyclic quinazoline analogues. The present paper reports a new tricyclic derivative: 5-(3-bromoanilino)-7,8-dimethoxytetrazolo[1,5-*a*]quinazoline. This compound was synthesized by two different pathways *via* a 1,3-dipolar cycloaddition of an azide at carbon 2 of the quinazoline ring.

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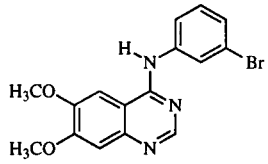
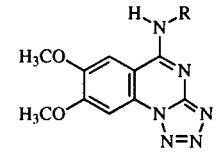
4-Anilinoquinazolines have been shown [1-4] to be potent and highly selective inhibitors of the tyrosine kinase activity of the Epidermal Growth Factor receptor. Among these compounds, the 4-(3-bromoanilino)-6,7-dimethoxyquinazoline is essential and was shown to bind competitively at the ATP site of the enzyme [5].



PD 153035

Tyrosine kinase enzymes are components of the growth signal transduction pathway. The overexpression of these enzymes has been noted in a lot of proliferative diseases such as cancer, restenosis and asthma, thus they have become crucial targets for drug design. Indeed, tyrosine kinase inhibitors are of potential interest as anticancer drugs because the Epidermal Growth Factor receptor is known to be overexpressed in a high percentage of cancers [6] and this overexpression is associated with poor prognosis [7].

Like the other protein kinases, the enzyme can be described as constituted of two binding sites - ATP and substrate. The ATP site has the overall geometry of a deep planar cleft. Based on this geometry, extended planarity could be a key element in the molecular recognition of the cleft [8]. For this reason, closely related fused tricyclic analogues of 4-(3-bromoanilino)-6,7-dimethoxyquinazoline [9,10] could be interesting probes to specify the sizes of the binding area. Moreover, the ATP site possesses a glycine rich loop containing a number of hydrophilic amino acids such as threonines and serines which interact with the adenine nitrogen of ATP through a hydrogen bond. Introducing additional nitrogen atoms into the tricyclic structure could provide compounds capable of inhibiting tyrosine kinase activity at the ATP site.



5-Aminotetrazolo[1,5-*a*]quinazolines

We therefore decided to synthesize 5-aminotetrazolo-[1,5-*a*]quinazolines. This heterocycle was prepared by two different pathways from 2-azidoquinazoline. The first one led to a tetrazoloquinazoline but this method was not specific with regard to the cyclisation direction. The second route was an unequivocal synthesis for 5-aminotetrazolo[1,5-*a*]quinazolines.

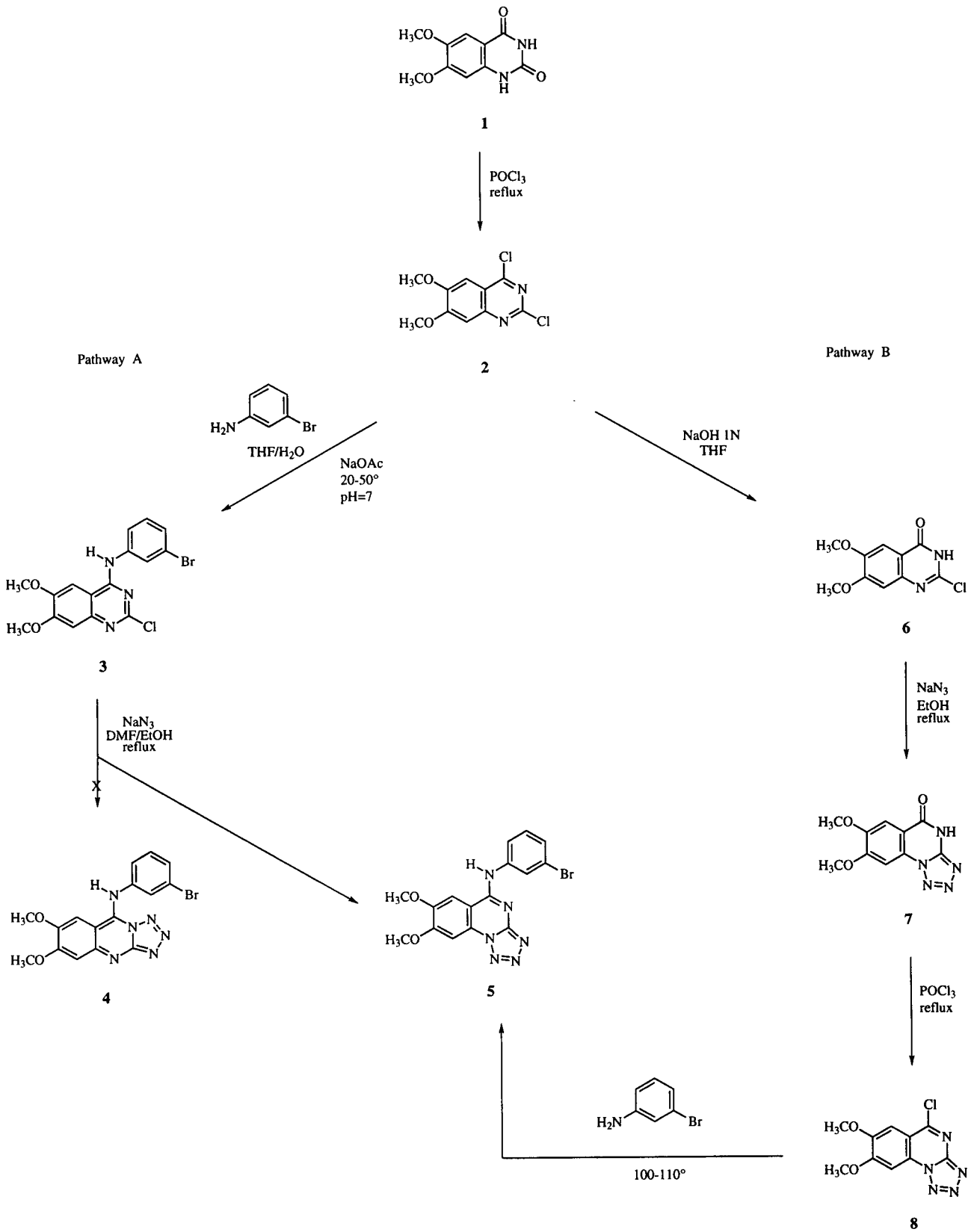
In the present paper we report two synthetic pathways to the tetrazolo analogues from 4-(3-bromoanilino)-6,7-dimethoxyquinazoline.

Chemistry.

The first synthetic pathway (pathway A) was accomplished as described in Scheme 1. With this strategy, the amino substitution on the quinazoline ring occurred before cyclisation.

6,7-Dimethoxyquinazolin-2,4-dione **1** is commercially available. Reaction of the dione with phosphoryl chloride gave a high yield of 2,4-dichloro-6,7-dimethoxyquinazoline **2**. Reaction with 3-bromoaniline, under carefully controlled conditions to prevent disubstitution, gave the 4-(3-bromoanilino) derivative **3** according to the method described by Ife *et al.* [11]. Monosubstitution occurred at carbon 4 of the quinazoline ring because (i) the 4-chlorine

Scheme I


1
POCl3
reflux
2
Pathway A
Pathway B
THF/H2O
NaOAc
20-50°
pH=7
NaOH 1N
THF
3
6
NaN3
DMF/EtOH
reflux
NaN3
EtOH
reflux
4
5
7
POCl3
reflux
100-110°
8

atom is more reactive [12] and (ii) 4-chloro-2-amino- and 4-amino-2-chloroquinazoline isomers can be easily and unequivocally differentiated by comparing their uv spectra [13,14]. Indeed, the uv spectrum of compound **3** exhibits a maximum absorption at 334 nm, in accordance with that of the 2-chloro-4-dialkylaminoquinazolines (330 nm) [13]. Finally, compound **3** was reacted with sodium azide at the reflux temperature of ethanol-dimethylformamide to give the tetrazolo derivative whose existence is only proved by the absence in ir of a characteristic band of the azide group at approximately 2120-2160 cm^{-1}.

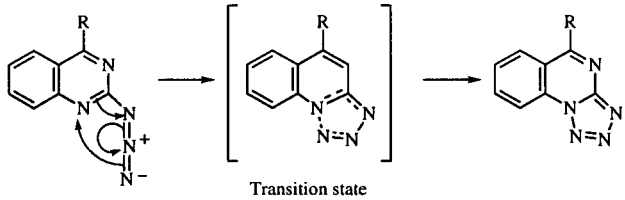
To ascertain the identity of the tetrazoloquinazoline formed at the time of cyclisation, an unequivocal synthesis was set up, and for this purpose, the tetrazolo[1,5-*a*] isomer was then prepared according to pathway B in Scheme 1.

Compound **2** was synthesized as described above. The 2-chloro-6,7-dimethoxyquinazolin-4-one **6** was unambiguously obtained [12] by selective alkaline hydrolysis at room temperature by the method described by Hess [15]. Sodium azide ethanol replaced the chlorine atom at position 2 of the quinazoline ring. The subsequent formation of the tetrazoloquinazoline **7** was confirmed by ir. The absence of the azide band at 2120-2160 cm^{-1} confirmed cyclisation and a strong band at 1690 cm^{-1} indicated the presence of a lactam group. Thus, this information enabled us to confirm the formation of the tetrazolo[1,5-*a*] derivative **7**. Refluxing in phosphoryl chloride gave 5-chloro-7,8-dimethoxytetrazolo[1,5-*a*]quinazoline **8** which in turn was reacted with 3-bromoaniline at 100° to give the required 5-bromoanilino compound **5**.

Comparison of the ir, mass spectra, nmr and tlc data indicates formation of 5-aminotetrazolo[1,5-*a*]quinazoline **5**, the condensed tricyclic system *via* pathway B.

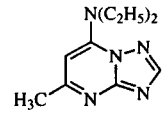
The reaction may be an intramolecular 1,3-dipolar cycloaddition. Such a cycloaddition could involve the addition of a 1,3-dipole (the azide) to a π-bond system (the -C=N bond of the quinazoline ring) leading to the adjacent five-membered heterocycle, very likely implying a concerted mechanism [16] rather than a diradical one [17] (Scheme 2).

Scheme 2



Conclusions.

Tetrazolo[1,5-*a*]quinazolines were shown to be available by successive substitutions on carbons 4 and 2 of 2,4-dichloroquinazoline. The product may be considered a tricyclic analogue of 4-(3-bromoanilino)-6,7-dimethoxyquinazoline. This approach may provide a potential anticancer drug able to inhibit the Epidermal Growth Factor receptor tyrosine kinase. The objective of our present efforts is to prepare imidazo[2,3-*b*] and triazolo[5,1-*b*]-quinazolines as tricyclic analogues of Trapidil, the only purine-like molecule claimed as a potential drug in the treatment of restenosis on the basis of clinical trials. These compounds, like 4-(3-bromoanilino)-6,7-dimethoxyquinazoline, could complete the family of the tyrosine kinase inhibitors by binding the hydrophobic pocket of the ATP site of the enzyme and may be considered as new antiproliferative agents.



Trapidil

EXPERIMENTAL

Melting points were determined on a Büchi 535 capillary melting point apparatus and are uncorrected. Analytical tlc was performed on precoated Kieselgel $60F_{254}$ plates (Merck). The spots were located by uv (254 and 366 nm); R_f values are given for guidance. Column chromatography was performed on silica gel 60 230-400 Mesh purchased from Merck. The ir spectra were determined as potassium bromide discs with a Perkin Elmer 1310 spectrophotometer; absorbances are reported in ν (cm^{-1}). The ^1H nmr spectra were recorded on a Bruker AC 300 spectrometer using tetramethylsilane as an internal standard. Chemical shifts are expressed in δ units (ppm) and the splitting patterns are designated as follows: s singlet, bs broad singlet, t triplet, dd doublet of doublets, m multiplet, bm broad multiplet. The spectra confirmed the proposed structures. The mass spectra were recorded on a quadripolar Finnigan Mat SSQ 710 instrument in the chemical ionisation or electron impact mode. The hplc analyses were performed on a Hewlett-Packard 1090 liquid chromatograph, using a Licrospher 60 RP-select B C8 5 μm 250 x 4.6 mm column (inverse phase) to estimate the purity of the products. Elution was performed with the following two systems: solution A (80% water, 5% PIC® B-8 low UV Reagent (Waters Part No WAT084283), 15% methanol) and solution B (10% water, 5% PIC® B-8, 85% methanol). In the isocratic mode, percentages of solutions A and B are noted. Elemental analyses for C,H,N were performed by the "Service Central d'Analyses" at the CNRS, Vernaison, France.

2,4-Dichloro-6,7-dimethoxyquinazoline **2** [11,15] and 2-chloro-6,7-dimethoxyquinazolin-4-one **6** [15] were prepared following procedures already published.

2-Chloro-4-(3-bromoanilino)-6,7-dimethoxyquinazoline (**3**).

2,4-Dichloro-6,7-dimethoxyquinazoline **2** (6.4 g, 24.7 mmoles) was stirred in a mixture of water (125 ml), tetrahydrofuran (250 ml), 3-bromoaniline (3 ml, 27.2 mmoles) and sodium acetate (3.1 g, 37.1 mmoles) for 6 days during which time the temperature was progressively raised to 60°. The reaction mixture was evaporated to dryness *in vacuo*. The residue was triturated in dichloromethane-methanol and the precipitate filtered. Crystallisation from methanol left the monosubstituted product as a white powder, 4.8 g (49%), mp 237-240°; tlc: dichloromethane-ethyl acetate, 7:3 (v/v) R_f, 0.71; ir (potassium bromide): 3320 (NH), 3000-2800 (CH), 1620 (C=N), 1590 (C=C); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 3.97 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 7.22 (s, 1H, H_8), 7.40 (m, 2H, $H_{5'}$ and $H_{6'}$), 7.84 (m, 2H, H_5 and $H_{4'}$), 8.06 (s, 1H, $H_{2'}$), 9.89 (s, 1H, NH); ms: (chemical ionisation) m/z 393 (MH^+)/395 (isotope), 360, 314, 280, 205, 157, 91; hplc: (isocratic 10% A, 90% B), λ = 220, 230, 254 and 280 nm, rt = 9.6 minutes 100% purity.

Anal. Calcd. for $C_{16}H_{13}BrClN_3O_2$: C, 48.86; H, 3.33; N, 10.69. Found: C, 48.63; H, 3.30; N, 10.93.

7,8-Dimethoxytetrazolo[1,5-*a*]quinazolin-5-one (**7**).

A mixture of 2-chloro-6,7-dimethoxyquinazolin-4-one **6** (5 g, 20.8 mmoles) and sodium azide (1.35 g, 20.8 mmoles) was refluxed by stirring for 18 hours in ethanol. The solvent was removed *in vacuo* to give the tricyclic compound which crystallized from dioxane as a white powder, 4.7 g (92%), mp 266-268°; tlc: dichloromethane-ethanol 9:1 (v/v) R_f, 0.75; ir (potassium bromide): 2900 (CH), 1690 (C=O), 1630 (C=N), 1600 (C=C); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 3.91 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 7.54 (s, 1H, H_9), 7.68 (s, 1H, H_6), 13.37 (s, 1H, NH).

Anal. Calcd. for $C_{10}H_9N_5O_3$: C, 48.59; H, 3.67; N, 28.33. Found: C, 48.31; H, 3.69; N, 28.52.

5-Chloro-7,8-dimethoxytetrazolo[1,5-*a*]quinazoline (**8**).

A mixture of **7** (3 g, 12.1 mmoles) and phosphoryl chloride (11.3 ml, 121 mmoles) was refluxed with stirring for 5 hours. Phosphoryl chloride was removed *in vacuo* to leave a compound which was slowly poured into ice-water. The mixture was stirred and alkalinized with ammonium hydroxide. The resulting precipitate was filtered and crystallized from cyclohexane, 2.4 g (76%); tlc: dichloromethane-ethyl acetate 9:1 (v/v) R_f, 0.70; ir (potassium bromide): 1605 (C=N), 1550, 1500; ^{1}H nmr (dimethyl sulfoxide-d_6): 3.99 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 7.30 (s, 1H, H_9), 7.39 (s, 1H, H_6); ms: (electron impact) m/z 258, 243, 215, 188, 154, 126, 76.

Anal. Calcd. for $C_{10}H_8ClN_5O_2$: C, 45.21; H, 3.04; N, 26.36. Found: C, 45.47; H, 2.98; N, 26.04.

5-(3-Bromoanilino)-7,8-dimethoxytetrazolo[1,5-*a*]quinazoline (**5**).

Method A.

A mixture of 2-chloro-4-(3-bromoanilino)-6,7-dimethoxyquinazoline **3** (1 g, 2.5 mmoles) and sodium azide (0.3 g, 5.1 mmoles) was refluxed in ethanol-dimethylformamide for 48 hours. The mixture was cooled to room temperature, filtered and washed with dichloromethane. Recrystallisation from dimethylformamide yielded **5** as a white powder, 0.6 g (58%).

Method B.

Compound **8** (0.8 g, 3 mmoles) was stirred with 3-bromoaniline (1.3 ml, 12 mmoles) and 2-propanol. The reaction mixture was heated at 100-110° for 1 hour. The solvent was removed. The residue was taken up in acetone-1*N* sodium hydroxide and filtered. The resulting precipitate was washed with dichloromethane and cyclohexane. Recrystallisation from dimethylformamide yielded **5** as a white powder, 0.8 g (67%), mp 284-286°; tlc: dichloromethane-ethanol 95:5 (v/v) R_f, 0.59; ir (potassium bromide): 3300 (NH), 1650 (C=N), 1620, 1610; ^{1}H nmr (dimethyl sulfoxide-d_6, 353K): 4.02 (s, 3H, OCH_3), 4.09 (s, 3H, OCH_3), 7.45 (m, 2H, $H_{5'}$ and $H_{6'}$), 7.84 (m, 2H, $H_{4'}$ and H_9), 8.12 (m, 2H, $H_{2'}$ and H_6), 9.86 (s, 1H, NH); ms: (chemical ionisation) m/z 401 (MH^+)/403 (isotope), 374 ($M-N_2$), 360, 329, 293, 265, 207, 189, 147, 97, 91, 57; hplc: (isocratic 25% A, 75% B), λ = 220, 230, 254 and 280 nm, rt = 7 minutes 100% purity.

Anal. Calcd. for $C_{16}H_{13}BrN_6O_2$: C, 47.90; H, 3.27; N, 20.95. Found (method A): C, 48.09; H, 3.25; N, 20.93. Found (method B): C, 48.25; H, 3.25; N, 20.59.

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