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Substituted Azoloquinolines and -quinazolines as New Potent Farnesyl Protein Transferase Inhibitors

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Abstract—A series of (4-chlorophenyl)-α-(1-methyl-1*H*-imidazol-5-yl)azoloquinolines and -quinazolines was prepared. These compounds displayed potent Farnesyl Protein Transferase inhibitory activity and tetrazolo[1,5-*a*]quinazolines are promising agents for oral in vivo inhibition.

The discovery of mutated forms of the Ras protein in approximately 30% of human cancers resulted in a growing interest in Farnesyl Protein Transferase (FPT) as a target for novel anti-cancer agents.[1,2] FPT not only plays a key role in enabling the Ras protein to acquire full biological activity as a signal transducer,[3–11] but its substrates also include several other proteins which are critical intermediates of cell signaling and cytoskeleton organization.[12–22] Thus the hypothesis that inhibition of FPT may be beneficial in the treatment of certain cancers is currently being clinically tested with some drug candidates undergoing phase II or phase III trials.[16,18,20,22]

R115777 **1** (ZARNESTRA™) is a 4-phenylquinolinone that is currently undergoing Phase II clinical trials for the treatment of hematological and solid tumors.[23–27] In an attempt to prevent some in vivo metabolism occurring at the quinolinone nitrogen, we envisioned the synthesis of analogues in which this nitrogen would be incorporated into an heterocycle. As the quinazolinone analogue of **1** has demonstrated potent in vitro enzymatic inhibition,[28] we decided to implement the above modifications not only in the quinoline but also in the quinazoline series.

Herein, we describe the synthesis and inhibitory profile of a series of (4-chlorophenyl)-α-(1-methyl-1*H*-imidazol-5-yl) azoloquinolines or quinazolines (Fig. 1),[29] comparing their potency with ZARNESTRA™.

[4-Amino-3-(3-chlorobenzoyl)phenyl]-(4-chlorophenyl) methanone **2**[28] was reacted with acetic anhydride to give amide **3** which was cyclized into quinolinone **4** using *t*-BuOK as a base (Scheme 1). Then, refluxing **4** in phosphorus oxychloride provided the 2-chloroquinoline **5**. In initial attempts we sought to build the azoloquinoline heterocycle prior to the introduction of the *N*-methylimidazolyl moiety as depicted below. Therefore, **5** was

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condensed with either 2,2-dimethoxyethanamine or hydrazinecarboxaldehyde to cyclize into respectively imidazo[1,2-*a*]quinoline **6** or [1,2,4]triazolo[4,3-*a*]quinoline **7** in acidic media. By action of *n*-butyllithium 1-methylimidazole was first deprotonated at C-2 and the resulting carbanion silylated. In the same pot, further deprotonation at C-5 by *n*-butyllithium and condensation onto ketones **6** and **7** provided **8** and **9**, respectively. The hydroxyl function was then converted into the corresponding chlorine by reaction with thionyl chloride which was substituted by ammonia to provide **12** and **13**, respectively.

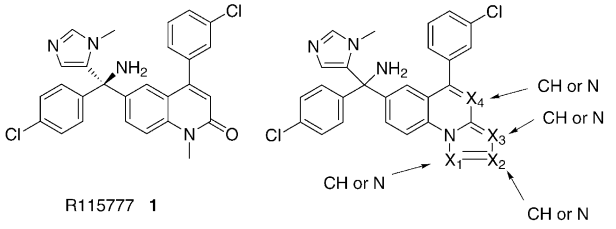
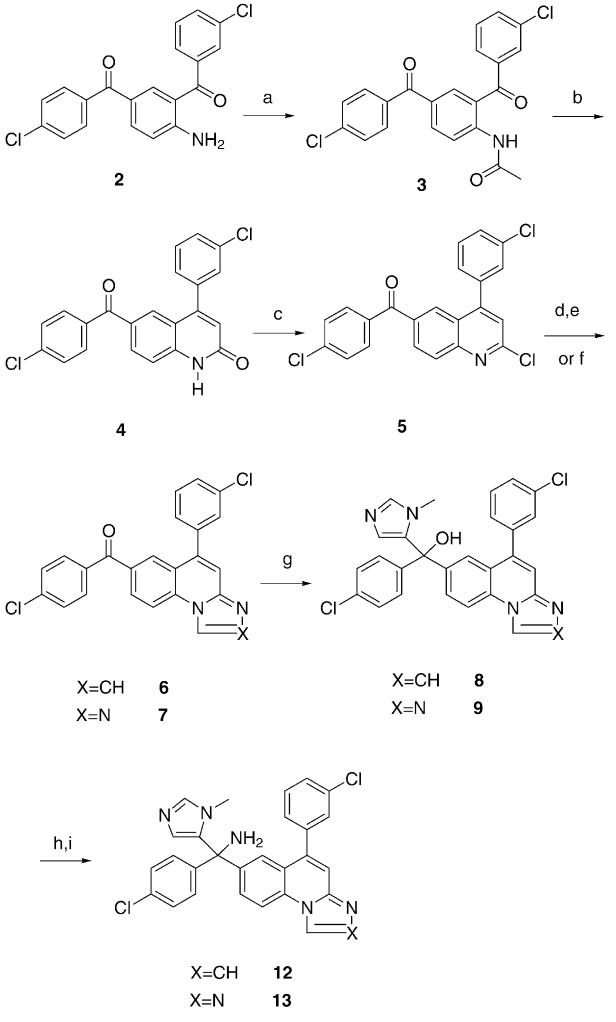


Figure 1. Structure of R115777 **1** and the tricyclic analogues envisioned.

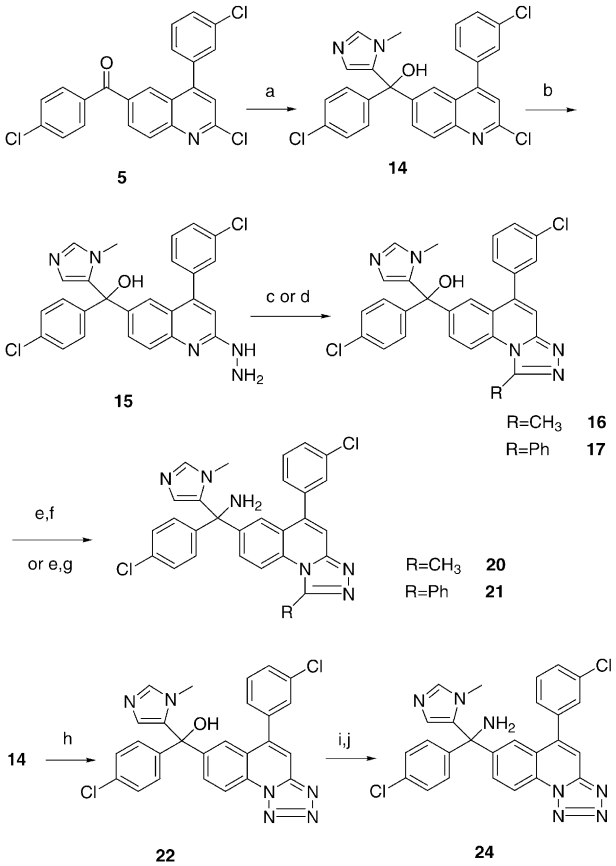


Scheme 1. Reagents and conditions: (a) Ac_2O, toluene, 110 °C; (b) *t*-BuOK, DME, rt, 86%; (c) $POCl_3$, 80 °C; (d) $H_2NCH_2CH(OCH_3)_2$, 120 °C; (e) AcOH, xylene, 140 °C, 56%; (f) hydrazinecarboxaldehyde, *n*-BuOH, 120 °C, then HCl 3 N, THF, 60 °C, 34%; (g) (1) 1-methylimidazole, $ClSiEt_3$, *n*-BuLi, THF, −70 °C; (2) *n*-BuLi, −70 °C, 48% **8**, 9% **9**; (h) $SOCl_2$, 40 °C; (i) (1) NH_4OH, THF, rt, 10% **12** or (2) NH_3/*i*-PrOH, 5 °C, 20% **13**.

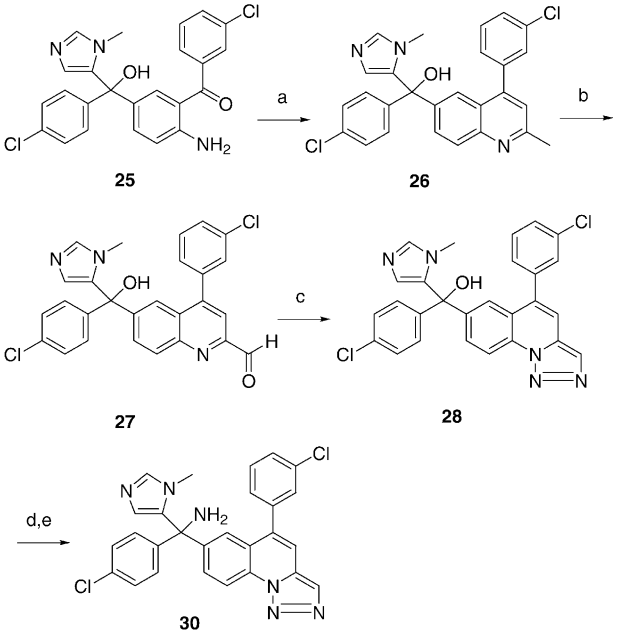
Condensation of 5-lithio-2-triethylsilyl-1-methylimidazole onto ketone **7** gave only a low yield. This may have resulted from the poor solubility of **7**. Therefore, we attempted to introduce this moiety sooner in the synthesis, namely on ketone **5** (Scheme 2). This was achieved with a better yield (71%) and also provided us with a common intermediate **14** for subsequent introduction of the triazole (**16**, **17**) and tetrazole (**22**) rings.

To access the [1,2,3]triazolo[1,5-*a*]quinoline **30**, *ortho*-aminobenzophenone **25**[28] was first reacted with 2-propanone in acidic media to provide the 2-methylquinoline **26** (Scheme 3). Oxidation of the methyl group with SeO_2 gave aldehyde **27** which was condensed with (*para*-tolylsulfonyl)hydrazine and cyclized by heating into **28**. The hydroxyl group was then converted into the amino moiety in two steps to give **30**.

The strategy depicted in Scheme 2 was applied to synthesis of azoloquinazolines using 2-chloroquinazoline **33** as central intermediate (Scheme 4). **33** was prepared in two



Scheme 2. Reagents and conditions: (a) (1) 1-methylimidazole, $ClSiEt_3$, *n*-BuLi, THF, −70 °C; (2) *n*-BuLi, −70 °C, 71%; (b) H_2NNH_2 50% in water, dioxane, 70 °C, 76%; (c) 1,1,1-triethoxyethane, *n*-BuOH, 100 °C, 53%; (d) triethoxymethylbenzene, *n*-BuOH, 100 °C, 72%; (e) $SOCl_2$, rt, R = Ph or $SOCl_2$, toluene, R = CH_3 ; (f) NH_4OH, THF, rt, 16% R = Ph; (g) NH_3/*i*-PrOH, toluene, 0 °C, 20% R = CH_3; (h) NaN_3, DMF, 90 °C, 41%; (i) $SOCl_2$, 40 °C; (j) NH_3/*i*-PrOH, THF, 5 °C, 39%.



Scheme 3. Reagents and conditions: (a) 2-propanone, H_2SO_4, AcOH, 100 °C, 60%; (b) SeO_2, dioxane, H_2O, 100 °C; (c) (*para*-tolylsulfonyl) hydrazine, MeOH, 60 °C, 21%; (d) $SOCl_2$, rt; (e) NH_3/*i*-PrOH, THF, 0 °C, 13%.

steps by chlorination of quinazolinone **31**[28] to provide **32** and then introduction of the *N*-methylimidazol-5-yl moiety as in Scheme 2 with still a good yield. Imidazole **35**, triazoles **36**, **39** and tetrazole **37** were obtained by following the experimental procedures depicted in Schemes 1 and 2.

The hydroxy substituent was also converted in two steps into the amino (Scheme 5).

These compounds were evaluated for inhibition of FPT in vitro and compared to **1**. The structure activity relationships are presented in Tables 1–3 (Figs 2–4).

Imidazoloquinoline **12** or imidazoloquinazoline **39** showed slight decreases in in vitro potency for inhibi-

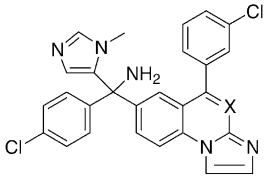
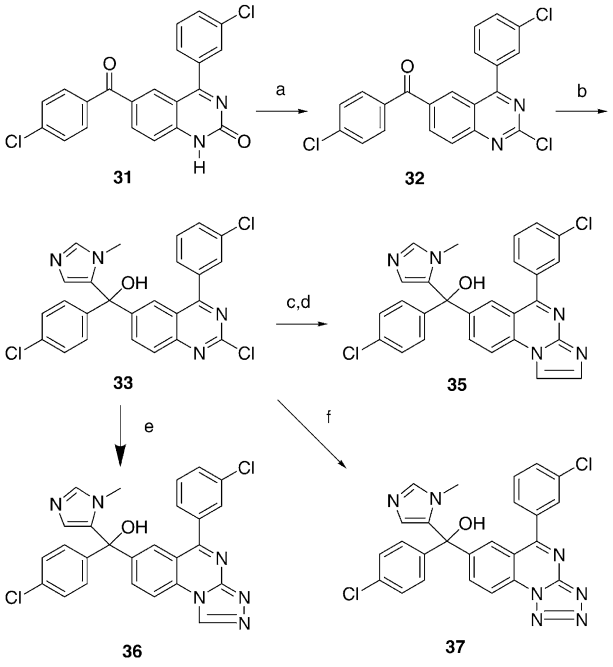


Figure 2.

Table 1. Comparison of FPT inhibition for imidazoloquinolines, -quinazolines and R115777

Compd	X	FPT (enz) IC_{50}, nM[a]	Cell proliferation IC_{50}, nM[b]
1	—	0.9	1.7
12	C	7	28
39	N	5	88

[a]The concentration required for a 50% reduction of the FPT-catalyzed incorporation of [3*H*]-farnesylpyrophosphate into a biotinylated laminB peptide.
[b]See ref 28.



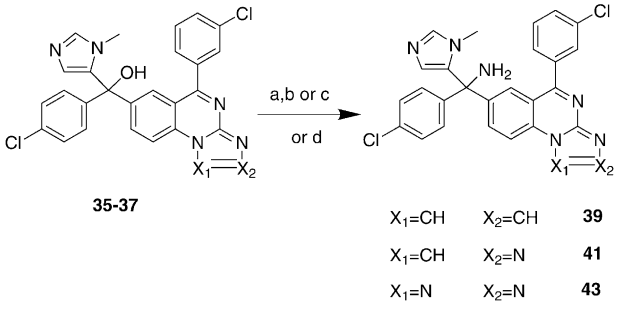
Scheme 4. Reagents and conditions: (a) $POCl_3$, 80 °C; (b) (1) 1-methylimidazole, $ClSiEt_3$, *n*-BuLi, THF, −70 °C; (2) *n*-BuLi, −70 °C, 61%; (c) $H_2NCH_2CH(OCH_3)_2$, 120 °C; (d) HCl (concd), toluene, 110 °C, 6.5%; (e) hydrazinecarboxaldehyde, 80 °C, 14%; (f) NaN_3, DMA, 50 °C, 75%.

tion of the isolated FPT enzyme compared to R115777 but were 16–50 times less potent in the cellular assay.

[1,2,4]Triazoloquinoline **13** or quinazoline **41** were not sufficiently active either in the isolated enzyme assay (**13**) or in the cellular assay (**41**) to be further studied (Table 2). Permuting a nitrogen and a carbon atom to get [1,2,3]triazoloquinoline **30** did not improve its potency.

Interestingly adding a methyl substituent on the triazole ring provided a compound with promising inhibitory profile (**20**) whereas a phenyl group (**21**) was not better than the hydrogen analogue **13**.

We moved to tetrazoloquinoline **24** (Table 3), a compound which still showed excellent enzymatic inhibition and maintained activity in the cellular assay. It also showed a moderate enzymatic inhibition of the enzyme



Scheme 5. Reagents and conditions: (a) $SOCl_2$; (b) NH_3/*i*-PrOH, toluene, 5 °C, 16%, **41**; (c) NH_3/*i*-PrOH, 5 °C, 22%, **39**; (d) sulfonylurea, 160 °C, 26%, **43**.

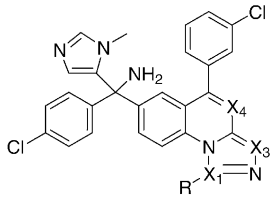


Figure 3.

Table 2. Comparison of FPT inhibition for triazoloquinolines, -quinazolines and R115777

Compd	X_4	X_3	X_1	R	FPT (enz) IC_{50}, nM[a]	Cell proliferation IC_{50}, nM[b]
1	—	—	—	—	0.9	1.7
13	C	N	C	H	65% inh. @ 0.1 μM	nt
41	N	N	C	H	3	186
20	C	N	C	CH_3	1.5	17.5
21	C	N	C	Ph	60% inh. @ 0.1 μM	304
30	C	C	N	—	68% inh. @ 0.1 μM	nt

nt, not tested.
[a]The concentration required for a 50% reduction of the FPT-catalyzed incorporation of [3*H*]-farnesylpyrophosphate into a biotinylated laminB peptide.
[b]See ref 28.

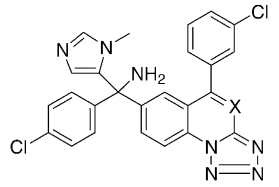


Figure 4.

Table 3. Comparison of FPT inhibition for tetrazoloquinolines, -quinazolines and R115777

Compd	X	FPT (enz) IC_{50}, nM[a]	Cell proliferation IC_{50}, nM[b]	GGPT IC_{50}, nM[c]
1	—	0.9	1.7	10,000
24	C	3.5	55	176
43	N	2.5	12	461
24a[d]	C	36% inh. @ 0.1 μM	>0.5 μM	55% inh. @ 10 μM
24b[e]	C	1.7	20	81
43a[f]	N	0.6	10	>1000
43b[g]	N	22	108	nt

nt, not tested.
[a]The concentration required for a 50% reduction of the FPT-catalyzed incorporation of [3*H*]-farnesylpyrophosphate into a biotinylated laminB peptide.
[b]See ref 28.
[c]The concentration required for a 50% reduction of the Geranylgeranylprotein transferase-I (GGPTase-I) catalyzed incorporation of [^3H]-geranylgeranylpyrophosphate into a biotinYRASNRSCAIL peptide.
[d]ee > 99%.[30]
[e]ee = 94%.[30]
[f]ee > 99%.[31]
[g]ee = 92%.[31]

Geranylgeranylprotein transferase I (GGPTase I). We had previously observed the importance of stereochemistry in R115777[27] chemistry. Since our analogues bore the same chiral center, **24** was also separated into its enantiomers **24a** and **24b**. As seen previously with R115777, activity mainly resides in one enantiomer.

Table 4. Comparison of in vivo results for compounds **1**, **20**, **24b** and **43a**

Compd	In vivo[a] % inhibition	Metabolism[b] (%)
1	37%	66%
20	Not significant	nt
24b	Not significant	nt
43a	97%	80%

nt, not tested.
[a]In vivo screening at 25 mg/kg in mice injected with T24 NIH 3T3 cells, percentage of tumor weight inhibition.[28] Compounds were administered orally once daily starting 2 days after tumor inoculation and tumor growth inhibition was measured 17 days after administration.
[b]Percentage of parent drug remaining after 120 min incubation with human liver microsomes.[28]

Interestingly, the corresponding quinazoline **43** showed a slightly better in vitro FTI potency but was less active on GGPTase I inhibition. Again, one enantiomer **43a** proved to be more potent than the other one.

20, **24b** and **43a** were in the same range of activity than R115777. This prompted us to perform preliminary in vivo experiments on these three compounds (Table 4).

The compounds **20** and **24b** did not show any significant inhibition of tumor growth when given orally at a dose of 25 mg/kg a day. However **43a** demonstrated improved in vivo antitumoral activity in murine xenografts and was also slightly more stable to metabolism by human microsomes than was R115777. It also showed selectivity for FPT over GGPTase I. Our attention is now focused on further evaluation of this compound.

We have examined a number of azoloquinolines and -quinazolines for inhibition of FPT in vitro and we have identified compounds that are in the same range of potency as R115777. Moreover, the tetrazolo[1,5-*a*]quinazoline template offers a promising avenue towards potent FTIs with improved in vivo potency.

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References and Notes

1. Rodenhuis, S. *Semin. Cancer Biol.* **1992**, *3*, 241.
2. Bos, J. L. *Cancer Res.* **1989**, *49*, 4682.
3. Barbacid, M. *Ann. Rev. Biochem.* **1987**, *56*, 779.
4. Casey, P. J.; Solski, P. A.; Der, C. J.; Buss, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 8323.
5. Kato, K.; Cox, A. D.; Hisaka, M. M.; Graham, S. M.; Buss, J. E.; Der, C. J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6403.
6. Der, C. J.; Cox, A. D. *Cancer Cells* **1991**, *3*, 331.

7. Seabra, M. C.; Reiss, Y.; Casey, P. L.; Brown, M. S.; Goldstein, J. L. *Cell* **1991**, *65*, 429.
8. Rowinsky, E. K.; Windle, J. J.; Von Hoff, D. D. *J. Clin. Oncol.* **1999**, *17*, 3631.
9. Rowinsky, E. K.; Patnaik, A. *Emerg. Drugs* **2000**, *5*, 161.
10. End, D. W. *Invest. New Drugs* **1999**, *17*, 241.
11. Johnston, S. *Lancet Oncol.* **2001**, *2*, 18.
12. Lebowitz, P. F.; Casey, P. J.; Prendergast, G. C.; Thissen, J. A. *J. Biol. Chem.* **1997**, *272*, 15591.
13. Feldkamp, M. M.; Lau, N.; Rak, J.; Kerbel, R. S.; Guha, A. *Int. J. Cancer* **1999**, *81*, 118.
14. Ashar, H. R.; James, L.; Gray, K.; Carr, D.; Black, S.; Armstrong, L.; Bishop, W. R.; Kirschmeier, P. *J. Biol. Chem.* **2000**, *275*, 30451.
15. Adnane, J.; Bizouarn, F. A.; Chen, Z.; Ohkanda, J.; Hamilton, A. D.; Munoz-Antonia, T.; Sebti, S. M. *Oncogene* **2000**, *19*, 5525.
16. Gibbs, R. A. *Curr. Opin. Drug Discov. Dev.* **2000**, *5*, 585.
17. Gibbs, R. A.; Zahn, T. J.; Sebolt-Leopold, J. S. *Curr. Med. Chem.* **2001**, *8*, 1437.
18. Karp, J. E.; Kaufmann, S. H.; Adjei, A. A.; Lancet, J. E.; Wright, J. J.; End, D. W. *Curr. Opin. Oncol.* **2001**, *13*, 470.
19. Tamanoi, F.; Kato-Stankiewicz, J.; Jiang, C.; Machado, I.; Thapar, N. *J. Cell. Biochem.* **2001**, *37*, 64.
20. Haluska, P.; Dy, G. K.; Adjei, A. A. *Eur. J. Cancer* **2002**, *38*, 1685.
21. Ohkanda, J.; Knowles, D. B.; Blaskovich, M. A.; Sebti, S. M.; Hamilton, A. D. *Cur. Top. Med. Chem.* **2002**, *2*, 303.
22. Ghobrial, I. M.; Adjei, A. A. *Hematol. Oncol. Clin. N. Am.* **2002**, *16*, 1065.
23. End, D. W.; Smets, G.; Todd, A. V.; Applegate, T. L.; Fuery, C. J.; Angibaud, P.; Venet, M.; Sanz, G.; Poignet, H.; Skrzat, S.; Devine, A.; Wouters, W.; Bowden, C. *Cancer Res.* **2001**, *61*, 131.
24. Norman, P. *Curr. Opin. Investing. Drugs* **2002**, *3*, 313.
25. Venet, M. G.; Angibaud, P. R.; Muller, P.; Sanz, G. C. W. O patent 97/21701, 1997.
26. Venet, M. *Abstract of papers*, 222nd National Meeting of the American Chemical Society, Chicago, IL, Aug. 26–30, 2001; American Chemical Society: Washington, DC, 2001; 69BUZP.
27. Venet, M.; End, D. W.; Angibaud, P. *Cur. Top. Med. Chem.* **2003**, *3*, 1095.
28. Angibaud, P.; Bourdrez, X.; Devine, A.; End, D. W.; Freyne, E.; Ligny, Y.; Muller, P.; Mannens, G.; Pilatte, I.; Poncelet, V.; Skrzat, S.; Smets, G.; Van Dun, J.; Van Remoortere, P.; Venet, M.; Wouters, W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1543.
29. (a) Angibaud, P. R.; Venet, M. G.; Boudrez, X. M. W. O. patent 00/39082, 2000. (b) Venet, M. G.; Angibaud, P. R.; End, D. W. O patent 01/98302, 2001.
30. **24** (4 g) was separated into its enantiomers by column chromatography over Chiralcel OD® (eluent: EtOH 100%). Two pure fractions were collected and the solvent was evaporated. Fraction 1 was converted into the ethanedioic salt (1:1) and crystallized from ethanol. The precipitate was filtered off and dried, yielding 1.59 g (34%) of (A)-5-(3-chlorophenyl)-α-(4-chlorophenyl)-α-(1-methyl-1*H*-imidazol-5-yl)-tetrazolo[1,5-*a*]quinoline-7-methanamine ethanedioate (1:1) **24a** (ee > 99% measured by HPLC). Fraction 2 was converted into the ethanedioic salt (1:1) and crystallized from ethanol. The precipitate was filtered off and dried, yielding 1.85 g (39%) of (B)-5-(3-chlorophenyl)-α-(4-chlorophenyl)-α-(1-methyl-1*H*-imidazol-5-yl)-tetrazolo[1,5-*a*]quinoline-7-methanamine ethanedioate (1:1) **24b**. This compound contains 3% of the (A) enantiomer as measured by HPLC.
31. **43** (7.5 g) was separated into its enantiomers and purified by column chromatography over Chiralpak AD® (eluent: hexane/EtOH 50/50; 15–35 μm). The pure first (A) fractions were collected and the solvent was evaporated yielding 3.3 g of residue which was crystallized from CH_3CN/DIPE. The precipitate was filtered off and dried, yielding 2.55 g of (−)-5-(3-chlorophenyl)-α-(4-chlorophenyl)-α-(1-methyl-1*H*-imidazol-5-yl)-tetrazolo[1,5-*a*]quinazoline-7-methanamine **43a**. This compound contains less than 0.5% of the (+) enantiomer as measured by HPLC. The second (B) fractions were collected and evaporated yielding 3.3 g of residue which was crystallized from CH_3CN/DIPE. The precipitate was filtered off and dried yielding 2.6 g of (+)-5-(3-chlorophenyl)-α-(4-chlorophenyl)-α-(1-methyl-1*H*-imidazol-5-yl)-tetrazolo[1,5-*a*]quinazoline-7-methanamine **43b**. This compound contains 4% of the (−) enantiomer as measured by HPLC.