Towards a New Role for Chemical Development in Drug Discovery and Development: Examples from GSK Verona

Tino Rossi

Preclinical Drug Discovery

GlaxoSmithKline S.p.A., Verona

Key Deliverables for Chemical Development

- Supply the quantities of Drug Substance required to support Preclinical and Clinical studies
  - on time!
  - right quality!
- Deliver a robust process for industrial manufacturing of Drug Substance
  - cost of manufacturing
  - reproducible quality
  - solid form and particle size control

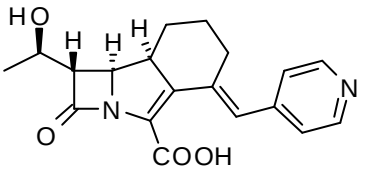
these two objectives are often in conflict!

Early Identification of Final Route

- Pros
  - Efficient and streamlined Drug Development Process
    - minimises number of studies required to Registration
    - reduces risks during technology transfer to manufacturing sites
    - early assessment of risks related to costs of manufacturing and solid form control
- Cons
  - Slow down of the Drug Development process in the early phases
  - Resource intensive/ineffective
    - focus on reduced number of Drug Candidates
    - 80-90% of the selected candidates do not reach the marketplace

Example 1: GV143253

GV143253



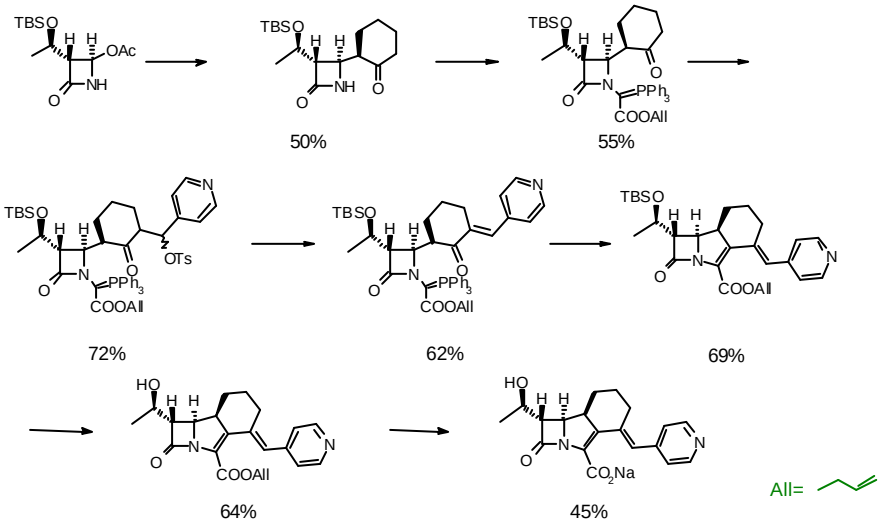
- Active against Gram positive pathogens including
  - MRSA/E, van-R Enterococci and PRSP
- Active against relevant Gram negative pathogens
  - *H.influenzae, K.pneumoniae*
- Stable to the most relevant β-lactamases
- Effective in several animal infection models
  - (septicaemia, thigh infection, endocarditis, abscess)
- Safety profile in animals superior to Vancomycin

Objectives for Chemical Development

- Preparation of ca. 350 g of Drug Substance
  - when: a.s.a.p.!!!!
  - purity ≥97%
- Develop a route suitable for industrial manufacturing of GV143253
  - economical
  - short
  - robust
  - reproducible

Expected requirements 10-30 tonnes per year

GV143253: Med. Chem. Route

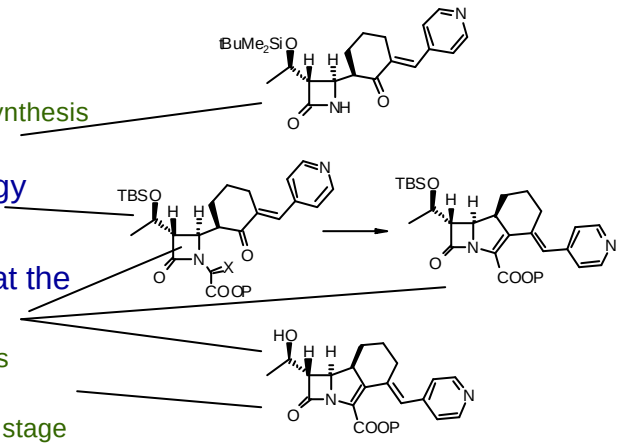


12 Chemical transformations, 5 Chromatographic purifications

Tot yield 2.4%

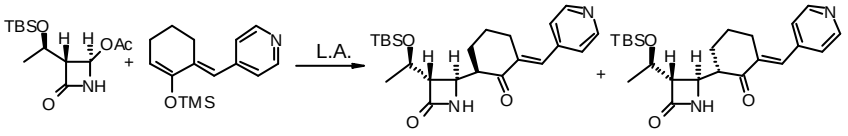
Issues to be Addressed

- Shorter and more convergent route
  - Stereoselective synthesis of
- Cyclisation strategy
- Protecting group at the carboxyl moiety
  - solid intermediates
  - avoid soluble Pd complexes at last stage



Cost of Goods issue: target >30% th. overall yield.

Convergent Route

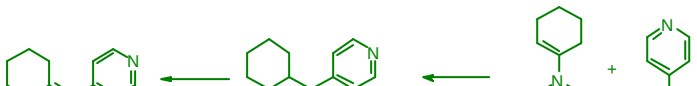


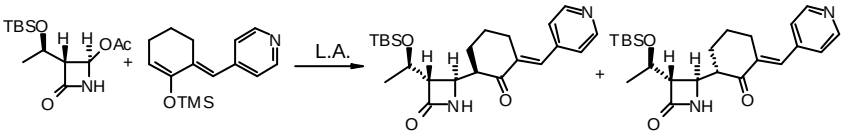
1 **2** **3** **4**

Entry	L.A.	Solvent	T (°C)	Sol. yield	Ratio 3/4
1	SnCl4?Et2O	Toluene	-20	25%	1/2.5
2	GaCl3?½DIPEA	CH_2Cl_2	0	27%	1/3
3	TMSOTf	CH_2Cl_2	-15	40%	1
4	TMSOTf	CH_3CN	-15	54%	1

other Lewis acids: $TiCl_4$, $BiCl_3$, $MgBr_2$; $ZnCl_2$, $FeCl_3$, LiCl, $BF_3 \cdot Et_2O$, InCl3, CeCl3, $Yb(OTf)_3$, $Sn(OTf)_2$, $SbCl_5$, Bu_2BOTf, Et_2AlCl did not give detectable amounts of reaction products

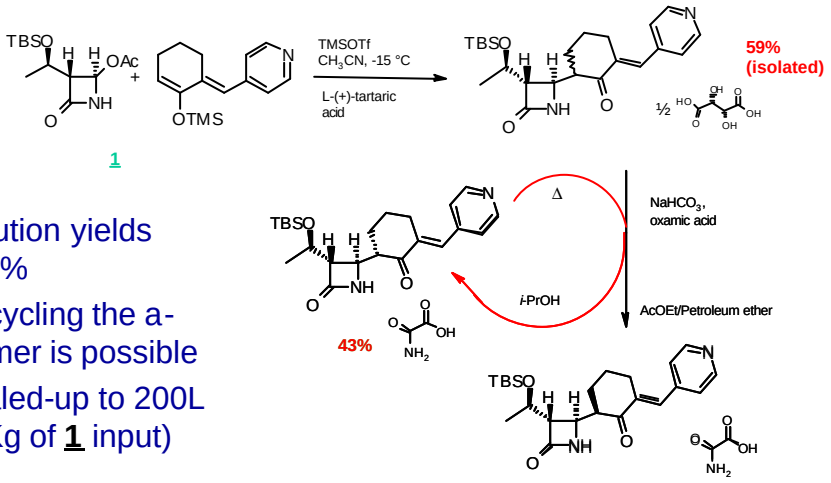
Convergent Route





1 **2** **3** **4**

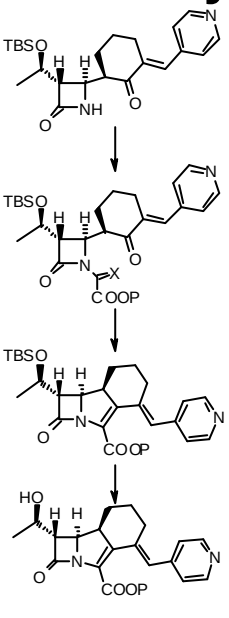
Isolation of ß-Isomer



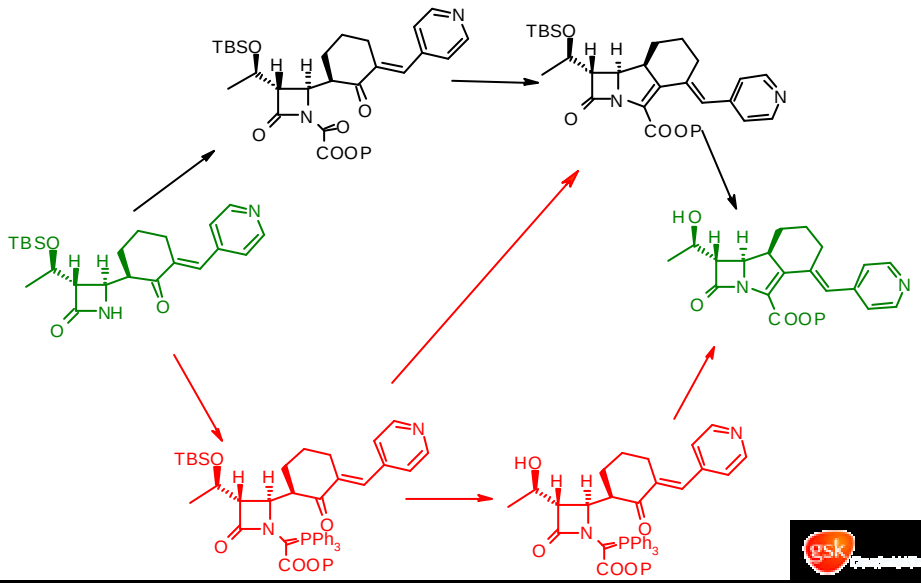
- Solution yields >70%
- Recycling the a-isomer is possible
- Scaled-up to 200L (5 Kg of **1** input)

Choice of the Ester Moiety

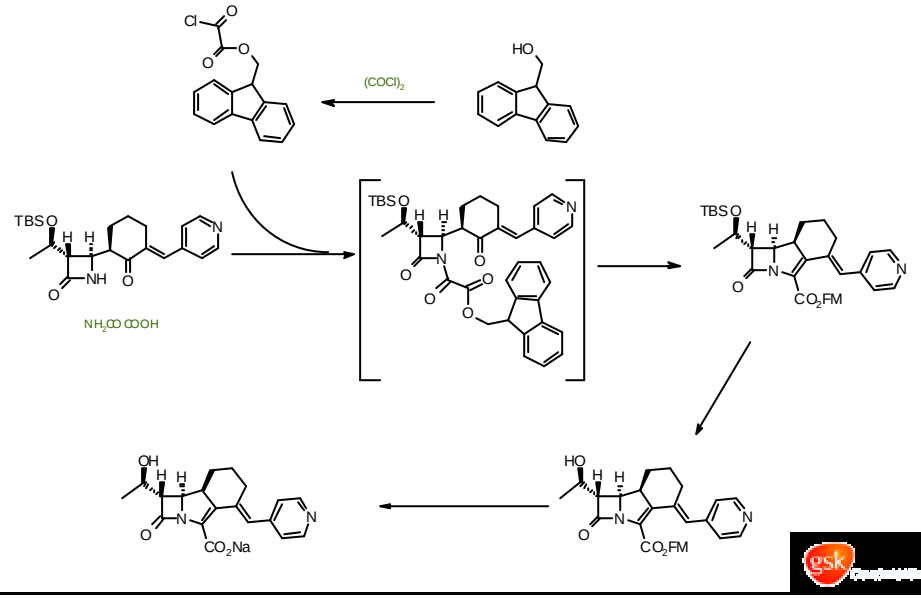
- Yields solid intermediates
  - allows control of batch to batch purity and homogeneity
- Does not affect the cyclisation process in terms of yield
- Infers stability to the structure
- Can be easily removed
  - high yields
  - mild conditions
  - robust reaction conditions
  - reproducible impurity profile
- Cost



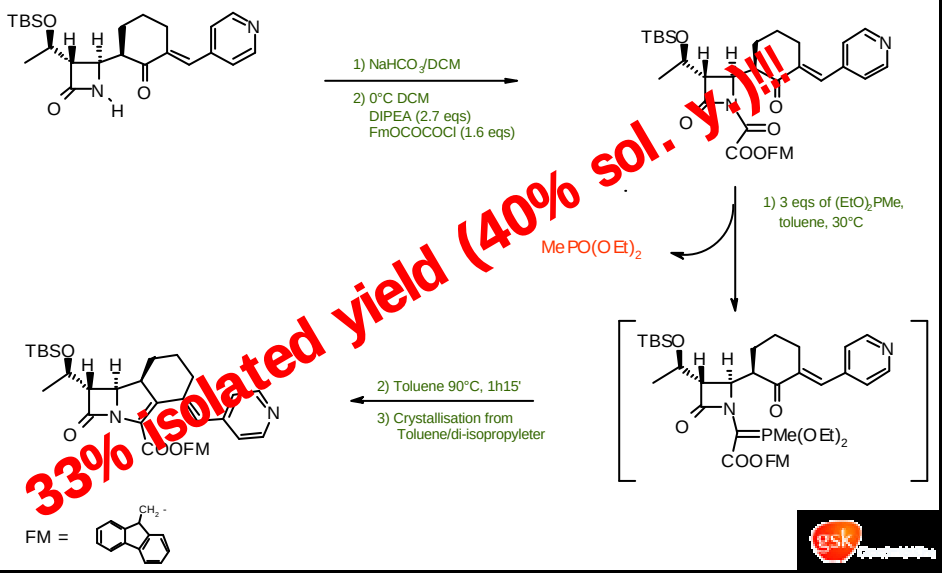
Ciclisation Strategy



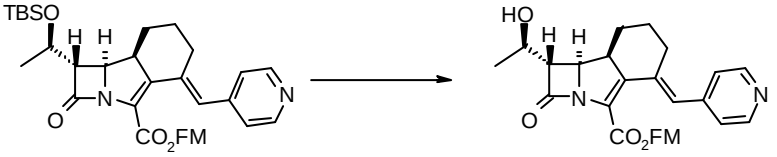
Fluorenyl Methyl Ester



Stage 3 Optimization



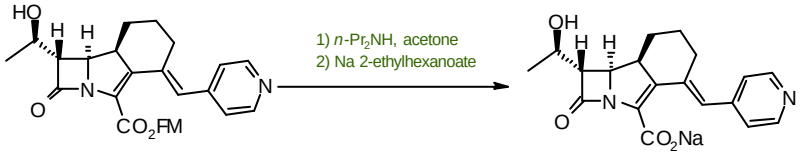
Silyl Protecting Group Removal



78%

- Several methods available
  - TBAF, AcoH
  - TBABr, CsF(KF), AcOH
  - $Et_3N{\cdot}3HF$, $Et(i\text{-}Pr)_2N{\cdot}3HF$
- $Et(i\text{-}Pr)_2N{\cdot}3HF$ selected after initial screening
- Statistical approach to process optimisation
  - Solvent, conc., T, eq. of $Et(i\text{-}Pr)_2N{\cdot}3HF$
  - DOE approach coupled with lab automation (DART)
- Optimisation of work-up and crystallisation

GV143253 formation

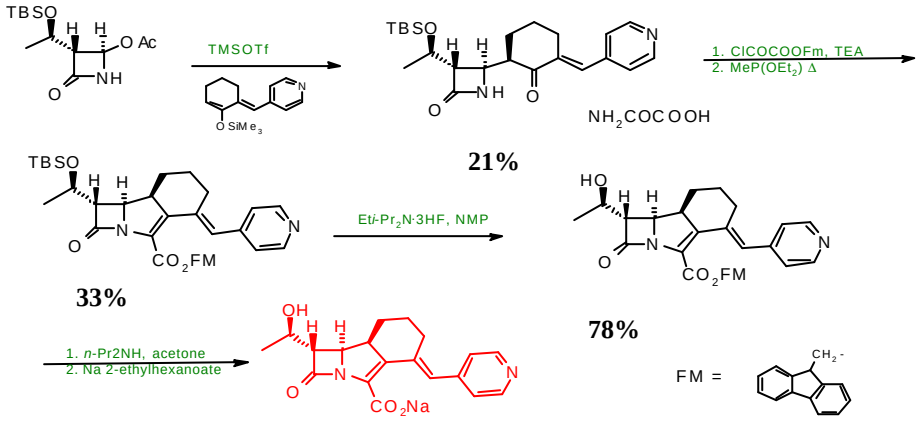


76%

- In excess of 30 amines screened
  - *n*-Pr_2NH chosen
- Selection of Solvent critical
  - In acetone GV143253 ammonium salts precipitates out and does not completely convert into sodium salt (observed on >5L scale)
  - Efficiency of stirring key to success

Suboptimal method selected for manufacturing

GV143253a in Pilot Plant



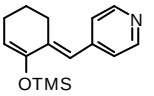
Overall yield 3.96%th, 5.0%w/w

Timelines

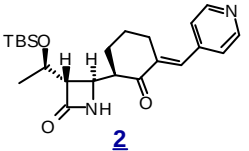
- Drug Substance for Preclinical studies made available 12 months after candidate selection

Critical analysis of the strategy followed

- A new process for the manufacture of silylenolether **1** and recycling of the α-isomer of ketoazetidinone to the desired β-isomer **2** had to be put in place
- β-ketoazetidinone **3** available from Sanfetrinem synthesis on multikilo scale
- a new cyclization procedure not strictly required



1



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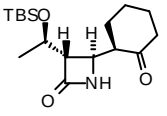

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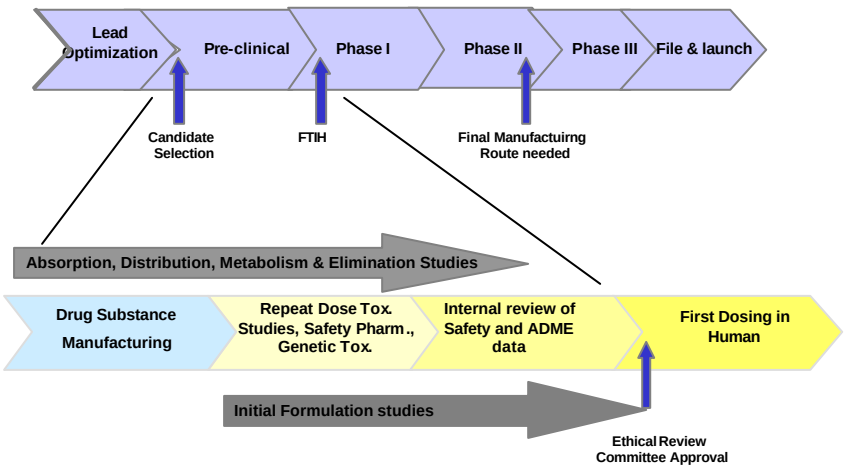
Drug Discovery Is A Complex Process

Our Experience in Drug Discovery and Development

- The Medicinal Chemistry route used for the initial synthesis of a Candidate is very rarely coincident with the final manufacturing route
  - but most of the times can be suitable for scale-up with some adjustments
- Relatively low amount of Drug Substance is required to support the early development of a Candidate (500-5000g)
- 80-90% of Candidates do not reach the market
- Final manufacturing route is needed to support long term preclinical studies (e.g. oncogenicity) and advanced (Phase III) clinical trials



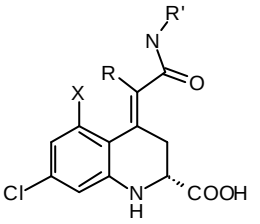
Novel Technologies Can Shorten Cycle Times

- Automation
  - Automated synthesizers coupled with high speed HPLC, LC-MS, LC-NMR; TLC
- Design Of Experiments
  - factorial design applied to process optimisation
- Continuous Flow Reactors
- Novel Purification Methods
  - Simulated Moving Bed Chromatography
- Solid Form and Particle Size design and control
  - continuous flow crystallisation
  - supercritical fluid crystallisation

Example 2:
Glycine Antagonists and GW 468816

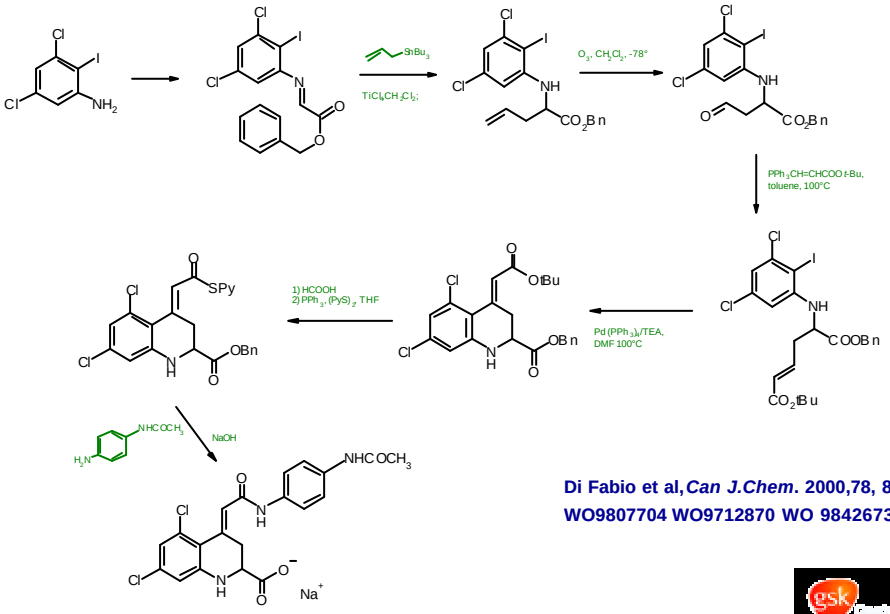
Tetrahydro Quinolines: A New Series of Glycine Antagonists

- Tetrahydro Quinolines a novel class of glycine antagonists
  - pain
  - migraine
  - drug depedency
- Biological activity concentrated in one single enantiomer



X= Cl, H

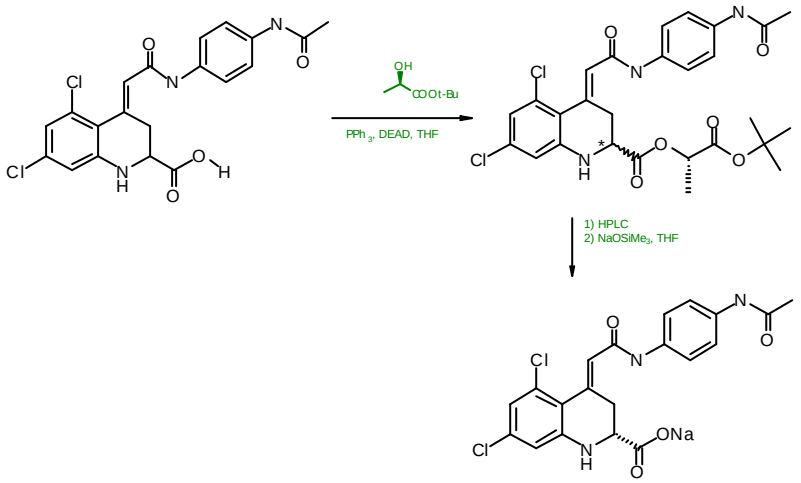
Med. Chem. Route to Initial Lead



Di Fabio et al, *Can J.Chem*. 2000,78, 809

WO9807704 WO9712870 WO 9842673

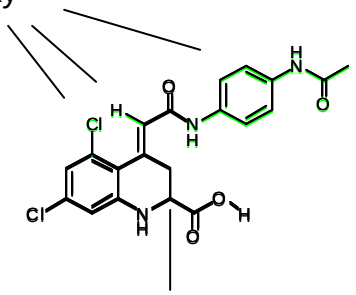
Use of *t*-Butyl(*R*)-(+)-lactate as Resolving Agent



Tranquillini, Bertani et al. XVIth International Symp on Med Chem

Early Involvement of Chem. Dev.: Issues and Opportunities

focus on route assessment and scale up or on advanced intermediates too risky

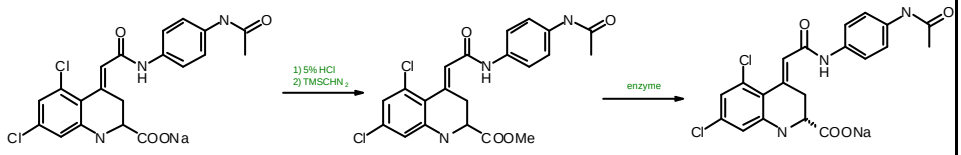


investigation on better methods for enantiomer separation couldresult in a more widely applicable process

Screening Approach ALTUSChiroScreen-EHkit

- 18 reactions vials each containing a different esterase, lipase or acylase
- a magnetic stirrer bar
- a convenient removable foam holder

Screening Approach ALTUS
ChiroScreen-EHkit

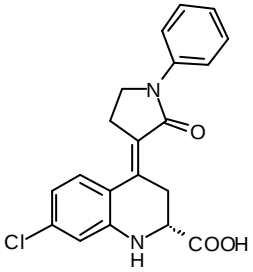


- A solution of ester in DMF the enzyme in the reaction solution
- The vials were stirred at room temperature and followed by HPLC at set intervals of 1.5h, 4.5h, 8h and 24h, making an estimate of the conversion to calculate the enantioselectivity

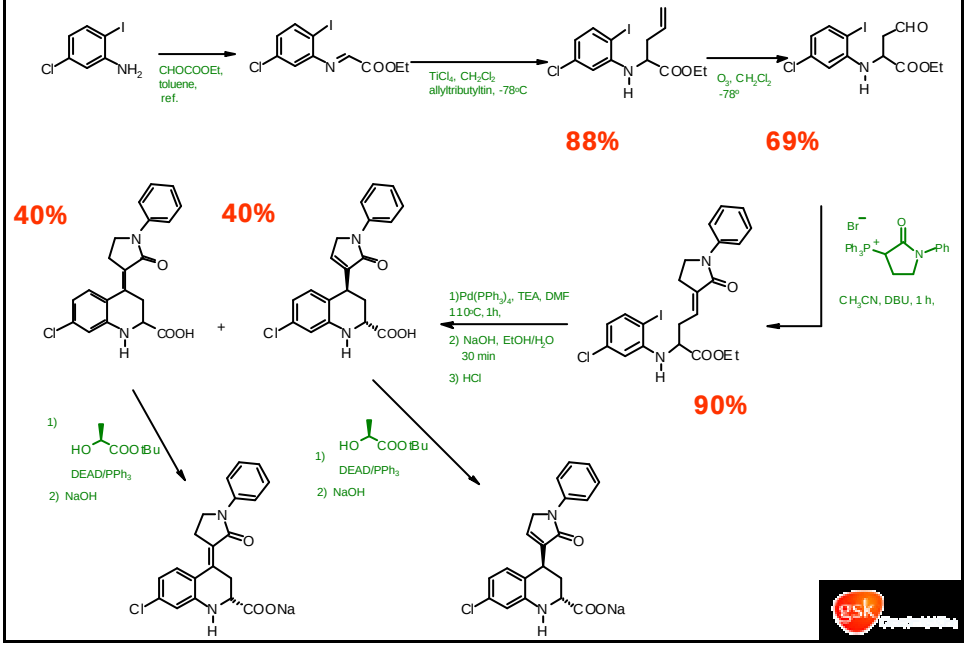
ChiroScreen-EH Result Summary

Catalyst name	% Product at 1.5h	% Product at 4.5h	% Product at 8h	% Product at 24h	Product ee at 24h*	
PorcineLiver esterase	50	55	60	80	0	
Candida rugosa lipase	5	8	9	20	40	
α-Chymotripsin	2	4	5	8	95	←
Aspergillus niger lipase	0	2	3	8	96	←
Mucor meihei lipase	0	2	3	8	0	
Chiro CLEC-CR (slurry)	0	2	8	8	34	
Subtilisin Carlsberg	0	3	4	8	95	←
Humicola lanuginosa lipase	0	0	1	2	60	
Bacillus species protease	7	6	20	30	80	
Chiro CLEC-BL (slurry)	4	3	3	4	95	←

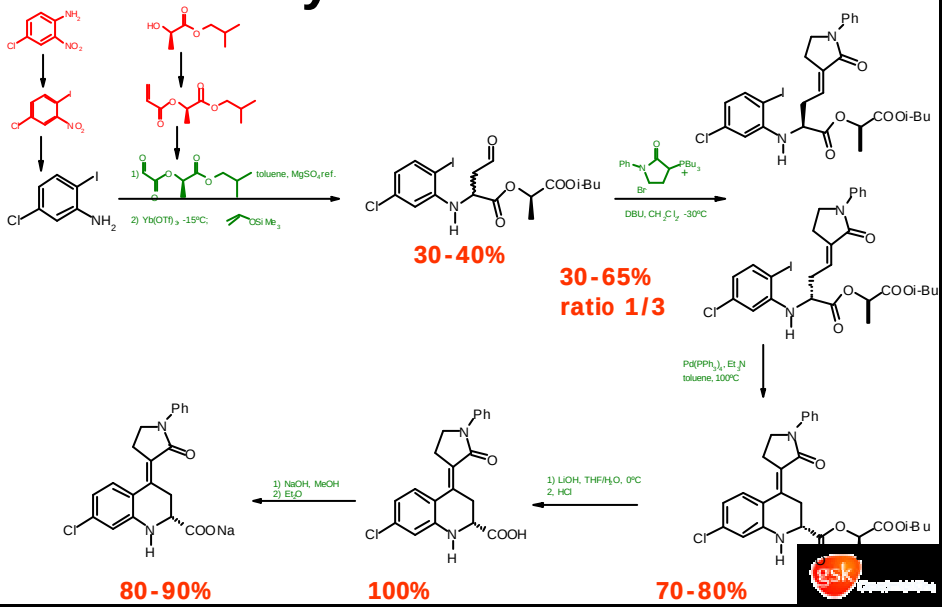
GW468816



Original Medicinal Chemistry Route

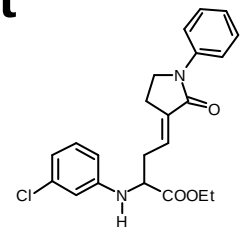


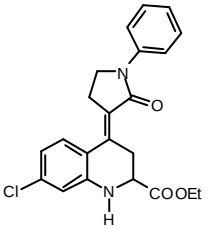
Advanced Medicinal Chemistry Route to GW468816



Initial Assessment

- Intermediates ethyl esters are solid, could be isolated by crystallisation
- ethylglioxylate commercially available
- the Medicinal Chemistry route could be reproduced on a lab scale on the ethyl ester analogue

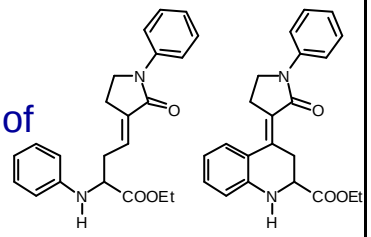


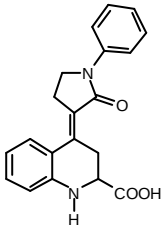


How could we obtain the desired enantiomer?

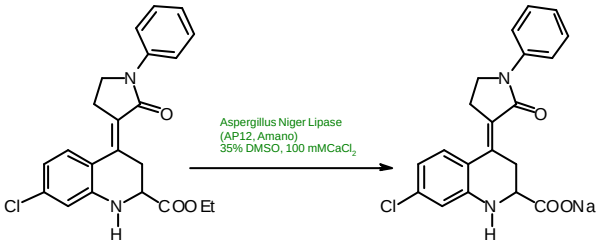
Separation of enantiomers

- chromatographic separation of enantiomers.
- enzymatic hydrolisis
- crystallisation of GW468816 with a resolving agent



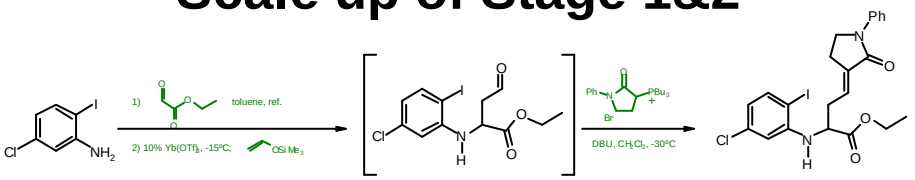


Payback from Previous investment

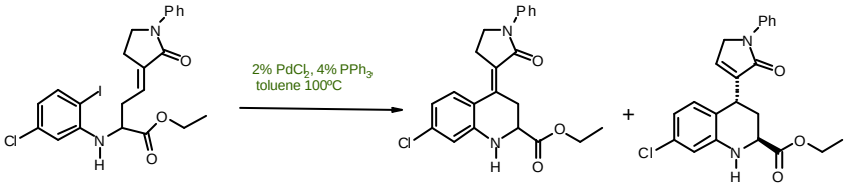


- 48% conversion, 40% yield (80% th.)
- *A. Niger* Lipase commercially available however large quantities required (ca 1.4 wt)
- Solvent mixture compromise between the solubility of starting material and reaction efficiency

Scale-up of Stage 1&2



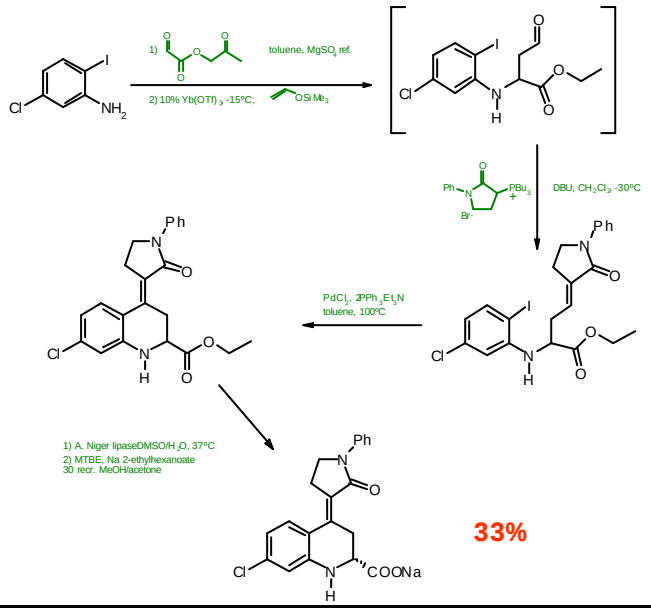
34%



ratio 10/1

75%
(Isolated)

Initial Manufacturing Route



75%

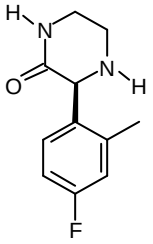
35%

33%

Timelines

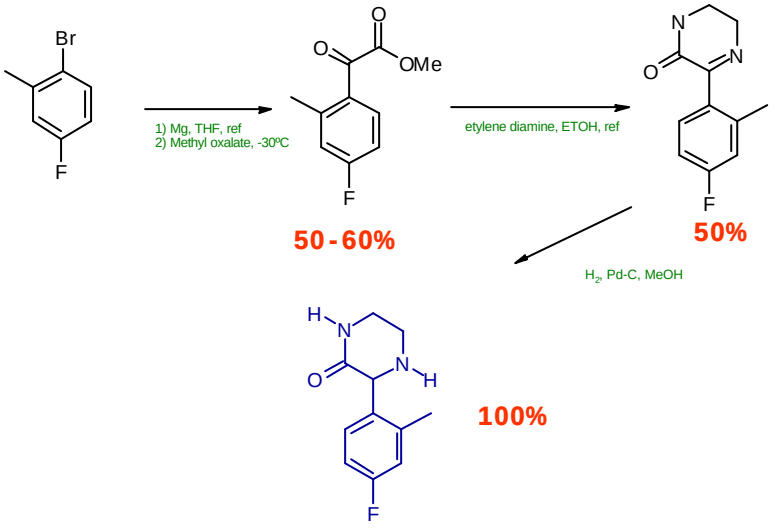
- Amounts to support the preclinical studies to First Time in Human (FTIH) produced within 4 months after selection of GW468186 as Development candidate

Example 3: Impact of Early Involvement of Chem Dev in Lead Opimization Projects



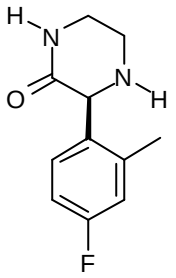
- Advanced Intermediate to a very important Project
- Med Chem could produce it in gram scale as racemate only
- Final active products single enantiomers produced in 8-12 chemical steps in <10 mg scale
  - Pure enantiomers obtained via chemical reaction with a resolving agent followed by HPLC or flash chromatography separation and deprotection

Med. Chem. Route to Ketopiperazine

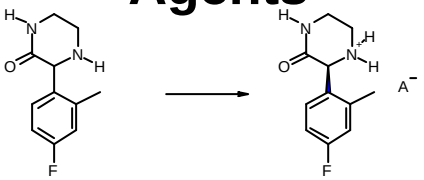


Chemical Development Objectives

- Identify a scaleable method to produce ketopyperazine intermediate in high (>98%) ee
  - support the Medicinal Chemistry strategy
  - Advanced intermediate available in case a candidate from the piperazine Lead series was identified

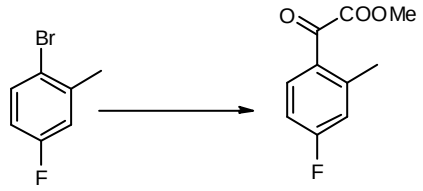


Initial Screen for Resolving Agents



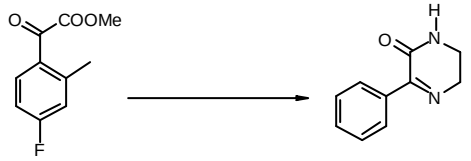
- (*R*)-camphorsulphonic, L-(+)-tartaric, (-)-di-O,O'-*p*-tolyl-L-tartaric, L-(+)-mandelic acid screened
- AcOEt, MeOH, EtOH, *i*-PrOH, *t*-BuOMe and their mixtures used
- L-(+)-mandelic in ethyl acetate gave a 86% ee at the initial screen
  - conditions optimized to 98% ee

Step 1



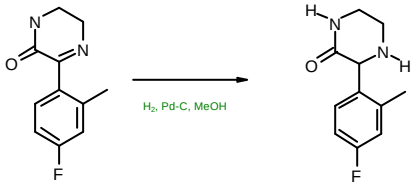
- Methyl oxalate
  - eq. of oxalate reduced from 3 to 1
  - reaction temperature from -30 to -50°C

Reaction product is not isolated and can be progressed to next stage



- no change introduced
- flash chromatography replaced with a simple filtration on a silica pad

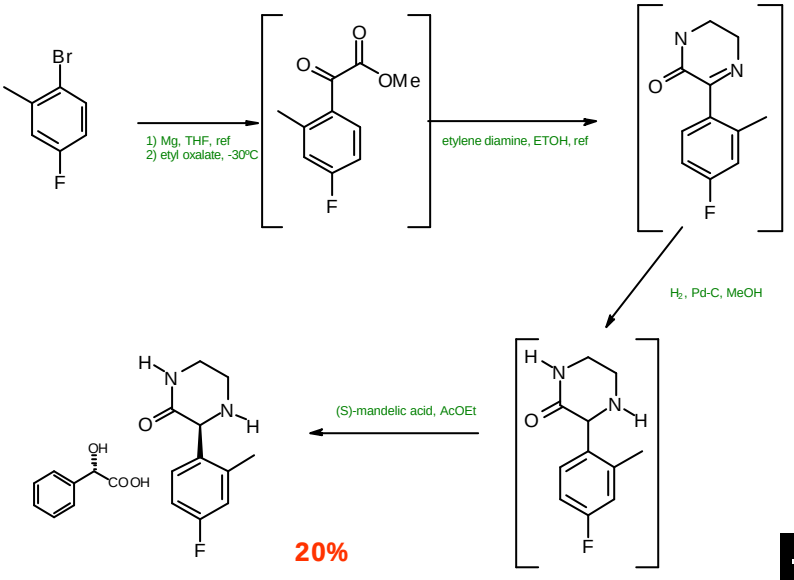
Intermediate not isolated and reaction mixture brought forward to next stage



- No change

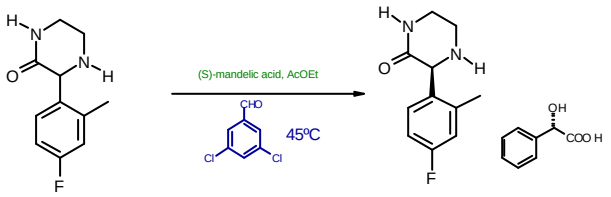
Intermediate not isolated and reaction mixture brought forward to next stage

Final Process



20%

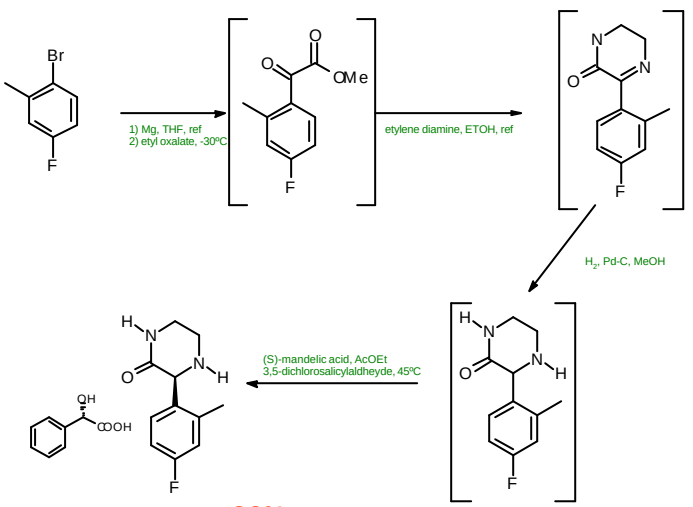
The Ice on the Cake!



73%

- dynamic deracemization catalized by 3,5-dichlorosalicylaldehyde

The Refined Process



32%

Impact on the Project

- Medicinal Chemistry
  - Productivity increase
    - from 10 new compounds per month (racemate) to 100 (single enantiomers)
    - from <10 mg per compound to >>100 mg.
- Drug Discovery process
  - efficiency gain
    - ca. 250 g of drug substance available <3 months after candidate selection