Structure-guided design for carbazole antagonists of toll-like receptors 7 and 8 (TLR7/8)

ACS MEDI Session: Diversity, Equity, and Inclusion in Medicinal Chemistry
August 23, 2022

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TLR 7/8 Antagonists for Treatment of Autoimmune Disorders

TLRs: pattern recognition receptors of the innate immune system

- Recognize signals of infection or cellular stress:
  - Pathogen-associated molecular patterns (PAMPs)
  - Damage-associated molecular patterns (DAMPs)
- TLR activation leads to the activation of pro-inflammatory pathways

Endosomal TLRs recognize nucleic acids

- TLR7/8 recognize single-stranded RNA from pathogens or tissue damage
- TLR3 recognizes dsRNA
- TLR9 recognizes dsDNA

Overactivation of TLRs has been linked to human autoimmunity

- TLR7/8 overactivation has been identified in lupus patients
- TLR7 implicated in lupus via mouse models
- TLR7 activation leads to type-I IFNs, anti-nuclear antibody production
- TLR8 activation leads to cytokine production, e.g. IL-6, IL-10, TNFα

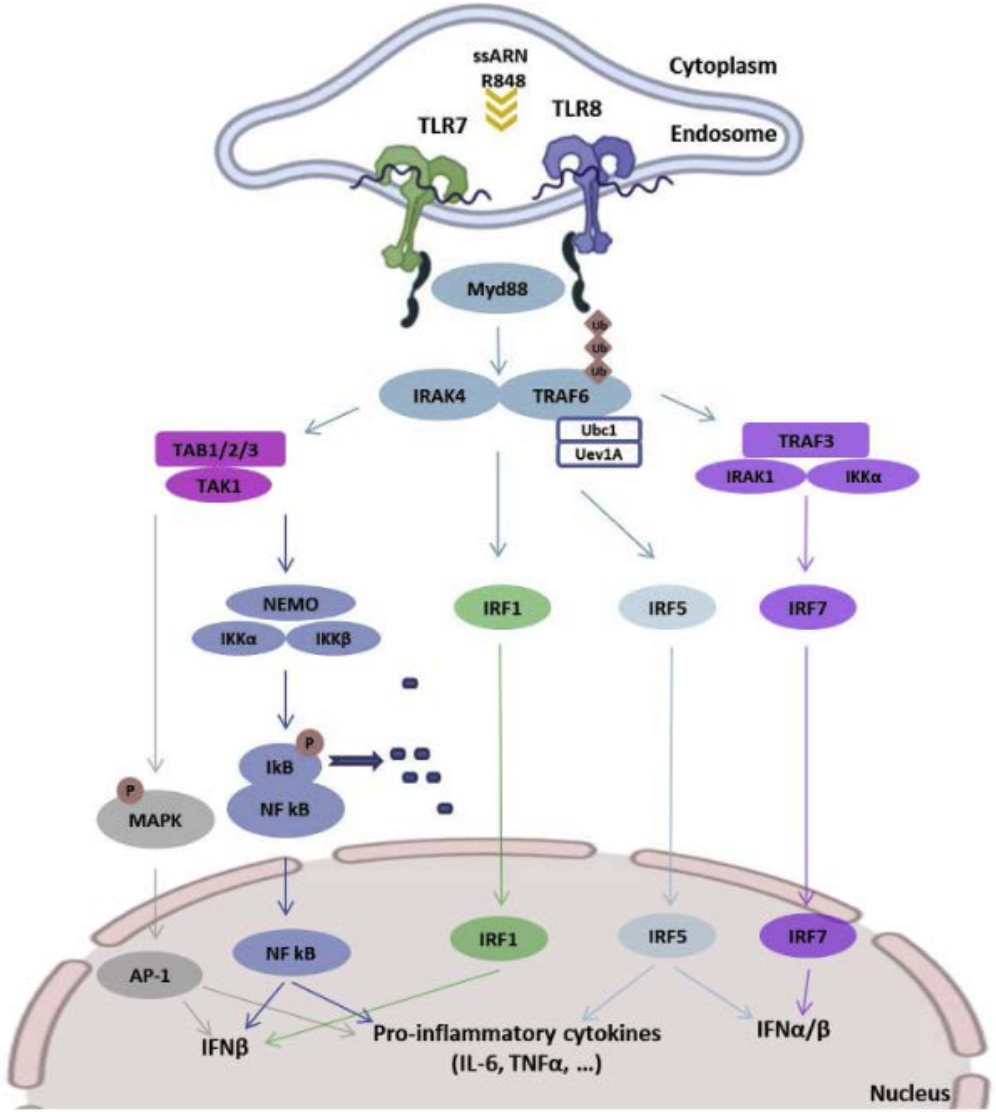


Image from *European Journal of Medicinal Chemistry*, 2020, 193, 112238

Select reviews on TLRs and autoimmunity:
(1) *Nat. Rev. Immunol.* 2006, 6, 823 - 835. (2) *Ann. Rev. Immunol.* 2007, 25, 419 - 441. (3) *Nat. Rev. Immunol.* 2013, 13, 453 - 460. (4) *Front. Pharmacol.* 2014, 5, 1 - 8.

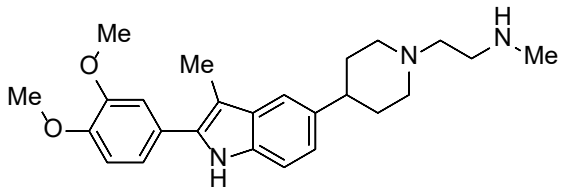
TLR 7/8 Antagonist: Evolution of lead indole series

HTS led to identification of indole 1

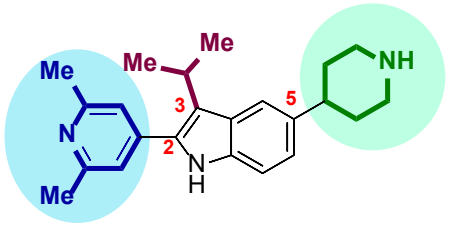
- Modest activity in TLR7/8 cellular reporter assay; selectivity against TLR3/4
- C-2, C-3, and C-5 optimization led to pyridine 2

Pyridine 2 promising in mouse models of lupus

- Liabilities include: high CNS exposure, very high V_{ss}, hERG inhibition
- Improvements in basicity and lipophilicity led to analogs like triazolopyridine (TriZP) 3

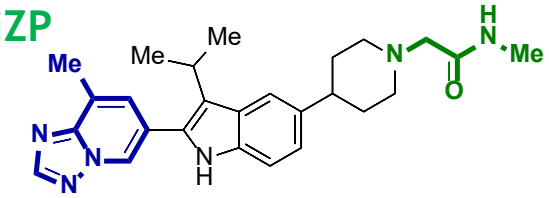


(1)
TLR7 reporter IC_{50} (nM): 290
TLR8 reporter IC_{50} (nM): 490



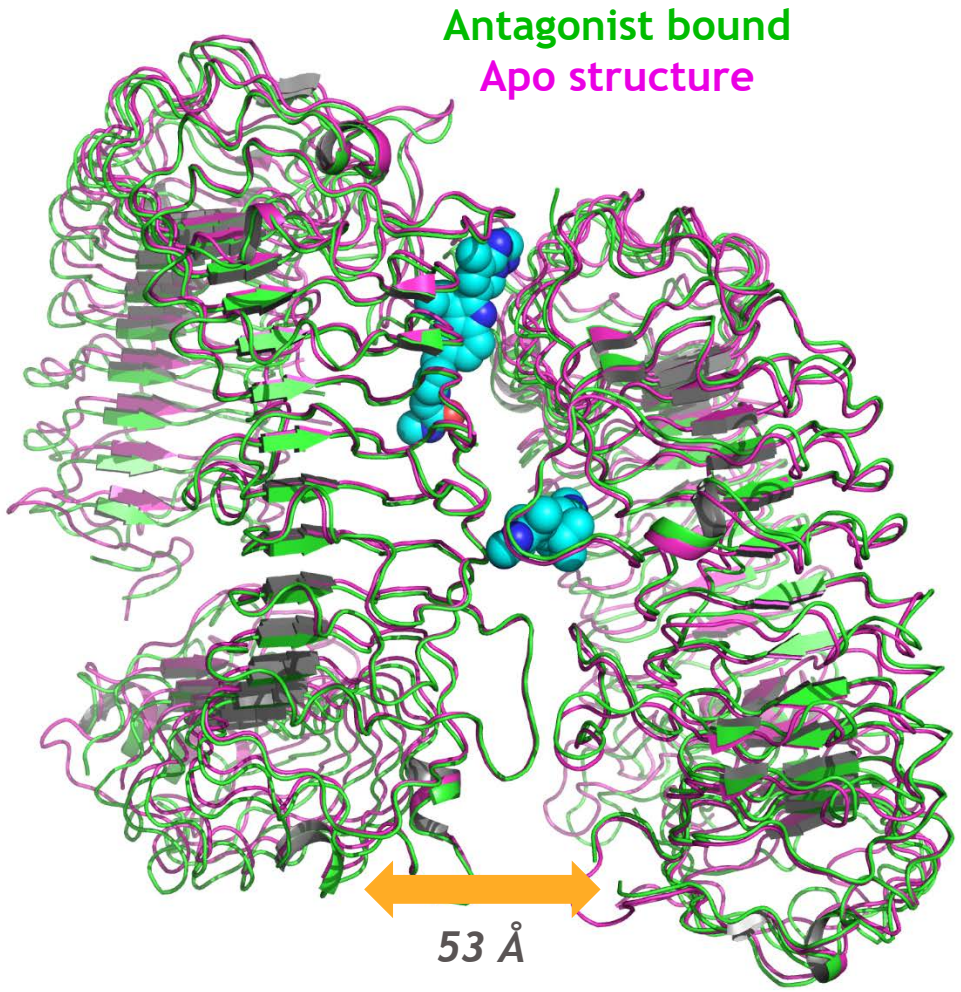
(2)
TLR7 reporter IC_{50} (nM): 0.7
TLR8 reporter IC_{50} (nM): 3.2
hWB TLR7/8 IC_{50} (nM): 1.4 / 4.2

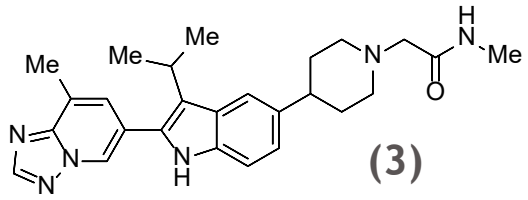
TriZP



(3)
TLR7 reporter IC_{50} (nM): 0.7
TLR8 reporter IC_{50} (nM): 4.7
hWB TLR7/8 IC_{50} (nM): 2.8 / 8.6
More balanced overall profile

X-ray Crystallography of Indole Antagonist Complexed to hTLR8

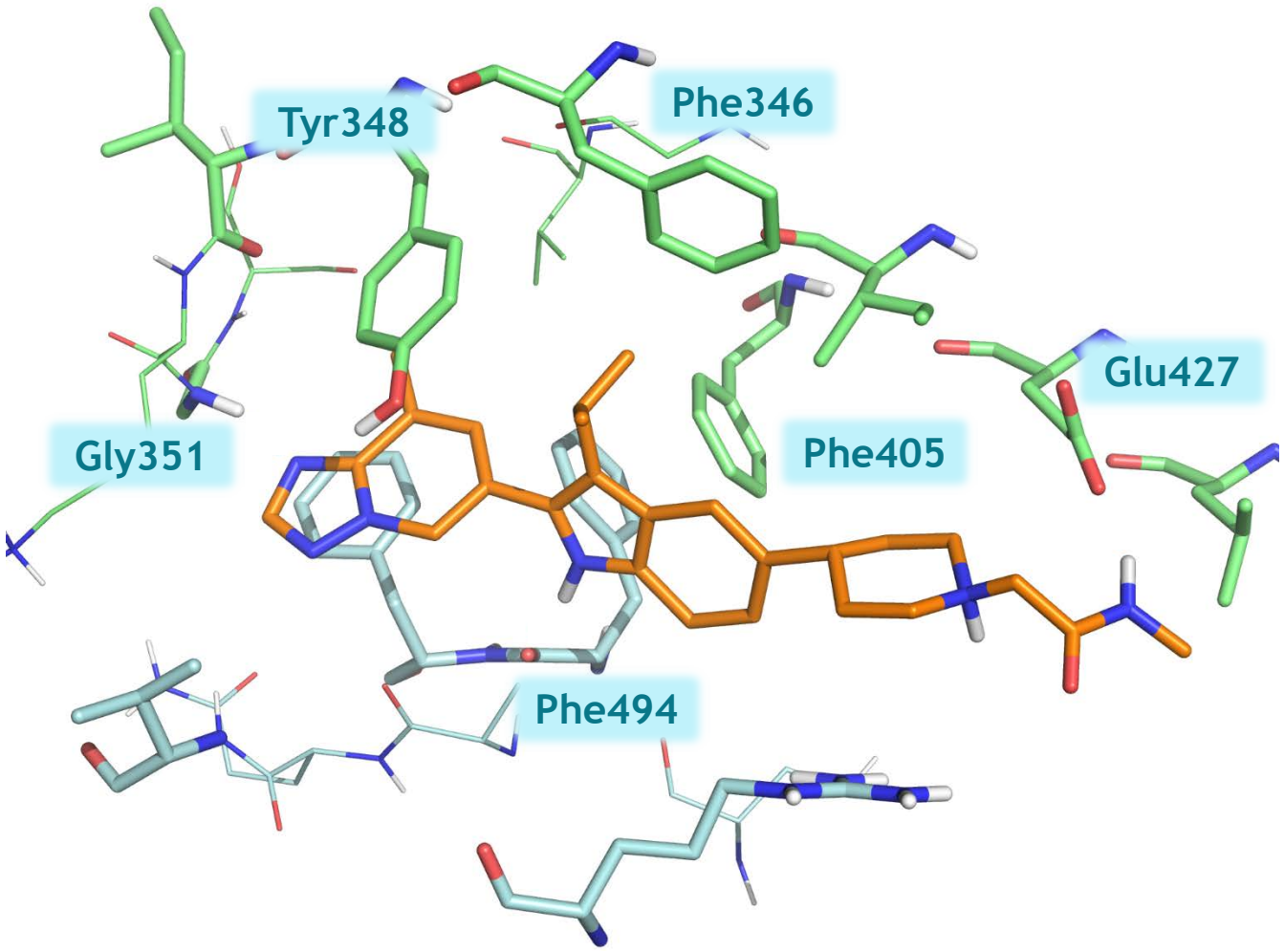


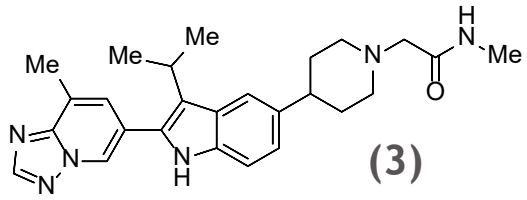


(3)

- Compound 1 binds 2:2 with hTLR8
- Locks in the Apo conformation
- Indole-based antagonists bind in agonist-binding site
- Agonist-bound structures show C-termini in closer proximity

X-ray Crystallography of Indole Antagonist Complexed to hTLR8



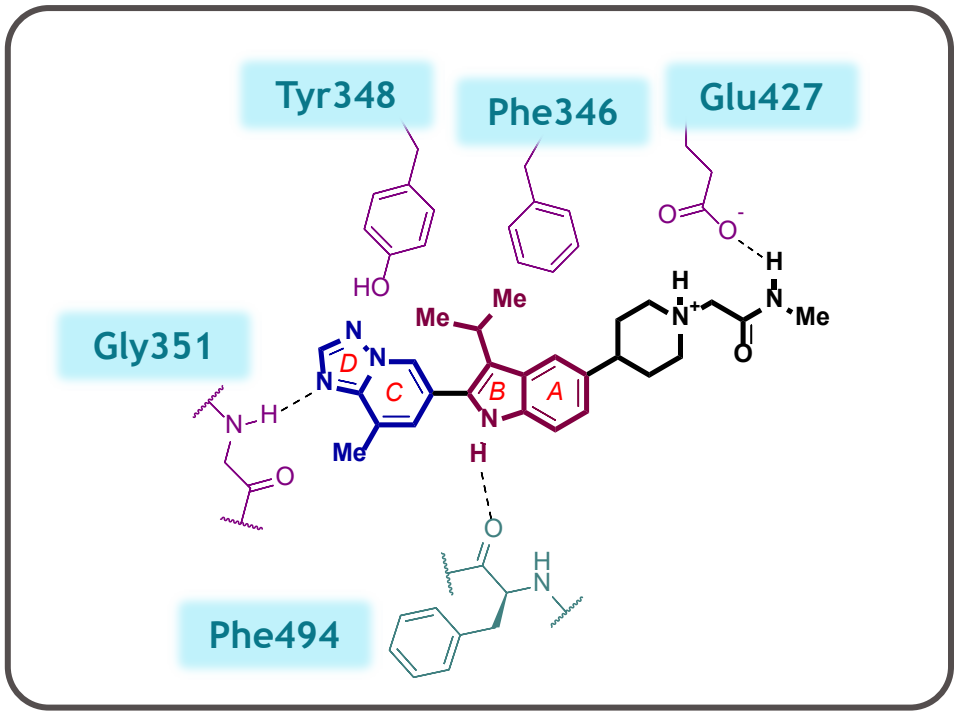


(3)

- TriZP forms H-bond with Gly351 NH
- Indole NH forms H-bond with backbone carbonyl of Phe494
- Glu427 forms H-bond with piperidine amide capping group
- C-3 iPr group fills hydrophobic pocket

Proposed evolution of indole to carbazole core

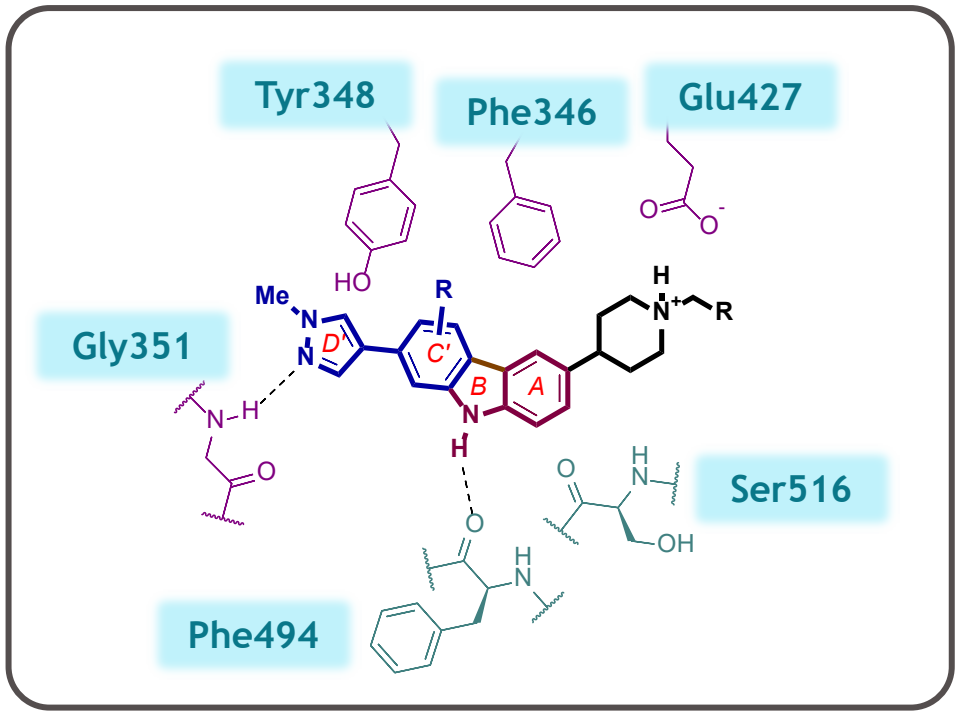
X-ray crystal structure of indole 3



Transpose "C-ring"

Core morph

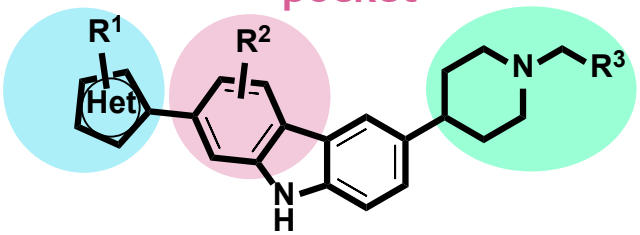
New series proposal



Carbazole divided into three regions for SAR exploration:

Heteroaryl substituent:
Gly351 H-bond

Lipophilic pocket

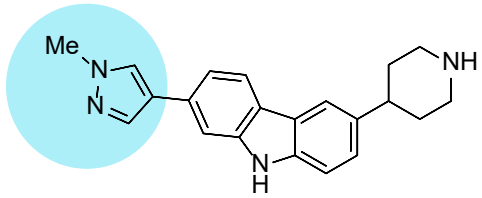


Saturated amine and *N*-capping group:
Glu427 interaction

Initial Carbazole SAR: Heteroaryl substituent

Series exploration commenced with unsubstituted core and piperidine

- Pyrazole **2** provided proof of concept for TLR7/8 antagonism with carbazole core
- Larger heteroarenes demonstrated increased potency
- Triazolopyridine (TriZP) was selected for advancement and series expansion
- X-ray crystal structure was obtained of pyridone **4** complexed human TLR8



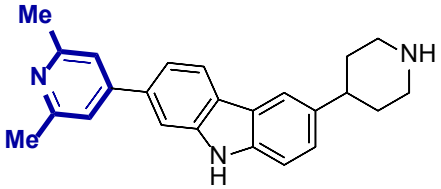
(2)

TLR7 reporter IC_{50} (nM): 1400

TLR8 reporter IC_{50} (nM): 860

hWB TLR7/8 IC_{50} (nM): >2000 / 1500

Larger P1 groups



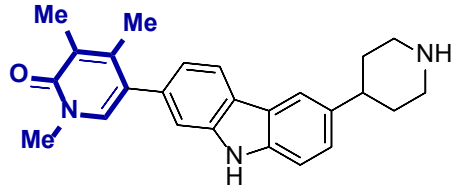
(3)

22

100

250 / 500

hTLR8 X-ray



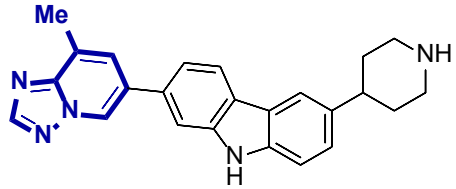
(4)

16

29

53 / 34

TriZP



(5)

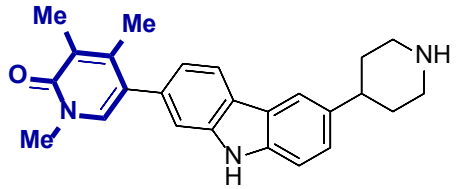
7.9

27

44 / 99

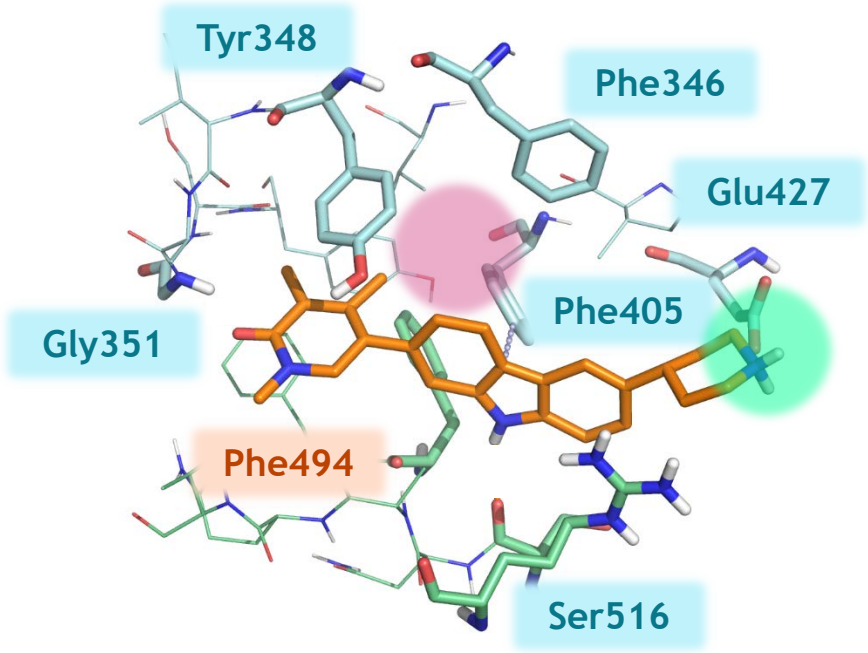
X-ray crystal structure of carbazole analog and human TLR8

X-ray structure of carbazole 4 bound to hTLR8:

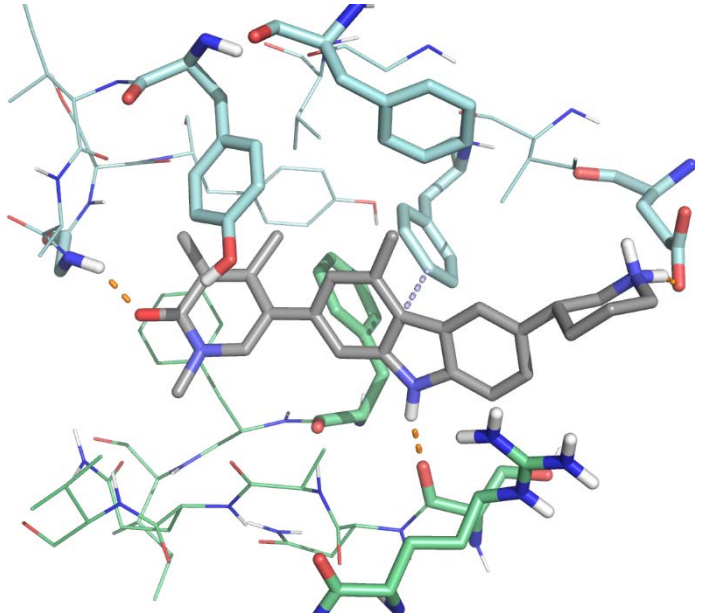


(4)

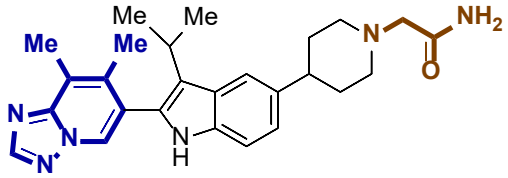
- Carbazole occupies indole binding pocket
- Core NH is shifted to engage Ser516 instead of Phe494



Modeling proposal:

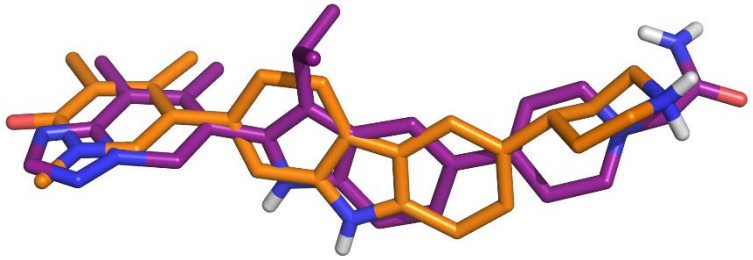


Indole with hTLR8 X-ray:

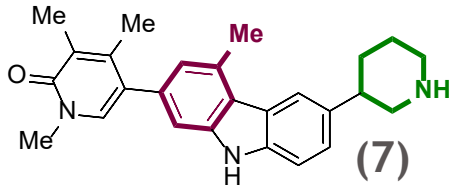


(6)

Overlay of 4 and 6 from hTLR8 X-ray structures



Proposed modifications to core and amine:

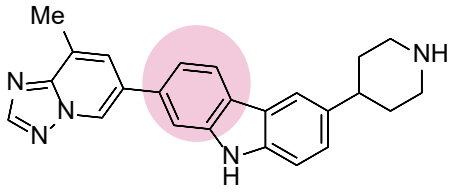


(7)

X-ray structure-based modifications

Carbazole core substitution and amine modifications explored in TriZP series

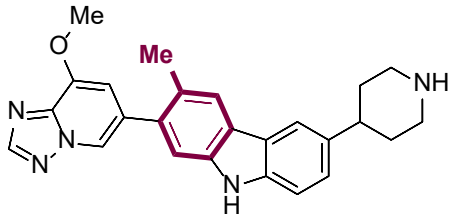
- Methyl groups on carbazole core provide enhanced potency
- Variations in saturated amine demonstrate similar potency to 4-piperidine



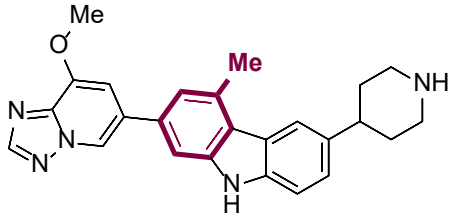
(5)

hWB TLR7/8 IC_{50} (nM): 44 / 99

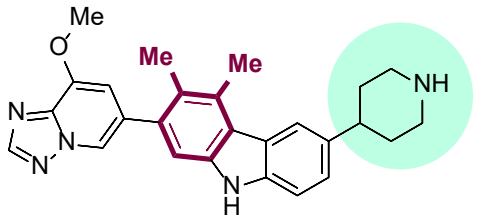
Methylation of carbazole



(8)
2.8 / 6.5

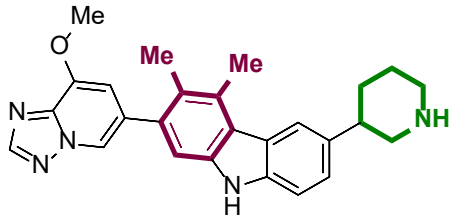


(9)
2.8 / 7.3

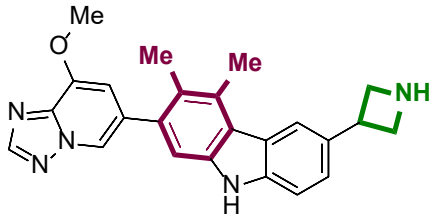


(10)
1.6 / 1.5

Amine modifications



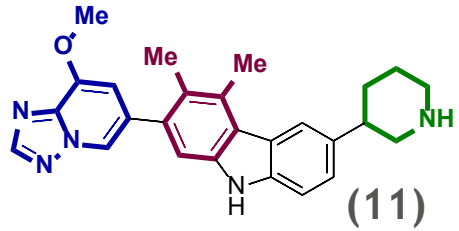
(11)
3.4 / 1.4



(12)
3.9 / 1.2

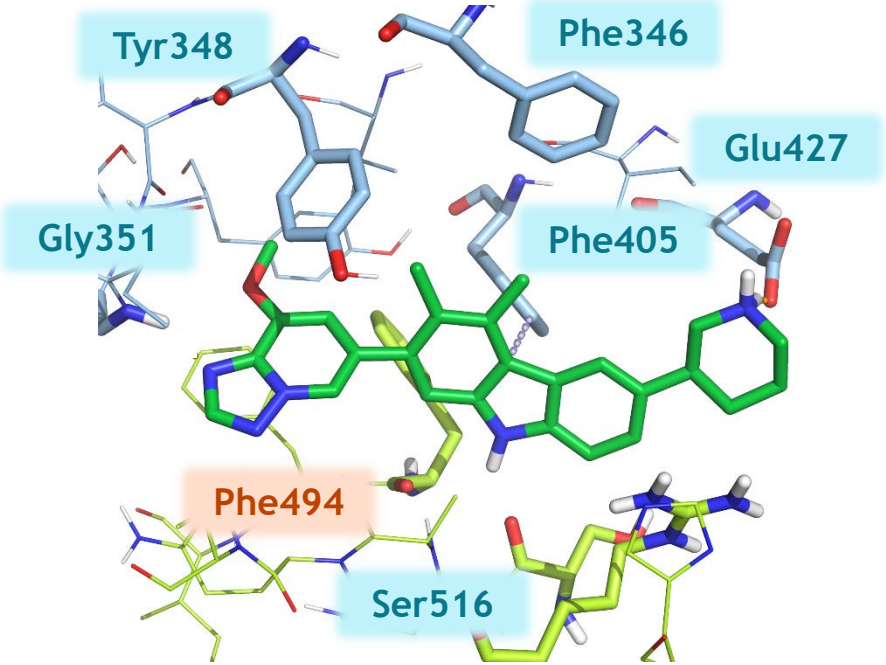
X-ray crystal structure: impact of core and amine changes

Carbazole with new design elements:

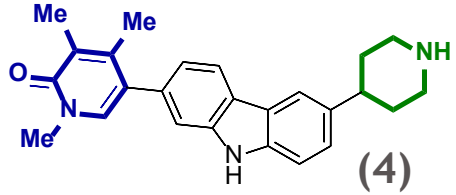


(11)

X-ray structure (S-isomer):

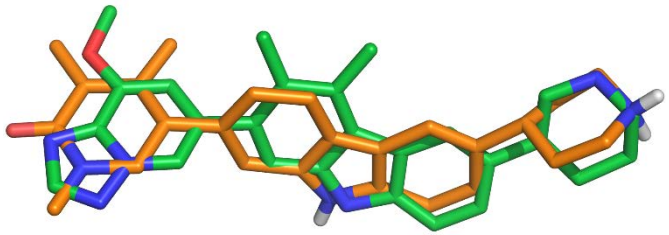


First carbazole with hTLR8 X-ray:



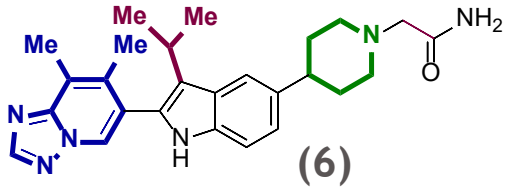
(4)

Carbazoles 4 and 11 hTLR8 X-ray overlay:



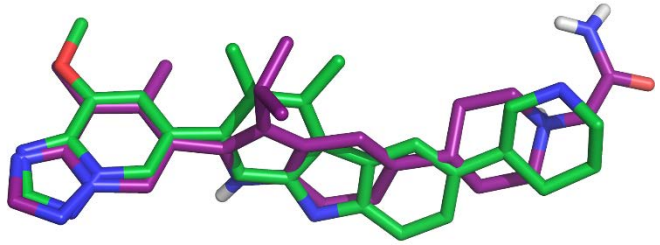
- Heteroaryl substituent and core occupy similar regions in 4 and 11
- Salt bridge with Glu427 observed in X-ray structure for 3-piperidine

Indole with hTLR8 X-ray:



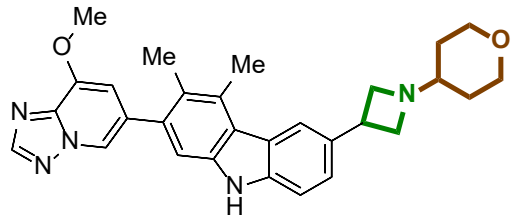
(6)

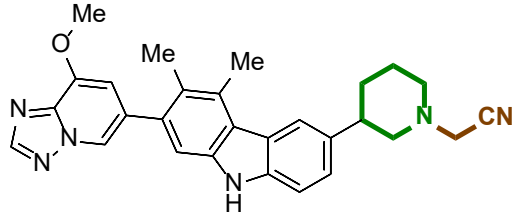
Indole 6 and carbazole 11 hTLR8 X-ray overlay:



- Dimethyl core (11) and iPr (6) occupy same pocket
- Core NH shifted in carbazole vs. indole
- 3-piperidine amine more closely overlaps with indole series amine

In vivo performance of select carbazole analogs



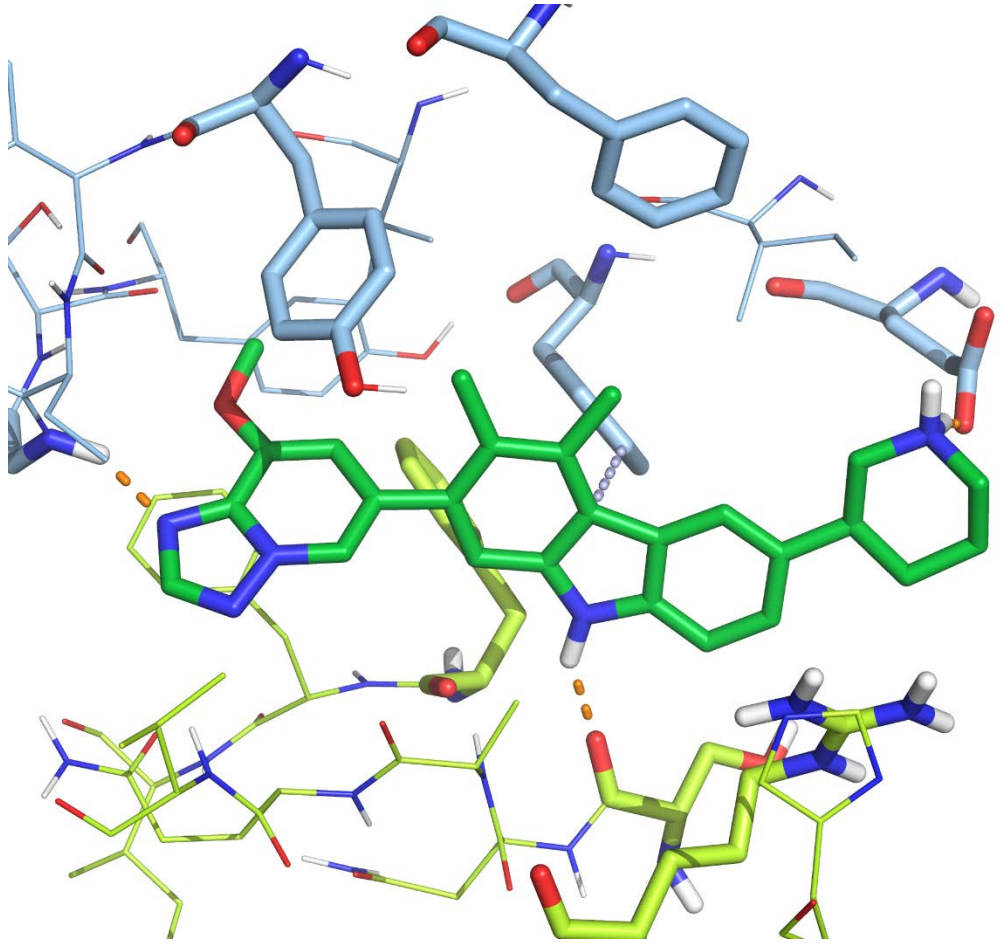


	(12)	(13)
TLR 7/8 hWB IC_{50} (nM)	15 / 2.1	16 / 7.3
Liver microsome $T_{1/2}$s (h/m) (min)	7 / 35	6 / 24
Caco-2 A->B (nm/sec), efflux ratio	79, 1.9	101, 0.5
Mouse PO PK (5 mpk): Cmax (nM) / AUC_{24h} (nM*h)	260 / ND (BLQ at 24 h)	1300 / ND (BLQ at 24 h)
TLR 7 in vitro mouse WB IC_{50} (nM)	15	65
TLR 7 in vivo (PD model) mouse WB IC_{50} (nM)	1.4	64

- Encouraging in vivo exposures as judged by Cmax observed in mouse PK (13)
- Carbazoles demonstrated good in vitro - in vivo correlation in TLR7 mouse PD model
- Future improvements would be aimed at extending duration of in vivo exposures

Conclusions

- **Carbazole chemical series was established starting from indole-based antagonists of TLR7/8**
- Carbazole antagonists of TLR7/8 maintained several key contacts displayed by indole series in hTLR8 orthosteric site
- Core NH in carbazole engaged an alternative residue for key H-bond (Ser516 vs Phe494)
- Amine repositioning in carbazole improved salt bridge interaction with Glu427 in X-ray crystal structure
- Carbazole-based antagonists of TLR7/8 demonstrated potency in mouse TLR7 PD model with good in vitro – in vivo correlation



Acknowledgements:

Chemistry (BMS*)
Alaric Dyckman
Dharmpal Dodd
Christopher Mussari
John Gilmore
Tasir Haque
Brian Whiteley
Michael Poss
David Tortolani
John Macor
Lou Lombardo
Percy Carter
Robert Borzilleri

CADD (BMS)
Shana Posy
Andy Tebben

Discovery Synthesis
Arvind Mathur
Rick Rampulla
Rulin Zhao
Bei Wang

Chemistry (BBRC*)
Sreekantha Ratna Kumar
Laxman Pasunoori
Subramanya Hegde
Anupama K.R. Reddy
D.K. Srinivas
P.V.V. Srinivas
Vikram B.
B.D.B. Raju
Rajeev Bhide
Ramakanth Sarabu

CADD (BBRC)
Ramesh Sistla

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Gary Schieven
Qihong Zhao
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Paul Davies
Julie Carman
Hongchen Qiu
Kim McIntyre
Luisa Salter-Cid

Biology (BBRC)
Shailesh Dudhgaonkar
Debarati Tagore
Manjunath Ramarao

PCO (BMS)
Stefan Ruepp
Ragini Vuppugalla
Kevin Stefanski
Qian Ruan
Ching Su
Jennifer Price
Celia D'Arienzo
Janet Caceres-Cortes
Georgia Cornelius
Zheng Yang

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Vishwanath Kurawattimath
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Ipsit Kundu
Michael Sinz
Sandhya Mandlekar
Mohan Pandey
Harinath Sale

Lead Disc. Opt. (BMS)
Mary Ellen Cvijic
Litao Zhang
Dave Harden
Ding-Ren Shen

Prot. Sciences (BMS)
Mark Witmer
Sophie Wu
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David Critton

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