Tripeptidyl-peptidase II (TPP II) Inhibitory Activity of (*S*)-2,3-Dihydro-2-(1*H*-imidazol-2-yl)-1*H*-indoles, a Systematic SAR Evaluation. Part 2

Henry J. Breslin,[a,*] Tamara A. Miskowski,[a] Michael J. Kukla,[a] Hans L. De Winter,[b]
Maria V. F. Somers,[b] Peter W. M. Roevens[b] and Robert W. Kavash[a]

[a]*Johnson & Johnson Pharmaceutical Research & Development, L.L.C., Welsh and McKean Roads, PO Box 776, Spring House, PA 19477-0776, USA*
[b]*Johnson & Johnson Pharmaceutical Research & Development, L.L.C., Turnhoutseweg 30, B-2340 Beerse, Belgium*

Abstract—We have systematically explored the structure–activity relationship (SAR) for a series of compounds **2** as inhibitors of tripeptidyl-peptidase II (TPP II), a serine protease responsible for the degradation of cholecystokinin-8 (CCK-8). This SAR evaluation of the core structure **2** suggest a fairly restrictive pharmacophore for such related structures, but has yielded a limited set of compounds (**2b**, **2c**, **2d**, **2s**, and **2t**) with potent TPP II inhibitory activity (IC_{50} 4-11 nM).
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As of 1998, 18% of the US population was defined as being obese,[1] which is a serious concern since obesity has been correlated as a causative contributor to numerous diseases and disorders.[2] The endogenous octa-peptide cholecystokinin-8 (CCK-8), as well as synthetic CCK agonists, exhibit pro-satiety effects, suggesting that CCK or related agents might prove useful in helping to address the obesity problem.[3] In 1996 Rose et al. identified Tripeptidyl-peptidase II (TPP II; EC 3.4.14.10) as an endogenous serine protease responsible for the degradation of CCK-8, and also identified butabindide, **1**, as a potent inhibitor of TPP II (IC_{50} = 7 nM).[4] Subsequently, Rose and coworkers demonstrated that treatment of mice with **1** resulted in a 50% reduced food intake over a 1-h period for treated animals relative to control animals,[4] which is consistent with the expected result if endogenous levels of CCK-8 were elevated.

1 (Butabindide)

*Corresponding author. Fax: +1-215-628-4985; e-mail: hbreslin@prdus.jnj.com
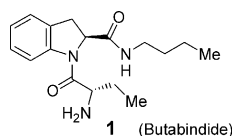
Recently we reported that **1** possesses some inherent chemical instability, with a propensity to intra-molecularly cyclize to yield butyl amine and **1**'s respective diketopiperazine (IC_{50} > 10 μM).[5] Based on this finding, we subsequently prepared a small set of compounds **2** in which an imidazole served as a bio-isostere equivalent for **1**'s labile amide moiety.

b R=Ala; X,R[I],R[III]=H; R[II]=n-Pr; n=1
c R=Abu; X,R[I],R[III]=H; R[II]=n-Pr; n=1
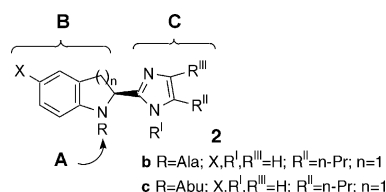
Imidazole **2c** (IC_{50} = 4 nM) proved equally potent to **1** as a TPP II inhibitor while being favorably void of the intra-molecular cyclization liability of **1**.[5] Our initially reported efforts included preparing only closely related derivatives of **1**, as a primary test of our hypothesis that compounds such as **2** may prove promising. Based on the favorable results for our initial analogues **2**, we have prepared additional heterocyclic analogues to more thoroughly evaluate the TPP II inhibitory structure–activity relationships (SARs). Below is described our related biological findings, as well as associated chemical synthesis.

To initiate our SAR work, we retro-synthetically evaluated our generic structure **2**. The resulting segmentation of the parent compound at good synthetic junction points led us to trisect the structure, which we arbitrarily labeled A–C, as outlined above for **2**.

Segment A variations of parent structure **2** proved generally the most simplistic and expedient to alter, relative to segments B and C. Changes explored in segment A were generally one to three steps from common intermediate noramines **5d** and **5f**, whose preparations and chemical characterizations we previously described in detail.[5] As shown in Scheme 1, we reacted secondary amines **5d** and/or **5f**, or their respective racemic mixtures **5e** and/or **5g**, under standard amide coupling conditions with generated mixed anhydrides of various commercially available *N*-BOC protected amino acids. Resulting amino acid adducts were simply *N*-deprotected to yield desired products **2a–i**, **2o**, and **2p**. Alternatively, noramine **5g** was reacted under standard Schotten–Baumann conditions with acid chlorides **6a**, **6b**, and **6c** to give products **2j**, **2k**, and **2m**, respectively. Anilines **2l** and **2n** were prepared via standard room temperature PtO$_2$/H$_2$ hydrogenation conditions from these respective nitro precursors **2k** and **2m**. Product **2q** was generated from **2p** under reductive diborane·THF conditions in refluxing THF. Full chemical characterizations (MS, ^{1}H NMR, TLC, LC) were completed for this set of final targets, as well as those described below for Segments B and C, and are consistent with expectations based on comparisons to related analogues previously described in detail.[5]

For segment B variations, we began by preparing a handful of 5-substituted indolines in lieu of our previously reported un-substituted indoline analogues.[5] As shown in Scheme 1, the synthesis followed to prepare the new 5-substituted indolines **2r–v** was virtually identical to our previously reported eight step route for the unsubstituted indolines,[5] with the simple modification of starting from 5-substituted starting material indolines **4a–c**. The 5-substituted indolines **4a–c** were prepared following a previously reported Mg/MeOH reduction of their respective indoles **3a–c**.[6] Indolines **4a–c** led to

racemic products **2r**, **2s**, and **2u** when Gly was used in the amide coupling step of the synthetic sequence. When L-Ala was used in the amide coupling step with **5b** and **5c**, mixtures of epimers were generated, which were *N*-BOC de-protected as mixtures with TFA. The resulting desired final products **2t** and **2v** were easily isolated as pure compounds from their respective epimers via preparative HPLC. The absolute configurations for **2t** and **2v** were assigned based on comparison of their NMRs to the unsubstituted indoline **2p**. Compound **2p**, and its respective epimer, were prepared via stereospecific syntheses.[5] For a cursory look at a different scaffold in place of the indoline scaffold of Segment B, we slightly altered the indoline synthesis to generate tetrahydroquinolines. As shown in Scheme 2, we formed our imidazole ring adjacent to the aromatic quinoline and then reduced **8** to its respective tetrahydroquinoline intermediate **9**. Noramine **9** was then coupled with *N*-BOC-L-Ala-F[7] **10**, and the resulting epimers **11a** and **11b** were separated by preparative HPLC. Subsequent *N*-BOC de-protection of these purified intermediates yielded epimers **2w** and **2x**. Absolute assignment of configurations for these two products was not pursued since these compounds lacked significant biological activity.
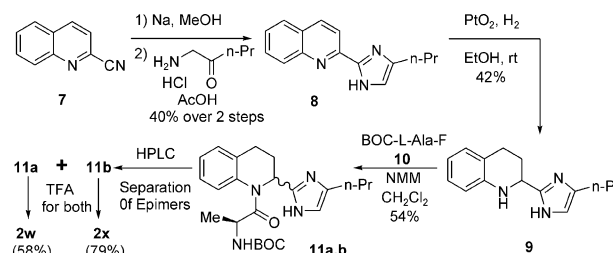
Scheme 2.

Work around segment C included substitutions on and for the imidazole heterocycle. As shown in Scheme 3, a simple iodomethane *N*-substitution reaction on imidazole intermediate **12**[5] yielded both regioisomers **13** and **14**. These regioisomer intermediates were separated via preparative HPLC and their absolute stereochemistries assigned based on comparison with similar *N*-sub-
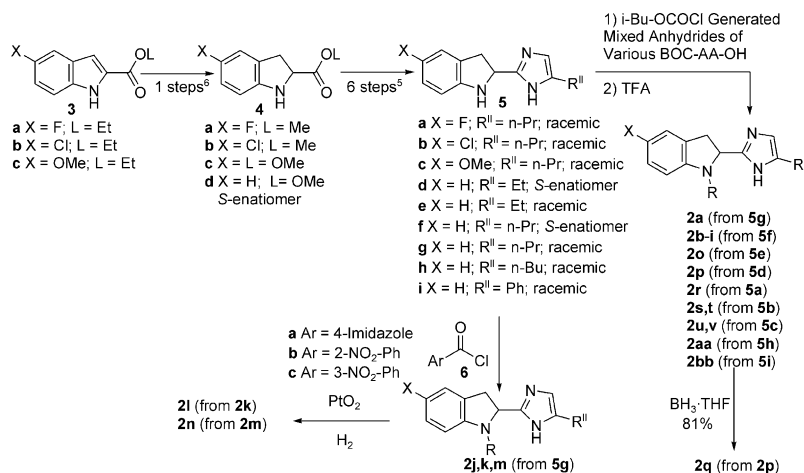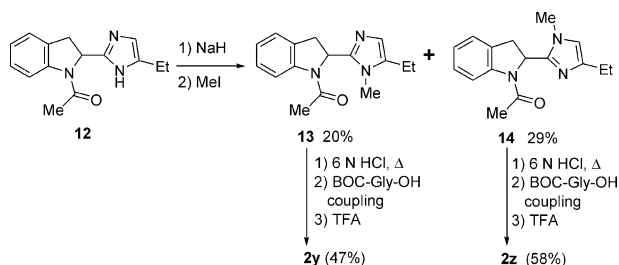
Scheme 1.

stituted imidazoles which have been well characterized.[8] Intermediates **13** and **14** were then independently hydrolyzed with refluxing 6 N aqueous HCl to their respective noramines, which were carried on to their final targets **2y** and **2z** following similar reaction conditions described above for segment A analogues. Trisubstituted imidazole analogues **2cc–2hh** were prepared by an alternate synthetic route, as depicted in Scheme 4. Indoline alcohol **15**[9] was oxidized to aldehyde **16**, which was subsequently subjected to Radziszewski reaction conditions[10] with diketones **17a** and **17b**[11] to yield respective imidazoles **18a** and **18b**. The BOC groups of **18a** and **18b** were removed with TFA, generating noramines **19a** and **19b**, which were coupled with BOC-Gly-F.[7] The resulting amides **20a** and **20b** were then treated with TFA to yield desired products **2cc** and **2dd**. Related analogues **2ee** and **2ff** were prepared following the same Scheme, after appropriate manipulation of ester **18b**. This entailed hydrolyzing ester **18b** with 1 N KOH in MeOH and subjecting the resulting acid **18c** to standard amide coupling conditions. The resulting 4-substituted amide-imidazoles **18d** were then carried on through their respective intermediates **19** and **20** to generate products **2ee** and **2ff**. Scheme 5 outlines the preparation of benzimidazole noramine **24**, the intermediate employed to prepare final targets **2gg** and **2hh**. Noramine **24** was subjected to coupling and deprotecting reactions similar to those described for analogues shown in Scheme 1. Also outlined in Scheme 5 is the preparation of some other heterocycles we prepared in lieu of an imidazole.

Related intermediates **26**, **27**, and **29** were hydrolyzed to their respective noramines, then also coupled and deprotected in a similar manner as described for the

imidazoles of Scheme 1, ultimately yielding final targets **2ii**, **2jj**, and **2kk**, respectively.

The related TPP II biological results for the diverse set of compounds prepared are summarized in Tables 1 and 2.[12] Evaluation of the SAR for analogues **2a–2f**, where the amino acid α appendage in segment A was the sole variation, suggest that limited lipophilicity is desirable to enhance activities [H (**2a**; IC_{50} 36 nM) < Me (**2b**; IC_{50} 6 nM) ∼ Et (**2c**; IC_{50} 4 nM) ∼ Pr (**2d**; IC_{50} 4 nM) > *i*-Pr (**2e**; IC_{50} 20 nM) > Bn (**2f**; IC_{50} 2300 nM)]. Chemical manipulations of the primary amine of **2b** (IC_{50} 6 nM) proved disastrous in terms of maintaining favorable biological activity. Explorations included a simple *N*-Me substitution (**2h**; IC_{50} > 1000 nM), a Pro-derived cyclic amine (**2g**; IC_{50} 1400 nM), and insertion of an extra methylene between the carbonyl and the amine (**2i**; IC_{50} > 10,000 nM). We also found that reduction of the amide carbonyl moiety of analogue **2p** led to a total loss of desired biological activity [**2q** (IC_{50} > 10,000 nM) << **2p** (IC_{50} 23 nM)]. Additional unnatural amino acids derivatives were also inactive, possessing IC_{50}s > 10,000 nM (**2j**, **2l**, **2n**). Evaluations of segment B of parent structure **2** included a cursory evaluation of 5-indoline substitutions. The related indoline SAR suggest that some variation is tolerable at the 5-position, although this substitution did not significantly enhance the desired biological activities [Set 1: Gly derivatives—OMe (**2u**; IC_{50} 70 nM) < H (**2a**; IC_{50} 36 nM) ∼ F (**2r**; IC_{50} 46 nM) < Cl (**2s**; IC_{50} 11 nM); Set 2: Ala derivatives—OMe (**2v**; IC_{50} 16 nM) ≤ H (**2b**; IC_{50} 6 nM) ∼ Cl (**2t**; IC_{50} 5 nM). Also related to segment B, we substituted the indoline heterocycle with a tetrahydroquinoline and lost most desired activity [**2w** (IC_{50} 400 nM) << **2b**; (IC_{50} 6 nM)]. For segment C of the parent molecule **2**, we found that a simple *N*-Me substitution at either imidazole nitrogen yielded significantly weaker TPP II inhibitors than their parent NH analogue **2o** [**2y** (IC_{50} > 10,000 nM) < **2z** (IC_{50} 2,100 nM) < **2o** (IC_{50} 194 nM)]. Replacement of the imidazole group with alternate five-membered ring heterocycles also proved less promising biologically, as readily seen by comparing **2ii** and **2kk** of Table 2 with imidazole **2a** (IC_{50} 36 nM), and **2jj** of Table 2 with imidazole **2o** (IC_{50} 194 nM). Fusing of the pendant propyl group with the imidazole in **2b** (IC_{50} 6 nM), to give benzimidazole derivative **2gg** (IC_{50} 1400 nM), also proved unfavorable for TPP II inhibitory activity.

Scheme 3.
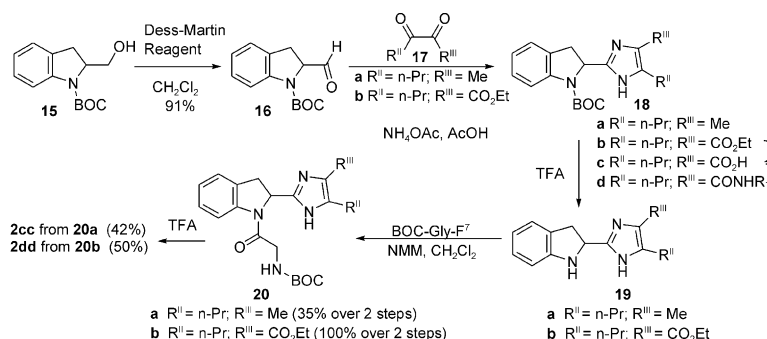
Scheme 4.
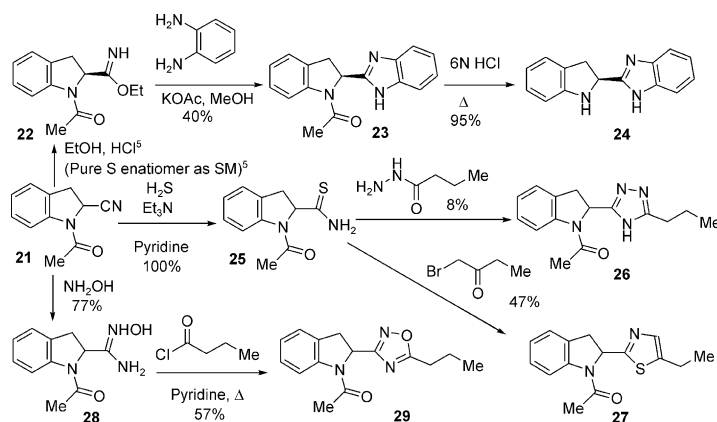
Scheme 5.

Table 1. Imidazoles, benzimidazoles and related TPP II IC$_{50}$'s

Compd	n	X	R	R^{I}	R^{II}	R^{III}	IC$_{50}$, (nM)[a]
2a	1	H	Gly[b,5]	H	n-Pr	H	36
2b	1	H	L-Ala[5]	H	n-Pr	H	6
2c	1	H	L-Abu[c,5]	H	n-Pr	H	4
2d	1	H	L-Norvaline	H	n-Pr	H	4
2e	1	H	L-Val	H	n-Pr	H	20
2f	1	H	L-Phe	H	n-Pr	H	2300
2g	1	H	L-Pro	H	n-Pr	H	1400
2h	1	H	L-N-Me-Ala	H	n-Pr	H	>10,000
2i	1	H	β-Ala	H	n-Pr	H	>10,000
2j	1	H	–CO-4–imidazole[b]	H	n-Pr	H	>10,000
2k	1	H	2-Nitro-benzoyl[b]	H	n-Pr	H	>10,000
2l	1	H	2-Amino-benzoyl[b]	H	n-Pr	H	>10,000
2m	1	H	3-Nitro-benzoyl[b]	H	n-Pr	H	>10,000
2n	1	H	3-Amino-benzoyl[b]	H	n-Pr	H	>10,000
2o	1	H	Gly[b]	H	Et	H	194
2p	1	H	L-Ala[5]	H	Et	H	23
2q	1	H	–CH$_2$–CH(Me)NH$_2$	H	Et	H	>10,000
2r	1	F	Gly[b]	H	n-Pr	H	46
2s	1	Cl	Gly[b]	H	n-Pr	H	11
2t	1	Cl	L-Ala	H	n-Pr	H	5
2u	1	OMe	Gly[b]	H	n-Pr	H	70
2v	1	OMe	L-Ala	H	n-Pr	H	16
2w	2	H	L-Ala[d]	H	n-Pr	H	400
2x	2	H	L-Ala[d]	H	n-Pr	H	800
2y	1	H	Gly	Me	Et	H	>10,000
2z	1	H	Gly	Me	H	Et	2100
2aa	1	H	Gly[b]	H	n-Bu	H	50
2bb	1	H	Gly[b]	H	Ph	H	>10,000
2cc	1	H	Gly[b]	H	n-Pr	Me	55
2dd	1	H	Gly[b]	H	n-Pr	CO$_2$Et	210
2ee	1	H	Gly[b]	H	n-Pr	CONH$_2$	200
2ff	1	H	Gly[b]	H	n-Pr	CO-Gly-OMe	135
2gg	1	H	L-Ala	H		Fused Ph	1400
2hh	1	H	L-Abu	H		Fused Ph	450

[a]IC$_{50}$ values were determined using Sigma plot C/R curves.
[b]Racemic and not S stereochemistry as depicted in Markush structure **2**.
[c]Abu, 2-aminobutyric acid.
[d]Separated epimers, absolute stereochemistries unassigned.

Table 2. Additional heterocycles and related TPP II IC$_{50}$'s

Compd	R	Imidazole replacement	R^{I}	R^{II}	R^{III}	IC$_{50}$, (nM)[a]
2ii (from **26**)	Gly[b]	Triazole	H	n-Pr	H	>10,000
2jj (from **27**)	Gly[b]	Thiazole	H	Et	H	7700
2kk (from **29**)	Gly[b]	Oxadiazole	H	n-Pr	H	2200

[a]IC$_{50}$ values were determined using Sigma plot C/R curves.
[b]Racemic.

We also explored the SAR for a small set of varied 2,4-di- and 2,4,5-tri-substituted imidazoles. For 2,4-di-substituted imidazoles, we found that limited aliphatic lipophilicity is desirable at the 4-imidazole position, based on comparison of a set of R^{II} mono varied analogues [Et (**2o**; IC_{50} 194 nM) < *n*-Pr (**2a**; IC_{50} 36 nM) ∼ *n*-Bu (**2aa**; IC_{50} 50 nM) > > Ph (**2d**; IC_{50} 1 > 10,000 nM)]. Our evaluations of 2,4,5-trisubstituted analogues suggest that some bulk, and even some polarity, is biologically tolerable for such associated analogues, although some gradations of desired activities were noticed. Again, this assessment was based on the SAR for a set of mono-varied analogues, where R^{II} was held constant as an *n*-Pr group [R^{III} = H (**2a**; IC_{50} 36 nM) ≥ R^{III} = Me (**2cc**; IC_{50} 55 nM) > R^{III} = CO–NH–Gly–OMe (**2ff**; IC_{50} 135 nM) ≥ R^{III} = CO–NH$_2$ (**2ee**; IC_{50} 200 nM) ∼ R^{III} = CO$_2$Et (**2dd**; IC_{50} 210 nM)].

In conclusion, we have systematically evaluated the SAR of our lead TPP II inhibitors **2**. We have found that for the maintenance of good TPP II inhibitory activity the latitude of structural variations on scaffold **2** is rather limited in nature, with our most potent TPP II inhibitors being identified as **2b**, **2c**, **2d**, **2s**, and **2t**. The limitations noted around the SAR of **2** are consistent with molecular modeling results that we have performed within our group, and these related modeling results will be published in detail in the near future.

References and Notes

1. Mokdad, A. H.; Serdula, M. K.; Dietz, W. H.; Bowman, B. A.; Marks, J. S.; Koplan, J. P. *JAMA* **1999**, *282*, 1519.
2. Must, A.; Spadano, J.; Coakley, E. H.; Field, A. E.; Colditz, G.; Dietz, W. H. *JAMA* **1999**, *282*, 1523.
3. Kordik, C. P.; Reitz, A. B. *J. Med. Chem.* **1999**, *42*, 181.
4. Rose, C.; Vargas, F.; Facchinetti, P.; Bourgeat, P.; Bambal, R. B.; Bishop, P. B.; Chan, S. M. T.; Moore, A. N. J.; Ganellin, C. R.; Schwartz, J.-C. *Nature* **1996**, *380*, 403.
5. Breslin, H. J.; Miskowski, T. A.; Kukla, M. J.; Leister, W. H.; De Winter, H. L.; Gauthier, D. A.; Somers, M. V. F.; Peeters, D. C. G.; Roevens, P. W. M. *J. Med. Chem.* **2002**, *45*, 5303.
6. Rose, C.; Vargas, F.; Bourgeat, P.; Schwartz, J.-C.; Bishop, P. B.; Bambal, R. B.; Ganellin, C. R.; Leblond, B.; Moore, A. N. J.; Chan, S.; Zhao, L. WO 9635805, 1996, CAN 136:72042.
7. Carpino, L. A.; Mansour, E.-S. M. E.; Sadat-Aalaee, D. *J. Org. Chem.* **1991**, *56*, 2611.
8. Kashima, C.; Harada, Y.; Hosomi, A. *Heterocycles* **1993**, *35*, 433.
9. Gross, K. M. B.; Jun, Y. M.; Beak, P. *J. Org. Chem.* **1997**, *62*, 7679.
10. Grimmett, M. R. In *Imidazole and Benzimidazole Synthesis*; Academic: San Diego, 1997; p 151.
11. Wasserman, H. H.; Vu, C. B. *Tetrahedron Lett.* **1990**, *31*, 5205.
12. TPP II enzyme isolation and related determination of inhibitory activity are described in detail in ref 5.