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Triazolyl tryptoline derivatives as β-secretase inhibitors

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ABSTRACT

Tryptoline, a core structure of ochrolifuanine E, which is a hit compound from virtual screening of the Thai herbal database against BACE1 was used as a scaffold for the design of BACE1 inhibitors. The tryptoline was linked with different side chains by 1,2,3-triazole ring readily synthesized by catalytic azide-alkyne cycloaddition reactions. Twenty two triazolyl tryptoline derivatives were synthesized and screened for the inhibitory action against BACE1. JJCA-140 was the most potent inhibitor (IC_{50} = 1.49 μM) and was 100 times more selective for BACE1 than for Cat-D.

Alzheimer's disease (AD) is a common neuro-degenerative disorder which affects 20–30 million individuals worldwide.[1] The patients' cognitive function slowly declines, leading to end-stage disease and death with-in 9 years after the diagnosis.[1,2] The deposition of aggregated β-amyloid peptides ($Aβ_{40,42}$) as plaques in brain is a hallmark of AD. Inhibition of the formation of amyloid plaques has been targeted in the new drug development. β-Secretase (BACE1) and γ-secretase are the key enzymes to generate these peptides from amyloid precursor protein (APP). The cleavage of APP by BACE1 is the initial step in Aβ formation; also, BACE1-knockout mice are deficient in Aβ production with no compensatory mechanism. Thus, inhibition of BACE1 activity becomes the promising target is an attractive target for AD drug development.[2,3]

BACE1 inhibitor was studied for more than a decade. Most of them were developed from nonhydrolyzable hydroxyethylene dipeptide isostere,[4–6] high-throughput screening (HTS),[7,8] and fragment-base screening.[9–11] In this study, new core structures of BACE1 inhibitors were identified via virtual screening of the Thai medicinal database. Ochrolifuanine E and its tryptoline core have not previously been described pharmacophores for BACE1 inhibition, and this discovery represents another direction in the design of BACE1 inhibitors.

Thai medicinal database (Chemiebase[12,13]) compounds were the source of various scaffolds for virtual screening (AutoDock 4.0)[14] and pharmacokinetic and toxicity filtering (Discovery Studio, Accelrys).[15] The Chemiebase covers the herbs in Thai Traditional Pharmacopoeias and the herbs used by the local practitioners for the preparation of traditional medicines. The flora in the database ranges from common to scarce and some plants are in danger of extinction in Thailand. Most of the plants are also common in neighboring countries or countries in other part of the world with the same climate. All identified compounds from the herbs reported under the same botanical names were collected and compiled. Currently, there are 2048 compounds in the database. Based on Thai traditional knowledge and the reduced search space, this database considered a knowledge-based database, and virtual screening of a knowledge-based database or focus library is recognized as an efficient strategy for lead identification. This database was selected on the basis of scaffold diversity of the natural compounds it contains. The protein template was constructed from two crystal structures of BACE1 bound to inhibitors (Protein Data Bank code: 2IRZ[16] and 1FKN[17]). In order to prepare the target protein as a template, the bound ligands (I02 and OM99-2) and crystallographic water were removed. The unbound proteins were superimposed (SwissPdb-Viewer)[18] to reconstruct the missing atoms (Leu152 and Ser173) in 2IRZ. The chain between residues 152 and 173 from 1FKN was cut and inserted in 2IRZ in the missing region to form a new template 2IRZ-F in a reasonable conformation. Hydrogens and Gasteiger charges were added to the fixed protein template, 2IRZ-F, by using AutoDockTools (ADT). During final preparations nonpolar hydrogens were merged. Grid maps

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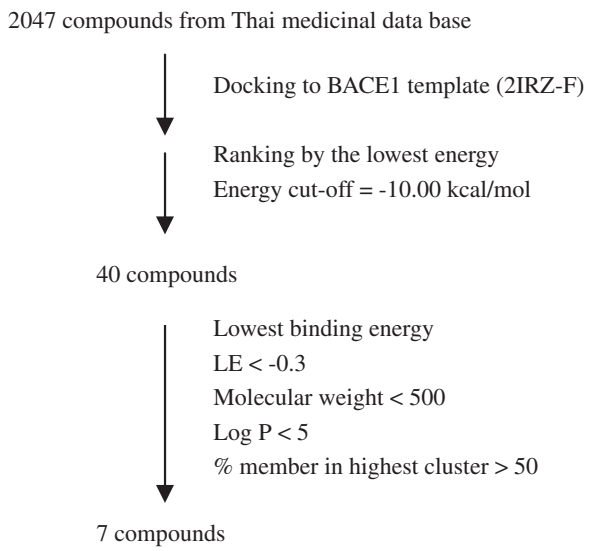


Figure 1. The procedure in virtual screening.

for each atom type in the ligands were set due to the centered on dimension 29.557, 40.506, 3.896 with 80 × 100 × 80 Å and 0.375 Å spacing between grids points were computed using AutoGrid 4.0. All ligands were generated and optimized with ChemDraw Ultra 9.0 and Chem3D Ultra 9.0. Gasteiger charge was assigned, nonpolar hydrogen was merged, aromatic carbons identified, and lastly, the rigid root and rotatable bonds were defined.

The constructed BACE1 template was validated by redocking with the native ligands, I02, I03 and 5HA (PDB code: 2IRZ, 2ISO and 2B8L). AutoDock 4.0 was employed to perform the docking calculation. The 3D configuration of I02, I03 and 5HA obtained from docking, or docked pose were compared with the crystallographic pose. Ligands docked in the binding pocket in the same configuration as found in crystal structure with RMSD <2.0 Å (Supplementary data). The redocking result or validation indicated that the prepared BACE1 template (2IRZ-F) is a good model for virtual screening.

For virtual screening, after each 100 docking runs per compound, the conformations that had the lowest binding energy to BACE1 were clustered and ranked. Each cluster consisted of conformers that had similar 3D structures (RMSD <2 Å). Fourty compounds that had the binding energy lower than −10 kcal/mol were selected

Table 1
Selected hit compounds from virtual screening

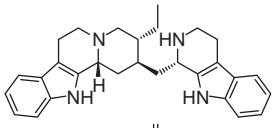
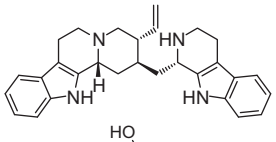
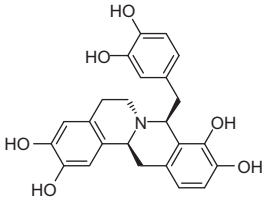
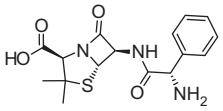
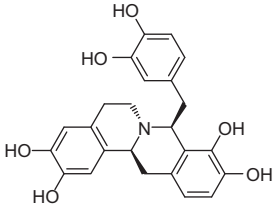
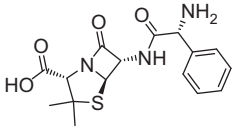
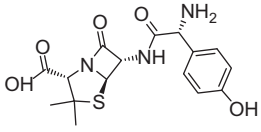
Compound	Structure	MW	Log *P*	Binding energy in highest clustering (kcal/mol)	% member in highest cluster	LE
JV5-40		438.61	4.06	−13.45	86	−0.41
JV5-42		436.59	3.79	−13.41	85	−0.41
JV2-697		421.44	3.30	−10.39	63	0.34
JV4-345		349.40	−0.20	−10.36	77	−0.43
JV2-695		421.44	3.30	−10.33	68	−0.33
JV4-346		349.40	−0.20	−10.26	86	−0.43
JV2-326		365.40	−0.58	−10.22	74	−0.41

Table 2
Binding energy, pharmacokinetic and toxicity of selected core structure tryptoline

Properties	JV5-40	Tryptoline
Binding energy (kcal/mol) in highest clustering	−13.45	−7.42
BBB level[a]	0	2
Absorption level[b]	1	0
Solubility level[c]	0	3
CYP2D6[d]	0	0
PPB level[e]	2	1
Hepatotoxicity[f]	1	0
Probability of mutagenicity	0	0.001
Rat oral LD_{50} (μg/kg)	235.5	701,500

[a] Blood–brain barrier penetration, 0 = very high, 2 = medium.
[b] Absorption level, 0 = good, 1 = moderate.
[c] Aqueous solubility, 0 = extremely low, 3 = good.
[d] Cytochrome P450 2D6, 0 = non-inhibitor.
[e] Plasma protein binding, 1 = binding is >90%, 2 = binding is >95%.
[f] Hepatotoxicity, 0 = nontoxic, 1 = toxic.

(Supplementary data). The 40 top ranked compounds were filtered by five parameters: binding energy, ligand efficiency[19–22] (<−0.3), molecular weight (<500), Log *P* (<5)[23] and % member in highest cluster (>50), resulting in seven hit compounds (Fig. 1 and Table 1). Pharmacokinetic and toxicity property of these compounds were evaluated in silico. Ochrolifuanine E found in the local plant *Dyera costulata*, the bark and leaves of *D. costulata* have been used in folk medicine for treatment for fever by traditional doctors in the South of Thailand.[24,25] The hit compound JV5-40, ochrolifuanine E was found to be most promising hit compound because it had the good binding (−13.45 kcal/mol) and the good pharmacokinetic properties: blood–brain barrier penetration (BBB) level = 0, absorption level = 1 and CYP2D6 = 0 (Table 2). While either other compounds had higher binding energy, no interaction with catalytic site Asp32 or Asp228 or low blood–brain barrier penetration property. However, the predicted toxicity of this compound was considerably high (LD_{50} of 235.5 μg/kg). The binding mode of ochrolifuanine E from docking consists of two hydrogen bondings with Asp32 and Gly230, the structure locates in pockets S2 and S1′ of the substrate binding site resulting in Van der Waals interaction. Tryptoline (2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole) part not only formed hydrogen bond with Asp32, the residue in the catalytic site (Fig. 2) but also gave better results in all parameters except the binding energy and BBB penetration when compared to ochrolifuanine E, (Table 2). Therefore, tryptoline was selected as the core nucleus for further design as the binding energy can be modified by the side chain. After tryptoline was docked with BACE1 template, phenyl ring could access the hydrophobic S1 pocket, and polar hydrogen NH at position 2 was hydrogen bonded with O=C of Asp228.

In order to locate the side chain in the S2 pocket, the side chain substitution was changed from C1 to C3. The 3-substituted tryptoline derivative in both *R* and *S*-configurations were docked with BACE1 template. The *S*-configuration at C3 position showed better binding energy and % member in the highest cluster than *R*-configuration as the side chain of *S*-compound can access to the S2 pocket better than *R*-compound. Therefore, (*S*)-3-(azidomethyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole was selected to be a new core structure for BACE1 inhibitor. The expected interaction of the designed structure was showed in Figure 3. The Cu(I)-catalyzed [3+2]azide-alkyne cycloaddition reaction is the method to connect the side chain (*R*) to the core nucleus due to the high yield and purity.

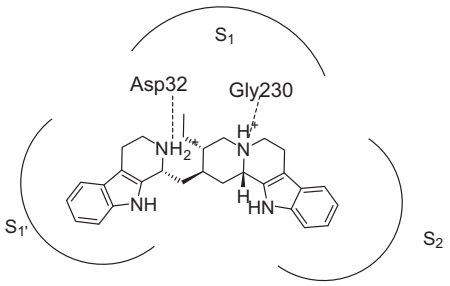


Figure 2. The binding mode of ochrolifuanine E.

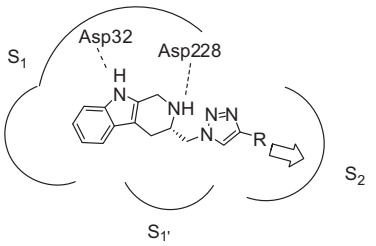


Figure 3. The expected interaction of the designed structure.

The designed compounds with various Rs were docked to the BACE1 template. Among 105 Rs including 29 alicyclics, 34 aromatics, 22 heterocyclics, 19 bicyclic of aromatics and heterocyclics and 1 tricyclic (Supplementary data), the top 22 compounds giving good docking results in binding energy and % member in the highest cluster were proceeded to the synthesis in 96 well plate for screening activity. The (*S*)-3-methyl tryptoline azide was prepared to react with 22 different alkynes (Fig. 4).

The general synthetic scheme for the preparation of azide is shown in Scheme 1 (Supplementary data). L-1,2,3,4-Tetrahydro norharman-3-carboxylic acid **1** was esterified to yield methyl ester **2** (4.68 g, 88%). Reduction of **2** with sodium borohydride gave (*S*)-(2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-3-yl) methanol **3** (3.49 g, 85%). The hydroxy group of **3** was converted to the nosylate, which was treated at 70 °C with sodium azide to give (*S*)-3-(azidomethyl)-2-(4-nitrophenyl sulfonyl)-2,3,4,9-tetrahydro-1*H*-pyrido-[3,4-*b*]indole **4** (2.76 g, 64%). After the removal of the nosyl group

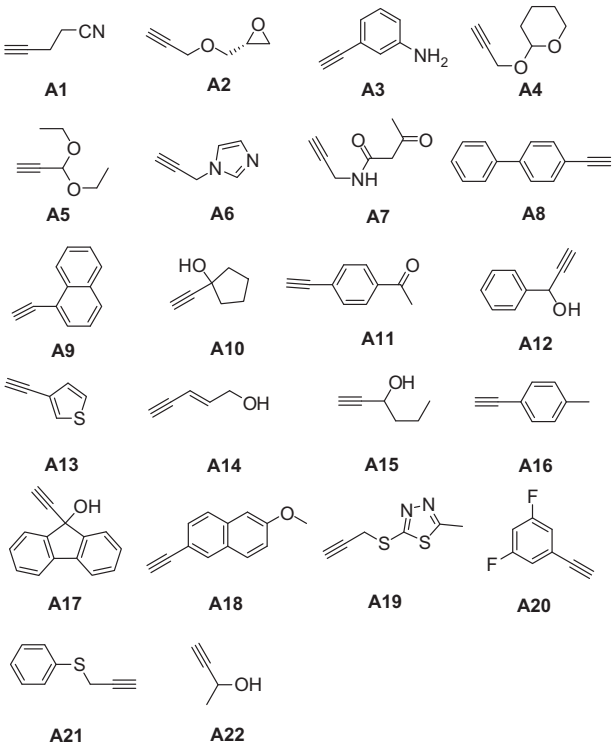
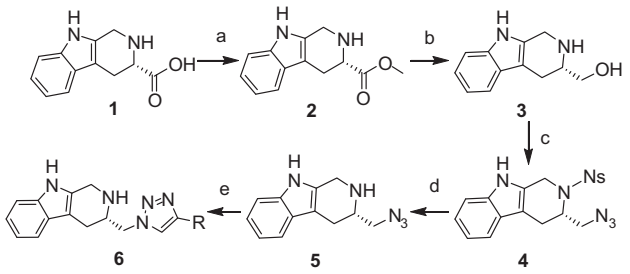


Figure 4. Alkynes used to synthesize the library.



Scheme 1. Synthesis of screening compounds. Reagents and conditions: (a) H_2SO_4, methanol, reflux, 18 h; (b) $NaBH_4$, ethanol, THF, reflux, 18 h; (c) (i) 4-nitrobenzenesulfonyl chloride, TEA, CH_2Cl_2, rt, 4 h; (ii) NaN_3, DMF, 70 °C, 6 h; (d) K_2CO_3, thiophenol, DMF, 50 °C, 2 h; (e) alkyne, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, DMSO, rt, 24 h.

with thiophenol and potassium carbonate, (*S*)-3-methyl tryptoline azide **5** (0.86 g, 57%) was obtained. Azides reacted in a 1:1 ratio with 22 alkynes to give the final triazole **6**. The final reactions were run in microtiter plates, Cu(I) was generated in situ using a combination of $CuSO_4$ and sodium ascorbate.[26] After the completion, reactions mixtures were diluted with 50 mM sodium acetate (pH 4.5) buffer to give a 25 mM stock solutions of screening candidates based on the amount of azides used in the cycloaddition reactions, and with the production of product more than 80% detected by LC–MS.

The 22 crude compounds were screened at 5 μM against the BACE1 ($n = 3$) (Fig. 5). Compound JJCA-140[27] corresponding to alkyne A18, showed inhibitory activity more than 50% at 5 μM, was resynthesized on a larger scale for complete characterization (melting point, FTIR, ^{1}H NMR, ^{13}C NMR, MS, and LC–MS). JJCA-140 was synthesized from azide **5** reacting with 2-ethynyl-6-methoxy-napthalene in *t*-BuOH/H_2O/EtOH. Cu(I) was generated in situ as described above, using a combination of 5 mol % $CuSO_4$ and 20 mol % sodium ascorbate. The pure compound was assayed for its activity against the BACE1 and Cat-D. BACE1 and Cat-D inhibitory activity of the compound were determined by enzymatic assay using a recombinant human BACE1 with FRET (fluorescence resonance energy transfer) substrate from PanVera® and Cat-D with FRET substrate from SensoLyte®. β-Secretase inhibitor IV (Calbiochem) and pepstatin A were the positive controls to evaluate the potency for BACE1 and Cat-D, respectively. Compound JJCA-140 showed potent inhibitory activity with BACE1 (IC_{50} = 1.49 μM) and more selective with BACE1 than Cat-D (IC_{50} >100 μM).

The binding mode of JJCA-140 to BACE1 was shown in Fig 6. The phenyl group of tryptoline fitted with hydrophobic S_1 pocket (Leu30, Tyr71, Phe108, and Trp115). Two polar hydrogens of tryptoline are involved in hydrogen bonding interactions in the S_1 domain, that is, NH to Asp32 and Asp228. The alkyne part was designed to increase activity by accessing S_2 sites (Gln73, Thr232 and Lys321) and providing dipole–dipole interaction. Triazole part has dipole–dipole interaction with Thr231 and Arg235 in S_2 pocket.

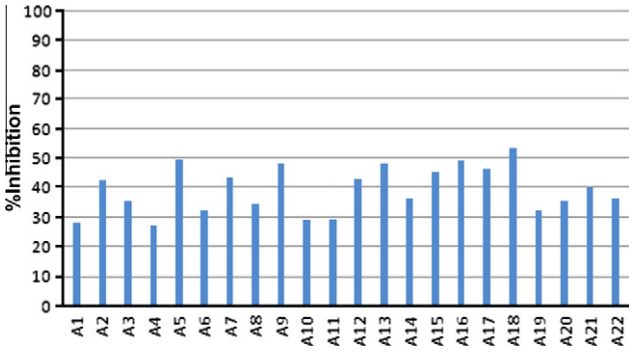


Figure 5. Assays of the library members at 5 μM against BACE1.

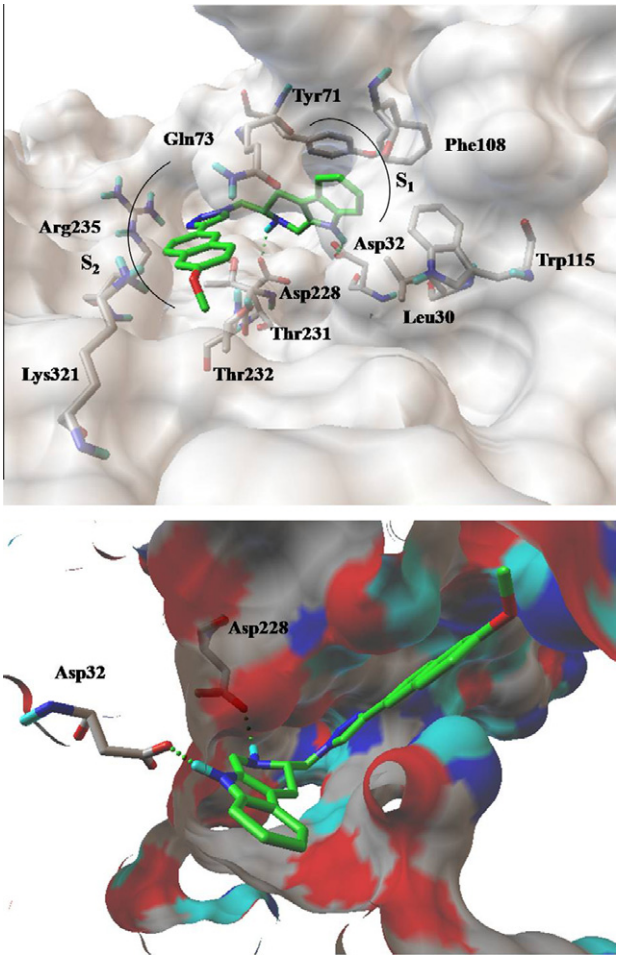


Figure 6. The binding modes of JJCA-140 (green) with BACE1.

In this study, Cu(I)-catalyzed [3+2] azide-alkyne cycloaddition reaction was used to synthesize a focused library from 22 different alkynes and an azide-containing pharmacophore. The compound JJCA-140 showed low micromolar inhibitory activity against BACE1 and 100 times more selective to BACE1 than Cathepsin-D in enzymatic assay. Tryptoline was the important part responsible for the binding to the catalytic site Asp32 and Asp228. Triazole was crucial for increasing the activity via S2 pocket interactions. The alkyne substitutent, methoxy naphthalene, appears to reach the S2 pocket thereby contributing to the increased activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.043.

References and notes

1. Heneka, M. T.; O'Banion, M. K. *J. Neuroimmunol.* **2007**, *184*, 69.
2. Citron, M. *Neuroscience* **2004**, *5*, 677.
3. Findeis, M. A. *Pharmacol. Ther.* **2007**, *116*, 266.

4. Stanton, M. G.; Stauffer, S. R.; Gregro, A. R.; Steinbeiser, M.; Nantermet, P.; Sankaranarayanan, S.; Price, E. A.; Wu, G.; Crouthamel, M. C.; Ellis, J.; Lai, M. T.; Espeseth, A. S.; Shi, X. P.; Jin, L.; Colussi, D.; Pietrak, B.; Huang, Q.; Xu, M.; Simon, A. J.; Graham, S. L.; Vacca, J. P.; Selnick, H. *J. Med. Chem.* **2007**, *50*, 3431.
5. Ghosh, A. K.; Kumaragurubaran, N.; Hong, L.; Kulkarni, S. S.; Xu, X.; Chang, W.; Weerasena, V.; Turner, R.; Koelsch, G.; Bilcer, G.; Tang, J. *J. Med. Chem.* **2007**, *50*, 2399.
6. Hom, R. K.; Gailunas, A. F.; Mamo, S.; Fang, L. Y.; Tung, J. S.; Walker, D. E.; Davis, D.; Thorsett, E. D.; Jewett, N. E.; Moon, J. B.; John, V. *J. Med. Chem.* **2004**, *47*, 158.
7. Malamas, M. S.; Erdei, J.; Gunawan, I.; Turner, J.; Hu, Y.; Wagner, E.; Fan, K.; Chopra, R.; Olland, A.; Bard, J.; Jacobsen, S.; Magold, R. L.; Pangalos, M.; Robichaud, A. J. *J. Med. Chem.* **2010**, *53*, 1146.
8. Cole, D. C.; Manas, E. S.; Stock, J. R.; Condon, J. S.; Jennings, L. D.; Aulabaugh, A.; Chopra, R.; Cowling, R.; Ellingboe, J. W.; Fan, K. Y.; Harrison, B. L.; Hu, Y.; Jacobsen, S.; Jin, G.; Lin, L.; Lovering, F. E.; Malamas, M. S.; Stahl, M. L.; Strand, J.; Sukhdeo, M. N; Svenson, K.; Turner, M. J.; Wagner, E.; Wu, J.; Zhou, P.; Bard, J. *J. Med. Chem.* **2006**, *49*, 6158.
9. Godemann, R.; Madden, J.; Krämer, J.; Smith, M.; Fritz, U.; Hesterkamp, T.; Barker, J.; Höppner, S.; Hallett, D.; Cesura, A.; Ebneth, A.; Kemp, J. *Biochemistry* **2009**, *48*, 10743.
10. Yang, W.; Fucini, R. V.; Fahr, B. T.; Randal, M.; Lind, K. E.; Lam, M. B.; Lu, W.; Lu, Y.; Cary, D. R.; Romanowski, M. J.; Colussi, D.; Pietrak, B.; Allison, T. J.; Munshi, S. K.; Penny, D. M.; Pham, P.; Sun, J.; Thomas, A. E.; Wilkinson, J. M.; Jacobs, J. W.; McDowell, R. S.; Ballinger, M. D. *Biochemistry* **2009**, *48*, 4488.
11. Congreve, M.; Aharony, D.; Albert, J.; Callaghan, O.; Campbell, J.; Carr, R. A. E.; Chessari, G.; Cowan, S.; Edwards, P. D.; Frederickson, M.; McMenamin, R.; Murray, C. W.; Patel, S.; Wallis, N. *J. Med. Chem.* **2007**, *50*, 1124.
12. Sangma, C.; Chuakheaw, D.; Jonkon, N.; Gadavanij, S. *Curr. Pharm. Des.* **2010**, *16*, 1753.
13. The structural database of chemical found in Thai medicinal plants. Available at: http://chemiebase.ku.ac.th.
14. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. *J. Comput. Chem.* **2009**, *30*, 2785.
15. O'Brien, S. E.; de Groot, M. J. *J. Med. Chem.* **2005**, *48*, 1287.
16. Rajapakse, H. A.; Nantermet, P. G.; Selnick, H. G.; Munshi, S.; McGaughey, G. B.; Lindsley, S. R.; Young, M. B.; Lai, M. T.; Espeseth, A. S.; Shi, X. P.; Colussi, D.; Pietrak, B.; Crouthamel, M. C.; Tugusheva, K.; Huang, Q.; Xu, M.; Simon, A. J.; Kuo, L.; Hazuda, D. J.; Graham, S. *J. Med. Chem.* **2006**, *49*, 7270.
17. Hong, L.; Koelsch, G.; Lin, X.; Wu, S.; Terzyan, S.; Ghosh, A. K.; Zhang, X. C.; Tang, J. *Science* **2000**, *290*, 150.
18. Guex, N.; Diemand, A.; Peitsch, M. C. *Trends Biochem. Sci.* **1999**, *24*, 364.
19. Kuntz, I. D.; Chen, K.; Sharp, K. A.; Kollman, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9997.
20. Hopkins, A. L.; Groom, C. R.; Alex, A. *Drug Discovery Today* **2004**, *9*, 430.
21. Abad-Zapatero, C.; Metz, J. T. *Drug Discovery Today* **2005**, *10*, 464.
22. Hetényi, C.; Maran, U.; García-Sosa, A. T.; Karelson, M. *Bioinformatics* **2007**, *23*, 2678.
23. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **2001**, *46*, 3.
24. Reanmongkol, W.; Subhadhirasakul, S.; Pairat, C.; Poungsawai, C.; Choochare, W.; Songklanakarin, J. *Sci. Technol.* **2002**, *24*, 227.
25. Mirand, C.; Men-Olivier, L.; Men, J. L.; Delaude, C. *Phytochemistry* **1983**, *22*, 577.
26. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
27. Melting point, IR, ^{1}H, and ^{13}C NMR, mass, and LC–MS determination. For JJCA-140, (*S*)-3-((4-(6-methoxynaphthalen-2-yl)-1*H*-1,2,3-triazol-1-yl)methyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole as a light brown solid: mp 232–234 °C; FTIR (KBr) (cm^{-1}): 3421.55 (pyrrole N–H, st.), 3302.33 (N–H, st.), 33109.19 (aromatic C–H, st.), 1613.26, 1481.43 (aromatic C–C, st.), 1266.38 (C–O–C, st.), 1215.99 (C–N, st), 1029.17 (C–O–C, st.), 743.52 (aromatic C–H, bending); ^{1}H NMR (300 MHz, DMSO-d_6) 10.70 (s, 1H, NH), 8.67 (s, 1H, H5jj), 8.33 (s, 1H, H1j), 7.96 (db, 1H, H5j), 7.88 (db, 2H, H3j, H4j) 7.34–7.32 (m, 2H, H7j, H8j), 7.26 (db, 1H, H8), 7.18 (db, 1H, H5), 6.99 (t, 1H, H7), 6.91 (t, 1H, H6), 4.58 (s, 2H, H1), 3.88 (s, 2H, H4), 3.88 (s, 3H, CH_3), 2.66 (db, 1H, CH_2), 2.49 (db, 1H, CH_2); ^{13}C NMR (300 MHz, DMSO-d_6); 157.53, 146.45, 135.90, 133.94, 129.60, 128.66, 127.45, 127.10, 126.20, 124.24, 123.49, 122.08, 120.48, 119.16, 118.36, 117.20, 110.95, 106.17, 106.02, 55.31, 54.16, 53.50, 41.96, 25.48; MS *m*/*z* 411.4 [M−H], 410.3 [M]; LC–MS *m*/*z* 410.1896 [M−H].