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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

A facile and single pot strategy for the synthesis of novel naphthyridine derivatives under microwave irradiation conditions using $ZnCl_2$ as catalyst, evaluation of AChE inhibitory activity, and molecular modeling studies

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Abstract A series of novel naphthyridine derivatives **3** and **4** was prepared from substituted pyridine **2** and ketones using $ZnCl_2$ as catalyst under microwave irradiation conditions. All the compounds were evaluated for AChE inhibitory activity and promising compounds **3d**, **3e**, **4b**, and **4g** was identified. Representative compounds **3d** and **3e** were found to show insignificant THLE-2 liver cell viability/toxicity. The binding mode between X-ray crystal structure of human AChE and compounds was studied using molecular docking method and fitness scores were found to be in good correlation with the activity data.

Keywords Acyclic ketones · Cyclic ketones · Microwave irradiation · Catalyst · Tacrine · Naphthyridine · AChE inhibitory activity · Liver cell viability · Molecular modelling

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder caused due to progressive loss of several cognitive abilities. Acetylcholinesterase, also known as AChE, is an enzyme that degrades the neurotransmitter acetylcholine, producing choline and an acetate group. It is mainly found at neuromuscular junctions and cholinergic nervous system, where its activity serves to terminate synaptic transmission.

In order to inhibit the acetylcholinesterase enzyme, some organic compounds have been identified that can block the function of AChE and thus cause excessive acetylcholine to accumulate in the synaptic cleft. Among the most common acetylcholinesterase inhibitors, phosphorus-based compounds are designed to bind to the active site of the enzyme. Further developments realized several new generation acetylcholinesterase inhibitors which have additional advantages. A rational strategy to treat the patients with acetylcholinesterase inhibitors (AChEI) can increase the acetylcholine levels in brain as a result the decrease in metabolic rate of released neurotransmitter thereby enhanced neurotransmission (Bartus *et al*., 1982). Tacrine (**1**) was the first AchE inhibitor approved in 1953; however, its clinical usefulness was limited due to hepatotoxicity (Balson *et al*., 1995; Stachlewitz *et al*., 1997). In recent past, the *ortho* amino nitriles are used as important building blocks for the synthesis of tacrine analogues via Friedlander reaction mainly using $AlCl_3$ as catalyst (Marco *et al*., 2001; Marco *et al*., 2004; Barreiro *et al*., 2003; Leon *et al*., 2005). Similarly Lewis acids (Da Costa *et al*., 2009; Proctor and Harvey, 2000; Gamba *et al*., 2008) like $InCl_3$, $BF_3{\cdot}Et_2O$, $FeCl_3$, $BiCl_3$, $SbCl_3$, $SnCl_2{\cdot}2H_2O$, $RuCl_3$, $CeCl_3$, $NiCl_2$, $CoCl_2{\cdot}2H_2O$, and CsCl are also used as catalysts for the synthesis of tacrine analogues. Apart from tacrine analogues, more recently, chiral tetrahydroacridine analogues (Pisoni *et al*., 2010) was also reported as AchE inhibitors. Based on the importance, the interest is continuously growing on the synthesis and identification of potential AChE inhibitors. In this study, we have

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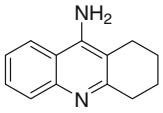
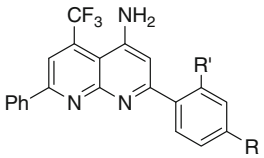
synthesized a series of novel naphthyridine derivatives, evaluated for AChE inhibitory activity and identified promising compounds **3d**, **3e**, **4b**, and **4g**.

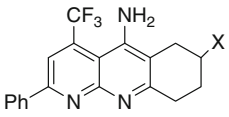


Tacrine **1**



3d, R = Cl, R'= H

3e, R = H, R'= Br



4b, X = H

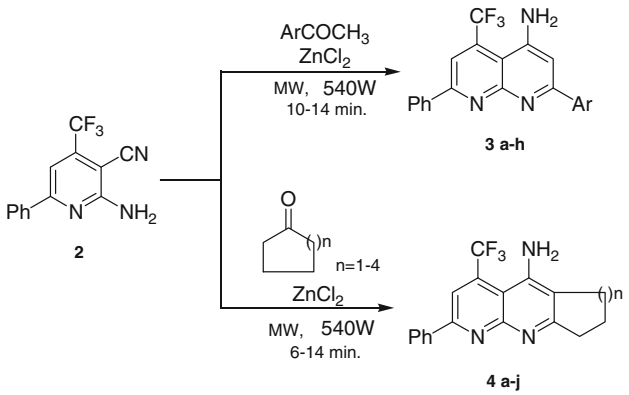
4g, X = CH_3

Chemistry

In continuation of our efforts (Sridhar *et al*., 2009; Chandra Shekhar *et al*., 2009; Shanthan Rao and Venkataratnam, 1991), we used $ZnCl_2$ as an inexpensive and environment friendly catalyst for the synthesis of novel tacrine analogues, isosteres of azaquinolines in single pot under microwave irradiation conditions. In a typical reaction, the 2-amino-3-cyano-4-trifluoromethyl-6-phenylpyridine **2** (Narsaiah *et al*., 1994; Guttsait *et al*., 1987) was reacted with acetophenone in DMF at 120–130°C for 18 h and found several products. To improve the selectivity, the same reaction was conducted under microwave irradiation conditions using synthos 3000 microwave reactor at 540 W, 130°C and 15 bar pressure using $ZnCl_2$ as catalyst and on subsequent workup furnished the desired products **3** in high yields. In order to see the versatility of the reaction, the compound **2** was further reacted with diverse substituted acetophenones/methyl heteroaryl/alkyl ketones in 10–14 min under the same conditions and obtained products in the range of 80–96% yield. In reaction of methyl isobutyl ketone with compound **2**, two products are formed in definite proportions. The participation of methyl protons in cyclization resulted major product **3h**, while methylene protons gave minor product **3i** in the ratio of 85:15, respectively. The ratio of the products was established after separation of each product and identification based on their intensity in ^1H NMR, CH_2 appeared as doublet at δ 2.75 in compound **3h** and CH_3 as singlet at δ 2.8 in compound **3i**. However, the reaction of compound **2** with β-ketoesters, β-1, 3-diketones, cyclic alkene, or pentafluorophenyl methylketone could not give the product and recovered the starting material. This may be attributed to hydrogen bonding due to chelation as a result no reaction. Further, the reaction did not proceed in the absence of zinc chloride under microwave irradiation conditions even after running for a long time, i.e., 20 min. The reaction sequence is outlined in Scheme 1 and products are tabulated in Table 1.

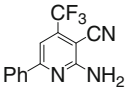
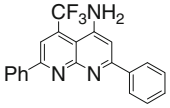
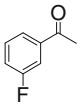
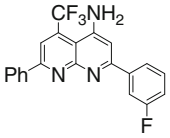
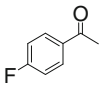
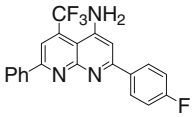
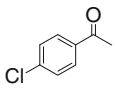
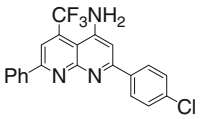
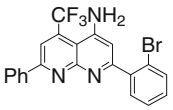
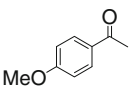
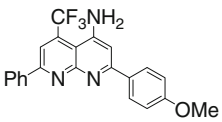
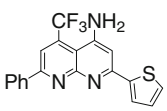
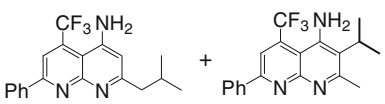
The mechanism of the reaction is mainly the formation of Schiff's base followed by intramolecular cyclization of enamine over to nitrile carbon which was complexed with zinc chloride. The complex on quenching with water resulted product as indicated in Scheme 2.

Similarly, when the compound **2** was reacted with diverse cyclic ketones in 6–14 min, tricyclic products **4** are formed (Scheme 1; Table 2). Ring strain cyclic ketones such as pentanone, octanone, indanone, and tetralone took longer reaction times, whereas 2,6-dimethylcyclohexanone could not give product even after 15 min. The possible reason assumed to be steric hindrance as a result no reaction.



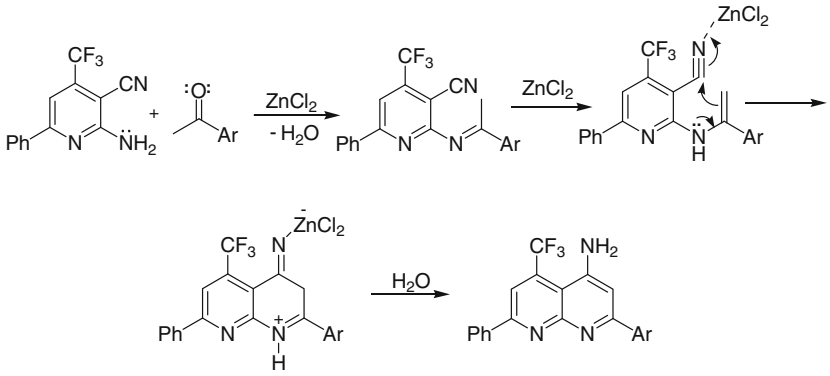
Scheme 1 Synthesis of 2-substituted-4-amino/2,3-fused-5-amino naphthyridine derivatives (**3a–i/4a–j**)

Table 1 Preparation of 2-substituted-4-amino naphthyridine derivatives (**3a–i**)

Entry	Compound	Substrate	Product **3**	Reaction time (min)	Yield (%)
1	 **2**		 **3a**	8	93
2	**2**		 **3b**	10	95
3	**2**		 **3c**	10	96
4	**2**		 **3d**	14	92
5	**2**		 **3e**	14	85
6	**2**		 **3f**	10	80
7	**2**		 **3g**	10	90
8	**2**		 85 : 15 **3h** **3i**	14	70

Scheme 2 A possible mechanistic pathway

Mechanism:



Results and discussion

AChE inhibition evaluation

To determine the potency of novel naphthyridine derivatives, AChE inhibitory activity was measured at different concentrations in vitro by the principle of Ellman method (Ellman *et al*., 1961) and tacrine **1** was used as the reference compound. Compounds **3d**, **3e**, **4b**, and **4g** were found to show high activity and on par with the standard tacrine. All other compounds show moderate activity and compound **4i** show least activity. The structure activity relationship analysis of compounds show that the fusion of cyclopentane, cycloheptane, or cyclooctane at 2,3-position of naphthyridine has no additional advantage in promoting activity. However, in compound **4b** with cyclohexane ring at 2,3-position and in compound **4g** methyl at a specific position of cyclohexane ring influenced the activity. Further fusion of phenyl ring over cyclopentane or cyclohexane and thiophene on 2nd position was found to be detrimental to the activity as experienced in compounds **3g** and **4h–i**. Further studies are underway to identify a lead compound and the activity data are tabulated in Table 3.

THLE-2 liver cell viability/toxicity

The promising AChE inhibitors **3d** and **3e** identified above were further screened for THLE-2 normal liver cell viability/toxicity at different concentrations using tacrine as standard. Either tacrine or compound **3e** did not show any significant cell death; however, compound **3d** at 10 μm caused a marginal 16% cell death as indicated in Table 4 and Fig. 1.

Molecular modelling studies

In order to revalidate the activity data, the binding mode of the compounds along with X-ray crystal structure of human AChE was studied using molecular docking with GOLD 3.2. The crystal structure of human AChE that was used contains two molecules of *N*-acetyl glucosamine (NAG). The active site of the protein analyzed with PDBSum reveals that the important active site residues are Ser347 and Asn350. The important active site residues along with the co-crystalized ligand are shown in Figs. 2 and 3. The co-crystal has close contact with the residue Asn350. The GOLD fitness function is made up of four components: protein–ligand hydrogen bond energy, protein–ligand van der Waals energy, ligand internal vdw energy, and ligand torsional strain energy and the fitness score is taken as the negative of the sum of the component energy terms (Jones *et al*., 1997). The docked poses of each ligand were visualized and the interactions were analyzed with silver. The best Fitness Scores for each ligand are tabulated along with the details of H-bonds and other interactions in Table 5. Compound **3d** has a fitness score 15.96 and it makes hydrogen bond with the active site residue Ser347. Compound **3e** has a fitness score of 18.78 and both **3d** and **3e** make close contacts with the active site residues Phe346 and Asp349. The fitness scores are in good correlation with the activity data as indicated in Fig. 4.

Conclusion

We have developed a novel and an efficient method for 1,8-naphthyridine derivatives in presence of inexpensive $ZnCl_2$ catalyst under microwave irradiation conditions. All the compounds were screened for AchE inhibitory activity at different concentrations and promising compounds were identified. Further, representative compounds **3d** and **3e** were found to show insignificant THLE-2 liver cell viability/toxicity. The fitness scores for all the compounds are calculated through molecular docking studies and are found to be in good correlation with the activity data.

Table 2 Preparation of 2,3-fused-5-amino naphthyridine derivatives (**4a–j**)

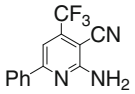
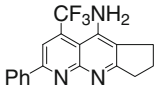
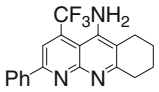
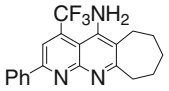
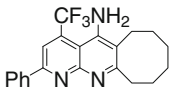
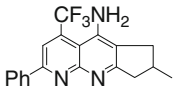
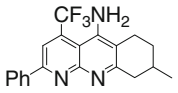
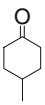
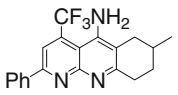
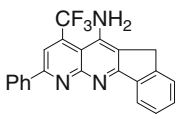
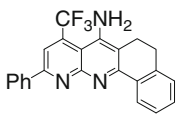
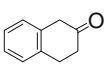
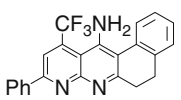
Entry	Compound	Substrate	Product **4**	Reaction time (min)	Yield (%)
1	 **2**		 **4a**	8	85
2	**2**		 **4b**	4	98
3	**2**		 **4c**	4	90
4	**2**		 **4d**	14	90
5	**2**		 **4e**	6	90
6	**2**		 **4f**	6	98
7	**2**		 **4g**	6	98
8	**2**		 **4h**	8	85
9	**2**		 **4i**	14	80
10	**2**		 **4j**	12	85

Table 3 IC_{50} (μM) values for the AChE inhibition

Entry	Compound no.	IC_{50} (μM)
1	Tacrine (**1**)	0.14
2	**3a**	7.10
3	**3b**	7.03
4	**3b**	6.58
5	**3d**	0.52
6	**3e**	0.89
7	**3f**	8.24
8	**3g**	10.36
9	**3h**	19.09
10	**4a**	8.05
11	**4b**	0.35
12	**4c**	10.13
13	**4d**	7.69
14	**4e**	9.37
15	**4f**	3.25
16	**4g**	0.51
17	**4h**	17.56
18	**4i**	23.14

Experimental section

Melting points were recorded on Casia-Siamia (VMP-AM) melting point apparatus and are uncorrected. All reactions were carried out in a synthos 3000 microwave reactor. IR spectra were recorded on a Perkin-Elmer FT-IR 240-C spectrophotometer using KBr optics. ^{1}H NMR spectra were recorded on Bruker AV 300 MHz in $CDCl_3$ using TMS as internal standard. Electron impact (EI) and chemical ionization mass spectra were recorded on a VG 7070 H instrument at 70 eV. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F_{254} (mesh); spots were visualized with UV light. Merck silica gel (60–120 mesh) was used for column chromatography. CHN analyses were recorded on a vario EL analyzer. AchE inhibitory activity was measured by Ellman's method and THLE-2 liver cell viability/toxicity was measured by MTT assay method.

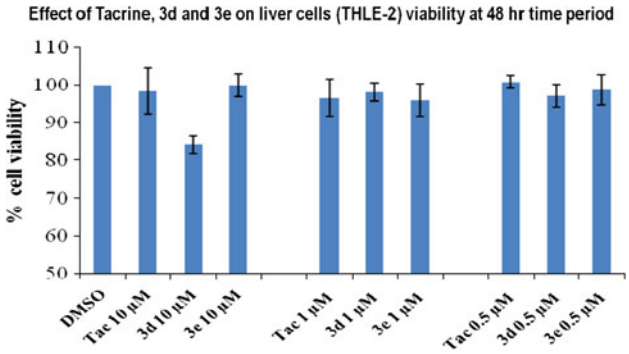


Fig. 1 THLE-2 liver cell viability/toxicity graph

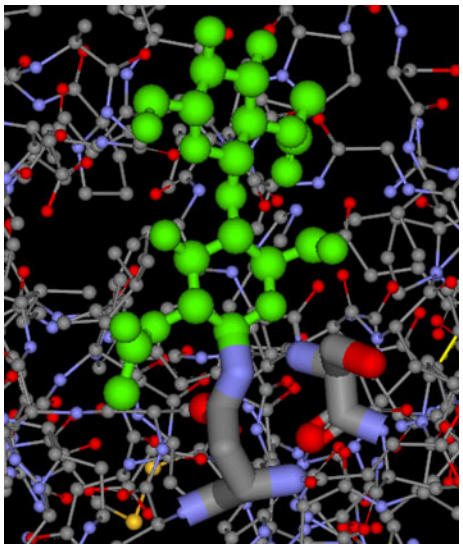


Fig. 2 The co-crystallized compound *N*-acetyl glucosamine (*green*) in the active site of human AChE. The key residues Ser347 and Asn350 are *highlighted* (Color figure online)

Preparation of 2-substituted-4-amino naphthyridine derivatives (**3a–i**) and 2,3-fused-5-amino naphthyridine derivatives (**4a–j**)

General procedure

The 2-amino-3-cyano-4-trifluoromethyl-6-phenylpyridine **2** (1.9 mmol) was reacted with cyclic ketones or

Table 4 THLE-2 liver cell viability/toxicity at 48 h time (%)

Compound No.	Micromolar concentration verses percentage of cell viability					
	0.5 μm	SD	1.0 μm	SD	10 μm	SD
Tacrine (standard)	100.73	1.67	96.59	5.00	98.45	6.13
3d	97.09	3.01	98.08	2.35	84.25	2.40
3e	98.66	4.01	95.92	4.35	99.94	2.94

SD standard deviation (values in percentage)

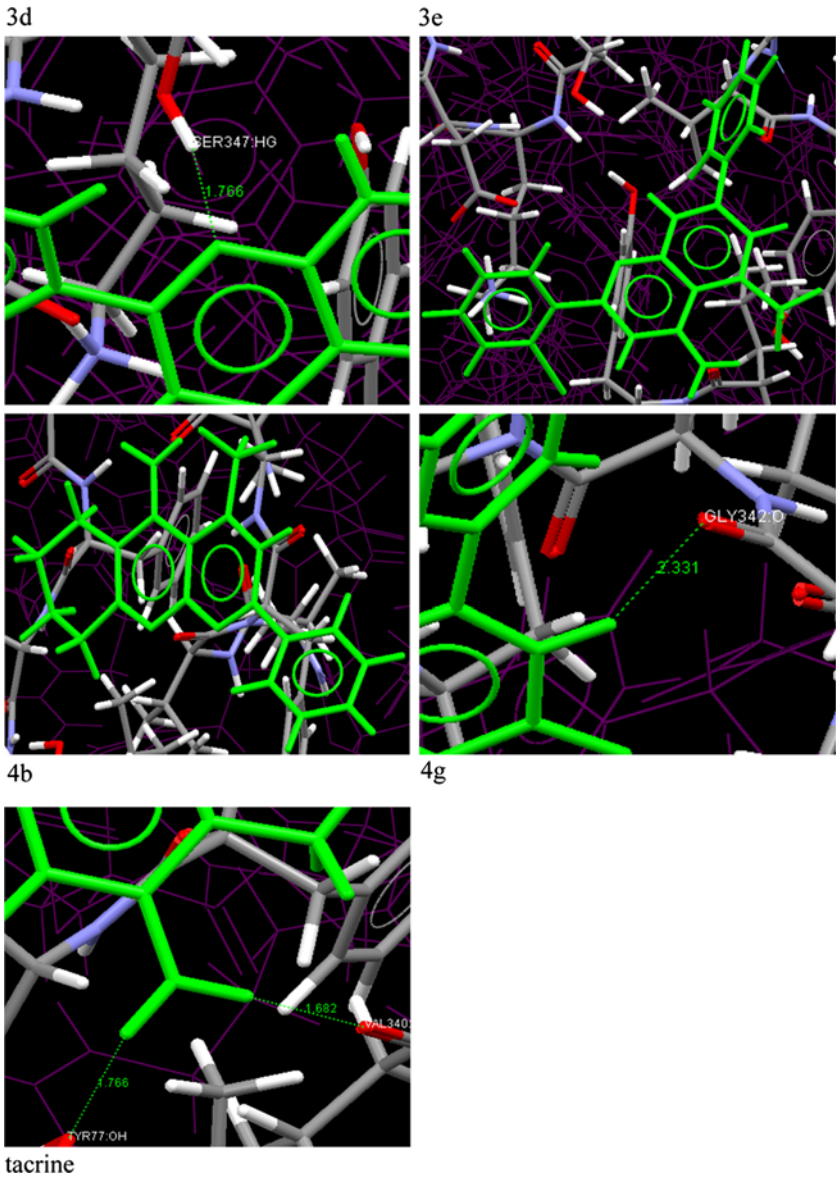


Fig. 3 Snapshots of the interaction of the ligands with human *AChE ligand* is show in *green*, the active site residues are *highlighted*, and the hydrogen bonds are shown in *green dotted lines* (Color figure online)

acetophenones (5.7 mmol) in presence of $ZnCl_2$ (0.73 mmol) under microwave irradiation conditions with 540 W power during 4–14 min and products were obtained in high yield. The product was purified by passing through a column packed with silica gel and *n*-hexane:ethylacetate (3:2) as eluents

*2,7-Diphenyl-5-(trifluoromethyl)-1,8-naphthyridin-4-amine (**3a**)* Yield: 0.65 g (93%); mp 203–205°C; I.R. (KBr, cm^{-1}): 3550, 3320 (–NH_2), 1652 (–C=N), 1323 (–C–F); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.4 (br., s, 2H, –NH_2), 7.18 (s, 1H, Ar–H–C(3)), 7.4–7.6 (m, 6H, Ar–H, Ar–H–C(6)), 8.1–8.2 (m, 3H, Ar–H), 8.3 (m, 2H, Ar–H); MS (EI, 70 eV): *m/z* 365 (M^+), 349 (M^+–NH_2), 296 (M^+–CF_3); Anal. Calcd. for $C_{21}H_{14}F_3N_3$: C, 69.04; H, 3.86; N, 11.50%. Found: C, 68.81; H, 3.76; N, 11.30%.

*2-(3-Fluorophenyl)-7-phenyl-5-(trifluoromethyl)-1,8-naphthyridin-4-amine (**3b**)* Yield: 0.69 g (95%); mp 210–212°C; I.R. (KBr, cm^{-1}): 3538, 3381 (–NH_2), 1634 (–C=N), 1323 (–C–F); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.38 (br., s, 2H, –NH_2), 7.10 (m, 2H, Ar–H), 7.4 (s, 1H, Ar–H–C(3)), 7.5 (m, 3H, Ar–H), 7.9 (m, 2H, Ar–H), 8.15 (s, 1H, Ar–H–C(6)), 8.3 (m, 2H, Ar–H); MS (EI, 70 eV): *m/z* 383 (M^+), 367 (M^+–NH_2), 314 (M^+–CF_3); Anal. Calcd. for $C_{21}H_{13}F_4N_3$: C, 65.80; H, 3.42; N, 10.96%. Found: C, 65.45; H, 3.32; N, 10.76%.

*2-(4-Fluorophenyl)-7-phenyl-5-(trifluoromethyl)-1,8-naphthyridin-4-amine (**3c**)* Yield: 0.70 g (96%); mp 204–206°C; I.R. (KBr, cm^{-1}): 3554, 3326 (–NH_2), 1657 (–C=N), 1327 (–C–F); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.30

Table 5 Docking results obtained with GOLD, indicating Fitness Score, its hydrogen bonding and Van der Waals components

Compound no.	Fitness	S (HB_ext)	S (vdw_ext)	S (int)	H-bond with residues	Close contacts with residues
Tacrine (**1**)	29.94	3.63	19.53	0.00	Tyr77,Val340	Tyr77
3a	29.89	0.17	24.73	−4.28	Phe346	Phe346,Val340
3b	15.16	0.03	23.95	−17.81	Phe346	Phe346, Asp349, Leu76
3c	14.70	0.69	23.39	−18.15	Ser347	Phe346, Asp349
3d	15.96	0.79	24.25	−18.17	Ser347	Phe346, Asp349
3e	18.78	0.00	26.27	−17.35	–	Leu76, Tyr77
3f	18.82	1.13	26.86	−19.23	Ser347, Leu76,Gly342	Phe346, Asp349,Ala343
3g	19.11	0.84	25.27	−16.47	Ser347	Ser347, Leu76
3h	15.30	0.00	23.71	−17.30	–	Leu76, Pro78
3i	3.84	0.00	22.53	−27.15	Gly345	Gly342,Phe346,Glu358
4a	12.46	0.00	23.70	−20.13	–	Val340
4b	11.30	0.00	25.57	−23.86	–	Val340, Gly342
4c	13.37	0.00	25.99	−23.36	–	Phe346, Gly345
4d	11.42	5.59	19.96	−21.61	Ser346	Val340, Phe346
4e	20.38	0.00	27.59	−17.56	–	Val340
4f	8.78	0.70	21.00	−20.80	Ser347	Phe346,Leu76
4g	12.45	0.00	24.54	−21.29	Gly342	–
4h	17.48	0.94	24.49	−17.13	Ser347	Phe346,Leu76
4i	13.53	0.00	24.99	−20.84	–	–
4j	15.73	0.00	27.34	−21.86	–	Phe346,Gly342,Val340

S (HB_ext) protein–ligand hydrogen bond energy, S (vdw_ext) protein–ligand Van der Waals (vdw) energy (external vdw), S (int) (internal torsion + internal vdw)

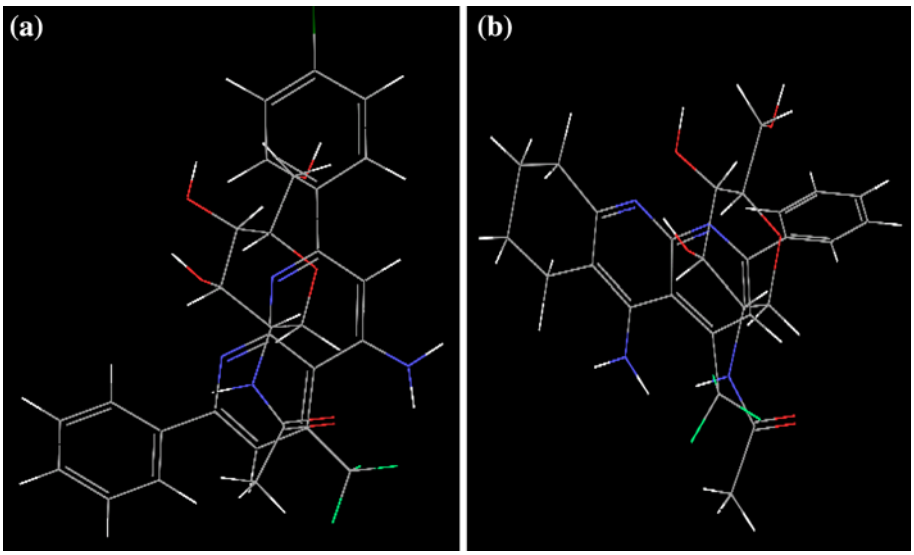


Fig. 4 Superposition of the synthesized drugs **a 3e** and **b 4b** with NAG

(br., s, 2H, $–NH_2$), 7.15–7.20 (m, 2H, Ar–H), 7.28 (s, 1H, Ar–H–C(3)), 7.5–7.6 (m, 3H, Ar–H), 8.10–8.20 (m, 3H, Ar–H), 8.35 (m, 2H, Ar–H); MS (EI, 70 eV): *m/z* 383 (M^+), 367 ($M^+–NH_2$), 314 ($M^+–CF_3$); Anal. Calcd. for $C_{21}H_{13}F_4N_3$: C, 65.80; H, 3.42; N, 10.96%. Found: C, 65.27; H, 3.32; N, 11.10%.

2-(4-Chlorophenyl)-7-phenyl-5-(trifluoromethyl)-1,8-naphthyridin-4-amine ***(3d)*** Yield: 0.69 g (92%); mp 218–220°C; I.R. (KBr, cm^{-1}): 3552, 3415 ($–NH_2$), 1655 (–C=N), 1326 (–C–F); ^1H NMR($CDCl_3$, 300 MHz): δ 5.35 (br., s, 2H, $–NH_2$), 7.18 (s, 1H Ar–H–C(3)), 7.4–7.6 (m, 5H Ar–H), 8.10–8.20 (m, 3H Ar–H), 8.3 (m, 2H Ar–H); MS

(EI, 70 eV): *m/z* 399 (M^+), 383 (M^+–NH_2), 364 (M^+–Cl), 330 (M^+–CF_3); Anal. Calcd. For $C_{21}H_{13}ClF_3N_3$: C, 63.09; H, 3.28; N, 10.51%. Found: C, 62.55; H, 3.20; N, 10.18%.

*2-(2-Bromophenyl)-7-phenyl-5-(trifluoromethyl)-1,8-naphthyridin-4-amine (**3e**)* Yield: 0.71 g (85%); mp 139–141°C; I.R. (KBr, cm^{-1}): 3552, 3325 (–NH_2), 1657 (–C=N), 1321(C–F); ^{1}H NMR($CDCl_3$, 300 MHz): δ 5.30 (br., s, 2H, –NH_2), 7.05 (m, 1H, Ar–H–C(3)), 7.25 (m, 1H, Ar–H), 7.4 (m, 1H, Ar–H), 7.5 (m, 3H, Ar–H), 7.65 (d, $J = 9.065$ Hz, 1H, Ar–H), 7.75 (dd, $J = 9.065$ Hz, 1H, Ar–H), 8.20 (s, 1H, Ar–H–C(6)), 8.3 (m, 2H, Ar–H); MS (EI, 70 eV): *m/z* 443 (M^+), 445 (M^++2), 427 (M^+–NH_2), 376 (M^+–CF_3), 364(M^+–Br); Anal. Calcd. for $C_{21}H_{13}BrF_3N_3$: C, 56.78; H, 2.95; N, 9.46%. Found: C, 56.55; H, 3.08; N, 9.82%.

*2-(4-Methoxyphenyl)-7-phenyl-5-(trifluoromethyl)-1,8-naphthyridin-4-amine (**3f**)* Yield: 0.60 g (80%); mp 194–196°C; I.R. (KBr, cm^{-1}): 3550, 3327 (–NH_2), 1657 (–C=N), 1328 (–C–F); ^{1}H NMR($CDCl_3$, 300 MHz): δ 3.85 (s, 3H, –OCH_3), 5.25 (br., s, 2H, –NH_2), 6.95 (d, $J = 8.687$ Hz, 2H, Ar–H), 7.15 (s, 1H, Ar–H–C(3)), 7.5 (m, 3H, Ar–H), 8.1–8.2 (m, 3H, Ar–H), 8.3 (m, 2H, Ar–H); MS (EI, 70 eV): *m/z* 395 (M^+), 379 (M^+–NH_2), 364 (M^+–OCH_3), 326 (M^+–CF_3); Anal. Calcd. for $C_{22}H_{16}F_3N_3O$: C, 66.83; H, 4.08; N, 10.63%. Found: C, 66.43; H, 4.00; N, 10.45%.

*7-Phenyl-2-(thiophin-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-amine (**3g**)* Yield: 0.63 g (90%); mp 219–221°C; I.R. (KBr, cm^{-1}): 3550, 3333 (–NH_2), 1650 (–C=N), 1326 (–C–F); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.25 (br., s, 2H, –NH_2), 7.1 (m, 2H, thiophine-H), 7.5 (m, 4H, Ar–H), 7.78 (d, $J = 3.588$ Hz, 1H, thiophine-H), 8.1 (s, 1H, Ar–H–C(6)), 8.3 (m, 2H Ar–H); MS (EI, 70 eV): *m/z* 371 (M^+), 302 (M^+–CF_3); Anal. Calcd. for $C_{19}H_{12}F_3N_3S$: C, 61.45; H, 3.26; N, 11.31%. Found: C, 61.15; H, 3.19; N, 11.09%.

*2-Isobutyl-7-phenyl-5-(trifluoromethyl)-1,8-naphthyridin-4-amine (**3h**)* Yield: 0.40 g (61%); mp 151–153°C; I.R. (KBr, cm^{-1}): 3556, 3315 (–NH_2), 1661 (–C=N), 1322 (–C–F); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.0 (d, $J = 6.610$ Hz, 6H, –$2CH_3$), 2.3 (m, 1H, –CH), 2.75 (d, $J = 7.176$ Hz, 2H, –CH_2), 5.25 (br., s, 2H, –NH_2), 6.65 (s, 1H, Ar–H–C(3)), 7.5 (m, 3H, Ar–H), 8.15 (s, 1H, Ar–H–C(6)), 8.3 (m, 2H, Ar–H); MS (EI, 70 eV): *m/z* 345 (M^+), 330 (M^+–CH_3), 275 (M^+–CF_3); Anal. Calcd. For $C_{19}H_{18}F_3N_3$: C, 66.08; H, 5.25; N, 12.17%. Found: C, 65.75; H, 5.19; N, 12.01%.

*2-Isopropyl-3-methyl-7-phenyl-5-(trifluoromethyl)-1,8-naphthyridin-4-amine (**3i**)* Yield: 0.08 g (12%); mp 150–152°C; I.R. (KBr, cm^{-1}): 3551, 3312 (–NH_2), 1620 (–C=N), 1321 (–C–F); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.5 (d, $J = 7.365$ Hz, 6H, –$2CH_3$), 2.8 (s, 3H, –CH_3), 3.6 (m, 1H, –CH), 5.25 (br., s, 2H, –NH_2), 7.5 (m, 3H, Ar–H), 8.15 (s, 1H, Ar–H–C(6)), 8.3 (m, 2H, Ar–H); MS (EI, 70 eV): *m/z* 345(M^+), 330 (M^+–CH_3), 275 (M^+–CF_3); Anal. Calcd. for $C_{19}H_{18}F_3N_3$: C, 66.08; H, 5.25; N, 12.17%. Found: C, 65.89; H, 5.15; N, 12.10%.

*2-Phenyl-4-(trifluoromethyl)-7,8-dihydro-6H-cyclopenta[b][1,8]naphthyridin-5-amine (**4a**)* Yield: 0.53 g (85%); mp 208–210°C; I.R. (KBr, cm^{-1}): 3540, 3339 (–NH_2), 1661 (–C=N), 1351 (–C–F); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.38 (m, 2H, –CH_2–), 2.98 (t, $J = 7.554$ Hz, 2H, –CH_2–), 3.25 (t, $J = 7.743$ Hz, 2H, –CH_2–), 4.95 (br., s, 2H, –NH_2), 7.5 (m, 3H, Ar–H), 8.15 (s, 1H, Pyridine-H), 8.3–8.4 (m, 2H, Ar–H); MS (EI, 70 eV): *m/z* 329 (M^+), 260 (M^+–CF_3); Anal. Calcd. for $C_{18}H_{14}F_3N_3$: C, 65.65; H, 4.28; N, 12.76%. Found: C, 65.68; H, 4.15; N, 12.56%.

*2-Phenyl-4-(trifluoromethyl)-6,7,8,9-tetrahydrobenzo[b][1,8]-naphthyridin-5-amine (**4b**)* Yield: 0.64 g (98%); mp 202–204°C; I.R. (KBr, cm^{-1}): 3468, 3386 (–NH_2), 1624 (–C=N), 1323 (–C–F); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.0 (m, 5H, –CH_2-), 2.5–2.7 (m, 3H, –CH_2–), 5.7 (br., s, 2H, –NH_2), 7.5 (m, 3H, Ar–H), 8.1 (s, 1H, Pyridine-H), 8.3 (m, 2H, Ar–H); MS (EI, 70 eV): *m/z* 343 (M^+), 274 (M^+–CF_3); Anal. Calcd. For $C_{19}H_{16}F_3N_3$: C, 66.46; H, 4.70; N, 12.24%. Found: C, 65.80; H, 4.50; N, 12.04%.

*2-Phenyl-4-(trifluoromethyl)-7,8,9,10-tetrahydro-6H-cyclohepta[b][1,8]-naphthyridin-5-amine (**4c**)* Yield: 0.61 g (90%); mp 210–212°C; I.R. (KBr, cm^{-1}): 3534, 3334 (–NH_2), 1671 (–C=N), 1324 (–C–F); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.8–2.0 (m, 6H, –CH_2–), 2.8–2.9 (m, 2H, –CH_2–), 3.3 (m, 2H–CH_2–), 5.1 (br., s, 2H, –NH_2), 7.5–7.6 (m, 3H, Ar–H), 8.15 (s, 1H, Pyridine-H), 8.3 (m, 2H, Ar–H); MS (EI, 70 eV): *m/z* 357 (M^+), 342 (M^+–NH_2), 288 (M^+–CF_3); Anal. Calcd. for $C_{20}H_{18}F_3N_3$: C, 67.22; H, 5.08, N, 11.76%. Found: C, 67.30; H, 5.10; N, 11.46%.

*2-Phenyl-4-(trifluoromethyl)-6,7,8,9,10,11-hexahydrocycloocta[b][1,8]-naphthyridin-5-amine (**4d**)* Yield: 0.63 g (90%); mp 208–210°C; I.R. (KBr, cm^{-1}): 3561, 3328 (–NH_2), 1657 (–C=N), 1329 (–C–F); ^{1}H NMR($CDCl_3$, 300 MHz): δ 1.4–2.0 (m, 8H, –CH_2-), 2.9–3.0 (t, $J = 6.592$ Hz, 2H, –CH_2–), 3.2 (t, $J = 6.592$ Hz, 2H, –CH_2–), 5.1 (br., s, 2H, –NH_2), 7.5 (m, 3H, Ar–H), 8.15 (s, 1H, Pyridine-H), 8.3 (m, 2H, Ar–H); MS (EI, 70 eV): *m/z* 371 (M^+), 356 (M^+–NH_2), 302 (M^+–CF_3); Anal. Calcd. For $C_{21}H_{20}F_3N_3$: C, 67.91; H, 5.43; N, 11.31%. Found: C, 67.51; H, 5.33; N, 11.15%.

7-Methyl-2-phenyl-4-(trifluoromethyl)-7,8-dihydro-6H-cyclopenta[b][1,8]-naphthyridin-5-amine ***(4e)*** Yield: 0.58 g (90%); mp 148–150°C; I.R. (KBr, cm^{-1}): 3524, 3420 ($–NH_2$), 1648 (–C=N), 1328 (–C–F); ^1H NMR($CDCl_3$, 300 MHz): δ 1.25 (d, $J = 6.830$ Hz, 3H, $–CH_3$), 2.47–2.53 (dd, $J = 5.855$ Hz, $J = 6.830$ Hz, 1H, –CH-), 2.7–2.8 (m, 1H, –CH–), 2.8–2.9 (dd, $J = 6.830$ Hz, $J = 7.806$ Hz, 1H, –CH–), 3.05–3.10 (dd, $J = 7.806$ Hz, $J = 8.782$ Hz, 1H, CH–), 3.35–3.42 (dd, $J = 7.806$ Hz, $J = 8.782$ Hz, 1H, –CH–), 4.9 (br., s, 2H, $–NH_2$), 7.45–7.55 (m, 3H, Ar–H), 8.15 (s, 1H, Pyridine-H), 8.25–8.35 (m, 2H, Ar–H); MS (EI, 70 eV): *m/z* 343 (M^+), 328 ($M^+–CH_3$), 274 ($M^+–CF_3$); Anal. Calcd. For $C_{19}H_{16}F_3N_3$: C, 66.46; H, 4.70; N, 12.24%. Found: C, 66.50; H, 4.58; N, 12.07%.

8-Methyl-2-phenyl-4-(trifluoromethyl)-6,7,8,9-tetrahydrobenzo[b][1,8]-naphthyridin-5-amine ***(4f)*** Yield: 0.66 g (98%); mp 160–162°C; I.R. (KBr, cm^{-1}): 3502, 3404 ($–NH_2$), 1625 (–C=N), 1317 (–C–F); ^1H NMR ($CDCl_3$, 300 MHz): δ 1.1 (d, $J = 6.043$ Hz, 3H, $–CH_3$), 1.9–2.1 (m, 2Hm, $–CH_2–$), 2.5–2.7 (m, 4H, $–CH_2–$), 3.2–3.3 (dd, $J = 1.511$ Hz, $J = 2.266$ Hz, 1H, –CH–), 5.35 (br., s, 2H, $–NH_2$), 7.4–7.5 (m, 3H, Ar–H), 8.1 (s, 1H, Pyridine-H), 8.2–8.3 (m, 2H, Ar–H); MS (EI, 70 eV): *m/z* 357 (M^+), 342 ($M^+–CH_3$), 288 ($M^+–CF_3$); Anal. Calcd. for $C_{20}H_{18}F_3N_3$: C, 67.22; H, 5.08, N, 11.76%. Found: C, 67.30; H, 4.99; N, 11.46%.

7-Methyl-2-phenyl-4-(trifluoromethyl)-6,7,8,9-tetrahydrobenzo[b][1,8]-naphthyridin-5-amine ***(4g)*** Yield: 0.66 g (98%); mp 200–202°C; I.R. (KBr, cm^{-1}): 3508, 3420 ($–NH_2$), 1638 (–C=N), 1327 (–C–F); ^1H NMR($CDCl_3$, 300 MHz): δ 1.2 (d, $J = 6.043$ Hz, 3H, $–CH_3$), 2.0–2.2 (m, 4H, $–CH_2–$), 2.68–2.74 (dd, $J = 3.777$ Hz, $J = 5.288$ Hz, 1H, –CH–), 3.0–3.1 (m, 1H, –CH–), 3.2–3.3 (m, 1H, –CH–), 5.1 (br., s, 2H, $–NH_2$), 7.4–7.5 (m, 3H, Ar–H), 8.1 (s, 1H, Pyridine-H), 8.2–8.3 (m, 2H, Ar–H); MS (EI, 70 eV): *m/z* 357 (M^+), 342 ($M^+–CH_3$), 288 ($M^+–CF_3$); Anal. Calcd. For $C_{20}H_{18}F_3N_3$: C, 67.22; H, 5.08; N, 11.76%. Found: C, 67.28; H, 4.99; N 11.46%.

2-Phenyl-4-(trifluoromethyl)-6H-indeno[1,2-b][1,8]-naphthyridin-5-amine ***(4h)*** Yield: 0.61 g (85%); mp 145–147°C; I.R. (KBr, cm^{-1}): 3520, 3424 ($–NH_2$), 1664 (–C=N), 1344 (–C–F); ^1H NMR ($CDCl_3$, 300 MHz): δ 4.0 (s, 2H, $–CH_2–$), 6.35 (br., s, 2H, $–NH_2$), 7.3–7.4 (m, 1H, Ar–H), 7.45–7.55 (m, 2H, Ar–H), 7.6–7.7 (m, 3H, Ar–H), 7.9 (d, $J = 7.365$ Hz, 1H, Ar–H), 8.2 (s, 1H, Pyridine-H), 8.3–8.4 (m, 2H, Ar–H); MS (EI, 70 eV): *m/z* 377 (M^+), 308 ($M^+–CF_3$); Anal. Calcd. For $C_{22}H_{14}F_3N_3$: C, 70.02; H, 3.74; N, 11.14%. Found: C, 69.68; H, 3.65; N, 11.01%.

10-Phenyl-8-(trifluoromethyl)-5,6-dihydronaphtho[1,2-b][1,8]-naphthyridin-5-amine ***(4i)*** Yield: 0.59 g (80%); mp 150–152°C; I.R. (KBr, cm^{-1}): 3526, 3425 ($–NH_2$), 1641 (–C=N), 1342 (–C–F); ^1H NMR ($CDCl_3$, 300 MHz): δ 2.9 (t, $J = 6.987$ Hz, 2H, $–CH_2–$), 3.1 (t, $J = 6.987$ Hz, 2H, $–CH_2–$), 5.2 (br., s, 2H, $–NH_2$), 7.15 (m, 1H, Ar–H), 7.25–7.35 (m, 2H, Ar–H), 7.50–7.55 (m, 3H, Ar–H), 8.15 (s, 1H, Pyridine-H), 8.2–8.3 (m, 2H, Ar–H), 8.6–8.7 (m, 1H, Ar–H); MS (EI, 70 eV): *m/z* 391 (M^+), 322 ($M^+–CF_3$); Anal. Calcd. for $C_{23}H_{16}F_3N_3$: C, 70.58; H, 4.12; N, 10.74%. Found: C, 70.38; H, 4.02; N, 10.64%.

9-Phenyl-11-(trifluoromethyl)-5,6-dihydronaphtho[2,1-b][1,8]-naphthyridin-5-amine ***(4j)*** Yield: 0.63 g (85%); mp 160–162°C; I.R. (KBr, cm^{-1}): 3522, 3421 ($–NH_2$), 1642 (–C=N), 1344 (–C–F); ^1H NMR($CDCl_3$, 300 MHz): δ 3.0(t, $J = 6.421$ Hz, 2H, $–CH_2–$), 3.3 (t, $J = 6.421$ Hz, 2H, $–CH_2–$), 5.3 (br., s, 2H, $–NH_2$), 7.2 (m, 1H, Ar–H), 7.3–7.4 (m, 2H, Ar–H), 7.45–7.55(m., 3H, Ar–H), 8.1 (s, 1H, Pyridine-H), 8.2–8.3 (m, 2H, Ar–H), 8.6–8.7 (m, 1H, Ar–H); MS (EI, 70 eV): *m/z* 391 (M^+), 322 ($M^+–CF_3$); Anal. Calcd. for $C_{23}H_{16}F_3N_3$: C, 70.58; H, 4.12; N, 10.74%. Found: C, 70.28; H, 4.10; N, 10.54%.

AChE inhibitory activity

Acetylcholine esterase (AChE) inhibitory activity was measured according to Ellman *et al*. (1961). AChE (5 mU) in 10 mM phosphate buffer at pH 7.4 was incubated with 200 µl of 100 mM phosphate buffer in the presence or absence of test compounds (0.1–10 µM) along with 0.8 mM acetylcholine iodide and 10 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) at 37°C and continuous absorbance was measured for 10 min at an interval of every 30 s at 412 nm using Tecan multimode reader Infinite M200 series.

THLE-2 liver cell viability/toxicity assay

To assess the cytotoxic effects of compound **3d**, **3e**, and Tacrine, normal liver cells (THLE-2) from ATCC were grown in bronchial epithelial growth medium bullet kit (Lonza) and treated with 0–10 µM of either Tacrine, compound **3d** or **3e** for a period of 48 h and cytotoxicity was measured by standard MTT assay. At the end of the treatments, medium was removed and cells were washed with Dulbecco's phosphate buffered saline (DPBS) and 10 µl of 5 mg/ml MTT solution in 200 µl of culture medium was added and incubated for 1 h at 37°C. Cells were solubilized with 200 µl of DMSO and absorbance was measured at 562 nm in a spectrophotometer.

Molecular modelling studies

To study the mode of interaction of the compounds with Human AChE, the compounds were docked to the protein using the GOLD 3.2 (Genetic Optimization for Ligand Docking) software. The following steps were followed for this study.

Protein and ligand preparation

The crystal structure of human acetylcholinesterase (1F8U) was obtained from Protein Data Bank and the active site was analyzed using PDBSum. The residues Asn350 and Ser347 found to be the key residues of the active site. The protein was prepared for docking study using the Protein Preparation Wizard of Maestro (Glide *et al*., 2008). Water molecules were removed, hydrogen was added, and the protein was minimized (only Hydrogen) using OPLS 2001 force field. The synthesized compounds along with the reference compound (Tacrine) was constructed and prepared for docking using the LigPrep Protocol of Maestro. Compound minimization was done using OPLS 2005 Force field. The protein and compound preparation by docking was done as per the earlier procedure (Srivani and Sastry, 2009).

Docking

The minimized protein and compounds was uploaded to GOLD 3.2 for docking. The active site radius was set to 10 Å from the atom number 5105, the Alpha Carbon Atom of one of the active site residue Ser347. All the default values for annealing parameters (Vander Waals = 4.0, H-bonding = 2.5) and Genetic Algorithm Parameters (population size = 100, selection pressure = 1.1, no. of operations = 10,000, no. of Islands = 5, niche size = 2, migrate = 10, mutate = 95, crossover = 95) were used for docking.

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