**Screening and identification of novel small molecule inhibitors against *Mycobacterium tuberculosis* Dihydrodipicolinate synthase enzyme using *in silico* and *in vitro* methods**

Anasuya Bhargav#1,2, Pratibha Chaurasia#1,2, Nikita V. Ivanisenko3, Vladimir A. Ivanisenko3, Bhupesh Taneja1,2, Srinivasan Ramachandran1,2\*

#equal contributors

\* corresponding author

1Council of Scientific and Industrial Research – Institute of Genomics and Integrative Biology (CSIR-IGIB), Mathura Road, New Delhi, 110025 India

2Academy of Scientific and Innovative Research (AcSIR), Ghaziabad- 201002, India

3Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia

Table S1: PubChem compounds shortlisted after AutoDock Vina docking (≥90% structure similarity to αKPA)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S. No.** | **PubChem ID** | **IUPAC Name** | **Binding Energy (kcal/mol)** | **Binding Residues** |
| **1** | 14155511  | 2,11-dioxododecanedioic acid  | -5.7  | ARG148, THR54, VAL113, GLY88, GLN278  |
| **2** | 54417948  | 2-oxotetradecanedioic acid  | -5.6  | ARG148, THR54, VAL113, GLY88, GLN278  |
| **3** | 21430879  | 2,10-dioxotridecanoic acid  | -5.5  | ARG148, THR54, GLY88  |
| **4** | 20227615  | 2-oxodecanedioic acid  | -5.3  | ARG148, TYR117, THR54  |
| **5** | 54438249  | 2,6-dioxoheptanoic acid  | -5.3  | THR54, GLY88  |
| **6** | 146903  | 2-oxooctanedioic acid  | -4.8  | LYS171, ARG148  |
| **7** | 25203457  | 2,6-dioxoheptanedioic acid  | -4.6  | LYS171, ARG148, GLY256  |
| **8** | 21611092  | 2-oxoundecanedioic acid  | -4.6  | LYS171, ARG148  |
| **9** | 13401222  | 2-oxononanedioic acid  | -4.5  | LYS171, ARG148  |
| **10** | 54219385  | 2,14-dioxopentadecanedioic acid  | -4.3  | GLY256, ARG148  |

Table S2: Top hits from PubChem shortlisted after AutoDock Vina docking (≥70% structure similarity to αKPA)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S. No.** | **PubChem ID** | **IUPAC Name** | **Binding Energy (kcal/mol)** | **Binding Residues** |
| **1** | 7177061  | 3-[(1R,3S)-3-(2-carboxyethyl)-2-oxocyclododecyl]propanoic acid  | -7.1  | ARG148, GLN278  |
| **2** | 18405634  | 5-cyclohexyl-2,3-dioxopentanoic acid  | -7  | THR54, GLY88, VAL113  |
| **3** | 90306765  | 2-[2-[(2R)-2-carboxycyclobutyl]ethyl]-6-oxoheptanedioic acid  | -6.7  | THR54, GLY88, VAL113, LEU111  |
| **4** | 122488915  | 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10-octadecafluoro-11-oxododecanoic acid  | -6.7  | ARG148, GLY88  |
| **5** | 91083884  | 3,3,4,4,5,5,6,6,7,7,8,8-dodecafluoro-2,9-dioxodecanedioic acid  | -6.6  | LYS171, ARG148  |
| **6** | 7657659  | (14S)-14-hydroxy-oxacyclohexadecan-2-one  | -6.6  | ARG148  |
| **7** | 40489622  | (2S)-2-hydroxycyclohexadecan-1-one  | -6.4  | ARG148  |
| **8** | 130483793  | 3-cyclooctyl-2-oxopropanoic acid  | -6.4  | THR54, GLY88  |
| **9** | 127020953  | 2-(4-methylcyclohexyl)-2-oxoacetic acid  | -6.3  | THR54, GLY88  |
| **10** | 21389374  | octadeca-8,10-diynedioic acid  | -6.2  | THR54, LYS66  |
| **11** | 57039662  | 1,2,3,4-tetraoxacyclotridecane-5,13-dione  | -6.2  | ARG148, GLY256  |
| **12** | 87284940  | 5-ethyl-6-methyl-2-oxohept-5-enoic acid  | -6.2  | SER52, THR54, GLY88  |
| **13** | 88383919  | 2-oxoicosa-5,8,11-triynoic acid  | -6.2  | THR54, GLY88, VAL113  |
| **14** | 356550  | 3,3,4,4,5,5,6,6-octafluoro-2,7-dihydroxyoctanedioic acid  | -6.1  | ARG148, GLY194  |
| **15** | 57564954  | 9,9,10,10,11,11,11-heptafluoro-6,8-dioxoundecanoic acid  | -6.1  | ARG148, GLY88  |
| **16** | 122488916  | 2,2,3,3,4,4,5,5-octafluoro-6-oxoheptanoic acid  | -6.1  | ARG148, GLY88  |
| **17** | 92151054  | (2R,7R)-3,3,4,4,5,5,6,6-octafluoro-2,7-dihydroxyoctanedioic acid  | -6.1  | LYS171, ARG148  |
| **18** | 131062031  | 3-(2,2-difluorocyclopentyl)-2-oxopropanoic acid  | -6.1  | THR54, GLY88  |
| **19** | 87917981  | 2,2-dihydroxy-16-methylheptadecanoic acid  | -6  | THR54, GLY88, VAL113  |
| **20** | 88655447  | 1,3-dihydroxypropan-2-yl 2-hydroxydodecanoate  | -6  | THR54, GLY88  |
| **21** | 18405541  | 6,6-dimethyl-2,3-dioxoheptanoic acid  | -6  | THR54, GLY88  |

Table S3: Compounds with tPSA 91.7Å2 PubChem shortlisted after AutoDock Vina docking (≥70% structure similarity to αKPA)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S. No.** | **PubChem ID** | **IUPAC Name** | **Binding Energy (kcal/mol)** | **Binding Residues** |
| **1** | 91257869  | 2,2-dimethyl-7-oxotridecanedioic acid  | -5.8  | GLY88, ARG148, GLN278  |
| **2** | 88089680  | 9-hydroxy-2,4-dioxononanoic acid  | -5.7  | THR54, SER52, GLY88  |
| **3** | 54423609  | 3,3-dihydroxyoctane-2,4,7-trione  | -5.5  | THR54, GLY88, SER52  |
| **4** | 88813284  | 2-acetylnonanedioic acid  | -5.5  | SER52, GLY88, ARG148  |
| **5** | 89661585  | 5,5,7,7-tetramethyl-2-oxononanedioic acid  | -5.5  | GLY88, ARG148, GLN278  |
| **6** | 12659740  | 4-acetyl-4-propylheptanedioic acid  | -5.4  | ARG148, GLN278  |
| **7** | 129988663  | 4-(carboxymethyl)-3-oxocycloheptane-1-carboxylic acid  | -5.4  | LYS171, ARG148  |
| **8** | 19907572  | 2,2-dimethyl-4-oxoheptanedioic acid  | -5.3  | ARG148, GLY88  |
| **9** | 21328269  | 2-acetylheptanedioic acid  | -5.3  | THR54, ARG148  |
| **10** | 88626271  | 2-acetyloctanedioic acid  | -5.3  | ARG148, GLY88  |
| **11** | 100975356  | 3-acetylnonanedioic acid  | -5.3  | GLY88, VAL113, ARG148  |
| **12** | 407651  | 4-acetyl-4-methylheptanedioic acid  | -5.2  | ARG148, GLN278  |
| **13** | 12527757  | 4-oxononanedioic acid  | -5.2  | GLY88, VAL113, ARG148  |
| **14** | 124003902  | 5-methyl-2-oxoheptanedioic acid  | -5.2  | ARG148, GLY88  |
| **15** | 12659739  | 4-acetyl-4-ethylheptanedioic acid  | -5.2  | ARG148, GLN278  |
| **16** | 13213508  | 4-oxododecanedioic acid  | -5.2  | ARG148, THR54  |
| **17** | 100975361  | 5-acetylundecanedioic acid  | -5.2  | ARG148, GLN278  |
| **18** | 101381692  | (1R,2R)-2-oxalocyclobutane-1-carboxylic acid  | -5.2  | LYS171, GLY194, ASP196  |
| **19** | 4575238  | 7-oxotridecanedioic acid  | -5.1  | THR54, SER52  |
| **20** | 11680217  | 2-oxo-4-pentylpentanedioic acid  | -5.1  | ARG148, LYS171, ASP196  |
| **21** | 13099240  | 5-oxododecanedioic acid  | -5.1  | ARG148, THR54  |
| **22** | 13854723  | 3-oxononanedioic acid  | -5  | GLY88, ARG148  |
| **23** | 14178864  | 3-oxooctanedioic acid  | -5  | THR54, GLY88  |
| **24** | 20290370  | 3-methyl-5-oxododecanedioic acid  | -5  | THR54, GLY88, ARG148  |
| **25** | 22902904  | 4-methyl-4-phosphorosoheptanedioic acid  | -5  | ARG148, GLN278  |
| **26** | 53928652  | 4-acetylnonanedioic acid  | -5  | THR54, GLY88  |
| **27** | 19907645  | 3-methyl-4-oxoheptanedioic acid  | -4.9  | ARG148, LYS171, ASP196  |
| **28** | 20358597  | 4-oxopentadecanedioic acid  | -4.9  | THR54, GLY88, GLN278  |
| **29** | 56978329  | 2-(2-ethylbutyl)-3-oxobutanedioic acid  | -4.9  | ARG148, ASP196  |
| **30** | 71309423  | dideuterio 2,2,3,3-tetradeuterio-4-oxopentanedioate  | -4.9  | THR54, GLY88, VAL113  |
| **31** | 87265194  | 2,2-diethyl-4-oxoheptanedioic acid  | -4.9  | ARG148, TYR117  |
| **32** | 10608739  | 5-oxodecanedioic acid  | -4.9  | GLY88, TYR117, ARG148  |
| **33** | 95084  | 4-oxoheptanedioic acid  | -4.8  | THR54, GLY88  |
| **34** | 20513076  | 2-octanoylpropanedioic acid  | -4.8  | ARG148, LYS171, GLY256  |
| **35** | 44150455  | 6-hydroxy-2,5-dioxohexanoic acid  | -4.8  | THR54, GLY88  |
| **36** | 91617210  | 4-hydroxy-2,3-dioxoheptanoic acid  | -4.8  | ARG148(2)  |
| **37** | 121220391  | 2,2,6,6-tetramethyl-4-oxoheptanedioic acid  | -4.8  | ARG148(2)  |
| **38** | 129809554  | 3,7-dimethyl-5-oxononanedioic acid  | -4.8  | ARG148, ALA173  |
| **39** | 93  | 3-oxohexanedioic acid  | -4.7  | ARG148, LYS171, ASP196  |
| **40** | 7313552  | 4-oxooctanedioic acid  | -4.6  | LYS171, ARG148  |
| **41** | 87517378  | 2,2-dimethyl-3-oxooctanedioic acid  | -4.6  | LYS171, ARG148  |
| **42** | 102113595  | 5-hydroxy-4,6-dioxoheptanoic acid  | -4.6  | ARG148, LYS171, GLY194  |
| **43** | 91971604  | 2,2-dideuterio-4-oxo(4,5-13C2)pentanedioic acid  | -4.5  | ARG148, LYS171, ASP196  |
| **44** | 88056543  | 2-deuterio-4-oxopentanedioic acid  | -4.3  | ARG148, LYS171, ASP196, GLY194  |
| **45** | 34179191  | 8-oxopentadecanedioic acid  | -4.1  | ARG148, GLN278  |
| **46** | 118388412  | 6-hydroxy-3,5-dioxohexanoic acid  | -4.1  | ARG148, LYS171  |

Table S4: Compounds shortlisted from ZINC database after AutoDock Vina docking (≥90% structure similarity to αKPA)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S. No.** | **ZINC ID** | **Popular Name** | **Binding Energy (kcal/mol)** | **Binding Residues** |
| **1** | ZINC00000012  | Adrafinil  | -6.5  | ARG148, GLN278  |
| **2** | ZINC85114010  | (3S)-4-amino-3-(4-chlorophenyl)-3-methyl-butanoic  | -5.5  | ARG148  |
| **3** | ZINC85114111  | (3S)-3-(aminomethyl)-3-(4-chlorophenyl)pentanoic  | -5.5  | ARG148  |
| **4** | ZINC00000038  | Amylocaine Hydrochloride  | -5.3  | ARG148  |

Table S5: Cross validation of selected compounds

|  |
| --- |
| **Control Dataset**  |
| **IUPAC Name**  | **XP Score**  | **% Inhibition**  |
| 2-oxoheptanedioic acid (α –KPA)  | -8.69031  | 88  |
| 2-oxohexanedioic acid  | -8.7216  | 40  |
| 2-oxopentanedioic acid  | -6.91423  | 15  |
| heptanedioic acid  | -6.65098  | 10  |
| Benzoic acid  | -5.05795  | 2  |
| p-amino benzoic acid  | -5.53097  | 8  |
| Methyl 3-oxohexanoate  | -2.97933  | 5  |
| 4-amino-2-hydroxy-benzoic acid  | -6.36964  | 10  |
| 2-phenoxyacetic acid  | -5.26183  | 4  |
| 5-butylpyridine-2-carboxylic acid  | -5.53599  | 5  |
| hydroxyheptanedioic acid  | -7.4505  | 74  |
| Methyl-oxo-ureidopentanoate  | -4.29451  | 65  |
| 5-(Carbamoylthio)pentanoic acid  | -6.11416  | 65  |
| amino-oxobutyl-methyl-amino oxoacetic acid  | -7.05484  | 44  |
| methyl-ethoxy-methyloxoacetamido-butanoate  | -3.05686  | 42  |
| oxo-ureidopentanoic acid  | -6.09969  | 10  |
| amino-oxobutylmethyloxalamide  | -4.29069  | 40  |
| carboxy-methylformamido butanoic acid  | -7.15949  | 39  |
| methyl-trioxoimidazolidin –butanoate  | -5.60094  | 38  |
| Ethoxy-dioxoheptanoic acid  | -5.21096  | 35  |
| methyl-oxo-tetrazol-hexanoate  | -2.18759  | 35  |
| Methyl-amino-oxoacetylbenzoate  | -3.50845  | 35  |
| Carboxy-hydroxy-methyl benzoic acid  | -7.33386  | 35  |
| Hydroxy-methoxycarbonyl pheny acetic acid  | -6.04421  | 35  |
| Ethyl-amino-oxobutyl-methylamino-oxoacetate  | -3.09054  | 35  |
| amino-oxoacetyl-benzoic acid  | -5.31471  | 34  |
| ethyl-carbamoylthio-pentanoate  | -2.1541  | 34  |
| Ethyl 4-((2-amino-2-oxoethyl)sulfinyl)butanoate  | -3.37479  | 31  |
| methoxycarbonyl-phenyl oxoacetic acid  | -7.17442  | 29  |
| 4-((2-amino-2-oxoethyl)sulfonyl) butanoic acid  | -6.24893  | 29  |
| Methyl 7-amino-6-hydroxy-7-oxoheptanoate  | -4.78141  | 28  |
| methyl 7-amino-6,7-dioxoheptanoate  | -3.14368  | 24  |
| carboxycarbonyl-benzoic acid  | -7.21914  | 35  |
| Ethyl 4-((2-amino-2-oxoethyl)thio)butanoate  | -2.63379  | 21  |
| 2-Hydroxyheptanediamide  | -4.60577  | 21  |
| 4-(2,4,5-Trioxoimidazolidin-1-yl)butanoic acid  | -5.96423  | 21  |
| 2-(4-Carbamoylphenyl)-2-oxoacetic acid  | -7.07315  | 15  |
| 4-((2-Amino-2-oxoethyl)thio)butanoic acid  | -5.79799  | 15  |
| Ethyl 4-((2-amino-2-oxoethyl)sulfonyl)butanoate  | -3.92565  | 12  |
| **Shortlisted Compounds**  |
| **IUPAC Name**  | **XP Score**  | **IC50**  |
| 2-oxo-4-pentylpentanedioic acid  | -8.78965  | NA  |
| 2,6-dioxoheptanedioic acid  | -8.48284  | NA  |
| 2,2-dideuterio-4-oxopentanedioic acid  | -7.9228  | NA  |
| 2-[2-[(2R)-2-carboxycyclobutyl]ethyl]-6-oxoheptanedioic acid  | -7.3082  | NA  |
| 3-methyl-4-oxoheptanedioic acid  | -6.97604  | NA  |
| 2-oxotetradecane dioic acid  | -6.40633  | NA  |
| 8-oxopentadecanedioic acid  | -6.17459  | NA  |
| 3,7-dimethyl-5-oxononanedioic acid  | -5.98559  | NA  |
| 3-oxononanedioic acid  | -5.63533  | NA  |
| 2-octanoylpropanedioic acid  | -5.61391  | NA  |
| 2-(2-ethylbutyl)-3-oxobutanedioic acid  | -5.57668  | NA  |