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

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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 |