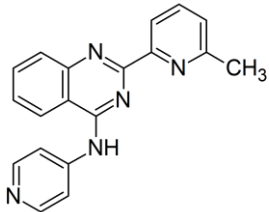
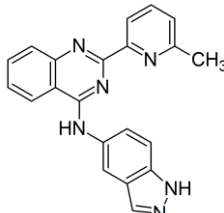
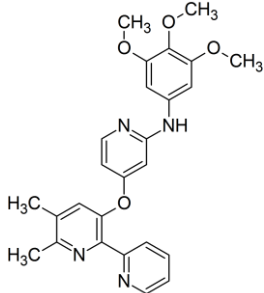
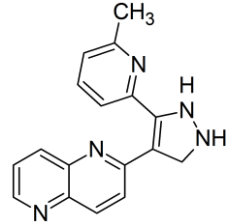
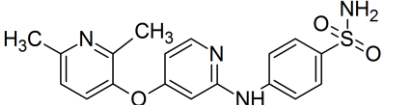
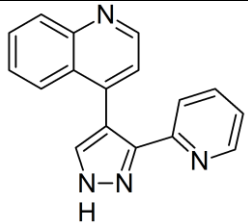
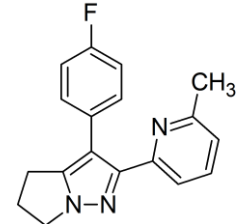
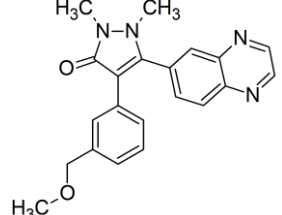


## Supplementary Material

**Table S1.** 3D structures of ALK-5 available at PDB, their main structural parameters (Resolution, R-value and R-free) and crystal ligands

|      | Resolution (Å) | R-value | R-Free | Ligand                                                                                   |                                                                                      |
|------|----------------|---------|--------|------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| 3HMM | 1.70           | 0.223   | 0.241  | 855: 2-(6-methylpyridin-2-yl)-N-pyridin-4-ylquinazolin-4-amine                           |   |
| 3GXL | 1.80           | 0.228   | 0.262  | QIG: N-1H-indazol-5-yl-2-(6-methylpyridin-2-yl)quinazolin-4-amine                        |   |
| 2WOT | 1.85           | 0.181   | 0.221  | ZZG: 4-[(5,6-dimethyl-2,2'-bipyridin-3-yl)oxy]-n-(3,4,5-trimethoxyphenyl)pyridin-2-amine |  |

|      |      |       |       |                                                                                              |                                                                                       |
|------|------|-------|-------|----------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| 1VJY | 2.00 | 0.223 | 0.275 | 460: 2-[5-(6-methylpyridin-2-yl)-2,3-dihydro-1h-pyrazol-4-yl]-1,5-naphthyridine              |    |
| 2WOU | 2.30 | 0.229 | 0.302 | ZZF: 4-({4-[(2,6-dimethylpyridin-3-yl)oxy]pyridin-2-yl}amino)benzenesulfonamide              |    |
| 1PY5 | 2.30 | 0.258 | 0.292 | PY1: 4-(3-pyridin-2-yl-1h-pyrazol-4-yl)quinoline                                             |    |
| 1RW8 | 2.40 | 0.260 | 0.291 | 580: 3-(4-fluorophenyl)-2-(6-methylpyridin-2-yl)-5,6-dihydro-4h-pyrrolo[1,2-b]pyrazole       |   |
| 3KCF | 2.80 | 0.237 | 0.278 | JZO: 4-[3-(methoxymethyl)phenyl]-1,2-dimethyl-5-quinoxalin-6-yl-1,2-dihydro-3H-pyrazol-3-one |  |

|                    |      |       |       |                                                                                                                      |     |
|--------------------|------|-------|-------|----------------------------------------------------------------------------------------------------------------------|-----|
| 3FAA               | 3.35 | 0.228 | 0.271 | 55F: N-[4-(5-fluoro-6-methylpyridin-2-yl)-5-quinoxalin-6-yl-1H-imidazol-2-yl]acetamide                               |     |
| 2X7O <sup>68</sup> | 3.70 | 0.267 | 0.273 | ZOP: (3z)-n-ethyl-n-methyl-2-oxo-3-(phenyl{[4-ylmethyl)phenyl]amino}methylidene)-2,3-dihydro-1h-indole-6-carboxamide |     |
| 1B6C               | 2.60 | 0.249 | 0.269 | ---                                                                                                                  | --- |
| 1IAS               | 2.90 | 0.255 | 0.284 | ---                                                                                                                  | --- |
| 3KFD <sup>69</sup> | 2.99 | 0.220 | 0.268 | ---                                                                                                                  | --- |

**Table S2.** Similarity values of each selected compound based on the crystal ligand

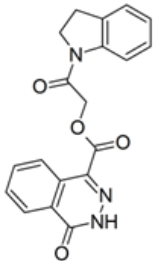
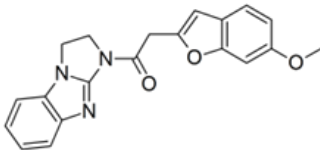
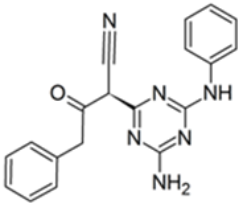
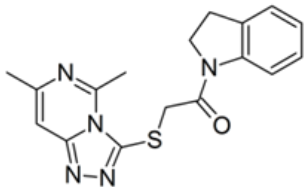
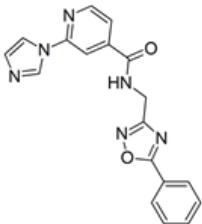
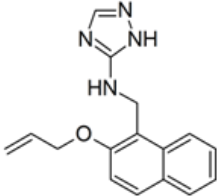
| <b>PDB</b> | <b>Ligand</b> | <b>Resolution (Å)</b> | <b>Cpd-16<br/>(pIC<sub>50</sub> = 7.92)</b> | <b>Cpd-15<br/>(pIC<sub>50</sub> = 7.82)</b> | <b>Cpd-33<br/>(pIC<sub>50</sub> = 7.80)</b> |
|------------|---------------|-----------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| 1RW8       | 580           | 2.40                  | 0.51                                        | 0.52                                        | 0.47                                        |
| 1PY5       | PY1           | 2.30                  | 0.42                                        | 0.41                                        | 0.57                                        |
| 1VJY       | 460           | 2.00                  | 0.40                                        | 0.41                                        | 0.44                                        |
| 2WOU       | ZZF           | 2.30                  | 0.33                                        | 0.34                                        | 0.35                                        |
| 2X7O       | ZOP           | 3.70                  | 0.49                                        | 0.48                                        | 0.34                                        |
| 3FAA       | 55F           | 3.35                  | 0.30                                        | 0.33                                        | 0.46                                        |
| 3KCF       | JZO           | 2.80                  | 0.55                                        | 0.59                                        | 0.38                                        |
| 3HMM       | 855           | 1.70                  | 0.31                                        | 0.32                                        | 0.78                                        |
| 3GXL       | QIG           | 1.80                  | 0.37                                        | 0.38                                        | 0.64                                        |
| 2WOT       | ZZG           | 1.85                  | 0.40                                        | 0.44                                        | 0.48                                        |

**Table S3.** Physicochemical properties of some ALK-5 ligands\* and each crystallographic ligand

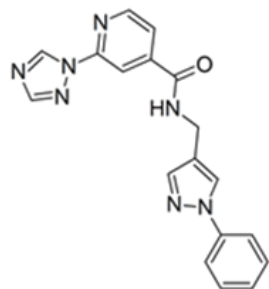
| PDB  | Ligand | nAtom | nC | nN | nO | CrippenLog | HybRatio | TopoPSA | MW      | nHBAcc | nHBDdon |
|------|--------|-------|----|----|----|------------|----------|---------|---------|--------|---------|
| -    | 15     | 52    | 23 | 5  | 1  | 3.905      | 0.217    | 62.97   | 385.180 | 5      | 0       |
| -    | 16     | 57    | 25 | 5  | 2  | 3.676      | 0.280    | 72.20   | 427.201 | 6      | 0       |
| -    | 33     | 39    | 18 | 5  | 0  | 5.921      | 0.111    | 91.83   | 333.105 | 5      | 1       |
| 1PY5 | PY1    | 33    | 17 | 4  | 0  | 4.112      | 0.00     | 54.46   | 272.106 | 4      | 1       |
| 1RW8 | 580    | 38    | 18 | 3  | 0  | 4.348      | 0.222    | 30.71   | 293.133 | 3      | 0       |
| 1VJY | 460    | 37    | 17 | 5  | 0  | 3.008      | 0.118    | 62.73   | 289.133 | 5      | 2       |
| 2WOU | ZZF    | 44    | 18 | 4  | 3  | 4.845      | 0.111    | 115.58  | 370.11  | 6      | 2       |
| 2X7O | ZOP    | 71    | 31 | 4  | 2  | 6.450      | 0.290    | 64.68   | 494.268 | 6      | 2       |
| 3FAA | 55F    | 42    | 19 | 6  | 1  | 2.207      | 0.118    | 91.63   | 362.129 | 7      | 2       |
| 3KCF | JZO    | 47    | 21 | 4  | 2  | 2.971      | 0.190    | 58.56   | 360.159 | 6      | 0       |
| 3HMM | 855    | 39    | 19 | 5  | 0  | 5.777      | 0.053    | 63.59   | 313.133 | 5      | 1       |
| 3GXL | QIG    | 45    | 21 | 6  | 0  | 3.354      | 0.143    | 61.14   | 354.159 | 6      | 2       |
| 2WOT | ZZG    | 60    | 26 | 4  | 4  | 7.171      | 0.192    | 87.62   | 458.195 | 4      | 1       |

\*Araujo, S. C., Maltarollo, V. G., & Honorio, K. M. (2013). Computational studies of TGF- $\beta$ RI (ALK-5) inhibitors: Analysis of the binding interactions between ligand–receptor using 2D and 3D techniques. *European Journal of Pharmaceutical Sciences*, 49(4), 542-549.

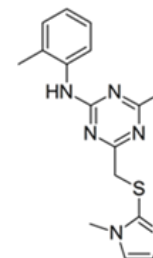
**Table S4.** Compounds selected from visual inspection regarding the main molecular interactions between some residues in the binding site and the studied ligands

| Cpd | Structure                                                                          | Cpd | Structure                                                                            |
|-----|------------------------------------------------------------------------------------|-----|--------------------------------------------------------------------------------------|
| 1   |   | 2   |   |
| 3   |   | 4   |   |
| 5   |  | 6   |  |

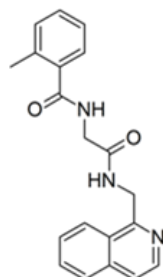
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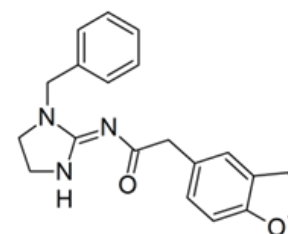
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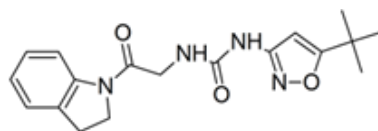
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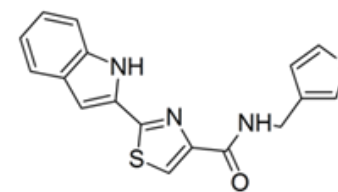
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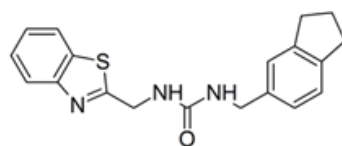
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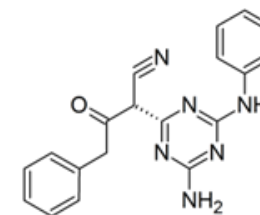
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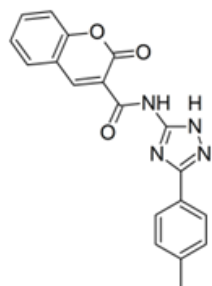
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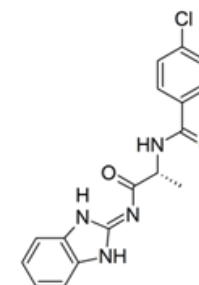
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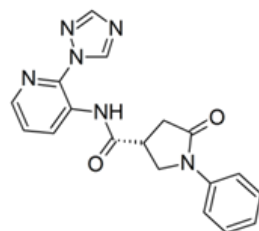
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16



17



**Table S5.** Prediction of absorption (A), distribution (D), metabolism (M) and excretion (E) for the selected compounds and the crystal ligand

| Property | Model Name                                             | Range          |         | Crystal | Predicted Value |        |        |        |        |
|----------|--------------------------------------------------------|----------------|---------|---------|-----------------|--------|--------|--------|--------|
|          |                                                        | Bad            | Good    |         | 1               | 2      | 3      | 4      | 5      |
| A        | Water solubility (log mol/L)                           | >1             | >-6     | -2.873  | -4.206          | -3.281 | -4.085 | -3.154 | -2.896 |
| A        | Caco2 permeability (log Papp in 10 <sup>-6</sup> cm/s) | <0.90          | >0.90   | 1.145   | 1.229           | 0.532  | 0.81   | 1.445  | 1.28   |
| A        | Intestinal absorption (human) (% Absorbed)             | <30%           | >30%    | 100     | 99.017          | 93.351 | 91.456 | 96.341 | 97.441 |
| A        | Skin Permeability (log Kp)                             | >-2.5          | <-2.5   | -2.735  | -2.74           | -2.735 | -2.755 | -2.795 | -2.735 |
| A        | P-glycoprotein substrate                               | No             | Yes     | No      | Yes             | No     | Yes    | No     | Yes    |
| A        | P-glycoprotein I inhibitor                             | No             | Yes     | Yes     | Yes             | Yes    | Yes    | No     | Yes    |
| A        | P-glycoprotein II inhibitor                            | No             | Yes     | Yes     | Yes             | Yes    | Yes    | No     | No     |
| D        | VDss (human) (log L/kg)                                | <-0.15         | >0.45   | 0.785   | 0.616           | 0.201  | -0.557 | -0.267 | 0.041  |
| D        | Fraction unbound (human) (FU)                          | >0             | 0       | 0.247   | 0               | 0.066  | 0      | 0.087  | 0.201  |
| D        | BBB permeability (log BB)                              | >0.3           | <-1     | -0.939  | -0.259          | 0.424  | -0.21  | -0.684 | -1.396 |
| D        | CNS permeability (log PS)                              | < -3           | > -2    | -2.996  | -2.748          | -2.079 | -2.739 | -2.634 | -2.79  |
| M        | CYP2D6 substrate                                       |                |         | No      | No              | No     | No     | No     | No     |
| M        | CYP3A4 substrate                                       |                |         | Yes     | Yes             | Yes    | Yes    | Yes    | Yes    |
| M        | CYP1A2 inhibitor                                       |                |         | No      | Yes             | Yes    | Yes    | No     | Yes    |
| M        | CYP2C19 inhibitor                                      | not<br>inhibit | inhibit | Yes     | Yes             | Yes    | No     | No     | Yes    |
| M        | CYP2C9 inhibitor                                       |                |         | Yes     | No              | Yes    | Yes    | No     | No     |
| M        | CYP2D6 inhibitor                                       |                |         | No      | No              | No     | No     | No     | No     |
| M        | CYP3A4 inhibitor                                       |                |         | Yes     | No              | Yes    | Yes    | No     | Yes    |
| E        | Total Clearance (log ml/min/kg)                        | <= -0.38       | >-0.38  | -0.104  | 0.468           | 1.144  | -0.116 | -0.016 | 0.478  |
| E        | Renal OCT2 substrate                                   | No             | Yes     | No      | Yes             | Yes    | No     | No     | Yes    |

**Table S6.** Prediction of toxicity for the new proposed compounds and the crystal ligand

| Model Name                                           | Range   |         | Crystal | Predicted Value |        |        |        |       |
|------------------------------------------------------|---------|---------|---------|-----------------|--------|--------|--------|-------|
|                                                      | Bad     | Good    |         | 1               | 2      | 3      | 4      | 5     |
| AMES toxicity                                        | Yes     | No      | No      | Yes             | Yes    | No     | No     | Yes   |
| Max. tolerated dose (human) (log mg/kg/day)          | >0.477  | <=0.477 | 0.211   | 0.302           | 0.391  | -0.045 | -0.033 | 0.653 |
| hERG I inhibitor                                     | Yes     | No      | No      | No              | Yes    | No     | No     | No    |
| hERGII inhibitor                                     | Yes     | No      | Yes     | No              | Yes    | Yes    | No     | Yes   |
| Oral Rat Acute Toxicity (LD 50) (mol/kg)             |         |         | 2.749   | 2.107           | 2.668  | 2.692  | 2.451  | 2.549 |
| Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day) |         |         | 0.9     | 2.368           | 1.169  | 1.774  | 1.029  | 0.572 |
| Hepatotoxicity                                       | Yes     | No      | Yes     | Yes             | No     | Yes    | Yes    | Yes   |
| Skin Sensitisation                                   | Yes     | No      | No      | No              | No     | No     | No     | No    |
| T. Pyriforms toxicity (log ug/L)                     | > 0.5   | < 0.5   | 0.285   | 0.302           | 0.285  | 0.313  | 0.34   | 0.285 |
| Minnow toxicity (log mM)                             | < - 0.3 | > - 0.3 | 2.273   | 0.918           | -1.183 | -0.256 | -1.298 | 2.294 |