Synthesis, Molecular Docking and Pharmacological Investigation of Heterocyclic Amine Derivatives as Potential Anticancer Agents

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Abstract

Cancer is a life-threatening disease that causes great damage to health worldwide. Studies have shown that hypoxia is the major contributor to tumor and cancer development due to overexpression of carbonic anhydrase. To encounter such cell abnormalities, demanding new drugs or novel analogs of currently in use. Therefore, the search for new pharmacoactive moieties with considerable effective activity against such tumors and cancers is needed. The implication of heterocyclic amine and acetamide derivatives well known as chemotherapeutic agents. Heterocyclic amine morpholine was taken as principal products and its new derivatives were synthesized after being designed computationally via molecular docking. A series of some of its new synthetic analogs i.e heterocyclic amine derivatives 1(a-o) were successfully synthesized and screened for their anticancer and carbonic anhydrase inhibitory potential. Most of the compounds showed good results possessing reasonable carbonic anhydrase inhibitory activity particularly compounds 1c, 1d, 1h and 1i showed very reasonable carbonic anhydrase inhibitory activity whereas compound 1h showed maximum inhibition comparable to acetazolamide. Similarly, four of the synthesized compounds showed good anticancer activity particularly compound 1b, 1c, 1h, and 1i showed reasonable, whereas compound 1h have better IC₅₀ value comparable to cisplatin when evaluated via in vitro MTT assay.

Introduction

Cancer has been an endless combat worldwide with a lot of advancement in remedies and defensive therapies. The disease is illustrated by cells frequently growing with the failure to be stopped, creating tumors with the potential to be metastatic. Latest studies in cancer therapy have shown that hypoxia is the major contributor to tumor and cancer development which is due to overexpression of carbonic anhydrase. Nevertheless, the instantaneous intensifying diversification of life threatening diseases like tumors and related cancers demanding new drugs or novel analogs of the drugs which are already in use. Therefore, the present time requires the search for new pharmacoactive moieties with considerable effective activity against such tumors and cancers. Carbonic anhydrase has 16 distinct isoforms that include membrane-bound isoforms (CA IV, CA IX, CA XIII), cytosolic isoforms (CA I, CA II, CA III, CA VII, and CA XIII), and mitochondrial isoforms (CA VA and CA VB); salivary isoforms (CA VI) are secreted isoforms. CA overexpression is a feature of certain physiological condition, Due to their high therapeutic potential, CAs have emerged as promising pharmacological candidates. Inhibitors of the CA enzyme are commonly used for diseases like mountain sickness, acidic ulcers, glaucoma, and epilepsy. Carbonic anhydrase is another significant therapeutic target in the treatment of obesity, and as anti-obesity medicines. Transmembrane enzymes have shown to be overexpressed in hypoxic tumors notwithstanding their poor distribution. Because of this, new compounds that can effectively inhibit tumor development and cancer proliferation are urgently needed. Telomerase is a ribonucleoprotein (RNP) reverse transcriptase responsible and Epacadostat (EPA) is inhibitor of indoleamine 2,3-dioxygenase.
Numerous unique strategies have been used in this respect, including the identification of novel targets, structural changes to existing compounds, the combination of two pharmacophores in a single molecule, and the use of structural variations such as spacers or linkers\textsuperscript{21}. The heterocyclic amine and its acetamide derivatives have long been recognised for their potency as chemotherapeutic agents, as evidenced by their numerous patents\textsuperscript{22,23}. Several activities are related with heterocyclic amine and its acetamide derivatives\textsuperscript{24–27}. Keeping in view the importance of heterocyclic amines, a series of some of its new synthetic analogs were synthesized and screened for anticancer and carbonic anhydrase activity. The heterocyclic amine morpholine was taken as principal products and its new derivatives were synthesized after being designed computationally via molecular docking.

**Material And Methods**

**Materials**

A digital Gallen-hamp (MPD-BM-3.5) equipment was used to record melting points (M.P) of synthesized compounds. Thin layer chromatography (TLC) was used to monitor the progress of the reactions. Proton NMR, \textsuperscript{13}C-NMR spectra were done on a Bruker AM300 in DMSO at 300 and 75 MHz respectively. FT-IR spectra were measured by using NICOLET IS10 spectrophotometer (\textit{v}_{\text{max}} \text{ in cm}^{-1}).

**Methods**

**Chemistry**

General procedure for the synthesis of 2-chloro-1-(morpholin-4-yl) ethanone (1)

To the solution of respective heterocyclic amine, morpholine (0.05 mol) in anhydrous dichloromethane, triethylamine (0.05 mol) was added. Then the addition of chloroacetyl chloride (0.05 mol) drop wise and continuously enthused at room temperature (rt) for 6–8 hrs. Progress of reaction was observed by TLC. Finally, reaction mixture was poured into ice cold water and extraction done by ethyl acetate, and solvent rotary evaporated. Afforded product was recrystallized with ethanol\textsuperscript{39}.

**General procedure for the synthesis of morpholine acetamide derivatives (1a-o)**

The compound 1 (0.05 mol) and anhydrous potassium carbonate in ethanol was stirred at rt for two hours with addition of heterocyclic amine, alcohol and thiol (a-o) (0.05 mol) in ethanol in dropwise manner. The mixture was further refluxed for 12hrs. The solid separated and washed with water, and recrystallized from ethanol\textsuperscript{28}.

**1,2-di(morpholin-4-yl) ethan-1-one (1a)**
Off white solid, mp 180°C; yield (85%), ethyl acetate/pet.ether (5:2), IR (KBr, cm\(^{-1}\)) : 3381 (CH), 1627 (CO, amide), 1258 (C-N), 1102 (C-O). \(^1\)H NMR (DMSO-d\(_6\), 300 MHz), \(^1\)H - NMR: δ 3.12 – 3.55 (8H), 3.56 – 3.65 (4H), 3.76 (4H) 2.73 (s). \(^13\)C - NMR (DMSO-d\(_6\), 300 MHz, δ ppm): 163.9, 66.4, 66.4, 66.3, 66.3, 53.7, 53.7, 49.8, 44.5, 44.5. Analysis for C\(_{10}\)H\(_{18}\)N\(_2\)O\(_3\) (214.26): C, 56.06; N, 13.07; H, 8.47; O, 22.40 %. Findings: C, 55.05; N, 12.06 ; H, 8.40; O, 21.39 %.

1-(morpholin-4-yl)-2-(pyrrolidin-1-yl) ethan-1-one (1b)

Dark brown liquid; yield (80%), ethyl acetate/pet.ether (5:2), IR (KBr, cm\(^{-1}\)) : 3377 (CH), 1635 (CO, amide), 1250 (C-N), 1109 (C-O). \(^1\)H NMR: δ 1.90 (4H), 2.99 (4H), 3.48 – 3.69 (8H), 3.62 (4H), 3.54 (2H, s). \(^13\)C - NMR (DMSO-d\(_6\), 300 MHz, δ ppm): 49.83, 23.45, 23.45, 163.90, 53.76, 53.76, 44.48, 44.48, 66.43, 66.43. Analysis for C\(_{10}\)H\(_{18}\)O\(_2\)N\(_2\) (198.26): C, 60.58; N, 14.13; H, 9.15. O, 16.14 % Findings: C, 59.58; H, 9.01. O, 15.13 %.

1-(morpholin-4-yl)-2-(pyridin-2-ylamino) ethan-1-one (1c)

Brown crystalline solid, m.p 178°C; yield (78%), ethyl acetate./pet.ether (5:2), IR (KBr, cm\(^{-1}\)) : 3417 (NH), 2917 (CH), 1625 (CO, amide), 1446 (C = N), 1512 (C = C), 1265 (C-N), 1111 (C-O). \(^1\)H - NMR: δ 3.48 – 3.69 (10H), 3.54 (1H), 6.97 (1H), 7.18 (1H), 7.63 (1H), 8.29 (1H). \(^13\)C - NMR (DMSO-d\(_6\), 300 MHz, δ ppm): 148.1, 158.6, 44.9, 167.7, 118.9, 107.7, 44.5, 44.5, 137.8, 66.5, 66.5. Analysis for C\(_{11}\)O\(_2\)H\(_{15}\)N\(_3\) (221.2): C, 59.70; H, 6.82; N, 18.97; O, 14.44%. Finding : C, 58.69; H, 6.72; N, 7.96; O, 13.39 % .

2-[(3-methoxyphenyl) amino]-1-(morpholin-4-yl) ethan-1-one (1d)

Yellowish brown solid, m.p 167°C; yield (86%), ethyl acetate/pet.ether (5:2), IR (KBr, cm\(^{-1}\)) : 3360 (NH), 2947 (CH), 1595 (CO, amide), 1480 (C = C), 1293 (C-N), 1152 (C-O), 1033 (C-O). \(^1\)H - NMR: δ 3.48 – 3.67 (10H), 3.64 (1H), 3.71 (3H), 6.56 (1H),, 6.72 (1H), 6.96 (1H), 7.17 (1H). \(^13\)C - NMR (DMSO-d\(_6\), 300 MHz, δ ppm): 106.23, 108.3, 119.2, 66.4, 66.4, 45.6, 45.6, 130.5, 65.92, 55.4, 144.3, 160.3, 167.8. Analysis for C\(_{13}\)H\(_{18}\)N\(_2\)O\(_3\) (250.3): C, 62.37; H, 7.24; N, 11.18; O, 19.17 %. Findings: C, 60.28; H, 6.12; N, 10.13; O, 18.9 % .

2-[(4-methoxyphenyl) amino]-1-(morpholin-4-yl) ethan-1-one (1e)

Blackish brown solid, mp 185°C; yield (77%), ethyl acetate/pet.ether (5:2), IR (KBr, cm\(^{-1}\)) : 3417 (NH), 2914 (CH), 1629 (CO, amide), 1504 (C = C), 1229 (C-N), 1111 (C-O), 1025 (C-O). \(^1\)H - NMR: δ 3.49 – 3.67 (8H), 3.73 (3H), 3.89 (2H, s), 6.84 (2H), 6.92 (2H). \(^13\)C - NMR (DMSO-d\(_6\), 300 MHz, δ ppm): 44.8, 167.7, 55.4, 156.8, 114.4, 114.4, 44.4, 44.4, 66.4, 66.4, 148.5. Analysis for C\(_{13}\)H\(_{18}\)N\(_2\)O\(_3\) (250.3): C, 62.36; H, 7.24; N, 11.18; O, 19.17 %. Findings: C, 61.26; H, 6.13; N, 10.16; O, 19.2 % .

2-[(2-methoxyphenyl) amino]-1-(morpholin-4-yl) ethan-1-one (1f)
Pale yellow crystalline solid, mp 177°C; yield (70%), ethyl acetate/pet.ether (5:2), IR (KBr, cm\(^{-1}\)): 3375 (NH), 2980 (CH), 1639 (CO, amide), 1450 (C = C), 1251 (C-N), 1107 (C-O), 1029 (C-O). \(^1\)H - NMR: δ 3.49–3.67 (8H), 3.81 (3H, s), 3.93 (2H, s), 6.84–6.98 (4H). \(^13\)C - NMR (DMSO-d\(_6\), 300 MHz, δ ppm): 66.4, 66.4, 121.8, 110.8, 123.2, 138, 44.8, 55.9, 167.7, 44.4, 44.4, 108.7, 147.6. Analysis for C\(_{13}\)H\(_{18}\)N\(_2\)O\(_3\) (250.3): C, 62.35; H, 7.25; N, 11.16; O, 19.14 %. Findings: C, 61.20; H, 6.25; N, 10.29; O, 18.0 %.

**1-(morpholin-4-yl)-2-phenoxy ethan-1-one (1g)**

Brown crystalline solid, mp 200°C; yield (78%), ethyl acetate/pet.ether (5:2), IR (KBr, cm\(^{-1}\)): 2855 (CH), 1632 (CO, amide), 1449 (C-C), 1255 (C-N), 1110 (C-O), 1013 (C-O). \(^1\)H - NMR: δ 3.51–3.66 (8H), 4.18 (2H, s), 6.90–7.00 (3H), 7.32 (2H, s). \(^13\)C - NMR (DMSO-d\(_6\), 300 MHz, δ ppm): 157.5, 123.6, 129.6, 129.6, 114.9, 114.9, 44.4, 44.4, 169.2, 68.8, 66.4, 66.4. Analysis for C\(_{12}\)H\(_{15}\)NO\(_3\) (221): C, 65.14; H, 6.83; N, 6.33; O, 21.69 %. Findings: C, 64.29; H, 6.25; N, 5.19; O, 21.0 %.

**2-[[5-methyl-2-(propan-2-yl)cyclohexyl]oxy]-1-(morpholin-4-yl) ethan-1-one (1h)**

Light yellowish crystalline solid, mp 215°C; yield (82%), ethyl acetate/pet.ether (5:2), IR (KBr, cm\(^{-1}\)): 3348 (CH), 1639 (CO, amide), 1403 (C-C), 1111 (C-N), 1016 (C-O). \(^1\)H - NMR: δ 0.85–0.90 (6H), 1.27–1.49 (3H), 1.46–1.85 (6H), 3.49–3.71 (9H), 3.94 (2H, s). \(^13\)C - NMR (DMSO-d\(_6\), 300 MHz, δ ppm): 48.1, 169.2, 44.4, 44.4, 21.7, 66.4, 66.4, 31.3, 23.3, 19.3, 80.6, 25.8, 68.8, 19.3, 39.9, 34.2. Analysis for C\(_{16}\)H\(_{29}\)NO\(_3\) (283.4): C, 67.81; H, 10.31; N, 4.94; O, 16.94 %. Findings: C, 66.01; H, 10.22; N, 4.53; O, 15.91 %.

**2-[[5-methyl-2-(propan-2-yl)phenoxy]-1-(morpholin-4-yl) ethan-1-one (1i)**

Light red crystalline solid, mp 172°C; yield (76%), ethyl acetate/pet.ether (5:2), IR (KBr, cm\(^{-1}\)): 2958 (CH), 1639 (CO, amide), 1450 (C-C), 1227 (C-N), 1157 (C-O), 1090 (C-O). \(^1\)H - NMR: δ 1.29 (6H), 4.15 (2H, s), 6.74 (1H), 7.01 (1H), 7.12 (1H). \(^13\)C - NMR (DMSO-d\(_6\), 300 MHz, δ ppm): 66.4, 66.4, 68.8, 123.4, 155.8, 169.2, 22.9, 22.9, 21.4, 25.8, 44.4, 44.4, 139.3, 140.8, 132.1, 113.4. Analysis for C\(_{16}\)H\(_{29}\)NO\(_3\) (277): C, 69.29; H, 8.36; N, 5.05; O, 17.31 %. Findings: C, 67.21; H, 8.31; N, 5.01; O, 17.22 %.

**2-[[4-chlorobenzyl] amino]-1-(morpholin-4-yl) ethan-1-one (1j)**

Pale yellowish crystalline solid, mp 190°C; yield (87%), ethyl acetate/pet.ether (5:2), IR (KBr, cm\(^{-1}\)): 3304 (NH), 2970 (CH), 1679 (CO, amide), 1628 (C-N), 1510 (C = C) 1470 (C-C), 1235 (C-N), 1112 (C-O), 880 (C-Cl). \(^1\)H - NMR: δ 3.48–3.67 (10H), 3.84 (2H, s), 7.34 (2H), 7.44 (2H). \(^13\)C - NMR (DMSO-d\(_6\), 300 MHz, δ ppm): 167.7, 129.2, 129.2, 135.6, 44.4, 44.4, 128.2, 128.2, 66.4, 66.4, 44.8, 139.1, 53.5. Analysis for C\(_{13}\)H\(_{17}\)ClN\(_2\)O\(_2\) (268): C, 58.10; H, 6.38; Cl, 13.19; N, 10.42; O, 11.91 %. Findings: C, 57.30; H, 6.08; Cl, 12.19; N, 9.42; O, 10.27 %.
2-[(4-fluorobenzyl) amino]-1-(morpholin-4-yl) ethan-1-one (1k)

Dirty white crystalline solid, mp 185°C; yield (79%), ethyl acetate/pet.ether (5:2), IR (KBr, cm\(^{-1}\)): 3304 (NH), 2970 (CH), 1679 (CO, amide), 1628 (C = N), 1510 (C = C) 1470 (C-C), 1235 (C-N), 1112 (C-O), 880 (C-F). \(^1\)H - NMR: \(\delta\) 3.48–3.67 (8H), 3.69 (2H, s), 3.83 (2H, s), 6.92 (2H), 7.27 (2H). \(^1^3\)C - NMR (DMSO-d\(_6\), 300 MHz, \(\delta\) ppm): 167.7, 129.2, 129.2, 163.4, 44.4, 44.4, 115, 115, 66.4, 66.4, 44.8, 139.1, 53.5. Analysis for C\(_{13}\)H\(_{17}\)FN\(_2\)O\(_2\) (252): C, 61.89; H, 6.79; F, 7.53; N, 11.10; O, 12.68 %. Findings: C, 60.09; H, 6.09; F, 7.40; N, 10.10; O, 12.08 %.

1-(morpholin-4-yl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl) amino]ethan-1-one (1l)

Whitish solid powder, mp 162°C; yield (91%), ethyl acetate/pet.ether (5:2), IR (KBr, cm\(^{-1}\)): 3382 (NH), 2920 (CH), 1676 (CO, amide), 1621 (C = N), 1512 (C = C). \(^1\)H - NMR: \(\delta\) 3.48–3.67 (8H), 3.78 (2H, s), 7.30 (1H), 7.47 (2H), 7.84 (2H). \(^1^3\)C - NMR (DMSO-d\(_6\), 300 MHz, \(\delta\) ppm): 44.4, 44.4, 44.8, 164.5, 161.1, 122.8, 66.4, 66.4, 128.9, 128.9, 126.9, 126.9, 167.7, 128.9. Analysis for C\(_{14}\)H\(_{16}\)N\(_4\)O\(_3\) (288): C, 58.31; H, 5.58; N, 19.41; O, 16.64 %. Findings: C, 58.0; H, 5.09; N, 19.02; O, 16.08 %.

2-(1,3-benzothiazol-2-ylsulfanyl)-1-(morpholin-4-yl) ethan-1-one (1m)

Whitish crystalline solid, mp 206°C; yield (89%), ethyl acetate/pet.ether (5:2), IR (KBr, cm\(^{-1}\)): 3284 (NH), 2920 (CH), 1676 (CO, amide), 1621 (C = N), 1512 (C = C), 902 (C-S). \(^1\)H - NMR: \(\delta\) 3.54–3.70 (8H), 3.90 (2H, s), 7.26–7.40 (2H), 7.74–7.85 (2H). \(^1^3\)C - NMR (DMSO-d\(_6\), 300 MHz, \(\delta\) ppm): 126.4, 32.1, 164.9, 66.4, 66.4, 121.8, 124.9, 44.4, 44.4, 122.4, 152.8, 166.8, 135.9. Analysis for C\(_{13}\)H\(_{14}\)N\(_2\)O\(_2\)S\(_2\) (294): C, 53.03; H, 4.73; N, 9.51; O, 10.86; S, 21.76%. Findings: C, 52.0; H, 4.74; N, 9.45; O, 10.77; S, 20.68%.

2-(benzylamino)-1-(morpholin-4-yl) ethan-1-one (1n)

White amorphous solid, mp 240°C; yield (74%), ethyl acetate/pet.ether (5:2), IR (KBr, cm\(^{-1}\)): 3354 (NH), 2920 (CH), 1673 (CO, amide), 1621 (C = N), 1512 (C = C) 1440 (C-C), 1245 (C-N), 1110 (C-O). \(^1\)H - NMR: \(\delta\) 3.48–3.67 (8H), 3.69 (2H, s), 3.83 (2H, s), 7.25–7.36 (5H). \(^1^3\)C - NMR (DMSO-d\(_6\), 300 MHz, \(\delta\) ppm): 140.1, 167.7, 44.8, 128.5, 128.5, 128.9, 66.4, 66.4, 53.5, 44.4, 44.4, 127.6, 127.6. Analysis for C\(_{14}\)H\(_{16}\)N\(_4\)O\(_3\) (234): C, 66.63; H, 7.73; N, 11.95; O, 13.65 %. Findings: C, 66.14; H, 7.24; N, 11.06; O, 13.43 %.

2-[(4-fluorophenyl)amino]-1-(morpholin-4-yl)ethanone (1o)

Dark yellow crystalline solid, mp 220°C; yield (71%), ethyl acetate/pet.ether (5:2), IR (KBr, cm\(^{-1}\)): 3354 (NH), 2920 (CH), 1673 (CO, amide), 1621 (C-N), 1512 (C = C) 1480 (C-C), 1245 (C-N), 1010 (C-O), 840 (C-F). \(^1\)H - NMR: \(\delta\) 3.49–3.67 (8H), 3.94 (2H, s), 6.86 (2H), 6.96 (2H). \(^1^3\)C - NMR (DMSO-d\(_6\), 300 MHz, \(\delta\) ppm):
117.1, 117.1, 118.7, 118.7, 44.4, 44.4, 160.4, 66.4, 66.4, 167.7, 44.8, 148.5. Analysis for 
$C_{12}H_{15}F_{2}N_{2}O_{2}$ (238): C, 60.48; H, 6.34; F, 7.96; N, 11.75; O, 13.42 %. Findings C, 60.42; H, 6.31; F, 7.67; N, 11.66; O, 13.03 %.

**In Vitro Carbonic Anhydrase Inhibition Assay**

CA inhibitory was performed according to previously described technique after standardizing reaction parameters such as enzyme and substrate concentrations, buffer(pH), and time of reaction. The Carbonic anhydrase(CA) catalyzed hydrolyzed product of p-nitrophenylacetate(PNPA). 60–65 microliters of 50–55 millimoles Tris-sulfate buffer(pH 7.6),(0.1 millimoles Zinc Chloride), 10–15 microliters (0.5 millimoles) test sample in 1% dimethyl sulfoxide(DMSO), and 10–15 litres (50 Units) enzyme(bovine) shifted per well. Contents were intermixed, and incubated at 36.5–37.5°C for 20–25 minutes. Absorbance was measured at 350 nm using a microplate reader. Results were articulated as % inhibitions by formula given:

$$\text{%inhibition} = \left[100 - \left(\frac{\text{absoltestcompound}}{\text{absolofcontrol}}\right) \times 100\right]$$

**MTT Assay**

The cytotoxic assay of compounds to be test was done by the MTT assay. Seeding of the cells into 96-well plates was performed and, after 24 h, treated with pre-designated compound concentrations or control. At 72 h-post drug addition, a chemical salt MTT {3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide} was added and re-suspended crystals of formazan were assayed. The % of cell growth inhibition was taken triplicate experiments and expressed as $[1-(A/B)] \times 100$, where A was absorbance value for experimental wells and B for control wells. The IC50 values calculated via Graph pad prism-6.

**Pharmacokinetics properties**

Molinspiration Cheminformatics online server was used for ADMET-pharmacokinetics properties of test compounds. As per Lipinski rule-5, compound have H-bond donor's $\leq 5$, H-bond acceptors $\leq 10$, M.W $< 500$ Da, and Partition coefficient (logP) $\leq 5$.

**Docking studies**

**Ligand selection and optimization**

The synthesized chemical structures (1a) to (1o) were used as ligand molecules and their structures were drawn in Discovery Studio Visualizer 2017, saved in PDB format which were further adapted to pdbqt format using Autodock Tools 1.5.6. The AutoDock Tools gave gasteiger charges to all atoms, added polar hydrogens and removed non-polar hydrogens from all the ligands.

**Accession of target protein**
The protein structures of bovine Tubulin (PDBID: 1JFF)\textsuperscript{43}, Telomerase Reverse Transcriptase (PDBID: 5CQG)\textsuperscript{20}, and Human carbonic anhydrase IX (PDBID: 6VKG)\textsuperscript{19} were selected for screening of ligands. 

**Target protein optimization**

For protein optimization the combined crystallized ligands and molecules of water and were removed from protein structures. The Zinc ion present in 6VKG was not removed as it can result information of ionic interactions between Ligand and target protein.

**Analysis of target active binding sites**

The binding site was defined and analyzed on the bases of co-crystallized ligands present in protein structures and search space was confined to get poses of ligands as much similar in conformation as possible to co-crystallized ligands. The docked poses along with score were obtained by using Auto Dock Vina. The poses with best score were rescored by help of x-score. The co-crystallized ligands were also docked as reference to compare and verify results. The binding energies of best poses in obtained by Auto Dock Vina in Kcal/mol and their rescored values by X-Score in PKd.

**Results And Discussion:**

**Chemistry**

Chemical reactions for the synthesis of the compounds have been don as per reported method\textsuperscript{28}. Scheme comprising of reaction of heterocyclic amine morpholine (1) with chloroacetyl chloride and the resulting intermediate chloroacetamide was reacted with different amines, alcohols and thiol to get heterocyclic amine derivatives 1(a-o) respectively\textsuperscript{28}. Compounds synthesized were of good yield. FTIR, \textsuperscript{1}H-NMR, \textsuperscript{13}C-NMR and elemental analysis used for characterization synthesized compounds.

**Pharmacological studies**

**In Vitro CA Inhibition Assay**

Results of CA assay were presented in as the mean triplicate experiments (± SEM) and expressed as % inhibitions:

\[
\%\text{inhibition} = [100 - (absoftest\text{compound}/absofcontrol) \times 100]
\]

(Table 1). Compound 1h has exhibited maximum percent enzyme inhibition (88.3%) in comparison to control acetazolamide. The increased inhibitory activity of compound 1h might be due to hydrophobic groups that leads to better binding interaction with inner hydrophobic pockets of CA\textsuperscript{29}. 
### Table 1
Percentage inhibition of synthesized compounds 1a-1o and control acetazolamide (AZM) *in-vitro* CA inhibition assay (n = 3)

| Compound | % Inhibition Carbonic Anhydrase |
|----------|---------------------------------|
| 1a       | 76.7 ± 4.41                     |
| 1b       | 75.0 ± 2.89                     |
| 1c       | 85.0 ± 5.13                     |
| 1d       | 81.7 ± 6.01                     |
| 1e       | 68.3 ± 1.67                     |
| 1f       | 73.3 ± 3.33                     |
| 1g       | 81.7 ± 6.01                     |
| 1h       | 88.3 ± 4.41                     |
| 1i       | 85.0 ± 2.89                     |
| 1j       | 76.7 ± 6.01                     |
| 1k       | 68.7 ± 1.86                     |
| 1l       | 61.7 ± 3.33                     |
| 1m       | 80.0 ± 5.77                     |
| 1n       | 73.3 ± 3.33                     |
| 1o       | 73.3 ± 3.33                     |
| Control  | 96.3 ± 1.33                     |

### MTT assay

The percentage of cell growth inhibition in triplicate experiments was expressed as \([1-(A/B)] \times 100\), and the IC50 values were calculated by using Graph-pad prism6 as presented in Table 2. Most of the synthesized compounds have shown better cell growth inhibition in MTT assay due to acetamide moiety present in all compounds30.
Table 2
The effects of compounds (1a-1o) on the viability of Resistant Ovarian cancer cell lines A2780\textsuperscript{cisR} (n = 3).

| Compounds | 10 µM % Cell proliferation inhibition | SEM | 25 µM % Cell proliferation inhibition | SEM | 50 µM % Cell proliferation inhibition | SEM | IC\textsubscript{50} Values |
|------------|---------------------------------------|-----|-------------------------------------|-----|-------------------------------------|-----|-------------------------|
| 1a         | 14.33                                 | 2.33| 28.33                               | 3.33| 43.33                               | 9.28| 23.86                  |
| 1b         | 13.67                                 | 2.96| 25.67                               | 2.33| 43.33                               | 7.69| 14.8                   |
| 1c         | 12.67                                 | 3.71| 22.33                               | 1.20| 36.00                               | 7.02| 15.68                  |
| 1d         | 15.00                                 | 2.89| 25.67                               | 2.33| 36.67                               | 6.01| 25.27                  |
| 1e         | 6.67                                  | 1.67| 28.33                               | 3.33| 50.00                               | 2.89| 26.45                  |
| 1f         | 7.67                                  | 1.45| 58.33                               | 6.01| 61.67                               | 1.67| 158.4                  |
| 1g         | 10.00                                 | 2.89| 53.33                               | 1.67| 57.33                               | 2.67| 146.1                  |
| 1h         | 5.33                                  | 1.45| 21.67                               | 1.67| 56.67                               | 6.01| 9.407                  |
| 1i         | 4.00                                  | 1.00| 25.00                               | 2.89| 63.33                               | 4.41| 11.2                   |
| 1j         | 5.33                                  | 1.33| 25.67                               | 2.19| 49.00                               | 3.21| 21.49                  |
| 1k         | 5.33                                  | 1.33| 41.67                               | 1.67| 58.33                               | 1.67| 64.17                  |
| 1l         | 11.67                                 | 1.67| 31.67                               | 1.67| 60.00                               | 2.89| 15.64                  |
| 1m         | 11.67                                 | 1.67| 36.67                               | 6.01| 58.33                               | 1.67| 32.39                  |
| 1n         | 10.67                                 | 0.67| 40.00                               | 2.89| 58.33                               | 1.67| 47.92                  |
| 1o         | 8.33                                  | 0.88| 36.67                               | 6.01| 58.33                               | 1.67| 38.08                  |
| Cisplatin  | 4.00                                  | 1.15| 20.67                               | 0.67| 60.33                               | 6.23| 8.502                  |

ADME and Pharmacokinetic studies

Table 3. present pharmacokinetic data of synthesized compounds analyzed by molinspiration and cheminformatics online servers\textsuperscript{31}. 
Table 3
Pharmacokinetic and ADMET parameters of synthesized compounds.

|     | miLogP | TPSA  | natoms | MWt   | HBA | HBD | nviolations | nrotb | volume  |
|-----|--------|-------|--------|-------|-----|-----|-------------|-------|---------|
| 1a  | 1.45   | 64.64 | 17     | 237.25| 5   | 1   | 0           | 6     | 219.11  |
| 1b  | -1.13  | 93.45 | 16     | 223.23| 6   | 4   | 0           | 4     | 200.45  |
| 1c  | 2.51   | 79.79 | 25     | 337.38| 6   | 2   | 0           | 7     | 311.25  |
| 1d  | 2.23   | 100.02| 24     | 327.34| 7   | 3   | 0           | 6     | 291.85  |
| 1e  | 4.24   | 93.46 | 32     | 427.46| 7   | 2   | 0           | 6     | 380.21  |
| 1f  | 4.61   | 76.47 | 34     | 454.53| 7   | 1   | 0           | 1     | 418.1   |
| 1g  | 4.51   | 73.23 | 31     | 411.46| 6   | 1   | 0           | 6     | 372.2   |
| 1h  | 3.42   | 35.54 | 17     | 230.26| 3   | 0   | 0           | 5     | 215.15  |
| 1i  | 0.83   | 64.36 | 16     | 216.24| 4   | 3   | 0           | 3     | 196.5   |
| 1j  | 4.05   | 50.36 | 24     | 318.38| 4   | 2   | 0           | 6     | 296.44  |
| 1k  | 4.19   | 70.92 | 24     | 320.35| 5   | 2   | 0           | 5     | 287.89  |
| 1l  | 6.21   | 64.36 | 32     | 420.47| 5   | 1   | 1           | 5     | 376.26  |
| 1m  | 6.58   | 47.37 | 34     | 447.54| 5   | 0   | 1           | 6     | 414.15  |
| 1n  | 6.47   | 44.13 | 31     | 404.47| 4   | 0   | 1           | 5     | 368.24  |
| 1o  | 0.83   | 64.36 | 16     | 216.24| 4   | 3   | 0           | 3     | 196.5   |

Docking study

The results of docking show that compounds have reasonably good binding with tubulin, carbonic anhydrase IX and very high affinity for telomerase reverse transcriptase enzyme. The compounds having bulkier heterocycle ring structure were more active as compared to compounds with simple phenyl and benzyl functional group\(^\text{32}\). Compounds 1h, 1i and 1l have the very promising binding affinity in case of binding with Tubulin (1JFF), Auto Dock vina has given highest score to 1h similarly in X-Score, 1h was scored to have highest binding affinity where as 1i and 1l also scored to have reasonable affinity. Binding affinity showed that the tubulin can be a good target for these compounds. The compounds were docked in taxol binding site which has a bulkier structure therefore the synthesized compounds with bulkier structure showed good binding affinity and most these compounds can be good candidates for tubulin inhibition, as whole or fragments\(^\text{33}\).

The compounds under study share a lot of characteristics with BIBR1532, a reported highly potent telomerase inhibitor. BIBR1532 have amide functional group along with naphthalene ring system\(^\text{34}\). The synthesized compounds have morpholine ring system which is more polar characteristics due to
presence of oxygen and nitrogen atoms\textsuperscript{35}. Similarly, most of the synthesized compounds have carbonyl functionality in the form of ester, amide and thioate esters, which having carbonyl functional group, can have same interactions in binding pocket as BIBR1532\textsuperscript{36}. The compound 1h, and 1l showed good activities but compound 1i shown highest affinity for Telomerase Reverse Transcriptase enzyme although it is slightly lower than BIBR1532. It is due to the fact that the binding pocket is highly hydrophobic and compound 1i contain toluene with 4- isopropyl group, being more hydrophobic, is a better candidate for further development of telomerase inhibitors\textsuperscript{37}. Using AutoDock vina the binding energy of the compound 1i was taken in the same docking pocket, resulted good binding energy value i.e. -9.8 Kcal/mol near to BIBR1532 i.e. -10.4 Kcal/mol as presented in Table 4. The results show that para-cymene functional group (4-isopropyltoluene) along with morphline ring can be a potent inhibitor of telomerase reverse transcriptase. The compound 1m has shown a high predicted binding affinity with carbonic Anyhydrase IX enzyme. The predicted value is even higher than that of epacadostat, an experimental inhibitor of same enzyme and was co-crystallized with protein structure used for docking\textsuperscript{38}. The compound 1d, 1f, 1h, 1g, 1n, 1o and also showed reasonably good hydrophobic and hydrophilic interactions. Zinc is also playing role in binding of Ligand to protein (Fig. 1, Fig. 2, Fig. 3). The results are suggestive that compound 1m should be evaluated for its carbonic anhydrase IX inhibitory activity through \textit{in-vitro} testing. The Docking results overall suggest that the compounds are pharmacologically active and have a very good potential for further testing as an inhibitor of Carbonic Anhydrase IX and they can also be considered as fragments or leads for further drug development.
Table 4
Binding energies and XScore of synthesized compounds against Tubulin (1jff), Telomerase Reverse Transcriptase (5cqg), Carbonic Anhydrase IX (6vkg).

| S.No | Ligand | Tubulin (1jff) |  | Telomerase Reverse Transcriptase (5cqg) |  | Carbonic Anhydrase IX (6vkg) |  |
|------|--------|----------------|---|-----------------------------------------|---|---------------------------------|---|
|      |        | Vina (kcal/mol) | XScore (pKd) | Vina (kcal/mol) | XScore (pKd) | Vina (kcal/mol) | X-Score (pKd) |
| 1.   | 1a     | -4.9           | 4.46         | -5.2           | 4.63         | -4.6             | 4.71          |
| 2.   | 1b     | -4.8           | 4.67         | -5.5           | 4.87         | -5.0             | 4.81          |
| 3.   | 1c     | -5.4           | 4.71         | -6.6           | 5.25         | -5.6             | 5.12          |
| 4.   | 1d     | -5.9           | 4.52         | -7.1           | 5.41         | -5.9             | 4.72          |
| 5.   | 1e     | -5.8           | 4.58         | -7.2           | 5.43         | -5.0             | 4.79          |
| 6.   | 1f     | -5.7           | 3.92         | -7.0           | 5.37         | -6.5             | 5.25          |
| 7.   | 1g     | -5.6           | 4.57         | -7.1           | 5.34         | -5.6             | 5.00          |
| 8.   | 1h     | -8.0           | 5.60         | -8.6           | 6.22         | -6.4             | 5.84          |
| 9.   | 1i     | -7.6           | 4.95         | -9.8           | 6.83         | -6.1             | 5.28          |
| 10.  | 1j     | -6.1           | 4.71         | -7.6           | 5.60         | -5.7             | 5.17          |
| 11.  | 1k     | -6.1           | 4.55         | -7.7           | 5.42         | -6.0             | 5.03          |
| 12.  | 1l     | -7.8           | 5.55         | -8.5           | 6.01         | -6.3             | 5.47          |
| 13.  | 1m     | -6.3           | 4.51         | -7.9           | 5.70         | -6.8             | 5.90          |
| 14.  | 1n     | -5.9           | 4.54         | -7.5           | 5.49         | -5.9             | 5.32          |
| 15.  | 1o     | -5.8           | 4.52         | -7.4           | 5.38         | -5.8             | 5.09          |
| 16.  | Ref    | -8.4           | 5.47         | -10.4 (BIBR)   | 7.00         | -6.4 (EPA)        | 5.54          |

(Taxol)

Declarations

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Author contributions
Conception and design of the study: Humaira Nadeem and Ahmed Sadiq Sheikh.; Design of the assays: Humaira Nadeem and Muhammad Umar Khayam Sahibzada; Data Acquisition: Ahmed Sadiq Sheikh, Nadia Shamshad Malik and Asif Mahmood Aslam; Analysis of results: Humaira Nadeem, Mahboob Alam, Ameer Khusro, Akhtar Aman, Muhammad Umar Khayam Sahibzada. and Muhammad Tariq Khan.; Drawing of figure images: Muhammad Tariq Khan and Ameer Khusro; Interpretation of the data: all authors; Drafting, revising and approval of the manuscript: all authors.

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**Figures**
Figure 1

Two-dimensional (2D) presentation of binding interactions of synthesized compounds with the amino acid residues of the binding site of Tubulin (PDB ID: 1JFF)
Figure 2

Two-dimensional (2D) presentation of binding interactions of synthesized compounds with the amino acid residues of the binding site of Telomerase Reverse Transcriptase (PDB ID: 5CQG)
Figure 3

Two-dimensional (2D) presentation of binding interactions of synthesized compounds with the amino acid residues of the binding site of Carbonic Anhydrase IX (PDB ID: 6VKG).

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