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NEW AMINOACETYLENIC-2-METHYLINDOLINE AS ANGIOGENESIS AND COX INHIBITORS

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ABSTRACT

A recent approach in cancer treatment is the angiogenesis inhibitors, as represented by the endothelial growth factor receptor inhibitors and vascular endothelial growth factor receptor inhibitors such as thalidomide and lenalidomide. We envision structural analogues to lenalidomide namely aminoacetylenic-2-methylindoline series as a novel and new angiogenesis inhibitors.

Aminoacetylenic-2-methylindoline derivatives were synthesized from reaction of 2-methylindoline with 3-bromoprop-1-yne to generate 2-methyl-1-(prop-2-yn-1-yl)-2,3-dihydro-1H-indole (AZ-1). A mixture of 2-methyl-1-(prop-2-yn-1-yl)-2,3-dihydro-1H-indole, paraformaldehyde, cyclic amine and cuprous chloride catalytic amount, in peroxide free dioxane through Mannich reaction yielded the desired aminoacetylenic-2-methylindoline derivatives (AZ2-7). The IR, DSC, ¹H-NMR, ¹³C-NMR and elemental analysis results were consistent with

the assigned structures. The docking results showed that all the designed compounds have good epidermal growth factor (EGF) receptor inhibition specifically **AZ-4** which scores -8.3 Kcal/mol, the invitro antiproliferative activity of the newly synthesized compounds exhibited some activity against breast and colon cancer cell lines. Further the COX-2 receptor inhibition specifically showed by **AZ-5** which scores -8.6 Kcal/mol suggested a promising effect in inhibiting the formation of new blood vessels, thereby inhibit tumor metastasis, diabetic retinopathy and rheumatoid arthritis.

Keywords: Aminoacetylenic; Antiproliferative; Angiogenesis; Epidermal growth factor

1. INTRODUCTION

Angiogenesis are generally suppressed in healthy adult organisms and is turned on temporarily in settings such as the female reproductive cycle or during tissue repair processes [1]. The “angiogenic switch” [2] is determined by opposing forces of proangiogenic and anti-angiogenic factors. Depending on the activity on each end of the balance, the “angiogenic switch” is turned “Off”, “On”, or is in a balance. The “angiogenic switch” is likely turned “On” in several diseases such as psoriasis, rheumatoid arthritis, diabetic retinopathy, and cancer [3]. Switching to an angiogenic phenotype likely requires both up-regulation of angiogenesis activators and downregulation of angiogenesis inhibitors [4]. Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are the most extensively studied angiogenesis inducers [5]. Among the various endogenous inhibitors of

angiogenesis [6] certain factors like Arresten [7], Canstatin [8] and Tumstatin [9] are derived from extracellular matrix (ECM). The ECM is a complex structure composed of many different glycoproteins, proteoglycans and hyaluronic acid.

Endogenous inhibitors of angiogenesis are defined as proteins or fragments of proteins that formed in the body and can inhibit the formation of blood vessels. Many endogenous inhibitors of angiogenesis are fragments of larger extracellular matrix (ECM) molecules. These fragments become released upon proteolysis of the ECM and the vascular basement membrane by enzymes, such as matrix metalloproteinases (MMP), cathepsins and elastases.

In addition to block cell cycle progression, most of the endogenous angiogenesis inhibitors induce intrinsic and/or extrinsic apoptotic pathways such as angiostatic/endostatic [10].

Drugs that block endothelial cell proliferation are:**Thalidomide**

Thalidomide inhibits angiogenesis induced by basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), inhibit tumor necrosis factor alpha (TNF- α), cyclooxygenase 2 (COX2), and modify the extracellular matrix [11]. The main side effects of thalidomide include fatigue, constipation, nausea, vomiting, peripheral neuropathy and drowsiness [12]. It has been suggested that the teratogenic effects of thalidomide on fetal limbs may be related to inhibition of blood vessels growth in the developing fetal limb bud [13]. Thalidomide's anti-angiogenic effects have been demonstrated in several animal angiogenesis models and there is evidence that the drug's anti-angiogenic effects may be species specific, and possibly may be related to a species-specific metabolite and/or metabolic activation. Thalidomide reduced the area of vascularization in a rabbit corneal model of induced neovascularization [14].

Thalidomide exists in two isoforms (Fig. 1), R and S. the R isomer is hypnotic while the S isomer is responsible for the teratogenic activity [15]. Thalidomide also inhibited angiogenesis in a rat aorta model, and in human aortic endothelial cells when human

or rabbit microsomes were present, but not when rat microsomes were present [12].

The mechanism of thalidomide's anti-angiogenic effects is through the inhibition of cytokine synthesis especially that of TNF- α . Although some evidence from animal models showed that thalidomide's effect on angiogenesis may result from a direct inhibitory effect on some components of angiogenesis [16].

Lenalidomide

Lenalidomide, thalidomide analogue, is an immunomodulatory agent with anti-angiogenic properties, lenalidomide has an asymmetric carbon atom and can exist as the optically active form S (-) and R (+), and is produced as a racemic mixture with a net optical rotation of zero. Indolone group instead of phthalimide in thalidomide [17]. Lenalidomide inhibits the secretion of pro-inflammatory cytokines and increases the secretion of anti-inflammatory cytokines from peripheral blood mono-nuclear cells. Lenalidomide inhibits cell proliferation with varying effectiveness (IC₅₀) in some but not all cell lines [18]. Lenalidomide (Fig. 2) inhibits the expression of cyclooxygenase -2 (COX-2) but not COX-1 in vitro [19].

Linomide

A quinolone-3-carboxamide with potential antineoplastic activity. Roquinimex inhibits

endothelial cell proliferation, migration, and basement membrane invasion; reduces the secretion of the angiogenic factor (tumor necrosis factor alpha) by tumor-associated macrophages (TAMs); and inhibits

angiogenesis. Linomide (Fig.3) is also an immune modulator that appear to alter cytokine profiles and enhance the activity of T cells, natural killer cells, and macrophages [20, 21].

Fig.1: (RS)-2-(2,6-dioxopiperidin-3-yl)-1H-isoindole-1,3(2H)-dione [15]

Fig. 2: 3-(4-amino-1-oxo-1,3-dihydro-2H-isoindol-2-yl)piperidine-2,6-dione (lenalidomide) [19].

Fig. 3: 4-hydroxy-N-1-dimethyl-2-oxo-N-phenyl-1,2-dihydroquinoline-3-carboxamide[21].

Veliparib, ABT-888

Veliparib is a poly (ADP-ribose) polymerase (PARP-1 and -2) inhibitor with chemosensitizing and antitumor activities.

With no anti-proliferative effects as single agents at therapeutic concentration, ABT-888 inhibits PARPs, thereby inhibiting DNA repair and potentiating the cytotoxicity of

DNA-damaging agents. PARP nuclear enzymes are activated by DNA single or double strand breaks, resulting in the poly ADP-ribosylation of other nuclear DNA-binding proteins involved in DNA repair, poly-ribosylation contributes to efficient DNA repair and to survival of proliferating cells exposed to mild genotoxic stresses as induced by oxidants, alkylating agents or ionizing radiation [22]. (Fig. 4).

In reviewing various structural features of different drugs in use and compounds under investigation as angiogenesis inhibitors, utilization of fractional base analysis in drug design, we envision the synthesis of aminoacetylenic derivatives of 2-methyl indoline as shown in Fig. 5 for the following reasons: 2-methylindoline is an isoster or fractional base analogue to phthalimide in thalidomide and indolone in lenalidomide. Replacement of glutarimide in thalidomide and lenalidomide by unique aminoacetylenic group to avoid teratogenicity and side effects associated with thalidomide and

lenalidomide. Aminoacetylenic moiety (Fig. 5) provide the functional groups to provide the required overlap with EGF receptor that generate antagonistic activity may be through the following:

- a- Amino group for ionic or hydrogen bonding with corresponding groups in different amino acid components of EGF receptor, as confirmed by molecular docking.
- b- Acetylenic group for electrostatic interaction with π -overlap located on the EGF receptor.
- c- 2-butyne to provide specific and appropriate distance between the indoline and the cyclic amino group.
- d- Non-bonding charge transfer between acetylenic bond and 2-methylindoline in our compounds with various hydrogen donating groups located in the EGF receptor.

Docking results and COX inhibitor activity with preliminary anticancer activity supporting our rationalization.

Fig. 4: 2-[(R)-2-methylpyrrolidine-2-yl]-1H-benzimidazole-4-carboxamide [22]

Fig. 5: Aminoacetylenic derivatives of 2-methyl indoline.

MATERIALS AND METHODS

Chemicals

The following chemicals and materials were used: sodium metal (C& S chemicals, USA), 2- methylindoline , propargyl bromide (Sigma-Aldrich, USA), piperdine 995 reagent plus (Sigma-Aldrich USA), 2- methylpiperidine 99% (Sigma-Aldrich, USA), 2,6-dimethylpiperidine, pyrrolidine 98% (Sigma-Aldrich, USA), N- methylpiperazine 99% (Sigma-Aldrich, USA), hexamethyleneimine 98% (Sigma-Aldrich, USA), tetrahydrofuran (Acros Organics,

GeelBelgium), 1,4-dioxan (Full Time, China), diethylether 99% (Lonover, England), benzene (MB laboratory, Sidney BC), chloroform (TEDIA, USA), acetonitrile (TEDIA, USA), potassium carbonate anhydrous (Gainland chemical company, UK), paraformaldehyde (BDH Chemicals, EU), cuprous chloride ((East Anglia chemicals, Hadleigh Ipswich).

Instrumentation

Infrared spectra (IR) were recorded using a Nicolet Impact 410 FT-IR spectrophotometer.¹H- and ¹³C-NMR were

acquired with the aid of Varian 300 MHz spectrometer and DMSO- d_6 as solvent, and TMS as standard. Differential scanning calorimetric were indicated using a Mettler Toledo STAR[®]System spectrum. The analysis was indicated by elemental analysis apparatus symbols of the elemental analyzed; the result obtained had a maximum deviation of $\pm 0.4\%$ from the theoretical value.

Methods and experimental molecular docking

The EGFR kinase domain used in this docking study was downloaded from the protein data bank (PDB: 1XKK) [23]. Then the co-crystallized ligand and all water molecules were removed from the protein structure. Protein atoms were given partial charges using Kollman united atom model in the Autodock Tool program [24, 25]. The ATP binding site of the EGFR kinase domain was identified by its own co-crystallized ligand (lapatinib) then a grid box of a 60 x 50 x 60 Å size was created using the Autogrid module [26, 27] with a grid spacing of 0.375 Å.

Ligand 3D structures were built then were energy minimized using Maestro program [28] and the OPLS force field [29] respectively. Atomic partial charges were given for all ligands by Gasteiger-Marsili model (tertiary amine groups were assigned

as protonated) [30]. Next, ligands were docked into the ATP binding site using the Autodock software (version 4.2) [26, 27] where the Lamarckian Genetic Algorithm [26] was employed for the conformational sampling process. Subsequently, docked poses were scored via the Autodock scoring function which includes terms for van der Waals, hydrogen bond, electrostatic interactions, and the ligand internal energy.

The 3D structures of COX-1 and COX-2 were downloaded from the protein data bank (RCSB Protein Data Bank) (PDB, ID: 3N8Z [31] and 3NT [32] and then all water molecules were removed. Partial charges were assigned to all atoms using Kollman united atom model that exists in the Autodock Tool software [24, 25]. Preparations of the COX-1 and COX-2 active site were completed by creating a grid box of a 50 x 50 x 50 Å size with a grid spacing of 0.375 Å using Autogrid (part of the Autodock software package [26, 27]). The active site was identified using the COX-1 and COX-2 co-crystallized ligands. After preparing the protein structures, the ligand preparation process was initiated by building their own 3D structures using the Maestro software [28]. A minimization process was carried out on each ligand structures using OPLS force field [29].

Subsequently, Gasteiger-Marsili model [30] was employed to assign partial charges for all prepared ligands. Tertiary amines of all ligands were set as protonated. The ligands were separately docked into the previously prepared active site using Autodock [26, 27] (version 4.2). Whereas the protein structure was treated as a rigid entity, the ligand structures were treated as flexible and a conformational sample process was carried out using Lamarckian Genetic Algorithm [26]. The Autodock scoring function was then used to score all docked poses. The Autodock score function includes different terms for van der Waals, hydrogen bond, electrostatic interactions, and the ligand internal energy.

Antiproliferative activity assay

Cells culture

The human breast adenocarcinoma MCF-7 and the human colon cancer Caco-II cell lines were purchased from the American Type Culture Collection (Rockville, MD, USA). The cells were grown and maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco, Waltham, MD, USA) supplemented with 1% of 2mM L-glutamine (Lonza), 10% fetal calf serum (Gibco, Paisley, UK), 50 IU/ml penicillin/streptomycin (Sigma, St. Louis, MO) and amphotericin B (Sigma, St. Louis,

MO) and were incubated in humidified atmosphere of 95% O₂ and 5% CO₂ at 37 °C. Cell cultures were passaged every 2-3 days or whenever the cells were reaching 80% confluent. Cells were seeded in 96-well micro-plates at density of 6-8 x 10³ cells/well and incubated for 24h to allow for adhesion.

Cell proliferation assay (MTT)

To evaluate cell proliferation, the MTT colorimetric assay was used as previously described [33]. The newly synthesized compound (AZ2-AZ7) at the desirable concentration were added to the wells. After the end of the exposure period (48 h), MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Dorset, UK) was applied into each well and incubated at 37 °C for 4 h. The yellow tetrazolium dye of MTT was reduced by metabolically active cells into an intracellular purple formazan product. Afterward, DMSO was added to each well to solubilize the purple formazan crystals formed. Then the absorbance was read using a micro-plate reader at 570 nm. Doxorubicin was used as the positive control and 0.1% DMSO in DMEM media as a solvent control.

Chemistry

Synthesis

Synthesis of 2-methyl-1-(prop-2-yn-1-yl)-2,3-dihydro-1H-indole (AZ-1)

To 2-methylindoline (1.75 g, 13.2 mmol) and K_2CO_3 (2.18 g, 15.8 mmol) in acetonitrile (20 ml), 3-bromoprop-1-yne (1.88 g, 15.8 mmol) in acetonitrile (10 ml) was added with continuous stirring, the mixture was refluxed for 80 min.

After cooling, the insoluble residue was removed by filtration and the filtrate then concentrated in vacuum. The resulting mixture was extracted with chloroform and distilled water using separatory funnel. The organic layer was treated with magnesium sulphate anhydrous, filtered and concentrated in vacuo to afford the desired brown crystal,

recrystallization from diethylether to afford compound (1.6 g, 93%), IR (NaCl, Cm^{-1}): 3020, 2970, (ArH, stretch), 3300 ($C\equiv C-H$, stretch), 2100 ($C\equiv C$, stretch), 1610 (Ar, $C=C$, stretch), 1250, 1100, 1010 (Ar, $C=c$ bending). 1H -NMR ($DMSO-d_6$): δ 1.22 (d, 3H, $C-CH_3$), 3.06 (d, 1H, CH_2-CH-N), 3.15 (s, 1H, $C\equiv CH$), 3.53, 3.92 (s, 2H, $CH_2-C\equiv CH$), 3.66 (d, 1H, CH_2-CH-N), 3.86 (m, 1H, $J=6.15$ Hz, $N-CH-CH_3$), 6.81-7.28 (m, 4H, ArH), Anal. Calcd, ($C_{12}H_{13}N$): C (84.3%); H (7.6%); N (8.1%). Found C (84.2%); H (7.58%); N (8.15%). DSC: melting point=58 °C.

Scheme 1 Synthesis of 2-methyl-1-(prop-2-yn-1-yl)-2,3-dihydro-1H-indole.

Compound **AZ-1** may be synthesized through other procedure: A solution of 2-methylindoline sodium (1.57 g, 0.012 mol) in 40 ml tetrahydrofuran was refluxed up to 60-65 °C. 3-bromoprop-1-yne (3 g, 0.03 mol) was added to the solution for 30 min as (10 ml every 10 min). The mixture was stirred for

70 min and filtered to give brown (10 ml) solution. The solvent was fractionated between chloroform and water, the chloroform layer was separated, dried with magnesium sulphate anhydrous and filtered. The solvent was removed under reduced pressure to afford the desired brown semi-

solid (1.8 g, 105.2%), and showed similar IR, ¹H-NMR and elemental analysis.

Synthesis of 2-methyl-1-[4-(2-methyl-1 λ⁴-amino-1-yl)but-2-yn-1-yl]-2,3-dihydro-1H-indole (AZ2-AZ7).

A mixture of 2-methyl-1-(prop-2-yn-1-yl)-2,3-dihydro-1H-indole (1.55 g, 0.01 mol) paraformaldehyde (0.45 g, 0.02 mol) and the cyclic amine (around 0.01 mol), and cuprous chloride catalytic amount (0.03 g), in peroxide-free dioxane (20 ml) was refluxed for 1 h. filtered and evaporated under reduced pressure. Diethyl ether was added to the residue resulting in the desired product (AZ2-AZ7) (Fig. 5).

Synthesis of 1-[4-(2,6-dimethyl-1λ⁴-pyridin-1-yl)but-2-yn-1-yl]-2-methyl-2,3-dihydro-1H-indole (AZ-2).

The titled compound was prepared following the general procedure for synthesis of 2-methyl-1-[4-(2-methyl-1λ⁴-pyridin-1-yl)but-2-yn-1-yl]-2-methyl-2,3-dihydro-1H-indole, (AZ2-AZ7). Yielded 3.45 g 116.5%. IR (NaCl, Cm⁻¹): 3048, 2964, 2926 (ArH, stretch), 1672, 1607, 1481, 1460 (Ar, C=C, stretch), 1091, 1057 (Ar, C=C, bending), 849, 749 (ArH, bending). ¹H-NMR (DMSO-D₆): δ, 1.16, 1.22, 1.34, 1.50, 1.66 (m, various protons of cyclicamine), 1.22, (d, 3H, CH-CH₃), 1.23-1.27 (q, 6H, N-C-CH₃) H splitting each one into double, 3.05 (m, 2H, N-CH, CH₃), 3.06

(d, 1H, CH-CH-N), 3.11, 3.51 (t, 2H, C-CH₂-N) due to long range coupling, 3.46, 3.85 (s, 2H, CH₂-C) due to long range coupling, 3.83 (m, 1H, J= 6.15Hz), 6.81-7.28 (m, 4H, ArH). ¹³C-NMR (DMSO-d₆): δ, 19 (C^{28, 39}), 21 (C^{17, 31}), 24 (C²¹), 35 (C⁷), 37 (C^{26, 27, 39}), 39 (C^{29, 30}), 41 (C¹⁴), 77 (C²⁴), 80 (C²⁵), 109 (C³), 118 (C⁴), 124 (C^{1, 5}), 127 (C⁶), 151 (C²). DSC: melting point = 109 °C. Anal. Calcd: (C₂₀H₂₈N₂) C, 81.08%; H, 9.46%; N, 9.45%. Found: C, 80.96%; H, 9.50%; N, 9.20%.

Synthesis of 2-methyl-1-[4-(4-methyl-1λ⁴,4λ⁴-pyrazin-1-yl)but-2-yn-1-yl]-2,3-dihydro-1H-indole (AZ-3).

The titled compound was prepared following the general procedure for synthesis of 2-methyl-1-[4-(2-methyl-1λ⁴-amino-1-yl)but-2-yn-1-yl]-2-methyl-2,3-dihydro-1H-indole, (AZ2-AZ7). Yielded 2.3 g 81.2%. IR (NaCl, Cm⁻¹): 3048, 2935, 2795 (ArH, stretch), 1607, 1482, 1456, (Ar, C=C, stretch), 1235, 1165, 1010 (Ar, C=C, bending), 817, 749 (ArH, bending). ¹H-NMR (DMSO-D₆): δ, 1.22 (d, 3H, C-CH₃), 2.14, 2.51, 2.82, 3.20 (m, various protons of cyclicamine), 2.24 (s, 3H, N-CH₃), 3.73 (t, 1H, C-CH₂-N), 4.13 (t, 1H, C-CH₂-N) due to long range coupling, 3.46 (t, 1H, CH₂-C), 3.77 (m, J= 6.15 Hz, 1H, N-CH-CH₃), 4.13 (t, 1H, CH₂-C) due to long range coupling, 6.81-7.28 (m, 4H, ArH). Anal. Calcd: (C₁₈H₂₅N₃) C, 76.32%; H,

8.83%; N, 14.84%. Found: C, 76.01%; H, 8.81%; N, 14.79%.

Synthesis of 2-methyl-1-[4-(2-methyl-1 λ^4 -pyridin-1-yl)but-2-yn-1-yl]-2,3-dihydro-1H-indole (AZ-4).

The titled compound was prepared following the general procedure for synthesis of 2-methyl-1-[4-(2-methyl-1 λ^4 -amino-1-yl)but-2-yn-1-yl]-2,3-dihydro-1H-indole, **AZ2-AZ7**. Yielded 2.4 g 120.5%. IR (NaCl, Cm^{-1}): 3048, 2963, 2929 (ArH, stretch), 1607, 1481, 1460, (Ar, C=C, stretch), 1374, 1234, 1065 (Ar, C=C, bending), 849, 749 (ArH, bending). $^1\text{H-NMR}$ (DMSO-D_6): δ , 1.22 (d, 3H, C-CH₃), 1.26 (d, 3H, J =, C-CH₃), 1.16, 1.33, 1.50, 1.51, 1.62, 1.72, 2.37, 2.95 (m, various protons of cyclicamine), 3.06 (d, 1H, CH₂-CH-N), 3.07, 3.47 (d, 2H, CH₂-N) due to long range coupling, 3.48, 4.87 (d, 2H, CH₂-C) due to long range coupling, 3.66 (d, 1H, CH₂-CH-N), 3.82 (m, 2H, J= 6.15 Hz, N-CH-CH₃), 6.81-7.28 (m, 4H, ArH). DSC: melting point = 98 °C.¹³ C-NMR (DMSO-D_6): δ , 19 (C^{28, 39}), 20 (C³¹), 24 (C¹⁷), 26 (C²¹), 34 (C⁷), 37 (C^{26, 27, 39}) 39 (C³⁰), 53 (C¹⁴), 78 (C²⁴), 80 (C²⁵), 109 (C³), 117 (C⁴), 124 (C^{1, 5}), 127 (C⁶), 151 (C²). Anal. Calcd: (C₁₉H₂₆N₂) C, 80.85%; H, 9.22%; N, 9.92%. Found: C, 80.65%; H, 9.01%; N, 9.89%.

Synthesis of 2-methyl-1-[4-(1 λ^4 -pyridin-1-yl)but-2-yn-1-yl]-2,3-dihydro-1H-indole (AZ-5).

The titled compound was prepared following the general procedure for synthesis of 2-methyl-1-[4-(2-methyl-1 λ^4 -amino-1-yl)but-2-yn-1-yl]-2,3-dihydro-1H-indole, **AZ2-AZ7**. Yielded 1.4 g 52.2%. IR (NaCl, Cm^{-1}): 3048, 2932, 2849 (ArH, stretch), 1607, 1481, 1460, (Ar, C=C, stretch), 1234, 1186, 1110 (Ar, C=C, bending), 852, 750, 718 (ArH, bending). $^1\text{H-NMR}$ (DMSO-D_6): δ , 1.22 (d, 3H, C-CH₃), 1.85, 1.96, 2.06, 2.28, 3.49 (m, various protons of cyclicamine), 3.06 (d, 1H, CH₂-C-N), 3.50, 3.89 (t, 2H, CH₂-C) due to long range coupling, 3.66 (d, 1H, CH₂-C-N), 3.81 (m, 1H, J= 6.15Hz, N-CH-CH₃), 3.73, 4.12 (t, 2H, C-CH₂-N) due to long range coupling, 6.81-7.28 (m, 4H, ArH). DSC: melting point = 99°C. Anal. Calcd: (C₁₈H₂₄N₃) C, 80.59%; H, 8.95%; N, 10.44%. Found: C, 80.62%; H, 9.15%; N, 10.81%.

Synthesis of 2-methyl-1-[4-(1 λ^4 -pyrrolidin-1-yl)but-2-yn-1-yl]-2,3-dihydro-1H-indole (AZ-6).

The titled compound was prepared following the general procedure for synthesis of 2-methyl-1-[4-(2-methyl-1 λ^4 -amino-1-yl)but-2-yn-1-yl]-2-methyl-2,3-dihydro-1H-indole, **AZ2-AZ7**. Yielded 2.8 g 110.2%. IR (NaCl,

Cm^{-1}): 2963 (ArH, stretch), 1607, 1460, 1431, (Ar, C=C, stretch), 1234, 1147, 1010 (Ar, C=C, bending), 749, 718 (ArH, bending). $^1\text{H-NMR}$ (DMSO-D_6): δ , 1.22 (d, 3H, N-C-CH₃), 1.89, 1.51, 2.98, 2.72, 3.15 (m, various protons of cyclicamine), 3.06 (d, 1H, CH₂-CH-N), 3.49, 3.89 (d, 2H, CH₂-C) due to long range coupling, 3.80 (m, 1H, J= 6.15 Hz, N-CH-CH₃), 3.73, 4.13 (d, 2H, CH₂-N) due to long range coupling, 6.81-7.28 (m, 4H, ArH). Anal. Calcd: ($\text{C}_{17}\text{H}_{22}\text{N}_2$) C, 80.32%; H, 8.66%; N, 11.02%. Found: C, 80.22%; H, 8.46%; N, 11.20%.

Synthesis of 1-[4-(azepam-1-yl)but-2-yn-1-yl]-2-methyl--2,3-dihydro-1H-indole (AZ-7).

The titled compound was prepared following the general procedure for synthesis of 2-methyl-1-[4-(2-methyl-1 λ^4 -amino-1-yl)but-2-yn-1-yl]-2,3-dihydro-1H-indole, **AZ2-AZ7**. Yielded 2.3 g 85.1%. IR (NaCl , Cm^{-1}): 3048, 2926, 2851 (ArH, stretch), 1607, 1481, 1460, (Ar, C=C, stretch), 1234, 1147 (Ar, C=C,

bending), 1088, 749 (ArH, bending). $^1\text{H-NMR}$ (DMSO-D_6): δ , 1.22 (d, 3H, N-CH-CH₃), 1.18, 1.46, 1.48, 1.62, 2.46, 2.89 (m, various protons of cyclicamine), 3.06 (d, 1H, CH₂-CH-N), 3.07, 3.47 (d, 2H, CH₂-N) due to long range coupling, 3.06 (d, 1H, CH₂-CH-N), 3.50, 3.89 (d, 2H, CH₂-C) due to long range coupling, 3.66 (d, 1H, CH₂-CH-N), 3.18 (m, 1H, J= 6.15 Hz, N-CH-CH₃), 3.73, 4.13 (d, 2H, C-CH₂-N), due to long range coupling, 6.8-7.28 (m, 4H, ArH). Anal. Calcd: ($\text{C}_{19}\text{H}_{26}\text{N}_2$) C, 80.85%; H, 9.22%; N, 9.92%. Found: C, 79.98%; H, 9.40%; N, 10.12%.

Statistical analysis

All experiments were done in triplicates and the results were expressed as mean $\pm$ standard deviation SD, being analyzed using a student's t-test. The analysis was carried out using IBM SPSS statistic for Window version 22.0 (IBM Corp., Armonk, NY, USA).

AZ-2 1-[4-(2,6-dimethyl-1 λ^4 -pyridin-1-yl)but-2-yn-1-yl]-2-methyl-2,3-dihydro-1H-indole)

AZ-3 2-methyl-1-[4-(4-methyl-1 λ^4 ,4 λ^4 -pyrazin-1-yl)but-2-yn-1-yl]-2,3-dihydro-1H-indole

AZ-4 2-methyl-1-[4-(2-methyl-1 λ^4 -pyridin-1-yl)-1H-but-2-yn-1-yl]-2,3-dihydro-1H-indole

AZ-5 2-methyl-1-[4-(1 λ^4 -pyridin-1-yl)but-2-yn-1-yl]-2,3-dihydro-1H-indole

AZ-6 2-methyl-1-[4-(1 λ^4 -pyrrolidin-1-yl)but-2-yn-1-yl]-2,3-dihydro-1H-indole

AZ-7 1-[4-(azepam-1-yl)but-2-yn-1-yl]-2-methyl-2,3-dihydro-1H-indole

Fig. 5: Aminoacetylenic compounds (AZ2-AZ7).

RESULTS AND DISCUSSION

Chemistry and molecular docking

The designed compounds were prepared according to schemes 1 and 2. The potassium salt of 2-methylindoline in acetonitrile was treated with a solution of 3-bromoprop-1-yne in acetonitrile. The mixture was heated up to 78 °C with continuous stirring for 80 min, cooled, filtered and the mixture was extracted with chloroform and distilled water using separatory funnel. The organic layer was concentrated in vacuum to afford the desired brown crystals, recrystallization from diethylether to afford the desired compound 2-methyl-1-(prop-2-yn-1-yl)-2,3-dihydro-1H-indole (**AZ-1**).

Compound **AZ-1** was generated through nucleophilic displacement to the bromine

located at 3-bromoprop-1-yne. The Mannich reaction of 2-methyl-1-(prop-2-yn-1-yl)-2,3-dihydro-1H-indole with paraformaldehyde, appropriate cyclic amine and catalytic amount of cuprous chloride. Yielded the desired compounds (**AZ2-AZ7**). The IR, ^1H -NMR, ^{13}C -NMR and elemental analysis were consistent with the assigned structures. The proposed mechanism for Mannich reaction is outlined in scheme 2 and 3. In order for Mannich reaction to proceed a reactive ammonium cation intermediates should be formed, from the condensation of formaldehyde and the appropriate amines (Schiff base formation). The attack of the carbon anion in 2-methyl-1-(prop-2-yn-1-yl)-2,3-dihydro-1H-indole cuprous salt on the

Schiff base, generate the desired Mannich products (AZ2-AZ7).

Scheme 2 Alkylation reaction of 2-methylindoline moiety.

Scheme 3: Proposed Mannich product

The aminoacetylenic-2-methylindoline compounds as EGFR antagonists are based on rationalization for the important criteria needed to overlap effectively with EGF receptor to induce antagonistic activity: Were the basic cyclic amino group for ionic interaction or for hydrogen bonding, the acetylenic group for electrostatic interaction, the 2-butyne provide specific and appropriate distance between the indoline and the cyclic amine, 2-methylindoline an isoster or fractional base analogue to thalidomide and

lenalidomide found in EGFR antagonists and many other biologically active compounds. Docking results in support of our assumption that kinase inhibitors are known to have cyclic system which has a nitrogen atom that is able to make an electrostatic interaction with the backbone amide of the ATP-binding site hinge region. Another amino group could exist in the kinase inhibitor which makes a water-mediated hydrogen bond with the Thr845 hydroxyl group (also exists in the purine binding region). Additionally, they

usually have a hydrophobic cyclic system that fits in a hydrophobic specificity determinant pocket on the other side of the binding site.

Our compounds were designed to have two heterocyclic linked with each other via rigid spacer (an acetylenic group), such a design should offer the ability to bind with the purine binding region via first cyclic system and with the specificity binding region via the second cyclic group. The rigid linker should boost compound binding via decreasing the entropic penalty (usually associated with flexible linker).

The **AZ** compounds have 2-methyl indoline as a core structure and it is linked with a heterocyclic system via an acetylenic linker. All dock compounds had favorable binding energies (Table 2) and showed interesting multiple binding modes where the two cyclic system had the ability to swap their positions and to make interesting electrostatic interactions. As shown in (Fig.7), the piperidine ring protonated nitrogen was one time bound with the Asp 855 and Asn 842 side chains and another time was bound to the Thr 845 hydroxyl group (which is involved in the usual water mediated hydrogen bond). In the first mode, which is more favorable (has less binding energy), the 2-methyl indoline ring was placed in the

hydrophobic specificity pocket, making extensive van der Waals interactions with the surrounding residue. Hence, these compounds are thought to have what it takes to be good binders for the EGFR-kinase enzyme.

Compound **AZ-4** had the best score among 2-methylindoline compounds (-8.3 Kcal/mol). **AZ-4** also had an electrostatic interaction with the ATP-binding site of the EGFR kinase domain with additional electrostatic interaction (Fig. 7). The aminoacetylenic 2-methylindoline derivatives also have favorable binding energies to COX-1 and COX-2 enzymes so they seem to have good fitting to these active sites (Table 2).

Compound **AZ-5** was predicted to have best binding (-8.0 Kcal/mol) with COX-1 active site. As shown in Fig.8). **AZ-5** has a good fitting and makes many van der Waals contacts with the surrounding hydrophobic residues (i.e. Val 349, Tyr 355, Leu 359, Trp 387, Phe 518, Met 522, Ile 523, and leu 531). Moreover, it makes a strong hydrogen bond with the backbone amide of Met 522. Compound **AZ-5** was also the best binder with the COX-2 active site (Fig.9) where it has a similar binding mode to that adopted in the COX-1 pocket. In addition to the extensive hydrophobic interactions made by **AZ-5**, an electrostatic interaction with the

Leu 352 backbone amide was made by its protonated nitrogen atom.

Antiproliferative activity

The newly synthesized derivatives were tested against breast (MCF-7) and colon cancer lines (Caco-2) for their antitumor

activities. Interestingly, these aminoacetylenicmethyldoline exhibited weak antiproliferative activities relative to known standard doxorubicin that has IC_{50} values of 2-10 $\mu\text{M/L}$ (Table 3). Further structural variation to enhance the antiproliferative activity will be investigated.

Fig. 7 Shows multiple binding modes demonstrated by AZ-4 (blue or pink sticks) in the ATP-binding site of the EGFR kinase domain (gold). The picture was generated by PyMol [31]. Electrostatic interactions are shown as yellow dotted lines. Some protein chains are not shown for clarity.

Table 1: Docking scores of different 2-methylindoline compounds in the EGF receptor active sites.

Molecule (Kcal/mol)	Autodock score
-13.8
AZ-2	-7.9
AZ-3	-8.1
AZ-4	-8.3
AZ-5	-7.6
AZ-6	-8.0
AZ-7	-7.9

Table 2: Docking scores of the synthesized compounds (AZ 2-7) into the COX-1 and COX-2 active site.

Compound	Autodock score in COX-1 (kcal/mol)	Autodock score in COX-2 (kcal/mol)
AZ-2	-7.6	-7.6
AZ-3	-7.7	-7.9
AZ-4	-7.7	-7.5
AZ-5	-8.0	-8.6
AZ-6	-7.7	-8.2
AZ-7	-8.0	-8.0
Flurbiprofen	-8.7	-8.0
Celecoxib	-8.5	-10.6

Fig. 8: Shows the binding mode of AZ-5 (orange) within the COX-1 active site (gray).

Fig. 9: Shows the binding mode of AZ-5 (orange) within the COX-2 active site (gray), respectively. Yellow dotted line indicate for hydrogen binding. The picture was generated by PyMol [31]. Some protein chains are not shown for clarity.

Table 3: The IC₅₀ values (μM/L) of the examined compounds

Compound	μM/L	
	MCF-7 x 10 ⁶	Caco-2 x 10 ⁶
AZ-2	0.473	0.446
AZ-3	0.571	0.593
AZ-4	0.593	0.635
AZ-5	0.466	0.515
AZ-6	0.501	0.535
AZ-7	0.475	0.457
Doxorubicin	2-10	2-10

Values are expressed as mean ± SD of three experiments

CONCLUSION

The synthesis and characterization of a new series of 2-methyl-1-(t-amino-2-butynyl)-2,3-dihydro-1H-indole, (AZ2-AZ7) were accomplished. Docking of the new aminoacetylenic 2-methylindoline compounds showed a promising approach in the treatment of cancer and other angiogenic related diseases like rheumatoid arthritis through inhibiting the activity of EGF receptor and COX receptors. The in vitro

antiproliferative activity of the newly synthesized compounds were tested against breast (MCF-7) and colon cancer cell lines (Caco-2). The design compounds exhibited weak activity relative to the well-known standard doxorubicin that has IC₅₀ values of 2-10 μM (Table 2). Further structural modifications to enhance the observed antiproliferative activity will be investigated.

CONFLICT OF INTEREST

The authors have disclosed no potential conflicts of interest, financial or otherwise.

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COMPLIANCE WITH ETHICAL STANDARDS

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