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Synthesis, molecular docking, antiproliferative and radical scavenging activities of vanillin derived 1,3,5-trisubstituted 2-pyrazolines

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Observing the good anticancer potential of 2-pyrazoline scaffold, a panel of 1,3,5- trisubstituted 2- pyrazolines namely, 5-(4-hydroxy-3-methoxyphenyl)-3-(4-substitutedphenyl)4,5-dihydro-1H-pyrazol-1 yl)(phenyl)methanones 2a-j and (5-(4-hydroxy-3-methoxyphenyl)-3-(4-substituted phenyl)4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanones 3a-j have been synthesized from the chalcone intermediates derived from the reaction between vanillin and substituted acetophenones, by condensing with benzhydrazide and isonicotinic acid hydrazide, respectively. The synthesized compounds have been characterized by spectral studies and screened for *in vitro* antiproliferative activity against human non small cell lung cancer cell line A549 by MTT assay and antioxidant activity by DPPH radical scavenging assay. The compound 2i has exhibited good antiproliferative activity followed by 2g and 2a. Compounds of both series 2a-j and 3a-j exhibited good radical scavenging activity. Molecular docking studies of 2h and 2j has revealed the good interaction with 3LCT (Crystal structure of anaplastic lymphoma kinase catalytic domain) receptor *via* hydrogen bonds, electrostatic and hydrophobic interactions. The drug-likeness properties of the compounds were also satisfactory leaving a good scope for further work.

Keywords: Chalcones; Benzhydrazide, Isonicotiniacid hydrazide, MTT assay, Antioxidant, *In Silico* ADME analysis

Despite significant progress made in molecular and cell biology in recent decades, cancer still remains an enigma[1]. There were 17 million new cases of cancer and an estimated 9.6 million deaths in 2018 worldwide. It is forecasted that, there will be 27.5 million new cancer cases each year by 2040 in the world. The lung, breast, bowel and prostate are the most common cancers occurring worldwide accounting for more than four in ten of all cancers diagnosed worldwide [2]. The incidence of lung cancer cases worldwide were 2.09 million with 1.76 million deaths in 2018. It is the leading cause of cancer-related deaths in the world with non-small cell lung cancer (NSCLC) making up about 85% of all lung cancer cases[3]. Resistance to anticancer drugs is a serious problem. Most of the anticancer drugs available today are selective against certain cancers and too toxic, but are tenable only because truly safe drugs are unavailable[1].

Free radical damage to macromolecules has been associated with cancer development. The increased level of antioxidants have shown to prevent such damage to macromolecules, Therefore, researchers have investigated the role of dietary antioxidant supplementation in lowering the risk of cancer development or mortality in humans[4].

Compounds embedded with 2-pyrazoline pendant are biodynamic agents and have been shown to display varied biological properties. The recent research on 2-pyrazolines established their promising role in cancer chemotherapy[5-7] and also in scavenging free radicals[8,9]. Further vanillin derivatives have also been shown to impart anticancer[10,11] and antioxidant potential[12].

All these aforementioned facts and our continuing interest on anticancer activity of 2-pyrazoline derivatives[13,14] triggered an interest to take up this investigation wherein the molecular architecture having 2-pyrazoline core derived from vanillin was developed with an anticipation of good anti-lung cancer and DPPH scavenging activity. Their spectral characterization, docking studies with anaplastic lymphoma kinase (ALK), *In silico* ADME parameter studies were also performed.

Experimental Details

Chemicals

The laboratory reagent grade chemicals were used without further purification. Vanillin, Substituted

acetophenones and DPPH were purchased from Loba Chemie Ltd, Mumbai, India. Ascorbic acid was purchased from SD Fine Chemicals Ltd, Mumbai, India. Eagles Minimum Essential Medium (MEM) and INH (Isonicotinicacid hydrazide) were purchased from HiMedia Laboratories Pvt. Ltd. Mumbai, India. Foetal Bovine serum (Thermo Fisher Scientific Waltham, USA) and 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide, MTT (Sigma Chemical Co., St. Louis, USA) were also purchased. Benzhydrazide was prepared as per the literature protocol[15-17]. Non small cell lung cancer cell line A549 was used to screen the compounds for antiproliferative activity.

Instrumentation

Melting points were determined using open capillary apparatus (Veego VMP-DS, Mumbai, India) and are uncorrected. Progress of the reaction and purity of the synthesized compounds was assessed by thin layer chromatography using aluminium sheets pre coated with 0.2 mm thick silica gel 60 F254 (Merck, Darmstadt, Germany) with various solvent combinations as mobile phase, the spots were resolved by iodine vapours. The UV spectra were taken in 99% ethanol on UV 1601 Shimadzu, Kyoto, Japan and Infrared spectra on Bruker Alpha-t, Massachusetts, USA as KBr disc. ^{1}H NMR was recorded in DMSO-d_6 on Agilent 400 MHz, California, USA. Chemical shifts were measured on δ scale in ppm downfield to TMS. ^{13}C NMR was recorded at 50 MHz. Mass spectra were recorded on Waters-SynaptG2, Massachusetts, USA by LCMS technique. The absorbance of MTT assay was read on Synergy TM HT Micro plate reader (Bio-tek Instruments Inc.,Winooski, USA).

Synthesis of chalcones 1a-j

Vanillin (0.1 mol, 15.2 g) and substituted acetophenones (0.1 mol) were dissolved in ethanol (95%). Dry HCl gas was passed into it for 40-45 min. The reaction mixture was left overnight at RT and then poured into crushed ice and neutralized with sodium bicarbonate if necessary. The solid so obtained was filtered and washed with water until excess acid is removed off. The chalcones were purified by recrystallization with aqueous alcohol (1:1).

(E)-3-(4-hydroxy-3-methoxyphenyl)-1-phenylprop-2-en-1-one (1a)

Dark brown powder; Yield 98%; m.p. 107-109°C; R_f 0.30 n-Hexane: Ethyl acetate (5:1); $\lambda_{max,}$ 294 nm; IR cm^{-1} 3432 (OH str), 3062 (Ar-CH str), 2935 (Ali-CH str), 1675 (C=O str), 1597 (C=C str), 1117 (C-O-C str)

(E)-3-(4-hydroxy-3-methoxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one (1b)

Greenish grey powder; Yield 74%; m.p. 170°C; R_f 0.18 n-Hexane : Ethyl acetate (5:1); $\lambda_{max,}$ 388 nm; IR cm^{-1} 3521 (OH str), 3101 (Ar-CH str), 3016 (Ali-CH str), 1647 (C=O str), 1569 (C-NO_2 str), 1519 (C=C str), 1122 (C-O-C str)

(E)-1-(4-bromophenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (1c)

Brown powder; Yield 94%; m.p. 100-103°C; R_f 0.30 n-Hexane : Ethyl acetate (5:1); $\lambda_{max,}$ 534 nm; IR cm^{-1} 3467 (OH str), 3033 (Ar-CH str), 2953 (Ali-CH str), 1672 (C=O str), 1598 (C=C str), 1120 (C-O-C str), 696 (C-Br str)

(E)-1-(4-chlorophenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (1d)

Dark brown powder; Yield 67%; m.p. 100-102°C; R_f 0.31 n-Hexane : Ethyl acetate (5:1); $\lambda_{max,}$ 535 nm; IR cm^{-1} 3457 (OH str), 3028 (Ar-CH str), 2966 (Ali-CH), 1669 (C=O str), 1592 (C=C str), 1092 (C-O-C str), 786 (C-Cl str)

(E)-3-(4-hydroxy-3-methoxyphenyl)-1-(4-iodophenyl)prop-2-en-1-one (1e)

Reddish brown powder; Yield 55%; m.p. 115-117°C; R_f 0.64 n-Hexane : Ethyl acetate (5:1); $\lambda_{max,}$ 378 nm ; IR cm^{-1} 3347 (OH str), 3068 (Ar-CH str), 2934 (Ali-CH str), 1676 (C=O str), 1577 (C=C str), 1121 (C-O-C str), 671 (C-I str)

***(E)-4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)benzonitrile* (1f)**

Yellowish orange powder; Yield 97%; m.p. 228-230°C; R_f 0.29 n-Hexane : Ethyl acetate (5:1); $\lambda_{max,}$ 382 nm; IR cm^{-1} 3345 (OH str), 3069 (Ar-CH str), 2991 (Ali-CH str), 2240 (CN m), 1657 (C=O str), 1574 (C=C str), 1117 (C-O-C str)

(E)-3-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (1g)

Orange powder; Yield 60%; m.p. 240-241°C; R_f 0.24 n-Hexane: Ethyl acetate (5:1); $\lambda_{max,}$ 362 nm; IR cm^{-1} 3733 (OH str), 3019 (Ar-CH str), 2973 (Ali-CH str), 1635 (C=O str), 1515 (C=C str), 1122 (C-O-C str)

(E)-3-(4-hydroxy-3-methoxyphenyl)-1-(p-tolyl) prop-2-en-1-one (1h)

Brown powder; Yield 90%; m.p. 128-130°C; R_f 0.28 n-Hexane: Ethyl acetate (5:1); $\lambda_{max,}$ 382 nm;

IR cm^{-1} 3508 (OH str), 3046 (Ar-CH str), 2972 (Ali-CH str), 1673 (C=O str), 1583 (C=C str), 1122 (C-O-C str)

(E)-3-(4-hydroxy-3-methoxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (1i)

Creamy yellow powder; Yield 47%; m.p. 160-161°C; R_f 0.15 n-Hexane : Ethyl acetate (5:1); $\lambda_{max,}$ 364 nm; IR cm^{-1} 3371 (OH str), 3071 (Ar-CH str), 2968 (Ali-CH str), 1650 (C=O str), 1597 (C=C str), 1119 (C-O-C str)

(E)-1-(4-aminophenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (1j)

Yellowish orange powder; Yield 48%; m.p. 220-222°C; R_f 0.32 n-Hexane : Ethyl acetate (5:1); $\lambda_{max,}$ 315 nm; IR cm^{-1} 3478 (NH_2), 3301 (OH str), 3046 (Ar-CH str), 3008 (Ali-CH str), 1662 (C=O str), 1581 (C=C str), 1173 (C-O-C str)

Synthesis of 1,3,5-trisubstituted 2-pyrazolines 2a-j and 3a-j

Chalcones **1a-j** (10 mmol) and benzhydrazide (10 mmol, 1.37 g) for **2a-j**/ INH (10 mmol, 1.5 g) for **3a-j** were dissolved in 10 mL glacial acetic acid and refluxed for 36-40 h. The progress of reaction was monitored by TLC. The mixture was poured into ice-cold water and was neutralized with sodium bicarbonate. The sticky nature was removed by treatment with brine if necessary. The solid so obtained was washed with ice-cold water and recrystallized with aqueous alcohol (1:1).

(5-(4-hydroxy-3-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)(phenyl)methanone (2a)

Brown powder; Yield 72%; m.p. 125-126°C; R_f 0.44 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 296 nm; IR cm^{-1} 3379 (OH str), 3060 (Ar-CH str), 2934 (Ali-CH str), 1687 (C=O str), 1595 (C=N str) , 1203 (C-O-C str); ^{1}H NMR δ: 8.92 (s, 1H, OH) 7.892-7.863 (t, *J*=11.6 Hz, 2H, ArH) 7.333-7.326 (d, *J*=2.8 Hz, 2H, ArH) 7.240-7.152 (m, 2H, ArH) 7.13 (s, 1H, ArH) 7.158-7.099 (m, 1H, ArH) 7.083-6.982 (m, 2H, ArH) 6.62 (s, 1H, ArH) 6.556-6.548 (d, *J*=3.2, 1H, ArH) 6.46 (s, 1H, ArH) 6.376-6.354 (d, *J*=8.8 Hz, 1H, CH Pyraz) 3.524-3.509 (d, *J*=6 Hz, 1H, CH_2 Pyraz) 3.324-3.386 (m, 3H, OCH_3) 3.269-1.251 (d, *J*=7.2, 1H, CH_2 Pyraz); ^{13}C NMR δ: 167.6, 151.2, 148.9, 148.1, 146.7, 141.3, 134.7, 134.6, 132.5, 131.1, 130.3, 128.4, 122.4, 118.5, 114.4, 112.1, 67.3, 56.2, 40.5; LC-MS *m/z*: 372.40 (M^+), 373.60 (M^++1).

(5-(4-hydroxy-3-methoxyphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazol-1 yl)(phenyl) methanone (2b)

Creamy yellow flakes; Yield 71%; m.p. 122-124°C; R_f 0.48 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 340 nm; IR cm^{-1} 3343 (OH str), 3076 (Ar-CH str), 2934 (Ali-CH str), 1739 (C=O str), 1645 (C=N str), 1572 (C-NO_2 str), 1120 (C-O-C str); ^{1}H NMR δ: 9.2 (s, 1H, OH) 8.386-8.375 (t, *J*=4.4 Hz, 2H, ArH) 8.105-8.082 (d, *J*= 10 Hz, 2H, ArH) 7.825-7.785 (m, 2H, ArH) 7.59 (s, 1H, ArH) 7.493-7.481 (m, 2H, ArH) 6.846-6.821 (m, 2H, ArH) 6.79 (s, 1H, ArH) 6.785-6.752 (d, *J*=13.2 Hz, H, ArH) 5.826-5.809 (d, *J*=6.8 Hz, 1H, CH Pyraz) 4.248-4.235 (d, *J*= 5.2 Hz, 1H, CH_2 Pyraz) 4.052-4.035 (d, *J*= 6.8 Hz, 1H, CH_2 Pyraz) 3.324-3.286 (m, 3H, OCH_3); ^{13}C NMR δ: 165.7, 151.9, 150.5, 149.3, 148.7, 143.8, 139.6, 133.8, 132.01, 128.3, 126.2, 119.8, 119.3, 117.2, 112.5, 63.56, 57.8, 40.24; LC-MS *m/z*: 417.00 (M^+), 418.23 (M^++1), 419.19 (M^++2).

[3-(4-bromophenyl)-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl](phenyl)methanone (2c)

Reddish brown powder; Yield 46%; m.p. 118-120°C; R_f 0.59 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 299nm ; IR cm^{-1} 3372 (OH str), 3065 (Ar-CH str), 2957 (Ali-CH str), 1734 (C=O str), 1646 (C=N str), 1127 (C-O-C str), 828 (C-Br str); ^{1}H NMR δ: 8.9 (s,1H,OH) 7.906-7.887 (dd, *J*= 7.6, 8.4 Hz, 2H, ArH), 7.692-7.676 (m, 2H, ArH) 7.653-7.613 (m, 2H, ArH) 7.552-7.449 (dd, *J*= 13.6, 11.2 Hz, 2H, ArH) 6.851-6.805 (t, *J*=18.4 Hz, 2H, ArH) 6.730-6.693 (m, 1H, ArH) 5.680-5.644 (dd, *J*= 3.6, 3.6 Hz, 1H, CH Pyraz) 3.560-3.509 (dd, *J*= 8.8, 6.8 Hz, 1H, CH_2 Pyraz) 3.508-3.492 (m, 3H, OCH_3) 3.428-3.418 (d, *J*=4 Hz, 1H, CH_2 Pyraz); ^{13}C NMR δ: 150.0, 138.1, 135.4, 134.0, 132.0, 131.6, 125.9, 119.8, 114.2, 57.4; LC-MS *m/z*: 451.00 (M^+), 452.99 (M^++1), 453.01 (M^++2).

(3-(4-chlorophenyl)-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)(phenyl)methanone (2d)

Yellowish brown powder; Yield 70%; m.p. 110-112°C; R_f 0.53 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 298 nm; IR cm^{-1} 3333 (OH str), 3060 (Ar-CH str), 2967 (Ali-CH str), 1674 (C=O str), 1588 (C=N str), 1125 (C-O-C str), 825.47 (C-Cl str); ^{1}H NMR δ: 9.84 (s, 1H, OH) 7.956-7.942 (dd, *J*= 6.8, 5.6 Hz, 2H, ArH) 7.848-7.791 (m, 2H, ArH) 7.639-7.602 (t, *J*=

14.8 Hz, 2H, ArH) 7.591-7.580 (d, *J*= 4.4 Hz, 1H, ArH) 7.498-7.367 (m, 2H, ArH) 6.839-6.832 (d, *J*= 2.8 Hz, 1H, ArH) 6.817-6.801 (d, *J*= 6.4 Hz, 1H, ArH) 6.795-6.773 (d, *J*= 8.8 Hz, 1H, ArH) 5.158-5.048 (dd, *J*= 10, 8.4 Hz, 1H, CH Pyraz) 3.803-3.784 (m, 3H, OCH_3) 3.549-3.511 (dd, *J*= 7.2, 6 Hz, 1H, CH_2 Pyraz) 3.439-3.391 (dd, *J*= 6, 4.4 Hz, 1H, CH_2 Pyraz); ^{13}C NMR δ: 167.2, 151.6, 148.1, 146.9, 137.2, 136.6, 134.8, 134.5, 128.9, 128.5, 128.2, 127.5, 120.1, 113.6, 111.2, 63.4, 58.3, 38.3; LC-MS *m/z*: 407.00 (M^+), 408.50 (M^++1).

(5-(4-hydroxy-3-methoxyphenyl)-3-(4-iodophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)(phenyl)methanone (2e)

Yellowish green powder; Yield 63%; m.p. 104-106°C; R_f 0.37 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 298 nm; IR cm^{-1} 3340 (OH str), 3061 (Ar-CH str), 2928 (Ali-CH str), 1690 (C=O str), 1585 (C=N str), 1123 (C-O-C str), 706 (C-I str); ^{1}H NMR δ: 8.92 (s, 1H, OH) 8.089-7.934 (m, 2H, ArH) 7.689-7.675 (m, 2H, ArH) 7.614-7.598 (m, 1H, ArH) 7.578-7.559 (m, 2H, ArH) 7.527-7.503 (m, 2H, ArH) 6.834-6.819 (d, *J*=6 Hz, 1H, ArH) 6.712-6.698 (d, *J*= 5.6 Hz, 1H, ArH) 6.683-6.667 (d, *J*= 5.6 Hz, 1H, ArH) 5.665-5.626 (dd, *J*= 4.4, 4 Hz, 1H, CH Pyraz) 3.772-3.756 (m, 3H, OCH_3) 3.534-3.517 (d, *J*= 6.8 Hz, 1H, CH_2 Pyraz) 3.161-3.104 (dd, *J*= 4.8, 4.4 Hz, 1H, CH_2 Pyraz); ^{13}C NMR δ: 167.8, 152.2, 147.8, 137.1, 135.4, 132.4, 130.8, 129.4, 128.0, 120.0, 115.1, 112.6, 99.0, 62.9, 58.0, 41.7; LC-MS *m/z*: 498.98 (M^+), 499.00 (M^++1).

4-(1-benzoyl-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)benzonitrile (2f)

Creamy yellow powder; Yield 83%; m.p. 140-142°C; R_f 0.40 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 320 nm; IR cm^{-1} 3372 (OH str), 3064 (Ar-CH str), 2937 (Ali-CH str), 2232 (CN str), 1724 (C=O str), 1649 (C=N str), 1120 (C-O-C str); ^{1}H NMR δ: 8.94 (s,1H, OH) 7.927-7.903 (m, 2H, ArH) 7.885-7.815 (m, 2H, ArH) 7.756-7.689 (m, 1H, ArH) 7.530-7.522 (m, 2H, ArH) 7.514-7.449 (m, 2H, ArH) 6.81 (s,1H, ArH) 6.737-6.653 (dd, *J*= 7.6, 7.2 Hz, 2H, ArH) 5.708-5.667 (dd, *J*= 4.8, 4.8 Hz, 1H, CH Pyraz) 3.894-3.818 (dd, *J*= 12.4, 12.4 Hz, 1H, CH_2 Pyraz) 3.749-3.677 (t, *J*= 28.8 Hz, 3H, OCH_3) 3.233-3.175 (dd, *J*= 5.2, 4.8 Hz, 1H, CH_2 Pyraz); ^{13}C NMR δ: 168.1, 150.0, 137.2, 135.0, 133.2, 129.6, 120.1, 117.9, 114.5, 112.6, 63.2, 58.0, 41.2; LC-MS *m/z*: 397.00 (M^+), 398.23 (M^++1), 340.40 (M^++2).

(5-(4-hydroxy-3-methoxyphenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)(phenyl)methanone (2g)

Brick red powder; Yield 37%; m.p. 120-122°C; R_f 0.46 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 310 nm; IR cm^{-1} 3369 (OH str), 3165 (Ar-CH str), 2938 (Ali-CH str), 1733 (C=O str), 1604 (C=N str), 1126 (C-O-C str) ^{1}H NMR δ: 9.96 (s, 1H, OH) 9.68 (s, 1H, OH) 8.035-8.019 (d, *J*= 6.4 Hz, 2H, ArH) 7.726-7.684 (m, 2H, ArH) 7.589-7.498 (m, 2H, ArH) 6.417-6.374 (m, 1H, ArH) 6.812-6.790 (d, *J*= 8.8 Hz, 1H, ArH) 6.658-6.632 (t, *J*= 10.4 Hz, 1H, ArH) 5.865-5.803 (dd, *J*= 9.6, 8.4 Hz, 1H, CH Pyraz) 3.781-3.662 (m, 3H, OCH_3) 3.489- 3.411 (dd, *J*= 6.8, 6.4 Hz, 1H, CH_2 Pyraz) 3.208-3.163 (dd, *J*= 6, 4.8 Hz, 1H, CH_2 Pyraz); ^{13}C NMR δ: 168.3, 161.3, 152.1, 148.1, 145.8, 138.4, 133.4, 129.4, 128.6, 120.6, 115.8, 114.9, 112.3, 63.8, 57.2, 40.9; LC-MS *m/z*: 389.09 (M^++1), 390.09 (M^++2).

(5-(4-hydroxy-3-methoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)(phenyl)methanone (2h)

Dark brown powder; Yield 76%; m.p. 88-90°C; R_f 0.54 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 296 nm; IR cm^{-1} 3378 (OH str), 3057 (Ar-CH str), 2929 (Ali-CH str), 1669 (C=O str), 1119 (C-O-C str); ^{1}H NMR δ: 8.69 (s, 1H, OH) 7.823-7.804 (m, 2H, ArH) 7.793-7.781 (m, 2H, ArH) 7.714-7.623 (m, 2H, ArH) 7.613-7.591 (d, *J*= 8.8 Hz, 1H, ArH) 7.285-7.268 (m, 2H, ArH) 6.767-6.753 (d, *J*= 5.6 Hz, 2H, ArH) 6.735-6.723 (d, *J*= 4.8 Hz, 1H, ArH) 6.280-6.223 (dd, *J*= 5.2, 4.4 Hz, 1H, CH Pyraz) 3.895-3.868 (t, *J*= 10.8 Hz, 1H, CH_2 Pyraz) 3.689-3.637 (dd, *J*= 6.8, 6.8 Hz, 1H, CH_2 Pyraz) 3.432-3.417 (m, 3H, OCH_3) 2.446-2.428 (m, 3H, CH_3); ^{13}C NMR δ: 151.8, 149.6, 147.3, 145.3, 139.2, 136.7, 129.1, 128.7, 118.3, 114.9, 64.3, 57.0, 41.2, 22.1; LC-MS *m/z*: 386.00 (M^+), 388.06 (M^++2).

(5-(4-hydroxy-3-methoxyphenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)(phenyl)methanone (2i)

Brownish green powder; Yield 53%; m.p. 90-92°C; R_f 0.41 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 309 nm; IR cm^{-1} 3369 (OH str), 3066 (Ar-CH str), 2938 (Ali-CH str), 1737 (C=O str), 1601 (C=N str), 1123 (C-O-C str); ^{1}H NMR δ: 8.96 (s, 1H, OH) 8.141-8.120 (d, *J*= 8.4 Hz, 1H, ArH) 7.842-7.826 (d, *J*= 6.4 Hz, 3H, ArH) 7.635-7.614 (d, *J*= 8.4 Hz, 2H, ArH) 7.717-7.701 (d, *J*= 6.4 Hz, 2H, ArH) 7.064-7.043 (d, *J*= 8.4 Hz, 2H, ArH) 6.841-6.805 (t, *J*= 6 Hz, 1H, ArH)

6.727-6.707 (d, *J*= 8 Hz, 1H, ArH) 6.656-6.636 (d, *J*= 8 Hz, 1H, ArH) 5.651-5.612 (dd, *J*= 4.4, 4 Hz, 1H, CH Pyraz), 3.851-3.788 (m, 3H, OCH_3) 3.760-3.705 (m, 3H, OCH_3) 3.433-3.384 (dd, *J*= 7.2, 5.6 Hz, 1H, CH_2 Pyraz) 3.150-3.094 (dd, *J*= 4.4, 4.4 Hz, 1H, CH_2 Pyraz); ^{13}C NMR δ: 168.0, 162.1, 151.1, 137.6, 134.8, 133.4, 131.0, 130.7, 118.7, 117.2, 67.3; LC-MS *m/z*: 402.00 (M^+), 403.99 (M^++1), 404.32 (M^++2).

(3-(4-aminophenyl)-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)(phenyl)methanone (2j)

Light brown flakes; Yield 67%; m.p. 147-148°C; R_f 0.36 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 316 nm; IR cm^{-1} 3326 (OH str), 3059 (Ar-CH str), 2948 (Ali-CH str), 1744 (C=O str), 1625 (C=N str), 1122 (C-O-C str); ^{1}H NMR δ: 10.16 (s, 1H, NH) 8.98 (s, 1H, OH) 7.932-7.906 (d, *J*= 10.4 Hz, 2H, ArH) 7.675-7.664 (m, 2H, ArH) 7.628-7.610 (m, 1H, ArH) 7.566-7.495 (m, 2H, ArH) 7.321-7.287 (m, 2H, ArH) 6.843-6.825 (d, *J*= 7.2 Hz, 2H, ArH) 6.730-6.711 (d, *J*= 7.6 Hz, 1H, ArH) 5.653-5.614 (dd, *J*= 4.4, 4.0 Hz, 1H, CH Pyraz) 3.449-3.365 (m, 3H, OCH_3) 3.142-3.087 (dd, *J*= 4.4, 4 Hz, 1H, CH_2 Pyraz) 2.260-2-215 (d, *J*= 18 Hz, 1H, CH_2 Pyraz) 2.073-2.037 (d, *J*= 14 Hz, 3H, CH_3) ^{13}C NMR δ: 170.9, 167.6, 149.9, 149.9, 148.1, 143.5, 137.2, 135.6, 132.9, 129.8, 129.6, 127.9, 121.1, 119.9, 117.8, 112.5, 62.6, 57.9, 41.2; LC-MS *m/z*: 387.00 (M^+), 388.23 (M^++1), 389.56 (M^++2).

(5-(4-hydroxy-3-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)(pyridin-4-yl)methanone (3a)

Dark brown powder; Yield 52%; m.p. 156-158°C; R_f 0.44 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 270 nm; IR cm^{-1} 3384 (OH str), 3059 (Ar-CH str), 2939 (Ali-CH str), 1695 (C=O str), 1597 (C=N str) , 1208 (C-O-C str) ^{1}H NMR δ: 8.87 (s, 1H, OH) 8.308-8.288 (t, *J*= 8 Hz, 2H, ArH) 7.804-7.791 (d, *J*= 5.2 Hz, 2H, ArH) 7.640-7.581 (m, 2H, ArH) 7.559-7.489 (m, 1H, ArH) 7.394-7.357 (m, 2H, ArH) 6.81 (s, 1H, ArH) 6.784-6.767 (d, *J*= 6.8 Hz, 1H, ArH) 6.69 (s, 1H, ArH) 6.367-6.347 (d, *J*= 8 Hz, 1H, CH Pyraz) 3.761-3.671 (m, 3H, OCH_3) 3.922-3.903 (d, *J*= 7.6 Hz, 1H, CH_2 Pyraz) 3.446-3.395 (dd, *J*= 6.8, 6.0 Hz, 1H, CH_2 Pyraz); ^{13}C NMR δ: 168.1, 150.9, 149.7, 148.2, 146.4, 140.8, 135.7, 134.2, 130.7, 129.3, 128.7, 122.1, 119.6, 115.8, 111.4, 68.7, 57.9, 40.1; LC-MS *m/z*: 373.00 (M^+), 374.86 (M^++1).

(5-(4-hydroxy-3-methoxyphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)(pyridin-4-yl)methanone (3b)

Light brown powder; Yield 72%; m.p. 150-152°C; R_f 0.22 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 335 nm; IR cm^{-1} 3330 (OH str), 3076 (Ar-CH str), 2938 (Ali-CH str), 1710 (C=O str), 1647 (C=N str), 1597 (C-NO_2 str), 1121 (C-O-C str); ^{1}H NMR δ: 8.9 (s, 1H, OH) 8.72 (s, 2H, ArH) 8.348-8.064 (m, 2H, ArH) 7.922-7.881 (dd, *J*= 8, 5.2 Hz, 2H, ArH) 7.713-7.705 (d, *J*= 3.2 Hz, 2H, ArH) 6.85 (s, 1H, ArH) 6.830-6.822 (t, *J*= 3.2 Hz, 1H, ArH) 6.753-6.723 (m, 1H, ArH) 5.709-5.692 (d, *J*= 6.8 Hz, 1H, CH Pyraz) 3.736-3.720 (m, 3H, OCH_3) 3.962-3.888 (dd, *J*= 12, 11.6 Hz, 1H, CH_2 Pyraz) 3.307-3.251 (dd, *J*= 4.4, 4.4 Hz, 1H, CH_2 Pyraz); ^{13}C NMR δ: 166.5, 152.1, 150.4, 150.0, 148.4, 144.2, 139.3, 134.7, 128.0, 126.4, 120.2, 119.9, 117.8, 112.6, 63.4, 58.0, 41.2; LC-MS *m/z*: 419.08 (M^++1), 420.08 (M^++2).

(3-(4-bromophenyl)-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)(pyridin-4-yl)methanone (3c)

Dark brown powder; Yield 51%; m.p. 160-162°C; R_f 0.57 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 298 nm; IR (KBr) cm^{-1} 3368 (OH str), 3066 (Ar-CH str), 2931 (Ali-CH str), 1755 (C=O str), 1640 (C=N str), 1122 (C-O-C str), 822 (C-Br str); ^{1}H NMR δ: 8.4 (s,1H,OH) 7.915-7.718 (dd, *J*= 7.6, 6.2 Hz, 2H, ArH) 7.654-7.639 (m, 2H, ArH) 7.593-7.528 (m, 2H, ArH) 7.516-7.484 (dd, *J*= 6, 4.4 Hz, 2H, ArH) 6.866-6.843 (t, *J*= 9.2 Hz, 2H, ArH) 6.728-6.694 (m, 1H, ArH) 5.478-5.432 (dd, *J*= 6, 5.2 Hz, 1H, CH Pyraz) 3.685-3.643 (dd, *J*= 7.6, 7.2 Hz, 1H, CH_2 Pyraz) 3.489-3.467 (m, 3H, OCH_3) 3.351-3.342 (d, *J*= 3.6 Hz, 1H, CH_2 Pyraz); ^{13}C NMR δ: 150.8, 138.1, 134.3, 133.9, 130.5, 125.9, 118.0, 114.2, 57.8; LC-MS *m/z*: 452.00 (M^+), 452.99 (M^++1), 453.01 (M^++2).

(3-(4-chlorophenyl)-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)(pyridin-4-yl)methanone (3d)

Yellowish brown powder; Yield 69%; m.p. 160-162°C; R_f 0.53 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 294nm; IR cm^{-1} 3354 (OH str), 3068 (Ar-CH str), 2956 (Ali-CH str), 1679 (C=O str), 1592 (C=N str), 1124 (C-O-C str), 829 (C-Cl str); ^{1}H NMR δ: 8.96 (s, 1H, OH) 8.71 (s, 2H, ArH) 7.940-7.917 (m, 2H, ArH) 7.830-7.809 (t, *J*= 8.4 Hz, 2H, ArH) 7.612-7.577 (m, 2H, ArH) 6.815-6.802 (d, *J*= 5.2 Hz, 2H, ArH) 6.703-6.673 (m, 1H, ArH) 5.546-5.531 (d, *J*= 6 Hz, 1H, CH

Pyraz) 3.733-3.705 (m, 3H, OCH_3) 3.613-3.564 (dd, *J*= 8.4, 6.4 Hz, 1H, CH_2 Pyraz) 3.451-3.400 (dd, *J*= 6.8, 6.8 Hz, 1H, CH_2 Pyraz); ^{13}C NMR δ: 166.1, 151.4, 147.1, 146.0, 140.1, 136.6, 134.9, 130.6, 130.0, 119.8, 115.2, 111.8, 62.9, 57.8, 41.2; LC-MS *m/z*: 408.06 (M^++1), 410.05 (M^++3).

(5-(4-hydroxy-3-methoxyphenyl)-3-(4-iodophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)(pyridin-4-yl)methanone (3e)

Ash blond powder; Yield 77%; m.p. 124-125°C; R_f 0.14 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 309nm ; IR cm^{-1} 3314 (OH str), 3060 (Ar-CH str), 2926 (Ali-CH str), 1752 (C=O str), 1640 (C=N str), 1126 (C-O-C str); ^{1}H NMR δ: 8.89 (s, 1H, OH) 7.982-7.976 (m, 2H, ArH) 7.621-7.593 (m, 2H, ArH) 7.484-7.362 (m, 2H, ArH) 7.376-7.243 (m, 2H, ArH) 6.773-6.762 (d, *J*= 4.4 Hz, 1H, ArH) 6.648-6.635 (d, *J*= 5.2 Hz, 1H, ArH) 6.606-6.594 (d, *J*= 4.8 Hz, 1H, ArH) 5.382-5.351 (dd, *J*= 4.8, 4.4 Hz, 1H, CH Pyraz) 3.724-3.712 (m, 3H, OCH_3) 3.504-3.492 (d, *J*= 4.8 Hz, 1H, CH_2 Pyraz) 3.086-3.052 (dd, *J*= 5.6, 4.8 Hz, 1H, CH_2 Pyraz); ^{13}C NMR δ: 167.2, 151.7, 148.1, 138.1, 134.8, 132.6, 131.1, 129.7, 128.5, 120.2, 115.4, 113.6, 97.2, 63.4, 57.3, 41.2; LC-MS *m/z*: 499.00 (M^+), 450.46 (M^++1), 451.05 (M^++2).

4-(5-(4-hydroxy-3-methoxyphenyl)-1-isonicotinoyl-4,5-dihydro-1*H*-pyrazol-3-yl)benzonitrile (3f)

Brownish yellow powder; Yield 59%; m.p. 114-116°C; R_f 0.40 Chloroform: Ethyl acetate (3:2); $\lambda_{max,}$ 319 nm; IR cm^{-1} 3365 (OH str), 3067 (Ar-CH str), 2931 (Ali-CH str), 2227 (CN str), 1720 (C=O str), 1645 (C=N str), 1126 (C-O-C str); ^{1}H NMR δ: 9.92 (s,1H, OH) 8.875-8.692 (m, 2H, ArH) 7.963-7.864 (m, 2H, ArH) 7.848-7.779 (m, 2H, ArH) 7.619-7.582 (m, 2H, ArH) 6.86 (s,1H, ArH) 6.810-6.764 (dd, *J*= 8, 7.2 Hz, 2H, ArH) 5.656-5.620 (dd, *J*= 4.8, 4.4 Hz, 1H, CH Pyraz) 3.992-3.938 (dd, *J*= 5.2, 5.2 Hz, 1H, CH_2 Pyraz) 3.735-3.704 (dd, *J*= 5.2, 4.8 Hz, 1H, CH_2 Pyraz) 3.693-3.617 (t, *J*= 30.4, 3H, OCH_3); ^{13}C NMR δ: 168.1, 152.8, 150.1, 147.9, 145.8, 141.1. 139.1, 135.6, 131.9, 130.3, 119.1, 118.9, 116.1, 111.4, 68.9, 57.3, 40.3; LC-MS *m/z*: 399.08 (M^++1), 400.08 (M^++2).

(5-(4-hydroxy-3-methoxyphenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)(pyridin-4-yl)methanone (3g)

Buff powder; Yield 67%; m.p. 90-92°C; R_f 0.42 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 308 nm; IR cm^{-1} 3362 (OH str), 3134 (Ar-CH str), 2932 (Ali-CH str), 1728 (C=O str), 1614 (C=N str), 1125 (C-O-C str); ^{1}H NMR δ: 9.96 (s, 1H, OH) 9.68 (s, 1H, OH) 7.724-7.715 (d, *J*= 3.6 Hz, 2H, ArH) 7.594-7.568 (m, 2H, ArH) 6.876-6.841 (m, 2H, ArH) 6.812-6.792 (m, 1H, ArH) 6.797-6.788 (d, *J*= 3.6 Hz, 1H, ArH) 6.654-6.634 (t, *J*= 8 Hz, 1H, ArH) 5.629-5.591 (dd, *J*= 4.4, 3.6 Hz, 1H, CH Pyraz) 3.783-3.753 (m, 3H, OCH_3) 3.846-3.783 (dd, *J*= 7.2, 6.8 Hz, 1H, CH_2 Pyraz) 3.150-3.095 (dd, *J*= 4.4, 4 Hz, 1H, CH_2 Pyraz); ^{13}C NMR δ: 169.3, 161.9, 151.9, 149.8, 148.0, 144.6, 135.2, 131.0, 130.7, 119.7, 117.8, 117.5, 112.5, 62.5, 58.0, 41.2; LC-MS *m/z*: 389.00 (M^+), 390.32 (M^++1), 391.42 (M^++2).

(5-(4-hydroxy-3-methoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)(pyridin-4-yl)methanone (3h)

Reddish brown powder; Yield 68%; m.p. 178-180°C; R_f 0.54 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 295 nm; IR cm^{-1} 3398 (OH str), 3025 (Ar-CH str), 2932 (Ali-CH str), 1753 (C=O str), 1669 (C=N str), 1118 (C-O-C str); ^{1}H NMR δ: 8.71 (s, 1H, OH) 7.883-7.860 (m, 2H, ArH) 7.837-7.791 (m, 2H, ArH) 7.721-7.673 (m, 2H, ArH) 7.305-7.286 (m, 2H, ArH) 6.791-6.782 (d, *J*= 3.6 Hz, 2H, ArH) 6.723-6.711 (d, *J*= 4.8 Hz, 1H, ArH) 6.380-6.342 (dd, *J*= 4.8, 4.4 Hz, 1H, CH Pyraz) 3.903-3.887 (t, *J*= 10.4 Hz, 1H, CH_2 Pyraz) 3.723-3.698 (dd, *J*= 4, 3.6 Hz, 1H, CH_2 Pyraz) 3.510-3.468 (m, 3H, OCH_3) 2.474-2.357 (m, 3H, CH_3); ^{13}C NMR δ: 151.1, 149.6, 147.0, 145.3, 136.7, 129.1, 128.8, 117.6, 114.7, 58.0, 41.2, 23.2; LC-MS *m/z*: 387.09 (M^+), 388.11 (M^++1).

(5-(4-hydroxy-3-methoxyphenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl) (pyridin-4-yl)methanone (3i)

Brown powder; Yield 34%; m.p. 96-98°C; R_f 0.12 Chloroform: Ethyl acetate (5:1) ; $\lambda_{max,}$ 307 nm; IR cm^{-1} 3273 (OH str), 3018 (Ar-CH str), 2939 (Ali-CH str), 1757 (C=O str), 1648 (C=N str), 1120 (C-O-C str); ^{1}H NMR δ: 9.84 (s, 1H, OH) 7.921-7.905 (d, *J*= 6.4 Hz, 2H, ArH) 7.866-7.849 (d, *J*= 6.8 Hz, 2H, ArH) 7.469-7.453 (d, *J*= 6.4 Hz, 2H, ArH) 7.142-7.128 (d, *J*= 5.6 Hz, 2H, ArH) 6.944-6.925 (d, *J*= 7.6 Hz, 2H, ArH) 6.905-6.888 (d, *J*= 7.2 Hz, 1H, ArH) 6.797-6.779 (t, *J*= 4.4 Hz, 1H, ArH) 5.426-5.378 (dd, *J*= 7.6, 6 Hz, 1H, CH Pyraz), 3.956-3.768 (m, 3H, OCH_3) 3.763-3.738 (m, 3H, OCH_3) 3.452-3.421 (dd, *J*= 4.8, 4.4 Hz, 1H, CH_2 Pyraz) 3.195-3.163 (dd, *J*= 5.2, 4.8 Hz, 1H, CH_2 Pyraz); ^{13}C NMR δ: 168.4, 162.9, 152.6, 148.8, 148.1, 146.9, 139.8, 133.7, 127.9, 130.7, 118.9, 116.2, 67.8, 57.3, 56.4, 40.1; LC-MS *m/z*: 404.10 (M^++1), 405.11 (M^++2).

(3-(4-aminophenyl)-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl) (pyridin-4-yl)methanone (3j)

Reddish brown powder; Yield 65%; m.p. 163-165°C; R_f 0.41 Chloroform: Ethyl acetate (5:1); $\lambda_{max,}$ 313 nm; IR cm^{-1} 3306 (OH str), 3053 (Ar-CH str), 2969 (Ali-CH str), 1740 (C=O str), 1635 (C=N str), 1124 (C-O-C str); ^{1}H NMR δ: 10.13 (s, 1H, NH) 8.95 (s, 1H, OH) 7.992-7.980 (d, *J*= 4.8 Hz, 2H, ArH) 7.641-7.562 (m, 2H, ArH) 7.524-7.484 (m, 2H, ArH) 7.308-7.279 (m, 2H, ArH) 6.896-6.881 (d, *J*= 6 Hz, 2H, ArH) 6.747-6.697 (dd, *J*= 7.6, 6.8 Hz, 1H, ArH) 5.428-5.394 (dd, *J*= 5.6, 5.2 Hz, 1H, CH Pyraz) 3.462-3438 (m, 3H, OCH_3) 3.160-3.137 (dd, *J*= 3.6, 3.2 Hz, 1H, CH_2 Pyraz) 2.303-2.274 (dd, *J*= 4.8, 4.4 Hz, 1H, CH_2 Pyraz) 2.040-2.023 (d, *J*= 6.8 Hz, 3H, CH_3); ^{13}C NMR δ: 169.5, 167.5, 149.8, 149.8, 148.1, 143.5, 137.6, 135.4, 132.2, 129.5, 129.2, 127.6, 120.8, 119.4, 117.2, 112.4, 62.5, 57.5, 41.2; LC-MS *m/z*: 388.40 (M^+), 389.51 (M^++1).

Antiproliferative activity

The cells were seeded at 1×10^5 cells/mL in 96 well micro titre plates in Eagle's MEM with 10% foetal bovine serum and incubated overnight at 37°C in 95% humidity and 5% CO_2 for attachment. Compound concentrations (ranging from 7.81-500 µMol/300 µL) in serial three fold dilutions were added in triplicate and incubated for 48 h at 5% CO_2 at 37°C. Thereafter, the cells were washed twice with phosphate buffer *p*H 7.4 and treated with 20 µL MTT (5 mg/mL in phosphate buffer *p*H 7.4) and further incubated for 4 h. The entire medium including formazan crystals were dissolved in 100 µL of DMSO and the absorbance was measured at 570 nm using a 96 well micro plate reader. The% cytotoxicity was determined using the untreated cells as negative control. 5-Fluorouracil was used as standard drug. The% cytotoxicity was determined using the untreated cells as negative control using the background corrected absorbance as follows[13].

$$\%\ \text{Cyctotoxicity} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

DPPH radical scavenging activity

Stock solution of DPPH (38 µg/mL) in ethanol (95%) was prepared. The various concentrations of 1,3,5-trisubstituted pyrazoline compounds were also prepared. For sample solutions ranging from 0.05 to 0.8 mL was added 2mL DPPH stock solution, further it was made up to 3mL with ethanol (95%). The mixtures were kept for 30 min in dark at RT and absorbance was recorded at 517 nm against reagent blank. Ascorbic acid was used as the reference compound. The DPPH scavenging activity was calculated as

$$\%\ \text{Scavenging} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

The IC_{50} of compounds was obtained by linear regression analysis between% inhibition and concentrations[9,18].

***In-silico* studies**

Using Chemdraw 16.0, the structure of the molecules were sketched and their MOL files were generated followed by subsequent generation of 3D structures by using Avogadro[19] and their energies were minimized with the MMFF94s force field and molecules were converted to SDF format. The cocrystal structure of ALK catalytic domain bound with adenosine diphosphate ligand (PDB ID: 3LCT) was retrieved from RCSB protein data bank[20]. The missing residues were corrected and the complexes bound to the receptor molecule were removed and PDB files were energy minimized using Autodock 4.2.6 [21]. The non-essential water molecules were removed and polar hydrogens were merged. Autodock 4.2.6 program supplied with Autogrid was used to produce grid maps. The docking analysis of 5-Flurouracil and synthesized molecules with ALK catalytic domain were subsequently carried out using AutoDock Vina[22] with the Lamarckian genetic algorithm (LGA). The scoring functions and hydrogen bonds formed with the surrounding amino acids of ALK catalytic domain were used to predict tested compound's binding modes. Finally, docking simulations of the compounds which have exhibited good binding affinity with the target were performed. The docking simulations were visualized in Discovery studio[23]. The ADME properties, pharmacokinetic properties, and drug-likeness of the compounds were also studied with the SwissADME[24] web server.

Results and Discussion

Chemistry

Chalcones or styryl ketones are not only versatile intermediates but also used as building blocks for the construction of five, six or seven membered heterocyclic rings. A series of chalcones **1a-j** were synthesized employing Claisen-Schimdt reaction[25] by condensing substituted acetophenones with vanillin in presence of dry HCl gas in good to excellent yields. Since the base catalysed reaction results into decrease in the activity of phenolic aldehyde by forming stabilized

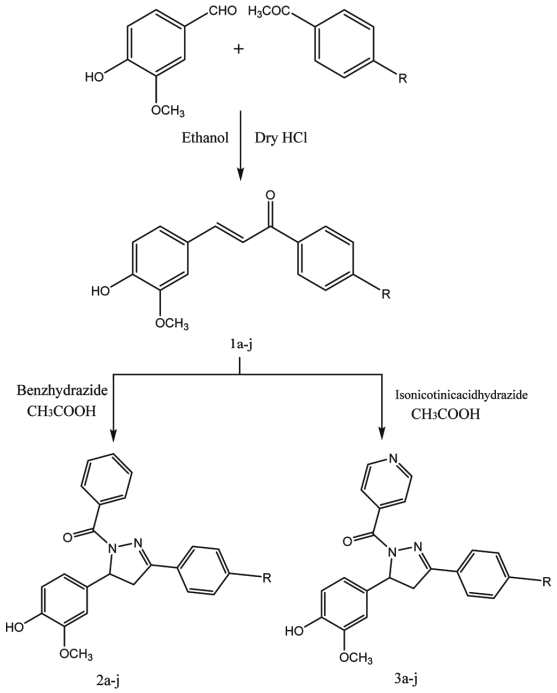
anion by delocalization[26], the reaction was carried out in acid medium. The 5-(4-hydroxy-3-methoxyphenyl)-3-(4-substituted phenyl)-4,5-dihydro-1H-pyrazol-1-yl)(phenyl)methanones **2a-j** and (5-(4-hydroxy-3-methoxyphenyl)-3-(4-substitutedphenyl)-4,5-dihydro-1H-pyrazol-1-yl) (pyridine-4-yl) methanones **3a-j** were synthesized by reacting chalcones **1a-j** with benzhydrazide/ INH in acetic acid respectively by Fischer and Knoevenagel condensation[27], a well-known synthetic method for the generation of 2-pyrazolines. The synthetic route is illustrated in Scheme 1.

The title compounds and the intermediates were characterized by FTIR, ^{1}H NMR, ^{13}C NMR and Mass spectral data. The IR spectra of compounds **2a-j** and **3a-j** showed C=N stretching characteristic of pyrazoline ring at 1585-1669 cm^{-1}, aromatic C-H stretching at 3018-3165 cm^{-1}, aliphatic C-H stretch at 2926-2969 cm^{-1}. In addition, compounds displayed C=O stretching at 1669-1757 cm^{-1}. In ^{1}H NMR spectra, the two protons of C-4 pyrazoline ring in compounds **2a-j** and **3a-j** resonated as two doublets in the range of δ 2.26-3.90. The proton at 5th position showed a doublet of doublet in most instances at δ 5.30-6.37 suggesting pyrazoline ring protons are diastereotopic. The OH protons and OCH_3 protons derived from vanillin resonated at δ 8.87-9.96 and 3.32-3.80, respectively. The aromatic protons appeared at δ 6.46-8.72. In ^{13}C NMR, the pyrazoline C-3 and C-4 signals appeared at δ ~150-152.2 and 41.2-41.7 whereas pyrazoline C-5 appeared at δ ~62.5-67.3. The methoxy carbon of vanillin resonated at δ ~57.4-58.07 and the carbon atom attached to hydroxyl group at δ ~144.6-148.4. The compounds **2a-j** and **3a-j** showed M^+, M^+ +1 and M^+ +2 in their respective *m/z* values in mass spectra which were in close agreement with their molecular weights. All these spectral features suggested the formation of intended 1,3,5-trisubstituted 2-pyrazolines.

The antiproliferative and DPPH radical scavenging activity of title compounds **2a-j** and **3a-j** is presented in Table 1. Compounds **2a-j** have shown lesser cytotoxicity than the standard 5-Fluorouracil (IC_{50} 10.23μM). The compound **2i** with 4-methoxy substitution displayed the moderate antiproliferative activity with an IC_{50} of 92.42 μM. Rest of the compounds exhibited very weak cytotoxic activity against A549 cell line with IC_{50} values >100 μM. It was observed that compounds with electron releasing substituents displayed good activity than those with electron withdrawing counterparts. Compounds **3a-j** have also exhibited very poor antiproliferative activity with only **3a** without any substitution displayed the weak activity (IC_{50} 108.45 μM). It was interesting that compounds **2a-j** were more active than compounds **3a-j**, suggesting the detrimental role of pyridine ring on the antiproliferative activity over phenyl ring. The

Table 1 — Cytotoxic and antioxidant activity of 1, 3, 5- trisubstituted 2-pyrazolines

Entry	R	IC_{50} (μM/mL)	
		Cytotoxicity A549 cell line	DPPH radical scavenging
2a	H	107.17	0.031
2b	NO_2	262.7	0.073
2c	Br	-	0.045
2d	Cl	149.44	0.030
2e	I	213.78	0.027
2f	CN	202.83	0.122
2g	OH	102.22	0.033
2h	CH_3	-	0.033
2i	OCH_3	92.42	0.018
2j	NH_2	141.33	0.036
3a	H	108.45	0.043
3b	NO_2	483.53	0.215
3c	Br	268.97	0.100
3d	Cl	-	0.102
3e	I	220.30	0.024
3f	CN	328.09	0.026
3g	OH	-	0.046
3h	CH_3	245.28	0.030
3i	OCH_3	-	0.036
3j	NH_2	304.00	0.032
5-Fluorouracil		10.23	-
Ascorbic acid		-	0.047



Scheme 1 — Synthetic route to compounds **2a-j** and **3a-j**

poor antiproliferative activity exhibited by these compounds could be attributed to more resistant nature of lung cancer cell lines towards the chemotherapeutic agents than other cancer cells[28]. Overall, compound **2i** emerged to be the good antiproliferative agent among the compounds tested.

The compounds **2a-j** have shown an excellent DPPH radical scavenging activity except compounds **2b** (nitro) and **2f (**cyano). The *4*-methoxy derivative **2i** displayed the remarkable activity (IC_{50} 0.018 μM/mL) followed by the derivatives having electron attracting 4-iodo **2e** and 4-chloro **2d**. The derivatives having electron releasing substituents such as 4-hydroxy **2g**, 4-acetamido **2j** exhibited little lower activity than compounds with electron attracting substituents. The compounds **3a**, **3e**, **3f**, **3h**, **3g**, **3i**, **3j** have shown the excellent radical scavenging activity when compared with standard ascorbic acid (IC_{50} 0.047 μM/mL). The 4-iodo derivative **3e** exhibited the highest scavenging activity with IC_{50} of 0.024 μM/mL, while the unsubstituted compound **3a** displayed least DPPH radical scavenging activity with an IC_{50} of 0.043 μM/mL. Compounds with electron attracting substituents namely 4-iodo **3e** and 4-cyano **3f** exhibited the highest radical scavenging activity, whereas compounds with 4-nitro **3b**, 4-bromo **3c** and 4-chloro **3d** have failed to show good activity than ascorbic acid suggesting milder electron attracting substituents favoured radical scavenging activity than the stronger ones. The presence of pyridine ring seems to favour the radical scavenging activity over phenyl ring. In both the series 4-iodo derivatives **2e** and **3e** have emerged to be the best radical scavenging agents. However, the good DPPH scavenging agents on the other hand did not show the expected good cytotoxic activity.

In-silico analysis

The physicochemical properties, ADME parameters, and violations of drug-likeness rules of the synthesized compounds are presented in Table 2. Calculated physicochemical and lipophilicity parameters were used by various filters to evaluate the drug-likeness of synthesized compounds. The filters generally assume that an orally active drug should not violate the required criteria more than once. The title compounds and the reference drug 5-Fluorouracil follow these criteria. However, The Fsp^3 (Ref 29) values of all compounds are lower than those of

Table 2 — Physicochemical and pharmacokinetic data of 1,3,5 trisubstituted pyrazolines

Entry	Physicochemical properties							Lipophillicity						Drug Likeness					Pharmacokinetics
	MW	Fsp3	RB	HBA	HBD	MR	TPSA	iLogP	XlogP3	Wlog P	Mlog P	Silicos IT	Consensus logP	Lipinski	Ghose	Veber	Egan	Muegge	F
2a	372.42	0.13	5	4	1	115.45	62.13	3.40	3.98	3.31	3.21	4.13	3.61	0	0	0	0	0	0.55
2b	417.41	0.13	6	6	1	124.27	107.95	2.98	3.80	3.21	2.29	1.96	2.85	0	0	0	0	0	0.55
2c	451.31	0.13	5	4	1	123.15	62.13	3.76	4.67	4.07	3.79	4.81	4.22	0	0	0	0	0	0.55
2d	406.86	0.13	5	4	1	120.46	62.13	3.62	4.6	3.96	3.68	4.77	4.13	0	0	0	0	0	0.55
2e	498.31	0.13	5	4	1	128.16	62.13	3.73	4.63	3.91	3.89	5.09	4.25	0	1	0	0	0	0.55
2f	397.43	0.12	5	5	1	120.16	85.92	2.96	3.69	3.18	2.53	4.17	3.31	0	0	0	0	0	0.55
2g	388.42	0.13	5	5	2	117.47	82.36	3.03	3.62	3.01	2.66	3.65	3.19	0	0	0	0	0	0.55
2h	386.44	0.17	5	4	1	120.41	62.13	3.66	4.34	3.61	3.42	4.66	3.94	0	0	0	0	0	0.55
2i	402.44	0.17	6	5	1	121.94	71.36	3.67	3.95	3.31	2.87	4.19	3.60	0	0	0	0	0	0.55
2j	429.47	0.16	7	5	2	129.76	91.23	3.30	3.15	3.07	2.62	3.81	3.19	0	0	0	0	0	0.55
3a	373.40	0.14	5	5	1	113.24	75.02	3.06	2.91	2.70	2.18	3.57	2.88	0	0	0	0	0	0.55
3b	418.40	0.14	6	7	1	122.06	120.84	2.59	2.73	2.61	1.30	1.40	2.12	0	0	0	0	0	0.55
3c	452.30	0.14	5	5	1	120.94	75.02	3.39	3.60	3.46	2.77	4.24	3.49	0	0	0	0	0	0.55
3d	407.85	0.14	5	5	1	118.25	75.02	3.26	3.53	3.35	2.66	4.2	3.40	0	0	0	0	0	0.55
3e	499.30	0.14	5	5	1	125.96	75.02	3.43	3.56	3.31	2.87	4.53	3.54	0	1	0	0	0	0.55
3f	398.41	0.13	5	6	1	117.96	98.81	2.93	2.62	2.57	1.52	3.6	2.65	0	0	0	0	0	0.55
3g	389.40	0.14	5	6	2	115.27	95.25	2.68	2.55	2.41	1.65	3.08	2.47	0	0	0	0	0	0.55

(*Contd*.)

Table 2 — Physicochemical and pharmacokinetic data of 1,3,5 trisubstituted pyrazolines (*Contd.*)

Entry	Physicochemical properties							Lipophillicity						Drug Likeness					Pharmacokinetics
	MW	Fsp3	RB	HBA	HBD	MR	TPSA	iLogP	XlogP3	WlogP	MlogP	Silicos IT	Consensus logP	Lipinski	Ghose	Veber	Egan	Muegge	F
3h	387.43	0.17	5	5	1	118.21	75.02	3.38	3.27	3.01	2.39	4.09	3.23	0	0	0	0	0	0.55
3i	403.43	0.17	6	6	1	119.73	84.25	3.30	2.88	2.71	1.86	3.62	2.87	0	0	0	0	0	0.55
3j	430.46	0.17	7	6	2	127.56	104.12	2.94	2.08	2.47	1.62	3.24	2.47	0	0	0	0	0	0.55
Accepted value	-	≤ 0.25	≤ 9	≤ 10	≤ 5	≤ 130	20-130Å												
5-Flurouracil	130.08	0	0	3	2	27.64	65.72	0.44	-0.89	-0.38	-0.73	1.78	0.05	0	3	0	0	2	0.55

Molecular weight: MW, topological polar surface area: tPSA, Molar Refractivity: MR, Fraction of sp3 carbon atoms: Fsp3, HBD: Hydrogen bonds donor, HBA: Hydrogen bond acceptor, RB: Rotatable bonds, LogP values: Indicator of Lipophilicity, F: Bioavailability Score.

The filters and criteria

Lipinski (Pfizer) filter [32]: MW ≤ 500; MLOGP ≤ 4.15; HBA ≤10; HBD ≤5

Ghose filter [33]: 160 ≤ MW ≤480; -0.4 ≤ WLOGP ≤ 5.6; 40 ≤ MR 130; 20 ≤ atoms ≤ 70

Veber (GSK) filter [34]: RB ≤ 10; TPSA ≤ 140

Egan (Pharmacia) filter [35]: WLOGP ≤ 5.88; TPSA ≤ 131.6

Muegge (Bayer) filter [36]: 200 ≤ MW ≤ 600, -2 ≤ XLOGP ≤ 5; TPSA ≤ 157; HBA ≤ 10; HBD ≤ 5; RB ≤ 15; number of rings ≤ 7; number of carbons >4; number of heteroatoms >1

Table 3 — Binding affinity, hydrogen bond, electrostatic and hydrophobic interactions between selected compounds and receptor

Entry	Binding affinity (kcal/mol)	Hydrogen bond	Electrostatic	Hydrophobic
2b	-8.2	ARG1275	-	LYS1150, LEU1256, LEU1196, ALA1148
2d	-8.3	ASP1203, LEU1122, GLY1202	-	LEU1196, VAL1180, LEU1256
2f	-8.4	ALA1200, ASN1254	-	LYS1150, LEU1256, LEU1196, ALA1148
2h	-8.7	ALA1200	GLU1132	LEU1256
2j	-8.6	ARG1253, ARG1275, GLU1167	-	LYS1150, LEU1256, LEU1196, VAL1130, ALA1148
3c	-8.3	GLY1201, ALA1200, SER1206	GLU1132	LEI1256, ALA1148, VAL1130, MET1199
3f	-8.3	GLY1269	ASP1270, ASP1203	VAL1130, LEU1256, ALA1148
3h	-8.4	ALA1200	GLU1132	LEU1256, LEU1122. VAL1130, MET1199
3j	-8.2	ARG1275, ASN1254	ASP1270	LEU1150, PHE1127
5-Fluoruracil	-4.7	ASP1160, LYS1285	ARG1275	PHE1127

5-Fluorouracil. The bioavailability score (F) [Ref 30] of the compounds signifies the probability of oral bioavailability in rats. It is interesting that the title compounds and 5-Fluorouracil have same F scores.

Anaplastic lymphoma kinase (ALK) is a tyrosine kinase, which can be aberrantly expressed in several tumour types. In non-small cell lung cancer (NSCLC), chromosomal rearrangements involving the *ALK* gene loci on chromosome 2 are found in approximately 5 percent tumours [31]. An ALK inhibitor is preferred as the initial therapy for patients whose tumour contains this genetic abnormality. Therefore, molecular docking studies to elucidate the mechanism of anti-lung proliferative action of the synthesized compounds by utilizing the crystal structure of the ALK catalytic domain as the target was undertaken. The binding affinity values obtained as a result of the docking studies are shown in Table 3. Compounds **2b, 2d, 2f, 2h, 2j, 3c, 3f, 3h** and **3j** displayed the good binding energy values. The compound **2b** has shown conventional hydrogen bond interaction with ARG1275, Pi-sigma interactions with LYS1150 and

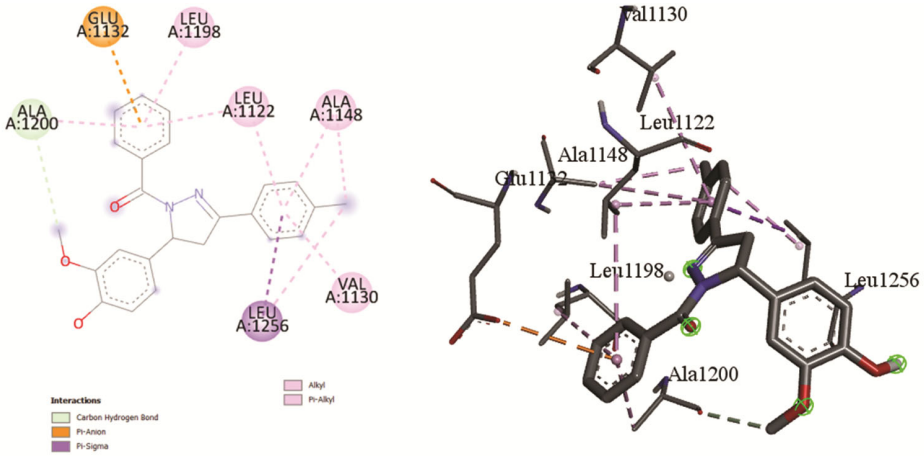


Fig. 1 — 2D and 3D representations of **2h** and its interaction with ALK

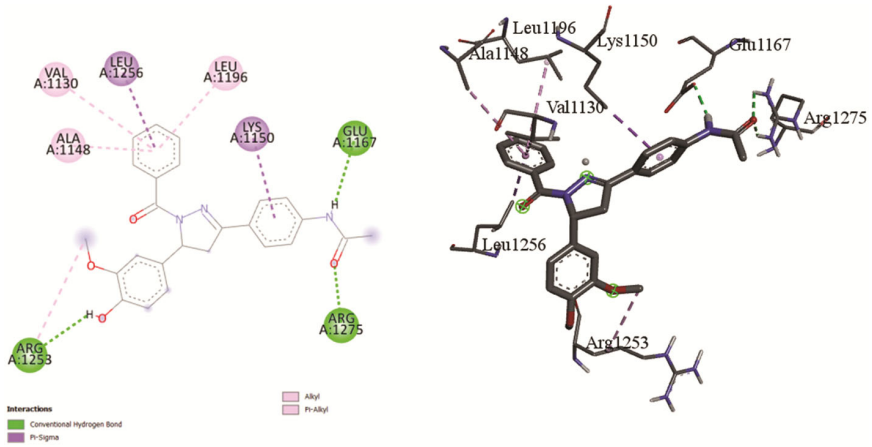


Fig. 2 — 2D and 3D representations of **2j** and its interaction with ALK

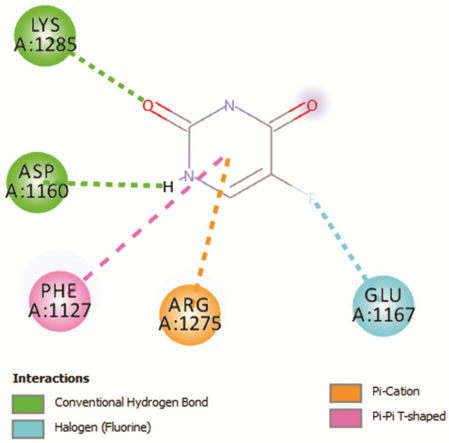


Fig. 3 — 2D and 3D representations of 5-Fluorouracil and its interactions with ALK

LEU1256 and also Pi-alkyl interactions with LEU1196 and ALA1148. All other compounds have also displayed good interaction with the target with hydrogen bond, Pi-sigma, Pi-alkyl, Pi-anion, amide pi stacked, Pi-cation interactions. The methoxy derivative **2i** that displayed good antiproliferative activity among the series has failed to show good binding interaction with ALK. Though all the docked compounds exhibited good binding affinity but did not interact with the same amino acids as that of 5- Fluorouracil. The 2D and 3D representations of **2h, 2j** and 5-Fluorouracil and its interaction with ALK are shown in Fig. 1, 2 and 3, respectively.

Conclusion

Understanding the growing need for anticancer therapeutics, a panel of compounds having 2-pyrazoline core namely, 5-(4-hydroxy-3-methoxyphenyl)-3 (4-substituted phenyl)-4,5-dihydro-1H-pyrazol-1-yl)(phenyl)methanones **2a-j** and (5-(4-hydroxy-3-methoxyphenyl)-3-(4-substituted phenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanones **3a-j** derived from vanillin chalcones were successfully synthesized by a facile protocol in

good yields. The structures of newly synthesized compounds and their intermediates were confirmed by IR, NMR and Mass spectral data. Most of the title compounds were found to possess poor activity against A549 lung cancer cells than the standard 5-Fluorouracil. On the other hand, compounds **3a-j** displayed remarkable radical scavenging activity than **2a-j** in DPPH radical scavenging assay when compared with reference standard ascorbic acid. The compounds which showed good DPPH scavenging activity on the other hand, did not show the good anti-lung proliferative activity. Only 4-iodo compound **3e** exhibited an excellent radical scavenging activity and a weak anti-lung proliferative activity. All the compounds were docked with target lung cancer protein ALK catalytic domain. Compounds **2h, 2j, 2f, 3h, 2d, 3c, 3f, 2b** and **3j** docked themselves with good binding energy. The compounds **2h** and **2j** displayed good interaction with the ALK catalytic domain. However, compound **2i** which possessed good antiproliferative activity among the title compounds failed to interact with ALK catalytic domain. The compounds have also met the criteria for drug likeness and ADME properties. In conclusion, the compounds synthesized in the study would be a fruitful architecture deserving further investigation and structural optimization to find a lead for the development of more potent anti-lung cancer compounds.

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