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SYNTHESIS OF THIAZOLYL-PYRAZOLE DERIVATIVES AS ANTI CANCER AGENTS

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ABSTRACT

In our last article we performed molecular modelling studies on a set of 42 molecules of thiazolyl-pyrazole derivatives. We deigned new chemical entities with Combilib:Vlife MDS software. We further performed docking studies to study the interaction of the designed new chemical entities with epidermal growth receptor factor (EGFR). We predicted 5 best compounds which fitted into the pocket of EGFR and may exhibit anticancer activity. In this article we present the synthesis and spectral evaluation of the synthesized compounds. We have further performed biological screening by Microculture Tetrazolium Test (MTT) assay for evaluation against breast cancer and lung cancer cell lines. Compounds KS-1, KS-3, KS-5, KS-6, KS-11 were found to have percentage inhibition of 82.12%, 22.66%, 69.57%, 8.09%,48.02%, at a concentration of 60μg/ml and 64.03%, 21.82%, 38.83%, 3.23%, 41.37%, at a concentration of 80μg/ml as against standard drug 5-flurouracil 1.94%, This indicated that these compounds exhibited excellent activity against cell lines in vitro.

KEYWORDS: *Synthesis, IR, NMR. EGFR.*

INTRODUCTION

Thiazole and pyrazole derivatives have been found to have excellent anti-tumour activity as illustrated by literature.[1],[2] Epidermal growth Factor Receptor is one of the very important and widely researched target in study of anti-cancer derivatives. Protein Kinases are important regulators of cell function that form one of the biggest and most functionally diverse gene families. They serve to orchestrate the activity of almost all cellular processes. Kinases play an important role particularly in signal transduction and co-ordination of complex functions

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such as the cell cycle.[3] EGFR is a 170 kDa protein containing approximately 20% of carbohydrate of its molecular mass and is heavily N-glycosylated.[4] Characteristically the proliferation and the survival of carcinoma cells are found to be sustained by a network of receptors/ligands of the ErbB family.

Thus this phenomenon is suggestive of therapeutic approaches, towards anti-EGFR agents and might depend on the total level of expression of ErbB receptors and ligands in tumor cells. On average, 50% to 70% of lung, colon and breast carcinomas have been found to express EGFR or ErbB-3. Research indicates that the overexpression of ErbB-4 in breast carcinoma is found in approximately 50% of the tumors. Expression of ErbB-4 has been recently shown to occur in 22% of human primary colon carcinomas. EGFR can play an anti-tumor role in late-stage breast cancer as is illustrated with loss of EGFR expression in metastatic outgrowth proficient D2. A1 breast cancer cells.[5] Cetuximab (IMC-C225/Erbitux) is an FDA approved human– murine chimeric anti-EGFR monoclonal antibody. Research proved that KRAS, a wild-type advanced colorectal cancer, responded positively to Cetuximab, an EGFR inhibitor. Cetuximab binds to the second (L2) domain of EGFR thereby inhibiting its downstream signaling by causing receptor internalization and restricting ligand-receptor interaction. It caused tumour regression by 10.8% and delayed the tumour by 1.5 months when used as single therapy. Similarly panitumumab is used either in combination or as monotherapy in treatment of EGFR-expressing metastatic colorectal cancer.[6]

EGFR STRUCTURE AND FUNCTION

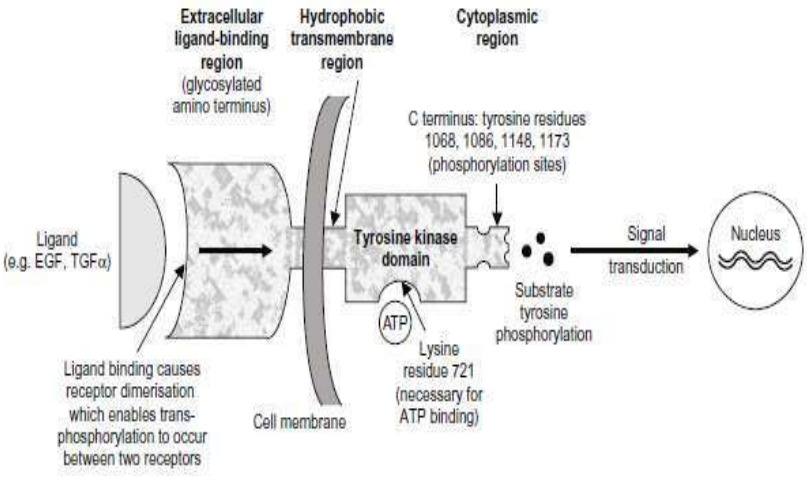

Fig: 1. Structure of EGFR

There are 3 major regions in EGFR. They are as follows: (i) the glycosylated N-terminus extracellular region (621 amino acids); (ii) the hydrophobic transmembrane region (23 amino acids); and (iii) the C-terminus cytoplasmic region (542 amino acids), which contains the tyrosine kinase domain (approximately 250 amino acids). The lysine residue 721 within this domain binds to ATP, generating phosphate for the tyrosine phosphorylation reaction. The major sites of phosphorylation are located at the C terminus (i.e. tyrosine residues 1068, 1086, 1148 and 1173).

Stimulation of EGFR tyrosine kinase following binding of a ligand [e.g. transforming growth factor-α (TGFα) or epidermal growth factor (EGF)] to a receptor leads to its transphosphorylation and to phosp.horylation of tyrosine residues from various cellular substrates. As a consequence, signalling pathways are activated such that extracellular signals are transduced to intracellular responses. The integrated biological responses to EGFR signalling are pleiotropic and include different processes such as mitogenesis or apoptosis, increase in cell moments, protein synthesis, and differentiation or dedifferentiation of cells. In addition to these processes upward EGFR signalling has also been associated with progress of tumor that eventually proceeds for invasion and metastasis.[7]

As stated earlier we have performed molecular modelling and drug design studies on a set of 42 molecules. We optimised the lead nucleus predicted 12 potent EGFR derivatives. Further we performed docking analysis and filtered 5 most potent anti-cancer compounds having drug like properties. In this paper we are presenting synthesis, spectral analysis and anticancer evaluation of these compounds.[8]

EXPERIMENTAL WORK

Synthesis of Substituted chalcone[9] Reaction: Synthesis of substituted chalcones.

Procedure (S_1-S_3)

Substitutes acetophenone or was added to

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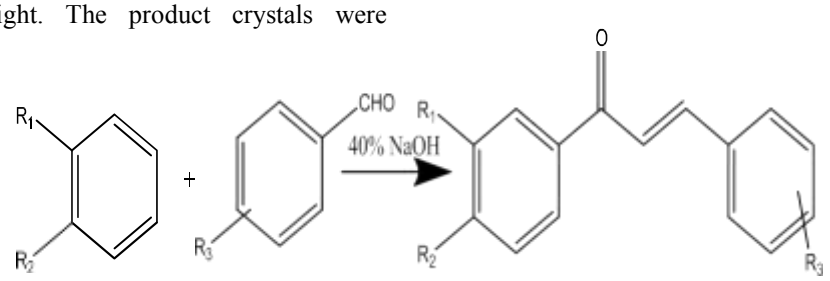
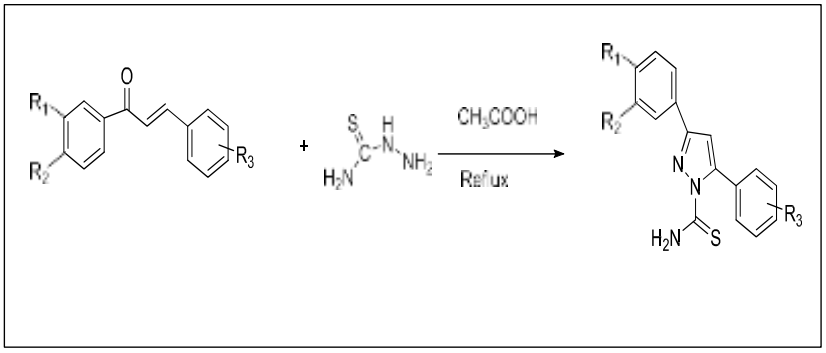
equimolar quantities of appropriate substituted aryl aldehyde, dissolved in ethyl alcohol. To this solution 40% sodium hydroxide was added dropwise and the reaction mixture was stirred for 40 min and then kept in refrigerator overnight. The product crystals were filtrated and washed carefully with ice water and cold methanol to neutral reaction. The resulting chalcones were purified by recrystallisation from methanol.



a- R_1 -COOH, R_2 -CH_3, R_3 -OH b- R_1 -CH_3, R_2 -CH_3, R_3 -OCH3 c- R_1- -CH_3, R_2 –OH, R_3 -OCH3
d- R_1- -CH_3, R_2 –$COCH_3$, R_3 –NH_2 e- R_1 -CH_3, R_2 -CH_3, R_3 -OCH3

Synthesis of Pyrazole derivative containing thiourea skeleton[10],[11] Synthesis of compounds SI1-SI3 Reaction

A mixture of substituted chalcone, thiosemicarbazide and acetic acid was heated at reflux for 3 hours, then poured onto crushed ice. The precipitate was separated by filtration, washed with water and crystallized from methanol to obtain the pyrazole derivative containing thiourea skeleton.



Synthesis of Thiazolyl –Pyrazole derivatives

Synthesis of compound KS1-KS11
Procedure

A mixture of pyrazole derivative containing thiourea skeleton and substituted 2-bromoacetophenone was heated at reflux for 4 h, then poured onto crushed ice. The precipitate was separated by filtration, washed with water, and crystallized from methanol to obtain the derivatives of thiazolyl-pyrazole.

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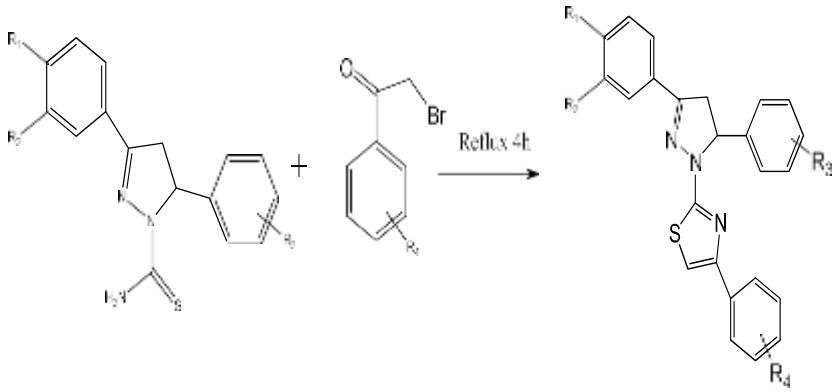

a. R_4- cyclopropyl d. R_4- cyclopropyl,
b. R_4 -CH_3 e. R_4- cyclopropyl,
c. R_4 -CH_3

5 compounds KS-1, KS-3, KS-5,KS-6, KS-11 were synthesized and spectrally evaluated.

Experimental

All the chemicals used were procured from Merck and SD fine chemical and purity of starting materials used for reactions was confirmed by checking their melting point or boiling point and by thin layer chromatography.

All the reactions were monitored using thin layer chromatography. The appropriate mobile phases (solvent systems) as applicable were developed using „silica gel G" as stationary phase. Melting points were determined in open capillary tube using Veego melting point apparatus and are uncorrected. FT-IR (KBr) spectra were recorded on "JASCO FT-IR V-460 plus" Spectrophotometer (Vmax in cm^{-1}). ^1H NMR spectra of synthesized compounds were recorded on "FT-NMR VARIAN MERCURY YH-300" Spectrometer at 300 MHz Frequency in $CDCl_3$ using TMS as internal standard (chemical shift δ in ppm) at Shimadzu Analytical Centre and NMR Facility, Dept. of Chemistry, University of Pune. LCMS were recorded on "2010EV LCMS Shimadzu" instrument by direct injection method. Purity of the compounds was checked on „Silica Gel G" coated on thin layer chromatographic plate procured from Merck, eluent was the mixture of different polar and non-polar solvents in varying proportions and detection was done either by observing in UV light or exposure to iodine vapours as required. The absence of TLC spots for starting materials and appearance of new TLC spot at different R_f value ensured the completion of reaction. The products of all the reactions were purified initially by different workup processes and recrystallization using suitable solvents. The absence of any impurity of starting material or possible by-product was ensure by performing qualitative organic analytical tests for various functional groups and by single TLC spot of product.[12-15]

RESULTS AND DISCUSSION

The thiazolyl pyrazole are known to be pharmacologically active compounds. The present study involves multistep synthesis of 4-acetyl-2-hydroxy benzoic acid, 1-(3,4- dimethylphenyl) ethanone, 1-(3-hydroxy 4-methylphenyl) ethanone derivatives of chalcones followed by synthesis of thiazolyl-pyrazole derivatives. As predicted by 2D and 3D QSAR , it was found upon synthesizing the compounds that Ring A and ring B- Dimethoxy substitution is important for activity on ring A along with chloro substitution at-R2 or -R4 (- meta position) on the ring is valuable for improving anticancer activity.. Steric substitution at R1 position of ring A decreases activity. The activity of compounds was found to be more than standard drugs.

Experimental Compound KS-1
4-(1-(4-(4-cyclopropylphenyl)thiazol-2-yl)-5-(4-hydroxyphenyl)-4,5-dihydro-1H- pyrazol-3-yl)-2-hydroxybenzoic acid

For compound KS-1, yield 70 % M.P-245^{0}C, IR(KBr) cm^{-1} 1685 (COOH), 1640.77
(Thiazole -C=N), 1371 (Phenolic OH) 1327 (-CN vibration), 1095 (Aromatic tertiary nitrogen), 116.78 (-C=S of thiazole ring), 3381 (CH_2 stretching of cyclopropane); 1H NMR($CDCl_3$) delta ppm, 11.3-12 (s, 1H of COOH of phenyl ring), 10-10.2 (s, 1H of OH of phenyl ring), 6.75-6.90 (s, 2H of C-H of phenyl ring), 7.8-8 (s, 2H of C-H of imidazole ring), 7.2-7.3 (s, 1H of thiazole ring), 3.0-4.0 (s, 1H of cyclopropyl ring).Elemental Analysis: C, 67.59; H, 4.66; N, 8.45; O, 12.86; S, 6.44

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Compound KS-3
3-(3,4-dimethylphenyl)-5-(4-methoxyphenyl)-1-(4-p-tolylthiazol-2-yl)-4,5-dihydro-1H- pyrazole
For compound KS-3 yield 75%, M.P 240^{0}C, IR(KBr) cm^{-1} 2920.23 (CH Ar), 1640 (Thiazole -C=N), 1016 (OCH_3 (-C-O-C)), 1438 ($-CH_3$); 1H NMR($CDCl_3$) delta ppm, 2.5-2.6 (s, 3H of CH_3 of phenyl ring), 4-4.1(s, 3H of OCH_3 of phenyl ring), 7-7.1 (s, 2H of C-H of phenyl ring), 7.8-8 (s, 2H of C-H of imidazole ring), 7.2-7.3 (s, 1H of thiazole ring).
Elemental Analysis: C, 74.14; H, 6.00; N, 9.26; O, 3.53; S, 7.07.

Compound KS-5
1-(5-(5-(4-aminophenyl)-1-(4-(4-cyclopropylphenyl)thiazol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-2-methylphenyl)ethanone
For compound KS-5 yield 69%, M.P 180^{0}C, IR(KBr) cm^{-1} 1720 (C=O of $COCH_3$), 3560 (-NH primary of NH_2), 1465 (-C=C aromatic stretching), 1087.85 (-CN tertiary amine), 1282 (- CN (vibration), 1H NMR($CDCl_3$) delta ppm, 3-3.1 (s, 3H of $COCH_3$ of phenyl ring), 3.55-4 (s, 3H of NH_2 of phenyl ring), 7-7.1 (s, 2H of C-H of phenyl ring), 7.8-8 (s, 2H of C-H of imidazole ring), 7.2-7.3 (s, 1H of thiazole ring).

Elemental Analysis: C, 73.14; H, 5.73; N, 11.37; O, 3.25; S, 6.51.

Compound KS-6
5-(5-(4-methoxyphenyl)-1-(4-p-tolylthiazol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-2- methylphenol
For compound KS-6 yield 75%, M.P 230^{0}C, IR (KBr) cm^{-1} 1371.39 (Phenolic-OH), 1012.6 (-C-O-C of OCH_3), 1689 (-C=N- of thiazole stretching), 2962.66 (CH-Ar), 1H NMR($CDCl_3$) delta ppm, 3.9-4 (s, 3H of OCH_3 of phenyl ring), 2.6-2.7 (s, 3H of CH_3 of phenyl ring), 1H NMR($CDCl_3$) delta ppm, 7-7.1(s, 2H of C-H of phenyl ring), 7.6-7.7 (s, 2H of C-H of imidazole ring), 7.2-7.3 (s, 1H of thiazole ring), 4-3-4.7 (s, 1H of OH of phenyl ring).
Elemental Analysis: C, 65.73; H, 5.24; N, 11.50; O, 8.76; S, 8.77

Compound KS-11
4-(4-cyclopropylphenyl)-2-(3-(3,4-dimethylphenyl)-5-(4-methoxyphenyl)-4,5-dihydro- 1H-pyrazol-1-yl)thiazole
For compound KS-11 yield 68.66%, M.P 182^{0}C, IR (KBr) cm^{-1}, 1469.76 ($-CH_3$), 2850.79 (Aromatic ring (sp^3 stretching), 3342.64 ((CH-Ar), 3381 (Cyclopropyl group), 1689 (-C=N- (thiazole stretching), 1H NMR($CDCl_3$) delta ppm, 3.9-4 (s 3H of OCH_3 of phenyl ring), 2.5-2.6 (s, 3H of CH_3 of phenyl ring), 7.1-7.2 (s, 2H of C-H of phenyl ring), 7.5-7.6 (s, 2H of C- H of imidazole ring), 7.3-7.4 (s, 1H of thiazole ring).

Elemental Analysis: C, 75.12; H, 6.09; N, 8.76; O, 3.34; S, 6.68

After spectral evaluation the compounds were further screened for anticancer activity.

Biology

The compounds were evaluated by MTT assay. MTT assay is a colorimetric assay, is based on the capacity of mitochondrial succinate dehydrogenase enzymes in living cells can reduce the yellow water soluble 3-(4,5-dimethyl thiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) into an insoluble coloured formazan product.[16,17] Two cell lines were taken lung cancer cell line NCI-H522 and breast cancer cell line (MCF-7). Following results were obtained.

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Sr No	Concentration	OD	Percentage of cell inhibition	Percentage of cell cytotoxicity	IC_{50}
1	Control (DMSO)	0.481	100	00	-
2	Std (5-flurouracil)	0.006	1.94	98.05	-
3	KS1-60 µg/ml	0.395	82.12	17.88	1.553
4	KS1-80 µg/ml	0.308	64.03	35.97	1.10
5	KS3-60 µg/ml	0.109	22.66	77.34	0.0665
6	KS3-80µg/ml	0.105	21.82	78.18	0.0455
7	KS5-60 µg/ml	0.215	69.57	30.43	1.239
8	KS5-80µg/ml	0.120	38.83	61.17	0.47
9	KS6-60 µg/ml	0.025	8.09	91.91	-0.297
10	KS6-80µg/ml	0.010	3.23	96.77	-0.419
11	KS11-60 µg/ml	0.231	48.02	51.98	0.70
12	KS11-80 µg/ml	0.199	41.37	58.63	0.53

CONCLUSION

We conclude that we synthesized compounds predicted in our last paper. The compounds were further spectrally evaluated and biologically screened. We found that these compounds exhibited excellent anti-EGFR activity as compared to standard compounds. The compounds were target specific and hence would have less toxicities as compared to current therapy.

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