Research Article

Synthesis and *In Vitro* Evaluation of Novel Thieno [2,3 *D*] Pyrimidines As Antiproliferative Agents

Suparna S. De and Manisha S. Phoujdar

Sinhgad College of Pharmacy, Pune, 411041, Maharashtra, India.

ABSTRACT

The present study was undertaken to design and develop series of potential, selective antiproliferative-anticancer condensed pyrimidines, bioisosteric with gefitinib. Seven compounds were synthesized in three steps, structurally characterized and evaluated for their antoproliferative activity on HT29, A549 and HeLa cell lines which are found to overexpress EGFR. Compounds 1_h and 2_f showed good activity against HT29 cell line whereas compounds 1i and 6i showed good activity against HeLa and A549 cell lines respectively amongst the group.

Keywords: Condensed pyrimidines, antiproliferative, bioisosteric, HT29, A549, HeLa.

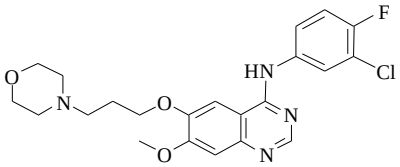
1. INTRODUCTION

Carcinogenesis or malignant transformation of normal cells results in uncontrolled proliferation of some cells, as well as their metastasis to secondary foci. These undifferentiated extra cells form a mass called as tumour which could be malignant or benign. Currently anticancer agents possessing DNA cleaving, antimitotic, antimetabolite, topoisomerase inhibitory or signal transduction inhibitory properties are used as effective drugs, in the chemotherapy of cancer [1].

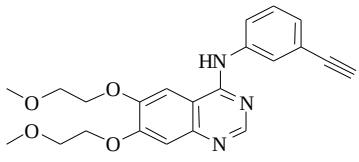
Several receptor tyrosine kinases [2] are known to be activated in the cancer cells and to drive tumour growth, angiogenesis, progression and metastasis. Inhibiting tyrosine kinase activity therefore represents a rational approach to cancer therapy[3].

A variety of small molecules exhibiting selective receptor tyrosine kinase inhibitory activities have been reported as potential therapeutic agents to control tumour growth. Notable amongst them is the class of 2-*H*-4-anilinoquinazolines, suitably substituted at the 5 and 6 positions. Noted antiproliferative drugs Gefitinib(Iressa) **1**[4,5], Erlotinib(Tarceva) **2**[6], Lapatinib[7], Canertinib[8] etc., fall in this class (Fig.1).

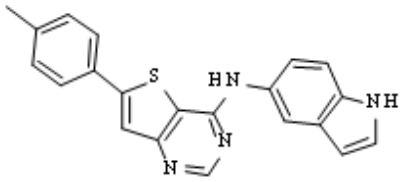
The bioisosterism between benzene and thiophene is well documented[9]. On these lines Munchhof *et al*.,[10] have reported the design, synthesis and VEGFR-2 kinase inhibitory activity of some thieno[3,2-*b*]pyrimidines **3** (Fig.1). The potential of thienopyrimidines as antiproliferative anticancer activity is yet to be fully explored. On this basis, we undertook the exploration in the bioisosteric 4-anilino-2*H*-5, 6-disubstituted thieno[2,3-*d*]pyrimidines. Total seven compounds thus synthesized in good yields have been characterized structurally, and when evaluated on HT 29 (Adenocarcinoma)[11], A549 (Lung Carcinoma)[12] and HeLa (Cervix Cancer)[13] cell lines for antiproliferative activity, have shown to have antiproliferative activity. These compounds have also been found non-cytotoxic to normal cells when tested on 3T3 normal mouse cell line.



1. Gefitinib



2. Erlotinib



3. Thieno[3,2-*b*]pyrimidines

Fig. 1: Structures of some antiproliferative drugs

2. MATERIALS AND METHODS

The design of the novel condensed 2*H*-pyrimidin-4-amines has been done on the basis of the knowledge of antiproliferative activity associated with the 2*H*-4-anilinoquinazoline pharmacophore and its SAR [14]. On this basis a series of compounds, comprising of suitably substituted 2*H*-4-substituted-anilinothieno[2,3-*d*]pyrimidines (**1_{f-i}, $2_{f,i}$ and 6_i**) were designed, for the synthesis and biological evaluation against various carcinoma cell lines (HT29 cell line, A549 cell line and HeLa cell line).

2.1 Chemistry

The starting materials, *o*-aminocarbonyl substrates were synthesized in house[15,16]. Their cyclocondensation with formamide in microwave, subsequent chlorination with $POCl_3$ and nucleophilic displacement reactions with various substituted anilines, to give a series of condensed 2*H*-pyrimidin-4-amines was done conventionally in three steps[17].

A general approach to synthesize the designed compounds (**1_{f-i}, $2_{f,i}$ and 6_i**) is shown in Fig.2.

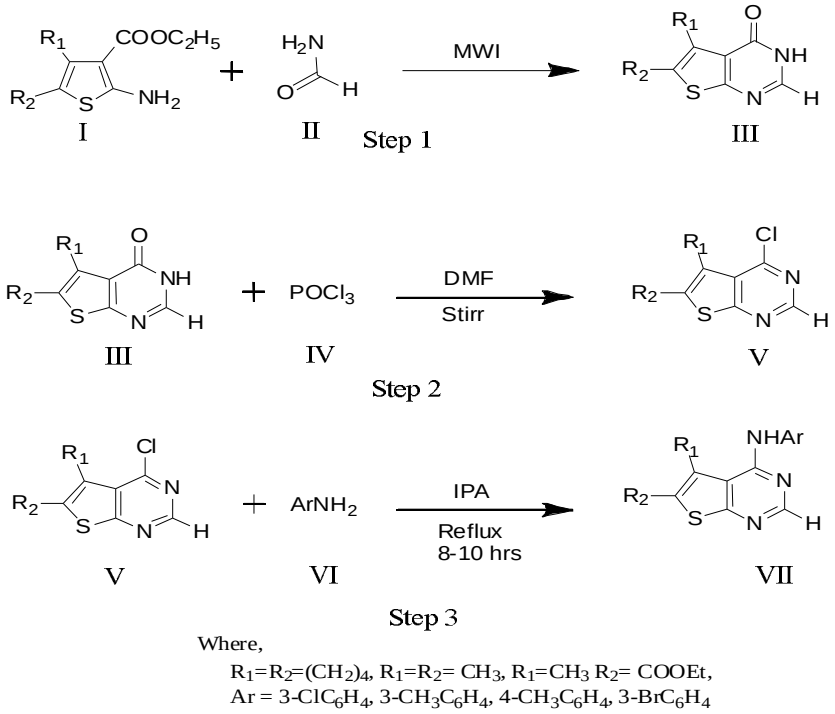


Fig. 2: Steps for synthesis of compounds (1_{f-i}, $2_{f,i}$ and 6_i)

The *o*-aminoesters (**I**) (0.02 mole) and formamide (**II**) (15 ml) were taken in 100 ml round bottom flask fitted with reflux condenser and irradiated in MWI at 40W for 25 min. The progress of the reaction was monitored by TLC. On completion, the reaction mixture was cooled to room temperature and poured into ice water. The solid obtained was filtered at suction, washed with chilled water and dried. The crude product (**III**) was recrystallised from methanol-dimethylformamide. This product (**III**) was then mixed with 40ml dimethyl formamide (DMF) and phosphorus oxychloride (**IV**) (10-12ml). The mixture was stirred for 1 hour at 0-5 ^{o}C and then stirring was continued at R.T for 8 hours. The progress of reaction was monitored by TLC. On completion of the reaction, excess of $POCl_3$ was distilled off under vacuum. The residue was then poured on crushed ice, neutralized by aq. $NaHCO_3$ and allowed to stand for one hour. The solid separated (**V**) was filtered by suction and washed with chilled water, dried and recrystallized from *n*-hexane. Thereafter, a solution of substituted aniline (**VI**) (0.02 mole) in isopropyl alcohol (20 ml) was added into the RBF containing compound **V** and the reaction mixture was refluxed conventionally for 8-10 hrs. The reaction mixture was cooled overnight. The solid separated (**VII**) was filtered at suction, washed with chilled water and dried. The crude product was recrystallised from *i*-propanol.
The synthesized compounds were characterized by melting point, I.R, NMR and Mass.
The list of synthesized compounds has been mentioned in Table 1.

2.1.1 Physical & spectral data for some representative compounds synthesized

N-(*m*-tolyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (1_f)

Melting Point: 128-130 ^{o}C
IR (KBr) cm^{-1}: 3456(γ_{NH}), 2921(γ_{C-H}), 1611($\gamma_{C=N}$), 2857(γ_{CH3}).
^{1}H NMR($CDCl_3$): 1.9 (4H, m, CH_2 at 6 and 7), 2.8 (2H, t, CH_2 at 8), 3.0 (2H, t, CH_2 at 5), 2.38 (3H, s, Ar-CH_3 at 3'), 6.9 (1H, d, Ar-H at 4'), 7.2 (1H, t, Ar-H at 5'), 7.44-7.48 (2H,d, Ar-H at 2' and 6'), 8.48 (1H,s, CH at 2)

N-(3-chlorophenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (1_h)

Melting Point: 130-135 ^{o}C
IR (KBr) cm^{-1}: 3450(γ_{NH}), 2921(γ_{C-H}), 1600($\gamma_{C=N}$), 764(γ_{C-Cl})
^{1}H NMR($CDCl_3$): 1.89-1.98 (4H, m, CH_2 at 6 and 7), 2.8 (2H, t, CH_2 at 8), 2.9-3.0 (2H, t, CH_2 at 5), 4.0 (1H, NH at 4), 7.0 (2H, Ar-H at 2' and 4'), 7.2 (1H, t, Ar-H at 5'), 7.4 (1H, d, Ar-H at 6'), 8.48 (1H,s, CH at 2)
MS m/z. 316 (M^+), 317 (M^+1), 318 (M+2), 288, 261, 189

2.2 Biological activity

The anti-proliferative potential of the compounds were tested on three different cell lines by MTT assay method.
The cell lines used were permanent cell lines obtained from National Centre for Cell Sciences (NCCS, Pune, India) and had origin from different cancers in humans. These were used as prototypes to find the potential of the compounds on different types of tumours. These cells have been characterized with the overexpression of EGFR[18-20].

2.2.1 Test Procedure

The HeLa, HT-29 and A-549 cell line were subcultured in a fresh tissue culture flask in DMEM (Dulbecco's Minimum Essential Medium) with 10% FBS (foetal bovine serum) and antibiotics, incubated in a 5% CO_2 incubator at 37^{o}C. The cells were treated with appropriate concentration of each compound in triplicate with a control without any compound. The plate was incubated in a CO_2 incubator for 72 hr. and then studied under the inverted microscope and the results were recorded. The cell morphology in each well was compared with that of the control to analyze the results. The cells were incubated with the test sample in appropriate concentrations (10, 25, 50, 100 and 200µg/ml). The results were observed after 72 hr. under inverted microscope. A control cell lines was also maintained for each set of tests, which had only the respective cell line and the growth promoting media without any test compound. The test was performed in 2 sets.
Concentrations of compounds showing 50% growth inhibition in cells were calculated as IC_{50} value.

3. RESULTS AND DISCUSSION

The activity comparision data of the synthesized compounds with the standard drugs (Gefitinib and Cyclophosphamide/CYPH) have been presented in Table 2. Data shows that all 7 compounds showed good inhibitory activity against three cell lines.

4. CONCLUSION

In present work, synthesis of a series of condensed 4-substituted anilinopyrimidines, unsubstituted at the position 2 of the pyrimidine ring has been undertaken. The moieties condensed to pyrimidine ring comprise of diverse structures like cyclohexane, dimethylthiophene and ethyl 3-methylthiophene-2-carboxylate.It was decided to try out these changes with respect to the ring attached to the pyrimidine ring, in order to obtain optimal activity. The starting materials, *o*-aminocarbonyl substrates were synthesized in house. Their cyclocondensation with formamide in microwave, subsequent chlorination with $POCl_3$ and nucleophilic displacement reactions with various substituted anilines was done conventionally. All the 7 compounds were found to have antiproliferative activity against the three cell lines. Amongst the group, compounds $\mathbf{1_h}$ and $\mathbf{2_f}$ have shown best activity against HT29 cell line whereas compounds $\mathbf{1_i}$ and $\mathbf{6_i}$ have shown best activity against HeLa and A549 cell lines respectively.

Conflict of interest statement

We declare that no benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article. We also declare that we have no conflict of interest in connection with this paper, other than any noted in the covering letter to the editor.

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