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Design, synthesis, in silico and in vitro studies of novel 4-methylthiazole-5-carboxylic acid derivatives as potent anti-cancer agents

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ABSTRACT

Since inhibitors of mucin onco proteins are potential targets for breast cancer therapy, a series of novel 4-methylthiazole-5-carboxylic acid (**1**) derivatives **3a**–**k** were synthesized by the reaction of **1** with $SOCl_2$ followed by different bases/alcohols in the presence of triethylamine. Once synthesized and characterized, their binding modes with MUC1 were studied by molecular docking analysis using Aruglab 4.0.1 and QSAR properties were determined using HyperChem. All synthesized compounds were screened for in vitro anti-breast cancer activity against MDA-MB-231 breast adenocarcinoma cell lines by Trypan-blue cell viability assay and MTT methods. Compounds **1**, **3b**, **3d**, **3e**, **3i** and **3f** showed good anti-breast cancer activity. Since **1** and **3d** exhibited high potent activity against MDA-MB-231 cell lines, they show could be effective mucin onco protein inhibitors.

It is almost counter-intuitive to believe that the complexity of a disease can be made even more complicated by the results of scientific research. Breast cancer is the most common malignancy in women and it accounts for nearly fifty per cent of cancer deaths in women. Like all chronic diseases breast cancer also poses a series of threats and difficulties, which may further lead to the development of mental problems in the patients. The incidence of breast cancer has been increasing steadily from one out of twenty in 1960 to one out of eight in women now.[1–4] Mammography is the most effective tool for screening and early detection of breast cancer in women.[5] Estrogens play crucial roles in breast cancer development and growth, and estrogen-stimulated growth in tumor cells requires estrogen receptors (ERs).[5–8] About two-thirds of human breast tumors reveal higher levels of ERs than normal breast tissues.[9] Inhibitions of proliferative pathways were considered an effective strategy to fight cancer and nowadays much attention has been paid to the discovery and development of new and more selective anti-cancer drugs.[9,10] A number of drug probables are preceded to clinical trials and few of them are in clinical use. It is a challenging target for synthetic chemists because of the complex structures of drug problems and handling of toxic starting materials and reagents. Under these circumstances, we aimed at the structural modification of 4-methylthiazole-5-carboxylic acid (**1**) to synthesize novel, safe and effective derivatives that can represent a promising pathway in search of new anti-cancer agents.

The present study is to predict and evaluate the efficacy of molecules against MUC1 onco protein, a member of mucin family. Mucins are the predominantly glycoproteins and they act as physical barriers and protect the apical borders of epithelial cells in adverse conditions. Mucins are largely unrecognized as effectors of carcinogenesis and intimately involved in breast malignancy. It also has been reported that mucins are predominantly over expressed in various human malignancies in addition to breast malignancy and their role in signaling cell growth and survival.

All of the mucin family proteins contain the tandem repeats of proline, threonine and serine residues which are called PTS domains and these domains are involved in the glycosylation process. MUC1 is the heavily glycosylated high molecular weight membrane protein comprising more than 50% of carbohydrate moiety

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and can be expressed by various epithelial cells. It is overexpressed in the entire cell membranes of carcinoma cells and allows them to interact with restricted receptors.[11] They contain large α sub unit and smaller β unit. The N-terminal region of α sub unit is fully exposed to cell surface and contains variable number of repeats containing PTS domains. Glycosylation of these repeats is altered in human carcinomas which in turn play a role in the immunosurveillance of cancer.[12,13] This N-terminal region is anchored to cell membrane through C-terminal region and blocks the cell–cell and cell–extracellular matrix interactions and when it is released the C-terminal region acts as a putative receptor which is engaged in signaling path ways related to tumor progression.[14]

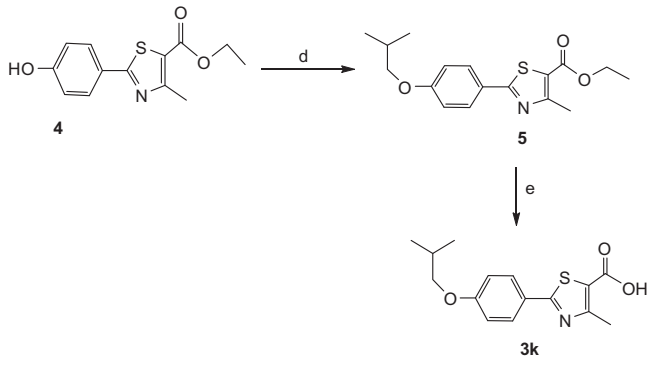
Hence, mucins are approved as therapeutic targets and adverse prognosis markers. In addition, Food and Drug Administration (FDA) approved MUC1 as a serum biomarker for breast cancer and targeting MUC1 is the ideal choice of controlling breast cancer.[15] Inhibitors of mucins function can become the promising agents to control and manage the breast cancerous condition.[14]

The derivatives (**3a**–**h**) of 4-methylthiazole-5-carboxylic acid (**1**) were prepared by the reaction of 4-methylthiazole-5-carboxylic acid (**1**) with thionyl chloride in the presence of catalytic amount of *N*,*N*-dimethyl formamide to form corresponding acid chloride **2** and its further reaction with substituted benzyl amines, aniline, 6-fluoro-3-(piperidin-4-yl)benzo[*d*]isoxazole and bioactive amines like cytosine, 5-fluoro-cytosine and alcohols in the presence of triehyl amine in toluene afforded the corresponding derivatives **3a**, **3b**, **3c**, **3d**, **3f**, **3g**, **3i**, **3j**, and **3e**, **3h** (Scheme 1 & Table 1). Substituted benzyl amines, 6-fluoro-3-(piperidin-4-yl)benzo[*d*] isoxazole[16] and aniline directly reacted with **2** in the presence of triethylamine as a base in toluene. But **2** did not react with cytosine and 5-fluoro-cytosine in the presence of triehylamine; hence they were prepared through silylated cytosine[17] and 5-fluoro-cytosine[17] in the presence of hexamethyldisilazane in toluene using catalytic amount of methane sulphonic acid and triethyl amine, to get **3i** and **3j**. Reaction of **4** with isobutyl bromide in the presence of K_2CO_3 and *N*-methyl-2-pyrrolidone produced **5** and it is further hydrolyzed to afford **3k** (Scheme 2).[18]

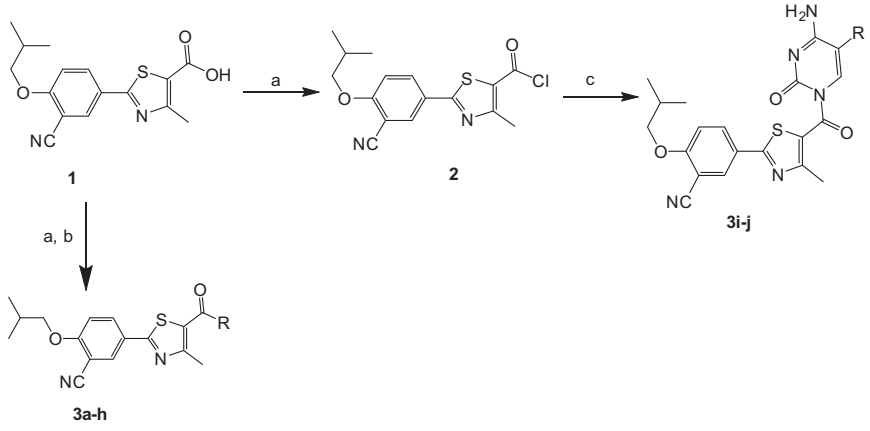
Characteristic IR stretching absorptions were observed in the regions 1245–1262 (O═C─N), 1530–1558 (N─H_{aliph}), 1685–1692 (C═O) for aliphatic and 1648–1655 for aromatic and 1661–1671 for piperazines, 2228–2232 (CN) cm^{-1}, respectively.[19,20] In the ^1H NMR spectra of compounds **3a**–**h**, NH proton chemical shift appeared in the region of 8.91–8.99 ppm[21] but this signal is not observed for **3e**, **3g**, **3h**, **3i** and **3j** indicating the amide group formation. In ^{13}C NMR spectra of compounds **3a**–**k**, chemical shifts were observed in the expected regions.[22] The chemical shift at 116.9–117.9 ppm is assigned to CN. ^{13}C chemical shift in the region 169.5–172.9 ppm is attributed to C═O of the amide group (see Schemes 1 and 2, Table 1).

The models were built for ligand molecules and molecular dynamics simulations were observed for a period of 10 ps. The total energy graphs of dynamics simulations showed that the confirmations were stabilized in the 10 ps dynamics run. The stabilized conformations of the ligands were saved and a QSAR study was carried out and their molecular descriptors were studied in HyperChem software tools.[23–25] All the ligand molecules were filtered with Lipinski filters. The drug likeness of the molecules was predicted from their molecular descriptors and Lipinski data.[23–25] Among all the molecules **3g** is showing a molecular weight of 518 Daltans which should be below 500 Daltans for any molecule to behave as a drug (Table 2). Hence **3g** may be antigenic to the host system. The remaining molecules are showing their descriptors in optimal range indicating their potential to behave as drugs.

The CASTp predicted binding site of MUC1 contain the amino acid residues Pro 1061, Tyr 1066, Gln 1070, Arg 1071, Ser 1074, Leu 1089, Ser 1090, Asn 1091 and Ile 1092. The molecular docking between the binding domain of mucin and stabilized conformations of the molecules showed that the lowest docking energy of −8.882 Kcal/mol was found with **3d** with the formation of two hydrogen bonds and highest docking energy of −4.387 Kcal/mol was found with **3j** with no hydrogen bond formation. Compounds **3c** & **3g** are not showing any docking energy, but forming single hydrogen bond which indicates the existence of weak interaction (Table 3). These docking results could explain that (except **3c** & **3g**)



Scheme 2. Synthetic route for the preparation of 2-(4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid. Reagents and conditions: (d) iso butyl bromide, K_2CO_3, *N*-methyl-2-pyrrolidone, (e) 5% NaOH, acetone, 5% HCl.



Scheme 1. Protocol for the synthesis of 4-methylthiazole-5-carboxylic acid derivatives. Reagents and conditions: (a) $SOCl_2$, toluene, DMF, 90–95 °C. (b) R-H, TEA, toluene, 70–75 °C. (c) TEA, *N*-(trimethylsilyl)-2-((trimethylsilyl)oxy)-1,2-dihydropyrimidin-4-amine, 55–60 °C, H_2O.