J. Chem. Sci. (2020)132:126
https://doi.org/10.1007/s12039-020-01834-w

REGULAR ARTICLE

Design, synthesis, molecular docking and cytotoxic activity of novel urea derivatives of 2-amino-3-carbomethoxythiophene

VENUGOPALARAO VIKRAM[a], SRINIVASA R PENUMUTCHU[b], RAVIRAJ VANKAYALA[c], SURESH THANGUDU[d], KARTEEK RAO AMPERAYANI[e] and UMADEVI PARIMI[e,*]

[a]Department of Chemistry, GITAM University, Rushikonda, Visakhapatnam, India
[b]Department of Chemistry, Case Western Reserve University, Cleveland, OH, USA
[c]Department of Bioscience and Bioengineering, Indian Institute of Technology Jodhpur, Jodhpur, India
[d]Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan
[e]Department of Chemistry, Gayatri Vidya Parishad College for Degree and PG Courses (A), Rushikonda, Visakhapatnam, India
E-mail: umadevichemistry@gmail.com

MS received 20 November 2019; revised 29 January 2020; accepted 24 July 2020

Abstract. An efficient feasible route for the one-pot synthesis of novel series of urea derivatives (2a–2j) from 2-amino-3-carbomethoxythiophene (1) via in situ isocyanate has been developed, and their corresponding anticancer activities were accomplished. The series of urea derivatives were characterized by using ^{1}H, ^{13}C nuclear magnetic resonance and mass spectroscopic analysis. The cytotoxic activities were evaluated against human cervical (HeLa) and human lung (NCI-H23) cancer cell lines. These studies revealed satisfactory activity for some of the compounds, which could potentially serve as lead compounds for drug discovery and development. Furthermore, molecular docking studies supported in identifying the potential binding sites between the urea derivatives and eukaryotic ribonucleotidereductase (RR). High ambiguity driven docking (HADDOCK) modelling was specifically employed to determine the model complex of RR and urea derivatives. The proposed model has provided a deep insight into the molecular level interactions of RR-urea model complexes in understanding the exact pharmacophore for designing highly potent RR inhibitors. Overall, the present work has shed light in developing a feasible and robust approach for the synthesis of novel urea derivatives of 2-amino-3-carbomethoxythiophene and identified a part of molecular structure that is responsible for a specific biological interaction leading to potential anticancer activities.

Keywords. One-pot synthesis; Urea derivatives; 2-Amino-3-carbomethoxythiophene; Cytotoxic activity; Docking study.

1. Introduction

According to the World Health Organization (WHO), cancer is the second leading cause of death globally and is responsible for an estimated 9.6 million deaths in 2018 (https://www.who.int/news-room/fact-sheets/detail/cancer). Therefore, there is a great demand to develop novel anticancer agents with higher efficacy and low toxicity.

Ribonucleotide reductase (RR) is an enzyme that catalyzes the formation of deoxyribonucleotides from ribonucleotides by *de novo* biosynthesis of DNA precursors in nature and maintains the tight control of dNTP pool that is essential for cellular homeostasis. The active form of human Ribonucleotide Reductase (hRR) consists of two protein subunits such as hRR1 and the smaller subunit hRR2. These protein subunits also exist in multimeric forms, such as $\alpha_2\beta_2$ and $(\alpha)_n(\beta_2)_m$ (where n = 4 or 6 and m = 1, 2, or 3). The hRR1 contains allosteric sites like specificity site (S-site) and the activity site (A-site) and one catalytic site (C-cite). The subunit hRR2 is associated with the diferric-oxygen centre, which generates stable tyrosyl free radical for catalysis.[1] RR is an attractive

*For correspondence

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s12039-020-01834-w) contains supplementary material, which is available to authorized users.

Published online: 12 September 2020

therapeutic target of cancer, and over the past years, many chemotherapeutic drugs inhibiting different subunits of RR have been developed and tested clinically for their potent anticancer activities and anti-HIV activities.[2–5] Previous studies have shown that peptidomimetic ligands such as EcR2pep, P7/ScR2pep, P6/ScR2pepheptapeptide, ScR4pep are well-known to have significant binding to RR and eventually induce inhibitory activity.[6] Recently, it was reported that benzyloxyurea derivatives bind to RR and induce anticancer activity on human leukemia cell K562 and murine leukemia cells L1210, respectively.[7] Hydroxyurea was the first RR inhibitor investigated as an antineoplastic agent in humans[8] and it was also reported that 1-(Benzyoloxy) urea and its derivatives bind to RR enzyme site 2EUD, and induce anticancer activity on human cancer cell HeLa.[9] The binding interface involved in RR- P7/ScR2pep and RR- P6/ScR2pep were shown in Figure 1. The SER691, GLN692 SER726 residues were mainly involved in the hydrogen bonding with the backbone imino group and carboxyl group of the P7/ScR2pep. In the case of P6/ScR2pep heptapeptides, SER691, GLN692 SER726 residues were involved in the hydrogen bonding with the acyl group and carboxyl group of peptide moiety.

In recent years, some of the kinase inhibitors with urea structure have been launched for the treatment of cancers. For example, linifanib, developed by Abbott, is used for the treatment of colorectal cancer.[10] Sorafenib [11] has been used for the treatment of advanced renal cell carcinoma,[12] hepatocellular carcinoma,[13] and differentiated thyroid carcinoma.[14] Lenvatinib, launched by Eisai, showed efficacy in the treatment of renal cell carcinoma, differentiated thyroid cancer and hepatocellular carcinoma.[15] Also, some of the urea derivatives were also reported to possess potent inhibiting effects against anticonvulsant,[16] and HIV, [17]antibacterial and antifungal agents[18] and CXCR3 antagonists.[19] They also served as an extensive application as agrochemicals, resin precursors as well as synthetic intermediates.[20]

Among various heterocyclic compounds, 2-aminothiophenes are important five-membered building blocks in organic synthesis and the chemistry of these small molecules are still developing based on the discovery of cyclization by Gewald.[21] Another attractive feature of 2-aminothiophene scaffolds is that their ability to act as synthons for the synthesis of biologically active thiophene-containing heterocycles.[22] 2-aminothiophene forms as a significant class of drugs which exhibit excellent biological activities such as, antimicrobial[23] and antiviral,[24] anti-tubercular[25] and particularly, anticancer[26–31] activities.

Keeping all these reports in view, we report herein, the experimental design, synthesis and characterization of a novel series of urea derivatives of 2-amino-3 carbomethoxythiophene with pyrimidine amine and benzylamine analogues as both derivatives which exhibited potential anticancer activity[32–34] *via* one-pot synthesis and subsequently studied the structure-activity relationships (SAR). The docking studies identified a part of the molecular structure that is responsible for a specific biological interaction leading to the destruction of cancer cells.

2. Experimental

2.1 *Materials and methods*

All chemicals were purchased from Sigma Aldrich and used directly. Reaction progress was monitored by thin-layer chromatography (TLC) using silica gel aluminium sheets (60F-254) and UV light. Melting points were recorded using Mettler Toledo MP70 model, ^{1}H & ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance 400 MHz spectrometer with tetramethylsilane (TMS) as an internal standard. Splitting patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), or doublet of doublet (dd) and multiplet (m). The broad (br) signals were also indicated. The value of chemical shifts (d) is given in ppm and coupling constants (J) in Hertz (Hz). Mass spectra were obtained using waters XEVO TQ LCMS instrument was used with an electrospray (ESI) positive and negative ionization modes.

2.2 *Synthesis*

Synthesis of 2-amino-3-carbomethoxythiophene (1): To the stirred solution of 1,4-dithiane-2,5-diol (1 g, 6.5 mmol) in methanol (7 mL) cooled to -5 °C, methyl cyanoacetate (0.88 g, 8.9 mmol) and triethylamine (0.33 g, 3.3 mmol) were slowly added at the same temperature. The reaction mixture was vigorously stirred at 25 °C for 1 h and then further stirred at 40 °C for another 3 h. The reaction progress was monitored by TLC. The obtained solids were filtered, and the filtrate was poured into ice-cold water (5 mL) and then stirred for 1 h. The obtained solid was filtered, and washed with ice-cold water (2 mL) and dried under reduced pressure. The purified compound was then obtained by crystallization in ethyl acetate and n-Heptane to afford compound (1).

Synthetic procedures for the synthesis of 2a-2j: To the stirred solution of methyl 2-aminothiophene-3-carboxylate (0.5 g, 3.2 mmol) in 1,4-Dioxane (5 mL) at below 0 °C, triphosgene (0.33 g, 1.1 mmol) was added in batch-wise at

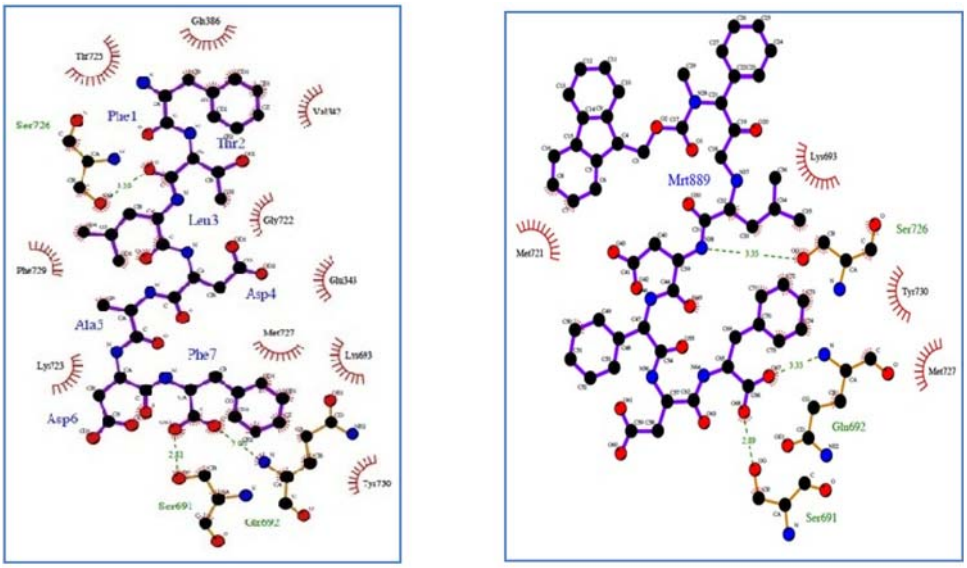


Figure 1. Binding interface involved in (**A**) RR- P7/ScR2pep and (**B**) RR- P6/ScR2pep.

below 10 °C. The reaction mass was then stirred at 45 °C for 4 h and the reaction progress was monitored by TLC. The reaction mixture was cooled to 25 °C and charged triethylamine (0.96 g, 9.5 mmol) and second amine (0.95 eq) and then stirred for 4-8 h. The reaction mixture was filtered and washed with 1,4-Dioxane and n-heptane followed by drying. If there is no product precipitate, the reaction mixture was then extracted thrice with isopropyl acetate. The organic layer was washed with water, dried over sodium sulphate, evaporated under reduced pressure, and purified by recrystallization in isopropyl acetate and n-Heptane.

2-amino-3-carbomethoxythiophene (1) White solid, yield: 77%, M.p. 72-73 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.25 (s, 2H, NH_2), 6.81 (d, J = 7.6 Hz, 1H, ThH), 6.27 (d, J = 7.6 Hz, 1H, ThH), 3.81 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) 165.8, 162.7, 125.8, 107.0, 106.9, 51.0; HRMS (m/z): Calculated mass for $C_6H_7NO_2S$ (M^++H): 158.0270 and measured mass for 158.0262.

Methyl 2-(3-(pyrimidin-4-yl)ureido)thiophene-3-carboxylate (**2a**) Light brown solid, yield: 82%, M.p. 174-175 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.96 (s, br, 1H, NH), 9.76 (s, 1H, NH), 8.13 (s, 1H, ArH), 7.15 (s, 1H, ArH), 6.98 (d, J = 5.76 Hz, 1H, ThH), 6.21 (d, J = 5.76 Hz, 1H, ThH), 3.13 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.43, 159.04, 158.06, 157.27, 154.30, 151.77, 125.49, 123.58, 114.47, 108.37, 52.32; HRMS (m/z): Calculated mass for $C_{11}H_{10}N_4O_3S$ (M^++H): 279.0546 and measured mass for 279.0544.

Methyl2-(3-(6-chloropyrimidin-4-yl)ureido) thiophene-3-carboxylate (**2b**) Light brown solid, yield: 82%, M.p. 181-182 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.35 (s, br, 1H, NH), 10.11 (s, 1H, NH), 8.84 (s, 1H, ArH), 7.59 (s, 1H, ArH), 7.22 (d, J = 5.84 Hz, 1H, ThH), 7.03 (d, J = 0.44 Hz, 1H, ThH), 3.46 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.43, 158.83, 158.09, 156.94, 154.18, 151.77, 125.49, 123.58, 114.47, 105.88, 52.32; HRMS (m/z): Calculated mass for $C_{11}H_9ClN_4O_3S$ (M^++H): 313.0157 and measured mass for 313.0155.

Methyl 2-(3-(6-chloropyrimidin-4-yl)-3-methylureido) thiophene- 3-carboxylate (2c) Brown crystalline solid, yield: 89%, M.p. 188-189 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.38 (s, br, 1H, NH), 8.91 (s, 1H, ArH), 7.24 (d, J = 5.84 Hz, 1H, ThH), 7.09 (s, 1H, ArH), 6.74 (d, J = 5.12 Hz, 1H, ThH), 3.90 (s, 3H, CH_3), 3.53 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.43, 159.98, 157.01, 155.43, 154.49, 152.26, 125.49, 123.58, 114.56, 107.63, 52.32, 29.51; HRMS (m/z): Calculated mass for $C_{12}H_{11}ClN_4O_3S$ (M^++H): 327.0313 and measured mass for 327.0279.

Methyl 2-(3-(6-methylpyrimidin-4-yl)ureido)thiophene-3-carboxylate (2d) Off white solid, yield: 85%, M.p. 154-155 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.09 (s, br, 1H, NH), 9.45 (s, br, 1H, NH), 8.90 (s, 1H, ArH), 7.25 (d, J = 4.6, 1H, ThH), 7.09 (s, 1H, ArH), 6.75 (d, J = 0.68 Hz, 1H, ThH), 4.54

(s, 3H, CH_3), 3.89 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.43, 164.34, 157.60, 157.06, 154.18, 151.77, 125.49, 123.58, 114.47, 105.80, 52.32, 24.60; HRMS (m/z): Calculated mass for $C_{12}H_{12}N_4O_3S$ (M^++H): 293.0703 and measured mass for 293.0703.

Methyl 2-(3-(6-methoxypyrimidin-4-yl)ureido)thiophene-3-carboxylate (2e) Off white solid, yield: 87%, M.p.

183-184 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.18 (s, br, 1H, NH), 9.24 (s,br,1H, NH), 8.90 (d, J = 0.76, 1H, ThH), 7.74 (s, 1H, ArH), 7.09 (d, J = 0.84 Hz, 1H, ThH), 6.75 (s, 1H, ArH), 4.50 (s, 3H, CH_3), 3.5 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.71, 164.43, 158.76, 156.22, 154.18, 151.77, 125.49, 123.58, 114.47, 91.17, 53.80, 52.32; HRMS (m/z): Calculated mass for $C_{12}H_{12}N_4O_4S$ (M^++H): 309.0652 and measured mass for 309.0647.

Methyl 2-(3-(6-cyanopyrimidin-4-yl)ureido) thiophene-3-carboxylate (2f) Off white solid, yield: 79%, M.p. 120-121 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.08 (s, br, 1H, NH), 9.24 (s, 1H, NH), 8.90 (d, J = 0.76, 1H, ThH), 7.74 (s, 1H, ArH), 7.09 (d, J = 0.84 Hz, 1H, ThH), 6.75 (s, 1H, ArH), 3.75 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.43, 159.64, 157.48, 154.18, 151.77, 141.88, 125.49, 123.58, 115.56, 114.47, 109.97, 52.32; HRMS (m/z): Calculated mass for $C_{12}H_9N_5O_3S$ (M^++H): 304.0499 and measured mass for 304.0491.

Methyl 2-(3-benzylureido)thiophene-3-carboxylate (2g) Yellow solid, yield: 89%, M.p. 135-136 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.33 (s, br, 1H, NH), 7.27-7.37 (m, 7H, ArH, ThH), 5.7 (s, br, 1H, NH), 4.52 (d, J = 6 Hz, 2H, CH_2), 3.86 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.43, 155.23, 151.39, 138.73, 128.56, 127.32, 127.24, 125.49, 123.58, 114.47, 52.32, 44.24; HRMS (m/z): Calculated mass for $C_{14}H_{14}N_2O_3S$ (M^++H): 291.0798 and measured mass for 291.0787.

Methyl 2-(3-benzyl-3-methylureido)thiophene-3-carboxylate (**2h**) Off white solid, yield: 88%, M.p. 201-202 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H, NH), 7.89-7.87 (m, 2H, ArH), 7.49-7.40 (m, 3H, ArH, ThH), 7.22 (d, J = 8.72 Hz, 2H, ArH, ThH), 5.24 (s, 2H, CH_2), 3.83 (s, 6H, N-CH_3,O-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.43, 155.19, 152.56, 137.02, 128.74, 127.82, 127.43, 125.49, 123.58, 114.56, 52.32, 51.55, 34.06.; HRMS (m/z): Calculated mass for $C_{15}H_{16}N_2O_3S$ (M^++H): 305.0954 and measured mass for 305.0948.

Methyl 2-(3-(4-methylbenzyl)ureido)thiophene-3-carboxylate (2i) Yellow solid, 83% yield, M.p. 180-181 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (s, br, 1H, NH), 7.38-7.13 (m, 5H,ArH, ThH), 6.21 (d, J = 5.76 Hz, 1H, ThH), 5.80 (s, br, NH),5.24 (s, 2H, CH_2), 3.83 (s, 3H, CH_3), 3.32(s, 3H, CH_3), ^{13}C NMR (100 MHz, CDCl3) δ 164.43, 155.23, 136.69, 136.59, 128.85, 127.34, 125.49, 125.38, 114.47, 52.32, 43.86, 21.00; HRMS (m/z): Calculated mass for $C_{15}H_{16}N_2O_3S$ (M^++H): 305.0954 and measured mass for 305.0952.

Methyl 2-(3-(4-cyanobenzyl)ureido)thiophene-3-carboxylate (2j) Off white solid, 90% yield, M.p. 122-124 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (s, br, 1H, NH), 7.37-7.28 (m, 6H, ArH, ThH), 5.50 (s, br, 1H, NH), 4.52 (d, J = 5.72 Hz, 2H, CH_2), 3.86 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.43, 155.23, 151.39, 141.60, 132.12, 128.35, 125.49, 123.58, 118.48, 114.47, 110.42, 52.32, 44.54; HRMS (m/z): Calculated mass for $C_{15}H_{13}N_3O_3S$ (M^++H): 316.0750 and measured mass for 316.0741.

2.3 *Cytotoxic evaluation*

Cell culture, materials and reagent: HeLa, and NCI-H23 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, and Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Thermo Fisher Scientific, USA), 2 mM L-glutamine, 100 μg/mL of penicillin and 100 U/mL of streptomycin. The cells were grown in a humidified incubator at 37 °C (95% humidity, 5% CO_2). Other chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA).

MTT Assay: One millilitre of HeLa, or NCI-H23 cells containing solution (2.0 x 10^4 cells/mL) were added to each well of a 24 well plate and incubated for 1 day to allow the cells to stick on the surface of the plate. Aliquots of DMSO solution containing different concentrations of compounds were added to the 24-well plate, and the cell solutions were incubated for another 3 days. A 50 μL amount of (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT) aqueous solution (0.5 mg/mL) was added to each well of the 24-wellplate and incubated for another 4 h before termination of the 3 days incubation. The upper layer of the solutions in the 24-well plate was discarded, and 1 mL of DMSO (10% final concentration) was added to each well to dissolve the violet color formazan product by pipette stirring. The optical absorbance measured at 570 nm were converted to cell viabilities based on a standard curve (absorbance vs. cell numbers) obtained from control experiments which were carried out under the same conditions except that no compounds were added during cell culture procedures.

2.4 *Molecular docking*

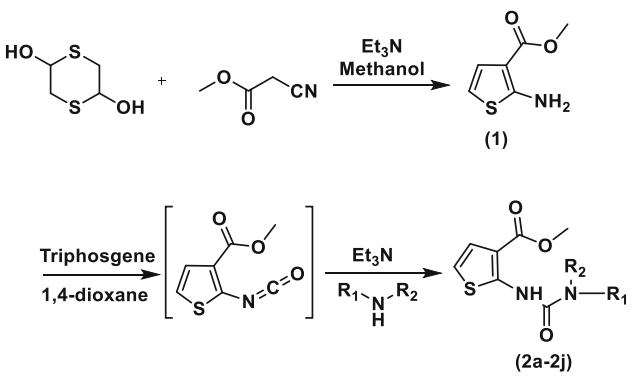
HADDOCK was used to dock urea derivatives of 2-amino-3-carbomethoxythiophene and RR to obtain the model complex of the RR-urea derivatives of 2-amino-3-carbomethoxythiophene. The structural coordinates for the Eukaryotic ribonucleotide reductase (RR) were obtained from the Protein Data Bank as input data (PDB ID: 2ZLF) and the structural coordinates for the urea derivatives of 2-amino-3-carbomethoxythiophene generated from the PRODRG server. The residues which involved in the interactions (VAL342, GLU343, GLN386, SER691, GLN692,

LYS693, GLY722, LYS723, THR725, SER726, MET727, PHE729 and TYR730) with P7/ScR2pep, P6/ScR2pepheptapeptide, ScR4pep with RR were defined as ambiguous interactions which restraint at the interface of the RR domain. The potent compounds 2e and 2g as well as the least active compounds 2b and 2c were docked into the active site of RR. A set of 2000 total complex structures were generated by rigid body minimization. The best 200 structures with the lowest water-refined interaction energies were used for subsequent analysis. Ligplus was used for graphical representations.

3. Results and Discussion

3.1 *Chemistry*

In the current work, we report an efficient synthetic route for the preparation of biologically active urea analogues. The steps involved in the proposed method for the synthesis of urea analogues are shown in Scheme 1. Previously, it was reported that isocyanate of 2-amino-3-carbomethoxythiophene was synthesized in two steps, including silylation of starting amine with hexamethyldisilane or chlorotrimethylsilane, followed by phosgenation of the resulting N-silyl-substituted amines[35] were isolated and treated with amine to prepare urea derivatives. However, in all the previously reported methodologies, isocyanate *in situ* reactions were less explored. In this work, the *in situ* generated isocyanate was reacted with amine, which completely avoided tedious and lengthy workup procedures to isolate isocyante.[36] This protocol is efficient to produce novel urea derivatives of 2-amino-3-carbomethoxythiophene. The formation of isocyanate was confirmed by adding methanol which in turn forms the corresponding methyl carbamate and its

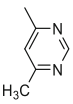
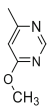
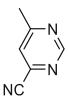
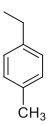
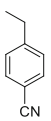


Scheme 1. Synthetic route of urea derivatives of 2-amino-3-carbomethoxythiophene.

^{1}H NMR was recorded. It has to be noted that there is no formation of homo urea in the reaction.

The 2-amino-3-carbomethoxythiophene (methyl 2-aminothiophene-3-carboxylate) (**1**) was synthesised by the reaction of l, 4-dithiane-2, 5-diol with methyl cyanoacetate in the presence of an alkali, triethylamine. Further, it was allowed to react with various pyrimidine and benzyl amines in the presence of

Table 1. Anticancer activities of synthesized compounds against HeLa and NCI-H23 cells.

Compound	R_1	R_2	*(IC_{50} ± SD) (μM) HeLa	NCI-H23
2a	H		55.0 ± 2.0	64.2 ± 0.8
2b	H		93.4 ± 1.2	146.5 ± 1.2
2c	-CH_3		55.5 ± 1.5	100.7 ± 1.4
2d	H		38.6 ± 0.8	54.5 ± 1.0
2e	H		1.2 ± 0.4	7.1 ± 0.7
2f	H		46.8 ± 1.1	60.7 ± 1.0
2g	H		8.5 ± 1.3	12.4 ± 0.5
2h	-CH_3		33.5 ± 0.7	50.7 ± 0.9
2i	H		33.0± 1.9	32.7± 0.6
2j	H		46.6 ± 1.1	83.5 ± 1.0

*IC_{50} values shown above are the concentrations for half-maximal inhibition of cell viability and were calculated from the data obtained from three independent experiments (n=3).

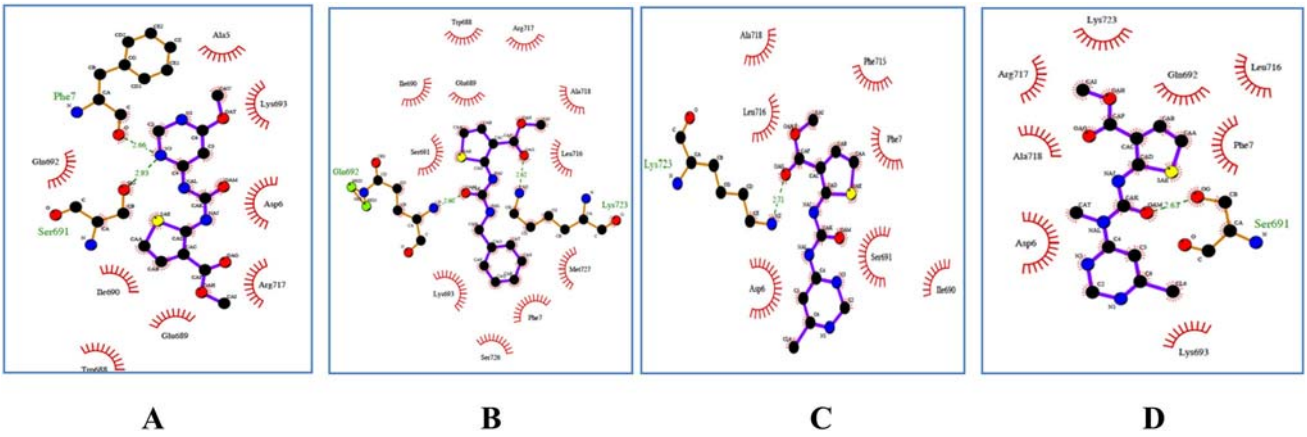


Figure 2. (**A**) RR and 2e complex, (**B**) RR and 2g complex, (**C**) RR and 2b complex and (**D**) RR and 2c complex.

triphosgene and triethylamine to afford different urea derivatives of 2-amino-3-carbomethoxythiophene, 2a-2j in good yields. All these analogues were confirmed by ^{1}H and ^{13}C NMR as well as mass spectrometry data.

3.2 *Evaluation of cytotoxic activity*

All the synthetic compounds (**2a**-**2j**) were evaluated for their cytotoxic activities against cervical (HeLa), and lung (NCI-H23) cancer cells, respectively. The inhibitory concentrations (IC_{50}) of tested compounds are given in Table 1. These synthesized compounds showed good activity against HeLa and NCI-H23 cancer cells. The highest inhibitory effect on the cell viabilities was observed for compounds **2e** and **2g** and lowest for compounds **2b** and **2c** in both the cancer cell lines. The highest inhibitory activity of **2e** and **2g** could be presumably due to the activation of the damage response pathway of the DNA, leading to the cell cycle arrest and induction of mitochondrial pathway of apoptosis.[37]

3.3 *Molecular docking analysis*

The HADDOCK modelling was employed to determine the model complex of the RR and novel urea derivatives of low and high cytotoxicity compounds. The residues which involved in the interactions (VAL342, GLU343, GLN386, SER691, GLN692, LYS693, GLY722, LYS723, THR725, SER726, MET727, PHE729 and TYR730) with P7/ScR2pep, P6/ScR2 pepheptapeptide, ScR4pep with RR defined as ambiguous interaction restraints at the interface of the RR domain. The potent compounds **2e** and **2g**, as well as the least active compounds **2b** and **2c** were docked into the active site of RR.

Our docking studies revealed that SER691, PHE7 are involved in the hydrogen bonding with the compounds **2g**, whereas GLN692, LYS723 involved in hydrogen bonding with acyl group of compound **2e**, in the case of compounds **2b** and **2c**, SER 691 and LYS 723 were involved with only one hydrogen bonding with acyl group shown in Figure 2.

Owing to the significant differences in the formation of the number of hydrogen bond as well as hydrophobic interactions between **2b**, **2c** and **2e**, **2g** compounds with RR. Further, the results are in good agreement with the cytotoxic activity of the phenyl group or methoxy substituted pyrimidine analogue of 2-amino-3-carbomethoxythiophene urea derivatives.

4. Conclusions

In summary, we have reported a facile and one-pot synthesis and design of urea derivatives of 2-amino-3-carbomethoxythiophene. Furthermore, these urea derivatives also exhibited promising *in vitro* cytotoxicity against a panel of two human cancer cell lines, HeLa and NCI-H23 were tested. Some of these analogues like **2d** and **2e** showed significant anticancer activity. Such significant anticancer activity could be presumably due to the activation of the damage response pathway of the DNA, leading to the cell cycle arrest and induction of mitochondrial pathway of apoptosis. In addition, the most potent and active compounds were studied for their docking studies in which these derivatives were proved to be effective binders of human ribonucleotidereductase protein. The proposed models have provided an understanding of the molecular level interactions of the eukaryotic

ribonucleoside reductase (RR)-novel urea derivatives complex and provide pharmacophores for designing highly potent RR inhibitors. These results provide an insight for future direction in the development of such molecules. Further investigations on the molecular and biological efforts of this series of compounds are in progress.

Acknowledgements

The docking studies were supported by grants from the National Institutes of Health (NIH): 1R01GM126833-01

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Supplementary Information (SI)

^{1}H NMR and ^{13}C NMR data is available at www.ias.ac.in/chemsci.

References

1. Xu H, Faber C, Uchiki T, Racca J and Dealwis C 2006 Structures of eukaryotic ribonucleotide reductase I define gemcitabine diphosphate binding and subunit assembly *PNAS* **103** 4028
2. Van der Donk W A, Yu G, Silva D J, Stubbe J, McCarthy J R, Jarvi E T, Matthews D P, Resvick R J and Wagner E 1996 Inactivation of ribonucleotide reductase by (E)-2'-Fluoromethylene-2'-deoxycytidine 5'-diphosphate: A paradigm for nucleotide mechanism-based inhibitors *Biochemistry* **35** 8381
3. Mayhew C N, Phillips J D, Greenberg R N, Birch N J, Elford H L and Gallicchio V S 1999 In vivo and in vitro comparison of the short-term hematopoietic toxicity between hydroxyurea and trimidox or didox, novel ribonucleotide reductase inhibitors with potential anti-hiv-1 activity *Stem Cells* **17** 345
4. Szekeres T, Fritzer-Szekeres M, Elford H L and Jayaram H M 1997 The enzyme ribonucleotide reductase: Target for antitumor and anti-HIV therapy *Crit. Rev. Clin. Lab. Sci.* **34** 503
5. Karp J E, Giles F J, Gojo I, Morris L, Greer J, Johnson B, Thein M, Sznol M and Low J 2008 A phase I study of the novel ribonucleotide reductase inhibitor 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, Triapine®) in combination with the nucleoside analog fludarabine for patients with refractory acute leukemias and aggressive myeloproliferative disorders *Leuk Res.* **32** 71
6. Xu H, Fairman J W, Wijerathna S R, Kreischer N R, Macchia J, Helmbrecht E, Cooperman B S and Dealwis C 2008 The structural basis for peptidomimetic inhibition of eukaryotic ribonucleotide reductase: A conformationally flexible pharmacophore *J. Med. Chem.* **51** 4653
7. Ren F, Zhong Y, Mai X, Liao Y J, Liu C, Feng L H, Sun W, Zen W B, Liu W M, Liu J and Jin N 2014 Synthesis and anticancer evaluation of benzyloxyurea derivatives *Chem. Pharm. Bull*. **62** 898
8. Moorthy N S H N, Cerqueira N M F S A, Ramos M J and Fernandes P A 2013 Development of ribonucleotide reductase inhibitor: A review on structure activity relationships *Mini Rev. Med. Chem.* **13** 1389
9. Hardjono S, Siswodihardjo S, Pramono P and Darmanto W 2016 Quantative structure cytotoxic activity relation 1-(Benzyoloxy)urea and its derivatives *Curr. Drug Discov. Technol*. **13** 101
10. Aversa C, Leone F, Zucchini G, Serini G, Geuna E, Milani A, Valdembri D, Martinello R and Montemurro F 2015 Linifanib: current status and future potential in cancer therapy *Exp. Rev. Anticancer Ther.* **15** 677
11. Wilhelm S, Carter C, Lynch M, Lowinger T, Dumas J, Smith R A, Schwartz B, Simantov R and Kelley S 2006 Discovery and development of sorafenib: A multikinase inhibitor for treating cancer *Nat. Rev. Drug Discov*. **5** 835
12. Kan R C, Farrell A T, Saber H, Tang S, Williams G, Jee J M, Liang C, Booth B, Chidambaram N, Morse D et al 2006 Sorafenib for the treatment of advanced renal cell carcinoma *Clin. Cancer Res*. **12** 7271
13. Keating G M and Santoro A 2009 Sorafenib *Drugs* **69** 223
14. Ferrari S M, Politti U, Spisni R, Materazzi G, Baldini E, Ulisse S, Miccoli P, Antonelli A and Fallahi P Sorafenib in the treatment of thyroid cancer *Exp. Rev. Anticancer Ther*. **15** 863
15. Capozzi M, De Divitiis C, Ottaiano A, von Arx C, Scala S, Tatangelo F, Delrio P and Tafuto S 2019 Lenvatinib, a molecule with versatile application: from preclinical evidence to future development in anti-cancer treatment *Cancer Manag. Res.* **11** 3847
16. Shimshomi J A, Bialer M, Wloderczyk B, Finnell R H and Yagen B 2007 Potent anticonvulsant urea derivatives of constitutional isomers of valproic acid *J. Med. Chem.* **50** 6419
17. Kumar M and Hosur M V 2003 Adaptability and flexibility of HIV-1 protease *Eur. J. Biochem*. **270** 1231
18. Zheng Q Z, Cheng K, Zhang X M, Liu K, Jiao Q C and Zhu H L 2010 Synthesis of some N-alkyl substituted urea derivatives as antibacterial and antifungal agents *Eur. J. Med. Chem.* **45** 3207
19. Watson R J, Daniel Allen R, Birch H L, Chapman G A, Galvin F C, Jopling L A, Knight R L, Meier D, Oliver K, Meissner J, David W G, Owen Elizabeth A, Thomas J, Tremayne N and Williams S C 2008 Development of CXCR3 antagonists. Part 3: Tropenyl and homotropenyl-piperidine urea derivatives. *Bioorg. Med. Chem. Lett.* **18** 147
20. Viana G M, Aguiara L C, Ferrao J A, Simas A B C and Vasconcelos M G 2013 The use of aqueous potassium dichloroiodate for the synthesis of ureas *Tetrahedron Lett*. **54** 936
21. Gewald K, Schinke E and Bottcher H 1966 Heterocyclen aus CH-aciden Nitrilen, VIII. 2-Amino-thiophene

aus methylenaktiven Nitrilen, Carbonylverbindungen und Schwefel *Chem. Ber.* **99** 94
22. Bozorov K, FeiNie L, Zhao J and Haji A 2017 2-Aminothiophene scaffolds: Diverse biological and pharmacological attributes in medicinal chemistry *Eur. J. Med. Chem.* **140** 465
23. Aly H M, Saleh N M and Elhady H A 2011 Design and synthesis of some new thiophene, thienopyrimidine and thienothiadiazine derivatives of antipyrine as potential antimicrobial agents *Eur. J. Med. Chem.* **46** 4566
24. Massari S, Nannetti G, Goracci L, Sancineto L, Muratore G, Sabatini S, Manfroni G, Mercorelli B, Cecchetti V, Facchini M., Palù G, Cruciani G, Loregian A and Tabarrini O 2013 Structural investigation of cycloheptathiophene-3-carboxamide derivatives targeting influenza virus polymerase assembly *J. Med. Chem*. **56** 10118
25. Lu X, Wan B, Franzblau S G and You Q 2011 Design, synthesis and anti-tubercular evaluation of new 2-acylated and 2-alkylated amino-5-(4-(benzyloxy)phenyl)thiophene-3-carboxylic acid derivatives *Eur. J. Med. Chem.* **46** 3551
26. Aguiar A C V, Moura R O, Mendonça-Junior J F B, Oliveira Rocha H A D, Gomes R B, Amara C and Schiavon M D S C 2016 Evaluation of the antiproliferative activity of 2-amino thiophene derivatives against human cancer cells lines *Biomed. Pharmacother.* **84** 403
27. Romagnoli R, Baraldi P G, Cruz-Lopez O, Tolomeo M, Cristina A D, Pipitone R M, Grimaudo S, Balzarini J, Brancale A and Hame E 2011 Synthesis of novel antimitotic agents based on 2-amino-3-aroyl-5(hetero)-arylethynyl thiophene derivatives *Bioorg. Med. Chem. Lett.* **21** 2746
28. Romagnoli R, Baraldi P G, Lopez-Cara C, Salvador M K, Preti D, Tabrizi M A, Balzarini J, Nussbaumer P, Bassetto M, Brancale A, Fu X-H, Yang G, Li J, Zhang S Z, Hamel E, Bortolozzi R, Basso G and Viola G 2014 Design, synthesis and biological evaluation of 3,5-disubstituted 2-amino thiophene derivatives as a novel class of antitumor agents *Bioorg. Med. Chem.* **22** 5097
29. Bozorov K, Zhao J Y, Fei Nie L, Ma H R, Bobakulov K, Hu R, Rustamova N, Huang G, Thomas E and Haji A A 2017 Synthesis and in vitro biological evaluation of novel diaminothiophene scaffolds as antitumor and anti-influenza virus agents *RSC Adv.* **7** 31417
30. Balzarini J, Thomas J, Liekens S, Noppen S, Dehaen W and Romagnoli R 2014 2-aminothiophene-3-carboxylic acid ester derivatives as novel highly selective cytostatic agents *Invest. New Drugs* **32** 200
31. Véras of Aguiar AC, of Moura RO, Bezerra Mendonça J F Junior, de Oliveira Rocha H A, Gomes Câmara R B and Dos Santos Carvalho Schiavon M 2016 Evaluation of the antiproliferative activity of 2-amino thiophene derivatives against human cancer cells lines *Biomed. Pharmacother.* **84** 403
32. Kaur R, Kaur P, Sharma S, Singh G, Mehndiratta S, Bedi P M S and Nepali K 2015 Anti-cancer pyrimidines in diverse scaffolds: a review of patent literature *Recent Pat. Anticancer Drug Discov.* **10** 23
33. Al-Issa S A 2013 Synthesis and anticancer activity of some fused pyrimidines and related heterocycles *Saudi Pharm. J.* **21** 305
34. Hackenberg F and Tacke M 2014 Benzyl-substituted metallocarbene antibiotics and anticancer drugs *Dalton Trans.* **43** 8144
35. Lebedev A V, Lebedeva A B, Sheludyakov V D et al 2006 Organosilicon synthesis of isocyanates: I. Synthesis of isocyanates of the furan, thiophene, and mono-and polyfluorophenyl series *Russ. J. Gen. Chem* **76** 110
36. Charalambides Y C and Moratti S C 2007 Comparison of base promoted and self catalyzed conditions in the synthesis of isocyanates from amines using triphosgene *Synth. Commun.* **37** 1037
37. Hempel J E, Cadar A G and Hong C C 2016 Development of thieno- and benzopyrimidinone inhibitors of the Hedgehog signaling pathway reveals PDE4-dependent and PDE4-independent mechanisms of action *Bioorg. Med. Chem. Lett.* **26** 1947