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RESEARCH ARTICLE

Microwave Assisted Synthesis, Molecular Docking and HIV-1 gp120 – CD4 Binding Inhibition Studies of Symmetrical *N, N'*-disubstituted Urea/Thiourea

SREE KANTH SIVAN, RADHIKA VANGALA and VIJJULATHA MANGA*

Molecular Modeling and Medicinal Chemistry Group, Department of Chemistry, University College of Science, Osmania University, Hyderabad-500007, India
vijjulathamanga@gmail.com

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Abstract: An effective and eco friendly procedure under microwave irradiation, for synthesis of symmetrical disubstituted alkyl and aryl ureas from amine hydrochlorides using water as solvent medium has been developed. Based on molecular docking studies on HIV-1 gp120, synthesized compounds were evaluated for HIV-1 gp120 – CD4 binding inhibition and these molecules showed micro molar range (0.047 μM to 4.07 μM) of inhibition.

Keywords: Disubstituted ureas, Amine hydrochlorides, Microwave irradiation, HIV-1 gp120, Docking

Introduction

Synthesis of substituted ureas and thioureas are important reactions in organic chemistry as they have important applications in agriculture, medicinal chemistry and chemical transformations[1-3]. Substituted ureas have been the subject of much attention owing to their biological activity as plant growth regulators, agro protective as well as tranquillizing and anticonvulsant agents[4]. The most important medicinal chemistry application of substituted urea is that they are the precursors for synthesis of anti HIV-1 compounds, substituted ureas with amino acid groups are reported as potent HIV-1 protease inhibitors[5,6]. Many reactions for synthesis of mono and disubstituted ureas have been extensively documented[7-9]. These commonly used methodologies are mainly based on usage of dangerous reagents such as isocyanates, phosgenes and triphosgenes[10,11]. Owing to the pressure for environmental protection and safety, cleaner synthetic routes have been developed as alternates for these toxic reagents. This includes solvent less synthesis using catalyst like triazabicyclodecene (TBD)[12], Pd-catalyzed oxidative carbonylation of amines[13].

Microwave irradiation methods have given an advantage for synthetic chemist for performing a cleaner and greener synthetic reaction. Mohammad. R. Saidi *et al.*,[14] reported synthesis of symmetrical disubstituted ureas by heating urea with aromatic amine under microwave irradiation without solvent. Mats Larhed *et al*.,[15] has reported a novel and very

fast gas-free carbonylation method for the preparation of ureas starting from primary amines. Under high intensity microwave heating with an in situ generation of intermediate isocyanates from $Co_2(CO)_8$ in presence of solvents like dimethylsulphoxide, tetrahydrofuran and acetonitrile. These microwave irradiation methods are exclusively used to synthesize either aryl or alkyl disubstituted ureas, using expensive reagents and solvents. We report here a novel microwave irradiated synthesis of symmetrical disubstituted alkyl and aryl ureas and thioureas, from amine hydrochlorides employing water as a solvent with no support of catalyst with in a time period of 3-5 minutes. Possible medicinal chemistry application of synthesized substituted urea were analyzed based on molecular docking studies on HIV-1 gp120.

Experimental

General procedure of preparation of disubstituted urea

An amine hydrochloride (2 mmol) was thoroughly grinded with urea or thiourea (1 mmol) in a borosil vessel, a paste of reaction mixture was made by adding few drops of water. Then the vessel was placed in synthetic microwave oven (Cata 2R) at 360W power until the mixture became dry. The maximum time taken was 5 minutes. The product was washed with water to remove any unreacted urea or amine hydrochloride. The solid was recrystallised from methanol-water (column purified where ever required), structures were confirmed from ^{1}H NMR and IR spectra.

Docking studies

X-ray crystal structure of HIV-1 gp120 envelope glycoprotein complexed with CD4 and induced neutralizing antibody 17b with 2.2 Å resolution was downloaded from RCSB Protein Data Bank (http:// www.rcsb.org/pdb/home/home.do) (pdb id: 1RZJ). GLIDE 5.6 was used for ligand preparation, protein preparation and induced fit docking. The neutralizing antibody and CD4 chains were deleted, except for the Ser42-Phe43-Leu44 chain that binds to the HIV-1 gp120 cavity. Protein was prepared using protein preparation module applying the default parameters; considering Ser42-Phe43-Leu44 as ligand moiety. A grid was generated around these residues of CD4 with receptor van der Waals scaling for non-polar atoms as 0.9. Ligands were built using Maestro build panel and prepared by LigPrep application in Schrödinger 2010 suite. Molecular docking of ligands into the generated grid was performed by using standard precision (SP) docking mode.

HIV-1 gp120–CD4 capture enzyme-linked immunosorbent assay (ELISA)

Commercially available CD4 Capture ELISA Kit was purchased from ImmunoDX, LLC. Several dilutions of positive reference CD4 (1000 ng/mL) in diluent buffer were prepared in Eppendorf tubes and labeled accordingly: 1000 ng/mL to 0.5 ng/mL in two fold serial dilutions. Test samples of compound to be analyzed were prepared using diluent buffer in 0.1–1000 ng/mL range. 100 µL of positive CD4 reference (1000 ng/mL)[22–24] was added into the wells of the 96-well plates, to this test samples of different dilution were added, few wells were left blank for standard reference with different concentrations of positive reference CD4. The plate was incubated at room temperature for 1 h. Contents of the wells were discarded and the wells were washed three times with wash buffer. To this 100 µL of Anti-CD4 Peroxidase/detector reagent, 1:100 in diluents buffer was added, and incubated at room temperature for 1 h. Plate was washed five times with 1x wash buffer (300 µL/well), contents of wells were discarded and 100 µL of TMB substrate was added to each well, a blue color was allowed to develop for a period of 10 min at room temperature, the development

of color was stopped by adding 50 µL of stop solution to each well, which resulted in change of color from blue to yellow. The absorption of contents of each well was read at 450 nm on Spectra Max M3 Multi-Mode Micro plate Reader from molecular devices within 15 min. Percent inhibition was calculated by using the following formula:

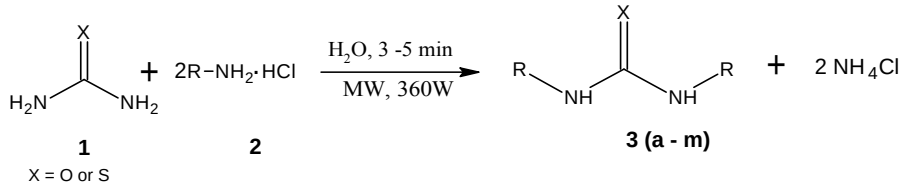
$$\% \text{ inh} = \frac{[(\text{Abs}^{450} \text{ CD4 reference (blank)}) - (\text{Abs}^{450} \text{ CD4 reference = test sample})]}{(\text{Abs450 CD4 reference (blank)})} \times 100$$

4 Parameter Logistic or 4PL nonlinear regression model was applied for calculating IC_{50} values using MasterPlex 2010 software.

Results and Discussion

Tenney and Blanchard reported a procedure for synthesis of symmetrical diphenylurea (carbanilide)[16], where aniline hydrochloride was boiled in water with urea for 1 to 2 hours to yield carbanilide. The reaction can be explained by the formation of an equilibrium mixture of urea and ammonium cyanate in boiling solution; the ammonium cyanate reacts at the moment of its formation with aniline hydrochloride, yielding phenylurea. Phenylurea also undergoes a secondary reaction, involving the intermediate formation of phenyl isocyanate, which reacts with aniline hydrochloride, forming carbanilide. Applying this procedure using different amine hydrochlorides yielded mono substituted ureas in many instance.

This encouraged us to apply microwave irradiation method to the reaction which should provide better yield with reduced time in an eco-friendly approach for synthesis of symmetrical *N*, *N*′ disubstituted ureas and thioureas.



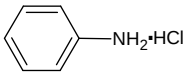
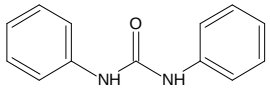
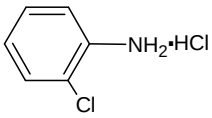
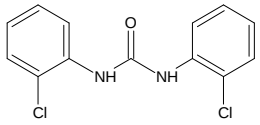
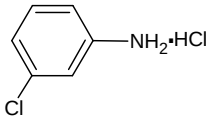
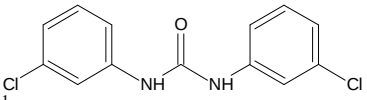
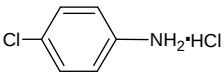
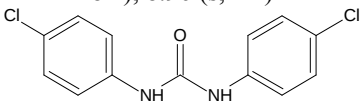
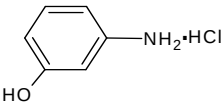
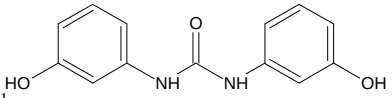
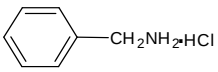
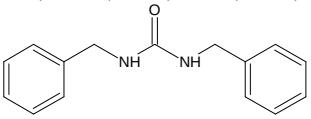
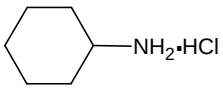
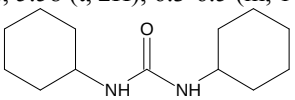
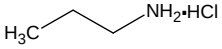
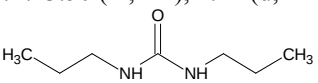
Scheme 1. Synthetic route for *N*, *N*′ symmetrical disubstituted urea/thiourea (**3a-m**)

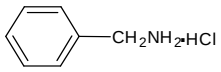
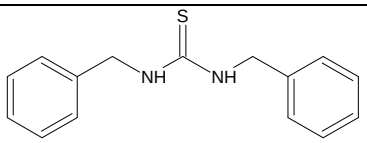
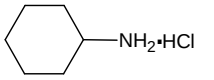
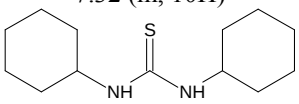
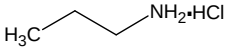
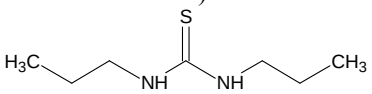
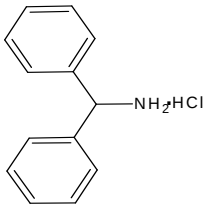
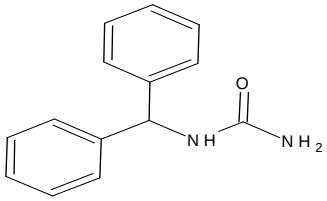
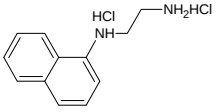
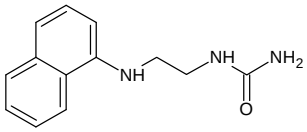
Reaction of an amine hydrochloride (2 mmol) with urea or thiourea (1 mmol) with a few drops of water as solvent in synthetic microwave oven (Cata 2R) at 360W power produced symmetrically disubstituted urea or thiourea in moderate to good yields (Scheme 1)[17]. The product is formed by loss of ammonium chloride after 3-5 minutes. Substituted ureas were easily recovered by adding water to the final reaction mixture, removing solid product by filtration and recrystallising the product from methanol-water. Yield and melting points of the synthesized compounds are given in Table 1. In comparison with reported conventional and microwave irradiation method, there is no need of any volatile organic compounds (VOC), toxic reagents, catalyst or solid support for the reaction.

Initially to begin with, aniline hydrochloride was reacted with urea in 2:1 ratio in presences of water under microwave radiation of 360W powers for 3 minutes, a gray coloured solid melting at 232 °C was obtained. A yield of 94% was obtained that was satisfactory enough to apply the scheme further on other substituted aniline hydrochlorides.

The yield for substituted aniline hydrochlorides ranged from 68% to 82%, an appreciable result, the scheme was further applied on primary aryl alkyl and alkylamine hydrochlorides; benzylamine hydrochloride was taken as the preliminary reactant that gave a yield of 96% as a white coloured solid.

Table 1. Amine hydrochlorides and products with their characterization and yields

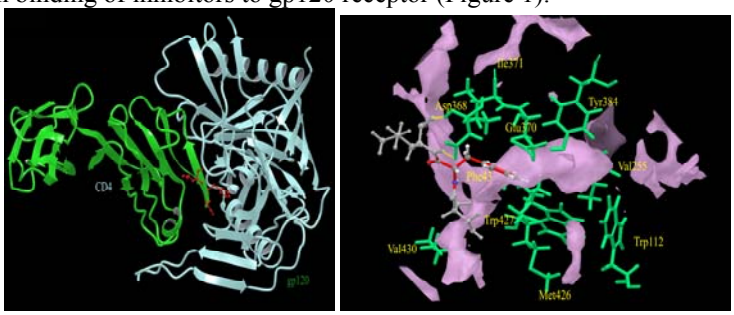
Entry	Amine hydrochloride	Product	Yield[a] %	Melting Point, °C
3a		 ^{1}H NMR (DMSO-D_6) δ = 6.4 – 7.0 (m, 10H), 8.2 (s, 2H)	94	230-232
3b		 ^{1}H NMR (DMSO-D_6) δ = 6.2(t, 2H), 6.4 (d, 2H), 6.8 (d, 2H), 7.4 (d, 2H), 8.4 (s, 2H)	72	236-238
3c		 ^{1}H NMR (DMSO-D_6) δ = 7.02(m, 8H), 8.96 (s, 2H)	78	242-244
3d		 ^{1}H NMR (DMSO-D_6) δ = 7.33(d, 4H), 7.47 (d, 4H), 8.87 (s, 2H)	68	254-258
3e		 ^{1}H NMR (DMSO-D_6) δ = 6.3-7.1(m, 8H), 8.3 (s, 2H), 9.0 (s, 2H)	82	218-220
3f		 ^{1}H NMR (DMSO-D_6) δ = 3.38(d, 4H), 5.58 (t, 2H), 6.3-6.5 (m, 10H)	96	146-148
3g		 ^{1}H NMR ($CDCl_3$) δ = 1.09-1.18(m, 8H), 1.31-1.41 (m, 6H), 1.93-1.97 (m, 6H), 3.47-3.50 (m, 2H), 4.12 (d, 2H)	38	188-190
3h		 ^{1}H NMR ($CDCl_3$) δ = 0.9 (t, 6H), 1.9 (m, 4H), 3.1 (m, 4H), 5.7 (d, 2H)	25	108-110

3i		 ^{1}H NMR (DMSO-D_6) decoupled δ = 4.62 (s, 4H), 6.07 (br, s, 2H), 7.23-7.32 (m, 10H)	48	148-150
3j		 ^{1}H NMR (CDCl3) δ = 1.219-1.276(m, 6H), 1.325-1.424 (m, 4H), 1.622-1.664 (m, 2H), 1.724-1.777 (m, 4H), 2.025-2.065 (m, 4H),3.854 (s, 2H), 5.594 (s, 2H)	42	156-158
3k		 ^{1}H NMR (CDCl3) δ = 0.972-1.009 (t,6H, *J1*=7.2 Hz, *J2*= 7.6Hz), 1.609-1.701(m, 4H), 3.39 (s, 4H), 5.8 (s, 2H)	35	62-64
3l		 ^{1}H NMR ($CDCl_3$) δ = 4.39 (s, 2H), 5.085 (d, 1H), 5.915 (d, 1H), 7.28-7.37 (m, 10H)	80	124-128
3m		 ^{1}H NMR ($CDCl_3$) δ = 4.39 (s, 2H), 5.085 (d, 1H), 5.915 (d, 1H), 7.28-7.37 (m, 10H)	60	138-142

[a]*Yields refer to isolated and chromatographically pure products*

The reaction with lower primary aliphatic amine hydrochlorides like propylamine and cyclohexylamine, gave a poor to moderate yield, methylamine and ethylamine hydrochlorides didn't yield disubstituted product. The reason for the poor reactivity of lower amines may be attributed towards their low boiling and decomposition points. Symmetrical *N*, *N*′- disubstituted thioureas were also synthesized following the same reaction conditions from the respective amine hydrochlorides and thiourea. Branched and bulky amine hydrochlorides where also used for synthesis that yielded mono substituted urea derivatives.

A computational protocol based on molecular docking was carried out to evaluate the possible medicinal chemistry application of synthesized substituted urea. All the synthesized symmetrical *N*, *N*' disubstituted aryl urea and thioureas were docked into CD4 binding site of HIV-1 gp120[18]. HIV-1 gp120 is a viral glycoprotein that makes a key interaction with host cell CD4 receptor[19]. This binding is the first step in virus entry and infection, inhibition of this binding is crucial step in antiviral therapy[20,21]. Phe43 of CD4 binding cavity in HIV-1 gp120 was chosen as binding site for computational simulations. The characteristic of this cavity is the presence of hydrophobic groups creating a hydrophobic cavity and plays a major role in binding of inhibitors to gp120 receptor (Figure 1).



(a) **(b)**

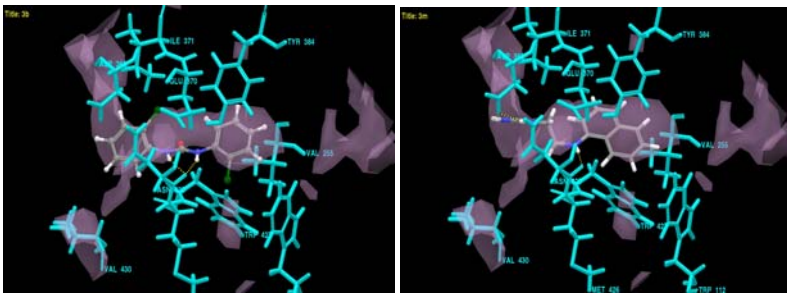
Figure 1. (a) Crystal structure of HIV-1 gp120 with soluble CD4 retrieved from the PDB (id:1RZJ), showing Phe43 of CD4 binding with HIV-1 gp120; (b) Focused view of Phe43 binding cavity of HIV-1 gp120, the hydrophobic region is represent in pink, Phe43 residue of CD4 is shown in red, it shows hydrogen bond interactions with Asp368 of HIV-1 gp120

A hydrogen bond interaction is seen with amino residue Trp427/Asp368. Based on these interactions it was summarized that molecules with hydrophobic groups that can be accommodated within the hydrophobic cavity and having some hydrogen bond donor groups that can interact with the hydrophilic region at the entry of the cavity comprising of residues Trp427, Asp368 would act as potential inhibitors. Substituted ureas have been reported as inhibitors of HIV-1 gp120 CD4 binding[17], it was a curious attempt to analyze the possible interaction of these small molecules with HIV-1 gp120.

All of the molecules showed hydrogen bond interaction with amino acid Asn 425, (Figure 2) the major contributory factor in binding is the hydrophobic interaction between the aromatic groups and the hydrophobic residues at the binding site. The binding affinity of these molecules encouraged us to check their *in vitro* activity against HIV-1 gp120. Synthesized symmetrical *N*, *N*' disubstituted aryl urea and thioureas were screened for their HIV-1 gp120 CD4 binding inhibition ability by HIV-1 gp120 CD4 capture Enzyme-linked immunosorbent assay (ELISA)[17]. All the screened compounds were found to inhibit HIV-1 gp120 CD4 binding in micro molar concentrations. Inhibitory activity (IC50) values are listed in Table 2. Based on the increase in hydrophobic character of 1,3-dibenzyl urea (**3f**) compared to 1,3-diphenyl urea (**3a**) there is an increase in activity by 0.3 μM.

In chloro substituted diphenyl urea molecules (**3b, 3c** and **3d**) showed ten folds decrease in activity from *ortho* to meta and *para* substitution respectively. *o*-Chloro diphenyl urea has two hydrogen bond interactions compared to one hydrogen bond interaction in case of *meta* and *para* chlorodiphenyl urea. Molecule **3e** has four fold reduced activity compared to **3a** due to the presence of hydrophilic hydroxyl substitution. Mono substituted benzhydril urea (**3l**) and *N*-1-Napthylethylene urea (**3m**) showed increase in activity due to bulky hydrophobic

groups and three hydrogen bond interactions. A correlation analysis between pIC_{50} of compounds and dockscore of molecules were performed that showed a significant r value of 0.679. Scatter plot of pIC_{50} and dockscore is shown in Figure 3.



(a) **(b)**

Figure 2. Dock pose of molecule (a) **3b** and (b) **3m** in the active site of HIV-1 gp120. The aromatic group is occupying the hydrophobic cavity. The molecule shows a hydrogen bond interaction with residue ASN 425 of HIV-1 gp120

Table 2. HIV-1 gp120 – CD4 binding inhibition (IC_{50} values), pIC_{50} and dockscore from SP docking for synthesized aryl substituted urea and thioureas

Compound	IC_{50} µM ± SD[a]	pIC50	Dockscore, kcal/mol
3a	0.966±0.04	6.015	-6.041
3b	0.047± 0.01	7.328	-7.807
3c	0.41±0.57	6.387	-5.787
3d	3.5±0.057	5.456	-5.743
3e	4.07±0.26	5.390	-5.738
3f	0.676±0.9	6.170	-5.876
3i	2.089±0.068	5.680	-5.969
3l	0.142±0.107	6.848	-5.641
3m	0.226±0.003	6.646	-6.764

[a]*values are mean of triplicate*

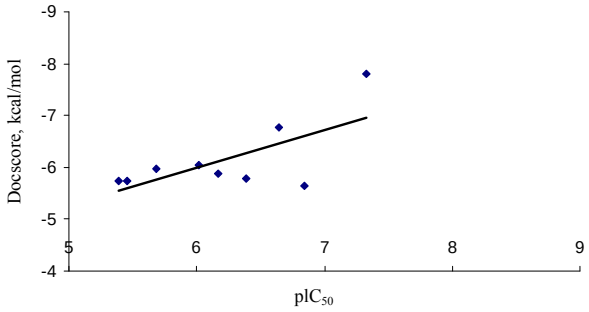


Figure 3. Scatter plot of dockscore (kcal/mol) versus biological activity in pIC_{50}

Conclusion

An effective and environmentally friendly methodology for the preparation of symmetric *N,N′* disubstituted urea that make use of microwave irradiation of amine hydrochloride with urea and water as solvent for affording a moderate to good yields in a VOC free aqueous

work-up. Plausible medicinal chemistry application of these substituted urea molecules were evaluated by molecular docking studies and the computational assumption was validated by HIV-1 gp120 – CD4 capture Enzyme-linked immunosorbent assay. These molecules showed micro molar range (0.047 μM to 4.07 μM) inhibition of HIV-1 gp120 – CD4 binding.

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