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Research Article

SYNTHESIS AND PHARMACOLOGICAL ACTIVITIES OF SOME NOVEL N,N′-DISUBSTITUTED UREA DERIVATIVES

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ABSTRACT

A series of N,N′-disubstituted urea derivatives (**1**-**10**) were synthesized and their structures were elucidated on the basis of analytical and spectral (IR, ^{1}H NMR) data. These synthesized compounds were assayed for their cytotoxicity against human breast cancer cell line (MCF-7) by using MTT assay with doxorubicin as a positive control. IC_{50} values for the tested compounds were higher than 50 µM except compound **8**. Compound **8** displayed moderate antiproliferative activity against MCF-7 cell line (with IC_{50} value of 41.27 ± 5.14 µM). All of the compounds were also evaluated for their antimicrobial effects against *Enterococcus hirae* (ATCC 10541), *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus aureus* (ATCC 6538) *and Candida albicans* (ATCC 10231). None of the compounds showed antimicrobial activity.

Keywords: Protein kinase, N,N′-disubstituted urea, cytotoxicity, anticancer, antimicrobial.

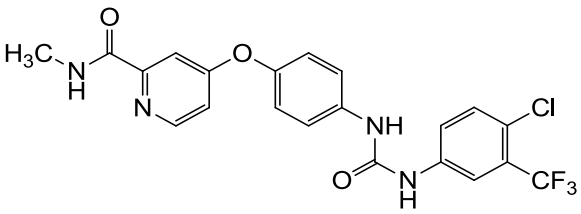
1. INTRODUCTION

Cancer is a major worldwide health problem. The current treatments for cancer include surgery, radiation therapy, chemotherapy, immunotherapy, targeted therapy, hormone therapy and stem cell transplant. Chemotherapy has been a cornerstone treatment among these therapies. Unfortunately, narrow therapeutic index and frequently acquired resistance to the current chemotherapeutics are the major reasons for the failure in cancer chemotherapy. Thereof, the development of more efficient and less toxic anticancer agents is still an ongoing area of interest[1,2].

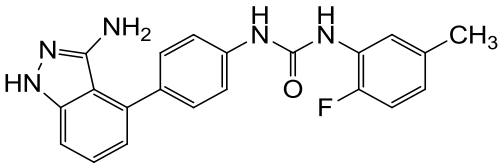
Protein kinase is an important enzyme family among various proteins and enzymes that plays significant role in processes such as cell growth, metabolism, apoptosis and inflammation. So, synthesizing molecules that inhibit protein kinases has been an attractive area in pharmaceutical industry since these proteins uncovered their detailed structure[3]. Imatinib (Gleevec®) is the first protein kinase inhibitor which entered clinical use in early 2000s [4]. Over a hundred protein kinase inhibitors have reached to advanced clinical trials over the last decade[4-7].

N,N′-disubstituted ureas are a class of protein-kinase inhibitors (**Figure 1**). Sorafenib

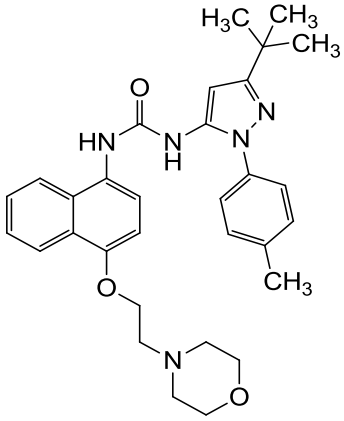
(Nexavar®) is one of the many structurally diverse multi-kinase inhibitors. It is approved by Food and Drug Administration (FDA) for hepatocellular carcinoma (hCC) and metastatic renal cell carcinoma (mRCC)[8-14]. Molecular docking results suggested that urea moiety of Sorafenib could form three critical hydrogen bonds with Asp-Phe-Gly (DFG) domain of VEGFR-2[15]. Linifanib (ABT-869), in clinical trials, is an another potent inhibitor of receptor tyrosine kinases (RTK), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF)[16]. Doramapimod (BIRB 796), N-pyrazole-N′-naphthly urea compound with strong p38 mitogen-activated protein kinase (MAPK) inhibitor activity, has been developed for mitigating rheumatoid arthritis and Crohn's disease (Figure 1)[17, 18].



Sorafenib



Linifanib (ABT-869)



Doramapimod (BIRB 796)

Fig. 1: Some protein kinase inhibitors possessing N,N′-disubstituted urea moiety

Protein kinase inhibitors such as sorafenib possess generally three main fragments; phenyl group (A), urea moiety (B) and big lipophilic heteroaromatic structure (C). It has been shown that the phenyl group is mainly responsible from kinase selectivity of sorafenib. The urea moiety of sorafenib contributes to the enzyme substrate interaction via H bonds. Heteroaromatic moiety interacts with kinase via hydrophobic interactions. Structural modification of C fragment of sorafenib has been targeted for the design of novel kinase inhibitors by many researchers[19-21] (**Figure 2**).

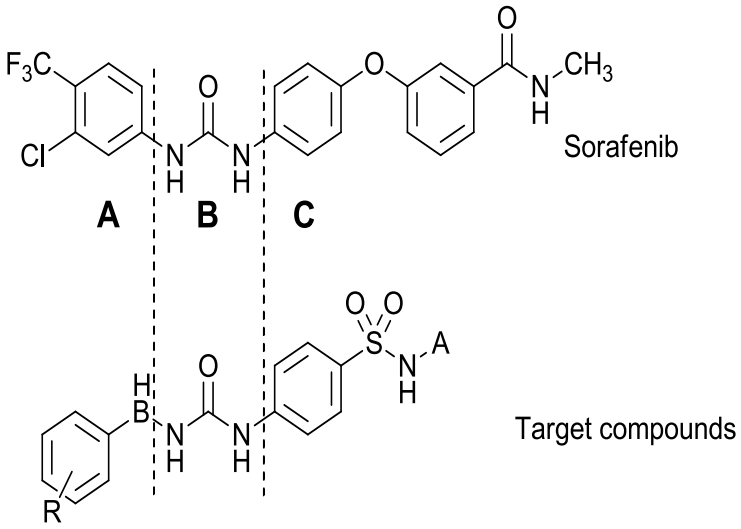


Fig. 2: Structure of sorafenib and target compounds of the present study

Sulfonamide derivatives which are known for their antibacterial activity also appeared as an important class of anticancer agents by interacting with different cellular targets[19,22,23]. In a study, in vitro inhibitory activities against A375, HepG2 and KETR3 cell lines of sulfonamide derivatives (I and II) were evaluated by MTT assay. IC_{50} values of these compounds were founded between 3.15 and 23.5 µM (**Figure 3)**[22]. It has been indicated that -SO_2-NH-Ar moieties of these molecules bind to the hinge region of kinase enzyme[19].

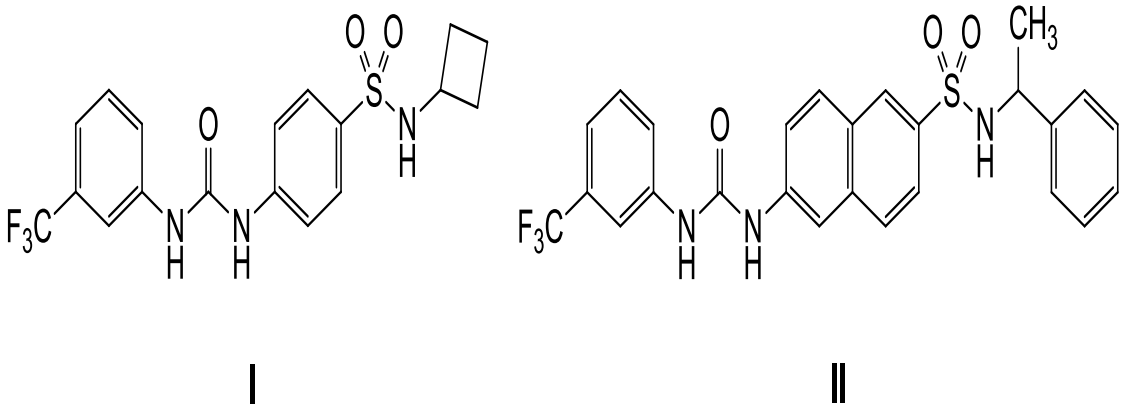
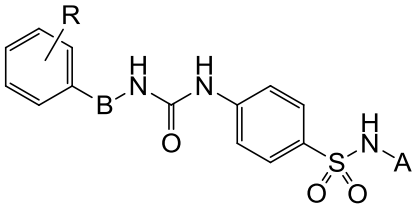


Fig. 3: Structure of the compounds I and II

In present study, a new series of N,N'-disubstituted urea derivatives having sulfonamide moiety were synthesized based on the structural features of sorafenib, with the aim of obtaining potent antitumor agents (**Table 1**).

Table 1: General formula of the target compounds 1-10s



Compound	A	B	R
1	thiazole	-	-H
2	acetyl	-	-H
3	benzoyl	-	-H
4	6-methoxy-3- pyridazinyl	-	-H
5	6-methoxy-3-pyridazinyl	$-CH_2$	-H
6	thiazole	-	2,5-dichloro
7	acetyl	-	2,5-dichloro
8	thiazole	$-CH_2$	-H
9	acetyl	$-CH_2$	-H
10	benzoyl	$-CH_2$	-H

2. EXPERIMENTAL

2.1. MATERIALS AND METHODS

All materials were commercially available and used without further purification. Sulfacetamide and sulfamethoxazole were purchased from Fluka. Sulfamethazine and sulfathiazole were purchased from Alpha Aesar GmbH & Co. Sulfabenzamide was purchased from Acros Organics. Phenyl isocyanate and benzyl isocyanate were purchased from Aldrich. Sulfamethoxypyridazine was purchased from Sigma. 2,6-Dichlorophenylisocyanate was purchased from Aldrich Chemistry. Melting points of the compounds were determined by an electrothermal melting point apparatus (Mettler Toledo FP62) in open capillary tubes and are uncorrected. Infrared (IR) spectra (10 T / cm^2 pressure applied potassium bromide discs) were recorded on a Perkin Elmer FT-IR 1720 spectrometer and the frequencies were expressed in cm^{-1}. ^1H NMR Spectra were obtained from 10% solution of the solution of the compounds in dimethlysulfoxide-d_6 by Bruker AC 400 MHz spectrometer. All chemical shift values were given in parts per million (ppm) relative to a tetramethlysilane reference.

2.2. Synthesis of N,N′-disubstituted urea derivatives (1-10)

The target compounds were prepared through refluxing 2.5 mmol of isocyanate derivative and 2 mmol of appropriate sulfonamide in 15 ml of THF (for compounds **1**, **2** and **3**) or stirring in DCM for 5-6 hours. Then, obtained crude products were filtered, dried and crystallized from ethanol.

2.2.1.N-Phenyl-N′-(4-(1,3-thiazole-2-yl)aminosulphonylphenyl)urea (1)

CAS Registry Number 349454-52-0

White crystals, yield 78%, mp >300 °C. FT-IR (KBr), cm^{-1}: 3378 (N-H, st), 3037 (C-H, aromatic, st), 1656 and 1519 (urea, st), 1317 and 1140 (RR′SO_2 [sulfone], st). ^1H NMR (DMSO-d_6 ppm): 12.6 (s, 1H, -SO_2N**H**-), 9.0 (s, 1H, -N**H**CO), 8.7 (s, 1H, -N**H**CO), 6.8-7.7 (m, 11 H, aromatic).

2.2.2.N-Phenyl-N′-(4-(acetyl)aminosulphonylphenyl)urea (2)

CAS Registry Number 356095-72-2

White crystals, yield 80%, mp: 227.1 °C. FT-IR (KBr), cm^{-1}: 3363 (N-H, st), 3130 (C-H, aromatic, st), 1686 (urea, st), 1315 and 1156 (RR′SO_2 [sulfone], st). ^1H NMR (DMSO-d_6 ppm): 11.9 (s, 1H, SO_2-**NH**), 9.2 (s, 1H, -**NH**CO), 8.8 (s, 1H, -**NH**CO), 6.9-7.8 (m, 10H, aromatic), 1.9 (s, 3H, C**H**$_3$CO).

2.2.3.N-Phenyl-N′-(4-(benzoyl)aminosulphonylphenyl)urea (3)

CAS Registry Number 176644-68-1

White crystals, yield 74%, mp: 179.2 °C. FT-IR (KBr), cm^{-1}: 3367 (N-H, st), 3269 (C-H, aromatic, st), 1684 and 1593 (urea, st), 1344 and 1160 (RR′SO_2 [sulfone], st). ^1H NMR

(DMSO-d_6 ppm): 12.1 (s, 1H, -SO_2NH), 9.2 (s, 1H, -NHCO), 8.8 (s, 1H, -NHCO), 6.2-7.9 (m, 14H, aromatic).

2.2.4.N-Phenyl-N′-(4-(6-methoxy-3-pyridazinyl)aminosulphonylphenyl)urea (4)

White crystals, yield 77%, mp: 231.6 °C. FT-IR (KBr), cm^{-1}: 3356 (N-H, st), 3308 (C-H, aromatic, st), 1630 and 1550 (urea, st), 1311 and 1139 (RR′SO_2 [sulfone], st), ^{1}H NMR (DMSO-d_6 ppm): 11.5 (s, 1H, SO_2-**NH**), 9.1 (s, 1H, -**NH**CO), 8.8 (s, 1H, -**NH**CO), 6.5-7.9 (m, 11H, aromatic), 4.1 (s, 3H, O**CH_3**).

2.2.5.N-Benzyl-N′-(4-(6-methoxy-3-pyridazinyl)aminosulphonylphenyl)urea (5)

White crystals, yield 72%, mp: 213.2 °C. FT-IR (KBr), cm^{-1}: 3333 (N-H, st), 3063 (C-H, aromatic, st), 1690 and 1593 (urea, st), 1395 and 1141 (RR′SO_2 [sulfone], st). ^{1}H NMR (DMSO-d_6 ppm): 9.0 (s, 1H, -SO_2**NH**), 9.2 (s, 1H, N**H**CO), 8.3 (s,1H,- CH_2**NHCO**), 6.8-7.7 (m, 11H, aromatic), 3.8 (s, 1H, -**OCH_3**), 4.1(d, 2H, C**H_2**-NH).

2.2.6.N-(2,6-dichlorophenyl)-N′-(4-(1,3-thiazole-2-yl)aminosulphonylphenyl)urea (6)

White crystals, yield 84%, mp: 213.7 °C. FT-IR (KBr), cm^{-1}: 3363 (N-H, st), 3112 (C-H, aromatic, st), 1679 and 1534 (urea, st), 1317 and 1148 (RR′SO_2 [sulfone], st). ^{1}H NMR (DMSO-d_6 ppm): 12.6 (s, 1H, -SO_2NH), 9.2 (s, 1H, -NHCO), 8.3 (s, 1H, -NHCO), 6.6-7.7 (m, 9H, aromatic).

2.2.7.N-(2,6-dichlorophenyl)-N′-(4-(acetyl)aminosulphonylphenyl)urea (7)

White crystals, yield 81%, mp: >300 °C. FT-IR (KBr), cm^{-1}: 3349 (N-H, st), 3184 (C-H, aromatic, st), 1658 and 1593 (urea, st), 1349 and 1157 (RR′SO_2 [sulfone], st). ^{1}H NMR (DMSO-d_6 ppm): 11.9 (s, 1H, SO_2-**NH**), 9.5 (s, 1H, -**NH**CO), 8.4 (s, 1H, -**NH**CO), 7.3-7.8 ppm (m, 7H, aromatic), 2.05 (s, 3H, CO**CH_3**)**.**

2.2.8.N-Benzyl-N′-(4-(1,3-thiazole-2-yl)aminosulphonylphenyl)urea (8)

White crystals, yield 71%, mp: >300 °C. FT-IR (KBr), cm^{-1}: 3312 (N-H, st), 3193 (C-H, aromatic, st), 1650 and 1521 (urea, st), 1313 and 1140 (RR′SO_2 [sulfone], st). ^{1}H NMR (DMSO-d_6 ppm): 12.6 (s, 1H, -SO_2**NH**), 9.0 (s, 1H, -**NH**CO), 6.9 (t, 1H, CH_2-**NH**), 4.2 (d, 2H, **-CH_2),** 6.4-7.7 (m, 11H, aromatic).

2.2.9.N-Benzyl-N′-(4-(acetyl)aminosulphonylphenyl)urea (9)

White crystals, yield 77%, mp: >300 °C. FT-IR (KBr), cm^{-1}: 3399 (N-H, st), 3061 (C-H, aromatic, st), 1677 and 1546 (urea, st), 1337 and 1160 (RR′SO_2 [sulfone], st). ^{1}H NMR (DMSO-d_6 ppm): 11.9 (s, 1H, SO_2-**NH**), 9.1 (s, 1H, -**NH**CO), 6.8 (t, 1H, CH_2-**NH**), 4.1 (d, 2H, **-CH_2)**, 6.4-7.7 (m, 9H, aromatic), 1.8 (s, 3H, **CH_3**).

2.2.10.N-Benzyl-N′-(4-(benzoyl)aminosulphonylphenyl)urea (10)

White crystals, yield 80%, mp: >300 °C. FT-IR (KBr), cm^{-1}: 3391 (N-H, st), 3063 (C-H, aromatic, st), 1666 and 1541 (urea, st), 1354 and 1165 (RR′SO_2 [sulfone], st). ^{1}H NMR (DMSO-d_6): 12.3 (s, 1H, SO_2-**NH**), 9.1 ppm (s, 1H, -**NH**CO), 6.8 (t, 1H, CH_2-**NH**), 4.2 (d, 2H, **-CH_2**NH), 6.4-7.8 (m, 14H, aromatic).

2.3. Biological activity procedures

2.3.1. Antiproliferative activity test procedure

Compounds **1**-**10** were assayed for their cytotoxicity against human breast cancer cell line (MCF-7, ATCC) by using the MTT assay with doxorubicin as a positive control. MCF-7 cells were grown in DMEM (Gibco, UK) supplemented with 10% FBS (Sigma-Aldrich Germany), 1% penicillin/strep (Gibco, UK) and 2 mM L-glutamine (Gibco, UK) and insulin (Sigma-Aldrich Germany).

Antiproliferative activities of the compounds were determined with using standard MTT colorimetric method. Briefly, cells were seeded at a density of 1×10^4 cells/well in 48-well microtiter plates. After 24 h, exponentially growing cells were exposed to the indicated compounds at final concentrations ranging from 1 to 100 µM. After 24 h, 100 µL of MTT (AppliChem, Germany) solution (1 mg/ml) were then added to each test well and the plates are further incubated for 2 h in the incubator at 37 °C. Then the MTT solution is discanted and 200 µL of isopropanol (Sigma-Aldrich, Germany) are added in each well and optical absorbance was measured at 570 nm (Microplate photometer, Multiskan Ascent, Finland) IC_{50} values were determined from replicates of 3 wells from three independent experiments.

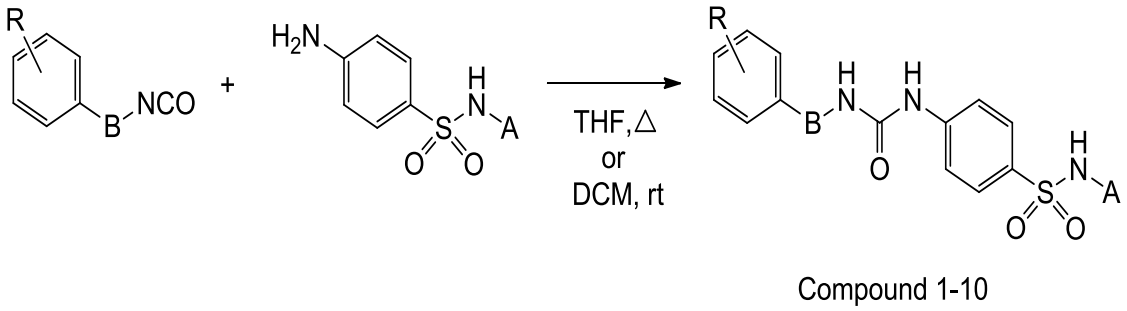
2.3.2. Antimicrobial Activity Test Procedure

General Disc Diffusion (Agar-Based) Method

Standard disc of Ofloxacin and Nystatin served as positive control and reference for antimicrobial activity. Filter discs impregnated with 10 µL of DMSO solvent were used as a negative control. MuellerHinton agar was used. Blank paper discs (Schleicher and Schuell, Spain) with a diameter of 8.0 mm were impregnated with 10 µL of the tested concentration of the stock solutions (1 mg / ml and 0.5 mg / ml in DMSO). When a filter paper disc impregnated with a tested chemical is placed on agar, the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area susceptible to the chemical around the disc. This area of no growth around the disc is the "zone of inhibition" or "clear zone". For disc diffusion, the zone diameters were measured with slipping calipers of the National Committee for Clinical Laboratory Standards (NCCLS)[24].

3. RESULTS AND DISCUSSION

The synthesis of N,N'-disubstituted urea derivatives was outlined in **Scheme**. Nucleophilic addition reactions of isocyanate derivatives to sulfonamide derivatives yield urea molecules. Reactions were carried out by refluxing the reactants in tetrahydrofurane (THF) or stirring in dichloromethane (DCM) at room temperature to give moderate yields of urea derivatives (70-80%). The chemical structures of the compounds were confirmed by IR, ^{1}H-NMR and results were presented in the experimental section.



Compound 1-10

A: thiazole-2-yl, acetyl, benzoyl, 6-methoxy-pyridazinyl.

B: -CH_2-, (-).

R: -Cl.

Scheme. General synthetic pathway of the target compounds 1-10

A series of novel N,N'-disubstituted urea derivatives bearing sulfonamide moiety (**1**-**10**) have been synthesized based on the structural features of sorafenib and their inhibitory activities were examined against human breast cancer cell line (MCF-7) by using MTT assay with doxorubicin as a positive control. The results are shown in **Table 2**. IC_{50} values of the tested compounds were higher than 50 µM except compound **8**. Compound **8** displayed the strongest antiproliferative activity against MCF-7 cell line (with IC_{50} value of 41.27 ± 5.14 µM). The results showed that N,N'-disubstituted urea derivatives bearing sulfonamide scaffold in the C fragment were not potent against MCF-7 cell line.

Table 2: IC_{50} values of the compounds 1-10 against MCF-7 cell line

Compound	IC_{50}	Compound	IC_{50}
1	> 50	**7**	> 50
2	> 50	**8**	**41.27 ± 5.14**
3	> 50	**9**	> 50
4	> 50	**10**	> 50
5	> 50	**Doxorubicin**	**0.72 ±0.06**
6	> 50		

Since sulfonamide derivatives known for their antibacterial activity, all compounds were also evaluated for their antimicrobial effects against *Enterococcus hirae* (ATCC 10541), *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus aureus* (ATCC 6538) *and Candida albicans* (ATCC 10231). None of the compounds showed antimicrobial activity.

Table 3: Zone of inhibition results of the compounds 1-10

Compound	Concentration of Test Compound (µM/µl)	Zone of Inhibition (diameter in mm)				
		E. hirae **ATCC 10541**	***E. coli*** **ATCC 11229**	***P. aureginosa*** **ATCC 15442**	***S. aureus*** **ATCC 6538**	***C. albicans*** **ATCC 10231**
Ofloxacin	5 µg	30	32	30	30	
Nystatin	100 units					28
1-10	10/5000	-	-	-	-	-

4. CONCLUSION

Synthesized compounds generally showed no in vitro antibacterial and cytotoxic activity except compound **8** that displayed low IC_{50} value 41.27 ± 5.14 µM against MCF-7 cell line. This result will lead us to synthesize more selective derivatives in our future studies.

5. ACKNOWLEDGEMENTS

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