Synthesis of *N*-Substituted Derivatives of *N*-(4-(*N*-(5-Chloro-2 methoxyphenyl)sulfamoyl)phenyl)acetamide with Potential Antiurease Activity

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Summary: In this study, a new series of *N*-alkyl/arakyl derivatives of *N*-(4-(*N*-(5-chloro-2-methoxyphenyl)sulfamoyl)phenyl)acetamide **(5a-k)** was synthesized and screened for enzyme inhibition activity. Target compounds, *N*-alkyl/arakyl sulfamoylacetamides (**5a-k**) were synthesized by stirring *N*-(4-(*N*-(5-chloro-2-methoxyphenyl)sulfamoyl)phenyl)acetamide (**3**) with different electrophiles (**4a-k**) in the presence of sodium hydride (NaH) and *N, N*-dimethylformamide (DMF). The structures of all the synthesized compounds have been deduced from their spectral (^{1}H-NMR, IR, EI-MS) data. All the new compounds displayed antiurease activity to varying degree.

Keywords: 2-amino-4-chloroanisol; 4-acetamidobenzenesulfonyl chloride; urease; ^{1}H-NMR; IR; EI-MS.

Introduction

Sulfonamides have been used as synthetic antibiotics in early times and are reported as first extensive antibacterial agents. They have important role in drug industry and act as anticonvulsant, anticancer, antifungal, antithyroid, antiviral, hypoglycemic, antiglaucoma and enzyme inhibitiors. Aryl and hetroaryl sulfonamides show promising inhibition against tumor [1-6]. They are involved in veterinary drugs and treat many diseases in animals. Sulfonamides also play vital part in animal husbandry. They are well absorbed from gastro-intestinal tract and metabolized in liver to inactive metabolites. They are excreted into bile, urine and feces. Sulfonamides have wide spectrum of antimicrobial activity and their mode of action is bacteriostatic [7-9].

Urease (urea amidohydrolase, EC 3.5.1.5) plays a vital role in many pathogenic processes in humans as well as in animals. It participate major role in kidney stone, peptic ulceration and pyelonephritis. Its role is also very promising in urinary catheter incrustation, urolithiasis and hepatic encephalopathy [10-13]. Therefore, the discovery of potent and safe urease inhibitors has been a very important area of pharmaceutical research. The present research work is an effort to synthesize new *N*-alkyl/aralkyl substituted sulfamoylacetamides with urease inhibitory activity.

Results and Discussion

New derivatives of *N*-alkyl/arakyl of *N*-(4-(*N*-(5-chloro-2-methoxyphenyl)sulfamoyl)phenyl) acetamide have been prepared (Scheme-1) with potential antiurease activity. The structures of all these compounds were characterized by spectral data. Parent compound *N*-(4-(*N*-(5-chloro-2-methoxy-phenyl)sulfamoyl)phenyl) acetamide (**3**) was prepared by the reported methods [14,15]. Further, *N*-alkyl/arakyl sulfamoylacetamides (**5a-k)** were synthesized by reacting the parent compound *N*-(4-(*N*-(5-chloro-2-methoxyphenyl) sulfamoyl)phenyl) acetamide (**3**) with the corresponding alkyl/aralkyl halides in the presence of DMF and NaH which acts as a base. The time of completion of alkylation reaction varies from 3 to 12 h. The precipitations were obtained by adding the cold water in the reaction mass, filtered off the solid and washed with distilled water. The parent compound **(3)** was obtained as a gray powder having melting point 195-200ºC. The molecular formula $C_{15}H_{15}ClN_2O_4S$ was established by EI-MS and counting the number of protons in the ^{1}H-NMR spectrum showing molecular ion peak at *m/z* 354 while the base peak appeared at *m/z* 141 corresponding to 4-chloroanisole cation fragment. The IR spectrum showed absorption bands at 3018 cm^{-1}, 1529 cm^{-1} and 1323 cm^{-1} which were assigned to, C-H (aromatic stretching), C=C (stretching of aromatic ring) and -SO_2 (stretching of sulfonyl group), respectively. In the aromatic region of the ^{1}H-NMR spectrum, signals appeared at δ 7.73 (d, J = 8.4 Hz, 2H, H-2', H-6') and 7.56 (d, J = 8.4 Hz, 2H, H-3',H-5'), due to downfield shifting and higher coupling constant showed that it is a di-substituted 4-acetamidobenzenesulfonyl ring and the

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signals resonated at δ 7.51 (d, J = 2.4 Hz, 1H, H-6), 6.97 (dd, J = 8.4, 2.4 Hz, 1H, H-4) and 6.64 (d, J = 8.4 Hz, 1H, H-3), which were assigned to the tri-substituted 2-amino-4-chloroanisole ring. In the aliphatic region of the ^{1}H-NMR spectrum, the two singlet signal appeared at δ 3.65 (s, 3H, -O$\underline{CH_3}$) and 2.17 (s, 3H, -CO$\underline{CH_3}$) which corresponds to methoxy group and the methyl of acetyl group, respectively. On the basis of above mentioned cumulative evidences, the structure of **3** was assigned as *N*-(4-(*N*-(5-chloro-2-methoxyphenyl)sulfamoyl)phenyl) acetamide. Similarly, the structures of other compounds were characterized by ^{1}H-NMR, IR and mass spectral data as described in experimental section.

As far as the biological activity is concerned, all the compounds were screened against urease enzyme and data is given in Table-1. All compounds showed varying degree of enzyme inhibition. Compounds **5e, 3** and **5a** were found to be good enzyme inhibitors with IC_{50} values of 62.36±0.19, 73.31±0.12 and 74.4±0.16 µmoles/L, respectively, relative to standard thiourea, IC_{50} value of 21.28±0.11 µmoles/L. The outstanding activity of these compounds is probably due to *N*-substitution of electrophiles in these molecules containing acetamide groups. However, only few compounds showed weak inhibition activity.

Table-1: Bioactivity studies of *N*-substituted derivatives of *N*-(4-(*N*-(5-chloro-2-methoxyphenyl) sulfamoyl)phenyl)acetamide.

Compound	Conc.(mM)	Urease Inhibition (%)	IC_{50}(µM)
3	**0.5**	**82.11±0.18**	**73.31±0.12**
5a	**0.5**	**78.97±0.63**	**74.4±0.16**
5b	**0.5**	**77.55±0.46**	**96.21±0.15**
5c	**0.5**	**81.98±0.42**	**84.55±0.11**
5d	**0.5**	**78.51±0.59**	**96.92±0.32**
5e	**0.5**	**82.20±0.69**	**62.36±0.19**
5f	**0.5**	**51.84±0.34**	**>300**
5g	**0.5**	**65.40±0.32**	**285.06±0.19**
5h	**0.5**	**63.43±0.51**	**293.06±0.11**
5i	**0.5**	**67.24±0.52**	**271.09±0.15**
5j	**0.5**	**77.42±0.59**	**115.09±0.21**
5k	**0.5**	**78.28±0.59**	**126.02±0.16**
Control	**Thiourea**	**98.92±0.15**	**21.28±0.11**

Note: IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

Experimental

General

Purity of all synthesized compounds was tested through thin layer chromatography (TLC) on plates coated with silica gel-G-25-UV_{254} under different solvent systems using ethyl acetate and *n*-hexane. Compounds were assigned as pure after appearing single spot through ultraviolet light. Melting points were determined with a Griffin-George melting point apparatus through open capillary tube method and are uncorrected. The I.R. spectra were registered on a Jasco-320-A spectrophotometer with potassium bromide (KBr) pellet technique (wave number in cm^{-1}). Nuclear magnetic resonance spectra were obtained on a Bruker spectrometers operating at 400 MHz, while $CDCl_3$ was used as solvent and chemical shifts were quoted in δ (ppm) units. Mass analysis (EIMS) was performed on JMS-HX-110-spectrometer through appropriate data system. 2-Amino-4-chloroanisol, 4-acetamidobenzenesulfonyl chloride and alkyl halides were purchased from Sigma Aldrich and Alfa Aesar. All the used solvents were of analytical grade.

Synthesis of N-(4-(N-(5-Chloro-2-methoxyphenyl) sulfamoyl)phenyl)acetamide **(3)**

Compound **3** was prepared according to the reported method [14-16].

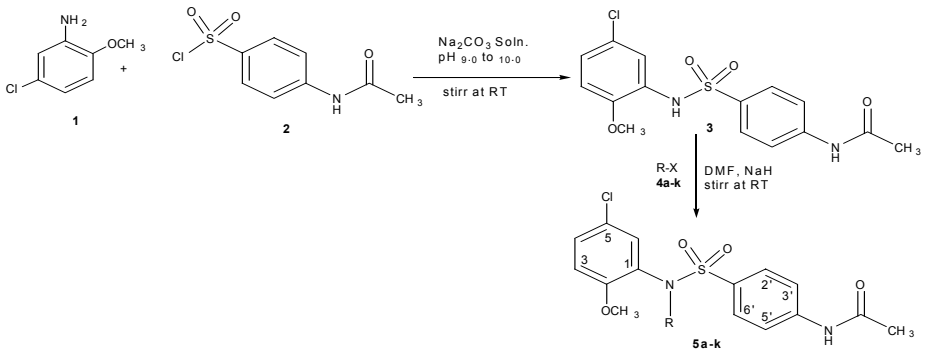
General Procedure for the Synthesis of Compounds **(5a-k).**

Compound (**3**) (0.56 mol, 0.20 g) was taken in round bottom flask and *N*,*N*-dimethyl formamide (5 ml) and sodium hydride (0.01g, 0.42 mmol) were added in it at room temperature. The reaction mixture was stirred for 15 min and then the corresponding electrophiles (**4a-k**, 0.56 mol) were added into the mixture. The reaction mass was further stirred and monitoring through TLC. After completion of the reaction, the reaction mixture was quenched with cold water (200 ml). The received solid was filtered, washed with water and dried to yield the corresponding *N*-substituted derivatives of *N*-(4-(*N*-(5-chloro-2-methoxyphenyl) sulfamoyl)phenyl) acetamide (**5a-k**).

Characterization of the Synthesized Compounds

N-(4-(N-(5-Chloro-2-methoxyphenyl)sulfamoyl) phenyl)acetamide **3**

Grey Powder, Yield 91%, m.p. 195-200ºC. Molecular Formula: $C_{15}H_{15}ClN_2O_4S$; Mol. Weight: 354 g. IR (KBr, cm^{-1}) ν_{max}: 3018 (C-H stretching of aromatic ring), 1529 (C=C stretching of aromatic ring), 1323 (-SO_2 stretching).^{1}H-NMR (400MHz, $CDCl_3$, δ/ppm): 7.73 (d, J = 8.4 Hz, 2H, H-2', H-6'), 7.56 (d, J = 8.4 Hz, 2H, H-3', H-5'), 7.51 (d, J = 2.4Hz, 1H, H-6), 6.97 (dd, J = 8.4, 2.4 Hz, 1H, H-4), 6.64 (d, J = 8.4 Hz, 1H, H-3), 3.65 (s, 3H, -OCH_3), 2.17 (s, 3H, -CO$\underline{CH_3}$). EIMS: *m/z* 354 $[M]^+$, 156 (64%), 141 (100%), 58 (10%); Anal. calcd for $C_{15}H_{15}ClN_2O_4S$: C 50.78, H 4.26, N 7.90, S 9.04; found C 50.69, H 4.32, N 7.85, S 9.11.



Compound	R	Compound	R
5a	$-CH_2-CH_3$ (1" 2")	**5g**	5" 1" 7" CH_2 3" Cl
5b	H, H_a, C=C, $-CH_2$ 2" 3" H_b 1"	**5h**	Cl 5" 1" 7" CH_2 3"
5c	2" CH_3, $-CH$ 1", CH_3 3"	**5i**	F 5" 1" 7" CH_2 3"
5d	5" 1" 7" CH_2-CH_2 8" 3"	**5j**	5" 1" 7" CH_2 3" CH_3
5e	Br 5" 1" 7" CH_2 3"	**5k**	$CH_3-CH_2-O-C(=O)-CH_2$ 1" 2"
5f	5" 1" 7" $CH_2-CH_2-CH_2$ 8" 9" 3"		

Scheme-1: Synthetic scheme of *N*-substituted derivatives of *N*-(4-(*N*-(5-chloro-2-methoxyphenyl) sulfamoyl)phenyl)acetamide.

N-(4-(N-(5-chloro-2-methoxyphenyl)-N-ethylsulfamoyl)phenyl)acetamide **5a**

Shiny white powder, Yield 74%, m.p. 98-100ºC. Mol. formula: $C_{17}H_{19}ClN_2O_4S$; Mol. Weight: 382 g. IR (KBr, cm^{-1})ν_{max}: 3018 (C-H stretching of aromatic ring), 1529 (C=C stretching of aromatic ring), 1324 (-SO_2- stretching). ^{1}H-NMR (400MHz, $CDCl_3$, δ/ppm): 7.71 (d, J = 8.4 Hz, 2H, H-2', H-6') 7.54 (d, J = 8.4 Hz, 2H, H-3', H-5'), 7.52 (d, J = 2.4Hz, 1H, H-6), 6.97 (dd, J = 8.4, 2.4 Hz, 1H, H-4), 6.64 (d, J = 8.4 Hz, 1H, H-3), 3.68 (s, 3H, -OCH_3), 3.58 (q, J = 6.8 Hz, 2H, H-1"), 2.12 (s, 3H, -$COCH_3$), 1.04 (t, J = 7.2 Hz, 3H, H-2"). EIMS: *m/z* 382 $[M]^+$, 156 (32%), 141 (100%), 58 (13%), 29 (31%); Anal. calcd for $C_{17}H_{19}ClN_2O_4S$: C 53.33, H 5.00, N 7.32, S 8.38; found C 53.29, H 5.06, N 7.36, S 8.41.

N-(4-(N-allyl-N-(5-chloro-2-methoxyphenyl) sulfamoyl)phenyl)acetamide **5b**

Pink powder, Yield 89%, m.p. 75-80ºC, Molecular formula: $C_{18}H_{19}ClN_2O_4S$; Mol. Weight: 394 g. IR (KBr, cm^{-1})ν_{max}: 3015 (C-H stretching of aromatic ring), 1525 (C=C stretching of aromatic ring), 1327 (-SO_2 stretching). ^{1}H-NMR (400MHz, $CDCl_3$, δ/ppm): 7.70 (d, J = 8.4 Hz, 2H, H-2', H-6'), 7.57 (d, J = 8.4 Hz, 2H, H-3', H-5'), 7.52 (d, J = 2.4 Hz, 1H, H-6), 6.97 (dd, J = 8.4, 2.4 Hz, 1H, H-4), 6.64 (d, J = 8.4 Hz, 1H, H-3), 5.73 (m, 1H, H-2"), 5.04 (dd, J = 11.6, 1.2 Hz, 1H, Ha -3"), 5.00 (dd, J = 6.2, 1.2 Hz, 1H, Hb -3"), 4.14 (d, J = 7.2 Hz, 2H, H-1"), 3.64 (s, 3H, -OCH_3), 2.16 (s, 3H, -$COCH_3$). EIMS: *m/z* 394 $[M]^+$, 156 (64%), 141 (100%), 58 (10%), 41 (33%); Anal. calcd for $C_{18}H_{19}ClN_2O_4S$: C 54.75, H 4.85, N 7.09, S 8.12; found C 54.62, H 4.92, N 7.14, S 8.15.

N-(4-(N-(5-chloro-2-methoxyphenyl)-N-isopropylsulfamoyl)phenyl)acetamide **5c**

Light pink powder, Yield 71%, m.p. 135-140ºC. Molecular formula: $C_{18}H_{21}ClN_2O_4S$; Mol. Weight: 396 g. IR (KBr, cm^{-1})ν_{max}: 3018 (C-H stretching of aromatic ring), 1529 (C=C stretching of aromatic ring), 1323 (-SO_2 stretching). ^{1}H-NMR

(400MHz, $CDCl_3$, δ/ppm): 7.72 (d, J = 8.4 Hz, 2H, H-2', H-6'), 7.59 (d, J = *8.4* Hz, 2H, H-3', H-5'), 7.53 (d, J = 2.4Hz, 1H, H-6), 6.97 (dd, J = 8.4, 2.4 Hz, 1H, H-4), 6.64 (d, J = 8.4 Hz, 1H, H-3), 4.34 (m, 1H, H-1''), 3.68 (s, 3H, -OCH_3), 2.19 (s, 3H, -$COCH_3$), 1.52 (d, J = 6.5 Hz, 6H, H-2'', H-3''). EIMS: *m/z* 396 $[M]^+$, 156 (51%), 141 (100%), 58 (12%), 43 (15%); Anal. calcd for $C_{18}H_{21}ClN_2O_4S$: C 54.47, H 5.33, N 7.06, S 8.08; found C 54.52, H 5.29, N 7.14, S 8.17.

N-(4-(N-(5-chloro-2-methoxyphenyl)-N-phenethylsulfamoyl)phenyl)acetamide **5d**

Dark brown powder, Yield 77%, m.p. 98-100ºC. Molecular formula: $C_{23}H_{23}ClN_2O_4S$; Mol. Weight: 458 g. IR (KBr, cm^{-1})ν_{max}: 3021 (C-H stretching of aromatic ring), 1529 (C=C stretching of aromatic ring), 1324 (-SO_2 stretching). ^{1}H-NMR (400MHz, $CDCl_3$, δ/ppm): 7.73 (d, J =8.4 Hz, 2H, H-2', H-6'), 7.56 (d, J =8.4 Hz, 2H, H-3', H-5'), 7.51 (d, J = 2.4Hz, 1H, H-6), 7.16-7.10 (m, 5H, H 2'' to 6''), 6.97 (dd, J = 8.4, 2.4 Hz, 1H, H-4), 6.64 (d, J = 8.4 Hz, 1H, H-3), 4.09 (t, J = 7.6 Hz, 2H, H-8''), 3.63 (s, 3H, -OCH_3), 2.79 (t, J = 7.6 Hz, 4H, H-7''), 2.13 (s, 3H, -$COCH_3$). EIMS: *m/z* 458 $[M]^+$, 156 (33%), 141 (100%), 105 (13%), 91 (16%), 58 (41%); Anal. calcd for $C_{23}H_{23}ClN_2O_4S$: C 60.19, H 5.05, N 6.10, S 6.99; found C 60.12, H 5.11, N 6.21, S 7.06.

N-(4-(N-(4-bromobenzyl)-N-(5-chloro-2-methoxyphenyl)sulfamoyl)phenyl)acetamide **5e**

Light brown powder, Yield 75%, m.p. 76-78ºC. Molecular formula: $C_{22}H_{20}BrClN_2O_4S$; Mol. Weight: 523 g. IR (KBr, cm^{-1}) ν_{max}: 3011 (C-H stretching of aromatic ring), 1525 (C=C stretching of aromatic ring), 1321 (-SO_2 stretching). ^{1}H-NMR (400MHz, $CDCl_3$, δ/ppm): 7.75 (d, J = 8.4 Hz, 2H, H-2', H-6'), 7.56 (d, J = 8.4 Hz, 2H, H-3', H-5'), 7.51 (d, J =2.4 Hz, 1H, H-6), 7.08 (d, J = 8.4 Hz, 2H, H-2'', H-6''), 7.05 (d, J = 8.2 Hz, 2H, H-3'', H-5''), 6.97 (dd, J = 8.4, 2.4 Hz, 1H, H-4), 6.64 (d, J = 8.4 Hz, 1H, H-3), 4.65 (s, 2H, H-7''), 3.66 (s, 3H, -OCH_3), 2.17 (s, 3H, -$COCH_3$). EIMS: *m/z* 523 $[M]^+$, 184 (10%), 156 (25%), 141 (100%), 90 (17%),70 (35%); Anal. calcd for $C_{22}H_{20}BrClN_2O_4S$: C 50.44, H 3.85, N 5.35, S 6.12; found C 50.32, H 3.92, N 5.31, S 6.23.

N-(4-(N-(5-chloro-2-methoxyphenyl)-N-(3-phenylpropyl)sulfamoyl)phenyl)acetamide **5f**

Brown sticky solid, Yield 75%, Molecular formula: $C_{24}H_{25}ClN_2O_4S$; Mol. Weight: 472 g. IR (KBr, cm^{-1})ν_{max}: 3018 (C-H stretching of aromatic ring), 1526 (C=C stretching of aromatic ring), 1326 (-SO_2 stretching), ^{1}H-NMR (400MHz, $CDCl_3$, δ/ppm): 7.77 (d, J = *8*.4 Hz, 2H, H-2', H-6'), 7.54 (d, J = 8.4 Hz, 2H, H-3', H-5'), 7.50 (d, J = 2.4Hz, 1H, H-6), 7.16-7.12 (m, 5H, H-2'' to 6''), 6.97 (dd, J = 8.4, 2.4 Hz, 1H, H-4), 6.64 (d, J = 8.4 Hz, 1H, H-3), 3.76 (t, J = 7.0 Hz, 2H, H-9''), 3.69 (s, 3H, -OCH_3), 3.55 (t, J = 7.1 Hz, 2H, H-7''), 2.63(m, 2H, H-2''), 2.10 (s, 3H, -$COCH_3$). EIMS: *m/z* 472 $[M]^+$, 156 (44%), 141 (100%), 119 (21%), 91 (19%), 70 (13%); Anal. calcd for $C_{24}H_{25}ClN_2O_4S$: C 60.94, H 5.33, N 5.92, S 6.78; found C 60.84, H 5.38, N 6.02, S 6.86.

*N-(4-(N-(5-chloro-2-methoxyphenyl)-N-(2-chlorobenzyl)sulfamoyl)phenyl)acetamide **5g***

Off white powder, Yield 59%, m.p. 58-60ºC. Molecular formula: $C_{22}H_{20}Cl_2N_2O_4S$; Mol. Weight: 479 g. IR (KBr, cm^{-1})ν_{max}: 3020 (C-H stretching of aromatic ring), 1527 (C=C stretching of aromatic ring), 1323 (-SO_2 stretching), ^{1}H-NMR (400MHz, $CDCl_3$, δ/ppm): 7.72 (d, J = 8.4 Hz, 2H, H-2', H-6'), 7.66 (d, J =8.4 Hz, 2H, H-3''), 7.61 (d, J =8.4 Hz, 2H, H-6''), 7.58 (d, J = 8.4 Hz, 2H, H-3', H-5'), 7.53 (d, J =2.4Hz, 1H, H-6), 6.97 (dd, J = 8.4, 2.4 Hz, 1H, H-4), 6.64 (d, J = 8.4 Hz, 1H, H-3), 6.63 (m, 2H, H-4'' & H-5''), 4.87(s, 2H, H-7''), 3.65 (s, 3H, -OCH_3), 2.12 (s, 3H, -$COCH_3$). EIMS: *m/z* 479 $[M]^+$, 156 (25%), 141 (100%), 124 (18%), 91 (25%), 86 (11%), 58 (46%); Anal. calcd for $C_{22}H_{20}Cl_2N_2O_4S$: C 55.12, H 4.21, N 5.84, S 6.69; found C 55.06, H 4.26, N 5.92, S 6.75.

N-(4-(N-(5-chloro-2-methoxyphenyl)-N-(4-chlorobenzyl)sulfamoyl)phenyl)acetamide **5h**

Gray powder, Yield 89%, m.p. 158-160, Molecular formula: $C_{22}H_{20}Cl_2N_2O_4S$; Mol. Weight: 479 g. IR (KBr, cm^{-1})ν_{max}:3016 (C-H stretching of aromatic ring), 1527 (C=C stretching of aromatic ring), 1322 (-SO_2 stretching. ^{1}H-NMR (400MHz, $CDCl_3$, δ/ppm): 7.69 (d, J = 8.4 Hz, 2H, H-2', H-6'), 7.57 (d, J = 8.4 Hz, 2H, H-3', H-5'), 7.50 (d, J = 2.4 Hz, 1H, H-6), 7.19 (d, J = 8.0 Hz, 2H, H-3'' & H-6''), 7.13 (d, J = 8.0 Hz, 2H, H-2'' & H-6''), 6.97 (dd, J = 8.4, 2.4 Hz, 1H, H-4), 6.64 (d, J = 8.4 Hz, 1H, H-3), 4.67 (s, 2H, H-7''), 3.60 (s, 3H, -OCH_3), 2.15 (s, 3H, -$COCH_3$). EIMS: *m/z* 479 $[M]^+$, 156 (21%), 141 (100%), 91 (45%), 85 (14%), 58 (30%); Anal. calcd for $C_{22}H_{20}Cl_2N_2O_4S$: C 55.06, H 4.12, N 5.49, S 6.35; found C 55.02, H 4.22, N 5.56, S 6.28.

N-(4-(N-(5-chloro-2-methoxyphenyl)-N-(4-fluorobenzyl)sulfamoyl)phenyl)acetamide **5i**

Dark gray sticky solid, Yield 76%, Molecular formula: $C_{22}H_{20}ClFN_2O_4S$; Mol. Weight:

462 g. IR (KBr, cm^{-1})ν_{max}: 3016 (C-H stretching of aromatic ring), 1527 (C=C stretching of aromatic ring), 1321 (-SO_2 stretching). ^{1}H-NMR (400MHz, $CDCl_3$, δ/ppm): 7.72 (d, *J* = 8.4 Hz, 2H, H-2', H-6'), 7.55 (d, *J* = 8.4 Hz, 2H, H-3', H-5'), 7.55 (d, *J* = 2.4Hz, 1H, H-6), 6.97 (dd, *J* = 8.4, 2.4 Hz, 1H, H-4), 6.91 (d, *J* = 8.4 Hz, 2H, H-3" & H-5"), 6.89 (d, *J* = 8.4 Hz, 2H, H-2" & H-6"), 6.64 (d, *J* = 8.4 Hz, 1H, H-3), 4.66 (s, 2H, H-7"), 3.67 (s, 3H, -OCH_3), 2.19 (s, 3H, -COC<u>H</u>$_3$). EIMS: *m/z* 462 $[M]^+$, 156 (33%), 141 (100%), 109 (27%), 90 (19%); Anal. calcd for $C_{22}H_{20}ClFN_2O_4S$: C 57.08, H 4.35, N 6.05, S 6.93; found C 56.99, H 4.39, N 6.12, S 6.97.

N-(4-(N-(5-chloro-2-methoxyphenyl)-N-(2-methylbenzyl)sulfamoyl)phenyl)acetamide **5j**

Brown powder, Yield 77%, m.p. 78-80ºC, Molecular formula: $C_{23}H_{23}ClN_2O_4S$; Mol. Weight: 458.9g. IR (KBr, cm^{-1})ν_{max}: 3017 (C-H stretching of aromatic ring), 1525 (C=C stretching of aromatic ring), 1321 (-SO_2 stretching), ^{1}H-NMR (400MHz, $CDCl_3$, δ/ppm): 7.73 (d, *J* = 8.4 Hz, 2H, H-2', H-6'), 7.54 (d, *J* = 8.4 Hz, 2H, H-3', H-5'), 7.52 (d, *J* = 2.4 Hz, 1H, H-6), 7.21 (d, *J* = 8.0 Hz, 2H, H-6"), 7.11 (d, *J* = 8.0 Hz, 2H, H-3"), 7.04 (m, 2H, H-4" & H-5"), 6.97 (dd, *J* = 8.4, 2.4 Hz, 1H, H-4), 6.64 (d, *J* = 8.4 Hz, 1H, H-3), 4.97(s, 2H, H-7"), 3.63 (s, 3H, -OCH_3), 2.18 (s, 3H, -COC<u>H</u>$_3$). EIMS: *m/z* 458 $[M]^+$, 156 (24%), 141 (100%), 105 (21%), 58 (16%), 90 (29%); Anal. calcd for $C_{23}H_{23}ClN_2O_4S$: C 60.19, H 5.05, N 6.10, S 6.99; found C 60.15, H 5.13, N 6.21, S 7.08.

Ethyl 2-(4-acetamido-N-(5-chloro-2-methoxyphenyl) phenylsulfonamido)acetate **5k**

Dark brown powder, Yield 64%, m.p. 84-90ºC Molecular formula $C_{19}H_{21}ClN_2O_6S$; Mol. Weight: 440 g. IR (KBr, cm^{-1})ν_{max}: 3018 (C-H stretching of aromatic ring), 1529 (C=C stretching of aromatic ring), 1323 (-SO_2 stretching), ^{1}H-NMR (400MHz, $CDCl_3$, δ/ppm): 7.71 (d, *J* = 8.4 Hz, 2H, H-2', H-6'), 7.53 (d, *J* = 8.4 Hz, 2H, H-3', H-5'), 7.50 (d, *J* = 2.4Hz, 1H, H-6), 6.97 (dd, *J* =8.4, 2.4 Hz, 1H, H-4), 6.64 (d, *J* =8.4 Hz, 1H, H-3), 4.14 (s, 2H, H-2"), 3.65 (s, 3H, -OCH_3), 2.20 (s, 3H, -COC<u>H</u>$_3$), 3.25 (q, 2H, -O<u>CH$_2$</u>CH_3), 0.75 (t, *J* = 6.2 Hz, 3H, -OCH_2<u>CH$_3$</u>). EIMS: *m/z* 440 $[M]^+$, 156 (32%), 141 (100%), 87 (24%), 58 (17%); Anal. calcd for $C_{19}H_{21}ClN_2O_6S$: C 51.76, H 4.80, N 6.35, S 7.27; found C 51.69, H 4.87, N 6.41, S 7.35.

Urease Inhibition Assay

The enzyme assay is the modified form of the commonly known Berthelot assay [17]. A total volume of 85 µl assay mixture contained 10 µl of phosphate buffer of pH 7.0 in each well in the 96-well plate followed by the addition of 10 µl of sample solution and 25 µl of enzyme solution (0.135 units). Contents were pre-incubated at 37ºC for 5 minutes. Then, 40 µl of urea stock solution (20 mM) was added to each well and incubation continued at 37ºC for further 10 min. After given time, 115 µl phenol hypochlorite reagents were added in each well (freshly prepared by mixing 45 µl phenol reagent with 70 µl of alkali reagent). For color development, incubation was done at 37ºC for another 10 min. Absorbance was measured at 625 nm using the 96-well plate reader Synergy HT BioTek, USA). The percentage enzyme inhibition was calculated by the following formula:

Inhibition (%) = 100 - (Abs of test sample / Abs of control) × 100

IC_{50} values (concentration at which 50% enzyme catalyzed reaction occurs) of compounds were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

Conclusion

The projected structures of the synthesized compounds are well supported by spectroscopic data. The newly synthesized compounds showed varying degree of antiurease activity.

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