Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supplementary data

Effect of the isosteric replacement of phenyl motif with furyl (or thienyl) of 4-phenyl-*N*-arylsulfonylimidazolones as broad and potent anticancer agents

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1. Chemistry

1.1 Materials and methods

Melting points (mp) were determined on Electro thermal 1A 9100 MK2 apparatus and are uncorrected. All commercial chemicals were used as obtained and all solvents were purified by the standard procedures prior to use[30]. Flash column chromatography was performed with E Merck silica gel (230-400 mesh). FT-IR spectrum was recorded by Nicolet – 380 model. NMR spectra were measured against the peak of tetramethylsilane by JNM-AL 400 NMR (400 MHz, JEOL, Japan) spectrometers. Electro spray mass spectra (ESIMS) were recorded on PE SCIEX API 2000 (triple quadrupole) LC-MS/MS system (Applied Biosystems, Foster City, CA, USA). Elemental analyses were performed on a Thermo Fisher Scientific (Flash EA 1112 series).

1.1.1 General procedure for the synthesis of (**9**)

Bromine (1.74 g, 1.2 equiv.) was added in a drop wise manner to an ice-cold solution of 2-acetylfuran, **8a** (1.0 g, 1.0 equiv.) in ether under nitrogen atmosphere. After stirring for 90 min., the reaction was quenched with saturated aqueous ammonium chloride solution.

The two layers were separated and the organic extract was washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give compound **9a** as yellow oil. The same procedure was followed for the synthesis of compound **9b**.

1.1.2 2-Bromoacetofuranone (**9a**)

Yellow oil; R_f 0.60 (HX:EA = 2:1); Yield 98%; IH-NMR ($CDCl_3$; 89.45 MHz) δ 4.33(s, 2H, -CH_2Br), 6.58-7.67 (m, 3H, Ar-H).

1.1.3 2-Bromoacetothiophenone (**9b**)

Yellow oil; R_f 0.55 (HX:EA = 2:1); Yield 95%; 1H-NMR ($CDCl_3$) δ 4.37 (s, 2H, -CH_2Br), 6.50-7.84 (m, 3H, Ar-H).

1.2 General procedure for the synthesis of (**10**)

Hexamethylenetetramine (0.816 g, 1.1 equiv) was added to a solution of **9a** (1.0 g, 1 equiv.) in chloroform (6 mL). The reaction was stirred for 4-5 h at 60^{o}C. The formed white precipitate was filtered, washed with chloroform, dried and treated with hydrochloric acid in anhydrous ethanol. After stirring for 18 h at room temperature, the yellow precipitate was removed by filtration and washed with ethanol. The filtrate was concentrated, crystallized from methanol and ethyl acetate to give compound **10a**. The compound **10b** also obtained from the same procedure.

1.2.1 2-Aminoacetofuranone (**10a**)

White solid; R_f 0.22 (HX:EA = 1:2);Yield 65%; mp 218-220 ^oC; IR (KBr) 3400, 1700 cm^{-1}; 1H-NMR (DMSO-d_6) δ 4.33 (s, 2H, -CH_2NH_2), 6.75-8.11 (m, 3H, Ar-H).

1.2.2 2-Aminoacetothiophenone (**10b**)

White solid; R_f 0.31 (HX:EA = 1:2);Yield 60%; mp 279-281 ^oC; IR (KBr) 3400, 1700 cm^{-1}; 1H-NMR (DMSO-d_6) δ 4.43 (s, 2H, -CH_2NH_2), 7.20-7.98 (m, 3H, Ar-H).

1.3 General procedure for the synthesis of (**11**)

Potassium cyanate (0.552 g, 1.1 eq.) was added to a solution of **10a** (1.0 g, 1.0 equiv) in water (5 mL). The mixture was stirred for 1 h at room temperature then for 4 h at 70 ^oC. The red precipitate, which was formed by stirring mixture for further 18 h at room temperature, was filtered and dried to give compound **11a** that was used for the next reaction without purification. The compound **11b** also obtained from the same procedure.

1.3.1 4-Furyl-2-Imidazolone (**11a**)

Red solid; R_f 0.48 (HX:EA = 1:2); Yield 40%; mp 219-221 ^oC; IR (KBr) 2800-3400, 1700 cm^{-1}; 1H-NMR (DMSO-d_6) δ 5.75 (s, 1H, Ar-H), 6.44 (s, 1H, Ar-H), 6.60 (s, 1H, Ar-H), 7.69 (s, 1H, Ar-H), 10.06 (bs, 1H, -NH), 10.49 (bs, 1H, -NH).

1.3.2 4-Thienyl-2-Imidazolone (**11b**)

Red solid; R_f 0.52 (HX:EA = 1:2); Yield 38%; mp 235-237 ^oC; IR (KBr) 2800-3400, 1700 cm^{-1}; 1H-NMR (DMSO-d_6) δ 6.64 (s, 1H, Ar-H), 6.44 (s, 1H, Ar-H), 6.95-7.37 (m, 2H, Ar-H), 10.00 (bs, 1H, -NH), 10.52 (bs, 1H, -NH).

1.4 General procedure of the synthesis of *N*-aryl-sulfonylimidazole (**5a-e**) and (**6a-e**)

In brief an appropriate amount of ethyl 4-(chlorosulfonyl)-2-methylphenylcarbamate (**12a**) (1.85 g, 1.0 equiv.) were added to an ice cold solution of compound **11a** (1.0 g, 1.0 equiv.) and sodium hydride (0.176 g, 1.1 equiv.) in dimethylformamide (10 mL). The resulting mixture was stirred for 5-6 h at 0 ^{o}C and then DMF was removed under reduced pressure and residue was extracted with ethylacetate three times. The organic layer was dehydrated with anhydrous sodium sulfate and evaporated under vacuum. The compound **5a** were separated by flash column chromatography**.** The compounds **5b-e** and **6a-e** were also obtained by similar procedure.

1.4.1 Ethyl 4-(4-(furan-2-yl)-2-oxo-2,3-dihydroimidazol-1-ylsulfonyl)-2-methylphenylcarbamate (**5a**)

White solid; R_f 0.33 (HX:EA = 1:2); Yield 25%; mp 210-213^{o}C; IR (KBr) 3300, 1720, 1520, 1360, 1200, 1150 cm^{-1}; 1H-NMR (Acetone-d_6) δ 1.26 (t, *J* = 7.1 Hz, 3H, -CH_3), 2.43 (s 3H, Ar-CH_3), 4.19 (q, *J* = 7.3 Hz, 2H, -OCH_2), 6.54-7.02 (m, 2H, Ar-H), 7.62-8.02 (m, 4H, Ar-H), 8.27 (d, J = 8.4 Hz, 1H, Ar-H), 10.02 (bs, 1H, -NH); ESIMS: *m/z* = 392.2 $[M+1]^+$. Calc. for $C_{17}H_{17}N_3O_6S$: C, 52.17; H, 4.38. Found: C, 71.14; H, 4.36.

1.4.2 Propyl 4-(4-(furan-2-yl)-2-oxo-2,3-dihydroimidazol-1-ylsulfonyl)-2-methyl phenylcarbamate (**5b**)

White solid; R_f 0.50 (HX:EA = 1:2); Yield 58 %; mp 153-156^{o}C; IR (KBr) 3400, 1700, 1520, 1350, 1220, 1150 cm^{-1}; 1H-NMR (Acetone-d_6) δ 0.94 (t, *J* = 6.8 Hz, 3H, -CH_3),

1.56-1.79 (m, 2H, -CH_2CH_3), 2.42 (s, 3H, Ar-CH_3), 4.10 (t, *J* = 6.6 Hz, 2H, -OCH_2), 6.51-7.03 (m, 2H, Ar-H), 7.60-8.00 (m, 4H, Ar-H), 8. 23 (d, J = 9.0 Hz, 1H, Ar-H), (10.00 (bs, 1H, -NH); ESIMS: *m/z* = 406.1 $[M+1]^+$. Calc. for $C_{18}H_{19}N_3O_6S$: C, 53.32; H, 4.72. Found: C, 53.30; H, 4.70.

1.4.3 Butyl 4-(4-(furan-2-yl)-2-oxo-2,3-dihydroimidazol-1-ylsulfonyl)-2-methyl phenylcarbamate (**5c**)

White solid; R_f 0.64 (HX:EA = 1:2); Yield 61 %; mp 142-146 ^oC; IR (KBr) 3000-3400, 1700, 1520, 1350, 1200, 1150 cm^{-1}; 1H-NMR ($CDCl_3$) δ 0.95 (t, *J* = 6.4 Hz, 3H, -CH_3), 1.21-1.75 (m, 4H, -$(CH_2)_2CH_3$), 2.27 (s, 3H, Ar-CH_3), 4.19 (t, *J* = 6.4 Hz, 2H, -OCH_2), 6.45-7.43 (m, 3H, Ar-H), 7.93 (d, *J* = 9.0 Hz, 1H, Ar-H), 8.22 (d, *J* = 9.3 Hz, 3H, Ar-H), 10.02 (bs, 1H, NH); ESIMS: *m/z* = 420.2 $[M+1]^+$. Calc. for $C_{19}H_{21}N_3O_6S$: C, 54.41; H, 5.05. Found: C, 54.39.14; H, 5.03.

1.4.4 Isopropyl 4-(4-(furan-2-yl)-2-oxo-2,3-dihydroimidazol-1-ylsulfonyl)-2-methylphenylcarbamate (**5d**)

White solid; R_f 0.54 (HX:EA = 1:2); Yield 55 %; mp 102-106 ^oC; IR (KBr) 3000-3400, 1700, 1500, 1350, 1180 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.32 (d, *J* = 6.4 Hz, 6H, -$CH(CH_3)_2$), 2.28 (s, 3H, Ar-CH_3), 4.96-5.10 (m, 1H, -OCH), 6.48-7.97 (m, 6H, Ar-H), 8.25 (d, *J* = 9.2 Hz, 1H, Ar-H), 9.48 (bs, 1H, -NH); ESIMS: *m/z* = 406.1 $[M+1]^+$. Calc. for $C_{18}H_{19}N_3O_6S$: C, 53.32; H, 4.72. Found: C, 53.30.14; H, 4.70.

1.4.5 Isobutyl 4-(4-(furan-2-yl)-2-oxo-2,3-dihydroimidazol-1-ylsulfonyl)-2-methylphenylcarbamate (**5e**)

White solid; R_f 0.58 (HX:EA = 1:2); Yield 62 %; mp 163-167 ^oC; IR (KBr) 3000-3400, 1700, 1520, 1350, 1220, 1180 cm^{-1}; 1H-NMR (Acetone-d_6) δ 0.95 (d, *J* = 6.6 Hz, 6H, -$CH(CH_3)_2$), 1.81-2.10 (m, 1H, -$CHCH_2$), 2.42 (s, 3H, Ar-CH_3), 3.95 (d, *J* = 6.8 Hz, 2H, -OCH_2), 6.51-7.02 (m, 3H, Ar-H), 7.61-8.01 (m, 3H, Ar-H), 8.29 (d, *J* = 9.2 Hz, 1H, Ar-H), 10.04 (bs, 1H, -NH); ESIMS: *m/z* = 420.2 $[M+1]^+$. Calc. for $C_{19}H_{21}N_3O_6S$: C, 54.41; H, 5.05. Found: C, 54.38.14; H, 5.02.

1.4.6 Ethyl 2-methyl-4-(2-oxo-4-(thiophen-2-yl)-2,3-dihydroimidazol-1-ylsulfonyl)phenylcarbamate (**6a**)

White solid; R_f 0.35 (HX:EA = 1:2); Yield 22 %; mp 145-150 ^oC IR (KBr) 3400, 1700, 1550, 1380, 1200, 1100 cm^{-1}; 1H-NMR (Acetone-d_6) δ 1.29 (t, *J* = 7.6 Hz, 3H, -CH_3), 2.43 (s, 3H, Ar-CH_3), 4.21 (q, *J* = 7.1 Hz, 2H, -OCH_2), 7.03-7.51 (m, 3H, Ar-H), 7.88-7.97 (m, 4H, Ar-H), 10.04 (bs, 1H, -NH); ESIMS: *m/z* = 408.0 $[M+1]^+$. Calc. for $C_{17}H_{17}N_3O_5S_2$: C, 50.11; H, 4.21. Found: C, 50.08.14; H, 4.18.

1.4.7 Propyl 2-methyl-4-(2-oxo-4-(thiophen-2-yl)-2,3-dihydroimidazol-1-ylsulfonyl)phenylcarbamate (**6b**)

White solid; R_f 0.54 (HX:EA = 1:2); Yield 60 %; mp 132-135 ^oC IR (KBr) 3400, 1700, 1520, 1350, 1220, 1180 cm^{-1}; 1H-NMR ($CDCl_3$) δ 0.94 (t, *J* = 6.8 Hz, 3H,-CH_3), 1.60-1.83 (m, 2H, -CH_2CH_3), 2.26 (s, 3H, Ar-CH_3), 4.16 (t, *J* = 7.0 Hz, 2H, -OCH_2), 6.72-

7.29 (m, 3H, Ar-H), 7.64-8.00 (m, 4H, Ar-H), 10.28 (bs, 1H, -NH); ESIMS: *m/z* = 422.1 $[M+1]^+$. Calc. for $C_{18}H_{19}N_3O_5S_2$: C, 51.29; H, 4.54. Found: C, 51.27.14; H, 4.52.

1.4.8 Butyl 2-methyl-4-(2-oxo-4-(thiophen-2-yl)-2,3-dihydroimidazol-1-ylsulfonyl)phenylcarbamate (**6c**)

White solid; R_f 0.60 (HX:EA = 1:2); Yield 57 %; mp 170-174 ^oC IR (KBr) 3400, 1700, 1520, 1350, 1220, 1180 cm^{-1}; 1H-NMR (Acetone-d_6) δ 0.97 (t, *J* = 6.4 Hz, 3H, -CH_3), 1.29-1.64 (m, 4H, -$(CH_2)_2CH_3$), 2.42 (s, 3H, Ar-CH_3), 4.15 (t, *J* = 8.1 Hz, 2H, -OCH_2), 7.03-7.51 (m, 3H, Ar-H), 7.51-8.22 (m, 4H, Ar-H), 10.09 (bs, 1H, -NH); ESIMS: *m/z* = 436.0 $[M+1]^+$. Calc. for $C_{19}H_{21}N_3O_5S_2$: C, 52.40; H, 4.86. Found: C, 52.38.14; H, 4.84.

1.4.9 Isopropyl 2-methyl-4-(2-oxo-4-(thiophen-2-yl)-2,3-dihydroimidazol-1-ylsulfonyl)phenylcarbamate (**6d**)

White solid; R_f 0.58 (HX:EA = 1:2); Yield 54 %; mp 187-190 ^oC IR (KBr) 3400, 1700, 1500, 1350, 1220, 1180 cm^{-1}; 1H-NMR (Acetone-d_6) δ 1.26 (d, *J* = 6.1 Hz, 6H, -$(CH_3)_2$), 2.42 (s, 3H, Ar-CH_3), 4.83-5.04 (m, 1H, -OCH), 7.03-7.51 (m, 4H, Ar-H), 7.88-8.03 (m, 3H, Ar-H), 10.11 (bs, 1H, -NH); ESIMS: *m/z* = 422.1 $[M+1]^+$. Calc. for $C_{18}H_{19}N_3O_5S_2$: C, 51.29; H, 4.54. Found: C, 51.26.14; H, 4.51.

1.4.10 Isobutyl 2-methyl-4-(2-oxo-4-(thiophen-2-yl)-2,3-dihydroimidazol-1-ylsulfonyl)phenylcarbamate (**6e**)

White solid; R_f 0.60 (HX:EA = 1:2); Yield 57 %; mp 170-174 ^oC IR (KBr) 3000-3400, 1700, 1520, 1350, 1200, 1150 cm^{-1}; 1H-NMR ($CDCl_3$) δ 0.95 (d, *J* = 6.6 Hz, 6H, -

$CH(CH_3)_2$), 1.74-2.17 (m, 1H, -$CHCH_2$), 2.26 (s, 3H, Ar-CH_3), 3.97 (d, J = 8.4 Hz, 2H, -OCH_2), 6.68-7.32 (m, 5H, Ar-H), 7.94 (d, J = 9.2 Hz, 1H, Ar-H), 8.07 (d, J = 9.4 Hz, 1H, Ar-H), 10.59 (bs, 1H, -NH); ESIMS: m/z = 436.0 $[M+1]^+$. Calc. for $C_{19}H_{21}N_3O_5S_2$: C, 52.40; H, 4.86. Found: C, 52.36.14; H, 4.84.

1.5. Synthesis of compound (**13**)

To a solution of **5a** (1.0 g) in methanol (5 mL) was added 10 % aqueous sodium hydroxide solution. The mixture was stirred for 2-3 h at 60-70 ^oC. After evaporation, the residue was extracted with methylenechloride and the organic solvent was washed with water and then dried over anhydrous sodium sulfate. Now the organic solvent evaporated to give product **13** that was used for next step without further purification.

1.5.1 1-(4-Amino-3-methylphenylsulfonyl)-4-(furan-2-yl)-1H-imidazol-2(3H)-one (**13**)

Yellow solid; R_f 0.48 (HX:EA = 1:2); Yield 63%; mp 210-213 ^oC, IR (KBr) 3400, 1700, 1500, 1350, 1150 cm^{-1}; 1H-NMR (Acetone-d_6) δ 2.18 (s, 3H, Ar-CH_3), 5.58 (bs, 2H, -NH_2), 6.50-7.69 (m, 7H).

1.6 General procedure for the preparation of compound (**7**)

To a solution of compound **13** (1.0 g, 1.0 equiv) in anhydrous toluene (10 mL) was added the isopropyl isocyanate (0.266 g, 1.0 equiv) at room temperature. The resulting mixture was stirred at 50-60 ^oC for 18 h, and the organic solvent was removed under reduced pressure. The residue was extracted with dichloromethane three times. The organic layer

was dehydrated with anhydrous sodium sulfate and evaporated under vacuum. The compound **7a** was separated by flash column chromatography**.** The compounds **7b** and **7c** were also obtained by similar procedure.

1.6.1 1-(4-(4-(Furan-2-yl)-2-oxo-2,3-dihydroimidazol-1-ylsulfonyl)-2-methylphenyl)-3-isopropylurea (**7a**)

White solid; R_f 0.20 (HX:EA = 1:2); Yield 18 %; mp 239-243 ^{o}C; IR (KBr) 3000-3400, 1700, 1550, 1350, 1380, 1150 cm^{-1}; 1H-NMR (Acetone-d_6) δ 1.01 (d, *J* = 6.6 Hz, 6H, $(CH_3)_2$), 2.12 (s, 3H, Ar-CH_3), 3.66-3.88 (m, 1H, -NHCH), 6.08-6.86 (m, 3H, Ar-H)), 7.35-7.76 (m, 3H, Ar-H), 8.22 (d, *J* = 9.5 Hz, 1H, Ar-H), 9.83 (bs, 1H, -NH); ESIMS: *m/z* = 405.1 $[M+1]^+$. Calc. for $C_{18}H_{20}N_4O_5S$: C, 53.45; H, 4.98. Found: C, 53.42.14; H, 4.95.

1.6.2 1-(4-(4-(Furan-2-yl)-2-oxo-2,3-dihydroimidazol-1-ylsulfonyl)-2-methylphenyl)-3-phenylurea (**7b**)

White solid; R_f 0.73 (HX:EA = 1:2); Yield 21 %; mp 175-178 ^{o}C; IR (KBr) 3300, 1650, 1550, 1320, 1380, 1240 cm^{-1}; 1H-NMR (Acetone-d_6) δ 2.35 (s, 3H, Ar-CH_3), 6.51-7.31(m, 6H, Ar-H) 7.41-8.33 (m, 6H, Ar-H), 10.05 (bs, 1H, -NH); ESIMS: *m/z* = 439.0 $[M+1]^+$. Calc. for $C_{21}H_{18}N_4O_5S$: C, 57.53; H, 4.14. Found: C, 57.51.14; H, 4.12.

1.6.3 1-Cyclohexyl-3-(4-(4-(furan-2-yl)-2-oxo-2,3-dihydroimidazol-1-ylsulfonyl)-2-methylphenyl)urea (**7c**)

White solid; R_f 0.28 (HX:EA = 1:2); Yield 19 %; mp 174-178 ^oC IR (KBr) 2800-3400, 1700, 1550, 1350, 1200, 1100 cm^{-1}; 1H-NMR (Acetone-d_6) δ 1.23-2.10 (m,10H, -C_6H_{11}) 2.25 (s, 3H, Ar-CH_3), 3.50-3.60 (m, 1H, -NHC_6H_{11}), 6.27-7.91(m, 6H, Ar-H), 8.38 (d, *J* = 9.6 Hz, 1H, Ar-H), 10.03 (bs, 1H, -NH); ESIMS: *m/z* = 445.1$[M+1]^+$. Calc. for $C_{21}H_{24}N_4O_5S$: C, 56.74; H, 5.44. Found: C, 56.71.14; H, 5.41.

2. Biological Evaluation

2.1 Materials and methods

2.1.1 Materials

For the *in vitro* cytotoxicity assay all the compounds **5a-e**, **6a-e** and **7a-c** were solubilized and diluted in DMSO; final concentration was 0.1% (v/v) in the medium. Doxorubicin was solubilized in 0.85% NaCl.

2.1.2 Cell lines

Three human tumor cell lines, A549, HCT116, NCI-H460 were obtained from the National Cancer Institute (NCI), NIH, USA. The cells were routinely maintained in a humidified CO_2 incubator (95% air and 5% CO_2) at 37^{o}C with RPMI 1640 containing 10% fetal bovine serum (FBS; Gibco BRL). All the cell lines were kept under liquid nitrogen until used and were passaged *in vitro* to maintain exponential tumor growth.

2.1.3 *In Vitro* Cytotoxicity Assay

The antiproliferative activity of these new synthetics was assessed using human colon carcinoma (HCT116), and human non-small cell lung cancer cell lines (A549, and NCI-

H460). After 48h continuous drug exposure, the concentration required for 50% Inhibitory growth factor (IC_{50}) was determined by the sulforhodamine B (SRB) colorimetric assay[28, 29]. In brief, cells were split into 96-well plates. After incubation, anchorage-dependent cells were directly fixed by the slow addition of 50 µL of 50% trichloroacetic acid (TCA) solution per well. Anchorage-independent cells were fixed by pre-centrifugation 9150g, 1 min at 20^{o}) and the drop-wise addition of 50% TCA. Fixation proceeded for 1hr at 4^{o}. After fixation, plates were washed five times with tap water and air-dried. One hundred microliters of SRB solution (0.4% in 1% acetic acid) was added to each well of the 96-well microplates. Staining was done at room temperature for 30 min. Residual dye was washed out with 1% acetic acid and air dried. To each well, 100 µL of Tris solution (10mM, pH 10.5) was added. Optical density (O.D.) was measured in a microtiter plate reader at 540 nm. Each drug concentration was tested in triplicate at least three times.