

Design, Green Synthesis and Biological Evaluation of Fluorinated *N*-Acylsulfonamides as Novel Anti-Inflammatory Agents: An *In Vivo* and *In Silico* Study

Zaineb Litim,^{*a} Ameni Ben Abdeljaoued,^a Amal Abdelhamid,^a Abderrahman Bouraoui, İsmail Özdemir,^b Naceur Hamdi,^c Mohamed Ali Soussi^a and Jamil Kraiem^{*a}

^aLaboratoire de Développement Chimique, Galénique et Pharmacologique des Médicament (LR12ES09)s, Faculté de Pharmacie de Monastir, Université de Monastir, Rue Avicenne, 5000, Monastir Tunisia. E-mail: jamil.kraiem@fphm.u-monastir.tn.

^bInonu University, Faculty of Arts and Sciences, Department of Chemistry, Malatya, 44280, Turkey.

^cResearch Laboratory of Environmental Sciences and Technologies (LR16ES09), Higher Institute of Environmental Sciences and Technology, University of Carthage, Hammam-Lif, Tunisia.

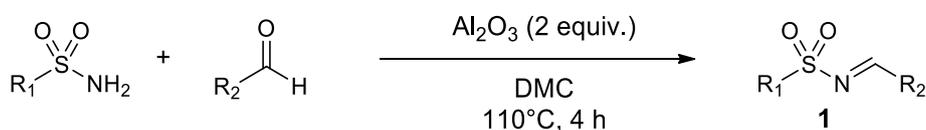
1. Chemistry

1.1. General data

All chemicals and solvents obtained from commercial suppliers were used without further purification. Analytical thin layer chromatography (TLC) was performed on Merck 60F-254 precoated silica gel sheets (0.25 mm). The plates were visualized by irradiation with UV light, followed by spraying with solution of KI. For column chromatography, 60–120 mesh silica gel and/or Celite 545 were used. Melting points were measured with Kofler heating bench and are uncorrected. ¹H NMR, ¹³C and ¹⁹F NMR spectra were recorded on a Bruker AV 500 MHz, AV 400 MHz or AV 300 MHz, using CDCl₃ or DMSO-*d*₆ as solvent. Chemical shifts (δ) are expressed in ppm relative to residual solvents (CDCl₃: δ_H = 7.26, δ_C = 77.16; DMSO-*d*₆: δ_H = 2.50, δ_C = 39.52). In all ¹H NMR spectra, the number of protons for each signal and coupling constants values (Hertz) are indicated. Multiplicity is also reported and designated by the following abbreviations: s (singlet), d (doublet), (dd) doublet of doublets, t (triplet), q (quartet) m (multiplet).

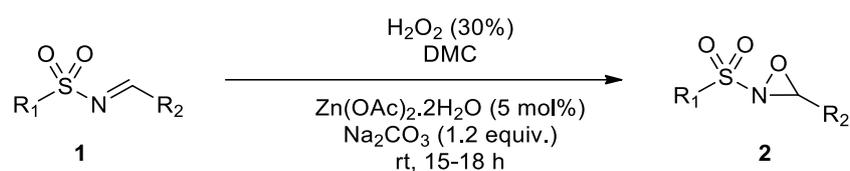
1.2. General procedure

1.2.1. Synthesis of *N*-sulfonylimines 1a-j



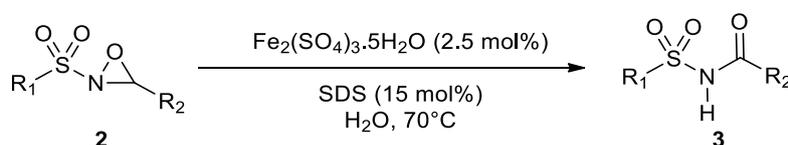
N-sulfonylimines were prepared according to the method developed by our group¹. A mixture of sulfonamide (1.2 mmol), aldehyde (1 mmol), and aluminum oxide (2 equiv.) in 1 mL of anhydrous DMC was stirred and heated in an 8 mL pressure tube at 110 °C for 4 hours. The progress of the reaction was monitored by TLC (cyclohexane/AcOEt (7:3)). After the reaction was complete, the crude mixture was filtered, and Al₂O₃ was thoroughly washed with 4 mL of hot DMC (60 °C). The collected filtrate was concentrated under reduced pressure and crystallized from ethanol/water to afford pure *N*-sulfonylimines **1**.

1.2.2. Synthesis of *N*-sulfonyloxaziridines **2a-j**



N-sulfonyloxaziridines were prepared according to the method developed by our group². To a solution of *N*-sulfonylimine **1** (0.5 mmol) in DMC (1.5 mL) was added Zn(OAc)₂·2H₂O (5mol%) and Na₂CO₃ (1.2 equiv). Then a 10 equiv. of 30 % hydrogen peroxide solution was added over a period of 5 min. The mixture was stirred at room temperature until disappearance of the imine (15-18h). The reaction was monitored by TLC (cyclohexane: AcOEt (8:2)). The reaction mixture was then diluted with AcOEt, washed with a solution of sodium sulfite and extracted with AcOEt (3×2 mL). The organic phase was dried over MgSO₄ and concentrated on a rotary evaporator to obtain an oily residue that usually solidifies at rest. This residue was then filtered through a short pad of silica gel (cyclohexane: AcOEt) to yield pure product **2**.

1.2.3. Synthesis of *N*-acylsulfonamides **3a-j**

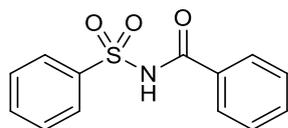


In a 8 mL sealed vial, Fe₂(SO₄)₃·5H₂O (2.5 mol%) and SDS (15 mol%) was stirred in 1 mL of H₂O at room temperature for 5 min. Thereafter, *N*-sulfonyloxaziridine **2** (0.5 mmol) was added and the reaction was carried out at 70°C until disappearance of the oxaziridine. The progress of the reaction was monitored by TLC (revealed with cyclohexane: AcOEt (7:3), and then sprayed with an aqueous solution of KI). After the completion of the reaction, 1mL of AcOEt was added and the mixture was centrifuged ((5min, 3000 rpm) ×3) to separate the organic and the

aqueous phases. Then, the residue was filtered through a short pad of silica gel and Celite. After evaporation of the solvent, the crude product was crystallized to yield the pure *N*-acylsulfonamides **3**.

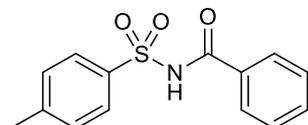
N-acylsulfonamides **3a**³, **3b**³, **3c**⁴, **3d**⁵, **3e**³, **3f**⁶, **3h**³, and **3j**⁷ are known compounds and previously reported in the literature. *N*-acylsulfonamides, **3g** and **3i** are new compounds.

1.2.3.1. Synthesis of *N*-(phenylsulfonyl)benzamide (**3a**)



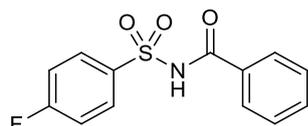
White solid (83%); crystallized from (ethanol/water); mp 147-149 °C; ¹H NMR: δ_H (300 MHz; CDCl₃) 9.37 (1H, s), 8.17 (2H, d, *J* = 7.8 Hz), 7.82 (2H, d, *J* = 7.8 Hz), 7.65 (1H, t, *J* = 7.3 Hz), 7.55 (3H, t, *J* = 7.7 Hz), 7.41 (2H, t, *J* = 7.6 Hz); ¹³C NMR: δ_C (75 MHz; CDCl₃) 164.5, 138.8, 134.2, 133.6, 131.3, 129.1, 129.0, 128.7, 128.0.

1.2.3.2. *N*-tosylbenzamide (**3b**)



White solid (87%); crystallized from (ethanol/water); mp 144-146 °C; ¹H NMR: δ_H (300 MHz; CDCl₃) 9.33 (1H, s), 8.05 (2H, d, *J* = 8.1 Hz), 7.82 (2H, d, *J* = 7.5 Hz), 7.54 (1H, t, *J* = 7.5 Hz), 7.41 (2H, t, *J* = 7.5 Hz), 7.34 (2H, d, *J* = 8.1 Hz), 2.43 (3H, s); ¹³C NMR: δ_C (75 MHz; CDCl₃) 164.5, 145.3, 135.8, 133.5, 131.4, 129.7, 129.0, 128.8, 128.0, 21.8.

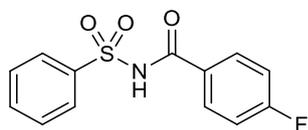
1.2.3.3. *N*-((4-fluorophenyl)sulfonyl)benzamide (**3c**)



White solid (96%); crystallized from (ethanol/water); mp 176-178 °C; ¹H NMR: δ_H (300 MHz; CDCl₃) 9.10 (1H, s), 8.19 (2H, dd, *J* = 8.6 Hz, *J* = 5.0 Hz), 7.80 (2H, d, *J* = 7.5 Hz), 7.57 (1H, t, *J* = 7.4 Hz), 7.44 (2H, t, *J* = 7.5 Hz), 7.23 (2H, t, *J* = 8.6 Hz); ¹³C NMR: δ_C (75 MHz; CDCl₃) 166.2 (d, *J*

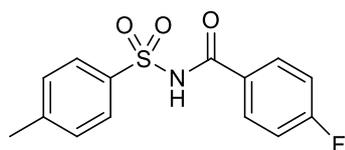
= 255.0), 164.4, 134.7 (d, $J = 3.0$ Hz), 133.8, 131.9 (d, $J = 9.8$ Hz), 131.3, 129.2, 128.0, 116.4 (d, $J = 22.9$ Hz); $^{19}\text{F NMR}$: δ_{F} (376 MHz, CDCl_3) -102.48.

1.2.3.4. 4-fluoro-*N*-(phenylsulfonyl)benzamide (3d)



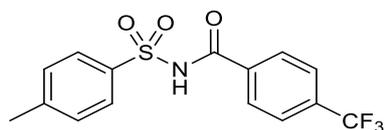
White solid (81%); crystallized from (ethanol/water); mp 158-160 °C; $^1\text{H NMR}$: δ_{H} (300 MHz; CDCl_3) 9.38 (1H, s), 8.16 (2H, d, $J = 7.8$ Hz), 7.86 (2H, dd, $J = 8.4$ Hz, $J = 5.4$ Hz), 7.66 (2H, t, $J = 7.4$ Hz), 7.56 (2H, t, $J = 7.6$ Hz), 7.09 (2H, t, $J = 8.4$ Hz); $^{13}\text{C NMR}$: δ_{C} (75 MHz; CDCl_3) 166.1 (d, $J = 254.3$ Hz), 163.5, 138.7, 134.3, 130.7 (d, $J = 9.0$ Hz), 129.2, 128.7, 127.6 (d, $J = 3.0$ Hz), 116.3 (d, $J = 22.5$ Hz); $^{19}\text{F NMR}$: δ_{F} (376 MHz, CDCl_3) -103.89.

1.2.3.5. 4-fluoro-*N*-tosylbenzamide (3e)



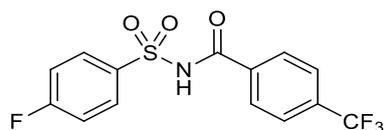
White solid (96%); crystallized from (ethanol/water); mp 160-162 °C; $^1\text{H NMR}$: δ_{H} (500 MHz; CDCl_3) 9.45 (1H, s), 8.04 (2H, d, $J = 8.4$ Hz), 7.87 (2H, dd, $J = 9.0$ Hz, $J = 5.0$ Hz), 7.36 (2H, d, $J = 8.0$ Hz) 7.09 (t, 2H, $J = 8.5$ Hz), 2.44 (3H, s); $^{13}\text{C NMR}$: δ_{C} (125 MHz; CDCl_3) 166.0 (d, $J = 254.3$ Hz), 163.5, 145.6, 135.4, 130.7 (d, $J = 9.5$ Hz), 129.8, 128.8, 127.4 (d, $J = 3.0$ Hz), 116.2 (d, $J = 22.0$ Hz), 21.9; $^{19}\text{F NMR}$: δ_{F} (376 MHz, CDCl_3) -104.08.

1.2.3.6. *N*-tosyl-4-(trifluoromethyl)benzamide (3f)



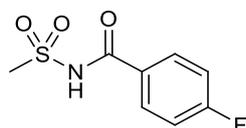
White solid (80%); crystallized from (ethanol/water); mp 187-189 °C; $^1\text{H NMR}$: δ_{H} (300 MHz; CDCl_3) 9.48 (1H, s), 8.04 (2H, d, $J = 8.1$ Hz), 7.95 (2H, d, $J = 8.1$ Hz), 7.68 (2H, d, $J = 8.1$ Hz), 7.36 (2H, d, $J = 8.1$ Hz), 2.45 (3H, s); $^{13}\text{C NMR}$: δ_{C} (75 MHz; CDCl_3) 163.5, 145.8, 135.4 (q, $J = 33.0$ Hz), 134.7, 129.9, 128.9, 128.8, 128.6, 126.1 (q, $J = 3.7$ Hz), 123.5 (q, $J = 271.1$ Hz), 21.8; $^{19}\text{F NMR}$: δ_{F} (282 MHz, CDCl_3) -63.35.

1.2.3.7. *N*-((4-fluorophenyl)sulfonyl)-4-(trifluoromethyl)benzamide (3g)



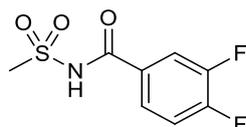
White solid (98%); crystallized from (ethanol/diethyl ether); mp 148-150 °C; $^1\text{H NMR}$: δ_{H} (400 MHz; CDCl_3); 9.44 (1H, s), 8.22 (2H, dd, $J = 8.8$ Hz, $J = 4.8$ Hz), 7.96 (2H, d, $J = 8.0$ Hz), 7.73 (2H, d, $J = 8.4$ Hz), 7.28 (2H, t, $J = 8.5$ Hz); $^{13}\text{C NMR}$: δ_{C} (100 MHz; CDCl_3) 166.2 (d, $J = 256.0$ Hz), 163.4, 135.3 (q, $J = 32.8$ Hz), 134.3, 134.0 (d, $J = 3.2$ Hz), 131.9 (d, $J = 9.8$ Hz), 128.5, 126.2 (q, $J = 3.6$ Hz), 123.4 (q, $J = 271.0$), 116.6 (d, $J = 22.7$ Hz); $^{19}\text{F NMR}$: δ_{F} (376 MHz, CDCl_3) -63.30, -101.70; HRMS (+ESI) calculated for $\text{C}_{14}\text{H}_9\text{F}_4\text{NO}_3\text{S}$ $[\text{M}+\text{H}]^+$: 348.0317, found: 348.0315.

1.2.3.8. 4-fluoro-*N*-(methylsulfonyl)benzamide (3h)



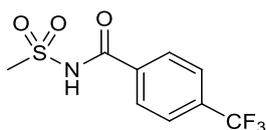
White solid (86%); crystallized from (ethanol/water); mp 188-190 °C; $^1\text{H NMR}$: δ_{H} (400 MHz; DMSO) 12.17 (1H, s), 8.03 (2H, dd, $J = 8.8$ Hz, $J = 5.6$ Hz), 7.36 (2H, t, $J = 8.8$ Hz), 3.38 (3H, s); $^{13}\text{C NMR}$: δ_{C} (100 MHz; DMSO); 165.3, 165.0 (d, $J = 250.0$ Hz), 131.4 (d, $J = 9.4$ Hz), 128.2 (d, $J = 3.0$ Hz), 115.7 (d, $J = 22.0$ Hz), 41.3; $^{19}\text{F NMR}$: δ_{F} (376 MHz, DMSO) -105.98.

1.2.3.9. 3,4-difluoro-*N*-(methylsulfonyl)benzamide (3i)



White solid (98%); crystallized from (ethanol/water); mp 143-145 °C; $^1\text{H NMR}$: δ_{H} (400 MHz; DMSO) 12.24 (1H, s), 8.10-7.96 (1H, m), 7.92-7.80 (1H, m), 7.69-7.55 (1H, m), 3.38 (3H, s); $^{13}\text{C NMR}$: δ_{C} (100 MHz; DMSO) 164.3 (d, $J = 1.0$ Hz), 152.5 (dd, $J = 252.0$ Hz, $J = 12.0$ Hz), 149.1 (dd, $J = 246.0$ Hz, $J = 13.0$ Hz), 129.1 (t, $J = 4.0$ Hz), 126.3 (dd, $J = 8.0$ Hz, $J = 4.0$ Hz), 118.0 (d, $J = 19.0$ Hz), 117.9 (d, $J = 18.0$ Hz), 41.3; $^{19}\text{F NMR}$: δ_{F} (376 MHz, DMSO) -131.32 (d, $J = 22.2$ Hz), -137.20 (d, $J = 22.3$ Hz); HRMS (+ESI) calculated for $\text{C}_8\text{H}_7\text{F}_2\text{NO}_3\text{S}$ $[\text{M}+\text{H}]^+$: 236.0192, found: 236.0185.

1.2.3.10. *N*-(methylsulfonyl)-4-(trifluoromethyl)benzamide (3j)



White solid (94%); crystallized from (ethanol/diethyl ether); mp 148-150 °C; $^1\text{H NMR}$: δ_{H} (400 MHz; DMSO) 12.43 (1H, s); 8.13 (2H, d, $J=8.0$ Hz), 7.91 (2H, d, $J = 8.0$ Hz) 3.40 (3H, s); $^{19}\text{F NMR}$: δ_{F} (376 MHz) -61.62.

2. Pharmacological evaluation: *In vivo* anti-inflammatory activity

2.1. Animals

Adult male Wistar rats (140-180 g) used in the anti-inflammatory screens were obtained from the Pasteur Institute (Tunis, Tunisia). They were housed at controlled temperature ($22 \pm 2^\circ\text{C}$) with free access to standard food pellets and water. Twelve hours before the experiments, the rats were fasted and allowed access to water *ad libitum*. All animal experiments were carried out in accordance with the European Union Regulations (Directive 86/609 CEE).

2.2. Method

Effects of *N*-acylsulfonamides **3a-j** on carrageenan-induced paw edema in rats were evaluated according to the method developed by Winter et al.⁸. Animals were divided into twelve groups of 6 animals each. The control group received normal saline (2.5 mL/kg, *i.p.*), the standard group received Diclofenac (25 mg/kg, *i.p.*) and the test groups received the synthesized compounds **3a-j** (50 mg/kg, *i.p.*). 30 minutes later, carrageenan suspension (1% w/v, in 0.9% saline solution) was subcutaneously injected into the left hind paw. The volume of paw edema was measured by plethysmometer (Ugo Basile 7150, Italy) before (V_0) and after injection of carrageenan at 1, 2, 3, 4 and 5 h (V_T). The percentage of paw edema inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(V_T - V_0) \text{ control} - (V_T - V_0) \text{ treated group}}{(V_T - V_0) \text{ control}} \times 100$$

3. ADMET profile

The evaluation of pharmacokinetics and toxicity proprieties of *N*-acylsulfonamide derivatives were assessed using web-based services ADMET-AI that relies on Chemprop-RDKit graph

neural network (GNN) architecture (<https://admet.ai.greenstonebio.com/>) and ProTox 3.0 (<https://tox.charite.de/protox3/>) that relies on molecular similarities.

References

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^1H NMR, ^{13}C NMR and ^{19}F NMR spectra of compounds 3a-j

