JOAB Series as Novel Pro-Inflammatory Cytokine Suppressants: Synthesis and *In-vitro* Pharmacological Evaluation

Ashley Bill, Christine Agudosi, Doreen E. Szollosi, Ivan Edafiogho, and Ola Ghoneim

University of Saint Joseph-School of Pharmacy, Hartford, CT

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Introduction

- The ability of enaminone (E121-Figure 1) to decrease the pro-inflammatory mediators (TNF-alpha and IL-6) in macrophages stimulated with lipopolysaccharide encouraged us to examine its structural scaffold as a novel anti-inflammatory agent.
- Pro-inflammatory mediators regulate the immune response when infection is present. However, overproduction cause severe tissue injury seen in autoimmune diseases such as Crohn's disease.
- JODI series were designed where an N-arylpiperazine motif was incorporated into the aromatic side of the enaminone (JODI 18 and 19; Figure 1)
- JODI 18 and 19 (Figure 1) has shown to suppress TNF-alpha and IL-6 and were more effective in reducing TNF-alpha after LPS stimulation when compared to dexamethansone (Figure 2).
- Positive results of JODI encouraged further exploration of the chemical scaffold, hence JOAB series was developed.

Figure 1: Enaminone E121 (yellow) and JODI series (green)

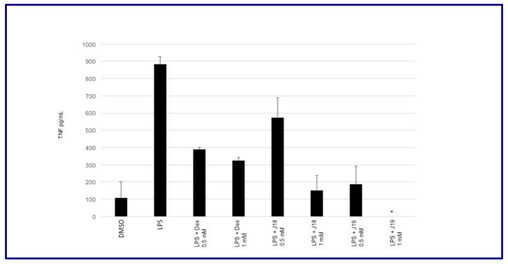
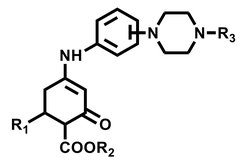


Figure 2: TNF alpha reduction by JODI 18 and 19 compared to dexamethasone

Objective

- To explore four possible chemical modification of the original enaminone scaffold to identify the structural features responsible for their activity

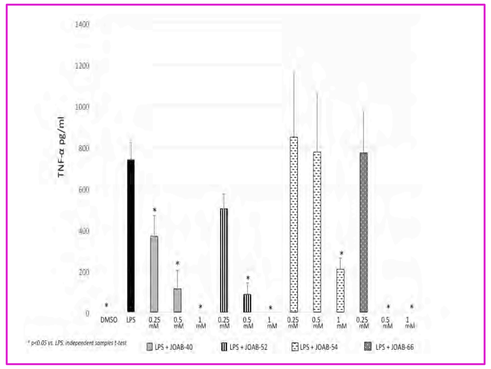
Chemical Scaffold & Biological Screening



R_1= CH_3, C_6H_5
R_2= CH_3, C_2H_5
R_3= CH_3, C_2H_5

Compound	Mol. Wt.	R_1	R_2	R_3	Piperazine Position	% Yield	Rxn time (h)	Log P
JODI-15	357.45	CH_3	CH_3	CH_3	Ortho	33	12	2.65
JODI-17	371.48	CH_3	C_2H_5	CH_3	Ortho	66	8	3.02
JODI-18	419.52	C_6H_5	CH_3	CH_3	Para	70	8	4.02
JOAB-40	433.54	C_6H_5	C_2H_5	CH_3	Para	91	8	4.39
JOAB-44	371.47	CH_3	C_2H_5	CH_3	Para	18	10	3.07
JOAB-48	371.47	CH_3	C_2H_5	CH_3	Meta	29	5	3.05
JOAB-52	447.57	C_6H_5	C_2H_5	C_2H_5	Para	95	6	4.77
JOAB-54	385.50	CH_3	C_2H_5	C_2H_5	Para	30	6	3.45
JOAB-56	371.47	CH_3	CH_3	C_2H_5	Para	80	6	3.07
JOAB-58	433.54	C_6H_5	CH_3	C_2H_5	Para	33	6	4.39
JOAB-60	357.45	CH_3	CH_3	CH_3	Para	35	6	2.70
JOAB-62	357.45	CH_3	CH_3	CH_3	Meta	47	6	2.67
JOAB-64	419.51	C_6H_5	CH_3	CH_3	Meta	84	24	3.99
JOAB-66	433.54	C_6H_5	C_2H_5	CH_3	Meta	79	8.5	4.37
JOAB-68	419.51	C_6H_5	CH_3	CH_3	Ortho	78	17	3.97
JOAB-70	433.54	C_6H_5	C_2H_5	CH_3	Ortho	15	10	4.35

Figure 3: Overall chemical scaffold and physicochemical properties of JODI/JOAB series



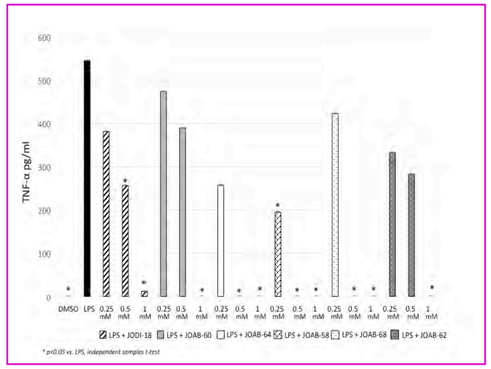
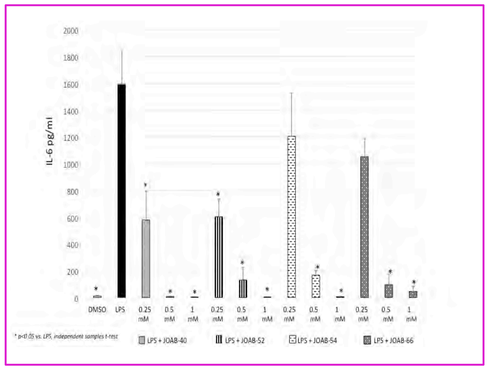


Figure 4: Effect of JODI/JOAB analogs on the release of TNF-alpha in LPS stimulated macrophages



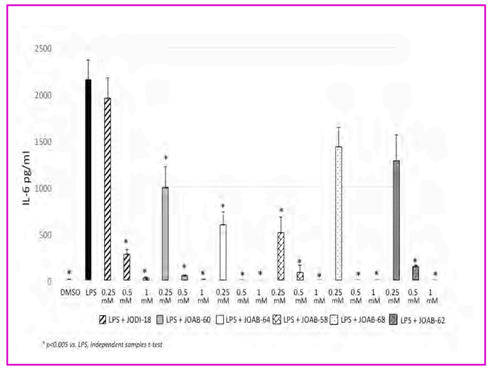
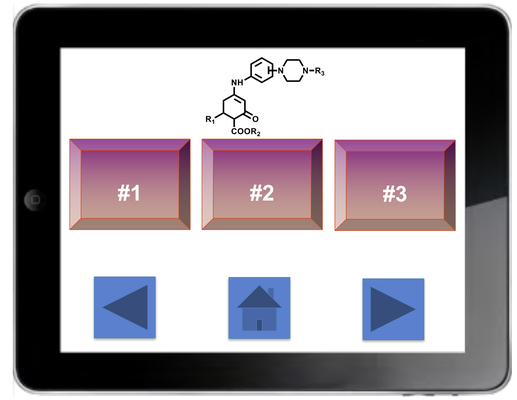


Figure 5: Effect of JODI/JOAB analogs on the release of IL-6 in LPS stimulated macrophages

Play Time!

- Click on one of the numbers below
- Identify the correct JOAB molecule
- Win prizes ☺



Results & Discussion

- Sixteen compounds were synthesized and tested on their ability to reduce TNF-alpha and IL-6 in LPS-stimulated macrophages (Figures 4 and 5).
- Size of R_1 play a major role of the ability of JOAB series to reduce TNF-alpha and IL-6.
- Phenyl ring in R_1 position is more favorable than its methyl counterpart.
- High lipophilicity at R1 play a major role on the ability to interact with the target site (s).
- R_3 size and lipophilicity might not be determining factors in the ability of the piperazino-enaminone to suppress the pro-inflammatory mediators.
- The effect of the position of the piperazine ring is less consistent. However, the para or meta positions are more favorable than the ortho counterpart.

Conclusion

- Piperazino-enaminones (JODI and JOAB series) represent potential pro-inflammatory mediator suppressants.
- Reduction of IL-6 seems to be more significant than reduction of TNF-alpha, indicative of an effect on a differential signaling mechanism or a transcription factor (future experiments).
- *Ghoneim et al., Bioorg. Med Chem, 2018, In press*