Novel non-peptide small molecules preventing IKKβ/NEMO association inhibit NF-κB activation in LPS-stimulated J774 macrophages

F. De Falco[1], C. Di Giovanni[1], A. Capuozzo[1], C. Irace[1], T. Iuvone[1], R. Santamaria[1], A. Lavecchia[1], R. Carnuccio[1]

[1]Dept. of Pharmacy, University of Naples Federico II

NF-κB is a transcription factor regulating several genes involved in important physiological and pathological processes (Ghosh and Hayden, 2012). NF-κB has been found constitutively activated in many inflammatory/immune diseases (Ghosh and Karin, 2002). Since the IKK (IκB kinase) activation is an indispensable component of all pro-inflammatory signaling pathways leading to NF-κB activation, considerable efforts have been done in order to develop novel anti-inflammatory therapeutics targeting IKK. Association of the IKK complex relies on critical interactions between the C-terminus NBD (NEMO binding domain) of the catalytic subunits IKKα and IKKβ, and the regulatory subunit NEMO (NF-κB Essential Modulator) (Karin and Ben-Neriah, 2000; Xu et al., 2011). Thus, this IKK/NEMO interacting region provides an attractive target to prevent the IKK complex formation and NF-κB activation. In this regard, cell permeable NBD peptides have been shown to prevent the inflammation in animal models by inhibiting the interaction of IKKβ with NEMO (May et al., 2000; Strickland and Ghosh, 2006). We have identified non-peptide small molecule disruptors of IKKβ/NEMO complex through structure-based virtual database screening of the NCI chemical library (Lavecchia and Di Giovanni, 2013). Preliminary, **31** compounds were evaluated for their ability to affect cell viability. Incubation of J774 murine macrophages for 24 h with different concentrations of all the compounds allowed us to exclude many of them, except for compounds **1**, **3**, **4**, **9**, **11**, **18**, **21**, **22** and **30**. Thus, we selected these compounds to evaluate their ability to inhibit NO_2^- production in LPS-stimulated J774 murine macrophages for 24 h. The results indicated that only the compound **22** [10-ethyl-10*H*-phenothiazine] was able to reduce significantly and in a concentration-dependent manner NO_2^- production. A subsequent hierarchical similarity search with the most active compound **22** led to the identification of an additional set of 10 close analogues. Then, we selected three of 10 analogues of compound **22** (**22.2**, **22.4** and **22.10**) that did not affect cell viability and were able to inhibit NO_2^- production in a concentration-dependent manner. Therefore, the aim of this study was to investigate whether these compounds were able to inhibit NF-κB activation by preventing IKK complex association. The results demonstrated that compound **22** and its three close analogues inhibited in a concentration-dependent manner IKKβ activity in J774 macrophages stimulated with LPS for 24h. Noteworthy, the IC_{50} value of the compound **22** was 0.0033μM, whereas the IC_{50} values of its analogues **22.2**, **22.4** and **22.10** were 0.1μM, 0.02 μM and 0.06 μM, respectively. The ability of the NBD reference peptide to inhibit IKKβ activity was also investigated and the IC_{50} value was 0.0025 μM compared to the IC_{50} value of compound **22**. Moreover, the reduced IKKβ activity by these compounds was associated with an inhibition of NF-κB/DNA binding activity as well as iNOS mRNA expression in these cells. Taken together, these findings demonstrate that compound **22** and its three close analogues inhibit NF-κB activation and iNOS mRNA expression by preventing IKKβ-NEMO interaction. These observations suggest that these compounds may represent an alternative strategy for pharmacological intervention in a number of chronic inflammatory diseases in which the IKK/NF-κB signaling pathway is dysregulated.

Ghosh and Hayden (2012). *Immunol Rev.* 246:5-13.
Ghosh and Karin M (2002). *Cell* 109:S81-S96.
Karin and Ben-Neriah (2000). *Annu Rev Immunol.* 18:621-635.
Xu et al. (2011). *Nature.* 472:325-30.
May et al. (2000). *Science.* 289:1550-4.
Strickland and Ghosh (2006). *Ann Rheum Dis.* 65 Suppl 3:iii75-82.
Lavecchia and Di Giovanni (2013). *Curr Med Chem* 20:2839-60.