Targeting the $P2X_7$ receptor in rheumatoid arthritis: biological rationale for $P2X_7$ antagonism

I.B. McInnes[1], S. Cruwys[2], K. Bowers[2], M. Braddock[2]

[1]Glasgow Biomedical Research Centre, University of Glasgow, Glasgow, United Kingdom; [2]AstraZeneca, Charnwood, Leicestershire, United Kingdom.

Abstract

Objective

This paper aims to explore the functional significance of the $P2X_7$ receptor in preclinical models of rheumatoid arthritis.

Methods

Preclinical studies in vivo were performed using the rat streptococcal cell wall (SCW) arthritis model. Ex vivo cultures of lipopolysaccharide (LPS)/benzoylbenzoyl adenosine triphosphate (BzATP)-stimulated human monocytes were generated to test the activities of a novel, highly specific inhibitor of human $P2X_7$, AZD9056, on interleukin (IL)-1 and IL-18 release.

Results

$P2X_7$ receptor expression was detected in inflamed synovial tissue after onset of SCW-induced arthritis in rats. Inhibition of $P2X_7$ therein led to reduced articular inflammation and erosive progression. No effect was noted on acute-phase responses. Ex vivo, AZD9056 inhibited IL-1 and IL-18 release to BzATP in LPS-primed human monocytes.

Conclusion

$P2X_7$ receptor inhibition could represent a novel approach to the treatment of inflammatory arthritis. However, confirmatory clinical studies are warranted to further explore this possibility.

Key words

rheumatoid arthritis, inflammation, ion channels, $P2X_7$ antagonism, AZD9056

Iain B. McInnes, MBChB, PhD
Simon Cruwys, PhD
Keith Bowers, PhD
Martin Braddock, PhD

Please address correspondence to:
Prof. Iain B. McInnes,
Glasgow Biomedical Research Centre,
University of Glasgow,
120 University Place,
Glasgow G12 8QQ, United Kingdom.
E-mail: iain.mcinnes@glasgow.ac.uk

Received on November 22, 2013; accepted in revised form on April 11, 2014.

Funding: this study was supported by AstraZeneca.

Competing interests:
I.B. McInnes has received grant funding and honoraria from AstraZeneca, F. Hoffmann-La Roche, Schering Plough, Bristol-Myers Squibb and Pfizer;
M. Braddock is an employee of AstraZeneca and owns stock;
S. Cruwys and K. Bowers are now former employees of AstraZeneca.

Introduction

Rheumatoid arthritis (RA) is an inflammatory disorder associated with chronic synovitis and progressive articular damage, significant co-morbidity and accelerated mortality (1-4). Pathogenesis-based therapeutic interventions have recently substantially altered the management of RA, primarily based on the advent of cytokine-blocking therapeutics targeting tumour necrosis factor-α and, subsequently, the introduction of B-cell-depleting therapy and co-stimulatory blockade. However, a significant proportion of patients exhibit partial or non-response to such biological agents, few patients achieve sustained remission, and protein-based biological agents remain expensive and must be delivered by parenteral routes (5, 6). Thus, substantial unmet clinical need remains. There is intense interest, therefore, in identifying novel pathological pathways in RA, particularly those that might be tractable to inhibitory small-molecule entities (SMEs) that are orally bioavailable. In particular, it would be desirable to generate SMEs that can inhibit cytokines known to be up-regulated and pro-inflammatory in RA synovitis.

Ion channels are increasingly implicated in multiple facets of host immune function in rodent models and *in vitro* systems (7-11). The P2X$_7$ receptor is a member of the P2 family of ligand-gated ion channels that has been implicated in numerous biological process involving immune regulation (reviewed in 12) and in bone biology (reviewed in 13, 14), making it a potentially interesting target for therapeutic intervention for the treatment of rheumatic disease. Recent studies further suggest a role for P2X$_7$ signalling in the pathogenesis of primary Sjögren's syndrome (15, 16), thus adding to a growing repertoire of autoimmune disease for which the biology may suggest P2X$_7$ receptor antagonism could provide effective therapies. The P2X$_7$ receptor binds extracellular nucleotides and is a low-affinity sensor of adenosine triphosphate (ATP). Extracellular ATP, in turn, represents a critical 'endogenous danger signal' of host tissue damage to the innate immune response (17, 18). P2X$_7$ agonism, acting together with pannexin-1 and via reduction of intracellular K^+ concentration, is permissive to NALP3 inflammasome assembly and subsequent caspase-1-dependent cleavage and release of interleukin (IL)-1β and IL-18 (17, 19, 20). The latter are up-regulated in rodent models of articular inflammation and in RA synovial membrane wherein they exhibit potent inflammatory properties (4, 21-23). We have explored the hypothesis that synovial P2X$_7$ receptor expression in RA synovial membrane provides a novel therapeutic target. We present *in vitro* and rodent *in vivo* model evidence for P2X$_7$ receptor expression and effector function.

Methods

In vitro culture methodology, streptococcal cell wall (SCW) rat arthritis model procedures and histology analyses are described online (see Supplementary Methods online).

Results and discussion

Validating P2X$_7$ as an inflammatory target in synovial membrane

We first confirmed the presence of P2X$_7$ protein expression in both the lining layer and interstitial regions of human RA synovial tissues (Supplementary Fig. 1). Similar patterns of expression were observed in synovial tissues derived from rats with established (day 6) SCW-induced arthritis (Supplementary Fig. 1).

To explore the functional importance of such expression, we used a novel, highly specific inhibitor of human P2X$_7$, namely AZD9056. AZD9056, a member of an adamantine amide series of compounds (Patent WO2004/074224) (24), was identified by high-throughput screening (25) and was further optimised to a highly potent and selective P2X$_7$ receptor antagonist. AZD9056 inhibited, in a concentration-dependent manner, IL-1β (Fig. 1a) and IL-18 (Fig. 1b) release induced in lipopolysaccharide (LPS)-primed human peripheral blood monocytes by the ATP analogue benzoylbenzoyl (Bz)ATP. Furthermore, IL-1β release from ATP-stimulated human blood (Fig. 1c) was also inhibited in a concentration-dependent manner by AZD9056. Crucially, in a

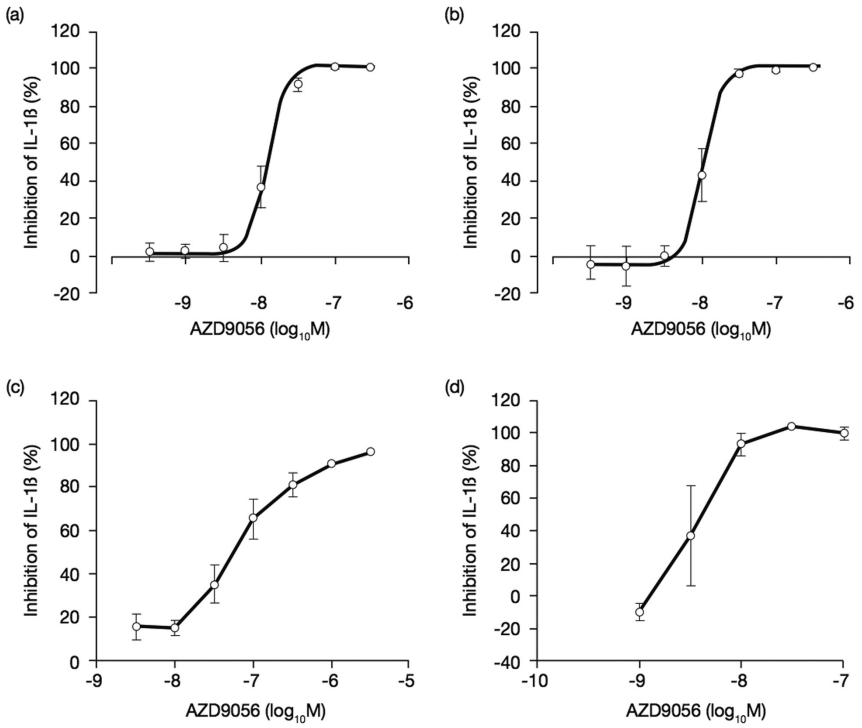

Fig. 1. Activity of P2X$_7$ antagonists against receptor and inhibition of ATP-stimulated cytokine release. **(a)** AZD9056 (0.3–300 nM) inhibited the BzATP (300 μM)-induced release of IL-1β from human isolated peripheral blood monocytes in a concentration-dependent manner with pIC$_{50}$ values (mean ± standard error of the mean [SEM]) of 7.9±0.1 (n=4). **(b)** AZD9056 (0.3–300 nM) inhibited the BzATP (300 μM)-induced release of IL-18 from human isolated peripheral blood monocytes in a concentration-dependent manner with pIC$_{50}$ values (mean ± SEM) of 8.0±0.1 (n=3). **(c)** AZD9056 (3–3000 nM) inhibited the ATP (3 mM)-induced release of IL-1β from human blood in a concentration-dependent manner with a pIC$_{50}$ value (mean ± SEM) of 7.2±0.1 (n=11). **(d)** AZD9056 (1–100 nM) inhibited the BzATP (1 mM)-induced release of IL-1β from human RA synovial cells in a concentration-dependent manner with a pIC$_{50}$ value (mean ± SEM) of 8.4±0.2 (n=3). This potency estimate was based on single determinations in three different donors to define a five-point concentration-response curve.

model using BzATP-stimulated human primary RA synovial cells, AZD9056 inhibited IL-1β release in a concentration-dependent manner (Fig. 1d). These *in vitro* results suggested that the presence of P2X$_7$ tissue expression could be of functional importance in sensing danger and in regulating effector cytokine production in RA.

P2X$_7$ antagonism ameliorates articular inflammation and damage in vivo

To explore the *in vivo* significance of this finding, we administered graded doses of the specific, orally bioavailable antagonist of rat P2X$_7$, AZ11657312, for 6 days after the induction of SCW arthritis in previously sensitised rats. Peak (2-hour) plasma concentrations for 10, 30 and 60 mg/kg treatment groups were 469±90, 1410±653 and 3363±105 ng/mL, respectively, representing satisfactory inhibitory concentrations (6-, 17.5- and 42-fold the pA2 for this compound, respectively). Trough (16-hour) plasma concentrations were markedly lower (30, 21, 35±19 and 69±118 ng/mL, respectively). Whereas a slight reduction in calliper ankle swelling (*vs*. vehicle control group; Fig. 2a), which primarily reflects oedema, was noted in AZ11657312 treatment groups, substantial and significant reduction in synovial membrane inflammation was evident by day 3 but particularly by day 6. Compared with controls, recipients of 30 or 60 mg/kg AZ11657312 exhibited reduction in synovitis, inflammation of synovial sub-lining, chondronecrosis and sub-chondral bone resorption, mainly in granulocytic precursors (Fig. 2b). Moreover, suppression of tissue inflammation and damage was dose-dependent. Consistent with this, radiographical examination of the tibiotarsal compartment indicated reduced articular damage in the 60 mg/kg group compared with controls (Fig. 2c). Importantly, we also observed dose-dependent inhibition of mechanical hyperalgesia (von Frey threshold) with 65% inhibition in the 60 mg/kg group (p<0.05, area under the curve *vs*. vehicle control; Fig. 2d), suggesting that the tissue and radiographical changes were clinically meaningful. No effect on plasma levels of the acute-phase reactant α1-acid glycoprotein (data not shown) was observed, consistent with predominant effects at a local tissue level. When administration of AZ11657312 was deferred until 1 day after onset of arthritis, we observed reduction in histologic scores compared with vehicle treated controls; these were generally of lesser magnitude and achieved significance only for the 60 mg/kg group for the synovial inflammation score, but not for chondronecrosis, or bone resorption.Commensurate with this radiographic damage scores were reduced but not by the same magnitude as noted with prophylactic dosing (data not shown). Together, these data indicate that P2X$_7$ mediates pro-inflammatory activity in the joint. It may also play a role in pain sensing in the joint although such effects cannot be separated from other direct central or peripheral effects operating via P2X$_7$ mediated pain pathways. Inhibition of this pathway however mediates optimal clinical benefit when initiated early.

The P2X$_7$ receptor is an ATP-gated ion channel primarily expressed on cells of the immune system. *In vitro*, it regulates monocyte release of pro-inflammatory IL-1 family cytokines via the NALP3 inflammasome (see Supplementary Fig. 2). Several effector cytokines of the IL-1 superfamily are present in RA synovium that exhibit pro-inflammatory potential in *ex vivo* model systems including IL-1, IL-18 and IL-33. In particular, IL-1β is a potent activator of synovial fibroblasts, chondrocytes and osteoclasts, and exhibits immune-regulatory activity especially in expanding Th17 cells that are also implicated in RA pathogenesis. However, IL-1 targeting alone, using a variety of biological inhibitors, has

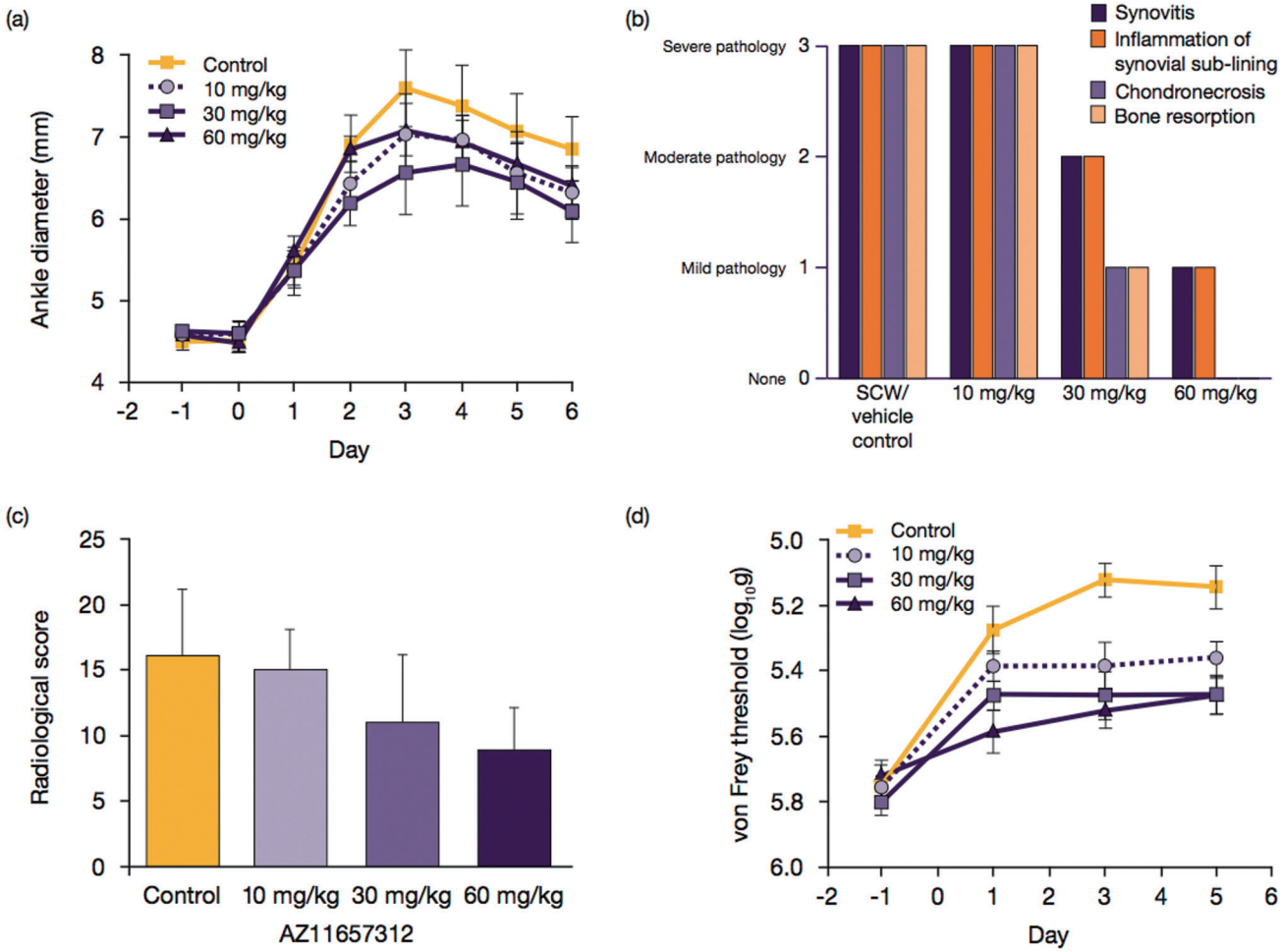

Fig. 2. Effect of a P2X$_7$ antagonist in the rat model of SCW-induced arthritis. Effect of AZ11657312 on: **(a)** ankle swelling; **(b)** pathology; **(c)** radiological outcome; and **(d)** mechanical hyperalgesia. Arthritis was induced as described in Supplementary Methods. AZ11657312 (10, 30 and 60 mg/kg) was administered by oral prophylactic dosing, starting 1 day before (day -1) induction of arthritis through to termination on day 3 or day 6 post-induction. **(a)** Ankle diameters were measured with vernier callipers on a daily basis from day -1. **(b)** Histological indices in SCW arthropathy were assessed on a four-point scale: normal, mild, moderate and severe pathology. This is defined in the methods section. Changes in histological indices were significant only at the 30 and 60 mg/kg dose levels. **(c)** For radiology, the tibio-tarsal compartment was examined. The articular compartment was divided into quandrants as described in Supplementary Methods online. Scoring was based on the presence or absence of a lesion in a quadrant and then a sum of scores made. The sum of radiographical scores were then combined for each joint and analysed by a Mann-Whitney U test. All other results are expressed as mean ± SEM. **(d)** Mechanical thresholds were assessed using von Frey filaments on days -1, 1, 3 and 5. The filaments were applied in increasing weights to the ankle region on the footpad of both feet. The first filament to induce a withdrawal response was considered to be the threshold. von Frey thresholds are described as the mean filament weight (g) and graphically presented as log10 of the force exerted.

not been clinically effective for reasons that are unclear. We hypothesised that the broader cytokine regulatory role of P2X$_7$ beyond IL-1, to include IL-18 and perhaps IL-33, might confer therapeutic utility in RA. This is a controversial area since intracellular inhibition of IL-1 family processing by caspase targeting has been disappointing. We considered that there remained merit in exploring additional approaches to modification of the IL-1 family *in vivo*, not least because caspase does not play an equally important role in processing distinct cytokine family members. Furthermore, whereas P2X7 would not likely directly influence TNF or IL-6 expression in monocyte series, indirect effects may occur to modify cytokines of proven therapeutic utility - P2X$_7$ expression on synovial fibroblasts was recently implicated in IL-6 release (26). Here, using a novel, specific antagonist of the human P2X$_7$ receptor AZD9056, which is a first-in-class agent, to target this ion channel, we provide evidence consistent with the notion that targeting the P2X$_7$ receptor could yield clinically meaningful improvement in a human autoimmune disorder.

SCW arthritis is a well-characterised model of inflammatory arthritis that integrates innate and adaptive immune components to drive articular damage. Due to exquisite species specificity of AZD9056, we used a synthologue inhibitor of rat P2X$_7$ to define the therapeutic potential of P2X$_7$ targeting in preclinical studies. P2X$_7$ antagonism suppressed synovial inflammation and radiographical damage and reduced mechanical hyperalgesia. Despite these significant effects on synovial tissues, no effect on the systemic acute-phase response was observed. These data are

consistent with known functions of IL-1, IL-18 and IL-33, including regulation of pathways integral to promoting synovial inflammation, articular matrix damage and nociception (19, 27) but limited effects on systemic IL-6 regulation. It was notable, however, that effects were diminished when inhibition was delayed until after the onset of substantial disease in the model. This is a rapid induction, aggressive monoarthritis model. The partial responses observed upon delayed inhibitor administration may reflect the multitude of inflammatory effector pathways operating in established disease, but indicate that P2X$_7$ at that stage mediates at least some, albeit reduced, contribution to events.

Recent advances in the treatment of RA have been remarkable, not least in the advent of biological agents that deliver substantial disease suppression and radiographical protection. There remains, however, considerable need for the development of novel SMEs that might reproduce the effects of biological cytokine blockade with advantages of convenience, tolerability or cost. These preclinical data provide evidence that were deemed sufficient to support targeting the P2X$_7$ pathway in human clinical trials (28).

Acknowledgements

The authors would like to acknowledge the following contributors: Alison Mackie, Jill Theaker, Mandy Lawson, Margaret McCormick, Malbinder Fagura, Elizabeth Rendall, Sandy Nicol and Anita Midha. The authors would like to thank Karen Brayshaw, PhD, at Complete HealthVizion for medical writing assistance in the preparation and revision of the draft manuscript, based on detailed discussion and feedback from all the authors. This medical writing support was funded by AstraZeneca.

References

1. KLARESKOG L, CATRINA AI, PAGET S: Rheumatoid arthritis. *Lancet* 2009; 373: 659-72.
2. GABRIEL SE: Cardiovascular morbidity and mortality in rheumatoid arthritis. *Am J Med* 2008; 121: S9-14.
3. SATTAR N, MCINNES IB: Vascular comorbidity in rheumatoid arthritis: potential mechanisms and solutions. *Curr Opin Rheumatol* 2005; 17: 286-92.
4. BRENNAN FM, MCINNES IB: Evidence that cytokines play a role in rheumatoid arthritis. *J Clin Invest* 2008; 118: 3537-45.
5. STRAND V, KIMBERLY R, ISAACS JD: Biologic therapies in rheumatology: lessons learned, future directions. *Nat Rev Drug Discov* 2007; 6: 75-92.
6. VAN VOLLENHOVEN RF: Treatment of rheumatoid arthritis: state of the art 2009. *Nat Rev Rheumatol* 2009; 5: 531-41.
7. HOGAN PG, LEWIS RS, RAO A: Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu Rev Immunol* 2010; 28: 491-533.
8. HAN J, KANG D: TRESK channel as a potential target to treat T-cell mediated immune dysfunction. *Biochem Biophys Res Commun* 2009; 390: 1102-5.
9. MATZA D, FLAVELL RA: Roles of Ca_v channels and AHNAK1 in T cells: the beauty and the beast. *Immunol Rev* 2009; 231: 257-64.
10. CAHALAN MD, CHANDY KG: The functional network of ion channels in T lymphocytes. *Immunol Rev* 2009; 231 :59-87.
11. FESKE S, PICARD C, FISCHER A: Immunodeficiency due to mutations in *ORAI1* and *STIM1*. *Clin Immunol* 2010; 135: 169-82.
12. WILEY JS, SLUYTER R, GU BJ, STOKES L, FULLER SJ: The human P2X$_7$ receptor and its role in innate immunity. *Tissue Antigens* 2011; 78: 321-32.
13. GROL MW, PANUPINTHU N, KORCOK J, SIMS SM, DIXON SJ: Expression, signalling and function of P2X$_7$ receptors in bone. *Purinergic Signal* 2009; 5: 205-21.
14. ROSENTHAL AK, GOHR CM, MITTON-FITZGERALD E, LUTZ MK, DUBYAK GR, RYAN LM: The progressive ankylosis gene product ANK regulates extracellular ATP levels in primary articular chondrocytes. *Arthritis Res Ther* 2013; 15: R154.
15. BALDINI C, ROSSI C, FERRO F *et al*.: The P2X7 receptor-inflammasome complex has a role in modulating the inflammatory response in primary Sjögren's syndrome. *J Intern Med* 2013; 274: 480.
16. LESTER S, STOKES L, SKARRATT KK *et al*.: Epistasis with HLA DR3 implicates the P2X7 receptor in the pathogenesis of primary Sjögren's syndrome. *Arthritis Res Ther* 2013; 15: R71.
17. DI VIRGILIO F: Liaisons dangereuses: P2X$_7$ and the inflammasome. *Trends Pharmacol Sci* 2007; 28: 465-72.
18. CHEN L, BROSNAN CF: Regulation of immune response by P2X$_7$ receptor. *Crit Rev Immunol* 2006; 26: 499-513.
19. DINARELLO CA: Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol* 2009; 27: 519-50.
20. MARIATHASAN S, WEISS DS, NEWTON K *et al*.: Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* 2006; 440: 228-32.
21. GABAY C, MCINNES IB: The biological and clinical importance of the 'new generation' cytokines in rheumatic diseases. *Arthritis Res Ther* 2009; 11: 230.
22. GRACIE JA, FORSEY RJ, CHAN WL *et al*.: A proinflammatory role for IL-18 in rheumatoid arthritis. *J Clin Invest* 1999; 104: 1393-401.
23. DAYER JM: The pivotal role of interleukin-1 in the clinical manifestations of rheumatoid arthritis. *Rheumatology* (Oxford) 2003; 42 (Suppl. 2): ii3-10.
24. WORLD INTELLECTUAL PROPERTY ORGANIZATION: (WO/2004/074224) Adamantane derivatives, processes for their preparation and pharmaceutical composition containing them. Available at: http://www.wipo.int/pctdb/en/wo.jsp?wo=2004074224&IA=SE2004000227&DISPLAY=DESC.
25. GUILE SD, ALCARAZ L, BIRKINSHAW TN *et al*.: Antagonists of the P2X$_7$ receptor. From lead identification to drug development. *J Med Chem* 2009; 52: 3123-41.
26. CAPORALI F, CAPECCHI PL, GAMBERUCCI A *et al*.: Human rheumatoid synoviocytes express functional P2X$_7$ receptors. *J Mol Med* 2008; 86: 937-49.
27. LIEW FY, PITMAN NI, MCINNES IB: Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat Rev Immunol* 2010; 10: 103-10.
28. KEYSTONE EC, MCINNES IB, WANG MW *et al*.: Clinical evaluation of the efficacy of the P2X$_7$ antagonist AZD9056 on the signs and symptoms of rheumatoid arthritis in patients with active disease despite treatment with methotrexate or sulphasalazine. *Ann Rheum Dis* 2012; 71: 1630-5.