An ADAM10 exosite inhibitor is efficacious in an in vivo collagen-induced arthritis model

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Abstract

Rheumatoid arthritis is a systemic autoimmune inflammatory disease which affects millions of people worldwide. There are multiple disease-modifying anti-rheumatic drugs available; however, many of the patients do not respond to any treatment. A disintegrin and metalloproteinase 10 has been suggested as a potential new target for RA due to its role in releasing of multiple pro- and anti-inflammatory factors from the cell surface of cells. In the present study we determined pharmacokinetic parameters and in vivo efficacy of a compound CID3117694 from a novel class of non-zinc-binding inhibitors.

Oral bioavailability was demonstrated in blood and synovial fluid after 10mg/kg dose. To test efficacy, we established the collagen-induced arthritis model in mice. CID3117694 was administered orally at 10, 30, and 50 mg/kg/day for 28 days. CID3117694 was able to dose-dependently improve disease score, decrease RA markers in blood and decrease signs of inflammation, hyperplasia, pannus formation, and cartilage erosion in affected joints as compared to the untreated control. Additionally, mice treated with CID 3117694 did not exhibit any clinical signs of distress, suggesting low toxicity. The results of this study suggest that ADAM10 exosite inhibition can be a viable therapeutic approach to RA.

INTRODUCTION

More than 1.3 million adults in the USA have been diagnosed with rheumatoid arthritis (RA) as of 2021 (1). RA is a systemic autoimmune inflammatory disease which manifests in multiple joints of the body and leads to their degradation. The inflammatory process primarily affects the lining of the joints (the synovial membrane) leading to irreversible bone erosion, joint deformity, and loss of function but can also affect other organs (2). This inflammatory process is driven by an imbalance of inflammatory (e.g., TNFα, IL-6, IL-1) (3, 4) and anti-inflammatory cytokines (e.g., IL-10, IL-Ra, soluble TNFR2, IL-11) (5–7) released from synoviocytes, cells that form the lining of the joints, and inflammatory cells (e.g., monocytes, neutrophils). The enzyme “a disintegrin and metalloproteinase 10” (ADAM10) has been identified as being involved in regulating the release (shedding) of cytokines from synoviocytes and other cell types (8, 9).

Expression of ADAM10 protein and mRNA was found to be elevated in the endothelial cells from RA synovial tissue, where it was shown to promote angiogenesis (9). ADAM10 was also shown to be upregulated in the serum of RA patients, with levels correlating with disease activity score (DAS28) (8). ADAM10 shedding of CD23 from B-cells prompts surface activated macrophages to release pro-inflammatory signals, which is proposed to lead to the progression of RA (10, 11). ADAM10 was found to positively mediate monocyte migration and adhesion to fibroblast-like synoviocytes (FLSs) (8), which were demonstrated to be a key cellular factor in RA (12). Loss of FLSs’ ability to secrete ADAM10 resulted in the decreased production of CX3CL1 and VEGF (8). Shedding of chemokines CX3CL1 and CXCL16 from human microvascular endothelial cells (HMVECs), FLS and macrophages by ADAM10 leads to the recruitment of immune and inflammatory cells thus promoting inflammation (9, 13, 14). ADAM10 was also shown to release TNFα and mediate release of IL-6 and IL-8 from FLS (13). ADAM10 siRNA
knockdown reduced RA-FLS proliferation, migration and invasion, secretion of VEGF-A and matrix metalloproteinase (MMP)-3 and -9 (13). In vivo, ADAM10 siRNA reduced RA score and VEGF-A, MMP-3, and MMP-9 levels in collagen-induced arthritis model (CIA) in mice (13). Based on these studies, ADAM10 represents an attractive target for RA drug development.

To the best of our knowledge there are no selective ADAM10-targeted RA therapies on the market or in development. RTD-1, a macrocyclic peptide inhibitor of ADAM17, is currently being evaluated by Oryn Therapeutics (15–17). Disease-modifying small molecule anti-rheumatic drugs (DMARDs) approved or under evaluation for therapy in RA include Janus kinase (JAK) inhibitors, TNF inhibitors, T cell costimulation blockers, IL-6 receptor inhibitors, promoters of B cell depletion, and interleukin 1 inhibitors. ACR70 score for these treatments has shown enhanced efficacy over methotrexate monotherapy (70% improvement, for responders), though the response rates are below 50% (18). Therefore, there is still an unmet need for drugs that would improve upon ACR70 efficacy and (especially) response rate alone or in combination with approved drugs. Based on this need, small molecule inhibitors of ADAM10 can be a novel and effective prevention or treatment therapy option.

Our group has previously reported the discovery of novel selective exosite-binding inhibitor of ADAM10, CID 3117694 (19). In the studies presented herein we report results of efficacy testing of CID 3117694 in the mouse CIA model of RA.

**MATERIALS AND METHODS**

**ADAM10 and ADAM17 assays.** Both assays were performed as published previously by us (19) Briefly, both assays followed the same general protocol. 5 µL of 2x enzyme solution (20 nM) in assay buffer (10 mM Hepes, 0.001% Brij-35, pH 7.5) were added to solid bottom black 384 plates (Nunc, cat# 264705). Next, test compounds and pharmacological controls were added to corresponding wells using a 384-pin tool device (V&P Scientific, San Diego). After 60 min incubation at RT, the reactions were started by addition of 5 µL of 2x solutions of glycosylated substrate DM2 (20 µM). Reactions were incubated at RT for 2 h, after which the fluorescence was measured using Biotek H1 multimode microplate imager ($\lambda_{\text{excitation}} = 360$ nm, $\lambda_{\text{emission}} = 460$ nm). $IC_{50}$ values were calculated by fitting normalized data to sigmoidal log vs. response equation utilizing non-linear regression analysis from GraphPad Prizm 6.

**Pharmacokinetics of CID 3117694.** All animal studies were conducted in accordance with NSU IACUC guidelines. Study protocol # 2021.04.DM2 was approved by NSU IACUC before the commencement of the studies. CID 3117694 was formulated in 10% DMSO, 40% PEG400, 30% PG, 20% H2O at 2 mg/mL and administered by intragastric (ig) gavage to Sprague-Dawley male rats at 10mg/kg. The animals were anesthetized by isoflurane. Blood and synovial fluid were collected at 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, and 24 h after administration of CID 3117694. Synovial fluid (SF) was collected using the 23-gauge needle connected to a mini peristaltic pump via perfusion tubing. Sterile saline was infused at a constant rate of 100 µl/min $^{-1}$. After infusion of 100 µl of vehicle (sterile saline), the outflow tubing was connected to the 25-gauge needle, to minimize pressure build-up within the joint space. Fluid was infused and
withdrawn at a constant rate until a 250 µl basal sample was collected in a 1.5 ml centrifuge tube. Samples were immediately frozen at -20°C. Blood was collected using 25G needle directly from the hearts. The samples were prepared for LC-MS/MS analysis using acetonitrile protein precipitation technique. Briefly, blank plasma and subject samples (plasma and SF samples) were retrieved from the deep freezer and allowed to thaw. The calibration curve standards (10 to 10,000 ug/mL of CID 3117694) and Quality Control samples were prepared using blank plasma. The thawed samples were vortexed to ensure complete mixing of contents. 50 µL of each sample were transferred to the vials and 200 µL of Internal Standard (IS, telmisartan) mixed with acetonitrile were added to all the samples and vortexed. Samples were kept on the shaker for 5 min to ensure complete mixing of contents. The samples were centrifuged at 4,000 rpm at 20°C for 10 min and the supernatant was transferred into auto injector vials and loaded into auto sampler. 5 µL was injected on to API-4500 Q TRAP LC-MS/MS system. The samples were separated on Phenomenex, Synergi C18 4.6 × 50 mm,4 µm HPLC column using 10mM ammonium acetate + 0.1% Formic acid buffer as solvent A and 100% Acetonitrile as solvent B. PK parameters were quantitated using WinNonLin (Certara, Princeton, NJ).

**In vivo efficacy in the CIA mouse model.** All animal studies were conducted in accordance with NSU IACUC guidelines. Study protocol # 2021.04.DM5 was approved by NSU IACUC before the commencement of the studies. CID 3117694 and indomethacin were ground with a small amount of the dispersing agent (0.5% HPMC (3 cP)–0.5% Tween 80), using a mortar and a pestle, and mixed repeatedly to form a smooth paste. The paste was moved to a graduated cylinder and filled up to the required volume with the dispersing agent to form a suspension as described elsewhere (20). Test compound (10, 30, & 50 mg/kg) and Indomethacin (2.5mg/kg) were administered with mouse gavage needle (gauge no: 18, BD, catalog number: 653902) through oral route as suspension with 5 mL/kg dose volume (from day 0 to day 28).

6-8-week-old C57 BL/6 mice from Charles River (male and female) were used in the study. Animals were randomly assigned to six groups: (1) normal (non-RA control), (2) RA control untreated, (3) RA control treated with 2.5 mg/kg/day indomethacin, and 3 test groups (CID 3117694 (10mg/kg/day, 30mg/kg/day, and 50mg/kg/day)) to provide an estimate for lead compound's efficacy range. Each group had three males and three females assigned to control for gender-based differences.

Arthritis was induced to right paw of the mice under anaesthesia. Left paw of the mice was used as a control. A total of 10 µl collagen from bovine (type II collagen) nasal septum emulsion (2 mg/ml) was injected into the right knee joint with glass Hamilton syringe and 25G needles for intraarticular (i.a.) injection, followed by a total of 100 µl Complete Freund’s adjuvant (CFA) emulsion injected at the back of mice using the syringe with 25G needles subcutaneously (s.c.). On day 14 after the first injection, the mice were injected i. a. with 10 µl of collagen from the bovine nasal septum (2 mg/kg) and s.c. with 100 µl CFA emulsion for boost injection.

Arthritis progression was monitored using a clinical arthritis scoring system (0-4) and paw swelling was measured with help of a plethysmograph on day 0, 14, 21 and 28 after induction of arthritis. To assign a
clinical score, interphalangeal, metacarpophalangeal, and carpal and tarsal joints were observed for swelling and redness. Normal joints were assigned score 0, in case when only one joint type was affected the score was 1, in case when two joint types were affected the score was 2, in case when three joint types were affected the score was 3, maximal redness and swelling of the entire paw with the loss of anatomic definition resulted in a score of 4.

After administration of primary dose of collagen from the bovine nasal septum and CFA scoring was assessed. Measurements were taken on day 0, 14, 21 and 28 after induction of arthritis in induced paws (right paw) and non-arthritis paws (left paw). CID 3117694 (10 mg/kg/day, 30 mg/kg/day, and 50 mg/kg/day) and pharmacological control (indomethacin 2.5 mg/kg/day) treatment was given every day for 28 days beginning from day 1 by oral gavage in hydroxy-propyl-methyl cellulose (HPMC) suspension.

Mice were weighed and observed every day and notes were made on the signs of distress. More specifically, mice were observed for posture, vocalization, ease of handling, lacrimation, chromodacryorrhea, salivation, coat condition, unsupported rearing, arousal, piloerection, motor movements, diarrhea, tail pinch reaction, constipation, and death.

The sample size for therapeutic evaluation was calculated by power analysis based on experimental data from similar studies. In a two-sided test and setting α value at 0.05 and desired power at 0.95, the sample size turned out to be 5 per group. We also expected deaths during experiments; therefore, we used the formula, Nt = N/1-q, where Nt is the number of mice initially used, N is the number required at the end of the study and q is the proportion of attrition which is generally 10%. Thus, at the end of the study period we expected to have data from at least 6 mice per group which is enough to attain statistical significance, if exists. Data was analyzed using a one-way ANOVA followed by Dunnett's test compared with Vehicle Control. At the end of the study, the mice were anesthetized and sacrificed by CO₂ euthanasia. The hind paws of the mice were subjected to histopathological examination and the serum was analyzed for CRP, TNF-α, IL-6, and IL-10 as described in below sections.

All the data was expressed as mean ± SEM and statistically analyzed by IBM-SPSS version 20 software using one-way ANOVA followed by post hoc Dunnett-t test at different variance levels. A p-value of 0.05 or less was considered statistically significant.

**RA serum marker analysis.**

Blood was collected from the tail vein, 100 µL was used for the assays. All assays were conducted using manufacturer’s instructions for the respective assay kits. The following assay kits were purchased: Mouse TNF-α GENLISA™ ELISA (Krishgen BioSystems cat# KB2145), Mouse IL-10 GENLISA™ ELISA (Krishgen BioSystems cat# KB2072), Mouse hsC-Reactive Protein, hs-CRP GENLISA™ ELISA (Krishgen BioSystems cat# KLM0318), Mouse IL-6 GENLISA™ ELISA (Krishgen BioSystems cat# KB2068).

**Histology.**
Limbs from euthanized animals were preserved in buffered formalin, decalcified, embedded, sectioned, and stained with hematoxylin and eosin (H & E). Microscopic images were acquired with Lx 400 microscope (Labomed, Fremont, CA).

RESULTS

CID 3117694 profiling suggests a high level of ADAM10 target selectivity. Searches of the PubChem database for biological activity of CID 3117694 revealed it to be inactive in 524 reported bioassays and active against only 3 targets, with ADAM10 being the top target (PubChem AID 743338). A second target was the cardiac ion channel hERG, though it is not a hERG inhibitor (a risk for cardiotoxicity). Rather, CID 3117694 was shown to be cardioprotective, diminishing the effects of pro-arrhythmic agents (PubChem AID 1511, no EC$_{50}$ value reported). The third known target of this molecule is DNA polymerase β (PubChem AID 485314) where CID3117694 was only very weakly active (IC$_{50}$ = 79 µM). Among a variety of drug safety targets studied, it is inactive against adrenergic (ADRB2), muscarinic (CHRM1) and opioid receptors (OPRK1, OPRM1, and OPRD1) (21). It lacks T-cell based effects, as reflected by being inactive in an assay for activators of T cell receptors (see PubChem bioassay 504894). It also does not affect caspase 8 activity. These data show that CID 3117694 is not target-promiscuous, a feature which should translate into low off-target in vivo toxicity, an attribute that is particularly noteworthy for potential RA therapeutic leads and in generally quite rare for inhibitors of Zinc metalloproteases.

Table 1. Structural analogs of CID 3117694 were acquired from Chembridge, with activity measured as indicated.
CID 3117694 structure-activity relationship (SAR) study. We purchased several commercially available analogs of CID 3117694 to determine the features important for ADAM10 binding. Substitutions of the aryl *meta* methoxy moiety in the R2 position (circled, Table 1, entry 1) for *para* tert-butyl, combined with various substitutions in the R1 position (entries 2–4) were not tolerated and resulted in the total loss of activity against ADAM10, suggesting an importance of this moiety. Introduction of a chlorine in the *para* position of R2 (Table 1, entry 7) did not rescue the activity against ADAM10, suggesting that future SAR studies should preserve the methoxy or a similar group in the *meta* position. Simultaneous introduction of an *ortho* methyl in R2 and 2-naphthyl group as R1 rescued activity of ADAM10; however, selectivity against ADAM17 was lost (Table 1, entry 5). Similar to entry 5, entry 6 substituted for o-Cl for *m*-OMe in R2 and an o-OMe in the R1 phenyl, and showed no selectivity and reduced affinity. These SAR data will guide our future efforts for optimization of CID 3117694.
Pharmacokinetics of CID 3117694 in Sprague-Dawley male rats. CID 3117694 structure was confirmed by H1 NMR (Fig. S1) and it was determined to be >95% pure by HPLC (Fig. S2). To determine if CID 3117694 can be used in vivo RA efficacy model studies, we conducted an in vivo pharmacokinetic (PK) study. CID 3117694 was formulated in 10% DMSO, 40% PEG400, 30% PG, 20% H2O at 2 mg/mL and administered by oral gavage to Sprague-Dawley male rats at 10 mg/kg. Blood and synovial fluid were collected at 15 min, 30 min, 1,2,4,8 and 24 h and quantitated by LC/MS. PK parameters were calculated by WinNonLin software. As can be seen in Fig. 1 and Table 2, Cmax (maximal concentration) in plasma and synovial fluid (SF) was reached at 1 hr after administration. Cmax in SF and plasma was 849 nM and 18.4 µM, respectively, and AUC (average drug exposure) was 3.04 µM and 91.4 µM respectively. The Ki value of CID 3117694 for ADAM10 inhibition is 883 nM (19) suggesting that the compound penetrates the synovium to reach a potentially therapeutically relevant concentration. As mentioned above, CID 3117694 was tested against multiple cell lines at a high concentration (10 µM and up to 100 µM in some cases) and showed no effect on cellular viability. This observation, in combination with the above-mentioned evidence for minimal off-target binding, suggest a suitable safety margin may exist for in vivouse.

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In vivo efficacy in the CIA mouse model. Encouraged by the results of the PK study, we proceeded to an efficacy and dose-finding study using the collagen-induced arthritis (CIA) model. To enable the delivery of CID 3117694 at doses 10 mg/kg, 30 mg/kg, and even 50 mg/kg doses, we identified a modified formulation that uses hydroxy-propyl-methyl cellulose (HPMC) to augment solubility.

We began administering both control and test compound simultaneously with RA induction on day 0. On day 14 after RA induction the clinical score was 3 for all groups, with exception of normal control, suggesting that no test dose was effective at this time point in preventing disease progression (Fig. 2A). By day 21, however, a divergence in efficacy was first noted. The disease score for vehicle control and for a 10 mg/kg dose of CID 3117694 had increased to 4, while indomethacin and a 50 mg/kg dose of CID 3117694 lowered the score to 2. The 30 mg/kg CID 3117694 dose animals were scored at 3. The 28-day timepoint showed more pronounced effects: the 10 mg/kg and 30 mg/kg doses of CID 3117694 lowered...
the disease score to 2, while indomethacin and a 50 mg/kg dose of CID 3117694 lowered the disease score to 1 (Fig. 2A, B, and C).

Targeting zinc-binding metalloproteases has historically been a difficult drug development strategy, especially when using Zn-binding small molecules, due primarily to the challenge of overcoming toxicity arising from off-target binding (22). We felt that CID 3117694, which does not bind zinc, may have advantages with respect to target fidelity and lowered demonstrated toxicity in animals. Body weight, a gross indicator of animal distress and toxicity, was monitored during the in vivo study, along with any other behavioral signs of distress. As evidenced by Fig. 3A and B, no significant differences in body weight between the vehicle control and treatment groups were observed, suggesting that CID 3117694 did not impact feeding behavior, which could arise from many effects, including the induction of nausea. We also observed no clinical signs of distress (e.g., change in behavior or appearance) that typically can be attributed to drug toxicity.

A hallmark of RA, paw swelling, was monitored on days 1, 14, 21, and 28 of the study (Fig. 3CD). CID 3117694 was able to time- and dose-dependently decrease paw swelling. Moreover, the highest dose of CID 3117694, 50 mg/kg/day, decreased the swelling to the level of the normal control (Fig. 3D).

To determine the effect of CID 3117694 on molecular markers of inflammation in RA in blood, we performed ELISA assays for TNFα, C-reactive protein (CRP), IL-6, and IL-10. As can be seen in Fig. 4, CID 3117694 dose-dependently decreased all four markers. Noteworthy, the highest tested dose (50mg/kg/day) had decreased the markers to the level of the healthy control.

We also evaluated the effect of CID 3117694 on the histology of articular joints affected by RA. Histopathological examination revealed significant inflammation, hyperplasia, pannus formation, and cartilage and bone erosion of the untreated RA mice as compared to healthy mice consistent with RA scores (Fig. 5AB). Indomethacin-treated mice exhibited only residual inflammation and hyperplasia, which was significantly reduced as compared to untreated RA mice (Fig. 5C). 10 mg/kg/day of CID 3117694 did not have a pronounced effect on RA markers (Fig. 5D). 30 mg/kg/day of CID 3117694 reduced erosion, but not the rest of the symptoms (Fig. 5E), while 50 mg/kg/day of CID 3117694 showed significant reduction of RA hallmarks in the majority of samples (Fig. 5F) comparable to indomethacin. Overall, CID 3117694 dose-dependently alleviated histopathological hallmarks of RA suggesting its potential for therapy.

**DISCUSSION**

CID 3117694 demonstrated efficacy in the treatment of RA mice as evidenced by clinical, molecular, and histological markers of RA. Its excellent selectivity profile against related metzincins (19) and limited off-target interactions (discussed above in “CID 3117694 profiling suggests a high level of ADAM10 target selectivity” section) suggest that its mechanism of action is based primarily on ADAM10 inhibition. This, in turn, means that CID 3117694 can be a highly useful in vivo probe to study the role of ADAM10 in RA progression using various models. As discussed in the Introduction, ADAM10 has been shown to shed
multiple substrates from the surface of inflammatory cells implicated in RA development and progression. Some of these substrates (e.g., TNFα, IL6) can also be shed by its closest analogue, ADAM17 (also known as TNFα Converting Enzyme (TACE) (23, 24), and, possibly, by other enzymes. Data presented here indicate that inhibition of ADAM10 is sufficient to stop RA-induced shedding of TNFα, which, in turn, suggests that ADAM10 is a primary sheddase of TNFα and other tested cytokines in this RA model. Therefore, studies using CID 3117694 can help determine which enzyme cleaves substrates with multiple cognate sheddases.

ADAM10 both sheds and mediates shedding of multiple proteins from the cell surface (25–30) some of which have a known role in RA, such as TNFα and IL6 (13), CXCL16 (31), to name a few. The present study demonstrates the ability of the ADAM10-selective inhibitor CID 3117694 to decrease levels of some of these molecules in blood, suggesting abrogation of shedding and, therefore, successful target modulation. One potential implication of this result is an ability to use these molecules as ADAM10 pharmacodynamic (PD) markers in future RA pre-clinical and clinical studies.

CID 3117694 is an exosite-targeting inhibitor that exhibited unusual substrate selectivity in in vitro experiments (19) suggesting that not all ADAM10 substrates might be protected by the administration of this compound in vivo. In the present proof-of-principle study we only assessed levels of some of the RA-relevant inflammatory molecules. To ascertain the full profile of substrates protected by CID 3117694, a larger proteomic study will be needed. However, despite potentially limited substrate-protective effect, CID 3117694 exhibited disease-modifying properties that warrants further studies.

A potential limitation of this study is the lack of detailed knowledge about how all ADAM10 substrates affect RA progression. As discussed in Introduction, ADAM10 sheds both pro- and anti-inflammatory molecules and knowing a compound-specific shedding inhibition profile could help determine the relative importance of these substrates in RA progression and identify opportunities for safe therapeutic intervention. While no signs of toxicity attributable to CID 3117694 were observed during the 28-day CIA study, more extensive toxicological evaluations are required, to gauge effects of longer periods of ADAM10 inhibitor administration.

Conclusions

The results of this study suggest that ADAM10 exosite inhibition can potentially be a viable therapeutic approach to the treatment of RA. Additionally, CID 3117694 can be a useful tool to study the role of ADAM10 in various in vitro and in vivo models of diseases.

Abbreviations

ADAM10 - A disintegrin and metalloproteinase 10

RA - rheumatoid arthritis
CIA - collagen-induced arthritis

TNFα - tissue necrosis factor α

IL-6 – interleukin 6

IL-1 – interleukin 1

IL-10 – interleukin 10

IL-Ra – interleukin receptor unit a

sTNFR2 - soluble tissue necrosis factor receptor 2

IL-11 – interleukin 11

DAS28 - disease activity score

Declarations

Ethical Approval and Consent to Participate: Arrive guidelines were followed in reporting animal study results. All animal studies were conducted in accordance with NSU IACUC guidelines. Study protocol # 2021.04.DM5 was approved by NSU Institutional Animal Care and Use Committee (IACUC) before the commencement of the studies.

Consent for publication: All authors reviewed and approved the manuscript.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Competing interests: The author(s) declare no competing interests.

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Author contributions:

Conceptualization: DM, MES

Investigation - SAR study: TBD, DM, JD

Investigation - PK study: DM, JD

Investigation - CIA study: DM, JD

Investigation - cheminformatics study: DM
References


**Figures**

**Figure 1**

CID3117694 oral dosing at 10 mg/kg in male SD Rats

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CID 3117694 penetrates synovium at therapeutically relevant concentration. 24 h time point not shown due to compound being below the limit of quantitation (LOQ).

Figure 2

CID 3117694 lowers RA clinical score. (A) RA clinical scores over time and on day 28 (B) Red arrows indicate the day of RA induction and booster injection. Note – all clinical scores were uniform within each group which resulted in SD = 0 which does not allow to perform statistical significance analysis. (C) Clinical score assignment on the last day of treatment (day 28). Panel 1 – normal control. Red arrows point to the joints used for clinical score assignment (A – interphalangeal, B – metacarpophalangeal, C – carpal and tarsal), panel 2 – disease vehicle control, panel 3 – disease treated with 2.5 mg/kg indomethacin, panel 4 - disease treated with 10 mg/kg CID 3117694, panel 5 - disease treated with 30 mg/kg CID 3117694, disease treated with 50 mg/kg CID 3117694.
Figure 3

C57BL/6 mice do not exhibit overt toxicity after treatment with CID 3117694. (A) Body weight measurements over the course of the treatment demonstrate the overall weight gain in all mice. Mice were treated perorally with indomethacin and CID 3117694 3x/week for 28 days. Black arrows indicate days of RA induction and booster injections. The red arrow indicates the day the mice were euthanized. (C) Paw thickness measurements over the course of the treatment demonstrate time-dependent improvement in all mice treated with CID 3117694; (D) Paw thickness measurements on the last day of the treatment (day 28) shows no significant differences between the vehicle control and treatment groups and dose-dependent improvement in all mice treated with CID 3117694. *** - p value < 0.001, ns - no significance (p value > 0.05). (B) CID 3117694 decreases thickness of arthritis-affected right paw at all
tested doses; Data show mean ± SD (n=6) analyzed using a one-way ANOVA followed by Dunnett's test compared with Vehicle Control.

Figure 4

CID 3117694 is efficacious in decreasing the molecular markers of RA in the collagen-induced model. (A) CID 3117694 lowers concentration of TNFα in blood of arthritic mice at all tested doses; (B) CID
3117694 lowers concentration of CRP in blood of arthritic mice at 30 and 50 mg/kg; **CID 3117694 lowers concentration of IL-6 in blood of arthritic mice at all tested doses; ***CID 3117694 lowers concentration of IL-10 in blood of arthritic mice at all tested doses. ns – no significance, * - p value < 0.05, ** - p value < 0.01, *** - p value < 0.005, **** - p value < 0.001. Data show mean ± SD (n=6) analyzed using a one-way ANOVA followed by Dunnett's test compared with Vehicle Control.

**Figure 5**

**CID 3117694 decreases histopathological markers of RA.** (A) Normal control; (B) Vehicle control; (C) Indomethacin 2.5 mg/kg/day; (D) 10 mg/kg/day CID 3117694; (E) 30 mg/kg/day CID 3117694; (F) 50 mg/kg/day CID 3117694. Shown images are representative H&E-stained sagittal sections of the arthritis joint of the hind paw examined by light microscopy at 100x magnification. 6 slides per test group were examined. White arrows: normal healthy bone & cartilage of arthritis joint; red arrows: synovial inflammation; black arrows: hyperplasia; green arrows: pannus formation; yellow arrows – cartilage erosion.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- MinondADAM10RAsuppl.docx