Bioorganic & Medicinal Chemistry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Structure–activity relationships of pyrazole derivatives as potential therapeutics for immune thrombocytopenias

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ARTICLE INFO

Article history:
Received 18 January 2014
Revised 1 March 2014
Accepted 10 March 2014
Available online xxxx

Keywords:
Immune thrombocytopenia
Pyrazole derivatives
Phagocytosis inhibitors
Structure–activity relationship

ABSTRACT

Idiopathic or immune thrombocytopenia (ITP) is a serious clinical disorder involving the destruction of platelets by macrophages. Small molecule therapeutics are highly sought after to ease the burden on current therapies derived from human sources. Earlier, we discovered that dimers of five-membered heterocycles exhibited potential to inhibit phagocytosis of human RBCs by macrophages. Here, we reveal a structure–activity relationship of the bis-pyrazole class of molecules with –C–C–, –C–N– and –C–O– linkers, and their evaluation as inhibitors of phagocytosis of antibody-opsonized human RBCs as potential therapeutics for ITP. We have uncovered three potential candidates, **37**, **47** and **50**, all carrying a different linker connecting the two pyrazole moieties. Among these compounds, hydroxypyrazole derivative **50** is the most potent compound with an IC_{50} of 14 ± 9 μM for inhibiting the phagocytosis of antibody-opsonized human RBCs by macrophages. None of the compounds exhibited significant potential to induce apoptosis in peripheral blood mononuclear cells (PBMCs). Current study has revealed specific functional features, such as up to 2-atom spacer arm and alkyl substitution at one of the N^1 positions of the bivalent pyrazole core to be important for the inhibitory activity.

1. Introduction

Immune cytopenias are clinical disorders characterized by the production of antibodies to specific hematopoietic cells in the blood.[1–4] Under these conditions, the cells become opsonized with antibodies and are subsequently recognized by the Fcγ-receptors (FcγR) on the mononuclear phagocyte membrane. Such recognition by monocyte–macrophages (Mφ) results in the phagocytosis and intracellular destruction of these cells. This process can create serious and sometimes life-threatening complications for these patients. Examples include warm autoimmune hemolytic anemia (AIHA; involving the phagocytosis of antibody-coated red blood cells), idiopathic/immune (autoimmune) thrombocytopenia (ITP; involving the phagocytosis of antibody-coated platelets) and alloimmune hemolytic anemia (involving the phagocytosis of donor red blood cells transfused into patients having alloantibodies to the donor red cell antigens (HTR) and infants at risk of hemolytic disease of the fetus and newborn (HDFN) due to the maternal antibodies crossing the placenta). In ITP, platelets are destroyed by autologous antibodies and/or the production of platelets is very low.[5]

Current therapies for the treatment of severe cases of ITP include administration of steroids, splenectomy, Rituximab (anti-CD20), thrombopoietin receptor agonists and administration of immunoglobulins.[6,7] Intravenous immunoglobulin (IVIg) and anti-D immunoglobulin are both used with varied success to treat immune cytopenias.[8,9] However, both IVIg and anti-D are a limited resource[10] due to their acquisition from human donations. Treatment with IVIg is also more expensive than that with anti-D,[11] primarily due to the large quantity of IVIg (several grams) that is required for effective therapy; the usual induction dose is 2 g IVIg/kg body weight, which may be followed by maintenance therapy as needed. IVIg cost per individual could be as much as $15,000 or higher, and shortage of IVIg is a major concern due to the need

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http://dx.doi.org/10.1016/j.bmc.2014.03.016
0968-0896/

for blood donation by thousands of individuals. Side effects of IVIg are usually mild, with headaches, sometimes severe, being the most debilitating; however, sometimes side effects can be severe and even life threatening.[12] IVIg preparations, because of their human source material, may also carry some risk of transferring blood-borne diseases, particularly any new, emerging blood-borne infections. An alternate therapy, anti-D is required in much less quantity in comparison to IVIg for successful treatment of ITP; however, the reason for this remains unknown. Serious complications from anti-D treatments, although rare, can be more dangerous than those that may occur with IVIg and include increased morbidity due to hemolysis, sometimes so severe that it had resulted in patient mortality.[13–16] Thus, development of novel, cheaper, safer and more efficient ways of treating immune cytopenias is warranted, and blood services agencies globally are in much need of such alternate therapies.

The pathophysiology of immune cytopenias, such as ITP, is due to extravascular phagocytosis of the antibody-opsonized blood cells which results in diminished levels of clinically important cells in the circulation.[16] As the cell destruction is due to recognition of the Fc portion of the opsonizing antibody by the FcγRs on phagocytes, a potential treatment modality would be to develop inhibitors of this antibody-driven FcγR-mediated phagocytosis.

Limited efforts were expended to find replacements for IVIg over the years despite an acute need for such therapeutic agents. A set of molecules carrying sulfhydryl and disulfide functional groups were evaluated for their ability to inhibit the phagocytosis of opsonized blood cells by macrophages.[17–22] It was hypothesized that sulfhydryl and disulfide functional groups found on the small molecules interact with the sulfhydryl or disulfide groups on the surface of phagocytes and thus inhibit the phagocytosis of opsonized red blood cells (RBC).[14,23,24] Among these compounds, a pyrazole derivative **1** (Chart 1) exhibited weak activity at 1 mM concentration inhibiting phagocytosis of the opsonized RBCs.[17] A library of derivatives designed based on **1** led to the isoxazole derivative **2**, with moderate activity (IC_{50} of 250 μM), and to the discovery that the homodimers **3–6** containing a disulfide bridge were more potent inhibitors of RBC phagocytosis.[25] It was observed that the disulfide bridge is more important than a thiol or other forms of polysulfide linkers to elicit the inhibition of phagocytosis.[25] Substitution at C4 of the pyrazole moiety in the disulfide-bridged dimers influenced the potency of these inhibitors. A novel scaffold **7** with no-substitution at the C4 position, exhibiting inhibitory activity with an IC_{50} of 0.1 μM for opsonized RBC phagocytosis, was identified as a potential candidate for further investigations.[25] This also led to the hypothesis to consider designing 'drug-like' chemical structures without a disulfide bridge linking the ligands to target ITP and other similar disorders. Here, we reveal the structure–activity relationships of the pyrazole class of molecules, including compounds replacing the disulfide bridge with –C–C–, –C–N– and –C–O– linkers, and their evaluation as inhibitors of phagocytosis of antibody-opsonized RBCs as therapeutics for ITP.

2. Chemistry and molecular design

A bioisosteric methodology was employed to build structure–activity relationships due to the lack of structural information on the target or large amount of data on the ligands diversity. We considered the data accumulated so far on the thiol and disulfide carrying pyrazole derivatives such as compounds **1–7** which could be modified in a number of ways to replace the disulfide linker, as well as improve the physicochemical properties of the compounds. As a first step for the structure–activity relationships, several modifications to structure **7** were considered: (a) substitution of the disulfide bridge to understand the tolerance and the necessity of this group, (Chart 1B, –X– = $–CH_2–$, $–C_2H_4–$, $–CH_2O–$, $–CH_2NHCH_2–$, –O–, $–OC_2H_4O–$), and (b) substitutions at N^1 on one of the pyrazole moieties to explore the role of substitutions ranging from simple hydroxyl group through cyclopentyl and cyclohexyl moieties (Chart 1B, R = $–CH_2OH$, –OH, –H, $–C_2H_4NH_2$, –Ph, –cyclopentyl, $–CH_2$-cyclohexyl). Thus, compounds **14**, **15**, **18**, **20**, **24**, **28**, **32**, **37**, **41**, **47–50**, and **53** were synthesized carrying the variations described above (Schemes 1–8).

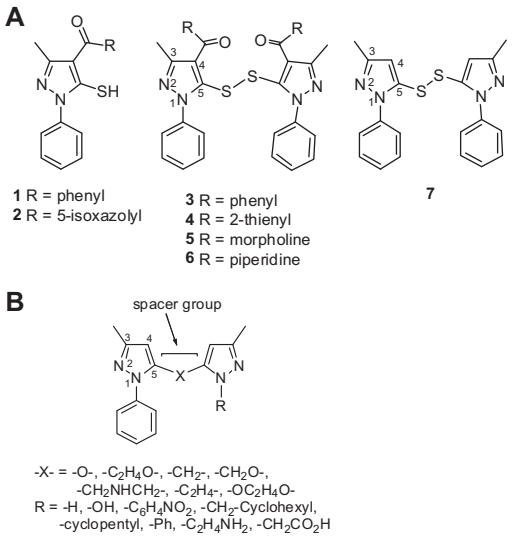
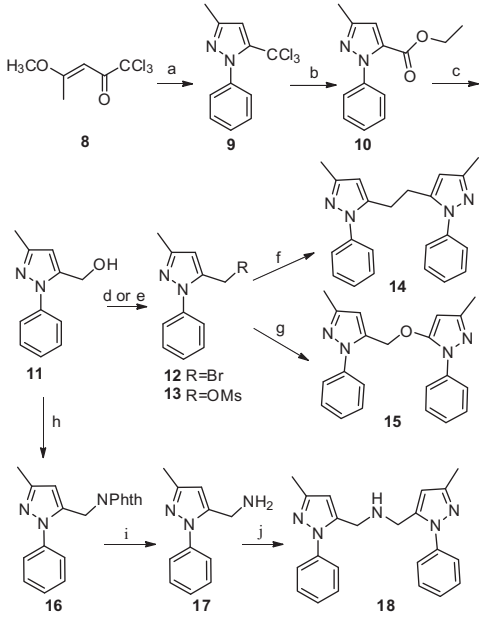


Chart 1. (A) Chemical structures of first generation derivatives inhibiting phagocytosis of opsonized human red blood cells. (B) Structural modifications incorporated into the pyrazole backbone.



Scheme 1. Synthesis of compounds **14**, **15** and **18**. Reagents and conditions: (a) Phenylhydrazine, toluene, μW at 85 °C, 5 min; (b) ethanol, pyridine, reflux, 2 h; (c) $LiAlH_4$, anhyd tetrahydrofuran, 0 °C to rt, 30 min; (d) PBr_3, toluene, reflux, 2 h; (e) methylsulfonyl chloride, Et_3N, anhydrous dichloromethane, reflux, 2 h; (f) magnesium turnings, anhyd tetrahydrofuran, μW, 100 °C, 1 h, additional stirring at rt, 42 h; (g) edaravone (**19**), Cs_2CO_3, anhydrous acetonitrile, 60 °C, 2 h, (h) PPh_3, DEAD, anhydrous tetrahydrofuran, 0 °C to rt, 48 h; (i) $NH_2NH_2.H_2O$, methanol, 0 °C to rt, 16 h; (j) compound **12**, K_2CO_3, acetonitrile, 60 °C, 2 h.