Synthesis and Evaluation of The Thrombolytic Activity of Novel Condensed Pyrimidine Sulfonamide Derivatives

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ABSTRACT
Pyrimidine-5-sulfonamides are new organic compounds of wide spectrum activity. A new trend is to incorporate a salicylate moiety in an attempt to enhance a thrombolytic activity. We started this strategy using 2-thiouracil 1 as a blocking unit by its ability to be chlorosulfonated at 5th position by refluxing with chlorosulfonic acid giving sulfonyl derivative 2 which was condensed with three common aromatic amines namely methyl-4-amino salicylate, methyl-3-aminobenzoate and methyl-4-aminobenzoate respectively yielding sulfonamide derivatives 3a-c. Moreover, 3a-c derivatives were reacted with chloroacetic acid-producing thiazolopyrimidines 4a-c, which in turn were reacted with p-nitrobenzaldehyde giving aryldine derivatives 5a-c. Also compounds 3a-c were cyclocondensed with anthranilic acid yielding pyrimidoquinazoline derivatives 6a-c. They also were condensed with ethanolamine forming imidazopyrimidine derivatives 7a-c. All the newly synthesized compounds were subjected for thrombolytic evaluation and some of them showed promising activity especially those bearing methyl salicylate moiety as compared to ticlopidine and clopidogrel. We performed different modifications in the thienopyridine chemical structure of ticlopidine and clopidogrel to conclude that the presence of second nitrogen atom in the pyridine ring (yielding pyrimidine moiety) and the presence of the other cycle instead of thiethyl ring would lead to enhanced the thrombolytic effect which was maximized by incorporation of a methyl salicylate moiety. We concluded that 4a, 6a, 8a, 7a, 3a compounds have more potent than both clopidogrel and ticlopidine respectively. They certainly deserve further studying in the future.

Key Words: Antiplatelet, condensed pyrimidine sulphonamides; synthesis and evaluation

INTRODUCTION
Interest has increased in the recent period to discover and synthesize new drugs as thrombolytic agents, and except for aspirin, few have come into clinical application (Joseph 2011). In 1978, Ticlopidine was firstly used as thrombolytic drug through blocking adenosine diphosphate (ADP) receptor (Panak et al. 1983) for treatment strokes and coronary stent occlusions (Gent et al. 1989; Robless et al. 2001). This effect can’t be observed in vitro and is detectable ex vivo only many hours after oral administration, this effect may be due to the formation of unstable metabolite. After that in 1996, it was observed that the ingestion of ticlopidine, at a dose of 500 mg by patients suffering from peripheral arterial diseases (Gryglewski et al. 1996; Gryglewski et al. 1978) was accompanied by an immediate fibrinolytic action shown by shortening of euglobulin clot lysis time. The rare side effects but it serious of aspirin such as neutropenia and thrombocytopenic purpura, the advent of newer and safer thrombolytic drugs such as clopidogrel and ticagrelor, its use remained limited (Warner 2011). In 1988, Clopidogrel was available as an antiplatelet drug for treatment and the prevention of cardiovascular diseases associated with atherosclerosis such as MI and angina. In some recent studies, a series of compounds where pyridine ring was replaced by a pyrimidine or pyrimidinone moieties left active derivatives of comparable thrombolytic activities to that of thienopyridine drugs (Committee 1996; Easton 1998). This was the aim of the present study where we describe the synthesis of a series of new compounds. Also, we replaced the thiophene ring with other isosteric rings to modify the thrombolytic activity. Also, methyl salicylate moiety was involved to give acceptable results.

MATERIALS AND METHODS

All materials used in this study have been described previously (Ahjel et al. 2020). The synthesis of new compounds was shown in the scheme I and II.

EXPERIMENTAL

2-Thiouracil-5-sulphonyl ethylamide (2)
Prepared as in literature (1).

Methyl-2-hydroxy-3-(4-oxo-2-thiazol-2-yl)-2,3,4,5-tetrahydroquinazolin-5-yl) sulphonyl) amino benzoate (3).

Methyl-4-(3,5-dioxo-2,3-dihydro-SH-[1,2]thiazolo[3,2-a] pyrimidine-6-yl) sulphonyl) amino benzeneacetic acid (4).

3,5-Dioxo-Substituted phenyl-2,3-dihydro-SH-[1,2]thiazolo[3,2-a] pyrimidine-6-sulphonamide (5a-c).

EXPERIMENTAL

2-Thiouracil-5-sulphonyl ethylamide (2)
Prepared as in literature (1).

4-Oxo-Substituted phenyl-2-thiazol-1,2,3,4,5-tetrahydroquinazolin-5-yl) sulphonamide (3a-c).

3,5-Dioxo-Substituted phenyl-2,3-dihydro-SH-[1,2]thiazolo[3,2-a] pyrimidine-6-sulphonamide (5a-c).

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3,5-Dioxo-Substituted phenyl-2,3-dihydro-SH-[1,2]thiazolo[3,2-a] pyrimidine-6-sulphonamide (5a-c).
Compounds preparation and administration

Stander’s drugs (donated by Sanofi Recherche) were prepared by dissolving them in distilled water at room temperature, while the new compounds were dissolved in 0.1% DMSO plus enough distilled water. The standard and new compounds were administration IV at 3-30 mg/kg.

Thrombolysis evaluation

Eighty-five Wistar rats weighting 350-450g were divided into seven groups, the first two groups for standers and the others five were subdivided into three subgroups each group and subgroup contain 5 rats. The rats were subjected to general anesthesia with thiopental (800U/kg IP). The right carotid artery was cannulated to delivering blood into a collagen strip then returned to the left femoral vein. After 20-30 minutes and during this circulation, a clot was weighting by using an auxotonic 386 Harvard transducer. The compounds were injected at a lower dose of 1 μg/kg then increased gradually until 30 μg/kg after one hour of injection, the thrombolytic effect of the compounds was recorded and ED50 was 30% of maximal thrombolysis (ED50) obtained from the equation of regression lines.

RESULTS

The antiplatelet activity of newly synthesized compounds was measured and compared with ticlopidine and clopidogrel as standard reference drugs. We found that the newly synthesized compounds showed a vary thrombolytic effect as compared with standers. However, 3a, 4a, 6a, 7a, and 8a have been lower ED30 than other new compounds as well as the standers. 4a and 6a (14.37 ± 0.94 and 22.89 ± 1.00 respectively) have been shown lowered ED30 than others. There is an insignificant difference (p>0.05) between 3a and 7a, 4a and 6a, and between 7a and 8a. There is a significant difference (p<0.05) between them and standers as well as other compounds. Table (1), figure (1), figure (2), and figure (3).

Figure 1: ED30 value of newly synthesized compounds and standers
**DISCUSSION**

Pyrimidine nucleus is a poor ring relative to most common electrophilic substitution reactions such as nitration, sulphonation, halogenation etc. The relative inertness is attributed to the –I and –M effects of the two nitrogen atoms. These reactions could be augmented by the introduction of electron releasing groups, thus for example in 2-thiouracil, the presence of –OH and SH groups counteract the deactivation caused by the two nitrogen atoms, consequently, 2-thiouracil undergoes most of these reactions. Chlorosulphonation is one of these reactions specially to prepare important pharmaceutical precursors or biologically active intermediates (*Ahjel 2020; Awad 2018*). Chlorosulphonation of thiouracil was one of the difficult interactions that embraced the scholars, and each time the result was not clear until it was prepared in the year 2002 by Fathalla OA (*Fathalla 2005*), who showed that the reaction is subjected to temperature gradient technique and the optimum temperature to obtain good yields was 120oC. In 2020 the yield was increased by the synergistic effect of thiouyl chloride when added with chlorosulphonic acid in this chlorosulphonation reaction. Thus 2-thiouracil-2-sulphonyl chloride was efficiently prepared and was used as a starting material to prepare a huge number of biologically active 2-thiouracil-5-sulphonamides by its reaction with series of aromatic amines in a SN2 reaction in presence of pyridine as an acid binder. Three aromatic amines were selected in this research namely, methyl-1-4-aminosalicylate, methyl-3-aminobenzoate, and methyl-3-aminobenzoate in an attempt to increase the thrombolytic effects. Also, to mimic thienopyridines of common thrombolytic effects, 2-thiouracil-sulphonamides 3a-c were cyclo-condensed with chloroacetic acid in acetic acid /acetic anhydride mixture yielding thiazolopyrimidinesulphonamides4a-c which were condensed with benzaldehyde due to the presence of active methylene group giving benzylidene derivatives 5a-c. Furthermore, 3a-c were condensed with ethanol amine, anthranilic acid, and 3-bromopropanoic acid to give imidazopyrimidines, pyrimidoquinazolines and pyrimidothiazines6a-c, 7a-c, and 8a-c respectively.

**Series I. 3a-c**

![Diagram of series I](image)

The optimum activity was obtained when the aryl group is methyl salicylate, the activity is decreased by removing the OH group (aryl group is methyl benzoate), the activity is dramatically decreased when m-substituted methyl benzoate is used (3c). Thus, salicylate augments the thrombolytic effect through inhibition of platelet prostaglandin synthesis a recently reported to stimulate nitric oxide synthesis in platelets that produce fibrinogen/fibrinolytic effect.

**Series II.4a-c**

![Diagram of series II](image)

Thiazolopyrimidine 4a represents the most potent compound of our research (aryl group is methyl salicylate; activity is decreased by the removal of OH group (aryl group is p-methylbenzoate) and by m-substitution.

**Series III.5a-c**

![Diagram of series III](image)

Benzyldiene formation (reaction of 3a-c with benzaldehyde) dramatically decrease or abolish the thrombolytic activity in a relative manner according to the aryl group.

**Series IV.6a-c**

![Diagram of series IV](image)

Analogous to 4a-c, maximum activity of imidazopyrimidines was obtained when the aryl group is methyl salicylate.
In imidazoquinazoline derivatives promising thrombolytic activity was obtained when the aryl group is methyl salicylate, the activity is gradually decreased by the removal of OH group and by m-


<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mean ± Std. Error</th>
<th>Std. Deviation</th>
<th>95% Confidence Interval for Mean</th>
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<tbody>
<tr>
<td>3a</td>
<td>91.39 ± 1.16</td>
<td>1.63</td>
<td>76.7093, 106.0607</td>
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<td>3b</td>
<td>5472.00 ± 85.00</td>
<td>120.21</td>
<td>4391.9726, 6552.0274</td>
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<tr>
<td>3c</td>
<td>10598.00 ± 160.00</td>
<td>226.27</td>
<td>8565.0072, 12630.9928</td>
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<tr>
<td>4a</td>
<td>14.37 ± 0.94</td>
<td>1.32</td>
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<tr>
<td>4b</td>
<td>1050.15 ± 20.05</td>
<td>28.35</td>
<td>795.3906, 1304.9094</td>
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<tr>
<td>4c</td>
<td>2057.60 ± 49.70</td>
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<td>5a</td>
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<td>5b</td>
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<td>6a</td>
<td>22.89 ± 1.00</td>
<td>1.41</td>
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<td>6b</td>
<td>6453.00 ± 200.00</td>
<td>282.84</td>
<td>3911.7591, 8994.2409</td>
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<td>9132.6031, 14218.8969</td>
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<tr>
<td>7a</td>
<td>74.85 ± 1.05</td>
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<td>7b</td>
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<td>282.84</td>
<td>6223.7591, 11306.2409</td>
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<tr>
<td>8a</td>
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<td>8b</td>
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<tr>
<td>Ticlopidine</td>
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<td>Clopidogrel</td>
<td>15745.00 ± 10.00</td>
<td>14.14</td>
<td>15617.9380, 15872.0620</td>
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</table>

Table 1: ED30 value of newly synthesized compounds and standers

Conondensed pyrimidine sulphonamides as newly synthesized heterocyclic compounds were prepared in an attempt to mimic the thrombolytic activity of thienopyridines such as ticlopidine and clopidogrel, they showed higher promising activity especially when incorporated with methyl salicylate nucleus in an attempt to augment the thrombolytic activity.

REFERENCES