Thiosemicarbazone and thiazolylhydrazones of 1-indanone: As a new class of nonacidic anti-inflammatory and antiplatelet aggregation agents

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Abstract: This research based on the anti-inflammatory and antiplatelet aggregation properties of some new thiazolyl hydrazone derivatives of 1-indanone. In this regard a thiosemicabazone and twelve thiazolyl derivatives of 1-indanone have been synthesized. Out of these synthetic compounds seven derivatives 1-3, 6, 11-13 exhibited varying degree of anti-inflammatory action with IC_{50} esteems going from 5.1±1.3 - 78.8±4.6μM/mL. Compound 1 (IC_{50} =5.1±1.9μM) displayed potent result than standard ibuprofen (IC_{50} = 11.2±1.9 μM). In antiplatelet aggregation assay, five compounds 1, 5, 6, 8 and 11 were observed to be dynamic with IC_{50} esteems observed in the range of 38.34-255.7±4.1μM, whereas, aspirin (IC_{50} = 30.3±2.6 μM) was used as standard. However, compound 11 was found to be good active for both anti-inflammatory and antiplatelet aggregation activities (IC_{50} = 13.9±4.9μg/mL) (IC_{50} = 38.60±3.1μM), respectively.

Keywords: 1-Indanone, thiosemicarbazone, thiazole, antiplatelet aggregation, anti-inflammatory.

INTRODUCTION

Over the years, several indanone derivatives, particularly indane, acidic subordinates, have been blended and their potential pain relieving and mitigating properties have been contemplated, including anti-inflammatory drug clidanac and sulindac (as 1 *H*-indene) with moderate antiplatelet function. Moreover, indan-1, 3-dione is an anticoagulant agent (Kalhor *et al*., 2015, Mirfazli *et al*., 2016, Henn *et al*., 1998). Indane nucleus subsequently being an appealing framework for therapeutic scientists (an isostere of indole ring, fig. 1) numerous indane based compounds (Bjarnason and Hayllar, 1993) are used clinically to treat various diseases including antiplatelet compounds (Palladino *et al*., 2003, Nigar *et al*., 2015). Furthermore, many other synthetic derivatives of indanone/indane ring system are medicinally important such as anti-inflammatory (Bepary *et al*., 2008, Kumar Pal *et al*., 2012, Saravanan *et al*., 2006), analgesic (Kumar Pal *et al*., 2012, Saravanan *et al*., 2006, Das *et al*., 2008), antiplatelet aggregation (Amidi *et al*., 2013), and thromboxane A_2 antagonist (Shinozaki *et al*., 1999). Out of these biological activities of indanone and their derivatives, anti-inflammatory activity is of particular interest.

Thiazole and its derivatives are the important class of heterocyclic compounds endowed with various pharmacological activities such as anti-cancer, antimicrobial, antiviral, anti-inflammatory analgesic and antioxidant activity. Thiazole scaffold is present in many drugs such as Sulfathiazol, Ritonavir and azofurin.(Gupta and Kant, 2013)

In addition to these facts, literature review also reveals that hydrazone and acyl hydrazone moieties are the most important pharmacophoric cores of several analgesic, anti-inflammatory and antiplatelet derivatives (Maia *et al*., 2009). In addition, specifically a large number of thiazolyl hydrazones now have attracted attention for researchers due to their diversified biological activities (Caputto *et al*., 2012) including anti-inflammatory activity (Bertez *et al*., 1984) (fig. 2).

In this study, we have synthesized thiazolyl hydrazone derivatives of 1-indanone by molecular hybridization of 1-indanone and thiosemicarbazide by synthesizing 1-indanone thiosemicarbazone (1) then introducing thiazole moieties to prepare 1-indanone thiazolyl hydrazone (2-13) in search of agents that mitigating exercises in blend with antiplatelet impacts is possibly helpful for the treatment of endless fiery conditions and pursuit of finding new

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molecules as another class of mitigating/antiplatelet compounds with dual action of anti-inflammatory/ antiplatelet aggregation effect having empowering results with minimum side effects usually associated with many NSAIDs drugs such as indomethacin, flufenamic acid, clindanac and sulindac are linked with interruption of gastrointestinal (GIT) tract which is seemingly of the contact of open carboxylic moiety (pKa = 3.5-5.5) in these drug to GIT tract (fig. 2) (Woods *et al*., 2001, Smith *et al*., 1998). Similarly antiplatelet agents also have certain serious limitations which include inhibition of platelet function (aspirin) (Singh and Rosen, 1998, Smith *et al*., 1998).

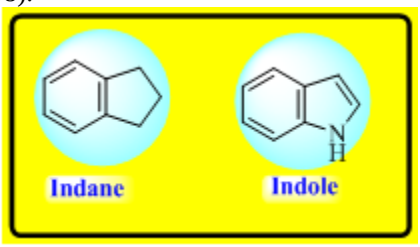


Fig. 1: Isosteres structures of Indane and indole ring.

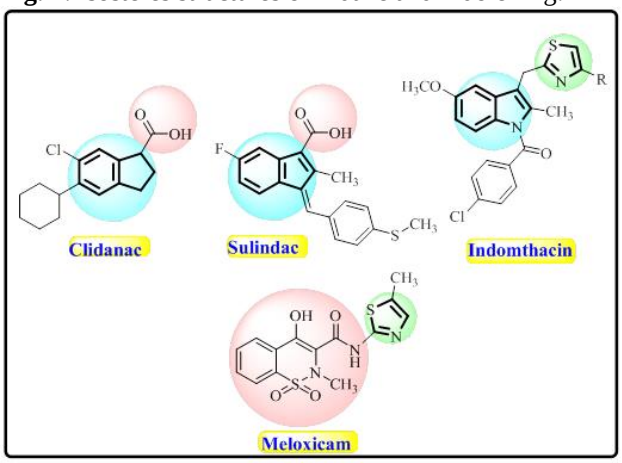


Fig. 2: Indane, indole and thiazole containing anti-inflammatory and antiplatelet drugs.

MATERIALS AND METHODS

General technique for amalgamation of compounds 1-13

Thiazolyl hydrazones 2-13 were synthesized by taking 1 mmol of 1-indanone with thiosemicarbazide 1 mmol, within the sight of reactant measures of acetic acid in 10 mL of ethanol as dissolvable to obtain intermediate thiosemicarbazone 1. The thiosemicarbazone 1 (1 mmol) was reacted with differently substituted phenacyl bromide (1 mmol) in the same solvent. All synthetic items were filtered by washing with petroleum ether, hexane, and diethyl ether (Scheme 1).

Mitigating action convention: Oxidative burst assay using chemiluminescence technique

Mitigating movement was performed by luminol-upgraded chemiluminescence measure. Entire blood HBSS++ (Hanks Balanced Salt Solution) was taken 25 µL in weakened frame and 25µL of three distinct groupings of compounds (1, 10 and 100µg/mL) were brooded with in triplicate way. Control wells comprise of HBSS++ and cells, barring mixes. All test were performed in 96 well plates, brooded it at 37ºC for 15 minutes in the indoor regulator council of luminometer. This brooding, sought after by 25µL of serum opsonized zymosan (SOZ) and 25 µL of intracellular responsive oxygen species recognizing test, clear wells (containing just HBSS++). Results were recorded by the level of the ROS which were estimated in luminometer in term of relative light units (RLU)(Helfand *et al*., 1982). Ibuprofen was used as standard IC_{50} = 11.2± 1.9μg/mL.

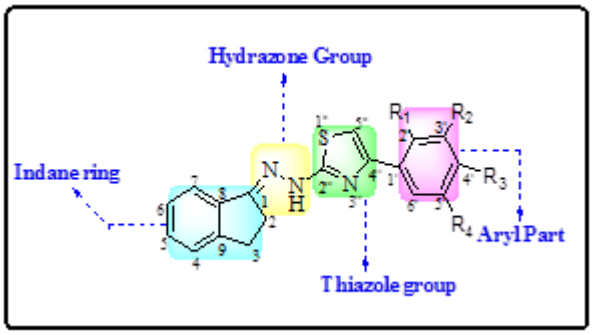


Fig. 3: General structure of synthetic thiazolyl hydrazone derivatives of 1-indanone

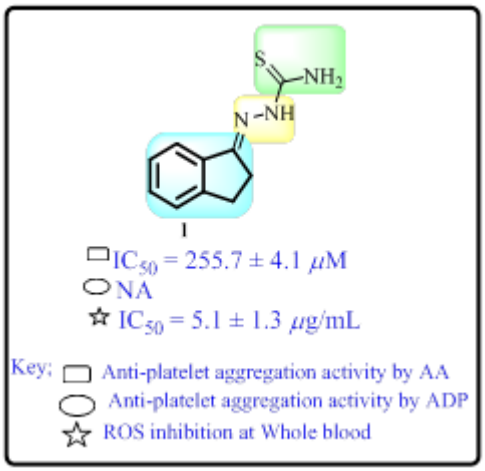
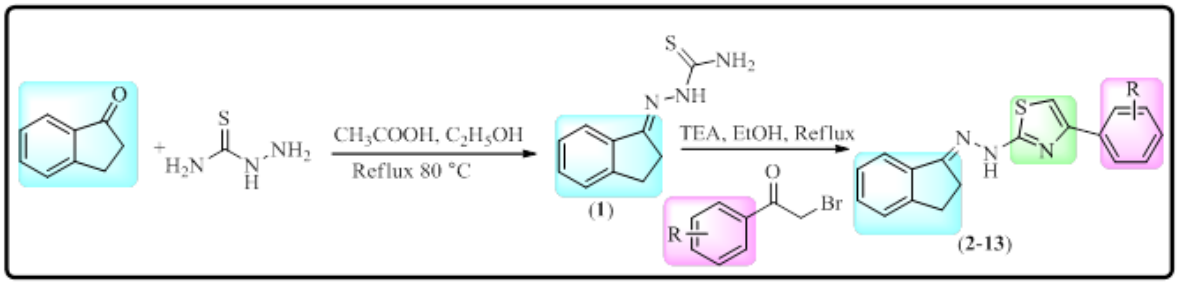


Fig. 4: Intermediate 1 (thisemicarbazone of 1-indanone).

Antiplatelet aggregation activity protocol

Turbidimetric method was employed for performing *in vitro* platelet aggregation using APACT 4004 aggregometer. Well fit volunteers were selected for whole blood. For obtaining platelet rich plasma (PRP), blood was easily blended with trisodium citrate dihydrate 3.8%. This was then centrifuged for 8min at 1000 rpm to attained PRP. After that centrifugation of blood was at 3000 rpm for 15min came about PPP (platelet poor plasma) this was taken as clear. The platelet tally utilized was 250000plts/mL. 1µL of the compounds broke down in dimethyl sulfoxide was blended with to 200µL of PRP



Scheme 1: Synthetic route for the synthesis of thiosemicarbazone and thiazolyl hydrazone of 1-indanone

and hatched for 5 min. Accumulation was brought by expansion of ADP (5µm) or arachidonic acid (1.25 mg/mL). Aggregometer (APACT 4004) was used for measuring aggregation. Aspirin was used as standard drugs and DMSO (0.5% v/v) was connected as negative control. The results were expressed as percent inhibition IC_{50} (Born and Cross, 1963).

RESULTS

The in vitro antiplatelet movement of all the synthetic compounds was carried out on platelet rich plasma (PRP) based turbidimetric technique created by Born (Born and Cross, 1963). The thiazolyl hydrazone derivatives of 1-indanone (5 and 11) demonstrated extensive movement against AA-prompted platelet collection, whereas no compound was found to be active at 250μM concentration when ADP was used as the aggregation inducer. Amongst thirteen synthetic compounds two compounds 5 (IC_{50} = 38.34±2.4 μM) and 11 (IC_{50} = 38.60 ±3.1μM) showed comparable activies. However, three compounds 1, 6, and 8 were found to be weakly active. Aspirin was used as standard (IC_{50} = 30.3±2.6 μM).

All synthesized derivatives were also tested to check their inhibitory potential on intracellular responsive oxygen species (ROS) delivered from human entire blood phagocytes at single centralization of 25µg/mL. Amongst them compounds 1 (IC_{50}=5.1±1.3μg/mL), 6 (IC_{50} =18.2± 4.1μg/mL), 2 (IC_{50}=14.6±2.6μg/mL), and 11 (IC_{50}= 13.9±4.9μg/mL) were found to be good active. However, compounds 3, 12, and 13 were found to be weakly active. Ibuprofen was utilized as standard (IC_{50}=11.2±1.9 µg/mL).

Compounds	R^1	R^2	R^3	R^4
2	H	H	OCH_3	H
3	H	Cl	H	Cl
4	H	H	Br	H
5	H	H	Cl	H
6	H	NO_2	H	H
7	H	H	C_6H_5	H
8	H	H	H	H
9	H	Br	H	H
10	H	H	NO_2	H
11	OH	H	H	H
12	Cl	H	Cl	H
13	H	H	CH_3	H

DISCUSSION

Intermediate compound 1 (fig. 4) was also screened for both activities and it was found to have good anti-inflammatory activity by whole blood phagocytes (IC_{50} = 5.1±1.3μg/mL) as well as weakly active for antiplatelet aggregation by AA.

In antiplatelet aggregation activity compound 5 having chloro substituent at 4′-position of aryl part was observed to be great dynamic for antiplatelet series and non-active for anti-inflammatory study. However, other dichloro substituted compounds 3 and 12 were found to have inactive antiplatelet aggregation activity but, moderately active for anti-inflammatory activity.

Compound 11 having hydroxyl substituted aryl part at position 2′ showed approximate compareable antiplatelet activity to compound 5. Compound 11 was also found to have good anti-inflammatory activity therefore it has dual inhibitory potential.

Compound 6 having nitro group at 3′-position of aryl part showed good anti-inflammatory potential but weak antiplatelet aggregation activity by AA. However, structural isomer 10 having nitro group at 4′-position of aryl part was found to be not active. Compound 2 having methoxy group at 4′-position of aryl part showed good antiinflammatory activity. However, replacing of methoxy group by methyl group at same position made it weakly active. Both of these compounds were inactive for antiplatelet aggregation activities.

To study the effect of carbon load, compound 8 having no any substituent at aryl part showed weak antiplatelet aggregation. While, 4′-phenyl substituted aryl part having compound 7 was found to be completly inactive. Compounds 9 and 4 having bromo substituted aryl part were found to be completely inactive.

Compound (11) was found to be dual inhibitor. It clearly shows the achievement of our work which was mainly focused to find out a new molecule (11) with dual activity of anti-inflammatory and antiplatelet aggregation action same as that of aspirin which if studied further may be proved to be a good molecule against a variety of chronic inflammatory diseases.

To the best of our insight just compounds 1, 5, 8, 13 (Caputto *et al*., 2012), 2 (CAS reg. no: 887352-02-5), 7 (CAS#887351-99-7) were previously reported while remaining analogs are new.

Structure-activity relationship

It should be noted that the Hydrazones ($C=N-NH_2$) have two nitrogen atoms of different nature and have C=N bond in conjugation with lone pair of electron of the terminal nitrogen atom. These structural specifications are generally responsible for chemical properties of the Hydrazones.

Both nitrogen atoms of the hydrazine group are nucleophilic, although the amino type nitrogen is most reactive (Tehrani *et al*., 2015). Despite the high structural similarity between Hydrazones and Thiosemicarbazone (1), the electronic characteristics of these compounds are quite different: The presence of thiocarbonyl, conjugated with the lone pair of electrons will change the delocalization pattern of electrons on Thiosemicarbazone.

In Thiosemicarbazone, the thioamide-type resonance between the nitrogen and thiocarbonyl alters the electron density so that the complexation behavior of these compounds differ substantially from that of hydrazones. Therefore the striking difference between the biological activities of hydrazine derivatives and their Thiosemicarbazone congeners could be on account of the different electronic interaction of these two group of compounds with their receptors. It is also worth mentioning that the companionship of thiocarbonyl moiety in Thiosemicarbazone derivatives will also change the shape and size of the molecules.

When thioamide group is changed into thiazole ring system due to presence of heterocyclic ring the structure/size of the molecule and electronic interactions of the groups with the receptors is entirely changed and make the molecule capable to produce antiplatelet as well as anti-inflammatory activity. Substitution of aryl (C_6H_5) or substituted aryl ($C_6HR^1R^2R^3R^4$) on thiazole ring may lead to increase or decrease the antiplatelet/anti-inflammatory activity.

Antiplatelet activity of compounds 5 and 11 may be due to interaction of lone pair of electron of electronegative elements Cl and O with the receptor. Compounds in which aryl is substituted by element less electronegative than Cl (4 and 9) or substituted by greater number of Cl atoms (3 and 12) or those in which aryl is substituted by groups have no lone pair of electron (6, 7, 8, 10 and 13) did not show remarkable antiplatelet activity.

Substitution of aryl with electron withdrawing group (2, 3 and 6) may lead to anti-inflammatory activity. Anti-inflammatory activity of 1 and 11 may be due to hydrogen bonding of NH_2 or OH group with the receptors.

CONCLUSION

In conclusion, compound 1 exhibited remarkable anti-inflammatory activity, however, compounds 2, 6 and 11 also possess less activity than standard and may be selected as novel lead molecule to optimize for various inflammatory conditions with minimum GIT side effects. Besides, compounds 5 and 11 may be of great interest in search of new antiplatelet aggregation agents by AA inducer. Moreover, compound 11 was found to be active in both anti-inflammatory and antiplatelet aggregation activity and consider to have dual potential and may prove to be a better drug molecule for the chronic inflammatory conditions associated with abnormal platelet activation and aggregation with less GI effects being non-acidic molecule as compare to other drugs such as aspirin having acidic moieties.

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