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LETTER

Design and one-pot synthesis of hybrid thiazolidin-4-one-1,3,5-triazines as potent antibacterial agent against human disease causing pathogens

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An efficient and general one-pot reaction to novel series of hybrid thiazolidine-4-one-1,3,5-triazine derivatives were developed. The easy work-up of the products, rapid reaction, and mild conditions are notable features of this protocol. These molecules were found to exhibit potent activity against the panel of Gram-positive and Gram-negative micro-organisms.

Treatment of infections caused by bacterial pathogens is always creating a peril to the healthcare. It becomes more acute due to the rapid development of resistance against the conventional chemotherapy.[1] Considering the magnitude of ever growing antibacterial resistance and high vulnerability against human population towards them, it is necessary to discover novel chemical entity (NCE) with improved pharmacological profile.

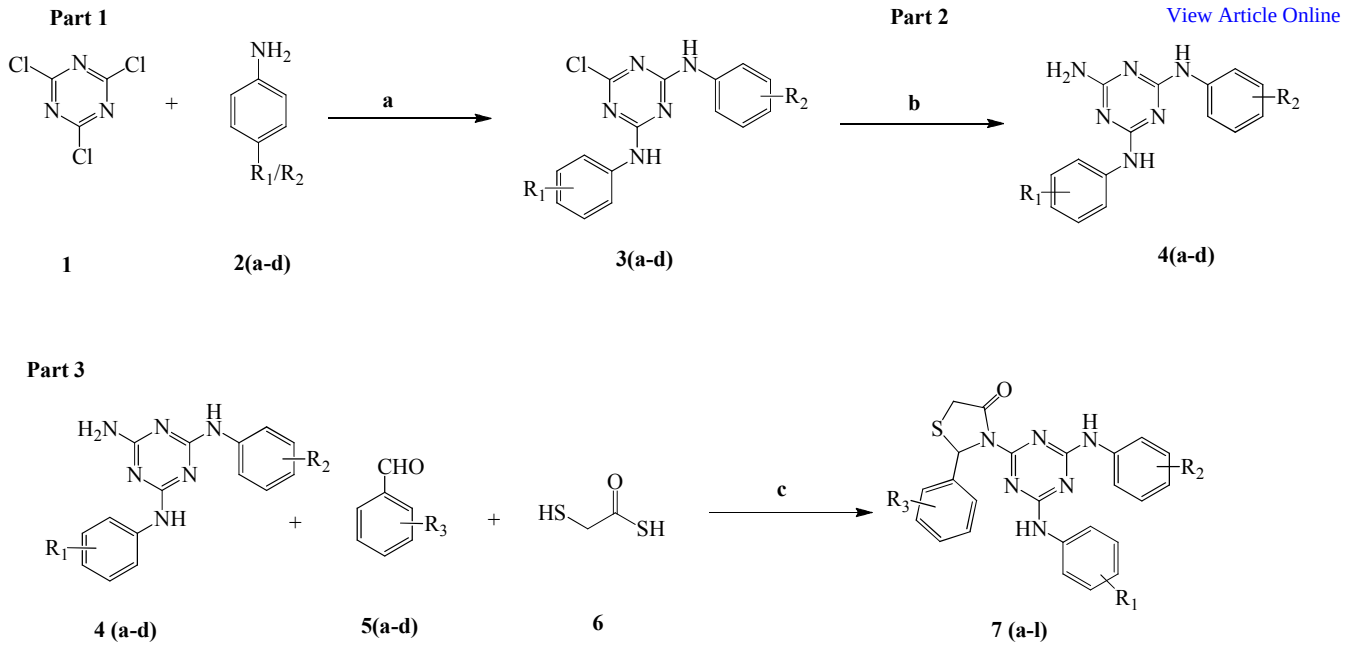
1,3,5-traizine, a pharmacophore found as a core skeleton in molecules with diverse biological activity such as antibacterial,[2] antifungal,[3] anticancer,[4] antimalarial[5] and antiviral[6] agents. In our aim to develop novel antibacterial agents derived from 1,3,5-triazine, we had reported a novel heterocyclic hybrid skeleton comprise of thiazole and 1,3,5-triazine as potent antibacterial agent.[7,8] Prompted by the results, we further carry out the modification on 1,3,5-triazine to deliver newer antibacterial agents with piperazine,[9] 1,3-thiazine[10] 1,3,4-thiadiazole[11] and 4-aminoquinoline[12]. More recently in our previous communication, we had disclosed that hybrid conjugates of 1,3,5-triazine were acted via arrest of bacterial translation. [10]

Based upon the our earlier observation and extensive investigation of the structure activity relationship for hybrid 1,3,5-triazine conjugates, it was indicated that 1,3,5-traizine core and its pendant substitution is crucial for generation and escalation of activity. It was further substantiated with the nature of the substituent on the other flanked portions of 1,3,5-traizine core.

As further study to develop newer hybrid conjugates of 1,3,5-triazine as antibacterial agent with diverse heterocyclic fragment on the pendant position, we report here the one-pot synthesis and antibacterial activity of conjugates derived from 1,3,5-triazine-thiazolidinone. Our primary objective was to optimize the potency of these compounds against Gram-positive and Gram-negative organisms.

The synthesis of designed target compounds were accomplished via a multi-step synthetic procedure and distributed in three parts. The part one was dealt with synthesis of di-substituted 1,3,5-triazine derivatives **3** (**a-d**), it was furnished by treating two equivalents of distinguished amine with commercially available 2,4,6-tri chlroro 1,3,5-triazine in the presence of base. It was a temperature dependent S_NAr nucleophilic reaction, thus, the reaction was carried out at 0-5 °C for the first substitution and then refluxing the same mixture to 40-45 °C for 3-4 h. The second part of synthesis deals with amination of mono chloro of di-substituted 1,3,5-triazine in the presence of ammonia to furnish 2,4-di substituted-6-amine-1,3,5-triazine derivative **4** (**a-d**). Whereas, the last part of the scheme illustrates the one-pot synthesis of hybrid thiazolidin-4-one-1,3,5-triazines derivatives **7** (**a-l**). It was afforded via condensation-cyclisation reaction between amine of di-substituted 1,3,5-triazines, corresponding aldehydes and 2-mercaptoethanethioic S-acid. These compounds were taken altogether in a flask and allowed to reflux at 110 °C in the presence of toluene. The completion of reaction was initially monitored via TLC and the structures of these molecules were ascertained on the basis of FT-IR, ^{1}H-NMR, mass spectral and elemental analysis (see supplementary information).

The final compounds were screened against panel of human disease causing pathogens consists of three Gram-positive and three Gram-negative strains. The MIC (minimum inhibitory concentration) of the compounds along with the activity of reference Cefixime was presented in Table 1. Compounds showed varying degree of antibacterial activity against Gram-positive pathogens; nevertheless, remarkable activity was also reported against the Gram-negative bacteria from the entire set of title hybrid compounds. Marked inhibition pattern was disclosed by the molecules having *m*-chloro substitution on the phenyl ring tethered to 1,3,5-triazine against entire set of bacterial strains **7** (**a-d**).Whereas, on isomeric replacement of chloro in aldehyde portion from *para* (**7a**) to *ortho* (**7b**) generate the molecule more potent towards Gram-negative and less active towards the Gram-positive. On the other hand, introduction of *p*-nitro group (**7c**) and its *ortho* isomeric counterpart (**7d**) in aldehyde by keeping the 1,3,5-triazine portion rigid showed noticeable variation in activity against the Gram-positive and Gram-negative bacterial strains. Out of which compound **7d** presented highly potent activity against *E. coli* than Cefixime as a standard. A marked variation in



Scheme 1 Reagents and condition: a. Various amines, stirring, aq. NaOH, 0-5 °C; Reflux, aq. NaOH 40-55°C: b. NH_3, reflux, 125-135 °C; c: Toluene, reflux, 110 °C.

antibacterial profile of the compounds were observed on introduction of *m*-fluoro in the place of *m*-chloro at the phenyl of 1,3,5-triazine. Compound **7e**, having *p*-chloro on the phenyl of thiazolidine-4-one showed significant to moderate activity against entire set of bacterial strains except potent in the case of *E. coli* than Cefixime. While, isomeric replacement of *p*-chloro to *o*-chloro (**7f**), makes the molecule more potent towards *S. aureus*, mild active towards *B. cereus, E. coli*, *P. vulgaris* and no change against *B. subtilis* and *P. aeruginosa*. Extremely potent antibacterial activity was disclosed by the compound **7g** resulted on introduction of *p*-NO_2 in the phenyl tethered to thiazolidine-4-one against *E. coli* and *P. vulgaris* than Cefixime as standard, while, no major difference in antibacterial profile was observed against rest of the strains except *B. cereus*. Further decline in antibacterial action was reported against *E. coli* and *P. vulgaris* by compound **7h**, with no significant change towards other microorganisms. On replacement of halogenated electron withdrawing groups with non-halogenated (NO_2) in the phenyl of 1,3,5-triazine fragment **7** (**i-n**) makes the molecule significantly active against the Gram-positive and mild to moderate against the Gram-negative microorganisms. While, significant activity was reported by the compounds **7i**, **7j** and even more potent than Cefixime against the *P. vulgaris* and *P. aeruginosa* by compound **7l**.

From the structure-activity relationship studies of hybrid thiazolidine-4-one-1,3,5-triazine derivatives, it was confirmed that isomeric replacements of the substituents may lead to marked difference in antibacterial profile of the compounds. It is noteworthy to mention that compounds consists of halogenated electron withdrawing groups were found as highly active against the Gram-negative pathogens and mild towards the Gram-positive. While, the compounds comprises of non-halogenated electron withdrawing group exert mild to moderate activity against the entire set of the bacterial strains. The increase in membrane permeability and alteration of volumetric and conformational state may be responsible for the pronounced antibacterial activity showed by halogen containing molecules. Additionally, these compounds perhaps tend to occupy the active site of molecular targets including the deeper pockets which is necessary to exert potent antibacterial action.

We had developed a novel series of hybrid thiazolidine-4-one-1,3,5-triazine derivatives as potent antibacterial agents via efficient synthetic methodology. Present study suggests the role of halogenated electron withdrawing groups to generate highly potent antibacterial agents from the title hybrid skeleton. Our optimisation studies on the hybrid derivatives of 1,3,5-triazine is underway and reported subsequently in future.

Experimental

All chemical reagents are commercial available, and used without further purification. Melting points of the synthesized compounds were determined in an open capillary tube Hicon Melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on silica gel-G coated plates to detect the completion of reaction. The diverse mobile phase was selected in different proportion according to the assumed polarity of the products. The spots was visualised by exposure to the Iodine vapour. Infra-Red (IR) spectra were recorded in KBr on Biored FTs spectrophotometer and the reported wave numbers are given in cm^{-1}. ^1H NMR spectra were recorded in DMSO on Bruker Model D9RX-400MHz spectrometer. Chemical shifts were reported as δ (ppm) relative to TMS as internal standard. Mass spectra were obtained on VG-AUTOSPEC spectrometer equipped with electrospray ionization (ESI) sources. Elemental analysis of C, H and N was performed on a Vario EL III CHNOS elemental analyzer.

Synthesis of the intermediate compounds **3** (**a-d**), **4** (**a-d**)[7] was performed in accordance with earlier reported procedures.

General procedure for the synthesis of title hybrid analogues 7(a-l).

2,4-Bis-(substituted-phenyl)-[1,3,5]-triazine-2,4,6-triamine **4(a-d)** (0.1mol), substituted benzaldehyde **5 (a-d)** (0.1mol) and 2-mercaptoethanethioic S-acid **6** (0.1mol) were refluxed in presence of toluene for 6-10 h. The product **7(a-l)** was filtered, washed with cold water and recrystallized with ethanol to afford the corresponding pure product.

Table 1 Minimum Inhibitory Concentration of designed target molecules.

Compound	Minimum Inhibitory Concentration (µg/mL)					
	Gram-positive bacteria			Gram-negative bacteria		
	S. aureus	*B. subtilis*	*B. cereus*	*E. coli*	*P. vulgaris*	*P. aeruginosa*
7a	62.5	31.25	31.25	62.5	125	15.625
7b	125	125	31.25	7.8125	15.625	3.91
7c	15.625	31.25	62.5	31.25	15.625	31.25
7d	31.25	62.5	31.25	1.95	62.5	15.625
7e	125	7.8125	31.25	1.95	7.8125	7.8125
7f	15.625	7.8125	125	62.5	62.5	7.8125
7g	31.25	15.625	15.625	1.95	3.91	31.25
7h	31.25	15.625	31.25	62.5	31.25	62.5
7i	15.625	31.25	62.5	1.95	15.625	15.625
7j	31.25	7.8125	15.625	31.25	125	31.25
7k	31.25	31.25	31.25	31.25	15.625	31.25
7l	31.25	62.5	31.25	15.625	1.95	1.95
Cefixime	7.8125	1.95	3.91	3.91	7.8125	3.91

[a] Footnote text.

Antibacterial Screening

Minimum Inhibitory Concentration

All the synthesized compounds were screened for their minimum inhibitory concentration (MIC, µg/mL) against selected Gram-positive organisms viz. *Bacillus subtilis* (NCIM-2063), *Bacillus cereus* (NCIM-2156), *Staphylococcus aureus* (NCIM-2079) and Gram-negative organism viz. *Escherichia coli* (NCIM-2065), *Proteus vulgaris* (NCIM-2027) and *Pseudomonas aeruginosa* (NCIM-2036), by the broth dilution method as recommended by the National Committee for Clinical Laboratory Standards with minor modifications.[13] Cefixime was used as standard antibacterial agent. Solutions of the test compounds and reference drug were prepared in dimethyl sulfoxide (DMSO) at concentrations of 125, 62.5, 31.25, 15.62, 7.81, 3.91, 1.95, 0.97 µg/mL. Ten tubes were prepared in duplicate with the second set being used as MIC reference controls (16–24 h visual). After sample preparation, the controls were placed in a 37 °C incubator and read for macroscopic growth (clear or turbid) the next day. Into each tube, 0.8 mL of nutrient broth was pipette (tubes 2–7), tube 1 (negative control) received 1.0 mL of nutrient broth and tube 10 (positive control) received 0.9 mL of nutrient. Tube 1, the negative control, did not contain bacteria or antibiotic. The positive control, tube 10, received 0.9 mL of nutrient broth since it contained bacteria but not antibiotic. The test compound were dissolved in DMSO (125 µg/mL), 0.1 mL of increasing concentration of the prepared test compounds which are serially diluted from tube 2 to tube 9 (tube 2–9 containing 125, 62.5, 31.25, 15.62, 7.81, 3.91, 1.95, 0.97 µg/mL, respectively). After this process, each tube was inoculated with 0.1 mL of the bacterial suspension whose concentration corresponded to 0.5 McFarland scale (9×10^8 cells/mL) and each bacterium was incubated at 37 °C for 24 h at 150 rpm. The final volume in each tube was 1.0 mL. The incubation chamber was kept humid. At the end of the incubation period, MIC values were recorded as the lowest concentration of the substance that gave no visible turbidity, i.e. no growth of inoculated bacteria.

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Notes and references

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†Electronic Supplementary Information (ESI) available: Analytical details are given in supplementary information

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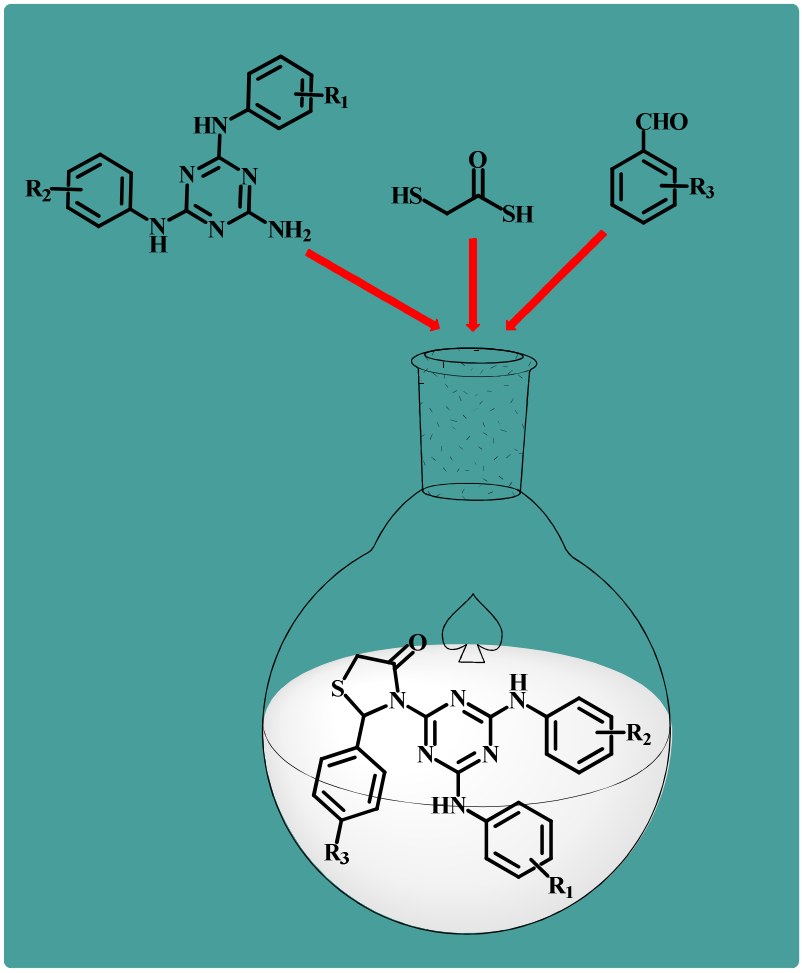
Graphical Abstract



A novel series of hybrid thiazolidine-4-one-1,3,5-triazine conjugates was developed as potent antibacterial agents through facile one-pot synthetic route.