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*Short Communication*

# Development of New Mulluscicide against *Lymnaea acuminita*: Synthesis and Biocidal Activity of Novel Fused Indino [1,2-d]-[1,3] Thiazin-5(4H)-one Derivatives

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Nowadays no bioactive agent plays a vital role to increase a product of agricultural crop, industrial production prolonging the utility of manufacture product controlling human and animal disease. Unfortunately, the number of effective and commercial bioactive agent is very limited. No biocidal agent developed till to date is so versatile to provide remedy against several microorganisms. Some have phytotoxic and several have residual toxicity. Therefore, suitable remedy is still essential to solve these problems. Thus, the basis of selecting heterocycles as the subject of this investigation was realization of the fact that heterocyclic compounds are in clinical use since a long time derived from natural source such as vitamins, hormones, and antibiotics [1-2]. Therefore, we have much attention to design the biologically active molecules [3-12]. Among the heterocyclic systems we have selecting the one class of heterocyclic compounds is 1,3 thiazine. The 1,3 thiazine are shows wide range of biocidal activity such as antibacterial [13], antifungal [14], antioxidant [15], herbicidal [16], antipyretic [17], calcium channel modulator [18-20], insecticidal [21] and antitumor [22]. Literature also reveals that compound containing thiazine fused system are shows more biocidal properties. Keeping above observation in mind and in continuation of our work on biologically active heterocycles and their increasing importance in pharmaceutical and biological field, it was planned to synthesize novel fused systems incorporating the two-active pharmacophore in a single molecular frame work and to evaluate their pharmacological activities. Here in we report the synthesis of numbers of fused indinothiazine derivatives together with their use in a series of heterocyclic transformations and evaluation as biocidal agents.

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*Apparatus and chemicals*

All reagents were purchased from Aldrich, solvents used were extra dried. Procedure for one typical case for each step has been described. All melting points were determined in open glass capillaries and are uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer-157 spectrophotometer ($cm^{-1}$), $^{1}H$ NMR and $^{13}CNMR$ spectra in DMSO-$d_6$ on a Varian EM-360 (200 MHz) spectrometer using TMS as internal reference (chemical shift in δ ppm). Elemental (C, H, N) analysis indicated that calculated and observed values were within acceptable limit. The purity of compounds checked by this layer chromatography on silica gel plate using ether and ethyl acetate as solvent system. Iodine chamber was used as developing chamber.

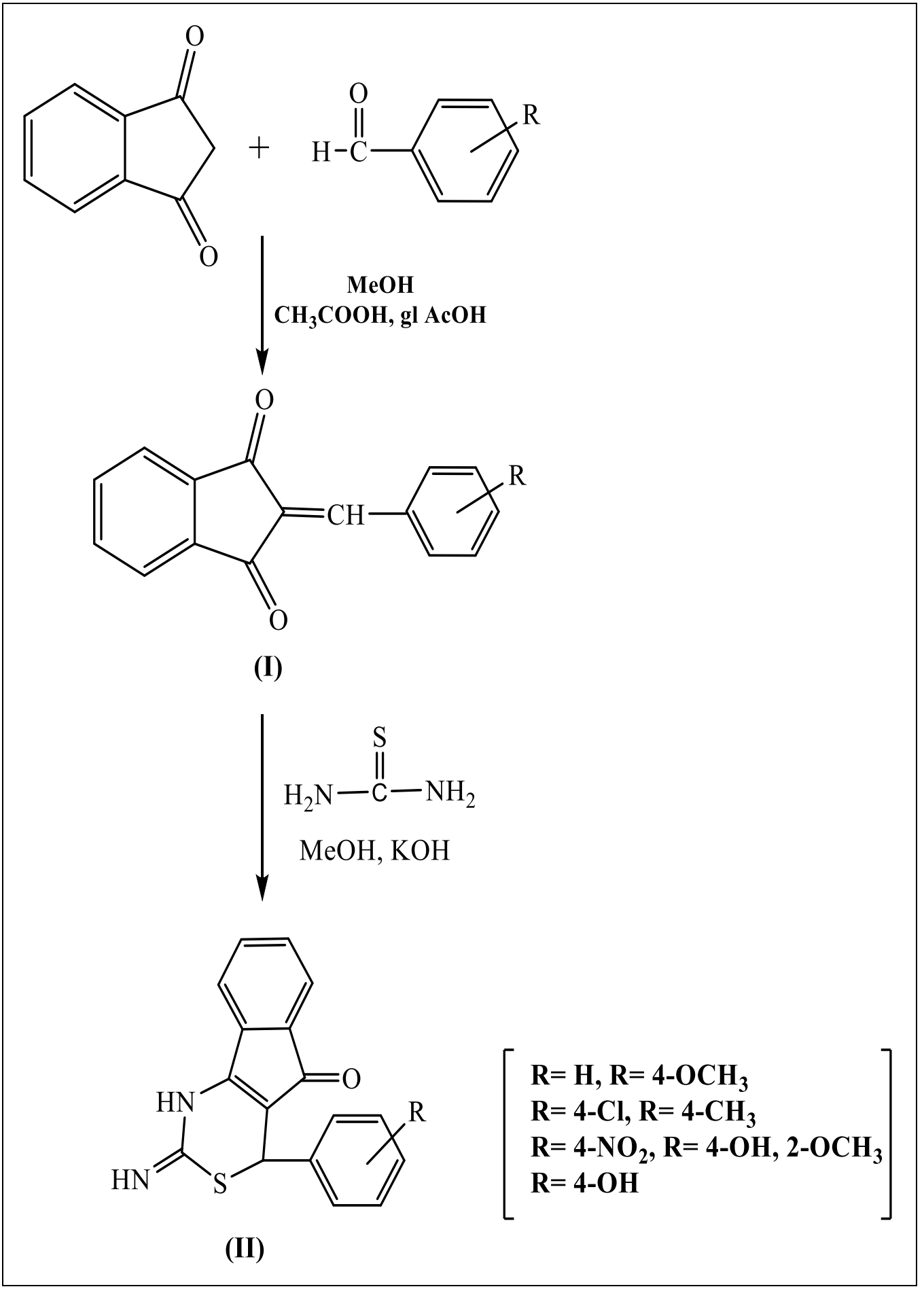
*General procedure for the preparation of 4-(Substitutedphenyl)-2-arylidenindan -1, 3-diones (I)*

A mixture indan -1, 3 -dione (0.01 M) substituted benzaldehyde (0.01 M) and fused sodium acetate (0.16 gm 0.02 M) were refluxed in glacial acetic acid in presence of methanol for four hours. The reaction mixture was cooled and poured in to water. The resulting solid mass was filtered, washed with water and recrystallised from aq. ethanol. All these prepared compounds are known and reported by us earlier [23-25].

*General procedure for the preparation of 4-(4-Substituted phenyl) 2 imino-1,2-dyhydro indeno-[1,2-d] [1,3] -thiazin-5-(4H)-ones (II)*

The cyclocondensation of 4- **(**Substitutedphenyl) -2-arylidenindane **(I)** (0.01M) with thiourea (0.01M) and KOH (0.62 gm, 0.011M) was refluxed in methanol for 4 hours furnished the 4-(4-Substituted phenyl) 2- imino-1,2-dyhydro indeno-[1,2-d] [1,3] -thiazin-5-(4H)-ones **(II).** The reaction mixture was cooled and poured into water. The resulting solid mass was filtered, washed with water and recrystallized from aq. ethanol gave the titled fused heterocycles **(II). (Scheme-1)**.

Other compounds of the type (IIa-IIg) were prepared similarly.




Scheme-1 Schematic representation synthesis of compound (I-II)

*4-(4-phenyl)-2-imino-1,2-dihydroindino-[1,2-d]-[1,3]-thiazin-5(4H)-ones (IIa)*
m. p. 123$^0$C, yield (69%). IR (KBr): 3240(-NH), 2220 (C≡N), 1690(C=O), 1495,1490,1525 (aromatic ring); $^1$H NMR (DMSO-$d_6$): 6.8-7.2 (m, 8H, Ar-H), 8.0(s,1H, NH), 3.6(dd, 1H, -CH-CN), 2.9(s, 1H, CHPh); $^{13}$C NMR (DMSO-$d_6$): 190.5, 170.8, 158.8, 141.6, 136, 135, 131, 128, 126, 123, 116, 114.2, 39, 26. Calcd. $C_{17}H_{12}N_2OS$: C, 69.84; H, 4.12; N, 9.58 Found: C, 69.60; H, 3.95; N, 9.45

*4-(4-methoxyphenyl)-2-imino-1,2-dihydroindino-[1,2-d]-[1,3]-thiazin-5(4H) ones (IIb)*
m. p. 155$^0$C, yield (55%). IR (KBr): 3250(-NH), 1690(C=O), 1620(-C=N)1495,1490,1525 (aromatic ring),1100 (C-S-S),$^1$H NMR (DMSO-$d_6$): 6.8-7.2 (m,8H, ArH),3.8(s,3H, $OCH_3$), 8.0(s,1H,NH),2.7(s,1H,CHPh);$^{13}$CNMR(DMSO$d_6$): 188.5,163,159,156.4,1936,135.7,132,130,128,126,123,114,55. 8,39.5Calcd. $C_{18}H_{14}N_2O_2S$: C 67.15; H 4.15; N 8.39 Found: C 67.00; H 4.37; N 8.21

*4-(4-chloroyphenyl)-2-imino-1,2-dihydroindino-[1,2-d]-[1,3]-thiazin-5(4H)-ones (IIc)*
m. p. 165$^0$C, yield (75%). IR (KBr): 3240(-NH), 1690(C=O), 1620(-C=N), 1105 (C-S-S),1495,1525 (aromatic ring); $^1$H NMR (DMSO-$d_6$): 6.86-7.5 (m, 8H, Ar-H), 3.8(s, 3H,$OCH_3$), 8.0(s,1H,NH), 3.0(s, 1H, CHPh); $^{13}$C NMR (DMSO-$d_6$) : 190.5, 170.8, 165, 141.6, 136, 135, 131, 128, 126, 123, 116, 114.2, 39, 26. Calcd. $C_{17}H_{11}N_2OSCl$: C, 59.78; H, 3.24 N, 8.20 Found: C, 66.09; H, 3.10; N, 8.03.

*4-(4-methylphenyl)-2-imino-1,2-dihydroindino-[1,2-d]-[1,3]-thiazin-5(4H)-ones (IId)*
m. p. 90$^0$C, yield (72%). IR (KBr): 3240(-NH), 1690(C=O), 1620(-C=N), 1194 (C-S-S) 1495,1525 (aromatic ring); $^1$H NMR (DMSO-$d_6$): 6.7-7.2 (m, 8H, Ar-H), 1.95(s, 3H,$CH_3$), 8.0(s,1H,NH), 2.5(s, 1H, CHPh); $^{13}$C NMR (DMSO-$d_6$) : 190.5, 170.8, 141.6, 136, 135, 131, 128, 126, 123, 116, 114.2, ,39, 21. Calcd. $C_{18}H_{14}N_2OS$ C, 66.14; H, 4.31; N, 8.56 Found: C, 66.09; H, 4.06; N, 8.60.

*4-(4-nitrophenyl)-2-imino-1,2-dihydroindino-[1,2-d]-[1,3]-thiazin-5(4H)-ones (IIe)*
m. p. 112$^0$C, yield (65%). IR (KBr): 3240(-NH), 1690(C=O), 1620(-C=N), 1115 (C-S-S),1500, 1495,1525 (aromatic ring); $^1$H NMR (DMSO-$d_6$): 7.3-7.8 (m, 8H, Ar-H), 8.0(s,1H,NH), 3.1(s, 1H, CHPh); $^{13}$C NMR (DMSO-$d_6$) : 190.5, 170.8, 158.8, 141.6, 136, 135, 131, 128, 126, 123, 116, 114.2, , 39, Calcd. $C_{17}H_{11}N_3O_3S$: C, 60.52; H, 3.28; N, 12.45 Found: C, 60.24; H, 3.18; N, 12.02.

*4-(4-hydroxy-2-methoxyyphenyl)-2-imino-1,2-dihydroindino-[1,2-d]-[1,3]-thiazin-5(4H)-ones (IIf)*
m. p. 125$^0$C, yield (85%). IR (KBr): 3490(-OH), 3240(-NH), 1690(C=O), 1620(-C=N), 1092 (C-S-S),1595, 1490, 1486, (aromatic ring); $^1$H NMR (DMSO-$d_6$): 9.3(s, -OH), 6.75-7.0 (m, 7H, Ar-H), 3.8(s, 3H,$OCH_3$), 8.0(s,1H,NH), 2.9(s, 1H, CHPh); $^{13}$C NMR (DMSO-$d_6$) : 190.5, 170.8, 158.8, 141.6, 136, 135, 131, 128, 126, 123, 116, 114.2, 39, 26. Calcd. $C_{18}H_{14}N_2O_3S$: C, 63.70; H, 4.45; N, 8.25 Found: C, 63.46; H, 4.35; N, 8.10.

*4-(4-hydroxyphenyl)-2-imino-1,2-dihydroindino-[1,2-d]-[1,3]-thiazin-5(4H)-ones (IIg)*
m. p. 135$^0$C, yield (60%). IR (KBr): 3490(-OH), 3240(-NH), 1690(C=O), 1620(-C=N), 1095 (C-S-S),1495, 14901486, (aromatic ring); $^1$H NMR (DMSO-$d_6$): 9.3(s, -OH), 6.75-7.0 (m, 7H, Ar-H), 8.0(s,1H,NH), 2.9(s, 1H, CHPh); $^{13}$C NMR (DMSO-$d_6$) : 190.5, 170.8, , 141.6, 136, 135, 131, 128, 126, 123, 116, 114.2, 39, 26. Calcd $C_{17}H_{12}N_2O_2S$: C, 69.46; H, 3.98; N, 9.08 Found: C, 69.40; H, 3.80; N, 8.76

*Molluscicidal activity*

The molluscicidal activity of the compounds (II) was evaluated against the snail *Lymnaea acuminata* which is a vector of the giant liver flukes *Fasciola gigantica* and *Fasciola hepatica*. It causes endemic fasciolosis in cattle population of eastern Uttar Pradesh.

Being herbivorous, these snails cause damage to submerged paddy crops especially in Terai region of U.P. In this region the water reservoirs and submerged paddy fields have become foci for such snail pests.

Adult *L. acuminata* were collected from ponds, lakes and low-lying submerged fields and were used as test animals. The snails were acclimatized for 72 hour in laboratory condition. Six sets of glass aquarium were used for each concentration of experiment. Ten adult snails were kept in each glass aquaria containing 3l dechlorinated tap water. Toxicity of different compounds was determined by the method of Rao and Singh [26]. The snails were treated with different concentrations of compounds. Mortality was recorded at 24 hr intervals up to 96 hour exposure periods. Control animals were kept in a similar manner without treatment. Dead snails were removed from the aquarium to avoid contamination of water. No response to the needle probe confirmed the death of the snail.

For one compound IIa (arbitrarily chosen) lethal concentration ($LC_{50}$) values, lower and upper confidence limits (LCL and UCL), g-values, t-ratio values, slope values and heterogeneity values were calculated according to the method of POLO computer program of Rusell *et al*. [27].

The molluscicidal data* indicates that all tested compounds showed strong to moderate activities. Compound **IIf** (24hr. $LC_{50}$ 1.91) has greater molluscicidal activity while compound **IIa, IId** and **IIg** moderate molluscicidal activity. The molluscicidal activity of the tested compounds is dose and time dependent. Nature of substituents is critical to molluscicidal activity. The electron donating substituents such as methyl, methoxy enhanced the molluscicidal activity. On the other hand the electron withdrawing groups such as chloro decreased the molluscicidal activity. The slope values were steep and separate estimation of $LC_{50}$ based on each of the six replicates was found to be within the 95% confidence limits of $LC_{50}$. The steep slope values indicated that even small increase in the concentration causes mortality in the snails. The 't' ratio is greater than 1.96, which indicates that regression is significant. Values of heterogeneity factor less than 1 denote that in the replicate of random sample the concentration response line would fall within 95%, confidence limit and thus the model fits the data adequately. The index of significance of potency estimation g-value indicates that the value of the mean is within limits at all probability (90, 95, and 99) as it is less than 0.5.

*The $LC_{50}$ values of all tested compounds can be obtained from authors on request.

## SUMMARY

Some new fused heterocyclic systems like 4-(4-Substitutedphenyl)-2-imino-1,2-dihydroindino-[1,2-d]-[1,3]

thiazin-5(4H)-ones **(2)** have been synthesized from key intermediate 4-Substituted-2-arylidenindan -1, 3-diones **(1)**. The key intermediate **(1)** afforded fused system **(2)** via cycloaddition reaction with thiourea in ethanol and potassium hydroxide. The structures of these compounds have been established on the basis of spectral data IR, $^{1}H$ NMR, $^{13}C$ NMR and elemental analysis. The molluscicidal activity of few the synthesized compounds has been screened on the snail *Lymnaea acuminate*. The $LC_{50}$ slope, t-ratio heterogeneity and g-values have been determined and discussed.

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