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Identification and optimization of novel pyrimido-isoxazolidine and oxazine as selective hydride donors

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ABSTRACT

Two novel carbon skeletons (3*S*,3a′*R*)-6′-(methylthio)-5′,7a′-dihydro-1′*H*-spiro[indoline-3,3′-isoxazolo[3,4-*d*]pyrimidine]-2,4′(3a′*H*)-dione (**11a**) and 3-(methylthio)-4a,5,7,11c-tetrahydropyrimido[4′,5′:3,4][1,2]oxazino[6,5-*b*]indol-1(2*H*)-one (**12a**) are characterized as hydride donors. The generation of these hydride sources during the spiroannulation reaction between isatin (**4**) and pyrimidine (**5**) through the free radical mechanism, was confirmed by (i) the increase in the stoichiometric yields of **11** and **12** when the same reaction was carried out in the presence of free radical initiators (e.g., *m*CPBA) and (ii) the formation of oxazepine (**14**) when AIBN was used as free radical initiator. The PKIE [K_H/K_D] values 4.5 and 4.9 obtained when deuterated **11a** (*d*) was used in the presence of TFA and TFA-*d*, respectively, suggest the hydride transfer step to be the rate determining step. These hydrides donors selectively reduce aldehyde in the presence of other reducible groups.

1. Introduction

Organic hydride donors are one class of compounds that can provide hydride anions in chemical and biochemical reactions.[1] As the naturally produced organic hydride donors, such as NAD(P)H,[2] FADH,[2,3] tetrahydrofolate,[4] and ascorbic acid (Vitamin C),[5] play very important roles in the processes of biological reductions and bio-antioxidations, the chemistry and biochemistry of naturally formed organic hydride donors have been a focus of attention of many chemists and biochemists in the past several decades.[6] Among all kinds of artificial organic hydride donors, five-membered heterocyclic compounds, such as 2,3-dihydrobenzodimidazoles (1*H*–5*H*), 2,3-dihydrobenzo-dthiazoles (6*H*), and 2,3-dihydrobenzodoxazoles (7*H*)[7–13] (Fig. 1) and some six-membered heterocyclic compounds, such as 1-benzyl-1,4-dihydronicotinamide (BNAH), Hantzsch ester (HEH), and 9,10-dihydroacridine ($AcrH_2$),[14–24] have drawn attention of chemists and biochemists. The systematic examination of the past publications reviewed by Cheng et al.[25] on the chemistry of the artificial five and six membered heterocyclic compounds as organic hydride donors suggest that the five-membered heterocyclic compounds with two nitrogen atoms as the heteroatoms (1*H*–5*H*) are the strong organic hydride donors. Their hydride donating abilities are generally higher than that of the model of NADH (BNAH) and most of them are used as reducing agents in organic syntheses. All the reported five-membered heterocyclic compounds with nitrogen and sulfur or oxygen as the heteroatoms (6*H* and 7*H*) possess hydride situated on the carbon between the two heteroatoms and are considered as weak organic hydride donors and their hydride-donating abilities are generally lower than that of HEH. The lower reactivity may play crucial role in providing selectivity in reduction. In view of the above and hitherto unknown isoxazoles and oxazines alone or fused with pyrimidines as hydride source (Fig. 1), we report 6-(methylthio)-1,3,3a,7a-tetrahydroisooxazolo [3,4-*d*]pyrimidin-4(5*H*)one (**11a**) and 7-(methylthio)-6,8a-dihydro-1*H*-pyrimido-[4,5c][1,2]oxazin-5(4a*H*)-one (**12a**) as novel and selective hydride donors discover during the spiroannulation reaction

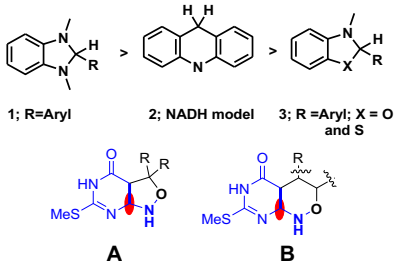

Fig. 1. The broad representation of hydride sources studied by Cheng and our synthesized isooxazoline (**A**) and oxazine (**B**).

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between isatin (**4**) and 6-amino-2-(methylthio)pyrimidin-4(3*H*)-one (**5**).

In view of the above and hitherto unknown skeletons incorporating pyrimidines fused with isoxazolidine or oxazines as hydride source, we report (3a′,7a′)-6′-(methylthio)-5′,7a′-dihydro-1′*H*-spiro[indoline-3,3′-isoxazolo[3,4-*d*]pyrimidine]-2,4′(3a′*H*)-dione **11a** and (4a,11c)-3-(methylthio)-4a,5,7,11c-tetrahydropyrimido[4′,5′:3,4][1,2]oxazino[6,5-*b*]indol-1-(2*H*)-one **12a** as novel and selective hydride donors formed during the spiroannulation reaction between isatin and 6-amino-2-(methylthio)pyrimidin-4(3*H*)-one.

2. Results and discussion

The reported synthesis of spiroannulated pyrimidines (**7** and **10**) by Bazgir et al.[26] from the cyclocondensation of 6-thiouracil (**4**) with isatin in the presence of acid catalyst supposed to proceed with the reported mechanism[26] to give unsymmetrically spiroannulated product **7** (Scheme 1). However, on repetition of this reaction in our laboratory, the spiroannulated product **7** was obtained in 40% yield instead of reported 78% along with the partially and completely reduced symmetrical spiroannulated products, i.e., dihydro- and tetrahydro-pyrimidine derivatives (**9** in 16% and **8** in 9% yields) along with trace amount of unreduced symmetrically spiroannulated substrate **10** and two unknown products **11** and **12**. The compound **8** and **9** with molecular ion peak two and four units higher than the M^+ of **7** were anticipated to be the dihydro- and tetrahydro-derivatives of **7**. In order to investigate and re-consider the proposed mechanism for the reaction, we first tried to confirm the structure of **8** and **9** by an unambiguous reduction of **7** and **10**.

The synthesis of **7** and **10** were achieved through their reported synthesis from isatin and *N*-benzylisatin through cyclocondensation with 6-amino-2-(methylthio) pyrimidin-4(3*H*)-one (**4**), respectively. The reduction of **7** with 1.2 equiv of LAH followed by dehydrogenation offered **7a**–**c** in 32, 20 and 16% yields, respectively, whereas, reduction of **10** under similar condition gave **8** in 25% overall yield as determined by ^{1}H NMR. It unambiguously confirmed the structures of **8** and **9** obtained during the cyclocondensation reaction described above in Scheme 1. The structures of **11** and **12** were determined on the basis of ^{1}H NMR of their respective reduced product, **11a** obtained on borane reduction,[27] which showed the peaks at 6.07 (*m*) and 3.42 (*d*) for H_1 and H_2, respectively, instead of the corresponding peaks at 5.87 (*dd*) and 3.38 (*d*) for **12a**, respectively (Scheme 1).

A three-step mechanism for the formation of **7** or **10** as reported by Bazgir (Fig. 2) consist of (i) *nucleophilic addition* of activated pyrimidine monomer (**5**) C-5 at C=O (C-3) of isatin followed by O-protonation; (ii) *nucleophilic substitution* of C-5 of **4** at C–OH (C-3) of 3-hydroxyl-3-pyrimidinyl isatin (**6**) and (iii) *cyclocondensation reaction*. In order to explain the formation of **8** and **9** in our case unlike Bazgir's observation it was initially inferred that it might have been due to the dismutation, but in that case the oxidation

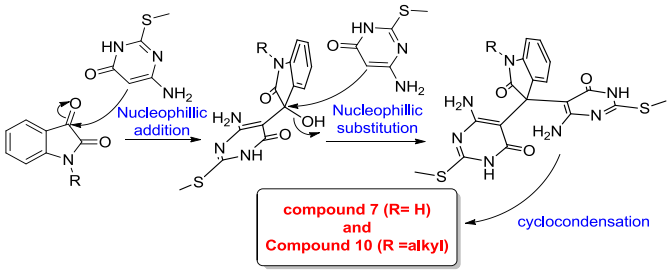
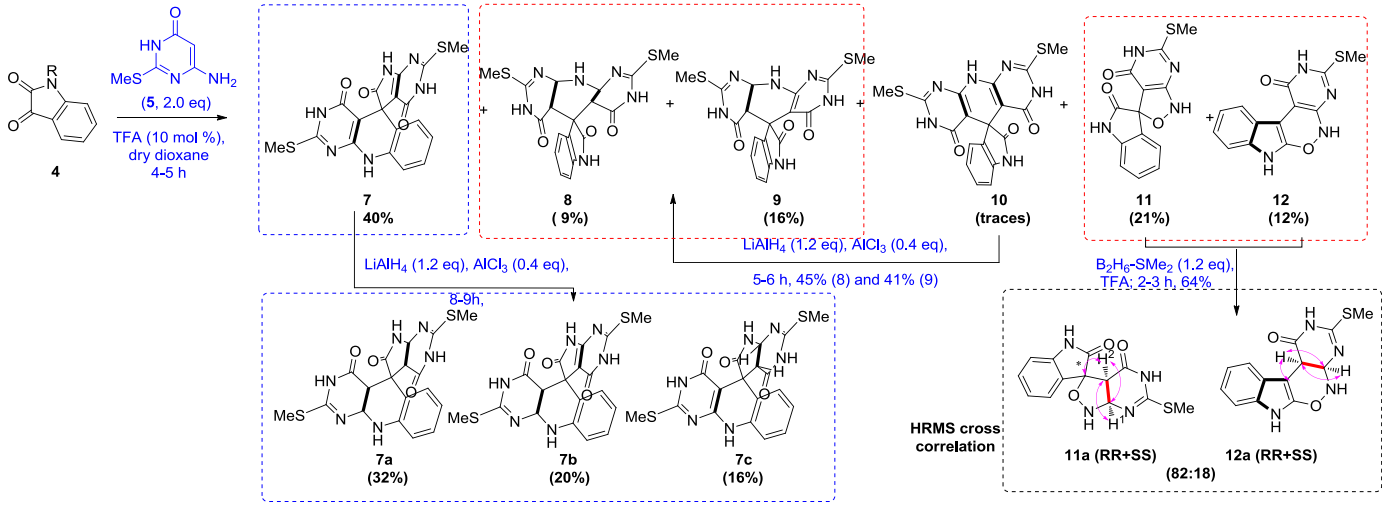


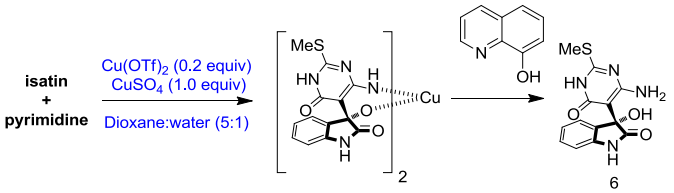
Fig. 2. The three step mechanism proposed by Bazgir et al.



Scheme 1. The formation of different products in the spiroindoline formation and confirmation of structures by unambiguous reduction of spiroindolines.

and reduction of same substrate should have occurred simultaneously, where the structure of **8** and **9** should have corresponded with the structure of **7**. The observed corroboration of **8** and **9** structures with **10** clearly discards this possibility of disproportion reaction. So, in order to investigate the mechanism for the formation of **8** and **9** the attention was focused on the separation of the proposed crucial intermediate **6** and was characterized (by UV and ^{13}C NMR) as its stable copper complex formed in 45% yield with copper triflate (0.2 equiv) and copper sulfate (1.2 equiv) (Scheme 2).

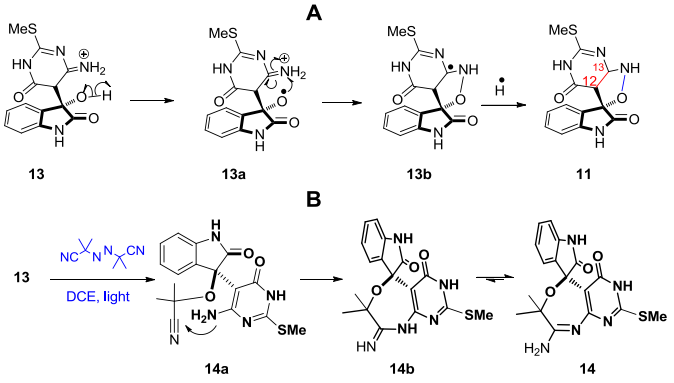
Since, the bimolecular reaction between **6** and **5** in 1:1 ratio would be simpler than the reported and experimented trimolecular reaction between isatin (**4**) and **5** in 1:2 ratios. The intermediate **6** was isolated from its copper complex with 8-hydroxyquinoline, and the **6** thus obtained was treated with 1.0 mol of **5** in the presence of acid catalyst to yield the expected products **7** and **10** but again the mixture of **7**–**12** was obtained confirming the involvement of intermediate **6** in the formation of the products (**7**–**12**). Since the proposed cationic mechanism did not explain the



Scheme 2. Formation of aminol **6**.

formation of **8**, **9** and **11**, **12** therefore different acid catalysts were screened, and it was found that the Brønsted acid catalyzes the reaction more efficiently than the Lewis acids towards the dominance for the formation of **7** and **10** in terms of stoichiometric ratio. Among the ten catalysts (six brønsted and four Lewis acids), TFA was the most effective catalyst for the reaction modulation towards the formation of reported products (**7** and **10**) as the rate of the reaction was 2.5 and 1.5 times faster than the *p*TSA and TfOH, respectively, while the rate with conventional protic acids was similar to that of *p*TSA. On the other hand all the four Lewis acids showed poor results except $FeCl_3$, which resulted in the increased stoichiometric yield of **8**, **9** (15 and 19%, respectively) and **11**, **12** (18% and 16%, respectively) under the similar reaction conditions in the presence of light.

While in the absence of light it leads in the formation of **7** as major product. The results obtained with Iron (**III**) chloride can be explained on the basis of the role of $FeCl_3$ as free radical initiator,[28] which leads the generation of free radical **13a** by homolytic cleavage of O–H (Scheme 3A). In order to prove the free radical mechanism in the formation of **11** and **12**, it appeared of interest to add the free radical initiators viz. *m*CPBA, AIBN etc. in the reaction. Among several conditions the one where *m*CPBA was used along with TFA (*m*CPBA:CF_3COOH=0.2:1) the stoichiometric yields of **11** and **12** superseded over the formation of **7** along with the increase in the yields of **8** and **9**. Thus, indicating that the formation of **8** and **9** is linked with the yields of **11** and **12**. This was further supported through NMR studies carried out on the reaction mixture using *m*CPBA:CF_3COOD (0.2:1) at different time intervals. Since the solvent of the reaction plays an important role in free radical stability and reactivity,[29] hence the role of the solvent in the favour of free radical mechanism[27] was also studied using six different solvents and the best results in terms of stoichiometric yields of **8**, **9** and **11**, **12** were obtained in dichloroethane (DCE) followed by *o*-dichlorobenzene (DCB) using TFA–*m*CPBA system in the presence of light (Table 1).

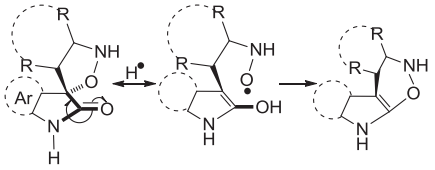


Scheme 3. A. The formation of spiroisoxazoline and depictation of 11; B. reaction of AIBN with resulting in the formation of oxetane ring (**14**).

Table 1
Effect of solvents on the stoichiometric yields of products with and without free radical initiator

Solvent	Ratio of products (**7**/(**8**+**9**)/(**11**+**12**) TFA	Ratio of products (**7**/(**8**+**9**)/(**11**+**12**) TFA:*m*CPBA
EtOH	36/20/30	31/20/28
Dioxane	32/18/26.5	28/traces
2-Me–2-PrOH	23/9/13.7	n.d.
DCE	28/12/24	8/40/60
DCB	26/11/21	10/32/48
H_2O	No reaction	No reaction

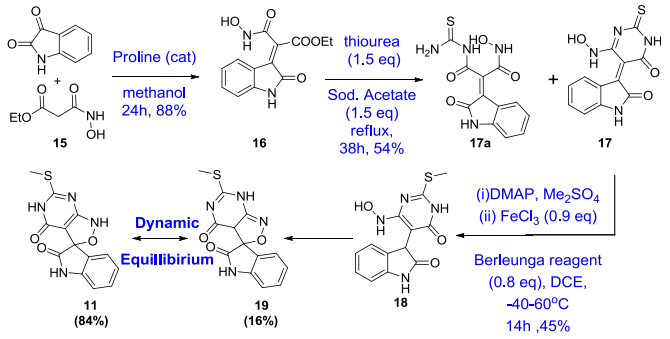
When AIBN was used as the free radical initiator instead of *m*CPBA, the lower yields of **8**, **9** and **11**, **12** were obtained possibly due to the formation of [1,4]-oxazepine derivative (**14**), which was isolated and characterized. The formation of **14** may be explained on the basis of the high reactivity of isopropylcyanide free radical formed during the reaction, which attacks preferably on **13a** to give **14a**. The intramolecular cyclization through nucleophilic attack of NH_2 on the nitrile in **14a** followed by the tautomerism in the **14b** thus formed leads in the formation of **14** (Scheme 3B). The formation of six-membered isooxazoline **12** and its dihydro-analogue **12a** may be explained through the acyl imine tautomerism in **11** and **11a**, respectively,[30] where homolytic cleavage of C–O bond followed by nucleophilic substitution at C2 of isatin and subsequent elimination of a H_2O molecule provides the desired six-membered isooxazoline **12** and **12a**, respectively (Scheme 4).



Scheme 4. Proposed mechanism for the formation of **12** and **12a**.

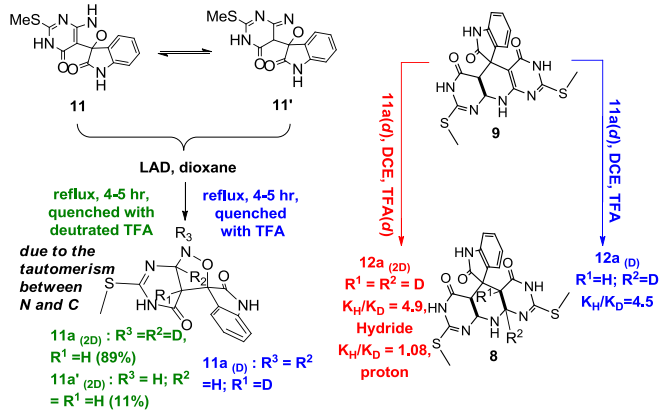
The alternative route for the synthesis of **11** was accomplished successfully commencing from ethyl 3-(hydroxyamino)-3-oxopropanoate (**15**) and isatin in the presence of NaOEt (1.4 equiv) under refluxing conditions to form unsaturated ester **16** in 89% yield. The **16** on reaction with thiourea in the presence of $FeCl_3$ (cat. amt.) gave **17** in 82% yield along with uncyclized intermediate **17a** in the formation of **17**. The S-methylation of **17** with dimethyl sulfate in the presence of DMAP afforded **18** in 45% yields. The treatment of **18** with Berluenga reagent (0.5 mol %) resulted to the formation of the desired spiroisoxazoline **11** in 54% yields. The **11** showed dynamic equilibrium with **19**, in the ratio of 3.7:1.1 (**11**:**19**), as revealed by NMR. In order to check that **11** is the precursor of **12** the **11** was converted to **12** by its treatment with 1.2 equiv of TFA in 72% yield (Scheme 5). The role of **11a** as hydride donor was further analyzed by reacting it with **10** in DCE in the presence of TFA (2.0 equiv) under inert atmosphere where it was found that 2.0 equiv of **11a** resulted the conversion of **10** to its tetrahydro- and dihydro-derivatives **8** and **9** in 34 and 52% yields, respectively.

As conversion of **10** to **8** requires two sequential hydride addition followed by proton transfer, therefore to simplify our study we only considered the second reduction on **9**, which led to the formation of **8**. In order to determine the PKIE[31] (primary kinetic isotope effect) the **11** was reduced with LAD (1.4 equiv) in dioxane under refluxing condition followed by quenching with TFA (1.0 equiv) and water (1.2 equiv). The isotope labelling was



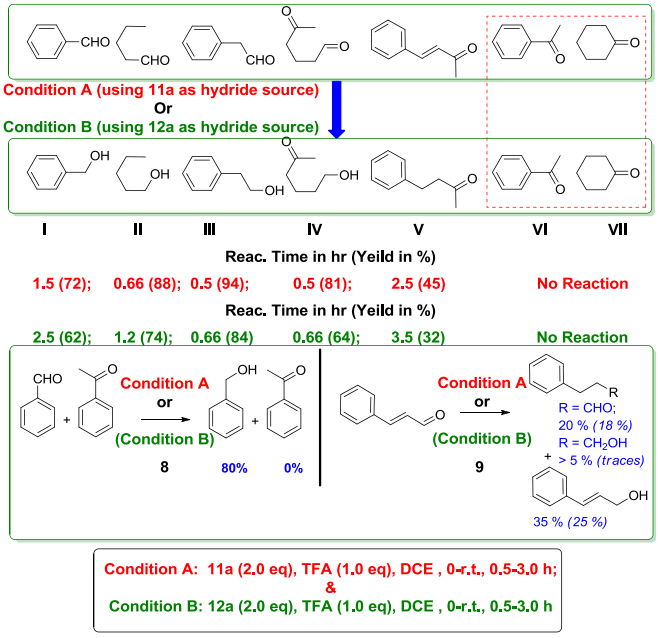
Scheme 5. Alternative route for the synthesis of **11**.

confirmed by the disappearance of H_6 proton signal at 6.07 ppm in ^{1}H NMR and appearance of triplet at 80.3 ppm in ^{13}C NMR. When TFA-*d* and D_2O solvents were employed for quenching, the peak at 54.1 ppm also got split into triplet suggesting the deuteration of the two protons (C–C fusion) of **11a** (d_2). The employment of **11a** (*d*) caused the reduction of **9** in the presence of CF_3COOH to offer **8** (*d*). The addition of deuteride ion took place at C6 and the primary kinetic isotope effect for the hydride transfer (K_H/K_D) was calculated to be 4.5, whereas K_H/K_D (solvent) value for the reaction replacing CF_3COOH from CF_3COOD (1.0 equiv) was found to be 4.9. So, the PKIE value (K_H/K_D) was calculated to be 1.08 thus signifying the Hydride transfer as the rate limiting step in the reduction of **9** to **8** (Scheme 6). This was further confirmed by the reduction of **9** with **11a**+**11a**′ (d_2), where the PKIE value was found to be 4.7. The PKIE value 5.1 obtained in the reduction of **9** by the six-membered oxazolidine [**12a** (*d*)] further confirmed that **11a** and **12a** act as hydride donors in the formation of **8** and **9** during the spiroannulation reaction between isatin and pyrimidine (**5**).



Scheme 6. Deuterium kinetic isotope effect.

In order to broaden the scope of theses novel isoxazolidine (**11a**) and oxazine (**12a**) as hydride source, attempts were made to reduce various aldehydes, ketones, and unsaturated esters. Where spiroisoxazolidine **11a** performed better than **12a** in the reduction of aldehyde but none of them reduced the ketones but they caused selective reduction of unsaturation in αβ-unsaturated systems (Scheme 7). Though the yields of the reduced unsaturated substrates were low but it is the first report of isoxazolidine as hydride source for the selective reduction of aldehyde and alkene in the presence of esters and ketones, as we have successfully reduced aldehydes in the presence of ketones. Thus, these primary scaffolds may be valuable in designing various other hydride donors with better effectivity and selectivity.



Scheme 7. Reduction of various substrates.

3. Conclusion

In summary we have found isoxazolidine as novel hydride source, which was explored to be the reason for the formation of dihydro and tetrahydro-derivative of spiroindoline compounds. We also found that the formation of these isoxazolidine compounds takes place through free radical mechanism and the formation of **11** and **12** can be enhanced by the addition of free radical initiator.

4. Experimental section

4.1. General method

Unless otherwise noted, all starting materials were obtained from commercial suppliers and used without purification. All reactions were carried out under an atmosphere of dry nitrogen. Solutions were evaporated under reduced pressure with a rotary evaporator and the residue was purified by silica gel flash chromatography using an ethyl acetate/hexane mixture as the eluent unless specified otherwise. NMR spectra were recorded on 300 MHz spectrometers. Chemical shifts were reported in parts per million relative to the residual solvent peak (7.26 ppm for $CHCl_3$) for ^{1}H spectra and (77.0 ppm for $CDCl_3$) for ^{13}C spectra. High resolution mass spectroscopy data in electronic impact were recorded with a resolution of 5000 RP at 5%. Electronic impact (EI) and chemical ionization (CI) mass spectroscopies were recorded on a HP5989B device. Infrared spectra were recorded on an FT IR spectrometer in neat for all compounds.

4.2. General procedure

Typical free radical procedure a mixture of 2,6-diaminopyrimidin-4(3*H*)-one (2.0 mmol), isatin (1.0 mmol), TFA (0.5 mmol), and *m*CPBA (1.0 mmol) in refluxing DCE (5 ml) was stirred for 8 h (TLC). After completion of reaction, the reaction mixture was filtered and the precipitate washed with water and then with EtOH to

afford the pure product **7** as a white powder (0.31 g, 40%). The filtrate was evaporated in vacuo and washed the with 10 ml water and extracted with EtOAc (2×20 ml). The organic layer was dried over sodium sulfate (anhyd) and concentrated under pressure to obtain crude substance, which was subjected to flash chromatography (EtOAc/hexane 60:40) to obtain compound **10**, **8**, and **9** at 24, 40 min, 84 min in 1–2%, 8% and 14% whereas the mixture of compound **11** and **12** was found to be contained in the fractions between 5 and 18 min. The fractions of 5–18 min was collected, concentrated, and subjected to flash chromatography (EtOAc/hexane 40:60) to obtain **11** and **12** in 19% and 11% at 20 and 32 min.

*4.2.1. 2,2′-Bis(methylthio)-3H-spiro[pyrimido[4,5-b]quinoline-5,5′-pyrrolo[2,3-d]pyrimidine]-4,4′,6′(3′H,7′H,10H)-trione (**7**).* Mp >240 °C; IR (KBr, ν_{max}, cm^{-1}): 3393, 3152, 1681, 1658, and 1637. ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 8.29–8.06 (s, 1H, NH), 7.38–7.20 (dd, J=5.5 Hz, 2.8, 1H, ArH), 7.20–7.02 (q, J=5.2, 3.5 Hz, 2H, ArH), 6.95–6.73 (m, 1H, ArH), 2.57–2.32 (d, J=5.6 Hz, 6H, 2SMe); ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 174.63, 173.40, 172.14, 167.56, 161.05, 157.95, 156.58, 137.59, 128.81, 126.91, 126.87, 126.15, 126.14, 126.10, 125.01, 115.07, 104.60, 101.02, 60.49, 13.26, 13.11. ESMS m/z (%): 427.1 (M+1); HRMS calculated for $C_{18}H_{14}N_6O_3S_2$: 426.0569. Found: 426.0549.

*4.2.2. 2′,8′-Bis(methylthio)-7′,9a′,10′,10a′-tetrahydro-3′H-spiro[indoline-3,5′-pyrido[2,3-d:6,5-d′]dipyrimidine]-2,4′,6′(4a′H,5a′H)-trione (**8**).* R_f=0.25 (EtOAc/methanol=99:1); Mp=190 °C. IR (KBr, ν_{max}, cm^{-1}): 3429.3, 3250.2, 1684, 1668, 1647.1, 1357.2, 1101.1, 946.1. ^1H NMR (300 MHz, DMSO-d_6, ppm) δ 9.71 (s, 1H, NH), 8.31–7.96 (m, 1H, ArH), 7.28–7.00 (m, 2H, 2ArH), 6.70–6.40 (m, 1H, ArH), 5.74 (dd, J=8.6, 3.9 Hz, 2H, 2NCHN), 3.35 (d, J=8.7 Hz, 2H, 2NCCH), 2.49 (s, 6H, 2SMe). ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 170.82, 165.32, 155.03, 139.21, 128.24, 127.67, 125.88, 120.87, 108.01, 64.03, 60.75, 40.66, 13.68. ESMS: 431.2 (M+1); HRMS calculated for $C_{18}H_{18}N_6O_3S_2$: 430.5039; found 430.5045 along with the molecular ion peak M+Na^+ was also obtained; HRMS calculated for $C_{18}H_{18}N_6O_3S_2Na$: 453.4937; found 453.4954.

*4.2.3. 2′,8′-Bis(methylthio)-10′,10a′-dihydro-3′H-spiro[indoline-3,5′-pyrido[2,3-d:6,5-d′]dipyrimidine]-2,4′,6′(4a′H,7′H)-trione (**9**).* Yellow solid; R_f=0.30 (EtOAc/methanol=99:1); Mp=190 °C. IR (KBr, ν_{max}, cm^{-1}): 3439.3, 3250.2, 1674, 1660, 1644.1, 1337.2, 1101.1, 946.1. ^1H NMR (300 MHz, DMSO-d_6, ppm) δ 10.48 (s, 1H, NH), 7.28 (d, J=7.8 Hz, 1H, ArH), 7.19 (t, J=7.5 Hz, 1H, ArH), 7.13–6.98 (m, 2H, ArH), 5.40 (dd, J=8.7, 3.7 Hz, 1H, NCHN), 3.10 (d, J=8.7 Hz, 1H, NCCH), 2.52 (s, 3H, SMe), 2.45 (s, 3H, SMe). ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 180.36, 167.31, 160.96, 157.61, 157.31, 157.03, 156.73, 150.18, 138.81, 131.78, 128.00, 123.85, 122.33, 107.75, 83.55, 60.72, 49.32, 44.04, 13.24, 12.81; ESI-MS 429.1 (M+1); HRMS calculated for $C_{18}H_{16}N_6O_3S_2$: 428.0725, found: 428.0731.

*4.2.4. 6′-(Methylthio)-1′H-spiro-[indoline-3,3′-isoxazolo[3,4-d]-pyrimidine]-2,4′(5′H)-dione (**11**).* Liquid; R_f=0.40 (EtOAc/methanol=99:1); IR (neat, ν_{max}, cm^{-1}): 3429.3, 3250.2, 1684, 1668, 1647.1, 1357.2, 1101.1, 946.1; ^1H NMR (300 MHz, DMSO-d_6, ppm) δ 7.14 (d, J=7.4 Hz, 2H, ArH), 7.04 (d, J=7.5 Hz, 1H, ArH), 6.93 (t, J=7.3 Hz, 1H, ArH), 2.36 (s, 3H, SMe); ^{13}C NMR (40 MHz, DMSO-d_6+$CDCl_3$, ppm) δ 172.17, 162.45, 159.44, 155.86, 144.09, 127.73, 127.44, 125.58, 122.93, 110.80, 88.88, 86.85, 12.54; ESI-MS 289.4 (M+1), 271 (M−18); HRMS calculated for $C_{13}H_{12}N_4O_2S$: 288.0681, found: 288.0675.

*4.2.5. 3-(Methylthio)-5,7-dihydropyrimido[4′,5′:-3,4][1,2]oxazine [6,5-b]indol-1(2H)-one (**12**).* Yellow liquid; R_f=0.45 (EtOAc/methanol=99:1); IR (KBr, ν_{max}, cm^{-1}): 3429.3, 3250.1, 1674, 1668, 1645.1, 1355.2, 1100.1, 947.1. ^1H NMR (300 MHz, DMSO-d_6, ppm) δ 7.50 (d, J=8.0 Hz, 1H, ArH), 7.47–7.36 (m, 1H, ArH), 7.28–7.14 (m, 2H, ArH), 2.47 (s, 3H, SMe); ^{13}C NMR (40 MHz, DMSO-d_6+$CDCl_3$, ppm) δ 164.15, 162.11, 159.21, 156.48, 138.55, 135.73, 121.53, 121.30, 115.44, 106.68, 106.52, 83.88, 13.01; ESI-MS 287.4 (M+1), 271 (M−18); HRMS calculated for $C_{13}H_{10}N_4O_2S$: 286.0524, found: 286.0521.

*4.2.6. 6′-(Methylthio)-5′,7a′-dihydro-1′H-spiro[indoline-3,3′-isoxazolo[3,4-d]pyrimidine]-2,4′(3a′H)-dione (**11a**).* A solution of borane dimethylsulfide complex (10 M, 0.4 ml) was added dropwise to a stirred solution of **11** (0.454 g, 2.0 mmol) in trifluoroacetic acid (6 ml) at 0 °C in an atmosphere of nitrogen for 10 min. The reaction mixture was stirred at 30 °C for another 3 h, and was then diluted with water (0.4 ml), concentrated and basified with ammonia solution. The aqueous solution was extracted with ethyl acetate (2×30 ml) dried over anhyd Na_2SO_4 on evaporating the organic under vacuo the crude yellowish liquid was purified from flash chromatography. Yield 48%; R_f 0.28 (60:40, EtOAc/hexane); IR (ν_{max}, neat, cm^{-1}): 3451.2, 3321.4, 2905.2, 1677.5, 1654.2, 1602.1, 1341.2, 1265.7, 1097.9, 954.2; ^1H NMR (300 MHz, DMSO-d_6, ppm) δ 10.23 (s, 3H, NH), 7.41 (d, J=7.4 Hz, 1H, ArH), 7.24 (t, J=7.5 Hz, 1H, ArH), 6.98 (t, J=7.3 Hz, 1H, ArH), 6.79 (d, J=7.1 Hz, 1H, ArH), 6.17–5.98 (m, 1H, NCHN), 5.88 (s, 1H, NH), 3.50–3.33 (m, 0.4H, *cis* CH), 3.24 (d, J=11.9 Hz, 0.5H, *trans* CH), 2.55 (s, 3H, SMe); ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 171.23–170.73, 162.38–161.88, 156.95–156.45, 144.01–143.51, 130.17–129.63, 127.66–127.16, 126.05–125.49, 123.40–122.90, 109.60–109.03, 82.28–81.62, 62.64–62.10, 14.04–13.54.; ESI-MS (M+1) 305.2; HRMS calculated for $C_{13}H_{12}N_4O_3S$: 304.0474, found: 304.0480.

*4.2.7. 3-(Methylthio)-4a,5,7,11c-tetrahydropyrimido [4′,5′:3,4] [1,2] oxazino[6,5-b]indol-1(2H)-one (**12a**).* Similar procedure of **11a** formation was carried out using **12**. Yield 36%; yellow liquid; IR (ν_{max}, neat, cm^{-1}); isolation with flash chromatography (EtOAc/hexane=90:10); R_f=0.25 (EtOAc/methanol=99:1); IR (KBr, ν_{max}, cm^{-1}): 3429.3, 3240.2, 1674, 1643, 1611.1, 1347.2, 1191.1, 936.1. ^1H NMR (300 MHz, DMSO-d_6, ppm) δ 7.69–7.45 (m, 1H, ArH), 7.34–7.16 (m, 1H, ArH), 7.13–6.92 (m, 2H, 2ArH), 6.07–5.83 (dd, J=7.5, 4.5 Hz, 1H, CH), 4.69–4.47 (br s, 1H, NH), 4.29–4.16 (d, J=7.8 Hz, 0.6H, CH), 4.14–4.03 (d, J=7.8 Hz, 0.4H, CH), 2.53–2.34 (s, 3H, SCH_3); ^{13}C NMR (75 MHz, DMSO-d_6, ppm). ^{13}C NMR (40 MHz, DMSO-d_6) δ 170.87, 160.80, 151.41, 146.86, 133.18, 124.24, 120.52, 114.67, 108.20, 102.87, 66.79, 66.75, 34.62, 13.83; ESI-MS (M+1) 289.5; HRMS calculated for $C_{13}H_{12}N_4O_2S$: 288.0681, found: 288.0688.

*4.2.8. Reduction of 2,2′-bis(methylthio)-3H-spiro[pyrimido[4,5-b]quinoline-5,5′-pyrrolo[2,3-d]pyrimidine]-4,4′,6′(3′H,7′H,10H)-trione (**7**).* The compound **7** (1.0 mmol) was added to a mixture of dry aluminium chloride (2.0 mmol) and LAH (0.5 mmol) in 25 ml of dry dioxane, which had been refluxed for 8–9 h, 0.25 ml of *tert*-butanol was added and refluxed for 1.5 h then the mixture was stirred overnight. A conventional workup was performed, yielding a product mixture, which immediately was taken for flash chromatography.

*4.2.8.1. 2,2′-Bis(methylthio)-7′,7a′,10,10a-tetrahydro-3H-spiro-[pyrimido[4,5-b]quinoline-5,5′-pyrrolo[2,3-d]pyrimidine]-4,4′,6′(3′H,4aH,4a′H)-trione (**7a**).* Yield=32%; ^1H NMR (300 MHz, DMSO-d_6, ppm) δ=8.14–7.86 (d, J=6.7 Hz, 1H, ArH), 7.86–7.58 (d, J=7.5 Hz, 1H, ArH), 7.25–7.10 (t, J=6.8 Hz, 2H, ArH), 7.08–6.92 (t, J=7.7 Hz, 1H, ArH), 6.52–6.36 (br s, 1H, NH), 6.25–6.07 (m, 1H, CH), 5.88–5.59 (d, J=3.3 Hz, 1H, CH), 4.06–3.83 (s, 1H, ArH), 3.41–3.23 (s, 6H, 2SMe), 2.56–2.31 (s, 6H, 2SMe); ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 173.87, 165.60, 165.34, 163.01, 155.34, 130.41, 126.55, 124.85, 117.87, 114.92, 76.47, 58.87, 53.28, 40.68, 13.56, 13.05; ESMS:

431.2 (M+1); HRMS calculated for $C_{18}H_{18}N_6O_3S_2$: 430.0882; found 430.0887.

*4.2.8.2. 2,2′-Bis(methylthio)-10,10a-dihydro-3H-spiro[pyrimido [4,5-b]quinoline-5,5′-pyrrolo[2,3-d]pyrimidine]-4,4′,6′(3′H,4aH,7′H)-trione (**7b**).* Yield: 20%; ^{1}H NMR (300 MHz DMSO-d_6, ppm) δ=8.14–7.86 (d, J=6.7 Hz, 1H, ArH), 7.86–7.60 (m, 1H, ArH), 7.36–7.15 (m, 2H, ArH), 6.24–6.04 (m, 1H, CH), 4.01–3.83 (s, 1H), 3.45–3.07 (s, 1H, NH), 2.56–2.36 (d, J=6.3 Hz, 6H, 2SMe); ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 173.51, 173.49, 167.71, 163.00, 162.39, 159.96, 157.31, 136.58, 126.34, 125.76, 124.20, 115.23, 83.03, 80.84, 56.30, 13.37, 13.22; ESMS: 429.2 (M+1); HRMS calculated for $C_{18}H_{16}N_6O_3S_2$: 428.0725; found 428.0735.

*4.2.8.3. 2,2′-Bis(methylthio)-7′,7a′-dihydro-3H-spiro[pyrimido [4,5-b]quinoline-5,5′-pyrrolo[2,3-d]pyrimidine]-4,4′,6′(3′H,4a′H,10H)-trione (**7c**).* Yield: 16%; ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ=7.19–7.07 (m, 1H, ArH), 7.05–6.86 (m, 3H, ArH), 6.47–6.32 (s, 1H, HN), 5.91–5.79 (d, J=9.4 Hz, 1H, CH), 3.15–2.95 (s, 1H, CH), 2.54–2.32 (d, J=12.4 Hz, 6H, SMe); ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 173.36, 171.21, 167.32, 157.39, 156.30, 155.90, 147.80, 126.85, 122.73, 120.01, 114.40, 91.12, 57.18, 49.33, 44.06, 13.41; ESMS: 429.2 (M+1); HRMS calculated for $C_{18}H_{16}N_6O_3S_2$: 428.0725; found 428.0739.

*4.2.9. Alternative synthesis of 6′-(methylthio)-1′H-spiro[indoline-3,3′-isoxazolo[3,4-d]pyrimidine]-2,4′(5′H)-dione (**11**)*

*4.2.9.1. Ethyl 3-(hydroxyamino)-3-oxo-2-(2-oxoindolin-3-ylidene)propanoate (**16**).* To a solution of isatin (1.0 mmol) in methanol was added ethyl 3-(hydroxyamino)-3-oxopropanoate (1.2 mmol) followed by the addition of proline (0.1 mmol) as catalyst and refluxed the reaction mixture for 5.5 h, after completion of the reaction, the solvent was evaporated, added water (5 ml) and extracted with ethyl acetate. The organic layer was dried over sodium sulfate, evaporated to obtain the crude solid, which was further purified through column chromatography, SiO_2 (60–120 mesh), with gradient polarity of 1–5% ethyl acetate/hexane to obtain **16**. Yield 92%; IR (ν_{max}, neat, cm^{-1}) 3405, 3312, 2800.2, 1723.2, 1645.2, 1618.2, 1425.1, 1105, 995.2; ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 10.67 (s, 1H), 10.43 (s, 1H), 8.33 (d, J=7.6 Hz, 1H, ArH), 8.21 (s, 1H, NH), 7.39 (t, J=7.5 Hz, 1H, ArH), 7.02 (t, J=8.0 Hz, 1H, ArH), 6.89 (d, J=8.9 Hz, 1H, CH), 4.19 (q, J=7.1 Hz, 1H, CH), 1.22 (t, J=7.1 Hz, 3H, SMe); ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 166.24, 162.79, 156.27, 141.97, 137.44, 130.19, 128.84, 124.78, 121.61, 121.55, 119.63, 109.76, 59.66, 14.04, 13.99; ESI-MS 277.5 (M+1); HRMS calculated for $C_{13}H_{12}N_2O_5$: 276.0746, found: 276.0741.

*4.2.9.2. (Z)-3-(4-(Hydroxyamino)-6-oxo-2-thioxo-1,6-dihydropyrimidin-5(2H)-ylidene)indolin-2-one (**17**).* The obtained solid of α,β unsaturated substrate (16, 1.0 mmol) was dissolved in 15 ml DMF, then added thiourea (1.5 mmol), to this reaction mixture followed by addition of sodium acetate (1.5 mmol), the reaction mixture was refluxed for 15 h. On completion of the reaction 50 ml water was added and the extracted with ethyl acetate (2×30 ml), dried over sodium sulfate (Na_2SO_4) and evaporated under vacuo. The obtained crude product was purified with flash column chromatography (55:45 ethyl acetate/hexane) to obtain **17** at 30–40 min flow rate 1.0 ml/min, yield 71%; isolation with flash chromatography (EtOAc/hexane=90:10); R_f=0.25 (EtOAc/methanol=99:1); IR (KBr, ν_{max}, cm^{-1}): 3429.3, 3250.2, 1684, 1668, 1647.1, 1357.2, 1101.1, 946.1. ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 10.67 (s, 1H, NH), 9.05 (d, J=7.6 Hz, 2H, ArH), 7.41 (t, J=7.6 Hz, 1H, ArH), 7.00 (t, J=7.6 Hz, 1H, ArH), 6.87 (d, J=8.1 Hz, 1H, CH), 2.63 (s, 1H, CH); ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 179.90, 165.15, 156.61, 155.84, 141.22, 140.93, 130.19, 124.85, 121.32, 117.26, 109.54; ESI-MS 289.4 (M+1); HRMS calculated for $C_{12}H_8N_4O_3S$: 288.0317, found: 288.0322.

*4.2.9.3. N1-Carbamothioyl-N3-hydroxy-2-(2-oxoindolin-3-ylidene)malonamide (**17a**).* Flow rate 1.0 ml/min; yield=18%; R_f=0.12 (EtOAc/methanol=99:1); IR (KBr, ν_{max}, cm^{-1}): 3429.5, 3254.2, 1674, 1658, 1617.1, 1347.2, 1121.1, 946.1. ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 10.78–10.61 (s, 1H, NH), 10.56–10.31 (s, 2H), 10.19–10.04 (s, 2H), 8.54–8.34 (d, J=8.1 Hz, 2H), 8.28–8.13 (s, 2H, NH_2), 8.09–7.88 (s, 1H, NH), 7.54–7.28 (t, J=7.8 Hz, 2H, ArH), 7.19–7.02 (t, J=7.7 Hz, 1H, ArH), 6.97–6.85 (dd, J=7.1, 1.9 Hz, 1H, ArH); ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 174.37, 166.60, 158.01, 155.72, 147.96, 138.88, 130.00, 126.44, 125.28, 121.54, 119.66, 109.51, 109.45; ESI-MS 307.6 (M+1); HRMS calculated for $C_{12}H_{10}N_4O_4S$: 306.0423, found: 306.0420.

*4.2.9.4. 6′-(Methylthio)-1′H-spiro[indoline-3,3′-isoxazolo[3,4-d]pyrimidine]-2,4′(5′H)-dione (**11**).* The isolated pure thiol (**17**) was dissolved in minimum DMSO and cooled the reaction mixture to 0 °C, 1.5 equiv potassium carbonate was added followed by the addition of Dimethyl sulfate (1.5 equiv) and heated the reaction mixture for 10–12 h after completion of the reaction, 30 ml of water was added and extracted the aqueous reaction mixture with ethyl acetate (2×30 ml), dried over anhyd sodium sulfate and evaporated the organic layer under vacuo. The crude product thus obtained was further purified by column chromatography (90:10 EtOAc/hexane). To the solution of thus obtained *S*-methylated **17** derivative (0.288 g, 1.0 mmol) in DCE (5.0 ml) and DMSO (2–3 drops to get the clear solution) at −40 °C, Iron(III) chloride (0.145 g, 0.9 mmol) was added followed by the addition of bis(pyridine) iodonium tetrafluoroborate (IPy_2BF_4, 0.297 g, 0.8 mmol) in Nitrogen atmosphere. The reaction was allowed to attain room temperature then heated to 60 °C and stirred for 14 h, after the completion of the reaction (as per TLC) 5 ml water was added at 0 °C and was stirred for 30–40 min then extracted with ethyl acetate (4×10 ml), dried and evaporated the organic layer to afford compound **19** along with compound **11**. The mixture on heating with SiO_2 under solvent free condition gets converted to **11** in 84% yields (^{1}H NMR and ^{13}C NMR matches with the previously obtained sample), alongside compound **18**.

*4.2.9.4.1. 6′-(Methylthio)-3a′H-spiro[indoline-3,3′-isoxazolo[3,4-d]pyrimidine]-2,4′(7′H)-dione (**19**).* Flow rate 1.0 ml/min; yield=18%; R_f=0.14 (EtOAc/methanol=99:1); IR (KBr, ν_{max}, cm^{-1}): 3423.3, 3249.2, 1694.3, 1587.1, 1357.2, 1101.1, 946.1; ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ=10.30–10.17 (s, 1H, NH), 9.99–9.91 (br s, 1H, NH), 7.51–7.34 (d, J=7.5 Hz, 1H, ArH), 7.30–7.15 (t, J=7.5 Hz, 1H, ArH), 7.08–6.90 (t, J=7.2 Hz, 1H, ArH), 6.85–6.64 (d, J=7.5 Hz, 1H, ArH), 4.09–3.78 (s, 1H, CH), 2.57–2.43 (s, 3H, SCH_3); ESI-MS 303.1 (M+1); HRMS calculated for $C_{13}H_{10}N_4O_3S$: 302.0474, found: 302.0482.

*4.2.9.4.2. (E)-2-(Methylthio)-3H-pyrimido[5′,4′:4,-5]furo[2,3-b]indol-4(9H)-one oxime (**18**).* Flow rate 1.0 ml/min; yield=11%; R_f=0.12 (EtOAc/hexane=99:1); IR (KBr, ν_{max}, cm^{-1}): 3429.3, 3250.2, 1647.1, 1611.2, 1131.1, 986.1; ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 10.09–9.86 (s, 1H, HN), 9.78–9.51 (s, 1H, OH), 8.28–8.01 (dd, J=7.4, 1.7 Hz, 1H, ArH), 7.76–7.61 (d, J=7.5 Hz, 1H, ArH), 7.61–7.30 (m, 3H, ArH), 2.86–2.31 (s, 3H, SMe); ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 160.97, 159.85, 159.15, 145.79, 139.63, 124.02, 123.47, 122.08, 121.12, 117.37, 105.33, 103.86, 13.88; ESI-MS 287.7 (M+1); HRMS calculated for $C_{13}H_{10}N_4O_2S$: 286.0524, found: 286.0528.

*4.2.10. (R)-3-(4-Amino-2-(methylthio)-6-oxo-1,6-dihydropyrimid in-5-yl)-3-hydroxyindolin-2-one (**6**).* A solution of isatin (1.0 mmol) and pyrimidine (5, 1.0 mmol) in dioxane/water (4:1.0 ml) was stirred along with gradual addition of $Cu(OTf)_2$ (0.1 mmol) and $CuSO_4$ (1.1 mmol) at −10 °C. After 2.5 h the solid precipitated was filtered, washed and dried under vacuum the obtained solid (244 mg, 0.8 mmol) was treated with 8-hydroxyquinoline (0.8 mmol) in dioxane: water system the yellow precipitate thus separated was

filtered through G4 sintered, the filtrate was evaporated under vacuo and used as such in the next step without further purification.

4.2.11. Procedure for the reaction in the presence of AIBN as free radical initiator. The compound **6** (1.0 mmol) was dissolved in DCE (5 ml) and DMSO (2–3 drops to obtain the clear solution), a solution of AIBN (1.5 mmol) in 3 ml of DCE was added dropwise in the presence of light at room temperature. The reaction was stirred for 50–75 min, and was evaporated under vacuo and was purified through flash column using EtOAc/hexane/ACN (50:50:0.005) to obtain a mixture of **14a** and **14** in 15 and 67% (determined by the ^{1}H NMR).

*4.2.11.1. 2-((3-(4-Amino-2-(methylthio)-6-oxo-1,6-dihydropyrimidin-5-yl)-2-oxoindolin-3-yl)oxy)-2-methylpropanenitrile (**14a**).* Flow rate 1.0 ml/min; yield=72%; R_f=0.12 (EtOAc/methanol=98:2); IR (KBr, ν_{max}, cm^{-1}): 3429.3, 3250.2, 1658, 1627.1, 1357.2, 1134.9, 946.1; ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 10.52–10.28 (s, 1H, NH), 7.35–7.06 (t, J=8.6 Hz, 2H, ArH), 7.02–6.88 (d, J=8.1 Hz, 1H, ArH), 6.83–6.74 (d, J=7.5 Hz, 1H, ArH), 6.62–6.41 (s, 2H, NH), 2.58–2.36 (s, 3H, SMe), 1.79–1.65 (s, 6H, 2Me); ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 175.32, 165.30, 162.85, 158.04, 142.24, 128.20, 128.18, 125.88, 123.14, 123.13, 115.51, 111.06, 83.95, 81.30, 63.20, 26.59, 12.93; ESI-MS 372.0 (M+1); HRMS calculated for $C_{17}H_{17}N_5O_3S$: 371.1052, found: 371.1051.

*4.2.11.2. 8′-Amino-7′,7′-dimethyl-2′-(methylthio)-3′H-spiro[indoline-3,5′-pyrimido[4,5-e][1,4]oxazepine]-2,4′(7′H)-dione (**14**).* Flow rate 1.0 ml/min; yield=68%; R_f=0.42 (EtOAc/hexane=60:40); IR (KBr, ν_{max}, cm^{-1}): 3429.3, 1654, 1347.2, 1281.1, 986.1; ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 10.54–10.29 (s, 1H, NH), 7.37–6.97 (dd, J=25.1, 7.5 Hz, 3H, ArH), 6.87–6.72 (d, J=8.7 Hz, 1H, ArH), 6.71–6.62 (t, J=7.5 Hz, 1H, ArH), 2.58–2.50 (s, 3H, SMe), 1.45–1.40 (s, 2H, Me), 1.39–1.35 (s, 3H, Me); ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 178.71, 171.04, 167.45, 162.68, 160.95, 142.08, 127.72, 127.70, 125.56, 122.59, 111.37, 110.53, 85.16, 81.07, 28.52, 26.53, 12.87; ESI-MS 372.8 (M+1); HRMS calculated for $C_{17}H_{17}N_5O_3S$: 371.1052, found: 371.1042.

*4.2.12. Formation of deuterated **11a** [**11a** (d) and **11a** (d_2)].* The stirred solution of **11** dissolved in DCE-d_2 was treated with the LAD (3.3 mg, 0.8 mmol) at 0 °C, the reaction mixture was refluxed for 3–4 h. After the completion of the reaction (as per TLC) the reaction quenched with the TFA (0.245 ml, 3.2 mmol) followed by the addition H_2O (0.05 ml, 3.2 mmol), the reaction mixture was filtered through sintered (G3) the precipitate was washed with dioxane (3×20 ml), thus obtained organic layer was dried, evaporated in vacuo and purified through flash column chromatography EtOAc/hexane/ACN (50:50:1).

*4.2.12.1. 7a′-Deutro-6′-(methylthio)-5′,7a′-dihydro-1′H-spiro[indoline-3,3′-isoxazolo[3,4-d]pyrimidine]-2,4′(3a′H)-dione (**11a**–**d**).* ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.23 (s, 1H, NH), 7.35 (d, J=7.6 Hz, 1H, ArH), 7.26 (t, J=7.5 Hz, 1H, ArH), 7.16–6.97 (m, 2H, ArH), 5.89 (br s, 1H), 4.21 (s, 1H, CH), 2.50 (s, 3H, SMe); ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 170.99, 162.11, 156.71, 143.77, 129.91, 127.41, 125.74, 123.16, 109.37, 109.33, 82.69, 82.04, 81.43, 61.04, 13.80; ESI-MS (M+1) 306.7; HRMS calculated for $C_{13}H_{11}DN_4O_3S$: 305.0693; found 305.0696.

*4.2.12.2. 1′,7a′-Dideutro-6′-(methylthio)-5′,7a′-dihydro-1′H-spiro[indoline-3,3′-isoxazolo[3,4-d]pyrimidine]-2,4′(3a′H)-dione (**11a**-d_2).* The stirred solution of 11 dissolved in DCE-d_2 was treated with the LAD (0.8 mmol) at 0 °C, the reaction mixture was refluxed for 3–4 h. After the completion of the reaction (as per TLC) the reaction quenched with the TFA-*d*, (3.2 mmol) followed by the addition D_2O (0.05 ml, 3.2 mmol), the reaction mixture was filtered through sintered (G4) the precipitate was washed with dioxane (3×20 ml), thus obtained organic layer was dried, evaporated in vacuo and purified through flash column chromatography EtOAc/hexane/ACN (50:50:1); yield=54%; ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 10.23 (s, 1H, NH), 7.35 (d, J=7.6 Hz, 1H, ArH), 7.26 (t, J=7.5 Hz, 1H, ArH), 7.14–7.02 (m, 2H, ArH), 5.89 (br s, 0.1H, NH), 4.26 (s, 0.89H, CH), 2.50 (s, 3H, SMe); ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 170.99, 162.11, 156.71, 143.77, 129.91, 127.41, 125.74, 123.16, 109.36, 82.69, 82.04, 81.47, 60.35, 59.85, 59.35, 58.66, 13.80; ESI-MS 307.1 (M+1) HRMS calculated for $C_{13}H_{10}D_2N_4O_3S$: 306.0756 found: 306.0764.

*4.2.13. Formation of deuterated **12a** (d) and **12a** (d_2): similar experimental procedure was repeated using **12** as starting material*

*4.2.13.1. 4a-Deutro-3-methyl-4a,5,7,11c-tetrahydropyrimido[4′,5′:3,4][1,2]oxazino[6,5-b]indol-1(2H)-one (**12a**–**d**).* ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 10.23 (s, 1H, NH), 7.35 (d, J=7.6 Hz, 1H, ArH), 7.26 (t, J=7.5 Hz, 1H, ArH), 7.16–6.97 (m, 2H, ArH), 5.89 (br s, 1H), 4.21 (s, 1H, CH), 2.50 (s, 3H, SMe); ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 170.99, 162.11, 156.71, 143.77, 129.91, 127.41, 125.74, 123.16, 109.37, 109.33, 82.69, 82.04, 81.43, 61.04, 13.80. ESI-MS 306.7 (M+1); HRMS calculated for $C_{13}H_{11}DN_4O_2S$: 289.0744; found 289.0751.

*4.2.13.2. 4a,11c-Dideutro-3-methyl-4a,5,7,11c-tetrahydropyrimido[4′,5′:3,4][1,2]oxazino[6,5-b]indol-1(2H)-one (**12a**-d_2).* Similar experimental procedure was repeated using **12** as starting material; yield=42%; ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 7.69–7.43 (m, 1H, ArH), 7.36–7.15 (m, 1H, ArH), 7.15–6.92 (m, 2H, ArH), 4.57 (br s, 0.5H, NH), 3.96 (s, 0.5H, CH), 2.47 (s, 3H, SMe); ^{13}C NMR (40 MHz, DMSO-d_6, ppm) δ 170.86, 160.79, 151.40, 146.86, 133.19, 129.57, 124.24, 120.54, 120.50, 114.68, 114.62, 108.19, 102.84, 69.81, 69.17, 68.57, 36.71, 36.18, 35.64, 35.13, 13.83; ESI-MS 307.1 (M+1) HRMS calculated for $C_{13}H_{10}D_2N_4O_2S$: 290.0806; found: 290.0812.

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Supplementary data

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