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Research Article

Biologically Significant Selenium-Containing Heterocycles; Selenium Redox Biochemistry of Zinc–Sulfur Coordination Sites in Proteins and Enzymes

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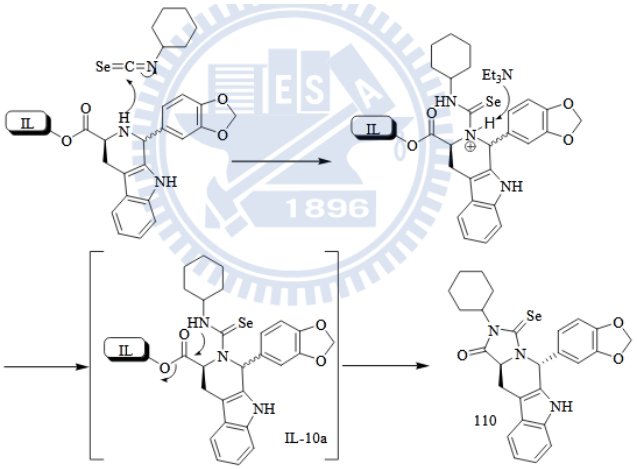
GRAPHICAL ABSTRACT



A mixture of cyclohexanyl isocyanide (0.1g,0.92mmol,1equiv)and selenium powder (0.21g,2.76mmol,3equiv) was dissolved by dichloromethane in 5 ml CEM reactor , The vial was sealed, immediately irradiated at 90 °C (by modulation of the power) for 3˜5 min. Column chromatography (hexane, Rf =0.4) ofthe crude residue yielded compound105 as a yelow oil. A mixture of N-(3-phenylpropyl) form amide (0.1 g, 0.61 mmol, 1equiv) and Cyanuric chloride (0.22g, 1.2 mmol, 2 equiv) was dissolved by dichloromethane in 5 ml CEM reactor, than triethylamine was added until to pH become 8˜9 in ice bath. The vial was sealed, immediately irradiated at 90 °C (bymodulation of the power) for 5˜10 min. The solution was cooled rapidly at room temperature by passing compressed air through the microwave cavity for 1 min, then diluted with CH2Cl2 and washed with a solution of buffer pH 8˜10 Na2CO3. Removal of the solvent under vacuum. Ethyl formate (120 mmol) was added dropwise to phenylpropanyl amine (40 mmol) at room temperature, and the resulting mixture was refluxed for 4-6 h. The excess ethyl formate was removed under reduced pressure to yield the colorless oil.

INTRODUCTION

Selenium has been increasingly recognized as an essential element in biology and medicine. Its biochemistry resembles that of sulfur, yet differs from it by virtue of both redox potentials and stabilities of its oxidation states. Selenium can substitute for the more ubiquitous sulfur of cysteine and as such plays an important role in more than a dozen selenoproteins. We have chosen to examine zinc–sulfur centers as possible targets of selenium redox biochemistry. Selenium compounds release zinc from zinc/thiolate-coordination environments,

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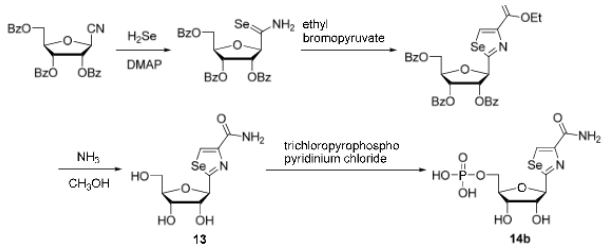
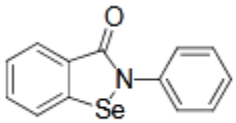
thereby affecting the cellular thiol redox state and the distribution of zinc and likely of other metal ions. Aromatic selenium compounds are excellent spectroscopic probes of the otherwise relatively unstable functional selenium groups. Zinc-coordinated thiolates, e.g., metallothionein (MT), and uncoordinated thiolates, e.g., glutathione, react with benzeneseleninic acid (oxidation state +2), benzeneselenenyl chloride (oxidation state 0) and selenocystamine (oxidation state −1). Benzeneseleninic acid and benzeneselenenyl chloride react very rapidly with MT and titrate substoichiometrically and with a 1:1 stoichiometry, respectively. Selenium compounds also catalyze the release of zinc from MT in peroxidation and thiol/disulfide-interchange reactions. The selenoenzyme glutathione peroxidase catalytically oxidizes MT and releases zinc in the presence of *t*-butyl hydroperoxide, suggesting that this type of redox chemistry may be employed in biology for the control of metal metabolism. Moreover, selenium compounds are likely targets for zinc/thiolate coordination centers *in vivo*, because the reactions are only partially suppressed by excess glutathione. This specificity and the potential to undergo catalytic reactions at low concentrations suggest that zinc release is a significant aspect of the therapeutic antioxidant actions of selenium compounds in antiinflammatory and anticarcinogenic agents.



The chemistry of organoselenium compounds has attracted much attention, not only because of strong interest in these compounds as synthetic tools[1–8]. but also as a result of their unique biological [9,10]. And medicinal activities. [11–19] Hatfield demonstrated the wide importance of organoselenium compounds in human health, especially in cancer chemoprevention, in food, and in plants. Although syntheses of thiazines and oxazines are well known, those of the corresponding selenazines have been limited, owing to difficulties in the preparation of the selenium-containing starting materials. Like many other syntheses of selenium containing heterocycles, they involve the use of toxic selenium reagents, which are often difficult to handle. Isoselenocyanates are very useful starting materials in heterocyclic chemistry because they are easy to prepare [4] and can be stored. Selenazines have attracted much attention not only in medicinal fields (antibacterial effects against Escherichia coli and Staphylococcus aureus, inhibitory effect on the proliferation of human HT-1080 fibrosarcoma cells, protein kinase inhibition, and as antitum or agents, but also as dyes. Nevertheless, the preparation of this ring system is not well described in the literature, and only recently have some reports and reviews on the synthesis and applications of selenazines been published.



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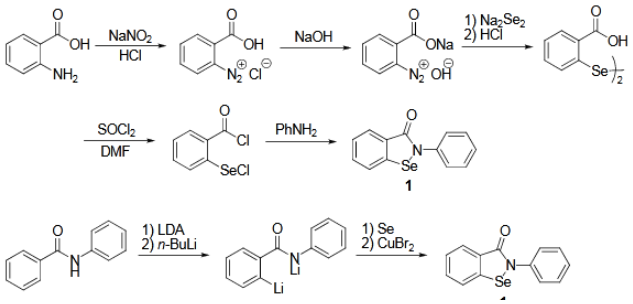
Mammalian metallothioneins (MT) are 7-kDa proteins in which 20 cysteines bind 7 zinc atoms in two clusters, constituting networks of zinc–sulfur interactions unique to biology [1]. This unusual coordination has now been explained in terms of a function of MT by the demonstration that the sulfur ligands and a variety of oxidizing agents interact with concomitant release of zinc. Thus, MT is a temporary zinc reservoir, whose metal content is controlled by redox reactions [2]. The redox potential of MT allows its ready reactions with mild cellular oxidants. In efforts to elucidate the compounds that might oxidize MT in the cell, we have established that both disulfides [3]. And selenium compounds [4]. such as ebselen (2-phenyl-1,2-benzisoselenazol-3(2*H*)-one) [5]. react with MT resulting in the prompt release of zinc at equimolar amounts of reactants. Thus, selenium can function in the redox regulation of thiols and may have a significant role by interacting with zinc coordinated cysteines in cellular zinc metabolism. It is an important aspect of this chemistry that selenium compounds can oxidize thiols under reducing conditions such as those found in the cytosol. Selenium compounds are redox fined-tuned owing to the many different oxidation states of selenium as well as the protein environment in which the element resides and in which it is found catalytically active in peroxidative reactions [6] thiol/disulfide interchange [7]. and reduction of cytochrome *c* [8] or molecular oxygen to superoxide [9]. by thiols. These multiple catalytic potentials may be among the reasons that selenium compounds are so effective while acting at concentrations much lower than the corresponding sulfur analogues.

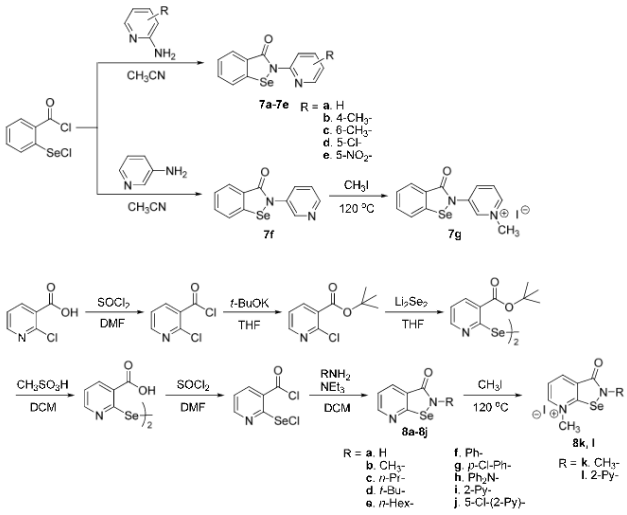


The chemistry of selenium qualitatively resembles that of its more abundant homologue, sulfur, but jointly encompasses much greater oxidoreductive potential, particularly when combined with zinc. A functional, catalytic role of selenocysteine has been investigated mainly in glutathione peroxidase, where the selenium atom changes its oxidation states in the course of the catalytic cycle. The biochemical potential of selenium compounds to undergo redox reactions with regard to MT as well as the release of zinc from its zinc–sulfur clusters has now been investigated by employing compounds displaying functional selenium groups that have been observed in vivo. This has been achieved through relatively stable phenyl derivatives that also serve as UV/VIS spectroscopic probes suitable for the characterization of the time-course of reactions.

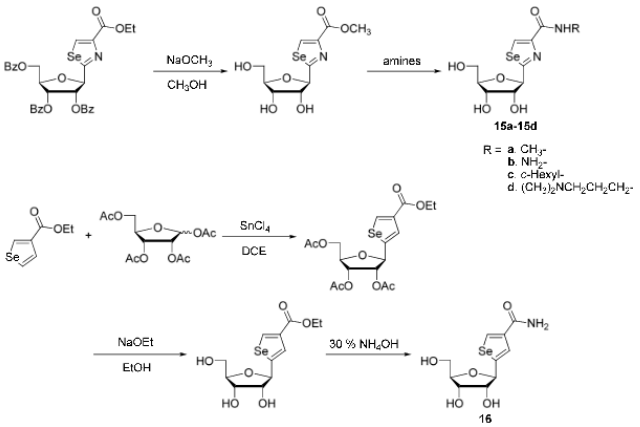
The present study demonstrates that selenium compounds can serve as rather specific, mild cellular oxidants of MT in an overall reducing environment and that they can act as catalysts for zinc release.

RESULTS AND DISCUSSIONS

The formation of selenol (-SeH) was monitored by using 1 mM 1-chloro-2, 4-dinitrobenzene (CDNB) as indicator [12]. in 20 mM Hepes-Na+, pH 7.5. CDNB (from a 100 mM stock solution in methanol) was added to the buffer before the reaction between MT and the selenium compound was initiated. A 10-fold excess of GSH over a given selenium compound served as a standard (100%) for the CDNB titration. An extinction coefficient of ε400 = 2,000 M−1·cm−1 for DNB–selenocysteamine was estimated by reacting 20 μM seleno-DL-cystine with 200 μM GSH for 1 h in the presence of CDNB. The reaction of 200 μM GSH with CDNB was used as a control [12]. Similarly, an extinction coefficient was estimated for DNB–benzeneselenol (ε400 = 8,700 $M^{-1}\cdot cm^{-1}$).



Although selenium has been recognized to be an essential micronutrient, the general chemical basis for its role in biochemistry has remained elusive. The redox biochemistry of selenium has been addressed predominantly in relation to the selenocysteine-containing enzyme glutathione peroxidase (Gpx). Neither the precise redox states of selenocysteine during the catalytic cycle of Gpx nor their redox potentials have been determined directly. Most investigations have focused on model compounds of selenium that exhibit peroxidase activity and among those, ebselen is prominent [6]. Yet the biological chemistry of selenium is neither limited to its reaction with hydrogen peroxide and GSH nor to the presence of selenocysteine. Glutathione selenotrisulfide, selenophosphate, and methylated selenium compounds, e.g., all occur *in vivo* The most striking feature of the redox chemistry of selenium, however, is the interaction with thiols even if and when they serve as metal ligands of zinc. We have now shown that a range of selenium compounds of different oxidation states react rapidly with the zinc–sulfur clusters in MT. The reactivity of benzeneselenenyl chloride, benzeneseleninic acid, and ebselen [5]. with MT or GSH far surpasses the reactivity of disulfides or strong oxidizing agents like ferricyanide or hydrogen peroxide.



The redox potential of diselenodiacetic acid is approximately −400 mV vs. the standard hydrogen electrode and, hence, there is little awareness that selenium compounds are oxidants toward thiols. Yet, most of the compounds examined here react specifically with thiols but not with any other amino acid side chain. According to the limited number of redox potentials of selenium compounds that are known, selenol should reduce disulfides, but thiols should not reduce diselenides [7]. Apart from questions about the accuracy of these indirectly measured redox potentials, selenium undergoes further reactions that shift the redox equilibria expected in the reactions studied. In catalytic reactions, selenol, e.g., is oxidized easily by traces of oxygen and by *t*-butyl hydroperoxide and disulfides. In such cases, a redox reaction followed by a chemical conversion of one of the products would explain the bias in redox potentials and would allow the reaction to proceed in the direction outlined. Such a mechanism explains the formation of selenol and the consumption of thiols in reactions of MT with selenocystamine, benzeneselenenyl chloride, and benzeneseleninic acid.

The redox chemistry of selenium compounds of oxidation states +2, 0 and −1 with zinc–sulfur clusters identifies these compounds as a prominent group of biological redox catalysts. Selenocysteine and other selenol derivatives undergo rapid peroxidation, forming highly reactive selenenic and seleninic acids. These compounds strongly interact with zinc–sulfur clusters of MT, while in contrast, their corresponding sulfur analogues are considerably less reactive.

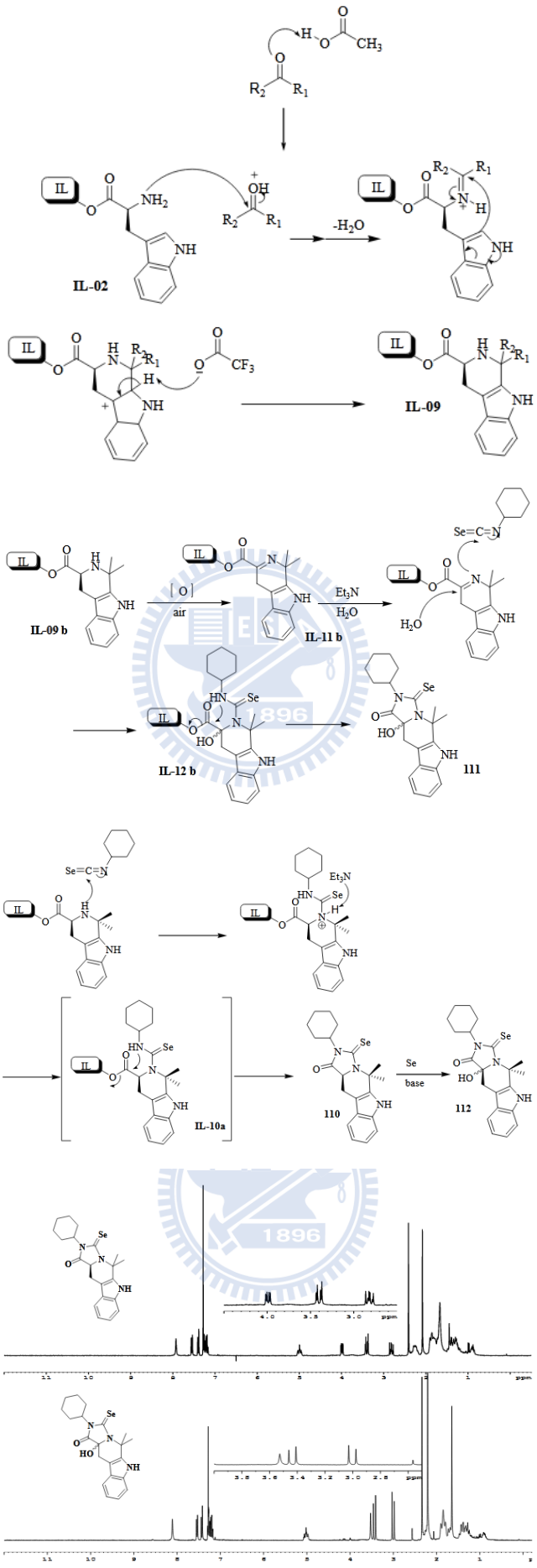
Although all selenium compounds investigated also react with GSH, zinc release from MT is observed even if the concentration of GSH exceeds that of the selenium compound 5-fold, corresponding to a 200- to 500-fold excess of GSH over MT. This ratio is similar to that encountered under physiological conditions [13]. Apparently, the reactivity of mixed selenodisulfides as reaction products between selenium compounds and GSH is sufficient to oxidize MT thiols. As a consequence, these selenium compounds constitute a class of thiol reagents that have great potential significance for biology because they are highly reactive toward zinc–sulfur clusters under overall reducing conditions and can enter catalytic cycles in the presence of other oxidizing agents. We are unaware of any

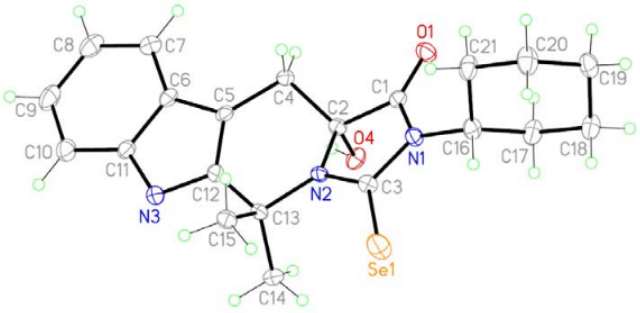
other class of compounds that exhibits these features to the extent detailed here.

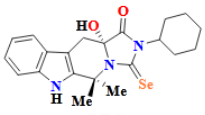
The selenium chemistry cited herein therefore enables the design of redox agents that can target zinc–sulfur bonds, not only in MT but in zinc fingers, zinc twists, and in many transcription factors and signaling proteins. The considerable potential of such compounds as antiviral and anticancer drugs is apparent, and the activity of some selenodrugs based on an interaction with zinc–sulfur centers can be anticipated but has not been systematized.

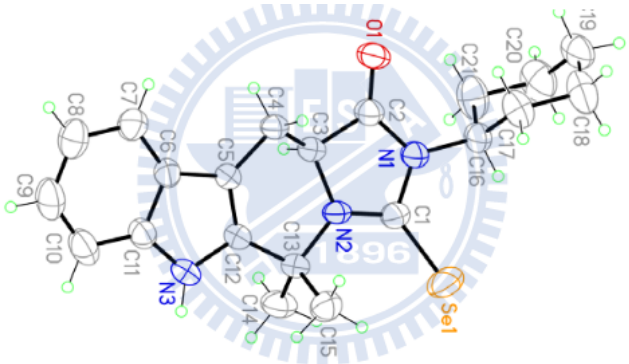
There are clearly numerous directions and approaches for the design of reagents and reactions for pharmaceutical exploitation of redox reactions employing the thiol/selenol, disulfide/diselenide and selenenic acid/sulfenic acid oxidation states. They cover a wide range of redox potentials that can be redox fine-tuned further by the protein environment in which they are placed. The redox potentials of biological selenium compounds are sufficiently low and their reactivities sufficiently high that they can and will act as oxidants even in the overall reducing environment of the cytosol. For all oxidation states, highly thiol-reactive species can be designed that can undergo biological redox reactions in conjunction with zinc–sulfur coordination sites.

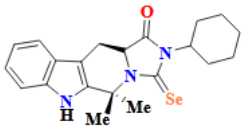
The close resemblance and interrelationships of the biological chemistries of selenium and sulfur have become apparent only recently as a part of selenocysteine-containing enzymes where selenium plays a pivotal part in the antioxidant defense mechanism of the cell. Peroxides are destroyed by using GSH as an "electron pool." However, the importance of this selenium chemistry for targeting zinc–sulfur clusters and thereby controlling zinc-dependent functions apparently has not been considered. Selenium compounds reduce peroxides while concomitantly releasing zinc very rapidly, thereby suggesting a potential role of selenium in cellular "metal trafficking." Thus, reactive oxygen species generated in oxidative stress release zinc from MT. Both zinc and thionein, T, the apoform of MT, can then function as cellular antioxidants, with the latter then becoming an important endogenous chelating agent of zinc [20]. Thus, the oxidant effect of selenium compounds on MT and zinc will likely be part of the antioxidant functions of selenium.

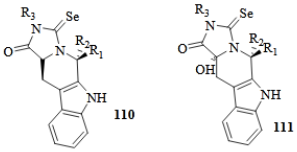


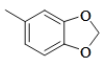
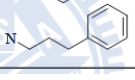




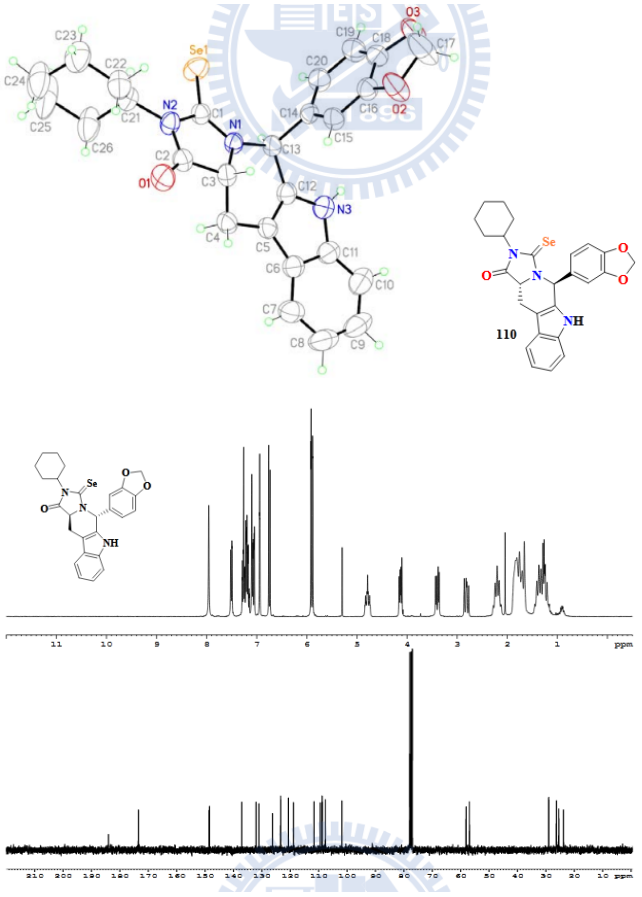






entry[a]	R_1	R_2	R_3	Isolated yield (%)[b]
110a		H		74%
110b	CH_3	CH_3		46%
111a	CH_3	CH_3		32%
111b	CH_3	CH_3		48%

2-Amino-4, 5, 6, 7-tetrahydro-1-benzoselenophene-3-carbonitrile (I) was prepared according to the proce-dure reported in [14] with some modifications: metallic selenium was added to cyclohexylidenemalono-dinitrile in ethanol in the presence of triethylamine as catalyst. Compound I was brought into reaction with ethylenediamine in the presence of carbon disulfide to obtain 2amino 3 (4,5 dihydro 1H imidazol 2 yl) 4,5,6,7 tetrahydro 1 benzoselenophene (II) and 2, 3, 5, 6, 8, 9, 10, 11 octahydroimidazo [2,1-c] [1] benzo-selenopheno [3,2-e] pyrimidine-5-thione (IV) as by product (Scheme 1). Compound II was subjected to heterocyclization to tetracyclic imidazobenzoseleno-phenopyrimidine systems in different ways. Treatment of II with triethyl orthoformate gave 2,3,8,9,10,11-hexahydroimidazo[2,1-c][1]benzoselenopheno[3,2-e]-pyrimidine (III). The reaction of II with benzaldehyde under conditions analogous to those reported in [18]. led to formation of 5-phenyl-2,3,5,6,8,9,10,11 octa hydroimidazo[2,1 c] [1]benzoselenopheno[3,2 e]pyri midine (V). Thione IV was also obtained by heating compound II with carbon disulfide in boiling dry pyr-idine. The same product was also formed together with II directly from nitrile I, ethylenediamine, and excess carbon disulfide at 60°C (reaction time 48 h); it can be isolated by fractional crystallization from ethanol. Samples of IV prepared by the two methods were identical in chemical and physical properties.



CONCLUSIONS

In summary, we have shown that 1, 3-selenazolidin-2- imines 9 and 1, 3-selenazin-2-imines 10 can easily be synthesized with excellent yields in one-pot reactions by starting from aryl and alkyl isoselenocyanates and ω-haloalkylamines in basic media. The analogous reaction with isothiocyanates opens a novel and efficient route to the corresponding sulfur heterocycles.

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